INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

MOLECULAR SYSTEMATICS OF *ARTEMISIA* SECTION *TRIDENTATAE* (ASTERACEAE)

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

By

AMY B. KORNKVEN

Norman, Oklahoma

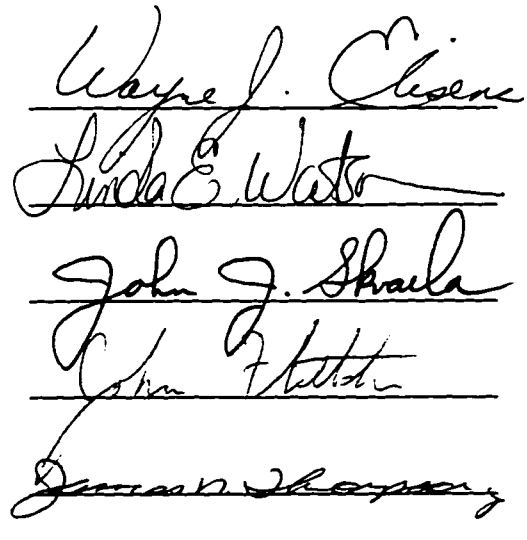
1997

MOLECULAR SYSTEMATICS OF *ARTEMISIA*

SECTION *TRIDENTATAE* (ASTERACEAE)

A DISSERTATION APPROVED FOR THE

DEPARTMENT OF BOTANY AND MICROBIOLOGY



DISSERTATION COMMITTEE

PREFACE

This dissertation consists of two manuscripts that have been organized according to the format specified for publication in Systematic Botany (Chapter 1) and American Journal of Botany (Chapter 2). The tables and figures are numbered independently in each chapter.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (Doctoral Dissertation Improvement Grant DEB-9311086 to Linda E. Watson and Amy B. Kornkven; DEB-9596274, DEB-9408019, and BSR-9110375 to Linda E. Watson), the American Society of Plant Taxonomists, and the University of Oklahoma.

Special thanks go to Linda Watson for providing laboratory facilities, expertise and assistance in designing and implementing my research, assistance in the preparation and critical review of this manuscript, and finally for her ongoing support throughout my years at the University of Oklahoma.

I thank James Estes for introducing me to plant systematics, directing me towards Artemisia sect. Tridentatae as a research project, and providing guidance and unfailing support.

I also thank Wayne Elisens for providing laboratory facilities, technical assistance, and support in all areas of my research; Leila Shultz for sharing her knowledge of Artemisia and providing valuable assistance in the field; Sara Hoot for providing the laboratory facilities enabling me to complete the ITS sequencing portion of my research; Tim Evans for both his expertise and assistance in the

manual sequencing of ITS and his critical review of this manuscript; Andrea Wolfe, Allan Nelson, and Patti Smith for their support throughout graduate school; Robert Jansen and J. Franscisco-Ortega for providing lettuce probes, ITS primers, and ITS sequence data; and the Oklahoma Biological Survey for providing a graduate assistantship, access to computers, and technical support.

I also thank my committee members Wayne Elisens, Linda Watson, John Fletcher, James Thompson, and John Skvarla for their time and critical review of this manuscript.

The Rocky Mountain Herbarium of the University of Wyoming provided invaluable access to Artemisia specimens in their collection.

I am also grateful to my husband, Chris Kornkven, for his continued support and understanding as I completed graduate school. My parents, Gerry and Jeanne Briggs, provided not only support and encouragement, but also accompanied me during my extensive fieldwork throughout western North America.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PART I

Molecular phylogeny of *Artemisia* section *Tridentatae* (Asteraceae) based on chloroplast DNA restriction site variation

ABSTRACT

Chloroplast DNA restriction site variation was used to examine phylogenetic relationships in Artemisia sect. Tridentatae, a complex of eleven species of woody shrubs dominant in the sagebrush communities of western North America. Twenty-seven endonucleases were utilized, and resulted in 82 variable site mutations, 27 of which were phylogenetically informative. The resulting cpDNA phylogeny indicates that sect. Tridentatae is monophyletic, with the exclusion of A. palmeri and the inclusion of A. bigelovii. A sister-group relationship between A. palmeri and three members of subg. Artemisia supports the exclusion of A. palmeri from sect. Tridentatae, and its inclusion within subg. Artemisia. Artemisia bigelovii, an anomalous species with heterogamous capitula, occurs within the Tridentatae clade, supporting its inclusion within the section. Introgression and subsequent chloroplast capture of the Tridentatae genome by A. californica and A. filifolia may explain the unexpected placement of these two species in the Tridentatae clade. Low cpDNA sequence divergence provides only limited resolution of phylogenetic relationships within sect. Tridentatae, indicative of a recently differentiated and/or hybridizing polymorphic species complex. In addition, the cpDNA data provides only equivocal evidence for either of two hypotheses regarding the origin and phylogenetic relationship of sect. Tridentatae within Artemisia.

Artemisia L. (sensu lato) (Asteraceae: Anthemideae) is a diverse genus, composed of approximately 400 species of annuals, herbaceous perennials, and shrubs, with a distribution primarily in the Northern Hemisphere (Heywood and Humphries 1977). Many of the species dominate shrub steppe regions throughout the world (Heywood and Humphries 1977). Section Tridentatae Rydb. is represented by eleven species of mostly xerophytic shrubs, which dominate much of the western landscape in North America (Fig. 1). These sagebrush communities cover over 68 million hectares throughout western North America (Beetle 1960; Shultz 1983). Artemisia tridentata Nutt. (big sagebrush) is one of the dominant shrubs at elevations of 1,500 to 3,000 m, and characterizes much of the Intermountain and Great Basin regions of the United States. Sagebrush has been an integral part of western North America since the late Pleistocene (Tidwell et al. 1972), and species in sect. Tridentatae have become increasingly more dominant in the vegetation during the last 100 years, as a result of extensive livestock grazing and reduced fire frequency (McArthur and Plummer 1978).

Subgeneric classification of Artemisia (s.l.) is based primarily on capitular morphology (Table 1). The subgeneric position and relationship of the North American sect Tridentatae to all other species of Artemisia has been the

subject of numerous systematic investigations (Rydberg 1916; Hall and Clements 1923; Ward 1953; Beetle 1960; McArthur and Plummer 1978; Shultz 1983; Ling 1991, 1995; Bremer and Humphries 1993). Most taxonomic treatments include sect. Tridentatae within Artemisia subg. Seriphidium (Besser) Rouy (Eurasian sagebrushes) (Rydberg 1916; Hall and Clements 1923; Ward 1953; Beetle 1960; Carlquist 1966), or treat Seriphidium Polj. as a segregate genus that includes sect. Tridentatae (Poljakov 1961; Ling 1991, 1995; Bremer and Humphries 1993). Seriphidium is segregated from Artemisia (s.s.) on the basis of four synapomorphies including: discoid, homogamous capitula; less than ten florets per capitulum; narrow lanceolate to linear apical anther appendages; and a specialized involucre of 4-7 rows of overlapping bracts (Poljakov 1961; Ling 1991, 1995; Bremer and Humphries 1993). In contrast, McArthur and Plummer (1978) propose that sect. Tridentatae differentiated from herbaceous ancestors of subg. Artemisia in North America, and that the similarity between subg. Seriphidium and sect. Tridentatae is an example of convergent evolution. Traditional data (morphology, anatomy, chemistry, and cytology) have not provided definitive support for either of these contrasting hypotheses for the origin and relationships of sect. Tridentatae.

Artemisia sect. Tridentatae has been studied extensively since the early 1900's. The first monograph of the genus Artemisia in North America was by Rydberg (1916), followed by several evolutionary treatments (Hall and Clements 1923; Beetle 1960), and cytogenetic (Ward 1953; McArthur and Plummer 1978; McArthur et al. 1981), anatomical (Diettert 1937; Moss 1940; Carlquist 1966; Shultz 1983), and numerous chemosystematic studies (Irwin 1971; Hanks et al. 1973; Geissman and Irwin 1974; Kelsey 1974). A lack of substantial morphological variation at the interspecific level, in combination with extensive morphological plasticity has created difficulties, not only in evaluating phylogenetic relationships within sect. Tridentatae, but also with the circumscription of the section. Although sect. Tridentatae has been previously defined on the basis of only a few morphological characters, i.e., the presence of homogamous capitula, the section appears to be a morphologically uniform and coherent group. However, several species remain problematic including A. bigelovii A. Gray, A. palmeri A. Gray, A. rigida (Nutt.) A. Gray, and A. pygmaeae A. Gray. For example, the presence of heterogamous capitula has led to the exclusion of A. bigelovii from sect. Tridentatae and its placement within subg. Artemisia (Hall and Clements 1923; Ward 1953; Shultz 1983; Ling 1992). Vegetative similarity to A. tridentata, in addition to

involucre and anther characters, supports inclusion of A. bigelovii within sect. Tridentatae (Beetle 1960; Poljakov 1961; McArthur and Plummer 1978; McArthur et al. 1981; Bremer and Humphries 1993). Bremer and Humphries (1993) interpret heterogamy in A. bigelovii either as a plesiomorphy or a secondary reversal.

Rydberg (1916) placed A. palmeri, an herbaceous perennial endemic to the California coast, into a segregate genus, Artemisiastrum Rydb., based on the presence of receptacular bracts. While the presence of homogamous flower heads, has led several authors to suggest its inclusion in sect. Tridentatae (Beetle 1960; Ward 1953). In contrast, several characters are used to support placement of A. palmeri into subg. Artemisia: presence of receptacular bracts; florets highly polymorphic in size and number, with approximately 12 to 25 perfect disk florets; herbaceous growth form; lack of interxylary cork; and other anatomical characters associated with species found in mesophytic habitats (Hall and Clements 1923; Moss 1940; McArthur and Plummer 1978; Shultz 1983). Bremer and Humphries (1993) place A. palmeri with the other North American Tridentatae species on the basis of three synapomophies: homogamous capitula; a 4-7 series of involucral bracts; and slender linear to lanceolate anther appendages. An understanding of the phylogenetic relationships of A. bigelovii and A.

palmeri within Artemisia (s.l.) may provide valuable information concerning the character state polarity of capitular morphology within the genus as a whole. A new interpretation of this suite of characters could have a significant impact on classification in Artemisia (s.l.). In addition, an understanding of the circumscription of sect. Tridentatae will help elucidate the evolution of capitulum morphology (i.e., heterogamy versus homogamy) within Artemisia (s.l.).

Interspecific relationships within sect. Tridentatae also have been studied extensively (Hall and Clements 1923; Ward 1953; Beetle 1960; McArthur et al. 1981; Shultz 1983). Two lineages have been proposed, based primarily on (1) leaf morphology, (2) habitat preference, and (3) ability to root-sprout after a fire (Table 2). The A. cana lineage (composed of A. cana Pursh. and A. tripartita Rydberg) is defined by linear to deeply three-lobed leaves, an ability to layer and root-sprout after a fire, and species that are associated with more mesophytic habitats. In contrast, species in the A. tridentata lineage (composed of A. tridentata, A. arbuscula Nutt., A. nova A. Nels., and A. longiloba (Osterh.) Beetle) have tridentate leaves, are located in xerophytic habitats, and are unable to root-sprout. Several species do not fit clearly into either proposed lineage (A. rigida, A. rothrockii A. Gray, and

A. pygmaea). Disagreement exists with the circumscription of the lineages, and relationships within each lineage remain unresolved (Table 2).

Previous interspecific and intrageneric classifications of Artemisia (s.l.), which have relied on data from morphology, chromosome numbers, and secondary metabolites, have provided little resolution of relationships within the genus. The only study using explicit cladistic methodology examines phylogenetic relationships at the tribal and subtribal levels (Anthemideae) (Bremer and Humphries 1993; Bremer 1994). Cladistic analysis of molecular data offers an opportunity to resolve phylogenetic relationships within sect. Tridentatae and its relationship to the other Artemisia subgenera and segregate genera. Therefore, we used cpDNA restriction site variation to address four primary objectives: (1) to examine the monophyly and circumscription of sect. Tridentatae, including placement of anomalous species; (2) to test previously proposed hypotheses of interspecific relationships within sect. Tridentatae; (3) to examine the phylogenetic relationships of sect. Tridentatae within Artemisia (s.l.), testing two conflicting hypotheses concerning its origin and placement; and (4) to interpret evolution of capitular morphology, a pivotal character used

to define sect. Tridentatae, using the molecular phylogeny as an independent framework.

MATERIALS and METHODS

All eleven species of Artemisia sect. Tridentatae, including representatives of all subspecific taxa, were analyzed for cpDNA restriction site variation. A total of 43 populations were included, with samples taken from throughout the geographic range of each species (Table 3). Thirteen outgroup species were included to represent the wide range of variation in Artemisia, including nine species from subg. Artemisia L., two species from subg. Dracunculus (Besser) Rydberg, and two species from subg. Seriphidium (Table 3). All of the North American taxa were collected from natural field populations, while the European and Asian collections were obtained primarily from botanic gardens (Table 3).

Fresh leaf material was stored on ice during transport to the laboratory and then placed in an ultra-low freezer at -80°C. The leaf material (~1.5 g) was ground into a fine powder using liquid nitrogen, with 4% wt/vol polyvinylpyrrolidone (PVP-40) added to each sample immediately after grinding. Total DNA was isolated using the CTAB isolation procedure, with further purification on cesium chloride/ethidium bromide (CsCl/EtBr) gradients

following standard protocols (Saghai-Maroof 1984; Doyle and Doyle 1987; Sambrook et al. 1989).

A pilot study of 43 populations (1 individual/ population; 3-9 populations/species) was conducted. DNA's were digested with ten restriction enzymes (AvaI, BamHI, BanI, BanII, BstXI, EcoRV, HaeII, HindIII, NcoI, NsiI), to determine the degree of intra- and interspecific variation. Since minimal polymorphisms were present, an additional 17 restriction enzymes were examined (AccI, AvaII, BclI, BglII, BstNI, ClaI, DraI, EcoRI, HaeIII, HincII, HphI, KpnI, MspI, RsaI, SspI, XbaI, XmnI). For the final analysis, 24 populations were sampled and mapped over all 27 restriction enzymes covering the entire cpDNA genome (listed above). The digested DNA fragments were separated by agarose gel electrophoresis (using 1% agarose gels), and transferred to nylon membranes (Sambrook et al. 1989; Southern 1975; Palmer 1986). Cloned homologous probes from lettuce (courtesy of R. Jansen) were used in the filter hybridizations. Chemiluminescent labeling (ECL kit: Amersham Co.) of the probes and hybridization were carried out following the manufacturer's instructions. Restriction site maps of the entire chloroplast genome were generated by comparison with previously constructed maps for members of the Asteraceae (Jansen and Palmer 1987, 1988; Jansen et al. 1992; Palmer 1986; Jansen and Palmer unpubl. data; Watson unpubl. data).

Phylogenetic relationships were analyzed with PAUP 3.1.1 (Swofford 1993), using Wagner parsimony to search for all equally most parsimonious trees (Farris 1970) using outgroup comparison (Maddison et al. 1984; Watrous and Wheeler 1981). Heuristic search options were performed and included TBR (tree-bisection-reconnection) branch swapping, with MULPARS on, and ACCTRAN optimization. Strict and semi-strict consensus trees were generated. Bootstrap (Felsenstein 1985) and decay analyses (Bremer 1988) were used to obtain estimates of support for each monophyletic clade on the resulting phylogenetic trees. The bootstrap option in PAUP 3.1.1 was run using the heuristic search option, stepwise random addition of taxa using 100 replicates, TBR branch swapping, MULPARS on, and ACCTRAN optimization. Autodecay 2.3 (Eriksson and Wilkstrom 1995), in conjunction with PAUP 3.1.1, was used to generate decay values for each clade. Interspecific relationships within sect. Tridentatae were examined by rooting the tree with representative species of subg. Artemisia (A. vulgaris L., A. abrotanum L., A. frigida Willd., and A. macrocephala Jacq.) using a global outgroup approach. Finally, subgeneric relationships were examined by rooting the tree with a single outgroup, Anthemis nobilis L. (Watson 1996).

RESULTS

Restriction Site Variation. The 27 restriction enzymes surveyed produced 82 variable site mutations, 27 of which are phylogenetically informative and 41 of which are autapomorphic (Table 4). Thirty-six of the 41 autapomorphies are present in outgroup species, compared to only five present in four of the Tridentatae species (A. arbuscula, A. rigida, A. nova, and A. palmeri). Fourteen mutations are polymorphic within species of sect. Tridentatae and were excluded from the analyses. The restriction site variation is distributed throughout the chloroplast genome, with the majority (61) located in the large single copy region. Only 15 mutations were found in the inverted repeat, with six in the small single copy region. Variation was scored for approximately 1,300 restriction sites, covering 95% of the chloroplast genome, and approximately 5% of the chloroplast nucleotide bases.

Intraspecific cpDNA variation is present in seven of the eleven Tridentatae species, including A. tridentata, A. cana, A. nova, A. arbuscula, A. rothrockii, A. rigida, and A. bigelovii (Table 4), and includes 14 site mutations. Four mutations are unique to single populations within three different species (A. rigida, A. arbuscula, and A. cana). The remaining 10 mutations occur in populations of more than two species. Single populations in each of five different

species (A. tridentata, A. nova, A. cana, A. arbuscula, and A. rothrockii) share three mutations. One mutation unites sympatric populations of A. tridentata and A. nova, which were both collected in San Juan County, Utah.

Two species, A. sublessingiana (Kell.) Krasch. ex Poljak. and A. maritima L., were included in the study from subg. Seriphidium (Table 3). Of these two species, only restriction sites from A. sublessingiana could be mapped for all 27 restriction enzymes. The incomplete maps for A. maritima were identical to the maps for A. sublessingiana. Therefore, maps of both Seriphidium species were combined and labeled as subg. Seriphidium on the cpDNA tree.

Phylogenetic Analysis. Phylogenetic analysis of the cpDNA data, including 68 characters and 23 taxa, produced 192 equally most parsimonious trees, 79 steps in length (CI=0.93). The data were reanalyzed to include two intraspecifically polymorphic characters (33, 47 of Table 4). This analysis resulted in 144 equally most parsimonious trees, 82 steps in length (CI=0.93; Fig. 2). The topologies of the two strict consensus trees are identical, with the exception that the addition of the two polymorphic characters provides additional resolution within sect. Tridentatae.

Three clades are strongly supported (bootstrap ≥ 90%) in the strict consensus tree (Fig. 2). The clade containing A. stelleriana Besser, A. gnaphalodes Nutt., A. ludoviciana Nutt., and A. palmeri is supported by six restriction site mutations with a bootstrap value of 98%. Three of these species are North American and one is a cultivated Asian species (A. stelleriana). Artemisia palmeri (sect. Tridentatae) is sister to A. ludoviciana and A. gnaphalodes (both subg. Artemisia), united by a single synapomorphy with weak bootstrap support (bootstrap = 67%). This clade is sister to A. stelleriana (subg. Artemisia).

The Tridentatae clade is supported by four synapomorphies with a bootstrap value of 96%. The A. californica Less. and A. rigida clade is also strongly supported and is sister to the remainder of the Tridentatae species. A close relationship between these two species is supported by three mutations, two of which represent homoplasious characters on the tree. The low level of divergence among the remaining Tridentatae species provides limited resolution at the interspecific level. In the strict consensus tree, the nine Tridentatae species and A. filifolia Torrey form an unresolved polytomy. The inclusion of the two polymorphic characters provides additional resolution within sect. Tridentatae. Artemisia filifolia, A. bigelovii, and A. longiloba form a weakly supported clade

with a bootstrap value of 55%. In the cpDNA phylogeny, the Tridentatae clade is paraphyletic, with the exclusion of A. palmeri and the inclusion of A. bigelovii, A. californica, and A. filifolia.

