FROM MOLECULAR PHYLOGENETICS TO THE EVOLUTION OF LIFE HISTORY AND EDAPHIC ENDEMISM: A COMPREHENSIVE APPRAISAL OF EVOLUTION IN *ERIOGONUM* (POLYGONACEAE)

By

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ABSTRACT

In the age of DNA sequencing, understanding relationships in groups of organisms and examining patterns of evolution of various characteristics of these organisms is closely tied to the study of molecular phylogenetics. This research project begins with an overall molecular phylogenetic study of the species-rich plant genus *Eriogonum* (Polygonaceae). DNA sequence data (plastid and nuclear) were used to reconstruct relationships in *Eriogonum,* with a special emphasis on the diverse subgenus *Eucycla.* Currently recognized subgenera are not monophyletic with clear evidence of multiple reversals from perennial to annual life histories. Members of *Eriogonum* subg*. Eucycla* belong to three distinct clades within the genus. Within the most diverse clade, here referred to as *Eucycla II,* numerous biological sources of gene tree discordance are impacting phylogenetic reconstructions based on the gene regions examined. Incomplete lineage sorting and/or hybridization and introgression are likely the main drivers of incongruence with the presented phylogenies. Specific evidence of hybridization is associated with certain species. In the widespread species *E. shockleyi,* extensive chloroplast haplotype diversity was recovered, indicative of a history of hybridization with other species, retention of ancestral haplotypes, or incomplete lineage sorting. Field, herbarium, and molecular work uncovered a new species of wild buckwheat, *Eriogonum domitum*, from west-central Utah. Taxonomic changes for three other taxa also resulted from this research.

Patterns of edaphic endemism were examined in a subset of *Eriogonum* species from the *Eucycla II* clade. Soil samples from 46 different populations were

collected and characterized according to numerous physical and chemical properties. Edaphic data were then compared to phylogenies inferred from chloroplast and nuclear sequences. Based on our results, the sampled species in the *Eucycla II* lineage fit the pattern of edaphic generalists, surviving on a wide variety of substrates in the absence of competition from adjacent species. Expanded sampling of capitate members of *Eriogonum* is warranted to further investigate patterns of narrow endemism in this diverse lineage.

INTRODUCTION

The flora of western North America is exceptionally diverse in so many ways. Heterogeneous landscapes, complex geological patterns and localized climate set the stage for a floristically diverse region. The process of speciation is impacted by many factors, but ecological conditions are of great interest (Kruckeberg 1986; Givnish 2010). Species distributions are impacted by abiotic and biotic factors, with climate and substrate type being proposed as two of the major drivers (Mason 1946; Kruckeberg 2002; Rajakaruna 2004). Recent phylogenetic studies have addressed the evolution of edaphic specialists in certain lineages, but they have largely focused on species tolerant of only one type of extreme substrate (e.g. serpentine, gypsum, etc.) (Mayer and Soltis 1994; Patterson and Givnish 2003; Moore and Jansen 2007; Anacker et al. 2012).

We selected the species rich and ecological diverse plant genus *Eriogonum* to address questions of phylogenetic relationships and patterns of edaphic endemism. The genus, one of the most species-rich genera in the Polygonaceae contains over 250 species and is limited in distribution to North America (Reveal 2005). Among these numerous species are both annual and perennial species ranging in size from a few centimeters tall to over 2 m in height. Ecological diversity also abounds in *Eriogonum.* Species can be found at some of the lowest and highest elevations in North America. Additionally, there are numerous rare species with distributions that are limited to extreme substrates. This lineage offers an excellent opportunity to examine patterns of narrow edaphic endemism across a broad range of soils.

A step-wise process was undertaken to begin to answer the questions put forth for this research project. Taxonomically, *Eriogonum* has been well studied over the years (Reveal 1978; 2005). Efforts to understand the evolutionary history of *Eriogonum* by using modern molecular phylogenetic techniques are still in the beginning phases (Sanchez and Kron 2008). The rapidly advancing field of molecular phylogenetics provides the necessary toolkit with which to infer relationships within a lineage, however an extensively sampled study of *Eriogonum* was lacking. We set out to investigate the subgeneric relationships in the species-rich genus *Eriogonum* by inferring phylogenies based on chloroplast (*trnL-trnF, rpS16, trnD-trnT*) and nuclear markers (*ITS, LEAFY*). We found that many of the previously recognized subgenera are non-monophyletic (Reveal 1969; 2005), with the largest subgenus, *Eucycla*, falling in three distinct lineages with other genera in the subfamily Eriogonoideae. An examination of the evolution of life history in this lineage also showed many reversals from the annual habit to the perennial habit, and vice versa. Based upon our reconstructions, there appear to be more shifts from perennial to annual in *Eriogonum* and relatives (Chapter 1).

The second stage of our research examined relationships within the species rich *Eriogonum* subg. *Eucycla* (*Eucycla II*). We sampled this perennial lineage extensively, both with multiple accessions per species and populations. An overall pattern of gene tree discordance was uncovered in this rapidly radiating clade (Chapter 2). Specific instances of hybridization followed by introgression and chloroplast capture are apparent with some species pairs. In addition to an overall signal of probable hybridization, factors such as incomplete lineage sorting and

retention of ancestral haplotypes are also likely affecting phylogenetic reconstruction. It appears that relationships in this lineage are highly reticulate. A discussion of species monophyly and comprehensive species concepts is also included (Knowles and Carstens 2007; Lega et al. 2012; Puillandre et al. 2012).

With a better understanding of the phylogenetic relationships in the *Eucycla II* clade, we investigated patterns of edaphic endemism by comparing data from various soil properties associated with each of the populations (Chapter 3). Widespread species, as well as putative narrow edaphic endemic species, were included in our sampling to compare patterns of generalist and specialist models in this lineage (Gankin and Major 1964; Meyer 1986). Data from measured soil properties from these sites were extremely variable, indicative of a broad range of edaphic conditions associated with these populations of wild buckwheat. Based on our phylogenetic reconstructions and subsequent comparisons of edaphic factors, it appears that this lineage is composed of edaphic generalists with species tolerating a variety of substrates (Chapter 3).

The final chapter of this thesis is a description of a new species from west-central Utah (Chapter 4). *Eriogonum domitum* Grady & Reveal, commonly known as the House Range wild buckwheat, is a geographically isolated and morphologically distinct species. Additionally, three new taxonomic combinations were put forth, based on genetic data (Grady and Reveal 2011).

These studies lay the groundwork for many additional research projects. Addressing the evolutionary history of a non-model organism poses many challenges, but with our work on *Eirogonum*, the stage is set to gain a better

understanding of speciation relating to ecological factors, the causes and consequences of phylogenetic discordance, and conservation of endangered and threatened plant species. The phylogenetic perspective presented here is essential to investigate these issues in a natural setting in a diverse and species rich lineage, such as *Eriogonum.*

LITERATURE CITED

Gankin, R. and J. Major. 1964. *Arctostaphylos myrtifolia,* its biology and relationship to the problem of endemism. *Ecology* 45: 792-808.

Givnish, T. J. 2010. Ecology of plant speciation. *Taxon* 59: 1326-1366.

Grady, B. R. and J. L. Reveal. 2011. New combinations and a new species of *Eriogonum* (Polygonaceae: Eriogonoideae) from the Great Basin Desert, United States. *Phytotaxa* 24: 33-38.

Knowles, L. L. and B. C. Carstens. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* 56: 887-895.

Kruckeberg, A. R. 1986. An essay: the stimulus of unusual geologies for plant speciation. *Systematic Botany* 11: 455-463.

Kruckeberg, A. R. 2002. Geology and plant life: the effects of landforms and rock types on plants. University of Washington Press, Seattle, Washington. pp. 117-155.

Lega, M., S. Foir, F. Prosser, A. Bertolli, M. Li, and C. Varotto. 2012. Application of the unified species concept reveals distinct lineages for disjuncts endemics of the *Brassica repanda* complex. *Biological Journal of the Linnean Society* 106: 482-497.

Mason, H. L. 1946. The edaphic factor in narrow endemism. I. The nature of environmental influences. *Madroño* 8: 209-226.

Mayer, M. S. and P. S. Soltis. 1994. The evolution of serpentine endemics – a chloroplast DNA phylogeny of the *Streptanthus-glandulosus* complex (Cruciferae). *Systematic Botany* 19: 557-574.

Meyer, S. E. 1986. The ecology of gypsophile endemism in the eastern Mojave Desert. *Ecology* 67: 1303-1313.

Moore, M. J. and R. K. Jansen. 2007. Origins and biogeography of gypsophily in the Chihuahuan Desert plant group *Tiquilia* subg. *Eddya* (Boraginaceae). *Systematic Botany* 32: 392-414.

Puillandre, N., M. V. Modica, Y. Zhang, L. Sirovich, M. C. Boisselier, C. Cruaud, M. Holford, and S. Samadi. 2012. Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology* 21: 2671-2691.

Rajakaruna, N. 2004. The Edaphic Factor in the Origin of Plant Species. *International Geology Review* 46: 471-478.

Reveal, J. L. 1969. The subgeneric concept in *Eriogonum* (Polygonaceae) Pp. 229-249 in *Current Topics in Plant Science.* ed. J. Gunckel. New York: Academic Press.

Reveal, J. L. 1978. Distribution and Phylogeny of Eriogonoideae (Polygonaceae). *Great Basin Naturalist Memoirs* 2: 169-190.

Reveal, J. L. 2005. Polygonaceae subfam. Eriogonoideae. Pp. 218-478 in *Flora of North America* Vol. 5 ed. Flora of North America Editorial Committee. New York: Oxford University Press.

Patterson, T. B. and T. J. Givnish. 2003. Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): evidence from a cpDNA phylogeny. *New Phytologist* 161: 253-264.

Sanchez A. and K. A. Kron. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Systematic Botany* 33: 87-96.

Phylogenetics and systematics of *Eriogonum* (Polygonaceae) with an emphasis on the species-rich subgenus *Eucycla* and an appraisal of life history evolution

Ben R. Grady and Kenneth J. Sytsma

Eriogonum, a diverse and charismatic genus of the American West, has been well-studied morphologically, but a densely sampled molecular phylogenetic approach has yet to be employed to study relationships within the genus. We sampled across the subfamily Eriogonoideae, with a focus on the large perennial subgenus *Eucycla* (79 of 110 species included). DNA sequence data were generated and analyzed from the chloroplast and nuclear genomes to assess subgeneric relationships and generic monophyly. The evolution of life history (annual or perennial) was then investigated using a phylogenetic approach under maximum parsimony and maximum likelihood. All of the gene regions we used provided phylogenetic resolution at various levels, with few major incongruencies for the major clades highlighted. The current subgeneric classification does not recognize monophyletic groups. The subg. *Eucycla* is polyphyletic, with members belonging to three distinct lineages. All three of these clades include annual members of other subgenera or genera (*Chorizanthe*). The ancestral state for *Eriogonum* and allied genera was likely an annual. A shift to perenniality occurred early in the evolution of *Eriogonum*, with multiple subsequent reversions to annual life history. Reclassification at the generic and subgeneric levels is necessary, with an emphasis on characters that show less convergence.

Key words: *Eriogonum*, Eriogonoideae, subg. *Eucycla*, life history reconstruction, convergence

The sheer number of species and the vast morphological diversity found in *Eriogonum* Michaux have captivated and confounded biologists since its recognition in the early 19th century. With 250+ species in North America, *Eriogonum* is exceeded in species number only by *Carex, Astragalus,* and *Penstemon* (Reveal 2005). Species of *Eriogonum* range from tiny annuals, no more than a few centimeters tall, to arborescent shrubs up to 2 meters high. The diversity of growth form in the perennial species is equally impressive. Habits include shrubs, sub-shrubs, pulvinate, caespitose, and erect herbaceous perennials (Figure 1). While all species of *Eriogonum* have small flowers, the overall floral display can vary dramatically. Some cymose inflorescences can be nearly a meter high, but some species have dense capitate inflorescences that barely exceed the cushion of basal leaves.

Some *Eriogonum* species are widespread across the western United States, occurring in as many as eleven states and parts of Canada (e.g. *E. ovalifolium* Nuttall and *E. umbellatum* Torrey). Others however, are extremely limited in distribution and are of conservation concern. *Eriogonum* also hosts a number of ecological specialists. There are taxa that occur only on continental islands and those that thrive above treeline on some of the tallest peaks in the Sierra Nevada Mountains (Reveal 2005). Countless species of *Eriogonum* have distributions that are apparently strongly influenced by local substrates. Species such as *E. gypsophilum, E. calcareum, E. pelinophilum,* and *E. ammophilum* are just a few examples whose names reflect their affinity to different soil types. Such diversity has led to varying

Figure 1. Diversity of growth form in *Eriogonum*. A. *E. wetherillii* (subg. *Ganysma*), basal rosette of leaves and mature inflorescence, annual, plant ca. 1.5 dm H (Grady 114). B. *E. alatum,* perennial, basal rosette and base of monocarpic inflorescence ca. 10 dm H (Grady 168). C. *E. lobbii* (subg. *Oligogonum*), basal leaves and prostrate inflorescences, plant <1 dm H (Grady 276). D. *E. acaule* (subg. *Eucycla*), densely mound-forming perennial growth form with cymose inflorescences, ca. 1 dm H (Grady 480). E. *E. viridulum* (subg. *Eucycla*), erect, herbaceous perennial growth form, ca. 3 dm H (Grady & Koopman 595). F. *E. rosense* (subg. *Eucycla*), caespitose perennial growth form with capitate inflorescences, plant ca. 2 dm H (Grady 283). G. *E. butterworthianum* (subg. *Eucycla*), perennial shrub, plant ca. 4 dm H (Grady 242). H. *E. umbellatum* (subg. *Oligogonum*), close up of umbellate inflorescence, basal leaf, and individual flowers with stipe-like base of perianth. I. *E. elongatum* (subg. *Eucycla*), close up of appressed involucre and flowers (Grady 238).

taxonomic treatments at all levels (Reveal 1969; 1978; 2005; Burke and Sanchez 2011).

Eriogonum is a member of the Eriogonoideae subfamily, distinguished from other members of the Polygonaceae by the lack of a well-defined ochrea and the presence of an involucre surrounding the numerous small flowers (Reveal 1978; Sanchez and Kron 2008). Members of Eriogonoideae are limited to North and South America, with nearly all of the species distributed throughout the arid portions of the western United States (Reveal 1978; Reveal 2005). *Eriogonum* accounts for approximately three quarters of the species richness in the subfamily, followed by *Chorizanthe* with 50 species. The remainder of the species in the subfamily are scattered throughout small segregate genera. Although tribal and subtribal classifications have been proposed for Eriogonoideae, a well-sampled phylogenetic hypothesis has yet to be published to support any such taxonomic groupings.

The most current taxonomic treatment divides *Eriogonum* into eight subgenera, based on morphological differences (Reveal 2005). Features such as flower shape, habit, involucre morphology, leaf pubescence, and achene shape are used to delineate the different subgenera. The distributions and number of species currently included in each subgenus is shown in Figure 2, with exact numbers of species per state in Appendix 1. Numbers are based upon Reveal's (2005) Flora of North America treatment and subsequently published species descriptions (Grady and Reveal 2011).

Ideally taxonomic groupings should reflect evolutionary history. Modern molecular systematics has provided an endless toolkit with which to infer

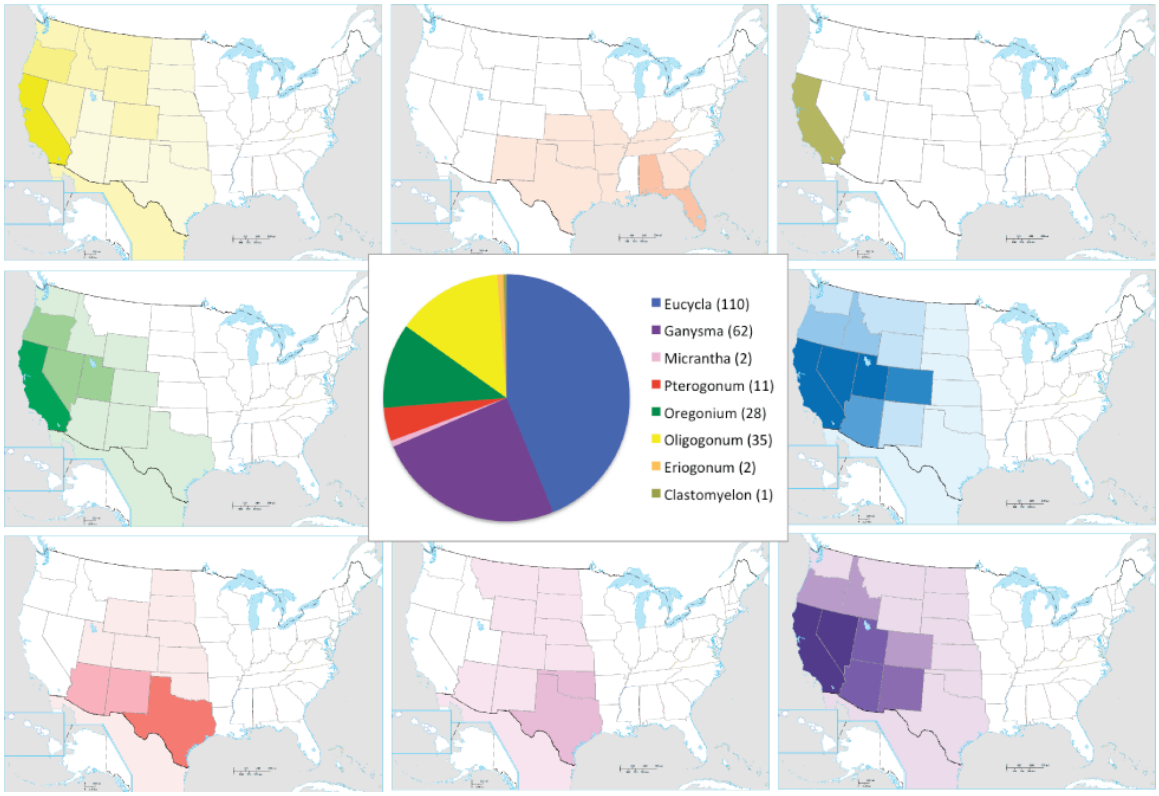


Figure 2. Taxonomic and geographic distribution of species in each of the currently recognized subgenera in *Eriogonum.* Colors of each pie in the chart correspond to an individual distribution map. The intensity of colors on the maps is indicative of relative number of species present in each state. Exact numbers of species per state for each subgenus is shown in Appendix 1.

taxonomic relationships. Molecular phylogenetic studies of the Polygonaceae have been underway for nearly a decade. Lamb Frye and Kron (2003) published the first extensive phylogenetic treatment of the family by utilizing the plastid gene *rbcL*. Additional studies have shown the utility of various gene regions in constructing family-wide evolutionary hypotheses (Sanchez and Kron 2008; Sanchez et al. 2009; Burke and Sanchez 2011; Schuster et al. 2011), and for generic relationships in the Polygonaceae (Wang et al. 2005; Kim and Donoghue 2008; Kim et al. 2008; Brinegar and Baron 2009; Sanchez and Kron 2009). Chloroplast regions and the nuclear

ribosomal *ITS* have been the most commonly employed markers in these studies, however *LEAFY* has been shown to be useful for inferring relationships at the generic level, and below (Kim et al. 2008; Sanchez and Kron 2008).

One component of the diversity exemplified within *Eriogonum* is in life history. It is one of three genera in the Eriogonoideae containing perennial species. *Dedeckera*, a perennial shrub, is a monotypic genus native to the Death Valley region of California (Reveal and Howell 1976). The perennial members of *Chorizanthe* are disjunct and limited to South America, leaving only annual species present in North America (Goodman 1934; Reveal and Hardham 1989). The remaining perennial species are all found within *Eriogonum.* Within *Eriogonum*, there are 162 perennial species from six of the eight subgenera, *Oregonium* and *Micrantha* being composed entirely of annual species (Reveal 2005). The bulk of the perennial species are found in subg. *Eucycla* (110/162).

Mechanisms and patterns of evolution in plant life histories have provided numerous questions for botanical evolutionary biologists. It has been postulated that genetic systems and climate change may be a driving force, causing shifts in life histories from perennial to annual, or vice versa (Stebbins 1950). Recent molecular studies in *Sidalcea* (Andreassen and Baldwin 2003; Andreasen 2012), *Nemesia* (Datson et al. 2008), *Lupinus* (Drummond et al. 2012), and *Oenothera* (Evans et al. 2005) have shown multiple instances of life-history shifts. Each of these shifts from perennial to annual life history tends to occur in arid-adapted groups, indicating a potential link with climate and niche influence on plant life history. Tank and Olmstead (2008) present phylogenetic evidence that the large perennial genus

Castilleja has evolved from annual ancestors, with a potential correlation with genome size and polyploidy.

We explore multiple gene regions from different genomic sources for phylogenetic utility in an extensively sampled *Eriogonum* dataset. We used these data to test the monophyly of the currently recognized subgenera in *Eriogonum*, with a focus on the hyper-diverse subgenus *Eucycla*. Additionally, we examine the evolution of annual and perennial habit across the genus.

MATERIALS AND METHODS

Taxonomic Sampling—Sampling across *Eriogonum* for subsequent phylogenetic analyses is focused on extensive coverage of the subgenus *Eucycla*, as circumscribed in the Flora of North America (Reveal 2005). Of the 110 species currently classified in subg. *Eucycla* (Reveal 2005; Grady and Reveal 2011), a total of 79 species are included in our phylogenetic assessment of *Eriogonum*. Representatives from other subgenera within *Eriogonum* and associated genera within Polygonaceae subfamily Eriogonoideae are included to assess the monophyly of the current subgeneric circumscriptions (Sanchez and Kron 2008). The only subgenus within *Eriogonum* not represented in our study is subg. *Clastomyelon*, which is composed of a single perennial species native to California (Reveal 2005). *Gilmania luteola* (Coville) Coville is used as the outgroup for all phylogenetic analyses based on the well-supported sister relationship to other members of the traditionally recognition of the subfamily Eriogonoideae (Sanchez and Kron 2008).

Appendix 2 includes all samples included, voucher information, and GenBank numbers for each accession and region included (well, not yet).

DNA Extraction, Amplification, and Sequencing—Total genomic DNA was extracted from field collected, silica-dried leaf tissue using the Qiagen DNeasy™ plant mini kit (Qiagen, Valencia, California). Undiluted total genomic DNA was then combined with 10x buffer, dNTPs and Ex Taq® taq polymerase from Takara (Otsu, Shiga, Japan) and DMSO, BSA, and forward and reverse primers of interest. All reactions were carried out in 12.5 μL total volume.

The internal transcribed spacer (*ITS*) of the nuclear ribosomal DNA was amplified using the *ITS4* and *ITS5* primers and protocol from White et al. (1990). Chloroplast introns and spacer regions (*trnL-trnF*, *rpS16*, and *trnD-trnT*) were amplified using primers from Taberlet et al. (1991) and Shaw et al. (2005). Internal primers were designed to amplify the entire *trnD-trnT* spacer region. The primer sequences, paired with the *trnD* and *trnT* primers respectively, are as follows: *trnE-m2* 5' GCTTTCTATATCGAATCGAATC 3' and *trnY-m2* 5' CATAGTATGAACAGTTTTTGG 3'. All chloroplast regions, except the *trnD* to *trnE* region, were successfully amplified with the following touchdown program: initial denaturation (94°C for 30 sec); amplification: 10 cycles 94°C 15 sec, 64°C 30 sec -1°C/cycle, and 68°C for 1 min; then 20 cycles 94°C 15 sec, 54°C 30 sec, 68°C 1 min, followed by extended elongation of 68°C for 5 min. The *trnD* to *trnE* spacer region was amplified using the following protocol: denaturation 94°C 1 min; amplification 30 cycles 94°C 15 sec, 44°C 30 sec, and 72°C 1 min; and final extension of 72°C for 5 min.

The second intron of the low-copy nuclear region *LEAFY* was initially amplified in a subset of *Eriogonum* taxa with the degenerate primers, *LFsxl-2* and *LFtxr*, from Frohlich and Meyerowitz (1997). Sanchez and Kron (2008) demonstrated the variability and utility of the second intron of *LEAFY*, finding only a single copy in the Eriogonoideae. To achieve more consistent amplification in the group of study, Eriogonoideae-specific primers were then designed from these initial sequences. The forward and reverse primer sequences are: *LFY71-F* 5' GCCTTGATTATCTCTTCCAC 3' and *LFY1369-R* 5' CCTGAACACCTGGTTTGTC 3'. PCR conditions for the modified *LEAFY* primers are: initial denaturation 94°C for 1 min; amplification 8 cycles 94°C for 20 sec, 59°C -1°C/cycle for 30 sec, 72°C for 1 min followed by 25 cycles of 94°C 20 sec, 52°C for 30 sec, and 72°C for 1 min; with a final extension of 72°C for 5 min.

All PCR products were diluted (30:1) in water and cycle sequenced using the same primers. These products were then purified by employing Agencourt magnetic beads (Agencourt, Beverly, Massachusetts). Sequencing reactions were carried out at the DNA Sequencing Facility at the University of Wisconsin Biotechnology Center using the ABI PRISM BigDye terminator on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems, Foster City, California).

Forward and reverse sequences were assembled and edited in Sequencher ver. 4.7 (Gene Codes, Ann Arbor, Michigan). Polymorphic sites in *ITS* and *LEAFY* were coded with IUPAC ambiguity codes. A site was coded as polymorphic if two peaks were present at the same site in both the forward and reverse read. If a region showed clear length variation in the chromatogram or if more than 1% of the

sequenced region was coded as polymorphic, bacterial cloning was implemented to separate potential alleles or copies. For samples to be cloned, the Promega pGEM-T vector protocol was followed, except the volume of all reagents and buffers was halved (Promega, Madison, Wisconsin). To obtain representative sequences of all copies/alleles present, 8 to 24 clones were sequenced, depending upon variation in the initial direct sequencing read. All cloned sequences were initially aligned and visually compared to assess variation present in recovered sequences, and to recognize chimeric sequences or sequences with obvious Taq errors (i.e. point mutations present in only one cloned sequence) (Edwards et al. 2008). Recombinant (chimeric) sequences and those with clear unique Taq errors were excluded from alignments.

Phylogenetic Analyses—Sequences from each respective region were manually aligned in MacClade 4.08 (Maddison and Maddison 2005) to maximize homologous site matches and minimize the placement of gaps in the alignments. Models of sequence evolution for Maximum Likelihood (ML) and Bayesian Inference (BI) were selected based on the BIC criterion from jModelTest and MrModelTest 2.3, respectively (Posada 2008; Nylander 2004). All basic phylogenetic analyses were carried out in the CIPRES Science Gateway v. 3.3 (Miller et al. 2010), using MrBayes v. 3.1.2 for BI (Huelsenbeck and Ronquist 2001) or RAxML v. 7.3.0 for ML bootstrapping (Stamatakis et al. 2008). Bayesian analysis of individual data sets was implemented with default settings, 10 million generations, with a 25% burnin. Maximum likelihood bootstrap values were gathered by using RAxML, with 1,000 bootstrap replicates and default settings.

Ancestral reconstruction of life history—The reconstruction of ancestral states of the annual vs. perennial habit for selected clades was carried out using maximum parsimony (MP) in MacClade (parsimony mapping) and maximum likelihood (ML) in BayesTraits ver. 1.0 (Pagel and Meade 2007). We used the single most likely tree from the BI analysis of the concatenated data set, rooted with *Gilmania luteola*. The most likely tree from our BI run had no zero-length branches, however some of the branches, especially in terminal clades, were very short. Terminals were coded as either annual or perennial based upon field observations and the most recent taxonomic treatment of the genus (Reveal 2005). We implemented the "trace character" option for the parsimony reconstruction of the annual vs. perennial habit across the most likely tree in MacClade. The MultiState model of evolution was employed to reconstruct ancestral states of life history in BayesTraits, with default parameters. One hundred ML runs (mltries=100) were performed in MultiState with a total of 53 nodes analyzed to find the probability of that node representing either an annual or perennial ancestor (Pagel 1999, Pagel and Meade 2007). Colored branches on the phylogram indicate life history MP reconstructions and the ML probabilities are represented by a pie chart on each node of interest.

RESULTS

Phylogenetic utility of included molecular regions—DNA sequence information for this study was generated from chloroplast and nuclear sources. Three chloroplast spacer/intron regions, the internal transcribed spacer (*ITS*)

region of the nuclear ribosomal DNA, and the second intron of *LEAFY* from the nuclear genome were sequenced and used for phylogenetic reconstruction in *Eriogonum* and related genera. Based upon our extensive sampling across *Eriogonum*, each of these regions shows utility at various taxonomic and phylogenetic levels. The summary statistics for the data matrices and all combined data sets are shown in Table 1 and Appendix 2.

Table 1. Summary statistics for all data matrices analyzed in this study of relationships *Eriogonum*. An * in the Accessions included column denotes additional cloned sequences present in the data matrix. Variable sites included, Parsimony informative sites, % variable, and % PIC are calculated with and without the outgroup, values for the ingroup only are shown in parentheses.

Region	Accessions included	Aligned length (bp)	Variable sites included (ingroup only)	Parsimony informative sites (ingroup only)	% variable (ingroup only)	% PIC (ingroup only)	Substitution model (BI)
nrITS	119*	771	315 (312)	221 (219)	40.8% (40.4%)	28.7% (28.4%)	GTR+G
LFY 2nd intron	93*	2266	958 (942)	513 (499)	42.2% (41.6%)	22.6% (22.0%)	GTR+I+G
trnL-trnF	111	1124	159 (150)	57 (54)	14.1% (13.3%)	5.0% (4.8%)	GTR+I+G
rpS16	116	1362	227 (222)	96 (93)	16.7% (16.3%)	7.0% (6.8%)	GTR+I+G
trnD-trnT	111	2423	412 (391)	177 (171)	17.0% (16.1%)	7.3% (7.1%)	GTR+I+G
Combined chloroplast	116	4909	798 (763)	330 (318)	16.2% (15.5%)	6.7% (6.5%)	GTR+I+G
Combined all	123	7938	2015 (1959)	998 (971)	25.4% (24.7%)	12.6% (12.2%)	GTR+G

The lowest levels of sequence variation were observed in the chloroplast datasets (Table 1). Manual alignment for each of the chloroplast markers was relatively straightforward. Because the chloroplast is maternally inherited, only one sequence was present for each region of each accession included. Large insertions and deletions were present in sequences for some taxa, but they were consistent with other sequence variation in related taxa, i.e. not homoplasious. Gaps were not

coded. Potentially homoplastic tandem repeats were observed in the *trnL-trnF* and *trnD-trnT* spacers, however these portions of the data matrix were excluded from the phylogenetic analyses. The combination of three-region data matrix resolved relationships of all major clades within the study group with strong PP and BS support (Figure 3, nodes B-H), however many lower-level relationships within these clades are ambiguous or poorly supported. The large polytomy within the densely sampled *Eucycla II* (clade D) is indicative of low sequence variation within these regions and consistent with a recently radiated group of species. Numerous clades within this polytomy are strongly supported, but branch lengths are relatively short, especially for perennial species.

The *ITS* alignment showed an intermediate level of variation when compared to the chloroplast and *LEAFY* sequence data, however the percentage of PIC's (parsimony informative characters) from this region is the highest in this study (Table 1). Manual alignment of the *ITS* region was unambiguous, therefore no portions of the alignment were excluded. Many of the major clades are strongly supported by the *ITS* sequence data, with the exception of clade C (Figure 4). Within these clades in the *ITS* genealogy, there is more internal structure with many more clades showing moderate to strong support. As with the chloroplast phylogeny, an extensive polytomy is present within the *Eucycla II* clade, however there are more supported nodes and topological structure with the *ITS* data. Unequal substitution rates are also evident with the *ITS* data, with annual species showing higher rates of sequence evolution, and thus longer branches in the phylogram (Figure 4, clade D and F). Many of the accessions were directly sequenced for the *ITS* region, however

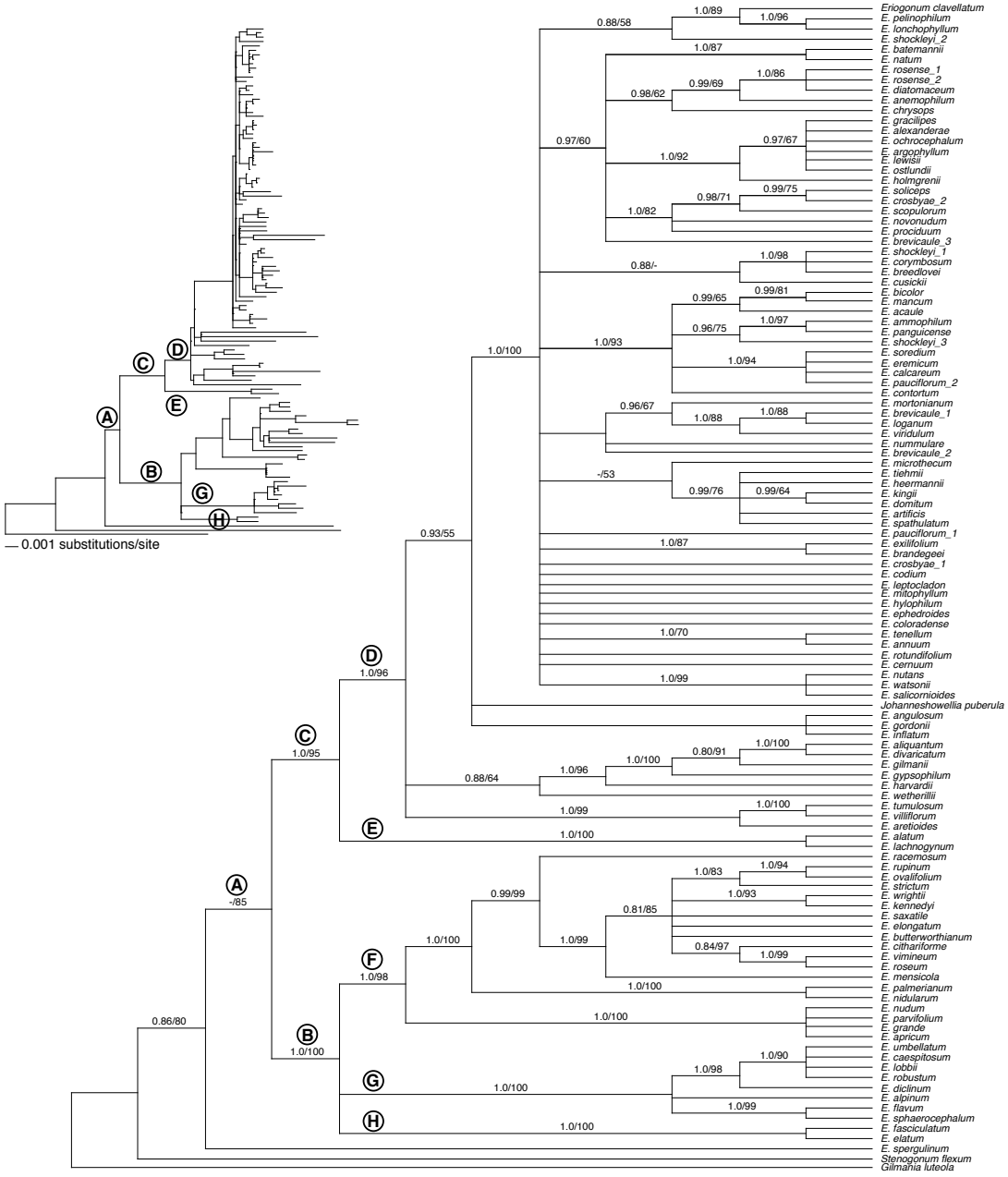


Figure 3. Consensus tree from Bayesian analysis of the combined three-region chloroplast data set (*trnL-trnF*, *rpS16*, and *trnD-trnT*) for 116 accessions of *Eriogonum* and related genera in Eriogoniodeae, with *Gilmania luteola* used as the outgroup. A phylogram representing the tree with the highest log likelihood from the Bayesian analysis is shown in the upper left corner. Major clades of interest are labeled with an encircled letter. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included.

a portion were cloned due to multiple polymorphisms or sequence length variation. The cloned sequences were aligned and used to infer phylogenies, in a parsimony framework. From these initial parsimony runs, cloned sequences that appeared in the same clade were reduced to one representative sequence to lessen the number of terminals in each resultant gene tree. In instances where multiple sequences could not be eliminated, two or more cloned sequences were retained in the phylogenetic analyses. Of those cloned, most were discarded due to minor variation that did not place the sequences in different parts of initial phylogenies. Nine accessions had two distinct forms of *ITS* after all cloned sequences were analyzed. All of these variant sequences are found in the *Eucycla II* clade (Figure 4, labeled c1 and c2).

Sequence data from the low-copy nuclear region of *LEAFY* show the highest variation of the regions used in this study. The percentage of parsimony informative characters, however, is slightly lower than those obtained from *ITS* (Table 1). Alignment of the *LEAFY* sequences was the most challenging of all of the regions used in this study. The length of this region varied considerably due to large insertions within the intron, for some of the accessions included. On the *LEAFY* gene tree, many of the deeper nodes within the phylogeny receive low support (Figure 5, nodes B, C, D, E, and F). The subg. *Oligogonum/*subg. *Eriogonum* clade is embedded within the *Eucycla I*/subg. *Oregonium* clade, unlike the other gene trees presented (Figure 5, clades G and F, respectively). There are many terminal clades that are cohesive and are strongly supported. The polytomy previously discussed in *Eucycla II* is still present in the *LEAFY* gene tree, however other clades within *Eucycla II*

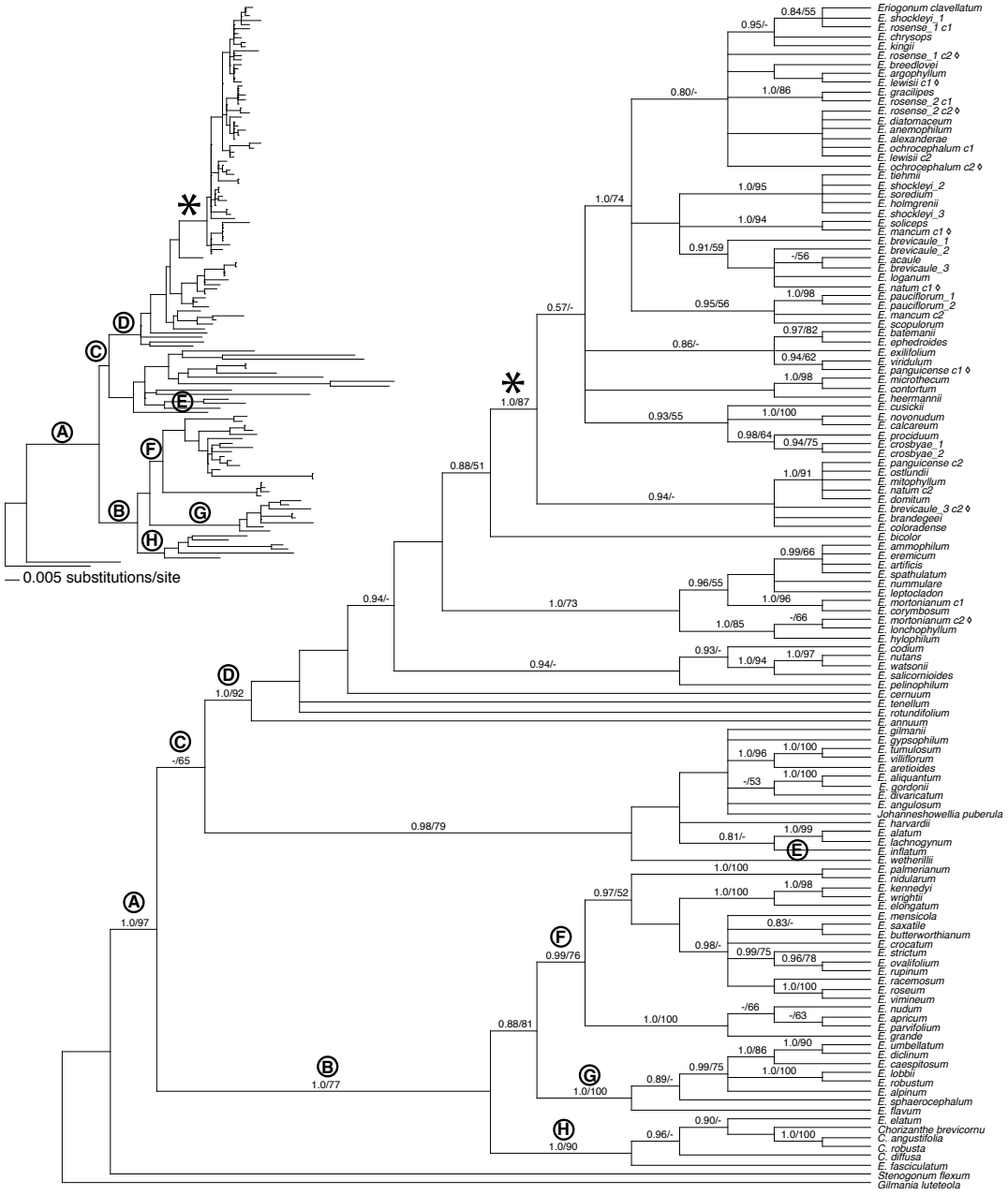


Figure 4. Consensus tree from Bayesian analysis of the *nrITS* region for 119 accessions of *Eriogonum* and related genera in Eriogoniodeae, with *Gilmania luteola* used as the outgroup. A phylogram representing the tree with the highest log likelihood from the BI analysis is shown in the upper left corner. Major clades of interest are labeled with an encircled letter. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Cloned accessions with more than one copy present are shown with a c1 and c2. Tips with a diamond (◊) are discarded in the combined analysis.

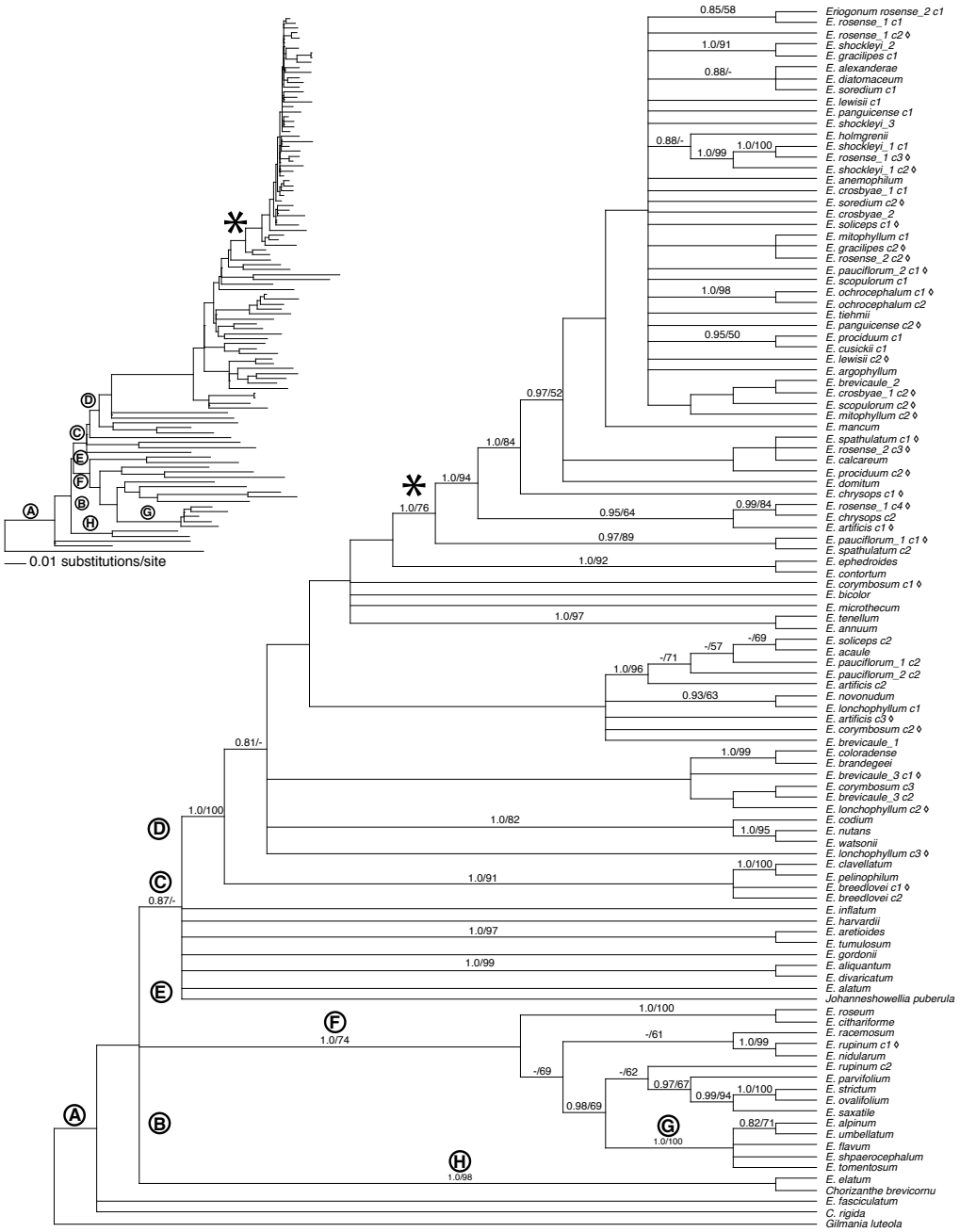


Figure 5. Consensus tree from Bayesian analysis of *LEAFY* for 93 accessions of *Eriogonum* and related genera in Eriogoniodeae, with *Gilmania luteola* used as the outgroup. A phylogram representing the tree with the highest log likelihood from the BI analysis is shown in the upper left corner. Major clades of interest are labeled with an encircled letter. BI posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only BI PP above 0.80 and ML bootstrap support values above 50 are included. Cloned accessions with more than one copy present are shown with a c1, c2, c3 or c4. Tip labels with a diamond (◊) are discarded in the combined analysis.

show moderate to strong support. Many of the *LEAFY* sequences used in this analysis are cloned. Most of the accessions are represented by just one sequence, however 23 accessions are represented by two, or more, distinct cloned sequences (tip label followed by a c1, c2, c3, or c4). All but one of the accessions with multiple sequences is from in the *Eucycla II* clade (Figure 5, clade D).

Although individual gene trees showed discordance, especially at shallower nodes, the data were concatenated to reduce the number of terminals and clarify subgeneric relationships and the reconstruction of evolution of annual and perennial habit. Accessions with more than one copy of *ITS* or *LEAFY* were reduced to one representative sequence for the combined analysis. After the initial winnowing of multiple cloned sequences, replicate sequences from identical accessions were randomly discarded for the combined analysis. These sequences have a diamond (◊) following the tip label on Figures 4 and 5. The exclusion of duplicate cloned sequences from identical accessions did not alter the topology of the combined phylogeny when compared to the individual gene trees (Figures 3-6).

