Phylogeny, biogeography, and chromosome evolution of the amphitropical genus Grindelia (Asteraceae) inferred from nuclear ribosomal and chloroplast sequence data
Author(s): Abigail J. Moore, Adriana Bartoli, Roberto D. Tortosa and Bruce G. Baldwin
Source: *Taxon*, Vol. 61, No. 1 (February 2012), pp. 211-230
Published by: Wiley
Stable URL: https://www.jstor.org/stable/23210325
Accessed: 08-03-2022 17:49 UTC

TAXON 61 (1) • February 2012: 211–230

Phylogeny, biogeography, and chromosome evolution of the amphitropical genus *Grindelia* (Asteraceae) inferred from nuclear ribosomal and chloroplast sequence data

Abigail J. Moore,[1,2] Adriana Bartoli,[3] Roberto D. Tortosa[3] & Bruce G. Baldwin[1]

1 *University of California, Berkeley, Jepson Herbarium and Department of Integrative Biology, 1001 Valley Life Sciences Building #2465, Berkeley, California 94720-2465, U.S.A.*
2 New Address: *Institut für Spezielle Botanik und Botanischer Garten, Johannes Gutenberg-Universität Mainz, Anselm-Franz-von-Bentzel Weg 9a, 55099 Mainz, Germany*
3 *Laboratorio de Botánica L. R. Parodi, Facultdad de Agronomia, Universidad de Buenos Aires, Av. San Martín 4453, 1417 Buenos Aires, Argentina*
Author for correspondence: *Abigail J. Moore, moorea@uni-mainz.de*

Abstract *Grindelia* is among the most taxonomically challenging groups of North American composites. The genus as a whole has an amphitropical distribution, with approximately half of the species native to North America and Mexico and the remainder native to South America. We used DNA sequence data from the nuclear ribosomal ITS and ETS and chloroplast *psaI-accD* regions to revisit hypotheses on biogeographic history across the genus. *Grindelia* as a whole is well-supported and is composed of two sister clades, one native to South America and the other native to North America including Mexico. The North American taxa constitute two clades that largely occur on different sides of the Continental Divide. The diverse radiation of *Grindelia* in the California Floristic Province appears to be most closely related to species from the Great Basin and Colorado Plateau and evidently descended from drought-adapted ancestors. Although Steyermark's hypotheses about the relationships of North American *Grindelia* are not all supported, we did recover a clade corresponding to his Pacific radiation and many of the Mexican and Texan species that he hypothesized to be basal in the genus represent early diverging lineages in our trees. Dunford's cytogenetic data on the North American species of *Grindelia* were also examined in a phylogenetic context.

Keywords amphitropical disjunction; Asteraceae; cytogenetics; *Grindelia*; long-distance dispersal; molecular phylogeny

Supplementary Material The alignment files are available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

In plant biogeography, certain disjunction patterns appear to occur more often than would be expected based on chance or on the distribution of suitable habitats (Thorne, 1972). Among these are the New World amphitropical patterns, with groups that occur in the temperate areas of North and South America, but not in the intervening tropics (Raven, 1963, 1972; Wen & Ickert-Bond, 2009). There are three major amphitropical disjunction patterns (Raven, 1963; Wen & Ickert-Bond, 2009): temperate, often (but not always) between the west coasts of North and South America (e.g., *Blennosperma* Less. and *Lasthenia* Cass., Ornduff, 1963; *Gilia* Ruiz & Pav., Morrell & al., 2000; *Sanicula* L., Vargas & al., 1998); desert, between the deserts of North and South America (e.g., *Larrea* Cav., Lia & al., 2001; *Tiquilia* Pers., Moore & al., 2006); and bipolar, between far-northern North America and far-southern South America (e.g., *Deschampsia* P. Beauv., Parodi, 1949; *Primula* L., Guggisberg & al., 2009). In addition, amphitropical disjuncts may differ in the closeness of relationship of the disjunct plants (Wen & Ickert-Bond, 2009). In some cases, the disjunction events are recent enough that the plants occurring on both continents (often in the California Floristic Province and the region of Chile with a Mediterranean climate) have been considered conspecific (e.g., *Osmorhiza berteroi* DC. and *O. depauperata* Phil., Wen & al., 2002; *Sanicula crassicaulis* Poepp. and *S. graveolens* Poepp., Vargas & al., 1998; *Tiquilia nuttallii* (Benth.) A.T. Richardson, Moore & al., 2006). In other cases, the plants have undergone substantial independent evolution, and sometimes diversification, on each continent (e.g., *Astragalus* L., Scherson & al., 2008; *Gentianella* Moench, Hagen & Kadereit, 2001; *Hoffmannseggia* Cav., Simpson & al., 2005).

Both vicariance and dispersal hypotheses have been advanced for such amphitropical disjunctions. Given the great distance between the temperate regions of North and South America, hypotheses have been invoked that involve vicariance during a period when the climate was cooler and suitable habitat may have occurred throughout the tropics (e.g., Solbrig, 1972) or that involve shorter-distance dispersal between suitable, mountain-top habitats (Cruden, 1966). While some so-called amphitropical disjuncts have limited diversity in mountainous areas throughout part of the tropics (e.g., *Epilobium* L., Seavey & Raven, 1977; *Phacelia* Juss., Heckard, 1963; *Trifolium amabile* Kunth, Ellison & al., 2006), many others would unlikely have been able to find suitable intermediate habitats at the time that their North and South American lineages diverged (Carlquist, 1983; e.g., *Gilia*, Morrell & al., 2000; *Tiquilia*, Moore & al., 2006). In addition, the paucity of disjunct animals between temperate North and South America (Simpson & Neff, 1985) suggests that most disjunct distributions are based on dispersal, not vicariance.

The California Floristic Province (CA-FP) is particularly rich in taxa that have disjunct, close relatives in central Chile. Both regions are characterized by having a Mediterranean climate, with cool, wet winters and hot, dry summers. Two factors may contribute to the prevalence of disjunctions between plants of the two areas: migratory birds may act as dispersal agents by feeding in one area before migrating to the other (e.g., Cruden, 1966). Additionally, it may be easier for plants from similar, but distant, climates (synclimatic, sensu Ackerly, 2009) to colonize the Mediterranean-climate areas of Chile or the CA-FP than it is for plants from adjacent areas with different climates (anticlimatic, sensu Ackerly, 2009).

Grindelia Willd. is a New World genus of the Asteraceae, tribe Astereae, with a high diversity of taxa in temperate regions of both North and South America (Figs. 1–2; distribution map in Fig. 6 on p. 223). Approximately 25 to 50 species (depending on the classification) are found in North America and Mexico, with centers of morphological and (depending on the classification) taxonomic diversity in California and Texas and northeastern Mexico (Steyermark, 1934; Nesom, 1990, 1992; Strother & Wetter, 2006). South America has 26 species, with a center of diversity in Argentina, though some species occur west of the Andes (Bartoli & Tortosa, 1999b, 2003b; Tortosa & Bartoli, 2001). Steyermark (1937) inferred a single disjunction between North and South American plants. No species have been hypothesized to occur on both continents; however, most studies and all recent treatments of the genus have focused exclusively on plants from one hemisphere or the other.

Given that *Grindelia* has species that occur in both Mediterranean-climate regions of the New World (CA-FP and Chile) and species that occur in dryland areas of both North and South America (in the southwestern United States and Mexico and in Argentina), it is unclear what type or types of disjunction have occurred in the history of the genus.

Differences in ploidy between plants of North and South America have been useful for understanding the direction of dispersal of some amphitropically distributed angiosperms (e.g., *Blennosperma*, Ornduff, 1963). In *Grindelia*, ploidy patterns are inconclusive about relationships: diploids and tetraploids are found on both continents, while hexaploids have only been found in two South American species (Whitaker & Steyermark, 1935; Dunford, 1964; Bartoli, 1993; Bartoli & Tortosa, 1998b). No dysploidy has been reported.

Relationships of *Grindelia* to other taxa of tribe Astereae are also not decisive about the historical biogeography of the genus. Recent molecular work (Morgan & Simpson, 1992; Morgan, 1997, 2003) has shown *Grindelia* to be sister to a clade composed of the North American genera *Isocoma* Nutt., *Rayjacksonia* R.L. Hartm. & M.A. Lane, and *Xanthocephalum* Willd. All members of this clade share the chromosome base number $x = 6$ with *Grindelia*. The North American *Hazardia* Greene, *Pyrrocoma* Hook., and *Lessingia* Cham. as well as the South American *Haplopappus* Cass. were part of a polytomy with the $x = 6$ group in the nrDNA trees, but were members of a separate clade in the cpDNA restriction site tree (Morgan, 2003). These results leave the continental origin of *Grindelia* ambiguous.

Understanding relationships within *Grindelia* is critical to resolving the biogeographic and ecological history of the group. There has been general agreement about the circumscription of *Grindelia*, with the exception of some South American taxa. All members of the genus have yellow ray and disc corollas, although ray florets are sometimes absent. As originally circumscribed, *Grindelia* was distinguished by having a pappus composed of 2–18 caducous awns. Recently, some species have been transferred to *Grindelia* that have pappi of many bristles: *G. ciliata* (formerly *Prionopsis ciliata* (Nutt.) Sprengel, Nesom & al., 1993), *G. anethifolia* (formerly *Haplopappus pectinatus* Phil., Bartoli & Tortosa, 1998a), and *G. prunelloides* (formerly *H. prunelloides* (Less.) DC., Bartoli & Tortosa, 1999a).

Klingenberg (2007) placed the South American species with pappi of many bristles (*G. anethifolia* and *G. prunelloides*, the latter of which she separated into two species) in their own genus, *Notopappus* Klingenb. She considered *Notopappus* to be intermediate between *Grindelia* and *Haplopappus*. *Notopappus* was distinguished from *Grindelia* s.str. by having a persistent pappus of 40–60 bristles instead of a deciduous pappus of 2–10 awns as well as by the shape of the cypselae and the presence of bristles on the involucre, a feature it shares with *Haplopappus*.

Delimitation of the species of *Grindelia* and understanding their ranges of morphological and ecological variation have proven to be far more controversial than the delimitation of the genus. All authors agree that *Grindelia* encompasses extensive morphological variation, which is at least loosely correlated with habitat. In his revision of North American *Grindelia*, Steyermark (1934) recognized 45 species and 66 additional varieties and forms in what he considered to be a recent radiation. The general trend of more recent authors has been to recognize fewer and fewer of Steyermark's taxa (e.g., Keck, 1959; Lane, 1993). This trend culminated in Strother & Wetter's (2006) treatment of the genus for *Flora of North America North of Mexico*, in which they combined 18 of Steyermark's species into a much expanded and morphologically variable *G. hirsutula*.

Grindelia tends to occupy relatively dry, open habitats, ranging from grasslands and shrublands to clearings in coniferous forests. Many taxa appear to be quite tolerant of xeric conditions and members of *Grindelia* are most commonly found in relatively dry habitats, although some taxa occur in saline or alkaline wetlands (*G. oolepis*, *G.* ×*paludosa* Greene pro. sp., and *G. stricta* var. *angustifolia* (A. Gray) M.A. Lane from North America and *G. aegialitis* Cabrera, *G. boliviana*, and *G. brachystephana* from South America). Variation in habit is often correlated with variation in habitat. The genus includes annuals, herbaceous perennials, and plants with varying degrees of woodiness up to true shrubs, with the greatest amount of variation occurring among the South American taxa (Bartoli & Tortosa, 2003a).