The cpDNA data does not support the monophyly of the three subgenera of Artemisia (Fig. 2). The nine species sampled from the subg. Artemisia do not form a monophyletic clade and possess no cpDNA synapomorphies. Artemisia dracunculus L. and A. filifolia, two species currently placed into subg. Dracunculus, also do not form a monophyletic clade. In fact, A. filifolia is part of the Tridentatae clade. Subgenus Seriphidium, including sect. Tridentatae, is not monophyletic, with the two species of subg. Seriphidium forming a polytomy with the Tridentatae clade, A. dracunculus, A. oelandica (Besser) V.Komarov, and the A. ludoviciana/A. palmeri/A. gnaphalodes/A. stelleriana clade.

DISCUSSION

Monophyly and Circumscription of sect. *Tridentatae*.

The molecular data strongly supports the monophyly of sect. Tridentatae, with the exclusion of A. palmeri and the inclusion of A. bigelovii (Fig. 2). The data clearly reveal a close relationship between A. palmeri, A. ludoviciana, A. gnaphalodes, and A. stelleriana. Artemisia palmeri, an

herbaceous species endemic to Baja California and southern California, has previously been included within sect. Tridentatae (subg. Seriphidium) based primarily on the presence of discoid, homogamous capitula (Hall and Clements 1923; Ward 1953; Ling 1991, 1995; Bremer and Humphries 1993). In all other characters, A. palmeri more closely resembles members of subg. Artemisia, in particular the A. vulgaris polyploid complex of North America (Keck 1940; Beetle 1963; Shultz 1983; McArthur and Plummer 1978; McArthur et al. 1981), of which both A. ludoviciana and A. gnaphalodes are members. A close relationship between A. stelleriana (an Asian species escaped from cultivation) and members of the North American A. vulgaris complex was first proposed by Hall and Clements (1923) and is supported by the molecular data. The cpDNA evidence indicates that A. palmeri should be excluded from sect. Tridentatae. Excluding capitular morphology, this is in agreement with conclusions based on vegetative and anatomical data that support the removal of A. palmeri from sect. Tridentatae (Beetle 1960; Shultz 1983).

Artemisia bigelovii is an anomalous species with heterogamous capitula. The presence of 1-2 marginal (ray) florets per head has been used to support the placement of A. bigelovii within subg. Artemisia (Hall and Clements 1923; Ward 1950; Shultz 1983). In contrast, based on involucral

and anther characters and overall appearance, A. bigelovii is included within sect. Tridentatae (subg. Seriphidium) (Beetle 1963; McArthur and Plummer 1978; McArthur et al. 1981; Bremer and Humphries 1993). The results from the molecular data are in agreement with the morphology-based phylogeny of Bremer and Humphries (1993), supporting the inclusion of A. bigelovii within sect. Tridentatae. This clade is strongly supported with a bootstrap value of 96%. In this context, heterogamy in A. bigelovii is either a secondary reversal or a plesiomorphic character state (Bremer and Humphries 1993). However, introgression and chloroplast capture of the Tridentatae genome by A. bigelovii is also possible; a few localities of hybrid zones with A. nova and A. tridentata exist, and A. bigelovii is sympatric throughout the northern half of its distribution with both species (Beetle 1960).

The cpDNA phylogeny strongly supports the inclusion of two highly specialized species, A. pygmaea and A. rigida, within sect. Tridentatae. Artemisia pygmaea, a dwarf sub-shrub restricted to calcareous soils in the Great Basin, is morphologically (Hall and Clements 1923; Beetle 1960; Ling 1995), anatomically (Moss 1940; Shultz 1981), and chemically distinct from the other species in sect. Tridentatae (Holbo and Mozingo 1965; Irwin and Geissman 1977). Artemisia rigida, a low spreading shrub found primarily on rocky

outcrops in eastern Oregon, Washington and into western Idaho, is also distinct within the section and is uniquely adapted to poor soil and extreme xerophytic conditions (Hall and Clements 1923; Holbo and Mozingo 1965). Most authors include A. pygmaea and A. rigida within the section based on the presence of discoid, homogamous capitula (Hall and Clements 1923; Ward 1950; Beetle 1960; McArthur and Plummer 1978; McArthur et al. 1981; Shultz 1983; Ling 1991, 1995; Bremer and Humphries 1993). However, Rydberg (1916) recognized two monotypic sections, sect. Pygmaea Rydb. and sect. Rigidae Rydb., in subg. Seriphidium, based on their specialized morphologies. Despite the distinct morphological and chemical characters that define these two species, both occur within the Tridentatae clade in the molecular phylogeny. There is some evidence that A. rigida may have diverged early in the evolution of the section, with the presence of two cpDNA autapomorphies and extensive morphological divergence. In contrast, A. pygmaea has no cpDNA autapomorphies and shares a common ancestor with the core Tridentatae species, despite its unique morphology and habit. Therefore, the cpDNA phylogeny supports the inclusion of both species within sect. Tridentatae.

In addition, the cpDNA phylogeny places A. californica and A. filifolia as members of the Tridentatae clade. A close relationship between these two taxa and sect.

Tridentatae is contrary to every classification system previously proposed for Artemisia (s.l.). Artemisia californica is currently placed in subg. Artemisia, whereas A. filifolia is a member of subg. Dracunculus. Hypotheses to explain this unexpected relationship include an inaccurate morphological classification, possible hybridization/introgression, or lineage sorting (Doyle 1992; Rieseberg and Brunsfeld 1992; Soltis et al. 1992; Soltis and Kuzoff 1995). The current classification is based primarily on capitular morphology which, as discussed below, may be under simple genetic control and subject to multiple origins and reversals. It is also possible that gene flow between members of sect. Tridentatae and both A. californica and A. filifolia, with subsequent chloroplast capture of the "Tridentatae" genome in each species, may explain the observed pattern. Artemisia filifolia is a widespread species, common on sandy soils from Nebraska to Arizona (Hall and Clements 1923). The range of A. filifolia overlaps with several Tridentatae species, and populations of each occur sympatrically. Artemisia californica is an important dominant species in the coastal regions of southern California. Although, A. californica occurs sympatrically with A. tridentata in California, its range does not overlap with A. rigida, which is located to the north in Washington and Oregon. Therefore, although

opportunities do exist for hybridization and subsequent transfer of the Tridentatae chloroplast genome into A. californica, this does not explain the sister relationship between A. californica and A. rigida in the cpDNA phylogeny.

Artemisia californica represents the only shrub placed into subg. Artemisia in North America, and closely resembles the more widespread A. tridentata in overall appearance and habit. If A. californica and sect. Tridentatae share a common polymorphic ancestor, the potential for past lineage extinctions and retention of polymorphisms may distort interspecific relationships. On the other hand, limited interspecific polymorphism in the chloroplast gnome within sect. Tridentatae does not support the presence of a highly polymorphic ancestor. Therefore, lineage sorting is theoretically possible, but difficult to detect without extensive population sampling and the use of different non-molecular and molecular markers (Avise 1989; Rieseberg and Brunsfeld 1992; Soltis et al. 1992a; Soltis and Kuzuff 1995).

In conclusion, the limited sampling of both A. californica and A. filifolia precludes unequivocally distinguishing between these competing hypotheses (i.e., chloroplast capture or lineage sorting). Adequate sampling and the combined use of both cytoplasmic and nuclear markers are essential to detect potential gene flow (Rieseberg and

Soltis 1991; Rieseberg and Brunsfeld 1992; Soltis et al. 1992).

Interspecific Relationships within sect. *Tridentatae*. The restriction site data are not in agreement with morphology, which has been used to support two lineages within sect. Tridentatae (Ward 1953; Beetle 1960; Shultz 1983). The molecular data do not support the recognition of either the A. tridentata lineage or the A. cana lineage (Table 2; Fig. 2). However, two different clades are present within the section: the A. californica/A. rigida clade and the Tridentatae clade. The A. californica and A. rigida clade is supported by two synapomorphies (bootstrap value = 93%) and is sister to the Tridentatae clade. The core Tridentatae species and A. filifolia (subg. Dracunculus) are united by two restriction site mutations. However, one of the synapomorphies that unites this clade is polymorphic in A. rigida. The other polymorphic character provides additional support for the clade containing A. tridentata, A. tripartita, A. filifolia, A. bigelovii, and A. longiloba, but this node is only weakly supported and collapses in the strict consensus tree (Fig. 2). The use of polymorphic characters is controversial, but the potential phylogenetic information present in these polymorphic characters justifies their careful inclusion in the data analysis (Nixon and Wheeler 1990; Wiens 1995).

Artemisia filifolia, A. bigelovii, and A. longiloba (Fig. 2) share one restriction site mutation. Interestingly, these three species represent all three distinct types of capitular morphology found in Artemisia (i.e.,, disciform, heterogamous flower heads with pistillate ray florets and either perfect, fertile disk florets or sterile disk florets; and discoid, homogamous flower heads with only perfect, fertile florets).

The lack of resolution within sect. Tridentatae is indicative of a recently differentiated and/or hybridizing polymorphic species complex. A pattern of low divergence between closely related species that have recently differentiated, has been observed in a number of different plant genera (Baldwin et al. 1990; Crawford et al. 1990, 1991, 1992; Rieseberg et al. 1991). Even though the conservative rate of chloroplast evolution at the interspecific level provides insufficient data to resolve phylogenetic relationships within sect. Tridentatae, intraspecific restriction site variation is present in seven Tridentatae species (Table 4). In fact, the level of intraspecific variation within the section is higher than the amount of variation present among species. A total of 23 mutations were detected in sect. Tridentatae, 14 of which are polymorphic in one or more species, five of which are autapomophic, and only four of which are phylogenetically

informative. Four mutations are restricted to single populations within three species (A. rigida, A. arbuscula, and A. cana). The presence of two autapomorphies in A. rigida provides additional support for the early divergence of this species from the rest of the Tridentatae. Artemisia rigida is morphologically and ecologically distinct from the other Tridentatae species, and is one of the few species in the section without reports of interspecific hybrids. In addition, unique mutations were detected in A. cana subsp. viscidula (Osterhout) Beetle and A. arbuscula subsp. thermopola Beetle, that are not present in the other populations sampled for these two taxa.

An example of possible interspecific gene flow, probably due to localized introgression, was found between two sympatric populations of A. tridentata (#2) and A. nova (#2). These two populations share one restriction site mutation, not found in any other population. Both species were collected from sympatric populations in Utah. It should be noted that hybridization between A. tridentata and A. nova has been documented for other sympatric populations (Ward 1950; Beetle 1960).

The intraspecific variation present, with the exception of the above example, does not appear to be closely correlated with geographic distribution. For example, three mutations characterize single populations in each of five

species (A. tridentata, A. nova, A. cana, A. arbuscula, and A. rothrockii). All five species, except A. rothrockii, are widespread and each of the above populations was collected in different states. Lineage sorting has been proposed to explain the non-concordance between species boundaries and cytoplasmic lineages in several plant taxa (Doebley 1990; Doyle et al. 1990). Section Tridentatae, a polymorphic species complex that has diverged relatively recently in response to climatic changes in western North America during the Quaternary (McArthur and Plummer 1978), may have undergone lineage sorting resulting in moderately high levels of intraspecific variation.

Relationship of sect. *Tridentatae* to *Artemisia* (s.l.). Ling (1991) proposes that subg. Seriphidium originated in Asia during the upper to middle Tertiary period and migrated across the Bering Strait into North America, and that the North American sect. Tridentatae shares a common ancestor with these Seriphidium progenitors. However, there are no cpDNA synapomorphies uniting sect. Tridentatae and the Seriphidium species, as would be expected based on Ling's hypothesis. The presence of six synapomorphies uniting the two species of subg. Seriphidium supports the distinctness of this subgenus. However, the relationship of subg. Seriphidium to sect. Tridentatae is unresolved in the cpDNA tree. Subgenus Seriphidium forms a polytomy with A.

oelandica, A. dracunculus, the North American subg. Artemisia group, and the Tridentatae clade.

If sect. Tridentatae were differentiated from herbaceous ancestors of subg. Artemisia in North America, as proposed by McArthur and Plummer (1978), then a common ancestor with North American species in subg. Artemisia would be expected. Because the unresolved polytomy at the base of the Tridentatae clade includes species from each of the three subgenera (Seriphidium, Dracunculus, and Artemisia), the cpDNA data provide only equivocal evidence for either of the two hypotheses regarding the origin and phylogenetic relationships of sect. Tridentatae.

The recognition of subg. Seriphidium as a segregate genus, as proposed by Poljakov (1961), Ling (1991; 1995), and Bremer and Humphries (1993), is not supported by the cpDNA restriction site data. The two European Seriphidium species sampled form a polytomy with A. oelandica, A. dracunculus, the North American subg. Artemisia clade containing A. palmeri, and the Tridentatae clade. The Seriphidium species sampled for this study clearly nest within Artemisia (s.l.). However, only a preliminary assessment of the relationship of Seriphidium to Artemisia can be made as a result of the limited number of Seriphidium species sampled.

Although only preliminary conclusions can be made at this time because of the limited sampling within Artemisia (s.l.), the cpDNA phylogeny is not concordant with current subgeneric classifications based on morphology. None of the three subgenera currently recognized are monophyletic in our cpDNA tree. Species of subg. Artemisia occur in three separate clades. Four species (A. vulgaris, A. frigida, A. abrotanum, and A. macrocephala) form an unresolved polytomy at the base of the entire tree. Artemisia abrotanum and A. macrocephala, however, do form a weakly supported clade which collapses in the strict consensus tree. Three of the species (A. vulgaris, A. abrotanum, and A. macrocephala) have a Eurasian distribution, but have also been introduced and naturalized in North America, while A. frigida has an extensive northern hemisphere distribution and extends through Alaska, Canada, and into the western United States. As discussed previously, A. californica and A. rigida have a sister relationship with the core Tridentatae species. Artemisia oelandica, an herbaceous species from Sweden, is part of a large unresolved polytomy.

Two representative species of subg. Dracunculus, A. dracunculus and A. filifolia, were included in this study. Artemisia dracunculus is a Eurasian species that has been naturalized in North America, whereas A. filifolia is endemic to deep sands of western North America. Both have

been previously placed in subg. Dracunculus based on the presence of heterogamous flower heads with pistillate outer florets and sterile (staminate) inner florets. In the cpDNA phylogeny, they occur in two separate clades, with A. filifolia nested in the Tridentatae clade. In the absence of hybridization, A. dracunculus and A. filifolia do not share a more recent common ancestor, as predicted on the basis of capitular morphology (Bremer and Humphries 1993). Further study of Artemisia (s.l.), a large and diverse genus, is essential for determining phylogenetic relationships within the genus as a whole.

Character Evolution. Artemisia sect. Tridentatae provides a unique opportunity to examine the evolution of capitular morphology within Artemisia (s.l.). The extensive variation in vegetative morphology, both within and among species, has resulted in few reliable characters for examining phylogenetic relationships. Therefore, subgeneric and sectional classification in Artemisia is based primarily on capitular morphology. The cpDNA phylogeny provides a framework for evaluating the circumscription of sect. Tridentatae. The phylogenetic relationships of A. bigelovii and A. palmeri are pivotal to understanding and defining subgeneric and sectional limits within Artemisia (s.l.), because both species possess character states that have been interpreted as plesiomorphic and/or secondary reversals

within the genus as a whole (Bremer and Humphries 1993). The cpDNA data provide strong support for the exclusion of A. palmeri and the inclusion of A. bigelovii within sect. Tridentatae. When the three types of capitula are mapped onto the cladogram (Fig. 3), a trend towards a reduction in both the number of florets per head (2-10 florets/head versus 10-35 florets/head) and the type of florets is apparent. This trend was first recognized by Hall and Clements (1923), when they proposed a phylogenetic treatment for the North American Artemisia species.

In contrast to most hypotheses of relationships within Artemisia, including that of Bremer and Humphries (1993), the presence of discoid, homogamous flower heads has arisen independently at least two and possibly three different times on the cpDNA tree: (1) subg. Seriphidium; (2) Artemisia palmeri; and (3) the Tridentatae clade. In addition, the presence of A. californica and A. filifolia in the Tridentatae clade requires an explanation of either two independent reversals to heterogamy or interspecific gene flow, such as introgression and chloroplast capture.

Several parallel gains and reversals of the presence of ray florets in Artemisia indicate that this character is unreliable in Artemisia. Furthermore, the presence or absence of ray florets has been shown to be under simple genetic control (one or two genes) in three different,

distantly related Asteraceae genera: Haplopappus Cass. (Jackson and Dimas 1983); Layia Hook and Arn. (Clausen et al. 1947; Clausen 1951); and Senecio L. (Ingram and Taylor 1982).

In conclusion, the cpDNA-based phylogeny provides strong support for the monophyly of sect. Tridentatae, with the exclusion of A. palmeri and the inclusion of A. bigelovii. The presence of both A. californica and A. filifolia in the sect. Tridentatae clade raises several interesting questions: (1) What is the potential for gene flow between these morphologically divergent taxa? (2) Is this pattern the result of lineage sorting from polymorphic ancestors in the subg. Artemisia? and (3) Does the current classification accurately reflect phylogenetic relationships within Artemisia (s.l.)? Examination of additional molecular markers (i.e., rapidly evolving regions) will almost certainly address these questions.

LITERATURE CITED

AVISE, J. C. 1989. Gene trees and organismal histories: A phylogenetic approach to population biology. Evolution 43: 1192-1208.

BALDWIN, B. G., D. W. KYHOS, and J. DVORAK. 1990. Chloroplast DNA evolution and adaptive radiation in the Hawaiian Silversword alliance (Asteraceae-Madiinae). Annals or the Missouri Botanical Garden 77: 96-109.

BEETLE, A. A. 1960. A study of sagebrush, the section Tridentatae of Artemisia. Bulletin 368. University of Wyoming Agricultural Experimental Station 368: 1-83.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795-803.

——— . 1994. Tribe Anthemideae, Chapter 19. Pp. 435-478 in Asteraceae Cladistics and Classification, K. Bremer. Timber Press: Portland, OR.

——— and C. HUMPHRIES. 1993. Generic monograph of the Asteraceae-Anthemideae. Bulletin of the Natural History Museum. Volume 23.

CARLQUIST, S. 1966. Wood Anatomy of Anthemideae, Ambrosieae, Calendulae, and Arctotideae (Compositae). Aliso 6: 1-23.

CLAUSEN, J. 1951. Stages in the Evolution of Plant Species. Cornell University Press, Ithaca, New York.

——— , D. D. KECK, and W. M. HIESEY. 1947. Heredity of geographically and ecologically isolated races. American Naturalist 81: 114-133.

CRAWFORD, D. J., J. D. PALMER, and M. KOBAYASHI. 1990. Chloroplast DNA restriction site variation and the phylogeny of Coreopsis section Coreopsis (Asteraceae). American Journal of Botany 77: 552-558.

——— , J. D. PALMER, and M. KOBAYASHI. 1991. Chloroplast DNA restriction site variation, phylogenetic relationships, and character evolution among sections of North American Coreopsis (Asteraceae). Systematic Botany 16: 211-224.

——— , T. F. STUESSY, M. B. COSNER, D. W. HAINES, M. SILVA, and M. BAEZA. 1992. Evolution of the Genus Dendroseris (Asteraceae: Lactuceae) on the Juan Fernandez Islands: Evidence from chloroplast and ribosomal DNA. Systematic Botany 17: 676-682.

DIETTERT, R. A. 1937. The morphology of Artemisia tridentata Nutt. Ph.D. Dissertation. State University of Iowa.

DOEBLEY, J. 1990. Molecular systematics of Zea (Gramineae). Maydica 35: 143-150.

DOYLE, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. Systematic Botany 17: 144-163.