Monophyly and Subgeneric relationships in Eriogonum—Molecular data indicate that the subgeneric relationships in *Eriogonum* are more complex than previously recognized and *Eriogonum,* as currently circumscribed is not monophyletic. Representatives included from other segregate genera (*Chorizanthe* and *Johanneshowellia*) are imbedded within *Eriogonum*. Based upon the reconstructed phylogenies of chloroplast and nuclear regions, *Eriogonum* diverged early into two main lineages (Figures 3-6; node A). One clade includes all sampled members of the following subgenera: *Eriogonum, Oligogonum,* and *Oregonium*; as

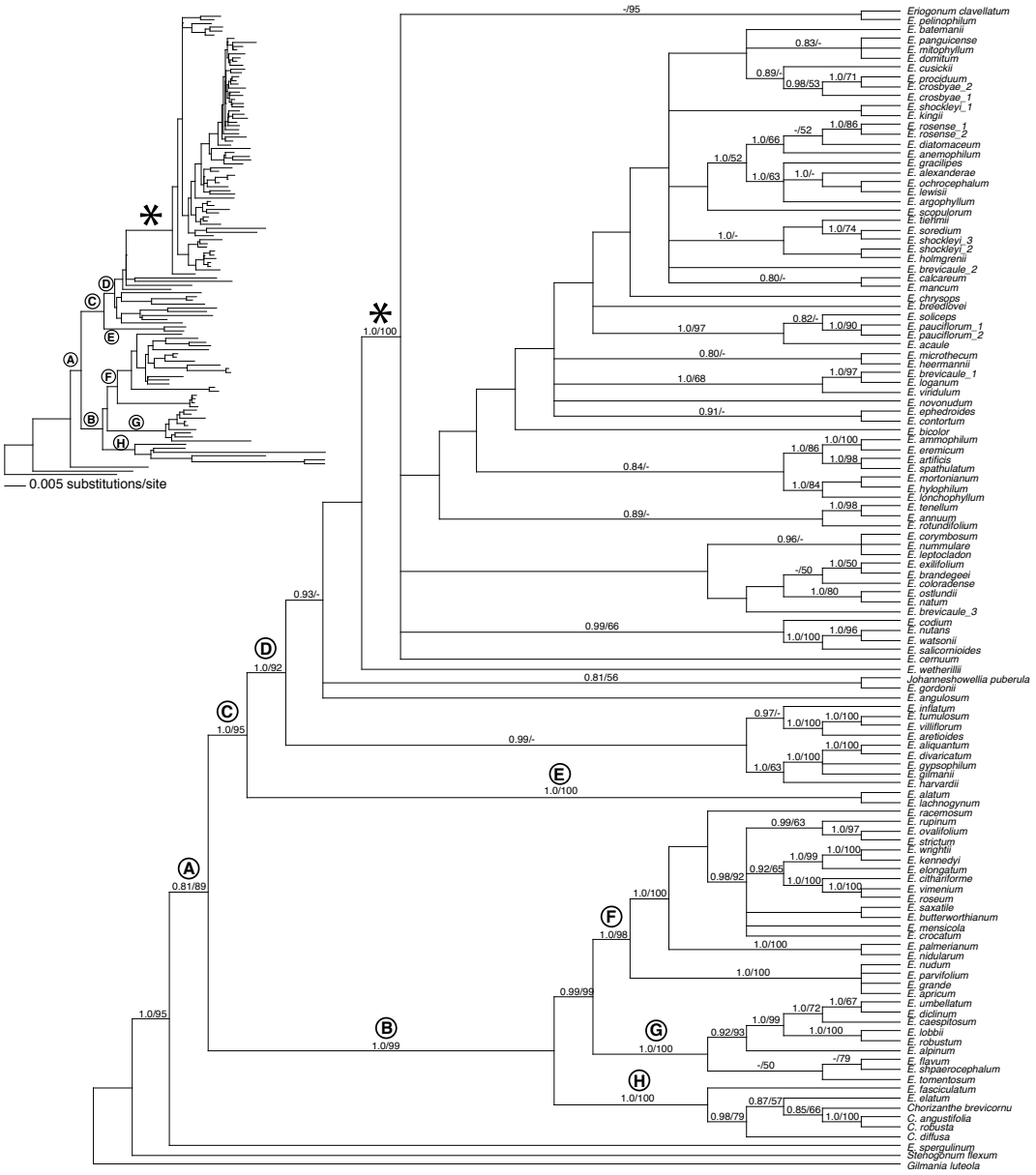


Figure 6. Consensus tree from Bayesian analysis of combined data set from five gene regions for 123 accessions of *Eriogonum* and related genera in Eriogoniodeae, with *Gilmania luteola* used as the outgroup. A phylogram representing the tree with the highest log likelihood from the BI analysis is shown in the upper left corner. Major clades of interest are labeled with an encircled letter. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Accessions with missing data for each gene region are included in Appendix 2.

well as a portion of the sampled members of the large subgenus *Eucycla* (17 of 79 sampled). *Chorizanthe,* the second most species-rich genus in Eriogonoideae, is sister to the other members of clade B (Figures 3-6; node B). The other major lineage (indicated by node C in Figures 3-6) includes all sampled members of subg. *Micrantha,* subg. *Ganysma,* and subg. *Pterogonum*, the remaining members of subg. *Eucycla* (62 of 79 sampled) and *Johanneshowellia*, a currently recognized segregate genus in Eriogonoideae.

The most species-rich subgenus, *Eucycla,* is clearly non-monophyletic with other recognized subgenera imbedded with it. One major group, which we call *Eucycla I,* is allied to subg. *Oregonium* with strong support in all of our individual analyses, except the phylogenetic reconstruction based on *LEAFY* (Figure 5). In the combined analysis of all of the regions, this clade is highly supported (Figure 6, node F). The clade containing the other members of subg. *Eucycla* (which we call *Eucycla II*) is clearly distinct from *Eucycla I*, although poorly supported nodes toward the base of the *ITS* and *LEAFY* gene trees confound the exact relationships within this clade. However, these nodes do receive stronger support in the combined analysis (Figure 6). Within this larger clade, southern representatives of *Eucycla II* share affinities with annual members of subg. *Ganysma* and subg. *Pterogonum*, and diverge prior to other more northerly-distributed species of *Eucycla II*. A summary tree, based on the combined molecular analysis is presented in Figure 7. Clades of interest are color-coded and subgeneric associations are highlighted.

Ancestral state reconstruction of life history—The earliest diverging lineages, relative to the bulk of *Eriogonum,* included in this phylogenetic analysis are

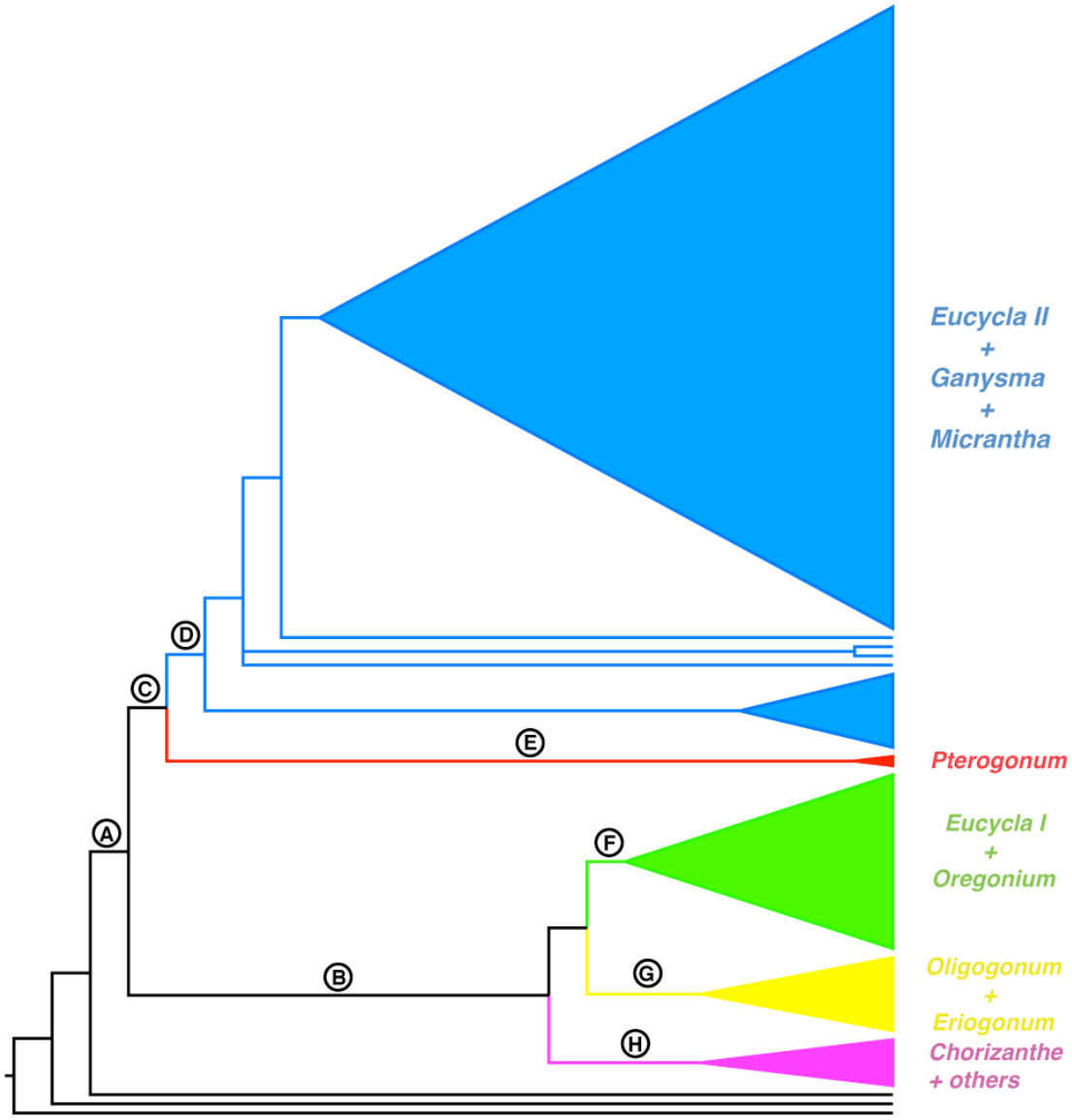


Figure 7. Summary tree from combined dataset showing relationships of major clades and subgenera in *Eriogonum.* The letters correspond to the clades in Figures 3-6.

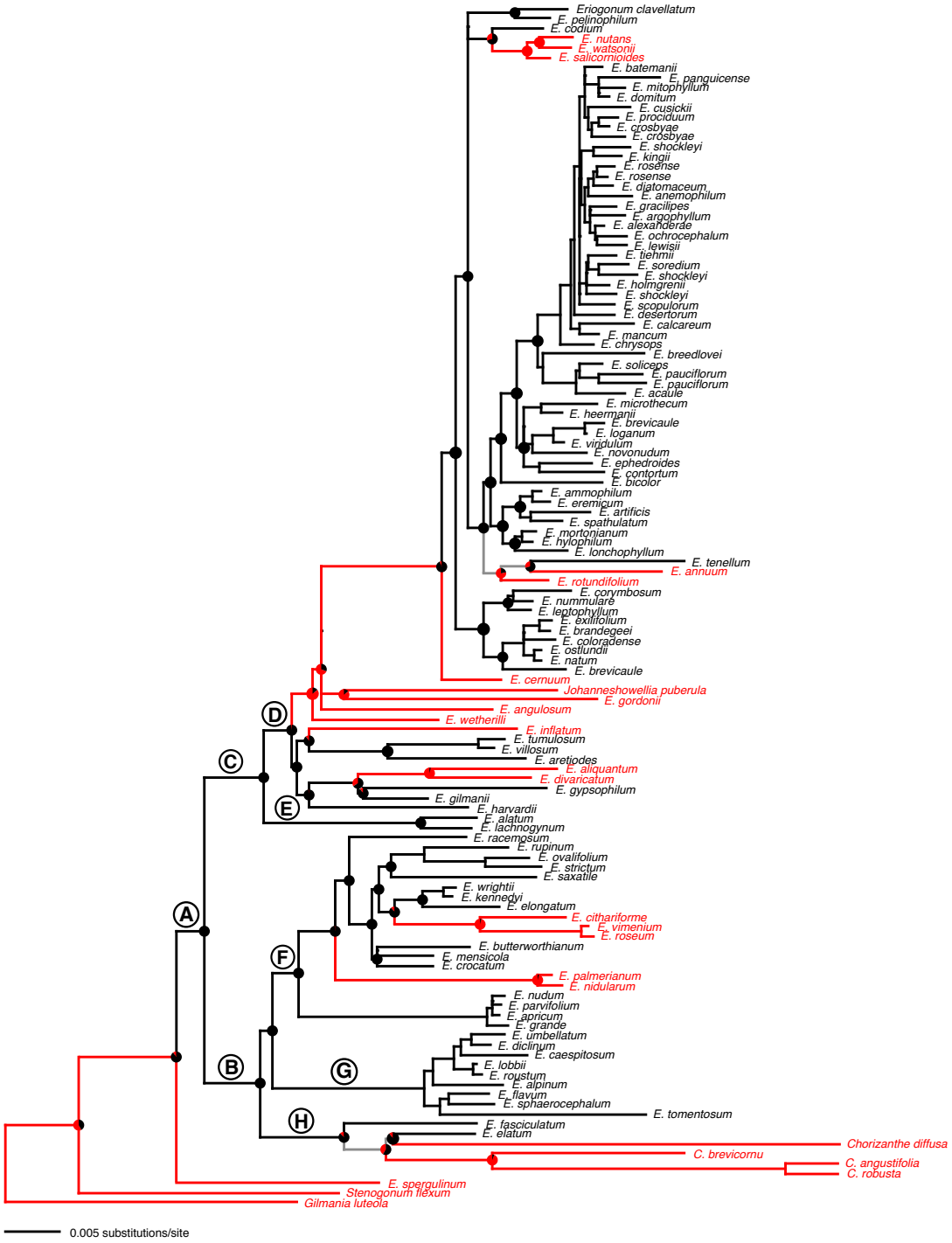


Figure 8. Bayesian Inferred phylogram from combined dataset; single tree from 10 million generations with highest log likelihood. Annual taxa are highlighted in red. Red branches indicate an annual ancestor as inferred from parsimony mapping, gray branches signify equivocal states. Pie charts at selected nodes indicate the likelihood of that node representing either an annual or a perennial; these reconstructions are based on a ML run in BayesTraits (MultiState).

tiny annual species (*Stenogonum flexum* and *Eriogonum spergulium*). Based upon parsimony and maximum likelihood ancestral reconstruction, there appears to have been an early shift from annual to perennial (base of the tree to node A, Figure 8) although there is uncertainty, with the root node having a 58% probability of the annual state versus 42% probability of being a perennial in the ML reconstruction. The ancestral state is reconstructed as annual under parsimony criterion. Node A (Figure 8) represents the ancestor of the bulk of the species of *Eriogonum* and related genera leading to linages B and C. Within these two lineages, both annual and perennial species are present. Annuals from subg. *Oregonium* are confined to clade B, while annual species from *Micrantha* and *Ganysma* are present in clade C (Figure 8). Within clade B the reconstructions recognize three separate shifts to the annual habit. The annual species in clade H are members of the genus *Chorizanthe*. The two distinct annual lineages present in clade F represent members of the subg. *Oregonium.* The *Eucycla II* lineage is more ambiguous at the base of the clade (Figure 8, clade C). Based upon parsimony and likelihood reconstructions, it appears that there could be as many as six life history shifts in clade C, or more conservatively, as few as three. These annual species represent two traditional subgenera, *Ganysma* and *Micrantha*.

DISCUSSION

Phylogenetic utility of included molecular regions—Determining phylogenetic relationships from only one gene region can provide a limited view of the actual evolutionary relationships of a group of organisms. Here we utilize

chloroplast and nuclear markers to reconstruct and compare gene trees in an extensively sampled phylogenetic study. Advantages and disadvantages were identified for each region, but a more complete understanding of deeper relationships of *Eriogonum* is apparent from a combination of these markers.

It is well known that using chloroplast markers, while relatively easy to sequence and analyze, only provides the maternal history of a group. Analysis of the chloroplast markers was fairly straightforward in this study. There were few problems with ambiguous alignments of the various regions used. When analyzed as a three-region combined dataset, the chloroplast markers provided strong support for nodes near the base of the phylogeny of *Eriogonum* (Figure 3). The topology of the plastid gene tree was congruent with the nuclear markers at these deeper nodes, allowing for uncertainty associated with the topologies in the nuclear (*ITS* and *LEAFY*) gene trees (nodes A, B, C, Figures 4 and 5). The chloroplast gene phylogeny does show a hard incongruence with the *ITS* tree concerning the placement of *E. alatum* and *E. lachnogynum* (node E, Figures 3 and 4). These two taxa, although being previously placed in different subgenera, show a sister relationship in both trees. In the combined chloroplast phylogeny, this clade is sister to the rest of *Eucycla II,* subg. *Ganysma,* and subg. *Micrantha*, whereas the *ITS* data places these two taxa within the larger subg. *Ganysma/Eucycla II* clade.

Direct sequencing of a portion of the samples for the *ITS* and *LEAFY* regions showed numerous polymorphisms and length variation in the trace files. Chimeric sequences were observed in some of the *LEAFY* sequences. These were apparent from comparison with other cloned sequences. Any chimeric sequences found, likely

the result of recombination (actual or PCR-mediated), were not included in any phylogenetic analysis. Many of these duplicate sequences likely represent allelic variation, however, there appear to be multiple copies of this locus in a small proportion of taxa, possibly the result of hybridization or polyploidy. For example, we recovered two copies of *ITS* and four alleles in the *E. rosense_1* accession (BRG180). Much like many members of *Eriogonum*, no chromosome counts exist for *E. rosense.* The multiple alleles/copies of *ITS* and *LEAFY* detected could be indicative of a history of recent polyploidy in *E. rosense.*

Phylogenetic relationships of the subgenera in Eriogonum—As currently recognized, the subgenera of *Eriogonum* are clearly not monophyletic. While one adequately sampled subgenus is monophyletic in our analysis, (subg. *Oligogonum*, clade G), other traditionally recognized subgenera do not correspond to strict monophyletic groups. Figure 7 is a simplified phylogeny, inferred from our combined dataset to portray the taxonomic findings of the current study. Additional taxonomic sampling across the entire subfamily seems necessary to depict subgeneric relationships and to subsequently reclassify these groups such that they more accurately reflect the evolutionary history of these lineages. Increased sampling of selected gene regions is also necessary to untangle some of the ambiguous topological results obtained from this study.

The most widespread and species-rich subgenus, *Eucycla*, is not monophyletic. Of the 79 sampled species previously circumscribed in this subgenus, members fall within three strongly supported clades (nodes D, F, H, Figures 3-7). *Eriogonum fasciculatum* and *E. elatum*, a shrub and herbaceous perennial

respectively, are closely related to sampled annual members of *Chorizanthe* in all gene trees presented here. Although there is uncertainty in the exact order of divergence, based upon the *ITS* and combined analyses, this clade appears to represent the sister to the rest of lineage B. This node (H) is not resolved in the chloroplast and *LEAFY* gene trees. Morphologically, *E. fasciculatum*/*E. elatum* and *Chorizanthe* share few obvious synapomorphies. Additional taxon sampling within this interesting clade could aid in understanding this unforeseen relationship and might lead to the discovery of morphological synapomorphies.

Another lineage containing members of *Eucycla* is the clade represented by node F in the included gene trees (Figures 3-7). This lineage includes widespread species such as *E. ovalifolium, E. nudum,* and *E. wrightii,* among other more narrowly distributed taxa. All sampled members of subg. *Oregonium* are also included in this clade, with strong PP and BS support in all analyses. The sampled annual species representing subg. *Oregonium* are not monophyletic however, forming two distinct clades within clade F. While there is a major distinction between annual and perennial species within this lineage, some morphological features are shared by the members of this clade. Sampled species within this clade, both annual and perennial, possess sessile involucres that are generally closely appressed to the inflorescence axis (Figure 1, image I). The leaf shape is also a uniting feature of members of this clade. When compared to other members of *Eriogonum,* these species tend to have wider leaf blades with a distinct petiole and chordate to truncate leaf base. The combination of these features may prove useful in placing other un-sampled members of the traditional subg. *Eucycla* in either *Eycycla I* or *Eucycla II.*

Geographically, species within this clade tend to have more extreme western distributions. However, there are exceptions. Widespread species, such as *E. ovalifolium* and *E. palmerianum*, can be found across the Great Basin and into the northern Rockies (Reveal 2005).

The bulk of the sampled members of subg. *Eucycla* fall within the densely sampled clade represented by node D (Figures 3-6). In addition to the remainder of the sampled subg. *Eucycla* species, members of subg. *Ganysma,* subg. *Pterogonum,* and subg. *Micrantha* fall in this a clade (node C, Figures 3-6). This clade is a diverse mixture of annual and perennial species from four previously recognized subgenera (Reveal 2005). Geographic distributions of sampled members of clade C show a general distribution that is most diverse in the Great Basin and the Colorado Plateau, with other members from southern deserts and the Northern Rockies. In the chloroplast tree (node E, Figure 3) and the phylogeny derived from the combined dataset (node E, Figure 6) the clade comprising *E. alatum* (subg. *Pterogonum*) and *E. lachnogynum* (subg. *Eucycla*) shows a sister relationship to a lineage represented by most members of subg. *Ganysma*, the only sampled representative of subg. *Micrantha*, and southern species from subg. *Eucycla* diverges. These members of subg. *Eucycla* are samples collected from the Chihuahuan Desert, Mohave Desert, and southern Colorado Plateau with characteristic strigose hairs covering the leaves, stems, and even the tepals. *Eriogonum gyposophilum* is an exception to this, being entirely glabrous. The remaining species in *Eucycla* fall within the large polytomy present, to some extent, in all presented gene trees (highlighted with an asterisk in Figures 4-6). However, relationships within this clade are more resolved with the

ITS, *LEAFY,* and combined datasets. The topologies and branch lengths exhibited by of each of the gene trees for these members of *Eucycla II* (asterisk in Figures 4-6) is highly indicative of a rapid radiation of species, although no tests of diversification are presented here. Exemplar widespread species present in this clade include *E. brevicaule, E. ochrocephalum, E. shockleyi, E. corymbosum,* and *E. microthecum.* Numerous narrowly endemic and species of conservation concern are also included here, *E. pelinophilum, E. soredium, E. mitophyllum,* and *E. diatomaceum.*

Patterns of life history evolution in Eriogonum and related genera—Many of the members of the subfamily Eriogonoideae are annual species. *Eriogonum* is a noteworthy genus within the subfamily because the majority of species within this large genus exhibit a perennial life history. Six of the eight previously recognized subgenera contain perennial species. There are 162 perennial species of the 251 total species recognized in *Eriogonum* (Reveal 2005). Based upon our MP and ML reconstructions of ancestral character state of annual vs. perennial habit, it seems likely that the ancestor of the subfamily was an annual species (Figure 8). Both the MP and ML reconstructions indicate a perennial ancestor for the two major lineages of *Eriogonum* and allied genera, signifying an early shift from annual to perennial in the subfamily (node A, Figure 8). The perennial state is maintained, with high probability, for the nodes leading to the major subgeneric splits (nodes B-H, Figure 8).

The terminal clades (nodes D-H, Figure 8), roughly corresponding to the previously recognized subgenera, contain multiple examples of shifts in life history. The "*Chorizanthe* + *E. fasciculata*" clade (node H, Figure 8) contains all sampled

members of *Chorizanthe* plus two members of the previously recognized subgenus *Eucycla*. The ancestor of this clade was most likely a perennial, with a shift to annual life history in members of *Chorizanthe*. No South American perennial representatives of *Chorizanthe* are included in the current study, however their inclusion would be important to further investigate patterns of life history evolution in this clade. It is important to note that no currently published molecular phylogenies have included any South American *Chorizanthe* species, so it is not certain that they would be members of this clade. The "*Oligogonum* + subg. *Eriogonum*" clade represents a perennial lineage, as sampled here (node G, Figure 8). Based on the unique structure of the flowers of species in these two subgenera (perianth basally attenuate with an un-winged stipe-like base, see Figure 1 Image H) the sister relationship of these two subgenera is supported morphologically. With additional sampling, it is likely that other members of these subgenera would be placed here as well. No annual species are present in either subg. *Oligogonum* or subg. *Eriogonum.*

The "*Eucycla I + Oregonium*" clade shows two clear shifts to the annual habit based on our sampling and reconstructions (node F, Figure 8). All of the included annual representatives in this clade are classified in the subg. *Oregonium.* Within this larger group, there is an early shift from perennial to annual leading to the clade containing *E. palmerianum and E. nidularum.* Both of these species are distributed throughout the Great Basin Desert and southward, at sites mostly below 2300 m elevations (Reveal 2005). The other shift to the annual habit in this group is represented by the branch leading to the *E. cithariforme*/*E. vimineum*/*E. roseum*

clade. *E. cithariforme* and *E. roseum* are found only in California. *Eriogonum vimineum* occurs in northern California and Nevada, north throughout Oregon (Reveal 2005). All three species inhabit elevations below 2200 m, and often much lower.

A preponderance of annual species is also present in the "*Eucycla II + Ganysma* + *Micrantha* clade (node D, Figure 8). Within this lineage, short branches, unsampled taxa, and unresolved nodes (Litsios and Salamin 2012) make it less clear just how many life history shifts have occurred, however our reconstructions show the ancestor of this lineage was likely a perennial. According to the tree topology and character mapping, there could be as many as six life history shifts within this large clade. Node D (Figure 8) shows a perennial to annual shift. The following nodes show uncertainty in the ML reconstruction, possibly due to low sequence variability, conflicting signal, short internal branches, or long terminal branches. We do, however, see strong signal for a shift back to perenniality in the core *Eucycla II* clade. Within this perennial-dominated clade, there are two annual clades present. One, which includes *E. annuum* and *E. rotundifloium*, shows uncertainty in both the MP and the ML reconstructions. The other annual clade (*E. nutans, E. watsonii, E. salicornioides*) is sister to a perennial mat-forming species from Washington (*E. codium*). These three annual species are all distributed in the northern Great Basin at low elevations (Reveal 2005).

One clear shift from perennial to annual occurs in clade E (Figure 8), leading to the clade containing *E. aliquantum* and *E. divaricatum.* These annual members of subg. *Ganysma* inhabit arid portions of the Colorado Plateau and Four-Corners

region. There could be another shift from perennial to annual in this clade. The presented topology and reconstruction shows an additional shift associated with *E. inflatum.* There is uncertainty associated with this as the phylogenetic position of *E. inflatum* is slightly different, or at the very least unresolved in the uncombined phylogenies (Figures 3-5). It seems likely that this shift may simply be a part of one of the other shifts discussed.

Conclusions

Here we demonstrate the utility of using multiple loci to infer the phylogenetic history of *Eriogonum* and related genera. All of the regions used in this study are useful for at varying levels within this group. Based on our molecular phylogenies, the current subgeneric classification in *Eriogonum* does not recognize monophyletic lineages. Taxonomic changes in the Eriogonoideae, both at the generic and subgeneric level, are warranted. It is clear that the most speciose subgenus, *Eucycla*, is polyphyletic. Members of the previous *Eucycla*, appear to represent at least three well-supported lineages, with members of other currently recognized subgenera imbedded within these groups. Focused, well-sampled phylogenetic studies of each of the clades recognized here should provide the means to rework the subgeneric classification of *Eriogonum*, such that the taxonomy reflects evolutionary history. A focus on utilizing morphological characters, in conjunction with molecular phylogenies will make generic and subgeneric classification more clear. One of the main criteria used to delineate subgenera, life history, has evolved numerous times within *Eriogonum*, thus confounding any classification which relies

on this trait. These shifts in life history may correspond to climate, as the annual species included in this study tend to occur in more xeric, low-elevation areas. Additional taxon sampling and inclusion of other gene regions would likely help to resolve any ambiguous relationships presented in our phylogenies. This study provides a necessary phylogenetic background for one of the most diverse and species-rich genera in the American flora.

Andreasen, K. 2012. Phylogeny, hybridization, and evolution of habit and breeding system in *Sidalcea* and *Eremalche* (Malvaceae). *International Journal of Plant Sciences* 173: 532-548.

Andreasen, K. and B. G. Baldwin. 2003. Reexamination of relationships, habital evolution, and phylogeography of checker mallows (*Sidalcea*; Malvaceae) based on molecular phylogenetic data. *American Journal of Botany* 90-436-444.

Burke, J. M. and A. Sanchez. 2011. Revised subfamily classification for Polygonaceae, with a tribal classification for Eriogonoideae. *Brittonia* 63: 510-520.

Datson, P. M., B. G. Murray, and K. E. Steiner. 2008. Climate and the evolution of annual/perennial life-histories in *Nemesia* (Scrophulariaceae). *Plant Systematics and Evolution* 270: 39-57.

Drummond, C. S., R. J. Eastwood, S. T. S. Miotto, and C. E. Hughes. 2012. Multiple continental radiations and correlates of diversification in *Lupinus* (Leguminosae): testing for key innovation with incomplete taxon sampling. *Systematic Biology* 61: 443-460.

Edwards, C. E., D. Lefkowitz, D. E. Soltis, and P. S. Soltis. 2008. Phylogeny of *Conradina* and related southeastern scrub mints (Lamiaceae) based on *GapC* gene sequences. *International Journal of Plant Sciences* 169: 579-594.

Evans, M. E. K., D. J. Hearn, W. J. Hahn, J. M. Spangle, and D. L. Venable. 2005. Climate and life-history evolution in evening primroses (*Oenothera*: Onagraceae): a phylogenetic comparative analysis. *Evolution* 59: 1914-1927.

Frohlich, M. W. and E. M. Meyerowitz. 1997. The search for homeotic gene homologs in basal angiosperms and Gnetales: a potential new source of data on the evolutionary origin of flowers. *International Journal of Plants Sciences* 158: S131-S142.

Goodman, G. J. 1934. A revision of the North American species of the genus *Chorizanthe*. *Annals of the Missouri Botanical Garden* 21: 1-102.

Grady, B. R. and J. L. Reveal. 2011. New combinations and a new species of *Eriogonum* (Polygonaceae: Eriogonoideae) from the Great Basin Desert, United States. *Phytotaxa* 24: 33-38.

Huelsenbeck J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.

Kim S. T. and M. J. Donoghue. 2008. Incongruence between cpDNA and nrITS trees indicate extensive hybridization within *Eupersicaria* (Polygonaceae). *American Journal of Botany* 95: 1122-1135.

Kim, S. T., S. E. Sultan, and M. J. Donoghue. 2008. Allopolyploid speciation in *Persicaria* (Polygonaceae): insights from a low-copy nuclear region. *Proceedings of the National Academy of Sciences* 105:12370-12375.

Lamb Frye, A. S. and K. A. Kron. 2003. *rbcL* phylogeny and character evolution in Polygonaceae. *Systematic Botany* 28: 326-332.

Litsios, G. and N. Salamin. 2012. Effects of phylogenetic signal on ancestral state reconstruction. *Systematic Biology* 61: 533-538.

Maddison, D. R. and W. P. Maddison. 2005. MacClade: analysis of phylogeny and character evolution, version 4.08. Sunderland: Sinauer Associates.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1 - 8.

Nylander, J. A. A. 2004. MrModelTest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612-622.

Pagel, M. and A. Meade. 2007. BayesTraits, version 1.0. Computer program and documentation distributed by the author, website: http:// www.evolution.rdg.ac.uk [accessed 17 October 2009].

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.

Reveal, J. L. 1969. The subgeneric concept in *Eriogonum* (Polygonaceae) Pp. 229-249 in *Current Topics in Plant Science.* ed. J. Gunckel. New York: Academic Press.

Reveal, J. L. 1978. Distribution and Phylogeny of Eriogonoideae (Polygonaceae). *Great Basin Naturalist Memoirs* 2: 169-190.

Reveal, J. L. 2005. Polygonaceae subfam. Eriogonoideae. Pp. 218-478 in *Flora of North America* Vol. 5 ed. Flora of North America Editorial Committee. New York: Oxford University Press.

Reveal, J. L. and C. B. Hardham. 1989. A revision of the annual species of *Chorizanthe* (Polygonaceae: Eriogonoideae). *Phytologia* 66: 98-198.

Reveal, J. L. and J. T. Howell. 1976. *Dedeckera* (Polygonaceae), a new genus from California. *Brittonia* 28: 245-251.

Sanchez A. and K. A. Kron. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Systematic Botany* 33: 87-96.

Sanchez, A. T. M. Schuster, and K. A. Kron. 2009. A large scale phylogeny of Polygonaceae based on molecular data. *International Journal of Plant Sciences* 170:1044-1055.

Schuster, T. M., J. L. Reveal, and K. A. Kron. 2011. Phylogeny of Polygoneae (Polygonaceae: Polygonoideae). *Taxon* 60: 1653-1666.

Shaw J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. S. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, R. L. Small. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

Stamatikis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758-771.

Stebbins, G. L. 1950. Variation and Evolution in Plants. Pp. 151-188. ed. L. C. Dunn. New York: Columbia University Press.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

Tank, D. C., and R. G. Olmstead. 2008. From annuals to perennials: Phylogeny of subtribe Castillejinae (Orobanchaceae). *American Journal of Botany* 95: 608-625.

Wang, A., M. Yang, and J. Liu. 2005. Molecular phylogeny, recent radiation and evolution of gross morphology of the rhubarb genus *Rheum* (Polygonaceae) inferred from chloroplast DNA *trnL-F* sequences. *Annals of Botany* 96: 489-498.

White T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pg: 315-322 in *PCR protocols: A guide to methods and application,* eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. New York: Academic Press.

Distributions for each currently recognized subgenus by state. Classification and number of species in each subgenus follows Reveal's treatment in the Flora of North America (2005).

	Eucycla	**Ganysma**	**Micrantha**	**Pterogonum**	**Oregonium**	**Oligogonum**	**Eriogonum**	**Clastomyelon**
Washington	7	2	0	0	2	9	0	0
Oregon	15	10	0	0	7	13	0	0
California	36	33	0	0	27	24	0	1
Arizona	24	23	0	1	6	4	0	0
Nevada	32	28	0	0	8	9	0	0
Utah	36	21	0	1	7	4	0	0
Idaho	13	7	0	0	4	8	0	0
Montana	9	2	1	0	0	6	0	0
Wyoming	9	4	1	1	1	5	0	0
Colorado	27	8	1	1	2	5	0	0
New Mexico	13	17	1	3	3	3	1	0
Texas	3	3	2	5	1	2	1	0
Oklahoma	1	1	2	1	0	1	1	0
Kansas	0	0	1	1	0	1	1	0
Nebraska	3	2	1	1	0	1	0	0
South Dakota	3	3	1	0	0	1	0	0
North Dakota	1	1	1	0	0	1	0	0
Missouri	0	0	0	0	0	0	1	0
Arkansas	0	0	1	0	0	0	1	0
Louisiana	0	0	1	0	0	0	1	0
Kentucky	0	0	0	0	0	0	1	0
Tennessee	0	0	0	0	0	0	1	0
Alabama	0	0	0	0	0	0	2	0
Florida	0	0	0	0	0	0	2	0
Georgia	0	0	0	0	0	0	1	0
South Carolina	0	0	0	0	0	0	1	0
Mexico	4	7	1	4	3	3	0	0

Voucher information, life history states, regions sequenced and included for accessions in this study.

Genus	species	Subgenus	Coll. #	Habit	Latitude	Longitude	State	County	*trnL-F*	*rpS16*	*trnD-T*	*ITS*	*LEAFY*
Chorizanthe	*angustifolia*	n/a	n/a	0	n/a	n/a	n/a	n/a	0	0	0	GB	0
Chorizanthe	*brevicornu*	n/a	n/a	0	n/a	n/a	n/a	n/a	0	0	0	GB	GB
Chorizanthe	*diffusa*	n/a	n/a	0	n/a	n/a	n/a	n/a	0	0	0	GB	0
Chorizanthe	*rigida*	n/a	JLR8464	0	36°20'22"N	117°28'47"W	California	Inyo	0	0	0	0	GB
Chorizanthe	*robustum*	n/a	n/a	0	n/a	n/a	n/a	n/a	0	0	0	GB	0
Eriogonum	*acaule*	*Eucycla*	480	1	41°33.795'N	108°16.089'W	Wyoming	Sweetwater	1	1	1	1	1
Eriogonum	*alexanderae*	*Eucycla*	413	1	38°48.428'N	119°13.308'W	Nevada	Lyon	1	1	1	1	1
Eriogonum	*ammophilum*	*Eucycla*	153	1	38°48.036'N	113°27.325'W	Utah	Millard	1	1	1	1	0
Eriogonum	*anemophilum*	*Eucycla*	402	1	40°25.006'N	118°08.626'W	Nevada	Pershing	1	1	1	1	1
Eriogonum	*apricum*	*Eucycla*	282	1	38°18.836'N	120°54.461'W	California	Amador	0	1	0	1	0
Eriogonum	*aretioides*	*Eucycla*	373	1	37°50.059'N	111°59.110'W	Utah	Garfield	1	1	1	1	1
Eriogonum	*argophyllum*	*Eucycla*	438	1	40°35.196'N	115°17.075'W	Nevada	Elko	1	1	1	1	1
Eriogonum	*artificis*	*Eucycla*	564	1	38°25.216'N	113°18.046'W	Utah	Beaver	1	1	1	1	1
Eriogonum	*batemanii*	*Eucycla*	124	1	39°57.813'N	108°46.733'W	Colorado	Rio Blanco	1	1	1	1	0
Eriogonum	*bicolor*	*Eucycla*	142	1	38°14.557'N	111°06.689'W	Utah	Wayne	1	1	1	1	1
Eriogonum	*brandegeei*	*Eucycla*	600	1	38°37.791'N	106°04.717'W	Colorado	Chaffee	1	1	1	1	1
Eriogonum	*breedlovei*	*Eucycla*	217	1	35°27.950'N	118°23.433'W	California	Kern	1	1	1	1	1
Eriogonum	*brevicaule_1*	*Eucycla*	357	1	40°58.470'N	109°27.780'W	Utah	Daggett	1	1	1	1	1
Eriogonum	*brevicaule_2*	*Eucycla*	380	1	41°04.010'N	115°01.189'W	Nevada	Elko	1	1	1	1	1
Eriogonum	*brevicaule_3*	*Eucycla*	482	1	41°38.473'N	108°29.046'W	Wyoming	Sweetwater	1	1	1	1	1
Eriogonum	*butterworthianum*	*Eucycla*	242	1	36°06.576'N	121°27.657'W	California	Monterey	0	1	0	1	0
Eriogonum	*calcareum*	*Eucycla*	443	1	43°18.129'N	116°34.817'W	Idaho	Owyhee	1	1	1	1	1
Eriogonum	*chrysops*	*Eucycla*	329	1	43°30.381'N	117°46.767'W	Oregon	Malhuer	1	1	1	1	1
Eriogonum	*clavellatum*	*Eucycla*	112	1	37°16.680'N	109°40.518'W	Utah	San Juan	1	1	1	1	1
Eriogonum	*codium*	*Eucycla*	534	1	46°36.722'N	119°45.905'W	Washington	Benton	1	1	1	1	1
Eriogonum	*coloradense*	*Eucycla*	601	1	38°10.846'N	106°42.019'W	Colorado	Sagauche	1	1	1	1	1
Eriogonum	*contortum*	*Eucycla*	635	1	38°51.351'N	109°48.264'W	Utah	Grand	1	1	1	1	1
Eriogonum	*corymbosum*	*Eucycla*	156	1	36°54.461'N	112°35.428'W	Arizona	Mohave	1	1	1	1	1
Eriogonum	*crocatum*	*Eucycla*	228	1	34°09.514'N	119°01.777'W	California	Ventura	1	1	1	1	0
Eriogonum	*crosbyae_1*	*Eucycla*	520	1	42°04.018'N	119°34.630'W	Oregon	Lake	1	1	1	1	1
Eriogonum	*crosbyae_2*	*Eucycla*	528	1	42°03.005'N	118°02.229'W	Oregon	Malheur	1	1	1	1	1
Eriogonum	*cusickii*	*Eucycla*	400	1	43°32.169'N	119°22.534'W	Oregon	Harney	1	1	1	1	1
Eriogonum	*diatomaceum*	*Eucycla*	290	1	39°13.891'N	119°16.881'W	Nevada	Lyon	1	1	1	1	1
Eriogonum	*domitum*	*Eucycla*	638	1	39°08.593'N	113°24.428'W	Utah	Millard	1	1	1	1	1
Eriogonum	*elatum*	*Eucycla*	183	1	38°47.347'N	116°52.382'W	Nevada	Nye	1	1	1	1	1
Eriogonum	*elongatum*	*Eucycla*	238	1	35°03.809'N	120°03.175'W	California	Santa Barbara	1	1	0	1	0
Eriogonum	*ephedroides*	*Eucycla*	596	1	40°08.605'N	109°01.428'W	Colorado	Rio Blanco	1	1	1	1	1
Eriogonum	*eremicum*	*Eucycla*	376	1	38°37.494'N	113°50.992'W	Utah	Millard	1	1	1	1	0
Eriogonum	*exilifolium*	*Eucycla*	351	1	40°47.604'N	106°28.396'W	Colorado	Jackson	1	1	1	1	0
Eriogonum	*fasciculatum*	*Eucycla*	170	1	35°15.037'N	114°43.766'W	Nevada	Clark	1	1	1	1	1
Eriogonum	*gilmanii*	*Eucycla*	263	1	37°14.026'N	117°40.603'W	California	Inyo	1	1	1	1	0
Eriogonum	*gracilipes*	*Eucycla*	268	1	37°22.980'N	118°10.888'W	California	Inyo	1	1	1	1	1
Eriogonum	*grande*	*Eucycla*	JC s.n.	1	33°44.000'N	119°43.000'W	California	Santa Barbara	0	1	1	1	0
Eriogonum	*gypsophilum*	*Eucycla*	100	1	32°33.797'N	104°25.301'W	New Mexico	Eddy	1	1	1	1	0
Eriogonum	*harvardii*	*Eucycla*	104	1	32°31.376'N	104°46.924'W	New Mexico	Eddy	1	1	1	1	1
Eriogonum	*heermannii*	*Eucycla*	253	1	36°31.005'N	117°49.552'W	California	Inyo	1	1	1	1	0
Eriogonum	*holmgrenii*	*Eucycla*	378	1	38°53.587'N	114°19.601'W	Nevada	White Pine	1	1	1	1	1
Eriogonum	*hylophilum*	*Eucycla*	589	1	39°52.979'N	110°13.889'W	Utah	Duchesne	1	1	1	1	0
Eriogonum	*kennedyi*	*Eucycla*	420	1	37°18.355'N	118°10.440'W	California	Inyo	1	1	1	1	0