Most previous phylogenetic hypotheses involving *Grindelia* concern the North American taxa. Within North America,

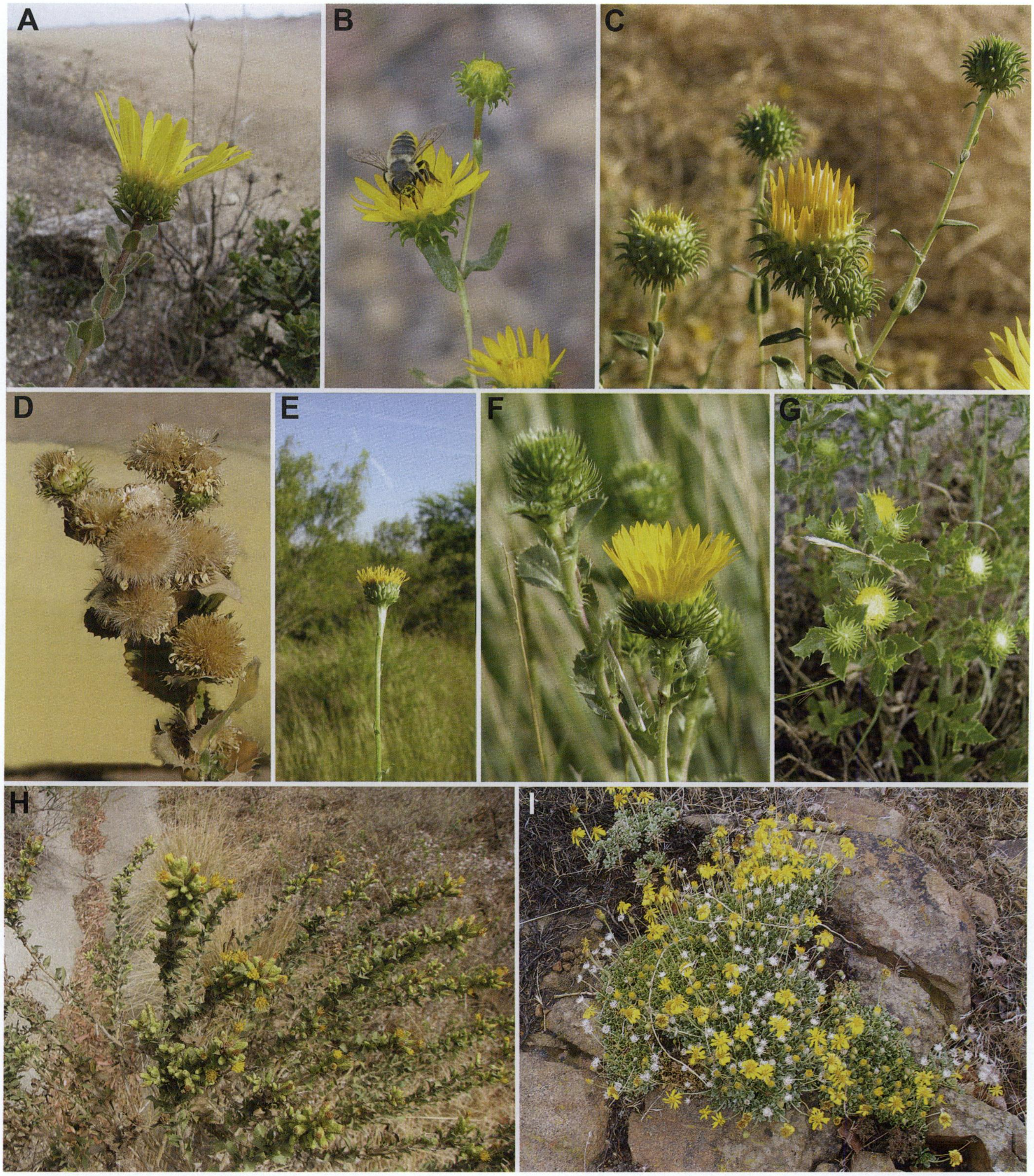


Fig. 1. Morphology of *Grindelia* and outgroup taxa (all photographs from the U.S.A. unless otherwise noted). **A,** *Grindelia hirsutula* showing radiate heads, Montara Mountain, San Mateo Co., California; **B,** *Megachile* Latreille subg. *Xanthosarus* Robertson (Megachilidae) collecting pollen from *G. squarrosa*, Leopard Creek Canyon, San Miguel Co., Colorado; **C,** *G. camporum* showing resin on young heads and recurved phyllaries, Jepson Prairie, Solano Co., California; **D,** *G. ciliata* showing pappus composed of many awns in this species, near Odessa, Ector Co., Texas; **E,** *G. oolepis* showing discoid heads, Welder Wildlife Refuge, San Patricio Co., Texas; **F,** *G. ciliata* showing awn-tipped phyllaries, near Roswell, Chaves Co., New Mexico; **G,** *G. pulchella* var. *discoidea* (Hook. & Arn.) Adr. Bartoli & Tortosa showing discoid heads and attenuate phyllaries, Valle de Punilla, Prov. Córdoba, Argentina; **H,** *Hazardia squarrosa* var. *grindelioides*, north of Santa Barbara, Santa Barbara Co., California; **I,** *Haplopappus glutinosus*, Andacollo, Prov. Neuquén, Argentina. — All photographs by A.J. Moore except G and I by A. Bartoli.

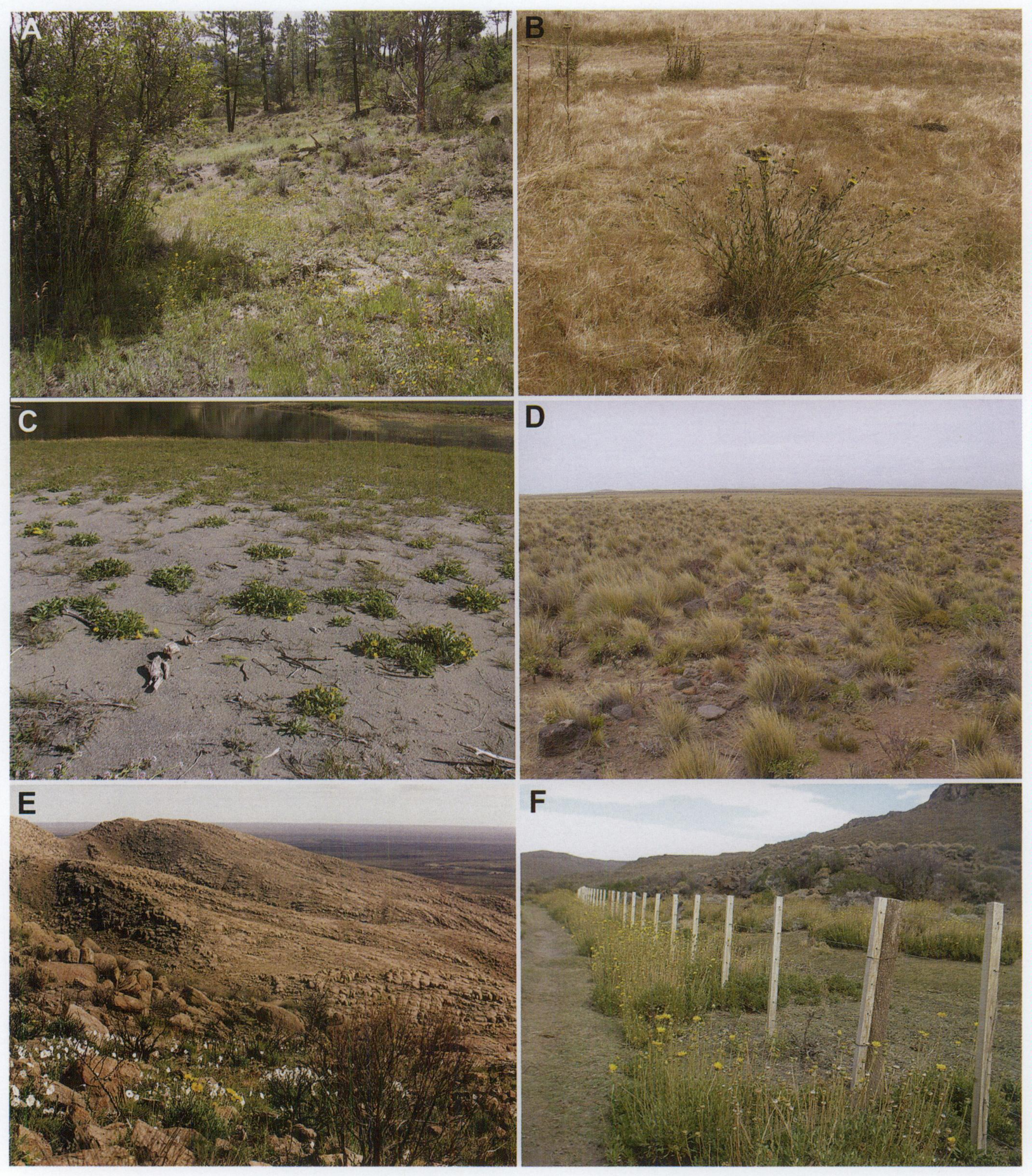


Fig. 2. Habitats in which *Grindelia* grows. **A,** *G. arizonica* in open *Pinus ponderosa* Lawson & C. Lawson woodland, near Durango, Archuleta Co., Colorado, U.S.A.; **B,** *G. hirsutula* in serpentine grassland, Redwood Regional Park, Alameda Co., California, U.S.A.; **C,** *G. stricta* var. *stricta* on a sand dune near Stone Lagoon, Humboldt Lagoons State Park, Humboldt Co., California, U.S.A.; **D,** *G. coronensis* in grassland, Mesta del Somuncurá, Cerro Corona, Dpto. 9 de Julio, Prov. Río Negro, Argentina; **E,** *G. covasii* in shrubland, cerro El Portal, Dpto. Lihue Calel, Prov. La Pampa, Argentina; **F,** *G. chiloensis* in a steppe community, Camarones, Dpto. F. Ameghino, Prov. Chubut, Argentina. — Photographs A–C by A.J. Moore, D by Héctor Troiani, E–F by A. Bartoli.

Steyermark (1937) considered the oldest lineages to be found in Mexico and the central part of the United States (the Ozark and Edwards plateaus). He suggested that multiple lineages from this central area colonized the western part of the continent. Steyermark considered the species of *Grindelia* along the Pacific Coast to have radiated quite recently because most of the habitats they occupy are of recent origin. He illustrated his hypotheses with an early phylogenetic tree (Steyermark, 1937: fig. 3).

Although Steyermark (1937) proposed an explicit phylogenetic hypothesis for *Grindelia*, he did not propose subgeneric taxa above the rank of species, nor has any other taxonomist. Steyermark (1937) considered the North American species to be too closely related and to show too much intergradation for subgenera or sections to be defensible, stating:

> With *Grindelia*, however, the species are so closely inter-related and give to the genus such a high degree of homogeneity that the establishment of sections would be artificial and well-nigh impossible. True, various species tend to form into little groups, but the lines are not sufficiently sharp to permit subgeneric or sectional groups. (p. 252)

No sectional classifications have been proposed for the South American species either (Cabrera, 1932; Bartoli & Tortosa, 1999b).

In the 1960s through the 1980s, Dunford (1964, 1970a, b, 1971, 1983, 1986) performed an extensive series of crosses between species of *Grindelia* native to North America. He documented chromosome pairing behavior in the resultant hybrids and used these data to infer the occurrence of reciprocal translocations that gave rise to distinct chromosome arrangements shared by groups of taxa. Dunford found at least four (possibly five) different chromosomal arrangements, which are each separated by one or more arm interchanges (Dunford, 1970a, 1986). He called these (1) the Hallii genome (present in the Californian species; Dunford, 1964), (2) the Havardii genome (present in various species from Texas as well as in the widespread *G. aphanactis*; Dunford, 1970a, b, 1971), (3) the Oxylepis genome (present in various species from Colorado and Mexico as well as in the widespread *G. squarrosa*; Dunford, 1970a, 1986), and (4) the Subalpina genome (detected only in *G. subalpina* from Colorado and Wyoming; Dunford 1986). In addition, two species, *G. oolepis* and *G. scabra* (Dunford, 1971, 1986), had a genome that was shown to differ from the Havardii and Oxylepis genomes, but was not completely characterized. The tetraploids that Dunford examined from California (with the Hallii genome) behaved as autotetraploids in both their extent of chromosome pairing during meiosis (e.g., quadrivalent formation) and in the lack of detected rearrangements differentiating their sets of chromosomes (Dunford, 1964, 1983). Although Dunford (1970a, 1986) found several different chromosomal arrangements, with associated impacts on interfertility, he did not investigate enough of the species to allow a sectional classification to be produced based on biosystematic and cytogenetic data.

We used sequence data from the nuclear ribosomal internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions as well as the chloroplast spacer region *psaI-accD* for phylogenetic analyses of *Grindelia*. All of these regions have been shown to be useful for fine-scale phylogenetic studies (e.g., Baldwin, 1992; Baldwin & Markos, 1998; Shaw & al., 2007). Our goals in this study were (1) to examine the biogeographic history of the genus, (2) to examine the evolution of morphology and habitat in a phylogenetic context, (3) to re-examine previous evolutionary hypotheses for *Grindelia*, and (4) to examine Dunford's genomic data in a phylogenetic context.

■ MATERIALS AND METHODS

A total of 111 plants from 62 species were sampled. Thirty-one of the approximately 45 species of North American *Grindelia* (excluding many Mexican species that were unavailable for sampling) and 17 of the 26 species of South American *Grindelia* were included. When possible, wide-ranging or morphologically variable species were sampled repeatedly. Outgroup taxa were chosen according to the phylogenies of Markos & Baldwin (2001) and Morgan (2003), with 19 additional outgroup accessions included from sequence data deposited in GenBank by those studies.

DNA samples were taken from fresh, frozen, or silica-dried material when possible and from herbarium material when newly-collected specimens could not be obtained (Appendix). DNA was extracted using the Qiagen Plant Mini Kit (Qiagen Inc., Valencia, California, U.S.A.). The samples were ground directly in the AP1 extraction buffer or in liquid nitrogen.

PCR of some samples (including those that were difficult to amplify) was carried out using AccuPower PCR PreMix (Bioneer Inc., Alameda, California, U.S.A.) using 0.375 μM concentration of each primer and 17 μl of genomic DNA that was diluted 1:50 from the original concentration upon extraction. The remaining samples were amplified using component-based PCR with 1× ThermoPol reaction buffer (New England Biolabs, Ipswich, Massachusetts, U.S.A.), 1.5 units of *Taq* polymerase (New England Biolabs), 0.4 μM each primer, 0.6 mM dNTPs, 0.5 μg BSA, and 3 μl genomic DNA at 1:10 dilution. The ITS region was amplified using the primers ITS4 (White & al., 1990) and either ITS-I (Urbatsch & al., 2000) or ITS5 (White & al., 1990) and sequenced using the primers ITS5 and ITS4. The 3′ end of the ETS region (411 base pairs) was amplified and sequenced using the primers Ast-1 (Markos & Baldwin, 2001) and 18S-ETS (Baldwin & Markos, 1998). The *psaI-accD* spacer was amplified as a whole using the primers psaI-72R and accD (Shaw & al., 2007) or in two pieces using the internal primers RforpsaI (GCC TAG TGA ATG AAA TTC GAA GAC) and FforaccD (GTG AGT ATA TAA TGT AGT TTT TCA TC), developed for this study. The PCR primers were used for sequencing, with the substitution of accDnew (GTG AAA TTG AGA CGA ATG GG) for accD when use of accD did not result in a clean sequence; however, this primer only proved effective for a limited number of samples.