——— and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

——— , J. L. DOYLE, and A. H. D. BROWN. 1990. Chloroplast DNA polymorphism and phylogeny in the B genome of Glycine subgenus Glycine (Leguminosae). American Journal of Botany 77: 772-782.

GEISSMAN, T. A. and M. A. IRWIN. 1974. Chemical constitution and botanical affinity in Artemisia. Pp. 135-143 in Chemistry in Botanical Classification, eds. G. Bendz and J. Santesson. Proceedings of the 25th Nobel Symposium. Academic Press, New York.

ERIKSSON, T. and N. WILKSTROM. 1995. Autodecay 3.0. Computer program distributed by T. Eriksson.

FARRIS, J. S. 1970. Methods for computing Wagner trees. Systematic Zoology 19: 83-92.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

HALL, H. M. and F. E. CLEMENTS. 1923. The phylogenetic method in taxonomy. The North American species of Artemisia, Chrysothamnus, and Atriplex. Carnegie Institution Washington Publication 326: 1-355.

HANKS, D. L., E. D. McARTHUR, R. STEVENS, and A. P. PLUMMER. 1973. Chromatographic characteristics and phylogenetic relationships of Artemisia, section Tridentatae. U.S.D.A. Forest Service Research Paper INT-141, 24 pp.

HEYWOOD, V. H. and C. J. HUMPHRIES. 1977. Anthemideae - Systematic review. Ch. 31, pp. 851-898 in The Biology and Chemistry of the Compositae, eds. V. H. Heywood, J. B. Harborne, and B. L. Turner. Academic Press, New York.

INGRAM, R. and L. TAYLOR. 1982. The genetic control of a nonradiate condition in Senecio squalidus and some observations on the role of ray florets in the Compositae. New Phytologist 91: 749-756.

IRWIN, M. A. 1971. Sesquiterpene lactones of Artemisia. Ph.D. Dissertation, University of California at Los Angeles.

JACKSON, R. C. and C. F. DIMAS. 1981. Experimental evidence for systematic placement of the Haplopappus phyllocephalus complex (Compositae). Systematic Botany 6: 8-14.

JANSEN, R. K. and J. D. PALMER. 1987. Chloroplast DNA from lettuce and Barnadesia (Asteraceae): structure, gene localization, and characterization of a large inversion. Current Genetics 11: 553-564.

——— and J. D. PALMER. 1988. Phylogenetic implications of chloroplast DNA restriction site variation in the Mutiseae (Asteraceae). American Journal of Botany 75: 753-766.

——— , H. J. MICHAELS, R. S. WALLACE, K. J. KIM, S. C. KEELEY, L. E. WATSON, and J. D. PALMER. 1992. Chloroplast DNA variation in the Asteraceae: Phylogenetic and evolutionary implications. Pp. 252-279 in Plant Molecular Systematics, eds. P. S. Soltis, D. E. Soltis, and J. J. Doyle. Chapman and Hall, New York.

KECK, D. D. 1946. A revision of the Artemisia vulgaris complex in North America. Proceedings of the California Academy of Sciences 17: 421-468.

KELSEY, R. G. 1974. The systematic usefulness of the sesquiterpene lactones in the genus Artemisia, section Tridentatae (sagebrush) of Montana. Ph.D. Dissertation, University of Montana.

LING, Y. R. 1991. A review of the classification, distribution and applications of Artemisia L. and Seriphidium (Bess.) Poljak. (Compositae) in China. Guihaia 11: 19-35.

——— . 1995a. 13. The new world Artemisia L. Pp. 255-281 in Advances in Compositae Systematics, eds. D. J. N. Hind, C. Jeffrey, and G. V. Pope. Royal Botanic Gardens, Kew.

——— . 1995b. 14. The new world Seriphidium (Besser) Fourr. Pp. 283-291 in Advances in Compositae Systematics, eds. D. J. N. Hind, C. Jeffrey, and G. V. Pope. Royal Botanic Gardens, Kew.

MADDISON, W. P., M. J. DONOGHUE, and D. R. MADDISON. 1984. Outgroup analysis and parsimony. Systematic Zoology 33: 83-103.

McARTHUR, E. D. and A. P. PLUMMER. 1978. Biogeography and management of native Western shrubs: a case study, section Tridentatae of Artemisia. Great Basin Naturalist Memoirs 2: 229-243.

——— , C. L. POPE, and D. C. FREEMAN. 1981. Chromosomal studies of subgenus Tridentatae of Artemisia: Evidence for autopolyploidy. American Journal of Botany 68: 589-605.

MOSS, E. H. 1940. Interxylary cork in Artemisia with reference to its taxonomic significance. American Journal of Botany 27: 762-768.

NIXON, K. C. and Q. D. WHEELER. 1990. An amplification of the phylogenetic species concept. Cladistics 6: 211-223.

PALMER, J. D. 1986a. Chloroplast DNA and phylogenetic relationships. In DNA Systematics. Volume II. Plants, ed. S. K. Dutta. CRC Press, Boca Raton, Florida.

——— . 1986b. Isolation and structural analysis of chloroplast DNA. Methods in Enzymology 118: 167-186.

POLJAKOV, P. P. 1961. Artemisia. Pp. 425-631 in Flora USSR vol. 26, ed. V. L. Komarov.

RIESEBERG, L. H. and S. J. BRUNSFELD. 1992. Molecular evidence and plant introgression. Pp. 151-176 in Molecular Systematics of Plants, eds. P. S. Soltis, D. E. Soltis, and J. J. Doyle. New York: Chapman and Hall.

——— and D.E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evolutionary Trends in Plants 5: 65-84.

——— , S. M. BECKSTROM-STERNBERG, A. LISTON, and D. M. ARIAS. 1991. Phylogenetic and systematic inferences from chloroplast DNA and isozyme variation in Helianthus sect. Helianthus (Asteraceae). Systematic Botany 16: 50-76.

RYDBERG, P. A. 1916. (Carduales), Carduaceae, Tageteae, Anthemideae. North American Flora 34. New York.

SAGHAI-MAROOF, M. A., K. M. SOLIMAN, R. A. JORGENSEN, and R. W. ALLARD. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences U. S. A. 81: 8014-8018.

SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press.

SHULTZ, L. M. 1983. Systematics and anatomical studies of Artemisia subgenus Tridentatae. Ph.D. Dissertation. Claremont Graduate School.

SOLTIS, D. E. and R. K. KUZOFF. 1995. Discordance between nuclear and chloroplast phylogenies in the Heuchera group (Saxifragaceae). Evolution 49: 727-742.

——— , P. S. SOLTIS, and B. G. MILLIGAN. 1992a. Intraspecific cpDNA variation: Systematic and phylogenetic implications. Pp. 117-150 in Molecular Systematics of Plants, eds. P. S. Soltis, D. E. Soltis, and J. J. Doyle. New York: Chapman and Hall.

——— , P. S. SOLTIS, J. N. THOMPSON, and O. PELLMYR. 1992b. Chloroplast DNA variation in Lithophragma (Saxifragaceae). Systematic Botany 17: 607-619.

SOUTHERN, W. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. Journal of Molecular Biology 98: 503-517.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1.1. Champaign: Illinois Natural History Survey.

TIDWELL, W. D., S. R. RUSHFORTH, and D. SIMPER. 1972. Evolution of floras in the Intermountain Region. Pp. 19-39 in Intermountain Flora: Vascular Plants of the Intermountain West Vol. 1, eds. A. Cronquist, A. H. Holmgren, N. H. Holmgren, and J. L. Reveal. Hafner Publ. Co., New York.

WARD, G. H. 1953. Artemisia section Seriphidium in North America: A cytotaxonomic study. Contributions from the Dudley Herbarium 4: 155-205.

WATROUS, L. E. and Q. D. WHEELER. 1981. The out-group comparison method of character analysis. Systematic Zoology 30: 1-11.

WATSON, L.E. 1996. Molecular systematics of the tribe Anthemideae. Pp. 341-348 in Compositae: Systematics. Proceedings of the International Compositae Conference, Kew 1994, eds. D. J. N. Hind and H. J. Beentje. Royal Botanic Gardens, Kew.

WIENS, J. J. 1995. Polymorphic characters in phylogenetic systematics. Systematic Biology 44: 482-500.

TABLE 1. Subgeneric classification of Artemisia L.

subg. Artemisia:	Disciform, heterogamous capitula; pistillate ray florets; perfect and fertile disk florets; receptacle either glabrous or hairy.
subg. Dracunculus:	Disciform, heterogamous capitula; pistillate ray florets; staminate disk florets (sterile, functionally male); receptacle glabrous.
subg. Seriphidium:	Discoid, homogamous capitula; all florets perfect and fertile; receptacle glabrous. [Recognized as segregate genus Seriphidium by Poljakov (1961), Ling (1991, 1995), and Bremer and Humphries (1993)]

TABLE 2. Comparison of three conflicting hypotheses of interspecific relationships within *Artemisia* sect. *Tridentatae*. Two lineages have been proposed: (1) *A. tridentata* lineage and (2) *A. cana* lineage.

	Ward (1953)	**Beetle (1960)**	**Shultz (1983)**
***A. tridentata* lineage**	*A. tridentata*	*A. tridentata*	*A. tridentata*
-seldom root sprouts or layers	*A. arbuscula*	*A. longiloba*	*A. nova*
-mostly tridentate leaves	*A. arbuscula* subsp. *nova*	*A. nova*	
-xerophytic	*A. arbuscula* subsp. *longiloba*	*A. bigelovii*	
	A. rigida	*A. pygmaeae*	
***A. cana* lineage**	*A. cana*	*A. cana*	*A. cana*
-root sprouts and layers	*A. tripartita*	*A. tripartita*	*A. tripartita*
-leaves entire or deeply divided		*A. rigida*	
-mesophytic			
questionable placement	*A. pygmaea*		*A. pygmaea*
	A. palmeri		*A. rigida*
reticulate taxa	*A. rothrockii*	*A. arbuscula*	*A. arbuscula*
		A. rothrockii	*A. rothrockii*
excluded taxa	*A. bigelovii*	*A. palmeri*	*A. bigelovii*

TABLE 3. Collection and locality data for Artemisia sect. Tridentatae and 13 outgroup species. Collection and voucher information includes: AK=Amy Kornkven, vouchers located at the University of Oklahoma (OKL); LS=Leila Shultz, vouchers at Utah State University (UTC); and LW=Linda Watson, vouchers at Uppsala Herbarium (UPS) or KEW unvouchered DNA's (Accession numbers).

Species	Voucher/Acc.	Locality (State: County)
Sect. Tridentatae		
1. A. tridentata		
subsp. tridentata	AK267	NM: Rio Arriba Co.
	LS11872	UT: San Juan Co.
	AK303	UT: Wayne Co.
subsp. vaseyana	AK207	WY: Albany Co.
	LS11852	WY: Teton Co.
	AK290	UT: San Juan Co.
subsp. wyomingensis	AK220	WY: Carbon Co.
	AK237	WY: Washakiie Co.
	AK249	WY: Crook Co.
2. A. nova	AK276	NM: Taos Co.
	LS11871	UT: San Juan Co.
	AK460	ID: Cassia Co.
	AK439	ID: Butte Co.
3. A. rigida	AK409	OR: Harney Co.
	AK384	WA: Yakima Co.
	AK418	OR: Malheur Co.

Table 3. Continued.

Species	Voucher/Acc.	Locality (State: County)
4. A. bigelovii	AK255	NM: Quay Co.
	AK286	NM: San Miguel Co.
	LS11874	WY: Teton Co.
	AK304	UT: Wayne Co.
5. A. cana		
subsp. cana	AK226	WY: Carbon Co.
	AK242	WY: Johnson Co.
	AK250	WY: Crook Co.
	AK232	WY: Natrona Co.
subsp. viscidula	AK214	WY: Carbon Co.
6. A. arbuscula		
subsp. arbuscula	AK403	OR: Klamath Co.
	AK419	ID: Ada Co.
	AK436	ID: Baine Co.
	AK535	NE: White Pine Co.
	AK531	NE: Lander Co.
subsp. thermopola	AK429	ID: Custer Co.
7. A. longiloba	AK426	ID: Camas Co.
	LS11953	WY: Teton Co.
8. A. tripartita		
subsp. tripartita	AK416	OR: Baker Co.
	AK449	ID: Bannock Co.
	AK370	WA: Douglas Co.
subsp. rupicola	AK208	WY: Albany Co.
9. A. palmeri	AK491	CA: San Diego Co.
	AK493	CA: San Diego Co.

Table 3. Continued.

Species	Voucher/Acc.	Locality (State: County)
10. A. pygmaea	AK533	NE: Eureka Co.
	AK543	NE: White Pine Co.
11. A. rothrockii	AK505	CA: Inyo Co.
	AK521	CA: Toulumne Co.
Subg. Artemisia		
12. A. vulgaris	LS11881	OK: Cultivated
13. A. ludoviciana	AK240	WY: Johnson Co.
14. A. abrotanum	LS11880	OK: Cultivated
15. A. californica	AK496	CA: San Diego Co.
16. A. frigida	AK221	WY: Carbon Co.
17. A. gnaphalodes	LW, s.n.	Uppsala Botanic Garden
18. A. oelandica	LW, s.n.	Uppsala Botanic Garden
19. A. macrocephala	LW, s.n.	Uppsala Botanic Garden
20. A. stelleriana	000-69-18228	Kew Botanic Garden
Subg. Dracunculus		
21. A. filifolia	LS11873	UT: Grand Co.
22. A. dracunculus	000-69-18226	Kew Botanic Garden
Subg. Seriphidium		
23. A. sublessingiana	LW, s.n.	Uppsala Botanic Garden
A. maritima	LW, s.n.	Uppsala Botanic Garden

TABLE 4. Chloroplast DNA restriction site variation. Ancestral fragments are given first, followed by derived fragments. Derived taxon numbers are listed in Table 3 and sect. Tridentatae (excluding A. palmeri) is listed as TRID. The probe regions are identified by LSC (large single copy region), IR (inverted repeat), and SSC (small single copy region). The numbers correspond to the 15 cloned fragments of lettuce cpDNA and to the size (kilobase pairs) of the probe region (Jansen and Palmer 1987). * indicates intraspecifically variable mutations within sect. Tridentatae, while (IV) indicates in which species intraspecific variation has been found.

#	Probe Region(kb)	RE	Mutation (kb)	Derived taxa
1	IR(12.3)	AccI	1.6+(0.7)=2.3	3
2	IR(12.3)	AccI	8.0=4.9+3.1	18
3	IR(12.3)	AccI	1.6+1.8=3.4	19
4	LSC(14.7)	AccI	4.8=2.0+2.8	1,15
5	LSC(10.6)	AccI	2.7+2.5=5.2	22
6	LSC(7.7,10.6)	AccI	1.9+(0.3)=2.2	12
7	LSC(4.6,5.4,6.3)	AvaI	11.6=4.9+6.7	TRID,9,13,15,17, 18,20,21,22,23
8	LSC(7.7,10.6)	AvaI	4.6+(1.2)=5.8	9,13,16,17,20
9	LSC(7.7,10.6)	AvaII	1.0+0.8=1.8	TRID,15,21
10	IR(12.3)	BamHI	1.6=1.0+(0.6)	9,13,17,20
11	LSC(7.7)	BamHI	6.7=5.0+1.7	3,11,15

Table 4. Continued.

#	Probe Region(kb)	RE	Mutation (kb)	Derived taxa
12*	SSC(18.8)	BamHI	8.6=4.0+4.6	3(IV)
13*	IR(12.3)	BanII	2.6=2.2+(0.4)	6(IV)
14*	SSC(18.8)	BanII	8.2=6.5+2.0	1(IV),2(IV),5(IV), 6(IV),10,11(IV)
15*	LSC(7.0,6.7)	BanII	5.5+(1.0)=6.5	1(IV),2(IV), 5(IV),11(IV)
16	LSC(3.8,6.9)	BclI	7.9+3.0=11.9	3,15,21
17	LSC(10.6)	BclI	8.0=4.0+3.8	TRID,15,21
18	SSC(18.8)	BclI	3.0=1.7+1.3	18
19	LSC(7.0,6.7)	BclI	12.3=7.9+4.4	19
20*	LSC(3.8,6.9)	BclI	7.9=6.9+1.0	5(IV)
21*	LSC(10.6)	BclI	4.2=3.8+(0.4)	1(IV),5(IV),6(IV)
22	IR(12.3)	BglII	1.6+(0.4)=2.0	23
23	LSC(14.7)	BglII	2.8=1.8+(1.0)	6
24	LSC(7.7)	BglII	4.0+1.4=5.4	TRID,15,21
25	LSC(14.7)	BstNI	3.0=2.0+(1.0)	12,16
26	LSC(7.7)	BstNI	4.8=3.0+1.8	9
27	LSC(7.7)	BstNI	4.8+(0.4)=5.2	14,18
28	LSC(7.7)	BstNI	4.8+1.9=6.7	19
29	LSC(10.6)	BstNI	2.4=2.2+(0.2)	9,13,16,17,20
30	IR(12.3)	BstNI	2.9+1.1=4.0	18
31	LSC(7.0,6.7)	BstNI	3.7=4.3	16
32	IR 12.3)	ClaI	4.5=2.3+2.2	9,13,17,20
33*	LSC(7.7)	ClaI	5.0=4.2+(0.8)	TRID,3(IV)
34	LSC(14.7)	ClaI	3.7+11.5=15.2	14
35*	SSC(18.8)	ClaI	7.8=5.3+2.5	1(IV),2(IV)
36	LSC(7.0, 6.7)	DraI	2.7+(0.3)=3.0	23

Table 4. Continued.

#	Probe Region(kb)	RE	Mutation (kb)	Derived taxa
37	LSC(14.7)	DraI	10.0=5.7+4.3	9,13,17
38*	LSC(7.7)	DraI	16=8.0+8.0	22,5(IV)
39*	LSC(7.7,10.6)	EcoRI	4.2=2.9+1.3	1,5(IV),6(IV),10
40	LSC(4.6,5.4,6.3)	EcoRI	1.5+0.6=2.1	3
41	LSC(4.6,5.4,6.3)	EcoRI	2.2=2.0+(0.2)	14
42	IR(12.3)	EcoRV	5.8=3.3+2.5	12
43	LSC(6.3)	EcoRV	5.8=5.4+(0.4)	23
44	LSC(10.6)	EcoRV	2.8+(0.5)=3.3	14,19
45	LSC(10.6)	EcoRV	6.7=5.7+(1.0)	15
46	LSC(4.6,5.4,6.3)	EcoRV	6.7=4.5+2.2	14
47*	LSC(4.6,5.4,6.3)	HaeII	4.3=3.2+1.1	1(IV),4,7,8,21
48	LSC(4.6,5.4,6.3)	HaeII	5.1=2.0+3.1	19
49	LSC(7.0,6.7)	HaeII	6.3+2.0=8.3	19,23
50	IR(12.3)	HaeIII	1.5+0.7=2.2	22
51	LSC(14.7)	HaeIII	1.8=1.3+(0.5)	18
52	LSC(7.0,6.7)	HaeIII	2.2=1.8+(0.4)	19
53*	LSC(14.7)	HaeIII	2.0+(0.3)=2.3	5(IV),9
54	SSC(18.8)	HincII	5.8=5.2+(0.6)	TRID,9,13,15,17, 18,20,21,22,23
55	LSC(4.6,5.4,6.3)	HincII	2.1+(0.3)=2.4	23
56	LSC(4.6,5.4,6.3)	HincII	2.1+1.8=3.9	19
57	LSC(4.6,5.4,6.3)	HincII	2.1+2.3=4.4	18
58	LSC(14.7)	HindIII	7.5=5.5+2.0	TRID,15,21
59	LSC(7.7)	HindIII	6.7=4.8+1.9	9,13,17,20
60	IR(12.3)	HphI	2.3=1.8+(0.5)	TRID,9,13,15,17,18, 20,21,22,23

Table 4. Continued.