Genus	species	Subgenus	Coll. #	Habit	Latitude	Longitude	State	County	*trnL-F*	*rpS16*	*trnD-T*	*ITS*	*LEAFY*
Eriogonum	*kingii*	*Eucycla*	435	1	40°35.782'N	115°23.708'W	Nevada	Elko	1	1	1	1	0
Eriogonum	*lachnogynum*	*Eucycla*	SLO8241	1	36°35.717'N	104°12.476'W	New Mexico	Colfax	1	1	1	1	0
Eriogonum	*leptocladon*	*Eucycla*	550	1	38°50.460'N	110°22.992'W	Utah	Emery	1	1	1	1	0
Eriogonum	*lewisii*	*Eucycla*	501	1	41°21.594'N	115°58.172'W	Nevada	Elko	1	1	1	1	1
Eriogonum	*loganum*	*Eucycla*	541	1	41°44.626'N	111°47.199'W	Utah	Cache	1	1	1	1	0
Eriogonum	*lonchophyllum*	*Eucycla*	649	1	38°24.468'N	107°47.924'W	Colorado	Montrose	1	1	1	1	1
Eriogonum	*mancum*	*Eucycla*	454	1	43°49.619'N	113°35.937'W	Idaho	Custer	1	1	1	1	1
Eriogonum	*mensicola*	*Eucycla*	256	1	36°31.991'N	117°44.765'W	California	Inyo	1	1	1	1	0
Eriogonum	*microthecum*	*Eucycla*	187	1	38°47.791'N	116°51.341'W	Nevada	Nye	1	1	1	1	1
Eriogonum	*mitophyllum*	*Eucycla*	557	1	38°53.683'N	111°53.920'W	Utah	Sevier	1	1	1	1	1
Eriogonum	*mortonianum*	*Eucycla*	155	1	36°54.461'N	112°35.428'W	Arizona	Mohave	0	1	1	1	0
Eriogonum	*natum*	*Eucycla*	581	1	39°03.648'N	113°14.700'W	Utah	Millard	1	1	1	1	0
Eriogonum	*novonudum*	*Eucycla*	401	1	42°48.352'N	117°43.907'W	Oregon	Malhuer	1	1	1	1	1
Eriogonum	*nudum*	*Eucycla*	220	1	35°30.042'N	118°23.973'W	California	Kern	1	1	1	1	0
Eriogonum	*nummulare*	*Eucycla*	173	1	37°27.730'N	115°21.590'W	Nevada	Lincoln	1	1	1	1	0
Eriogonum	*ochrocephalum*	*Eucycla*	418	1	39°18.402'N	119°34.292'W	Nevada	Storey	1	1	1	1	1
Eriogonum	*ostlundii*	*Eucycla*	556	1	38°36.144'N	112°05.362'W	Utah	Sevier	1	1	1	1	0
Eriogonum	*ovalifolium*	*Eucycla*	192	1	37°47.759'N	117°48.274'W	Nevada	Esmerelda	1	1	1	1	1
Eriogonum	*panguicense*	*Eucycla*	371	1	38°58.617'N	111°57.675'W	Utah	Sevier	1	1	1	1	1
Eriogonum	*parvifolium*	*Eucycla*	236	1	34°30.837'N	120°28.948'W	California	Santa Barbara	1	1	1	1	1
Eriogonum	*pauciflorum_1*	*Eucycla*	347	1	45°02.675'N	108°25.035'W	Montana	Carbon	1	1	1	1	1
Eriogonum	*pauciflorum_2*	*Eucycla*	604	1	40°44.248'N	104°04.055'W	Colorado	Weld	1	1	1	1	1
Eriogonum	*pelinophlium*	*Eucycla*	626	1	38°39.266'N	107°55.411'W	Colorado	Montrose	1	1	1	1	1
Eriogonum	*prociduum*	*Eucycla*	428	1	41°18.943'N	119°53.590'W	Nevada	Washoe	1	1	1	1	1
Eriogonum	*racemosum*	*Eucycla*	109	1	36°06.332'N	108°48.220'W	New Mexico	San Juan	1	1	1	1	1
Eriogonum	*rosense_1*	*Eucycla*	180	1	38°07.392'N	116°54.334'W	Nevada	Nye	1	1	1	1	1
Eriogonum	*rosense_2*	*Eucycla*	283	1	39°20.590'N	119°54.979'W	Nevada	Washoe	1	1	1	1	1
Eriogonum	*rupinum*	*Eucycla*	184	1	38°47.571'N	116°51.662'W	Nevada	Nye	1	1	1	1	1
Eriogonum	*saxatile*	*Eucycla*	234	1	34°43.771'N	119°58.253'W	California	Santa Barbara	1	1	1	1	1
Eriogonum	*scopulorum*	*Eucycla*	532	1	45°11.764'N	117°12.230'W	Oregon	Wallowa	1	1	1	1	1
Eriogonum	*shockleyi_1*	*Eucycla*	128	1	40°19.778'N	109°29.166'W	Utah	Uintah	1	1	1	1	1
Eriogonum	*shockleyi_2*	*Eucycla*	262	1	37°07.616'N	118°03.555'W	California	Inyo	1	1	1	1	1
Eriogonum	*shockleyi_3*	*Eucycla*	494	1	41°37.316'N	114°50.161'W	Nevada	Elko	1	1	1	1	1
Eriogonum	*soliceps*	*Eucycla*	340	1	44°47.656'N	113°14.815'W	Idaho	Lemhi	1	1	1	1	1
Eriogonum	*soredium*	*Eucycla*	375	1	38°26.979'N	113°16.960'W	Utah	Beaver	1	1	1	1	1
Eriogonum	*spathulatum*	*Eucycla*	583	1	38°58.748'N	112°22.947'W	Utah	Millard	1	1	1	1	1
Eriogonum	*strictum*	*Eucycla*	312	1	41°19.154'N	122°28.784'W	California	Siskiyou	1	1	1	1	1
Eriogonum	*tiehmii*	*Eucycla*	193	1	37°49.032'N	117°51.391'W	Nevada	Esmerelda	1	1	1	1	1
Eriogonum	*tumulosum*	*Eucycla*	356	1	40°45.497'N	108°50.008'W	Colorado	Moffatt	1	1	1	1	1
Eriogonum	*villiflorum*	*Eucycla*	566	1	38°31.017'N	113°32.722'W	Utah	Beaver	1	1	1	1	0
Eriogonum	*viridulum*	*Eucycla*	359	1	40°24.299'N	109°35.442'W	Utah	Uintah	1	1	1	1	0
Eriogonum	*wrightii*	*Eucycla*	216	1	35°32.257'N	118°26.983'W	California	Kern	1	1	1	1	0
Eriogonum	*aliquantum*	*Ganysma*	91	0	36°29.727'N	104°55.827'W	New Mexico	Colfax	1	1	1	1	1
Eriogonum	*angulosum*	*Ganysma*	222	0	35°13.863'N	119°38.857'W	California	Kern	1	1	1	1	0
Eriogonum	*cernuum*	*Ganysma*	133	0	39°58.678'N	109°59.431'W	Utah	Duchense	1	1	1	1	0
Eriogonum	*gordonii*	*Ganysma*	126	0	40°08.461'N	108°56.432'W	Colorado	Rio Blanco	1	1	1	1	1
Eriogonum	*inflatum*	*Ganysma*	111	0	37°16.937'N	109°30.384'W	Utah	San Juan	1	1	1	1	1
Eriogonum	*nutans*	*Ganysma*	415	0	38°34.236'N	119°11.189'W	Nevada	Lyon	1	1	1	1	1
Eriogonum	*rotundifolium*	*Ganysma*	107	0	33°20.719'N	106°04.985'W	New Mexico	Otero	1	1	1	1	0
Eriogonum	*salicornioides*	*Ganysma*	539	0	43°44.275'N	117°40.767'W	Oregon	Malheur	1	1	1	1	0
Eriogonum	*spergulinum*	*Ganysma*	286	0	39°19.812'N	119°55.685'W	Nevada	Washoe	0	1	0	0	0
Eriogonum	*tenellum*	*Ganysma*	87	1	37°12.595'N	103°38.396'W	Colorado	Los Animas	1	1	1	1	1
Eriogonum	*watsonii*	*Ganysma*	434	0	40°53.875'N	117°24.149'W	Nevada	Humboldt	1	1	1	1	1
Eriogonum	*wetherillii*	*Ganysma*	114	0	37°15.865'N	109°55.942'W	Utah	San Juan	1	1	1	1	0

Genus	species	Subgenus	Coll. #	Habit	Latitude	Longitude	State	County	*trnL-F*	*rpS16*	*trnD-T*	*ITS*	*LEAFY*
Eriogonum	*annuum*	*Micrantha*	605	0	40°46.113'N	102°56.510'W	Colorado	Logan	1	1	1	1	1
Eriogonum	*alpinum*	*Oligogonum*	313	1	41°19.018'N	122°28.813'W	California	Siskiyou	1	1	1	1	1
Eriogonum	*caespitosum*	*Oligogonum*	175	1	37°34.844'N	115°46.327'W	Nevada	Lincoln	1	1	0	1	0
Eriogonum	*diclinum*	*Oligogonum*	323	1	41°54.262'N	122°56.574'W	California	Siskiyou	1	1	1	1	0
Eriogonum	*flavum*	*Oligogonum*	469	1	45°01.363'N	108°26.114'W	Montana	Carbon	1	1	1	1	1
Eriogonum	*lobbii*	*Oligogonum*	276	1	38°03.165'N	119°15.927'W	California	Mono	1	1	1	1	0
Eriogonum	*robustum*	*Oligogonum*	289	1	39°23.161'N	119°42.526'W	Nevada	Washoe	1	1	1	1	0
Eriogonum	*sphaerocephalum*	*Oligogonum*	591	1	39°52.979'N	110°13.889'W	Utah	Duchesne	1	1	1	1	1
Eriogonum	*umbellatum*	*Oligogonum*	132	1	40°36.891'N	109°34.079'W	Utah	Uintah	1	1	1	1	1
Eriogonum	*cithariforme*	*Oregonium*	233	0	34°43.133'N	119°56.170'W	California	Santa Barbara	1	1	1	0	1
Eriogonum	*divaricatum*	*Oregonium*	629	0	37°18.862'N	109°39.230'W	Utah	San Juan	1	1	1	1	1
Eriogonum	*nidularium*	*Oregonium*	191	0	37°45.364'N	117°42.256'W	Nevada	Esmerelda	1	1	1	1	1
Eriogonum	*palmerianum*	*Oregonium*	159	0	36°51.865'N	112°18.956'W	Arizona	Coconino	1	1	1	1	0
Eriogonum	*roseum*	*Oregonium*	515	0	40°19.406'N	120°32.661'W	California	Lassen	1	1	1	1	1
Eriogonum	*vimineum*	*Oregonium*	305	0	41°06.944'N	120°48.148'W	California	Lassen	1	1	1	1	0
Eriogonum	*alatum*	*Pterogonum*	168	1	35°20.682'N	112°57.626'W	Arizona	Yavapai	1	1	1	1	1
Eriogonum	*tomentosum*	*Eriogonum*	s.n.	1	n/a	n/a	n/a	n/a	0	0	0	0	GB
Gilmania	*luteola*	n/a	JLR8465	0	36°20'49"N	116°42'59"W	California	Inyo	1	1	1	1	GB
Johanneshowellia	*puberula*	n/a	579	0	39°09.582'N	113°24.681'W	Utah	Millard	1	1	1	1	1
Stenogonum	*flexum*	n/a	365	0	39°37.550N	110°52.000'W	Utah	Carbon	1	1	1	1	0

Sources of incongruence in *Eriogonum*: Challenging phylogenetic reconstruction in a hyper diverse radiation of species

Ben R. Grady & Kenneth J. Sytsma

Empirical studies of gene tree discordance in thoroughly sampled clades are of great importance to understand processes such as lineage sorting, hybridization, and concerted evolution. We present an extensively sampled molecular phylogenetic study of a diverse clade in *Eriogonum* (Polygonaceae). We compare gene trees from chloroplast (*trnL-trnF, rps16, trnD-trnT*), nuclear-ribosomal internal transcribed spacer (*ITS*), and low-copy nuclear regions (*LEAFY*, *RPB2*) to decipher gene-to-gene discord and to examine species monophyly in narrowly and widely distributed taxa in *Eriogonum*. Genetic resolution from the regions used is reasonable for such a diverse clade, however lack of resolution in the core of the *Eucycla II* clade results in a polytomy, evident in all gene trees. Selected species are highlighted to discuss the potential of various evolutionary processes that lead to gene tree incongruence. Accessions of *E. shockleyi*, a widespread species of the Colorado Plateau and the Great Basin, are largely monophyletic in the nuclear gene trees. Multiple, divergent chloroplast haplotypes are recovered from populations of *E. shockleyi*, indicating a history of extensive hybridization or incomplete lineage sorting in this clade. We highlight a likely case of hybridization in *E. clavellatum* and an adjacent population of *E. shockleyi* from New Mexico, with likely introgression of the *ITS* region, as well as the chloroplast DNA. Chloroplast haplotype diversity is recognized in three geographically restricted species: *E. gracilipes*, *E. domitum*, and *E. soredium*. We finish with a discussion on the importance of using multiple lines of evidence (morphological, genetic, ecological, geographic) in recognizing and

defining species, especially in phylogenetically challenging groups such as *Eriogonum.*

Key words: incomplete lineage sorting, hybridization, species monophyly, species concepts, *Eriogonum,* gene tree incongruence

The reconstruction and inference of gene trees allows evolutionary biologists to gain insight into the history of a group of organisms. The comparison of multiple gene trees, especially in plants, often reveals discordant topologies. While this discordance poses practical problems, it is essential to compare multiple genealogies to recreate an accurate representation of a species history, which may or may not be reflect a strict tree-like history (Pamilo & Nei 1988, Maddison 1997; Degnan and Rosenberg 2009). Incongruent gene trees can arise from a variety of different processes, both technical and biological (Wendel and Doyle 1998). Technical causes of gene tree incongruence may include selecting DNA regions without sufficient levels of variation, sequencing error, and/or sparse taxon sampling. Wendel and Doyle (1998) also propose a number of biological reasons for obtaining discordant gene trees, such as incomplete lineage sorting, hybridization, gene duplication, recombination, and concerted molecular evolution of loci. In empirical studies of plant lineages, gene tree incongruence appears to be the rule, rather than the exception, particularly in studies with a focus at or near the level of species (e.g. Popp and Oxelman 2001; Mason-Gamer 2004; Kim and Donoghue 2008; van der Niet and Linder 2008; Hung et al. 2009; Gurushidze et al. 2010; Koopman and Baum 2010; Goodson et al. 2012). Inferring species relationships, especially those that are either in the process of speciating or are members of a lineage that has a history of recent and/or rapid radiations, can pose many challenges (Wendel and Doyle 1998; Knowles and Chan 2008). The aforementioned processes leading to gene tree discordance are amplified in closely related species

groups, especially when incomplete lineage sorting, hybridization, unrecognized polyploid species, and lack of genetic variation are recognized as probable issues (Popp and Oxelman 2001; Jakob and Blattner 2006; Maddison and Knowles 2006; Kim and Donoghue 2008; Hung et al. 2009; Albach and Muedt 2010; Gurushidze et al. 2010; Koopman and Baum 2010; Muedt 2011).

There has been much discussion as to whether phylogenetic reconstructions can be improved by increasing the number of taxa sampled or by the addition of more gene regions (Mitchell et al. 2000; Rokas et al. 2003; Rokas and Carroll 2005; Knowles and Carstens 2007; McCormack et al. 2009). It is widely acknowledged that a gene tree does not equal a species tree, and multiple loci are necessary to infer a more accuarate evolutionary history of a lineage or species (Maddison 1997). In model organisms, obtaining and generating data from numerous gene regions is simplified. Phylogenetic work in yeast has shown that overcoming incongruence in gene trees to accurately infer a species tree is best accomplished with many loci, rather than extensive taxon sampling (Rokas et al. 2003; Rokas and Carroll 2005). In non-model organisms or groups for which we have a limited knowledge of evolutionary history, gathering data from multiple genic regions is much more difficult. In this case, extensive taxon sampling is typically preferred (Knowles and Carstens 2007, Heath et al. 2008; Reeves and Richards 2011).

Recognition and reconciliation of these issues leads to a better understanding of the evolutionary history of a species, and ultimately to a relevant circumscription of a species. Few issues have caused so much discussion and controversy as has the “species concept” issue (e.g. Darwin 1859; Mayr 1942; Levin

1979; Sites and Marshall 2003; Hey 2006; Riesberg et al. 2006; Rieppel 2009). Historically the basis for defining a species has largely drawn from morphological characteristics, ecological preferences, and reproductive isolation, with a recent emphasis on phylogenetic concepts and monophyly (Mayr 1942; Baum 1992; de Queiroz 1992; Baum and Donoghue 1995; Riesberg et al. 2006). With advances in molecular techniques and phylogenetic analyses, well sampled systematic studies are showing that non-monophyly of previously recognized species is common (Riesberg and Brouillet 1994; Hung et al. 2009; Koopman and Baum 2010). Integrative approaches to incorporate multiple lines of evidence are still being developed and evaluated (Reeves and Richards 2011; Bacon et al. 2012; Lega et al. 2012; Puillandre et al. 2012).

Eriogonum (Polygonaceae), commonly known as wild buckwheat, provides an excellent model system to investigate discordant gene genealogies and to test various hypotheses of incongruence. Based upon morphological characters and geographic distribution, species in *Eriogonum* have been well characterized and circumscribed (Reveal 1969; 1978; 2005). Despite the long taxonomic history and prevalence of species with conservation concerns in this genus, a well-sampled molecular phylogenetic analysis of species relationships is currently lacking. Although no specific dates of divergence have been proposed, it has been postulated that certain clades within *Eriogonum* are the result of recent, rapid species radiations (Reveal 2005). All *Eriogonum* species occur in North America, with the vast majority distributed across arid regions of the western United States. Many of these species occur in distinct, isolated populations, however there are a few

examples of widespread species that occur across various floristic regions (e.g. *Eriogonum brevicaule, E. corymbosum, E. microthecum* and *E. ovalifolium*). Within *Eriogonum*, a diverse clade of perennial species, here referred to as the *Eucycla II* clade, offers an excellent opportunity to study biological processes that shape gene histories, and the subsequent untangling of species evolutionary histories.

The goal of this study is to demonstrate that extensive taxon and distributional sampling are necessary to recognize and untangle species' relationships in a complex, recently radiated clade of plant species. We compare sequence data from the chloroplast and nuclear gene regions to highlight differential evolutionary processes that have shaped relationships within *Eriogonum* subgenus *Eucycla II*. Our specific aims are to: 1) assess species relationships and species monophyly within *Eriogonum* subg. *Eucycla II*; 2) explore and highlight potential sources of incongruence from nuclear and plastid gene regions; and 3) discuss geographic patterns relating to differential genealogies and networks.

MATERIALS AND METHODS

Taxonomic Sampling—Relationships within *Eriogonum* subg. *Eucycla II* are explored through extensive taxon sampling and comparison of multiple gene regions. We expanded the sampling within the *Eucycla II* clade and included multiple accessions from across the geographic range of many species included in our Chapter 1 sampling regime. We included from 1-3 different accessions of narrowly endemic species, and as many as 17 accessions of geographically

widespread species (i.e. *E. shockleyi*). We made every effort to broadly sample across the geographic range of each species and to include samples of all recognized varieties of each species. Voucher information and included accessions with DNA sequence representation are included in Appendix 1. Based upon our gene trees in Chapter 1, *Eriogonum alatum, E. tumulosum,* and *E. aliquantum* are appropriate outgroup species to study relationships within the *Eucycla II* clade.

DNA Extraction, Amplification, and Sequencing—Total genomic DNA was extracted from field collected, silica-dried leaf tissue using the Qiagen DNeasy™ plant mini kit (Qiagen, Valencia, California). Undiluted total genomic DNA was then combined with 10x buffer, dNTP's and Ex Taq taq polymerase from Takara (Otsu, Shiga, Japan) and DMSO, BSA, and forward and reverse primers of interest. All reactions were carried out in 12.5 uL total volume.

The internal transcribed spacer (*ITS*) of the nuclear ribosomal DNA was amplified using the ITS4 and ITS5 primers and protocol from White et al. (1990). Chloroplast introns and spacer regions (*trnL-trnF*, *rpS16*, and *trnD-trnT*) were amplified using primers from Taberlet et al. (1991) and Shaw et al. (2005). Additional internal primers were designed for this study to amplify the entire *trnD-trnT* spacer region. The primer sequences, paired with the *trnD* and *trnT* primers respectively, are as follows: *trnE-m2* 5' GCTTTCTATATCGAATCGAATC 3' and *trnY-m2* 5' CATAGTATGAACAGTTTTTGG 3'. All chloroplast regions, excepting the *trnD* to *trnE* region, were successfully amplified with the following touchdown program: initial denaturation (94°C for 30 sec); amplification: 10 cycles 94°C 15 sec, 64°C 30 sec -1°C/cycle, and 68°C for 1 min; then 20 cycles 94°C 15 sec, 54°C 30 sec, 68°C 1

min, followed by extended elongation of 68°C for 5 min. The *trnD* to *trnE* spacer region was amplified using the following protocol: denaturation 94°C 1 min; amplification 30 cycles 94°C 15 sec, 44°C 30 sec, and 72°C 1 min; and final extension of 72°C for 5 min.

The second intron of the low-copy nuclear region *LEAFY* was initially amplified in a subset of *Eriogonum* taxa with the degenerate primers, *LFsxl-2* and *LFtxr*, from Frohlich and Meyerowitz (1997). To achieve more consistent amplification in the group of study, Eriogonoideae specific primers were then designed from these initial sequences. The forward and reverse primer sequences are: *LFY71-F* 5' GCCTTGATTATCTCTTCCAC 3' and *LFY1369-R* 5' CCTGAACACCTGGTTTGTC 3'. PCR conditions for the modified *LEAFY* primers are: initial denaturation 94°C for 1 min; amplification 8 cycles 94°C for 20 sec, 59°C - 1°C/cycle for 30 sec, 72°C for 1 min followed by 25 cycles of 94°C 20 sec, 52°C for 30 sec, and 72°C for 1 min; with a final extension of 72°C for 5 min.

Eriogonum-specific primers were developed for a portion of the second exon, second intron, third exon, third intron, and a portion of the fourth exon of the RNA polymerase II second largest subunit nuclear locus, *RPB2*. Available Caryophyllales *RPB2* sequences were obtained from GenBank and aligned. The forward primer sequence, anchored in the second exon is 5' CAAAGTTTTYATTGGGAAGG 3' and the reverse primer, located in the fourth exon of *RPB2* is 5' TGAATYACAAATGCCTCTTC 3'. A touchdown program was used to amplify this portion of *RPB2*. The conditions are: initial denaturation (94°C for 1 min); amplification: 10 cycles 94°C 20 sec, 59°C

30 sec -1°C/cycle, and 72°C for 1 min; then 25 cycles 94°C 20 sec, 49°C 30 sec, 72°C 1 min, followed by extended elongation of 72°C for 5 min.

PCR products were diluted (30:1) in water and cycle sequenced using the same primers. Agencourt magnetic beads were used to purify the cycle sequencing products prior to sequencing (Agencourt, Beverly, Massachusetts). Sequencing reactions were carried out at the DNA Sequencing Facility on the University of Wisconsin campus using ABI PRISM BigDye terminator on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems, Foster City, California).

Forward and reverse sequences were assembled and edited in Sequencher ver. 4.7 (Gene Codes, Ann Arbor, Michigan). Polymorphic sites in *ITS*, *LEAFY* and *RPB2* were coded with IUPAC ambiguity codes. A site was coded as polymorphic if two peaks were present at the same site in both the forward and reverse read. If a region showed clear length variation in the chromatogram or if more than 1% of the sequenced region was coded as polymorphic, cloning was implemented to separate potential alleles or copies. For samples to be cloned, the Promega pGEM-T vector protocol was followed, except the volume of all reagents and buffers was halved (Promega, Madison, Wisconsin). To obtain representative sequences of all copies/alleles present, 8 to 24 clones were sequenced, dependent upon variation in the initial direct sequencing read. All cloned sequences were initially aligned and visually compared to assess variation present in recovered sequences, and to recognize chimeric sequences or sequences with obvious Taq errors (i.e. point mutations present in only one cloned sequence) (Edwards et al. 2008). Recombinant

(chimeric) sequences and those with clear unique Taq errors were excluded from alignments.

Phylogenetic Analyses—Sequences from each respective region were manually aligned in MacClade 4.08 (Maddison and Maddison 2005) to maximize homologous site matches and minimize the placement of gaps in the alignments. Models of sequence evolution for Maximum Likelihood (ML) and Bayesian Inference (BI) were obtained from jModelTest and MrModelTest 2.3, respectively (Posada 2008, Nylander 2004). All basic phylogenetic analyses were carried out in the CIPRES Science Gateway v. 3.3 (Miller et al. 2010), using MrBayes v. 3.1.2 for BI (Huelsenbeck & Ronquist 2001) or RAxML v. 7.3.0 for ML bootstrapping (Stamatakis et al. 2008). Bayesian analysis of individual data sets was implemented with default settings, 10 million generations, with a 25% burnin. Maximum likelihood bootstrap values were gathered using RAxML, with 1,000 bootstrap replicates and default settings.

Network analysis of DNA sequence data—Sequence alignments for each of the four datasets were analyzed by employing the Neighbor-Net algorithm in SplitsTree4 ver. 4.12.3 with default settings and uncorrelated p-distances (Bryant & Moulton 2004; Huson & Bryant 2011). This method creates connected networks using distance-based methods to highlight and to help visualize evolution in a group that may not reflect simple branching relationships, and to detect conflicting signal in a dataset (Bryant and Moulton 2004). The outgroup taxa used in the previous phylogenetic tree analyses were eliminated from the network analyses, as were five

accessions from other subgenera due to extremely divergent genetic distances (long branch lengths) apparent in other trees.

RESULTS

Individual datasets and phylogenetic utility—The molecular phylogenies and networks constructed here provide useful information regarding the relationships in this species-rich clade of *Eriogonum*. None of the gene trees were fully resolved and there is substantial uncertainty and lack of resolution associated with certain parts of each phylogeny and network presented. The results from each of the respective gene regions and datasets are presented below and the summary statistics are included in Table 1.

Table 1. Summary statistics for all data matrices analyzed in this study of relationships *Eriogonum*. An * in the Accessions included column denotes additional cloned sequences present in the data matrix. Variable sites included, Parsimony informative sites, % variable, and % PIC are calculated with and without outgroups, values for the ingroup only are shown in parentheses.

Region	Accessions included	Aligned length (bp)	Variable sites included (ingroup only)	Parsimony informative sites (ingroup only)	% variable (ingroup only)	% PIC (ingroup only)	Substitution model (BI)
nrITS	137*	734	226 (189)	124 (108)	30.7% (25.7%)	17.0% (14.7%)	GTR+I+G
LFY 2nd intron	89*	2806	741 (679)	374 (341)	26.4% (24.5%)	13.3% (12.2%)	GTR+G
RPB2	85*	711	267 (258)	152 (145)	37.5% (36.3%)	21.4% (20.4%)	HKY+G
Combined chloroplast	137	4430	378 (306)	130 (112)	8.5% (6.9%)	2.9% (2.5%)	GTR+I

The combination of the spacers from *trnL-trnF* and *trnD-trnT* regions, and the intron associated with *rpS16* provided strong support for a monophyletic *Eucycla II* clade. Plastid sequence variation is the lowest among all of the loci utilized (Table1), thus we see relatively short branches in the presented BI phylogram (Figure 1). A large polytomy is apparent at the base of the *Eucycla II* clade, off of which emerge numerous strongly supported (>0.90 BI PP & >50 ML BS) clades (Figure 1, clades A-O) as well as unresolved placements for several accessions. This basal uncertainty is further highlighted when visualized as a splits graph (Figure 2). The center of the network is highly reticulate with relatively long branches leading to the edges. The labeled nodes from the combined chloroplast phylogeny (Figure 1) are also indicated on the splits graph (Figure 2).

The internal transcribed spacer (*ITS*) from the nuclear ribosomal genome amplified well across all accessions and provided the second highest percentage of parsimony informative characters of the loci used in this study (Table 1, Appendix 1). High levels of polymorphic sites and sequence length variation necessitated bacterial cloning to separate alternate copies of *ITS* in some samples in the *Eucycla II* clade. After eliminating paralogous and duplicate copies, we recovered two unique copies of the *ITS* region in 23 accessions. Each of the variant copies was retained to examine potential traces of hybridization (labeled c1 and c2, Figure 3). All other included accessions had only one copy of *ITS* present. There are numerous well-supported clades in the *ITS* gene tree (>0.90 BI PP & >50 ML BS), although many relationships remain unresolved (Figure 3). Especially noteworthy is the large polytomy containing many of the capitate members of *Eriogonum* (gray star, Figure

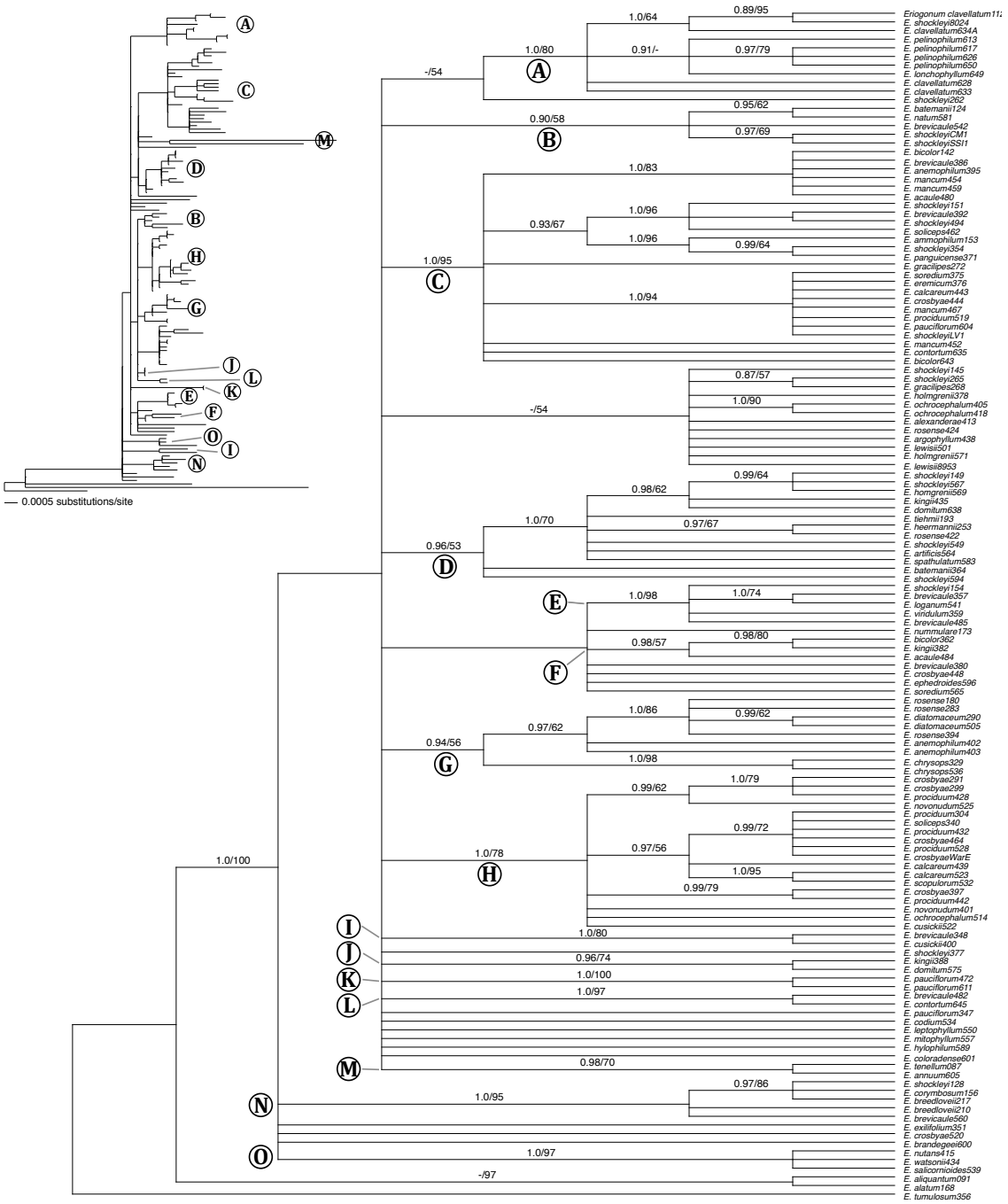


Figure 1. Consensus tree from Bayesian analysis of the combined three-region chloroplast data set (*trnL-trnF*, *rpS16*, and *trnD-trnT*) for 137 accessions of *Eriogonum* subg. *Eucycla II*. A phylogram representing the tree with the highest log likelihood from the Bayesian analysis is shown in the upper left corner. Major clades of interest are labeled with an encircled letter. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included.

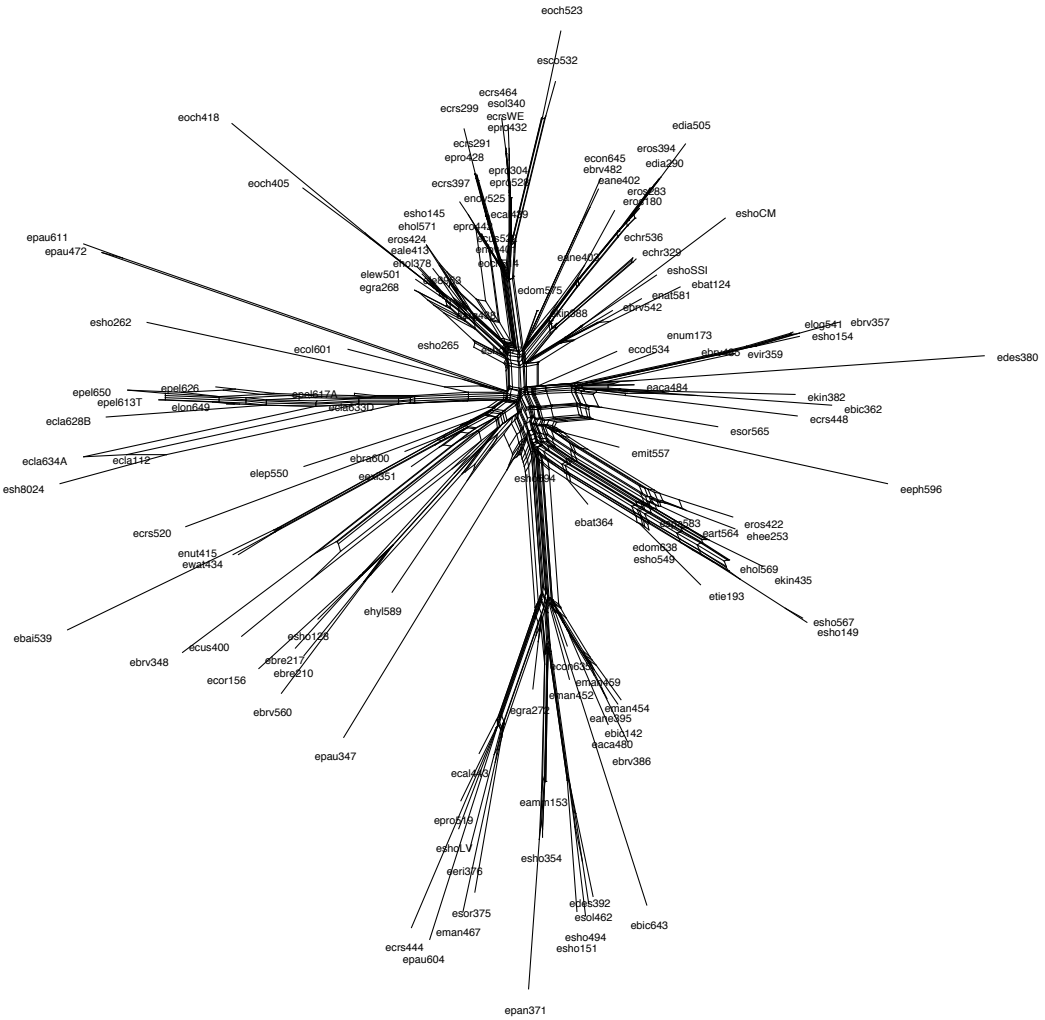


Figure 2. NeighborNet splits graph derived from the combined chloroplast data set with the outgroups excluded. Tip labels correspond to codes included in Appendix 1.

3). More resolution, especially for deeper nodes, is obtained from the *ITS* gene tree when compared to the combined plastid phylogeny (Figures 1 and 3). The network created from the *ITS* data set shows many connections near the center of the network, indicative of conflicting signal, potentially resulting from a variety of biological processes (Figure 4).

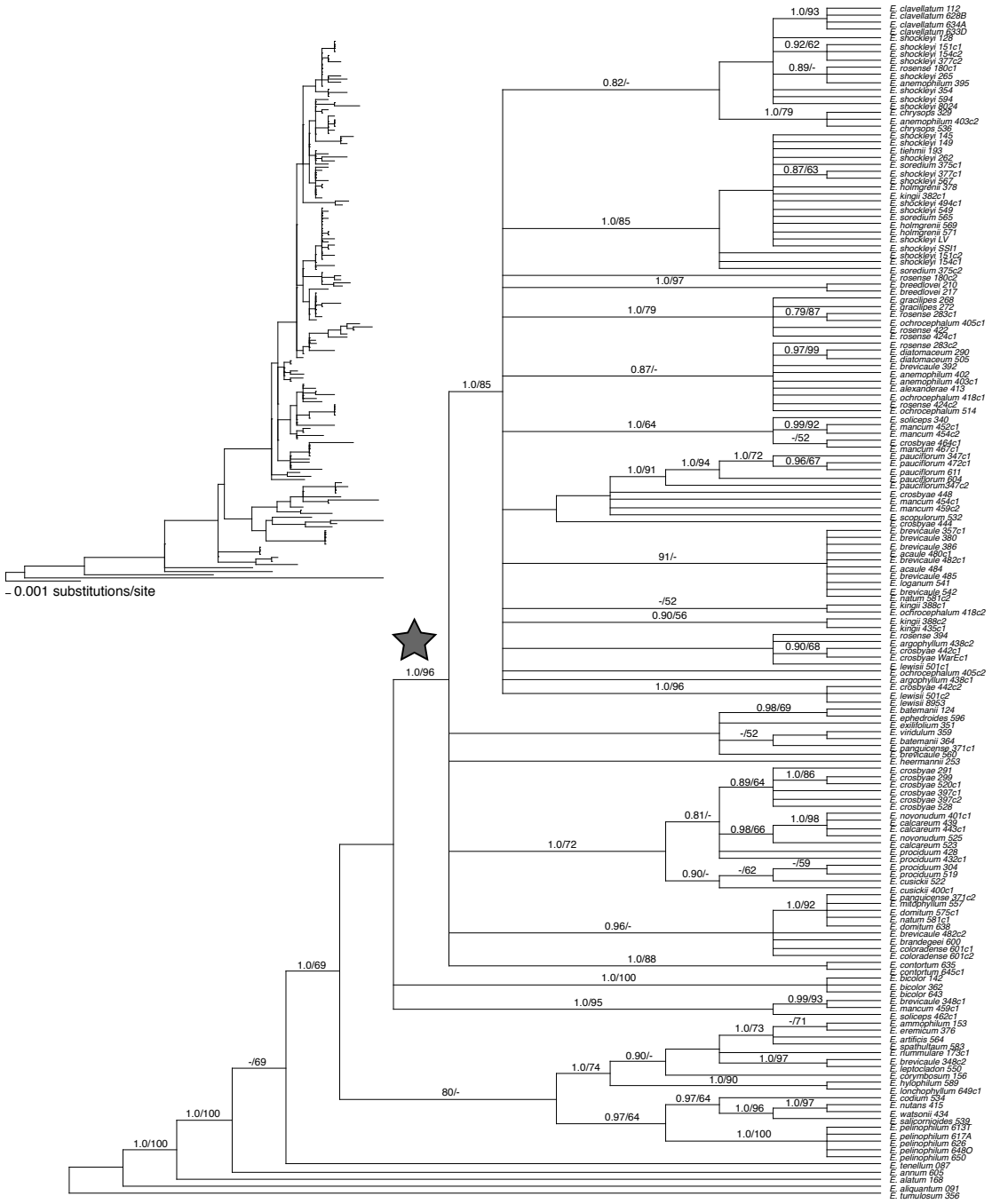


Figure 3. Consensus tree from Bayesian analysis of *ITS* for 137 accessions of *Eriogonum* subg. *Eucycla*. A phylogram representing the tree with the highest log likelihood from the Bayesian analysis is shown in the upper left corner. The discussed polytomy is indicated by a gray star. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Numbers following species are collection numbers and cloned accessions have a c1 or c2.

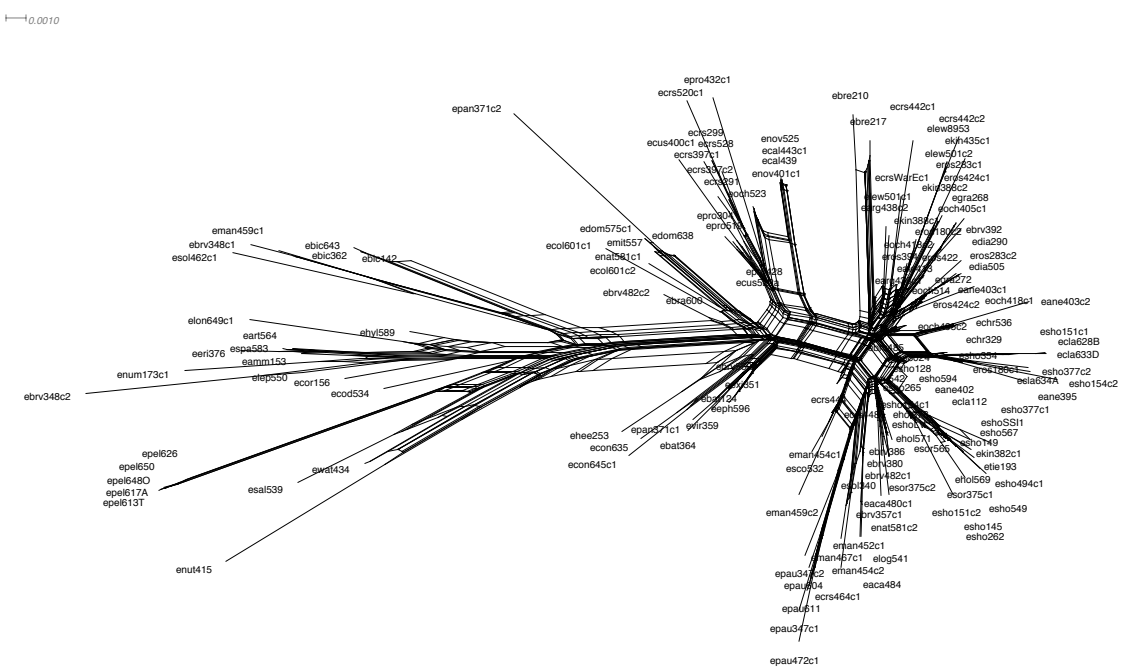


Figure 4. NeighborNet splits graph derived from the *ITS* data set with the outgroups excluded. Tip labels correspond to codes included in Appendix 1.

We were able to successfully amplify the second intron of the *LEAFY* locus for most of the accessions included in our expanded sampling of the *Eucycla II* clade (89 of 137 total). The order of divergence for major clades is largely similar to that observed in the *ITS* gene tree, and the large polytomy (gray star, Figure 5) is present in the core *Eucycla II* group. Allelic and potentially gene copy variation was observed from direct sequencing of *LEAFY*. Cloning revealed the presence of multiple alternate forms (divergent alleles or copies) of *LEAFY* present in 26 samples. We recovered three alternate sequences in the following accessions: BRG193 (*E. tiehmii*), BRG405 (*E. ochrocephalum*), BRG522 (*E. cusickii*), and BRG564

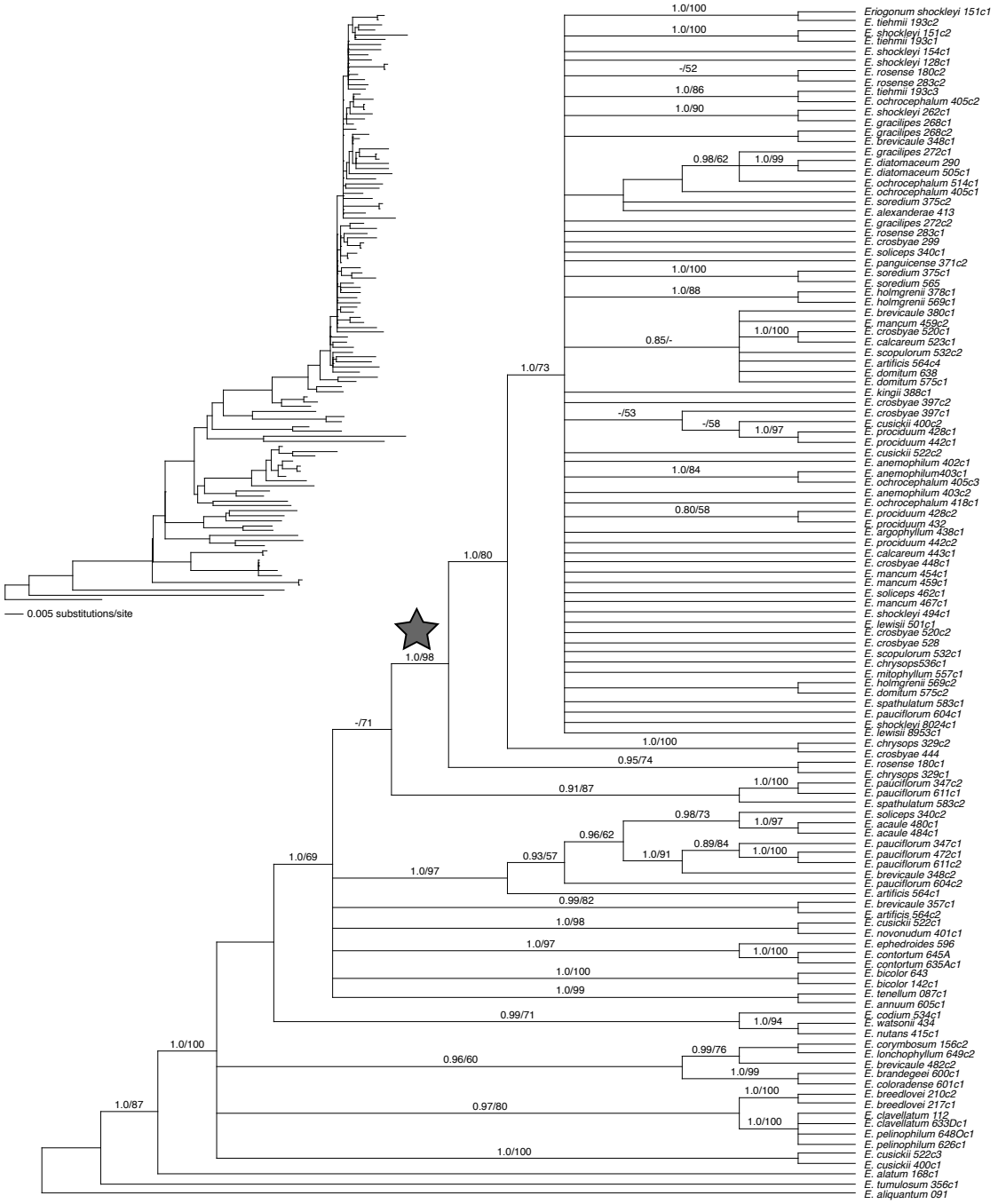


Figure 5. Consensus tree from Bayesian analysis of *LEAFY* for 89 accessions of *Eriogonum* subg. *Eucycla*. A phylogram representing the tree with the highest log likelihood from the Bayesian analysis is shown in the upper left corner. The discussed polytomy is labeled with a gray star. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Numbers following species are collection numbers and cloned accessions have a c1, c2, c3, or c4.

(*E. artificis*). Twenty-two samples showed two forms, and all other included accessions showed only sequence. The vast majority of these alternate sequences were placed in the large, unresolved clade (Figure 5), however alternate sequences of *E. cusicki* (BRG400 and BRG522), *E. artificis* (BRG564), *E. pauciflorum* (BRG604), *E. soliceps* (BRG340), *E. brevicaule* (BRG348), and *E. spathulatum* (BRG583) are present both within this large polytomy, and in one of the basal clades of the phylogeny (Figure 5). The Neighbor-Net derived splits graph from the *LEAFY* data indicates extensive reticulation (Figure 6). Long terminal branches roughly correspond to early diverging lineages, and the polytomy discussed previously is easily visualized (gray star, Figure 5).