PCR products were cleaned using the Exo-SAP PCR Product Pre-Sequencing Kit (USB Corp., Cleveland, Ohio, U.S.A.) and were cycle-sequenced using Big Dye v.3.1 (Applied Biosystems Inc., Foster City, California, U.S.A.). Sequencing products were resolved on ABI 377, ABI 3730, or ABI 3730xl automated sequencers (Applied Biosystems). Sequences were corrected using ChromasPro v.1.5 and earlier versions (Technelysium Pty. Ltd., Tewantin, Queensland, Australia) and aligned by eye in SeaView (Galtier & al., 1996; Gouy & al., 2010) following Simmons' (2004) similarity criterion. Positions 132–137, 149–186, 203–213, 355–363, 401–490, and 525–592 were removed from the original *psaI-accD* alignment prior to analysis due to the difficulty of assessing sequence homology across clades given the many insertions and deletions in those regions.

Nuclear ribosomal DNA data (nrDNA; ITS and ETS sequences) and chloroplast DNA data (cpDNA; *psaI-accD* sequences) were only analyzed separately due to the presence of strongly supported, conflicting clades in the nrDNA and cpDNA trees. The nrDNA dataset was slightly larger than the cpDNA dataset (130 sequences instead of 106 sequences), because cpDNA sequences could not be obtained from some herbarium specimens and only ITS and ETS sequences were available for the outgroup taxa sampled from GenBank. In addition, a useable ITS sequence could not be obtained for one accession of *G. boliviana*, so only its ETS sequence was included in the nrDNA matrix.

Parsimony heuristic searches were performed in PAUP* v.4.0b10 (Swofford, 2002) with random taxon addition (5000 replicates for cpDNA and 20,000 replicates for nrDNA), tree bisection-reconnection (TBR) branch-swapping, and gaps treated as missing data. MulTrees was turned off, but rearrangements per replicate were not limited. Parsimony bootstrap searches were conducted with 1000 bootstrap replicates, simple taxon addition, MulTrees on, and rearrangements limited to 10,000,000 per replicate for nrDNA and 200,000 per replicate for cpDNA analyses.

Maximum likelihood heuristic searches were performed using RAxML v.7.2.7: HPC2 on teragrid for cpDNA and RAxML v.7.2.8: HPC2 on teragrid for nrDNA (Stamatakis, 2006; Stamatakis & al., 2008) in the Cipres Portal (Miller & al., 2009). The searches were run with 10,000 rapid bootstrap replicates. The GTRCAT model with 25 rate categories was used for the bootstrap search and the GTRGAMMA model was used for the final tree as recommended by Stamatakis & al. (2008). Bootstrap values were obtained by constructing majority-rule consensus trees in PAUP*.

Bayesian analyses were run using Mr. Bayes v.3.1.2 (Huelsenbeck & Ronquist, 2001) on teragrid in the Cipres Portal (Miller & al., 2009). Two runs were performed with four chains each; the chains were run for 15,000,000 generations and sampled every 1000 generations. The GTR plus invgamma model of sequence evolution was used based on the output of MrModeltest v.2.3 (Nylander, 2004). Posterior probabilities were derived from the set of post-burn-in trees found after the standard deviation of the split frequencies dropped below 0.01 (generations 4,363,000–15,000,000 for cpDNA and 5,641,000–15,000,000 for nrDNA).

Estimations of ancestral character states for ploidy were performed in Mesquite v.2.5 (Maddison & Maddison, 2008). Ploidy was coded by taxon, not by individual, as the ploidy was not known for all accessions. This method of coding led some terminals to have polymorphic character states, because some taxa have two or more ploidy levels. Only parsimony reconstructions were performed due to the polymorphic data.

Tree searches were performed in BEAST v.1.6.0 (Drummond & Rambaut, 2007) on the nrDNA dataset to provide ultrametric trees for biogeographic reconstruction. Four separate analyses were run for 50,000,000 generations, each with the first 10,000,000 generations removed as burn-in. The GTR substitution model was used with empirical base frequencies, invariant sites, and four gamma categories based on the output of MrModeltest v.2.3 (Nylander, 2004). An uncorrelated lognormal relaxed clock was used with no calibration (to obtain an uncalibrated ultrametric tree) and branching according to the Yule speciation process. The priors and operators were at their default settings, with the exception of weights of Tree:subtreeSlide, Tree:narrowExchange, UCLD mean and heights:upDown, and Internal node heights:uniform, which were set to 65 (half the number of taxa) and Tree:wilsonBalding and Tree:wideExchange, which were set to 1.0, all following the suggested values from the BEAST manual (Drummond & al., 2007).

Maximum likelihood estimations of biogeographic evolution were performed on individual post-burn-in trees from the BEAST analysis using *lagrange* (Ree & al., 2005, Ree & Smith 2008). Areas were coded according to collection location, not according to taxon range, for the biogeographic analyses, because all taxa were not monophyletic in the analyses. Five areas were included in the analyses (see map in Fig. 6 on p. 223): South America east of the crest of the Andes, South America west of the crest of the Andes, North America east of the crest of the Rockies, North America west of the crest of the Rockies but east of the crest of the Sierra Nevada/Cascade axis, and North America west of the crest of the Sierra Nevada/Cascade axis (the California Floristic Province). Taxon ranges were limited to two of these areas. Ranges were allowed to be composed of any two areas except for North America west of the crest of the Sierra Nevada/Cascade axis and North America east of the crest of the Rockies, as it was considered improbable for a taxon to exist in those two areas but to be absent from the intervening area.

Parsimony analyses were performed in PAUP* to examine the evolution of Dunford's (1986) genomic characters. Only the North American taxa were included in these analyses, along with the South American *G. anethifolia* 2 as the outgroup. Analysis parameters were identical to those used in the other parsimony analyses but with 10,000 random addition replicates in the original heuristic searches and bootstrap rearrangements limited to 1,000,000 per replicate. In these analyses, a character representing the genome was added to the nrDNA matrix. A step matrix was constructed for the genome character with the number of steps between genomes equal to the number of chromosomal rearrangements that separated them (diagram of rearrangements in Fig. 7 on p. 224). The genome character was treated as unordered. Analyses were performed with the genome character weighted 1, 5, 7, 10, 15, and 20 times as heavily as an individual position in the sequence alignment using simple character weighting. Although it is known that *G. oolepis* and *G. scabra* have the same genomic arrangement, the relationship of this genomic arrangement to the others was not fully determined. Therefore, these plants were coded as having an unknown genome. When the so-called Scabra genome was included in the analysis and was separated from the remaining genomes by 1, 2, or 3 steps, the tree topologies were not significantly different from those found in the analyses in which the genome was coded as unknown.

■ RESULTS

Of the three regions sequenced—ITS and ETS from nrDNA and the *psaI-accD* spacer from cpDNA—ETS had the highest proportion of parsimony-informative characters (Table 1). However, *psaI-accD* had the most variable and the most parsimony-informative characters because it was more than three times as long as the ETS segment that was sequenced and more than twice as long as the ITS region.

The topologies from the different analyses (parsimony, maximum likelihood, and Bayesian) of each region were generally congruent. The only clade that was strongly supported in one analysis (parsimony bootstrap > 75% or Bayesian posterior probability > 0.95) and not present in the maximum likelihood trees (and thus not shown in the figures) was the placement of *G. arizonica* as sister to the clade formed by *G. aphanactis* 2 and *G. fastigiata* 2 in the cpDNA Bayesian trees, which was supported with a posterior probability of 0.97.

Grindelia was well-supported as monophyletic in trees from nrDNA (Fig. 3) and cpDNA (Fig. 4) data. The remaining members of Morgan & al.'s (Morgan & Simpson, 1992; Morgan, 1997, 2003) $x = 6$ clade (i.e., *Isocoma*, *Rayjacksonia*, *Xanthocephalum*) were also strongly supported as a monophyletic group. *Haplopappus* was resolved as monophyletic in the nrDNA tree, but paraphyletic in the cpDNA tree. None of the trees resolved a well-supported sister group to *Grindelia*.

Within *Grindelia*, the North and South American taxa formed sister clades in the nrDNA tree. In the cpDNA tree, *Grindelia* was composed of three clades in a polytomy: (1) a clade containing all of the North American species, (2) a clade composed of both accessions of the South American *G. covasii* plus *G. patagonica*, and (3) a strongly-supported clade comprising the remaining species of South American *Grindelia*.

The North American clade was divided into three groups in the nrDNA tree: the two accessions of *G. nuda* on a branch by themselves (weakly supported as sister to the Western Clade), plus two major clades. The first major clade (the Eastern Clade; see Fig. 3 for clade labels) contained taxa from the eastern part of the range of *Grindelia*, east of the Continental Divide, as well as the widespread species *G. aphanactis* and *G. squarrosa* and the western *G. arizonica*. Within the Eastern Clade, there were several small clades containing one to three species as well as one large clade, which contained the widespread *G. squarrosa* and *G. aphanactis* as well as a few species with more limited ranges, all minimally divergent in sequence. The second major clade within North American *Grindelia* (the Western Clade) contained taxa that are native to the area west of the Continental Divide, as well as *G. nana*, which extends east into Montana. Within the Western Clade, *Grindelia* from the Pacific states (California, Oregon, and Washington) formed a clade with *G. howellii* from Idaho and Montana (the Pacific Clade). Within the Pacific Clade, the plants collected in the California Floristic Province grouped together in a subclade.

None of these clades within North American *Grindelia* was present in the cpDNA tree. Chloroplast data resolved only a few relationships, due in part to low sequence divergence in North America, but mainly due to a high level of autapomorphic changes. Only three of these relationships were also found in the nrDNA tree: the sister relationship between the two accessions of *G. oolepis*, the sister relationship between the two accessions of *G. ciliata*, and the sister relationship between the two accessions of *G. ciliata* and *G. adenodonta* 2 (the only accession of that species present in the cpDNA dataset).

In the nrDNA tree, the South American clade was divided at the base into two well-supported clades. One consisted of *G. brachystephana*, one accession of *G. pulchella*, and *G. scorzonerifolia*. The other contained all of the remaining species of South American *Grindelia*, including those species segregated as the genus *Notopappus* (i.e., *G. anethifolia* and *G. prunelloides*) by

Table 1. Characteristics of the sequences used in this study. The numbers for ITS include ITS1, ITS2, and the 5.8S region.

Region	Number of sequences	Number of characters	Number of variable characters	Proportion of variable characters	Number of parsimony-informative characters	Proportion of parsimony-informative characters
cpDNA; nrDNA sequences with a corresponding cpDNA sequence						
psaI-accD	106	1430	229	0.160	92	0.064
ITS	105	645	116	0.180	53	0.082
ETS	106	412	98	0.238	61	0.148
Complete set of nrDNA sequences						
ITS	129	645	142	0.221	81	0.126
ETS	130	412	111	0.269	76	0.184
Combined	129	1057	252	0.238	157	0.149

Klingenberg (2007). Within the latter clade, the two accessions of *G. buphthalmoides* constituted a clade as did the two accessions of *G. boliviana*, while all of the accessions of *G. chiloensis* and *G. anethifolia* formed a clade together with *G. coronensis* and *G. mendocina*. *Grindelia prunelloides* grouped separately from *G. anethifolia*, in a clade with *G. pygmaea*.

Resolution and sequence divergence in the cpDNA tree were both markedly higher for South American *Grindelia* than they were for North American *Grindelia*. However, the relationships that were resolved with cpDNA data were mainly different from those resolved with nrDNA data for South America as well. The only relationship recovered in both trees was the clade uniting the two accessions of *G. buphthalmoides*, although the clade composed of *G. covasii* and *G. patagonica*, which was found in the cpDNA tree, was not contradicted in the nrDNA tree.

