

DISSERTATION

A PRICKLY PUZZLE:
PHYLOGENY AND EVOLUTION OF THE *CARDUUS-CIRSIUM* GROUP (CARDUEAE:
COMPOSITAE), AND UNTANGLING THE TAXONOMY OF *CIRSIUM* IN NORTH
AMERICA

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ABSTRACT

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PHYLOGENY AND EVOLUTION OF THE *CARDUUS-CIRSIUM* GROUP (CARDUEAE: COMPOSITAE), AND UNTANGLING THE TAXONOMY OF *CIRSIUM* IN NORTH AMERICA

Generic delimitations within the cosmopolitan *Carduus-Cirsium* group (i.e., “thistles”) have a long history of taxonomic confusion and debate. We present the most comprehensive molecular phylogeny of the group to date to test generic limits, reconstruct the evolution of pappus type, and elucidate the role of chromosomal evolution. We offer two solutions for the recognition of monophyletic genera: (1) consolidate all taxa into one large genus (*Carduus* or *Cirsium*), or (2) recognize each major clade as a genus (*Carduus*, *Cirsium*, *Eriolepis*, *Notobasis*, *Picnomon*, *Silybum*, and *Tyrimnus*). Under the second proposal, the cryptic genus *Eriolepis* is segregated from *Cirsium*, and the African *Carduus* are included within *Cirsium*. The best diagnosable morphological character to delimit the genera is pollen type, which is not practical in field-based application. We caution that prior to implementing either solution, a thorough, comprehensive morphological analysis of all current members of *Cirsium* sect. *Epitrachys* (= genus *Eriolepis*) be completed. Future morphological studies may find additional achene or leaf surface characters that could be used for practical field identification of the segregate genera. The data show that the plumose pappus state is symplesiomorphic for the group, with one transition to barbellate pappus, likely followed by a reversal to its ancestral state as the group colonized Eurasia. The data are consistent with a North African origin in the region of the

Mediterranean and a single colonization event to North America. An ancestral chromosome state of $n = 17$ is hypothesized for the group, and a descending dysploidy series in *Carduus* is hypothesized to correspond with the aridification of the Mediterranean region. The *Carduus-Cirsium* group highlights the difficulty of delimiting morphologically similar, cryptic genera.

Cirsium is one of the most taxonomically challenging groups of Compositae in North America. This study represents the first attempt to infer a broadly sampled phylogeny of *Cirsium* in North America. The two main objectives are to: (1) test whether currently hypothesized species variety complexes (*C. arizonicum*, *C. clavatum*, *C. eatonii*, and *C. scariosum*) constitute monophyletic lineages, and (2) recircumscribe any taxa that are identified as problematic. Phylogeny reconstructions based on DNA sequence data from two nuclear ribosomal regions and four plastid markers were used to infer evolutionary lineages and test species' delimitations. Eight species varietal complexes were resolved as polyphyletic. We recircumscribed these complexes and in doing so found evidence to support the recognition of six new taxa. We hypothesize that the extensive taxonomic difficulty within *Cirsium* is the result of several factors: 1) previously undescribed taxa, 2) inadequate representation of taxa from herbarium specimens, 3) phenotypic convergence, 4) hybridization, and 5) incipient speciation. While we can provide evidence to support the recircumscription of some taxa, others remain unresolved.

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Herbarium Endowment, Colorado Native Plant Society Marr Grant and Steinkamp Fund, American Society of Plant Taxonomists Graduate Student Research Award, Colorado Mountain Club Foundation Research Grant, and Smithsonian Institution Predoctoral Fellowship. Lastly, I thank all of my friends and family, especially my children, for their support and understanding during the PhD journey.

PREFACE

I have long been fascinated by our native thistles. So much so, that when I began my Master's studies in 1998, I was asked by my advisor, Dr. Jun Wen, which group I would like to study. I emphatically replied "thistles!" to which she adamantly replied "no!". Although she was right to reject this idea at the time, I never gave up wanting to study this fascinating, yet prickly, group. Thus, when I returned to graduate school for my PhD in 2015, I immediately thought of finally studying the taxonomy and evolution of the native thistles. In particular, I was never satisfied with the treatment of the alpine thistles in Colorado and the southern Rocky Mountains. You may wonder, why the long gap between completing a Master's and beginning a PhD? Well, life had other plans for me in between my studies. I was married, had twins, and even underwent treatment for breast cancer during those years. But, I never gave up on my pursuit of a PhD.

In 2018, I serendipitously met Smithsonian Institute Senior Curator of Compositae, Dr. Vicki Funk. She was out for a workgroup at the USGS Powell Center, and asked Dr. Mark Simmons if he knew anyone that was familiar with the local flora, and if he knew of anyone studying *Cirsium*. Well, that person turned out to be me! Once we met, I knew immediately that I must ask Vicki to be on my graduate committee. Her wealth of knowledge on Compositae as well as her infectious enthusiasm and never-ending support were invaluable to this project. Vicki also introduced me to several other Compositae researchers, including Dr. Alfonso Susanna and Dr. David Keil, whom I worked closely with on these chapters. Vicki supported me financially too, even offering her own apartment for me to stay in during my visits to Washington, D.C. to do lab work. In 2019, I received a Smithsonian Institution Predoctoral Fellowship and lived in Washington, D.C. for four months. This was an amazing, educational experience for me and one

that I'll never forget. Unfortunately, Vicki passed away before my PhD was officially completed. I would not have been able to complete this project without her. I honor her legacy by continuing to study Compositae and mentor the next generation of botanists.

This thesis is comprised of three chapters: 1) generic delimitations in the *Carduus-Cirsium* group, 2) the taxonomy of *Cirsium* in North America, and 3) conclusions and next steps. The first chapter is a broad overview of the *Carduus-Cirsium* group. This work uncovers evidence for the resurrection of a cryptic genus, *Eriolepis*, long disguised as *Cirsium*. The second chapter focuses on untangling the taxonomy of *Cirsium* in North America. Thistles are one of the most taxonomically difficult genera in North America. I conclude that this is due to: 1) convergence, 2) previously undescribed species, 3) hybridization, 4) inadequate representation of taxa from herbarium specimens, and 5) incipient speciation. The final chapter wraps up the first two chapters as well as discusses next steps in sorting out thistle taxonomy.

While I was the primary researcher and writer for each chapter, several coauthors also contributed: David J. Keil, Wendy C. Hodgson, Shannon D. Fehlberg, Alfonso Susanna, Dean J. Kelch, Daniel S. Park, Mark P. Simmons, Vicki A. Funk, Andrew H. Thornhill, Bayram Yildiz, Turan Arabaci, and Tuncay Dirmenci. Specific author contributions are as follows. For Chapter One, I conceived and designed the project. Fieldwork was conducted by myself, DSP, DK, BY, TA, TD, and VAF. VAF provided additional funding. DK, and BY, TA, and TD provided sequence data for specimens from Turkey and Europe. I performed lab work for all North American taxa as well as data alignment, phylogenetic inferences, and character state reconstruction analyses. I produced all figures with input from AS, DSP, and VAF. AHT performed the divergence time analysis. The manuscript was written by myself with contributions from AS, DSP, DK, MPS, VAF, and AHT.

For Chapter Two, I conceived and designed the project. Fieldwork was conducted by myself, DJK, WCH, and VAF. VAF provided additional funding. SDF provided sequence data for specimens extracted at Desert Botanical Garden. I performed lab work for all remaining North American taxa as well as data alignment, phylogenetic inferences, and morphological analyses. MPS assisted with phylogenetic inferences. I produced all figures. The manuscript was written by myself with contributions from DJK, MPS, WCH, and SDF.

The work presented here represents the most comprehensive study for the evolution of the *Carduus-Cirsium* group worldwide and for *Cirsium* in North America to date. The resulting taxonomic clarity will aid in the production of dichotomous keys for *Cirsium* in North America. However, the challenges are many for thistle taxonomy in North America. Although I was able to provide evidence to support some taxonomic delimitations, others remain unanswered. In fact, each discussion section for *Cirsium* in North America could be another Master's or even PhD project. I will pass these projects on to my future graduate students and mentees. In short, the thistles turned out to be a life project for me, and I am ok with that. Thistle be fixed, eventually!

DEDICATION

This manuscript is dedicated to my National Museum of Natural History (NMNH), Smithsonian Institution, Washington, D.C. advisor Dr. Vicki Funk. Her mentorship, guidance, and unending support made this work possible.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
PREFACE.....	vi
DEDICATION.....	ix
Chapter One – A Prickly Puzzle: Generic delimitations in the <i>Carduus-Cirsium</i> group (Compositae: Cardueae: Carduinae).....	1
Introduction.....	1
Taxonomic history	3
Morphology.....	6
Chromosomes	9
Biogeography.....	10
Aims.....	10
Materials and methods	11
Sampling and outgroup selection.....	11
DNA extraction, amplification, and sequencing.....	12
Phylogenetic analyses	14
Character state and biogeographic area reconstruction	17
Chromosomal evolution.....	17
Divergence time analysis	17
Results.....	18
Phylogenetic analysis.....	18
The <i>Carduus-Cirsium</i> group.....	21
Clade one	21
Clade two	22
Divergence time analysis	22
Discussion.....	26
Generic delimitations	26
Solution one	26
Solution two.....	27
Ancestral character states.....	31
Chromosomal evolution.....	32
Biogeographic implications	33
Conclusions.....	36
Chapter Two – Thistle be a mess: Untangling the taxonomy of <i>Cirsium</i> (Cardueae: Compositae) in North America	38
Introduction.....	38
Aims.....	44
Materials and methods	45
Taxon sampling.....	45
DNA extraction, amplification, and sequencing.....	45
Phylogenetic analyses	47
Taxonomic evaluations	49

Results.....	49
Phylogenetic analysis.....	49
Incongruence.....	55
Discussion.....	57
Species delimitation of North American thistles	57
<i>Cirsium arizonicum</i> complex.....	57
<i>Cirsium clavatum</i> complex.....	60
<i>Cirsium cymosum</i> complex.....	64
<i>Cirsium eatonii</i> complex.....	65
<i>Cirsium fontinale</i> complex and the CA-FP adaptive radiation clade	77
<i>Cirsium mohavense</i> and <i>C. virginense</i>	80
<i>Cirsium rydbergii</i>	82
<i>Cirsium scariosum</i> complex	83
Conclusions.....	86
New combinations	88
Chapter Three – Conclusions and Next Steps	90
References.....	103
Appendices.....	124

CHAPTER ONE –

A PRICKLY PUZZLE: GENERIC DELIMITATIONS IN THE *CARDUUS-CIRSIIUM* GROUP (COMPOSITAE: CARDUEAE: CARDUINAE)¹

And yet it must be allowed that this very case of the *Thistles* is one which has given to the [...] genus-makers one of the most difficult of problems—a problem actually no nearer its successful solution [...] than it was two centuries ago as Tournefort [1694] left it. — Greene (1892a: 202)

Introduction

Cardueae is one of the largest tribes in Compositae (nom. alt.: Asteraceae), with approximately 74 genera and 2500 species. Members are commonly referred to as “thistles” because of the presence of spines on their leaves and/or involucre bracts (Funk et al., 2005; Susanna & Garcia-Jacas, 2007, 2009). Some members of Cardueae are cultivated (e.g., artichoke, *Cynara cardunculus* L., or safflower, *Carthamus tinctorius* L.), while others are noxious weeds (e.g., Canada thistle, *Cirsium arvense* (L.) Scop., or yellow star thistle, *Centaurea solstitialis* L.). Within Cardueae, the informally recognized *Carduus-Cirsium* group (Fig. 1.1) is comprised of six genera and approximately 566 species: *Carduus* L. (~93), *Cirsium* Mill. (~468), *Notobasis* (Cass.) Cass. (1), *Picnomon* Adans. (1), *Silybum* Adans. (i.e., milk thistle; 2), and *Tyrinnus* Cass. (1; retrieved 1 May 2017 from the Integrated Taxonomic Information System online database, <http://www.itis.gov>). The complex belongs to the subtribe Carduinae, which is currently limited to the *Carduus-Cirsium* group plus the genera *Cynara* L., *Galactites* Moench, *Lamyropsis* M.Dittrich, and *Ptilostemon* Cass. (Herrando-Moraira et al., 2019).

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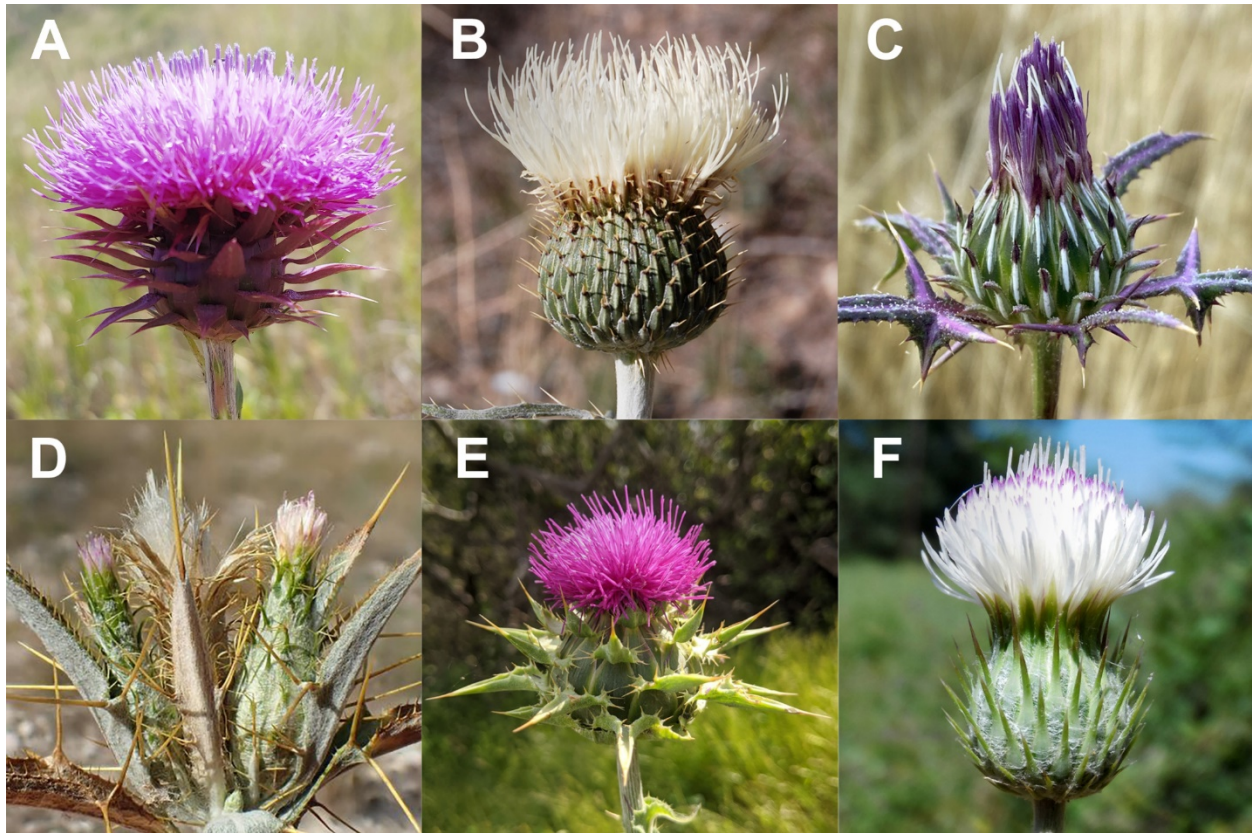


Figure 1.1. Capitula in the *Carduus-Cirsium* group. A, *Carduus nutans* L.; B, *Cirsium canescens* Nutt.; C, *Notobasis syriaca* Cass.; D, *Picnomon acarna* (L.) Cass.; E, *Silybum marianum* (L.) Gaertn.; F, *Tyrimnus leucographus* Cass. — Photos A & B by Jennifer Ackerfield, C by Uriah Resheff, D by Hugues Tinguy, E by Nora Bales, and F by Stavros Apostolou.

The *Carduus-Cirsium* group is monophyletic and united by several synapomorphies, including cylindrical basal pappus tissue and a sclerified apical pericarp epidermis (Häffner, 2000; Susanna & Garcia-Jacas, 2009). The majority of previous work has been completed within Cardueae to recognize only natural, monophyletic genera (Garcia-Jacas & al., 2000, 2002, 2008; Vilatersana et al., 2000; Wagenitz & Hellwig, 2000; Susanna et al., 2006; López-Vinyallonga & al., 2011; Barres et al., 2013; Herrando-Moraira et al., 2019), leaving the delineations within the *Carduus-Cirsium* group remaining as problematic. Indeed, a “successful solution” to the thistle problem, as Greene (1892a) so eloquently stated, is long overdue.

Taxonomic history

There is a long history of taxonomic controversy regarding generic delimitations in the *Carduus-Cirsium* group, particularly for the genus *Cirsium*. Since its inception, *Cirsium* has been recognized by various authors as at least 16 different genera (Table 1.1). The name *Cirsium* was

Table 1.1 Generic delimitations of the *Carduus-Cirsium* group in early treatments.

	<i>Carduus</i> L.	<i>Cirsium</i> Mill.	<i>Notobasis</i> (Cass.) Cass.	<i>Picnomon</i> Adans.	<i>Silybum</i> Adans.	<i>Tyrimnus</i> Cass.
Type	<i>Carduus nutans</i> L.	<i>Carduus heterophyllus</i> L.	<i>Carduus syriacus</i> L.	<i>Carduus acarna</i> L.	<i>Carduus marianus</i> L.	<i>Carduus leucographus</i> L.
Tournefort (1694)	<i>Carduus</i>	<i>Cirsium</i>	<i>Carduus</i>	<i>Cnicus</i>	<i>Carduus</i>	<i>Cirsium</i>
Linnaeus (1753)	<i>Carduus</i>	<i>Carduus Serratula</i> L.	<i>Carduus</i>	<i>Carduus</i>	<i>Cnicus</i> <i>Carduus</i>	<i>Carduus</i>
Miller (1754)	<i>Carduus</i>	<i>Cirsium</i>	<i>Carduus</i>	<i>Carduus</i>	<i>Carduus</i>	<i>Carduus</i>
Adanson (1763)	<i>Carduus</i>	<i>Cirsium</i>	<i>Carduus</i>	<i>Picnomon</i>	<i>Silybum</i>	<i>Silybum</i>
Necker (1790)	<i>Carduus</i>	“ <i>Cephalonoplos</i> Neck.” (not validly published) <i>Cnicus</i> L. <i>Onotrophe</i> Cass. <i>Serratula</i> <i>Xylanthema</i> Neck.	Not listed	Not listed	Not listed	Not listed
Sweet (1825)	<i>Carduus</i>	<i>Erythrolaena</i> Sweet	Not listed	Not listed	Not listed	Not listed
Cassini (1826)	<i>Carduus</i>	<i>Cirsium Echenais</i> Cass. <i>Eriolepis</i> Cass. <i>Lophiolepis</i> (Cass.) Cass. <i>Onotrophe</i> Cass. <i>Orthocentron</i> (Cass.) Cass.	<i>Notobasis</i>	<i>Picnomon</i>	<i>Silybum</i>	<i>Tyrimnus</i>
Candolle & Duby (1828)	<i>Carduus</i>	<i>Cirsium</i>	<i>Carduus</i>	<i>Cirsium</i>	<i>Silybum</i>	<i>Carduus</i>
Lessing (1832)	<i>Carduus</i>	<i>Cirsium Breea</i> Less. <i>Spanioptilon</i> Less.	<i>Notobasis</i>	<i>Picnomon</i>	<i>Silybum</i>	<i>Tyrimnus</i>
Candolle (1837)	<i>Carduus Clavena</i> DC.	<i>Cirsium</i>	<i>Notobasis</i>	<i>Picnomon</i>	<i>Silybum</i>	<i>Tyrimnus</i>
Koch (1851)	<i>Carduus</i>	<i>Cirsium Echenais</i> <i>Epitrachys</i> (DC. ex Duby) K.Koch	<i>Notobasis</i>	<i>Picnomon</i>	<i>Silybum</i>	Not listed

Fourreau (1869)	<i>Carduus</i>	<i>Cirsium</i> <i>Cephalonoplos</i> (Neck. ex DC.) Fourr. <i>Eriolepis</i>	Not listed	<i>Picnomon</i>	<i>Silybum</i>	<i>Tyrimnus</i>
Gray (1874)	Not listed	<i>Cnicus</i>	Not listed	Not listed	Not listed	Not listed
Greene (1892b)	<i>Carduus</i>	<i>Carduus</i>	Not listed	Not listed	Not listed	Not listed

adopted by Andreas of Carystus as early as 230 B.C. (Dioscorides, 1554) and subsequently used by botanists in the pre-Linnean period (Mattioli & Du Pinet, 1572; Tournefort, 1694) to describe thistles traditionally used to treat diseases of the veins. In fact, *Cirsium*, from the Greek “kirsos”, means “swollen veins” and was referred to as “hemorrhoidal thistle” because the coloration and swelling of the stem resembled varicose veins or hemorrhoids (Cassini, 1826). Although Tournefort (1694) also recognized the genus *Carduus*, Linnaeus (1753) chose to use *Carduus* and *Serratula* L. to treat those species formerly called *Cirsium*. A year later, Miller (1754) validly published *Cirsium* but also accepted *Carduus* as “the true thistle”. However, in Miller’s 1768 edition, he adopted Linnaeus’s view and merged *Cirsium* with *Carduus*.

In his *Familles des plantes*, Adanson (1763) recognized four genera: *Carduus*, *Cirsium*, *Picnomon*, and *Silybum*. Adanson was also the first to provide morphological evidence for the separation of *Cirsium* (plumose pappus) from *Carduus* (barbellate pappus). Cassini (1818, 1823, 1825a,b, 1826) was the first to delimit the genera of the group as they are currently circumscribed, but also described five additional genera (*Echenais* Cass., *Eriolepis* Cass., *Lophiolepis* (Cass.) Cass., *Onotrophe* Cass., *Orthocentron* (Cass.) Cass.) as distinct from *Cirsium*. Cassini first described *Lophiolepis* and *Orthocentron* as subgenera, elevating them to generic level in his later treatment (Cassini, 1826). Concurrently with Cassini, Sweet (1825) described a new genus, *Erythrolaena* Sweet, for a thistle from Mexico (*Erythrolaena conspicua* Sweet, now recognized as *Cirsium conspicuum* (Sweet) Sch.Bip.). Later, Lessing (1832), in his

influential treatise of Compositae (*Synopsis generum Compositarum*), also recognized the genera *Breea* Less. and *Spanioptilon* Less. apart from *Cirsium*. To add further confusion, subsequent treatments by some early North American botanists (Gray, 1874; Jones, 1895) incorrectly treated all thistles as *Cnicus* L. Other North American botanists (Greene, 1892b; Rydberg, 1900) recognized only the genus *Carduus*, regarding it as inseparable from *Cirsium*.

Candolle provided the first sectional treatment for *Cirsium* (Candolle & Duby, 1828). In his *Botanicon gallicum*, Candolle recognized four sections: *C. sect. Acarna* DC. (including *Picnomon*), *C. sect. Chamaeleon* DC. (including *Notobasis*), *C. sect. Erysithales* DC., and *C. sect. Epitrachys* DC. ex Duby. In his second revision of the group, Candolle (1837) recognized the genera *Picnomon* and *Notobasis* per Cassini (Cassini, 1826). In this revision, Candolle (1837) also took the genera that Cassini had distinguished as distinct from *Cirsium* and subsumed these as sections of the genus: *C. sect. Eriolepis* (Cass.) DC., *C. sect. Lophiolepis* (Cass.) DC., *C. sect. Onotrophe* (Cass.) DC., and *C. sect. Orthocentron* (Cass.) DC. Candolle (1837) synonymized his earlier section *Epitrachys* under section *Eriolepis* to correspond to Cassini's generic delimitations. Candolle (1837) also named two additional sections (*C. sect. Cephalonoplos* (Neck.) DC., *C. sect. Corynotrichum* DC.), and retained the genus *Echenais*. In addition, Candolle (1837) recognized some species of *Carduus* with thicker hair on the involucre bracts as a separate genus, *Clavena* DC.

Most current treatments (Davis & Parris, 1975; Werner, 1976) recognize three sections in *Cirsium*: *C. sect. Cephalonoplos*, *C. sect. Epitrachys*, and *C. sect. Cirsium*. Section *Cephalonoplos* is comprised of a single species, *C. arvense* (L.) Scop. (i.e., Canada thistle) and is characterized by the presence of dioecious, although sometimes imperfectly so, heads (Werner, 1976). Originally described by Linnaeus (1753) as *Serratula arvensis* L., the species was

removed from the genus upon separation of the type, *S. tinctoria* L. (Linnaeus's "Serratula no. 1"), and placed in *Cirsium* by Scopoli (1772). Occasionally, *C. sect. Cephalonoplos* is recognized at the generic rank as *Breea* (Lessing, 1832) or *Cephalonoplos* (Neck. ex DC.) Fourr. (Necker, 1790; Fourreau, 1869). Cassini (Cassini, 1826) placed *Cirsium arvense* in the genus *Cirsium*, despite previously providing a description of the dioecious heads (Cassini, 1823).

Cirsium section *Epitrachys* is comprised of approximately 100 species, including *C. cephalotes* Boiss., *C. italicum* DC., and *C. vulgare* (Savi) Ten. This section is characterized by the presence of rigid setae (i.e., "hispid hairs") on the adaxial leaf surface (Davis & Parris, 1975). Included within *C. sect. Epitrachys* are all species previously assigned to the genera *Lophiolepis* and *Eriolepis* by Cassini (Cassini, 1823, 1826). All remaining thistles belong to section *Cirsium*. Kazmi (1963) provided the most recent sectional treatment for *Carduus* and subdivided the genus into two subgenera, subg. *Carduus* and subg. *Afrocarduus* Kazmi. Subgenus *Afrocarduus* contains all species found in the mountains of tropical eastern Africa, whereas subgenus *Carduus* contains all Eurasian species (Kazmi, 1963).

Morphology

Members of the *Carduus-Cirsium* group share numerous morphological affinities (Table 1.2). Pappus type (barbellate or plumose) is the primary character used to subdivide the group. Barbellate bristles are present in *Carduus*, *Silybum*, and *Tyrimnus*, whereas feathery, plumose bristles are present in *Cirsium*, *Notobasis*, and *Picnomon* (Werner, 1976). *Carduus* and *Cirsium* are usually separated by this single morphological character (Bentham, 1873; Kazmi, 1963; Keil, 2006). One unique character is seen in *Picnomon*, having the terminal spine of involucre bracts pinnately divided as well as deflexed (Werner, 1976). *Silybum* has involucre bracts with terminal spines that are pinnately lobed at the base but not pinnately divided

Table 1.2. Morphological characteristics of the *Carduus-Cirsium* group.

	<i>Carduus</i>	<i>Cirsium</i>	<i>Notobasis</i>	<i>Picnomon</i>	<i>Silybum</i>	<i>Tyrimnus</i>
Cauline leaves	Decurrent	Sessile, amplexicaul, or decurrent	Amplexicaul	Decurrent	Amplexicaul	Decurrent
White-veined leaves	Absent	Absent	Present	Absent	Present	Present
Involucral bract apex	Entire spine	Entire spine; sometimes margins erose	Entire spine	Pinnately divided throughout, deflexed spine	Pinnately lobed at base	Entire spine
Dorsal corolla lobe epidermal cells	Undulate (straight in subg. <i>Afrocarduus</i> Kazmi)	Straight	Straight	Straight	Undulate	Undulate
Anther basal appendages	Short-sagittate	Short-sagittate	Short-sagittate	Entire	Short-sagittate	Entire
Filaments	Distinct	Distinct	Distinct	Distinct	Monadelphous	Monadelphous
Pappus	Barbellate	Plumose	Plumose	Plumose	Barbellate	Barbellate
Achene pericarp	10–15 longitudinal grooves (4 lines in subg. <i>Afrocarduus</i>)	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines
Achene apical elaiosome	Present	Present	Absent	Present	Present	Present

throughout or deflexed (Werner, 1976). All other members have an undivided or sometimes erose terminal spine at the apex of the involucral bracts.

Features of the achenes and pollen provide a few unique character combinations (Table 1.2). Most notably, *Notobasis* is the only member with achenes lacking an apical elaiosome (a yellowish-white tissue body on the apical plate of the achene which is visible only after the pappus has fallen; Dittrich, 1970). The pericarp surface also differs among the genera (Dittrich, 1970; Häffner, 2000). In *Cirsium*, *Notobasis*, *Picnomon*, *Silybum*, and *Tyrimnus*, the pericarp surface is smooth with four vertical lines that are lighter in color but neither raised nor sunken. *Carduus* achenes are subdivided into two groups. Members of *Carduus* subg. *Carduus*

have vertical vessels that consist of 10–15 longitudinal grooves sunken into the pericarp, while members of *Carduus* subg. *Afrocarduus* have four vertical lines as in *Cirsium*. Members of *Carduus* subg. *Afrocarduus* share other morphological similarities to *Cirsium* including a persistent achene pericarp that does not disintegrate at maturity, straight versus undulate cell walls in the outer epidermis of the corolla lobes, and shortly pilose versus densely pilose filaments (Häffner, 2000).

Pollen morphology has proven to be a useful source of taxonomic evidence in Compositae (Skvarla et al., 1977; Romaschenko et al., 2004; López-Vinyallonga et al., 2011). Punt & Hoen (2009) found that pollen of *Cirsium* sect. *Cirsium* (sexine one layer of short, simple columellae), *Cirsium* sect. *Epitrachys* (sexine one layer of stout columellae; echinae partially filled with columellae), and *Carduus* (*Carduus* subg. *Carduus*; sexine one layer of stout columellae; echinae completely filled with columellae) were unique. Furthermore, they noted that the pollen of *Cirsium* sect. *Epitrachys* more closely resembled the pollen of *Carduus* than *Cirsium* sect. *Cirsium*. Although *Cirsium vulgare* was placed in *Cirsium* sect. *Epitrachys* (Davis & Parris, 1975) based on the presence of rigid setae on the adaxial leaf surface, Punt & Hoen (2009) noted that the pollen of this species fell into the type for *Cirsium* sect. *Cirsium*.

Carduus, *Cirsium*, *Silybum*, and *Tyrimnus* otherwise overlap morphologically. For example, *Carduus* are often distinguished from *Cirsium* by the presence of leaves decurrent as spiny wings on the stem, but some *Cirsium* (e.g., *C. italicum* DC., *C. vulgare* (Savi) Ten.) as well as *Tyrimnus* also share this character (Werner, 1976; Keil, 2006). As first noted by Cassini, both *Tyrimnus* and *Silybum* have filaments coherent at the margins (i.e., monadelphous), but *Tyrimnus* has peripheral sterile florets in the capitula (Cassini, 1826). *Silybum* has traditionally been separated from the other genera by the presence of white-veined leaves that produce a milky sap

(Werner, 1976). However, *Tyrimnus* and *Notobasis* also have white-veined leaves (Susanna & Garcia-Jacas, 2007). *Tyrimnus* and *Silybum* also share another feature in common with Eurasian *Carduus*—the presence of undulate versus straight cells in the walls of the dorsal corolla lobe epidermis (Häffner, 2000). Lastly, *Picnomon* and *Tyrimnus* both have entire anther basal appendages, while all other genera have short-sagittate basal appendages.

Chromosomes

Changes in chromosome number can play a major role in evolution (Levin, 2002; Doyle et al., 2004). While polyploidy events (e.g., whole-genome duplication) result in the doubling of chromosome sets, dysploidy events result in a reduction of chromosome number through chromosomal rearrangements or the loss or gain of a centromere (Lysak, 2014). Both polyploidy and dysploidy can have phenotypic consequences, increase reproductive isolation, and thus drive diversification and ultimately speciation (Husband, 2004; Yakimowski & Rieseberg, 2014; Winterfeld et al., 2018).

Dysploidy is common in Compositae (Semple & Watanabe, 2009). It has been hypothesized that a haploid number of $n = 17$ ($2n = 34$) is the ancestral chromosome number for the *Carduus-Cirsium* group (Keil, 2006). However, this number may have arisen via dysploid reduction from $n = 18$. Within the group there are relatively few instances of polyploidy ($2n = 4x = 68$). However, there are several instances of descending dysploidy in North American *Cirsium* from $n = 17$ ($2n = 34$) to $n = 16$ ($2n = 32$) and in Eurasian *Carduus* from $n = 11$ ($2n = 22$) to $n = 8$ ($2n = 16$). Reconstructing the ancestral chromosome state in the group may not provide an accurate number given the loss and subsequent doubling of chromosomes in ancestral species. Therefore, our main goals regarding chromosomal evolution are to: (1) map the changes in chromosome number along the branches of our phylogeny and (2) determine if dysploidy or

polyploidy events occurred primarily at speciation events (i.e., cladogenetic) or within lineages (i.e., anagenetic).

Biogeography

The *Carduus-Cirsium* group is primarily distributed in the Northern Hemisphere, with a few species found in the Southern Hemisphere in the mountains of tropical eastern Africa (Susanna & Garcia-Jacas, 2009). The largest genus, *Cirsium*, is found throughout Europe, western Asia, and eastern Asia, and is the only genus with species native to North America (Werner, 1976; Keil, 2006; Shi & Greuter, 2011). *Carduus* is also native to Eurasia and northern Africa, and it is the only genus with species native to the mountains of tropical eastern Africa (Kazmi, 1963; Jeffrey, 1968). *Notobasis*, *Picnomon*, *Silybum*, and *Tyrinnus* are primarily found in the Mediterranean region and northern Africa (Susanna & Garcia-Jacas, 2007). Within the two largest genera, *Cirsium* and *Carduus*, there are many narrowly endemic species, while relatively few are naturally widely distributed, despite their notoriety of comprising highly invasive taxa.

Previous studies (Kelch & Baldwin, 2003; Barres et al., 2013) hypothesized a single migration of *Cirsium* to North America during the Pliocene, but the sister clade to New World *Cirsium* remained unresolved. Barres et al. (2013) also hypothesized that a sister group from Middle Asia dispersed into North America via migration across the Bering Land Bridge. Inferring the sister clade to North American *Cirsium* will aid in determining the direction of dispersal from Eurasia. A more comprehensive sampling will also determine if *Cirsium* dispersed to North America once as hypothesized by Kelch & Baldwin (2003), or multiple times.

Aims

A systematic revision of the *Carduus-Cirsium* group to clarify generic boundaries is long overdue. Preliminary research has shown conflicting results for delimiting *Cirsium* and *Carduus*

as monophyletic genera (Häffner & Hellwig, 1999; Kelch & Baldwin, 2003; Susanna et al., 2006; Barres et al., 2013; Park & Potter, 2013, 2015). However, sample sizes were relatively small in these studies. This study represents the first attempt to reconstruct a broadly sampled phylogeny of the *Carduus-Cirsium* group to test for natural segregate genera as well as infer morphological evolution and migration patterns.

The four main objectives are to: (1) delimit monophyletic genera and provide unique character combinations, (2) reconstruct the evolution of pappus type and assess its taxonomic significance, (3) elucidate the role of chromosomal evolution in thistle diversification, and (4) infer the dispersal route(s) to North America.

Materials and Methods

Sampling and outgroup selection

A total of 173 accessions were used in phylogenetic inferences, including all genera of the subtribe Carduinae sensu Herrando-Moraira et al. (2019) (Appendix 1). Of these accessions, 60 were previously published on GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) by Garcia-Jacas et al. (2002), Kelch & Baldwin (2003), Robba et al. (2005), Hidalgo et al. (2006), Susanna et al. (2006), Wang et al. (2007), Soininen et al. (2009), Gao et al. (2010), Pelsner et al. (2010), Barres et al. (2013), Park & Potter (2013), Galimberti et al. (2014), and Aust et al. (2015) (Appendix 1). Due to the taxonomic complexity of the genera and high degree of misidentifications in herbaria, taxa on GenBank that could not be verified with voucher specimens were excluded from this analysis. Accessions from Kelch & Baldwin (2003) were not included in this analysis, with the exception of two *Carduus* taxa (*C. nutans* L., *C. tenuiflorus* Curtis) and seven *Cirsium* taxa (*C. discolor* (Muhl. ex Willd.) Spreng., *C. hydrophilum* (Greene) Jeps., *C. monspessulanum* Hill., *C. palustre* (L.) Scop., *C. quercetorum* (A.Gray) Jeps.,

C. rhotophilum S.F.Blake, *C. spinosissimum* (L.) Scop.), which could not be resampled and had verifiable identifications. Remaining accessions were obtained from herbarium specimens or fresh material collected in the field (see Appendix 1).

Accessions were included in the analysis if they met the following criteria of having either two transcribed spacer regions of the nuclear ribosomal (ETS, ITS), or one nuclear ribosomal DNA region and two additional plastid markers. The exception to this was the inclusion of nine *Carduus* GenBank taxa for which only the ITS region was available. Inclusion of these nine taxa did not alter the tree topology. All accessions included in the analysis had the ITS region amplified. Nomenclature for North American taxa follows the treatment in *Flora of North America* (Keil, 2006).

Outgroups were selected using the Cardueae phylogeny in Herrando-Moraira et al. (2018). Outgroups consisted of taxa from all genera sister to the *Carduus-Cirsium* group (*Cynara cardunculus* L., *Galactites tomentosa* Moench, *Lamyropsis carpini* Greuter, *Ptilostemon afer* Greuter), and two species from the sister subtribe Onopordinae (*Onopordum tauricum* Willd., *Syreitschikovia spinulosa* Pavlov). Six additional outgroups were used in the dating analysis: *Carlina acanthifolia* All. (subfam. Carduoideae, tribe Cardueae), *Brachylaena discolor* DC. (subfam. Carduoideae, tribe Tarchonantheae), *Gerbera piloselloides* Forssk. (subfam. Mutisioideae), *Fulcaldea stuessyi* Roque & V.A.Funk (subfam. Barnadesioideae), *Chuquiraga avellanadae* Lorentz (subfam. Barnadesioideae), and *Nastanthus patagonicus* Speg. (Calyceraceae).

DNA extraction, amplification, and sequencing

DNA extractions were performed using DNeasy Plant MiniKits (Qiagen, Germantown, Maryland, U.S.A.) following the manufacturer's instructions. PCR products were generated for

two transcribed spacer regions of the nuclear ribosomal DNA (ITS, ETS) because of their known usefulness in Compositae studies (Baldwin, 1992), and four plastid markers (*matK*, *ndhF*, *psbA-trnH*, *trnL-trnF*; Table 1.3). DNA extractions of 50 thistle taxa from Europe and Asia were only

Table 1.3. Gene regions and primers used in amplification.

Gene region	Primer sequences	Reference	Approximate size (bp)
ITS	ITS4: TCC TCC GCT TAT TGA TAT GC ITS5A: GGA AGG AGA AGT CGT AAC AAG G ITS5_C3: GGA AGT AAA AGT CGT AAC AAG C	White & al. (1990) Downie & Katz-Downie (1996)	643
ETS	18S-ETS: ACT TAC ACA TGC ATG GCT TAA ETS-Car-1: TTC GTA TCG TTC GGT	Kelch & Baldwin (2003)	583
<i>matK</i>	trnK-710F: GTA TCG CAC TAT GT[T/A] TCA TTT GA AST-1R: CCG CAC ACT TGA AC[G/C] ATA ACC CAG	Susanna & al. (2006)	980
<i>ndhF</i>	ndhF+607: ACC AAG TTC AAT GYT AGC GAG ATT AGT C ndhF1603: CCT YAT GAA TCG GAC AAT ACT ATG C	Jansen (1992)	636
<i>psbA-trnH</i>	psbA3f: GTT ATG CAT GAA CGT AAT GCT C psbAHf: CGC GCA TGG TGG ATT CAC ATT CC	Sang & al. (1997)	524
<i>trnL-trnF</i>	trnLC: CGA AAT CGG TAG ACG CTA CG trnLF: ATT TGA ACT GGT GAC ACG AG	Taberlet & al. (1991)	736

amplified for ETS and ITS. The sequencing of these 50 taxa was performed prior to that of the other 62 taxa by D. Kelch, B. Yildiz, T. Dirmenci, and T. Arabaci in 2008. I could not perform amplification of the cpDNA markers in these samples because of the age of the extracted DNA and inability to re-extract from the deposited specimens. The protocol for extraction and amplification for these samples was the same as for the 62 additional samples, with the exception of being performed at the Berkeley, California laboratory facility. Although ribosomal DNA is known to be affected by concerted evolution, there is a documented low occurrence of paralogs within Cardueae (Herrando-Moraira et al., 2019). Therefore, I did not perform cloning to confirm that a single repeat type was present.