#	Probe Region(kb)	RE	Mutation (kb)	Derived taxa
61	IR(12.3)	HphI	3.4=3.2+(0.2)	14
62	LSC(14.7)	HphI	3.5=3.0+(0.5)	12
63	LSC(4.6,5.4,6.3)	HphI	3.5=2.3+(0.5)	23
64	LSC(4.6,5.4,6.3)	KpnI	16.0=12.5+3.5	Trid,9,13,15,17, 18,20,21,22,23
65	LSC(10.6)	KpnI	12.5+(1.0)=13.5	14,16,19
66	LSC(7.0,6.7)	MspI	2.5+(0.2)=2.7	4,7,21
67	LSC(7.7,10.6)	MspI	1.2+(0.7)=1.9	9,13,16,17,20
68	IR(12.3)	MspI	2.5+(0.2)=2.7	2
69	LSC(14.7)	MspI	1.9+(1.5)= 3.4	16
70	LSC(14.7)	MspI	2.4+(2.2)=4.6	18
71	LSC(7.0,6.7)	NcoI	17.0=2.8+14.2	13
72	LSC(7.7,10.6)	NcoI	6.3+6.4=12.7	12
73	IR(12.3)	RsaI	1.7+(0.1)=1.8	1,2,5,6,7,8,10,11,21
74	SSC(18.8)	RsaI	0.9+(0.4)=1.3	23
75	LSC(4.6,5.4,6.3)	RsaI	1.6=1.3+(0.3)	20
76	LSC(4.6,5.4,6.3)	SspI	2.0=1.1+0.9	12
77*	IR(12.3)	SspI	4.5+(0.7)=5.2	1(IV),2(IV), 5(IV),11(IV)
78*	LSC(7.0,6.7)	SspI	1.7=1.5+(0.2)	3(IV)
79	LSC(14.7)	XbaI	3.3+1.7=5.0	TRID,9,15,17,18, 21,22,23
80	LSC(14.7)	XbaI	1.9+3.3=5.2	22
81	LSC(7.7)	XmnI	3.0=1.8+1.2	18,22
82	LSC(10.6)	XmnI	3.5+2.2=5.7	14

FIG. 1. Distributions of the eleven Tridentatae species. (A) A. tridentata; (B) A. cana; (C) A. nova and A. rigida; (D) A. arbuscula; (E) A. bigelovii and A. tripartita; and (F) A. palmeri, A. pygmaea, A. longiloba, and A. rothrockii.


A.tridentata


A. cana


A. nova


A. arbuscula


A. tripartita
A. bigelovii


A. longiloba
A. rothrockii
A. pygmaea
A. palmeri

FIG. 2. Strict consensus of 144 equally most parsimonious trees from analysis of cpDNA restriction site variation among Artemisia sect. Tridentatae and outgroup species (CI=0.93, RI=0.93, RC=0.86, and tree length=82 excluding invariant characters). Anthemis nobilis was used as an outgroup. Bootstrap and decay values are indicated above and below each node, respectively. Restriction site changes supporting each node are indicated above the line, with non-homoplastic changes in parentheses. The two polymorphic characters included in the analysis are indicated on the tree (#33, 47). The subgeneric classification is indicated by color. The species currently included within sect. Tridentatae are identified in bold. * indicates possible chloroplast capture events.

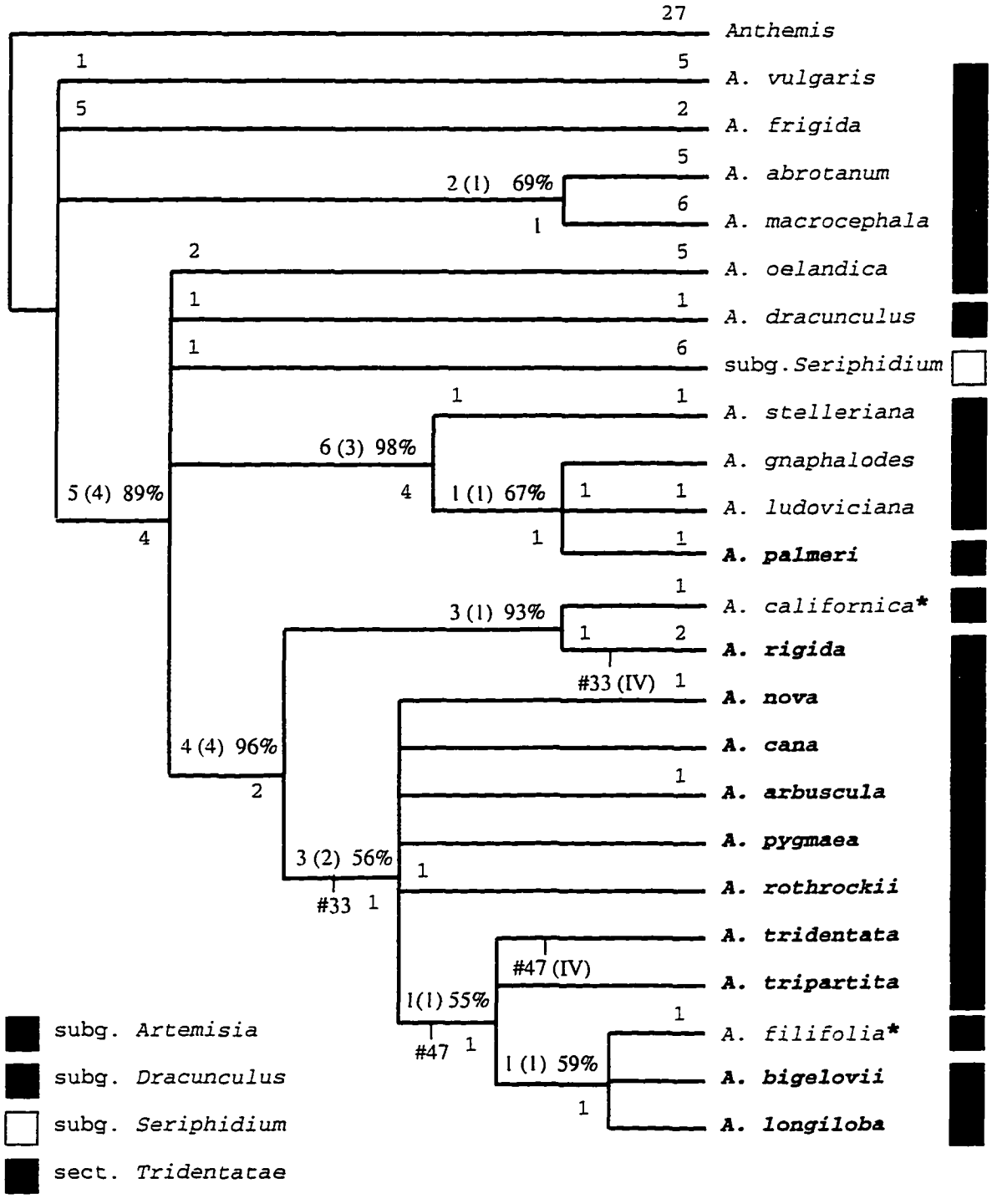
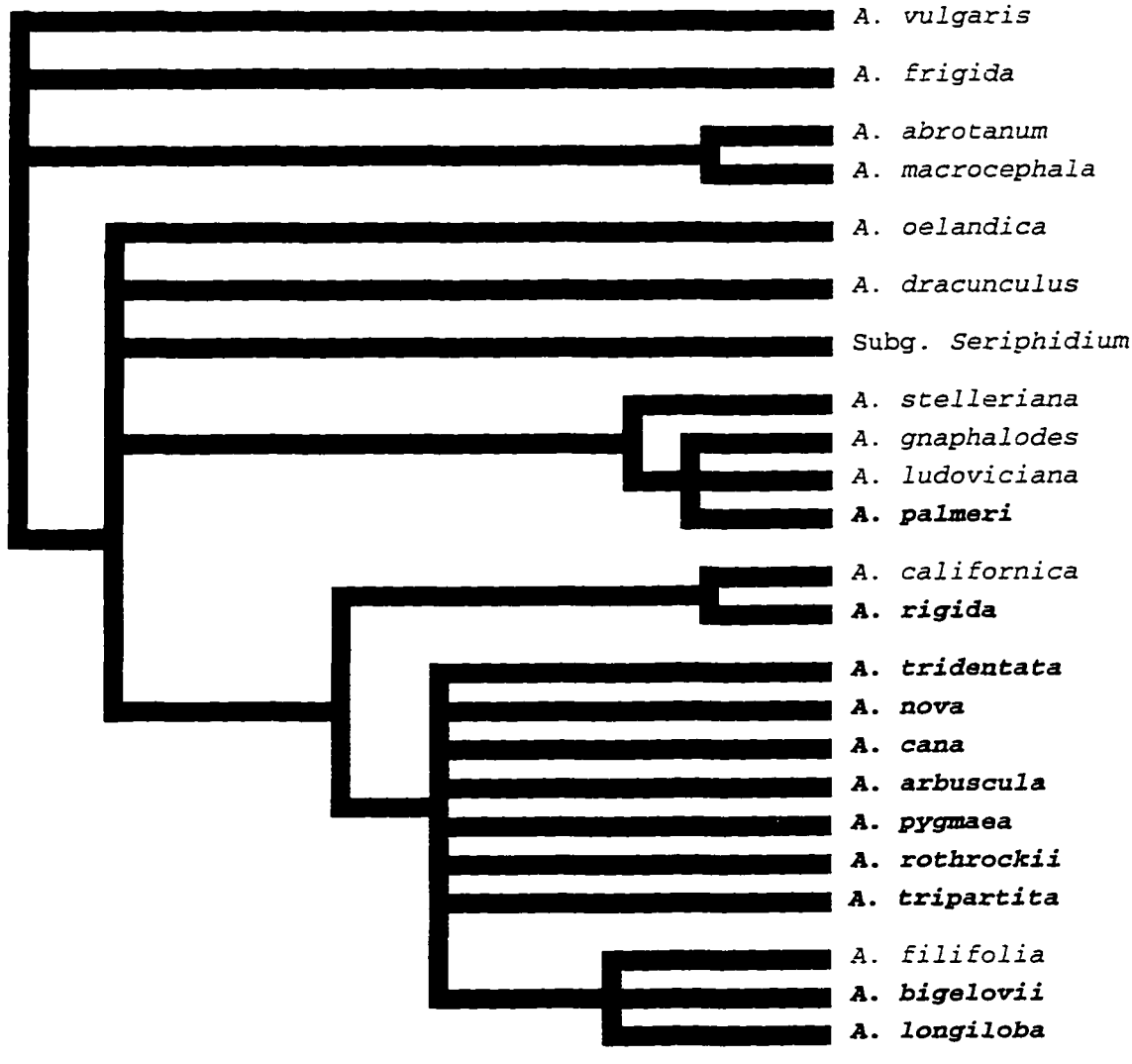

Anthemis
A. vulgaris
A. frigida
A. abrotanum
A. macrocephala
A. oelandica
A. dracunculus
subg. Seriphidium
A. stelleriana
A. gnaphalodes
A. ludoviciana
A. palmeri
A. californica*
A. rigida
A. nova
A. cana
A. arbuscula
A. pygmaea
A. rothrockii
A. tridentata
A. tripartita
A. filifolia*
A. bigelovii
A. longiloba
27
2 (1) 69%
5 (4) 89%
6 (3) 98%
1 (1) 67%
3 (1) 93%
#33 (IV)
4 (4) 96%
3 (2) 56%
#33
#47 (IV)
1 (1) 55%
#47
1 (1) 59%
subg. Artemisia
subg. Dracunculus
subg. Seriphidium
sect. Tridentatae

FIG. 3. Distribution of capitulum morphology mapped on the chloroplast DNA restriction site phylogeny for Artemisia (s.l.). The three types of capitula found in Artemisia (s.l.) are indicated in color.



Heterogamous flower heads, with pistillate ray florets, and perfect disk florets.

Heterogamous flower heads, with pistillate ray florets, and sterile disk florets.

Homogamous flower heads, with perfect disk florets.

PART 2

Phylogenetic analysis of *Artemisia* section *Tridentatae* (Asteraceae) based on sequences from the internal transcribed spacers (ITS) of nuclear ribosomal DNA

ABSTRACT

Artemisia sect. Tridentatae is composed of eleven species of xerophytic shrubs, which dominate much of western North America. Phylogenetic relationships were examined by sequencing the internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA). ITS sequences were generated for the eleven Tridentatae and eight outgroup species, with 249 to 253 bp and 222 to 223 bp in ITS1 and ITS2, respectively. Pairwise divergence values within sect. Tridentatae (s.s.) range from 0.0 to 4.1%. Divergence values between sect. Tridentatae and the anomalous species range from 2.8 to 5.1% for A. bigelovii and 5.8 to 8.0% for A. palmeri. Phylogenetic analyses of ITS sequence data support the monophyly of sect. Tridentatae, with the exclusion of A. bigelovii and A. palmeri. The core Tridentatae species form a clade, with A. rigida and A. pygmaea as sister taxa to the remainder of the Tridentatae. Both ITS sequence and cpDNA restriction site data strongly support the exclusion of A. palmeri from sect. Tridentatae and its inclusion in subg. Artemisia. Although cpDNA data support the inclusion of A. bigelovii within sect. Tridentatae, the ITS data support a close relationship between A. bigelovii and subg. Dracunculus (A. dracunculus and A. filifolia). Interspecific gene flow and subsequent chloroplast capture may explain the discordance observed between the nuclear and chloroplast phylogenies regarding the placement of three taxa, A. bigelovii, A. filifolia, and A. californica.

Artemisia sect. Tridentatae Rydb. contains some of the most ecologically and economically dominant shrub species in western North America. Section Tridentatae, as currently circumscribed, encompasses eleven species of mostly xerophytic shrubs with an extensive distribution that ranges from the Rocky Mountains, west into the foothills of the Sierra Nevada, south into northern New Mexico and Arizona, and north to Canada. Despite being extensively studied since the early 1900's, sect. Tridentatae presents numerous taxonomic difficulties (Rydberg, 1916; Hall and Clements, 1923; Ward, 1953; Beetle, 1960; McArthur and Plummer, 1978; Shultz, 1983). Little is known about phylogenetic relationships both within the section and at the subgeneric level in Artemisia L. (sensu lato).

Historically, capitular and reproductive morphology has been the pivotal character used to divide Artemisia (s.l.) into three subgenera: subg. Artemisia L. (disciform, heterogamous capitula, with pistillate ray florets, and perfect fertile disk florets); subg. Dracunculus (Besser) Rydb. (disciform, heterogamous capitula with pistillate ray florets and staminate disk florets); and subg. Seriphidium (Besser) Rouy (discoid, homogamous capitula with perfect, fertile disk florets). However Bremer and Humphries (1993), in their morphologically based cladistic analysis of Anthemideae, narrowly define Artemisia, segregating several

genera, including Seriphidium Polj. and include all eleven species of Tridentatae within Seriphidium. Several synapomophies support this placement including the presence of discoid homogamous flower heads with perfect disk florets; less than ten florets per head; 4-7 rows of involucral bracts; and slender, narrowly lanceolate to linear apical anther appendages (Poljakov, 1961; Bremer and Humphries, 1993; Bremer, 1994; Ling, 1991, 1995).

Two conflicting hypotheses concern the origin and placement of sect. Tridentatae within Artemisia (s.l.). Ling (1991, 1995) suggests that the progenitors of sect. Tridentatae are Asian Seriphidium species that migrated over the Bering Strait. In contrast, McArthur and Plummer (1978) suggest that sect. Tridentatae originated from herbaceous members of subg. Artemisia and differentiated in situ in North America during the Pleistocene, in response to the extreme climatic changes occurring throughout the west. Chloroplast DNA restriction site data provide only equivocal evidence for either of these two hypotheses regarding the origin and phylogenetic relationship of sect. Tridentatae within Artemisia (s.l.) (Kornkven, 1997).

Previous studies, including morphology (Rydberg, 1916; Hall and Clements, 1923; Beetle, 1960), anatomy (Moss, 1940; Carlquist, 1966; Shultz, 1983), cytology (Ward, 1953; McArthur and Plummer, 1978; McArthur et al., 1981), and

chemistry (Irwin, 1971; Hanks et al., 1973; Geissman and Irwin, 1974; Kelsey, 1974), support the monophyly and circumscription of the section with two important exceptions. Two species, A. palmeri A. Gray and A. bigelovii A. Gray, have been variously included or excluded from sect. Tridentatae. Artemisia palmeri, an herbaceous species endemic to southern California, is morphologically similar to members of subg. Artemisia (Moss, 1940; Beetle, 1960; McArthur et al., 1981; Shultz, 1983). However, the presence of homogamous flower heads led most authors to include A. palmeri within sect. Tridentatae (Hall and Clements, 1923; Ward, 1953; Bremer and Humphries, 1993; Ling, 1995).

In contrast, A. bigelovii is included within the section based primarily on overall morphological similarity to A. tridentata Nutt., in addition to the presence of 4-7 rows of involucral bracts and slender, lanceolate to linear apical anther appendages (Beetle, 1960; Poljakov, 1961; McArthur and Plummer, 1978; McArthur et al., 1981; Bremer and Humphries, 1993). However, the presence of some heterogamous flower heads supports its exclusion and placement into subg. Artemisia (Hall and Clements, 1923; Ward, 1953; Shultz, 1983; Ling, 1995). Traditional data provide conflicting evidence for the placement of these two species.

The cpDNA restriction site data support the monophyly of the section, with the exclusion of A. palmeri and inclusion of A. bigelovii (Kornkven, 1997). Unexpectedly, both A. californica Less., and A. filifolia Torrey nested within the Tridentatae clade in the cpDNA tree. These two species are currently placed in two different subgenera, subg. Artemisia (A. californica) and subg. Dracunculus (A. filifolia), on the basis of capitular morphology (Hall and Clements, 1923; Poljakov, 1961; Cronquist, 1972; Bremer and Humphries, 1993; Ling, 1991, 1995). Several different hypotheses can be proposed to explain this unexpected placement including interspecific gene flow, lineage sorting, or a current classification that does not accurately reflect phylogenetic relationships (Kornkven, 1997).

In addition, interspecific relationships within sect. Tridentatae remain unresolved. Two lineages (A. tridentata and A. cana Pursh.) have been proposed based on leaf morphology, habitat preference, and ability to root sprout (Ward, 1953; Beetle, 1960; Shultz, 1983; Table 1). There has been little agreement concerning the circumscription of the two lineages and interspecific relationships within each lineage remain unresolved. The lack of significant morphological divergence within sect. Tridentatae has made it difficult to examine phylogenetic relationships within

this highly polymorphic group. Relationships among the Tridentatae species also remain unresolved on the cpDNA based phylogeny, with a polytomy at the base of the Tridentatae clade (Kornkven, 1997). The relatively recent and rapid expansion of sect. Tridentatae throughout western North America during the Pleistocene, in conjunction with the conservative rate of evolution of the chloroplast genome may account for the low variation observed within Artemisia. The lack of phylogenetically informative cpDNA variation within sect. Tridentatae and possible introgression and chloroplast capture events, emphasizes the importance of examining a more rapidly evolving region within the nuclear genome. The internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA) have been widely used to examine phylogenetic relationships within many plant taxa (reviewed in Baldwin, 1992; Baldwin et al., 1995). ITS sequences are easily amplified and sequenced directly across a wide range of plant taxa and the level of variation has generally been higher than that observed in comparable cpDNA restriction site studies. In particular, ITS sequence data has provided valuable insight into potential examples of interspecific gene flow, based on discordance between nuclear and chloroplast-based phylogenies (Soltis and Kuzoff, 1995; Bayer et al., 1996; Soltis et al., 1996).