In the nrDNA tree, *Haplopappus* was divided into three major clades. One clade was composed of *H. anthylloides*, a monophyletic *H. glutinosus*, and *H. paucidentatus*. The second clade was composed of *H. macrocephalus*, *H. setigerus*,

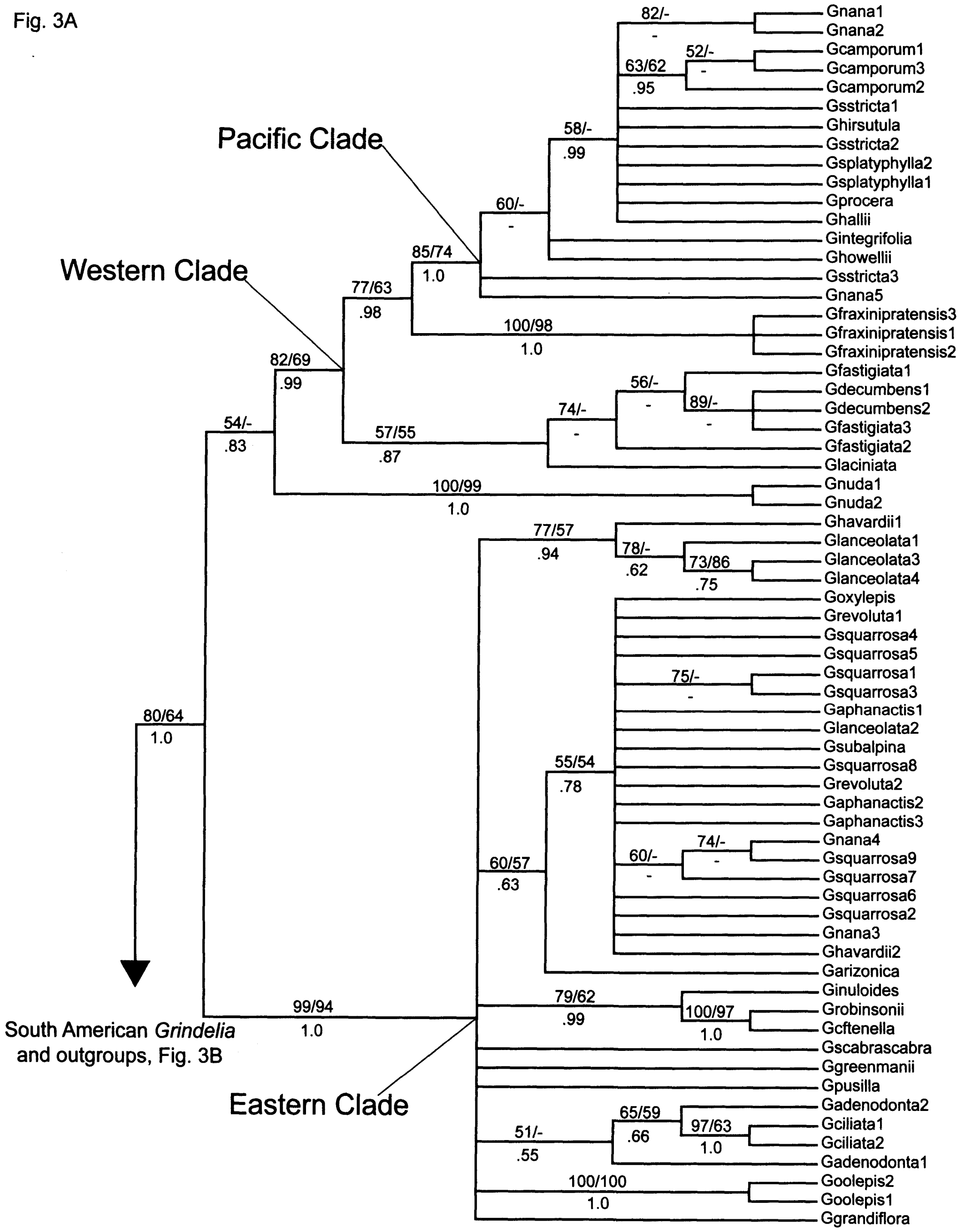


Fig. 3. Maximum likelihood bootstrap tree from the RAxML analysis of the nrDNA data. Maximum likelihood and parsimony bootstrap values are above the branches, Bayesian posterior probabilities are below the branches. *Grindelia* accessions are indicated by a G followed by the specific epithet. *Haplopappus* accessions are indicated by an H followed by the specific epithet. The varieties of *G. stricta* are designated by Gs followed by the varietal epithet. Taxa represented by more than one individual are numbered following the Appendix.

H. uncinatus, and *H. velutinus*. The third clade was composed of *H. foliosus*, *H. marginalis*, a paraphyletic *H. multifolius*, and *H. undulatus*. The only *Haplopappus* clade that was resolved in the cpDNA tree was identical to one of the three clades in the nrDNA tree, with the removal of two taxa for which cpDNA data were not available.

The remainder of the results and the discussion section focus on the results of the nrDNA analyses due to strongly supported incongruities between the nrDNA and cpDNA trees and the empirically supported prediction that relative levels of infraspecific and interspecific gene flow in cpDNA make it less likely to track the history of the bulk of plant genes than a rapidly coalescing nuclear gene region (see Petit & Excoffier, 2009). Tetraploidy appears to have arisen at least three times in North American *Grindelia*, once in the Eastern Clade and two or three times in the Western Clade, depending on whether or not one assumes that *G. camporum*, which putatively has both diploid and tetraploid members, is monophyletic (Fig. 5). In South America, tetraploidy also appears to have arisen multiple times. Only one of the two South American hexaploid taxa was sampled in the molecular trees.

Biogeographic analyses were not able to resolve the areas occupied by the ancestors of most of the clades, as could be expected given the fact that most of the major clades were from

Fig. 3B

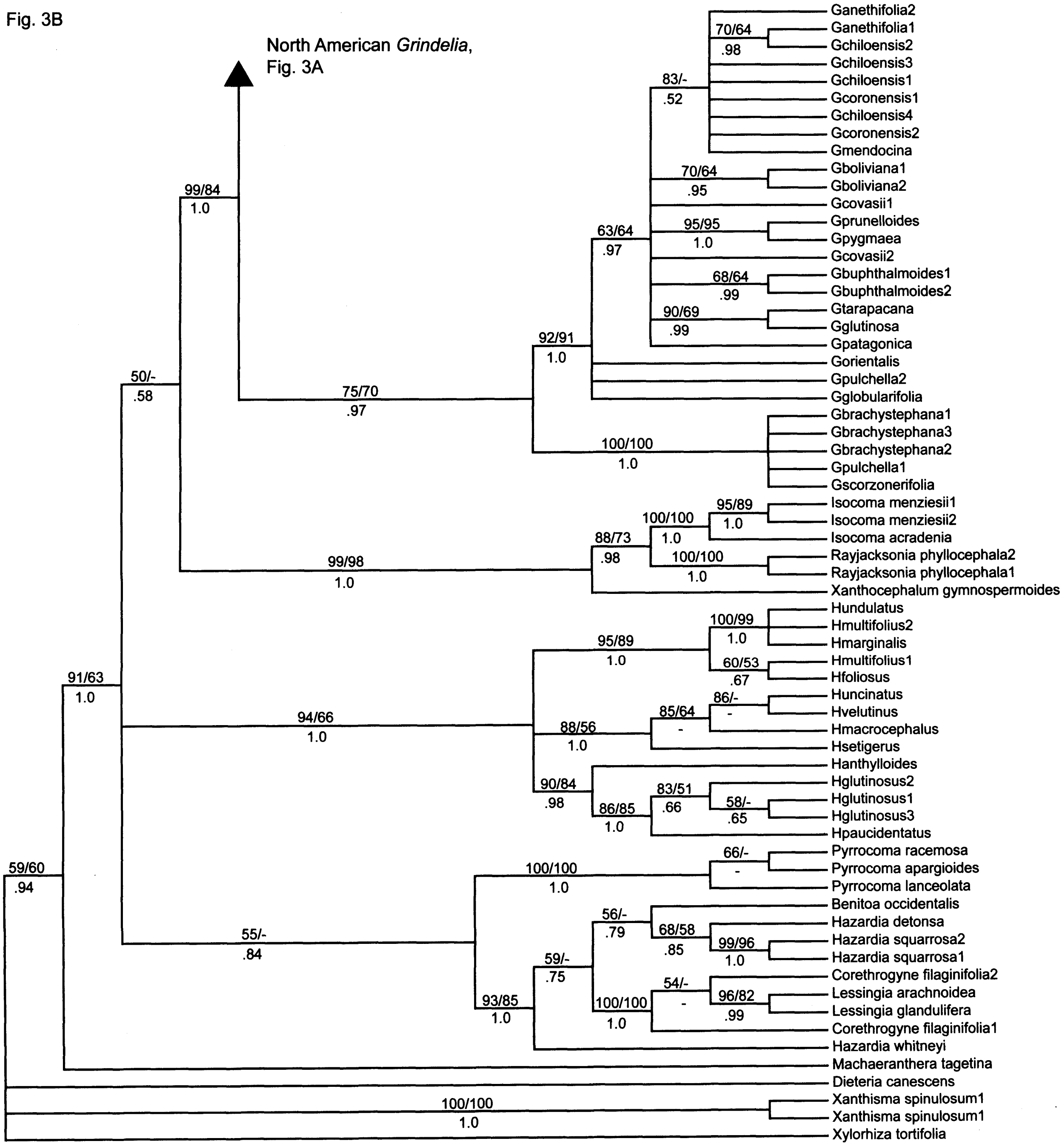
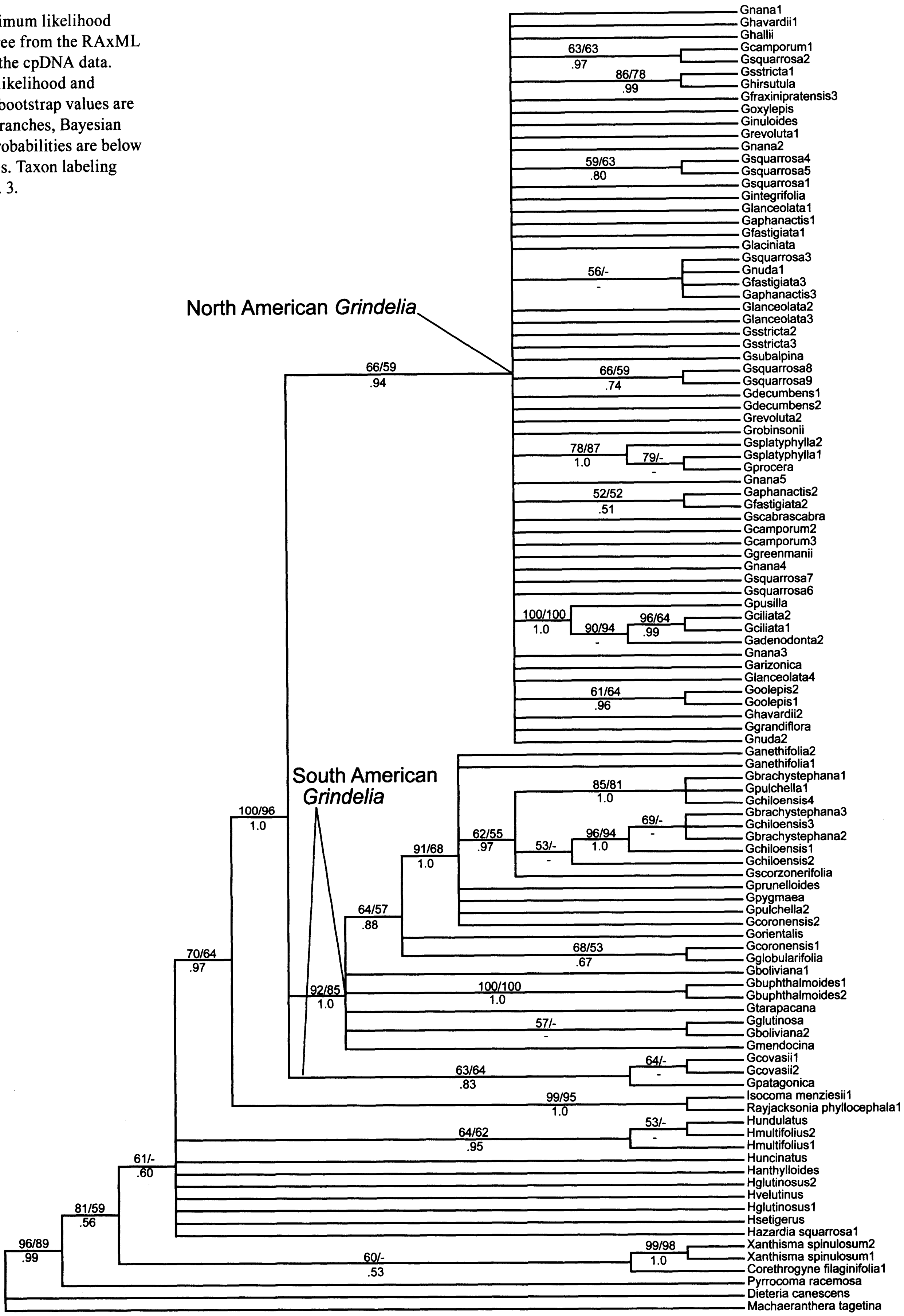


Fig. 4. Maximum likelihood bootstrap tree from the RAxML analysis of the cpDNA data. Maximum likelihood and parsimony bootstrap values are above the branches, Bayesian posterior probabilities are below the branches. Taxon labeling follows Fig. 3.