PCR reactions were performed with 25 µl of reaction containing 10.5 µl of sterile water, 5 µl of 10× PCR reaction Buffer A (Promega, Madison, Wisconsin, U.S.A.), 2 µl of 10 mM

dNTPs (Pharmacia Biotech, Piscataway, New Jersey, U.S.A.), 2.5 μ l of 50 mM MgCl₂, 0.5 μ l of 10 mg/ml Bovine Serum Albumin (Sigma, St. Louis, Missouri, U.S.A.), 1 μ l of 10 mM of each of the two primers, 0.1 μ l *Taq* DNA polymerase enzyme (Bioline, Taunton, Massachusetts, U.S.A.), and 2.5 μ l of DNA template. The amount of DNA template was adjusted to generate sufficient PCR products for DNA sequencing when necessary. Amplification was performed on a Bio-Rad thermal cycler c1000 (Bio-Rad, Hercules, California, U.S.A.). The PCR program consisted of an initial preheating at 95°C for 3 min; followed by 37 cycles of (94°C, 45 s; 54°C, 45 s; 72°C, 2 min), with a final 72°C, 7 min elongation step and holding at 10°C. ExoSAP-IT (Affymetrix, Cleveland, Ohio, U.S.A.) was used to purify PCR products for sequencing. The enzymatic removal of primers and excess dNTPs involved mixing 10 μ l of the PCR product with 1 μ l of ExoSAP-IT, incubating the mixture at 37°C for 30 min, and then raising the temperature to 80°C for 15 min to denature the ExoSAP-IT enzymes. Unincorporated dye terminators were removed using Sephadex gel filtration (GE Healthcare, Piscataway, New Jersey, U.S.A.) using MultiScreen plates (Millipore, Billerica, Massachusetts, U.S.A.). Cycle sequencing was performed using BigDye v.3.1 (Applied Biosystems, Foster City, California, U.S.A.) at the Smithsonian Institution on a Hitachi 3730xl DNA Analyzer (Applied Biosystems). Sequence reads of each PCR product were assembled and edited in Geneious (v.5.6.3).

Phylogenetic analyses

All nucleotide sequences were aligned independently using MAFFT v.7 (Katoh et al., 2017). The iterative refinement method of Q-INS-i, which considers the secondary structure information of rDNA, was used for alignments of ETS and ITS. The G-INS-I algorithm was used for the plastid gene regions. The default gap opening penalty (1.53) was applied, and the gap offset value was set to 0.1 for all alignments. All nucleotide sequences were further aligned

manually in AliView v.3.0 (Larsson, 2014) using the procedure outlined in Simmons (2004) following Zurawski & Clegg (1987). Gaps were treated as missing data.

Characters were analyzed using several alternative potential process partitions as a means of data exploration (Bull et al., 1993). Each of the six gene regions was analyzed independently to resolve their respective gene trees. Gene trees for the two nrDNA regions and the four plastid loci were analyzed independently to examine incongruence and evidence of potential introgression or lineage sorting (Doyle, 1992; Wendel et al., 1995). Regions were compared visually for topological heterogeneity among regions, using a 75% bootstrap cut-off value. Topological incongruence was not expected for the plastid regions because they are all part of the uniparentally inherited chloroplast genome (Gastony & Yatskievych, 1992).

Best-fit likelihood models for each partition were selected using the Bayesian information criterion in the PartitionFinder (Lanfear et al., 2012) algorithm as implemented in IQ-TREE v.1.6.10 (option -m MFP+MERGE; Nguyen et al., 2015). This option also merges partitions to reduce potential model overfitting and allows concurrent searches of model space and tree space (Kalyaanamoorthy et al., 2017). The merge option resulted in a final partition of three character sets and corresponding models: ETS (TVM+F+G4), ITS (TIM3e+R3), and plastid (*matK*, *ndhF*, *psbA-trnH*, *trnL-trnF*; TVM+F+R2). A relaxed clustering algorithm was used to reduce computations by only examining the top 10% of the partitioning schemes (option -recluster 10).

Maximum likelihood (ML) analyses (Felsenstein, 1973) were performed in IQ-TREE v.1.6.10 (Nguyen et al., 2015) using the best partition scheme and substitution models identified and described above. For concatenation-based species tree inference, IQ-TREE has been shown to be comparable to or outperform other maximum likelihood programs (i.e., RAxML/ExaML)

and yield the best-observed likelihoods for matrices with 200 or less taxa (Zhou et al., 2017). In addition, the relatively fast computational time and ease of use allows for a thorough exploration of tree space in IQ-TREE. Branch lengths were linked across loci (IQ-TREE edge-proportional model, option -spp) to allow each partition to have its own evolution rate but share the same set of branch lengths (Duchene et al., 2018). Node support was determined by nonparametric bootstrapping using IQ-TREE's ultrafast bootstrap approximation (option -bb; Hoang et al., 2017) with 5000 pseudoreplicates. I also inferred the SH-like aLRT support values for each node. Following Simmons & Norton (2014), any clades receiving a high likelihood-based bootstrap support but 0% SH-like aLRT support were collapsed.

Bayesian inference (BI) analyses (Yang & Rannala, 1997) were implemented in MrBayes (Huelsenbeck & Ronquist, 2001) via the Cyber Infrastructure for Phylogenetic Research online portal (CIPRES; <http://www.phylo.org/>). BI was performed using the best-fit partitioning scheme recommended by PartitionFinder (Lanfear et al., 2012). The “greedy” algorithm with branch lengths estimated as linked and the BIC were used to search for the best-fit partitioning scheme. This resulted in the the following partitioning scheme: GTR (General time reversible) substitution model (nst=6) with gamma-distributed rate variation across sites and a proportion of invariable sites (=invgamma) for the ETS, ITS *ndhF*, *psbA-trnH*, and *trnL-trnF* partitions, and the F81 substitution model (nst=1) for the *matK* partition. The concatenated dataset was subsequently subjected to Markov Chain Monte Carlo (MCMC) sampling using two replicates of four chains (one cold, three hot). Fifty million generations total were completed with a sampling frequency of every 1000 generations. Tracer v.1.5 (Rambaut & Drummond, 2013) was used to visualize and analyze the MCMC trace files using a 25% burn-in value. All tree topologies were viewed in FigTree v.1.4.3 (Rambaut, 2016).

Character state and biogeographic area reconstruction

A character matrix was assembled based on the data matrix provided in Table 1.2. All characters scored were treated as discrete and unordered, with equal probability for any particular character change. Mesquite v.2.7.5.2011 (Maddison & Maddison, 2015) was used to map characters onto the ML phylogeny. The Markov k -state 1 (Mk1) parameter model of evolution was used for the ML reconstructions. For the biogeographic area reconstruction, branches and internodes of the ML phylogeny were colored according to their distribution per Funk et al. (2009). I also included one additional area of Asia Minor (highlighted in blue).

Chromosomal evolution

Chromosome count data was obtained from the *Index to plant chromosome numbers* (Goldblatt & Johnson, 1991) and Chromosome Counts Database (CCDB; Rice et al., 2015). Chromosome counts were recorded for a total of 137 taxa, representing 82% of the taxa in our phylogeny (Appendix 1). Chromosome reconstruction was performed under the ChromEvol v.2.0 model (Glick & Mayrose, 2014) as implemented and expanded in RevBayes v.1.0.2 (Höhna et al., 2016; Freyman & Höhna, 2017) only on taxa with verified chromosome counts. In ChromEvol, the chromosomal evolution along a phylogeny is represented as a continuous-time Markov process (Mayrose et al., 2010). Root frequencies were treated as free parameters of the model and estimated from the observed data. A stochastic character map was used to visualize the evolution of chromosomes along the branches of the phylogeny. The R package *phytools* v.0.7-47 (Revell, 2012) was used to visualize the output from RevBayes.

Divergence time analysis

Divergence times were estimated using BEAST v.2.4.5 (Drummond & Rambaut, 2007; Bouckaert et al., 2014). Three calibration points (CP) were set up for a BEAST run using

BEAUti v.2.4.5 (Drummond & Rambaut, 2007). Calibration point one (CP1) was a secondary dated node corresponding to the origin of the Compositae family (83.5 Myr with a 95% CI) per Mandel et al. (2019). The two other calibration points were based on fossil records. CP2 was set to 47.5 Myr to constrain the node of clade Mutisioideae and subfamily Carduoideae, based on the macrofossil capitulescence of *Raiguenrayun cura* Barreda et al. (Barreda et al., 2012). CP3 was set to 14 Myr based on achenes identified as *Cirsium* (Mai, 2001) and placed at the stem node of the *Carduus-Cirsium* group clade. The BEAST analysis was run for 40 million generations under a Yule relaxed-clock model with individual tree models applied to the nuclear and plastid subsets. Trees were logged every 1000 generations. The maximum clade credibility tree was summarized using TreeAnnotator v.2.1.2 (Rambaut & Drummond, 2014) using a 20% burn-in of logged trees.

Results

Phylogenetic analysis

The phylogeny of the combined dataset, with node support for both the maximum likelihood (ML) and Bayesian inference (BI) is shown in Fig. 1.2A, B. Gene trees for the ETS, ITS, and each plastid region are shown in supplementary Fig. 1–6. Of the 407 parsimony-informative sites found in the combined analysis, 349 (86%) were found in the rapidly evolving ETS and ITS regions (Table 1.4).

There was no significant ($BS \geq 75$ and $PP \geq 0.75$) incongruence between the nuclear and plastid gene trees (Suppl. Fig. 1–6). This is either the result of a lack of parsimony-informative characters in the plastid regions, or missing sequence data for the four plastid markers. One weakly supported incongruence was found between the ITS and three plastid gene trees (*matK*, *ndhF*, *trnL-trnF*) for the position of the African *Carduus* (*C. keniensis* R.E.Fr.,

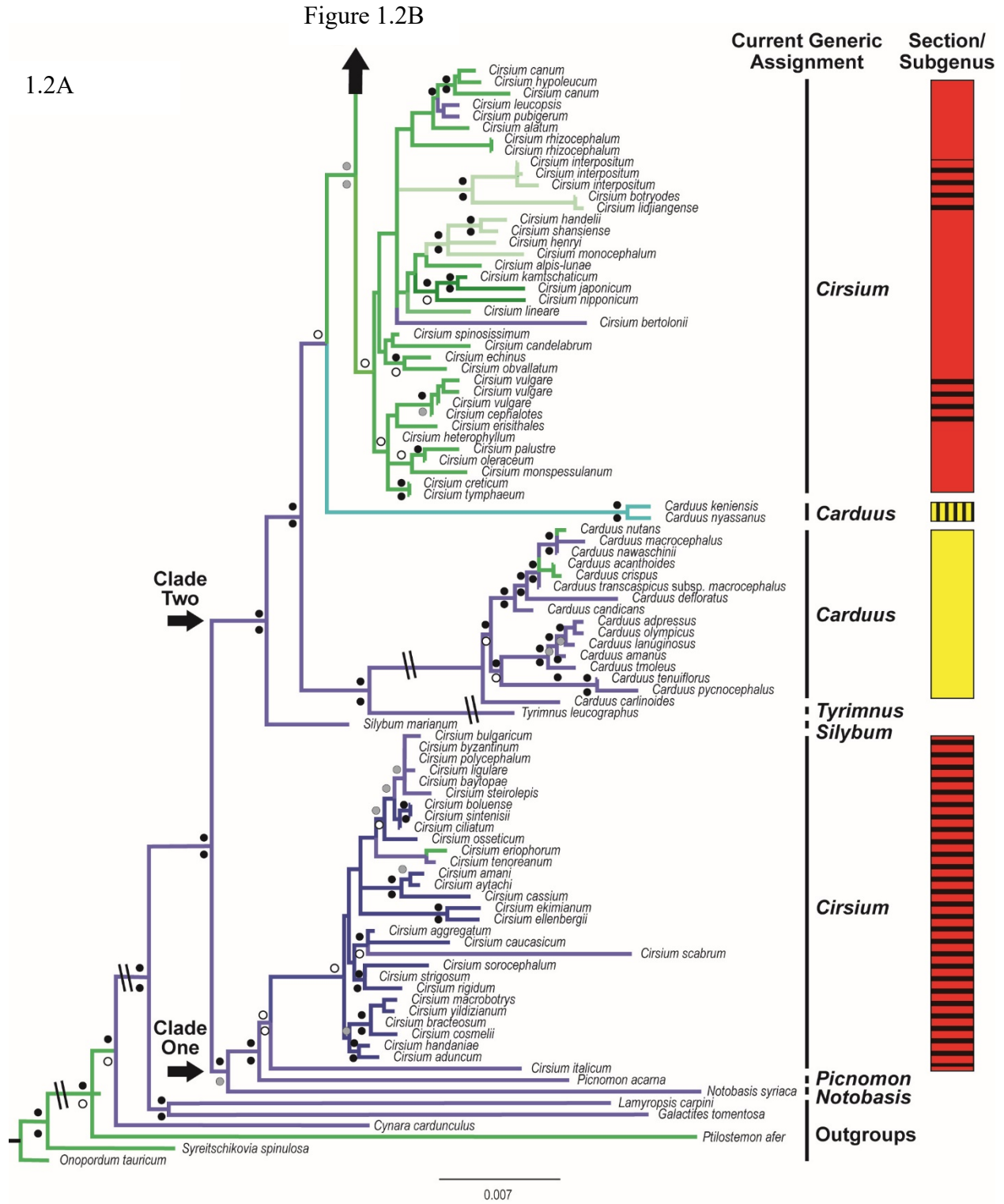


Figure 1.2A, B. Maximum likelihood phylogenetic reconstruction for the *Carduus-Cirsium* group and outgroups, and distribution map.

1.2B

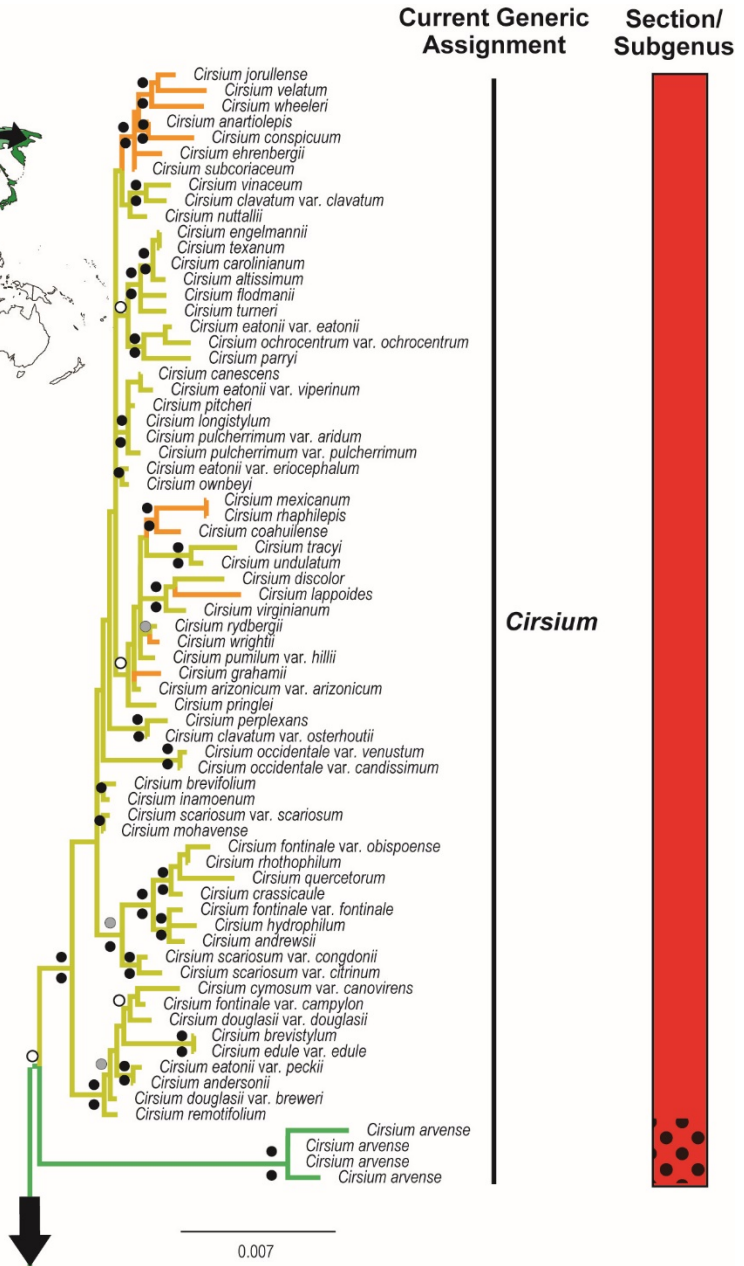
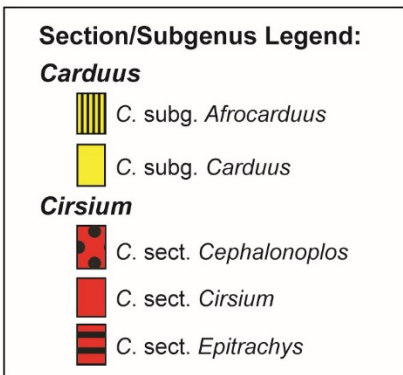
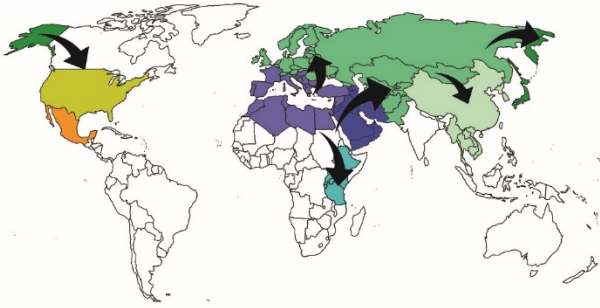


Figure 1.2A

Figure 1.2A, B. Maximum likelihood phylogenetic reconstruction for the *Carduus-Cirsium* group and outgroups, and distribution map. Circles above nodes represent ML bootstrap support (BS) and those below nodes represent Bayesian inference posterior probability (PP); values are: black circles $\geq 95\%$ (BS)/0.95 (PP); grey circles $\geq 85\%$ (BS)/0.85 (PP); white circles $\geq 75\%$ (BS)/0.75 (PP). Branches shortened for fit are indicated on the phylogeny by two diagonal lines. Phylogeny branch colors correspond to the distribution map geographic ranges.

Table 1.4. Statistics for each phylogenetic analysis.

	ETS	ITS	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>trnL-trnF</i>	Plastid	Nuclear	Combined
Accessions	137	167	83	62	77	108	111	167	167
Characters	583	644	964	636	525	735	2860	1227	4087
Missing data (%)							17%	8%	25%
Parsimony-informative sites	147	202	12	11	19	16	58	349	407
Invariable sites	319	341	897	611	488	695	2691	660	3351

C. nyassanus (S.Moore) R.E.Fr.). In the ITS gene tree, the African *Carduus* are sister to the remainder of the *Carduus-Cirsium* group (BS = 99, PP = 0.72; Suppl. Fig. 2). However, the African *Carduus* are strongly supported as included within the *Carduus-Cirsium* group polytomy in the *matK* (BS = 99, PP = 1.0; Suppl. Fig. 3), *ndhF* (BS = 93, PP = 1.0; Suppl. Fig. 4), and *trnL-trnF* gene trees (BS = 91, PP = 1.0; Suppl. Fig. 6).

The Carduus-Cirsium group

The *Carduus-Cirsium* group is resolved as monophyletic with good support (Fig. 1.2A; BS = 100, PP = 1.00). Within the group, a division into two main clades (hereafter referred to as Clade One and Clade Two) is found. Both *Carduus* and *Cirsium* as they are currently delimited are resolved as polyphyletic.

Clade One

The first main clade (BS = 98, PP = 0.83) consists of taxa currently assigned to *Notobasis*, *Picnomon*, and *Cirsium* (Fig. 1.2A). The monotypic genera *Notobasis* and *Picnomon* are resolved as consecutive sisters to a clade of Eurasian *Cirsium* belonging to *Cirsium* sect. *Epitrachys* (BS = 75; PP = 0.75), with the exception of *C. cephalotes* and *C. vulgare* which are resolved in Clade Two.

Clade Two

The second main clade (BS = 98, PP = 1.00) consists of taxa currently assigned to *Silybum*, *Tyrimnus*, *Carduus*, and *Cirsium* (Fig. 1.2A, B). *Silybum* is resolved as sister to all other taxa in Clade Two (BS = 100, PP = 1.00). Clade Two is then subdivided into two subclades. Subclade one consists of *Tyrimnus* and Eurasian *Carduus* (BS = 99, PP = 0.98). Within subclade one, *Tyrimnus* is resolved as sister to Eurasian *Carduus* (BS = 100, PP = 1.00).

Subclade two consists of African *Carduus*, Eurasian *Cirsium* (including *C. vulgare* and *C. cephalotes* from *Cirsium* sect. *Epitrachys*), and all North American *Cirsium* (BS = 75). At the backbone of subclade two, the Eurasian *Cirsium* and African *Carduus* are unresolved and only weakly supported in the ML phylogeny. African *Carduus* are nested within *Cirsium* in our Bayesian analysis (Fig. 1.3A, B). Also, in subclade two, *Cirsium* sect. *Cephalonoplos* is nested within *Cirsium* sect. *Cirsium*. Although not previously assigned to a section, there are three southeastern Asian *Cirsium* taxa included in our sampling (*C. botryodes* Petr., *C. interpositum* Petr., *C. lidjiangense* Petr. & Hand.-Mazz.) also reported to have rigid setae on the adaxial leaf surface that I am including in *Cirsium* sect. *Epitrachys* (Shi & Greuter, 2011). These taxa are resolved in our phylogeny as belonging to Clade Two, along with *C. cephalotes* and *C. vulgare*. A well-supported clade (BS = 99, PP = 1.00) of taxa exclusive to North America is resolved in subclade two.

Divergence time analysis

The divergence time estimates resolved the stem age of the *Carduus-Cirsium* group as 20.2 Myr old (Fig. 1.4). Using the generic delimitations in Solution Two (see discussion below), the divergences that established *Notobasis*, *Picnomon*, *Cirsium* sect. *Epitrachys*, *Silybum*, *Tyrimnus*, *Carduus*, and *Cirsium* are estimated to have occurred approximately 16 Myr ago (12.7–19.5 Myr

Figure 1.3B

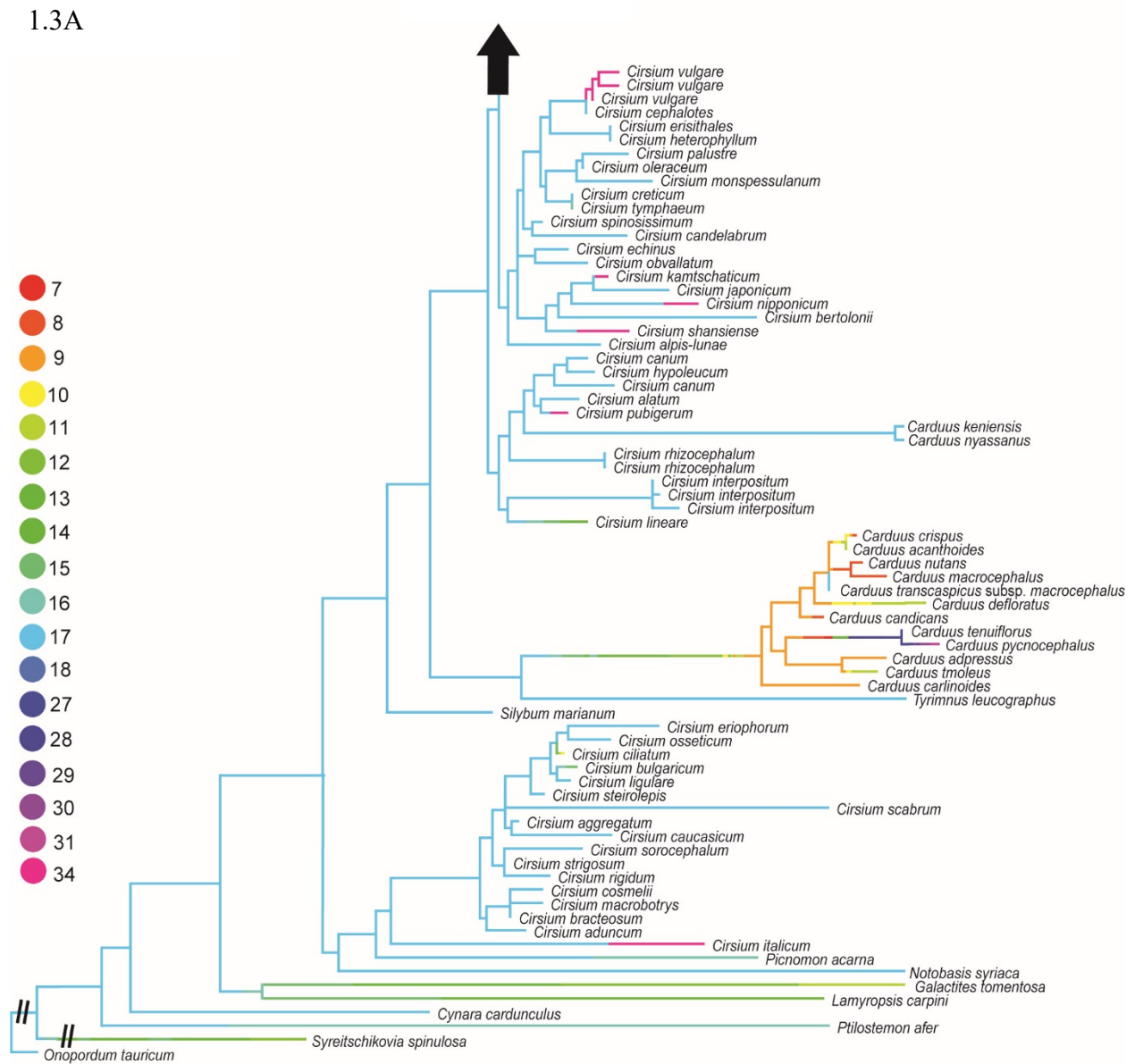


Figure 1.3A, B. Chromosomal evolution inferred by ChromEvol for the *Carduus-Cirsium* group. Phylogenetic tree based on a BI analysis using RevBayes. Stochastic character mapping as applied to chromosome number evolution is shown on each branch. Tree branches are colored according to the legend to show the hypothesized ancestral chromosome state and evolution to the current chromosome state for each taxon. Branches shortened for fit are indicated on the phylogeny by two diagonal lines.

1.3B

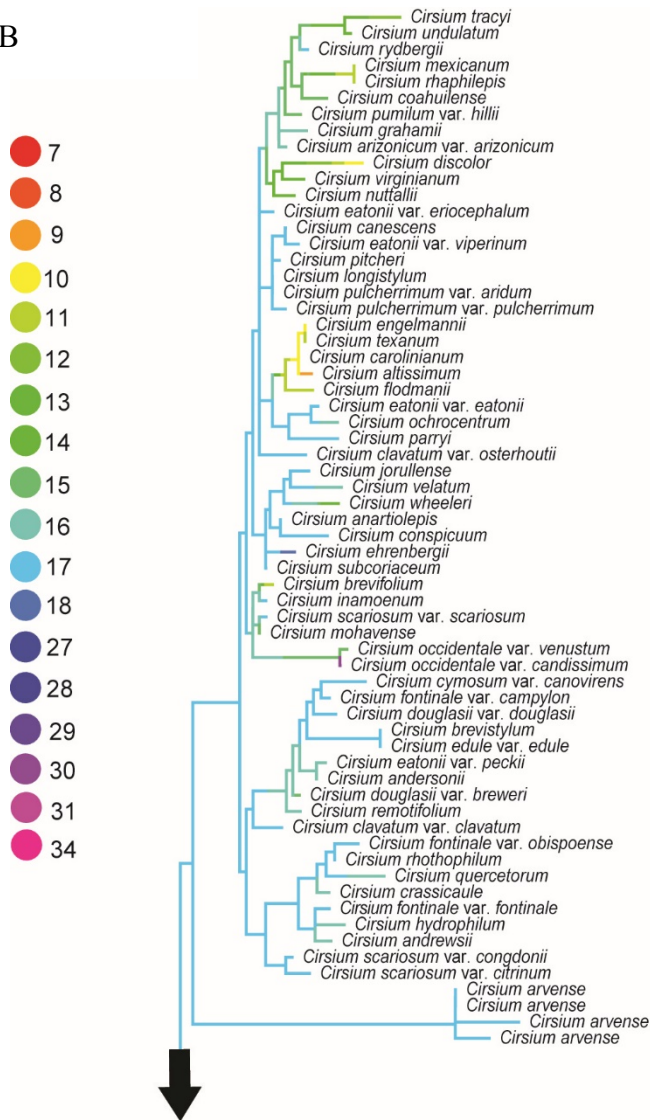


Figure 1.3A

95% CI). Clade One (*Notobasis*, *Picnomon*, *Cirsium* sect. *Epitrachys*) is estimated to have diverged 14.3 Myr (10.5–17.8 95% CI) at approximately the same time as Clade Two (*Silybum*, *Tyrinnus*, *Carduus*, *Cirsium*; 14.1 Myr; 11.0–17.6 95% CI). The *Cirsium* sect. *Epitrachys* lineage split from *Picnomon* approximately 11.9 Myr ago (8.3–15.3 95% CI) and diversified within the last 10.6 Myr (7.5–13.9 95% CI).

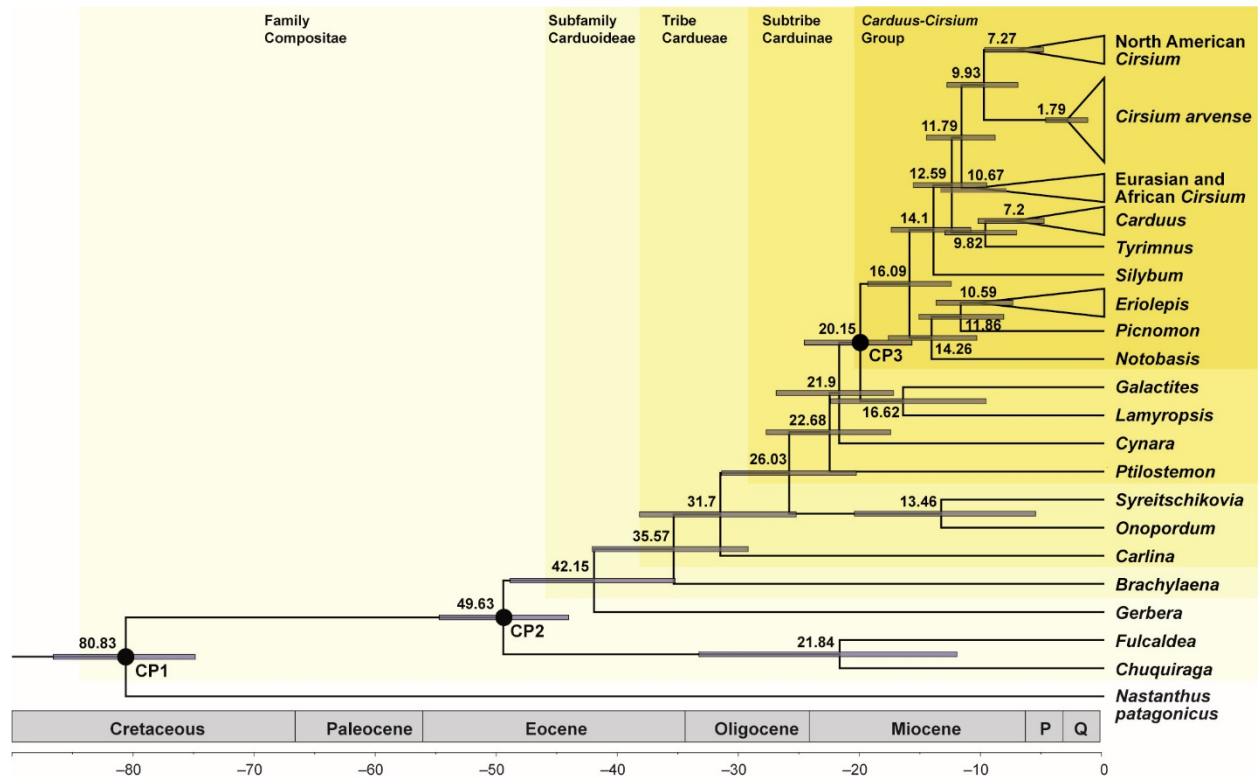


Figure 1.4. Time-calibrated phylogeny of the *Carduus-Cirsium* group within Compositae. Median age is shown at each node. Purple bars on nodes indicate the 95% confidence intervals (CI). Black circles on nodes represent the calibration points (CP) used in the dating analysis. In the scale axis, “P” and “Q” correspond to Pliocene and Quaternary, respectively.

Subclade one (*Carduus*, *Tyrimnus*) and subclade two (*Cirsium*) diverged approximately 12.6 Myr (7.3–20.0 95% CI). Within subclade one, *Carduus* diverged from *Tyrimnus* approximately 9.8 Myr (7.2–13.2 95% CI). Within subclade two, Eurasian and African *Cirsium* diverged from *Cirsium arvense* and North American *Cirsium* approximately 11.8 Myr (9.0–14.7 95% CI). *Cirsium arvense* and North American *Cirsium* diverged approximately 9.9 Myr (5.2–16.8 95% CI). The divergence that established North American *Cirsium* is estimated to have occurred 7.3 Myr (5.0–9.9 95% CI).

Discussion

Generic delimitations

Both *Carduus* and *Cirsium* as they are currently circumscribed are polyphyletic. No support was found for the recognition of four smaller genera—*Cephalonoplos*, *Echenais*, *Erythrolaena*, and *Spanioptilon* (Table 1.1). *Cirsium arvense* is nested within the Eurasian *Cirsium* (Clade Two, subclade two), providing no support for the recognition of *Cephalonoplos*. Although occasionally treated as a distinct genus (Koch, 1851), I found no support for the recognition of *Echenais* as the representative taxon (*Cirsium echinus* Hand.-Mazz.), is nested in the Eurasian *Cirsium* clade (Clade Two, subclade two). No support was also found for the recognition of *Erythrolaena* (Sweet, 1825) as the only member of the genus (*Cirsium conspicuum*) is nested within the North American *Cirsium* clade (Clade Two, subclade two). Last, the only member of *Spanioptilon* (*S. lineare* (Thunb.) Less; *Cirsium lineare* (Thunb.) Sch.Bip.) is nested within the *Cirsium* sect. *Epitrachys* clade (Clade One). I propose two alternative solutions for the delimitation of natural, monophyletic genera.

Solution One

The first solution is to combine all genera into a single genus. A current lack of unique character combinations to delimit among Eurasian *Carduus*, African *Carduus*, *Cirsium*, *Silybum*, and *Tyrimnus* supports this combination. It may be argued that the inclusion of 566 taxa into a single genus may result in a cumbersome approach. This solution also presents an interesting dilemma. The *International Code of Nomenclature for algae, fungi, and plants*' rule of priority would dictate that all *Cirsium*, *Notobasis*, *Picnomon*, *Silybum*, and *Tyrimnus* should be subsumed into *Carduus* which was proposed in 1753, one year prior to the formal designation of *Cirsium* (Turland et al., 2018). In addition, *Carduus* is the type for the tribe Cardueae. However, *Cirsium*

holds significant cultural and broader public appeal. *Cirsium* is the symbolic flower of Scotland—images of thistles appear on Scotland’s coat of arms, and the order of the thistle is a prestigious ranking. Additionally, within North America and Asia, all *Carduus* are invasive species, while only three non-native *Cirsium* (*C. arvense*, *C. palustre* (L.) Scop., *C. vulgare*) occur in North America (Keil, 2006) and Asia (Shi & Greuter, 2011), and assigning all *Cirsium* to *Carduus* would only result in reinforcing prevailing misconceptions that all thistles are invasive, noxious weeds. Lastly, preserving *Cirsium* would result in significantly fewer taxonomic changes. However, a previous attempt (Briquet, 1905) to conserve *Cirsium* (Miller, 1754) over *Carduus* (Linnaeus, 1753) failed.

Solution Two

An alternative solution is to recognize each major clade of the phylogeny as a genus. This would result in the recognition of seven genera: *Carduus*, *Cirsium*, *Eriolepis*, *Notobasis*, *Picnomon*, *Silybum*, and *Tyrimnus* (Fig. 1.5). Under this scenario, Cassini’s (1826) genus *Eriolepis* would be reinstated. Although Cassini described the conspecific *Lophiolepis* first (Cassini, 1823), he originally treated it at the subgeneric level (*Cirsium* subg. *Lophiolepis* Cass.). Cassini (1826) later granted *Lophiolepis* generic rank, but had already named *Eriolepis* at the generic level (Cassini, 1825a), thus giving priority to *Eriolepis* for subsequent generic delimitations. Koch (1851) considered the rigid setae a distinct enough character to recognize members of *Cirsium* sect. *Epitrachys* at the generic rank. Unfortunately, Koch incorrectly used Candolle’s sectional name *Epitrachys* instead of Cassini’s previously described *Eriolepis* for the generic name, thus making any species assignments to the genus *Epitrachys* (DC. ex Duby) K.Koch superfluous and therefore illegitimate.

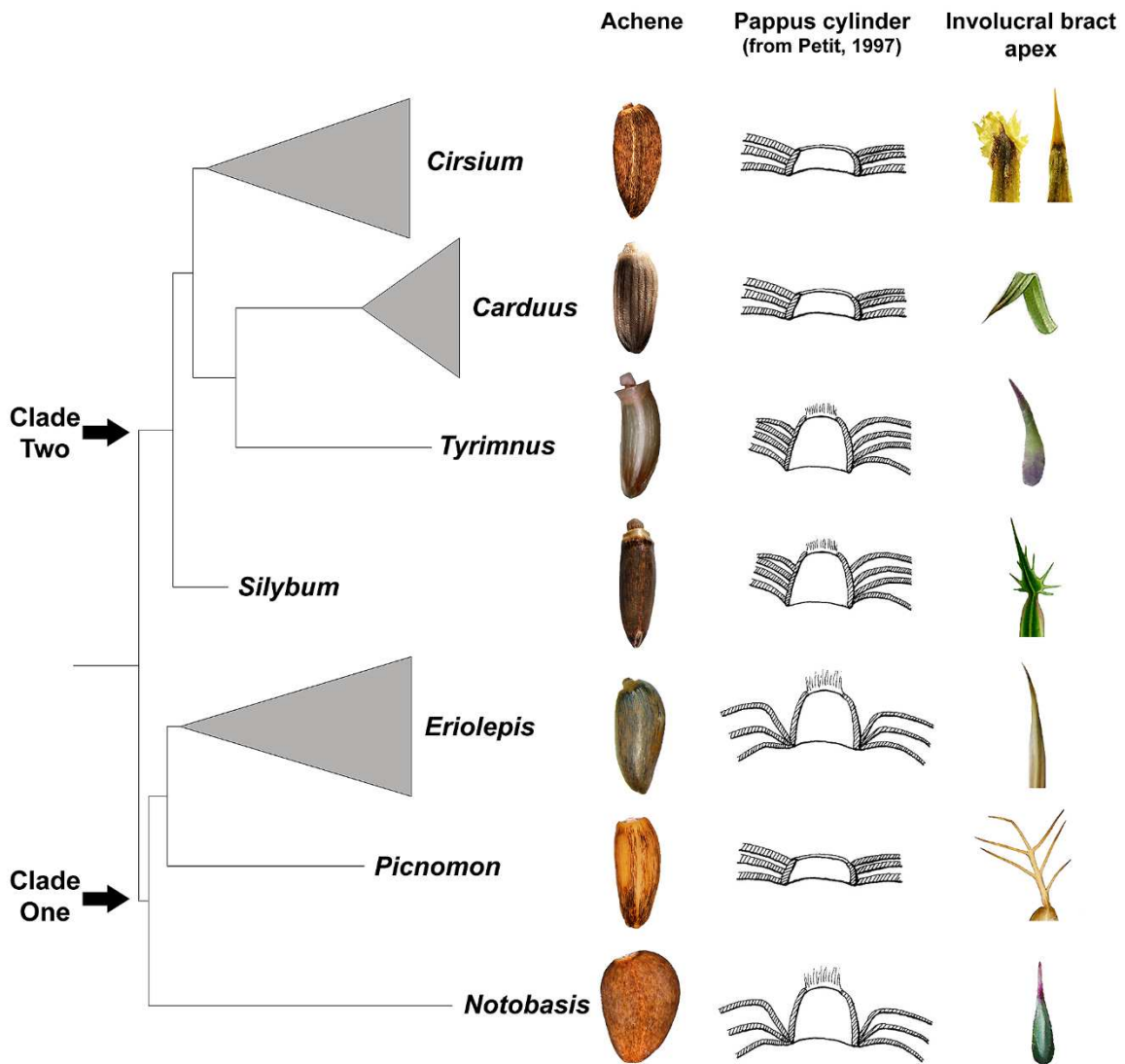


Figure 1.5. Maximum likelihood phylogenetic reconstruction for generic delimitations of Solution Two. Achene photographs taken by Jennifer Ackerfield at the Smithsonian Institution, Washington, D.C., except for that of *Tyrimnus* which was taken by Alfonso Susanna. Pappus cylinder type is from Petit (1997). Photographs of the involucral bract apices were taken by Jennifer Ackerfield.