The objective of this study is to construct a phylogeny of Artemisia sect. Tridentatae using sequence data from ITS regions of nrDNA. The data are used to (1) re-examine the monophyly and circumscription of the section, (2) resolve interspecific phylogenetic relationships, (3) examine the congruence between nuclear ITS and chloroplast DNA phylogenies to assess possible interspecific gene flow, and (4) evaluate two conflicting hypotheses on the origin and placement of sect. Tridentatae within Artemisia (s.l.).

MATERIALS AND METHODS

All eleven species of sect. Tridentatae were included in this study, including the two anomalous species, A. bigelovii and A. palmeri (Table 2). Multiple populations of each species were not sequenced due to the low level of sequence divergence observed for ITS during the early stages of the study. Because relationships between sect. Tridentatae and other members of Artemisia are unclear, eight outgroup species were sampled including representatives from each subgenus (five species from subg. Artemisia, two from subg. Dracunculus, and one from subg. Seriphidium; Table 2). All North American taxa were collected from natural field populations; the European and Asian taxa were obtained primarily from botanic gardens. Voucher specimens are deposited at the University of

Oklahoma Robert Bebb Herbarium (OKL), unless otherwise noted (Table 2).

Fresh leaf material was stored on ice in the field and then stored in an ultra-low freezer (-80°C). Total DNA was isolated from approximately 1.5 g of frozen leaf tissue. The leaves were ground into a fine powder using liquid nitrogen with 4% w/v polyvinylpyrrolidone (PVP-40) added to each sample. Total DNA was extracted using the 2X CTAB isolation procedure, and further purified on cesium chloride/ethidium bromide (CsCl/EtBr) gradients (Sambrook et al., 1986; Palmer, 1986; Doyle and Doyle, 1987).

A single fragment containing both ITS regions, as well as the 5.8S coding region of nrDNA, was amplified by polymerase chain reaction (PCR). The DNA was amplified using primers ITS4 and ITS5 of White et al. (1990) and Baldwin (1992). The PCR reaction mixture consisted of 1X reaction buffer, 2.5mM magnesium chloride solution, 10 mM dNTP solution in equimolar ratio, 10 μM primers, 10-50 ng of template DNA, and 2.5 U of AmpliTaq DNA Polymerase (Perkin Elmer Corp.). The samples were heated to 94°C for 2 minutes, followed by 30 cycles of denaturation (94°C for 30 seconds), primer annealing (48°C for 2 min.), and extension (72°C for 2 min.). A final extension phase of 7 min. at 72°C terminated the PCR reaction. Each amplification

product was verified on 1% agarose gels using 1X Tris Acetate (TAE) buffer stained with EtBr.

The double stranded PCR products were purified by electrophoresis on 2% low melting point agarose (NuSieve GTG). The DNA bands were then excised from the gel and melted at 65-75°C for approximately 10 minutes. Either glassmilk (GeneClean) or Wizard columns (Promega) were used to further purify and concentrate the amplified DNA.

ITS sequences were obtained by either direct manual sequencing (A. tripartita Rydb., A. pygmaea Gray, A. longiloba (Oster.) Beetle, A. cana, A. rigida (Nutt.) Gray, A. arbuscula Nutt., and A. dracunculus L.), automated sequencing (A. tridentata, A. nova A. Nels., A. palmeri, A. abrotanum L., A. californica, A. ludoviciana Nutt., A. rupestris L., and A. sublessingiana (Kell.) Krasch. Ex Poljak.), or a combination of both (A. bigelovii, A. filifolia, A. rothrockii Gray, and A. vulgaris L.).

Manual sequencing -- The purified double stranded PCR products were sequenced directly using Sequenase 2.0 (U. S. Biochemical Corp.) and ^{35}S dATP labeling. The standard dideoxy chain-termination method was followed using two forward (ITS1 and ITS3) and two reverse (ITS2 and ITS4) primers to sequence the entire ITS regions (Sanger et al., 1977; White et al., 1990; Baldwin, 1992; Francisco-Ortega et

al., in press). Primer concentrations of 2.5 μM generated optimal sequencing product.

The DNA/primer mixture was denatured by boiling the double stranded DNA; the annealing mixture was then flash-frozen in liquid nitrogen, and thawed at the beginning of the extension reaction (Gyllensten, 1989; Conti et al., 1993; Rodman et al., 1993; Baum et al., 1994). The labeled fragments were separated by gel electrophoresis on 6% polyacrylamide with 1X Tris Borate buffer (TBE). Both short and long gels were run to completely sequence both strands. The gels were fixed for 30 minutes in 10% acetic acid/12% ethanol and transferred to 3-MM Whatman paper. The gels were dried under vacuum at 80°C, and exposed to Kodak XAR film for 24-72 hours.

Automated sequencing -- PCR products were purified on 2% low-melting point agarose. The excised DNA bands were further purified and concentrated using Wizard Columns (Promega). The purified double stranded PCR products were sequenced using AmpliTaq DNA Polymerase FS (Perkin Elmer), in a dye terminator mix, on an ABI automated sequencer (Model 373A). The two forward (ITS1 and ITS3) and two reverse (ITS2 and ITS4) primers (3.2 μM) were used to sequence both ITS regions and part of the 5.8S gene (White et al., 1990; Baldwin, 1992). Sequencher 2.1 (Gene Codes

Corp. Inc.) was used to examine the resulting chromatograms and to align the sequences.

All ITS sequences for Artemisia species, including sect. Tridentatae and outgroup species, were further aligned manually using sequential pairwise comparisons. The presence of several small insertions and deletions was not a significant factor in aligning the sequences. Each indel was excluded from the phylogenetic analysis and coded as missing data. The indels were then mapped onto the consensus tree to examine their phylogenetic distribution. In addition, to assess the phylogenetic impact of the indels, each indel was included in the data matrix as a binary character.

The complete sequence for both ITS1 and ITS2 of A. tridentata was used as a reference sequence, with only the differences between the remaining sequences indicated (Appendix 1). The boundaries of both ITS regions were determined by comparison with published and unpublished sequences from other Asteraceae taxa (Baldwin, 1992, 1993; Kim and Jansen, 1994; Bain and Jansen, 1995; Sang et al., 1994, 1995; Bayer et al., 1996; Francisco-Ortega et al., in press).

Phylogenetic analysis -- Complete ITS sequences of the eleven Tridentatae and eight Artemisia species were included in the phylogenetic analysis. In addition, five outgroups

from the Anthemideae were included to examine the origin and phylogenetic relationships of sect. Tridentatae. The sequence data were analyzed with PAUP 3.1.1 (Swofford 1993). A modified island search was conducted using TBR (Tree-Bisection-Reconnection) branch swapping, MULPARS on, ACCTRAN optimization, and 1000 replicate tree searches with random taxon addition and order (Madison, 1991; Conti et al., 1993). Both bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988) were used to obtain estimates of support for each monophyletic clade on the resulting phylogenetic trees. The bootstrap option in PAUP was run with 100 replicates, simple taxon addition, and TBR branch-swapping. AutoDecay 2.3 (Eriksson and Wilkstrom, 1995), in conjunction with PAUP 3.1.1, was used to generate decay values for each clade. Strict and semi-strict consensus trees were generated from each set of equally parsimonious trees. Sequence divergence values were calculated for all pairwise comparisons over all characters using the data distance matrix option in PAUP 3.1.1.

Indels were coded as missing characters and mapped onto the strict consensus tree to assess their phylogenetic content (or support). The gap regions also were excluded from the data matrix and recoded as two binary characters (Baldwin, 1995).

Interspecific relationships within sect. Tridentatae were examined by outgroup analysis, using two species of subg. Artemisia, A. canariensis (Besser) Less. and A. vulgaris. In addition, a global outgroup approach was used to examine subgeneric relationships (Watrous and Wheeler, 1981; Maddison et al., 1984), including representative genera from five subtribes within the Anthemideae including: Anthemidinae (Anthemis L.); Leucantheminae (Nippoanthemum Kitam.); Artemisiinae (Dendranthema (DC.) Des Moul.); Matricariinae (Cymbopappus B. Nord.); and Ursiniinae (Ursinia Gaertn). These outgroup genera were selected on the basis of previous studies of phylogenetic relationships utilizing molecular data (Watson et al., 1996; Francisco-Ortega et al., in press).

To examine congruence between the nuclear and organellar phylogenies, the cpDNA restriction site variation was reanalyzed, including the same taxa used in the ITS study. For comparison of topologies between the cpDNA and ITS trees, the cpDNA trees were rerooted with A. dracunculus, based on results of the ITS analysis, which placed A. dracunculus in a basal position. In addition, a phylogenetic analysis of the combined ITS sequence and cpDNA restriction site data sets was run using the heuristic search option of PAUP with TBR branch swapping, MULPARS on, and ACCTRAN optimization. The combined data set included 18

taxa and 541 characters, 486 characters for ITS and 55 for cpDNA data, with A. dracunculus as the outgroup.

RESULTS

ITS sequences -- The ITS spacers of Artemisia sect. Tridentatae are 253 and 222 base pairs (bp) in length for ITS1 and ITS2, respectively (Appendix 1). Within Artemisia (s.l.), ITS1 ranges from 249 to 253 bp, while ITS2 ranges from 222 to 223 bp. The size of both ITS spacers in Artemisia (s.l.) fall within the range of sizes observed for other Asteraceae taxa (Baldwin, 1992, 1995; Kim and Jansen, 1994; Sang et al., 1995; Bayer et al., 1996). The manually aligned sequences require four gaps of 1-2 bp each for ITS1, while the alignment of ITS2 requires only a single gap of 1 bp (Appendix 1). Three indels are autapomorphic, while two are potentially phylogenetically informative. One indel, a 2 bp deletion, unites A. sublessingiana and A. canariensis. The second indel, a single bp deletion, was present in three Artemisia species (A. canariensis, A. rupestris, and A. dracunculus) and four of the five outgroup species (except Anthemis).

Ambiguous nucleotide positions were infrequent in both ITS regions, with 10 and 13 in ITS1 and ITS2, respectively. Two ITS length variants were observed within several populations of A. tridentata and A. nova. After further

examination of voucher material, it was determined that polyploidy may have been a factor in these populations. Several additional populations of both species were then surveyed with no apparent length variation. Length variation was not present in A. rothrockii, a hexaploid species of unknown origin.

In ITS1 78 (30.5%) nucleotide base positions are variable, 20 of which are potentially phylogenetically informative, and 58 of which are autapomorphic. In ITS2, 59 (26.4%) positions are variable, 22 of which are potentially phylogenetically informative, and 37 of which are autapomorphic. Overall, ITS1 is more variable than ITS2, but the number of phylogenetically informative positions is slightly higher in ITS2. Focusing on sect. Tridentatae (s.s.), if the two anomalous species, A. bigelovii and A. palmeri, are excluded, 14 positions (5.5%) are variable in ITS1 and 20 (9.0%) are variable in ITS2. The inclusion of A. bigelovii and A. palmeri increases the amount of variation to 35 (ITS1) and 32 (ITS2) positions, respectively. These numbers do not include indels or undetermined polymorphisms. Almost all of the variation observed among sequences was due to point mutations, and was not a result of indels or other length variants.

Pairwise divergence values within sect. Tridentatae (s.s.) range from 0.0 to 4.1% (Table 3). Divergence values

between A. bigelovii and sect. Tridentatae range from 2.8 to 5.1%, while the values between A. palmeri and sect. Tridentatae range from 5.8 to 8.0%. Divergence values within Artemisia (s.l.) range from 0.0 to 8.1% and 4.2 to 22% between Artemisia and the five Anthemideae outgroups (Anthemis, Dendranthema, Cymbopappus, Nippoanthemum, and Ursinia).

Phylogenetic analysis -- Phylogenetic analysis of the two ITS sequences, with A. canariensis as the outgroup, generated 392 equally most parsimonious trees. The strict consensus tree has a consistency index (CI) of 0.734, retention index (RI) of 0.541, rescaled consistency index (RC) of 0.397, and tree length of 188 steps, excluding invariant characters (Fig. 1). Three clades are weakly supported (bootstrap values < 50%; decay values ≤ 2) on the strict consensus tree including: (1) the core Tridentatae forming a clade with A. rigida and A. pygmaea as sister taxa to the remainder of the Tridentatae (decay = 2); (2) subg. Dracunculus, A. dracunculus and A. filifolia, forming a strongly supported clade with A. bigelovii; and (3) subg. Artemisia, A. vulgaris and A. ludoviciana, forming a clade with A. palmeri. A sister relationship between A. palmeri and A. ludoviciana was first supported by cpDNA restriction site data (Kornkven, 1997) and is also supported by the ITS sequence data. Parsimony analysis supports the monophyly of

sect. Tridentatae, with the exclusion of the two anomalous species, A. bigelovii and A. palmeri. However, phylogenetic resolution within sect. Tridentatae is limited by low sequence divergence at the interspecific level, and relationships within the section are only partially resolved.

The inclusion of two indels as binary characters in the analysis generated a strict consensus of 112 equally most parsimonious trees (not shown) (CI=0.74). Treating indels as either missing data or as binary characters generated strict consensus trees with identical topologies.

Separate analysis of ITS1 generated over 4000 trees with a length of 115 steps. The strict consensus tree (not shown) was almost completely unresolved, with the exception of three minor clades: (1) A. bigelovii and A. dracunculus; (2) A. palmeri and A. rupestris; and (3) A. abrotanum and A. filifolia. Analysis of ITS2 generated 107 trees of 111 steps. The strict consensus tree of ITS2 (not shown) has an identical topology to the combined ITS1 and ITS2 analysis, with slightly less resolution.

Overall, the topologies of the rerooted cpDNA and ITS trees are similar with two important exceptions (Fig. 3). Although cpDNA restriction site data support the inclusion of A. bigelovii within sect. Tridentatae, the ITS phylogeny supports a close relationship between A. bigelovii, A.

dracunculus, and A. filifolia. In addition, A. filifolia is found within the Tridentatae clade in the cpDNA phylogeny, and with A. bigelovii and A. dracunculus in the ITS tree. Secondly, A. californica is basal and sister to sect. Tridentatae in the cpDNA tree and unresolved in the ITS tree.

The combined analysis of cpDNA and ITS datasets produced 183 equally most parsimonious tree with two clades supported on the strict consensus tree (not shown)(CI=0.78, RI=0.64, RC=0.50, and tree length=231, excluding invariant characters). Section Tridentatae forms a monophyletic clade, with A. rigida and A. californica sister to the remainder of the Tridentatae species including both A. bigelovii and A. filifolia. The four species in subg. Artemisia also form a clade, with A. vulgaris and A. abrotanum as sister to A. ludoviciana and A. palmeri. Artemisia dracunculus forms a polytomy with A. sublessingiana, the sect. Tridentatae clade, and the subg. Artemisia clade. The combined ITS and cpDNA phylogeny has a topology most closely resembling the cpDNA based phylogeny.

The inclusion of the five Anthemideae genera as a global outgroup, produced a strict consensus of 392 equally most parsimonious trees, 356 steps in length (CI=0.71, RI=0.54, RC=0.38; Fig. 2). Based on this analysis, the subg. Dracunculus clade (including A. dracunculus and A.

filifolia) and A. bigelovii, is basal within Artemisia (s.l.). Among the other 17 species of Artemisia, two subclades are supported, including sect. Tridentatae (s.s.) and the North American A. vulgaris species complex. A close relationship between A. vulgaris, A. ludoviciana, and A. palmeri is supported (bootstrap < 50%). In addition, six species from subg. Artemisia and A. sublessingiana (subg. Seriphidium) form a weakly supported clade (2 bp substitutions; bootstrap < 50%) on the semi-strict consensus tree. This clade collapses on the strict consensus tree and forms an unresolved polytomy with the Tridentatae clade.

DISCUSSION

Monophyly and circumscription of sect. *Tridentatae* --

The ITS sequence-based phylogeny supports the monophyly of sect. Tridentatae (s.s.), with the exclusion of two anomalous species, A. bigelovii and A. palmeri (Fig. 1). The phylogenetic relationship of these two species to sect. Tridentatae has been historically problematic, with both species possessing character states that have been variously interpreted as either plesiomorphic or secondary reversals (Hall and Clements, 1923; Ward, 1953; Beetle, 1960; McArthur and Plummer, 1978; McArthur et al., 1981; Shultz, 1983; Bremer and Humphries, 1993; Ling, 1991, 1995). An understanding of the placement of these anomalous species

may provide valuable information concerning the character state polarity of capitular morphology, a pivotal character to understanding and defining subgeneric and sectional limits within Artemisia (s.l.).

Artemisia palmeri, an herbaceous species endemic to southern California, has been previously included with the other North American Tridentatae (subg. Seriphdidium) on the basis of several synapomophies including: the presence of homogamous flower heads, with perfect fertile disk florets; a 4-7 series of involucral bracts; and narrowly linear to lanceolate anther appendages (Hall and Clements, 1923; Ward, 1953; Bremer and Humphries, 1993; Ling, 1991, 1995). However, the inclusion of A. palmeri in subg. Artemisia is supported by the presence of receptacular bracts, florets highly polymorphic in size and number, with approximately 12 to 25 disk florets per head, herbaceousness, lack of interxylary cork, and other anatomical characteristics associated with species found in mesophytic habitats (Moss, 1940; McArthur and Plummer, 1978; Beetle, 1960; Shultz, 1983). Artemisia palmeri is sister to A. ludoviciana and A. vulgaris in a monophyletic clade in the ITS tree (Fig. 1). Chloroplast DNA restriction site data (Kornkven, 1997), also strongly support a sister relationship between A. palmeri and A. ludoviciana, and support the inclusion of A. palmeri in subg. Artemisia.

Artemisia bigelovii has been previously excluded from sect. Tridentatae, based on the presence of at least some heterogamous flower heads, typically with one pistillate ray floret and two perfect disk florets (Hall and Clements, 1923; Ward, 1953; Shultz, 1983; Ling, 1991, 1995), while cpDNA restriction site data, chromosomal studies (karyotype analysis), and vegetative similarity to A. tridentata support its inclusion (Beetle, 1963; McArthur and Plummer, 1978; Kornkven, 1997). Bremer and Humphries (1993) concur and include A. bigelovii in the segregate genus Seriphidium (including all eleven Tridentatae species) on the basis of several involucral and anther characters and suggest that heterogamy in A. bigelovii is either plesiomorphic or a secondary reversal. In contrast, A. bigelovii is nested within subg. Dracunculus clade in the ITS tree, supporting a sister group relationship between A. bigelovii, A. dracunculus, and A. filifolia (Fig. 1). Although capitula in A. bigelovii are heterogamous, the disk florets are perfect and fertile, in contrast to the heterogamous capitula of subg. Dracunculus, which are characterized by sterile, functionally male disk florets. Therefore, molecular data and floral morphology provide conflicting evidence on the placement of A. bigelovii within Artemisia.

The rerooted cpDNA-based phylogeny differs from the ITS sequence-based phylogeny in the placement of both A.

bigelovii and A. filifolia (Fig. 3). On the cpDNA tree, A. bigelovii is sister to A. filifolia and A. longiloba and is clearly embedded within the Tridentatae clade, with a bootstrap value of 96%. However, in the ITS tree there is support for a clade composed of A. bigelovii, and A. filifolia, with A. dracunculus, resulting in conflict between the two topologies regarding the relationships of A. bigelovii and A. filifolia (Fig. 3).