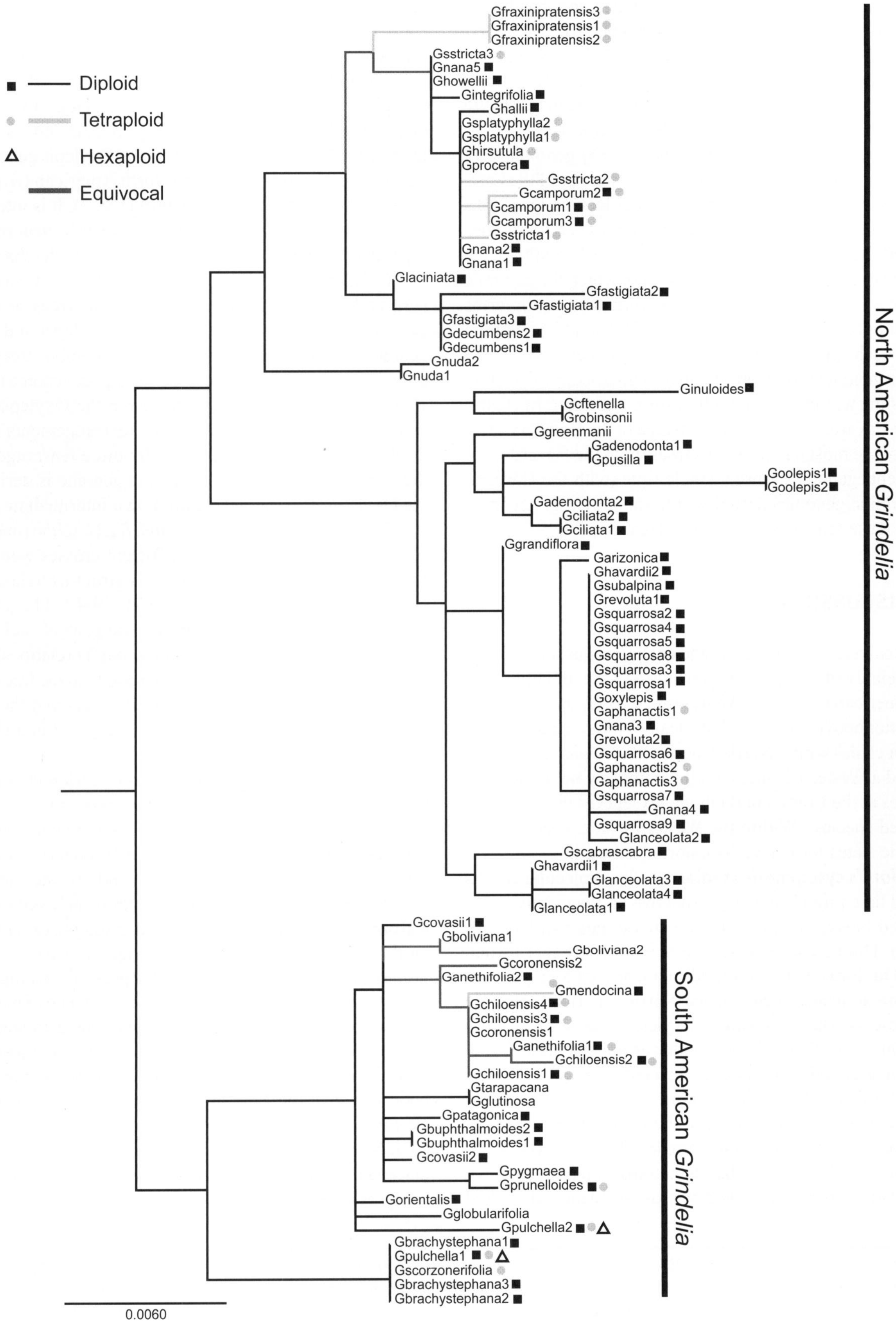


Fig. 5. Maximum likelihood tree from the RAxML analysis of the nrDNA data (lnL = −4557.92) with branches shaded according to the parsimony reconstruction of ancestral ploidy and symbols at the tips of the branches indicating the ploidy of the taxa when known. Taxon labeling follows Fig. 3.

one or sometimes two adjacent areas and had ranges that did not overlap with those of their sister clade(s) (Fig. 6). The ancestors of the Western Clade were resolved as being from North America between the Rocky Mountains and the Cascade/Sierra Nevada axis, while the ancestors of South American *Grindelia* were resolved as being from eastern South America.

In the analyses in which Dunford's (1986) genomic data were combined with the nrDNA data, the topology of the Eastern Clade was less resolved than in the trees lacking Dunford's genomic data. However, the differences between topologies concerned exclusively poorly supported clades. In addition, the topologies of the trees from the analyses in which the genomic character was given different weights were identical in terms of well-supported clades (Fig. 7). The Hallii and Subalpina genomes each appeared to have arisen only once; however, the Subalpina genome is only present in a single species. The Oxylepis genome was present in both Eastern and Western clades, while the Havardii genome was confined to the Eastern Clade. In some of the most parsimonious trees, both the Havardii and the Oxylepis genomes arose a single time (with the Havardii and Subalpina genomes nested within the Oxylepis genome), although these branches lacked bootstrap support.

■ DISCUSSION

All analyses of both nrDNA and cpDNA sequences support the monophyly of *Grindelia* as a whole and the monophyly of North American *Grindelia*. Analyses of nrDNA data also support the monophyly of South American *Grindelia* and support two major clades within North American *Grindelia*: an Eastern Clade and a Western Clade, with the boundary between those two clades at the Continental Divide (except for three widely-distributed species). Within the Western Clade, species from the Pacific states form a well-supported subclade.

Dunford's cytogenetic studies. — The molecular results presented here provide a new perspective on the extensive experimental biosystematic and cytogenetic data available for *Grindelia*. Through his observations of meiosis in experimental hybrids, Dunford (1986) found and characterized four different chromosomal arrangements in the North American taxa. He called these the Hallii, Havardii, Oxylepis, and Subalpina genomes (Dunford, 1986). These genomic arrangements are separated from one another by one to three reciprocal translocations.

Dunford's (1970a) Hallii genome was documented only from taxa shown here to belong to the Pacific Clade (Fig. 7); all members of the clade that he sampled shared the Hallii genome. Dunford determined that the tetraploid members of the Pacific Clade were autotetraploid by crossing them with diploid members. Each tetraploid appeared to possess two copies of the Hallii genome that had not undergone extensive rearrangement following tetraploidization (Dunford, 1964, 1983).

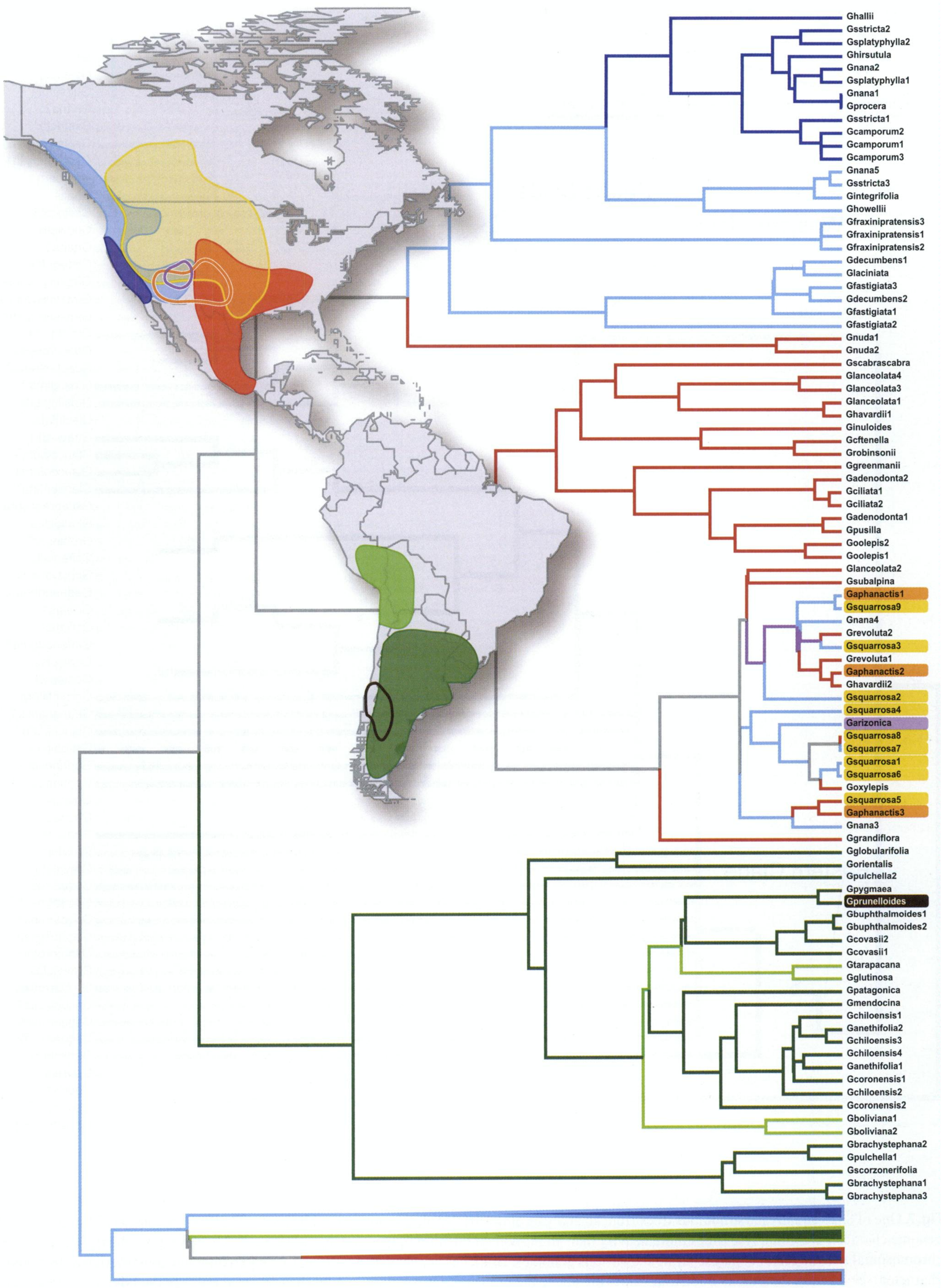
The Oxylepis genome (Dunford, 1970a, 1986) occurs in two places on the tree: in the Western Clade in *G. fastigiata* and in the Eastern Clade in *G. squarrosa* and its relatives. Dunford (1970a) hypothesized that the Oxylepis genome could be the ancestral arrangement in North American *Grindelia*, as resolved here without good support (Fig. 7). It is intermediate in structure between the Hallii and Havardii genomes and is separated from each of them by a single arm-interchange event.

Grindelia subalpina is part of the polytomy with *G. squarrosa* and relatives in the strict-consensus trees or variously grouped with plants that possess the Oxylepis and Havardii genomes in the individual equally parsimonious trees. It is the only species known to possess the Subalpina genome (Dunford, 1986). This genome is separated from the Oxylepis genome possessed by *G. squarrosa* by two rearrangements and from the Hallii and Havardii genomes by three rearrangements. It thus seems likely that the Subalpina genome is derived from the Oxylepis genome, via an unknown intermediate genome.

Two remaining species of *Grindelia*, *G. scabra* and *G. oolepis*, have a fifth genome, but sufficient crosses were not performed to completely determine its structure relative to the remaining genomes (Dunford, 1971, 1986). The genome of these two species differs by one rearrangement each from the Havardii and the Oxylepis genomes, but its relationship to the Hallii and Subalpina genomes is unknown. In the trees from the combined analysis of nrDNA sequence data and the genomic data, *G. oolepis* and *G. scabra* were resolved in a clade with plants of uncharacterized genomes.

In one instance, representatives of different populations that are morphologically assignable to one species, *G. nana*, were resolved as members of clades that are characterized by different genomes, with samples from Montana (*G. nana* 3) and Shasta County, California (*G. nana* 1 and 2) nested among taxa with the Hallii genome and the samples from Siskiyou County, California (*G. nana* 4 and 5) nested among taxa with the Havardii and Oxylepis genomes. Although these particular populations have not been examined for genomic arrangement(s), these results appear to reflect either an instance of past hybridization involving taxa of differing genomic arrangement or a taxonomic problem (e.g., cryptic diversity) that awaits resolution concerning *G. nana*. Putative hybrid individuals have been found in the field and herbarium where the ranges of *G. nana* and *G. squarrosa* overlap in northwestern California (A.J. Moore, pers. obs.). Other similar examples of disparate placement of putatively conspecific samples in the phylogenetic analyses are only weakly supported and warrant more study.