The second through fourth exons and associated introns of *RPB2* amplified well for many of the taxa included in this study (85 of a total of 137). Levels of sequence variation were the highest for all regions utilized here (Table 1). Unlike the other low-copy region investigated, *LEAFY*, there was little sequence length variation detected in the samples sequenced. A portion of the samples sequenced revealed many nucleotide polymorphisms, up to as many as 3.5% of the sequence. Any directly sequenced samples of *RPB2* that contained more than 1% of the region as polymorphic necessitated cloning to separate potential copy variation. Multiple copies were detected in thirty-five of the samples sequenced, with one accession containing four distinct forms of *RPB2* (BRG348). Five others had three distinct forms present: *E. eremicum* (BRG376), *E. calcareum* (BRG443), *E. mancum* (BRG459), *E. soliceps* (BRG462), and *E. spathulatum* (BRG583). In the constructed gene tree from the *RPB2* dataset, very few of the deeper nodes receive PP or BS

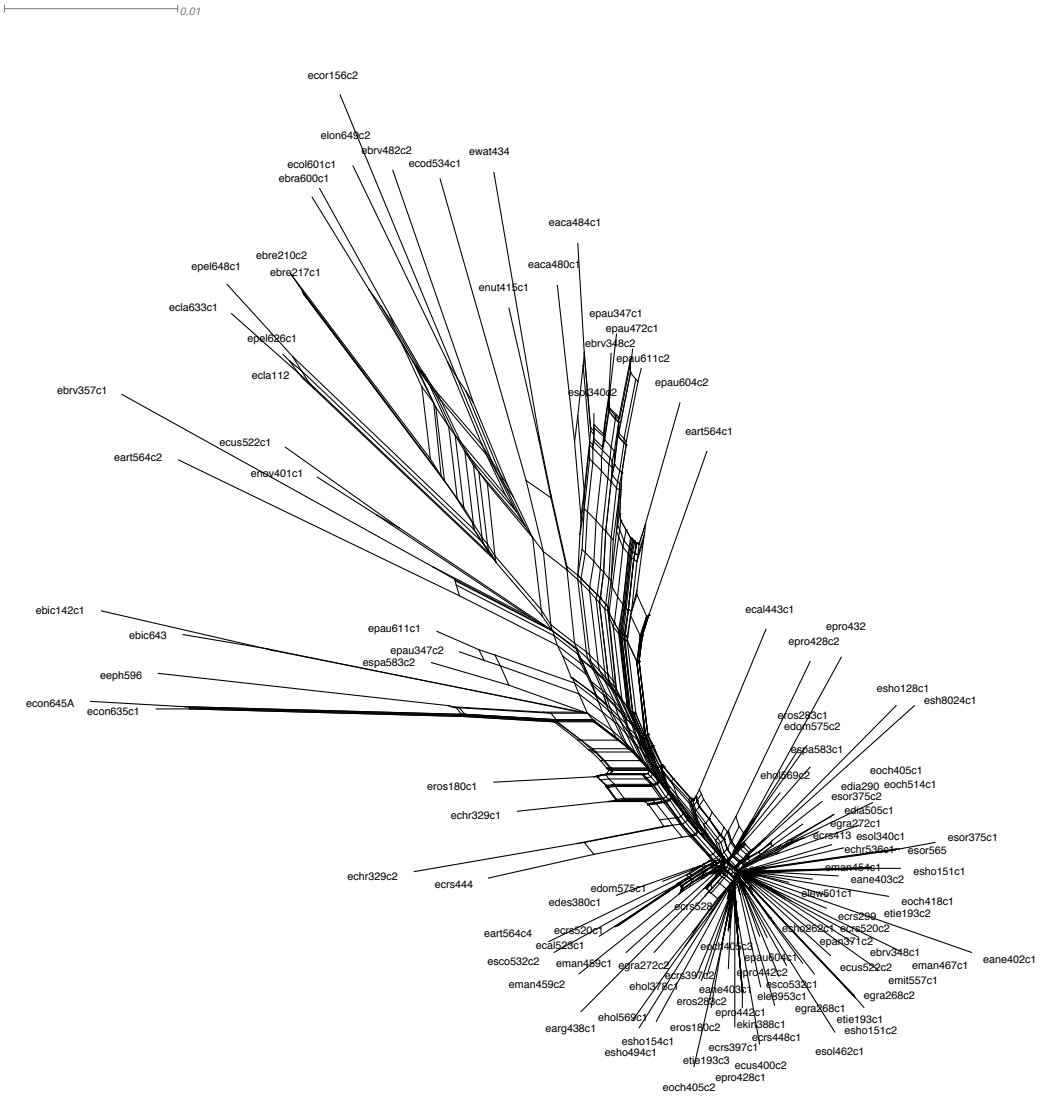


Figure 6. NeighborNet splits graph derived from the *LEAFY* data set with the outgroups excluded. Tip labels correspond to codes included in Appendix 1.

support. There are a number of terminal clades that receive higher support (>0.90 BI PP & >50 ML BS). Within these clades, however very little resolution is apparent (Figure 7). The network analysis of the *RPB2* dataset reveals patterns similar to the other datasets. Multiple alternate connections are present at the base of the network, with divergent clusters emerging (Figure 8).

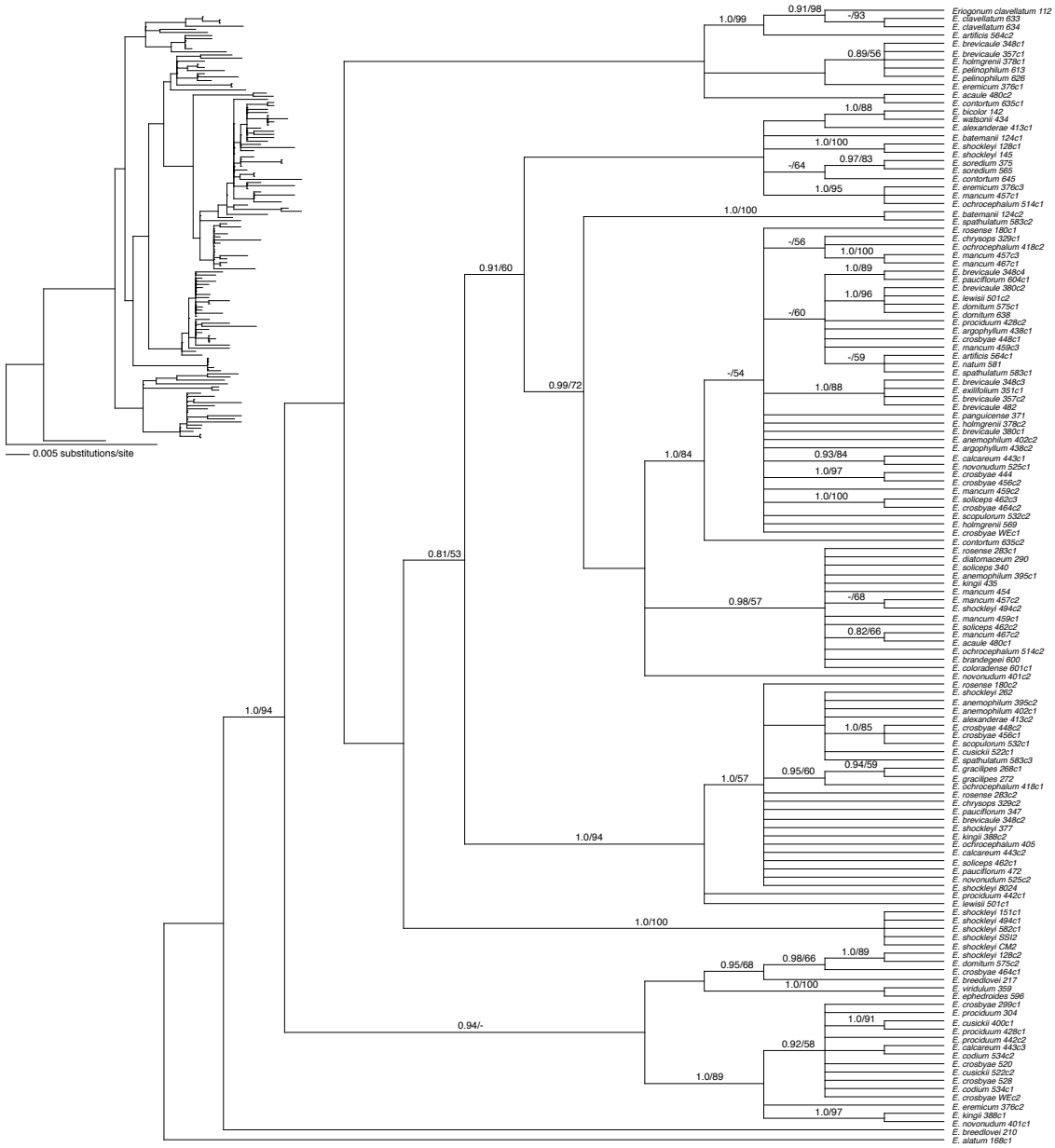


Figure 7. Consensus tree from Bayesian analysis of *RPB2* for 85 accessions of *Eriogonum* subg. *Eucycla*. A phylogram representing the tree with the highest log likelihood from the Bayesian analysis is shown in the upper left corner. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Numbers following species are collection numbers and cloned accessions have a c1, c2, c3, or c4.

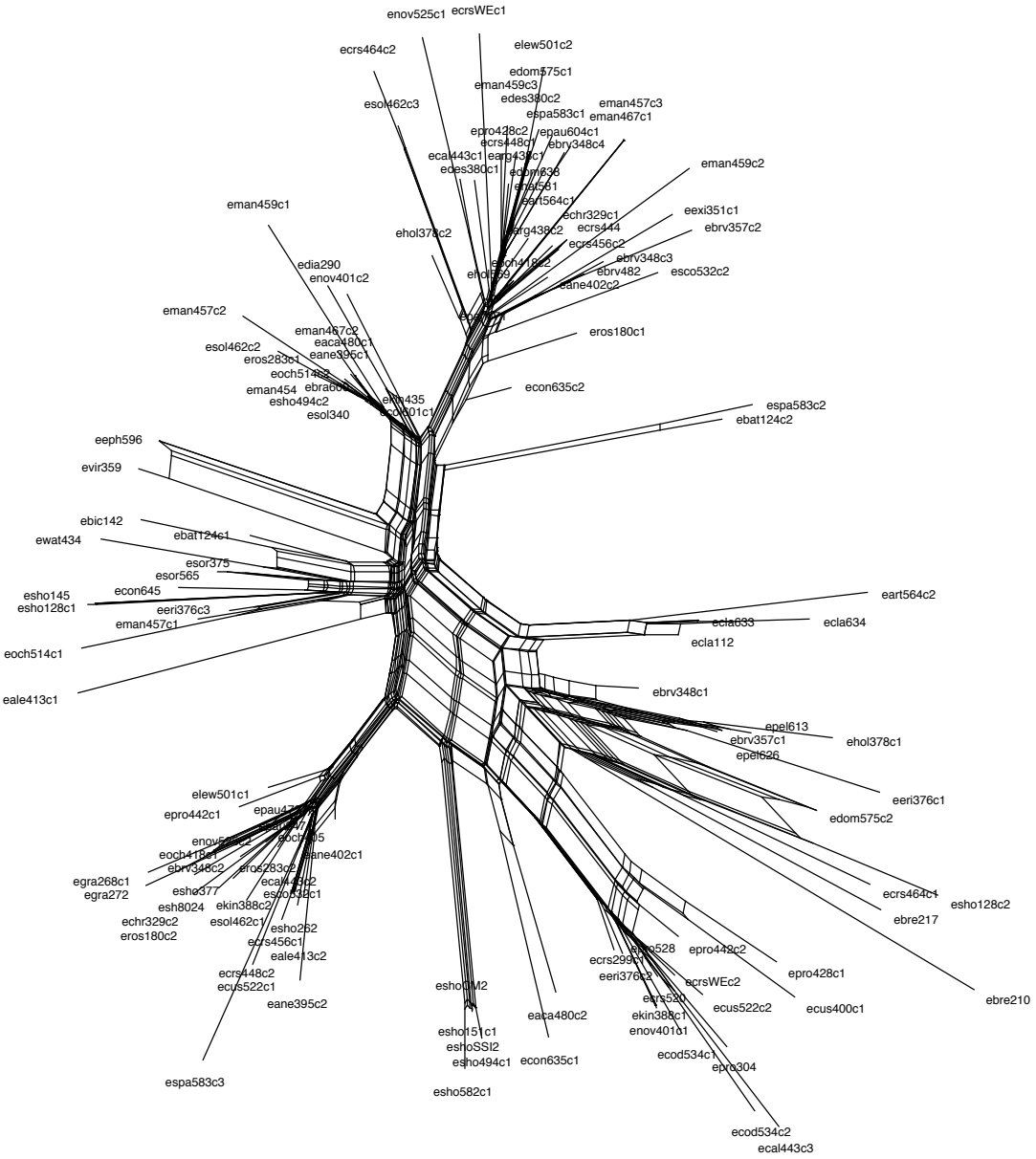


Figure 8. NeighborNet splits graph derived from the *RPB2* data set with the outgroups excluded. Tip labels correspond to codes included in Appendix 1.

General phylogenetic patterns and implications—The three individual gene trees and one combined plastid phylogeny from this lineage of *Eriogonum* highlight a number of important evolutionary processes that affect molecular phylogenetic reconstructions. Extensive taxon sampling is important to fully understand the history of a diverse group of organisms, such as this clade in *Eriogonum.* If only one individual is sampled per species, any anomalous placements would not be apparent, as there would be no basis for comparison. Even narrowly distributed species show some level of non-monophyly, especially in our plastid phylogeny (e. g. *E. soredium, E. holmgrenii,* and *E. domitum*). The comparison of multiple gene trees is also necessary to perceive the unusual placement of a taxon or clade in an individual phylogeny. We highlight examples below of gene tree comparisons, which allow specific hypotheses of evolutionary processes to be proposed. These processes, namely incomplete lineage sorting, hybridization with introgression and/or subsequent changes in ploidy level, concerted evolution, and recent, rapid radiations are all likely impacting the phylogenetic trees inferred in this study.

The phylogeny derived from the three-region plastid dataset highlights a number of these biological processes. A general signature of a rapid radiation of species within this clade is apparent from our results. Nearly all of the species included in this study are included in a large, basal polytomy in the plastid phylogeny (Figure 1). Numerous haplotypes were recovered and some well-supported clades included species both morphologically and geographically distinct.

One such clade is indicated by node N (Figure 1). This clade includes samples from across the western U. S. The two included accessions of *E. breedlovei* (BRG210 and BRG217), a California endemic from the southern Sierra Nevadas, are members of this clade. A large and widespread shrubby species, *Eriogonum corymbosum* (BRG156 from northern Arizona) possesses a chloroplast haplotype essentially identical to the sampled *E. breedlovei* accessions. This clade also includes representatives of two other widespread species collected from eastern Utah, *E. shockleyi* (BRG128) and *E. brevicaule* (BRG560). Based on this and the presence of many shared haplotypes in different species, it seems plausible that extensive hybridization, incomplete lineage sorting and retention of ancestral haplotypes may explain many of the general relationships observed in our plastid phylogeny. Additional examples, relating to selected species of interest, are highlighted below.

Our other inferred gene trees also point to a recent, rapid radiation of *Eriogonum* species sampled here. We see cases of lack of resolution even with rapidly evolving regions such as *ITS* and *LEAFY*. There are more well resolved clades with strong PP and BS support in both the *ITS* and *LEAFY* gene trees, especially in earlier diverging lineages (Figures 3 and 5). Many of the species in these basal clades are shrubby species with open, cymose inflorescences. Both of the presented phylogenies from *ITS* and *LEAFY* have a large, unresolved polytomy with short internal branches (gray star, Figures 3 and 5). This unresolved clade includes many of the mat-forming capitate species, formally recognized as *Eriogonum* sect. *Capitata* (Reveal 1969), or informally recognized as the "*E. ochrocephalum* complex" or the "*E.*

brevicaule complex" (Reveal 2005). Many of the recovered multiple copies of *ITS* or alternate alleles from *LEAFY* fall within this polytomy (Figures 3 and 5).

The results gathered from our analysis of *RPB2* are best handled conservatively, for the moment. When successfully amplified, the sequence alignment was unambiguous. However, it is apparent from cloning efforts and subsequent phylogenetic reconstruction that multiple copies were recovered. This accounts for the high level of sequence variation, when compared to the other regions used in this study (Table 1). Multiple copies were not recovered for all samples; some sequences showed only clean single peaks in the resultant trace files. It is possible that there was an early duplication of the *RBP2* locus in *Eriogonum*, and selected copies were lost in certain species. Alternatively, the presence of more than one copy of *RPB2* could be due to unrecognized polyploid species present in this lineage of *Eriogonum.* Another possibility is the preferential amplification of one copy of *RBP2* or the other, in certain samples. Our inclusion and interpretation of the *RPB2* gene tree is precautionary and limited. Some useful information can be gleaned from this region, however more effort needs to be put forth to sort out potential copy number for this region, and to optimize primers and PCR conditions to equally amplify all potential copies and alleles. If all copies were amplified with an equal rate of success, this could be used as an initial proxy to recognize the prevalence of polyploidy (Kim et al. 2008).

Relationships and phylogenetic position of selected species—Some of the most intriguing phylogenetic results from this study are those associated with *E. shockleyi*. This widespread species grows on a variety of substrates across seven

states in the western U.S., with the majority of the populations occurring below 2000 m in the Colorado Plateau or Great Basin region (personal observation; Reveal 2005). While there have been a number of intraspecific varieties proposed, the morphology of *E. shockleyi* is consistent across the range. Varying morphological features include: involucre shape, leaf shape, and tepal color (Smith and Bateman 2002) although tepal color has been observed to vary from white to yellow to pink within populations (personal observation). A study of ISSR variation in Idaho populations of two proposed varieties of *E. shockleyi* found no distinguishing genetic markers and overlapping morphological features within populations (Smith and Bateman 2002). An extra effort was made to collect *E. shockleyi* from populations across its range, with the inclusion of seventeen samples in the plastid dataset. Of those included, accessions of *E. shockleyi* were present in six distinct, well-supported clades along the unresolved backbone of the chloroplast phylogeny (nodes A, B, C, D, E, and N; Figure 9). Clade C, for instance, contains four different accessions of *E. shockleyi* (BRG151 from western Utah; BRG494 from northeastern Nevada; BRG354 from western Colorado; and JFS-LV1 from southern Idaho) from across the northern part of its range. The lineage indicated by node D (Figure 9), has representatives of four different populations as well, all collected from either eastern or western Utah (Appendix 1). One sample collected from the western edge of its range in California (BRG265), is sister to an adjacent California collection of *E. gracilipes* (BRG268), although support values are somewhat lower (0.87 PP and 57 BS). Two other samples (BRG262 and BRG377) were not resolved as members of any clades (Figure 9). The multiple chloroplast haplotypes recovered for *E. shockleyi*

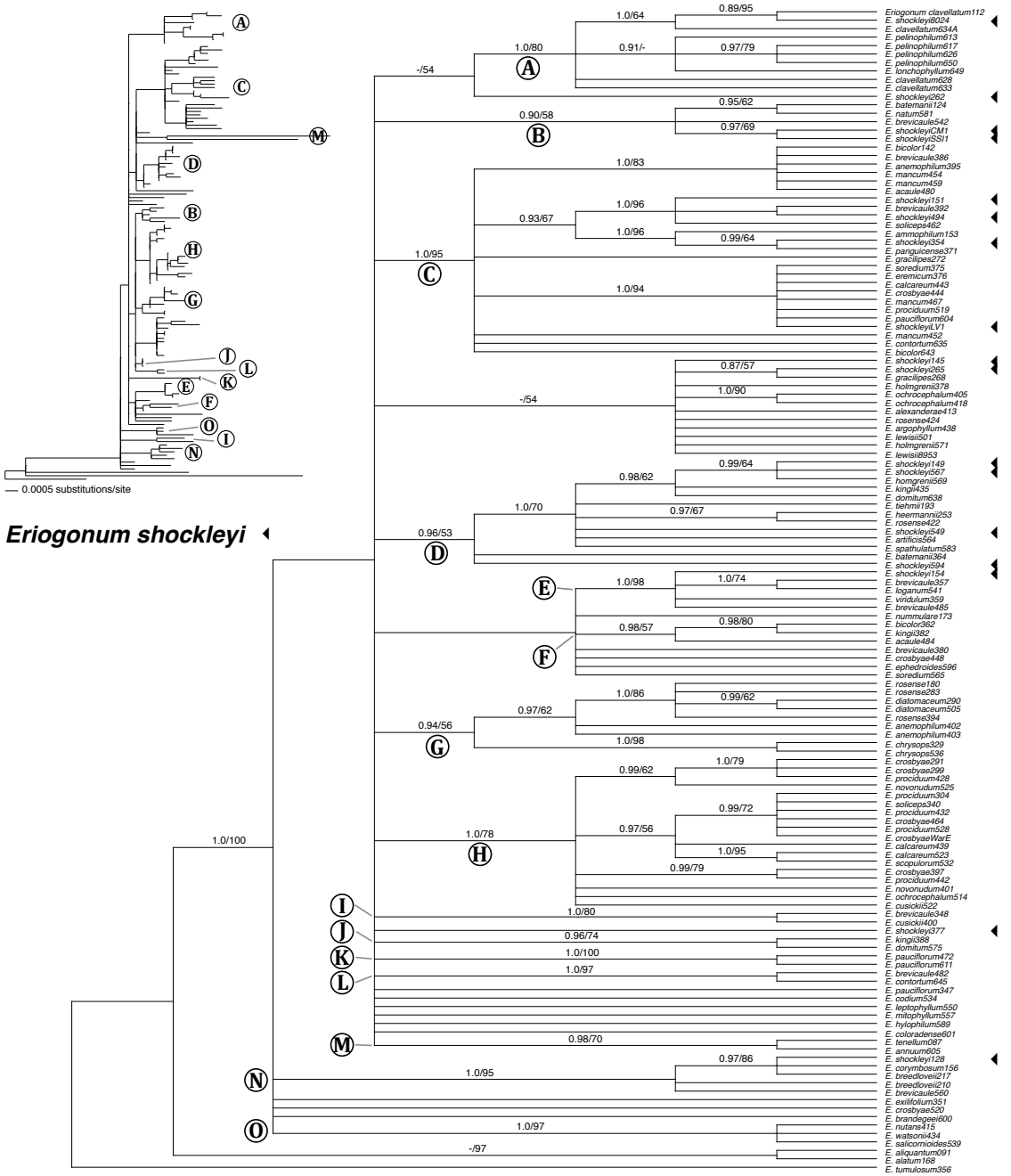


Figure 9. Consensus tree from Bayesian analysis of the combined three-region chloroplast data set (*trnL-trnF*, *rpS16*, and *trnD-trnT*) highlighting the topological positions of *E. shockleyi* accessions (same tree a Figure 1). Seventeen accessions of *E. shockleyi* (corresponding to an arrow) are found throughout the phylogeny. Collection information for each sample is included in Appendix 1. Major clades of interest are labeled with an encircled letter. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included.

can be explained by incomplete lineage sorting of a once widespread, genetically diverse ancestor, or repeated hybridization events with multiple species in the *Eucycla II* clade.

Accessions of *E. shockleyi* are much closer to monophyly in the *ITS* and *LEAFY* gene trees. The inferred *ITS* phylogeny places all accessions of *E. shockleyi* in one of two clades within the large terminal polytomy (Figure 10). There is a general, although not exclusive, geographic pattern associated with these two clades. The top clade tends to include more eastern collections, while the lower clade includes more western and northern samples. We recovered two alternate copies of *ITS* in BRG151 and BRG154 with one copy corresponding to the eastern clade and one falling in the more western clade. All sequences obtained from the *E. shockleyi* accessions for the *LEAFY* locus were included in the large terminal polytomy (Figure 11). Interestingly, the *LEAFY* sequence obtained from the other California population of *E. shockleyi* is virtually identical to an allele from *E. gracilipes* (BRG268). We recovered multiple divergent sequences of *E. shockleyi* for *RPB2* (Figure 12). Although the potential issues of the utility of *RPB2* in *Eriogonum* have already been discussed, we do recover a seemingly *E. shockleyi*-specific version of *RPB2*. It is possible that this locus could be useful in helping to untangle the evolutionary history of this complex, genetically diverse species.

Interesting phylogenetic relationships emerge with an examination of a pair of putative sister species within this clade, *E. clavellatum* and *E. pelinophilum*. These two species, restricted endemics of the Colorado Plateau, are thought to be closely related due to similarities in morphology and geographic range (Reveal 1973;

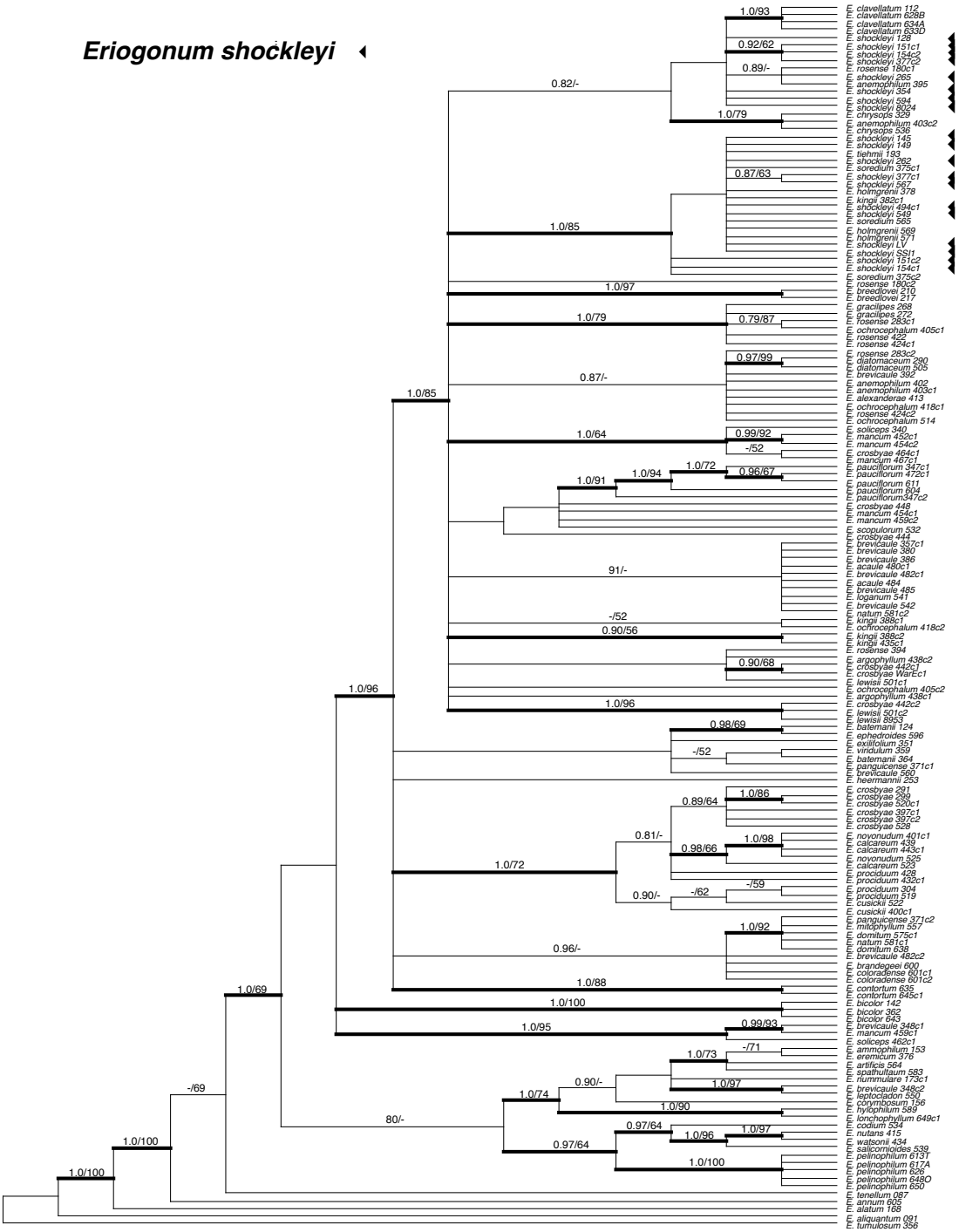


Figure 10. Consensus tree from Bayesian analysis of *ITS* highlighting the topological positions of *E. shockleyi* accessions (same tree a Figure 3). Sixteen accessions of *E. shockleyi* (corresponding to an arrow) are included, with three samples possessing two copies of *ITS* (BRG151, BRG154, BRG377). Collection information for each sample is included in Appendix 1. BI posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only BI PP above 0.80 and ML bootstrap support values above 50 are included. Branches with >0.90 BI PP and >50 ML BS are in bold.

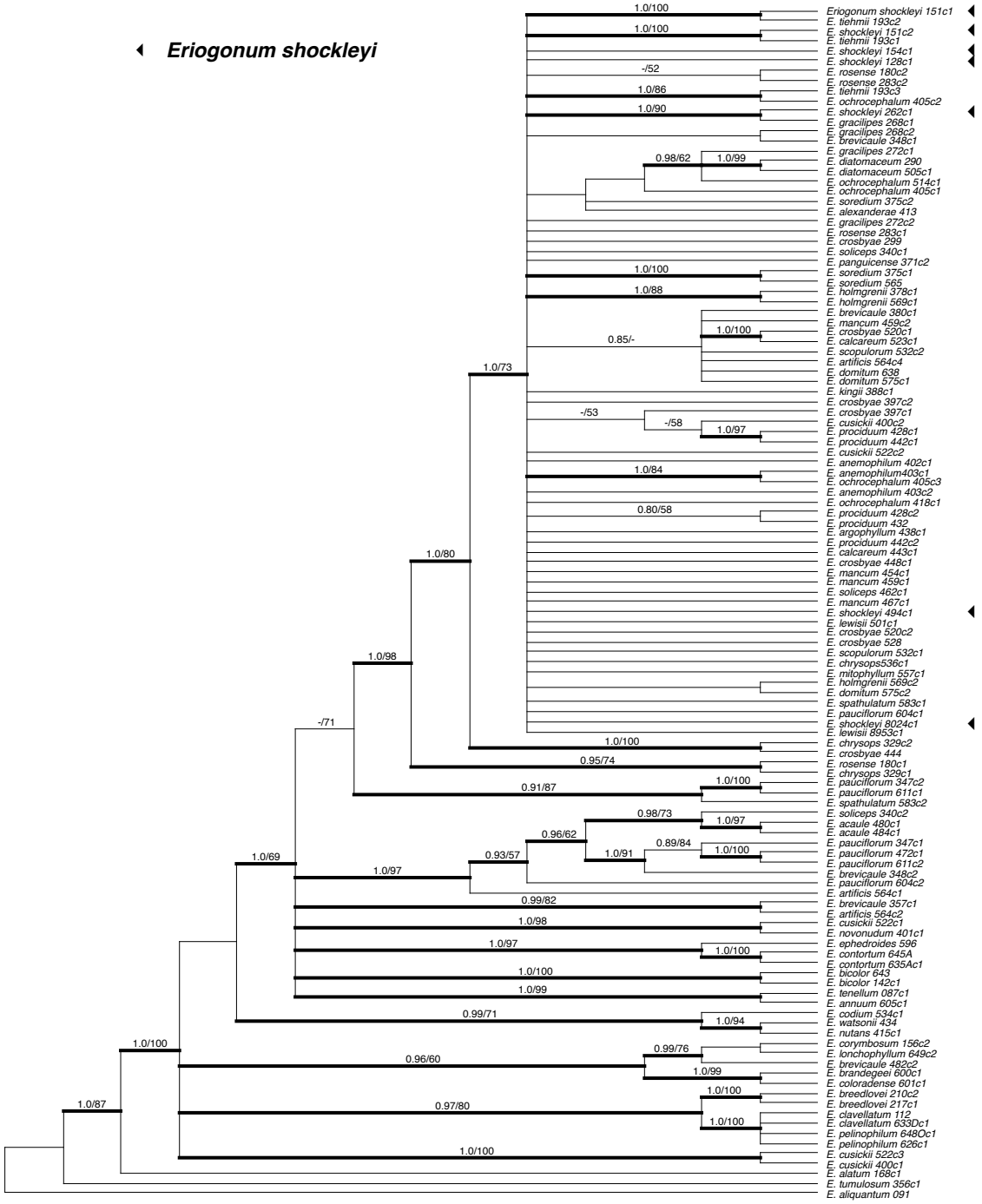


Figure 11. Consensus tree from Bayesian analysis of *LEAFY* highlighting the topological positions of *E. shockleyi* accessions (same tree a Figure 5). Six accessions of *E. shockleyi* (corresponding to an arrow) are included, with one sample possessing two copies of *LEAFY* (BRG151). Collection information for each sample is included in Appendix 1. BI posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only BI PP above 0.80 and ML bootstrap support values above 50 are included. Branches with >0.90 BI PP and >50 ML BS are in bold.

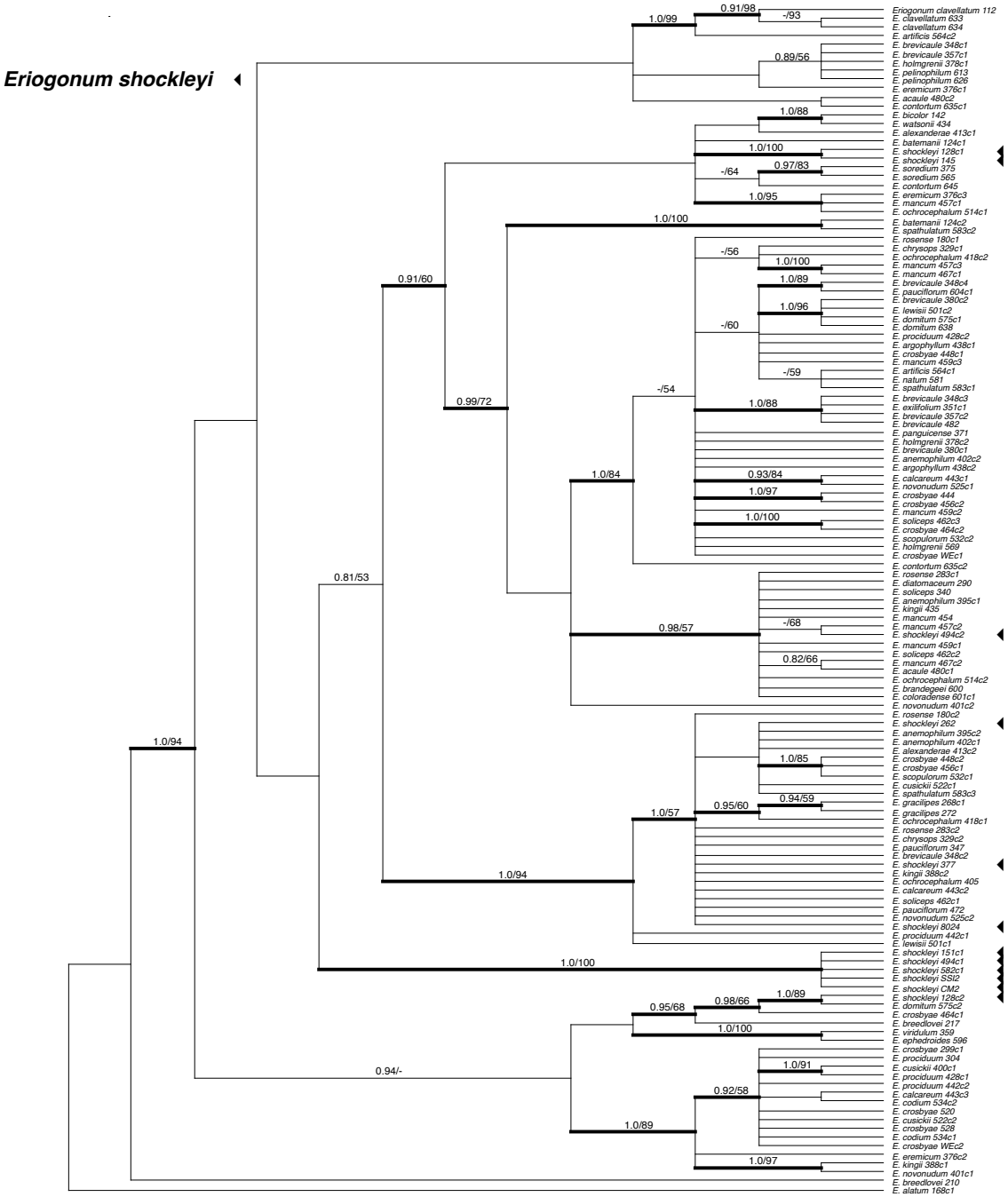


Figure 12. Consensus tree from Bayesian analysis of *RPB2* highlighting the topological positions of *E. shockleyi* accessions (same tree a Figure 7). Ten accessions of *E. shockleyi* (corresponding to an arrow) are included, with two samples possessing two copies of *ITS* (BRG128, BRG494). Collection information for each sample is included in Appendix 1. BI posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only BI PP above 0.80 and ML bootstrap support values above 50 are included. Branches with >0.90 BI PP and >50 ML BS are in bold.

Reveal 2005). Additional figures of the individual gene trees were created to more easily highlight the phylogenetic positions of these two species, and any associated taxa (Figures 13-16). Relevant clades are highlighted in yellow, with *E. pelinophilum* accessions shown in pink font and *E. clavellatum* accessions shown in green font. The two low-copy nuclear regions (*LEAFY* and *RPB2*) support this close relationship, and indicate that these two species fall within an early diverging clade outside of the main radiation of the core *Eucycla II* (Figures 15 and 16). While each species is individually monophyletic in the *ITS* tree, *E. pelinophilum* and *E. clavellatum* are well separated in this phylogenetic reconstruction (Figure 14). The phylogenetic placement of the *E. pelinophilum* accessions in the *ITS* tree is more consistent with other gene trees (Figures 13-16). Thus it seems probable that recent hybridization, possibly with widespread *E. shockleyi*, or retention and fixation of an ancestral copy of *ITS,* explains the anomalous position of *E. clavellatum* (Figure 14). The clade containing *E. pelinophilum* and *E. clavellatum* is located on the large basal polytomy in the chloroplast gene tree (Figure 13). In the chloroplast phylogeny, two other accessions are also members of this clade (PP 1.0, BS 80), *E. shockleyi* (SLO8024) and *E. lonchophyllum* (BRG649). Other genes from this collection of *E. lonchophyllum*, a widespread species in the Colorado Plateau region, do not show a close relationship to *E. clavellatum* and *E. pelinophilum* (Figures 14 and 15). The included collection of *E. lonchophyllum* was collected from a population that was sympatric with *E. pelinophilum* (BRG650) in west-central Colorado. Chloroplast capture of the maternal plastid from *E. pelinophilum* is the likely explanation for this anomalous relationship. However, since only one accession of *E. lonchophyllum* is

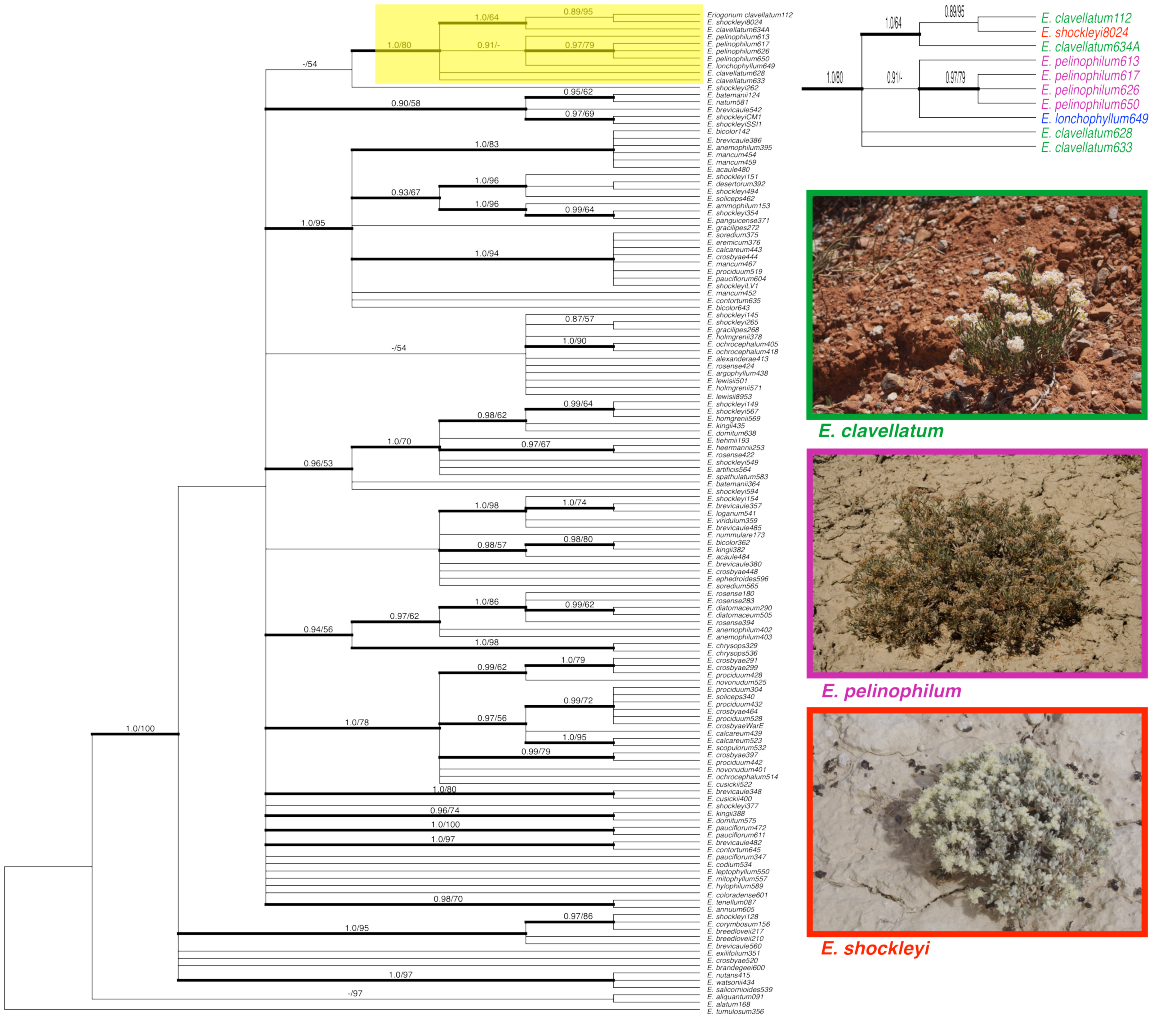


Figure 13. Combined chloroplast phylogeny highlighting relationships of *E. clavellatum* and *E. pelinophilum*, as well as other species. The yellow clade is magnified to illustrate relationships within this clade of interest. *E. clavellatum* is highlighted in green (4 accessions), *E. pelinophilum* is highlighted with pink (4 accessions), *E. shockleyi* (SLO8024) is red, and *E. lonchophyllum* (BRG649) is blue. Branches in bold indicate a node receiving both >0.90 BI PP and >50 ML BS support. The images at the right show differences in growth form of these species.

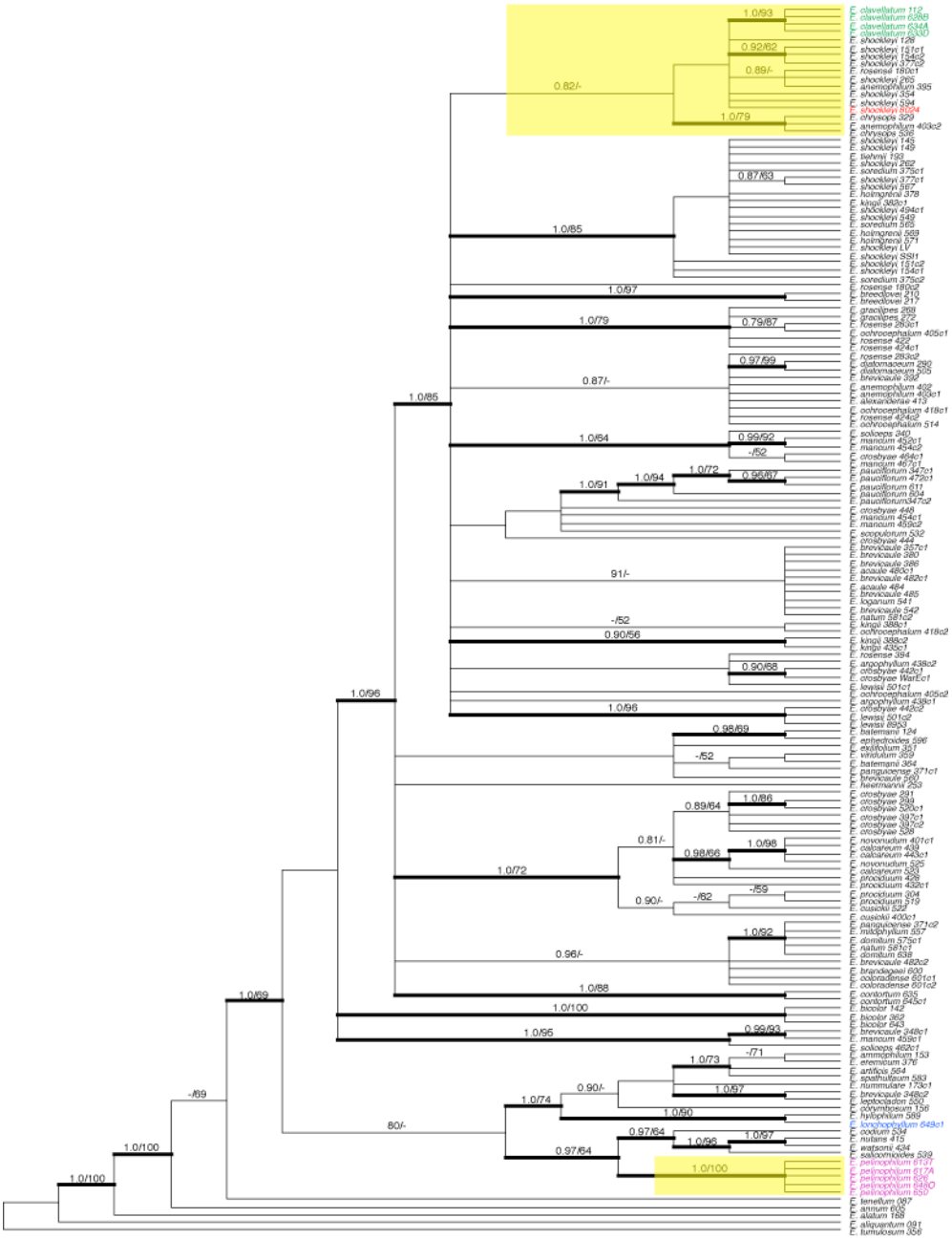


Figure 14. *ITS* phylogeny highlighting relationships of *E. clavellatum* and *E. pelinophilum*, as well as other species. The yellow clades illustrate clades of interest. *E. clavellatum* is highlighted in green (4 accessions), *E. pelinophilum* is highlighted with pink (5 accessions), *E. shockleyi* (SLO8024) is red, and *E. lonchophyllum* (BRG649) is blue. Branches in bold indicate a node receiving both >0.90 BI PP and >50 ML BS support.