Interspecific hybridization and transfer of a nrDNA repeat type from a species in subg. Dracunculus to A. bigelovii, followed by the concerted evolution and fixation of the repeat in the nuclear genome of A. bigelovii, would result in a gene tree that did not accurately represent organismal phylogenetic relationships. If this occurred without any corresponding morphological changes, it would explain the unexpected ITS placement of A. bigelovii. Concerted evolution, resulting from either unequal crossing-over and/or gene conversion, may result in the fixation of a gene within a population and ultimately within a species (Zimmer et al., 1980; Appels and Dvorak, 1982; Arnheim, 1983; Avise, 1989; Doyle, 1992; Wendel et al., 1995). The presence of seven autapomorphies in the nrDNA repeat of A. bigelovii suggests past introgression, perhaps occurring during the early diversification of Artemisia in North America. On the other hand, interspecific gene flow between

A. bigelovii and a species not sampled in our study is also a possibility, although the sister relationship of A. bigelovii and A. filifolia in both molecular trees supports gene flow between these two species.

In addition, introgression and chloroplast capture of the Tridentatae chloroplast genome by A. filifolia may explain the anomalous placement of A. filifolia within the Tridentatae clade in the cpDNA tree. In the past few years, numerous examples of chloroplast capture have been documented while examining discordance between nuclear and organellar phylogenies (Rieseberg and Soltis, 1991; Rieseberg and Brunsfeld, 1992; Soltis and Kuzoff, 1995; Bayer et al., 1996; Soltis et al., 1996; Campbell et al., 1995).

Therefore, interspecific gene flow between A. bigelovii and A. filifolia, involving exchange of both nuclear and chloroplast genomes can be invoked to explain the sister relationship and anomalous placement of both species in the cpDNA and ITS tree. These two taxa occur sympatrically throughout much of their distribution, although no hybridization has been reported between the extant species. Both hybridization and introgression are significant factors in many plant species (Anderson, 1949; Stebbins, 1950, 1969; Grant, 1981), and are common phenomena within Artemisia

(Hall and Clements, 1923; Keck, 1946; Ward, 1953; Beetle, 1960; McArthur et al., 1981).

In addition, two divergent species, A. rigida and A. pygmaea, are united with the Tridentatae in the ITS sequence-based phylogeny. Both species are uniquely adapted to restricted habitats in western North America and exhibit specialized modifications to extreme conditions of aridity in both anatomy and morphology (Hall and Clements, 1923; Carlquist, 1966; Shultz, 1983). Artemisia rigida is restricted primarily to rocky outcrops along the Snake River plains in western Oregon and Washington. Artemisia pygmaea, a dwarf sub-shrub, is found in isolated populations on calcareous soils in the cold deserts of Nevada, Utah, and northern Arizona. Rydberg (1916) placed the species into two separate sections, sect. Rigidae Rydb. and sect. Pygmaea Rydb. in subg. Seriphidium, based on their specialized morphologies. However, the ITS data are in agreement with morphology in supporting a basal position of A. rigida and A. pygmaea within sect. Tridentatae (Fig. 1).

In the cpDNA-based phylogeny A. californica is sister to A. rigida and is basal within the Tridentatae clade (Fig. 3). Artemisia californica, a low shrub endemic to the cismontane region of California, is included in subg. Artemisia based on the presence of heterogamous flower heads with pistillate ray florets and perfect, fertile disk

florets (Hall and Clements, 1923). Several possible explanations have been proposed for the placement of A. californica in the cpDNA tree including a classification that does not accurately reflect relationships, lineage sorting, and introgression with subsequent chloroplast capture (Kornkven, 1997). In contrast to the cpDNA tree, the relationship of A. californica to sect. Tridentatae is unresolved on the ITS tree, with A. californica, the remaining six species of subg. Artemisia (including A. palmeri), sect. Tridentatae, and A. sublessingiana forming a polytomy. Artemisia californica and A. rigida do not share a sister relationship in the ITS tree; sequence divergence between the two species is 3.4%, including nine and five autapomorphies, respectively.

In summary, based on the ITS phylogeny, sect. Tridentatae is monophyletic, with exclusion of A. palmeri and A. bigelovii. The inclusion of A. rigida and A. pygmaea in sect. Tridentatae is overwhelmingly supported by both molecular and non-molecular data (Hall and Clements, 1923; Ward, 1950; Beetle, 1960; McArthur and Plummer, 1978; McArthur et. al., 1981; Shultz, 1983; Bremer and Humphries, 1993; Ling, 1991, 1995; Kornkven, 1997), and therefore both species should remain in sect. Tridentatae. Interspecific gene flow is one possible explanation for the conflicting placement of A. bigelovii and A. filifolia on the ITS and

cpDNA phylogenies. The placement of A. californica is unresolved based on the molecular data.

Phylogenetic relationships within sect. *Tridentatae* -- While the ITS sequence data support the monophyly of sect. Tridentatae, the results do not support either of the two previously proposed lineages within the Tridentatae (Ward, 1953; Beetle, 1960; Shultz, 1983; Table 1; Fig. 1). Although, a close relationship between A. cana and A. tripartita has been previously proposed based on non-molecular data (A. cana lineage; Table 1), the two species share only a single base pair substitution for ITS. Furthermore, no cpDNA restriction site mutations unite these two species, and no further evidence supports either of the two proposed lineages within sect. Tridentatae (Kornkven, 1997). However, both molecular and non-molecular data support A. rigida and A. pygmaea as basal within the section.

The difficulty in clearly defining species boundaries in sect. Tridentatae, has resulted in both A. nova and A. longiloba being variously included as subspecies of either A. arbuscula (i.e., A. arbuscula subsp. nova (Nels.) Ward and A. arbuscula subsp. longiloba (Osterh.) Shultz) or A. tridentata (i.e., A. tridentata subsp. nova (Nels.) Hall and Clements)(Hall and Clements, 1923; Ward, 1953; Shultz, 1983; Ling, 1991, 1995; Table 1). However, the molecular data

support the distinctness of these two taxa, in that they possess eleven and four autapomorphies, respectively, accounting for 37 and 13% of all autapormophies in the Tridentatae. Therefore, the ITS data support the recognition of these two taxa as two distinct species, and not as infraspecific taxa.

In conclusion, neither ITS sequence nor cpDNA restriction site data have sufficient levels of variation to resolve interspecific relationships within sect. Tridentatae, indicating that additional sampling within the section would not provide further resolution of interspecific relationships. The level of phylogenetic resolution observed in the cpDNA and ITS phylogenies is similar, although some relationships in the cpDNA tree are more strongly supported with bootstrap values ranging from 55 to 98%, compared to bootstrap values of less than 50% on the ITS tree, indicating that the cpDNA restriction site data provide greater resolution at the interspecific level than the ITS sequence data, probably due at least partially to the higher number of nucleotides examined for the cpDNA study.

A relatively recent and rapid radiation of sect. Tridentatae throughout the Intermountain region of western North America, is one possible explanation for the low sequence divergence seen in both the nuclear and organellar

genomes. Pollen records indicate that Artemisia is a relative newcomer to this region, with Artemisia pollen first evident in the Upper Miocene, but only becoming widespread and significant during the Pleistocene (Gray, 1964; Mehringer, 1965; Tidwell et al., 1972). Therefore, sect. Tridentatae (s.s.) appears to have diverged relatively recently in response to changing climatic conditions in the west, which has resulted in a group of closely related shrub species that differ only slightly in morphology and habitat preference, but are well-defined morphologically from the other Artemisia species. Furthermore, the Tridentatae are monophyletic on the basis of molecular data.

Origin and relationship of sect. *Tridentatae* within *Artemisia* (s.l.) -- Two contrasting hypotheses for the origin of sect. Tridentatae have been proposed. Based on floral morphology, Bremer and Humphries (1993) and Ling (1991, 1995) support the inclusion of sect. Tridentatae within the segregate genus Seriphidium and propose that progenitors of the North American Tridentatae (Asian Seriphidium species) migrated over the Bering Strait and subsequently underwent rapid speciation in response to available habitats and climatic conditions throughout the west. In contrast, McArthur and Plummer (1978) propose that sect. Tridentatae evolved from North American progenitors in subg. Artemisia and developed in situ in western North

America during the Pleistocene in response to a rapidly changing environment. Both the ITS sequence and cpDNA restriction site data provide only equivocal evidence for either of these two hypotheses regarding the origin and relationship of the North American sect. Tridentatae within Artemisia (s.l.). Despite numerous systematic investigations utilizing molecular and non-molecular data, the origin and relationship of sect. Tridentatae within Artemisia remains unresolved (Rydberg, 1916; Hall and Clements, 1923; Ward, 1953; Carlquist, 1966; McArthur and Plummer, 1978; Shultz, 1983; Bremer and Humphries, 1993; Ling, 1991, 1995; Kornkven, 1997).

Although the monophyly of Artemisia (s.l.) is supported by the ITS sequence data (bootstrap < 50%; decay = 1), further studies using additional representatives of subtribe Artemisiinae are clearly needed to fully assess the monophyly of Artemisia (s.l.), and also to examine subgeneric relationships within this large and important genus. Still, several preliminary comments can be made concerning relationships within Artemisia (s.l.). Hall and Clements (1923), in the first phylogenetic treatment of the genus, consider subg. Artemisia (as sect. Abrotanum) as ancestral and the reduction of the capitulum in both subg. Dracunculus and subg. Seriphidium as advanced. In a more recent evolutionary treatment of Artemisia, Ling (1991,

1995) concurs and proposes a complicated scenario concerning the evolution of the group, treating members of both subg. Seriphidium and subg. Dracunculus as advanced. In particular, Ling suggests that members of sect. Abrotanum were the progenitors of subg. Seriphidium and that there was a reduction in both ray florets and number of disk florets within subg. Seriphidium. In sharp contrast, subg. Dracunculus is basal in the ITS sequence-based phylogeny, raising questions concerning the polarity and evolution of capitular morphology within Artemisia (s.l.)(Figs. 2 and 3). The presence of sterile disk florets in subg. Dracunculus can therefore be interpreted as either plesiomorphic within the genus or as an independent loss of perfect, fertile disk florets to sterile florets. In Bremer and Humphries (1993) cladistic analysis, the presence of disciform, heterogamous capitula with pistillate ray florets and perfect, fertile disk florets is plesiomorphic within Artemisiinae, supporting multiple origins of heterogamous capitula with sterile disk florets in the subtribe.

The segregation of subg. Seriphidium from Artemisia (s.l.) is not supported by either cpDNA restriction site-based or ITS sequence-based phylogenies (Figs. 1 and 2). This is in contrast to Bremer and Humphries (1993) morphologically based cladistic analysis of the tribe Anthemideae, which narrowly defines Artemisia and segregates

numerous genera, including Seriphidium, closely following two earlier treatments of the subtribe (Poljakov, 1960; Ling, 1991, 1995).

Character evolution -- The ITS and cpDNA phylogenies provide an independent framework in which to examine the evolution of capitular morphology within Artemisia (s.l.). Capitular morphology encompasses at least four different, variable characters including: (1) the presence or absence of ray florets in the capitula; (2) disk florets that are either perfect and fertile or sterile and functionally staminate, with reduced ovaries; (3) a glabrous or hairy receptacle; and (4) a reduction in the total number of florets per head. Historically, sectional classification in Artemisia (s.l.) has been based on the presence of four types of capitula, with more recent studies either combining these into three subgenera or narrowly defining Artemisia (s.l.) and segregating out several genera (Rydberg, 1916; Hall and Clements, 1923; Poljakov, 1961; Bremer and Humphries, 1993; Ling, 1991, 1995). The complete reduction of marginal ray florets (discoid, homogamous flower heads) and an overall reduction in the number of disk florets (i.e., two to fifteen florets per head) is derived within Artemisia (s.l.) (Hall and Clements, 1923; Ling, 1991, 1995), a conclusion further supported by the molecular data (Fig. 3). Bremer and Humphries (1993) support a single

origin of homogamy in the segregate genus Seriphidium, and include all eleven Tridentatae species, with A. palmeri, and consider heterogamy in A. bigelovii as either plesiomorphic or a secondary reversal. In contrast, molecular data support at least two independent origins of homogamy, with the placement of A. palmeri in subg. Artemisia. The relationship of the North American sect. Tridentatae to the Eurasian subg. Seriphidium remains unresolved, with sect. Tridentatae (s.s.), A. sublessingiana (subg. Seriphidium), and representatives from subg. Artemisia forming an polytomy in both molecular phylogenies. Further studies are clearly needed to examine not only subgeneric relationships within Artemisia (s.l.), but also to examine generic relationships within subtribe Artemisiinae.

LITERATURE CITED

ANDERSON, E. 1949. Introgressive Hybridization. New York: John Wiley.

APPELS, R. and J. DVORAK. 1982. Relative rates of divergence of spacer and gene sequences within the rDNA region of species in the Triticeae: Implications for the maintenance of homogeneity of a repeated gene family. Theoretical and Applied Genetics 63: 361-365.

ARNHEIM, N. 1983. Concerted evolution of multigene families. In M. Nei and R. K. Koehn [eds.], Evolution of genes and proteins, 38-61. Sinauer, Sunderland, MA.

AVISE, J. C. 1989. Gene trees and organismal histories: A phylogenetic approach to population biology. Evolution 43: 1192-1208.

BAIN, J. and R. K. JANSEN. 1995. A phylogenetic analysis of the aureoid Senecio (Asteraceae) complex based on ITS sequence data. Plant Systematics and Evolution 195: 209-219.

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Molecular Phylogenetics and Evolution 1: 3-16.

—— . 1993. Molecular phylogenetics of Calycadenia (Compositae) based on ITS sequences of nuclear ribosomal DNA: Chromosomal and morphological evolution reexamined. American Journal of Botany 80: 222-238.

—— , M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, and M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden 82: 247-277.

BAUM, D. A., K. J. SYTSMA, and P. C. HOCH. 1994. A phylogenetic analysis of Epilobium (Onagraceae) based on nuclear ribosomal DNA sequences. Systematic Botany 19: 363-388.

BAYER, R. J., L. HUFFORD, and D. E. SOLTIS. 1996. Phylogenetic relationships in Sarraceniaceae based on rbcL and ITS sequences. Systematic Botany 21: 121-134.

BEETLE, A. A. 1960. A study of sagebrush, the section Tridentatae of Artemisia. Bulletin 368. University of Wyoming Agricultural Experimental Station 368: 1-83.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42: 795-803.

—— . 1994. Tribe Anthemideae. In Asteraceae, Chapter 19, 435-478. Timber Press, Portland, OR.

—— and C. HUMPHRIES. 1993. Generic monograph of the Asteraceae-Anthemideae. Bulletin of the Natural History Museum. Volume 23.

CAMPBELL, C. S., M. J. DONOGHUE, B. G. BALDWIN, and M. F. WOJCIECHOWSKI. 1995. Phylogenetic relationships in Maloideae (Rosaceae): Evidence from sequences of the internal transcribed spacers of nuclear ribosomal DNA and its congruence with morphology. American Journal of Botany 82(7): 903-918.

CARLQUIST, S. 1966. Wood Anatomy of Anthemideae, Ambrosieae, Calendulae, and Arctotideae (Compositae). Aliso 6: 1-23.

CONTI, E., A. FISCHBACH, and K. J. SYTSMA. 1993. Tribal relationships in Onagraceae: Implications from rbcL sequence data. Annals of the Missouri Botanical Garden 80: 672-685.

CRONQUIST, A. 1972. Volume five, Asterales, Artemisia. In A. Cronquist, A. H. Holmgren, N. H. Holmgren, J. L. Reveal, and P. K. Holmgren [eds.], Intermountain Flora, 146-165. The New York Botanical Garden, New York.

DOYLE, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. Systematic Botany 17: 144-163.

—— and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.

GEISSMAN, T. A. and M. A. IRWIN. 1974. Chemical constitution and botanical affinity in Artemisia. In G. Bendz and J. Santesson [eds.], Chemistry in botanical classification, 135-143. Proceedings of the 25th Nobel Symposium. Academic Press, New York.

GRANT, V. 1981. Plant Speciation. New York: Columbia University Press.

GRAY, J. 1964. Northwest American Tertiary palynology: the emerging picture. Pacific Science Congress Series 19: 21-30.

GYLLENSTEN, K. 1989. Direct sequencing of in vitro amplified DNA. In H. A. Erlich [ed.], PCR Technology: Principles and Applications for DNA Amplification, 45-60. Stockton Press, New York.

ERIKSSON, T. and N. WILKSTROM. 1995. Autodecay 3.0. Computer program distributed by T. Eriksson.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

FRANSCISCO-ORTEGA, J., A. SANTOS-GUERRA, A. HINES, and R.K. JANSEN. In Press. Molecular evidence for a Mediterranean origin of the Macaronesian endemic genus Argyranthememum (Asteraceae). American Journal of Botany.

HALL, H. M. and F. E. CLEMENTS. 1923. The phylogenetic method in taxonomy. The North American species of Artemisia, Chrysothamnus, and Atriplex. Carnegie Institution Washington Publication 326: 1-355.

HANKS, D. L., E. D. MCARTHUR, R. STEVENS, and A. P. PLUMMER. 1973. Chromatographic characteristics and phylogenetic relationships of Artemisia, section Tridentatae. U.S.D.A. Forest Service Research Paper INT-141, 24 pp.

HEYWOOD, V. H. and C. J. HUMPHRIES. 1977. Anthemideae - Systematic review. In V. H. Heywood, J. B. Harborne, and B. L. Turner [eds.], The Biology and Chemistry of the Compositae, 851-898. Academic Press, New York.

IRWIN, M. A. 1971. Sesquiterpene lactones of Artemisia. Ph.D. Dissertation, University of California, Los Angeles.

KECK, D. D. 1946. A revision of the Artemisia vulgaris complex in North America. Proceedings of the California Academy of Sciences 17: 421-468.

KELSEY, R. G. 1974. The systematic usefulness of the sesquiterpene lactones in the genus Artemisia, section Tridentatae (sagebrush) of Montana. Ph.D. Dissertation, University of Montana.

KIM, K-J. and R. K. JANSEN. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (Krigia): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. Plant Systematics and Evolution 190: 157-185.

KORNKVEN, A. B. 1997. Molecular systematics of Artemisia sect. Tridentatae (Asteraceae). Ph.D. Dissertation, University of Oklahoma, Norman.

LING, Y. R. 1991. A review of the classification, distribution and applications of Artemisia L. and Seriphidium (Bess.) Poljak. (Compositae) in China. Guihaia 11: 19-35.

—— . 1995a. 13. The new world Artemisia L. In D. J. N. Hind, C. Jeffrey, and G. V. Pope [eds.], Advances in Compositae Systematics, 255-281. Royal Botanic Gardens, Kew.

—— . 1995b. 14. The new world Seriphidium (Besser) Fourr. In D. J. N. Hind, C. Jeffrey, and G. V. Pope [eds.], Advances in Compositae Systematics, 283-291. Royal Botanic Gardens, Kew.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most-parsimonius trees. Systematic Zoology 40: 315-328.

—— , M. J. DONOGHUE, and D. R. MADDISON. 1984. Outgroup analysis and parsimony. Systematic Zoology 33: 83-103.

McARTHUR, E. D. and A. P. PLUMMER. 1978. Biogeography and management of native Western shrubs: a case study, section Tridentatae of Artemisia. Great Basin Naturalist Memoirs 2: 229-243.

McARTHUR, E. D., C. L. POPE, and D. C. FREEMAN. 1981. Chromosomal studies of subgenus Tridentatae of Artemisia: Evidence for autopolyploidy. American Journal of Botany 68: 589-605.

MEHRINGER, P. J. 1965. Late Pleistocene vegetation in the Mojave Desert of southern Nevada. Journal of Arizona Academy of Science 3: 172-187.

MOSS, E. H. 1940. Interxylary cork in Artemisia with reference to its taxonomic significance. American Journal of Botany 27: 762-768.

PALMER, J. D. 1986a. Chloroplast DNA and phylogenetic relationships. In S. K. Dutta [ed.], DNA Systematics. Volume II. Plants. CRC Press, Boca Raton, Florida.

—— . 1986b. Isolation and structural analysis of chloroplast DNA. Methods in Enzymology 118: 167-186.