Fig. 6. Ultrametric tree obtained from BEAST with areas (coded according to collecting locality, not the range of the species) mapped according to *lagrange*. The branch colors correspond to the areas on the map, with purple branches having a reconstructed ancestral area of both eastern and western North America and intermediate green branches having a reconstructed ancestral area of both eastern and western South America. The names of the taxa that occur in more than one area or that have an otherwise unusual distribution are colored and their ranges are individually outlined on the map. Branches for which an ancestral area was not recovered with greater than 0.70 confidence are colored gray. Outgroup clades are condensed. The outside of the outgroup triangles are colored with the range of the basal taxa, while the inside of the triangle is colored with the range of other taxa within the clade. Taxon labeling follows Fig. 3. The base map is from www.planiglobe.com. ▶


Ghallii
Gsstricta2
Gsplatyphylla2
Ghirsutula
Gnana2
Gsplatyphylla1
Gnana1
Gprocera
Gsstricta1
Gcamporum2
Gcamporum1
Gcamporum3
Gnana5
Gsstricta3
Gintegrifolia
Ghowellii
Gfraxinipratensis3
Gfraxinipratensis1
Gfraxinipratensis2
Gdecumbens1
Glaciniata
Gfastigiata3
Gdecumbens2
Gfastigiata1
Gfastigiata2
Gnuda1
Gnuda2
Gscabrascabra
Glanceolata4
Glanceolata3
Glanceolata1
Ghavardii1
Ginuloides
Gcftenella
Grobinsonii
Ggreenmanii
Gadenodonta2
Gciliata1
Gciliata2
Gadenodonta1
Gpusilla
Goolepis2
Goolepis1
Glanceolata2
Gsubalpina
Gaphanactis1
Gsquarrosa9
Gnana4
Grevoluta2
Gsquarrosa3
Grevoluta1
Gaphanactis2
Ghavardii2
Gsquarrosa2
Gsquarrosa4
Garizonica
Gsquarrosa8
Gsquarrosa7
Gsquarrosa1
Gsquarrosa6
Goxylepis
Gsquarrosa5
Gaphanactis3
Gnana3
Ggrandiflora
Gglobularifolia
Gorientalis
Gpulchella2
Gpygmaea
Gprunelloides
Gbuphthalmoides1
Gbuphthalmoides2
Gcovasii2
Gcovasii1
Gtarapacana
Gglutinosa
Gpatagonica
Gmendocina
Gchiloensis1
Ganethifolia2
Gchiloensis3
Gchiloensis4
Ganethifolia1
Gcoronensis1
Gchiloensis2
Gcoronensis2
Gboliviana1
Gboliviana2
Gbrachystephana2
Gpulchella1
Gscorzonerifolia
Gbrachystephana1
Gbrachystephana3

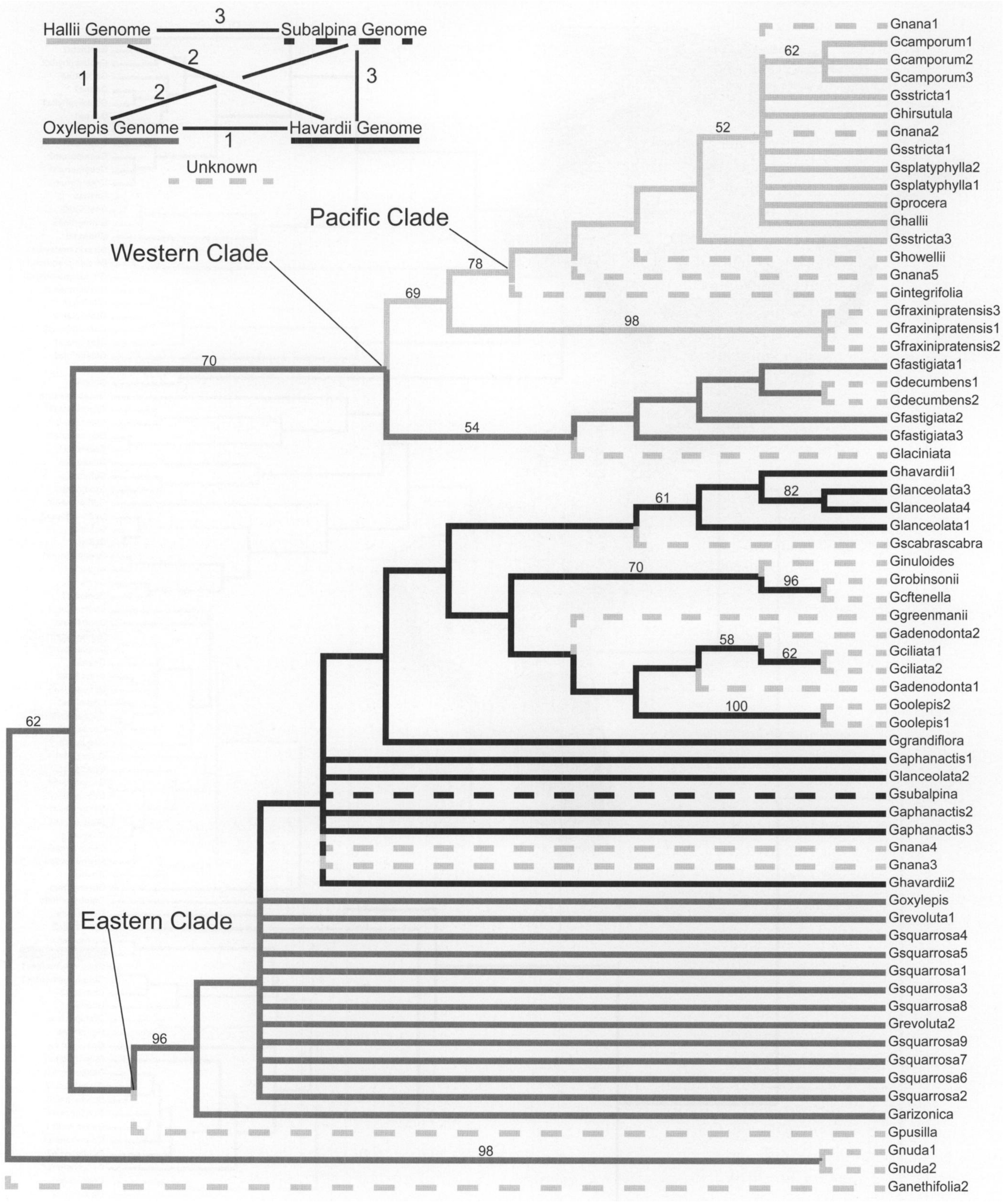


Fig. 7. One of 8325 equally parsimonious trees from an analysis of the nrDNA data combined with Dunford's (1986) genomes (120 steps), with the genome character weighted five times more heavily than an individual position in the sequence alignment. The diagram indicates the number of chromosomal rearrangements that separate the various genomes. Branches are shaded according to the parsimony reconstruction of the genome character (see legend). Parsimony bootstrap values are above the branches. Only values greater than 50 are shown. Taxon labeling follows Fig. 3.

Ploidy level. — Although diploids, tetraploids, and hexaploids are all present within *Grindelia*, the ancestors of each clade, and hence the plant(s) that underwent amphitropical dispersal unequivocally appear to have been diploid based on the molecular trees (Fig. 5).

Tetraploidy appears to have arisen three or four times in North American *Grindelia* based on the molecular data: once in *G. aphanactis* and two or three times in the Western Clade (see Raven & al., 1960; Dunford, 1970b for chromosome numbers). Independent origins of tetraploidy in the Pacific Clade and *G. aphanactis* are supported by Dunford's cytogenetic studies of artificial hybrids, which showed that *G. aphanactis* was separated from the Pacific species by two chromosomal rearrangements. The tetraploid sister to the Pacific Clade, *G. fraxinipratensis*, was not included in Dunford's studies.

Ploidy level is more variable in South American *Grindelia*, with three ploidy levels present: diploid, tetraploid, and hexaploid (Bartoli & Tortosa, 1998b). Tetraploidy appears to have arisen a minimum of three times, with multiple origins possible in each of the following cases: *G. anethifolia* and *G. chiloensis*, *G. prunelloides*, and *G. scorzonerifolia* and *G. pulchella*. Although we were able to sample only one of the hexaploid species, hexaploidy likely arose twice, as one of the hexaploid species, *G. pulchella*, also has diploid and tetraploid members.

Steyermark's lineages. — Steyermark (1937) considered some taxa, mainly those that are found in Mexico and Texas, to be basal within *Grindelia* and to have existed in their present locations for a long period of time. He considered other taxa, namely those on the Pacific Coast and *G. squarrosa* in the interior of the continent, to have radiated recently into new habitats. He expressed his phylogenetic hypotheses in a tree diagram (Steyermark, 1937: fig. 3).

Although the molecular trees do not correspond closely to Steyermark's, there are some notable similarities. The species that he considered to be basal (i.e., not resulting from recent radiations) are *G. arizonica*, *G. grandiflora*, *G. havardii*, *G. lanceolata*, *G. scabra* var. *neomexicana* (Wooton & Standl.) Steyerm., and the Mexican species. In the molecular tree, these taxa represent early diverging lineages of the Eastern Clade and tend to have at least some sequence differences to distinguish them from other species, in keeping with a longer independent history.

Steyermark (1937) also hypothesized that *G. squarrosa* spread rapidly throughout the central-western part of North America. This suggestion is borne out by the molecular finding that all accessions of *G. squarrosa* are part of a (more diverse) clade with very few nucleotide substitutions and no indels differentiating its members. Although Steyermark did not discuss in detail the relationships of the other species resolved here as relatives of *G. squarrosa*, he did place them near *G. squarrosa* in his tree.

The only one of Steyermark's (1937) lineages that is found intact in the molecular tree corresponds to the Pacific Clade, resolved here. Steyermark was the only previous investigator to put this particular group of taxa together as close relatives while simultaneously excluding all other taxa. He hypothesized that the Pacific species radiated recently onto newly exposed or newly formed soils. This hypothesis of recent radiation is supported by the very short molecular branches that distinguish the taxa of the Pacific Clade from one another and from their most recent common ancestor. Steyermark attributed the great morphological variability of the Pacific taxa to their youth, stating, "the many variations have not yet had time to differentiate themselves, nor have the geographic barriers been great enough to have accomplished this" (Steyermark, 1937: 246).

Other treatments. — All three species that have been included in other genera based on their pappus morphology—*G. ciliata* [*Prionopsis ciliata*, Nesom & al., 1993], *G. anethifolia* [*Haplopappus pectinatus*, Bartoli & Tortosa 1998a, or *Notopappus pectinatus* (Phil.) Klingenb., Klingenberg 2007], and *G. prunelloides* [*H. prunelloides*, Bartoli & Tortosa, 1999a, or *N. ameghinoi* (Speg.) Klingenb., *N. andinus* (Phil.) Klingenb., *N. chryseus* (Kuntze) Klingenb., and *N. prunelloides* (Less.) Lingenb., Klingenberg, 2007]—are nested well within *Grindelia*. In addition, the two sampled members of Klingenberg's (2007) genus *Notopappus* did not form a monophyletic group within *Grindelia*. The position of *G. ciliata* resolved here is congruent with the close relationship of *Prionopsis ciliata* and the single representative of *Grindelia*, *G. lanceolata*, in the higher-level trees of Morgan & Simpson (1992) and Morgan (1997, 2003).

Lane (1992) considered *G. camporum*, *G. hirsutula*, and *G. stricta* to form a clade. She hypothesized that *G. nana* and *G. integrifolia* were more closely related to *G. squarrosa* than to *G. camporum*, *G. hirsutula*, and *G. stricta*. The molecular trees do show some evidence of potential hybridization between *G. nana* and *G. squarrosa* (see discussion of Dunford's studies above). However, it appears that the affinities of *G. nana* and *G. integrifolia* lie with the members of the Pacific Clade rather than with *G. squarrosa*, which is a member of the Eastern Clade.

Strother & Wetter (2006) took a much broader view of the circumscription of many *Grindelia* species than had previous authors. Most of the plants that Strother & Wetter classified as *G. hirsutula* (*G. camporum*, *G. fastigiata*, *G. nana*, and *G. stricta*), as well as several species they considered to be allied to *G. hirsutula* (*G. decumbens*, *G. howellii*, and *G. integrifolia*) were resolved in the Western Clade. However, other members of the Western Clade (*G. fraxinipratensis*, *G. laciniata*) were not considered by Strother & Wetter to be allied with *G. hirsutula*. One member of Strother & Wetter's *G. hirsutula* was resolved in the Eastern Clade, *G. revoluta*. It appears to be closely related to *G. squarrosa*.

In addition, Strother & Wetter (2006) expanded *G. arizonica* to include *G. laciniata*. Molecular data indicate that the similarity of those two species may be caused by convergence, as *G. arizonica* was resolved in the Eastern Clade, whereas *G. laciniata* was resolved in the Western Clade. *Grindelia aphanactis*, treated by Strother & Wetter as part of *G. squarrosa*, was part of a polytomy with *G. squarrosa* in the molecular trees; the sequences of the two species were nearly identical. More extensive sampling with more rapidly evolving markers will be necessary before the hypotheses presented in Strother & Wetter's (2006) treatment can be fully tested.

Biogeography and ecology: North America. — The two major clades of North American *Grindelia* have overlapping but distinctive distributions (Fig. 6). One of the clades contains all of the species that are endemic to the Pacific states/provinces as well as species native to Nevada, Utah, western New Mexico, and Colorado west of the crest of the Rocky Mountains. The other clade consists of all of the species found in Texas, Mexico, Wyoming, eastern New Mexico, and Colorado east of the crest of the Rockies, as well as *G. arizonica*, which occurs on and south of the Colorado Plateau, and the widespread *G. squarrosa* and *G. aphanactis*. As *Grindelia* is largely absent from both forested and alpine areas, it is likely that the Rocky Mountains presented a barrier to dispersal that kept the two lineages separate. Given that the Rocky Mountains have been at approximately their current elevation since the end of the Laramide Orogeny in the Eocene (Dickinson & al., 1988; McMillan & al., 2006) and that the origin of the asteroid tribes (including Astereae) has been dated to approximately 30 million years ago (Kim & al., 2005, Torices 2010), dispersal is more likely than vicariance to explain this distribution. In addition, the more northern taxa are nested well within the two clades, as would be expected from a pattern of colonization from the south, but not from a pattern of vicariance due to the uplift of the Rocky Mountains.

The plants sampled from the California Floristic Province (CA-FP) form a subclade within the Pacific Clade, which otherwise consists mainly of taxa long considered to be closely related to, or even conspecific with, the plants in the CA-FP. The Pacific Clade in turn appears to have descended from species native to the desert southwest. This interpretation is congruent with Raven & Axelrod's (1978) hypothesis that *Grindelia* is a desert element in the California flora.