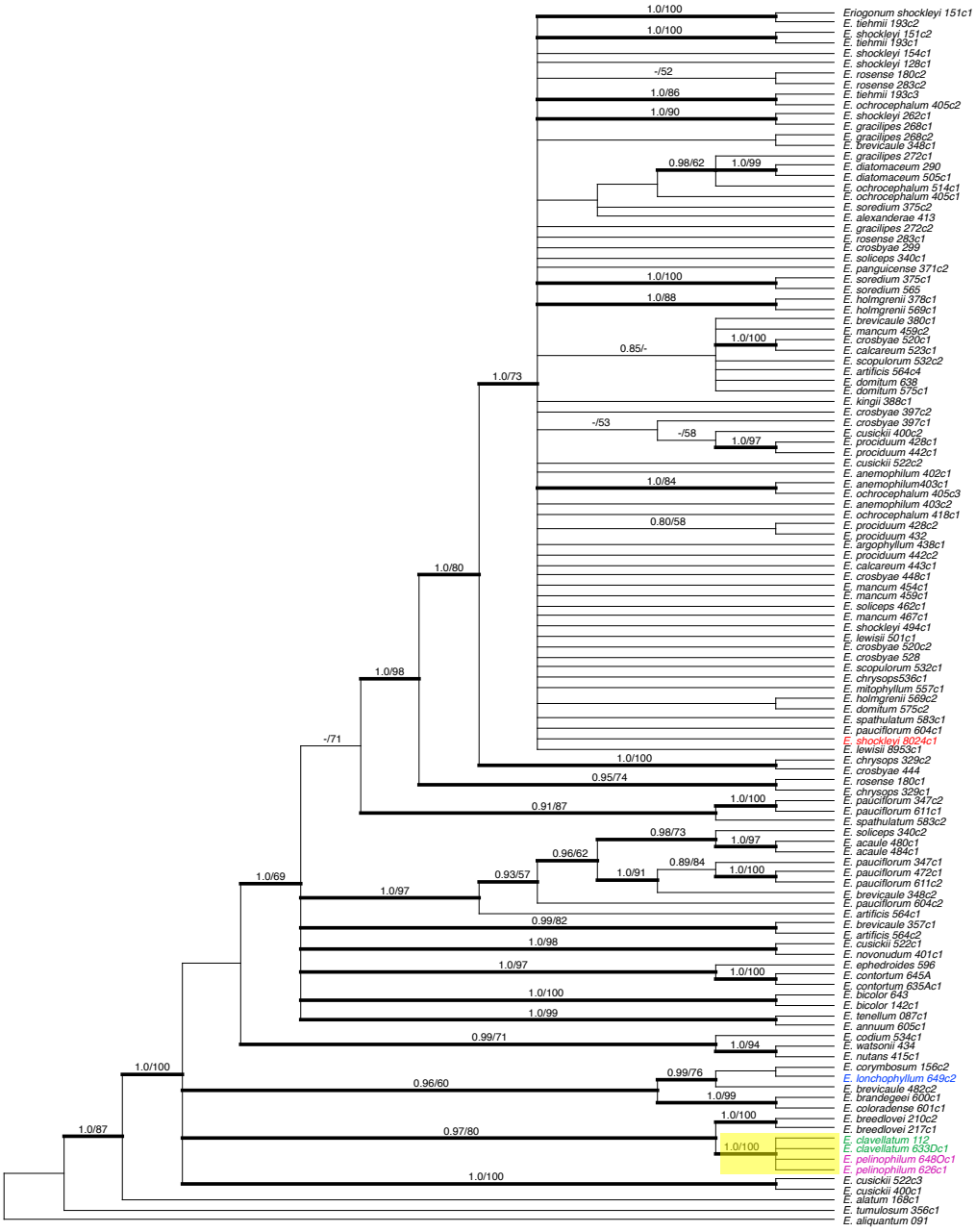


Figure 15. *LEAFY* phylogeny highlighting relationships of *E. clavellatum* and *E. pelinophilum*, as well as other species. The yellow clade illustrates the clade of interest. *E. clavellatum* is highlighted in green (2 accessions), *E. pelinophilum* is highlighted with pink (2 accessions), *E. shockleyi* (SLO8024) is red, and *E. lonchophyllum* (BRG649) is blue. Branches in bold indicate a node receiving both >0.90 BI PP and >50 ML BS support.

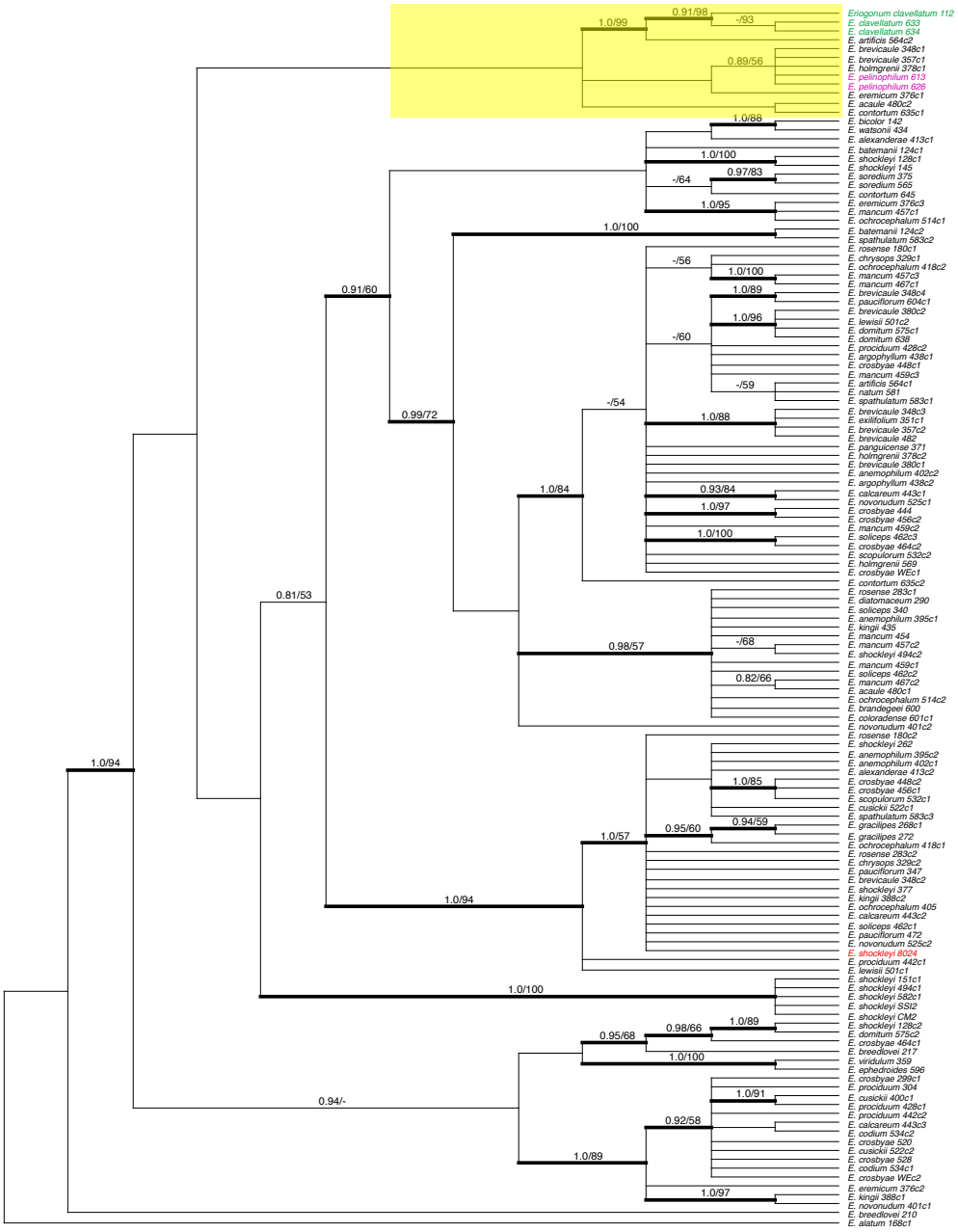


Figure 16. *RPB2* phylogeny highlighting relationships of *E. clavellatum* and *E. pelinophilum*, as well as other species. The yellow clade illustrates the clade of interest. *E. clavellatum* is highlighted in green (3 accessions), *E. pelinophilum* is highlighted with pink (2 accessions), *E. shockleyi* (SLO8024) is red, and *E. lonchophyllum* is not included. Branches in bold indicate a node receiving both >0.90 BI PP and >50 ML BS support.

included in our sampling, this is not conclusive. Additional samples of *E. lonchophyllum* from across its range, and especially those that are not sympatric with *E. pelinophilum*, are necessary to confirm this hypothesis and rule out incomplete lineage sorting and other biological sources of genetic discordance.

Of the numerous narrowly distributed species in the *Eucycla II* clade, we highlight and discuss the phylogenetic positions of eight selected species. Of those selected, all except *E. calcareum* and *E. gracilipes* have distributions that are limited to one state; however neither of these taxa are considered widespread across the western United States. The others are mostly limited to a single mountain range, or even a single peak, as in *E. soredium.* Detailed descriptions of morphological characters and geographic distributions are included in Reveal's most recent treatment of the genus in the Flora of North America (2005).

Eriogonum gracilipes, a high elevation species of the White Mountains in California, is monophyletic in the *ITS*, *LEAFY*, and *RPB2* gene trees, at least for one of the alleles recovered (Figures 18-20). The two accessions show two distinct haplotypes in the combined plastid gene tree and are members of different, strongly supported clades (Figure 17). A morphologically and ecologically similar species, *E. holmgrenii,* shows comparable phylogenetic patterns. Two of the three included accessions of *E. holmgrenii* possess very similar chloroplast haplotypes (BRG378 and BRG571), with the other sample (BRG569) falling in a distinct clade (Figure 17). *Eriogonum holmgrenii* is monophyletic in the nuclear gene trees, with the exception of one recovered copy of BRG378 in the *RPB2* gene tree (Figures 18-20). Both *E. holmgrenii* and *E. gracilipes* are members of the large polytomy in the *ITS* gene tree

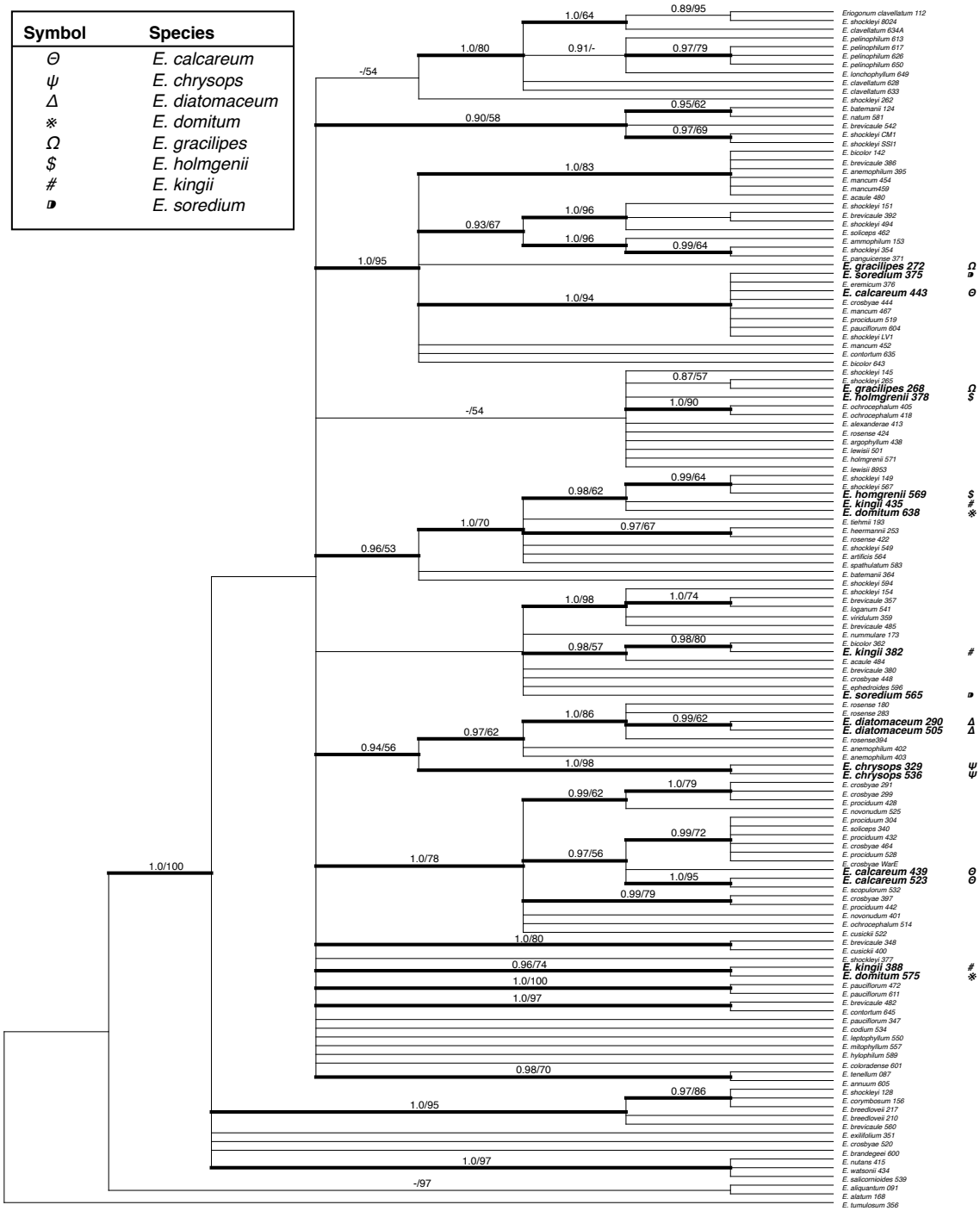


Figure 17. Chloroplast phylogeny highlighting positions of narrowly distributed species in *Eriogonum.* Symbols in the legend are used to mark each species in the topology.

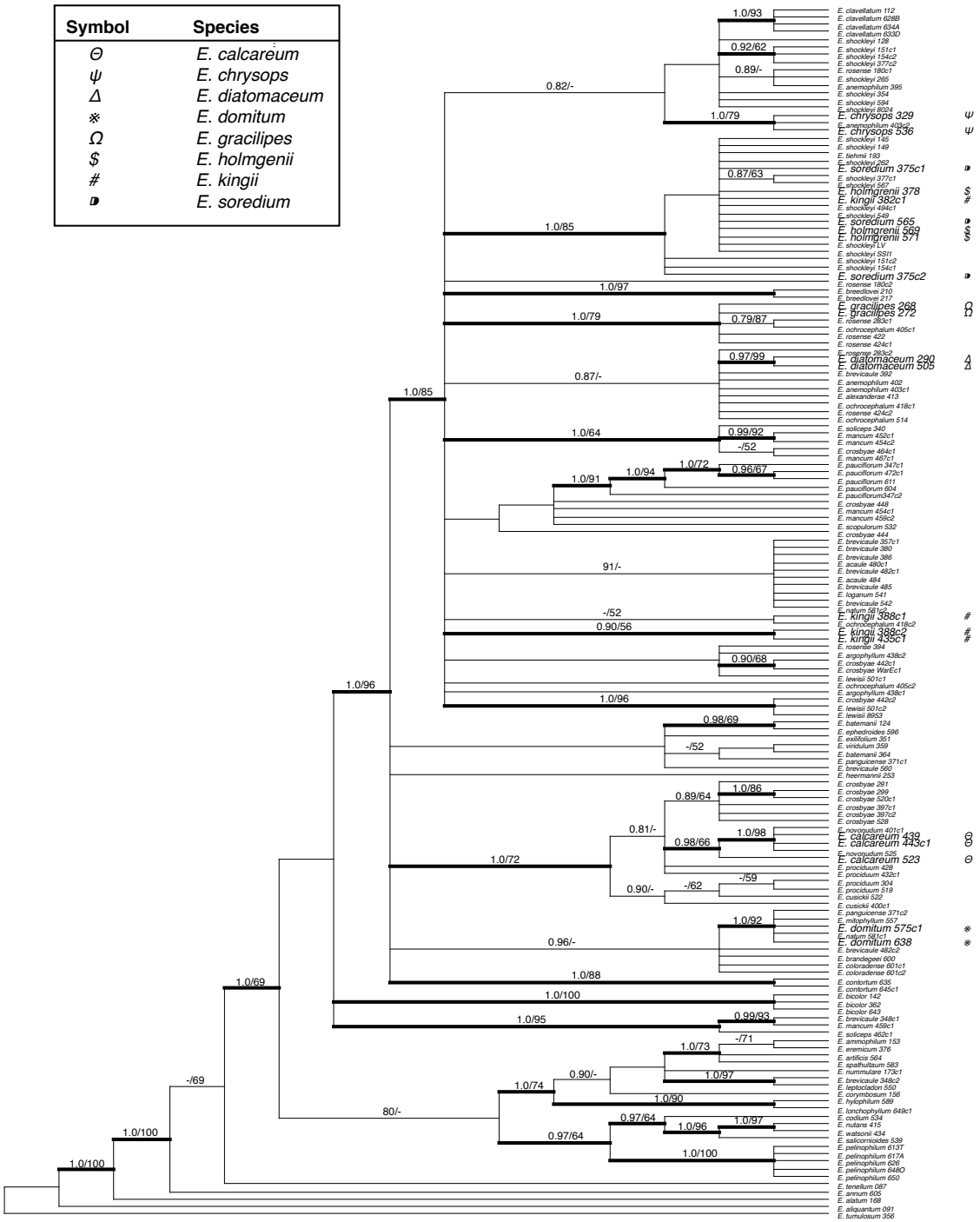


Figure 18. *ITS* phylogeny highlighting positions of narrowly distributed species in *Eriogonum.* Symbols in the legend are used to mark each species in the topology.

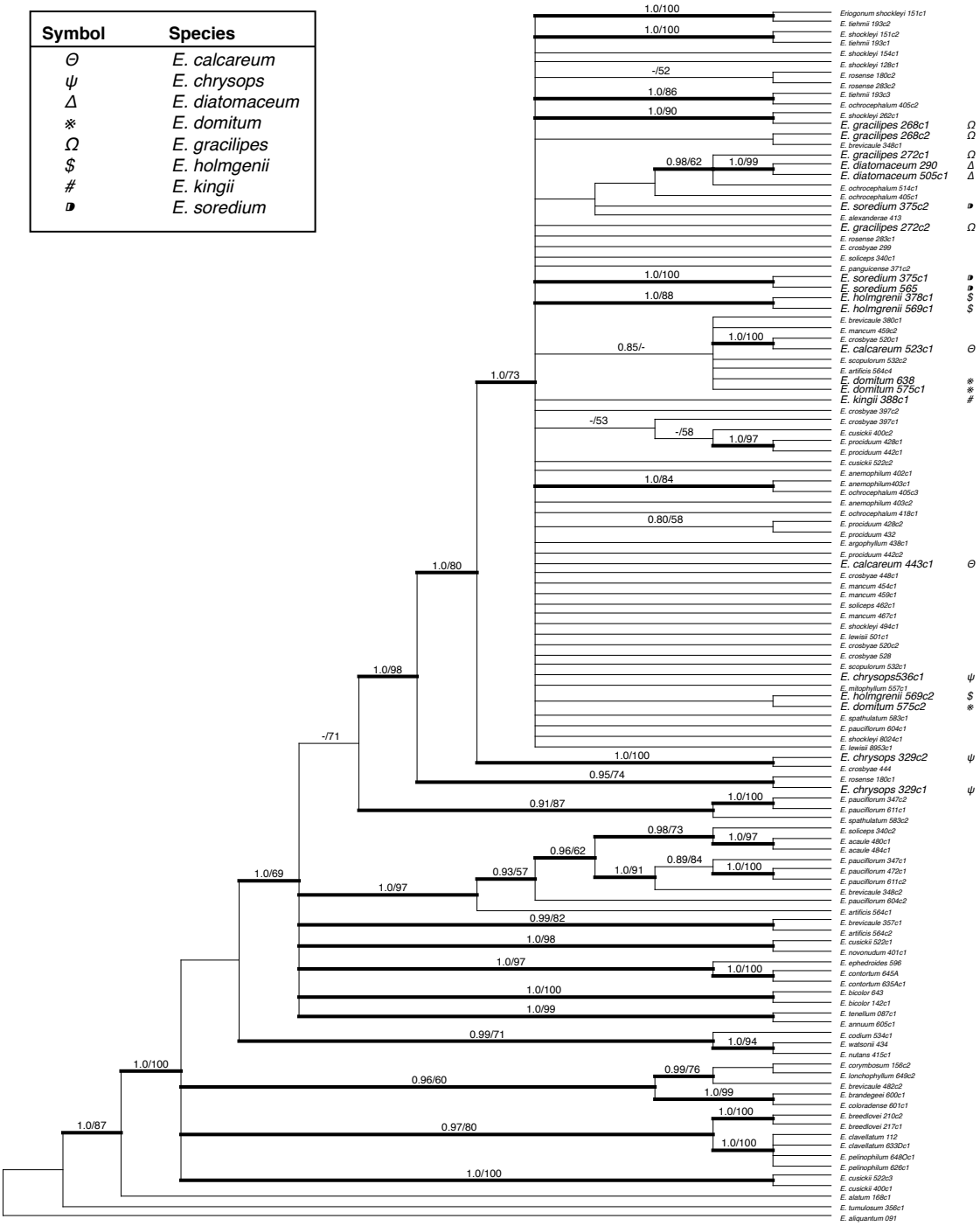


Figure 19. *LEAFY* phylogeny highlighting positions of narrowly distributed species in *Eriogonum.* Symbols in the legend are used to mark each species in the topology.

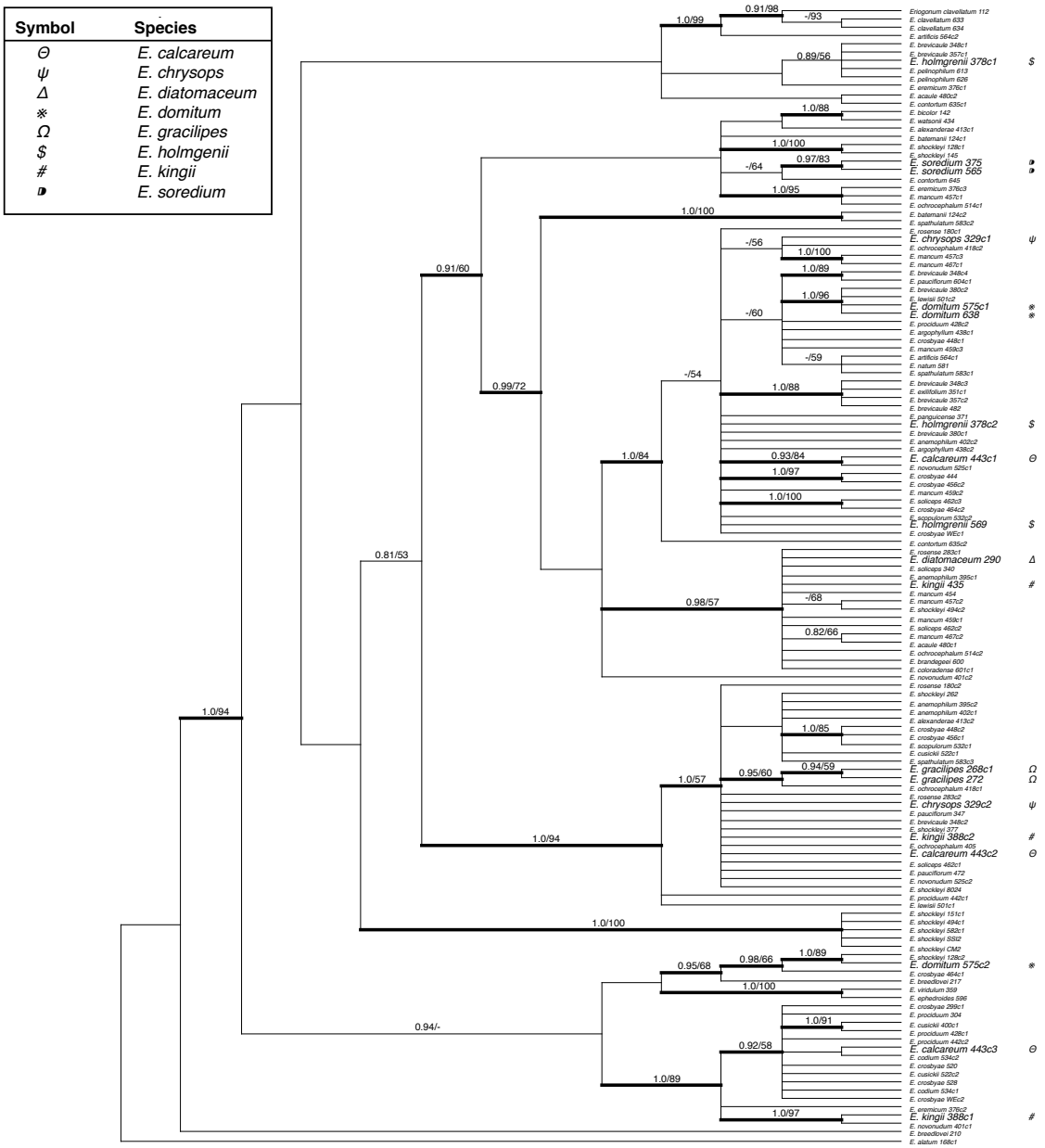


Figure 20. *RPB2* phylogeny highlighting positions of narrowly distributed species in *Eriogonum.* Symbols in the legend are used to mark each species in the topology.

(Figure 18), however these two species are included in separate, strongly-supported clades (PP 1.0, BS 85; PP 1.0, BS 79, respectively). This may indicate that these two species are not closely related sister species, as is hypothesized (Reveal 2005).

(calcareous) at high elevations, may explain our results for *E. holmgrenii* and *E. gracilipes.*

Eriogonum domitum, a newly described species from the House Range in western Utah (Grady & Reveal 2011), is monophyletic in the *ITS*, *LEAFY*, and *RPB2* genealogies (Figures 18-20). An additional copy of *RPB2* for BRG 575 is distinct from the other accessions sampled, although there is no guarantee that all copies were amplified equally. For the two samples included in the plastid dataset, two distinct haplotypes were recovered for *E. domitum*, despite their collection from two adjacent mountain peaks in the same range (Figure 17). *Eriogonum soredium*, an equally rare and geographically limited species, exhibits analogous phylogenetic patterns in the included gene trees (Figures 17-20). Population-level sampling of *E. domitum* and *E. soredium* is necessary to ascertain an estimate of haplotype diversity present in these narrowly endemic species. Although currently geographically isolated from other members of this clade, it is possible that historical distributions of these isolated species were more widespread due to past climatic fluctuations in the Great Basin, thus offering the opportunity for hybridization and chloroplast capture with other members of *Eriogonum.* This retention of haplotype diversity may also be a result of a very recent speciation event and insufficient time for fixation of haplotype diversity in these two species.

Two species, *E. chrysops* and *E. diatomaceum,* formed strongly supported monophyletic clades in the combined chloroplast phylogeny, indicating a single haplotype recovered in each of the samples included in this study. *Eriogonum*

diatomaceum (BRG290 and BRG505) is also monophyletic in the *ITS* and *LEAFY* gene trees (Figure 18 and 19). One sample (BRG505) failed to amplify for the *RPB2* locus, thus only one accession of *E. diatomaceum* is included in this tree (Figure 20). Reciprocal monophyly is also observed in the *ITS* phylogeny for *E. chrysops* (Figure 18), however the two alleles of *LEAFY* for BRG329, are sister to the large polytomy that includes BRG536 (Figure 19). It is possible that additional genetic diversity would be uncovered with increased sampling in these narrowly distributed species.

We included three accessions of *E. kingii*, collected from three isolated ranges in northeast Nevada. Three distinct plastid haplotypes were recovered for these accessions (Figure 17). Collections BRG388 and BRG435 are monophyletic in the *ITS* phylogeny, with BRG382 falling in a different clade, albeit in the same large polytomy as the other accessions of *E. kingii* (Figure 18). Only one accession of *E. kingii* was successfully amplified for the *LEAFY* locus, so no indication of monophyly can be made. The *RPB2* region was amplified in two of the *E. kingii* samples (BRG388 & BRG435) however these sequences are extremely divergent (Figure 20). Explanations for the relative genetic diversity observed in this species may be explained by a number of processes. These isolated populations from northeastern Nevada may be showing convergent morphologies (membranceous involucres, pale yellow tepals, and tomentose scapes) or *E. kingii* may be the product of recent polyploidization. Hybridization and introgression could also explain the patterns observed in the chloroplast phylogeny (Figure 17). Acquiring additional samples of *E. kingii* and complete sequences for low-copy regions are necessary to uncover the true evolutionary history of this taxon.

Eriogonum calcareum is a low elevation species, distributed across xeric portions of Idaho and Oregon. Of the three accessions sampled here, two distinct chloroplast haplotypes were recovered, making this taxon paraphyletic in the plastid analysis (Figure 17). The two accessions with similar haplotypes (BRG439 & BRG523) are members of a clade composed of northwestern Great Basin species (clade H, Figure 1 and Figure 17). The other accession (BRG443) belongs to a geographically and morphologically diverse subclade within clade C (Figure 1 and Figure 17), possibly a result of introgression with one of these other species. These same samples are not paraphyletic in either the *ITS* or *LEAFY* gene trees (Figures 18 and 19). Only one of these accessions was successfully amplified for the *RPB2* region, however three distinct copies were present (Figure 20).

Recognizing and defining species in Eriogonum—Defining and circumscribing species in a hyper diverse, recently radiated lineage is challenging. Monophyly of all species in all gene trees is unlikely (Knowles and Carstens 2007; Knowles and Chan 2008; Koopman and Baum 2010). This study provides the initial framework to thoroughly evaluate species relationships and circumscriptions in an exceptionally diverse lineage of *Eriogonum*. Some species are monophyletic in nearly all of the gene trees presented here (e.g. *E. clavellatum, E. chrysops, E. diatomaceum, E. pelinophilum*, and *E. soredium*). Other narrowly distributed species appear to retain multiple chloroplast haplotypes, likely the result of hybridization and/or incomplete lineage sorting with the plastid data. Admittedly, more thorough sampling is necessary for nearly all species included in this study to determine the extent of haplotype diversity held in some of these rare species with small

population sizes. Based on our multiple gene trees, which include many accessions of *Eriogonum* species, the strict recognition of monophyletic species is impractical. Morphological and ecological diversity abounds within this clade. There are numerous examples of currently isolated, independently evolving lineages worthy of species recognition. Factors such as incomplete lineage sorting, possible polyploidization, and hybridization may blur species boundaries or violate a strictly bifurcating pattern of speciation in this group. A holistic approach to defining species seems more applicable for a phylogenetically challenging lineage such as this.

Albach, D. C. and H. M. Muedt. 2010. Phylogeny of *Veronica* in the southern and northern hemispheres based on plastid, nuclear ribosomal, and low-copy nuclear DNA. *Molecular Phylogenetics and Evolution* 54: 457-471.

Bacon, C. D., M. J. McKenna, M. P. Simmons, and W. L. Wagner. 2012. Evaluating multiple criteria for species delimitation: an empirical example using Hawaiian palms (Arecaceae: Pritchardia). *BMC Evolutionary Biology* 12: 23.

Baum, D. A. 1992. Phylogenetic species concepts. *TRENDS in Ecology and Evolution* 7:1-2.

Baum, D. A. and M. J. Donoghue. 1995. Choosing among alternative "Phylogenetic" species concepts. *Systematic Botany* 20: 560-573.

Bryant, D. and V. Moulton. 2004. Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution* 21: 255-265.

Darwin, C. 1859. *On the Origin of Species by Means of Natural Selection*, Murray.

Degnan, J. H. and N. A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *TRENDS in Ecology and Evolution* 24: 332-340.

de Queiroz, K. 1992. Phylogenetic definitions and taxonomic philosophy. *Biology and Philosophy* 7: 295-313.

Edwards, C. E., D. Lefkowitz, D. E. Soltis, and P. S. Soltis. 2008. Phylogeny of *Conradina* and related southeastern scrub mints (Lamiaceae) based on *GapC* gene sequences. *International Journal of Plant Sciences* 169: 579-594.

Frohlich, M. W. and E. M. Meyerowitz. 1997. The search for homeotic gene homologs in basal angiosperms and Gnetales: a potential new source of data on the evolutionary origin of flowers. *International Journal of Plants Sciences* 158: S131-S142.

Goodson, B. E., S. K. Rehman, and R. K. Jansen. 2012. Molecular systematics and biogeogreaphy of *Descuriainia* (Brassicaceae) based on nuclear and non-coding chloroplast DNA. *Systematic Botany* 36: 957-980.

Grady, B. R. and J. L. Reveal. 2011. New combinations and a new species of *Eriogonum* (Polygonaceae: Eriogonoideae) from the Great Basin Desert, United States. *Phytotaxa* 24: 33-38.

Gurushidze, M., R. M. Fritsch, and F. R. Blattner. 2010. Species-level phylogeny of *Allium subgenus Melanocrommyum*: Incomplete lineage sorting, hybridization, and *trnF* gene duplication. *Taxon* 59: 829-840.

Heath, T. A., S. M. Hedtke, and D. M. Hillis. 2008. Taxon sampling and the accuracy of phylogenetic analyses. *Journal of Systematics and Evolution* 46: 239-257.

Hey, J. 2006. On the failure of modern species concepts. *TRENDS in Ecology and Evolution* 21: 447-450.

Huelsenbeck J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.

Hung, K., B. A. Schall, T. Hsu, Y. Chung, C. Peng, and T. Chiang. 2009. Phylogenetic relationships of diploid and polyploid species in *Ludwigia* sect. *Isnardia* (Onagraceae) based on chloroplast and nuclear DNAs. *Taxon* 58: 1216-1225.

Huson, D. H. and D. Bryant. 2011. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254-267 (software available from www.splitstree.org/).

Jakob, S. S. and F. R. Blattner. 2006. A chloroplast genealogy of *Hordeum* (Poaceae): long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. *Molecular Biology and Evolution* 23: 1602-1612.

Kim S. T. and M. J. Donoghue. 2008. Incongruence between cpDNA and nrITS trees indicate extensive hybridization within *Eupersicaria* (Polygonaceae). *American Journal of Botany* 95: 1122-1135.

Kim, S. T., S. E. Sultan, and M. J. Donoghue. 2008. Allopolyploid speciation in *Persicaria* (Polygonaceae): insights from a low-copy nuclear region. *Proceedings of the National Academy of Sciences* 105: 12370-12375.

Knowles, L. L. and B. C. Carstens. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* 56: 887-895.

Knowles, L. L. and Y. Chan. 2008. Resolving species phylogenies of recent evolutionary radiations. *Annals of the Missouri Botanical Garden* 95: 224-231.

Koopman, M. M. and D. A. Baum. 2010. Isolating nuclear genes and identifying lineages without monophyly: an example of closely related species from southern Madagascar. *International Journal of Plant Sciences* 171: 761-771.

Lega, M., S. Foir, F. Prosser, A. Bertolli, M. Li, and C. Varotto. 2012. Application of the unified species concept reveals distinct lineages for disjuncts endemics of the Brassica repanda complex. *Biological Journal of the Linnean Society* 106: 482-497.

Levin, D. A. 1979. The nature of plant species. *Science* 204: 381-384.

Maddison, W. P. 1997. Gene trees in species trees. *Systematic Biology* 46: 523-536.

Maddison, W. P. and L. L. Knowles. 2006. Inferring phylogeny despite incomplete lineage sorting. *Systematic Biology* 55: 21-30.

Maddison, D. R. and W. P. Maddison. 2005. MacClade: analysis of phylogeny and character evolution, version 4.08. Sunderland: Sinauer Associates.

Mason-Gamer, R. J. 2004. Reticulate evolution, introgression, and intertribal capture in an allohexaploid grass. *Systematic Biology* 53: 25-37.

Mayr, E. 1942. *Systematics and the Origin of Species.* Columbia University Press, New York.

McCormack, J. E., H. Huang, and L. L. Knowles. 2009. Maximum likelihood estimates of species trees: How accuracy of phylogenetic inference depends upon the divergence history and sampling design. *Systematic Biology* 58: 501-508.

Miller, M.A., Pfeiffer, W., and Schwartz, T. (2010) "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1 - 8.

Mitchell, A., C. Mitter, and J. C. Regier. 2000. More taxa or more characters revisited: combing data from nuclear protein-encoding genes for phylogenetic analyses of Noctuoidea (Insecta: Lepidoptera). *Systematic Biology* 49: 202-224.

Muedt, H. M. 2011. Amplified fragment length polymorphism data reveal a history of auto- and allopolyploidy in New Zealand endemic species of *Plantago* (Plantaginaceae): new perspectives on a taxonomically challenging group. *International Journal of Plant Sciences* 172: 220-237.

Nylander, J. A. A. 2004. MrModelTest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pamilo, P. and M. Nei. 1988. Relationships between gene trees and species trees. *Molecular Biology and Evolution.* 5: 568-583.

Popp, M. and B. Oxelman. 2001. Inferring the history of the polyploid *Siliene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 20: 474-481.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.

Puillandre, N., M. V. Modica, Y. Zhang, L. Sirovich, M. C. Boisselier, C. Cruaud, M. Holford, and S. Samadi. 2012. Large-scale species delimitation method for hyperdiverse groups. *Molecular Ecology* 21: 2671-2691.

Reeves, P. A. and C. M. Richards. 2011. Species delimitation under the general lineage concept: an empirical example using wild North American hops (Cannabaceae: *Humulus lupulinus*). *Systematic Biology* 60: 45-59.

Reveal, J. L. 1969. The subgeneric concept in *Eriogonum* (Polygonaceae) Pp. 229-249 in *Current Topics in Plant Science.* ed. J. Gunckel. New York: Academic Press.

Reveal, J. L. 1973. A new subfruticose *Eriogonum* (Polygonaceae) from western Colorado. *Great Basin Naturalist* 33: 120-122.

Reveal, J. L. 1978. Distribution and Phylogeny of Eriogonoideae (Polygonaceae). *Great Basin Naturalist Memoirs* 2: 169-190.

Reveal, J. L. 2005. Polygonaceae subfam. Eriogonoideae. Pp. 218-478 in *Flora of North America* Vol. 5 ed. Flora of North America Editorial Committee. New York: Oxford University Press.

Rieppel, O. 2009. Species monophyly. *Journal of Zoological Systematics and Evolutionary Research* 48: 1-8.

Riesberg, L. H. and L. Brouillet. 1994. Are many plant species paraphyletic? *Taxon* 43: 21-32.

Riesberg, L. H., T. E. Wood, and E. J. Baack. 2006. The nature of plant species. *Nature* 440: 524-527.

Rokas, A. and S. B. Carroll. 2005. More genes or more taxa? The relative contribution of gene number and taxon number to phylogenetic accuracy. *Molecular Biology and Evolution* 22: 1337-1344.

Rokas, A., B. L. Williams, and S. B. Carroll. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425: 798-804.

Sites, J. W. and J. C. Marshall. 2003. Delimiting species: A renaissance issue in systematic biology. *TRENDS in Ecology and Evolution* 18: 462-470.

Shaw J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. S. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, R. L. Small. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

Smith, J. F. and T. A. Bateman. 2002. Genetic differentiation of rare and common varieties of *Eriogonum shockleyi* (Polygonaceae) in Idaho using ISSR variability. *Western North American Naturalist* 62: 316-326.

Stamatikis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758-771.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

van der Niet, T., and H. P. Linder. 2008. Dealing with incongruence in the quest for the species tree: A case study from the orchid genus *Satyrium. Molecular Phylogenetics and Evolution* 47: 154-174.

Wendel, J. F. and J. J. Doyle. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. Pp. 265-296. in *Molecular Systematics of Plants.* ed. D. E. Soltis, P. E. Soltis, and J. J. Doyle. Boston: Kluwer Academic Publishers.

White T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pg: 315-322 in *PCR protocols: A guide to methods and application,* eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. New York: Academic Press.

Voucher and locality information for the accessions of *Eriogonum* included in the phylogenetic analyses. A number 1 indicates inclusion of the gene region (some samples have a 2, 3, or 4 indicating the number of copies/alleles included) and a 0 indicates the gene region is not used for that sample. All collection numbers refer to Ben R. Grady collections except otherwise indicated: JLR=James L. Reveal, SLO=Steve L. O'Kane, Jr., JFS=James F. Smith.

Coll. #	Genus	species	code	Latitude	Longitude	State	County	Chloroplast	*ITS*	*LEAFY*	*RPB2*
87	*Eriogonum*	*tenellum*	eten087	37°12.595'N	103°38.396'W	Colorado	Los Animas	1	1	1	0
91	*Eriogonum*	*aliquantum*	eali091	36° 29.727'N	104° 55.827'W	New Mexico	Colfax	1	1	1	0
112	*Eriogonum*	*clavellatum*	ecla112	37°16.680'N	109°40.518'W	Utah	San Juan	1	1	1	1
124	*Eriogonum*	*batemanii*	ebat124	39°57.813'N	108°46.733'W	Colorado	Rio Blanco	1	1	0	2
128	*Eriogonum*	*shockleyi*	esho128	40°19.778'N	109°29.166'W	Utah	Uintah	1	1	1	0
142	*Eriogonum*	*bicolor*	ebic142	38°14.557'N	111°06.689'W	Utah	Wayne	1	1	1	1
145	*Eriogonum*	*shockleyi*	esho145	38°16.474'N	111°07.925'W	Utah	Wayne	1	1	0	1
149	*Eriogonum*	*shockleyi*	esho149	38°26.047'N	113° 08. 022'W	Utah	Beaver	1	1	0	0
151	*Eriogonum*	*shockleyi*	esho151	38°26.627'N	113°16.300'W	Utah	Beaver	1	2	2	1
153	*Eriogonum*	*ammophilum*	eamm153	38°48.036'N	113°27.325'W	Utah	Millard	1	1	0	0
154	*Eriogonum*	*shockleyi*	esho154	36°54.841'N	112°29.682'W	Arizona	Coconino	1	2	1	0
156	*Eriogonum*	*corymbosum*	ecor156	36°54.461'N	112°35.428'W	Arizona	Mohave	1	1	1	0
168	*Eriogonum*	*alatum*	eala168	35°20.682'N	112°57.626'W	Arizona	Yavapai	1	1	1	1
173	*Eriogonum*	*nummulare*	enum173	37°27.730'N	115°21.590'W	Nevada	Lincoln	1	1	0	0
180	*Eriogonum*	*rosense*	eros180	38°07.392'N	116°54.334'W	Nevada	Nye	1	2	2	2
193	*Eriogonum*	*tiehmii*	etie193	37°49.032'N	117°51.391'W	Nevada	Esmerelda	1	1	3	2
210	*Eriogonum*	*breedlovei*	ebre210	36°06.621'N	118°29.185'W	California	Tulare	1	1	1	1
217	*Eriogonum*	*breedlovei*	ebre217	35°27.950'N	118°23.433'W	California	Kern	1	1	1	1
253	*Eriogonum*	*heermannii*	ehee253	36°31.005'N	117°49.552'W	California	Inyo	1	1	0	0
262	*Eriogonum*	*shockleyi*	esho262	37°07.616'N	118°03.555'W	California	Inyo	1	1	1	1
265	*Eriogonum*	*shockleyi*	esho265	37°09.653'N	118°02.341'W	California	Inyo	1	1	0	0
268	*Eriogonum*	*gracilipes*	egra268	37°22.980'N	118°10.888'W	California	Inyo	1	1	2	1
272	*Eriogonum*	*gracilipes*	egra272	37°33.065'N	118°13.464'W	California	Mono	1	1	2	1
283	*Eriogonum*	*rosense*	eros283	39°20.590'N	119°54.979'W	Nevada	Washoe	1	2	2	2
290	*Eriogonum*	*diatomaceum*	edia290	39°13.891'N	119°16.881'W	Nevada	Lyon	1	1	1	1
291	*Eriogonum*	*crosbyae*	ecrs291	41°05.930'N	119°24.421'W	Nevada	Washoe	1	1	0	0
299	*Eriogonum*	*crosbyae*	ecrs299	41°14.687'N	119°28.354'W	Nevada	Washoe	1	1	1	1
304	*Eriogonum*	*prociduum*	epro304	41°05.141'N	120°44.307'W	California	Lassen	1	1	0	1
329	*Eriogonum*	*chrysops*	echr329	43°30.381'N	117°46.767'W	Oregon	Malhuer	1	1	2	2
340	*Eriogonum*	*soliceps*	esol340	44°47.656'N	113°14.815'W	Idaho	Lemhi	1	1	2	1
347	*Eriogonum*	*pauciflorum*	epau347	45°02.675'N	108°25.035'W	Montana	Carbon	1	2	2	1
348	*Eriogonum*	*brevicaule*	ebrv348	45°02.675'N	108°25.035'W	Montana	Carbon	1	2	2	4
351	*Eriogonum*	*exilifolium*	eexi351	40°47.604'N	106°28.396'W	Colorado	Jackson	1	1	0	1
354	*Eriogonum*	*shockleyi*	esho354	40°43.317'N	108°45.895'W	Colorado	Moffatt	1	1	0	0
356	*Eriogonum*	*tumulosum*	etum356	40°45.497'N	108°50.008'W	Colorado	Moffatt	1	1	1	0
357	*Eriogonum*	*brevicaule*	ebrv357	40°58.470'N	109°27.780'W	Utah	Daggett	1	1	1	2
359	*Eriogonum*	*viridulum*	evir359	40°24.299'N	109°35.442'W	Utah	Uintah	1	1	0	1
362	*Eriogonum*	*bicolor*	ebic362	39° 37.550'N	110° 52.000'W	Utah	Carbon	1	1	0	0
364	*Eriogonum*	*batemanii*	ebat364	39° 37.550'N	110° 52.000'W	Utah	Carbon	1	1	0	0
371	*Eriogonum*	*panguicense*	epan371	38°58.617'N	111°57.675'W	Utah	Sevier	1	2	1	1
375	*Eriogonum*	*soredium*	esor375	38°26.979'N	113°16.960'W	Utah	Beaver	1	2	2	1
376	*Eriogonum*	*eremicum*	eere376	38°37.494'N	113°50.992'W	Utah	Millard	1	1	0	3
377	*Eriogonum*	*shockleyi*	esho377	38°37.494'N	113°50.992'W	Utah	Millard	1	2	0	1
378	*Eriogonum*	*holmgrenii*	ehol378	38°53.587'N	114°19.601'W	Nevada	White Pine	1	1	1	2
380	*Eriogonum*	*brevicaule*	ebrv380	41°04.010'N	115°01.189'W	Nevada	Elko	1	1	1	2
382	*Eriogonum*	*kingii*	ekin382	41°01.584'N	115°04.393'W	Nevada	Elko	1	1	0	0
386	*Eriogonum*	*brevicaule*	ebrv386	41°05.719'N	114°20.763'W	Nevada	Elko	1	1	0	0
388	*Eriogonum*	*kingii*	ekin388	41°41.872'N	114°44.483'W	Nevada	Elko	1	2	1	2