There is some preliminary morphological support for this solution (Table 1.5; Fig. 1.5). First, *Notobasis* is the only genus in which the achenes lack an apical elaiosome. Second, *Picnomon* is the only genus in which the involucral bracts exhibit a pinnately divided terminal

spine. Third, after separating out the African *Carduus* taxa, *Carduus* is the only genus in which the achenes exhibit 10–15 longitudinal grooves (versus 4 longitudinal lines in all other genera). Fourth, *Silybum* and *Tyrimnus* both have monadelphous stamens, but *Tyrimnus* differs in having peripheral sterile florets in the capitula and achenes with a deep apical crown.

However, the placement of *Cirsium cephalotes*, *C. vulgare*, and the three discordant Asian species (*C. botryodes*, *C. interpositum*, *C. lidjiangense*) in our phylogeny is problematic for the recognition of *Eriolepis* from *Cirsium*. Although *C. cephalotes* and *C. vulgare* have been traditionally placed in *Cirsium* sect. *Epitrachys*, in our phylogeny they are nested in *Cirsium* sect. *Cirsium*. Historically, the rigid setae used to delimit *Cirsium* sect. *Epitrachys* have been described under a variety of terms including “coarsely hispid hairs”, “scabrous-hispid hairs”, “setose-spinulose hairs”, and “prickly-hairy” by various authors (Keil, 2006). This warranted further anatomical studies to determine if these “rigid setae” were indeed homologous among *C. vulgare* and other members of *Cirsium* sect. *Epitrachys*. A previous examination of the cleared leaves of *C. vulgare* determined that the “rigid setae” were not epidermal outgrowths, but true spines with an enlarged base emerging from the veinlets within the leaf tissues (Keil, 2006). I analyzed leaves of *C. vulgare* and *C. eriophorum*, and noted that the true spines of *C. vulgare* could be distinguished from the rigid setae of *Eriolepis* by their stouter form and enlarged base. Although the true spines and rigid setae (i.e., “hispid hairs”) are superficially similar, they are in fact not homologous. This character must currently be used with caution, and sampled across a larger number of taxa prior to its utilization.

Two additional characters can be used to discern *Eriolepis* from *Cirsium*, although these characters are difficult to observe and not practical in application. First, according to Punt & Hoen (2009), there are three main pollen types in the *Carduus-Cirsium* group: *Carduus*

Table 1.5. Main diagnostics characters of the genera proposed for Solution Two.

	<i>Carduus</i>	<i>Cirsium</i> ^a	<i>Eriolepis</i> ^b	<i>Notobasis</i>	<i>Picnomon</i>	<i>Silybum</i>	<i>Tyrinnus</i>
Achene apical elaiosome	Present	Present	Present	Absent	Present	Present	Present
Achene pericarp	10–15 longitudinal grooves	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines	4 longitudinal lines
Achene terminal crown	Shallow	Shallow to moderate	Shallow to moderate	Absent	Shallow	Shallow	Deep
Involucral bract apex	Entire spine	Entire spine; sometimes margins erose	Entire spine	Entire spine	Pinnately divided throughout, deflexed spine	Pinnately lobed at base	Entire spine
Filaments	Distinct	Distinct	Distinct	Distinct	Distinct	Monadelphous	Monadelphous
Outer disk florets	Perfect	Perfect or sterile	Perfect	Perfect	Perfect	Perfect	Sterile
Dorsal corolla lobe epidermal cells	Undulate	Straight	Straight	Straight	Straight	Undulate	Undulate
Adaxial leaf surface	No rigid setae	No rigid setae; true spines	Rigid setae	No rigid setae	No rigid setae	No rigid setae	No rigid setae
Pappus cylinder	Not fimbriate	Not fimbriate	Fimbriate	Fimbriate	Not fimbriate	Fimbriate	Fimbriate
Pollen type	<i>Carduus crispus</i>	<i>Cirsium palustre</i>	<i>Cirsium eriophorum</i>	Unknown	Unknown	Unknown	Unknown

a – including *Carduus* subg. *Afrocarduus*

b – formerly *Cirsium* sect. *Epitrachys*

crispus L. pollen type, *Cirsium eriophorum* Scop. pollen type, and *Cirsium palustre* (L.) Scop. pollen type. These types correspond exactly to the clades in our analysis: *Carduus crispus* type is limited to European *Carduus*, *Cirsium eriophorum* type is circumscribed to *Cirsium* sect. *Epitrachys* (i.e., *Eriolepis*), and *Cirsium palustre* type is limited to *Cirsium* sect. *Cirsium*. Indeed, the pollen type of *C. vulgare* was found to correspond to the *C.* sect. *Cirsium* type and not the *C.* sect. *Epitrachys* type. Additionally, Petit (1997) found that the pappus cylinder was fimbriate in *C.* sect. *Epitrachys* but not in *C.* sect. *Cirsium* (Fig. 1.5). Examination of a fresh

C. vulgare pappus cylinder found that it was smooth, fitting the *C. sect. Cirsium* type. The pappus cylinder of *C. eriophorum* (in *C. sect. Epitrachys*) had fimbriate hairs, but these were difficult to see. Each of these three characters must be used with caution as they have not been sampled widely across the two proposed genera.

This solution leaves all African *Carduus*, and Eurasian and North American *Cirsium* to be designated as *Cirsium*. Although contained within *Cirsium* in this solution, the African *Carduus* are only weakly supported for inclusion. Morphologically, the African *Carduus* are intermediate between the Eurasian *Carduus* and *Cirsium*, lending support for their inclusion within *Cirsium* (Table 1.2). However, the position of the African taxa is not well-resolved in our concatenated phylogeny (Fig. 1.2A). The discordance between the ITS gene tree and plastid gene trees indicates a history of incomplete lineage sorting and/or introgression. A future phylogenomic study of the group will provide additional informative characters to resolve the position of the African taxa. This study may even provide support for recognition of the African taxa as a distinct genus (“*Afrocarduus*”).

There are several advantages to this solution. First, this circumscription results in smaller, more manageable genera instead of one large, rather unwieldy genus. Second, it retains the generic name *Cirsium* for all North American and Asian taxa. Lastly, this solution would result in significantly fewer nomenclatural changes. However, I strongly suggest that prior to accepting Solution Two for generic delimitations, a thorough morphological analysis of all species with “rigid setae” be performed.

Ancestral character states

The maximum likelihood reconstruction of the ancestral pappus character state suggests that having a plumose pappus is the symplesiomorphic state for the *Carduus-Cirsium* group

(Fig. 1.6). The barbellate pappus type was derived once in the lineage (Clade Two: *Silybum* and subclade one), followed by a reversal back to this ancestral state (Clade Two: subclade two). The pappus type is thus not phylogenetically informative and cannot be used solely to distinguish *Cirsium* or *Carduus* species. The presence of imperfectly dioecious heads represents an apomorphy that has arisen once within the *Cirsium arvense* lineage.

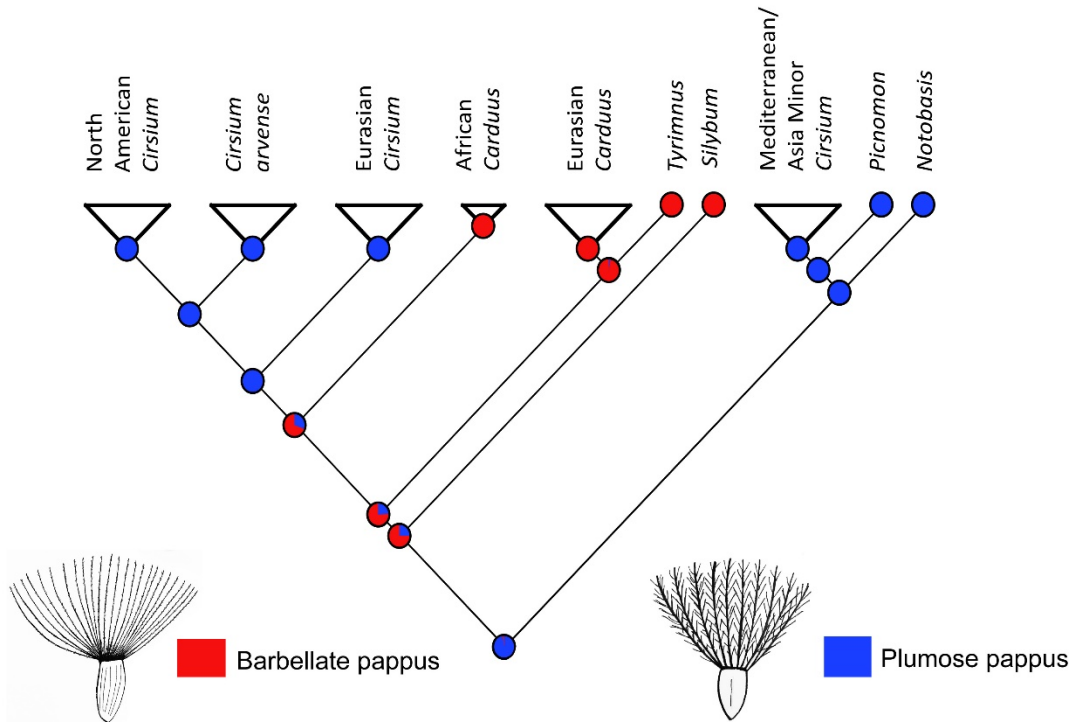


Figure 1.6. Maximum likelihood phylogeny with pappus type ancestral state reconstruction in the *Carduus-Cirsium* group.

Chromosomal evolution

An ancestral chromosome state of $n = 17$ is hypothesized for the *Carduus-Cirsium* group, although I acknowledge that this may have arisen through dysploidy reduction (Fig. 1.3A, B). Polyploidy events occur within lineages, and are found on a few terminal branches of our phylogeny. I conclude that these events are therefore not associated with increases in diversification. Alternatively, dysploidy events occur primarily at speciation events, and I

therefore surmise that these events correspond to increases in diversification. Descending dysploidy is common throughout the evolutionary history of Compositae (Mota et al., 2016). Most notably, the Eurasian *Carduus* clade has undergone a dramatic dysploidy reduction from $n = 11$ to $n = 8$.

I hypothesize that the chromosomal reduction corresponds to the aridification of the Mediterranean. Dysploidy may allow for rapid radiation in novel environments (Burt, 2000) and is common in the Mediterranean flora (Vilatersana et al., 2000). The drying of the Mediterranean has been accompanied by rapid diversification mediated by dysploidy in other lineages, including many Compositae (Garnatje et al., 2004; Fiz-Palacios & Valcárcel, 2013; Escudero et al., 2018). Dysploidy may promote speciation by facilitating the accumulation of locally adaptive alleles via suppression of chromosomal recombination (Kirkpatrick & Barton, 2006; De Storme & Mason, 2014).

In North American *Cirsium*, there is one series of descending dysploidy from $n = 15$ to $n = 10$ and one series of descending dysploidy from $n = 15$ to $n = 9$ (Fig. 1.3A, B). Most notable, the clade of *C. altissimum* (L.) Spreng., *C. carolinianum* (Walter) Fernald & B.G.Schub., *C. engelmannii* Rydb., *C. flodmanii* (Rydb.) Arthur, and *C. texanum* Buckley has a dysploid series of $n = 12$ to $n = 9$. However, in contrast to Mediterranean *Carduus*, the dysploid series in North America do not follow occupation of arid environments. *Cirsium altissimum* and *C. flodmanii* are often found in damp soil, while the other species in the clade are found in grasslands or prairie (Keil, 2006).

Biogeographic implications

Our phylogenetic data shows strong biogeographic structure with a pattern of dispersal events followed by subsequent localized radiations (Fig. 1.2A, B). Our results support a

hypothesis of the Mediterranean region of North Africa as the center of origin for the *Carduus-Cirsium* group during the mid-Miocene. The large number of outgroups as well as the current distribution of the extant genera *Notobasis* and *Picnomon* in North Africa and the Mediterranean lends weight to this hypothesis. From North Africa, subsequent dispersal events occurred to Asia Minor, the Mediterranean region of southern Europe, tropical eastern Africa, Eurasia, and ultimately North America.

Dispersal in Compositae is most often associated with the pappus, which allows for effective wind dispersal (Sheldon & Burrows, 1973). However, in *Cirsium*, the pappus detaches from mature seeds as a single unit (Susanna & Garcia-Jacas, 2009), and thus wind dispersal may be limited, especially if the achenes are heavy. Secondary dispersal by ants is also found in *Cirsium*, which are attracted to the apical elaiosome (Weiss, 1908; Pemberton & Irving, 1990; Alba-Lynn & Henk, 2010). Dispersal can also be achieved through hydrochory in some thistles (Craddock & Huenneke, 1997), making water-dispersal a third possible means of dispersal. I offer two hypotheses for dispersal of *Cirsium* to North America. First, the ancestor of the *Carduus-Cirsium* group dispersed from North Africa to Asia Minor, probably via stepping-stones on the Tethyan coast (Barres et al., 2013) either by wind or ant dispersal. From here, the Mediterranean Basin was colonized multiple times. From the Mediterranean Basin, ancestors of the group simultaneously colonized Eurasia and eastern Africa, becoming isolated in the tropical mountains during aridification of the continent (Fig. 1.2B). Finally, a single dispersal event from Eurasia to North America occurred (Fig. 1.2B), supporting the original hypothesis of Kelch & Baldwin (2003). This migration event most likely occurred over the Bering Land Bridge, which was a corridor for dispersal until the Pliocene (5.3–2.5 Myr; Gladenkov et al., 2002). Our analysis dates the dispersal of *Cirsium* into North America during the Miocene (Fig. 1.4; 7.3

Myr), which agrees with the timing of the Bering Land Bridge as a corridor of dispersal. A previous study of *Plectocephalus*, one of only three Cardueae genera native to North America, also found strong evidence for the Bering Land Bridge as a corridor of dispersal from the Mediterranean during the Miocene (Susanna et al., 2011). The majority of extant taxa in North America then diversified to the present extent during the Pleistocene.

Alternatively, a long-distance dispersal from Africa to North America is a possibility, albeit an unlikely one. Long-distance dispersals from Africa to Australia have been observed in other plant groups (Bergh & Linder, 2009; Li et al., 2009), but these typically have origins in South Africa and not the mountainous regions of tropical eastern Africa. Although the seeds of some *Cirsium* can float and their dispersal has been noted to be mediated by water, the distance from Africa to North America is approximately 12,500 km and it is unlikely that seeds would have maintained buoyancy for the extended period of time it would have taken to reach North America.

Within Clade One, the diversification of *Carduus* corresponds with the onset of the Mediterranean climate and Quaternary glacial cycles during the Messinian Salinity Crisis approximately 5.6–6.0 Myr ago (Barrón et al., 2010). Although the *Carduus* lineage split from *Tyrimnus* approximately 9.8 Myr ago (Fig. 1.4) the majority of the extant taxa diversified to the present day extent within the last 2–6 Myr (Suppl. Fig. 7). Within Clade Two, *Cirsium* dispersed into Africa in the Miocene approximately 7.75 Myr ago (Fig. 1.4; 4.6–11.1 95% CI), although present day taxa (*Carduus keniensis* and *Carduus nyassanus* in our phylogeny) only diversified to the present extent within the last 1.9 Myr. Colonization of Africa was probably more widespread during cooler periods, with species finding refugia in the mountainous regions of tropical eastern Africa during periods of aridization which may have led to extinction of some

lineages (Barres et al., 2013). Although one of the most widespread and economically devastating agricultural weeds in the world (Schroeder et al., 1993), *Cirsium arvense* diversified to the present extent only within the last 1.8 Myr (Fig. 1.4; 0.5–4.0 95% CI). Within the North American *Cirsium* clade, most taxa have diversified to the present extent within the last 3 Myr (Suppl. Fig. 7). While Clade One in our phylogeny is relatively well-resolved, Clade Two has poor backbone resolution. This could be the result of insufficient sampling of informative regions or indicate recent radiations within the group.

Conclusions

A “successful solution” for delimiting generic boundaries in the *Carduus-Cirsium* group is long overdue, but a stable taxonomy for the group at the generic level is only just beginning to emerge. This is the first study to widely sample from *Carduus* and *Cirsium*. I provide two solutions to segregate the *Carduus-Cirsium* group into natural, monophyletic genera. Although I show that both *Carduus* and *Cirsium* do not form monophyletic groups, a definitive generic delimitation solution remains elusive at this time. However, I do offer two alternative solutions that may be adopted once additional sampling of both morphological and phylogenetic informative characters is complete.

Prior to adopting either of the solutions presented for delimiting generic boundaries, I suggest waiting for the results of an upcoming study in the *Carduus-Cirsium* group. The authors are currently working on a phylogenomic study using targeted enrichment of highly informative nuclear regions designed specifically for Compositae by Mandel et al. (2014, 2015, 2017). The high-throughput sequencing techniques provided through this method will significantly increase the informative characters available for phylogenetic inference. In addition, this method has been shown to be useful at all taxonomic levels within Compositae. This will aid in resolving the

topology (in particular at the backbone of Clade Two) and provide additional clade support for the placement of the African species. In addition, this upcoming study will allow us to infer a more complete biogeographic history. For the present, I must be satisfied that I am much nearer a “successful solution” in the case of the thistles.

CHAPTER TWO –

THISTLE BE A MESS: UNTANGLING THE TAXONOMY OF CIRSIUM (CARDUEAE: COMPOSITAE) IN NORTH AMERICA¹

Introduction

Cirsium Mill. (Compositae), otherwise known as the “thistles,” is a genus of herbaceous biennials or perennials that are widely distributed in the temperate regions of Eurasia (~370 taxa; Werner, 1976; Ackerfield et al., 2020), eastern tropical Africa (~10 taxa; Beentje, 2000; Ackerfield et al., 2020), and North America (~118 taxa; Keil, 2006). They are referred to as “thistles” because of the presence of spines on the leaves and/or involucre bracts. Within North America, *Cirsium* has undergone a continental wide radiation (Kelch & Baldwin, 2003; Ackerfield et al., 2020), with many narrowly distributed endemics and few widespread taxa (Keil, 2006). The greatest taxon richness of *Cirsium* occurs in the western half of North America, particularly in the Rocky Mountains, Great Basin, desert Southwest, California-Floristic Province (CA-FP), and Mexico. Only two species occur in both the Old World and North America. *Cirsium kamtschaticum* Ledeb. ex DC. is found in Japan, Siberia, and the Alaskan Aleutian Islands (Werner, 1976; Keil, 2006). *Cirsium heterophyllum* (L.) Hill is found in Greenland and Eurasia. However, neither of these species is native to the North American mainland.

Canada thistle [*C. arvense* (L.) Scop.] is one of the worst agricultural weeds in the world (Guggisberg et al., 2012). Unfortunately, the prevalence and destructive nature of Canada thistle has led to widespread misconceptions that all thistles are invasive plants. For example, Iowa lists

¹ Published as: Ackerfield, J.R., Keil, D.J., Hodgson, W.C., Simmons, M.P., Fehlbeg, S.D. and Funk, V.A. 2020. Thistle be a mess: Untangling the taxonomy of *Cirsium* (Cardueae: Compositae) in North America. *Journal of Systematics and Evolution*. In Press.

all *Cirsium* species as noxious weeds irrespective of whether or not they are native (Eckberg et al., 2017). However, thistles are sources of food for bumble bees, hawk moths, flies, beetles, butterflies, and even hummingbirds. Thistles also provide important nesting resources for cavity nesting bees, and their seeds are sources of food for songbirds and small mammals (Eckberg et al., 2017). Thirty-five taxa in North America are considered rare or of conservation concern (Keil, 2006). Six of these taxa are also listed as federally threatened or endangered under the U.S. Fish and Wildlife Service Endangered Species Act.

Despite its abundance and importance, *Cirsium* remains one of the most taxonomically challenging groups of Compositae in North America, particularly in the western states (Cronquist, 1994; Keil, 2006). These taxonomic difficulties have been hypothesized to be the result of limited morphological differentiation, incipient speciation, and/or hybridization among taxa (Ownbey et al., 1975; Cronquist, 1994; Kelch & Baldwin, 2003; Keil, 2006). Early North American botanists such as Asa Gray (1863) acknowledged the taxonomic problems within the genus. But given the lack of available current evidence for species delimitations, Gray (1863: 69) concluded, “I could not pretend to name the thistles of the Rocky Mountains and am not disposed to add to the existing confusion.” Later botanists attempted to resolve these taxonomic issues but were similarly frustrated. Harold Harrington, author of the *Manual of the Plants of Colorado* (1954: 625) stated that “[*Cirsium*] is a variable and difficult genus with numerous intergradations in [Colorado].” Stanley Welsh (1982: 199), co-author of the *Flora of Utah* (Welsh et al., 2003) wrote that “The thistles of Utah have long constituted one of the most difficult problems in the plant taxonomy of the state.” Arthur Cronquist, author of the *Intermountain Flora* treatment for *Cirsium* (1994: 389) wrote “I am still not satisfied with my

treatment of *Cirsium* here, but it is the best I can do in the time available for the preparation of a flora.” Keil (2006: 96) in his treatment for the *Flora of North America* stated that “many problems remain to be worked out in North American *Cirsium*... the field is open and the challenges are many.” Most recently, Peter Lesica, author of the *Manual of Montana Vascular Plants* (2012: 512) stated that “*Cirsium* gets my vote for the most confusing genus in Montana.”

Gray (1874) completed the first North American treatment for *Cirsium*, recognizing 28 species and five varieties. However, Gray incorrectly treated all *Cirsium* as *Cnicus* L. Greene (1892) later rectified Gray’s (1874) use of the genus *Cnicus* and added 10 new species and three varieties to North American *Cirsium*. However, Greene transferred all North American species to the genus *Carduus* L., which he considered conspecific with *Cirsium*. In Petrak’s (1917) comprehensive treatment of the genus for North America, he recognized 77 species. A comprehensive regional treatment was completed by Rydberg (1917, 1922) for the Rocky Mountains and adjacent plains. In his later treatment, Rydberg (1922) recognized 58 native and one introduced species.

With the advent of the *Flora of North America* series, an effort was made yet again to provide a comprehensive treatment of *Cirsium* for North America. In this treatment, Keil (2006) recognized 62 species and 56 varieties. Many formerly recognized species were broadly circumscribed and synonymized with or placed as varieties of a more widespread species. In particular, the *C. arizonicum* (A. Gray) Petrak, *C. clavatum* (M.E. Jones) Rydb., *C. eatonii* (A. Gray) B.L. Rob., and *C. scariosum* Nutt. varietal complexes (i.e., species divided into two or more infraspecific varieties) underwent significant taxonomic changes (Table 2.1).

Previous phylogenetic work on North American *Cirsium* has been limited. Kelch and Baldwin (2003) reconstructed the only molecular phylogeny of North American *Cirsium* using characters from the external transcribed spacer (ETS) and internal transcribed spacer (ITS)

Table 2.1. Major taxonomic treatments of the *C. arizonicum* (A. Gray) Petr., *C. clavatum* (M.E. Jones) Rydb., *C. eatonii* (A. Gray) B.L. Rob., and *C. scariosum* Nutt. varietal complexes.

Basionym and Author (Year of publication in parentheses)	Petrak (1917) <i>Systematische Übersicht der amerikanischen Arten der Gattung Cirsium</i>	Rydberg (1922) <i>Flora of the Rocky Mountains and Adjacent Plains</i>	Cronquist (1994) <i>Intermountain Flora</i>	Welsh et al. (2003) <i>Utah Flora</i>	Keil (2006) <i>Flora of North America</i>	Ackerfield et al. (This study)
<i>Cirsium arizonicum</i> (A. Gray) Petr.						
<i>Carduus pulchellus</i> Greene ex Rydb. (1906)	<i>C. pulchellum</i> (Greene ex Rydb.) Wooton & Standl.	<i>C. pulchellum</i>	<i>C. calcareum</i> (M.E. Jones) Wooton & Standl.	<i>C. calcareum</i> var. <i>pulchellum</i> (Greene ex Rydb.) S.L. Welsh	<i>C. arizonicum</i> var. <i>bipinnatum</i> (Eastw.) D.J. Keil	<i>C. pulchellum</i>
<i>Carduus truncatus</i> Greene (1906)	<i>C. pulchellum</i>				<i>C. arizonicum</i> var. <i>bipinnatum</i>	<i>C. pulchellum</i>
<i>Cirsium</i> “ <i>aleatorum</i> ” Barlow-Irick						<i>C. arizonicum</i> var. <i>tenuisectum</i>
<i>Cirsium arizonicum</i> var. <i>tenuisectum</i> D.J. Keil (2004)					<i>C. arizonicum</i> var. <i>tenuisectum</i>	<i>C. arizonicum</i> var. <i>tenuisectum</i>
<i>Cirsium chellyense</i> R.J. Moore & Frankton (1974)					<i>C. arizonicum</i> var. <i>chellyense</i> (R.J. Moore & Frankton) D.J. Keil	<i>C. chellyense</i>
<i>Cirsium chuskaense</i> R.J. Moore & Frankton (1974)					<i>C. arizonicum</i> var. <i>chellyense</i>	<i>C. chellyense</i>
<i>Cirsium navajoense</i> R.J. Moore & Frankton (1974)					<i>C. arizonicum</i> var. <i>chellyense</i>	<i>C. chellyense</i>
<i>Cirsium</i> “ <i>surrepticum</i> ” Barlow-Irick						Unresolved
<i>Cnicus arizonicus</i> A. Gray (1874)	<i>C. arizonicum</i>	<i>C. arizonicum</i>	<i>C. arizonicum</i>	<i>C. arizonicum</i>	<i>C. arizonicum</i> var. <i>arizonicum</i>	<i>C. arizonicum</i> var. <i>arizonicum</i>
<i>Cnicus bipinnatus</i> Eastw. (1898)	<i>C. pulchellum</i> var. <i>bipinnatum</i> (Eastw.) Petr.	<i>C. bipinnatum</i> (Eastw.) Rydb.	<i>C. calcareum</i>	<i>C. calcareum</i> var. <i>bipinnatum</i> (Eastw.) S.L. Welsh	<i>C. arizonicum</i> var. <i>bipinnatum</i>	<i>C. calcareum</i>
<i>Cnicus calcareus</i> M.E. Jones (1895)	<i>C. calcareum</i>		<i>C. calcareum</i>	<i>C. calcareum</i> var. <i>calcareum</i> (Eastw.) S.L. Welsh	<i>C. arizonicum</i> var. <i>bipinnatum</i>	<i>C. calcareum</i>

<i>Cnicus diffusus</i> Eastw. (1898)	<i>C. pulchellum</i> ssp. <i>diffusum</i> (Eastw.) Petr.				<i>C. arizonicum</i> var. <i>bipinnatum</i>	<i>C. calcareum</i>
<i>Cnicus drummondii</i> (Torr. & A. Gray) A. Gray var. <i>bipinnatus</i> Eastw. (1893)	<i>C. pulchellum</i> var. <i>bipinnatum</i>	<i>C. bipinnatum</i>	<i>C. calcareum</i>	<i>C. calcareum</i> var. <i>bipinnatum</i>	<i>C. arizonicum</i> var. <i>bipinnatum</i>	<i>C. pulchellum</i>
<i>Cnicus rothrockii</i> A. Gray (1882)	<i>C. rothrockii</i> (A. Gray) Petr.		<i>C. rothrockii</i>	<i>C. rothrockii</i>	<i>C. arizonicum</i> var. <i>rothrockii</i> (A. Gray) D.J. Keil	<i>C. arizonicum</i> var. <i>rothrockii</i>
<i>Cnicus rothrockii</i> var. <i>diffusus</i> Eastw. (1896)	<i>C. pulchellum</i> ssp. <i>diffusum</i>	<i>C. diffusum</i> (Eastw.) Rydb.	<i>C. rothrockii</i>	<i>C. rothrockii</i>	<i>C. arizonicum</i> var. <i>bipinnatum</i>	<i>C. arizonicum</i> var. <i>rothrockii</i>
<i>Cnicus nidulus</i> M.E. Jones (1895)	<i>C. nidulum</i> (M.E. Jones) Petr.	<i>C. nidulum</i> (M.E. Jones) Rydb.	<i>C. arizonicum</i>	<i>C. arizonicum</i> var. <i>nidulum</i> (M.E. Jones) S. Welsh	<i>C. arizonicum</i> var. <i>arizonicum</i>	<i>C. arizonicum</i> var. <i>arizonicum</i>
<i>Cirsium clavatum</i> (M.E. Jones) Rydb.						
<i>Carduus araneosus</i> Osterh. (1905)	<i>C. griseum</i> x <i>C. parryi</i>	<i>C. araneosus</i> Rydb.			<i>C. clavatum</i> var. <i>osterhoutii</i> (Rydb.) D.J. Keil	<i>C. griseum</i> var. <i>osterhoutii</i> (Rydb.) Ackerfield & D.J. Keil, comb. nov.
<i>Carduus centaureae</i> Rydb. (1901)	<i>C. americanum</i> (A. Gray) K. Schum	<i>C. centaureae</i> (Rydb.) K. Schum.	<i>C. centaureae</i>	<i>C. centaureae</i>	<i>C. clavatum</i> var. <i>americanum</i> (A. Gray) D.J. Keil	<i>C. centaureae</i>
<i>Carduus griseum</i> Rydb. (1901)	<i>C. griseum</i> (Rydb.) K. Schum.	<i>C. griseum</i>			<i>C. clavatum</i> var. <i>americanum</i>	<i>C. griseum</i> var. <i>griseum</i>
<i>Carduus laterifolius</i> Osterh. (1906)	<i>C. laterifolium</i> (Osterh.) Petr.	<i>C. laterifolium</i> (Osterh.) Rydb.			<i>C. clavatum</i> var. <i>americanum</i>	<i>C. centaureae</i>
<i>Carduus modestus</i> Osterh. (1913)	<i>C. modestum</i> (Osterh.) Rydb.	<i>C. modestum</i>			<i>C. clavatum</i> var. <i>americanum</i>	<i>C. griseum</i> var. <i>griseum</i>
<i>Carduus oreophilus</i> Rydb. (1901)		<i>C. oreophilum</i> (Rydb.) Rydb.			<i>C. clavatum</i> var. <i>americanum</i>	<i>C. griseum</i> var. <i>griseum</i>
<i>Carduus osterhoutii</i> Rydb. (1905)	<i>C. osterhoutii</i> (Rydb.) Petr.	<i>C. osterhoutii</i>			<i>C. clavatum</i> var. <i>osterhoutii</i>	<i>C. griseum</i> var. <i>osterhoutii</i>
<i>Carduus spathulatus</i> Osterh. (1905)	<i>C. scapanolepis</i> Petr.	<i>C. spathulifolium</i> Rydb.			<i>C. clavatum</i> var. <i>americanum</i>	<i>C. scapanolepis</i>
<i>Cirsium clavatum</i> var. <i>markaguntensis</i> S.L. Welsh (2003)				<i>C. clavatum</i> var. <i>markaguntensis</i> S.L. Welsh	<i>C. clavatum</i> var. <i>clavatum</i>	<i>C. markaguntense</i> (S.L. Welsh) Ackerfield & D.J. Keil, comb. nov.
<i>Cnicus clavatus</i> M.E. Jones (1895)	<i>C. clavatum</i> (M.E. Jones) Petr.	<i>C. clavatum</i>	<i>C. clavatum</i>	<i>C. clavatum</i>	<i>C. clavatum</i> var. <i>clavatum</i>	<i>C. clavatum</i>
<i>Cirsium eatonii</i> (A. Gray) B.L. Rob.						
<i>Carduus polyphyllus</i> Rydb. (1910)	<i>C. polyphyllum</i> (Rydb.) Petr.	<i>C. polyphyllum</i>			<i>C. eatonii</i> var. <i>murdockii</i> S.L. Welsh	<i>C. eatonii</i> var. <i>murdockii</i>
<i>Carduus tweedyi</i> Rydb. (1900)	<i>C. tweedyi</i> (Rydb.) Petr.	<i>C. tweedyi</i> (Rydb.) Rydb.			<i>C. eatonii</i> var. <i>murdockii</i>	<i>C. eatonii</i> var. <i>murdockii</i>

<i>Cirsium clokeyi</i> S.F. Blake (1938)					<i>C. eatonii</i> var. <i>clokeyi</i> (S.F. Blake) D.J. Keil	<i>C. eatonii</i> var. <i>clokeyi</i>
<i>Cirsium eatonii</i> var. <i>harrisonii</i> S.L. Welsh (1982)				<i>C. eatonii</i> var. <i>harrisonii</i> S.L. Welsh	<i>C. eatonii</i> var. <i>eatonii</i>	<i>C. harrisonii</i> (S.L. Welsh) Ackerfield & D.J. Keil, comb. nov.
<i>Cirsium eatonii</i> var. <i>murdockii</i> S.L. Welsh (1982)			<i>C. murdockii</i> (S.L. Welsh) Cronquist	<i>C. murdockii</i>	<i>C. eatonii</i> var. <i>murdockii</i>	<i>C. eatonii</i> var. <i>murdockii</i>
<i>Cirsium eatonii</i> var. <i>viperinum</i> D.J. Keil (2004)					<i>C. eatonii</i> var. <i>viperinum</i> D.J. Keil	<i>C. viperinum</i> (D.J. Keil) Ackerfield & D.J. Keil, comb. nov.
<i>Cirsium eriocephalum</i> A. Gray (1863)	<i>C. scopulorum</i> (Greene) Cockerell	<i>C. scopulorum</i>	<i>C. scopulorum</i>	<i>C. scopulorum</i>	<i>C. eatonii</i> var. <i>eriocephalum</i> (A. Gray) D.J. Keil	<i>C. scopulorum</i>
<i>Cirsium peckii</i> L.F. Hend. (1939)			<i>C. peckii</i>		<i>C. eatonii</i> var. <i>peckii</i> (L.F. Hend.) D.J. Keil	<i>C. peckii</i>
<i>Cnicus eatonii</i> A. Gray (1884)	<i>C. eatonii</i>	<i>C. eatonii</i>	<i>C. eatonii</i>	<i>C. eatonii</i> var. <i>eatonii</i>	<i>C. eatonii</i> var. <i>eatonii</i>	<i>C. eatonii</i> var. <i>eatonii</i>
<i>Cnicus hesperius</i> Eastw. (1898)	<i>C. hesperium</i> (Eastw.) Petr.	<i>C. hesperium</i> (Eastw.) Rydb.			<i>C. eatonii</i> var. <i>hesperium</i> (Eastw.) D.J. Keil	<i>C. hesperium</i>
<i>Cirsium scariosum</i> Nutt.						
<i>Carduus butleri</i> Rydb. (1910)	<i>C. butleri</i> (Rydb.) Petr.	<i>C. butleri</i>	,		<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>
<i>Carduus coloradensis</i> Rydb. (1905)	<i>C. coloradense</i> (Rydb.) Cockerell ex Daniels	<i>C. coloradense</i>	<i>C. scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>coloradense</i> (Rydb.) D.J. Keil	<i>C. coloradense</i>
<i>Carduus erosus</i> Rydb. (1901)	<i>C. erosus</i> (Rydb.) K. Schum.	<i>Cirsium centaureae</i> × <i>C. coloradense</i>	<i>C. scariosum</i>		<i>C. scariosum</i> var. <i>coloradense</i>	<i>C. coloradense</i>
<i>Carduus lacerus</i> Rydb. (1910)	<i>C. lacerum</i> (Rydb.) Petr.	<i>C. lacerum</i>	<i>C. scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>
<i>Carduus magnificus</i> A. Nelson (1912)	<i>C. magnificum</i> (A. Nelson) Rydb.	<i>C. magnificum</i>	<i>C. scariosum</i>		<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>
<i>Carduus oreophilus</i> Rydb. (1901)	<i>C. oreophilum</i> (Rydb.) Rydb.	<i>C. oreophilum</i>				Unresolved
<i>Carduus validus</i> Greene (1897)	<i>C. quercetorum</i> (A. Gray) Jeps. var. <i>citrinum</i> Petr.				<i>C. scariosum</i> var. <i>citrinum</i> (Petr.) D.J. Keil	<i>C. validum</i> (Greene) Ackerfield & D.J. Keil, comb. nov.
<i>Cirsium acaule</i> (L.) Scop. var. <i>americanum</i> A. Gray (1863)	<i>C. coloradense</i> (Rydb.) Cockerell ex Daniels ssp. <i>acaulescens</i> (A. Gray) Petr.	<i>C. acaulescens</i> (A. Gray) K. Schum.	<i>C. scariosum</i>		<i>C. scariosum</i> var. <i>americanum</i> (A. Gray) D.J. Keil	<i>C. tioganum</i>
<i>Cirsium congdonii</i> R.J. Moore & Frankton (1967)					<i>C. scariosum</i> var. <i>congdonii</i> (R.J. Moore & Frankton) D.J. Keil	<i>C. congdonii</i>

<i>Cirsium loncholepis</i> Petr. (1917)	<i>C. loncholepis</i>				<i>C. scariosum</i> var. <i>citrinum</i> (Petr.) D.J. Keil	Unresolved
<i>Cirsium quercetorum</i> (A. Gray) Jeps. var. <i>citrinum</i> Petr. (1917)	<i>C. quercetorum</i> var. <i>citrinum</i>				<i>C. scariosum</i> var. <i>citrinum</i>	<i>C. validum</i>
<i>Cirsium scariosum</i> Nutt. (1841)	<i>C. scariosum</i>	<i>C. scariosum</i>	<i>C. scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>	<i>C. scariosum</i> var. <i>scariosum</i>
<i>Cirsium scariosum</i> var. <i>robustum</i> D.J. Keil (2004)					<i>C. scariosum</i> var. <i>robustum</i> D.J. Keil	Unresolved
<i>Cirsium scariosum</i> var. <i>thorneae</i> S.L. Welsh (1982)				<i>C. scariosum</i> var. <i>thorneae</i> S.L. Welsh	<i>C. scariosum</i> var. <i>thorneae</i> S.L. Welsh	Unresolved
<i>Cirsium scariosum</i> var. <i>toyabense</i> D.J. Keil (2004)					<i>C. scariosum</i> var. <i>toyabense</i> D.J. Keil	Unresolved
<i>Cnicus drummondii</i> (Torr. & A. Gray) A. Gray var. <i>acaulescens</i> A. Gray (1874)	<i>C. coloradense</i> ssp. <i>acaulescens</i>	<i>C. acaulescens</i> (A. Gray) K. Schum.	<i>C. scariosum</i>		<i>C. scariosum</i> var. <i>americanum</i>	<i>C. tioganum</i>
<i>Cnicus tioganus</i> Congd. (1899)	<i>C. tioganum</i> (Congd.) Petr.		<i>C. scariosum</i>		<i>C. scariosum</i> var. <i>americanum</i>	<i>C. tioganum</i>

nuclear ribosomal DNA (nrDNA) regions. However, this work had a small sample size (35 North American taxa) and did not include multiple accessions from throughout species' geographic ranges. Kelch and Baldwin (2003) did recover a clade constituting adaptive radiation in the California Floristic Province. They also noted low levels of sequence divergence and hypothesized that *Cirsium* either recently radiated in North America or that rDNA is highly conserved in the group.

Aims

This study represents the first attempt to infer a broadly sampled phylogeny of nearly every species *Cirsium* in North America, in which populations of widespread species are sampled across their geographic and ecological range. The two main objectives of this study are to: (1) test whether currently hypothesized species varietal complexes (*C. arizonicum*, *C. clavatum*, *C. eatonii*, and *C. scariosum*) constitute monophyletic lineages, and (2) recircumscribe

any taxa that are identified as problematic using the general lineage (De Queiroz, 2007) and phenophyletic (Freudenstein et al., 2017) species concepts.

Materials and methods

Taxon sampling

A total of 168 accessions were sampled. Of these accessions, 18 were previously posted on GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and 68 were included in a previous study by Ackerfield et al. (2020; Appendix 2). Because of the taxonomic complexity of the genus and high frequency of *Cirsium* specimen misidentifications in herbaria, accessions from Kelch and Baldwin (2003) that could not be verified with voucher specimens were excluded. Our 168 accessions consist of 105 taxa from North America, representing 89% of the total taxa (118) sensu Keil (2006). Several taxa were sampled from multiple populations across their geographic and ecological ranges. Outgroups [*Carduus nutans* L., *C. arvensis*, and 13 Eurasian *Cirsium*] were selected using the *Carduus-Cirsium* group phylogeny in Ackerfield et al. (2020).

DNA extraction, amplification, and sequencing

DNA extractions were performed using DNeasy Plant MiniKits (Qiagen, Germantown, Maryland, U.S.A.) following the manufacturer's instructions. PCR products were generated for two transcribed spacer regions of the nuclear ribosomal DNA (ETS and ITS), and four plastid markers (*matK*, *ndhF*, *psbA-trnH*, and *trnL-trnF*; Table 2.2).

Table 2.2. Gene regions and primers used in amplification.