It has been hypothesized that desert plants would be preadapted to the summer drought of the CA-FP, given their ability to survive without rain for multiple hot months (Axelrod, 1975; Raven & Axelrod, 1978; Ackerly, 2009). *Grindelia* belongs to a subtribe (Machaerantherinae) of tribe Astereae that mainly occurs in desert or other dry-land habitats in western North America (e.g., Morgan, 2003). Other clades within the Machaerantherinae have either radiated in the CA-FP (*Lessingia*/*Corethrogyne* DC., Markos & Baldwin, 2001), or have CA-FP endemic taxa (e.g., *Hazardia*, *Isocoma*, Markos & Baldwin, 2001; *Pyrrocoma*, Morgan, 2003).

Although many of the taxa in the Pacific Clade are quite drought-tolerant and some flower in late summer or early fall after extended periods without rain, the Pacific Clade also includes taxa that are adapted to both salt- and fresh-water wetland habitats. Tolerance to physical drought may have helped plants adapt more easily to the physiological drought caused by growing in saline soils. One of these, *G. stricta* var. *angustifolia*, is the only shrub in North American *Grindelia*. Other taxa are suffrutescent, with short woody stems at the base from which herbaceous shoots grow each year, but *G. stricta* var. *angustifolia* has woody stems that are up to two meters in length.

The sister group of the Pacific Clade is *G. fraxinipratensis*, which is endemic to alkaline meadows in the Amargosa Valley in southwestern Nevada and adjacent California, principally in the Ash Meadows National Wildlife Refuge in Nevada. Twenty-four species of plants and animals are endemic to the Ash Meadows area (Trammell & al., 2008) and none of the other endemic plants that has been studied in a phylogenetic context is sister to such a diverse non-desert clade. For example, *Zeltnera namophila* (Reveal & al.) G.Mans. is nested well within the California Clade of *Zeltnera* G. Mans. (Mansion & Zeltner, 2004). *Cordylanthus tecopensis* Munz & J.C. Roos belongs to a clade composed of the remaining members of *Cordylanthus* Benth. subgenera *Hemistegia* (A. Gray) Jeps. and *Dicranostegia* (A. Gray) T.I. Chuang & Heckard, which are native to alkaline wetlands throughout western North America (Tank & Olmstead, 2008).

Amphitropical disjunction. — *Grindelia* appears to fit the pattern of a desert amphitropical disjunct. Within North America, species from Texas, Mexico, and the dry-land areas of the southwestern United States diverge basally in both clades. The taxa that grow along the Pacific coasts of North and South America are well nested within the North and South American clades, respectively. The fact that both North and South American clades are old enough to have undergone significant independent diversification is also congruent with the pattern seen in many other desert disjuncts (e.g., *Astragalus*, Scherson & al., 2008; *Tiquilia*; Moore & Jansen, 2006, Moore & al., 2006). *Grindelia* is somewhat unusual among desert disjuncts, however, in representing only a single amphitropical dispersal, as does, for example, *Ephedra* L. (Ickert-Bond & al., 2009). Many clades of desert disjuncts have undergone multiple amphitropical dispersal events (Wen & Ickert-Bond, 2009; e.g., *Hoffmannseggia*, Simpson & al., 2005; *Lycium* L., Levin & Miller, 2005; *Tiquilia*, Moore & Jansen, 2006, Moore & al., 2006).

Grindelia does not appear to have any adaptations for long-distance dispersal. Its fruits (cypselae) do have a pappus, but the pappus falls off so readily that removing a cypsela from a head with the pappus still attached is difficult (A.J. Moore, pers. obs.). Birds readily consume the fruits (A.J. Moore, pers. obs.), but as the seed is the main nutritive component, endozoochory seems to be an unlikely dispersal mechanism. In some species, the resins on the developing flower heads are still present in the fruiting stage, causing the fruits to be sticky (A.J. Moore, pers. obs.). Intact fruits could thus potentially become stuck to the bills or other parts of birds that are eating the fruits or that otherwise come in contact with the plants. Lack of obvious means for long-distance dispersal may explain why only one successful amphitropical dispersal is evident in *Grindelia*.

The pattern of relationships between North and South American *Grindelia* by itself cannot rule out the possibility of vicariance instead of dispersal as an explanation for the intercontinental disjunction. However, vicariance has been considered to be less likely for most species with a desert disjunction pattern, given a lack of suitable habitat in the intervening regions and a lack of animals shared between the two areas, as would be expected under a vicariance scenario (Carlquist, 1983; Simpson & Neff, 1985; Morrell & al., 2000; Moore & al., 2006).

The direction of amphitropical dispersal in *Grindelia* is not evident from the phylogenetic data, but a North American origin with subsequent dispersal to South America is consistent with a North American center of diversity of the Machaerantherinae, and with western North American endemism of most of the close relatives of *Grindelia* (Nesom & Robinson, 2007). If this hypothesis is correct, then the ancestors of the South American genus *Haplopappus* underwent an independent dispersal from North to South America. If *Haplopappus* were ultimately resolved to be the sister group of *Grindelia*, then it would be equally parsimonious to assume two independent dispersals from North to South America (one for *Haplopappus* and one for South American *Grindelia*) or to assume one dispersal from North to South America (for the ancestor of *Haplopappus* and *Grindelia*), followed by a dispersal from South to North America (for North American *Grindelia*). Regardless of its direction, the amphitropical dispersal of *Grindelia* must have either occurred before the crown groups diversified, or early enough in the diversification of the crown groups that monophyly could be re-established by coalescent processes.

■ ACKNOWLEDGEMENTS

We would like to thank T. Blankenship, R. Byrne, D. Canestro, C. Gonzàlez, S. Laymon, R. Lutzy, J.E. Moore, J.L. Moore, W.L. Moore, M.S. Park, G.H. Rua, R.L. Welch, P. Williams, and E.H. Zacharias for assistance in the field; C.M. Guilliams, M.S. Park, B L. Wessa, and E.H. Zacharias for assistance in the laboratory; Cleveland National Forest, Kern National Wildlife Refuge, University of California Natural Reserve System Kenneth S. Norris Rancho Marino Reserve, and Welder Wildlife Refuge for permission to collect; C. di Vittorio, C. Hobbs, D.G. Kelch, P. Lessica, M.S. Park, F. Ratto, S. Teiller, and E.H. Zacharias for specimens; the curators of the herbaria CAS/DS and UC/JEPS for permission to sample leaf material; C.M. Guilliams, D.T. Harbaugh, C. Hobbs, D.G. Kelch, R. Kirkpatrick, M.S. Park, G.H. Rua, D. Stone, J.L. Strother, G. Walden, R.L. Welch, and E.H. Zacharias for helpful discussions; D. Franke for preparing Fig. 6; J.S. Ascher and M. Leong for bee identification; the Lawrence R. Heckard Endowment Fund of the Jepson Herbarium, the Department of Integrative Biology, and the American Society of Plant Taxonomists for funding; and D.D. Ackerly, R. Byrne, B.D. Mishler, J.L. Moore, T.F. Stuessy, and one anonymous reviewer for helpful comments on earlier drafts of the manuscript.

■ LITERATURE CITED

Ackerly, D.D. 2009. Evolution, origin and age of lineages in the Californian and Mediterranean floras. *J. Biogeogr.* 36: 1221–1233.

Axelrod, D.I. 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. *Ann. Missouri Bot. Gard.* 62: 280–334.

Baldwin, B.G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: An example from the Compositae. *Molec. Phylogenet. Evol.* 1: 3–16.

Baldwin, B.G. & Markos, S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: Congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molec. Phylogenet. Evol.* 10: 449–463.

Bartoli, A. 1993. Presencia de hexaploides ($2n = 36$) en *Grindelia chiloensis* (Corn.) Cabrera (Asteraceae). *Bol. Soc. Argent. Bot.* 29: 139–142.

Bartoli, A. & Tortosa, R.D. 1998a. Intermediacy between *Grindelia chiloensis* and *Haplopappus pectinatus* (Astereae, Asteraceae). *Taxon* 47: 337–345.

Bartoli, A. & Tortosa, R.D. 1998b. Estudios cromosómicos en especies sudamericanas de *Grindelia* (Asteraceae, Astereae). *Kurtziana* 26: 165–171.

Bartoli, A. & Tortosa, R.D. 1999a. Novedades en *Grindelia* (Asteraceae, Astereae). *Hickenia* 3: 1–4.

Bartoli, A. & Tortosa, R.D. 1999b. Revisión de las especies sudamericanas de *Grindelia* (Asteraceae: Astereae). *Kurtziana* 27: 327–359.

Bartoli, A. & Tortosa, R.D. 2003a. Architecture of the genus *Grindelia* (Asteraceae: Astereae). *Flora* 198: 106–111.

Bartoli, A. & Tortosa, R.D. 2003b. A new species of *Grindelia* (Asteraceae, Astereae) from the Meseta del Somuncura (Patagonia), Argentina. *Brittonia* 55: 146–149.

Cabrera, Á.L. 1932. Revisión de las especies sudamericanas del género *Grindelia. Revista Mus. La Plata* 33: 207–249.

Carlquist, S. 1983. Intercontinental dispersal. *Sonderb. Naturwiss. Vereins Hamburg.* 7: 37–47.

Cruden, R.W. 1966. Birds as agents of long-distance dispersal for disjunct plant groups of the temperate Western Hemisphere. *Evolution* 20: 517–532.

Dickinson, W.R., Klute, M.A., Hayes, M.J., Janecke, S.U., Lundin, E.R., McKittrick, M.A. & Olivares, M.D. 1988. Paleogeographic and paleotectonic setting of Laramide sedimentary basins in the central Rocky Mountain region. *Bull. Geol. Soc. Amer.* 100: 1023–1039.

Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analyses by sampling trees. *B. M. C. Evol. Biol.* 7: 214. doi: 10.1186/1471-2148-7-214.

Drummond, A.J., Ho, S.Y.W., Rawlence, N. & Rambaut, A. 2007. A rough guide to BEAST 1.4. http://beast-mcmc.googlecode.com/files/BEAST14_Manual_6July2007.pdf (accessed March 2011).

Dunford, M.P. 1964. A cytogenetic analysis of certain polyploids in *Grindelia* (Compositae). *Amer. J. Bot.* 51: 49–56.

Dunford, M.P. 1970a. Interchange heterozygosity in diploid interspecific hybrids in *Grindelia* (Compositae). *Amer. J. Bot.* 57: 623–628.

Dunford, M.P. 1970b. Triploid and tetraploid hybrids from diploid × tetraploid crosses in *Grindelia* (Compositae). *Amer. J. Bot.* 57: 856–860.

Dunford, M.P. 1971. Chromosome characteristics of Texas species of *Grindelia* (Compositae) and some interspecific hybrids. *S. W. Naturalist* 16: 131–142.

Dunford, M.P. 1983. Chromosome analysis of hybrids between 4N *Grindelia camporum* and 2N *G. grandiflora* (Compositae). *Amer. J. Bot.* 70: 1320–1324.

Dunford, M.P. 1986. Chromosome relationships of diploid species of *Grindelia* (Compositae) from Colorado, New Mexico, and adjacent areas. *Amer. J. Bot.* 73: 297–303.

Ellison, N.W., Liston, A., Steiner, J.J., Williams, W.M. & Taylor, N.L. 2006. Molecular phylogenetics of the clover genus (*Trifolium*—Leguminosae). *Molec. Phylogenet. Evol.* 39: 688–705.

Galtier, N., Gouy, M. & Gautier, C. 1996. SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. *Bioinformatics* 12: 543–548.

Gouy, M., Guindon, S. & Gascuel, O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molec. Biol. Evol.* 27: 221–224.

Guggisberg, A., Mansion, G. & Conti, E. 2009. Disentangling reticulate evolution in an Arctic-Alpine polyploid complex. *Syst. Biol.* 58: 55–73.

Hagen, K.B. von & Kadereit, J.W. 2001. The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the Southern Hemisphere as revealed by nuclear and chloroplast DNA sequence variation. *Organisms Diversity Evol.* 1: 61–79.

Heckard, L.R. 1963. The Hydrophyllaceae. *Quart. Rev. Biol.* 38: 117–123.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Ickert-Bond, S.M., Rydin, C. & Renner, S.S. 2009. A fossil-calibrated relaxed clock for *Ephedra* indicates an Oligocene age for the divergence of Asian and New World clades and Miocene dispersal into South America. *J. Syst. Evol.* 47: 444–456.

Keck, D.D. 1959. *Grindelia*. Pp. 1163–1166 in: Munz, P.A., *A California Flora*. Berkeley: University of California Press.

Kim, K.-J., Choi, K.-S. & Jansen, R.K. 2005. Two chloroplast DNA inversions originated simultaneously during the early evolution of the sunflower family (Asteraceae). *Molec. Biol. Evol.* 22: 1783–1792.

Klingenberg, L. 2007. Monographie der südamerikanischen Gattungen *Haplopappus* Cass. und *Notopappus* L. Klingenberg (Asteraceae—Astereae). *Biblioth. Bot.* 157: 1–331.

Lane, M.A. 1992. Transcordilleran transgressions: Effects of translation and hybridization in California and Pacific Northwest *Grindelia* (Compositae: Astereae). *Amer. J. Bot.* 79(Suppl.): 150–151.

Lane, M.A. 1993. *Grindelia*. Pp. 271–274 in: Hickman, J.C. (ed.), *The Jepson Manual: Higher Plants of California*. Berkeley: University of California Press.