Coll. #	Genus	species	code	Latitude	Longitude	State	County	Chloroplast	*ITS*	*LEAFY*	*RPB2*
392	*Eriogonum*	*brevicaule*	ebrv392	40°43.464'N	116°08.831'W	Nevada	Elko	1	1	0	0
394	*Eriogonum*	*rosense*	eros394	39°30.306'N	117°22.976'W	Nevada	Lander	1	1	0	0
395	*Eriogonum*	*anemophilum*	eane395	39°53.193'N	117°06.766'W	Nevada	Lander	1	1	0	2
397	*Eriogonum*	*crosbyae*	ecrs397	41°51.699'N	119°00.508'W	Nevada	Humboldt	1	2	2	0
400	*Eriogonum*	*cusickii*	ecus400	43°32.169'N	119°22.534'W	Oregon	Harney	1	1	2	1
401	*Eriogonum*	*novonudum*	enov401	42°48.352'N	117°43.907'W	Oregon	Malhuer	1	1	1	2
402	*Eriogonum*	*anemophilum*	eane402	40°25.006'N	118°08.626'W	Nevada	Pershing	1	1	1	2
403	*Eriogonum*	*anemophilum*	eane403	40°16.452'N	118°44.796'W	Nevada	Pershing	1	2	2	0
405	*Eriogonum*	*ochrocephalum*	eoch405	39°35.415'N	119°53.211'W	Nevada	Washoe	1	2	2	1
413	*Eriogonum*	*alexanderae*	eale413	38°48.428'N	119°13.308'W	Nevada	Lyon	1	1	1	2
415	*Eriogonum*	*nutans*	enut415	38°34.236'N	119°11.189'W	Nevada	Lyon	1	1	1	0
418	*Eriogonum*	*ochrocephalum*	eoch418	39°18.402'N	119°34.292'W	Nevada	Storey	1	2	1	2
422	*Eriogonum*	*rosense*	eros422	37°10.743'N	118°32.612'W	California	Inyo	1	1	0	0
424	*Eriogonum*	*rosense*	eros424	39°19.897'N	119°38.028'W	California	Alpine	1	2	0	0
428	*Eriogonum*	*prociduum*	epro428	41°18.943'N	119°53.590'W	Nevada	Washoe	1	1	1	2
432	*Eriogonum*	*prociduum*	epro432	41°35.174'N	120°25.973'W	California	Modoc	1	1	1	0
434	*Eriogonum*	*watsonii*	ewat434	40°53.875'N	117°24.149'W	Nevada	Humboldt	1	1	1	1
435	*Eriogonum*	*kingii*	ekin435	40°35.782'N	115°23.708'W	Nevada	Elko	1	1	0	1
438	*Eriogonum*	*argophyllum*	earg438	40°35.196'N	115°17.075'W	Nevada	Elko	1	2	1	2
439	*Eriogonum*	*calcareum*	ecal439	42°46.839'N	115°54.803'W	Idaho	Owyhee	1	1	0	0
442	*Eriogonum*	*crosbyae*	ecrs442	42°44.367'N	116°54.767'W	Idaho	Owyhee	1	2	2	2
443	*Eriogonum*	*calcareum*	ecal443	43°18.129'N	116°34.817'W	Idaho	Owyhee	1	1	1	3
444	*Eriogonum*	*mancum*	eman444	44°06.644'N	114°51.671'W	Idaho	Custer	1	1	1	1
448	*Eriogonum*	*mancum*	eman448	44°16.850'N	114°09.753'W	Idaho	Custer	1	1	1	2
452	*Eriogonum*	*mancum*	eman448	44°17.911'N	113°29.232'W	Idaho	Custer	1	1	0	0
454	*Eriogonum*	*mancum*	eman454	43°49.619'N	113°35.937'W	Idaho	Custer	1	2	1	1
459	*Eriogonum*	*mancum*	eman459	44°28.266'N	113°14.707'W	Idaho	Lemhi	1	2	2	3
462	*Eriogonum*	*soliceps*	esol462	44°47.639'N	113°14.828'W	Montana	Beaverhead	1	1	1	3
464	*Eriogonum*	*crosbyae*	ecrs464	46°30.702'N	114°14.637'W	Montana	Ravali	1	1	0	2
467	*Eriogonum*	*mancum*	eman467	46°42.314'N	112°05.614'W	Montana	Lewis & Clark	1	1	1	2
472	*Eriogonum*	*pauciflorum*	epau472	44°34.652'N	104°41.786'W	Wyoming	Crook	1	1	1	1
480	*Eriogonum*	*acaule*	eaca480	41°33.795'N	108°16.089'W	Wyoming	Sweetwater	1	1	1	2
482	*Eriogonum*	*brevicaule*	ebrv482	41°38.473'N	108°29.046'W	Wyoming	Sweetwater	1	2	1	1
484	*Eriogonum*	*acaule*	eaca480	41°52.555'N	110°26.389'W	Wyoming	Lincoln	1	1	1	0
485	*Eriogonum*	*brevicaule*	ebrv485	41°52.555'N	110°26.389'W	Wyoming	Lincoln	1	1	0	0
494	*Eriogonum*	*shockleyi*	esho494	41°37.316'N	114°50.161'W	Nevada	Elko	1	1	1	2
501	*Eriogonum*	*lewisii*	elew501	41°21.594'N	115°58.172'W	Nevada	Elko	1	2	1	2
505	*Eriogonum*	*diatomaceum*	edia505	39°13.946'N	119°18.062'W	Nevada	Lyon	1	1	1	0
514	*Eriogonum*	*ochrocephalum*	eoch514	39°52.893'N	120°00.934'W	California	Lassen	1	1	1	2
519	*Eriogonum*	*prociduum*	epro519	41°24.262'N	120°13.493'W	California	Modoc	1	1	0	0
520	*Eriogonum*	*crosbyae*	ecrs520	42°04.018'N	119°34.630'W	Oregon	Lake	1	1	2	1
522	*Eriogonum*	*cusickii*	ecus522	43°20.985'N	120°42.150'W	Oregon	Lake	1	1	3	2
523	*Eriogonum*	*calcareum*	ecal523	43°55.857'N	117°39.874'W	Oregon	Harney	1	1	1	0
525	*Eriogonum*	*novonudum*	enov525	43°36.567'N	117°05.276'W	Oregon	Malheur	1	1	0	2
528	*Eriogonum*	*crosbyae*	ecrs528	42°03.005'N	118°02.229'W	Oregon	Malheur	1	1	1	1
532	*Eriogonum*	*scopulorum*	esco532	45°11.764'N	117°12.230'W	Oregon	Wallowa	1	1	2	1
534	*Eriogonum*	*codium*	ecod534	46°36.722'N	119°45.905'W	Washington	Benton	1	1	1	2
536	*Eriogonum*	*chrysops*	echr536	43°30.387'N	117°46.770'W	Oregon	Malheur	1	1	1	0
539	*Eriogonum*	*salicornioides*	esal539	43°44.000'N	117°41.000'W	Oregon	Malheur	1	1	0	0
541	*Eriogonum*	*loganum*	elog541	41°44.626'N	111°47.199'W	Utah	Cache	1	1	0	0
542	*Eriogonum*	*brevicaule*	ebrv542	41°24.994'N	111°58.038'W	Utah	Box Elder	1	1	0	0
549	*Eriogonum*	*shockleyi*	esho549	39°25.153'N	110°25.288'W	Utah	Emery	1	1	0	0
550	*Eriogonum*	*leptocladon*	elep550	38°50.460'N	110°22.992'W	Utah	Emery	1	1	0	0
557	*Eriogonum*	*mitophyllum*	emit557	38°53.683'N	111°53.920'W	Utah	Sevier	1	1	1	0
560	*Eriogonum*	*brevicaule*	ebrv560	39°22.331'N	111°23.736'W	Utah	Sanpete	1	1	0	0
564	*Eriogonum*	*artificis*	eart564	38°25.216'N	113°18.046'W	Utah	Beaver	1	1	3	2
565	*Eriogonum*	*soredium*	esor565	38°27.137'N	113°17.021'W	Utah	Beaver	1	1	1	1

Coll. #	Genus	species	code	Latitude	Longitude	State	County	Chloroplast	*ITS*	*LEAFY*	*RPB2*
567	*Eriogonum*	*shockleyi*	esho567	38°31.083'N	113°32.753'W	Utah	Beaver	1	1	0	0
569	*Eriogonum*	*holmgrenii*	ehol569	38°54.637'N	114°18.679'W	Nevada	White Pine	1	1	2	1
571	*Eriogonum*	*holmgrenii*	ehol571	38°53.800'N	114°18.800'W	Nevada	White Pine	1	1	0	0
575	*Eriogonum*	*domitum*	edom575	39°09.988'N	113°24.555'W	Utah	Millard	1	1	2	2
581	*Eriogonum*	*natum*	enat581	39°03.648'N	113°14.700'W	Utah	Millard	1	2	0	1
583	*Eriogonum*	*spathulatum*	espa583	38°58.748'N	112°22.947'W	Utah	Millard	1	1	2	3
589	*Eriogonum*	*hylophilum*	ehyl589	39°52.979'N	110°13.889'W	Utah	Duchesne	1	1	0	0
594	*Eriogonum*	*shockleyi*	esho594	40°18.427'N	109°41.399'W	Utah	Uintah	1	1	0	0
596	*Eriogonum*	*ephedroides*	eeph596	40°08.605'N	109°01.428'W	Colorado	Rio Blanco	1	1	1	1
600	*Eriogonum*	*brandegeei*	ebra600	38°37.791'N	106°04.717'W	Colorado	Chaffee	1	1	1	1
601	*Eriogonum*	*coloradense*	ecol601	38°10.846'N	106°42.019'W	Colorado	Sagauche	1	2	1	1
604	*Eriogonum*	*pauciflorum*	epau604	40°44.248'N	104°04.055'W	Colorado	Weld	1	1	2	1
605	*Eriogonum*	*annuum*	eann605	40°46.113'N	102°56.510'W	Colorado	Logan	1	1	1	0
611	*Eriogonum*	*pauciflorum*	epau611	43°45.033'N	101°56.968'W	South Dakota	Jackson	1	1	1	0
613	*Eriogonum*	*pelinophlium*	epel613	38°30.037'N	107°48.338'W	Colorado	Montrose	1	1	0	1
617	*Eriogonum*	*pelinophlium*	epel617	38°26.715'N	107°47.926'W	Colorado	Montrose	1	1	0	0
626	*Eriogonum*	*pelinophlium*	epel626	38°39.266'N	107°55.411'W	Colorado	Montrose	1	1	1	1
628	*Eriogonum*	*clavellatum*	ecla628	37°19.486'N	109°39.961'W	Utah	San Juan	1	1	0	0
633	*Eriogonum*	*clavellatum*	ecla633	37°16.040'N	109°48.435'W	Utah	San Juan	1	1	1	1
634	*Eriogonum*	*clavellatum*	ecla634	37°18.590'N	109°51.300'W	Utah	San Juan	1	1	0	1
635	*Eriogonum*	*contortum*	econ635	38°51.351'N	109°48.264'W	Utah	Grand	1	1	1	2
638	*Eriogonum*	*domitum*	edom638	39°08.593'N	113°24.428'W	Utah	Millard	1	1	1	1
643	*Eriogonum*	*bicolor*	ebic643	39°20.752'N	109°01.231'W	Colorado	Mesa	1	1	1	0
645	*Eriogonum*	*contortum*	econ645	39°15.231'N	108°55.730'W	Colorado	Mesa	1	1	1	1
648	*Eriogonum*	*pelinophilum*	epel648	38°24.468'N	107°47.924'W	Colorado	Montrose	0	1	1	0
649	*Eriogonum*	*lonchophyllum*	elon649	38°24.468'N	107°47.924'W	Colorado	Montrose	1	1	1	0
650	*Eriogonum*	*pelinophilum*	epel650	38°48.156'N	107°52.850'W	Colorado	Delta	1	1	0	0
8024 (SLO)	*Eriogonum*	*shockleyi*	esho8024	36°40.335'N	107°59.480'W	New Mexico	San Juan	1	1	1	1
8953 (JLR)	*Eriogonum*	*lewisii*	elew8953	n/a	n/a	Nevada	Elko	1	1	1	0
CM-1 (JFS)	*Eriogonum*	*shockleyi*	eshoCM1	n/a	n/a	Idaho	Owyhee	1	0	0	1
LV-1 (JFS)	*Eriogonum*	*shockleyi*	eshoLV1	n/a	n/a	Idaho	Owyhee	1	1	0	0
SSI-1 (JFS)	*Eriogonum*	*shockleyi*	eshoSSI1	n/a	n/a	Idaho	Elmore	1	1	0	1
WarE (JLR)	*Eriogonum*	*crosbyae*	ecrsWE	n/a	n/a	Idaho	Owyhee	1	1	0	2

A Jack-of-all-trades or master of one? Evolutionary patterns of edaphic endemism in capitate members of *Eriogonum* (Polygonaceae)

Ben R. Grady & Kenneth J. Sytsma

Ecological and geographic patterns of narrow soil endemism in plants are striking, with azonal soils, such as calcareous, gypsum, and serpentine often hosting specialized suites of endemic species. We examine evolutionary patterns of edaphic endemism in a phylogenetic framework in a clade of ecologically diverse species of *Eriogonum* (Polygonaceae) across a wide range of azonal soils. We used DNA sequences from chloroplast (*trnL-trnF, rpS16, trnD-trnT*) and nuclear markers (*nrITS* and *LEAFY*) to infer phylogenies of 45 'capitate' species of *Eriogonum*, including both narrow soil endemics and wide-ranging species. Strong geographic signal was detected in both sources of molecular data (plastid and nuclear) by employing the Mantel Test. Soil samples were characterized and classified by a multivariate cluster analysis of measured physical and chemical soil properties, recognizing four distinct edaphic groups. These edaphic groups were compared across the plastid and nuclear phylogenies, and visually inspected for general and clade-specific patterns. The ML reconstructions of measured pH and edaphic groups across the plastid and nuclear trees show uncertainty at many nodes, especially the deeper nodes. Some clustering of edaphic groups is evident in terminal clades, however most species occurring in defined edaphic groups were scattered throughout the phylogenies. Widespread species also occur on soils belonging to distinct edaphic groups. For these reasons, we propose a 'generalist model' of edaphic endemism for this clade of *Eriogonum* species, with potential shifts to more specialized soil types occurring in distinct lineages. It is likely that species in this

clade in *Eriogonum* can tolerate a variety of substrates, in the absence of competition from surrounding vegetation.

Key words: azonal soils, edaphic endemism, *Eriogonum,* Mantel Test, soil generalists

Many interacting processes shape distributions and patterns of endemism in plants. Climate is a major driving force in determining the distribution of vegetation and flora. Some authors have ranked the edaphic factor as the second most important factor influencing plant distributions, though it is impossible to fully separate edaphic conditions from local and regional climates (Jenny 1941, 1980; Mason 1946; Kruckeberg 1986; 2002; Rajakaruna 2004). Regardless, some plant species and vegetation types seem to show strong preferences to certain specialized soil types (Meyer 1986; Kruckeberg 2002; Safford et al. 2005). Edaphic endemism, distributional restriction caused by substrate properties, has drawn much attention from ecologists and evolutionary biologists. The ecology of various substrates has directly influenced the evolution and formation of species limited by edaphic conditions (Mason 1946; Raven 1964; Kruckeberg 1967; 1986; 2002; Macnair and Gardner 1998; Rajakaruna 2004; Givnish 2010). There are some lineages of plants with seemingly higher proportions of species limited largely by substrate preference. Studies of the serpentine outcrops in the California Floristic Province provide the most detailed view of patterns of edaphic endemism in North America (Kruckeberg 1967; 1984; Hickman 1993; Safford et al. 2005; Anacker et al. 2010). In the Safford et al. study (2005), a small number of genera accounted for a disproportionate number of the serpentine endemic species in the California Flora. *Streptanthus* (Brassicaceae), *Eriogonum* (Polygonaceae), *Hesperolinon* (Linaceae), and *Arctostaphylos* (Ericaceae) are four genera recognized in which greater than 4% of the species are serpentine endemics (Safford et al. 2005). Other examples of

azonal substrates with recognized suites of edaphic endemic species are calcareous (Misra and Tyler 1999; Kruckeberg 2002) and gypsum substrates (Meyer 1986; Palacio et al. 2007). The marked contrast from one soil type to another is often striking, as are the resulting vegetation patterns (Kruckeberg 2002).

Phylogenetic investigations into the patterns of edaphic endemism and speciation are increasing, with studies of serpentine tolerance being the most prevalent. Serpentine tolerance in the genus *Caulanthus* (Brassicaceae) is shown to have evolved several times in various species, based on plastid and ITS-based phylogenies (Mayer and Soltis 1994; 1999; Pepper and Norwood 2001). The speciose genus, *Calochortus* (Calochortaceae) also appears to contain numerous independent lineages that have moved onto serpentine substrates in California (Patterson and Givnish 2003). A widespread phylogenetic approach, incorporating multiple plant lineages taken by Anacker et al. (2010) shows repeated shifts in the evolution of serpentine specialists, however they also detected lower rates of diversification once serpentine tolerance evolves. Similar patterns of repeated evolution of obligate serpentine endemics occur in Old World members of Boraginaceae (Cecchi and Selvi 2009; Cecchi et al. 2011). Repeated evolution of gypsophily in *Tiquilia* (Moore and Jansen 2007), as well as in the Nyctaginaceae (Douglas and Manos 2007) is also apparent from recently published phylogenetic studies. All of these phylogenetic studies examine patterns of evolution onto one type of azonal substrate (e.g. serpentine or gypsum). To date, no study has attempted to address evolutionary patterns of edaphic endemism across a multitude of soil conditions within a single plant genus.

One of the exceptional genera identified by Safford et al. (2005) as contributing a disproportionate number of serpentine endemics is *Eriogonum* (Polygonaceae). *Eriogonum*, commonly know as wild buckwheat, is well known in the western American flora as a remarkably speciose genus with many rare taxa. In fact, of the 250+ species currently included in *Eriogonum,* approximately one third are considered rare across their range (Reveal 1978, 2005). The United States Fish and Wildlife Service lists a total of thirteen taxa (species or varieties) as either Endangered, Threatened, or as a Candidate for listing on the Endangered Species List (USFWS Species Reports). Many other species are listed by various state and federal agencies, acknowledging some level of rarity.

One of the more intriguing lineages of *Eriogonum* is the *Eucycla II* clade (see Chapter 1), composed mostly of 'capitate' members of the formerly recognized subg. *Eucycla* (Reveal 1969, 2005). While this group does not include any serpentine endemic species, either strict or broad (Safford et al. 2005), it does include numerous species that only occur on other azonal substrates. *Eriogonum diatomaceum*, commonly referred to as the Churchill Narrows wild buckwheat, is one such exceptional species in the 'capitate' lineage. *Eriogonum diatomaceum* was only recently discovered and described (Reveal et al. 2002). This isolated species is limited to chalky, diatomaceous substrates at elevations between 1300-1400 m in west-central Nevada (personal observation; Reveal 2005). The USFWS lists *E. diatomaceum* as a Candidate for listing on the Endangered Species list (USFWS Species Reports). Another noteworthy strict edaphic endemic species is *E. soredium.* This extremely rare species of west-central Utah, is found only on limestone

outcrops at the southern end of the San Francisco Mountains (personal observation; Reveal 2005). This densely-mound forming species was recently listed as a Candidate species for the Endangered Species list (USFWS Species Reports). The final example of an edaphic specialist mentioned here is *E. calcareum.* The distribution of the Harper wild buckwheat is more widespread than the previous two species mentioned, occurring in six counties in Idaho and Oregon, however it has a strong affinity for fine-grained calcareous soils (Grady and Reveal 2011).

Examining the phylogenetic patterns of narrow endemism relating to edaphic factors raises another pertinent question. Why are there so many edaphic endemic species in certain plant lineages? Various models have been proposed for the ecology and evolution of edaphic endemism. Gankin and Major outline the "refuge model" (also referred to as the "generalist model"), in which a stress-tolerant generalist species or population is able to proliferate by growing on marginal soils in the absence of competition from other stress-intolerant species (1964). Alternatively, some plant species may be better adapted to unusual chemical and physical properties associated with a certain soil types, i.e. the "specialist model" proposed by Meyer (1986). This model states that "specialist" plant species are physiologically better adapted to special azonal soils, than they are to more typical zonal soils. If a plant lineage is composed of edaphic generalists, than we would expect to see little pattern of edaphic similarity when compared to the evolutionary history. Soil characteristics, when mapped on a phylogeny of a group, would show strong, clade-specific affinities if the "specialist model" applies.

We examine patterns of endemism and models of edaphic specialization within a clade of *Eriogonum* species by 1) examining the edaphic specialist vs. generalist model by mapping and reconstructing ancestral "states" of soil property clusters on two phylogenies from the plastid genome (*trnL-trnF, rpS16,* and *trnD-trnT*) and *ITS*/*LEAFY*, and 2) comparing geographic distances to distances generated from DNA sequences of the plastid and nuclear genomes to determine if either show a strong geographic correlation.

Materials and Methods

Taxonomic Sampling—A subset of capitate members from the *Eucycla II* clade were included for testing hypotheses regarding edaphic and geographic endemism (see Figures 1, 3, and 5, Chapter 2). We measured soil properties for a total of 46 different accessions, representing 23 different species of 'capitate' members of *Eriogonum*. We included multiple samples of wide ranging species, such as *E. shockleyi, E. mancum,* and *E. pauciflorum*. Each of these species were collected from sites with varying substrates from across their ranges in the western U.S (Figure 1). Narrowly distributed endemic species were also included in these analyses. *Eriogonum soredium, E. holmgrenii, E. gracilipes,* and *E. diatomaceum* are prime examples of species with distributions that coincide with a particular soil substrate, i.e. edaphic endemic species (Reveal 2005). Other species within this clade were included in these analyses to thoroughly sample this lineage. Different outgroup species were used to root the different phylogenies used in this study, based upon previous phylogenetic results (Chapter 2, Figures 1, 3, and 5).

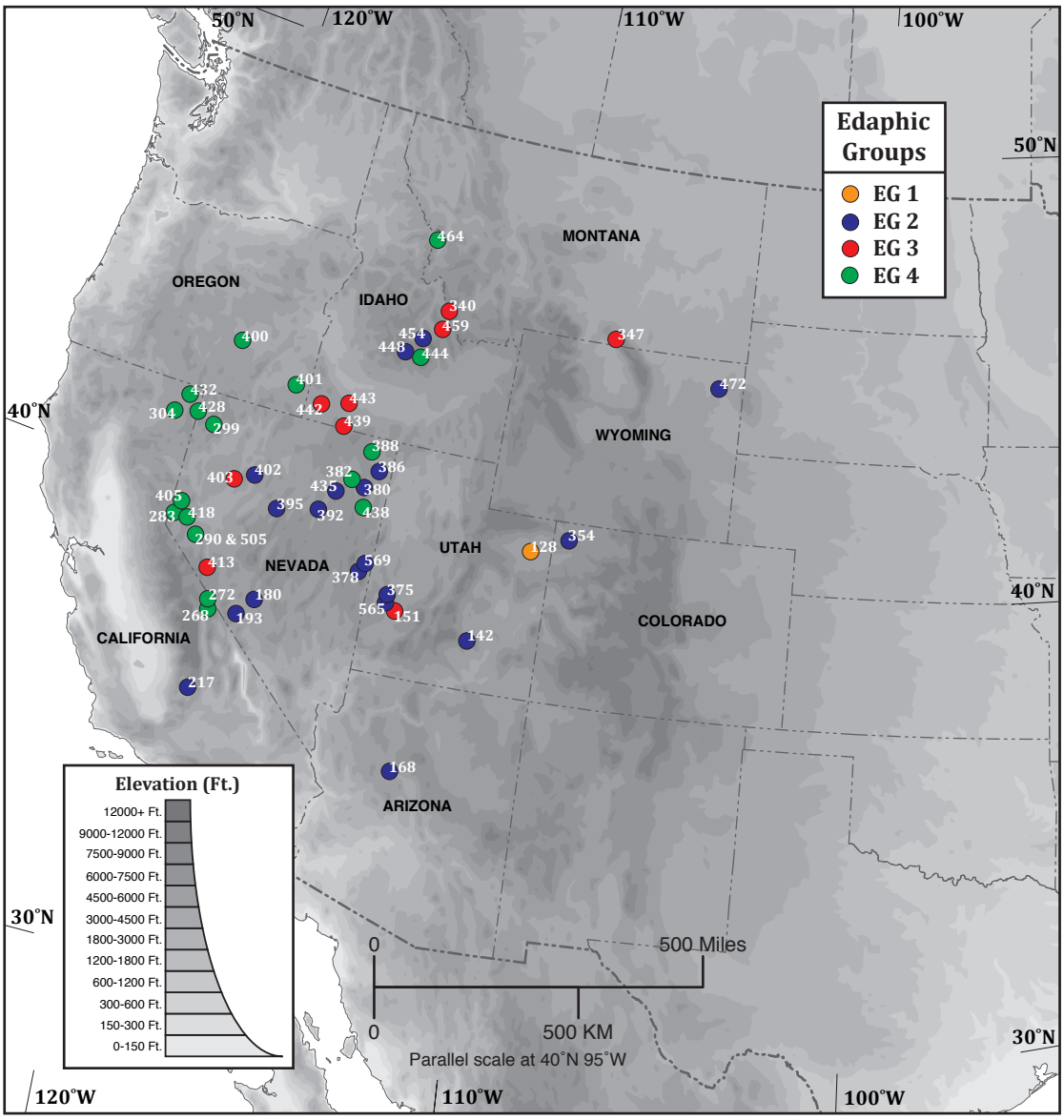


Figure 1. Distribution map of *Eriogonum* accessions included in geographic and edaphic analyses. Numbers associated with colored circles refer to collection numbers and correspond to samples in Appendix 1.

***DNA Extraction, Amplification, and Sequencing*—**Total genomic DNA was extracted from field collected, silica-dried leaf tissue using the Qiagen DNeasy™ plant mini kit (Qiagen, Valencia, California). Undiluted total genomic DNA was then

combined with 10x buffer, dNTP's and Ex Taq taq polymerase from Takara (Otsu, Shiga, Japan) and DMSO, BSA, and forward and reverse primers of interest. All reactions were carried out in 12.5 uL total volume.

The internal transcribed spacer (*ITS*) of the nuclear ribosomal DNA was amplified using the *ITS4* and *ITS5* primers and protocol from White et al. (1990). Chloroplast introns and spacer regions (*trnL-trnF*, *rpS16*, and *trnD-trnT*) were amplified using primers from Taberlet et al. (1991) and Shaw et al. (2005). Special internal primers were designed to amplify the entire *trnD-trnT* spacer region. The primer sequences, paired with the *trnD* and *trnT* primers respectively, are as follows: *trnE-m2* 5' GCTTTCTATATCGAATCGAATC 3' and *trnY-m2* 5' CATAGTATGAACAGTTTTTGG 3'. All chloroplast regions, excepting the *trnD* to *trnE* region, were successfully amplified with the following touchdown program: initial denaturation (94°C for 30 sec); amplification: 10 cycles 94°C 15 sec, 64°C 30 sec - 1°C/cycle, and 68°C for 1 min; then 20 cycles 94°C 15 sec, 54°C 30 sec, 68°C 1 min, followed by extended elongation of 68°C for 5 min. The *trnD* to *trnE* spacer region was amplified using the following protocol: denaturation 94°C 1 min; amplification 30 cycles 94°C 15 sec, 44°C 30 sec, and 72°C 1 min; and final extension of 72°C for 5 min.

The second intron of the low-copy nuclear region *LEAFY* was initially amplified in a subset of *Eriogonum* taxa with the degenerate primers, *LFsxl-2* and *LFtxr*, from Frohlich and Meyerowitz (1997). Sanchez and Kron (2008) demonstrated the variability and utility of the second intron of *LEAFY*, finding only a single copy in Eriogonoideae. To achieve more consistent amplification in the group

of study, Eriogonoideae specific primers were then designed from these initial sequences. The forward and reverse primer sequences are: *LFY71-F* 5' GCCTTGATTATCTCTTCCAC 3' and *LFY1369-R* 5' CCTGAACACCTGGTTTGTC 3'. PCR conditions for the modified *LEAFY* primers are: initial denaturation 94°C for 1 min; amplification 8 cycles 94°C for 20 sec, 59°C -1°C/cycle for 30 sec, 72°C for 1 min followed by 25 cycles of 94°C 20 sec, 52°C for 30 sec, and 72°C for 1 min; final extension of 72°C for 5 min.

All PCR products were diluted (30:1) in water and cycle sequenced using the same primers. These products were then purified by employing Agencourt magnetic beads (Agencourt, Beverly, Massachusetts). Sequencing reactions were carried out at the DNA Sequencing Facility on the University of Wisconsin Biotechnology Center using the ABI PRISM BigDye terminator on an Applied Biosystems 3730xl automated DNA sequencer (Applied Biosystems, Foster City, California).

Forward and reverse sequences were assembled and edited in Sequencher ver. 4.7 (Gene Codes, Ann Arbor, Michigan). Polymorphic sites in *ITS* and *LEAFY* were coded with IUPAC ambiguity codes. A site was coded as polymorphic if two peaks were present at the same site in both the forward and reverse read. If a region showed clear length variation in the chromatogram or if more than 1% of the sequenced region was coded as polymorphic, bacterial cloning was implemented to separate potential alleles or copies. For samples to be cloned, the Promega pGEM-T vector protocol was followed, except the volume of all reagents and buffers was halved (Promega, Madison, Wisconsin). To obtain representative sequences of all copies/alleles present, 8 to 24 clones were sequenced, dependent upon variation in

the initial direct sequencing read. All cloned sequences were initially aligned and visually compared to assess variation present in recovered sequences, and to recognize chimeric sequences or sequences with obvious Taq errors (i.e. point mutations present in only one cloned sequence) (Edwards et al. 2008). Recombinant (chimeric) sequences and those with clear unique Taq errors were excluded from alignments.

Phylogenetic analysis—Sequences from each respective region were manually aligned in MacClade 4.08 (Maddison and Maddison 2005) to maximize homologous site matches and minimize the placement of gaps in the alignments. Models of sequence evolution for Maximum Likelihood (ML) and Bayesian Inference (BI) were obtained from jModelTest and MrModelTest 2.3, respectively (Posada 2008, Nylander 2004). All basic phylogenetic analyses were carried out in the CIPRES Science Gateway v. 3.3 (Miller et al. 2010), using MrBayes v. 3.1.2 for BI (Huelsenbeck & Ronquist 2001) or RAxML v. 7.3.0 for ML bootstrapping (Stamatakis et al. 2008). Bayesian analysis of individual data sets was implemented with default settings, 10 million generations, with a 25% burnin. Maximum likelihood bootstrap values were gathered by using RAxML, with 1,000 bootstrap replicates and default settings. We used GARLI ver. 2.0 (Zwickl 2006) to generate a ML tree that was fully bifurcating for ancestral state reconstruction in BayesTraits. Default parameters were implemented and the selected model of sequence evolution was used for each respective dataset.

To eliminate redundant terminals in the *ITS*/*LEAFY* dataset, we compared initial phylogenies generated from all included sequences (alternative cloned

sequences included) and selected sequences that were the most congruent between each gene region (see Appendix 2 for sequences removed for each region). While we acknowledge that this may introduce phylogenetic bias, it is necessary to reduce the number of terminals in our trees to correspond to the number of data points used for the geographic and edaphic analyses. The GTR+G model of sequence evolution was implemented for the combined *ITS*/*LEAFY* data set because it was the simpler of the two models (fewer parameters) selected for each individual data partition (Nylander 2004).

Geographic and genetic distance comparisons—To assess geographic signal in our phylogenetic trees, point-to-point distances were calculated for each population. Exact latitude and longitude coordinates were taken from each associated field site with a hand-held WAAS-enabled GPS unit (Garmin eTrex HCx). The program GeographicDistanceMatrixGenerator ver. 1.2.3 was used to create a distance matrix, in km, from the coordinates for each site using the WGS84 reference spheroid calculation. Genetic distance matrices were generated in PAUP* ver. 4.0b10 (Swofford 2002) using uncorrected p distances. Mantel Tests of correlation between data matrices were performed by comparing geographic distances between collection sites to genetic distances, for both the combined chloroplast matrix and the combined *ITS*/*LEAFY* matrix (Mantel 1967). The matrices were tested for correlation in R using APE with 10,000 permutations.

Soil sampling and analysis—Many of the sites where these species occur have substrates that are extremely patchy, therefore soil survey maps were too coarse for specific data collection for each of the species' distributions (USDA, Web

Soil Survey). Soil samples for analysis of chemical and physical properties were collected from the field at each respective site. For each site, we located an estimate of the center of each population of the *Eriogonum* species of interest. An individual plant was located near the center and used as a representative for soil sample collection. All organic matter on the surface was cleared from the immediate area and four different samples of the top 30 cm of the substrate were collected. The subsamples, collected at each cardinal direction and ca. 30-40 cm from the plant, were pooled into one representative soil sample for each site. Samples were stored in the dark at room temperature until physical and chemical analyses were performed (up to 4 months).

The soil properties selected for inclusion in our analysis cover a wide range of physical and chemical properties useful for characterization of a variety of different substrates from field collected soil samples. Soil chemical analysis of soil pH, exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}), and concentration of sulfur/sulfate were performed by the Soil and Plant Analysis Lab (SPAL) at the University of Wisconsin. Soil pH values were measured in a 1:1 soil:solution ratio by using a pH meter (Fisher Scientific Accumet Model no. AR25) according to the procedure outlined in Watson and Brown (1998). We also chose to analyze essential plant macronutrients in the form of exchangeable cations to further characterize the chemical properties of our field collected soil samples. Concentrations of Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+} were obtained by atomic absorbtion (AA) spectrophotometry in the UW Soil and Plant Analysis Lab following procedures described in Thomas (1982) and Warncke and Brown (1982). Available phosphorus, also measured from each

sample by the UW SPAL, was determined by spectrophotometry of the soil in solution (Bray and Kurtz 1945; Frank et al. 1998) To assess concentrations of sulfur (measured in the form of SO_4) in our soil samples analytical methods described by Combs et al. (1998) were employed by the UW SPAL. Soil texture and bulk density, measures of particle size distribution, porosity, and particle density, were carried out in the Balster Lab at the University of Wisconsin (Gee and Or 2002). To assess the effectiveness of phylogenetically examining just one measured soil property, we chose soil pH. Soil pH is a commonly measured variable in soil analysis and considered a master variable that affects physiological responses in the plant/soil interface in many ways (Jenny 1980). Values of soil pH were grouped according to acidic (<6.8), neutral (6.8-7.2), and alkaline (>7.2) values. Total data from our soil analyses were synthesized (see Appendix 3 for properties analyzed) into discrete groups by a multivariate cluster analysis using Euclidian distance and Ward's linkage measures in PC-ORD ver. 6 (McCune and Mefford 2011). Natural groups were recognized from visualization of the cluster dendrogram. These edaphic groups (EG, henceforth) were used to visually examine phylogenetic patterns of edaphic endemism in *Eriogonum*.

Phylogenetic examination of soil properties—To visualize phylogenetic patterns relating to soil properties (edaphic groups and pH) we used maximum parsimony (MP) in MacClade (parsimony mapping) (Maddison and Maddison 2005) and maximum likelihood (ML) in BayesTraits ver. 1.0 (Pagel and Meade 2007) to trace these "characters" and find associated probabilities at nodes of interest. We used the single most likely tree from the ML analysis for both the combined

chloroplast and the combined nuclear (*ITS*/*LEAFY*). Terminals were coded as acidic, neutral, or alkaline for soil pH, and 1, 2, 3, or 4 for the associated edaphic group, in each respective analysis. We implemented the "trace character" option for the parsimony reconstruction of soil pH or the EG's across the most likely tree in MacClade. The MultiState model of evolution was employed to reconstruct ancestral "states" associated with soil pH and our characterization of different clustered substrates (EG) in BayesTraits, with default parameters. One hundred ML runs (mltries=100) were performed in MultiState with a total of 21 nodes analyzed in the chloroplast phylogeny and 31 nodes of interest in the *ITS/LEAFY* tree, to find probabilities relating to soil characteristics associated with each node (Pagel 1999, Pagel and Meade 2007). Colored branches on the phylogram indicate MP reconstructions and the ML probabilities are represented by a pie chart on each node of interest.

RESULTS

Phylogenetic relationships of sampled species—Phylogenetic reconstruction of the evolutionary history of the 45 ingroup accessions is inferred with data from three genomic sources. The three chloroplast regions (*trnL-trnF*, *rpS16*, *trnD-trnT*) were easily amplified and aligned for all accessions included in this sampling regime. Although sequence variation was relatively low (Table 1), a number of clades are resolved and well supported with PP and BS, including three strongly supported clades emanating from a basal polytomy (labeled A, B, and C, Figure 2). Within each of these major chloroplast lineages, there are numerous well-

Table 1. Summary statistics for all data matrices analyzed in this study of relationships *Eriogonum*. An * in the Accessions included column denotes additional cloned sequences present in the data matrix. Variable sites included, Parsimony informative sites, % variable, and % PIC are calculated with and without the outgroup, values for the ingroup only are shown in parentheses.

Region	Accessions included	Aligned length (bp)	Variable sites included (ingroup only)	Parsimony informative sites (ingroup only)	% variable (ingroup only)	% PIC (ingroup only)	Substitution model (BI)
nrITS	46	716	89 (80)	40 (39)	12.4% (11.1%)	5.6% (5.4%)	GTR+I+G
LFY 2nd intron	36	1320	243 (-)	79 (-)	18.4% (-)	6.0% (-)	GTR+G
Combined ITS + LEAFY	46	2038	335 (326)	120 (119)	16.4% (16.0%)	5.9% (5.8%)	GTR+G
Combined chloroplast	46	3989	175 (129)	44 (44)	4.3% (3.2%)	1.1% (1.1%)	GTR+I

supported clades. As mentioned in Chapter 2, incomplete lineage sorting and introgression are likely issues in the phylogenetic history in this lineage of *Eriogonum*, although there are species that are monophyletic based on our plastid data. The following species are monophyletic sister species with more than one accession included: *E. diatomaceum, E. rosense,* and *E. ochrocepham*. There are some narrowly distributed species included here that show chloroplast haplotype variation within populations. The sampled individuals of *E. soredium, E. holmgrenii,* and *E. gracilipes* show different haplotypes, although these samples were collected in close proximity to each other. Interestingly, the three samples of *E. kingii*, collected from different mountain ranges in northeast Nevada, are placed in each of the three earliest diverging clades (A, B, and C Figure 2). Of the widespread species sampled here, *E. shockleyi* appears fairly cohesive, contrary to our results in Chapter 2, however far fewer accessions are included here. The three accessions of the widespread *E. brevicaule* all show different plastid haplotypes.

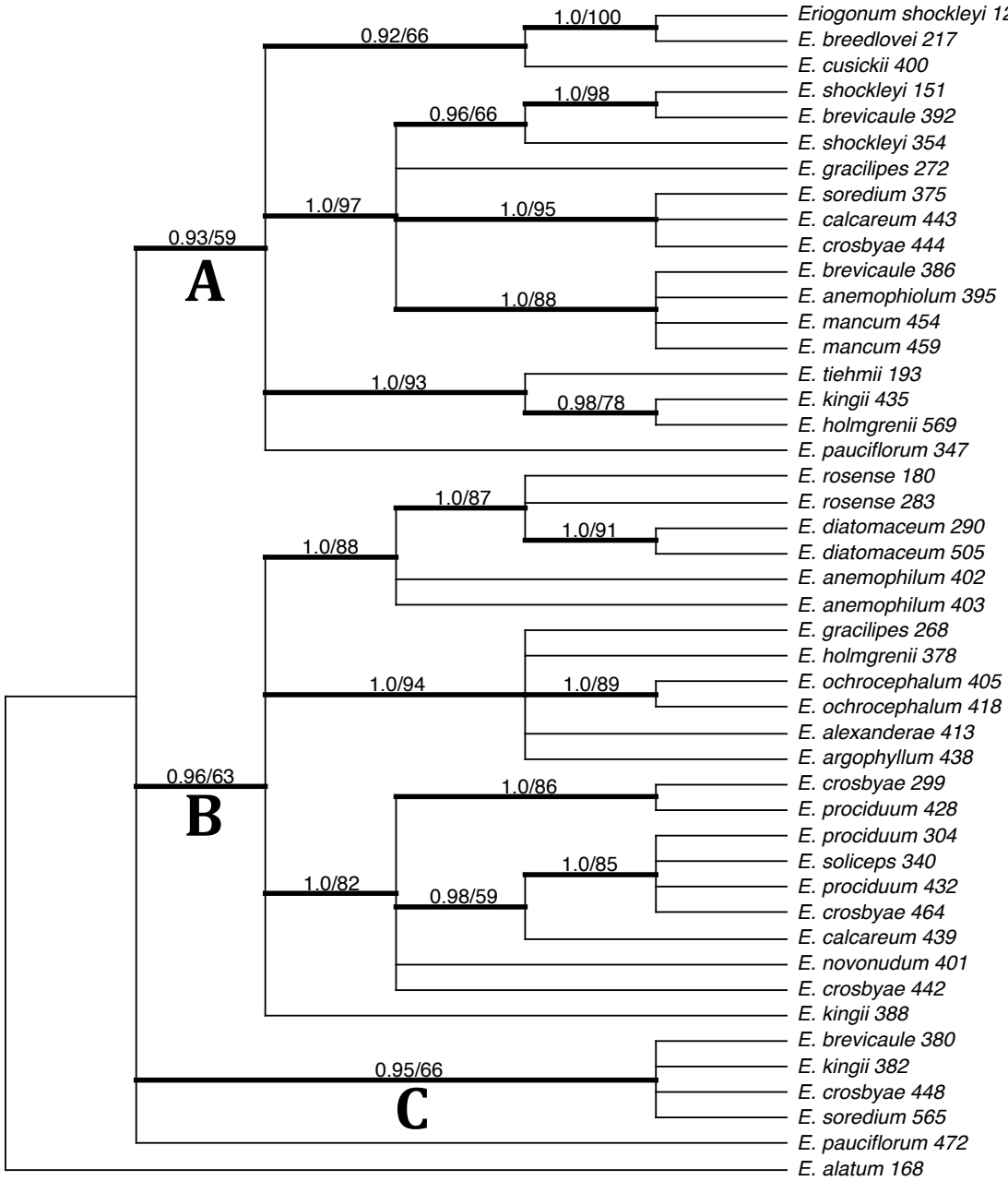


Figure 2. Consensus tree from Bayesian analysis of the combined three-region chloroplast data set (*trnL-trnF*, *rpS16*, and *trnD-trnT*) for 45 ingroup accessions of *Eriogonum* subg. *Eucycla II*. Major clades of interest are labeled with either A, B, or C. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Branches in bold have >0.90 BI PP support and >50% ML BS support.

Sequences from *ITS* and *LEAFY* exhibited higher levels of variation, when compared to plastid sequences. The percentage of parsimony informative characters for the ingroup for the two regions are 5.4% and 6.0%, respectively (Table 1). Multiple sequences with numerous nucleotide polymorphisms and length variation were detected from direct sequencing. Cloning and subsequent sequencing revealed samples with more than one copy or divergent allele present for both *ITS* and *LEAFY.* The reasons for this are discussed at length in Chapter 2, but hybridization, possibly resulting in allopolyploidy, and concerted evolution are likely impacting these genealogies. Because we are conducting numerous comparisons of soil properties and geographic distances with these data, additional copies had to be removed from the phylogenetic analyses of both *ITS* and *LEAFY*, to allow one terminal per accession included. All recovered sequences for both of these regions were included in an initial BI analyses. The individual gene trees were compared for congruent placement of accessions. Extraneous sequences, possibly previously unrecognized paralogs, were then eliminated from the subsequent alignment to limit one sequence per sample. The initial *ITS* and *LEAFY* gene trees are included in Appendix 2 with the removed sequences highlighted in gray. After removal of extra sequences for *ITS* and *LEAFY*, the datasets were concatenated to simplify analysis of patterns of endemism. The *ITS*/*LEAFY* combined data set resolved many relationships within this clade, however there are unresolved relationships at the base of the tree involving species of the northern Great Basin (Figure 3). Many of the species included, including some that are widely distributed,

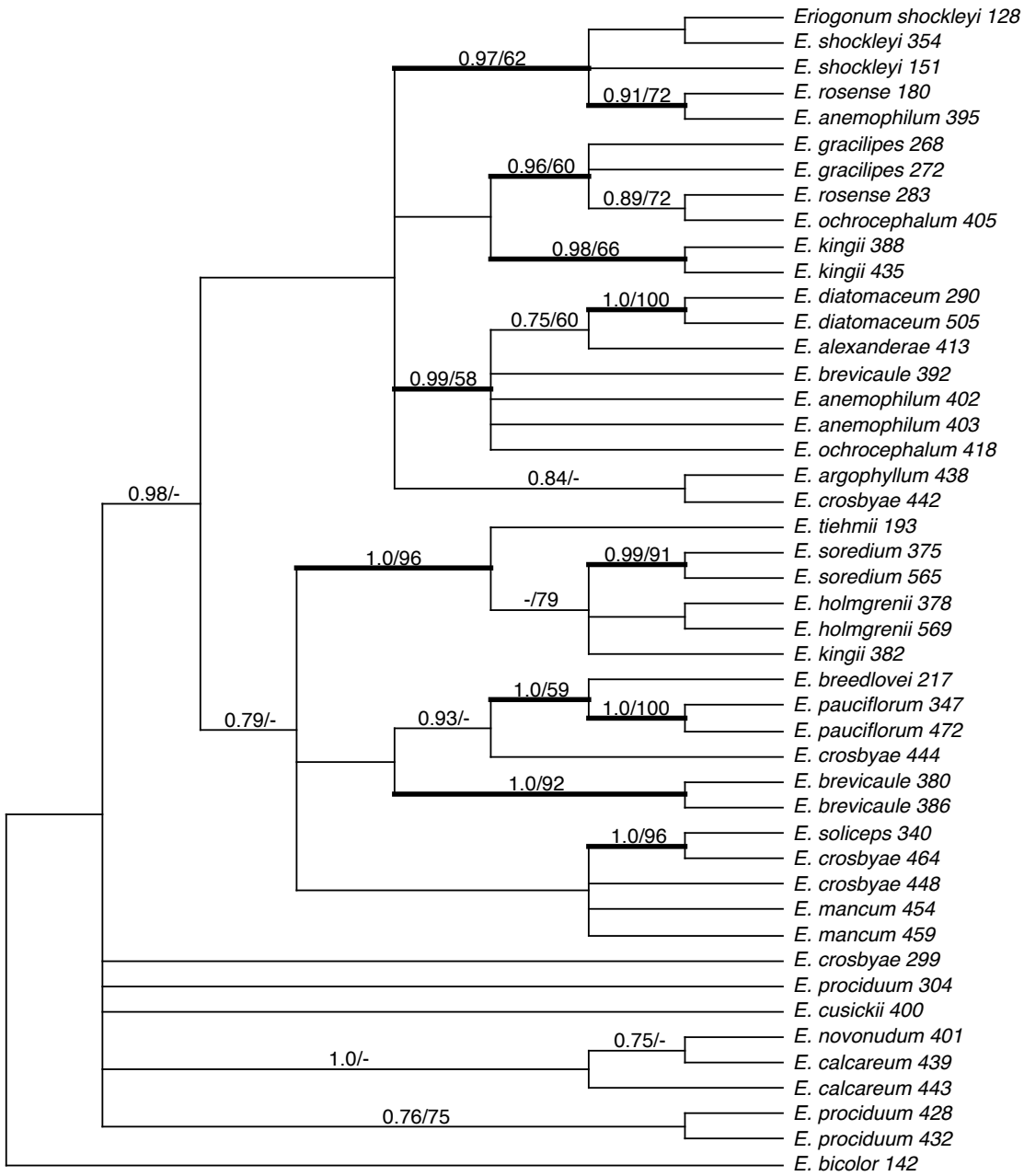


Figure 3. Consensus tree from Bayesian analysis of the combined nuclear (*ITS*/*LEAFY*) datasets for 45 ingroup accession and the outgroup, *E. bicolor*. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Branches in bold have >0.90 BI PP support and >50% ML BS support.

show reciprocal monophyly in the combined analysis (e.g. *E. soredium, E. holmgrenii, E. diatomaceum, E. shockleyi, E. pauciflorum, E. mancum,* and *E. calcareum*).