Gene region	Primer sequences	Reference	Approximate size (bp)
ITS	ITS4: TCC TCC GCT TAT TGA TAT GC ITS5A: GGA AGG AGA AGT CGT AAC AAG G ITS5_C3: GGA AGT AAA AGT CGT AAC AAG C	White & al. (1990) Downie & Katz-Downie (1996)	643
ETS	18S-ETS: ACT TAC ACA TGC ATG GCT TAA ETS-Car-1: TTC GTA TCG TTC GGT	Kelch & Baldwin (2003)	583
<i>matK</i>	trnK-710F: GTA TCG CAC TAT GT[T/A] TCA TTT GA	Susanna & al. (2006)	980

AST-1R: CCG CAC ACT TGA AC[G/C] ATA ACC CAG

<i>ndhF</i>	ndhF+607: ACC AAG TTC AAT GYT AGC GAG ATT AGT C ndhF1603: CCT YAT GAA TCG GAC AAT ACT ATG C	Jansen (1992)	636
<i>psbA-trnH</i>	psbA3f: GTT ATG CAT GAA CGT AAT GCT C psbAHf: CGC GCA TGG TGG ATT CAC ATT CC	Sang & al. (1997)	524
<i>trnL-trnF</i>	trnLC: CGA AAT CGG TAG ACG CTA CG trnLF: ATT TGA ACT GGT GAC ACG AG	Taberlet & al. (1991)	736

PCR reactions were performed in 25.1 μ l reactions containing 10.5 μ l of sterile water, 5 μ l of 10 \times PCR reaction Buffer A (Promega, Madison, Wisconsin, U.S.A.), 2 μ l of 10mM dNTPs (Pharmacia Biotech, Piscataway, New Jersey, U.S.A.), 2.5 μ l of 50 mM MgCl₂, 0.5 μ l of 10mg/ml Bovine Serum Albumin (Sigma, St. Louis, Missouri, U.S.A.), 1 μ l of 10mM of each of the two primers, 0.1 μ l Taq DNA polymerase enzyme (Bioline, Taunton, Massachusetts, U.S.A.), and 2.5 μ l of DNA template. The amount of DNA template was adjusted to generate sufficient PCR products for DNA sequencing when necessary. Amplification was performed on a Bio-Rad thermal cycler c1000 (Bio-Rad, Hercules, California, U.S.A.). The PCR program consisted of an initial denaturation at 95°C for 3 min; followed by 37 cycles of (94°C, 45 s; 54°C, 45 s; 72°C, 2 min), with a final 72°C, 7 min elongation step. ExoSAP-IT (Affymetrix, Cleveland, Ohio, U.S.A.) was used to purify PCR products before sequencing. The enzymatic removal of primers and excess dNTPs involved mixing 10 μ l of the PCR product with 1 μ l of ExoSAP-IT, incubating the mixture at 37°C for 30 min, and then raising the temperature to 80°C for 15 min to denature the ExoSAP-IT enzymes. Unincorporated dye terminators were removed using Sephadex gel filtration (GE Healthcare, Piscataway, New Jersey, U.S.A.) using MultiScreen plates (Millipore, Billerica, Massachusetts, U.S.A.). Cycle sequencing was performed using BigDye v.3.1 (Applied Biosystems, Foster City, California) at the Smithsonian Institution on a Hitachi 3730xl DNA Analyzer (Applied Biosystems, Foster City, California). Sequence reads of each PCR product were assembled and edited in Geneious v.5.6.3.

Phylogenetic analyses

All nucleotide sequences were aligned using MAFFT v.7 (Kato et al., 2017). The iterative refinement method of Q-INS-i, which considers the secondary structure information of rDNA, was used for ITS and ETS alignments. The G-INS-I algorithm was used for the plastid gene regions. The default gap opening penalty (1.53) was applied and the gap offset value was set to 0.1 for all alignments. Nucleotide sequences were further aligned manually in AliView (Larsson, 2014) using the procedure outlined in Simmons (2004) following Zurawski and Clegg (1987). Gaps were treated as missing data.

Characters were analyzed using several alternative potential process partitions as a means of data exploration (Bull et al., 1993). Each of the six gene regions was analyzed independently to resolve their respective gene trees. Gene trees for the two combined nrDNA regions and the four combined plastid loci were analyzed independently to check for mutually well-supported topological incongruence and hence evidence of potential introgression or lineage sorting (Doyle, 1992; Wendel et al., 1995). Gene trees were compared visually for topological incongruence, using a 75% bootstrap cut-off value. Topological incongruence was not expected among plastid regions because they are all part of the typically uniparentally inherited plastid genome (Gastony & Yatskievych, 1992).

As implemented in IQ-TREE v.1.6.10 (Nguyen et al., 2015), PartitionFinder (Lanfear et al., 2012) was used to find the best-fit likelihood models for each partition. Models were selected using the Bayesian Information Criterion (BIC). Based on these results, the following substitution models were chosen for each partition: ETS (HKY+R2), ITS (TNe+I+G4), *matK* (F81+I), *ndhF* (F81+I+G4), *psbA-trnH* (TPM2u+I+G4), and *trnL-trnF* (TPM2u+I+G4).

Maximum likelihood (ML) analyses (Felsenstein, 1973) were performed in IQ-TREE v.1.6.10 (Nguyen et al., 2015) using the substitution models described above. For concatenation-based species-tree inference, IQ-TREE has been shown to be comparable to or outperform other maximum likelihood programs (e.g. RAxML/ExaML) for matrices with 200 or fewer taxa (Zhou et al., 2017). Branch lengths were linked across partitions using an edge-proportional partition model with proportional branch lengths (IQ-TREE edge-proportional model, option `-spp`). This option accommodates different evolutionary rates between partitions (Duchene et al., 2018). Node support was determined by nonparametric bootstrapping using IQ-TREE's ultrafast bootstrap approximation (option `-bb`; Hoang et al., 2018) with 5000 pseudoreplicates. Near zero-length branches (with bootstrap support less than 50) were collapsed to polytomies in the final tree (option `-czb`).

Bayesian inference (BI) analyses (Yang & Rannala, 1997) were implemented in MrBayes (Huelsenbeck & Ronquist, 2001) via the Cyber Infrastructure for Phylogenetic Research online portal (CIPRES; <http://www.phylo.org/>). BI was performed using the best-fit partitioning scheme recommended by PartitionFinder (Lanfear et al., 2012). The “greedy” algorithm with branch lengths estimated as linked and the BIC were used to search for the best-fit partitioning scheme. This resulted in the the following partitioning scheme: GTR (General time reversible) substitution model (`nst=6`) with gamma-distributed rate variation across sites and a proportion of invariable sites (`=invgamma`) for the ETS, ITS *ndhF*, *psbA-trnH*, and *trnL-trnF* partitions, and the F81 substitution model (`nst=1`) for the *matK* partition. The concatenated dataset was subsequently subjected to Markov Chain Monte Carlo (MCMC) sampling using two replicates of four chains (one cold, three hot). Fifty million generations total were completed with a sampling frequency of every 1000 generations. Tracer v.1.5 (Rambaut & Drummond, 2013) was used to

visualize and analyze the MCMC trace files using a 25% burn-in value. All tree topologies were viewed in FigTree v.1.4.3 (Rambaut, 2016).

Taxonomic evaluations

Approximately 2500 herbarium specimens were examined from the following herbaria: ALA, ARIZ, ASU, BRY, CS, DAV, DES, MEXU, MONTU, OBI, OSC, RENO, RM, RSA, TEX, UNM, USCH, UTC, and WTU (Thiers, 2016). Type specimens were viewed on JSTOR Global Plants (<http://plants.jstor.org>) when not available for loan. I applied the general lineage species concept (De Queiroz, 2007) and phenophyletic concept (Freudenstein et al., 2017) to provide an objective framework for species delimitations. By also applying the phenophyletic view of Freudenstein et al. (2017), I allowed for species resolved as paraphyletic to be treated as the same species (Rieseberg & Brouillet, 1994) as long as they shared the same morphological phenotype and thus the same inferred ecological role. I used both concepts because the general lineage concept does not explicitly state that species can be paraphyletic. Species resolved as polyphyletic are not considered to be the same species under both concepts. All accessions from which DNA was sampled were verified by myself and delimited sensu Keil (2006).

Results

Phylogenetic analysis

The phylogenetic tree from the concatenated dataset, with node support for both the ML and BI is shown in Figures 1D, H, and K. Branches with less than 75 BS are collapsed to correspond to the discussion below. The BI tree is shown in Supplemental Figure 8. All ML trees for the combined plastid, combined nuclear, and individual gene regions are shown in Supplemental Figures 9–16. Of the 176 total parsimony-informative sites, 82% were found in the more rapidly evolving nrDNA ITS and ETS regions (Table 2.3). North American *Cirsium* taxa

are resolved as a clade with strong support (BS=98, PP=1.0; Fig. 2.1D, H). *Cirsium arvense* is resolved as sister to the North American clade. Neither *C. kamtschaticum* nor *C. heterophyllum* (the only two species occurring in both the Old and New Worlds) are resolved in the North American clade (Fig. 2.1K).

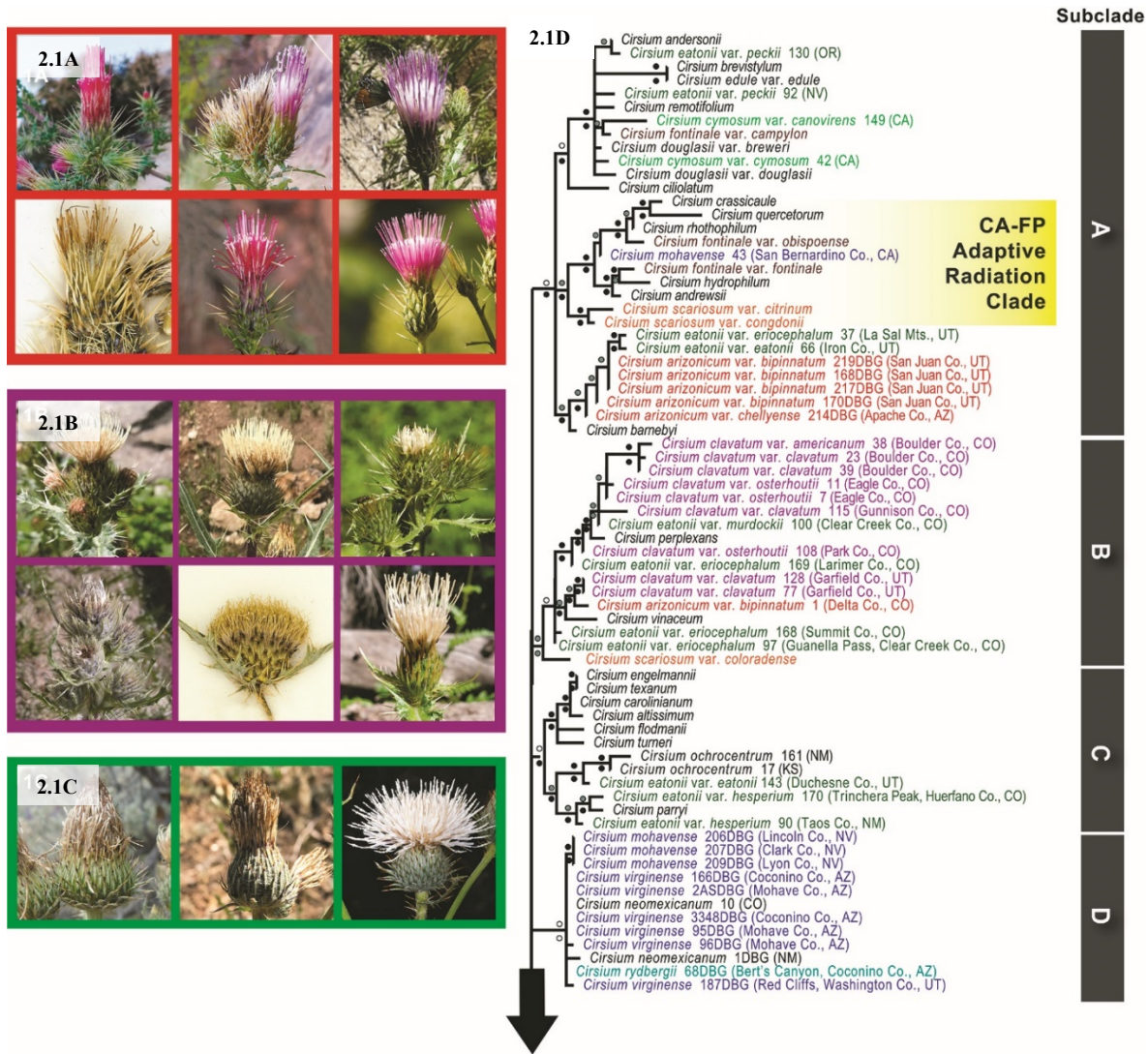


Figure 2.1H

Figure 2.1 A–D. Maximum likelihood phylogenetic reconstruction. Circles above nodes represent ML bootstrap support (BS) and those below nodes represent BI posterior probability (PP), values are: black circles $\geq 95\%$ (BS)/0.95 (PP); dark grey circles $\geq 85\%$ (BS)/0.85 (PP); white circles $\geq 75\%$ (BS)/0.75 (PP). Branches with less than 75% BS are collapsed. If multiple accessions of the same taxon are present, accession number and geographic locality are indicated in parentheses. Nomenclature for photographs follows Keil (2006) with our proposed taxon name in parentheses if different. Colors around photos correspond to taxon names in the

phylogeny. Photos by J. Ackerfield except where noted. A, *C. arizonicum* varietal complex, top row: var. *arizonicum*, var. *bipinnatum* (*C. pulchellum*), var. *bipinnatum* (*C. calcareum*; photo by Al Schneider), bottom row: var. *chellyense* [*C. chellyense*; U.S.A., Arizona: Canyon de Chelly, I.W. Clokey 680 (DAO)], var. *rothrockii* (photo by Bob Nieman), and var. *tenuisectum* (photo by Lonny Holmes). B, *C. clavatum* varietal complex, top row: var. *clavatum* (*C. centaureae*), var. *clavatum* (*C. clavatum*; photo by Chuck Smith), var. *clavatum* (*C. griseum* var. *griseum*), bottom row: var. *osterhoutii* (*C. griseum* var. *osterhoutii*), var. *clavatum* [(*C. markaguntense*; U.S.A., Utah: Iron Co., N.D. Atwood & L.C. Higgins 5918 (BRY)], var. *clavatum* (*C. scapanolepis*; photo by Sean Mallory). C, *C. cymosum* varietal complex, from left to right: var. *canovirens* (*C. canovirens*; photo by Matt Lavin), var. *canovirens* (*C. cymosum*; photo by Morgan Stickrod), var. *cymosum* (*C. cymosum*; photo by Terry Gosliner).

Figure 2.1D

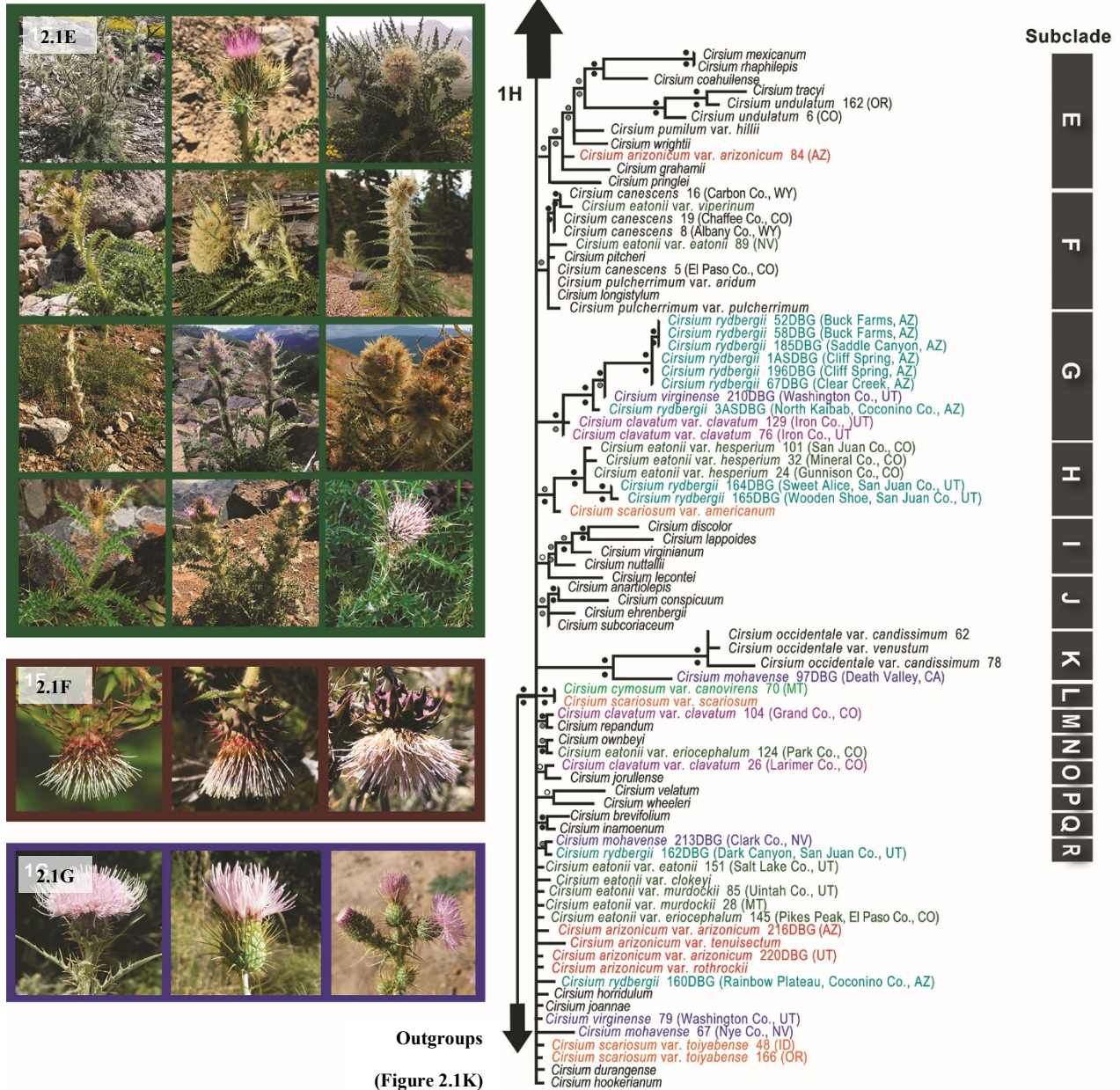


Figure 2.1 E–G. E, *C. eatonii* varietal complex, top row from left to right: var. *clokeyi* (photo by Dr. Tom Armbruster), var. *eatonii* (photo by H. Tracy), var. *eriocephalum* (*C. scopulorum*), second row: var. *eriocephalum* (*C. griseum* var. nov.; photo by Jim Bromberg), var. *eriocephalum* (*C. sp. nov.*), var. *eriocephalum* (Pike’s Peak; *C. scopulorum*), third row: var.

eriocephalum (La Sal Mts.; *C. sp. nov.*; photo by Hannah Wacker), var. *hesperium* (San Juan Mts.; *C. hesperium*), var. *hesperium* (Culebra Range; *C. sp. nov.*), bottom row: var. *murdockii* (*C. eatonii* var. *murdockii*), var. *peckii* (*C. peckii*; photo by iNaturalist user rachell1976), var. *viperinum* (*C. viperinum*; photo by Corey Lange). F, *C. fontinale* varietal complex, from left to right: var. *campylon* (photo by David Greenberger), var. *fontinale* (photo by Angela Pai), and var. *obispoense* (photo by Paul Excoffier). G, *C. mohavense* (from left to right): *C. mohavense* (San Bernardino Co.; photo by Ron Vanderhoff), *C. mohavense* (Nevada; photo by Lonny Holmes) and *C. virginense* (Utah).

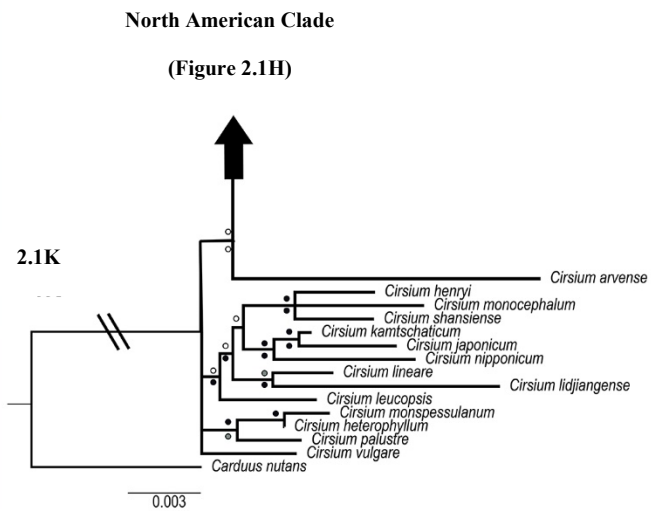


Figure 2.1 I–K. I, *C. rydbergii*, from left to right: *C. rydbergii* (*C. rydbergii*; photo by Isaac Marck), *C. rydbergii* Buck Farms population (*C. sp. nov.*; photos by W. Hodgson), *C. rydbergii* Cliff Springs population (*C. sp. nov.*; inset by W. Hodgson). J, *C. scariosum* varietal complex, top row: var. *americanum* (*C. tioganum*), var. *citrinum* (*C. validum*; photo by Alice Abela), var. *coloradense* (*C. coloradense*), bottom row: var. *congdonii* (*C. congdonii*; photo by iNaturalist user leptonia), var. *scariosum*, var. *toyabense* (photo by iNaturalist user dawnvla).

The North American clade is divided into 18 subclades (A–R; Fig. 2.1D, H). Eighteen accessions are resolved as part of the polytomy in the North American clade after the collapse branches with ≤ 75 BS. Some subclades are well-supported in the ML analysis but poorly supported (with posterior probabilities less than 0.75) in the BI tree. There is some biogeographic structure (Bailey, 1998) among subclades but no resolution at the backbone of the North

Table 2.3. Statistics for each data matrix.

	ETS	ITS	<i>matK</i>	<i>ndhF</i>	<i>psbA-trnH</i>	<i>trnL-trnF</i>	Plastid	Nuclear	Combined
Number of accessions	164	167	146	80	152	126	125	168	168
Number of characters	580	644	964	634	500	724	2822	1224	4046
Missing data (%)							16	1	17
Number of singleton sites	61	73	26	4	12	7	49	134	183
Number of parsimony informative characters	67	77	4	4	17	7	32	144	176

American clade. Subclade A (BS=78, PP=0.99) is further divided into three smaller clades. The first smaller clade consists of taxa from the Pacific Northwest and California, the second smaller clade consists of taxa endemic to the California Floristic Province, and the third smaller clade consists of taxa from the Colorado Plateau. Subclade B (BS=88, PP=0.98) consists of taxa from the Rocky Mountains. Subclade C (BS=79, PP=0.97) is further divided into two smaller clades. The first clade consists of taxa from the Great Plains and southeast, and the second clade consists of taxa from the Great Plains and Rocky Mountains. Subclade D (BS=76, PP=0.84) consists of taxa from the desert Southwest. Subclade E (BS=85, PP=0.5) consists of taxa mostly from the desert Southwest and Mexico. Subclade F (BS=88, PP=0.53) consists mostly of taxa from the Rocky Mountains and Intermountain Region. Subclade G (BS=98, PP=0.91) consists of taxa from the desert Southwest and Colorado Plateau. Subclade H (BS=94, PP=0.99) consists of taxa from the Rocky Mountains and Colorado Plateau. Subclade I (BS=84, PP=0.64) consists of taxa from the southeastern U.S. Subclade J (BS=91, PP=0.86) consists of taxa from Mexico. Subclade K (BS=98, PP=1.0) consists of taxa from California.

Seven small subclades of only two taxa are resolved in the ML analysis. Three of these subclades (O, P, and R) are not resolved in the BI. Subclade L (BS=100, PP=0.96) consists of *C.*

cymosum (Greene) J.T. Howell var. *canovirens* (Rydb.) D.J. Keil (accession 70) and *C. scariosum* var. *scariosum* (accession 164) from the Rocky Mountains. Subclade M (BS=97, PP=0.89) consists of *C. clavatum* var. *clavatum* (accession 104) from the Rocky Mountains and *C. repandum* Michx. from the southeast. Subclade N (BS=89, PP=0.96) consists of *C. ownbeyi* S.L. Welsh and *C. eatonii* var. *eriocephalum* (A. Gray) D.J. Keil (accession 124) from Colorado. In the BI, this clade also contains *C. arizonicum* var. *tenuisectum* D.J. Keil. Subclade O (BS=78, PP=0.58) consists of *C. clavatum* var. *clavatum* (accession 26) and *C. jorullense* (Kunth) Spreng. Subclade P (BS=77) consists of *C. wheeleri* (A. Gray) Petr. and *C. velatum* (S. Watson) Petr. Subclade Q (BS=98, PP=1.0) consists of *C. brevifolium* Nutt. and *C. inamoenum* (Greene) D.J. Keil from the Pacific Northwest and Rocky Mountains. Lastly, subclade R (BS=93) consists of *C. mohavense* (Greene) Petr. (accession 213DBG) and *C. rydbergii* Petr. (accession 162DBG). Only relationships supported by both the BI and ML analyses with bootstrap and posterior probability support values equal to or higher than 75 and 0.75 respectively were considered for the discussion below.

Incongruence

Six instances of strongly supported incongruence between the nuclear gene tree (consisting of two linked gene regions) and plastid gene tree (consisting of four linked gene regions) were recovered. First, the plastid gene tree shows a clade containing *C. eatonii* var. *hesperium* (Eastw.) D.J. Keil (accession 32), *C. eatonii* var. *hesperium* (accession 170), *C. pulcherrimum* (Rydb.) K. Schum. var. *pulcherrimum*, *C. parryi* (A. Gray) Petr., and *C. grahamii* A. Gray (BS=99, PP = 0.94; Fig. S1B). However, in the nuclear gene tree *C. eatonii* var. *hesperium* (accession 32) is resolved in a clade with *C. eatonii* var. *hesperium* (accessions 24, 101) and *C. rydbergii* (accessions 164DBG, 165DBG; BS=95, PP=0.99; Fig. S1C). *Cirsium*

pulcherrimum var. *pulcherrimum* is resolved in a clade with *C. canescens* Nutt. in the nuclear gene tree (BS=99, PP=0.85; Fig. S1C). *Cirsium parryi* is resolved in a clade with *C. eatonii* var. *hesperium* (accession 90), *C. eatonii* var. *eatonii* (accession 143), and *C. ochrocentrum* A. Gray (BS=95, PP=0.89; Fig. S1C).

Second, the plastid gene tree shows a clade containing *C. crassicaule* (Greene) Jeps., *C. ownbeyi*, *C. eatonii* var. *eriocephalum* (accession 124), and *C. arizonicum* var. *tenuisectum* (BS=96, PP=0.8; Fig. S1B). However, *C. crassicaule* is resolved in the CA-FP clade in the nuclear gene tree (BS=100, PP=1.0; Fig. S1C). Third, the plastid gene tree shows strong support for a clade of *C. brevifolium* and *C. undulatum* (Nutt.) Spreng. (BS=99, PP=0.94; Fig. S1B), while in the nuclear gene tree *C. undulatum* is sister to *C. tracyi* (Rydb.) Petr. (BS=100, PP=1.0) and *C. brevifolium* is sister to *C. inamoenum* (BS=100, PP=0.87; Fig. S1C). Fourth, the plastid gene tree recovers a clade containing *C. clavatum* var. *clavatum* (accession 26), *C. velatum*, and *C. jorullense* (BS=98, PP=0.99; Fig. S1B). However, the nuclear gene tree places *C. clavatum* var. *clavatum* (accession 26) in a clade with *C. canescens* (BS=99, PP=0.85; Fig. S1C). The hypothesized causes of the incongruence for each of the above are presented in the discussion.

Lastly, the *psbA-trnH* gene tree shows a strongly supported sister-species relationship between *Carduus nutans* (outgroup) and *C. discolor* (Muhl. ex Willd.) Spreng. (BS=100, PP=1.0; Fig. S1H). However, *C. discolor* is resolved in the North American *Cirsium* clade in the ETS (BS=85, Fig. S1D), ITS (BS=93, PP=1.0; Fig. S1E), and *trnL-trnF* gene trees (BS=85, PP=1.0; Fig. S1I). All *C. discolor* sequences were obtained from GenBank. I therefore suspect that either contamination for the *psbA-trnH* locus or misidentification is the cause of incongruence. *Cirsium discolor* is resolved in the North American clade in our concatenated phylogeny.

Discussion

Species Delimitation of North American Thistles

Cirsium is one of the most taxonomically difficult genera of Compositae in North America. The molecular phylogenetic results presented here provide novel insights for species delimitations using the general lineage and phenophyletic species concepts. However, within *Cirsium* the taxonomic challenges are many and in some instances, additional morphological and molecular work must be completed prior to recircumscription. The circumscriptions of eight species (*C. arizonicum*, *C. clavatum*, *C. cymosum*, *C. eatonii*, *C. fontinale* (Greene) Jeps., *C. mohavense*, *C. rydbergii*, and *C. scariosum*) sensu Keil (2006) are resolved as polyphyletic. In the following discussion, the term lineage is used in reference to subclades A-R and not to accessions that are part of the North American clade polytomy.

Cirsium arizonicum complex

Cirsium arizonicum is widely distributed throughout the Colorado Plateau and deserts of southwestern U.S. and northern Mexico. Members of this varietal complex share unique morphological traits compared to other *Cirsium* taxa including short (1–4.5 mm long) stigmatic tips and corolla lobes over 10 mm long and about twice as long as the throat (i.e., the portion of the corolla between the base of the lobes and the level of filament attachment; Moore & Frankton, 1974; Barlow-Irick, 2003; Fig. 2.1A). These taxa are also primarily pollinated by hummingbirds (Barlow-Irick, 2003; Eckberg et al., 2017). There are currently five varieties recognized within this complex: *arizonicum*, *bipinnatum* (Eastw.) D.J. Keil, *chellyense* (R.J. Moore & Frankton) D.J. Keil, *rothrockii* (R.J. Moore & Frankton) D.J. Keil, and *tenuisectum* (Keil, 2006). These varieties are further subdivided by corolla color. Varieties *arizonicum* and

rothrockii have red corollas, whereas varieties *bipinnatum*, *chellyense*, and *tenuisectum* have pink or purple corollas (Fig. 2.1A).

Historically, there has been much disagreement over species delimitations within this complex (Table 1.1). Petrak (1917) initially recognized five species: *C. arizonicum*, *C. calcareum* (M.E. Jones) Wooton & Standl., *C. nidulum* (M.E. Jones) Petr., *C. pulchellum* (Greene ex Rydb.) Wooton & Standl., and *C. rothrockii* (A. Gray) Petr. Moore and Frankton (1974) later revised the group and recognized these same species albeit they synonymized *C. pulchellum* with *C. calcareum*. Moore and Frankton (1974) also described three new species: *C. chellyense* R.J. Moore & Frankton, *C. chuskaense* R.J. Moore & Frankton, and *C. navajoense* R.J. Moore & Frankton. Barlow-Irick (2003) performed a multivariate analysis of the *Cirsium arizonicum* complex and found support for the recognition of six species: *C. arizonicum*, *C. calcareum*, *C. chellyense*, *C. pulchellum*, as well as two new species that were never formally described, “*C. aleatorium*” and “*C. surrepticum*.” Barlow-Irick (2003) synonymized *C. nidulum* and *C. rothrockii* with *C. arizonicum*, and *C. chuskaense* with *C. chellyense*. Keil (2006) likewise considered *C. arizonicum* var. *nidulum* (M.E. Jones) S.L. Welsh conspecific with *C. arizonicum* var. *arizonicum*.

Cirsium arizonicum sensu Keil (2006) is resolved as polyphyletic in our inferred phylogeny, consisting of at least three distinct evolutionary lineages (i.e., mono- or paraphyletic groups; Fig. 2.1D, H). The first lineage consists of accession 84 of variety *arizonicum*. Subclade E (BS=85) is not strongly supported in our BI (PP=0.50), but within this subclade *C. arizonicum* var. *arizonicum* is well-supported as sister to *C. grahamii* in both analyses (BS=87, PP=0.83; Fig. S1A, B). All other accessions of varieties *arizonicum* and *rothrockii* are part of the North American clade polytomy. However, these are the only varieties that exhibit red corollas (versus

purple or pink; Fig. 2.1A; Keil, 2006). In addition, these two varieties are sympatric in distribution (Moore & Frankton, 1974; Barlow-Irick, 2003; Keil, 2006). The only phenotypic difference among the taxa noted by Keil (2006) is in leaf pubescence (variety *rothrockii* glabrous vs. variety *arizonicum* with abaxial tomentum). Therefore, for now I recommend continued use of the treatment proposed by Keil (2006), recognizing *C. arizonicum* var. *arizonicum* (including var. *nidulum*) and *C. arizonicum* var. *rothrockii*.

Second, accessions corresponding to variety *bipinnatum* sensu Keil (2006) are resolved as polyphyletic in two distinct evolutionary lineages. Accession 1 from Colorado is resolved in subclade B (BS=88, PP=0.98). However, accessions 168DBG, 170DBG, 217DBG, and 219DBG from southeastern Utah are resolved in subclade A (BS=78, PP=0.99). Barlow-Irick (2003) separated *C. calcareum* from *C. pulchellum* based on the presence of longer corolla lobes (13–18 mm vs. 8–13 mm) and mostly shorter style tips (1–3 mm vs. 2–4.5 mm). Accession 1 from Colorado corresponds phenotypically to *C. pulchellum*, while the accessions from southern Utah and northern Arizona correspond phenotypically to *C. calcareum* sensu Barlow-Irick (2003). Given the unique phenotypes and evolutionary lineages of each, I recommend recognition of both *C. calcareum* and *C. pulchellum* as distinct species.

Lastly, although I only included one accession (214DBG) of variety *chellyense*, this variety is phenotypically distinct from its sister taxa in subclade A. In contrast to *C. calcareum*, *C. chellyense* has long-decurrent leaf bases (10–20 mm versus shortly decurrent to 8 mm) and multicellular hairs present on the stem (versus glabrous to tomentose; Moore & Frankton, 1974; Barlow-Irick, 2003). I therefore recommend recognition of *C. chellyense* as distinct from *C. calcareum* given the unique phenotypes of each taxon.

Our single accession of variety *tenuisectum* is resolved within subclade N in our BI with good support (PP=0.96; Fig. S1B) but is part of the North American-clade polytomy in our ML analysis (Fig. 2.1H). I hypothesize that this taxon arose by hybridization of *C. arizonicum* with *C. mohavense*. In our *trnL-trnF* gene tree, variety *tenuisectum* is resolved in a clade with *C. arizonicum* (BS=75, PP=0.97; Fig. S1J). However, in our ETS gene tree, variety *tenuisectum* is resolved in a clade with *C. mohavense*, albeit with low support (BS=67; Fig. S1E). Additional evidence can be found by comparing the morphologies of the three taxa. Phenotypically, variety *tenuisectum* is intermediate between *C. arizonicum* and *C. mohavense*, having dark pink corollas (vs. light pink in *C. mohavense* and red in *C. arizonicum*) and exerted styles (as in *C. arizonicum*; Fig. 2.1A, 2.1G). Until additional evidence suggests otherwise, I recommend continued use of this taxon as a variety within the *C. arizonicum* complex sensu Keil (2006). Based on the type locality and original descriptions of *C. arizonicum* var. *tenuisectum* and “*C. aleatorium*,” I believe that these binomials are referencing the same individuals.

Cirsium clavatum complex

Cirsium clavatum is a polymorphic complex of thistles occurring throughout montane forests and alpine ecosystems in Utah and Colorado. *Cirsium clavatum* is characterized by the presence of sessile to shortly decurrent leaf bases, white corollas, and lower involucre bracts with lateral spines (Keil, 2006; Fig. 2.1B). Rydberg (1922) initially recognized eight species that are now considered synonymous with *C. clavatum* (Keil, 2006): *C. araneans* Rydb., *C. centaureae* (Rydb.) K. Schum., *C. griseum* (Rydb.) K. Schum., *C. laterifolium* (Osterh.) Rydb., *C. modestum* (Osterh.) Rydb., *C. oreophilum* (Rydb.) Rydb., *C. osterhoutii* (Rydb.) Petr., and *C. spathulifolium* (Osterh.) Rydb. In his *Manual of the Plants of Colorado*, Harrington (1954) recognized only two of Rydberg’s species (*C. centaureae* and *C. spathulifolium*). Harrington

(1954) considered *C. griseum* and *C. modestum* as synonymous with *C. spathulifolium*.

Harrington (1954) also erroneously included *C. eatonii* as present in Colorado. A later treatment by Weber & Wittmann (2011) continued to erroneously include *C. eatonii* for Colorado, and recognized only *C. centaureae* out of Rydberg's original eight species. Specimens in Colorado that would have corresponded to *C. spathulifolium* were subsequently identified as *C. eatonii*.

Keil (2006) attempted to rectify the resulting taxonomic confusion by recognizing *C. clavatum* as present in Colorado and restricting *C. eatonii* (as var. *eatonii*) to populations in Utah. Keil (2006) also recognized three varieties within *C. clavatum* to account for some of the variation in the group: *clavatum*, *americanum* (A. Gray) D.J. Keil, and *osterhoutii* (Rydb.) D.J. Keil (Keil, 2006; Table 2.1). However, Keil (2006) noted that as currently circumscribed, *C. clavatum* remained polymorphic across its range.

Cirsium clavatum is resolved as polyphyletic in our inferred phylogeny in at least five distinct evolutionary lineages (Fig. 2.1D, H). Although I did not sample directly from the type locality of *C. clavatum* [U.S.A., UT: Fish Lake, Sevier Co., M.E. Jones s.n. (BRY)], I did sample from nearby populations in Garfield Co., UT (accessions 77 and 128) that phenotypically correspond to the type specimen. These accessions are resolved in subclade B (BS=88, PP=0.98). A second evolutionary lineage from Utah was resolved consisting of *C. clavatum* accessions 76 and 129 (Subclade G, BS=98, PP=0.91). These specimens are morphologically distinct from the *C. clavatum* type specimens in with dark-infused involucre bracts (Fig. 2.1B). Specimens with this phenotype and from this geographic area were previously described as *C. clavatum* var. *markaguntense* S.L. Welsh (Welsh et al., 2003). I therefore propose making a new combination and recognizing this taxon at the specific rank as *C. markaguntense* (S.L. Welsh) Ackerfield & D.J. Keil, comb nov.

The Colorado accessions of *C. clavatum* are resolved in at least three distinct evolutionary lineages. Each of these lineages corresponds to a species previously recognized by Rydberg (1917, 1922). The first lineage consists of accessions 23, 38, and 39 (Subclade B, BS=88, PP=0.98; Fig. 2.1H). These accessions correspond to *C. clavatum* var. *clavatum* (accessions 23, 39) and var. *americanum* (accession 38) sensu Keil (2006). However, they differ from *C. clavatum* sensu stricto in having more than one head clustered at the tips of stems, and erose, fringed, or dilated and twisted inner involucre bract apices (Fig. 2.1B). Plants corresponding to this phenotype were previously described as *C. centaureae* (Rydberg, 1901). I believe that *C. laterifolium* is synonymous with *C. centaureae* based on several shared morphological features including involucre bracts fringed or erose and similar habit.

Closely related to *C. centaureae* are accessions assigned to multiple different taxa sensu Keil (2006). Accessions 7, 11, and 108 correspond to *C. clavatum* var. *osterhoutii*, while accession 115 corresponds to *C. clavatum* var. *clavatum*. Two additional accessions correspond to *C. eatonii* var. *murdockii* S.L. Welsh (accession 100) and *C. eatonii* var. *eriocephalum* (accession 169). I propose that these accessions are all varieties of Rydberg's (1901) *C. griseum*. *Cirsium griseum* is unique among the other "*C. clavatum*" lineages in having involucre bracts that are subequal (vs. imbricate) and tipped with flat, stout spines (vs. rounded, shorter spines; Fig. 2.1B). Our accession 115 corresponds morphologically and geographically to the type specimen of *Carduus griseus* Rydb. [(U.S.A., CO: Telluride, San Miguel Co., F. Tweedy 321 (US)], that was later transferred to the genus *Cirsium*. I believe Rydberg's *C. modestum* and *C. oreophilum* also correspond morphologically to and are thus conspecific with *C. griseum*.

I propose that variety *osterhoutii* be recognized within the *C. griseum* varietal complex as *C. griseum* var. *osterhoutii* (Rydb.) Ackerfield & D.J. Keil, comb nov. Variety *osterhoutii* is

separable from the other varieties by the presence of densely pubescent involucre bracts (Keil, 2006; Ackerfield, 2015; Fig. 2.1B). Variety *osterhoutii* is endemic to Colorado where it occurs at mostly higher elevations (10,500–13,500 ft.) than variety *griseum* (Ackerfield, 2015). However, one of us (JRA) has observed intermediate forms between variety *osterhoutii* and variety *griseum* where the two overlap in elevation. I believe that Rydberg's *C. araneans* (G.E. Osterhout 2169, 26 Jun 1900, Red Cliff, Eagle Co., CO, NY) is conspecific with *C. griseum* var. *osterhoutii*. This specimen is morphologically similar to and collected at the type locality for *C. osterhoutii* [U.S.A., CO: Red Cliff, Eagle Co., G.E. Osterhout 2706 (RM)]. Our sequenced specimen of *C. eatonii* var. *murdockii* (accession 100) from Colorado also corresponds to the interpretation of Rydberg's *C. griseum* var. *osterhoutii* presented here.