POLJAKOV, P. P. 1961. Artemisia. In V. L. Komarov [ed.], Flora USSR, vol. 26, 425-631.

RIESEBERG, L. H. and S. J. BRUNSFELD. 1992. Molecular evidence and plant introgression. In P. S. Soltis, D. E. Soltis, and J. J. Doyle [eds.], Molecular Systematics of Plants, 151-176. Chapman and Hall, New York.

—— and D.E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evolutionary Trends in Plants 5: 65-84.

RODMAN, J., R. PRICE, K. KAROL, E. CONTI, K. SYTSMA, and J. PALMER. 1993. Nucleotide sequences of the rbcL gene indicate monophyly of mustard oil plants. Annals of the Missouri Botanical Garden 80: 686-699.

RYDBERG, P. A. 1916. (Carduales), Carduaceae, Tageteae, Anthemideae. North American Flora 34: 244-285. New York.

SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press.

SANG, T., D. J. CRAWFORD, S. C. KIM, and T. F. STUESSY. 1994. Radiation of the endemic genus Dendroseris (Asteraceae) on the Juan Fernandez Islands: Evidence from sequences of the ITS regions of nuclear ribosomal DNA. American Journal of Botany 81: 1494-1501.

—— , D. J. CRAWFORD, T. F. STUESSY, and M. SILVA O. 1995. ITS sequences and the phylogeny of the genus Robinsonia (Asteraceae). Systematic Botany 20: 55-64.

SANGER, F., S. NICKLEN, and A. R. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences, U.S.A. 74: 5463-5467.

SHULTZ, L. M. 1983. Systematics and anatomical studies of Artemisia subgenus Tridentatae. Ph.D. Dissertation. Claremont Graduate School.

SOLTIS, D. E. and R. K. KUZOFF. 1995. Discordance between nuclear and chloroplast phylogenies in the Heuchera group (Saxifragaceae). Evolution 49: 727-742.

—— , L. A. JOHNSON, and C. LOONEY. 1996. Discordance between ITS and chloroplast topologies in the Boykinia group (Saxifragaceae). Systematic Botany 21: 169-185.

STEBBINS, G. L. 1950. Variation and Evolution in Plants. New York: Columbia University Press.

—— . 1969. The significance of hybridization for plant taxonomy and evolution. Taxon 18: 26-35.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1.1. Champaign: Illinois Natural History Survey.

TIDWELL, W. D., S. R. RUSHFORTH, and D. SIMPER. 1972. Evolution of floras in the Intermountain Region. In A. Cronquist, A. H. Holmgren, N. H. Holmgren, and J. L. Reveal [eds.], Intermountain Flora: Vascular Plants of the Intermountain West, Vol. 1, 19-39. Hafner Publ. Co., New York.

WARD, G. H. 1953. Artemisia section Seriphidium in North America: a cytotaxonomic study. Contributions from the Dudley Herbarium 4: 155-205.

WATROUS, L. E. and Q. D. WHEELER. 1981. The out-group comparison method of character analysis. Systematic Zoology 30: 1-11.

WATSON, L. E. 1996. Molecular systematics of the tribe Anthemideae. In D. J. N. Hind and H. J. Beentje [eds.], Compositae: Systematics. Proceedings of the International Compositae Conference, Kew 1994, 341-348. Royal Botanic Gardens, Kew.

WENDEL, J. F., A. SCHNABEL, and T. SEELANAN. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (Gossypium). Proceedings of the National Academy of Science, U.S.A. 92: 280-284.

WHITE, T.J., T. BRUNS, S. LEE, and J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 315-322. Academic Press, San Diego.

ZIMMER, E. A., S. L. MARTIN, S. M. BEVERLEY, Y. W. KAN, and A. C. WILSON. 1980. Rapid duplication and loss of genes coding for the alpha chains of hemoglobin. Proceedings of the National Academy of Science, U. S. A. 77: 2158-2162.

APPENDIX 1. Aligned ITS sequences from 20 Artemisia species. Artemisia tridentata is the reference taxon and matching base pairs are indicated by dots (.). Coding of ambiguous positions follow IUPAC nomenclature; hyphens=gaps, M=A/C, R=A/G, S=C/G, W=A/T, Y=C/T, K=G/T, ?=nucleotides of unknown identity, and blanks represent sequence not determined. Species abbreviations are as follows: A. tridentata (TRID), A. cana (CANA), A. nova (NOVA), A. tripartita (TRIP), A. arbuscula (ARBU), A. longiloba (LONG), A. rigida (RIGD), A. rothrockii (ROTH), A. pygmaea (PYGM), A. bigelovii (BIGL), A. palmeri (PALM), A. ludoviciana (LUDV), A. californica (CALF), A. vulgaris (VULG), A. abrotanum (ABRO), A. rupestris (RUPE), A. dracunculus (DRAC), A. filifolia (FILI), A. sublessingiana (SUBL), and A. canariensis (CANR).

ITS 1 (50)

```
TRID  TCGAACCCTG CAAAGCAGAA CGACCCGTGA ACACGTAAAA ACAACCGAGT
CANA  .......... .......... .......... .......... ..........
NOVA  .......... .......... .......... .......... ..........
TRIP                                  . .......... ..........
ARBU                                       .... ..........
LONG  .?........ .......... .......... ...?...... ..........
RIGD                             .?.. .......... ..........
ROTH                                  . .......... ..........
PYGM                        .......... .......... ..........
BIGL  .......... .......... .......A.. ...T...... ..........
PALM  .......... .......... .......... ..G....... C....T...C
LUDV  .......... .......A.. .......... .......... ..........
CALF  ???....... .....????? ?????..... .......... .....T....
VULG                                  . .......... .....T....
ABRO  .......... .......A.. .......... .......... ..........
RUPE  .??.....C. ......?... .......C.. .......... .........C
DRAC                                       .... ..........
FILI  ???....... .......C.. .......... .......... ..........
SUBL  .......... .......... .......... .......... .........C
CANR  .......... .......... .....Y.Y.. .......... ....Y-...C
```

(100)

```
TRID  GTCGATTGGA TCAAGCGCTT GTTTGATCCT CTCGACGCTT TGTCGATGCG
CANA  .......... .......... .......... .......... ..........
NOVA  .......... .......... .......... .......... ..........
TRIP  .......... .......... .......... .......... ..........
ARBU  .......... .......... .......... .......... ..........
LONG  ..?....... .......... .......... .......... ..........
RIGD  .......... .......... .......... .......... ..........
ROTH  .......... .......... .......... .......... ..........
PYGM  .......... .......... .......... .......... ..........
BIGL  ..T....... .......... .......... .......... .........A
PALM  ......C... .......... .......... .......... ..C.......
LUDV  .......... .......... .......... .......... ..........
CALF  ........A. .......... .......... .......... ..C.....A.
VULG  ....???... .......... .....G.... .......... ......C...
ABRO  ......Y... .......... .......... .......... ..........
RUPE  .......... ..C....... .......... .......... ..........
DRAC  ..?.T..... .......... .......... .......... ........T.
FILI  .......... .......... .......... .......... ......C...
SUBL  ....G..... .......... .......G.A A....T...C ..........
CANR  .......... .T.G...... .......... .......... ......C...
```

```
                                                                    (150)
TRID  CGTTCACTCG AGTTCTTTTG GAC--CGTGT GAATGTGTYG TYGGCGCATT
CANA  .......... .......... ...--..... .........C. CC.A...T..
NOVA  .......... .......... ...--..... .......... .C........
TRIP  .......... .......... ...--..... .........C. .C........
ARBU  .......... .......... ...--..... .........CR ..........
LONG  ....G..... .......... ...--..... .........C. .T........
RIGD  .A........ .......... ...--.T... ..G.....CA .T........
ROTH  .......... .......... ...--..... .........CR YT........
PYGM  .A...R.... .......... ...--.T..Y .........C. .T........
BIGL  .A........ .......... ...--.T... .........CA .C....M...
PALM  .A...G.... ....S...C. ...--.T..C ..G.....CR .T........
LUDV  .........C .......... ...--..... .........C. YC........
CALF  .A........ .......... ...--..... .........C. .T........
VULG  .......... .......... ...--.T... .....C..C. .T........
ABRO  .A.......S .........C. ...TT.AA.. .........C. .C........
RUPE  .A........ ......C... ...--.T... ..G..C..C. .T........
DRAC  ....TGT... .....C.C.. ...--.TY.. .........CA .C......C.
FILI  .A...G.... .........C. ...--.T..C .........C. .C........
SUBL  .A........ --..YG.Y.. ...C-.T... ..T.....C. .T........
CANR  .A........ --........ ...--.T... ..??????C. .T........
```

```
                                                                    (200)
TRID  AACAACCCCC GGCACAATGT GTGCCAAGGA AAACTAAACT CTAGAAGGCT
CANA  .......... .......... .......... .......... ..........
NOVA  .......... .......... .......... .......... Y.........
TRIP  .......... .......... .......... .......... ..........
ARBU  .......... .......... .......... .......... ..........
LONG  .......... .......... .......... .......... TA........
RIGD  .......... .......... .......... ....A..... ..........
ROTH  .......... .......... .......... .......... ..........
PYGM  .......... .......... .......... .......... ..........
BIGL  .......... .....:.... ....?..... .......... .AR.......
PALM  .......... .........C .......... .......... T.........
LUDV  .......... .......... .......... .......... ..........
CALF  .......... .......... .......... .......... ..........
VULG  .......... .......... .....?.... .......... ..........
ABRO  .......... ......T... .......... .......... ..........
RUPE  .......... .......... .......... ....C..... .G........
DRAC  .......... .......... .......... .......... .A........
FILI  .......... .......... .......... .......... ..........
SUBL  .......... .......... .......... .......... ..........
CANR  .......... .......... .......... .......... ..........
```

```
                                                                    (250)
TRID  CGTTTTCATG TTGCCCCCGT TCGCGGTGTG CTCATGGGAT GTGGCTTCTT
CANA  .......... .......... .......... .......... ..........
NOVA  Y......... .......... .......... .......... .......Y..
TRIP  .......... .......... .......... .......... ..........
ARBU  .......... .......... .......... .......... ..........
LONG  .......G.. .......... .......... .......... .......?..
RIGD  .......... .......... .......... .......... ..........
ROTH  .......... .......... .......... .......... ..........
PYGM  .......... .......... .......... .......... ..........
BIGL  T......... .......... C?.....K.. .C........ ......C...
PALM  ....C..... .......... .......... .........C .C........
LUDV  .......... .......... .......... .......... ..........
CALF  ..G....... .......... .......... .......... ..........
VULG  .......... .......... .....?...? .........C ..........
ABRO  ........-.. .......... .......... .......... ..........
RUPE  .....-.G.. .......... .......... .......... .C........
DRAC  .....-.G.. .A........ .....?...C .........C .C........
FILI  .......... .......... .......... .......... ..........
SUBL  Y..Y...... .......... .......... .......... ..........
CANR  M....-.... ....A..... .......... .......... ..........
```

```
      (255)       ITS 2                                         (40)
TRID  TATAA       ATCGCGTCGC CCCCCACAAC TCTCCGTAAA GGGAGCTTGT
CANA  .....       .....?.... ...??...G. .......... ..........
NOVA  .....       ..T....... ...?...... .T........ ....A.....
TRIP  .....       .....?.... ...??..... .......... ..........
ARBU  .....       .....?.... ...??..... ...Y...... ....R.....
LONG  .....       .....?.... ...??..... .......... ..........
RIGD  .....       .....?.... ...??..... .......T.. ....A.....
ROTH  .....       .......... .......... .......... ..........
PYGM  .....       ...?.?.... ...??G.... .......... ..........
BIGL  .....       .....?..R. .Y.??..... ....T..... ....A.....
PALM  .....       .......... .........T ...T..C... .A..A.....
LUDV  .....       .......... .........T ......C... .A..A.....
CALF  .....       .......... .........T .......... ....A.....
VULG  .....       .......... .........T ......C... ....A.C...
ABRO  .....       ....T..... ...C.....T .Y...TC... ....A.....
RUPE  .....       ..T.G..T.. ...?....GT ......C... ....A.....
DRAC  .....       .....?.... ...??..... .......... ....A.....
FILI  .....       .....?.AYG G....G.C.A .......... ....A.....
SUBL  .....       .......... .......... .Y.......G ....A..C..
CANR  .....       .......... .........GT ......C... ....A..GT.
```

```
                                                                    (90)
TRID  GTTTTGGGGG CGGATATTGG TCTCCCGTGC TCAT-GGCGT GGTTGGCCGA
CANA  .......... .......... .......... ....-..... ..........
NOVA  .......... .......... .T........ ....-..... ..........
TRIP  .......... .......... .......... ....-..... ..........
ARBU  .......... .......... .......... ....-..... ..........
LONG  .......... .......... .......... ....-...K. ..........
RIGD  .......... .......... .......... ....-..... ..........
ROTH  .......... .......... .......... ....-..... ..........
PYGM  .......... .......... .......... ....-..... ..........
BIGL  .......... .......... .......... ....-..... .......Y..
PALM  .C........ .......... .......... G...-..... ..........
LUDV  .C........ .......... .......C.. ....-..... ..........
CALF  .......... .......... .......... ....-..... ..........
VULG  .......... .......... .......... ....-..... ..........
ABRO  ....C..... .......... .......... ....-..... ..........
RUPE  .......... ......C... .......... ....-..... ..........
DRAC  .......... .......... .......... ....-..... ..........
FILI  .......... .......... .......... ....-..... ..........
SUBL  .......... .......... .......... ....-..... ..........
CANR  .......... .......... .......... ....T..... ..........


                                                                    (140)
TRID  AATAGGAGTC CCTTCGATGG ACGCACGAAC TAGTGGTGGT CGTAAAAACC
CANA  .......... .T........ .......... .......... ..........
NOVA  .......... .......... .......... .......... ..........
TRIP  ..Y....... .Y........ .........A .......... T.........
ARBU  .......... .......... .......... .......... ..........
LONG  .......... .......... .......... .......... ..........
RIGD  ..C....... .......... .......... .......... ..........
ROTH  .......... .......... .......... .......... ..A.......
PYGM  .......... .......... .......... .......... ..........
BIGL  .......... .Y........ .......... .......... ..........
PALM  .......... .......... .......... .......... ..........
LUDV  .......... .......... .......... .......... ..........
CALF  ..C....... ......G... .......... .......... ..........
VULG  .......... .T.....C.. .......... .......... ..........
ABRO  .......... .......... .......... .......... ..........
RUPE  .......... .......... .......R.. .......... ..........
DRAC  .......... .......... .......... .......... ..........
FILI  .......... .......... .......... .......... ..........
SUBL  ......R... .....S.... .......... ..R....... ..........
CANR  .......... .......... .......... .......... ..........
```

```
                                                                    (190)
TRID  CTCGTCTTTT GCTTCGTGCC GTTAGTCGCA AGGGAAACTC TTAGAAAACC
CANA  .......... .Y.GT..... .......... .......... ..........
NOVA  ..T..A.... .......... .......... .......A.T ..........
TRIP  .......... .......... .......... .......... ..........
ARBU  .......... .......... .......... .......... ..........
LONG  .....M.... ?......... .......... .......... ..........
RIGD  .......... .......... .......... .......... ..........
ROTH  ..Y....... .......... .......... ....G..... .W........
PYGM  .......... .......... .......... .......... ..........
BIGL  .......... .........T .......... .......... ..........
PALM  .......... .T.......T ..C....... .......... ..........
LUDV  .......... .T.......T ..C....... .......... ..........
CALF  .......... .......... .....?..T. .......... ..........
VULG  .......... .T........ ..C....... .A........ ..........
ABRO  .......... .T........ .......... .......... ..........
RUPE  .......... .T........ .......... .......... ..........
DRAC  .......... .T.......T .........G .......... ...?......
FILI  .......... .........T .......... .......... ..........
SUBL  ...S...... .T...S.... .......S.. .......... .A........
CANR  .......... .T.GT..... .......... .......... ...?......
```

```
                                                        (223)
TRID  CCAACGTGTC GTCTCTCGAC GACGCTTCGA CCG
CANA  ..?????... .......... ..???..... ...
NOVA  .........T .......... .......... ...
TRIP  ..???..... ..?....... .......... ...
ARBU  ..???..... ..?....... .......... ...
LONG  .......... ..??...... .......... ...
RIGD  ..???..... ..??...... .......... ...
ROTH  .......... .......... .......... ...
PYGM  ...??..... .......... .......... ...
BIGL  ....?..... ......T... .......... ...
PALM  .......... ....M.W..T .......... ...
LUDV  .......... ......T..T .......... ...
CALF  ....T..... ......T..T .......... ...
VULG  .........T ......T..T .......... ...
ABRO  ...G...... ....T.T... .G........ ...
RUPE  .......... ....T.T... .G........ ...
DRAC  .A..T..... ......T... ..G....... ...
FILI  .......TG. ......T... ..A....... ...
SUBL  .......K.. ....T.T..M .GS....... ...
CANR  .......... ......T... ..G....... ...
```

TABLE 1. Comparison of three conflicting hypotheses of interspecific relationships within *Artemisia* sect. *Tridentatae*. Two lineages have been proposed: (1) *A. tridentata* lineage and (2) *A. cana* lineage.

	Ward (1953)	**Beetle (1960)**	**Shultz (1983)**
***A. tridentata* lineage**	*A. tridentata*	*A. tridentata*	*A. tridentata*
-seldom root sprouts or layers	*A. arbuscula*	*A. longiloba*	*A. nova*
-mostly tridentate leaves	*A. arbuscula* subsp. *nova*	*A. nova*	
-xerophytic	*A. arbuscula* subsp. *longiloba*	*A. bigelovii*	
	A. rigida	*A. pygmaeae*	
***A. cana* lineage**	*A. cana*	*A. cana*	*A. cana*
-root sprouts and layers	*A. tripartita*	*A. tripartita*	*A. tripartita*
-leaves entire or deeply divided		*A. rigida*	
-mesophytic			
questionable placement	*A. pygmaea*		*A. pygmaea*
	A. palmeri		*A. rigida*
reticulate taxa	*A. rothrockii*	*A. arbuscula*	*A. arbuscula*
		A. rothrockii	*A. rothrockii*
excluded taxa	*A. bigelovii*	*A. palmeri*	*A. bigelovii*

TABLE 2. Collection and locality data for 19 Artemisia and outgroup species included in the ITS sequence study. Collection and voucher information includes: AK=Amy Kornkven, vouchers located at the University of Oklahoma (OKL); LS=Leila Shultz, vouchers at Utah State University (UTC); and LW=Linda Watson, vouchers at Uppsala Herbarium (UPS; Botanic Garden Material) or KEW unvouchered DNA's (Accession numbers). Sequences for the six Anthemideae outgroups were obtained from Francisco-Ortega et al. (in press), with the GenBank accession numbers listed.

SPECIES	**VOUCHER/ACC.**	**LOCALITY (State: County)**
sect. Tridentatae		
A. tridentata	AK305	UT: San Juan Co.
A. cana	AK226	WY: Carbon Co.
A. nova	AK460	ID: Cassia Co.
A. bigelovii	AK300	UT: Emery Co.
A. rigida	AK384	WA: Yakima Co.
A. arbuscula	AK419	ID: Ada Co.
A. longiloba	AK426	ID: Camas Co.
A. tripartita	AK444	ID: Clark Co.
A. pygmaea	AK543	NE: White Pine Co.
A. rothrockii	AK505	CA: Inyo Co.
A. palmeri	AK491	CA: San Diego Co.