Genetic distances for each of the data partitions were tested for correlation to geographic distance using the Mantel Test. We wanted to see if the phylogenetic signal present in our molecular datasets was largely influenced by geographic proximity of sampled collections for each of the species included in our study. Computed p-values for each genetic matrix were significant (at $\alpha=0.05$), thus we reject the null hypothesis of no correlation between geographic and genetic distance in our molecular data sets. The combined *ITS*/*LEAFY* dataset appears to be slightly more correlated to geographic distance than our plastid data ($p<0.0001$ and $p<0.0007$, respectively).

Soil properties—Values of chemical properties and physical characteristics of the soils analyzed varied dramatically. The texture analysis revealed samples that ranged from sand (97% sand, 1% silt, 2% clay) to silt loam (30% sand, 52% silt, 18% clay) to clay (7% sand, 33% silt, 60% clay). Bulk density measurements ranged from 0.54 g/cm^3 to 1.58 g/cm^3. For the chemical properties of the soils analyzed, values obtained for each of the variables cover a broad spectrum. Soil pH values, for instance, ranged from 5.2 to 9.5. Of the 47 total samples included, seven samples were acidic (pH <6.8), four were considered neutral (pH 6.8-7.2), and thirty-six were alkaline (pH >7.2). Differences in soil calcium were some of the most striking of the cations measured. The sample associated with the *E. shockleyi* 128 collection contained 16,290 ppm of calcium. This value is more than twice the concentration of calcium measured in any of the other samples. With an associated concentration of

99 ppm magnesium, the Ca:Mg for this sample is 165. The lowest measured concentration of calcium (94 ppm) came from a sulfur hot spring, near the lone population of *E. argophyllum* 438. The magnesium concentration for this sample is 14 ppm, giving this sample a Ca:Mg of 7, nearly 25 times less than the Ca:Mg ratio calculated for the soil at the *E. shockleyi* 128 site. Complete measured values for physical and chemical soil properties are included in Appendix 3.

The cluster analysis of the physical and chemical properties of the included soil samples indicated four distinct edaphic groups (EG's), based on multivariate distance measures (Figure 4). The soil sample associated with *E. shockleyi* 128 is recognized as the sole member of EG 1 (highlighted in orange). EG 2 (blue) includes 18 soil samples, with textures that are generally more coarse and soil pH values tending to be higher. Nine samples are included EG 3 (red), mostly fine-grained samples with high pH and high levels of Ca. The largest cluster, with 19 included samples, is EG 4 (green). Samples in this group are generally more coarsely-grained soils with measured pH values from 5.3 to 9.5. Of the four soil groups characterized, EG 4 encompasses the greatest diversity in soil properties (Figure 4, Appendix 3).

Phylogenetic comparisons of soil characteristics—Two different soil factors were used in the phylogenetic mapping and node reconstruction for this group of *Eriogonum.* We selected soil pH as a single measured property and mapped acidic, neutral, and basic soil pH values for each soil examined on the chloroplast and combined nuclear phylogenies. We wanted to evaluate the potential edaphic signal based on a single measured soil property. Multivariate soil groupings from

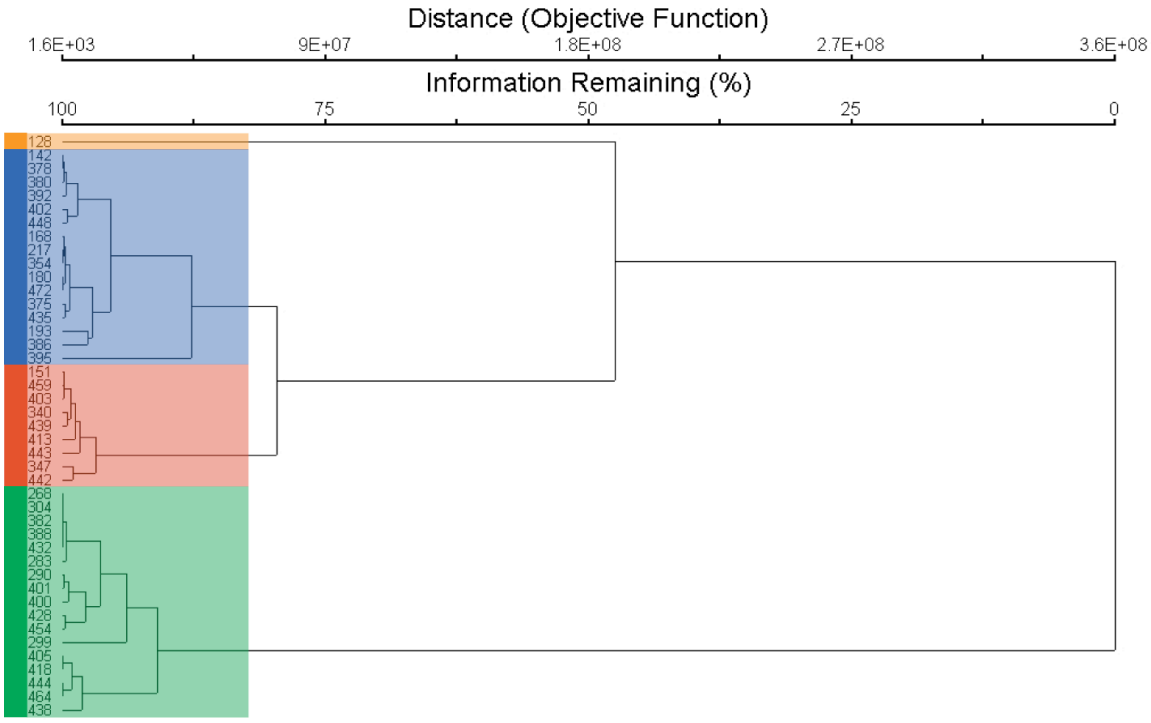


Figure 4. Edaphic groups (EG's) based on cluster analysis of 11 measured physical and chemical properties of soil samples collected from *Eriogonum* species sites. Four clusters were defined based on visual inspection of the cluster dendrogram. EG 1 is shown in orange, EG 2 is blue, EG 3 is highlighted in red, and EG 4 is shown in green.

eleven measured soil values are also used to visually inspect any phylogenetic patterns relating to a more holistic view of edaphic conditions in *Eriogonum*.

Soil pH values appear to show some phylogenetic structure when mapped on the chloroplast phylogeny (Figure 5). Of the three major clades radiating from the basal polytomy, clade A (containing *E. shockleyi 128*, *E. crosbyae 444*, *E. pauciflorum 347*, and others) consists mostly of samples collected from alkaline substrates. The ML reconstructions show uncertainty for values of pH in these nodes or high rates of changes between states, however most of the nodes represent a higher probability

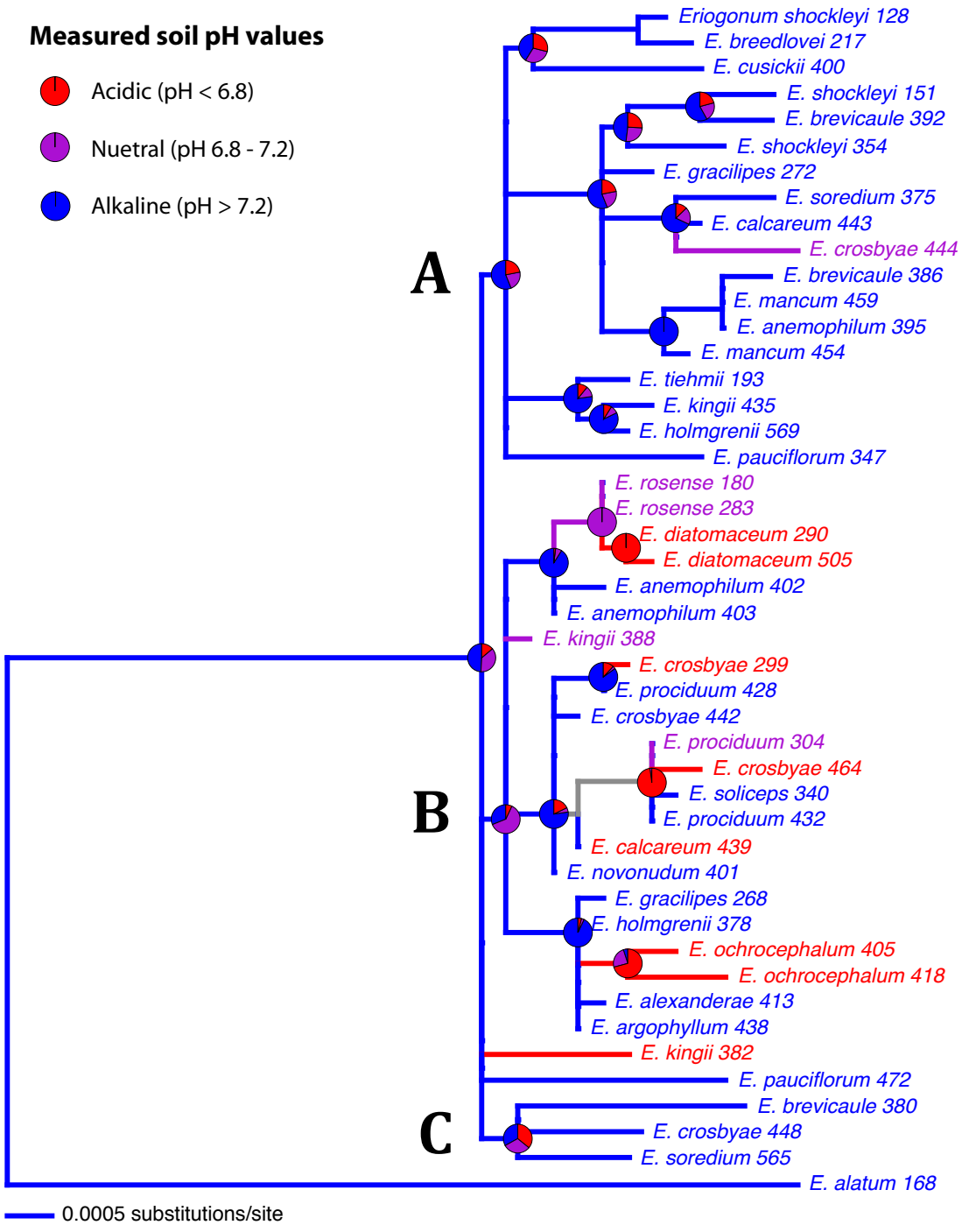


Figure 5. Parsimony and Maximum Likelihood reconstructions based on measured values of soil pH for each associated collection of *Eriogonum.* Phylogram of single most likely tree topology from ML analysis of the combined three-region chloroplast data set (*trnL-trnF*, *rpS16*, and *trnD-trnT*) for 45 ingroup accessions of *Eriogonum*, plus the outgroup *E. alatum*. See Figure 1 for support values associated with nodes. Branches are colored according to MP mapping and the colored pie charts at nodes of interest are probabilities from the ML reconstruction.

of alkaline soils (Figure 5). Clade B (*E. rosense* 180, *E. crosbyae* 299, *E. argophyllum* 438, and others) includes more of a diversity of pH values associated with the populations and shows a basal node with a higher probability of a neutral pH value. Seven of the eight *Eriogonum* populations collected from acidic soils belong to this clade (highlighted in red, Figure 5). More uncertainty is present in the *ITS*/*LEAFY* soil pH phylogeny (Figure 6). Maximum parsimony mapping of soil pH shows mostly ancestral states of growing on alkaline substrates, however ML reconstructions for most nodes show an equal probability for all classes of soil pH present.

The multivariate clustering of soil properties and subsequent phylogenetic mapping resulted in basal uncertainty regarding edaphic groups, however major clades show distinct patterns, especially in the plastid reconstruction (Figure 7). Clade A in the chloroplast phylogeny (Figure 7) is mostly composed of species growing on substrates classed in EG 2 (blue), however representatives growing on each of the other EG's are found in this clade as well. Clade B includes the majority of samples associated with EG 4 (green). Maximum likelihood reconstruction of EG's show the highest probabilities associated with EG 4 (green) and EG 3 (red). Parsimony mapping of EG's indicate equivocal states at the base of the combined *ITS*/*LEAFY* tree (Figure 8), with a shift toward ancestral species likely associated with substrates similar to EG4 (green). EG's associated with the nodes from the ML reconstruction show much uncertainty throughout the topology, except toward the tips of the branches (Figure 8). There are two noteworthy clades from the EG mapping and reconstruction from the combined *ITS*/*LEAFY* tree. One clade, which includes *E. gracilipes* 268, *E. gracilipes* 272, *E. rosense* 283, and *E. ochrocephalum*

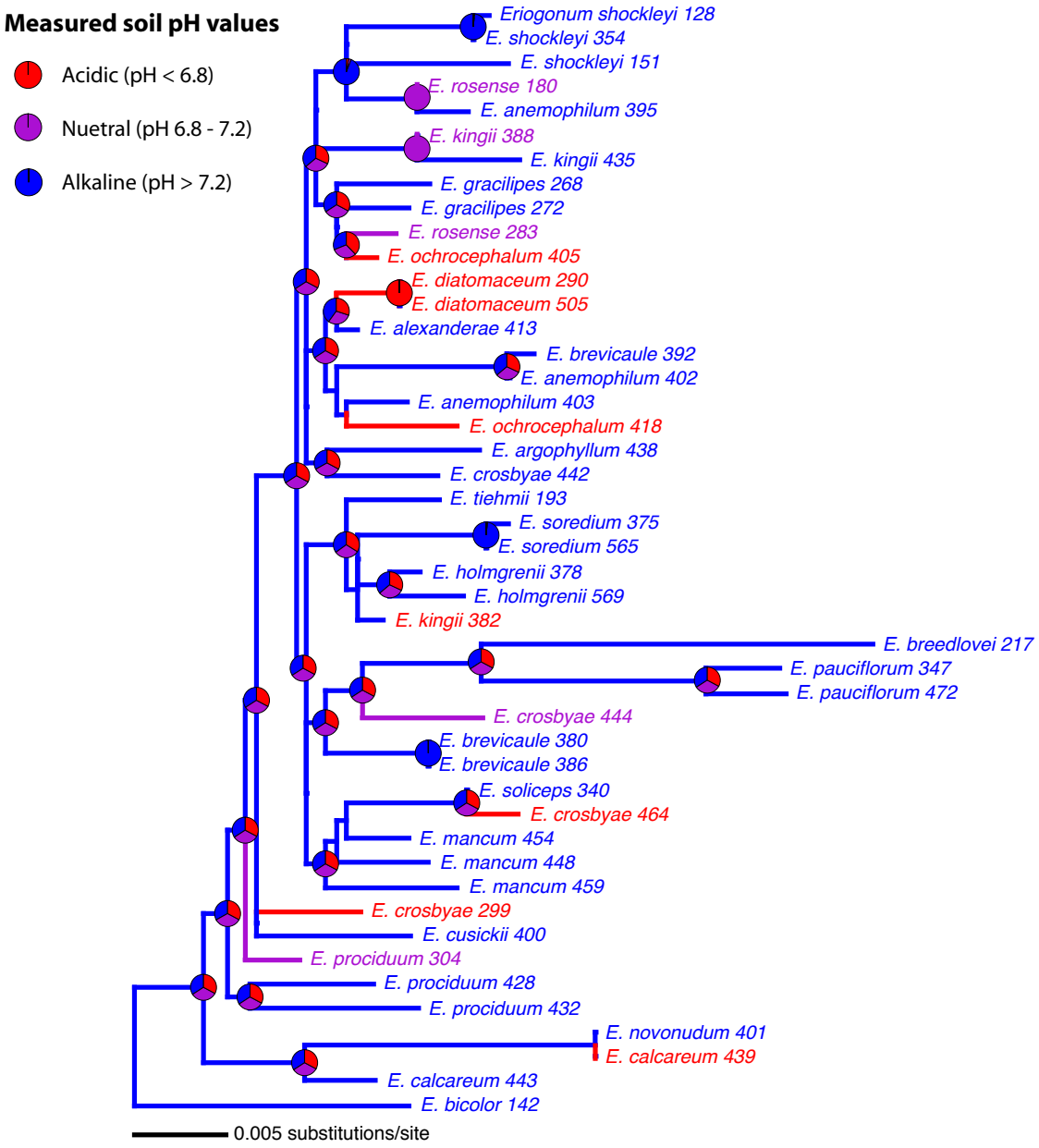


Figure 6. Parsimony and Maximum Likelihood reconstructions based on measured values of soil pH for each associated collection of *Eriogonum.* Phylogram of single most likely tree topology from ML analysis of the combined nuclear (*ITS*/*LEAFY*) dataset for 45 ingroup accessions of *Eriogonum* species, plus the out group *E. bicolor*. See Figure 2 for support values associated with nodes. Branches are colored according to MP mapping and the colored pie charts at nodes of interest are probabilities from the ML reconstruction.

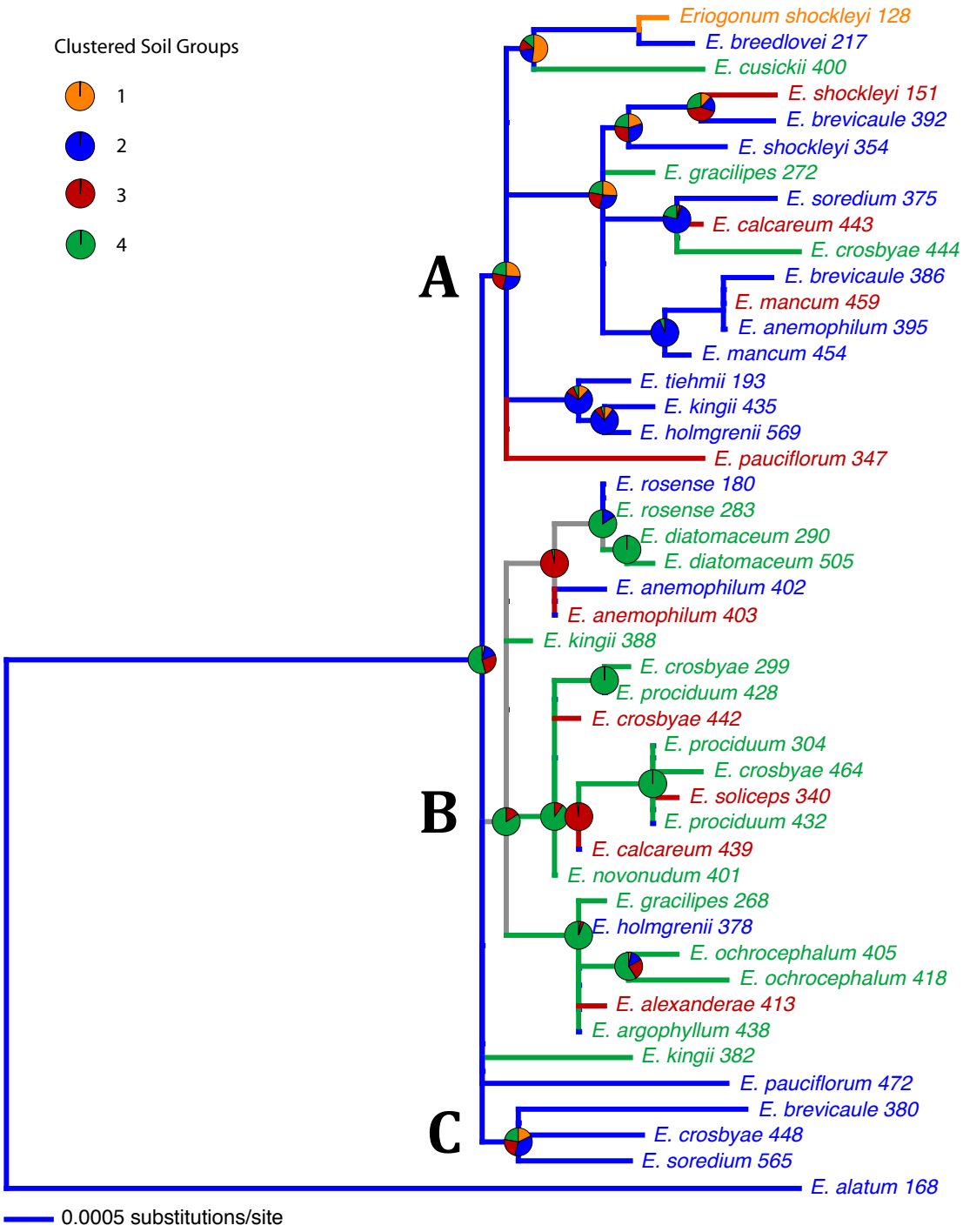


Figure 7. Parsimony and Maximum Likelihood reconstructions based on edaphic groups (EG's) for each associated collection of *Eriogonum.* Phylogram of single most likely tree topology from ML analysis of the combined three-region chloroplast data set (*trnL-trnF*, *rpS16*, and *trnD-trnT*) for 45 ingroup accessions of *Eriogonum*, plus the outgroup *E. alatum*. See Figure 1 for support values associated with nodes. Branches are colored according to MP mapping and the colored pie charts at nodes of interest are probabilities from the ML reconstruction.

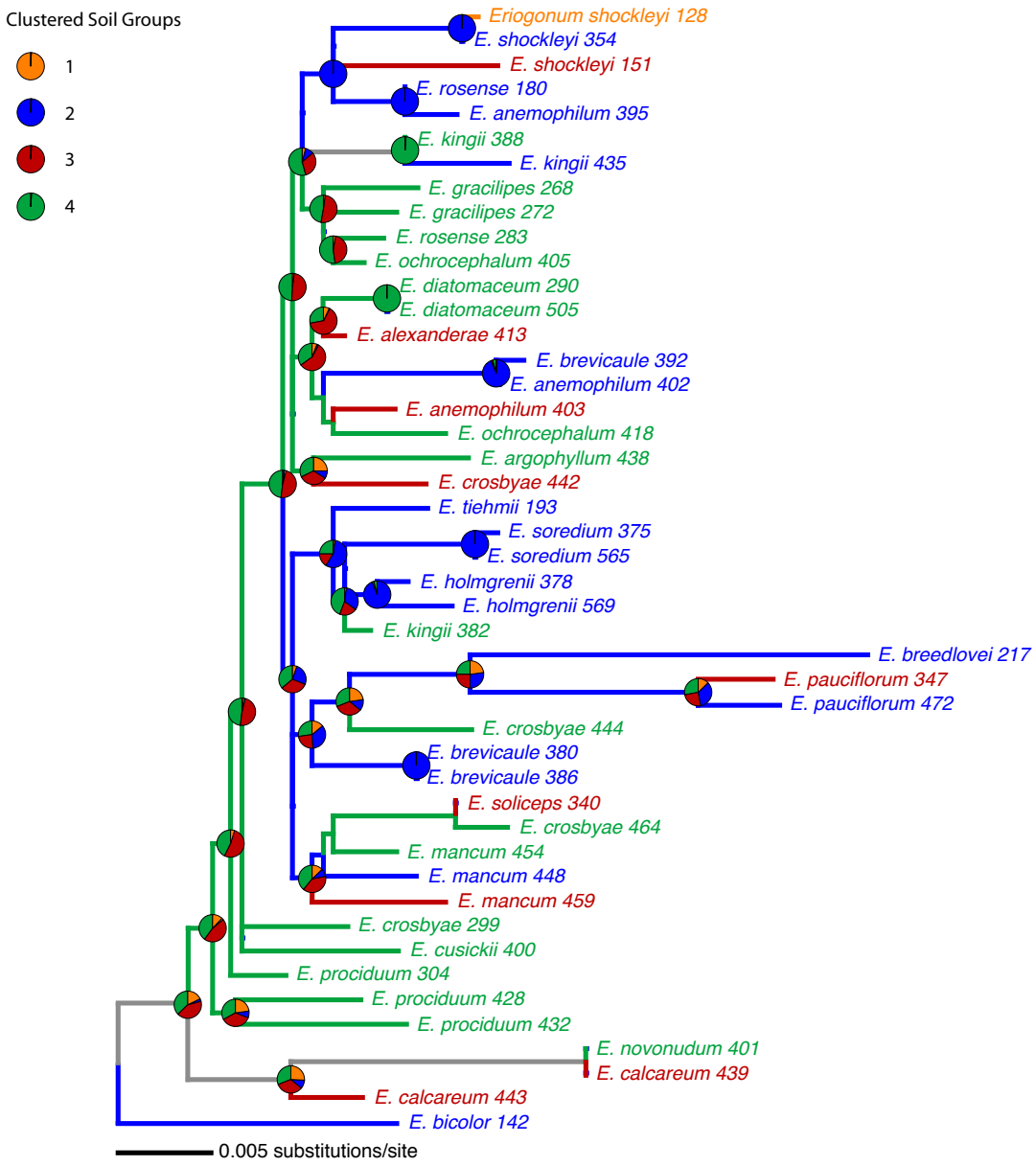


Figure 8. Parsimony and Maximum Likelihood reconstructions based on edaphic groups (EG's) for each associated collection of *Eriogonum.* Phylogram of single most likely tree topology from ML analysis of the combined nuclear (*ITS*/*LEAFY*) dataset for 45 ingroup accessions of *Eriogonum* species, plus the out group *E. bicolor*. See Figure 2 for support values associated with nodes. Branches are colored according to MP mapping and the colored pie charts at nodes of interest are probabilities from the ML reconstruction.

405, is composed entirely of species growing on the EG 4-type substrate (Figure 8).
We also see a clade dominated by samples from the EG 2-type substrate (blue), composed of the following species: *E. tiehmii* 193, *E. soredium* 375, *E. soredium* 565, *E. holmgrenii* 378, and *E. holmgrenii* 569 (Figure 8). Few other clear patterns emerge from these reconstructions.

DISCUSSION

Species relationships and phylogenetic structure of topologies—

Numerous biological processes confound phylogenetic inference in closely related groups of species, especially those that are likely the result of a recent, rapid radiation (see Chapter 2). Obtaining a fully resolved, well-supported species tree from a group such as *Eriogonum* is a daunting task. In spite of this, numerous relationships and clades of interest are present in our reconstructions, allowing us to examine phylogenetic patterns of edaphic endemism in this *Eriogonum* lineage. The overall topology and species relationships were not fully resolved in either of our combined chloroplast or *ITS*/*LEAFY* phylogenies (Figures 2 and 3). Many of the nodes at or near the base of the trees are not supported either in the BI or ML analyses, and branch lengths are short. There are, however, numerous well-supported clades throughout the topology in both phylogenies presented. Our combined *ITS*/*LEAFY* phylogeny (Figure 3) shows unresolved basal positions of species from the northwestern Great Basin (*E. crosbyae, E. prociduum, E. cusickii,* and *E. calcareum*). Many of these same accessions are included in clade B of the plastid tree (Figure 2), with *E. cusickii* (BRG400) and *E. calcareum* (BRG443) being

the notable exceptions. Many more of the species pairs included in this study are monophyletic in the *ITS*/*LEAFY* tree, when compared to the chloroplast tree, although see the Results section for a discussion on duplicate sequence pruning from our dataset (Appendix 2). Species that are not monophyletic in our combined *ITS*/*LEAFY* tree are *E. rosense, E. anemophilum, E. ochrocephalum, E. kingii, E. brevicaule* and *E. crosbyae.* The following species, *Eriogonum tiehmii, E. argophyllum, E. alexanderae, E. breedlovei, E. soliceps, E. cusickii,* and *E. novonudum,* are only represented by one accession in our reduced dataset so no indication of monophyly can be made. Many of the cases of species non-monophyly in our plastid phylogeny can likely be attributed to incomplete lineage sorting or recent introgression, (see Chapter 2 Discussion).

Based on the results of our comparison of genetic and geographic distance, we see significant correlations in both of our DNA sources, plastid and nuclear (Figures 2 and 3). As expected, species that occur near each other are more genetically similar. The highly significant p-values calculated from the Mantel Test ($p<0.0001$ for the nuclear and $p<0.0007$ for the plastid comparisons) are likely influenced by the close proximity of collections for the narrowly endemic species. For example, *E. soredium* is limited in range to no more than a few hectares, thus our two collections were only separated by 0.3 km, the smallest distance in our geographic distance matrix. Similarly, our two accessions each of *E. holmgrenii* and *E. gracilipes*, were both collected within 20 km of each other (Figure 1). In spite of these examples of narrowly distributed species, it is clear that there is a strong

connection between geographic proximity and genetic relatedness in this group of *Eriogonum* species.

Eriogonum species as edaphic generalists—Physical and chemical properties measured in this study varied widely for the soils analyzed (Appendix 3, Figure 4), which may indicate a diverse set of edaphic conditions associated with this lineage of *Eriogonum* species. The lone soil sample included in EG 1 is from a barren outcrop in northeastern Utah. High concentrations of calcium (16,290 ppm) and sulfate (199 ppm) were measured from this soil sample, indicative of a soil composed of high levels of gypsum ($CaSO_4 \cdot 2H_2O$). For comparison, a soil sample measured from a site in southern New Mexico, associated with a population of *E. gypsophilum* growing on gypsum outcrops contained similar, although higher concentrations for these properties. We obtained values of 26,485 ppm Ca and 369 ppm S from this gypsum outcrop (data not included in Appendix 3). Based on these comparisons from soil sampled at a known gypsum outcrop associated with *E. gypsophilum*, the *E. shockleyi 128* site in northeastern Utah contains high levels of gypsum in the substrate. The Ca:Mg is also the highest calculated for any of the soil samples included in our analysis (165:1). Based on chemical properties measured here, this is an extreme substrate however a wide-ranging species, *E. shockleyi,* appears to tolerate these conditions.

The soil samples included in EG 2 are associated with *Eriogonum* collections from seven different states. The soil texture tends to be more coarse than others measured and pH values range from neutral to alkaline (Appendix 3). This EG, highlighted in blue in Figures 7 and 8, is the edaphic group for both outgroups in

each molecular analysis. Members of clades A and C in the plastid EG reconstruction mostly occur on substrates clustered in EG 2 (Figure 7). Relationships in this clade could be driven more by geographic proximity, rather than shared evolutionary preference of substrate conditions. The phylogenetic associations of EG 2 (blue) with the *ITS*/*LEAFY* phylogeny appears to be unstructured when examined visually (Figure 8), however short branches and low nodes support values along the backbone of this tree create substantial uncertainty in the ML reconstructions of EG preference in the history of this group of *Eriogonum* species. An additional source of uncertainty in the ML probabilities may come from the long terminal branches leading to some of the tips, thus allowing more chance for the character in question to change states in the divergent lineage. There is a clade composed of three narrowly endemic species (*E. tiehmii, E. soredium,* and *E. holmgrenii*) that does show some EG similarity (Figure 8). This may point to a lineage that is preferentially adapted to specific substrates, however additional sampling and testing is necessary to confirm this specific hypothesis.

The edaphic group which contains soil samples with the highest pH levels (more alkaline) and fine-grained textures tend to make up EG 3 (red). The soil samples collected from the *E. calcareum* populations belong to this group, and as its name implies, this EG includes soil samples with the highest measured concentrations of Ca cations (excepting *E. shockleyi 128*), ranging from 5994-7677 ppm. In both the chloroplast and *ITS*/*LEAFY* trees, accessions growing on EG 3-type soil are spread throughout the trees, indicating no phylogenetic pattern to species occurring on fine-grained calcareous substrates (Figures 7 and 8). Geographically,

these populations tend to occur in northern and western parts of the Great Basin and into the Northern Rockies.

The final edaphic group, EG 4 (green), corresponds to coarse-grained soils, collected from across the Great Basin and north into Montana. Three edaphic endemic species occur on substrates belonging to this edaphic group, *E. argophyllum, E. diatomaceum,* and *E. gracilipes.* Both samples of *E. gracilipes,* and collections of *E. rosense* and *E. ochrocephalum* form a well-supported clade (PP 0.96 and 60 BS) in the *ITS*/*LEAFY* tree. They all occur on substrates that are classified in this EG (Figure 8). This could be a lineage with an adaptation to a specific substrate type. Conversely, geographic proximity may provide the explanation, as all of these populations are found within 300 km of each other. The plastid phylogeny shows much more structure in relation to sample associated with the EG 4-type soil. Clade B is predominately composed of species occurring on this type of substrate, with 14 of the 22 accessions belonging to the EG 4 group (Figure 7).

Eriogonum shockleyi, a widespread species discussed at length in Chapter 2, occurs on three different EG's in this study. Three accessions are included in our reduced sampling and examination of patterns of soil endemism. From the data presented here and many field observations, *E. shockleyi* can tolerate vastly different edaphic conditions, from the most extreme gypsum outcrops (BRG128) to coarse-textured soils with high measured pH (BRG151). This species may fit the pattern of an edaphic generalist. Across its range, it can be found in a variety of different ecological settings, although most populations occur in very open sites with little vegetation cover (personal observation). Other widespread species in this study

show similar patterns, namely *E. mancum, E. crosbyae,* and *E. pauciflorum* (Figures 7 and 8). Conversely, *E. anemophilum* is a relatively restricted species that occurs in isolated areas in northern Nevada. Collections of *E. anemophilum* included here were made from areas belonging to different EG's (Figures 7 and 8).

Many of the edaphic endemic species discussed above are narrowly distributed across the landscape. Some of the strictest endemics, *E. soredium, E. argophyllum,* and *E. tiehmii,* are so limited in distribution that they seemingly only occur on the same substarte and do not have patchy distributions (personal observation). These populations are exceedingly small, with each having distributions smaller than 10 hectares, and estimated populations of fewer than 21,000 individuals (fewer than 5,000 in *E. argophyllum*) (Morefield 1992; 1995). In our analysis presented here, *E. soredium* is represented by two samples (BRG375 and BRG565), and because of rarity, *E. argophyllum* and *E. tiehmii* are represented by only one sample each (BRG438 and BRG193, respectively). All three taxa are morphologically distinct, geographically isolated, and ecologically specialized. Genetically, *E. soredium* shows reciprocal monophyly for all nuclear regions analyzed, although sampling of additional accessions or loci could prove otherwise. The two accessions possess different chloroplast haplotypes, however incomplete lineage sorting in recently diverged species is likely the cause (Chapter 2). No implications of monophyly can be made for the other two species mentioned above because of the inclusion of only a single accession. These three species, as well as others in the 'capitate group' of *Eriogonum* warrant more in-depth, population-level studies to examine genetic structure and ecological specialization. However with

our current knowledge they each fit the definition of a strict edaphic endemic species.

Overall patterns observed in our phylogenies of this group in *Eriogonum*, and subsequent soil comparisons, indicate little evolutionary structure to edaphic groups, assuming the soil properties we measured accurately characterize each substrate. ML reconstructions of pH and EG's show much uncertainty at deeper nodes in the chloroplast and the *ITS*/*LEAFY* phylogenies (Figures 5-8). Higher probabilities for certain EG's associated with species are evident in shallower nodes (Figures 7 and 8). It is likely that phylogenetic reconstructions and subsequent conclusions regarding well-supported nodes may be hampered by lack of sequence divergence in this diverse clade of *Eriogonum.* However, for a group such as *Eriogonum*, the phylogenetic structure and support, associated with our inferences is reasonable. We propose the generalist model regarding edaphic tolerance in this clade of *Eriogonum* species, with more recently diverged species moving towards edaphic specialization. Evidence includes little phylogenetic clustering of EG's in the overall topology with equivalent probabilities at many nodes, widespread (*E. shockleyi* and *E. mancum*) and narrowly distributed species (*E. anemophilum*) occurring on substrates belonging to different EG's, and the likelihood of a rapid radiation of these species, coupled with extended generation times of these long-lived perennials, leading to a lower chance of genetic adaptation to specific substrates.

Anacker, B. L., J. B. Whitall, E. E. Goldberg, and S. P. Harrison. 2010. Origins and consequences of serpentine endemism in the California flora. *Evolution* 65: 365-376.

Bray, R. H. and L. T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soil. *Soil Science* 59: 39-45.

Cecchi, L. and F. Selvi. 2009. Phylogenetic relationships of the monotypic genera *Halacsya* and *Paramoltkia* and the origins of serpentine adaptation in circum-Mediterranean Lithospermae (Boraginaceae): Insights from *ITS* and *matK* DNA sequences. *Taxon* 58: 700-714.

Cecchi, L., A. Coppi, and F. Selvi. 2011. Evolutionary dynamics of serpentine adaptation in *Onosma* (Boraginaceae) as revealed by *ITS* sequences. *Plant Systematics and Evolution* 297: 185-199.

Combs, S. M., J. L. Denning, and K. D. Frank. 1998. Sulfate-sulfur In: *Recommended Chemical Soil Test Procedures for the North Central Region* (revised). Ed. J. R. Brown. Missouri Agricultural Experimental Station SB1001. Columbia, MO. Pp. 35-40.

Douglas, N. A. and P. S. Manos. 2007. Molecular phylogeny of Nyctaginaceae: Taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America. *American Journal of Botany* 94: 865-872.

Edwards, C. E., D. Lefkowitz, D. E. Soltis, and P. S. Soltis. 2008. Phylogeny of *Conradina* and related southeastern scrub mints (Lamiaceae) based on *GapC* gene sequences. *International Journal of Plant Sciences* 169: 579-594.

Ersts, P. J. Geographic Distance Matrix Generator (version 1.2.3). American Museum of Natural History, Center for Biodiversity and Conservation. Available from http://biodiversityinformatics.amnh.org/open_source/gdmg. [Accessed on June 2012].

Frank, K., D. Beegle, and J. Denning. 1998. Phosphorus. In: *Recommended Chemical Soil Test Procedures for the North Central Region* (revised). Ed. J. R. Brown. Missouri Agricultural Experimental Station SB1001. Columbia, MO. Pp. 21-26.

Frohlich, M. W. and E. M. Meyerowitz. 1997. The search for homeotic gene homologs in basal angiosperms and Gnetales: a potential new source of data on the evolutionary origin of flowers. *International Journal of Plants Sciences* 158: S131-S142.

Gankin, R. and J. Major. 1964. *Arctostaphylos myrtifolia,* its biology and relationship to the problem of endemism. *Ecology* 45: 792-808.

Gee, G. W. and D. Or. 2002. Particle-size analysis. In Dane J. H. and Topp G. C. (ed.) Methods of Soil Analysis part 4. SSSA Book Series no. 5. Madison, WI.

Givnish, T. J. 2010. Ecology of plant speciation. *Taxon* 59: 1326-1366.

Grady, B. R. and J. L. Reveal. 2011. New combinations and a new species of *Eriogonum* (Polygonaceae: Eriogonoideae) from the Great Basin Desert, United States. *Phytotaxa* 24: 33-38.

Hickman, J. C. (ed.). 1993. The Jepson Manual: Higher Plants of California. University of California Press, Berkeley, CA.

Huelsenbeck J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754-755.

Jenny, H. 1941. Factors of Soil Formation, a System of Quantatative Pedology: McGraw Hill, New York.

Jenny, H. 1980. The Soil Resource: Origin and Behavior. Springer-Verlag New York, USA pp. 56-67.

Kruckeberg, A. R. 1967. Ecotypic response to ultramafic soils by some plant species of northwestern United States. *Brittonia* 19:133-151.

Kruckeberg, A. R. 1984. California Serpentines: Flora, Vegetation, Geology, Soils, and Management Problems. University of California Publications in Botany, vol. 78. Berkeley: University of California Press.

Kruckeberg, A. R. 1986. An essay: the stimulus of unusual geologies for plant speciation. *Systematic Botany* 11: 455-463.

Kruckeberg, A. R. 2002. Geology and plant life: the effects of landforms and rock types on plants. University of Washington Press, Seattle, Washington. pp. 117-155.

Macnair, M. R. and M. Gardner. 1998. The evolution of edaphic endemics, in Endless Forms: Species and Speciation, eds. D. J. Howard and S. H. Berlocher Oxford University Press, New York. Pp. 157-171

Maddison, D. R. and W. P. Maddison. 2005. MacClade: analysis of phylogeny and character evolution, version 4.08. Sunderland: Sinauer Associates.

Mantel, N. 1967. Detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209.

Mason, H. L. 1946. The edaphic factor in narrow endemism. I. The nature of environmental influences. *Madroño* 8: 209-226.

Mayer, M. S. and P. S. Soltis. 1994. The evolution of serpentine endemics – a chloroplast DNA phylogeny of the *Streptanthus-glandulosus* complex (Cruciferae). *Systematic Botany* 19: 557-574.

Mayer M. S., and P. S. Soltis. 1999. Intraspecific phylogeny analysis using ITS sequences: insights from studies of the *Streptanthus glandulosus* complex (Cruciferae). *Systematic Botany* 24: 47-61.

McCune, B., and M. J. Mefford. 2011. PC-ORD. Multivariate analysis of ecological data. Version 6. MjM Software, Gleneden Beach, Oregon, U.S.A.

Meyer, S. E. 1986. The ecology of gypsophile endemism in the eastern Mojave Desert. *Ecology* 67: 1303-1313.

Meyer, S. E. and A. Paulsen. 2000. Chilling requirements for seed germination of 10 Utah species of perennial wild buckwheat (*Eriogonum* Michx. [Polygonaceae]). *Native Plants Journal* 1: 18-24.

Miller, M.A., Pfeiffer, W., and Schwartz, T. 2010 "Creating the CIPRES Science Gateway for inference of large phylogenetic trees" in Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA pp 1 - 8.

Misra, A. and G. Tyler. 1999. Influence of soil moisture on soil solution chemistry and concentrations of minerals in the calcicoles *Phleum phleoides and Veronica spicata* grown on a limestone soil. *Annals of Botany* 84: 401-499

Moore, M. J. and R. K. Jansen. 2007. Origins and biogeography of gypsophily in the Chihuahuan Desert plant group *Tiquilia* subg. *Eddya* (Boraginaceae). *Systematic Botany* 32: 392-414.

Morefield, J. D. 1992. Unpublished status report: *Eriogonum argophyllum*. U. S. Fish and Wildlife Service, Great Basin Field Office, Reno, NV.

Morefield, J. D. 1995. Current knowledge and conservation status of *Eriogonum tiehmii* Reveal (Polygonaceae), Tiehm buckwheat. U. S. Fish and Wildlife Service, Carson City, Nevada

Nylander, J. A. A. 2004. MrModelTest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612-622.

Pagel, M. and A. Meade. 2007. BayesTraits, version 1.0. Computer program and documentation distributed by the author, website: http:// www.evolution.rdg.ac.uk [accessed June 2010 and after].

Palacio S., A. Escudero, G. Montserrat-Marti, M. Maestro, R. Milla, M. J. Albert. 2007. Plants living on gypsum: beyond the specialist model. *Annals of Botany* 99: 333-343.

Patterson, T. B. and T. J. Givnish. 2003. Geographic cohesion, chromosomal evolution, parallel adaptive radiations, and consequent floral adaptations in *Calochortus* (Calochortaceae): evidence from a cpDNA phylogeny. *New Phytologist* 161: 253-264.

Pepper, A. E. and L. E. Norwood. 2001. Evolution of *Caulanthus amplexicaulis* var. *barbarae* (Brassicaceae), a rare serpentine endemic plant: a molecular phylogenetic approach. *American Journal of Botany* 88: 1479-1489.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253-1256.

Rajakaruna, N. 2004. The Edaphic Factor in the Origin of Plant Species. *International Geology Review* 46: 471-478.

Raven, P. H. 1964. Catastrophic selection and edaphic endemism. *Evolution* 18:336-338.

Reveal, J. L. 1969. The subgeneric concept in *Eriogonum* (Polygonaceae) Pp. 229-249 in *Current Topics in Plant Science.* ed. J. Gunckel. New York: Academic Press.

Reveal, J. L. 1978. Distribution and Phylogeny of Eriogonoideae (Polygonaceae). *Great Basin Naturalist Memoirs* 2: 169-190.

Reveal, J. L. 2005. Polygonaceae subfam. Eriogonoideae. Pp. 218-478 in *Flora of North America* Vol. 5 ed. Flora of North America Editorial Committee. New York: Oxford University Press.

Reveal, J. L., J. Reynolds, and J. Picciani. 2002. *Eriogonum diatomaceum* (Polygonaceae: Eriogonoideae), a new species from western Nevada, U.S.A. *Novon* 12: 87-89.

Safford, H. D., J. H. Viers, and S. P. Harrison. 2005. Serpentine endemism in the California Flora: a database of serpentine affinity. *Madroño* 52: 222-257.

Sanchez A. and K. A. Kron. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. *Systematic Botany* 33: 87-96.

Shaw J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. S. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, R. L. Small. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142-166.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/. Accessed [October 2007 and after].

Stamatikis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758-771.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), ver. 4.0b10. Sunderland: Sinauer Associates.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105-1109.

Thomas, G. W. 1982. Exchangeable cations. In: *Methods of Soil Analysis, part 2. 2nd edition*. Eds. Page, A. L. et al. Agronomy Monographs 9. ASA and SSSA, Madison, WI.

United States Fish and Wildlife Service, Department of the Interior. Endangered Species Reports. Available online at http://ecos.fws.gov/tess_public/SpeciesReport.do. [Accessed March 2007 and after].

Warncke, D. and J. R. Brown. 1982. Potassium and other basic cations. In: *Recommended Chemical Soil Test Procedures for the North Central Region* (revised) ed. J. R. Brown. Missouri Agricultural Experimental Station Columbia, MO. Pp. 31-33.

Watson, M. E. and J. R. Brown. 1998. pH and lime requirement. In: *Recommended Chemical Soil Test Procedures for the North Central Region* (revised). Ed. J. R. Brown. Missouri Agricultural Experimental Station SB1001. Columbia, MO. Pp. 13-16.