Accession 169 was originally identified as *C. eatonii* var. *eriocephalum* based on a shared alpine habitat and similar morphology (i.e., strongly undulate leaves and heads in a dense terminal cluster; Fig. 2.1E). However, upon closer inspection this specimen differs from *C. eatonii* var. *eriocephalum* in having pink to white corollas (vs. purple; Fig. 2.1E). This lineage is resolved here within the *C. griseum* complex. Further work is necessary to ascertain if this lineage constitutes part of the *C. griseum* complex, is a hybrid between *C. griseum* and *C. scopulorum*, or is a distinct (yet cryptic) species separate from either *C. griseum* or *C. scopulorum*. For now, I propose that this be recognized as a new variety within the *C. griseum* complex, as it may hybridize freely with other *C. griseum* varieties.

The third evolutionary lineage consists of accession 104 from Grand Co., Colorado (Subclade M, BS=97, PP=0.89; Fig. 2.1D). This accession corresponds morphologically and geographically to Rydberg's (1917, 1922) *C. spathulifolium*. *Cirsium spathulifolium* exhibits imbricate involucre bracts, lack lateral spines on the lower involucre bracts, and entire

involucral bract apices (Fig. 2.1B). This species was named from Osterhout's type specimen of *Carduus spathulatus* Osterh. [U.S.A., CO: North Park, Saw Mill, Larimer Co., G.E. Osterhout 2254 (RM)]. Although Rydberg used the epithet *spathulifolium* in his *Cirsium* species descriptions, in his key he inadvertently listed the species as *C. spathulatum*. However, Petrak (1917) transferred Osterhout's species from *Carduus* to *Cirsium* as *C. scapanolepis* (Osterh.) Petr. just prior to Rydberg's *C. spathulifolium* description, thus rendering Rydberg's name invalid.

Keil (2006) hypothesized that variety *osterhoutii* may share a close relationship with the alpine *C. eatonii* var. *eriocephalum* based on the presence of densely pubescent involucral bracts and strongly undulate leaves observed in both taxa. However, accessions 124 of *C. eatonii* var. *eriocephalum* and 108 of variety *osterhoutii* were both collected from the same locality. These accessions are not resolved in the same clade, indicating that although variety *osterhoutii* and *C. eatonii* var. *eriocephalum* are morphologically similar in having densely woolly involucral bracts, they are distinct evolutionary lineages. One author (JRA) also notes that variety *osterhoutii* and the yellow form of *C. eatonii* var. *eriocephalum* co-occur with no intermediates.

Cirsium cymosum complex

Cirsium cymosum var. *cymosum*, *C. cymosum* var. *canovirens*, *C. brevifolium*, and *C. inamoenum* are a taxonomically difficult group distributed in California, the Pacific Northwest, and northern Rocky Mountains (Lesica, 2012). These taxa also have several shared morphological features including solitary pedunculate heads, white to pale lavender corollas, and leaves auriculate-clasping to decurrent on the stem to 3 cm (Keil, 2006; Fig. 2.1C). The differences among the taxa are subtle and often not well represented on herbarium specimens. For instance, *C. cymosum* var. *cymosum* and *C. inamoenum* have an inconspicuous glutinous

dorsal ridge on the involucre bracts (Greene, 1897) while *C. brevifolium* and *C. cymosum* var. *canovirens* exhibit a prominent dorsal ridge (Keil, 2006). However, the glutinous dorsal ridge can be difficult to see on herbarium specimens and usually dries brown, making this a difficult criterion to use post collection.

Cirsium cymosum and *C. inamoenum* were concurrently described (as *Carduus*) by Greene (1897). Greene (1897) did not list type specimens in his descriptions, but noted that *Carduus inamoenus* Greene was conspecific with his *Carduus undulatus* Nutt. var. *nevadensis* Greene specimen [U.S.A., CA: Truckee Valley, Greene s.n. (F)]. Additional specimens listed in his description [U.S.A., CA: West Humboldt, Greene s.n. (NDG); U.S.A., WA: Mill Plain, Howell s.n. (NDG)] were later annotated to *C. neomexicanum* A. Gray and *C. brevifolium*, respectively. *Cirsium canovirens* (Rydb.) Petr. was later described (as *Carduus*; Rydberg, 1900) from Montana [U.S.A., MT: Yellowstone Park, Jack Creek Canon, P.A. Rydberg & E.A. Bessey 5213 (K)]. Although *C. canovirens* was sometimes treated at the specific level (Cronquist, 1994; Welsh et al., 2003), this taxon was considered a variety of *C. cymosum* by Keil (2006). Other authors have considered *C. canovirens* conspecific with *C. inamoenum* (Dorn, 2001; Lesica, 2012). Cronquist (1994) synonymized *C. inamoenum* under *C. subniveum* Rydb. (Rydberg, 1917).

I recovered three distinct evolutionary lineages for these taxa in our inferred phylogeny (Fig. 2.1D, H). The first lineage consists of California accessions of *C. cymosum* var. *cymosum* and *C. cymosum* var. *canovirens* (Subclade A, BS=78, PP=0.99; Fig. 2.1H). The second lineage consists of accessions of *C. brevifolium* and *C. inamoenum* (Subclade Q, BS=98, PP=1.0; Fig. 2.1D). The third lineage consists of an accession of *C. cymosum* var. *canovirens* from near the type locality for *C. canovirens* in Montana (Subclade L, BS=100, PP=0.96; Fig. 2.1D).

The California lineage of *C. cymosum* consists of accessions of varieties *cymosum* and *canovirens* sensu Keil (2006). Therefore, I first propose that *C. cymosum* be recircumscribed to include specimens also attributable to variety *canovirens* from California and Oregon.

Additionally, I propose that *C. canovirens* be recognized at the specific rank but restricted in range to Idaho, Montana, and Nevada.

Cirsium brevifolium and *C. inamoenum* are distinct evolutionary lineages from *C. cymosum* and *C. canovirens* despite sharing many morphological features. The type specimen of *C. inamoenum* was collected in California, whereas our sequenced accession of *C. inamoenum* was collected in Nevada. Therefore, I cannot conclusively state that this accession corresponds to Greene's *C. inamoenum*. Alternatively, our Nevada accession may correspond to Rydberg's *C. subniveum* [U.S.A., WY: Jackson Hole, A Nelson 1070 (US)]. Additional sampling of *C. inamoenum* from California and Wyoming are necessary to clarify species boundaries among these taxa.

I hypothesize that *C. brevifolium* is the result of past hybridization between *C. undulatum* and *C. inamoenum*. Our plastid gene tree shows a well-supported clade containing *C. undulatum* and *C. brevifolium* (BS=99, PP=0.94; Suppl. Fig. 9). However, *C. undulatum* is sister to *C. tracyi* in our nuclear gene tree (BS=100, PP=0.87; Suppl. Fig. 10). *Cirsium undulatum* has a wide range throughout the Great Plains and Rocky Mountains (Keil, 2006). Therefore, it is possible that *C. undulatum* could have been sympatric with *C. inamoenum* in the past.

Cirsium eatonii complex

The *C. eatonii* varietal complex is a polymorphic assemblage of thistles distributed on mountain peaks throughout the southern Rocky Mountain and Intermountain Regions (Fig. 2.1E). *Cirsium eatonii* is also one of the most problematic, taxonomically challenging complexes

of thistles in North America (Keil, 2006; Lesica, 2012; Ackerfield, 2015). Most recently, Keil (2006) divided the complex into seven varieties: *clokeyi* (S.F. Blake) D.J. Keil, *eatonii*, *eriocephalum*, *hesperium*, *murdockii*, *peckii* (L.F. Hend.) D.J. Keil, and *viperinum* D.J. Keil. These varieties are in part distinguished based on their involucre bract surface. Varieties *eatonii*, *clokeyi*, and *viperinum* have glabrous involucre bracts with conspicuous lateral spines present on the outer bracts. Alternatively, varieties *eriocephalum*, *hesperium*, *murdockii*, and *peckii* have tomentose involucre bracts that mostly lack lateral spines.

Cirsium eatonii was first described as *Cnicus eriocephalus* A. Gray var. *leiocephalus* D.C. Eaton by Gray (1874) from a Summit Co., UT collection made by Daniel C. Eaton and Sereno Watson in 1869. Gray (1883) later elevated this variety to *Cnicus eatonii* A. Gray in honor of Eaton. Heller (1898) transferred the species to *Carduus* as *Carduus leiocephalus* (D.C. Eaton) A. Heller. However, it was Robinson (1911) who transferred the species to *Cirsium* [*Cirsium eatonii* (A. Gray) B.L. Rob.]. Welsh (1982) subsequently subdivided *Cirsium eatonii* into three varieties: *eatonii*, *harrisonii* S.L. Welsh, and *murdockii*. Although Welsh (1982) described variety *harrisonii* as distinct from variety *eatonii* based on the presence of dark purple involucre bracts, it is considered conspecific with variety *eatonii* by Keil (2006).

The other two varieties with glabrous involucre bracts, *clokeyi* and *viperinum*, are each endemic to mountain peaks in Nevada (Keil, 2006). When Blake (1938) described *C. clokeyi* from the Charleston Mountains (Clark Co., NV), he noted the similarity of this new species to *C. eatonii*. However, Blake (1938) separated *C. clokeyi* from *C. eatonii* by the presence of larger heads and stouter, longer spines on the involucre bracts in *C. clokeyi*. Blake (1938: 10) also noted that *C. clokeyi* was “the most savagely armed of all the United States species of *Cirsium*.”

Keil (2004) more recently described variety *viperinum*, which is endemic to the upper elevations of the Snake Range (White Pine Co., NV).

The four varieties with tomentose involucre bracts have undergone significant taxonomic change since their inceptions. The charismatic megaflores thistles found on mountain peaks in the southern Rocky Mountains are currently classified as varieties *eriocephalum* and *hesperium* (Keil, 2006). Variety *eriocephalum* was originally described as *C. eriocephalum* by Gray (1863) based on a Charles Parry collection from 1861. Unfortunately, Gray was unaware that this epithet had already been used to describe a species of thistle from Europe (Wallroth, 1840) thus rendering Gray's *C. eriocephalum* invalid. Gray (1874) was still unaware of the prior use of this binomial when he transferred the epithet to *Cnicus* (as *Cnicus eriocephalus* A. Gray). Greene (1892) later transferred the species to *Carduus* as *Carduus scopulorum* Greene, using the specific epithet *scopulorum* as originally suggested by Parry (Parry & Gray, 1861). Lastly, Cockerell (in Daniels, 1911) placed the species back into *Cirsium*, transferring Greene's *scopulorum* epithet [*Cirsium scopulorum* (Greene) Cockerell]. Nelson (1909) was the first to use the varietal name of *eriocephalus*, but incorrectly treated the epithet as a variety of *Carduus hookerianus* Nutt. Keil (2006) placed this taxon as variety *eriocephalum* within the *C. eatonii* complex, using Nelson's validly published varietal epithet.

There are three morphologically distinct phenotypes of variety *eriocephalum* (Fig. 2.1E). Plants growing in northern Colorado in the Front and Gore Ranges have purple anther tubes and white style branches, and generally have fewer terminal heads in a spreading to nodding array. Plants growing in middle and southern Colorado as well as outside of Santa Fe, New Mexico have white anther tubes and yellow style branches. This form generally has numerous terminal heads in a nodding array. Variety *eriocephalum* is also disjunct in the La Sal Mountains of Utah.

These plants have pinkish-purple anther tubes and white style branches, and the heads are arranged in a spiciform, erect arrangement.

When Eastwood (1898) described *Cnicus hesperius* Eastw. from the San Juan Mountains of southwestern Colorado, she noted that it differed from *C. eriocephalus* phenotypically by the presence of erect heads and stamens with pubescent filaments and anthers. However, the pubescent filaments on the type specimen were later determined to be fungal mycelium (Moore & Frankton, 1965). Based on this finding, Moore and Frankton (1965) subsumed *C. hesperius* into synonymy with *C. scopulorum*. Keil (2006) later reinstated the taxon but placed it in the *C. eatonii* complex as variety *hesperium*.

Varieties *murdockii* and *peckii* sensu Keil (2006) have tomentum on the involucre bracts, but it is not so dense as to obscure the bracts as in varieties *eriocephalum* and *hesperium*. Although described by Rydberg (1900, 1910) as separate species, *C. tweedyi* (Rydb.) Petr. and *C. polyphyllum* (Rydb.) Petr. were found to be conspecific by Moore and Frankton (1965). *Cirsium murdockii* (S.L. Welsh) Cronquist was first described (Welsh, 1982) as a variety of *C. eatonii*, only differing from var. *eatonii* by the presence of densely pubescent involucre bracts and ochroleucous disk florets. Keil (2006) synonymized *C. murdockii*, *C. polyphyllum*, and *C. tweedyi* with *C. eatonii* var. *murdockii*. Henderson (1939) noted the close affinity of *C. peckii* L.F. Hend. to *C. scopulorum* and *C. clokeyi*. *Cirsium peckii* is endemic to Steens Mountain and the Pueblo Mountains (Harney Co., OR) and adjacent Humboldt Co., NV.

Not only is the *C. eatonii* complex sensu Keil (2006) resolved as polyphyletic, but varieties *eatonii*, *eriocephalum*, and *hesperium* are resolved as polyphyletic as well (Fig. 2.1D, H). I will first suggest solutions to parse out the polyphyletic variety *eatonii*. There are two distinct lineages of variety *eatonii* recovered: 1) accession 143 (Subclade C, BS=79, PP=0.95),

and 2) accession 66 (Subclade A, BS=78, PP=0.99). A third possible lineage consisting of accession 89 is resolved in Subclade F (BS=88, PP=0.53), but this resolution is not well-supported in our BI.

Of these three accessions, 143 from Duchesne Co., UT is geographically closest to the type locality for *C. eatonii*. All other accessions of varieties *eatonii* and *murdockii* from Utah are part of the North American clade polytomy. Morphologically, varieties *eatonii* and *murdockii* in Utah are nearly indistinguishable, with the only exception of variety *murdockii* possessing tomentum on the involucre bracts (Welsh, 1982; Cronquist, 1994). However, given the current morphological and molecular evidence, I am unable to discern if variety *murdockii* from Utah should be recognized as a distinct species or variety of *C. eatonii*. Therefore, I recommend retention of Keil's (2006) treatment for these taxa.

The second lineage (accession 66) of variety *eatonii* was collected in the Tushar Mountains in Piute Co., UT. This collection was originally assigned to *C. eatonii* var. *harrisonii*, a taxon that Keil (2006) subsumed under variety *eatonii*. However, this accession is a distinct evolutionary lineage from our other *C. eatonii* accessions (Fig. 2.1D). I therefore propose recognizing collections of variety *eatonii* from the Tushar Mountains under the new combination of *C. harrisonii* (S.L. Welsh) Ackerfield & Keil, comb. nov. *Cirsium harrisonii* exhibits marked morphological differences from *C. eatonii* in having dark purple involucre bracts and lower involucre bracts lacking lateral spines.

Accession 89 of variety *eatonii* was collected in Elk Co., NV. This accession is supported in the same clade as our accession of *C. eatonii* var. *viperinum* in our ML analysis but is weakly supported for inclusion in our BI (Fig. 2.1H). However, both specimens are morphologically similar in having glabrate leaves, longer corollas (29–35 mm), and longer pappus bristles (20–25

mm), that are characteristic of variety *viperinum* sensu Keil (2006). In addition, variety *viperinum* is endemic to mountain tops in Nevada near the collection site for accession 89 of variety *eatonii*. I therefore propose recognizing Nevada collections corresponding to *C. eatonii* under the new combination of *C. viperinum* (D.J. Keil) Ackerfield & D.J. Keil, comb. nov. Therefore, *C. eatonii* as circumscribed here is now restricted to Utah.

Second, I suggest solutions to parse out the polyphyletic variety *eriocephalum*. The lineage of *C. eatonii* var. *eriocephalum* consisting of accession 169 is now considered part of the *C. griseum* varietal complex (see discussion under *C. clavatum*). The remaining accessions corresponding to variety *eriocephalum* are resolved in three distinct evolutionary lineages (Fig. 2.1D, H). First, accessions 97 and 168 from Colorado with purple anther tubes and white style branches are resolved in subclade B (BS=88, PP=0.93). Second, accession 124 from Colorado with white anther tubes and yellow style branches is resolved in subclade N (BS=89, PP=0.96). Lastly, accession 37 from the La Sal Mountains of Utah is resolved in subclade A (BS=78, PP=0.99). I suggest that these three phenotypes are in fact three different species that have been erroneously lumped under the broad umbrella of *C. eatonii* var. *eriocephalum* (i.e., *C. scopulorum*) as a result of shared morphology (densely woolly involucral bracts), similar alpine habitat, and/or examination of incomplete or faded herbarium specimens.

I also find conflicting lines of evidence for which of the two Colorado lineages to recognize as the true *C. scopulorum*. The *C. eriocephalum* type specimens consist of mixed collections from both Charles Parry and Elihu Hall (Gray, 1863). These specimens have lost their corolla color and are thus currently unassignable to the purple or yellow form upon initial examination. However, the original description by Gray (1863) lists the corolla color as yellow. Based on this description, I could be inclined to assign the yellow form to *C. scopulorum*.

However, additional research into Parry's collection sites reveals a stronger line of evidence for the purple form to be recognized as *C. scopulorum*. Charles Parry first visited the mountains of central Colorado in 1861, collecting alpine and subalpine plants for Asa Gray from what he labeled the "headwaters of Clear Creek and the alpine ridges lying east of Middle Park, Colorado Territory." Parry returned to Colorado in 1862 with Hall and Harbour. The collection locality listed for all specimens from the 1862 expedition is much less informative: "Colorado Territory, lat. 39°–41°, alpine and subalpine." However, through Parry's letters to Gray I obtained a more accurate record of their 1862 collection destinations (Parry & Gray, 1861). Thanks to Parry's notes, I found that this subsequent expedition began at the upper waters of the Platte near South Park (Park Co.). From there, they returned to Denver by way of Pike's Peak (El Paso Co.), ascending the mountain peak on July 1st, 1862. From Denver, the expedition returned to Parry's original collection site at the headwaters of Clear Creek (Clear Creek Co.) to determine the altitude of Torrey's, Gray's, and Engelmann peaks. They finished the expedition in the vicinity of Long's Peak (Boulder Co.).

Parry's collections of *C. eriocephalum* include the designated lectotype specimen [U.S.A., CO Territory: from the headwaters of Clear Creek and the alpine ridges lying east of "Middle Park," C. Parry s.n. (HU)], and syntype and isosyntype specimens [U.S.A., CO Territory: lat. 39°–41°, alpine and subalpine, C. Parry s.n. (HU)]. Hall's specimens include two collections designated as syntypes [U.S.A., CO Territory: Rocky Mountain Flora Lat 39°–41°, Hall & Harbour s.n. (HU)]. Hall collected specimens independently of Parry, to which Parry later assigned his own collection numbers. One of Hall's specimens corresponds morphologically to Parry's collections of *C. eriocephalum*. This specimen also contains a handwritten note from Hall in which he described the high alpine thistle as "dense, many headed yellow flowered

species but too young perhaps Parry got it in [a] better state.” Hall’s other collection is an atypical form and is most likely a collection of *C. parryi*. This specimen corresponds morphologically to Parry’s collection no. 340 of *C. edule* Nutt.? that Gray (1863) later described as a new species for Parry (*C. parryi* A. Gray; Gray, 1863). Parry may have inadvertently included Hall’s atypical collection in his own *C. eriocephalum* collections.

Gray (1863) completed an enumeration of Parry’s collections, from which he described the new species *C. eriocephalum* for the alpine thistle. Although Gray (1863: 69) described *C. eriocephalum* as having “heads of yellow flowers...crowded into a capitate cluster as large as a man’s fist,” the only record from Parry or Hall of the yellow corolla color is Hall’s handwritten note on an immature specimen lacking visible corollas. Presumably, Hall recorded the corolla as yellow in reference to the yellow spines. In Parry’s letters to Gray (Parry & Gray, 1861), Parry noted the corolla color as “white.” Parry (Parry & Gray, 1861) further suggested “If new a very good name would be *Cirsium scopulorum*.” This reference to a “white” corolla is most likely referencing the white, tomentose hairs densely covering the involucre bracts. Therefore, neither type specimen was mature enough to determine the corolla color. The corolla color was therefore erroneously reported as yellow by Hall and then incorporated into the original species description by Gray.

The only line of evidence strong enough to use to determine which form corresponds to the type of *C. scopulorum* is the locality of Parry’s first alpine thistle collection from Middle Park. The distribution of the purple form of *C. eatonii* var. *eriocephalum* lies well within the boundary of Middle Park (Fig. 2.2). Therefore, I propose that the binomial *C. scopulorum* be applied to the high alpine thistle of Colorado with purple anther tubes and white style branches, despite the original species description indicating the corolla as yellow.

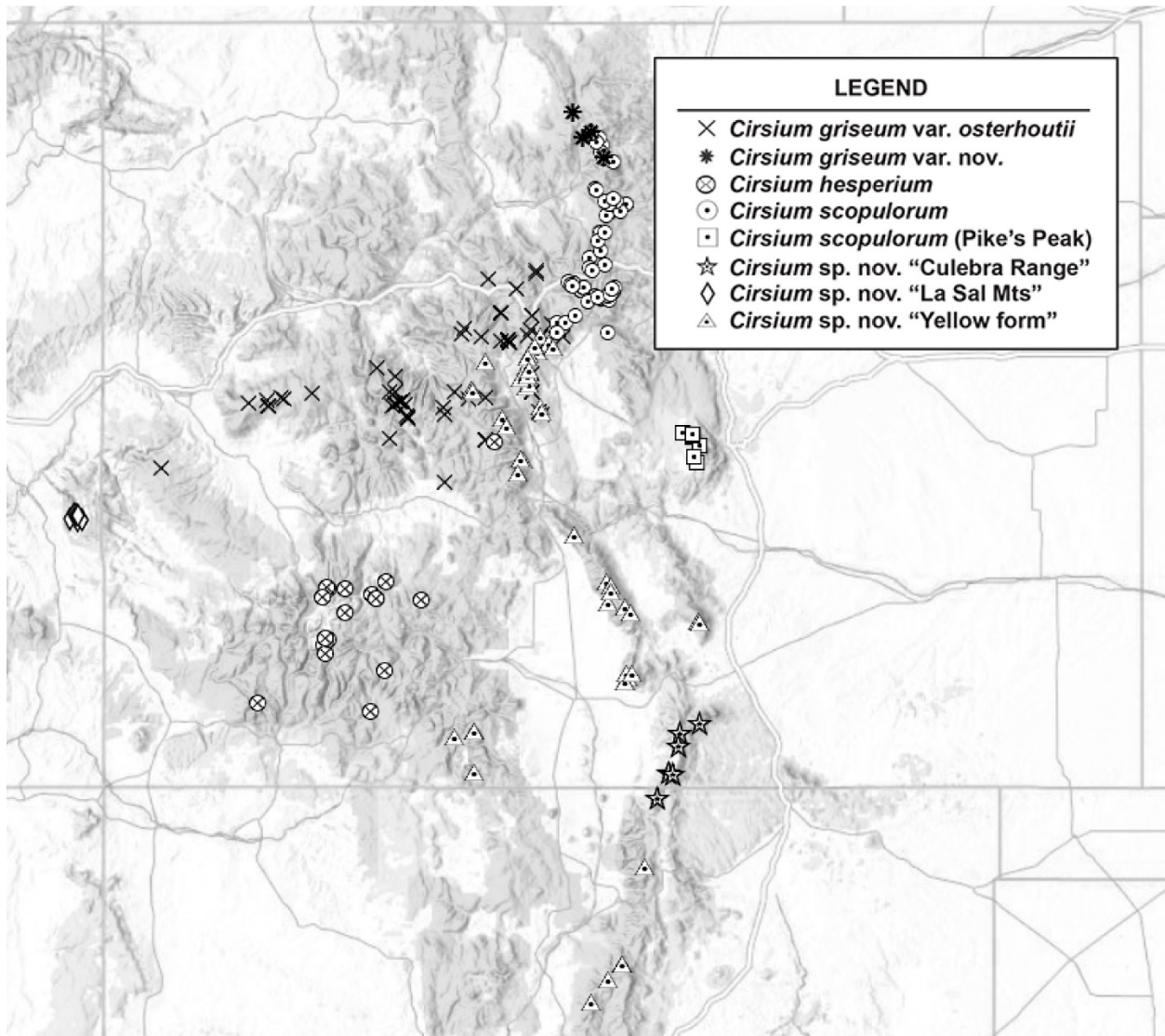


Figure 2.2. Distribution map of alpine *Cirsium* taxa in the southern Rocky Mountains. *C. griseum* var. *osterhoutii* (X), *C. griseum* var. nov. (asterisk), *C. hesperium* (circle with X), *C. scopulorum* (circle with dot), *C. scopulorum* from Pike's Peak (square with dot), *C. sp. nov.* 'Culebra range' (star with dot), *C. sp. nov.* 'La Sal Mts.' (open diamond), and *C. sp. nov.* 'yellow form' (triangle with dot).

Accession 124 of *C. eatonii* var. *eriocephalum* from Colorado with white anther tubes and yellow style branches is a distinct evolutionary lineage with a distinct geographic boundary separate from the purple form (now recognized as *C. scopulorum*; Fig. 2.2). I therefore propose that the yellow form be recognized as a new species. The presence of variety *eriocephalum* in

Utah has been limited to one disjunct population in the La Sal Mountains (Fig. 2.2; Welsh et al., 2003). However, our accession from this locality is a distinct evolutionary lineage (Fig. 2.1H). Morphologically, specimens from this location do not correspond to any known described *Cirsium* species (Fig. 2.1E). Therefore, I propose that this population also be recognized as a new species. Accession 145 of variety *eriocephalum* from the Pike's Peak, El Paso Co., Colorado population unfortunately is part of the North American-clade polytomy (Fig. 2.1H). These plants are markedly different morphologically from the other three phenotypes in having numerous heads tightly packed along the stem nearly to the ground (Fig. 2.1E). They are also geographically isolated from other populations (Fig. 2.2). Additional research may provide evidence that these represent yet another undescribed species, but for now I suggest using *C. scopulorum* for these plants.

Lastly, I suggest solutions to resolve the polyphyletic variety *hesperium*. Our phylogenetic analysis indicates that variety *hesperium* as it is currently circumscribed consists of at least two independent evolutionary lineages (Fig. 2.1D, H). The first lineage (accessions 24, 32, 101) is resolved in subclade H (BS=94, PP=0.99; Fig. 2.1H). These accessions were collected nearest to the type locality for *C. hesperium* (San Juan Mts., Colorado). I therefore propose that *C. hesperium* be reinstated for plants corresponding to variety *hesperium* from the San Juan Mountains of southwestern Colorado.

The second lineage of variety *hesperium* (accessions 90 and 170) is resolved in subclade C (BS=79, PP=0.97; Fig. 2.1D). These accessions were collected in the Culebra Range of northern New Mexico and southern Colorado. These accessions represent yet another undescribed species (Fig. 2.2; *C. sp. nov.* 'Culebra Range'). Upon initial examination, these accessions differ morphologically from *C. hesperium* sensu stricto in having green rather than

maroon-red stems (Fig. 2.1E). I will formally describe these two new species in a future publication.

I also provide evidence for past and/or present hybridization of *C. hesperium* with *C. parryi*. *Cirsium parryi* is resolved in a clade with *C. sp. nov.* ‘Culebra Range’ (accession 170) and *C. eatonii* var. *hesperium* (accession 32) from the San Juan Mountains in our plastid gene tree (BS=99, PP=0.94; Fig. S1D). However, in our nuclear gene tree *C. parryi* is resolved in a clade with *C. sp. nov.* ‘Culebra Range’ (accession 90) from northern New Mexico (BS=100, PP=1.0; Fig. Suppl. Fig. 10). I hypothesize that *C. hesperium* migrated down mountain peaks during times of Pleistocene glaciation, hybridizing with lower elevation *C. parryi* prior to retreating back to mountain tops as glaciers receded.

Variety *peckii* is a distinct evolutionary lineage from the other *C. eatonii* varieties sensu Keil (2006; Subclade A, BS=78, PP=0.99; Fig. 2.1D). I therefore propose reestablishing the binomial *C. peckii* L.F. Hend. Our single accession of variety *clokeyi* from Nevada is part of the North American-clade polytomy in our inferred phylogeny (Fig. 2.1H). Variety *clokeyi* does subtly differ morphologically from *C. eatonii* var. *eatonii* and is narrowly endemic to the Spring Range of Clark Co., Nevada (Fig. 2.1E; Keil, 2006). However, I do not currently have sufficient evidence to warrant recognition of this variety as a species (*C. clokeyi* S.F. Blake). I therefore recommend continued use of *C. eatonii* var. *clokeyi* for this taxon. Variety *murdockii* from Montana (accession 28) is also part of the North American-clade polytomy in our inferred phylogeny. Although separated geographically from variety *murdockii* in Utah, I cannot currently provide morphological or molecular evidence to support recognition of Montana accessions as a unique species. I therefore recommend continued use of Keil’s (2006) treatment for these taxa.

Keil (2006) hypothesized that the *C. eatonii* varietal complex constituted an alpine radiation. He postulated that during Pleistocene glacial episodes, the ancestor to the complex occupied lower elevations with a contiguous distribution. Subsequent isolation on mountain tops allowed them to morphologically diversify to the present extent. However, polyphyly of the *C. eatonii* varietal complex indicates that lowland congeners have undertaken multiple, independent dispersals to mountain tops.

Cirsium fontinales complex and the CA-FP adaptive radiation clade

The California Floristic Province (CA-FP) is characterized by a Mediterranean-like climate of cool, wet winters and hot, dry summers, and has been isolated for millions of years by major climatic and dispersal barriers (Ackerly, 2009; Baldwin, 2014). Coupled with the Mediterranean-like climate, stressful abiotic conditions such as serpentine soil, sand dunes, and brackish marshes are found in the CA-FP. This combination of unique climatic and abiotic conditions has facilitated high levels of plant endemism (Baldwin, 2014). Many groups of plants as well as animals have undergone recent diversification within the CA-FP (Moore et al., 2014), including thistles (Kelch & Baldwin, 2003). Within the CA-FP, 20 thistle taxa are endemic and an additional one (*Cirsium praeteriens* J.F. Macbr.) is presumed extinct (Keil & Turner, 1993).

Kelch and Baldwin (2003) resolved a clade of the following seven extant taxa that they hypothesized underwent adaptive radiation in the CA-FP: *C. andrewsii* (A. Gray) Jeps., *C. douglasii* DC., *C. fontinale* var. *fontinale*, *C. fontinale* var. *obispoense* J.T. Howell, *C. hydrophilum* (Greene) Jeps., *C. quercetorum* (A. Gray) Jeps., and *C. rhothophilum* S.F. Blake. Most of these taxa are narrowly restricted in range and are remarkable for their ecological specialization in habitat use. For instance, members of the *C. fontinale* complex strictly occur on serpentine soil, a stressful ecological habitat in which plants must adapt to low levels of many

essential macronutrients, high levels of heavy metals, and low water-holding capacity (Kruckeberg, 1951; 1954; Brady et al., 2005). *Cirsium fontinale* is a tall (to 2.2 m), succulent perennial herb characterized by strongly undulate leaf margins, a mixture of non-glandular hairs and glandular-papillate hairs on the adaxial leaf surface, nodding heads, and greenish-purple to purple involucre bracts (Keil & Turner, 1992; Fig. 2.1F).

Cirsium fontinale is currently subdivided into three varieties (Keil, 2006): *campylon* (H. Sharsm.) Pilz ex D.J. Keil & C.E. Turner, *fontinale*, and *obispoense* J.T. Howell. Each variety is narrowly restricted in range: variety *campylon* is known from the Mount Hamilton Range (Alameda, Santa Clara, and Stanislaus Cos.), variety *fontinale* is only known from the vicinity of Crystal Springs Reservoir (San Mateo Co.), and variety *obispoense* is only known from the southern Santa Lucia and San Luis Ranges (San Luis Obispo Co.; Keil & Turner, 1993; California Native Plant Society, 2019). Variety *campylon* was originally described as a *C. campylon* (Sharsmith, 1939) but was subsumed into *C. fontinale* by Keil & Turner (1992) based on phenotypic similarity among the three taxa and their shared affinity for serpentine soil.

The CA-FP adaptive radiation clade is resolved in our inferred phylogeny within subclade A (BS=78, PP=0.99; Fig. 2.1D), but with the inclusion of four additional taxa (*C. crassicaule*, *C. mohavense*, *C. scariosum* var. *citrinum* (Petr.) D.J. Keil, and *C. scariosum* var. *congdonii* (R.J. Moore & Frankton) D.J. Keil. Also, contrary to the results of Kelch and Baldwin (2003), *C. douglasii* was not resolved in the CA-FP adaptive radiation clade in our inferred phylogeny. This species is not endemic to the CA-FP, so this result is not surprising. See discussions on the *C. mohavense* and *C. scariosum* complexes for discussion on these taxa.

Cirsium fontinale is resolved as polyphyletic within subclade A. While varieties *fontinale* and *obispoense* are resolved in the CA-FP adaptive radiation clade, variety *campylon* is resolved

in a clade with other taxa from the Pacific Northwest (Fig. 2.1D). This result indicates that variety *campylon* is a distinct evolutionary lineage from the remainder of the *C. fontinale* complex. However, prior to recognizing this variety as a distinct species, I recommend waiting for the upcoming results of a population level study on the *C. fontinale* complex.

Morphologically, variety *campylon* is intermediate with the other *C. fontinales* varieties. Because of the rarity of these taxa, I was only able to sample one specimen of each variety. Therefore, hybridization or contamination could be skewing these results. Additionally, the ETS and ITS gene trees conflict in the placement of variety *campylon*. In the ETS gene tree, variety *campylon* is resolved in a clade with the other *C. fontinale* varieties, albeit with low support (BS=69, PP=0.61; Fig. S1E). However, in the ITS gene tree, variety *campylon* is resolved in the same clade as seen in our concatenated results (BS=86, PP=0.89; Fig. S1F). I can rule out misidentification as a potential source of conflict, as the specimen of variety *campylon* used in our analysis was collected and verified by one of the authors (DJK).

Cirsium occidentale (Nutt.) Jeps. is currently subdivided into seven varieties (Keil, 2006), two of which [var. *candissimum* (Greene) Petr. and var. *venustum* (Greene) Petr.] were included in our phylogenetic analysis. Although some varieties are endemic to the CA-FP, *C. occidentale* is not a member of the adaptive radiation clade (Subclade K, BS=100, PP=1.0; Fig. 2.1D). This is not surprising given that *C. occidentale* varieties are typically not restricted to the CA-FP or to specialized habitats.

While *C. crassicaule* is resolved in the CA-FP adaptive radiation clade in the nuclear gene tree (BS=100, PP=1.0; Fig. S1C), in the plastid gene tree it is resolved in a strongly supported clade with *C. arizonicum* var. *tenuisectum*, *C. eatonii* var. *eriocephalum* (accession 124), and *C. ownbeyi* (BS=96, PP=0.8; Fig. S1D). I hypothesize that the discrepancy among

gene trees was the result of incomplete lineage sorting (ILS). The discordant geographical distribution and morphological dissimilarity of *C. crassicaule* to these other taxa make an alternative hypothesis of potential hybridization unlikely.

Cirsium mohavense and *C. virginense*

Cirsium mohavense and *C. virginense* S.L. Welsh are distributed in the southwestern U.S. near desert springs and seeps. These species share morphological features of a glutinous dorsal ridge on the involucre bracts, leaves decurrent as spiny wings, and corollas ranging from white to pale pink or lavender (Keil, 2006; Fig. 2.1G). There has been considerable disagreement among authors concerning the taxonomic treatment of *C. virginense*. When Welsh (1982) first described *C. virginense*, he did not attempt to distinguish *C. virginense* from *C. mohavense* because the latter was not known to occur in Utah. Cronquist (1994) provided the only known distinction between the two taxa in his treatment for the *Intermountain Flora*. Although Cronquist kept the two taxa separate, the only character he used to distinguish between them was life span (*C. mohavense* biennial vs. *C. virginense* perennial with creeping roots). Based on a lack of morphological distinction between the two taxa, Keil (2006) subsumed *C. virginense* within a broader *C. mohavense*. *Cirsium virginense* is a species of conservation concern in Utah (Utah Rare Plant Guide, 2003-2020). Therefore, the recognition of *C. virginense* apart from *C. mohavense* has land management and conservation effort implications.

Together, *C. mohavense* and *C. virginense* are resolved in at least four distinct evolutionary lineages (Fig. 2.1D, H). First, accession 43 of *C. mohavense* from San Bernardino Co., CA is geographically closest to the type locality [U.S.A., CA: Rabbit Springs, Mohave Desert, S.B. Parish & W.F. Parish 1834 (UC)]. This accession is resolved in the CA-FP adaptive radiation clade (Subclade A, BS=78, PP=0.99; Fig. 2.1D). Second, accession 97DBG of *C.*

mohavense from Death Valley, CA is resolved in subclade K (BS=100, PP=1.0; Fig. 2.1H). This accession is well-supported as sister to *C. occidentale*, but unfortunately the herbarium specimen is incomplete and only consists of a single head and a short stem with few leaves. Therefore, I cannot make any further taxonomic conclusions concerning this specimen beyond its determination as *C. mohavense*.

Third, accessions of *C. mohavense* from Nevada (206DBG, 207DBG, and 209DBG) are resolved in subclade D (BS=76, PP=0.84) with *C. virginense* accessions 166DBG, 2ASDBG, 3348DBG, 95DBG, and 96DBG from Arizona and accession 187DBG from near the type locality in Utah (Fig. 2.1D). Lastly, another accession of *C. virginense* (210DBG) from near the type locality is resolved in subclade G with *C. rydbergii* (BS=98, PP=0.91; Fig. 2.1H). All other *C. mohavense* and *C. virginense* accessions are either resolved in clades only supported in our ML analysis or part of the North American-clade polytomy (Fig. 2.1H).

These results of four distinct evolutionary lineages indicate that *C. mohavense* sensu Keil (2006) should be restricted to southern California in distribution. However, I do not immediately discern any morphological differences among the *C. mohavense* California specimen and specimens from Nevada. Additionally, although accessions 187DBG and 210DBG of *C. virginense* are resolved in different subclades, both specimens were collected nearest the type locality for *C. virginense* [U.S.A., UT: St. George, Washington Co., S.L. Welsh 21234 (MO)]. I re-extracted DNA from both samples and re-amplified the ITS and ETS regions to eliminate contamination as a potential cause of this discrepancy. Given these conflicting results, I cannot currently discern if *C. virginense* should be recognized apart from *C. mohavense*. Therefore, I am working on a phylogenomic and morphometric study to clarify species boundaries in this group.

Cirsium rydbergii

Hanging gardens occur on cliff faces or alcoves undercut along canyon walls, and are formed by perched aquifers that seep through the permeable sandstone (May et al., 1995). These unique environments occur throughout northwestern Colorado, northern Arizona, and southern Utah (Welsh, 1989; Sada & Lutz, 2016), forming a patchwork of “islands” along the canyon walls throughout the Colorado Plateau (Welsh, 1989). These desert oases also support many rare and endemic plants, including *C. rydbergii* (Welsh, 1989; Fig. 2.1I).

Cirsium rydbergii was described by Petrak (1917; *C. rydbergii* Petr.) and Rydberg (1917; as *C. lactucinum* Rydb.) from Rydberg’s San Juan Co., Utah collection [U.S.A., UT: Along San Juan River, near Bluffs, P.A. Rydberg 10001 (NY)]. Since then, the binomial *C. rydbergii* has been applied to any large-leaved thistle found in hanging gardens throughout Utah and Arizona, with the exception of the more recently described *C. joannae* S.L. Welsh, N.D. Atwood & L.C. Higgins (Welsh et al., 2003). However, in our inferred phylogeny, *C. rydbergii* is resolved as polyphyletic in at least three distinct evolutionary lineages (Fig. 2.1D, H).

The first lineage resolved consists of the two accessions (164DBG and 165DBG) that are geographically closest to the type locality (San Juan Co., UT). These accessions are resolved in subclade H with good support (BS=94, PP=0.99; Fig. 2.1H). Additionally, these specimens differ morphologically from the other accessions identified as *C. rydbergii* by having yellowish-green leaves, involucre bracts mostly ascending, and a flowering scape bearing long branches at the base (Fig. 2.1I).

The second lineage consists of accessions DBG52, DBG58, and DBG185 from the north rim of the Grand Canyon, Coconino Co., Arizona at Buck Farms and Saddle Canyons. These accessions are resolved in our inferred phylogeny in subclade G (BS=98, PP=0.91; Fig. 2.1H).