TABLE 2. Continued

subg. Artemisia		
A. abrotanum	LS11880	OK: Cultivated
A. californica	AK496	CA: San Diego Co.
A. ludoviciana	AK240	WY: Johnson Co.
A. rupestris	LW,s.n.	Uppsala Botanic Garden
A. vulgaris	LS11882	OK: Cultivated
subg. Dracunculus		
A. dracunculus	000-69.18218	Kew Botanic Garden
A. filifolia	LS11873	UT: Grand Co.
subg. Seriphidium		
A. sublessingiana	LW,s.n.	Uppsala Botanic Garden

Anthemideae outgroups	**(GenBank Accession Number)**
Artemisia canariensis	L77740
Anthemis arvensis	L77773
Dendranthema coreanum	L77802
Nippoanthemum nipponicum	L77772
Cymbopappus adenosolen	L77759
Ursinia anthemoides	L77783

TABLE 3. Pairwise divergence values between sequences of 20 Artemisia species. Values are based on the distance matrix option of PAUP 3.1.1 over the combined ITS1 and ITS2 regions. Values above diagonal represent sequence divergence values, values below diagonal represent nucleotide substitution differences.

		1	2	3	4	5	6	7	8	9	10
1	A. tridentata	-	0.015	0.023	0.000	0.000	0.009	0.018	0.004	0.007	0.034
2	A. cana	7	-	0.039	0.014	0.016	0.026	0.037	0.021	0.025	0.048
3	A. nova	11	18	-	0.025	0.023	0.030	0.041	0.029	0.033	0.051
4	A. tripartita	0	6	11	-	0.000	0.012	0.018	0.007	0.009	0.034
5	A. arbuscula	0	7	10	0	-	0.009	0.014	0.005	0.007	0.028
6	A. longiloba	4	12	14	5	4	-	0.028	0.014	0.016	0.037
7	A. rigida	8	16	18	8	6	12	-	0.021	0.016	0.037
8	A. rothrockii	2	9	13	3	2	6	9	-	0.011	0.039
9	A. pygmaea	3	11	15	4	3	7	7	5	-	0.036
10	A. bigelovii	16	22	24	15	12	17	16	17	16	-
11	A. palmeri	29	36	38	30	26	31	28	31	26	35
12	A. ludoviciana	13	18	22	12	11	18	19	15	16	23
13	A. californica	14	21	24	13	12	18	15	16	14	24
14	A. vulgaris	18	23	26	18	17	22	22	20	19	27
15	A. abrotanum	17	22	25	15	14	21	19	19	16	26
16	A. rupestris	25	30	33	24	22	26	22	25	23	32
17	A. dracunculus	24	28	33	23	21	24	26	26	24	24
18	A. filifolia	20	26	28	19	18	25	22	22	15	26
19	A. sublessingiana	19	26	28	20	18	23	20	20	18	27
20	A. canariensis	18	18	28	19	17	22	19	20	17	27

Table 3. cont.

		11	12	13	14	15	16	17	18	19	20
1	A. tridentata	0.061	0.027	0.031	0.041	0.036	0.053	0.056	0.042	0.040	0.039
2	A. cana	0.078	0.039	0.047	0.054	0.048	0.065	0.066	0.056	0.056	0.040
3	A. nova	0.080	0.047	0.053	0.059	0.053	0.070	0.077	0.060	0.059	0.061
4	A. tripartita	0.068	0.027	0.030	0.042	0.034	0.055	0.054	0.043	0.046	0.044
5	A. arbuscula	0.060	0.025	0.028	0.040	0.032	0.051	0.049	0.042	0.042	0.040
6	A. longiloba	0.067	0.039	0.040	0.051	0.045	0.056	0.056	0.054	0.050	0.048
7	A. rigida	0.064	0.043	0.034	0.051	0.043	0.050	0.061	0.050	0.046	0.044
8	A. rothrockii	0.070	0.034	0.036	0.045	0.043	0.056	0.060	0.049	0.045	0.046
9	A. pygmaea	0.058	0.036	0.032	0.044	0.036	0.051	0.056	0.033	0.040	0.039
10	A. bigelovii	0.075	0.049	0.053	0.062	0.056	0.069	0.056	0.056	0.058	0.059
11	A. palmeri	-	0.048	0.063	0.059	0.066	0.072	0.083	0.070	0.074	0.062
12	A. ludoviciana	23	-	0.041	0.039	0.036	0.062	0.065	0.053	0.057	0.047
13	A. californica	29	19	-	0.050	0.050	0.068	0.075	0.059	0.059	0.051
14	A. vulgaris	26	17	22	-	0.055	0.062	0.073	0.066	0.064	0.047
15	A. abrotanum	31	17	23	24	-	0.051	0.074	0.055	0.053	0.050
16	A. rupestris	34	29	31	27	24	-	0.081	0.077	0.058	0.048
17	A. dracunculus	36	28	32	31	32	35	-	0.074	0.074	0.078
18	A. filifolia	33	25	27	29	26	36	32	-	0.066	0.059
19	A. sublessingiana	35	27	27	28	25	27	32	31	-	0.047
20	A. canariensis	29	22	23	20	23	22	33	27	22	-

FIG. 1. Strict consensus of 392 equally most parsimonious trees from analysis of ITS sequence data for 20 Artemisia species. (CI=0.76, RI=0.61, RC=0.46, and tree length=188 excluding invarient characters; CI=52, RC=32, and tree length=110, excluding autapomorphies) Subgeneric delimitation indicated by color, sect. Tridentatae indicated in bold, * indicates outgroup species, and *** indicates anomalous species.

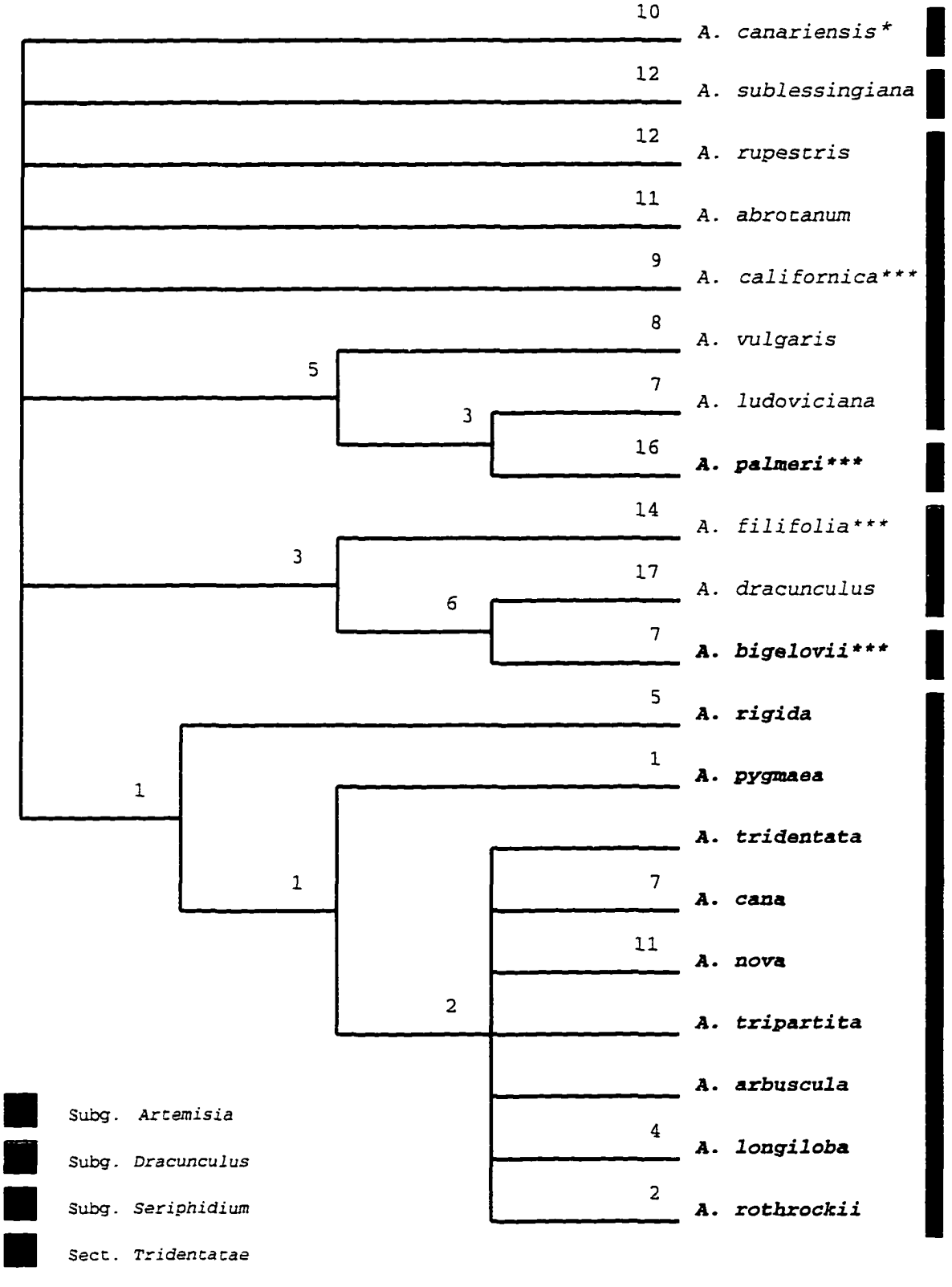

10
A. canariensis*
12
A. sublessingiana
12
A. rupestris
11
A. abrotanum
9
A. californica***
8
A. vulgaris
5
7
A. ludoviciana
3
16
A. palmeri***
14
A. filifolia***
3
17
A. dracunculus
6
7
A. bigelovii***
5
A. rigida
1
1
A. pygmaea
A. tridentata
1
7
A. cana
11
A. nova
2
A. tripartita
A. arbuscula
4
A. longiloba
2
A. rothrockii
Subg. Artemisia
Subg. Dracunculus
Subg. Seriphidium
Sect. Tridentatae

FIG. 2. Strict consensus of 392 equally parsimonious trees from analysis of ITS sequence data for 20 Artemisia and five outgroup species. (CI=0.71, RI=0.54, RC=0.38, and tree length=356; CI=0.50, RC=0.54, RI=0.27, and tree length=224) Subgeneric delimitation indicated by color, sect. Tridentatae indicated in bold, * indicates outgroup species, and *** indicates anomalous species.

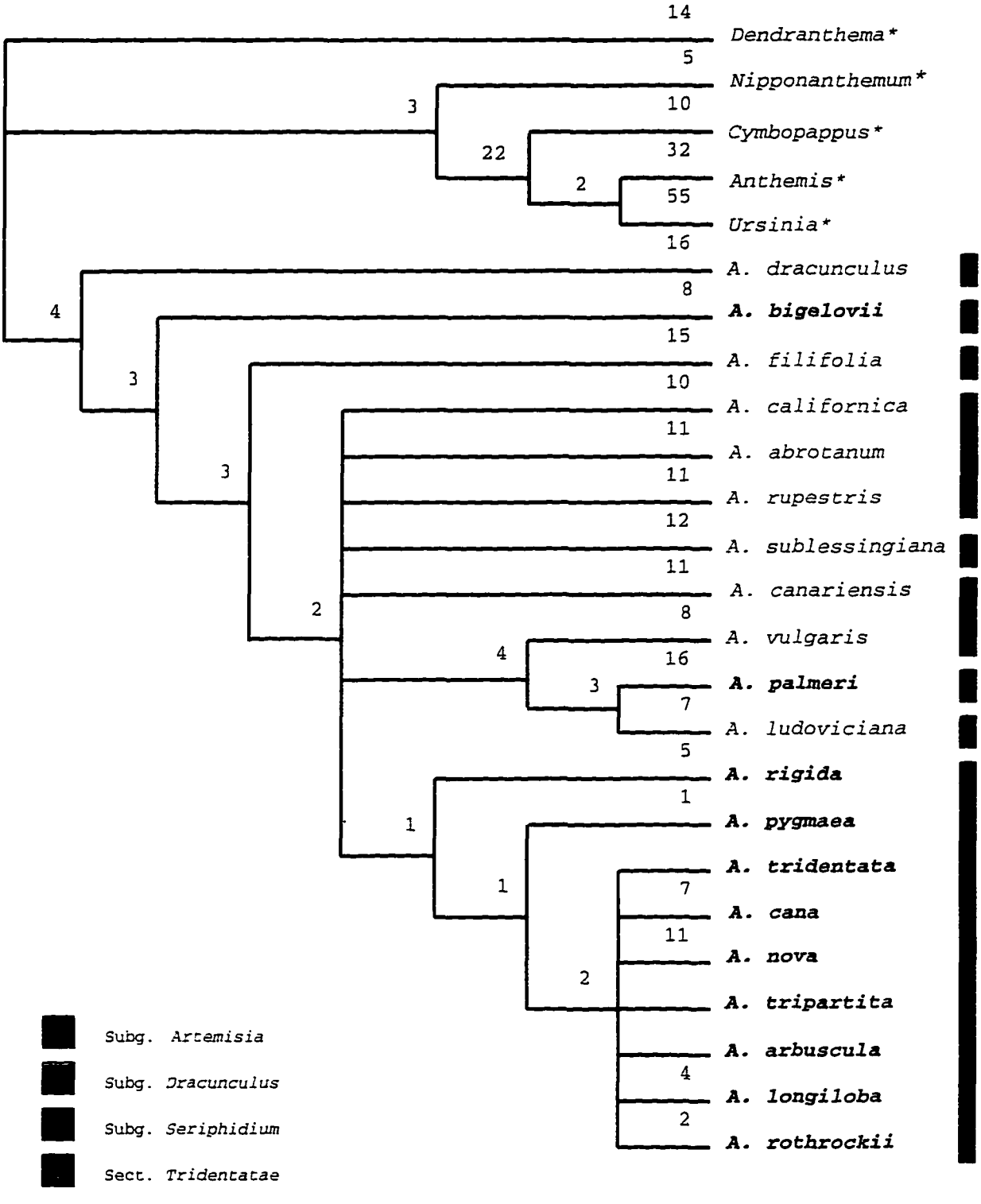
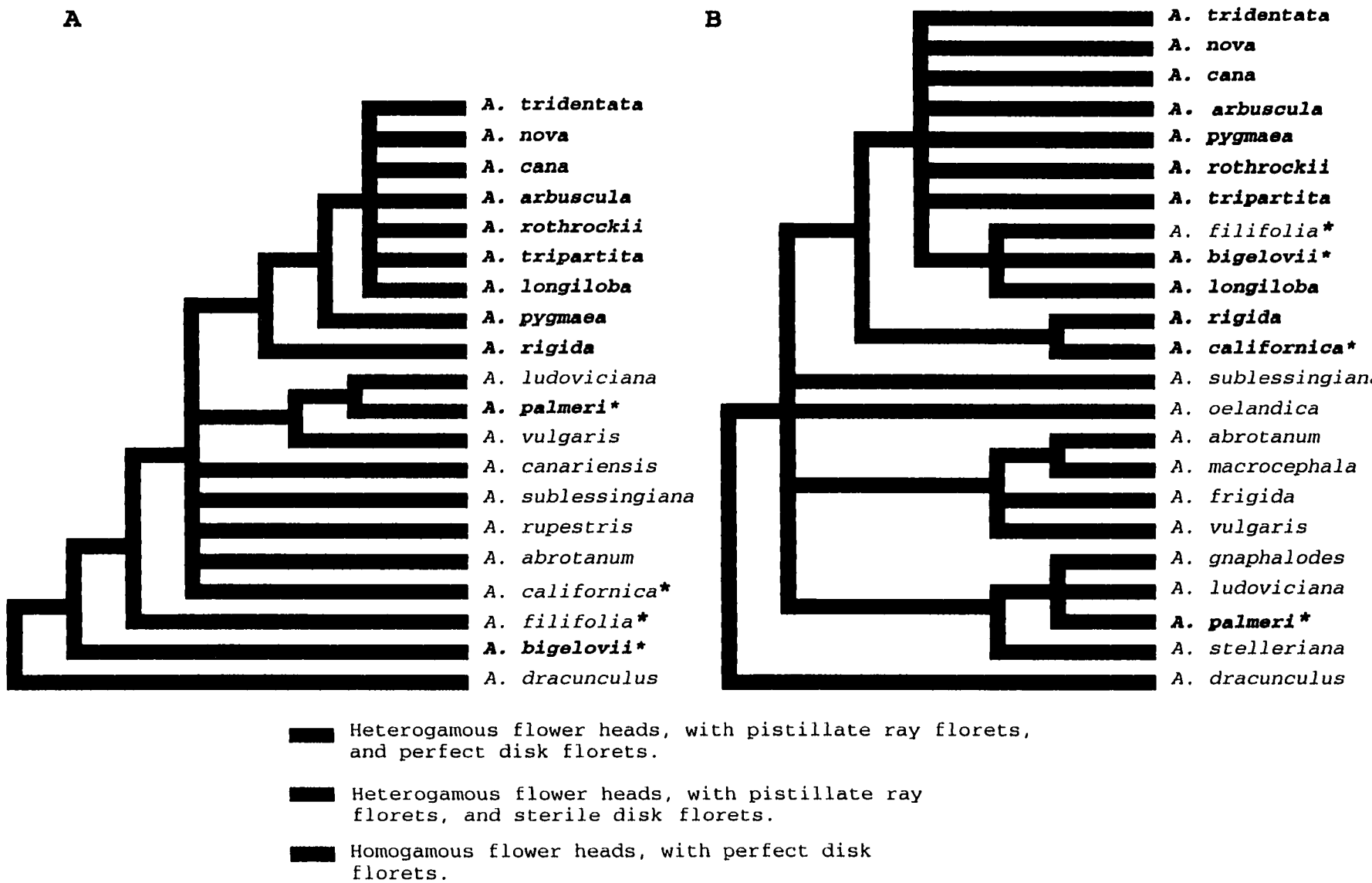

14
Dendranthema*
5
Nipponanthemum*
3
10
Cymbopappus*
22
32
Anthemis*
2
55
Ursinia*
16
A. dracunculus
8
A. bigelovii
4
15
A. filifolia
3
10
A. californica
11
A. abrotanum
3
11
A. rupestris
12
A. sublessingiana
11
A. canariensis
2
8
A. vulgaris
4
16
A. palmeri
3
7
A. ludoviciana
5
A. rigida
1
A. pygmaea
1
A. tridentata
1
7
A. cana
11
A. nova
2
A. tripartita
A. arbuscula
4
A. longiloba
2
A. rothrockii
Subg. Artemisia
Subg. Dracunculus
Subg. Seriphidium
Sect. Tridentatae

FIG. 3. Comparison of strict consensus of analysis from both ITS sequence and cpDNA restriction site data, with the distribution of capitular morphology mapped onto each tree. (A) Strict consensus of 392 tree for the ITS sequence analysis (CI=0.73, RI=0.54, RC=0.397, and tree length=188). (B) Strict consensus of 144 trees for cpDNA analysis, rerooted with A. dracunculus (CI=0.93, RI=0.93, RC=0.86, and tree length=82). Capitular morphology indicated by color, sect. Tridentatae indicated in bold, and * indicates problematic species.


A
A. tridentata
A. nova
A. cana
A. arbuscula
A. rothrockii
A. tripartita
A. longiloba
A. pygmaea
A. rigida
A. ludoviciana
A. palmeri*
A. vulgaris
A. canariensis
A. sublessingiana
A. rupestris
A. abrotanum
A. californica*
A. filifolia*
A. bigelovii*
A. dracunculus
B
A. tridentata
A. nova
A. cana
A. arbuscula
A. pygmaea
A. rothrockii
A. tripartita
A. filifolia*
A. bigelovii*
A. longiloba
A. rigida
A. californica*
A. sublessingiana
A. oelandica
A. abrotanum
A. macrocephala
A. frigida
A. vulgaris
A. gnaphalodes
A. ludoviciana
A. palmeri*
A. stelleriana
A. dracunculus
Heterogamous flower heads, with pistillate ray florets, and perfect disk florets.
Heterogamous flower heads, with pistillate ray florets, and sterile disk florets.
Homogamous flower heads, with perfect disk florets.