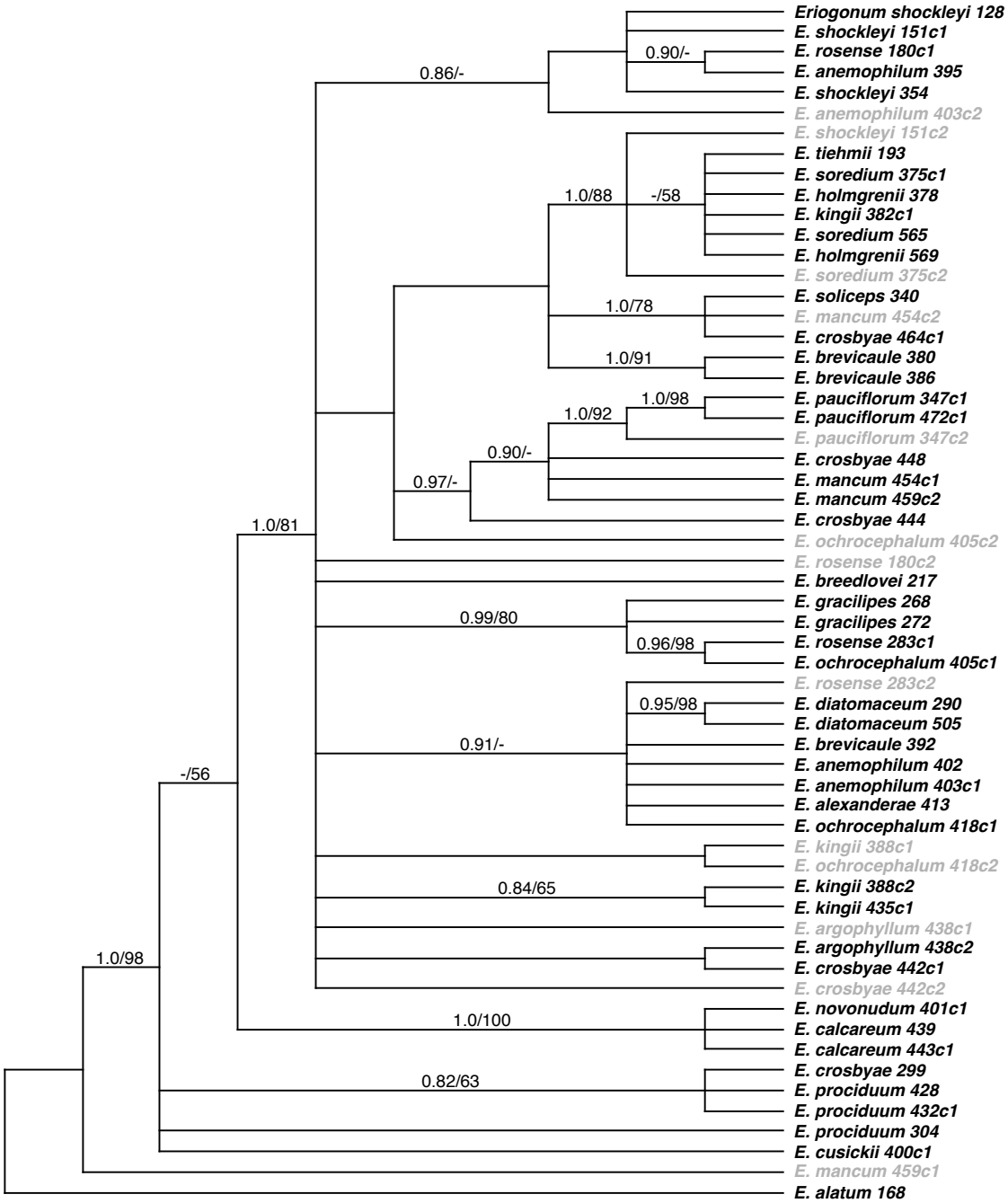
White T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pg: 315-322 in

PCR protocols: A guide to methods and application, eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. New York: Academic Press.

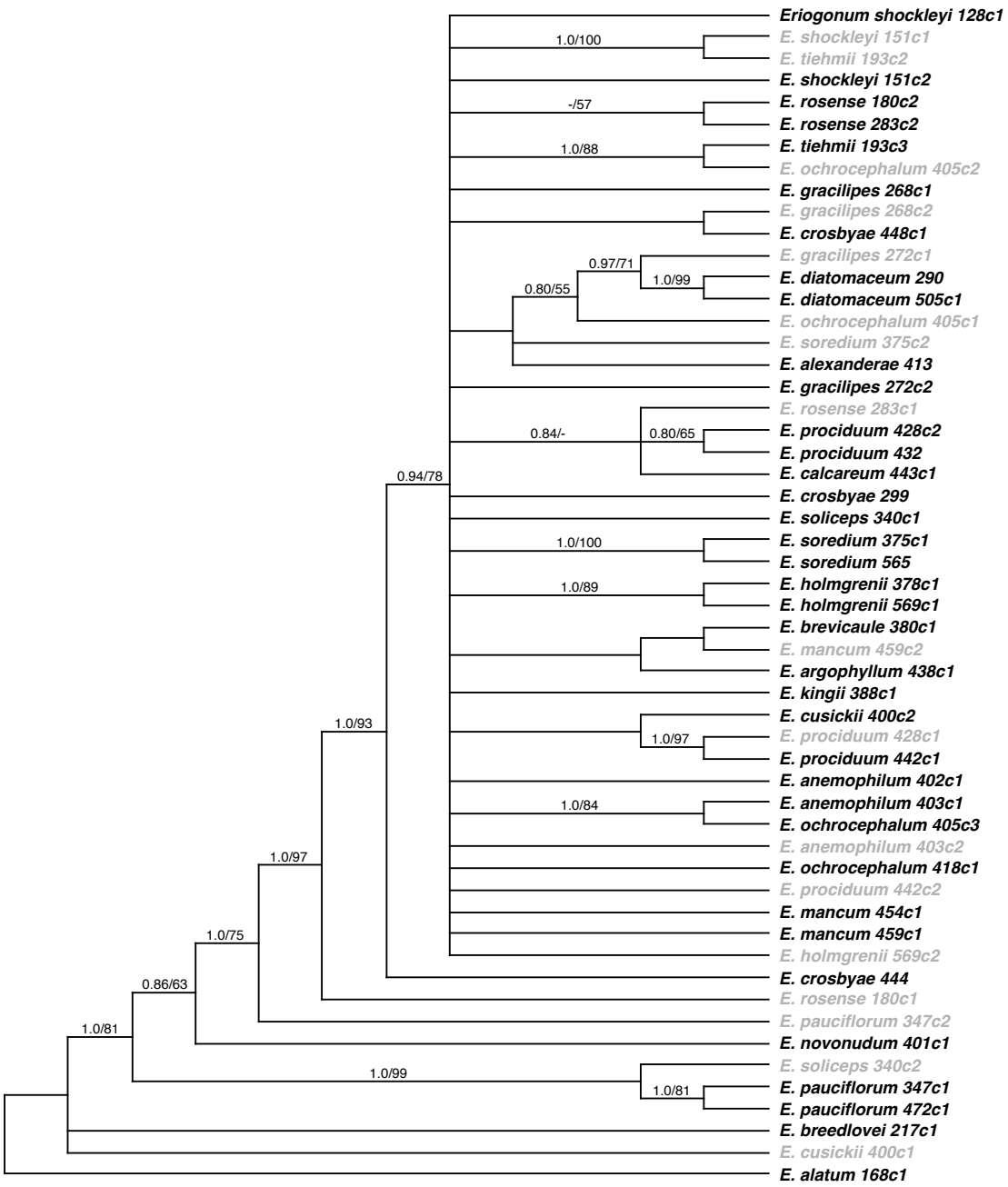
Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.

Voucher and locality information for the accessions of *Eriogonum* included in the phylogenetic and edaphic analyses. A number 1 indicates inclusion of the gene region and a 0 indicates the gene region is not used for that sample.

Coll. #	Genus	species	Latitude	Longitude	Elevation (m)	State	County	trnLF	trnDT	rpS16	ITS	LFY
128	*Eriogonum*	*shockleyi*	40°19.778'N	109°29.166'W	1522	Utah	Uintah	1	1	1	1	1
142	*Eriogonum*	*bicolor*	38°14.557'N	111°06.689'W	1583	Utah	Wayne	0	0	0	1	0
151	*Eriogonum*	*shockleyi*	38°26.627'N	113°16.300'W	1911	Utah	Beaver	1	1	1	1	1
168	*Eriogonum*	*alatum*	35°20.682'N	112°57.626'W	1602	Arizona	Yavapai	1	1	1	0	0
180	*Eriogonum*	*rosense*	38°07.392'N	116°54.334'W	1937	Nevada	Nye	1	1	1	1	1
193	*Eriogonum*	*tiehmii*	37°49.032'N	117°51.391'W	1825	Nevada	Esmerelda	1	1	1	1	1
217	*Eriogonum*	*breedlovei*	35°27.950'N	118°23.433'W	2514	California	Kern	1	1	1	1	1
268	*Eriogonum*	*gracilipes*	37°22.980'N	118°10.888'W	3068	California	Inyo	1	1	1	1	1
272	*Eriogonum*	*gracilipes*	37°33.065'N	118°13.464'W	3570	California	Mono	1	1	1	1	1
283	*Eriogonum*	*rosense*	39°20.590'N	119°54.979'W	3278	Nevada	Washoe	1	1	1	1	1
290	*Eriogonum*	*diatomaceum*	39°13.891'N	119°16.881'W	1311	Nevada	Lyon	1	1	1	1	1
299	*Eriogonum*	*crosbyae*	41°14.687'N	119°28.354'W	1634	Nevada	Washoe	1	1	1	1	1
304	*Eriogonum*	*prociduum*	41°05.141'N	120°44.307'W	1551	California	Lassen	1	1	1	1	0
340	*Eriogonum*	*soliceps*	44°47.656'N	113°14.815'W	2145	Idaho	Lemhi	1	1	1	1	1
347	*Eriogonum*	*pauciflorum*	45°02.675'N	108°25.035'W	1451	Montana	Carbon	1	1	1	1	1
354	*Eriogonum*	*shockleyi*	40°43.317'N	108°45.895'W	1770	Colorado	Moffatt	1	1	1	1	0
375	*Eriogonum*	*soredium*	38°26.979'N	113°16.960'W	2207	Utah	Beaver	1	1	1	1	1
378	*Eriogonum*	*holmgrenii*	38°53.587'N	114°19.601'W	2960	Nevada	White Pine	1	1	1	1	1
380	*Eriogonum*	*brevicaule*	41°04.010'N	115°01.189'W	2039	Nevada	Elko	1	1	1	1	1
382	*Eriogonum*	*kingii*	41°01.584'N	115°04.393'W	2521	Nevada	Elko	1	1	1	1	0
386	*Eriogonum*	*brevicaule*	41°05.719'N	114°20.763'W	1800	Nevada	Elko	1	1	1	1	0
388	*Eriogonum*	*kingii*	41°41.872'N	114°44.483'W	2472	Nevada	Elko	1	1	1	1	1
392	*Eriogonum*	*brevicaule*	40°43.464'N	116°08.831'W	1553	Nevada	Elko	1	1	1	1	0
395	*Eriogonum*	*anemophilum*	39°53.193'N	117°06.766'W	1639	Nevada	Lander	1	1	1	1	0
400	*Eriogonum*	*cusickii*	43°32.169'N	119°22.534'W	1356	Oregon	Harney	1	1	1	1	1
401	*Eriogonum*	*novonudum*	42°48.352'N	117°43.907'W	1084	Oregon	Malhuer	1	1	1	1	1
402	*Eriogonum*	*anemophilum*	40°25.006'N	118°08.626'W	2045	Nevada	Pershing	1	1	1	1	1
403	*Eriogonum*	*crosbyae*	40°16.452'N	118°44.796'W	1706	Nevada	Pershing	1	1	1	1	1
405	*Eriogonum*	*ochrocephalum*	39°35.415'N	119°53.211'W	1911	Nevada	Washoe	1	1	1	1	1
413	*Eriogonum*	*alexanderae*	38°48.428'N	119°13.308'W	1427	Nevada	Lyon	1	1	1	1	1
418	*Eriogonum*	*ochrocephalum*	39°18.402'N	119°34.292'W	1431	Nevada	Storey	1	1	1	1	1
428	*Eriogonum*	*prociduum*	41°18.943'N	119°53.590'W	2174	Nevada	Washoe	1	1	1	1	1
432	*Eriogonum*	*prociduum*	41°35.174'N	120°25.973'W	1382	California	Modoc	1	1	1	1	1
435	*Eriogonum*	*kingii*	40°35.782'N	115°23.708'W	3095	Nevada	Elko	1	1	1	1	0
438	*Eriogonum*	*argophyllum*	40°35.196'N	115°17.075'W	1841	Nevada	Elko	1	1	1	1	1
439	*Eriogonum*	*calcareum*	42°46.839'N	115°54.803'W	857	Idaho	Owyhee	1	1	1	1	0
442	*Eriogonum*	*prociduum*	42°44.367'N	116°54.767'W	2371	Idaho	Owyhee	1	1	1	1	1
443	*Eriogonum*	*calcareum*	43°18.129'N	116°34.817'W	707	Idaho	Owyhee	1	1	1	1	1
444	*Eriogonum*	*mancum*	44°06.644'N	114°51.671'W	2000	Idaho	Custer	1	1	1	1	1
448	*Eriogonum*	*mancum*	44°16.850'N	114°09.753'W	2096	Idaho	Custer	1	1	1	1	1
454	*Eriogonum*	*mancum*	43°49.619'N	113°35.937'W	1937	Idaho	Custer	1	1	1	1	1
459	*Eriogonum*	*mancum*	44°28.266'N	113°14.707'W	2128	Idaho	Lemhi	1	1	1	1	1
464	*Eriogonum*	*mancum*	46°30.702'N	114°14.637'W	2858	Montana	Ravali	1	1	1	1	0
472	*Eriogonum*	*pauciflorum*	44°34.652'N	104°41.786'W	1251	Wyoming	Crook	1	1	1	1	1
505	*Eriogonum*	*diatomaceum*	39°13.946'N	119°18.062'W	1368	Nevada	Lyon	1	1	1	1	1
565	*Eriogonum*	*soredium*	38°27.137'N	113°17.021'W	2115	Utah	Beaver	1	1	1	1	1
569	*Eriogonum*	*holmgrenii*	38°54.637'N	114°18.679'W	3460	Nevada	White Pine	1	1	1	1	1



Appendix 2 Figure 1. Consensus tree from Bayesian analysis of the *nrITS* of *Eriogonum* subg. *Eucycla II* sampling for this study. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Accession labels in light gray were discarded for the combined *ITS*/*LEAFY* analysis.



Appendix 2 Figure 2. Consensus tree from Bayesian analysis of *LEAFY* for the *Eriogonum* subg. *Eucycla II* sampling for this study. Bayesian posterior probabilities (PP) and RAxML bootstrap support values are shown above branches. Only Bayesian PP above 0.80 and ML bootstrap support values above 50 are included. Accession labels in light gray were discarded for the combined *ITS*/*LEAFY* analysis.

Measured soil properties for all included samples in this study. We included 11 properties in the multivariate cluster analysis. They are pH, P, K, Ca, Mg, S, bulk density, NA, % clay, % silt, and % sand. O.M% (soil organic matter %) was not included. The final column is the Edaphic Group (EG) placement for each sample based on the multivariate clustering analysis.

coll. #	sample	soil pH	O.M. %	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Ca:Mg	S (ppm)	density	Na (ppm)	%clay	%silt	%sand	texture	EG
128	esho128	8.0	0.4	1	70	16290	99	165	199	1.4	7	18.3	9.2	72.5	sandy loam	1
142	ebic142	8.2	0.6	1	147	4956	93	53	107	1.4	30	35.0	17.3	47.6	sandy clay	2
168	eala168	8.0	1.1	1	61	4304	92	47	4	1.2	7	8.1	20.9	71.0	sandy loam	2
180	eros180	7.0	1.9	27	326	4162	407	10	7	1.2	23	37.9	19.0	43.2	clay loam	2
193	etie193	9.2	1.4	1	217	3994	161	25	37	1.1	1144	7.6	36.3	56.1	sandy loam	2
217	ebre217	8.0	2.8	21	61	4017	28	143	2	1.4	10	6.1	8.1	85.9	loamy sand	2
354	esho354	8.5	1.1	1	153	4089	205	20	3	1.2	16	6.4	11.8	81.8	loamy sand	2
375	esor375	8.8	1.2	1	61	3718	542	7	3	1.4	11	7.8	13.7	78.5	loamy sand	2
378	ehol378	8.1	6.7	11	82	4961	80	62	1	0.8	9	21.1	48.4	30.5	loam	2
380	ebrv380	8.3	1.6	2	224	5189	106	49	2	1.4	43	8.0	15.9	76.1	sandy loam	2
386	ebrv386	8.8	2.1	1	1084	4514	466	10	285	1.0	285	24.2	49.7	26.2	loam	2
392	ebrv392	9.0	1.8	5	174	5099	226	23	51	0.8	481	12.6	16.7	70.7	sandy loam	2
395	eane395	9.1	1.4	3	976	4752	307	15	2	0.8	3587	38.5	16.2	45.3	sandy clay	2
402	eane402	8.7	2.0	14	285	5355	796	7	60	1.2	51	18.1	52.2	29.6	silt loam	2
435	ekin435	8.3	1.9	21	89	3723	46	81	14	1.5	8	6.0	13.2	80.8	loamy sand	2
448	ecrs448	8.5	1.1	1	132	4709	987	5	6	1.5	16	16.3	16.6	67.1	sandy loam	2
472	epau472	8.5	0.7	1	121	4270	386	11	49	1.4	10	16.1	44.5	39.5	loam	2
565	esor565	9.0	n/a	3	61	3718	550	7	4	1.3	11	8.3	14.5	77.3	loamy sand	2
569	ehol569	8.0	n/a	10	79	5002	83	60	2	0.9	11	19.1	50.4	30.5	loam	2
151	esho151	8.6	1.5	1	275	6548	573	11	3	1.4	15	16.1	20.3	63.5	sandy loam	3
340	esol340	8.3	3.7	18	686	6769	202	34	3	1.1	16	19.5	29.2	51.3	loam	3
347	epau347	8.5	1.3	1	195	6078	1030	6	129	1.1	51	59.8	33.0	7.3	clay	3
403	eane403	7.7	1.3	12	201	6455	284	23	3	1.1	181	48.5	22.3	29.2	clay	3
413	eale413	9.0	1.7	1	143	6972	166	42	7	1.2	911	29.0	19.0	52.0	sandy clay loam	3
439	ecal439	6.3	3.3	49	326	7213	118	61	254	0.9	62	31.3	36.7	32.0	clay loam	3
442	ecrs442	8.3	3.5	28	350	5994	94	64	2	1.5	35	10.2	28.4	61.5	sandy loam	3
443	ecal443	7.8	2.5	7	330	7677	679	11	294	0.8	79	32.9	49.0	18.1	salty clay loam	3
459	eman459	8.3	3.4	6	247	6892	500	14	2	0.9	17	6.1	28.7	65.2	sandy loam	3
268	egra268	8.0	2.7	21	199	1814	375	5	35	1.2	8	10.1	38.5	51.4	loam	4
272	egra272	8.1	n/a	21	173	1789	370	5	34	1.2	7	9.3	35.6	55.1	loam	4
283	eros283	6.9	1.2	13	127	1727	721	2	4	1.5	30	10.1	14.3	75.6	sandy loam	4
290	edia290	6.1	2.1	44	282	2232	658	3	195	0.7	660	35.5	20.9	43.6	clay loam	4
299	ecrs299	6.3	2.8	5	469	1804	1644	1	412	0.5	2205	48.8	46.1	5.1	silty clay	4
304	epro304	7.1	2.5	18	210	1801	331	5	3	1.1	27	8.0	29.8	62.2	sandy loam	4
382	ekin382	6.7	2.8	12	227	1782	284	6	3	1.1	16	21.1	23.3	55.6	sandy clay loam	4
388	ekin388	7.2	2.4	19	172	1915	248	8	1	1.4	14	8.1	19.1	72.8	sandy loam	4
400	ecus400	7.6	2.6	39	890	2295	539	4	3	1.4	241	10.0	17.6	72.5	sandy loam	4
401	enov401	9.1	0.8	2	443	2355	271	9	6	1.0	598	20.1	21.8	58.2	sandy clay loam	4
405	eoch405	5.3	2.9	52	135	790	246	3	6	1.2	14	21.9	34.9	43.2	loam	4
418	eoch418	5.2	1.2	5	104	916	233	4	13	1.0	17	19.6	9.8	70.6	sandy loam	4
428	epro428	7.3	1.2	9	117	2625	725	4	1	1.4	54	13.7	23.5	62.9	sandy loam	4
432	epro432	7.6	2.3	4	45	1945	334	6	2	1.4	54	2.0	21.9	76.0	loamy sand	4
438	earg438	9.5	2.5	6	317	94	14	7	16	0.9	935	6.2	12.3	81.5	loamy sand	4
444	ecrs444	7.1	0.4	22	57	347	46	8	2	1.3	11	1.9	0.8	97.3	sand	4
454	eman454	7.9	1.0	9	75	3147	600	5	1	1.6	31	4.1	38.8	57.1	sandy loam	4
464	ecrs464	6.0	1.0	233	48	208	28	7	2	1.4	6	1.9	1.0	97.1	sand	4
505	edia505	6.4	n/a	40	278	2300	660	4	195	0.8	700	34.5	20.9	44.6	clay loam	4

New combinations and a new species of *Eriogonum* (Polygonaceae: Eriogonoideae) from the Great Basin Desert, United States

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Three new species of *Eriogonum* have been identified from field research and ongoing molecular systematic analysis of selected members of *Eriogonum* subg. *Eucycla*. *Eriogonum alexanderae*, formerly recognized as a variety of *E. ochrocephalum* or subsumed within *E. crosbyae*, warrants species-level designation. *Eriogonum calcareum*, also previously recognized as a variety of *E. ochrocephalum*, shares many affinities with the narrowly distributed *E. novonudum*, however range and morphology do not overlap between these two species. *Eriogonum crosbyae* var. *mystrium* shares a more similar evolutionary history to *E. crosbyae*, rather than *E. prociduum,* thus a new combination is proposed. *Eriogonum domitum* is described as a new species. This isolated taxon from west-central Utah is no longer thought to be a member of *E. mancum*, differing in morphological characters, geographic distribution and molecular information.

Key words: Edaphic endemism, subgenus *Eucycla*, wild buckwheat

While much of the vegetation throughout the Great Basin Desert is relatively homogenous, the rich geological history and heterogenous edaphic substrates create islands of potential evolutionary endemism. *Eriogonum* Michx. (1803) is a prime example of a plant genus that matches this landscape diversity with species diversity. Consisting of over 250 species, *Eriogonum* is one of the most speciose genera found in North America (Reveal 2005). As more and more field and molecular research is conducted, additional revised and previously undescribed species of *Eriogonum* come to light. Here we propose three species, *Eriogonum alexanderae* and *Eriogonum calcareum,* formerly recognized as varieties of *E. ochrocephalum* S. Watson (1880), and *Eriogonum domitum* previously confused with *E. mancum* Rydb. (1917). In addition, a novel combination is proposed. *Eriogonum crosbyae* var. *mystrium* is more closely related to *E. crosbyae* Reveal (1981) than it is to *E. prociduum* Reveal (1972), based on DNA sequence data. Because these taxa are perennial and possess flowers that lack a simple, stipe-like extension of the perianth, they are classified in *Eriogonum* subg. *Eucycla* (Nutt.) Kuntze.

TAXONOMIC TREATMENT

Eriogonum alexanderae **(Reveal) Grady & Reveal,** ***comb. & stat. nov.,*** based on *E. ochrocephalum* S. Watson var. *alexanderae* Reveal, Great Basin Naturalist 45: 276. 1985. TYPE—U.S.A. **Nevada**, *Lyon Co.*: Wilson Canyon, 12.8 mi NE of Smith, Lyon Co., Nevada, 21 Jun 1978, *J. L. Reveal et al. 4737* (holotype: US, isotypes:

ASU, BRY, CAS, COLO, DUKE, F, GH, MEXU, MICH, MIN, MO, MONTU, NY, OKL, RENO, RM, RSA, TEX, US, UTC, WS).

Eriogonum alexanderae, a robust caespitose perennial, is sufficiently different from other capitate members of *Eriogonum* subg. *Eucycla* to be recognized as a distinct species. Initially, this taxon was included in *E. ochrocephalum* as a variety (Reveal 1985). The treatment of the genus for the *Flora of North America* included *E. alexanderae* in the broadly circumscribed *E. crosbyae* as these taxa share similar growth forms, tomentose flowering stems, and similar floral morphologies (Reveal 2005). *Eriogonum alexanderae* differs from both *E. crosbyae* and *E. ochrocephalum* in a number of respects, including morphology, distribution, and genetic identity.

Eriogonum alexanderae occurrences are limited to four western Nevada counties (Douglas, Lyon, Mineral and Washoe) in the southwestern Great Basin Desert. Here it can be found in discrete populations at elevations of 1400 m to 2100 m on clay hills and slopes. The Alexander wild buckwheat flowers from May to July. As such the species occurs to the southeast of the current distribution of *E. ochrocephalum.* While these two entities can be found in the same general geographic region, their ranges do not overlap. The tomentose flowering stem of *E. alexanderae* is the most distinguishing feature when comparing it to *E. ochrocephalum* as the latter has glabrous to slightly glandular flowering stems that are never tomentose. The involucre shape also differs with those of *E. alexanderae* being turbinate, while those of *E. ochrocephalum* are generally campanulate.

Prior to this taxonomic treatment, *Eriogonum alexanderae* was considered to be a variety of the more widespread species, *E. crosbyae* (Reveal 2005). DNA sequence data, both chloroplast and nuclear, indicate that *E. alexanderae* is not as closely related to *E. crosbyae* as previously thought (Grady & Sytsma, unpublished data). Clear morphological distinctions can also be made between the two species. *Eriogonum crosbyae*, as now defined, is a more diminutive plant, with a compact, mat-forming habit. *Eriogonum alexanderae* is a larger, more robust caespitose species. Along these same lines, leaf size varies noticeably with the leaf blades of *E. alexanderae* 5–15 mm wide, whereas the blades of mature leaves of *E. crosbyae* are narrower than 5 mm (Reveal 1981, 1985). The involucre length is another feature that distinguishes *E. alexanderae* (>4 mm) from *E. crosbyae* (<3.5 mm).

Additional specimens examined:—U.S.A. **Nevada**, ***Douglas Co.***: Pine Nut Mtns, Hoye Canyon at extreme SE end of the range, 1.6 rd mi S of Jack Wright Summit on Nevada Hwy 208, on a dirt rd to Walker River, growing with pinyon-juniper on light-colored clay hills, T10N, R23E, Sec. 9, 5250 ft elev., 9 Jun 1998, *Tiehm 12380* (NY, UTC). ***Lyon Co.***: W of Wilson Canyon on the S side of Nevada Hwy 208, 5.9 rd mi E of Smith, associated with *Atriplex, Ephedra,* and *Camissonia,* 38°48′25.7″N, 119°13′18.5″W, 1427 m elev., 26 Jun 2008, *Grady 413* (NY, WIS); along Nevada Hwy 338, 0.6 mile NW of U.S. Forest Service Road 028 (toward Conway Stage Station), 8.2 miles N of the California state line, on clay slopes associated with *Artemisia,* 38°26′58″N, 119°08′12″W, T7N, R25E, Sec. 24, ca. 6000 ft elev., 18 Aug 1992, *Reveal 7292* (MARY); same location, 5 Jun 2007, *Reveal 8845* (MARY); Aldrich Grade along

Nevada Hwy 3C, N of Fletcher Springs, growing with pinyon-juniper on white clay hills, T7N, R27E, Sec. 12, 6300 ft elev., 6 Jun 1981, *Tiehm 6558* (CAS, MO, NY, UTC); 0.5 rd mi NW on Nevada Hwy 22 [now 338] from the rd E along the East Walker River, growing with pinyon pine and *Artemisia* on whitish clay hillsides, T7N, R25E, Sec. 24, 6000 ft elev., 6 Jun 1981 *Tiehm 6527* (CAS, MO, NY, UTC); NE of Pine Nut Mtns, Bull Canyon, 2.7 rd mi SSW of the main pole line rd, growing on brown clayey slopes, T16N R23E, Sec. 28, 5200 ft elev., 21 May 1999, *Tiehm 12805* (NY, UTC). ***Mineral Co.***: Bodie Hills, 1.3 rd mi from Bodie Road on the rd to Aurora, growing with pinyon-juniper on white-colored ash deposits, T5N, R28E, Sec. 6, 6700 ft elev., 12 July 1983, *Tiehm & Lavin 8111* (CAS, NY, UTC); Garfield Hills, 9.1 rd mi S of Lucky Boy Pass Road on Nevada Hwy 359 to Mono Lake, then 4.6 rd mi on a dirt rd, growing on light-colored clay outcrops, T6N, R31E, Sec. 23, 6000 ft elev., 20 Jun 1998, *Tiehm & Nachlinger 12517* (NY, UTC).

Eriogonum calcareum **(S. Stokes) Grady & Reveal, *comb. & stat. nov.***, based on *E. ochrocephalum* S. Watson subsp. *calcareum* S. Stokes, *Eriogonum*: 92. 1936. TYPE—U.S.A. Oregon, Malheur Co.: near Harper Ranch, 13 Jun 1896, *J.B. Leiberg 2254* (lectotype of var. *calcareum*: GH, designated by Reveal in Harvard Pap. Bot. 9: 188. 2004; duplicates of the lectotype: F, K, NY, ORE, P, POM, S, US).

Eriogonum ochrocephalum S. Watson var. *sceptrum* Reveal, Phytologia 66: 252. 1989.—U.S.A. Idaho, Owyhee Co.: 11 mi SW of Bruneau, 7 Jul 1974, *J. L. Reveal 3687*. Holotype: US; isotypes: ARIZ, ASU, BRY, COLO, DAV, GH, IDS, GH, NY, RM, RSA, TEX, UTC.

Eriogonum calcareum occurs on fine-grained substrates scattered across the north-central Great Basin at elevations of 700 to 1000 m in Elmore, Owyhee, Payette, and Twin Falls counties of Idaho, and in Baker and Malheur counties in Oregon. The Harper wild buckwheat flowers from May to June. Recent molecular work (Grady & Sytsma, unpublished data) has shed new light on the closest relatives of *E. calcareum,* confirming that *E. calcareum* is most closely related to the Oregon endemic, *E. novonudum* M. Peck (1945). While it is clear now that these two species are closely related, they do not grow sympatrically. Additionally, the inflorescence-type is sufficient to differentiate these two species, with *E. calcareum* having its involucres in a capitate or subcapitate cluster atop its flowering stem, while the involucres of *E. novonudum* are arranged in a cymose inflorescence.

Eriogonum calcareum, and the related *E. novonudum,* are not closely allied to *E. ochrocephalum*, thus it is no longer included as a variety. The Harper wild buckwheat occurs well to the north of other populations of *E. ochrocephalum*; the closest known populations being separated by a distance of at least 400 km. The longer (3–6 mm) narrowly turbinate involucres of *E. calcareum*, as compared to shorter (3–5 mm) campanulate involucres of *E. ochrocephalum,* distinguish the two.

Representative specimens examined:—U.S.A. **Idaho**, ***Elmore Co.***: 1 mi W of King Hill, T5S R10E, Sec. 12, 2600 ft elev., 12 Jun 1989, *DeBolt 1146* (NY); Glenn's Ferry Formation above Rosevear Gulch S of Glenn's Ferry, on heavy clay hillsides, 43°01′N, 115°15′W, 3000 ft elev., 13 May 1980, *Grimes et al. 1544* (CAS, NY); along U.S. Hwy

30 near Glenn's Ferry, 5 mi W of King Hill at milepost 151.5 on a steep clay road bank above the Snake River, T5S, R10E, Sec. 21, 2600 ft elev., 13 Jul 1975, *Reveal 3898* (BRY, CAS, DUKE, F, GH, MICH, MO, NY, OKL, RSA, TEX, UTC); SE of Payette, 2100 ft elev., 3 Jun 1945, *Ripley & Barneby 6546* (NY). ***Owyhee Co.***: Ca 1.5 mi N of Little Jacks Creek via Vaught Rd, white sandy-clay lacustrine soils, 42°49′N, 116°00′W, 6 Jun 1998, *Atwood 23785* (BRY, MO, NY); 12 km NW of Murphy along Idaho Hwy 78, T1S, R2W, Sec. 28, 785 m, 25 Jun 1991, *DeBolt 1518* (NY); clay hills along Idaho Hwy 51, 1.9 rd mi S of Shoofly Rd & 8.2 rd mi S of Idaho Hwy 78, 9 air mi SSW of Bruneau, on heavy clay hills and flats, 42°46′50.3″N, 115°54′48.1″W, 857 m, 9 Jul 2008, *Grady 439* (NY, WIS); near ORV trail S of Idaho Hwy 78, 6.8 rd mi NW of Murphy, on white silty tuffaceous badlands, 43°18′07.7″N, 116°34′49.0″W, 707 m, 9 Jul 2008, *Grady 443* (NY, WIS); 10 mi S of Bruneau, 29 May 1946, *Maguire & Holmgren 26236* (BRY, CAS, DS, NY, UTC); Hart Creek Canyon W of Oreana, 43°01′00″N, 116°29′00″W, 3200 ft elev., 8 Aug 1978, *Rosentreter 268* (NY); Sugar Valley Badlands W of Bruneau, 42°48′N, 115°47′W, 2600 ft elev., 3 Jun 1983, *Rosentreter 3133* (NY); Little Jack Creek, T7S, R4E, Sec. 19, 3000 ft elev., 16 Jun 1982, *Smithman et al. LS-0844* (NY). ***Twin Falls Co.***: Along Yahoo Creek ca 8 air mi S of Hagerman near Thousand Springs, 42°45′00″N, 114°55′00″W 3200 ft elev., 14 May 1980, *Grimes et al. 1562* (NY); 10 mi N of Twin Falls, 30 May 1946, *Maguire & Holmgren 26239* (BRY, CAS, DS, MO, NY, TEX, UC, UTC). **Oregon**, ***Baker Co.***: 1.5 mi from Durkee along Burnt River Rd, 24 Jul 1976, *Ertter et al.* 2019 (BSU, NY); foothills of Iron Mtn along Burnt River Canyon Rd just N of I-84, ca. 1 air mi NW of Durkee, on steep tan-white powdery-limy hillsides, 44°36′04.2″N, 117°28′25.4″W, 829 m, 9 Jul

2009, *Grady 535* (NY, WIS); Huntington, 25 Jun 1930, *M.E. Jones 25236* (CAS).

Malheur Co.: 3-4 mi N of Harper, 3000 ft elev., 14 Jun 1955, *Cronquist 7831* (CAN, CAS, DS, GH, ILL, KANS, MICH, MIN, MONTU, NY, RM, RSA, TEX, UC, UTC); 6.7 mi N of Harper along the Harper-Westfall Rd, 19 May 1978, *Grimes & Packard 1132* (CIC, NY, UTC); chalky cliffs and hills along Harper/Westphal Rd, 7.1 rd mi N of U.S. Hwy. 20, 43°55′51.4″N, 117°39′52.4″W, 846 m, 4 Jul 2009, *Grady & Flores 523* (NY, WIS); McCarty Ridge E of Jamison, on bare white eroding hillside, 44°14′00″N, 117°23’00″W, 3 Jun 1978, *Packard 781113* (NY); Dry Creek Rd, 43°54′00”N, 117°18′00”W, 6 Jun 1978, *Packard & Grimes 78157* (BRY, CIC, NY, UTC); 11 mi SW of Vale, 19 Jun 1942, *M.E. Peck 21297* (CAS, UC, WILLU); 7.2 mi N of U.S. Hwy 20 n of Harper, 2850 ft elev., 17 Jun 1971, *Reveal 2390* (BRY, CAS, GH, NY, OKL, RENO, RM, RSA, SD, UC, US, UTC).

Eriogonum crosbyae **Reveal var. *mystrium* (Reveal) Grady & Reveal, *comb. nov.***, based on *E. prociduum* Reveal var. *mystrium* Reveal in Phytologia 86: 142. 2004. TYPE— U.S.A., **Oregon**, *Harney Co.*: Little Cottonwood Creek, Pueblo Mtns., 14 mi N of Denio, T39N, R35E, Sec. 31, 4500-5000 ft elev. 14 Jun 1959, *A. Cronquist 8439* (holotype: NY, isotypes: GH, MICH, OSC, RSA, TEX, UTC, WS, WTU).

Eriogonum crosbyae var. *mystrium* is now considered to be more closely allied to *E. crosbyae,* rather than *E. prociduum,* as previously treated (Reveal 2004). The Pueblo Mountains wild buckwheat, a low, matted perennial, can be found in isolated populations across the north-central Great Basin. Most populations occur in extreme

southeastern Oregon (Malheur & Harney Cos.), however additional collections have been made in the Santa Rosa Range, Humboldt Co., Nevada and remote areas of Owyhee Co., Idaho. This variety can be found growing at elevations from 1500 m to 2440 m. It flowers from May to July depending on the elevation at which the plants grow. *Eriogonum crosbyae* var. *mystrium* does not seem to have a specific substrate preference, although it tends to be associated with more coarse-grained (sandy-gravelly) soils than its counterpart, *E. crosbyae* var. *crosbyae*.

Molecular data indicate that this variety is closely allied to *E. crosbyae*, hence the new combination proposed here. The Pueblo Mountains wild buckwheat also shares a suite of characters with *E. crosbyae*. Both varieties share similar compact growth forms and have membranous, campanulate involucres with floccose teeth. They differ with var. *mystrium* having glabrous flowering stems, whereas those of var. *crosbyae* are floccose to tomentose.

Eriogonum prociduum occurs in the same general portion of the Great Basin, however it and *E. crosbyae* var. *mystrium* never co-occur. These two taxa share similar growth forms with var. *mystrium* having more robust features (larger leaves and inflorescences). It is the size and appearance of the flowering stems that best differentiate these taxa, as var. *mystrium* has longer and thicker flowering stems, 0.6–1.1(–1.2) dm × >1 mm, which are generally green in color. The flowering stems of *E. prociduum* have shorter and narrower flowering stems, these being 0.2–0.6(–0.7) dm × <1 mm, and reddish in color.

Representative specimens examined:—U.S.A. **Idaho, *Owyhee Co.*:** near summit of South Mountain near abandoned BLM lookout tower, crest of windswept cobbley-gravelly ridge, soil derived from light-colored volcanic parent material, 42°44′22.1″N, 116°54′46.0″W, T8S, R5W, Sec. 10, 2371 m elev., 9 Jul 2008, *Grady 442* (NY, WIS), ditto, 28 Jun 2005, *Mansfield 5268* (CIC, NY); ditto, 13 Aug 1995, *Moseley 2901* (NY); ditto, 2 Jun 1995, *Reveal 7445* (MARY); ditto, 4 Jul 1992, *Smithman & Smithman LS-2554* (CIC, NY). **Nevada, *Humboldt Co.* :** Santa Rosa Range, SE side of Auto Hill, NW of Buckskin Mtn., T46N, R39E, Sec. 33, 6500 ft elev., 29 May 1987, *Tiehm 11128* (NY); Santa Rosa Range, Auto Hill, 0.25 rd mi NNW of National Mine rd jct. on main ridge rd, 41°49′59″N, 117°34′57″W, 6400 ft (1951 m) elev., 6 Jun 2008, *Tiehm & Nachlinger 15589* (NY); **Oregon**, ***Harney Co.*:** Wild Horse Creek Canyon, E side of Steens Mts., 22 May 1929, *Applegate 5644* (DS); Little Cottonwood Cr., Pueblo Mts., 1.5 mi past the Trout Creek/Fields-Denio Rd, T40S, R34E, secs. 9/10, 6000 ft elev., 10 Jun 1981, *Grimes 2086* (BRY, CAS, MARY, NY, OSC, UTC); Machine Meadow-Roschense Place, head of Willow Cr, Pueblo Mts., on barren gravel, T39S, R34E, Sec 27, 6000 ft elev., 22 Jul 1980, *Wrighti 1407* (MARY, OSC); ditto, 26 Jun 1980, *Wright & Franklin 1356* (MARY, OSC); ca 1 mi E of McLean Hunting Cabin along rd to Ten Cent Meadow, Pueblo Mts., on bare gravel, T40S, R34E, Sec. 2, 22 Jul 1980, *Wright 1408* (MARY, OSC); ridgetop above Denio Basin, Pueblo Mts., on gravel, T41S, R34E, Sec. 10, 7000 ft elev., 23 Jul 1980, *Wright 1411* (MARY); ridgetop above Denio Basin, Pueblo Mts., on gravelly basaltic scree, T41S, R34E, Sec. 15, 6900 ft elev., 23 Jul 1980, *Wright 1410* (MARY); ditto, 6800 ft elev., 23 Jul 1980, *Wright 1413* (MARY); ditto, 11 Jun 1980, *Wright & Price 1321* (MARY, OSC). ***Malheur Co.*:** Oregon

Canyon Mtns., along Disaster Peak Rd 18.4 rd mi W of McDemitt, just W of Cottonwood/Mine Creek ca. 1 air mi SSW of Opalite Mine, gravelly calcareous substrate, 42°01′16″N, 118°01′54.2″W, 1523 m elev., 5 Jul 2009, *Grady & Flores 526* (WIS); Oregon Canyon Mtns. Brett (Opalite) Mine, 2.5 rd mi N of Disaster Peak Rd, 22.8 air mi W of McDermitt, growing mostly on steep hill of red, gravelly-sandy mine tailings, 42°03′00.3″N, 118°02′13.7″W, 1591 m elev., 5 Jul 2009, *Grady & Flores 528* (WIS).

Eriogonum domitum* Grady & Reveal *sp. nov.

A Eriogono manco Rydb. *foliis ellipticis ad orbiculatis* (*nec oblanceolatis ad spatulatis*) *et involucris rigidis* (*nec membranceis*) *differt.*

Type:**—**U.S.A.: Utah: Millard Co., House Range, NE slope of Notch Peak ca. 100 m below summit, exposed limestone cliff edges in thin, silty, limy soil, associated with *Cercocarpus* and *Ephedra,* 39°08′35.6″N, 113°24′25.7″W, T19S, R14W, Sec. 22, 2832 m (9293 ft) elev., 6 Jul 2010, *B.R. Grady & K.E. Heyduk 638* (holotype: NY, isotypes: BH, BRY, US, UTC, WIS, and elsewhere).

Plants low, pulvinate perennial herbs forming mats to 1 dm across from underground branching caudex; *leaves* basal, semi-erect, fasciculate in terminal tufts, the petioles (1–)3–6 mm long, tomentose, the leaf-blades elliptic to orbicular, (5–)6–12 x (3–)5–9 mm, densely grayish tomentose abaxially, less so and greenish

adaxially, the apex and leaf base rounded, the margins plane; *flowering stems* scapelike, erect to semi-erect, 2–5(–5.5) cm, tomentose, grayish; *inflorescences* capitate, (0.8–)1–1.5 cm wide; *bracts* 5–6, scalelike, 1–2.5 mm long, narrowly triangular; *peduncles* lacking; *involucres* congested, 4–7, campanulate, rigid, (1.5–)2–3 x 1.5–2(–2.5) mm, tomentose, the teeth 5, 0.6–1.0(–1.2) mm long; *flowers* 2.5–3.5 mm long on pedicels 2–3 mm long, the perianth white to pink to rose or magenta with red midvein, glabrous, the hypanthium 1/3–1/2 length of perianth, the tepals monomorphic; *stamens* exserted, 2–3 mm long, the filaments usually sparsely pilose basally; *achenes* trigonous, light brown, 2–2.5(–3) mm long, glabrous except for a minutely papillate beak.

Distribution and habitat:—Like many other capitate members of *Eriogonum* subg. *Eucycla*, *E. domitum* is a narrowly distributed edaphic endemic species. As such, *E. domitum* is known only from high limestone ridges in the House Range of west-central Utah. The House Range wild buckwheat occurs in a fairly narrow elevation band of 2760–2900 m, higher than the pinyon-juniper woodland. Individuals are found most often in a thin limy soil that accumulates in fractures of Notch Peak limestone. This species has only been observed by the authors flowering in July, but presumably produces flowers in June, continuing into August.

Etymology:—The specific epithet, *domitum*, means “of the house”, referring to the House Range, to which this species is endemic.

Observations:—Morphologically, *Eriogonum domitum* resembles *E. mancum* Rydb., the former previously considered to be a relict population of that more northerly species (Reveal 2005). Ongoing molecular studies indicate that *E.*

domitum has a distinct evolutionary history from that of *E. mancum* (Grady & Sytsma, unpublished data). In the field, these species can be distinguished by the leaf blade shape and the appearance of the involucres. The leaves of *E. domitum* are elliptic to orbicular on a well-defined petiole, while those of *E. mancum* are oblanceolate to spatulate with the blade grading into the petiole. The involucres of *E. domitum* are distinctly rigid and turbinate in shape, as compared to the membranous, open-campanulate involucres of *E. mancum.* Geographically, these two species are separated by nearly 500 km.

Eriogonum holmgrenii Reveal (1965), another capitate member of subg. *Eucycla,* also occurs on high-elevation calcareous substrates in the eastern portion of the Great Basin Desert. Although *E. holmgrenii* and *E. domitum* are separated by only ca. 80 km, much of the intervening area is low-elevation basin, with a drastically different moisture regime and geology than either species currently occupies. Though similar ecologically, these two species are easy to distinguish based on pubescence. Glandular trichomes cover the flowering stems, leaves and the perianth of *E. holmgrenii*. All trichomes on the vegetative and floral structures of *E. domitum* are simple and non-glandular.

Additional specimens examined:—U.S.A. **Utah, *Millard Co.*,**: House Range, summit of Pine Peak ca. 1.5 air mi N of Notch Peak, limestone outcrops and gravel, gray silty-limy soil, 39°09′59.3″N, 113°24′33.3″W, T19S, R14W, Sec. 10, 2820 m (9250 ft) elev., 22 Jul 2009, *Grady 575* (NY, WIS); ca. halfway between Pine Peak and Notch Peak, steep ridgeline of limestone gravel, gray silty-limy soil, associated with

39°09′23.2″N, 113°24′38.5″W, T19S, R14W, Sec. 15, 2830 m (9285 ft) elev., 22 Jul 2009, *Grady 578* (WIS); House Range, limestone ridge ca. ½ mi ESE of Notch Peak, silty, limy soil and limestone gravel and cobbles, 39°08′29.2″N, 113°24′06.1″W, T19S, R14W, Sec. 22, 2765 m (9072 ft) elev., 6 Jul 2010, *Grady & Heyduk 636* (WIS).

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Michaux, A. (1803) *Flora Boreali-Americana*. Parisiis, pp. 246.

Peck, M.E. (1945) Some interesting plants of Malheur County, Oregon. *Leaflets of Western Botany* 4: 177–196.

Reveal, J.L. (1965) A new alpine *Eriogonum* from Nevada. *Leaflets of Western Botany* 10: 183–186.

Reveal, J.L. (1972) Two new species of *Eriogonum* (Polygonaceae) from California and adjacent states. *Aliso* 7: 415-419.

Reveal J.L. (1981) Notes on endangered buckwheats (*Eriogonum:* Polygonaceae) with three newly described species from the western United States. *Brittonia* 33: 441–448.

Reveal, J.L. (1985) New Nevada entities and combinations in *Eriogonum* (Polygonaceae). *Great Basin Naturalist* 45: 276–280.

Reveal, J.L. (2004) New entities in *Eriogonum* (Polygonaceae: Eriogonoideae). *Phytologia* 86: 121–159.

Reveal, J.L. (2005) Polygonaceae Jussieu subfam. Eriogonoideae Arnott. *Flora of North America North of Mexico* 5: 218–478.

Rydberg, P.A. (1917) *Flora of the Rocky Mountains and Adjacent Plains*. Hafner Publishing, New York, New York, pg: 220–221.

Watson, S. (1880) *Botany Volume II.* John Wilson and Son, University Press, Cambridge, Massachusetts, pp. 480.