They are also morphologically distinct from *C. rydbergii* sensu stricto in having dark green leaves, flowering stems unbranched, and longer involucral bract spines (Fig. 2.1I). Also in this lineage are accessions 196DBG, 67DBG, 1ASDBG, and 3ASDBG from Cliff Springs and Clear Creek Canyon also from the north rim of the Grand Canyon, Coconino Co., AZ. However, these plants differ markedly from the Buck Farms population in having dark green leaves that are densely spinose, leaves deeply pinnately dissected into narrow lobes, and involucral bract spines that are ascending versus spreading (Fig. 2.1I). Some of these collections have even been assigned to *C. arizonicum* var. *bipinnatum* because of their unique morphology and ambivalent identification. Although the Buck Farms and Cliff Springs populations may appear close geographically, they are separated by steep canyon walls and are thus reproductively isolated. Given the distinct phenotypes and geographic isolation of each population, I suggest that *C. rydbergii* as it is currently delimited is comprised of at least three different species. I will provide descriptions of the two new species from the Grand Canyon in a future publication.

Another lineage consisting of accession 68DBG is weakly resolved in subclade D (BS=76, PP=0.84) with accessions of *C. mohavense* and *C. virginense* (Fig. 2.1D). This accession corresponds to the phenotype from Buck Farms in the Grand Canyon and was collected near the Buck Farms population. I hypothesize that this discrepancy is either the result of insufficient molecular character evidence or cryptic speciation.

Cirsium scariosum complex

The *C. scariosum* varietal complex is a polymorphic assemblage of eight infraspecific varieties sensu Keil (2006): *americanum*, *citrinum*, *coloradense* (Rydb.) D.J. Keil, *congdonii*, *robustum* D.J. Keil, *scariosum*, *thorneae* S.L. Welsh, and *toiyabense* S.L. Welsh. Taxa within this complex have been variously treated as species, or even erroneously treated as *C.*

drummondii Torr. & A. Gray or *C. foliosum* (Hook.) DC. in the past (Table 2.1; Moore & Frankton, 1967). In Moore and Frankton's (1967) detailed treatment of the complex four species were recognized: *C. acaulescens* (A. Gray) K. Schum., *C. coloradense* (Rydb.) Cockerell ex Daniels, *C. congdonii* R.J. Moore & Frankton, and *C. scariosum*. However, they later revised this treatment to acknowledge the priorable binomial *C. tioganum* (Congdon) Petr. over *C. acaulescens*. Cronquist (1994) later subsumed all variation within the group within a broadly delimited, polymorphic *C. scariosum*. Keil (2006) ultimately combined both Cronquist's (1994) and Moore and Frankton's (1967) treatments by subdividing *C. scariosum* into the eight varieties listed above.

Although polymorphic, taxa within the complex are united together morphologically by the presence of a dense cluster of sessile heads typically overtopped by crowded, distal leaves (Fig. 2.1J). The complex is subdivided by habit (acaulescent vs. caulescent). Varieties *americanum* and *congdonii* are acaulescent, while varieties *coloradoense*, *thorneae*, and *toiyabense* are caulescent. However, two varieties (*citrinum* and *scariosum*) may have acaulescent to caulescent plants within the same population (Cronquist, 1994; Keil, 2006). Five varieties (*americanum*, *coloradoense*, *scariosum*, *thorneae*, and *toiyabense*) are widespread in meadows and along streams throughout the Rocky Mountains and Intermountain Basin. Three other varieties (*citrinum*, *congdonii*, and *robustum*) are narrowly distributed in California and adjacent Oregon.

Cirsium scariosum as delimited by Keil (2006) is resolved as polyphyletic in four distinct evolutionary lineages in our inferred phylogeny (Fig. 2.1D, H). First, variety *scariosum* is resolved in subclade L (BS=100, PP=0.96), sister to *C. canovirens* as delimited here (Fig. 2.1H). Second, the California endemic taxa (varieties *citrinum* and *congdonii*) are resolved in subclade

A (BS=78, PP=0.99) in the CA-FP adaptive radiation clade (Fig. 2.1D). Although sister in our inferred phylogeny, these taxa differ markedly in morphology and thus are not representative of the same species (Fig. 2.1J; Keil, 2006). I therefore propose recognition of each taxa as a distinct species: *C. congdonii* and a new combination of *C. validum* (Greene) Ackerfield & D.J. Keil, comb. nov. Variety *citrinum* was originally described as *Carduus validus* by Greene (1897), but this epithet was never transferred to *Cirsium*. It should be noted that I was not able to include *C. loncholepis* in our analysis because of scarcity of material available. This taxon is considered synonymous with *C. scariosum* var. *citrinum* by Keil (2006). It is currently listed as Threatened by the State of California and Endangered by the Federal Government (California Native Plant Society, 2019).

Third, variety *americanum* is resolved in subclade H (BS=94, PP=0.99; Fig. 2.1D). Besides California varieties *citrinum* and *congdonii*, variety *americanum* is the only remaining member of the complex that is exclusively acaulescent. I therefore propose reinstatement of the binomial *C. tioganum* for specimens previously assigned to variety *americanum*. Lastly, variety *coloradense* is resolved in subclade B (BS=88, PP=0.93; Fig. 2.1D). I therefore propose reinstatement of the binomial *C. coloradense* for specimens previously assigned to variety *coloradense*.

While I have remedied some of the varieties within the *C. scariosum* complex, the delineation of varieties *robustum*, *thorneae*, and *toyabense* remains unclear. Accessions of variety *toyabense* are resolved as part of the North American-clade polytomy (Fig. 2.1H) and I was unable to sample varieties *robustum* and *thorneae*. I therefore recommend continued use of Keil's (2006) treatment for these varieties for the time being. I will include additional sampling

from this complex in a future phylogenomic study to further resolve relationships among these taxa as well as evaluate the status of *C. loncholepis*.

Conclusions

The assessment and ultimate preservation of biodiversity is reliant upon a well-delineated taxonomy based on a robust morphological and molecular systematic framework. This work is the first step in providing a broadly sampled systematic framework to inform species delimitations within North American *Cirsium*. I found that eight species as currently delimited (Keil, 2006) were resolved as polyphyletic. I have provided recircumscriptions, including evidence for the recognition of six new taxa, based on both morphological and molecular evidence. A sound taxonomy also provides a baseline for assessment of a species' conservation status and helps inform protection policies. I expect to see an increase in number of species of conservation concern as the narrowly endemic species proposed here are described and population sizes quantified. Lastly, without a well-delineated taxonomy, previously undescribed species may be lost before they are ever recognized. This nearly occurred at the Grand Canyon Cliff Springs population in 2018, when a weed crew almost extirpated what is treated here as a newly recognized, narrowly endemic species. Increased awareness and plans to monitor and protect the population resulted from botanists' collaboration with park staff, illustrating how important it is for botanists to work closely with land managers.

I propose that the extensive taxonomic difficulty within *Cirsium* is the culmination of several factors. First, I suggest that some of the polymorphic variation can be attributed to previously undescribed taxa. *Cirsium* plants are extremely "prickly," large in stature, and overall difficult to collect. Thus, herbarium specimens are often either few altogether or incompletely sampled. To counter this, I coupled our analysis of herbarium specimens and extensive field

work with iNaturalist (<http://www.inaturalist.org>) observations. These observations allowed us to easily discern variation in morphology across a species' geographic range that I could not observe using herbarium specimens alone.

Second, I propose that multiple lines of phenotypic convergence have complicated the taxonomy of the group. For example, *Cirsium* plants growing at high alpine elevations in the southern Rocky Mountains and adjacent Utah were previously identified under the broad umbrella of *C. eatonii* var. *eriocephalum* (i.e., *C. scopulorum*). However, I provide evidence that *C. eatonii* var. *eriocephalum* is an artificial assemblage of at least four different taxa (*C. griseum*, *C. scopulorum*, *C. sp. nov.* 'La Sal Mts.', and *C. sp. nov.* 'Yellow Form') that share an alpine distribution. I hypothesize that woolly involucre bracts evolved independently in these lineages in response to cold alpine conditions. Additionally, I infer a convergence of characters (narrowly campanulate heads, short stigmatic tips, and corolla lobes about twice as long as the throat) in thistles treated as *C. arizonicum* varieties sensu Keil (2006). I hypothesize that this convergence is in response to selection in favor of the hummingbird pollination syndrome.

Third, I postulate that *Cirsium* has undergone a recent continental-wide radiation, and as such some species of *Cirsium* in North America may still be in the process of diversification (i.e., incipient speciation). Ackerfield et al. (2020) inferred that *Cirsium* in North America originated approximately 7.2 Myr, with most species diversifying within the last 2.0–1.0 Myr. Here I have been able to provide molecular and morphological evidence to support some taxonomic delineations, but others remain unresolved. Therefore, I am working on a phylogenomic study of North American *Cirsium*, using targeted enrichment of highly informative nuclear regions designed specifically for Compositae (Mandel et al. 2014, 2015,

2017). This analysis will provide significantly more informative molecular characters available for phylogenetic inference and aid in resolving the remaining problematic taxa.

Lastly, I propose that hybridization has played a role in diversification of *Cirsium* across North America. Hybridization is known to play a role in the diversification of plant lineages, especially in those that are younger and have had less time for establishment of reproductive barriers (Soltis & Soltis, 2009). While I provide some evidence for hybridization, the lack of resolution in the plastid region hinders identification of possible hybrids. Our phylogenomic study will provide additional insight into the role of hybridization in *Cirsium* diversification.

This work offers important insights into the evolution of a recently radiated group in North America. This work also highlights the importance of observations, either through field studies or with the use of iNaturalist, apart from analysis of herbarium specimens. In future manuscripts I will formally describe the six new taxa proposed here and include dichotomous keys for identification. Our future phylogenomic study will further aid in resolving remaining taxonomic issues and elucidating evolutionary relationships. This study will also provide a robust framework to infer a biogeographic history of *Cirsium* in North America. In short, species delimitations in the thistles have been a mess. While I have made great strides to untangle delineations within *Cirsium*, the taxonomic challenges are many and additional questions remain unanswered. But, thistle be fixed!

New combinations

Cirsium griseum (Rydb.) K. Schum. var. *osterhoutii* (Rydb.) Ackerfield & D.J. Keil, comb. nov.
≡ *Carduus osterhoutii* Rydb., Bull. Torrey Bot. Club 32: 131. 1905. – Holotype: U.S.A.,
Colorado, Eagle Co., Red Cliff, 17 Jul 1902, *G.E. Osterhout 2706* (NY!).

Cirsium harrisonii (S.L. Welsh) Ackerfield & D.J. Keil, comb. nov. ≡ *Cirsium eatonii* (A.
Gray) B.L. Rob. var. *harrisonii* S. L. Welsh, Great Basin Naturalist 42(2): 200. 1982. –
Holotype: U.S.A., Utah, Piute Co., Tushar Mts., T28S R4W S8, alpine meadow, talus slope,
3416 m elev., 16 Aug 1978, *Welsh & Henroid 18084* (BRY!).

Cirsium markaguntense (S.L. Welsh) Ackerfield & D.J. Keil, comb. nov. ≡ *Cirsium clavatum*
(M.E. Jones) Petr. var. *markaguntensis* S.L. Welsh, Utah Fl. (ed. 3): 168. 2003. – Holotype:
U.S.A., Utah, Iron Co., 10 mi up Cedar Canyon east of Cedar City, Spruce-fir community, 18
Aug 1973, *N.D. Atwood & L.C. Higgins 5918* (BRY!).

Cirsium validum (Greene) Ackerfield & D.J. Keil, comb. nov. ≡ *Carduus validus* Greene, Flora
Franciscana 4: 479. 1897. – Lectotype: U.S.A., California, Kern Co.: Tehachapi, 24 Jun 1889,
E.L. Greene sn (ND!).

Cirsium viperinum (D.J. Keil) Ackerfield & D.J. Keil, comb. nov. ≡ *Cirsium eatonii* (A. Gray)
B.L. Rob. var. *viperinum* D.J. Keil, Sida 21(1): 212–213. 2004. – Holotype: U.S.A., Nevada,
White Pine Co.: Snake Range, Humboldt National Forest, Snake Creek Canyon, above Johnson
Lake, steep rocky slope, granite, 3353 m, 10 Aug 1964, *Holmgren & Reveal 1588* (UTC!).

CHAPTER THREE – CONCLUSIONS AND NEXT STEPS

Since its inception, *Cirsium* has been a center of taxonomic confusion, both at the generic and species levels. These studies represent the most comprehensive systematic assessments of *Cirsium* and the *Carduus-Cirsium* group to date. And while great strides have been made in untangling the taxonomy of the *Carduus-Cirsium* group and *Cirsium* in North America, many questions remain unanswered. The generic study provides well-supported evidence that *Cirsium*, as it is currently delimited, does not form a natural, monophyletic group. However, before the *Carduus-Cirsium* group can be segregated into the genera proposed in solution two, additional morphological work must be done. First, examination of the rigid setae in additional members of *Cirsium* sect. *Epitrachys* must be performed. Sampling this character across a larger number of taxa is necessary prior to its utilization. Second, additional pollen analyses should be performed on the three discordant Asian species (*C. botryodes*, *C. interpositum*, and *C. lidjiangense*) to see if they have the *C. palustre* pollen type as well. These analyses will determine if these characters are stable to use for generic delimitations. The placement of the African *Carduus* remains unclear as well. Whether or not these taxa should be included within *Cirsium* or delimited as their own genus (*Afrocarduus*) is currently unclear.

North American *Cirsium* is one of the most taxonomically confusing and challenging groups of Compositae, especially in the western states. Misinterpretation of faded or incomplete herbarium specimens, and the perpetuation of misapplied taxonomy have exacerbated this problem for well over 100 years. It was indeed a challenge to untangle the resulting thistle nomenclatural mess. The molecular phylogenetic results here provide novel, important insights for species delimitations. However, in *Cirsium* the taxonomic challenges are many. In the following instances, additional morphological and molecular work must be completed.

First, the *C. arizonicum* complex needs additional work, as the species remains polymorphic across its range as it is delimited here. Within this varietal complex, it is still unclear whether variety *tenuisectum* should be recognized at the species rank, continue to be included as a variety of *C. arizonicum*, or is the result of hybridization between *C. arizonicum* and *C. mohavense*. This taxon is of conservation concern, and only known from two localities (New York Mts., CA and Spring Mts., NV; Keil, 2006). Thus, recognition of this taxon has land management implications. In addition, as it is only known from two localities, this taxon may be eligible for protection under the Endangered Species Act. Likewise, I was only able to sample one accession of *C. arizonicum* var. *chellyense*. Additional accessions of this variety must be included in a future phylogenetic study. Lastly, I was only able to include one accession of *C. pulchellum*. Therefore, additional samples of *C. pulchellum* must be carefully analyzed in a phylogenetic context to determine the ranges of *C. pulchellum* and the morphologically similar *C. calcareum*.

Second, great strides were made in untangling the *C. clavatum* taxonomic mess. *Cirsium clavatum* is now restricted to Utah in distribution, and all Colorado '*C. clavatum*' are assigned to previously described taxa (*C. centaureae*, *C. griseum*, and *C. scapanolepis*). However, the lineage of *C. griseum* proposed as a new variety from Rocky Mountain National Park may represent introgression between *C. scopulorum* and lower elevation *C. griseum*. A thorough genetic study of the high alpine thistles of Rocky Mountain National Park is necessary to determine if these populations represent a cryptic species or hybrids. Questions remain such as: Have these thistles picked up some genes from lower down the mountain, retreated back up the mountain, and become genetically and/or morphologically differentiated enough to call a new species? A third future research project is to increase sampling of *C. brevifolium*, *C. canovirens*,

C. cymosum, and *C. inamoenum*. It is currently unclear as to the ranges of each species, as well as if *C. subniveum* should be recognized apart from *C. inamoenum*.

Fourth, the alpine thistles of the Rocky Mountains require additional systematic work. Again, while great strides were made to sort out the taxonomic mess of the *C. eatonii* varietal complex, some conclusions could not be made given the evidence at hand, leaving several questions remaining. One question that remains is: What is the common ancestor of the alpine thistles in Colorado? The backbone of the North American *Cirsium* clade was unresolved because of insufficient character evidence. Therefore, it is unclear how these alpine thistles are related to each other, only that they are distinct evolutionary lineages. Do the alpine thistles share a common ancestor, or do they represent independent colonization's of mountain tops? Resolving the backbone of the North American clade would shed important insight onto the evolution of the alpine thistles. Because thistles in North America are hypothesized to have relatively recently radiated within the last 1–2 MYR, reconstructing the evolutionary history of the alpine thistles could shed important insight onto the formation of the alpine flora in the Rocky Mountains. Second, var. *clokeyi* and var. *eriocephalum* (i.e., *C. scopulorum*) restricted to Pike's Peak were unresolved in this analysis. It is currently unclear whether these should be treated as distinct species. Each of these varieties is narrowly distributed in range, and endemic to a single mountain top. If they are treated as distinct species, this would have land management implications as they would mostly likely be considered species of conservation concern. Third, only one lineage of *C. eatonii* from Utah was resolved in this analysis. Additional accessions should be analyzed, including accessions of var. *murdockii* from throughout its geographic range. It is still unclear whether var. *murdockii* is a separate lineage from *C. eatonii* in Utah, or

whether var. *murdockii* in the northern part of the range (Montana, Wyoming) is distinct from the Utah populations.

Survival at high alpine altitudes is not easy, and several species have adapted to these harsh growing conditions in different ways. This includes cushion, low-growing, compact growth habits that are adapted to slow growth in nutrient-poor environments, anthocyanic leaves which absorb more heat, and dense coverings of hairs which protect plant reproductive structures from exposure to the cold (Billings, 1974). In the alpine thistles, a dense covering of hairs is produced on the involucre bracts, creating a warm environment for floral development. However, another morphological aspect that may have led to alpine thistle's survival in these harsh climates is their duration as a monocarpic perennial. Monocarpic perennials persist as basal rosettes, and send up a flowering stalk only once prior to dying. Alpine thistles are remarkable in the alpine because they stand tall against a landscape of low-growing plants. Could the monocarpic perennial habit be an adaptation that allowed thistles to occupy and persist in the alpine environment, despite their height? If so, other questions remain such as how long do basal rosettes persist prior to flowering? Are there specific environmental conditions that plants favor for flowering?

Fifth, additional sampling of species in the CA-FP should be performed, in particular to include multiple accessions of all varieties of *C. fontinale*. *Cirsium fontinale* is a federally listed endangered species. It is so rare that I was only able to obtain material from variety *campylon*, and had to rely on previously published ETS and ITS sequences on GenBank for varieties *fontinale* and *obispoense*. Although *C. fontinale* was resolved as polyphyletic, additional samples of each variety must be examined prior to elevation of variety *campylon* as a distinct species from the other *fontinale* varieties. The three varieties are morphologically similar and all occur

on serpentine seeps, but in different locations within the CA-FP. Whether or not these results indicate convergence of variety *campylon* is debatable, as there is conflicting evidence among the gene trees as well.

Sixth, the placement of *C. mohavense* in the CA-FP adaptive radiation clade needs careful examination and further study. It is odd that this species should be in the CA-FP clade, as it is the only taxon in the clade that does not occur strictly in the CA-FP. *Cirsium mohavense* and *C. virginense* remain taxonomical contentious given the current evidence as well. Although both species are resolved as polyphyletic, the support for this is not high and the morphological evidence does not support breaking up either respective taxon into separate species. *Cirsium virginense* is a taxon of conservation concern. Therefore it is important that I provide evidence for or against its recognition as a species distinct from *C. mohavense*.

Seventh, there is much remaining work to be done in the *C. scariosum* varietal complex. I was not able to resolve or include all varieties within this complex in the analysis. In particular, varieties *robustum* and *thorneae* should be examined in a phylogenetic context to see if they are part of the *C. scariosum* varietal complex or would be better treated as distinct species. *Cirsium loncholepis* was also not included in this analysis because of its rarity. It is currently unclear if this taxon should be recognized as a distinct species or remain synonymous with *C. validum*. *Cirsium loncholepis* is listed as endangered under the Endangered Species Act, and thus recognition of this taxon has significant land management and conservation implications.

Lastly, a phylogeographic analysis of hanging garden thistles (*C. ownbeyi*, *C. rydbergii*, and the two new species from the Grand Canyon) could aid in understanding mechanisms driving speciation on the Colorado Plateau. One way that I can better understand these mechanisms is by reconstructing the impact of historical and/or biogeographical processes

driving diversification in extant taxa. By incorporating phylogenetic, geologic, and paleoclimatic data, I can investigate the mechanisms driving present day distributions. Recently diverged sister taxa found in disparate habitats are of interest for examining the processes driving speciation. In particular, I can ask what climatic fluctuations and/or geologic events of the past shaped the species' current distribution and led to the occupation of such disparate habitats.

The Colorado Plateau of southwestern North America is a physiographic province of the Intermontane Plateaus region encompassing approximately 130,000 square miles of southeastern Utah, extreme western and southwestern Colorado, northwestern New Mexico, and the northern half of Arizona (Coats et al., 2008). It is bounded by the Rocky Mountains to the north and east, Great Basin to the west, and Sonoran Desert to the south. The Colorado Plateau ranges from 2,000–12,700 ft. in elevation, and is comprised of high mountains deeply cut by the Colorado River canyon system, the most notable of which is the Grand Canyon. The origin and assembly of the flora of the Colorado Plateau has not been well studied despite the dynamic geologic and climatic history of the area and high levels (10-15%; Stohlgren et al., 2005) of plant endemism (Fowler et al., 2007). Thus, it is relatively unknown how the distributions of extant species on the Colorado Plateau have been shaped by habitat preference as well as Pleistocene glaciations and climatic oscillations (Talbot et al., 2013; Krause et al., 2015). Understanding the effects of climatic oscillations of the Last Glacial Maximum (LGM) on present day species' and community distribution is also essential for predicting their future responses to global climate change.

One of the most unique habitats of the Colorado Plateau is that of perennially wet hanging gardens. These hanging gardens are markedly different from the surrounding arid desert communities of the Colorado Plateau in having perennially wet rock walls or soils (Welsh, 1989;

Sada & Lutz, 2016). These desert oases are found in alcoves undercut along canyon walls, and are formed by perched aquifers that seep through the permeable sandstone (Fowler et al., 2007). These oases form a patchwork of “islands” along the canyon walls throughout the Colorado Plateau (Welsh, 1989). Much of our understanding of evolutionary processes is the result of island research (Santos et al., 2016). However, little research has been conducted on the origin or phylogeography of vegetation in hanging garden communities. In particular, these “island” habitats are useful for studying how the Colorado River has provided a corridor for dispersal from the Grand Canyon to higher-elevation regions in eastern Utah and western Colorado.

As expected, such unique habitats also support many rare and endemic plants (Welsh, 1989), including members of the genus *Cirsium*. Four species of *Cirsium* are endemic to hanging gardens of the Colorado Plateau: *C. ownbeyi*, *C. rydbergii*, and the two new species presented here from the north rim of the Grand Canyon. Although not previously discussed, *Cirsium ownbeyi* is found in the northern range of the Colorado Plateau in canyons in northwestern Colorado and adjacent Utah, while *C. rydbergii* and the two new hanging gardens species are found in the southern part of the Colorado Plateau in southeastern Utah and northcentral Arizona.

Cirsium is an excellent system to study the origin and assembly of the flora of the Colorado Plateau, and the influence of Pleistocene glaciation and climatic fluctuations on present day species’ distributions for two reasons. First, although no one species occupies all hanging garden communities, *Cirsium* is found in over 75% of these unique habitats (Malanson, 1980). Second, the phylogeny of North American *Cirsium* indicates three well-supported instances of sister-species pairs comprised of taxa found in hanging gardens of the Colorado Plateau together with other species growing montane or alpine habitats of the surrounding mountains. For

example, *C. ownbeyi* is sister to a new species of thistle (*Cirsium* sp. nov. ‘Yellow form’), a common alpine species in the adjacent Colorado Rocky Mountains. The two new species of hanging garden thistles from the north rim of the Grand Canyon are sister to *C. clavatum* from the mountains of southern Utah. And *C. rydbergii*, found in hanging gardens in southeastern Utah, is sister to *C. hesperium* from the alpine habitat of the Colorado San Juan Mountains.

To date, no studies of species’ responses to Late Quaternary glacial cycles have been conducted for the Colorado Plateau. Distributional responses to past climate change of each species and possible Pleistocene refugia sites on the Colorado Plateau will be evaluated by using ecological niche models (ENMs) in conjunction with paleoclimatic reconstructions from the LGM (Waltari et al., 2007). ENMs take known occurrences of species in combination with high resolution climate and soil data to predict the species’ inferred environmental requirements or fundamental niches (Guisan & Zimmerman, 2000). ENMs will be generated for each of these species to characterize the current spatial distribution of suitable conditions for each, and to reconstruct their predicted distribution in the LGM.

Two alternative hypotheses that could explain the disparate habitats of these sister taxa are as follows. First, vicariance, wherein a common ancestor is unable to persist in a changing, warming climate and its distribution becomes isolated and fragmented, separates into two species. Second, dispersal between geographically isolated glacial refugia followed by *in situ* diversification. BioGeoBEARS (Matzke, 2013, 2014) can be used to test how different models of dispersal, vicariance, and founder event speciation may have evolved (Matzke, 2013, 2014).

The phylogeny for *Cirsium* presented here has low resolution at the backbone of the North American clade. This is presumably because of the low DNA sequence divergence in the recently radiated North American thistles. Fortunately, resolution of recently evolved groups is

becoming more feasible with the advent of high-throughput DNA sequencing, Through high throughput sequencing (HTS) techniques, we are now able to analyze millions of basepairs of DNA characters versus the relatively few (generally 4000–10,000) available using traditional Sanger sequencing technology. This has resulted in a dramatic increase in character evidence necessary for reconstructing robust, well-resolved phylogenies. In particular, the Hyb-Seq approach has shown great utility in resolving recently radiated groups (Mandel et al., 2014). Hybridization-sequencing (Hyb-Seq) is a combination of target enrichment and genome skimming. Hyb-Seq uses ‘baits’ (probes) to enrich specific target loci from DNA. This method allows for data collection of low-copy nuclear genes and high-copy genomic targets for evolution and phylogenetic studies. Low-copy nuclear genes are especially important in phylogenetic reconstruction as they contain more informative characters than high-copy plastid genes, and are thus useful for studying species level relationships. Low-copy genes are also important for minimizing orthology issues in downstream analyses. In addition to recovery of the target loci, in the Hyb-Seq method off-target reads are also usually recovered. These off-target reads include plastid and mitochondrial DNA, repetitive DNA, and regions adjacent to the target loci which often include intronic regions (Mandel et al., 2017).

Mandel et al. (2017) successfully used Hyb-Seq data to reconstruct relationships across major lineages of Compositae as well as among closely related species belonging to the genus *Helianthus* (sunflowers). In a future research project, I will use this established Hyb-Seq protocol to reconstruct a well-resolved phylogeny for *Cirsium* in North America. I selected this protocol for following three reasons. First, this method has been shown to resolve evolutionary relationships at all taxonomic levels within the Compositae (Mandel et al., 2017). Second, this protocol is amenable to sequencing DNA from herbarium specimens, because it is robust to

DNA degradation. This is particularly useful for studying *Cirsium* diversification in North America. For example, additional sampling of *C. rydbergii* from throughout its range is necessary to include populations from unsampled geographic areas. Because many of these localities are difficult to access or inaccessible other than by rafting, many samples will be taken from previously collected herbarium specimens. Third, the data will be readily combinable with other worldwide studies of Compositae using the same bait probe set. This method will allow for development of a phylogeny with additional resolution among North American *Cirsium*.

A well-resolved phylogeny is also critical to inferring the biogeographic history of *Cirsium* in North America. A major goal of evolutionary biology is to understand the abiotic (e.g., climate change, mountain uplift, soil chemistry) and biotic (e.g., pollinator preference, fruit dispersal, growth form, chemical defenses, floral morphology) factors that facilitate and/or promote diversification (the rate that new species form and other species go extinct) within lineages and shape patterns of species' distribution. The interplay of abiotic and biotic factors generates selective pressures, stimulating morphological diversity and adaptive innovations. Speciation occurs when sufficient ecological, functional, or reproductive differences have accumulated, resulting in evolutionary independence from progenitors (De Queiroz, 2007). Over time, species must either isolate in unaltered ecosystems, or diversify and adapt to changing abiotic and biotic pressures. Ultimately, failure to either isolate or adapt puts species at risk of extinction.

The factors influencing diversification are often studied in lineages that have undergone radiations (Givnish et al., 2009). The term 'radiation' has several differing conceptual definitions (e.g., adaptive radiations, non-adaptive radiations, rapid radiations, exaptive radiations; Rundell & Price, 2009; Losos & Mahler, 2010), but essentially each concept incorporates two processes:

the adaptation of a lineage to changing biotic and/or abiotic conditions, and lineage (species) diversification. In particular, in an adaptive radiation, a single lineage rapidly diversifies into multiple lineages which are specialized to inhabit unique ecological niches (Givnish et al., 2009).

I hypothesize that *Cirsium* has undergone a recent continental-wide radiation across North America, with most extant taxa originating 1–2 Myr. The distributions of North American *Cirsium* are undoubtedly influenced by edaphic and topographic conditions, climate and moisture availability, and substrate specificity. Most native thistles are restricted to specific ecological niches including prairies, salt marshes, sand dunes, pine barrens, shale barrens, alpine tundra, limestone cliffs, hanging gardens, and desert seeps (Keil, 2006). The evolutionary history and origin of alpine plants in NA mountain systems is in particular not well understood. It is unclear whether alpine species originate from multiple lowland progenitors to mountain tops, or constitute alpine radiations assisted by long-distance dispersal across geographic barriers.

Within North America, numerous unique ecological niches are present, with the majority of ecological diversity occurring in the western states and Mexico, including the California Floristic Province (CA-FP), a known biodiversity hotspot (Baldwin 2014). The diversity of younger lineages, especially in western NA, has been hypothesized to be the result of several factors. First, the wide array of diverse biomes, climates, and topography across NA have undoubtedly contributed to the observed floristic abundance by providing ecological opportunities that facilitated rapid diversification. Second, aridification of the west during the Quaternary provided ecological opportunity for many lineages to expand and diversify. Third, hybridization has also long been known to play a role in the diversification of plant lineages, especially in those that are younger and have had less time for establishment of reproductive barriers (Kleinkopf et al., 2019). Evidence suggests that introgression is common in the

evolutionary history of many plant lineages (Mallet, 2005). However, it is often difficult to separate hybridization from incomplete lineage sorting (ILS), especially in recently radiated lineages (Kleinkopf et al., 2019).

Despite the abundance and diversity of plant lineages throughout western NA, the factors influencing their diversification have been relatively unstudied. Because these regions have, at least in a geologic timescale, only recently been opened for occupation, lineages that have taken advantage of ecological opportunity are also more recently diverged. Thus, these younger lineages generally lack sufficient character evidence to resolve phylogenetic relationships among species given traditional Sanger sequencing methods. Therefore, studying the factors effecting the diversification and biogeography of these lineages, especially in western NA, has been greatly hindered.

Use of the Hyb-Seq method will greatly enhance our understanding of thistle diversification and speciation in North America by providing significantly more informative characters with which to build a well-resolved phylogeny. This phylogeny will then be used to reconstruct the biogeographic history of North American thistles. The biogeographic study will address questions such as: Was the ancestral biome for *Cirsium* in North America xeric or mesic? Is there an association with the expansion of thistles and an increase in arid environments? Are there hotspots of diversity, such as the CA-FP, that have had a longer time for speciation? Has dispersal away from these hotspots been limited by phylogenetic niche conservatism and an inability to adapt to different niches? How did Quaternary glaciation cycles affect the distribution of modern North American *Cirsium* distributions?

The future study of the hanging garden thistles will provide valuable insight into how hanging garden and alpine plant species are related, and serve as a predictor for other non-

Cirsium plant lineages. Coupling high throughput sequencing with ecological-niche modeling will provide additional insights into the past distributions of extant plant lineages and the current genetic variation across the Colorado Plateau.

A robust phylogeny is also necessary to determine if lineages may have arisen through hybridization. The impact of incomplete lineage sorting in the evolutionary history of the group can also be determined by examining discordant gene trees. In combination with additional morphological analyses, a robust phylogeny built from Hyb-Seq data will also aid in sorting out any contentious or remaining taxonomic issues, such as those mentioned above.

In short, there are many questions remaining in the study of the thistles. These future studies will aid in resolving remaining taxonomic issues, inferring the biogeographic history of thistles in North America, and enhancing our understanding of the processes influencing speciation. I look forward to working on these future projects and continuing to untangle the taxonomy of *Cirsium*.

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APPENDICES

Appendix 1

Voucher data, sources of material, and GenBank accession numbers for the 173 taxa included in this work. Voucher data are in the following format: taxon name, country, locality, collection and collection number, herbarium of deposition and unique specimen identifier, ITS, ETS, *matK*, *ndhF*, *psbA-trnH*, *trnL-trnF* GenBank accession numbers, and chromosome count (*n*). An “–” indicates missing data; an asterisk (*) indicates new sequence data.

Brachylaena discolor DC., AY826236, –, AY85090, AF233828, –, AY772280; *Carduus acanthoides* L., JX867641, JX867669, KT249935, –, –, KC969560, *n*=11; *Carduus adpressus* C.A.Meyer, KT013056, –, –, –, –, –, *n*=9; *Carduus amarus* Rech.fil., KT013057, –, –, –, –, –; *Carduus candicans* Waldst.& Kit., KT013061, –, –, –, –, –, *n*=8; *Carduus carlinoides* Gouan, AY826240, –, AY013527, KC589931, –, AY772284, *n*=9; *Carduus crispus* L., GU188570, –, JN894376, –, AY914835, AY914855, *n*=8; *Carduus defloratus* L., AY826241, –, AY785091, KC589932, HG800511, AY772285, *n*=11; *Carduus keniensis* R.E.Fr., KC590040, –, KC590013, KC589933, –, KC590047, *n*=17; *Carduus lanuginosus* Willd., KT013065, –, –, –, –, –; *Carduus macrocephalus* Desf., KY242485, –, –, –, –, –, *n*=8; *Carduus nawaschirii* Bordz., KT013069, –, –, –, –, –; *Carduus nutans* L., AF443678, JX867670, KC969472, KT176826, AF129839, AF129825, *n*=8; *Carduus nyassanus* R.E.Fr., KC590041, –, KC590014, KC589934, –, KC590048, *n*=17; *Carduus olympicus* Boiss., KT013085, –, –, –, –, –; *Carduus pycnocephalus* L., EF123105, –, AY013528, –, –, KC969561, *n*=31; *Carduus tenuiflorus* Curtis, AF443679, AF443731, KC969473, –, –, KC969562, *n*=27; *Carduus tmoleus* Boiss., KT013085, –, –, –, –, –, *n*=11; *Carduus transcaspicus* Gand. subsp. *macrocephalus* Kazmi, KT013066, –, –, –, –, –, *n*=17;

Carlina acanthifolia All., KF301216, KF301145, AY013529, KC589935, –, KF301173;
Chuiraga avellanadae Lorentz, KJ789754, –, EU841333, –, EU841281, EF530276; *Cirsium*
aduncum Fisch. & C.A.Meyer ex DC., Turkey: *Yildiz sn* (CDA Yildiz pers. herb.), MN335104*,
MN230923*, –, –, –, MN314878*, $n=17$; *Cirsium agregatum* Ledeb., Turkey: Trabzon, Caykara,
20 Aug 2006, *Yildiz 16466* (CDA Yildiz pers. herb.), MN335058*, MN230878*, –, –, –, –;
Cirsium alatum (S.G.Gmel.) Bobrov, Turkey: Erzurum, 3 Aug 2006, *Yildiz 16280* (CDA),
MN335057*, MN230877*, –, –, –, MN314880*; *Cirsium alpis-lunae* A.J.B.Brilli-Cattarini &
L.Gubellini, KC969542, –, KC969495, –, –, KC969579, $n=17$; *Cirsium altissimum* (L.) Hill,
U.S.A.: Kansas, Harvey Co., Corner of Rock Rd.and NE 36th St., Newton, 5 Aug 2016,
Ackerfield 16-103 (CS Ackerfield pers. herb.), MN335120*, MN230941*, MN275358*,
MN275377*, MN275444*, MN314901*, $n=9$; *Cirsium amani* Post, Turkey: Osmaniye, *Yildiz*
16421 (CDA Yildiz pers. herb.), MN335056*, MN230876*, –, –, –, –; *Cirsium anartiolepis*
Petr., Mexico: Unam, Rumbo al llano de los Tres Gobernadores, 20 Mar 2005, *M.Tenorio 994*
(MEXU 1172327), MN335122*, MN230945*, MN275345*, MN275380*, MN275442*,
MN314938*, $n=17$; *Cirsium andersonii* (A.Gray) Petr., U.S.A.: Nevada, Washoe Co., Hwy 431
at overlook area, 28 Aug 2009, *L.Rowe sn* (RENO 49919), MN335131*, MN230959*, –,
MN275370*, MN275432*, MN314910*, $n=16$; *Cirsium andrewsii* (A.Gray) Jeps., U.S.A.:
California, Santa Clara Co., off the side of Marine Dr., 28 Apr 2012, *D.Park 327-1* (DAV
210229), MN335133*, MN230965*, MN275320*, MN275391*, MN275421*, MN314913*,
 $n=16$; *Cirsium arizonicum* (A.Gray) Petr.var.*arizonicum*, U.S.A.: Arizona, Coconino Co.,
Coconino National Forest N rim of West Fork Canyon, 14 Jun 2001, *E.Gilbert 712* (ASU
246878), MN335141*, MN230979*, MN275327*, MN275404*, MN275465*, MN314929*,
 $n=16$; *Cirsium arvense* (L.) Scop., AY826264, AF443734, KC969499, –, HQ596645,

KC969583, $n=17$; *Cirsium arvense* (L.) Scop., U.S.A.: Illinois, Cook Co., 12 Jun 1998, *D.Kelch s.n.*(UC), MN335070*, MN230890*, –, –, MN275410*, MN314882*, $n=17$; *Cirsium arvense* (L.) Scop., France: Montpellier, ex seed distributed ARC, MN335071*, MN230891*, –, –, MN275411*, MN314883*, $n=17$; *Cirsium arvense* (L.) Scop., JX867618, AF443734, –, –, –, –, $n=17$; *Cirsium aytachii* H.Duman & R.R.Mill, Turkey: 16 Jul 2007, *Yildiz 16439* (CDA *Yildiz pers. herb.*), MN335073*, MN230893*, –, –, –, –; *Cirsium baytopae* Davis & Parris, KC969545, –, KC969500, –, –, KC969584; *Cirsium bertolonii* Spreng., KC969546, –, KC969501, –, –, KC969585, $n=17$; *Cirsium boluense* Davis & Parris, Turkey: 22 Aug 2007, *Yildiz 16612* (CDA *Yildiz pers. herb.*), MN335074*, MN230894*, –, –, –, –; *Cirsium botryodes* Petr.ex Hand.-Mazz., MH710807, –, –, –, –, –; *Cirsium bracteosum* DC., Turkey: Agri, 12 Aug 2007, *T.Dirminci 3551* (CDA *Dirminci pers. herb.*), MN335076*, MN230896*, –, –, –, –, $n=17$; *Cirsium brevifolium* Nutt., U.S.A.: Idaho, Adams Co., Hells Canyon Rd, 26 Jun 2011, *D.William 2011-30* (SRP 191909), MN335106*, MN230925*, MN275356*, –, MN275417*, MN314887*, $n=11$; *Cirsium brevistylum* Cronquist, U.S.A.: Oregon, Yamhill Co., Walker Flat, 5 Jul 1995, *R.Halse 4944* (OSC 183033), MN335132*, MN230963*, MN275335*, MN275389*, MN275408*, MN314912*, $n=17$; *Cirsium bulgaricum* DC., Turkey: Giresun, 22 Aug 2006, *Yildiz 16391* (CDA), MN335077*, MN230897*, –, –, –, –, $n=15$; *Cirsium byzantinum* Steud., Turkey: *B.Yildiz sn* (CDA), MN335078*, MN230898*, –, –, –, –; *Cirsium candelabrum* Griseb., Turkey: Kırklarell, *Yildiz BY16525* (CDA), MN335079*, MN230899*, –, –, –, –, $n=17$; *Cirsium canescens* Nutt., U.S.A.: Wyoming, Carbon Co., Muddy Gap, 7 Aug 2016, *Ackerfield 16-113* (CS *Ackerfield pers. herb.*), MN335146*, MN230938*, MN275350*, MN275367*, MN275447*, MN314898*, $n=17$; *Cirsium canum* All., AF443689, AF443740, KT250105, –, –, –, $n=17$; *Cirsium canum* All., France: Montpellier, ex seed distributed ARC, *D.Kelch DGK*

01.030 (UC), MN335063*, MN230883*, -, -, -, -, n=17; *Cirsium carolinianum* (Walter) Fernald & B.G.Schub., U.S.A.: South Carolina., Lancaster Co., Langley Branch, 21 May 2014, *A Pittman sn* (USCH 05211401), MN335109*, MN230928*, MN275354*, -, MN275454*, MN314890*, n=10; *Cirsium cassium* Davis & Parris, Turkey: Hatay, *Yildiz 16420* (CDA Yildiz pers. herb.), MN335080*, MN230900*, -, -, -, -; *Cirsium caucasicum* (Adams) Petr., Turkey: Ardahan, 17 Aug 2006, *Yildiz 16329* (CDA Yildiz pers. herb.), MN335059*, MN230879*, -, -, -, -; *Cirsium cephalotes* Boiss., Turkey: Giresun, 5 Jul 2007, *Yildiz 16386* (CDA Yildiz pers. herb.), MN335060*, MN230880*, -, -, -, -; *Cirsium ciliatum* Moench, Turkey: Van, between Ercis & Patnos, 13 Aug 2017, *T.Dirmenci 3564* (CDA Yildiz pers. herb.), MN335061*, MN230881*, -, -, -, -; *Cirsium clavatum* (M.E.Jones) Petr. var. *clavatum*, U.S.A.: Utah, Garfield Co., Allen Well & Pelham Draw, 21 Jul 2004, *M.Madsen & H.Lovell 1853* (BRY 472985), MN335158*, MN230975*, MN275329*, MN275401*, MN275463*, MN314930*, n=17; *Cirsium clavatum* (M.E.Jones) Petr. var. *osterhoutii* (Rydb.) D.J.Keil, U.S.A.: Colorado, Eagle Co., off Hwy 24 south of Tennessee Pass, 26 Jun 2016, *Ackerfield 16-38* (CS Ackerfield pers. herb.), MN335139*, MN230976*, MN275328*, MN275402*, MN275464*, MN314917* n=17; *Cirsium coahuilense* G.B.Ownbey & Pinkava, Mexico: Coahuila, At Hermanas at Hot Springs W of Hwy 57, 9 Jun 2004, *Henrickson 23575* (MEXU 1439450), MN335164*, MN230961*, -, -, MN275468*, -, n=15; *Cirsium conspicuum* (G.Don) Sch.Bip., Mexico: Guerrero, Taxco, 28 May 1998, *R.Duran 2302* (MEXU 901981), MN335125*, MN230946*, -, -, MN275441*, MN314926*, n=17; *Cirsium cosmellii* Adams ex DC., Turkey: *Yildiz sn* (CDA Yildiz pers. herb.), MN335064*, MN230884*, -, -, -, -, n=17; *Cirsium crassicaule* (Greene) Jeps., U.S.A.: California, Kern Co., along west bank of outlet canal near Adobe, 30 May 2004, *Moe 2591* (RSA 709002), MN335121*, MN230944*, MN275346*, MN275379*, MN275443*,

MN314903*, n=16; *Cirsium creticum* (Lam.) D'Urv., Turkey: Cannakale, 31 Jul 2007, *Yildiz 16495* (CDA Yildiz pers. herb.), MN335081*, MN230901*, -, -, -, -, n=17; *Cirsium cymosum* (Greene) T.Howell var. *canovirens* (Rydb.) D.J.Keil, U.S.A.: California, Kern Co., 9 May 2004, *D.Keil 30302-8* (OBI 67343), MN335114*, MN230934*, MN275314*, -, MN275448*, MN314894*, n=17; *Cirsium discolor* (Muhl.ex Willd.) Spreng., AF443692, AF443744, KP643137, GU817845, GU818355, GU817988, n=10; *Cirsium douglasii* DC. var. *breweri* (A.Gray) D.J.Keil & C.E.Turner, U.S.A.: California, Trinity Co., 58.3 mi W of Red Bluff along rt 36, 25 Jun 2003, *Funk 12450* (RSA 736179), MN335126*, MN230948*, -, MN275382*, MN275439*, MN314904*, n=15; *Cirsium douglasii* DC. var. *douglasii*, U.S.A.: Nevada, Ormsby Co., Kings Canyon, 15 Jul 2001, *A.Tiehm 13690* (RENO 49971), MN335159*, MN230980*, MN275326*, MN275405*, MN275466*, MN314933*, n=17; *Cirsium eatonii* (A.Gray) B.L.Rob. var. *eatonii*, U.S.A.: Utah, Duchesne Co., Uinta Mountains Hades Canyon, 19 Aug 2009, *S.Goodrich 27674* (USU 478715), MN335113*, MN230933*, MN275317*, -, MN275449*, MN314893*, n=17; *Cirsium eatonii* (A.Gray) B.L.Rob. var. *eriocephalum* (A.Gray) D.J.Keil, U.S.A.: Colorado, Park Co., Mount Sherman west of Fairplay, 9 Aug 2015, *Ackerfield 15-127* (CS Ackerfield pers. herb.), MN335108*, MN230927*, MN275321*, -, MN275455*, MN314889*, n=17; *Cirsium eatonii* (A.Gray) B.L.Rob. var. *peckii* (L.F.Hend.) D.J.Keil, U.S.A.: Oregon, Harney Co., Steen's Mountain, 20 Jul 2007, *Y.Yuan 07-19* (WTU 386209), MN335110*, MN230929*, MN275322*, -, MN275453*, MN314891*, n=16; *Cirsium eatonii* (A.Gray) B.L.Rob. var. *viperinum* D.J.Keil, U.S.A.: Nevada, White Pine Co., Schell Creek Range, 25 Aug 1998, *A.Tiehm 12690* (RENO 51636), MN335137*, MN230983*, MN275324*, MN275369*, MN275412*, MN314919*, n=17; *Cirsium echinus* (M.Bieb.) Hand.-Mazz., AY826263, -, AY013535, KC589950, -, AY772305, n=17; *Cirsium edule*

Nutt.var.*edule*, U.S.A.: Oregon, Lane Co., Along FS Rd 35, 29 Jul 2008, *R.Halse* 7484 (OSC 219119), MN335143*, MN230964*, MN275334*, MN275390*, MN275409*, MN314922*, *n*=17; *Cirsium ehrenbergii* Sch.Bip., Mexico: Oaxaca: Dt.Miahuatlan, 1.4 km antes de Zapotitlan, 3 Aug 2011, *R.Martinez* 433 (MEXU 1303352), MN335157*, MN230958*, MN275310*, MN275372*, MN275433*, –, *n*=18; *Cirsium ekimianum* Yildiz & Dirmenci, Turkey: Erzurum, ca.10 km from Aşkale, 12 Aug 2007, *Yildiz* 16270 (CDA Yildiz pers. herb.), MN335082*, MN230902*, –, –, –, –; *Cirsium ellenbergii* Bornm., Turkey: Maraş, Göksun, 2 Sep 2006, *Yildiz* 16430 (CDA), MN335083*, MN230903*, –, –, –, –; *Cirsium engelmannii* Rydb., U.S.A.: Texas, Tarrant Co., Tandy Hills Natural Area, 10 Jun 2016, *M.B.Best* 346a (CS Ackerfield pers. herb.), MN335148*, MN230947*, MN275344*, MN275381*, MN275440*, MN314925*, *n*=10; *Cirsium eriophorum* (L.) Scop., Austria: South Wien, 24 Jul 2005, *Yildiz sn* (CDA), MN335084*, MN230904*, JN895546, –, –, MN314877*, *n*=17; *Cirsium erisithales* (Jacq.) Scop., KC969550, –, KC969508, –, –, KC969592, *n*=17; *Cirsium flodmanii* (Rydb.) Arthur, U.S.A.: Colorado, Larimer Co., Soapstone Prairie Natural Area, 7 Aug 2012, *J.McAleer* 430a (CS 110317), MN335107*, MN230926*, MN275357*, –, MN275456*, MN314888*, *n*=11; *Cirsium fontinale* (Greene) Jeps.var. *campylon* (H.Sharsm.) Pilz ex Keil & C.Turner, U.S.A.: California, Santa Clara Co., N of Morgan Hill on US 101, 11 Aug 1998, *D.Keil* 27922 (OBI 57723), *fontinale* (Greene) Jeps. var. *fontinale*, AF443695, AF443747, KC969509, –, –, –, *n*=17; *Cirsium fontinale* (Greene) Jeps. var. *obispoense* T.Howell, AF443696, AF443748, –, –, –, *n*=17; *Cirsium grahamii* A.Gray, U.S.A.: New Mexico, Catron Co., Gila Cliff Dwellings, 23 Aug 2002, *E.Bennett* 185 (ARIZ 364086), MN335150*, MN230971*, MN275332*, MN275397*, MN275413*, MN314941*, *n*=16; *Cirsium handaniae* Yildiz, Dirmenci & Arabaci, Turkey: Ardahan, 17 Aug 2006, *B.Yildiz* 16333 (CDA Yildiz pers. herb.), MN335085*,

MN230905*, -, -, -, -; *Cirsium handelii* Petr.ex Hand.-Mazz., China: Yunnan, 12 Jul 2000
 (CAS Kelch pers. herb.), MN335086*, MN230906*, -, -, -, -; *Cirsium henryi* (Franch.) Diels,
 AF443697, AF443749, -, -, -, -; *Cirsium heterophyllum* (L.) Hill, KX166058, -, JN895548, -, -
 , GQ244802, n=17; *Cirsium hydrophilum* (Greene) Jeps., AF443698, AF443750, KC969510, -,
 -, KC969594, n=16; *Cirsium hypoleucum* DC., Turkey: Kastamonu, Ilgaz, 23 Aug 2006, *Yildiz*
16409 (CDA Yildiz pers. herb.), MN335087*, MN230907*, -, -, -, -, n=17; *Cirsium*
inamoenum (Greene) D.J.Keil, U.S.A.: Nevada, Mineral Co., Bodie Hills, 4 Jun 2013, *A.Tiehm*
16540 (RENO 23124), MN335136*, MN230981*, MN275325*, MN275406*, MN275459*,
 MN314918*, n=17; *Cirsium interpositum* Petr., China: Yunnan, 3 Nov 1990 (CAS Kelch pers.
 herb.), MN335065*, MN230885*, -, -, -, -, n=17; *Cirsium italicum* DC., Italy (CAS Kelch pers.
 herb.), MN335089*, MN230909*, -, -, -, -, n=34; *Cirsium japonicum* Fisch.ex DC.,
 KM051436, -, HM989744, -, GU724249, -, n=17; *Cirsium jorullense* (Kunth) Spreng., Mexico:
 Michoacan, Mun.Anganguero, 13 Aug 2014, *D.Alvarez 13227* (MEXU 1417986), MN335124*,
 MN230969*, MN275313*, MN275395*, MN275419*, MN314936*, n=17; *Cirsium*
kamtschaticum Ledeb.ex DC., U.S.A.: Alaska, Aleutian Islands, Oct 2000, *S.Talbot 001-6* (ALA
 1010423), AB035987, MN230982*, MN275316*, MN275366*, MN275425*, MN314920*,
 n=34; *Cirsium lappoides* (Less.) Sch.Bip., Mexico: La Paz, Santa Rita, 28 May 2015, *A.Naraez*
799 (MEXU 1423856), MN335128*, MN230950*, MN275342*, MN275384*, MN275416*,
 MN314905*; *Cirsium leucopsis* DC., Turkey: Asparta, 6 Sep 2006, *B.Yildiz 16457* (CDA Yildiz
 pers. herb.), MN335088*, MN230908*, -, -, -, -; *Cirsium lidjiangense* Petr.ex Hand.-Mazz.,
 AY914828, -, -, -, AY914836, AY914856; *Cirsium ligulare* Boiss., Turkey: Afyon, 10 Aug
 2006, *Yildiz 16223* (CDA Yildiz pers. herb.), MN335090*, MN230910*, -, -, -, -, n=17;
Cirsium lineare Sch.Bip., AF443727, AF443779, KX526545, -, -, -, n=14; *Cirsium longistylum*

W.Moore & Frankton, U.S.A.: Montana, Meagher Co., Kings Hill Pass, 12 Aug 2016, *Ackerfield 16-132* (CS Ackerfield pers. herb.), MN335119*, MN230940*, MN275348*, MN275376*, MN275445*, MN314900*, *n*=17; *Cirsium macrobotrys* (C.Koch) Boiss., Turkey: Sivas, İmranlı, 11 Aug 2006, *Yildiz 16237* (CDA Yıldiz pers. herb.), MN335091*, MN230911*, –, –, –, –, *n*=17; *Cirsium mexicanum* DC., Mexico: Oaxaca, 3.5 km al N de Tamazulapan, 19 Mar 1997, *J.Villaseñor 1399* (MEXU 1367917), MN335161*, MN230962*, JQ586816, –, MN275430*, –, *n*=11; *Cirsium mohavense* (Greene) Petr., U.S.A.: Nevada, Nye Co., 1.5 mi E of 376, 18 Jul 2001, *B.Niell sn.*(RENO 51668), MN335149*, MN230970*, MN275319*, MN275396*, MN275460*, MN314915*, *n*=15; *Cirsium monocephalum* (Vaniot) H.Lev., AF443701, AF443753, –, –, –, KF301170; *Cirsium monspessulanum* (L.) Hill, AF443717, AF443769, KC969512, –, –, KC969597, *n*=17; *Cirsium nipponicum* (Maxim.) Mak., KC590042, –, KC590016, KC589951, –, KC590049, *n*=34; *Cirsium nuttallii* DC., U.S.A.: South Carolina, Barnwell Co., South Edisto River on E side of SC 39, 2 Jul 2011, *J.Nelson 29687* (USCH 110742), MN335123*, MN230968*, MN275362*, MN275394*, MN275407*, MN314921*, *n*=13; *Cirsium obvallatum* (M.Bieb.) M.Bieb., Turkey: *Yildiz sn* (CDA Yıldiz pers. herb.), MN335092*, MN230912*, –, –, –, MN314885*, *n*=17; *Cirsium occidentale* (Nutt.) Jeps. var. *candidissimum* (Greene) F.Macbr., U.S.A.: Nevada, Churchill Co., Fondaway Canyon, 8 May 2015, *A.Tiehm 16913* (RENO 61409), MN335144*, MN275333*, MN275333*, MN275392*, MN275429*, MN314940*, *n*=30; *Cirsium occidentale* (Nutt.) Jeps. var. *venustum* (Greene) Jeps., U.S.A.: California, Solano Co., Stebbins cold canyon reserve, 18 Jun 1999, *E.Dean 511* (DAV 149192), MN335145*, MN230953*, MN275360*, MN275385*, MN275436*, MN314944*, *n*=15; *Cirsium ochrocentrum* A.Gray var. *ochrocentrum*, U.S.A.: Kansas, Logan Co., off of I-70 at the Oakley exit, 5 Aug 2016, *Ackerfield 16-104* (CS Ackerfield pers. herb.), MN335118*,

MN230939*, MN275349*, MN275375*, MN275446*, MN314899*, n=16; *Cirsium oleraceum* (L.) Scop., KT249784, –, KT249982, –, –, –, n=17; *Cirsium osseticum* (Adams) Petr., Turkey: NE Turkey, *Yildiz sn* (CDA Yildiz pers. herb.), MN335093*, MN230913*, –, –, –, –, n=17; *Cirsium ownbeyi* S.L.Welsh, U.S.A.: Colorado, Moffat Co., Hackings Draw, 29 Jun 1988, *T.Naumann 254* (CS -33), MN335138*, MN230984*, –, –, MN275467*, MN314931*; *Cirsium palustre* (L.) Scop., AF443704, AF443756, AY013536, KC589953, –, AY772307, n=17; *Cirsium parryi* (A.Gray) Petr., U.S.A.: Colorado, Chaffee Co., East side of Cottonwood Pass, 23 Jul 2016, *Ackerfield 16-96* (CS Ackerfield pers. herb.), MN335151*, MN230942*, MN275359*, MN275378*, MN275415*, MN314902*, n=17; *Cirsium perplexans* (Rydb.) Petr., U.S.A.: Colorado, Montrose Co., Uncompahgre Basin hills SE of Cedar Creek, 21 Jun 2011, *L.Brummer 4320* (CS Brummer pers. herb.), MN335155*, MN230943*, MN275347*, MN275368*, MN275469*, MN314924*; *Cirsium pitcheri* (Torr. ex Eaton) Torr. & A.Gray, U.S.A.: Wisconsin, Door Co., South Sturgeon Bay Canal dune complex, 28 Sep 2007, *S.Zager 2007-72* (WIS v0253040WIS), MN335105*, MN230924*, –, –, MN275422*, MN314886*, n=17; *Cirsium polycephalum* DC., Turkey: Kirklarell, 2 Aug 2007, *Yildiz 16530* (CDA Yildiz pers. herb.), MN335094*, MN230914*, –, –, –, –; *Cirsium pringlei* (S.Watson) Petr., Mexico: Coahuila, Ladera alta de la Sierra La Veleta en el Ejido Nuncio, 13 Jul 2014, *J.Encina 3814* (MEXU 141662), MN335160*, MN230974*, MN275363*, MN275400*, MN275462*, MN314923*; *Cirsium pubigerum* (Desf.) DC., Turkey: Erzurum, Tortum, 16 Aug 2006, *Yildiz 16291* (CDA Yildiz pers. herb.), MN335095*, MN230915*, –, –, –, –, n=34; *Cirsium pulcherrimum* (Rydb.) K.Schum. var. *aridum* (Dorn) D.J.Keil, U.S.A.: Wyoming, Carbon Co., Chalk Mt.NW edge of Shirley Basin, 15 Jul 2010, *R.Dorn 10679* (RM 906929), MN335135*, MN230956*, MN275338*, MN275387*, MN275434*, MN314909*, n=17; *Cirsium*

pulcherrimum (Rydb.) K.Schum.var. *pulcherrimum*, U.S.A.: Wyoming, Carbon Co., Exit 267 off Hwy I-80 near Elk Mountain, 7 Aug 2016, *Ackerfield 16-111* (CS Ackerfield pers. herb.), MN335115*, MN275351*, MN275351*, MN275374*, MN275414*, MN314895*, *n*=17; *Cirsium pumilum* (Nutt.) Spreng. var. *hillii* (Canby) B.Boivin, JX867632, JX867660, MK519920, –, –, –, *n*=15; *Cirsium quercetorum* (A.Gray) Jeps., AF443706, AF443758, KC969516, –, –, KC969601, *n*=16; *Cirsium remotifolium* (Hook.) DC., U.S.A.: Oregon, Polk Co., McTimmonds Valley about 3.5 air mi N of Pedee, 1 Jul 1995, *R.Halse 4926* (WTU 333611), MN335153*, MN230960*, MN275336*, MN275388*, MN275431*, MN314911*, *n*=16; *Cirsium raphilepis* (Hemsl.) Petr., Mexico: Oaxaca, Santiago Tilantongo, 24 Apr 2013, *A.Piestryznska AP229* (MEXU 1415892), MN335142*, MN230967*, MN275361*, MN275393*, MN275428*, MN314914*, *n*=11; *Cirsium rhizocephalum* C.A.Mey., Turkey: Erzurum, 13 Aug 2006, *Yildiz 16279* (CDA Yildiz pers. herb.), MN335055*, MN230875*, –, –, –, MN314879*, *n*=17; *Cirsium rhotophilum* S.F.Blake, AF443709, AF443761, KC969518, –, –, KC969603, *n*=17; *Cirsium rigidum* DC., Turkey: 16 Aug 2006, *Yildiz 16345* (CDA Yildiz pers. herb.), MN335096*, MN230916*, –, –, –, –, *n*=17; *Cirsium rydbergii* Petr., U.S.A.: Utah, San Juan Co., Rainbow Plateau, *W.Hodgson 11740* (DES DES00044724), MN335054*, MN230874*, MN275355*, –, MN275427*, –, *n*=17; *Cirsium scabrum* (Poir.) Bonnet & Barratte, KC969554, –, KC969519, –, –, KC969604, *n*=17; *Cirsium scariosum* Nutt. var. *citrinum* (Petrak) D.J.Keil, U.S.A.: California, Ventura Co., Cuddy Valley, 30 Jun 2001, *D.Keil 29634F* (OBI 60342), MN335162*, MN230952*, –, –, MN275437*, MN314907* *n*=17; *Cirsium scariosum* Nutt.var. *congdonii* (R.J.Moore & Frankton) D.J.Keil, U.S.A.: California, San Bernardino Co., Transverse Ranges, 9 Jun 2010, *Fraga 3468* (RSA 60342), MN335127*, MN230949*, MN275343*, MN275383*, MN275458*, MN314942*, *n*=17; *Cirsium scariosum*

Nutt.var. *scariosum*, Idaho, Boise Co., Boise National Forest south of Lowman, 21 Jul 2001, *D.Keil 29682C* (OBI 60356), MN335117*, MN230937*, MN275318*, MN275457*, MN275457*, MN314897*, *n*=17; *Cirsium shansiense* Petr., China: Yunnan, 4 Oct 1998 (CAS Kelch pers. herb.), MN335097*, MN230917*, –, –, –, –, *n*=34; *Cirsium sintenisii* Freyn, Turkey: Kastamonu: Ilgaz, 23 Aug 2006, *Yildiz 16408* (CDA Yildiz pers. herb.), MN335098*, MN230918*, –, –, –, –; *Cirsium sorocephalum* Fisch.& C.A.Mey., Turkey: *Yildiz sn* (CDA Yildiz pers. herb.), MN335099*, MN230919*, –, –, –, –, *n*=17; *Cirsium spinosissimum* (L.) Scop., AF443720, AF443772, KC969521, –, –, KC969606 *n*=17; *Cirsium steirolepis* Petr., Turkey: Cannakale, 31 Jul 2007, *Yildiz 16496* (CDA Yildiz pers. herb.), MN335100*, MN230920*, –, –, –, –, *n*=17; *Cirsium strigosum* (M.Bieb.) M.Bieb., Turkey: *Yildiz sn* (CDA Yildiz pers. herb.), MN335101*, MN230921*, –, –, –, –, *n*=17; *Cirsium subcoriaceum* (Less.) Sch.Bip., Mexico: Estado de Mexico, Valle de Bravo, Cerro Gordo, 19 Mar 2011, *J.Corrall 1560* (MEXU 1383975), MN335156*, MN230973*, MN275330*, MN275399*, MN275461*, MN314937*, *n*=17; *Cirsium tenoreanum* Petr., KC969556, –, KC969522, –, –, KC969607; *Cirsium texanum* Buckley, U.S.A.: Texas, Tarrant Co., Tandy Hills Natural Area, 10 Jun 2016, *M.B.Best 346b* (CS Best pers. herb.), MN335129*, MN230954*, MN275340*, MN275386*, MN275435*, MN314927*, *n*=11; *Cirsium tracyi* (Rydb.) Petr., U.S.A.: Colorado, Garfield Co., 5 miles northwest of De Beque, 25 Jun 2016, *Ackerfield 16-29* (CS Ackerfield pers. herb.), MN335152*, MN230931*, MN275352*, MN275373*, MN275451*, MN314892*, *n*=12; *Cirsium tymphaeum* Hausskn., Denmark: Seed ex Grakenland, *D.Kelch DGK 01.037* (UC Kelch pers. herb.), MN335102*, –, –, –, –, –, *n*=16; *Cirsium undulatum* (Nutt.) Spreng., U.S.A.: Montana, Meagher Co., East of White Sulphur Springs, 12 Aug 2016, *Ackerfield 16-125* (CS Ackerfield pers. herb.), MN335134*, MN230972*, MN275331*, MN275398*, MN275424*,

MN314916*, n=13; *Cirsium velatum* (S.Watson) Petr., Mexico: San Jose del Rincon, A 3.76 km al WSW de San Jose del Rincon, 30 Aug 2014, *D.Alvarez 13787* (MEXU 1407190), MN335154*, MN230978*, MN275312*, MN275403*, MN275420*, MN314935*, n=16; *Cirsium vinaceum* (Woot.& Standl.) Woot.& Standl., U.S.A.: New Mexico, Otero Co., 5.5 mi E of Sunspot Scenic Byway, 7 Jul 2006, *R.Gutiérrez 1171* (ASU 263869), MN335147*, MN230957*, MN275337*, –, MN275470*, MN314943*; *Cirsium virginianum* (L.) Michx., U.S.A.: South Carolina, Lee Co., Longleaf Pine Heritage Preserve, 17 Sep 2011, *A.Pittman 09171117* (USCH 112590), MN335111*, MN230930*, MN275353*, –, MN275452*, MN314932*, n=14; *Cirsium vulgare* (Savi) Ten., U.S.A.: California, Contra Costa Co., Crockett, 21 Jun, 2001, *D.Kelch DGK 01.038* (CDA Kelch pers. herb.), MN335068*, MN230888*, –, –, –, –, n=34; *Cirsium vulgare* (Savi) Ten, France: Seed ex Bas-Rhin, *D.Kelch DGK 01.038* (CDA), MN335069*, MN230889*, –, –, –, –, n=34; *Cirsium vulgare* (Savi) Ten, U.S.A.: Wyoming, Carbon Co., Muddy Gap off Hwy 287, 7 Aug 2016, *Ackerfield 16-112* (CS Ackerfield pers. herb.), MN335116*, MN230936*, MN275315*, MN275364*, MN275426*, MN314896*, n=34; *Cirsium wheeleri* (A.Gray) Petr., U.S.A.: New Mexico, Catron Co., E of Mogollon, 15 Aug 2010, *R.Sivinski 7733* (UNM 124841), MN335130*, MN230955*, MN275339*, MN275365*, MN275418*, MN314908*, n=14; *Cirsium wrightii* A.Gray, U.S.A.: New Mexico, Socorro Co., Ojo Caliente Spring, 5 Sep 2006, *R.Sivinski 6132* (UNM 111590), MN335140*, MN230977*, MN275323*, MN275371*, MN275423*, MN314934*; *Cirsium yildizianum* Arabaci & Dirmenci, Turkey: *Yildiz 16 333* (CDA Yildiz pers. herb.), MN335103*, MN230922*, –, –, –, –; *Cynara cardunculus* L., JX867643, JX867671, KC969525, –, AF129842, AF129828, n=17; *Fulcaldea stuessyi* Roque & V.A.Funk, KF989504, –, KF989831, KF989720, JF920289, JF920294; *Galactites tomentosa* Moench, AY780403, –, AY013541, –, AF129845, AY772328,

n=11; *Gerbera piloselloides* Forssk., GU126788, MG661595, EU385355, EU385163, –, MG661653; *Lamyropsis carpini* Greuter, GU907724, GU907742, KC590027, KC589978, –, KC590055, *n*=13; *Nastanthus patagonicus* Speg., KF989503, MH049386, KF989830, KF989719, KF989921, KF989611; *Notobasis syriaca* Cass., AY780405, –, AY013545, KC589981, AF129847, AY772340, *n*=17; *Onopordum tauricum* Willd., AY826309, –, KC969530, KC589987, –, KC969609, *n*=17; *Picnomon acarna* (L.) Cass., AY826311, –, AY013549, KC589989, AF129849, AY772349, *n*=16; *Ptilostemon afer* Greuter, AY780407, GU907746, AY013550, KC589992, AF129850, AY772354, *n*=16; *Silybum marianum* (L.) Gaertn., AY826329, AM267320, AY013551, KC589999, AF129851, AY772364, *n*=17; *Syreitschikovia spinulosa* Pavlov, AY826339, –, AY785122, KC590004, –, AY772374, *n*=12; *Tyrimnus leucographus* Cass., AY826343, –, AY013554, KC590007, AF129852, AY772378, *n*=17.

Appendix 2

Voucher data, sources of material, and GenBank accession numbers for the 168 taxa included in this work. Voucher data are in the following format: taxon name (sensu Keil, 2006), extraction accession number (only listed if multiple accessions of the same taxa are present), country, state, county, locality, collection and collection number, ITS, ETS, *matK*, *ndhF*, *psbA-trnH*, and *trnL-trnF* GenBank accession numbers. An “–” indicates missing data; an asterisk (*) indicates new sequence data.

Carduus nutans L., AF443678, JX867670, KC969472, KT176826, AF129839, AF129825; *Cirsium altissimum* (L.) Hill, MN335120, MN230941, MN275358, MN275377, MN275444, MN314901; *Cirsium anartiolepis* Petr., MN335122, MN230945, MN275345, MN275380, MN27544*, MN314938; *Cirsium andersonii* (A. Gray) Petr., MN335131, MN230959, –, MN275370, MN275432, MN314910; *Cirsium andrewsii* (A. Gray) Jeps., MN335133, MN230965, MN275320, MN275391, MN275421, MN314913; *Cirsium arizonicum* (A. Gray) Petr. var. *arizonicum*, 84, MN335141, MN230979, MN275327, MN275404, MN275465, MN314929; *Cirsium arizonicum* (A. Gray) Petr. var. *arizonicum*, 216DBG, U.S.A.: Arizona. Coconino Co.: Off the Jump-Up Nail Trail No. 8, 27 Sep 1998, *Hodgson 11167* (DES), MN604503*, MN604527*, MN604655*, –, MN617205*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *arizonicum*, 220DBG, U.S.A.: Utah. Beaver Co.: Wah Wah Mts., *N Holmgren 3731* (DES), MN604506*, MN604535*, MN604656*, –, MN617212*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *bipinnatum* (Eastw.) D.J. Keil, 1, U.S.A.: Colorado. Delta Co.: Escalante Canyon, 4 Aug 2012, *L Brummer 10952* (CS), MN604444*, MN604532*, MN604682*, MN617197*, –, MN617289*; *Cirsium arizonicum* (A. Gray) Petr. var. *bipinnatum* (Eastw.) D.J. Keil, 168DBG, U.S.A.: Utah. San Juan Co.: Glen Canyon National Recreation Area, 12 Jun 1996, *Hodgson*

9933 (DES), MN604505*, MN604529*, MN617172*, –, MN617207*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *bipinnatum* (Eastw.) D.J. Keil, 170DBG, U.S.A.: Utah. San Juan Co.: Navajo Reservation, Rainbow Plateau, 19 Jul 1999, *Hodgson 11748* (DES), MN604442*, MN604530*, MN617173*, –, MN617208*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *bipinnatum* (Eastw.) D.J. Keil, 217DBG, U.S.A.: Utah. San Juan Co.: Along trail leading from Cathedral Bluff to Salt Creek, 16 Jun 2009, *Hodgson 24049* (DES), MN604501*, MN604533*, MN604659*, –, MN617210*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *bipinnatum* (Eastw.) D.J. Keil, 219DBG, U.S.A.: Utah. San Juan Co.: Rainbow Plateau, Navajo Nation just south of trail to Rainbow Bridge, 19 Jul 1999, *Hodgson 11746* (DES), MN604504*, MN604528*, MN604611*, –, MN617206*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *chellyense* (R.J. Moore & Frankton) D. J. Keil, 214DBG, U.S.A.: Arizona. Apache Co.: Navajo Reservation, 15 Jul 1961, *D Demaree 44628* (ASC), MN604502*, MN604534*, MN604683*, –, MN617211*, –; *Cirsium arizonicum* (A. Gray) Petr. var. *rothrockii* (A. Gray) D.J. Keil, 55, U.S.A.: Arizona. Gila Co.: Carol Site, off State Rt 77, 17 Jul 2003, *D Damrel V 830* (ASU), MN604525, MN604536, MN604657*, MN617174*, MN617213*, MN617290*; *Cirsium arizonicum* (A. Gray) Petr. var. *tenuisectum* D.J. Keil, 148, U.S.A.: Nevada. Clark Co.: Toiyabe National Forest, Spring Mountains, 17 Aug 2002, *Keil 29912C* (OBI), MN604445*, MN604609*, MN604612*, –, MN617214*, MN617291*; *Cirsium arvense* (L.) Scop., AY826264, AF443734, KC969499, –, HQ596645, KC969583; *Cirsium barnebyi* S.L. Welsh & E. Neese, U.S.A.: Colorado. Garfield Co.: Logan Wash, 28 Jun 2016, *D Malone sn* (CS), MN604446*, MN604597*, MN604658*, MN617175*, MN617215*, MN617292*; *Cirsium brevifolium* Nutt., MN335106, MN230925, MN275356, –, MN275417, MN314887; *Cirsium brevistylum* Cronquist, MN335132, MN230963, MN275335, MN275389, MN275408, MN314912; *Cirsium canescens* Nutt., 5,

U.S.A.: Colorado. Chaffee Co.: Farish natural area, 28 Jun 2016, *P Smith sn* (CS), MN604448*, MN604598*, MN604684*, MN617199*, MN617217*, MN617294*; *Cirsium canescens* Nutt., 8, U.S.A.: Wyoming. Albany Co.: West of Laramie just before mile marker 306 off of Hwy I-80, 7 Aug 2016, *Ackerfield 16-106* (CS), MN604449*, MN604538*, MN604614*, MN617176*, MN617218*, MN617295*; *Cirsium canescens* Nutt., 16, MN335146, MN230938, MN275350, MN275367, MN275447, MN314898; *Cirsium canescens* Nutt., 19, U.S.A.: Colorado. Chaffee Co.: Hwy 24/285 approximately 9 miles east of Buena Vista, 14 Jun 2016, *Ackerfield 16-25* (CS), MN604447*, MN604537*, MN604613*, MN617198*, MN617216*, MN617293*; *Cirsium carolinianum* (Walter) Fernald & B.G. Schub., MN335109, MN230928, MN275354, –, MN275454, MN314890; *Cirsium ciliolatum* (Henderson) J.T. Howell, U.S.A.: Oregon. Jackson Co.: Dead Indian Rd, 16 July 2001, *Keil 29646* (OBI), MN604450*, MN604539*, MN604661*, –, MN617219*, MN617296*; *Cirsium clavatum* (M.E. Jones) Petr. var. *americanum* (A. Gray) D.J. Keil, U.S.A.: Colorado. Boulder Co.: Along the Middle St. Vrain trail by Camp Dick Campground, 7 Aug 2010, *Ackerfield 3810* (CS), MN604451*, MN604540*, MN604664*, MN617177*, MN617220*, MN617297*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 23, U.S.A.: Colorado. Boulder Co.: West of Ward along road to Brainard Lake recreation area off of the road at Red Rock Lake, 7 Jul 2016, *Ackerfield 16-45* (CS), MN604456*, MN604544*, MN604620*, MN617200*, MN617225*, ---- MN617302*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 26, U.S.A.: Colorado. Larimer Co.: Off of Hwy 14 just west of the Big Bend Campground near Kinikinik, 29 Jun 2016, *Ackerfield 16-43* (CS), MN604457*, MN604545*, MN604685*, MN617201*, MN617226*, MN617303*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 39, U.S.A.: Colorado. Boulder Co.: Along the Middle St. Vrain trail by Camp Dick Campground, 7 Aug 2010, *Ackerfield 3808*

(CS), MN604458*, MN604546*, MN604662*, MN617178*, MN617227*, MN617304*;
Cirsium clavatum (M.E. Jones) Petr. var. *clavatum*, 76, U.S.A.: Utah. Iron Co.: Thunder Ridge Boy Scout Camp, Cedar Breaks formation, 16 Jul 2010, *L Higgins 29189* (BRY), MN604459*, MN604608*, MN604663*, MN617179*, MN617228*, MN617339*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 77, MN335158, MN230975, MN275329, MN275401, MN275463, MN314930; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 104, U.S.A.: Colorado. Grand Co.: Roosevelt National Forest, in meadows surrounding Dumont Lake, 28 June 2016, *Ackerfield 16-42* (CS), MN604452*, MN604541*, MN604616*, –, MN617221*, MN617298*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 115, U.S.A.: Colorado. Gunnison Co.: Gunnison National Forest: trail to cement caves off of Cement Creek Rd south of Crested Butte, 10 July 2017, *Ackerfield 6005* (CS), MN604453*, MN604606*, MN604617*, –, MN617222*, MN617299*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 128, U.S.A.: Utah. Garfield Co.: Teasdale Ranger District, Dixie National Forest, 18 July 2007, *M Madsen 3670* (BRY), MN604454*, MN604542*, MN604618*, –, MN617223*, MN617300*; *Cirsium clavatum* (M.E. Jones) Petr. var. *clavatum*, 129, U.S.A.: Utah. Iron Co. 1 mi. NE of Hancock Peak on Markagunt Plateau, 3 Aug 2005, *M Madsen 2612* (BRY), MN604455*, MN604543*, MN604619*, –, MN617224*, MN617301*; *Cirsium clavatum* (M.E. Jones) Petr. var. *osterhoutii* (Rydb.) D.J. Keil, 7, MN335139, MN230976, MN275328, MN275402, MN275464, MN314917; *Cirsium clavatum* (M.E. Jones) Petr. var. *osterhoutii* (Rydb.) D.J. Keil, 11, U.S.A.: Colorado. Eagle Co.: Off of Hwy 24 south of I-70 at Red Cliff along Shrine Pass Rd., 26 Jun 2016, *Ackerfield 16-34* (CS), MN604461*, MN604603*, MN604632*, MN617202*, MN617288*, MN617306*;
Cirsium clavatum (M.E. Jones) Petr. var. *osterhoutii* (Rydb.) D.J. Keil, 108, U.S.A.: Colorado. Park Co.: Weston Pass west of Fairplay, 8 Aug 2015, *Ackerfield 15-78* (CS), MN604460*,

MN604547*, MN604631*, –, MN617229*, MN617305*; *Cirsium coahuilense* G.B. Ownbey & Pinkava, MN335164, MN230961, –, –, MN275468, –; *Cirsium conspicuum* (G. Don) Sch. Bip., MN335125, MN230946, –, –, MN275441, MN314926; *Cirsium crassicaule* (Greene) Jeps., MN335121, MN230944, MN275346, MN275379, MN275443, MN314903; *Cirsium cymosum* (Greene) J.T. Howell var. *canovirens* (Rydb.) D.J. Keil, 70, U.S.A.: Montana. Madison Co.: Along Jack Creek 1.5 mi above the footbridge, 19 Jul 2011, *P Lesica 10640* (MONTU), MN604463*, MN604548*, MN604660*, MN617203*, MN617231*, MN617308*; *Cirsium cymosum* (Greene) J.T. Howell var. *canovirens* (Rydb.) D.J. Keil, 149, MN335114, MN230934, MN275314, –, MN275448, MN314894; *Cirsium cymosum* (Greene) J.T. Howell var. *cymosum*, 42, U.S.A.: California. Mono Co.: Eastern Sierra Nevada Mts., E of Hwy 89 0.2 mi S of Mono/Alpine county line, 2 Aug 2011, *Howald 2762* (RSA), MN604462*, MN604607*, MN604615*, MN617180*, MN617230*, MN617307*; *Cirsium discolor* (Muhl. ex Willd.) Spreng., AF443692, AF443744, KP643137, GU817845, GU818355, GU817988; *Cirsium douglasii* DC. var. *breweri* (A. Gray) D.J. Keil & C.E. Turner, MN335126, MN230948, –, MN275382, MN275439, MN314904; *Cirsium douglasii* DC. var. *douglasii*, MN335159, MN230980, MN275326, MN275405, MN275466, MN314933; *Cirsium durangense* (Greenm.) G.B. Ownbey, Mexico: Zacatecas: Miguel Auza, por la carretera de terraceria a Ramon Corona Dgo., 12 Sept 1997, *J Balleza 6896* (MEXU), MN604464*, MN604593*, –, –, MN617232*, –; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *clokeyi* (S.F.Blake) D.J. Keil, U.S.A.: Nevada. Clark Co.: Spring Mountains, Toiyabe National Forest, 17 Aug 2002, *Keil 29915A* (OBI), MN604465*, MN604549*, MN604665*, –, MN617233*, MN617309*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eatonii*, 66, U.S.A.: Utah: Piute Co.: 1/2 mi NE of Buillion Pasture at old ASPE transect/plots, Tushar Mt. Range, 1 Aug 2002, *M Madsen 1471* (BRY), MN604466*,

MN604599*, MN604667*, MN617181*, MN617235*, MN617311*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eatonii*, 89, U.S.A.: Nevada. Elko Co.: Ruby Mts., cirque area NE of Pearl Peak, S of Harrison Pass, 6 Aug 2013, *A Tiehm 16641* (RENO), MN604467*, MN604551*, MN604668*, MN617182*, MN617236*, MN617312*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eatonii*, 143, MN335113, MN230933, MN275317, –, MN275449, MN314893; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eatonii*, 151, U.S.A.: Utah. Salt Lake Co.: Wasatch Mts., Snowbird Resort east of Salt Lake City, 31 July 2004, *Keil 30328* (OBI), MN604514*, MN604550*, MN604666*, –, MN617234*, MN617310*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eriocephalum* (A. Gray) D.J. Keil, 124, MN335108, MN230927, MN275321, –, MN275455, MN314889; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eriocephalum* (A. Gray) D.J. Keil, 168, U.S.A.: Colorado. Summit Co.: Above Georgia Pass on Continental Divide, 19 Aug 2018, *L Yeatts sn* (CS), MN604469*, MN604553*, MN604678*, –, MN617238*, MN617314*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eriocephalum* (A. Gray) D.J. Keil, 169, U.S.A.: Colorado. Larimer Co.: Rocky Mountain National Park, *J Bromberg sn* (CS), MN604470*, MN604554*, MN604640*, –, MN617239*, MN617315*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eriocephalum* (A. Gray) D.J. Keil, 37, U.S.A.: Utah. Grand Co.: LaSal mountains, 6 Aug 2009, *J Fowler 6425* (RM), MN604471*, MN604555*, MN604641*, MN617204*, MN617240*, MN617316*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eriocephalum* (A. Gray) D.J. Keil, 97, U.S.A.: Colorado. Clear Creek Co.: Guanella Pass south of Georgetown, 15 Jul 2017, *C Jones sn* (CS), MN604472*, MN604556*, MN604642*, –, MN617287*, MN617317*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *eriocephalum* (A. Gray) D.J. Keil, 145, U.S.A.: Colorado. El Paso Co.: Pike's Peak below summit, 18 Jul 2018, *Ackerfield 6514* (CS), MN604474*, MN604557*, –, MN617242*, MN604622*, MN617319*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *hesperium*

(Eastw.) D.J. Keil, 24, U.S.A.: Colorado. Gunnison Co.: Cottonwood Pass summit, 23 Jul 2016, *Ackerfield 16-76* (CS), MN604475*, MN604558*, MN604624*, MN617183*, MN617243*, MN617321*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *hesperium* (Eastw.) D.J. Keil, 32, U.S.A.: Colorado: Mineral Co.: Hunter Lake US Hwy 160 W 9 air mi from Beaver Creek Rd to Forest Rd 419, 5 Jul 2003, *J Flaig 346* (RM), MN604476*, MN604559*, MN604625*, MN617184*, MN617283*, MN617322*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *hesperium* (Eastw.) D.J. Keil, 90, U.S.A.: New Mexico. Taos Co.: Sangre de Cristo Mts. trail into Serpent Lake, 31 Jul 2013, *R Sivinski 8592* (UNM), MN604477*, MN604560*, MN604669*, MN617185*, MN617244*, MN617323*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *hesperium* (Eastw.) D.J. Keil, 101, U.S.A.: Colorado. San Juan Co.: Stony Pass east of Silverthorn, 20 Jul 2017, *Ackerfield 6010* (CS), MN604473*, MN604602*, MN604621*, –, MN617241*, MN617318*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *hesperium* (Eastw.) D.J. Keil, 170, U.S.A.: Colorado. Huerfano Co.: Trinchera Peak, 8 Aug 2018, *Ackerfield 6546* (CS), MN604526*, –, MN604623*, –, MN617284*, MN617320*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *murdockii* S.L. Welsh, 28, U.S.A.: Montana. Gallatin Co.: Palisade Falls, 12 Aug 2016, *Ackerfield 16-123* (CS), MN604478*, MN604561*, MN604627*, MN617186*, MN617245*, MN617324*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *murdockii* S.L. Welsh, 85, U.S.A.: Utah. Uintah Co.: S slope of Uinta Mts., 26 Jul 2005, *G Brown & S Goodrich 159* (BRY), MN604479*, MN604604*, MN604670*, MN617187*, MN617246*, MN617325*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *murdockii* S.L. Welsh, 100, U.S.A.: Colorado. Clear Creek Co.: Approximately 1 mile up the trail to Shelf Lake off of Guanella Pass road, 18 Jul 2017, *Ackerfield 6009* (CS), MN604468*, MN604552*, MN604626*, –, MN617237*, MN617313*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *peckii* (L.F. Hend.) D.J. Keil, 92, U.S.A.: Nevada. Humboldt Co.: Jackson Mts., 2.3 rd miles

NE of the Jackson & Trout Creek rds junction, 16 Jun 2009, *A Tiehm 15885* (RENO), MN604480*, MN604562*, MN604671*, MN617188*, MN617247*, MN617338*; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *peckii* (L.F. Hend.) D.J. Keil, 130, MN335110, MN230929, MN275322, –, MN275453, MN314891; *Cirsium eatonii* (A. Gray) B.L. Rob. var. *viperinum* D.J. Keil, MN335137, MN230983, MN275324, MN275369, MN275412, MN314919; *Cirsium edule* Nutt. var. *edule*, MN335143, MN230964, MN275334, MN275390, MN275409, MN314922; *Cirsium ehrenbergii* Sch. Bip., MN335157, MN230958, MN275310, MN275372, MN275433, –; *Cirsium engelmannii* Rydb., MN335148, MN230947, MN275344, MN275381, MN275440, MN314925; *Cirsium flodmanii* (Rydb.) Arthur, MN335107, MN230926, MN275357, –, MN275456, MN314888; *Cirsium fontinale* (Greene) Jeps. var. *campylon* (H. Sharsm.) Pilz ex Keil & C. Turner, MN335163, MN230951, MN275341, –, MN275438, MN314906; *Cirsium fontinale* (Greene) Jeps. var. *fontinale*, AF443695, AF443747, KC969509, –, –, –; *Cirsium fontinale* (Greene) Jeps. var. *obispoense* J.T. Howell, AF443696, AF443748, –, –, –, –; *Cirsium grahamii* A. Gray, MN335150, MN230971, MN275332, MN275397, MN275413, MN314941; *Cirsium henryi* (Franch.) Diels, AF443697, AF443749, –, –, –, –; *Cirsium heterophyllum* (L.) Hill, KX166058, –, JN895548, –, –, GQ244802; *Cirsium hookerianum* Nutt., U.S.A.: Washington. Kittitas Co.: Sun East development on Robbins Rd, 26 April 2010, *Knoke 2004* (WTU), MN604481*, MN604563*, MN604628*, –, MN617248*, MN617326*; *Cirsium horridulum* Michx., U.S.A.: South Carolina. Lexington Co.: Approximately 100 m from 1021 Indian Mound Rd. residence, Lexington, 10 Apr 2007, *J Brannon 27* (USCH), MN604482*, MN604564*, MN604686*, MN617189*, MN617249*, MN617342*; *Cirsium hydrophilum* (Greene) Jeps., AF443698, AF443750, KC969510, –, –, KC969594; *Cirsium inamoenum* (Greene) D.J. Keil, MN335136, MN230981, MN275325, MN275406, MN275459, MN314918;

Cirsium japonicum Fisch. ex DC., KM051436, –, HM989744, –, GU724249, –; *Cirsium joannae* S.L. Welsh, N.D. Atwood & L.C. Higgins, U.S.A.: Utah. Washington Co.: Zion National Park, Iceberg Canyon NE of Kolob Arch on top second fall, 14 Aug 2002, *N Atwood et al.* 28867 (BRY), MN604483*, MN604605*, MN604687*, MN617196*, MN617250*, MN617327*; *Cirsium jorullense* (Kunth) Spreng., MN335124, MN230969, MN275313, MN275395, MN275419, MN314936; *Cirsium kamtschaticum* Ledeb. ex DC., MN230982, MN275316, MN275366, MN275425, MN314920; *Cirsium lappoides* (Less.) Sch. Bip., MN335128, MN230950, MN275342, MN275384, MN275416, MN314905; *Cirsium lecontei* Torr. & A. Gray, U.S.A.: South Carolina. Dorchester Co.: N side of Old Pond Rd about 7 air mi ESE of Harleyville, 26 Aug 2006, *J Nelson* 26077 (USCH), MN604484*, MN604565*, –, MN617190*, MN617251*, MN617340*; *Cirsium leucopsis* DC., MN335088, MN230908, –, –, –, –; *Cirsium lidjiangense* Petr. ex Hand.-Mazz., AY914828, –, –, –, AY914836, AY914856; *Cirsium lineare* Sch. Bip., AF443727, AF443779, KX526545, –, –, –; *Cirsium longistylum* J.W. Moore & Frankton, MN335119, MN230940, MN275348, MN275376, MN275445, MN314900; *Cirsium mexicanum* DC., MN335161, MN230962, JQ586816, –, MN275430, –; *Cirsium mohavense* (Greene) Petr., 43, U.S.A.: California. San Bernardino Co.: Bighorn Mts Wilderness, unnamed spring WSW of Mount Spring, 30 Jul 2014, *S DeGroot* 7219 (RSA), MN604485*, MN604594*, MN604672*, MN617191*, MN617256*, MN617328*; *Cirsium mohavense* (Greene) Petr., 67, MN335149, MN230970, MN275319, MN275396, MN275460, MN314915; *Cirsium mohavense* (Greene) Petr., 97DBG, U.S.A.: California. Inyo Co.: Death Valley, 27 Sep 1979, *M Kurzius* 1237 (DES), MN604516*, MN604570*, –, –, MN617257*, –; *Cirsium mohavense* (Greene) Petr., 206DBG, U.S.A.: Nevada. Lincoln Co.: Ash Springs Pahrnagat Valley, *Niles* 5344 (DES), MN604512*, MN604566*, MN604645*, –, MN617252*, –; *Cirsium mohavense* (Greene) Petr.,

207DBG, U.S.A.: Nevada. Clark Co.: Red Rock Springs, *Niles 4707* (DES), MN604513*, MN604567*, MN604646*, –, MN617253*, –; *Cirsium mohavense* (Greene) Petr., 209DBG, U.S.A.: Nevada. Lyon Co.: Honey Bee Pond, *Niles 4138* (DES), MN604515*, MN604568*, MN604629*, –, MN617254*, –; *Cirsium mohavense* (Greene) Petr., 213DBG, U.S.A.: Nevada. Clark Co.: Red Spring, *Leary 4240* (DES), MN604517*, MN604569*, MN604630*, –, MN617255*, –; *Cirsium monocephalum* (Vaniot) H.Lev., AF443701, AF443753, –, –, –, KF301170; *Cirsium monspessulanum* (L.) Hill, AF443717, AF443769, KC969512, –, –, KC969597; *Cirsium neomexicanum* A. Gray, 10, U.S.A.: Colorado. Delta Co.: Grand Mesa, Petrie Mesa ca 7 air mi NNW of Delta, 2 Jun 2011, *L Brummer 3594* (RM), MN623372*, MN623370*, MN629935*, MN629937*, MN629938*, MN629940*; *Cirsium neomexicanum* A. Gray, 1DBG, seed from New Mexico (DES), MN623373*, MN623371*, MN629936*, –, MN629939*, –; *Cirsium nipponicum* (Maxim.) Mak., KC590042, –, KC590016, KC589951, –, KC590049; *Cirsium nuttallii* DC., MN335123, MN230968, MN275362, MN275394, MN275407, MN314921; *Cirsium occidentale* (Nutt.) Jeps. var. *candidissimum* (Greene) J.F. Macbr., 62, MN335144, MN275333, MN275333, MN275392, MN275429, MN314940; *Cirsium occidentale* (Nutt.) Jeps. var. *candidissimum* (Greene) J.F. Macbr., 78, U.S.A.: Nevada. Pershing Co.: Humboldt Range, NW end of Spring Valley, 25 Jun 2011, *A Tiehm 16386* (RENO), MN604486*, MN604600*, MN604673*, MN617192*, MN617258*, MN617337*; *Cirsium occidentale* (Nutt.) Jeps. var. *venustum* (Greene) Jeps., MN335145, MN230953, MN275360, MN275385, MN275436, MN314944; *Cirsium ochrocentrum* A. Gray var. *ochrocentrum*, 17, MN335118, MN230939, MN275349, MN275375, MN275446, MN314899; *Cirsium ochrocentrum* A. Gray var. *ochrocentrum*, 161, U.S.A.: New Mexico. Socorro Co.: 6 miles southeast of Bingham, 16 Aug 2001, *Keil 29703* (OBI), MN604487*, MN604571*,

MN604674*, –, MN617259*, MN617341*; *Cirsium ownbeyi* S.L. Welsh, MN335138,
 MN230984, –, –, MN275467, MN314931; *Cirsium palustre* (L.) Scop., AF443704, AF443756,
 AY013536, KC589953, –, AY772307; *Cirsium parryi* (A. Gray) Petr., MN335151, MN230942,
 MN275359, MN275378, MN275415, MN314902; *Cirsium perplexans* (Rydb.) Petr.,
 MN335155, MN230943, MN275347, MN275368, MN275469, MN314924; *Cirsium pitcheri*
 (Torr. ex Eaton) Torr. & A. Gray, MN335105, MN230924, –, –, MN275422, MN314886;
Cirsium pringlei (S. Watson) Petr., MN335160, MN230974, MN275363, MN275400,
 MN275462, MN314923; *Cirsium pulcherrimum* (Rydb.) K. Schum. var. *aridum* (Dorn) D.J.
 Keil, MN335135, MN230956, MN275338, MN275387, MN275434, MN314909; *Cirsium*
pulcherrimum (Rydb.) K. Schum. var. *pulcherrimum*, MN335115, MN275351, MN275351,
 MN275374, MN275414, MN314895; *Cirsium pumilum* (Nutt.) Spreng. var. *hillii* (Canby) B.
 Boivin, JX867632, JX867660, MK519920, –, –, –; *Cirsium quercetorum* (A. Gray) Jeps.,
 AF443706, AF443758, KC969516, –, –, KC969601; *Cirsium remotifolium* (Hook.) DC.,
 MN335153, MN230960, MN275336, MN275388, MN275431, MN314911; *Cirsium repandum*
 Michx., U.S.A.: South Carolina. Lee Co.: Both sides of Lynches River Rd and 0.66 air mi SW of
 jct of Sec Hwys 187 and 13, 27 Jun 2014, *J Nelson 33174* (USCH), MN604488*, MN604601*,
 MN604681*, –, MN617260*, MN617329*; *Cirsium raphilepis* (Hemsl.) Petr., MN335142,
 MN230967, MN275361, MN275393, MN275428, MN314914; *Cirsium rhothophilum* S.F.
 Blake, AF443709, AF443761, KC969518, –, –, KC969603; *Cirsium rydbergii* Petr., 52DBG,
 U.S.A.: Arizona. Coconino Co.: Grand Canyon National Park, Buck Farm Canyon, 16 Sep 2009,
Hodgson 24398 (DES), MN604519*, MN604576*, MN604649*, –, MN617265*, –; *Cirsium*
rydbergii Petr., 58DBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon National Park, Buck
 Farm Canyon, 27 May 2004, *Hodgson 18219* (DES), MN604520*, MN604577*, MN604635*, –

, MN617266*, –; *Cirsium rydbergii* Petr., 67DBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon National Park, Clear Creek, 11 Sep 2005, *G Rink 4479* (DES), MN604521*, MN604578*, MN604650*, –, MN617267*, –; *Cirsium rydbergii* Petr., 68DBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon National Park, Bert's Canyon, 28 May 2004, *K Watters 20* (DES), MN604522*, MN604579*, –, –, MN617268*, –; *Cirsium rydbergii* Petr., 160DBG, MN335054, MN230874, MN275355, –, MN275427, –; *Cirsium rydbergii* Petr., 162DBG, U.S.A.: Utah. San Juan Co.: Dark Canyon Plateau, 11 May 2008, *Hodgson 22997* (DES), MN604509*, MN604572*, MN604675*, –, MN617261*, –; *Cirsium rydbergii* Petr., 164DBG, U.S.A.: Utah. San Juan Co.: Sweet Alice Canyon, 7 Jul 1994, *Hodgson 8432* (DES), MN604510*, MN604573*, MN604647*, –, MN617262*, –; *Cirsium rydbergii* Petr., 165DBG, U.S.A.: Utah. San Juan Co.: Woodenshoe Canyon, 6 Aug 1993, *Hodgson 7434* (DES), MN604511*, MN604574*, MN604689*, –, MN617263*, –; *Cirsium rydbergii* Petr., 185DBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon National Park, Kanab Canyon, 2 May 2013, *M McMaster sn* (DES), MN604518*, MN604575*, MN604648*, –, MN617264, –; *Cirsium rydbergii* Petr., 196DBG, Arizona. Coconino Co.: Grand Canyon, Cliff Spring, 21 Sep 2010, *Hodgson 25702* (DES), MN604443*, MN604531*, MN604633*, –, MN617209*, –; *Cirsium rydbergii* Petr., 1ASDBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon National Park, Cliff Spring, *Hodgson sn* (DES), MN604489*, MN604580*, MN604636*, –, MN617269*, –; *Cirsium rydbergii* Petr., 3ASDBG, U.S.A.: Arizona. Coconino Co.: North Kaibab, *Hodgson sn* (DES), MN604495*, MN604585*, MN604634*, –, MN617275*, –; *Cirsium scariosum* Nutt. var. *americanum* (A. Gray) D.J. Keil, U.S.A.: Colorado. Park Co.: High Creek Fen, 23 Jul 2016, *Ackerfield 16-102* (CS), MN604490*, MN604581*, MN604637*, MN617193*, MN617270*, MN617330*; *Cirsium scariosum* Nutt. var. *citrinum* (Petrak) D.J. Keil, MN335162, MN230952,

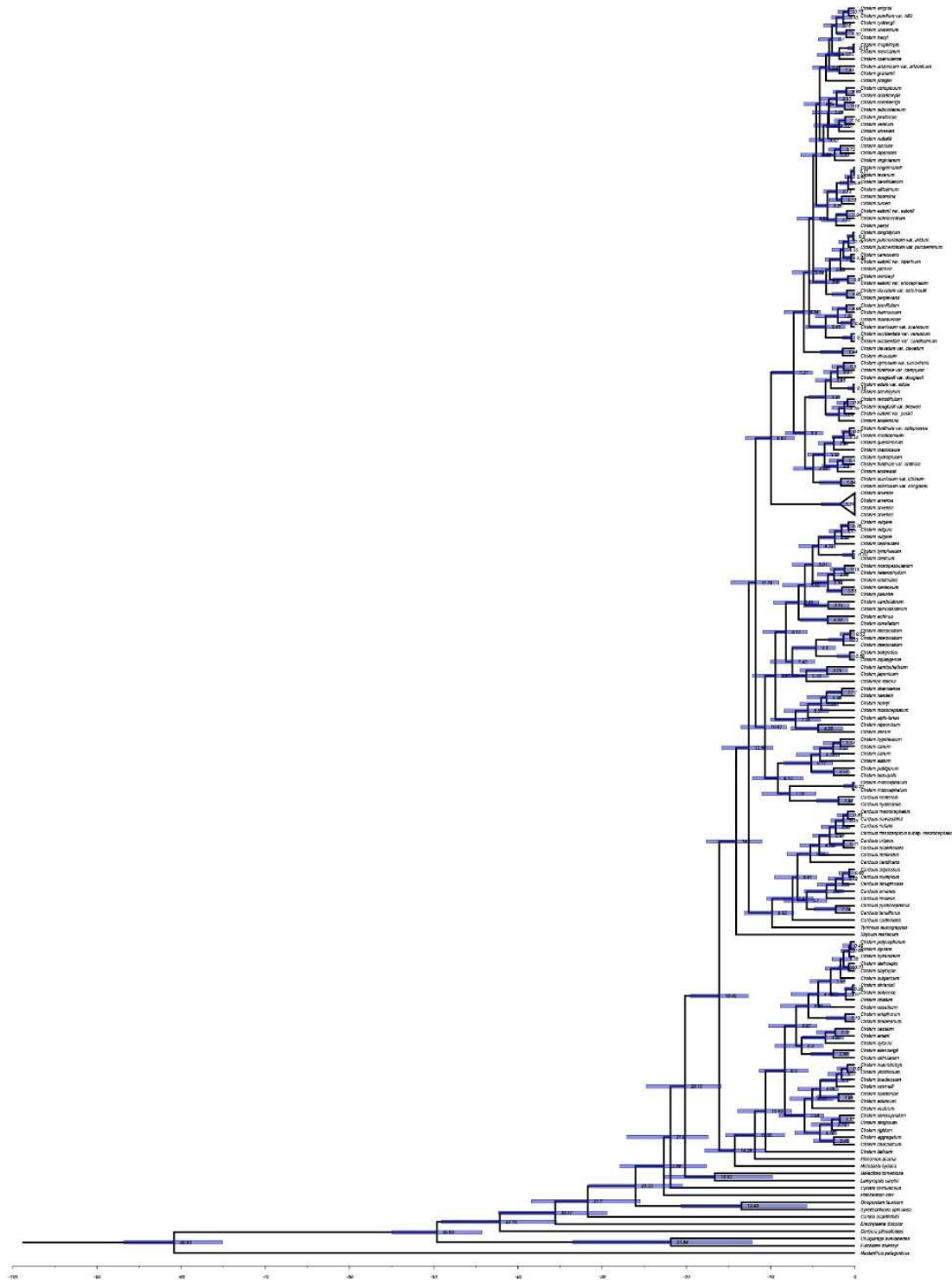
–, –, MN275437, MN314907; *Cirsium scariosum* Nutt. var. *coloradense* (Rydb.) D.J. Keil,
 U.S.A.: Colorado. Jackson Co.: Approximately 6 mi west of Walden along Hwy 14, 28 Jun
 2016, *Ackerfield 16-41* (CS), MN604491*, MN604595*, MN604676*, MN617194*,
 MN617271*, MN617331*; *Cirsium scariosum* Nutt. var. *congdonii* (R.J. Moore & Frankton)
 D.J. Keil, MN335127, MN230949, MN275343, MN275383, MN275458, MN314942; *Cirsium*
scariosum Nutt. var. *scariosum*, U.S.A.: Idaho. Boise Co.: Boise National Forest south of
 Lowman, 21 July 2001, *Keil 29682C* (OBI), MN604492*, MN604582*, MN604638*, –,
 MN617272*, MN617332*; *Cirsium scariosum* Nutt. var. *toiyabense* D.J. Keil, 48, U.S.A.:
 Idaho. Custer Co.: Pioneer Mts., Fall creek trail toward Moose Lake, 11 Jul 2009, *J Smith et al.*
8326 (BRY), MN604494*, MN604584*, MN604677*, MN617195*, MN617274*, MN617334*;
Cirsium scariosum Nutt. var. *toiyabense* D.J. Keil, 166, U.S.A.: Oregon. Harney Co.: Vicinity of
 Malhuer Field Station, 11 Aug 2000, *Keil 29092* (OBI), MN604493*, MN604583*,
 MN604639*, –, MN617273*, MN617333*; *Cirsium shansiense* Petr., MN335097, MN230917,
 –, –, –, –; *Cirsium subcoriaceum* (Less.) Sch. Bip., MN335156, MN230973, MN275330,
 MN275399, MN275461, MN314937; *Cirsium texanum* Buckley, MN335129, MN230954,
 MN275340, MN275386, MN275435, MN314927; *Cirsium tracyi* (Rydb.) Petr., MN335152,
 MN230931, MN275352, MN275373, MN275451, MN314892; *Cirsium turneri* Warnock,
 MN230932, MN335112, MN275311, –, MN275450, MN314939; *Cirsium undulatum* (Nutt.)
 Spreng., 6, MN335134, MN230972, MN275331, MN275398, MN275424, MN314916; *Cirsium*
undulatum (Nutt.) Spreng., 162, U.S.A.: Oregon. Sherman Co.: Columbia River Gorge, 19 July
 2001, *Keil 29662A* (OBI), MN604496*, MN604610*, MN604651*, –, MN617276*,
 MN617335*; *Cirsium velatum* (S. Watson) Petr., MN335154, MN230978, MN275312,
 MN275403, MN275420, MN314935; *Cirsium vinaceum* (Woot. & Standl.) Woot. & Standl.,

MN335147, MN230957, MN275337, –, MN275470, MN314943; *Cirsium virginense* S.L.
 Welsh, 2ASDBG, U.S.A.: Arizona. Mohave Co.: Three Springs, 11 Aug 2011, *G Rink 10834*
 (DES), MN604498*, MN604589*, MN604643*, –, MN617286*, –; *Cirsium virginense* S.L.
 Welsh, 79, U.S.A.: Utah. Washington Co.: northern saline seep W of Grapevine Pass Wash, 18
 May 2004, *Alexander 1690* (RENO), MN604497*, MN604596*, MN604688*, –, MN617285*,
 MN617336*; *Cirsium virginense* S.L. Welsh, 95DBG, U.S.A.: Arizona. Mohave Co.: Hualapai
 Indian Reservation, Travertine Falls, 10 Sep 1994, *Hodgson 8514* (DES), MN604499*,
 MN604591*, MN604654*, –, MN617281*, –; *Cirsium virginense* S.L. Welsh, 96DBG, U.S.A.:
 Arizona. Mohave Co.: Hualapai Indian Reservation, Bridge Canyon, 11 Sep 1994, *Hodgson*
8517 (DES), MN604507*, MN604592*, MN604680*, –, MN617282*, –; *Cirsium virginense*
 S.L. Welsh, 166DBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon, Hualapai Indian
 Reservation, Medicine Spring, 22 Sep 2009, *Hodgson 24441* (DES), MN604524*, MN604588*,
 MN604679*, –, MN617279*, –; *Cirsium virginense* S.L. Welsh, 187DBG, U.S.A.: Utah.
 Washington Co.: St. George on Red Hill, 6 Oct 1984, *S Welsh 23224* (BRY), MN604508*,
 MN604586*, MN604652*, –, MN617277*, –; *Cirsium virginense* S.L. Welsh, 210DBG, U.S.A.:
 Utah. Washington Co.: Seep along road ca 0.5 mi east of Danish Ranch, 19 Aug 1986, *R Warrick*
2961 (BRY), MN604523*, MN604587*, MN604653*, –, MN617278*, –; *Cirsium virginense*
 S.L. Welsh, 3348DBG, U.S.A.: Arizona. Coconino Co.: Grand Canyon, Hualapai Nation, near
 Medicine Spring, 7 Jun 2015, *Hodgson 30437* (DES), MN604500*, MN604590*, MN604644*,
 –, MN617280*, –; *Cirsium virginianum* (L.) Michx., MN335111, MN230930, MN275353, –,
 MN275452, MN314932; *Cirsium vulgare* (Savi) Ten, MN335116, MN230936, MN275315,
 MN275364, MN275426, MN314896; *Cirsium wheeleri* (A. Gray) Petr., MN335130, MN230955,

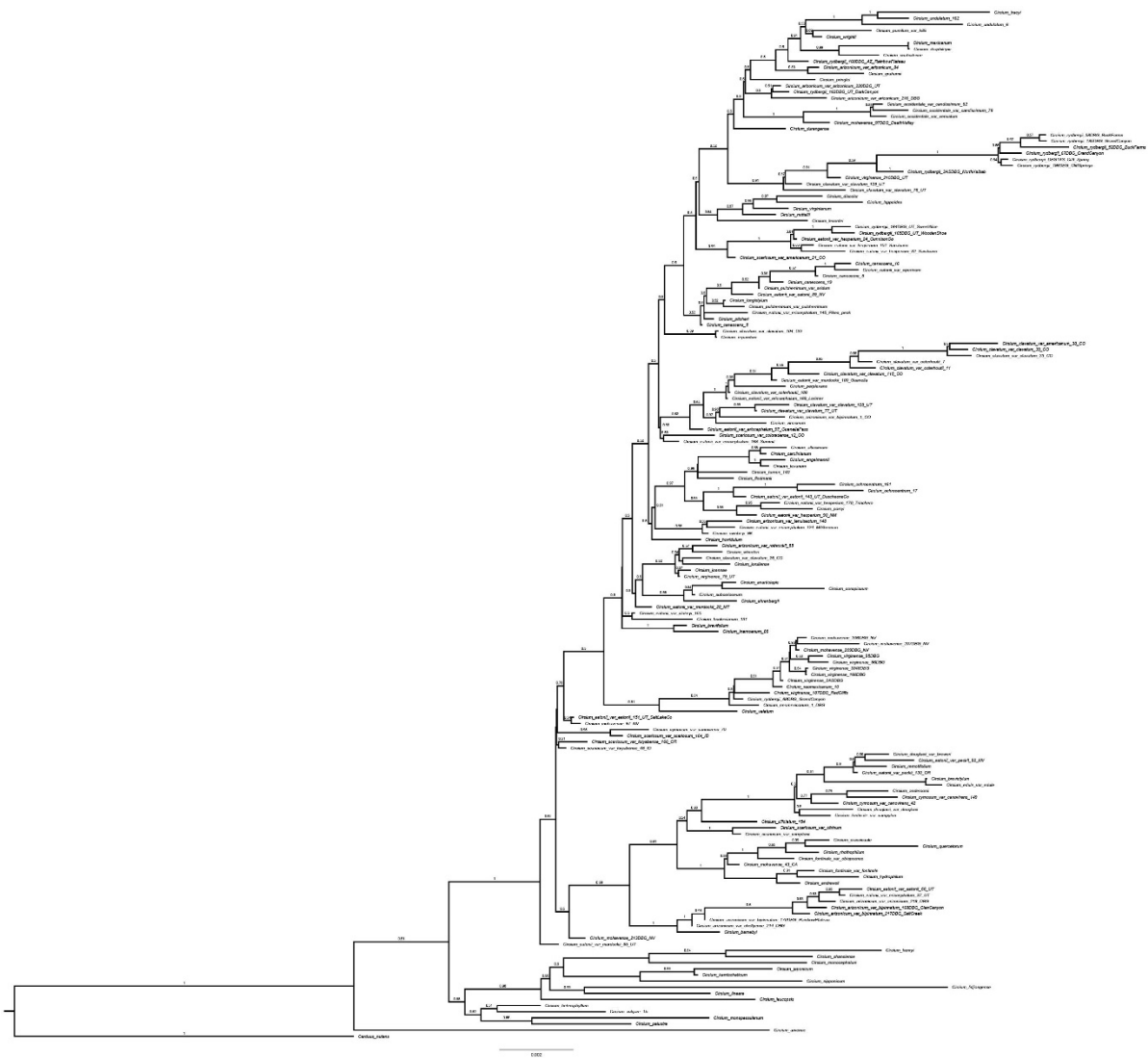
MN275339, MN275365, MN275418, MN314908; *Cirsium wrightii* A. Gray, MN335140,
MN230977, MN275323, MN275371, MN275423, MN314934.



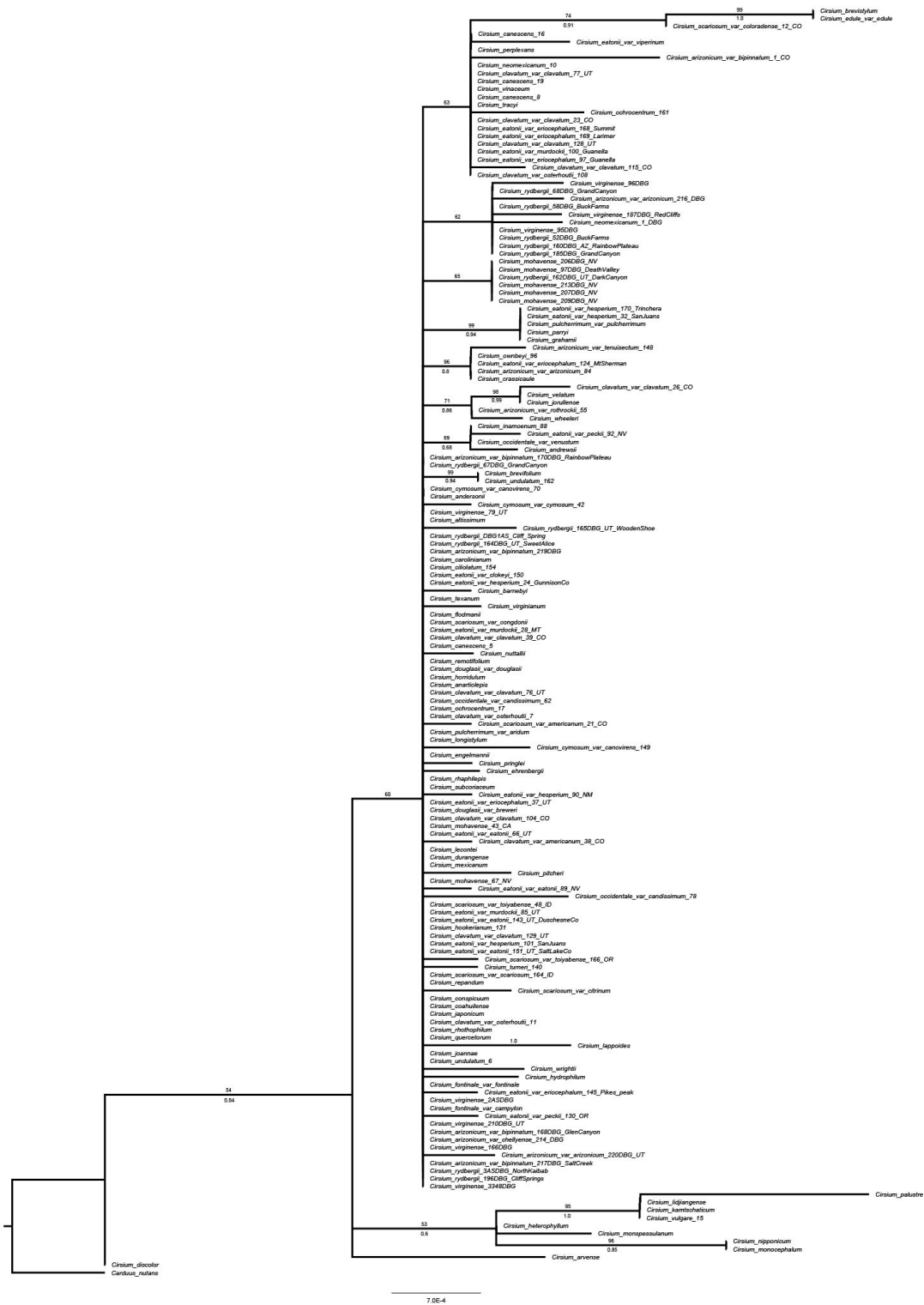
Supplemental Figure 1. ETS region



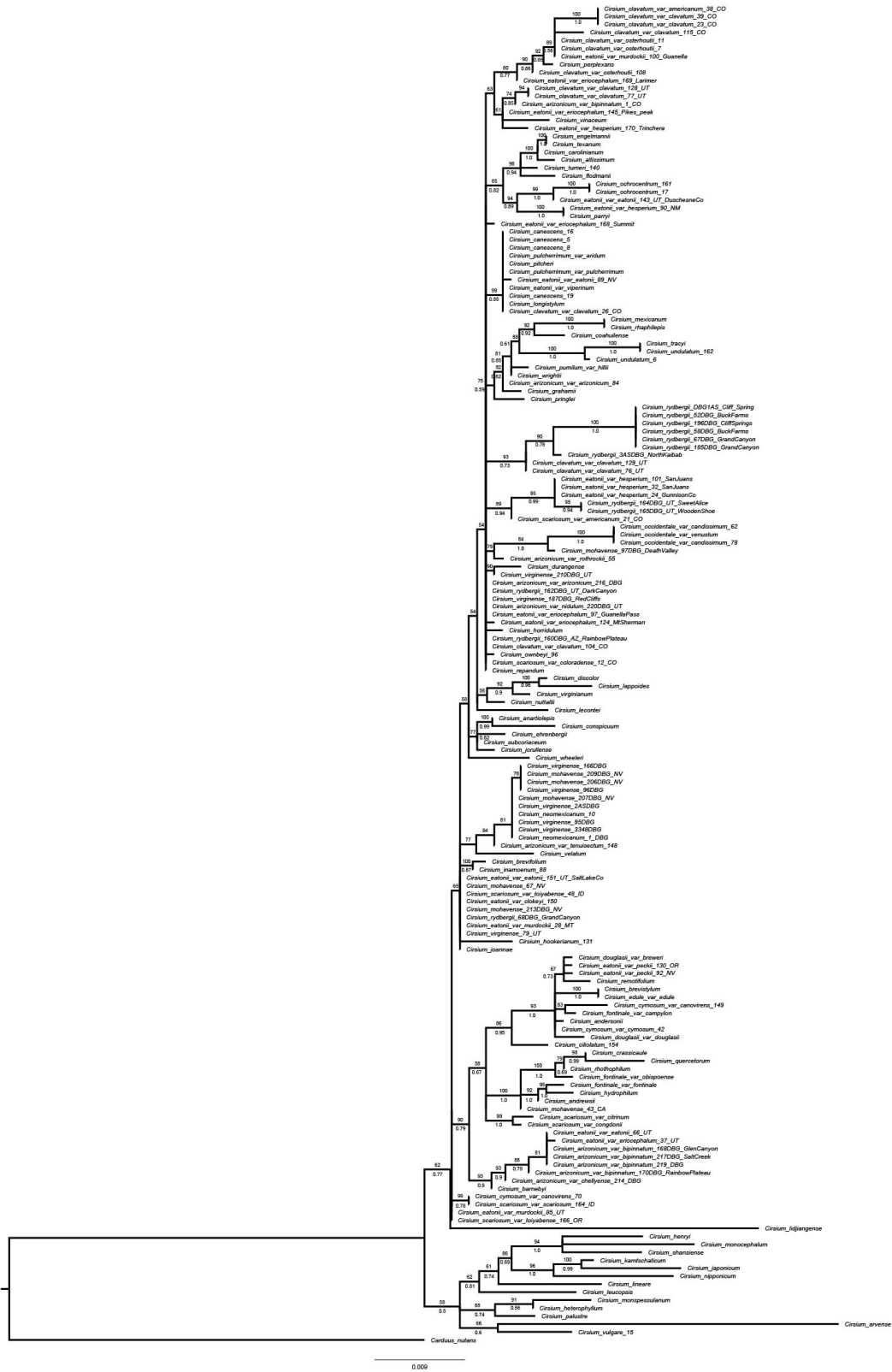
Supplemental Figure 7. Time-calibrated phylogeny of the *Carduus-Cirsium* group (expanded to show all tips) within the family Compositae. Median age is shown at each node. Purple bars on nodes indicate the 95% confidence intervals (CI).



Supplemental Figure 8. Bayesian inference. Posterior probabilities are listed above each branch.



Supplemental Figure 9. Combined plastid regions



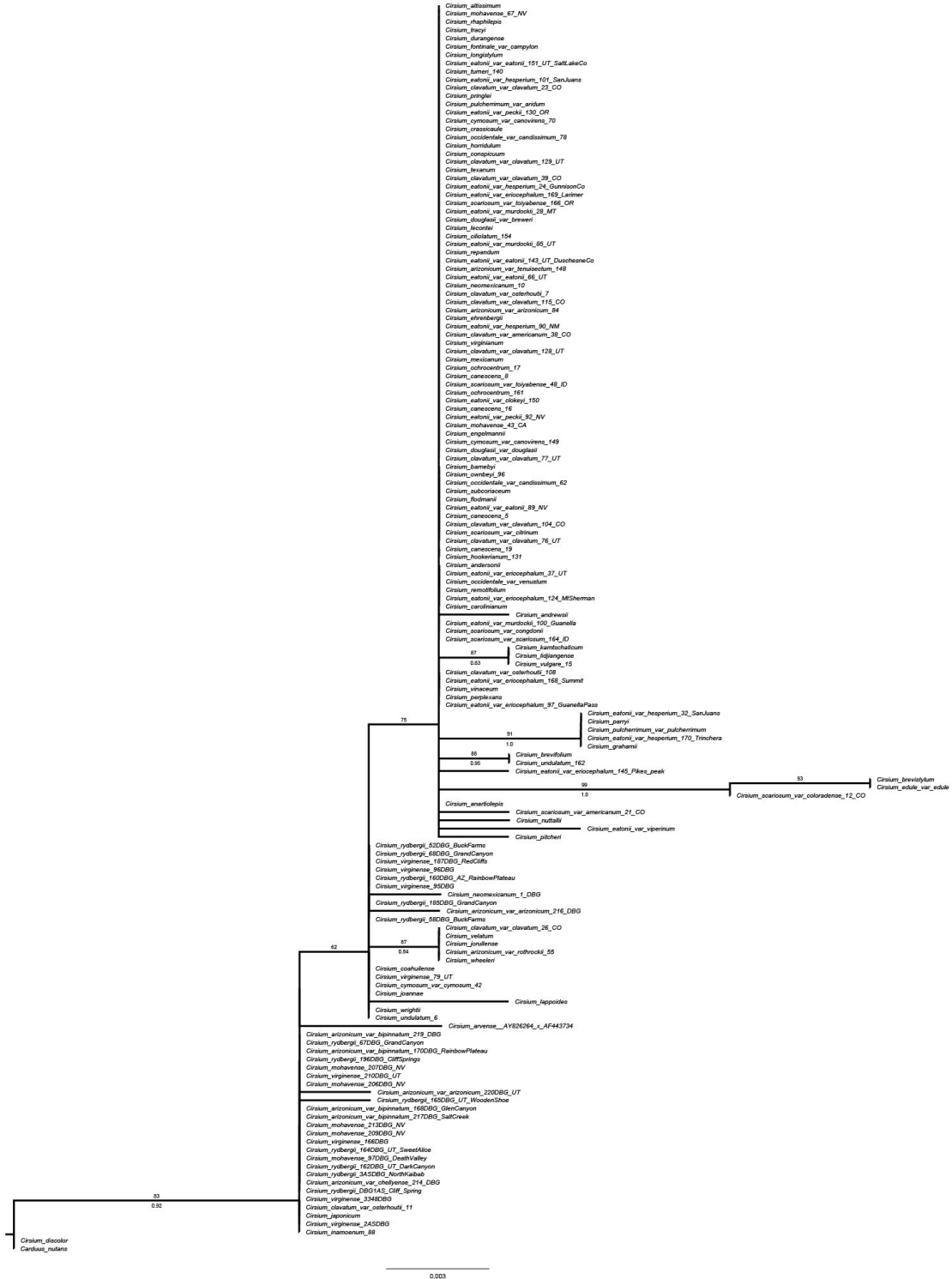
Supplemental Figure 10. Combined nuclear regions



Supplemental Figure 13. *matK* plastid region



Supplemental Figure 14. *ndhF* plastid region



Supplemental Figure 15. *psbA-trnH* plastid region