Levin, R.A. & Miller, J.S. 2005. Relationships within tribe Lycieae (Solanaceae): Paraphyly of *Lycium* and multiple origins of gender dimorphism. *Amer. J. Bot.* 92: 2044–2053.

Lia, V.V., Confalonieri, V.A., Comas, C.I. & Hunziker, J.H. 2001. Molecular phylogeny of *Larrea* and its allies (Zygophyllaceae): Reticulate evolution and the probable time of creosote bush arrival to North America. *Molec. Phylogenet. Evol.* 21: 309–320.

Maddison, W.P. & Maddison, D.R. 2008. Mesquite: A modular system for evolutionary analysis. Version 2.5. http://mesquiteproject.org.

Mansion, G. & Zeltner, L. 2004. Phylogenetic relationships within the New World endemic *Zeltnera* (Gentianaceae-Chironiinae) inferred from molecular and karyological data. *Amer. J. Bot.* 91: 2069–2086.

Markos, S. & Baldwin, B.G. 2001. Higher-level relationships and major lineages of *Lessingia* (Compositae, Astereae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. *Syst. Bot.* 26: 168–183.

McMillan, M.E., Heller, P.L. & Wing, S.L. 2006. History and causes of post-Laramide relief in the Rocky Mountain orogenic plateau. *Bull. Geol. Soc. Amer.* 118: 393–405.

Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T., Chan, L., Hoover, P. & Warnow, T. 2009. The CIPRES Portals. CIPRES. http://www.phylo.org/sub_sections/portal (accessed: 4 Aug. 2009). (Archived by WebCite(r) at http://www.webcitation.org/5imQlJeQa)

Moore, M.J. & Jansen, R.K. 2006. Molecular evidence for the age, origin, and evolutionary history of the American desert plant genus *Tiquilia* (Boraginaceae). *Molec. Phylogenet. Evol.* 39: 668–687.

Moore, M.J., Tye, A. & Jansen, R.K. 2006. Patterns of long-distance dispersal in *Tiquilia* subg. *Tiquilia* (Boraginaceae): Implications for the origins of amphitropical disjuncts and Galápagos Islands endemics. *Amer. J. Bot.* 93: 1163–1177.

Morgan, D.R. 1997. Reticulate evolution in *Machaeranthera* (Asteraceae). *Syst. Bot.* 22: 599–615.

Morgan, D.R. 2003. nrDNA external transcribed spacer (ETS) sequence data, reticulate evolution, and the systematics of *Machaeranthera* (Asteraceae). *Syst. Bot.* 28: 179–190.

Morgan, D.R. & Simpson, B.B. 1992. A systematic study of *Machaeranthera* (Asteraceae) and related groups using restriction site analysis of chloroplast DNA. *Syst. Bot.* 17: 511–531.

Morrell, P.L., Porter, J.M. & Friar, E.A. 2000. Intercontinental dispersal: The origin of the widespread South American plant species *Gilia laciniata* (Polemoniaceae) from a rare California and Oregon coastal endemic. *Pl. Syst. Evol.* 224: 13–32.

Nesom, G.L. 1990. Studies in the systematics of the Mexican and Texan *Grindelia* (Asteraceae: Astereae). *Phytologia* 68: 303–332.

Nesom, G.L. 1992. *Grindelia villarrealii* (Asteraceae: Astereae), a new species from northeastern Mexico. *Phytologia* 73: 264–266.

Nesom, G.L. & Robinson, H. 2007. Astereae. Pp. 284–342 in: Kadereit, J.W. & Jeffrey, C. (eds.), *The families and genera of vascular plants*, vol. 8, *Flowering plants: Eudicots; Asterales*. Berlin: Springer.

Nesom, G.L., Suh, Y. & Simpson, B.B. 1993. *Prionopsis* (Asteraceae: Astereae) united with *Grindelia*. *Phytologia* 75: 341–346.

Nylander, J.A.A. 2004. MrModeltest, version 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Ornduff, R. 1963. Experimental studies in two genera of Helenieae (Compositae): *Blennosperma* and *Lasthenia*. *Quart. Rev. Biol.* 38: 141–150.

Parodi, L.R. 1949. Las gramíneas sudamericanas del género *Deschampsia*. *Darwiniana* 8: 415–475.

Petit, R.J. & Excoffier, L. 2009. Gene flow and species delimitation. *Trends Ecol. Evol.* 24: 386–393.

Raven, P.H. 1963. Amphitropical relationships in the floras of North and South America. *Quart. Rev. Biol.* 38: 151–177.

Raven, P.H. 1972. Plant species disjunctions: A summary. *Ann. Missouri Bot. Gard.* 59: 234–246.

Raven, P.H. & Axelrod, D.I. 1978. Origin and relationships of the California flora. *Univ. Calif. Publ. Bot.* 72: 1–134.

Raven, P.H., Solbrig, O.T., Kyhos, D.W. & Snow, R. 1960. Chromosome numbers in Compositae. I. Astereae. *Amer. J. Bot.* 47: 124–132.

Ree, R.H. & Smith, S.A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57: 4–14.

Ree, R.H., Moore, B.R., Webb, C.O. & Donoghue, M.J. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* 59: 2299–2311.

Scherson, R.A., Vidal, R. & Sanderson, M.J. 2008. Phylogeny, biogeography, and rates of diversification of New World *Astragalus* (Leguminosae) with an emphasis on South American radiations. *Amer. J. Bot.* 95: 1030–1039.

Seavey, S.R. & Raven, P.H. 1977. Chromosomal differentiation and the sources of the South American species of *Epilobium* (Onagraceae). *J. Biogeogr.* 4: 55–59.

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Amer. J. Bot.* 94: 275–288.

Simmons, M.P. 2004. Independence of alignment and tree search. *Molec. Phylogenet. Evol.* 31: 874–879.

Simpson, B.B. & Neff, J.L. 1985. Plants, their pollinating bees, and the Great American Interchange. Pp. 427–452 in: Stehli, F.G. & Webb, S.D. (eds.), *The Great American Biotic Interchange*, New York: Plenum.

Simpson, B.B., Tate, J.A. & Weeks, A. 2005. The biogeography of *Hoffmannseggia* (Leguminosae, Caesalpinioideae, Caesalpinieae): a tale of many travels. *J. Biogeogr.* 32: 15–27.

Solbrig, O.T. 1972. The floristic disjunctions between the "Monte" in Argentina and the "Sonoran Desert" in Mexico and the United States. *Ann. Missouri Bot. Gard.* 59: 218–223.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrapping algorithm for the RAxML web-servers. *Syst. Biol.* 57: 758–771.

Steyermark, J.A. 1934. Studies in *Grindelia*. II. A monograph of the North American species of the genus *Grindelia*. *Ann. Missouri Bot. Gard.* 21: 433–608.

Steyermark, J.A. 1937. Studies in *Grindelia*. III. *Ann. Missouri Bot. Gard.* 24: 225–262.

Strother, J.L. & Wetter, M.A. 2006. *Grindelia*. Pp. 424–436 in: Flora of North America Editorial Committee (eds.), *Flora of North America North of Mexico*, vol. 20. New York and Oxford: Oxford University Press.

Swofford, D. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer.

Tank, D.C. & Olmstead, R.G. 2008. From annuals to perennials: Phylogeny of subtribe Castillejinae (Orobanchaceae). *Amer. J. Bot.* 95: 608–625.

Thorne, R.F. 1972. Major disjunctions in the geographic ranges of seed plants. *Quart. Rev. Biol.* 47: 365–411.

Torices, R. 2010. Adding time-calibrated branch lengths to the Asteraceae supertree. *J. Syst. Evol.* 48: 271–278.

Tortosa, R.D. & Bartoli, A. 2001. *Haplopappus ameghinoi* y *H. chryseus* pertenecen al género *Grindelia* (Asteraceae, Astereae). *Bol. Soc. Argent. Bot.* 36: 141–142.

Trammell, E.J., Berry, K., Bassett, S. & Sada, D.W. 2008. Distribution and recovery of vegetational assemblages in Ash Meadows National Wildlife Refuge, Nevada. *S. W. Naturalist* 53: 326–334.

Urbatsch, L.E., Baldwin, B.G. & Donoghue, M.J. 2000. Phylogeny of the coneflowers and relatives (Heliantheae: Asteraceae) based on nuclear rDNA internal transcribed spacer (ITS) sequences and chloroplast DNA restriction site data. *Syst. Bot.* 25: 539–565.

Vargas, P., Baldwin, B.G. & Constance, L. 1998. Nuclear ribosomal DNA evidence for a western North American origin of Hawaiian and South American species of *Sanicula* (Apiaceae). *Proc. Natl. Acad. Sci. U.S.A.* 95: 235–240.

Wen, J. & Ickert-Bond, S.M. 2009. Evolution of the Madrean-Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *J. Syst. Evol.* 47: 331–348.

Wen, J., Lowry, P.P., II, Walck, J.L. & Yoo, K.-O. 2002. Phylogenetic and biogeographic diversification in *Osmorhiza* (Apiaceae). *Ann. Missouri Bot. Gard.* 89: 414–428.

Whitaker, T.W. & Steyermark, J.A. 1935. Cytological aspects of *Grindelia* species. *Bull. Torrey Bot. Club* 62: 69–73.

White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.), *PCR Protocols: A guide to methods and applications*. San Diego: Academic Press.

Appendix. Voucher information for the sequences used in this study. *Grindelia* accessions are listed first followed by all other taxa. For each accession, information is listed in the following order: taxon name, taxon number (used in phylogenetic trees to differentiate individuals of species for which more than one individual was sampled), DNA number (in parentheses); country, state/province, and county (for specimens collected in the United States) or locality (for specimens collected outside of the United States) where specimen was collected; voucher information; ITS GenBank number, ETS GenBank number, and *psaI-accD* GenBank number if available.

Grindelia adenodonta (Steyerm.) G.L. Nesom, 1 (254); U.S.A., Texas, Hays; *Moore & Steyermark 3000* (UC); JQ011985, JQ011874, none. ***Grindelia adenodonta***, 2 (293); U.S.A., Texas, Jackson; Wetter 605 (CAS); JQ011939, JQ011828, JQ012051. ***Grindelia anethifolia*** (Phil.) Adr. Bartoli & Tortosa, 1 (231); Argentina, Neuquén, Zapala; *Moore & al. 7* (BAA); JQ011947, JQ011836, JQ012065. ***Grindelia anethifolia***, 2 (232); Argentina, Neuquén, Pucunches; *Moore & al. 8* (BAA); JQ011946, JQ011835, JQ012064. ***Grindelia aphanactis*** Rydb., 1 (11); U.S.A., AZ, Cochise; *Moore & Park 367* (JEPS); JQ011909, JQ011798, JQ012020. ***Grindelia aphanactis***, 2 (98); U.S.A., CO, Las Animas; *Moore & Moore 589* (JEPS); JQ011927, JQ011816, JQ012038. ***Grindelia aphanactis***, 3 (144); U.S.A., NM, Santa Fe; *Moore & Moore 614* (JEPS); JQ011928, JQ011817, JQ012039. ***Grindelia arizonica*** A. Gray (100); U.S.A., CO, Archuleta; *Moore & Moore 609* (JEPS); JQ011943, JQ011832, JQ012055. ***Grindelia boliviana*** Rusby, 1 (209); Peru, Puno,; *Soukup 56* (UC); JQ011949, JQ011838, JQ012067. ***Grindelia boliviana***, 2 (353); Bolivia, Cochabamba, Quillacollo; *Rua & Aageseu 345* (BAA); none, JQ011887, JQ012089. ***Grindelia brachystephana*** Griseb., 1 (204); Argentina, Corrientes, San Luis del Palmar; *Schinini 16051* (UC); JQ011948, JQ011837, JQ012066. ***Grindelia brachystephana***, 2 (245); Argentina, Buenos Aires, Bahía Blanca; *Marzaro & Ratto s.n.* (BAA 26034); JQ011954, JQ011843, JQ012072. ***Grindelia brachystephana***, 3 (246); Argentina, Chubut, Puerto Madryn; *Bartoli & Tortosa 57-02/1* (BAA); JQ011951, JQ011840, JQ012069. ***Grindelia buphthalmoides*** DC., 1 (237); Argentina, Buenos Aires, Sierra de la Ventana; *Moore & al. 72* (BAA); JQ011959, JQ011848, JQ012077. ***Grindelia buphthalmoides***, 2 (250); Argentina, Buenos Aires, Tornquist; *Marzaro & Ratto s.n.* (BAA 26016); JQ011968, JQ011857, JQ012086. ***Grindelia camporum*** Greene, 1 (6); U.S.A., CA, Alameda; *Moore & Zacharias 433* (JEPS); JQ011896, JQ011785, JQ012007. ***Grindelia camporum***, 2 (22); U.S.A., CA, San Joaquin; *Moore 381* (JEPS); JQ011930, JQ011819, JQ012041. ***Grindelia camporum***, 3 (42); U.S.A., CA, Merced; *Zacharias 947* (JEPS); JQ011931, JQ011820, JQ012042. ***Grindelia chiloensis*** (Cornel.) Cabrera, 1 (5); Argentina, Mendoza; *UCBG 95.0506* (UC); JQ011957, JQ011846, JQ012075. ***Grindelia chiloensis***, 2 (230); Argentina, La Pampa, Cura-Co; *Moore & al. 5* (BAA); JQ011967, JQ011856, JQ012085. ***Grindelia chiloensis***, 3 (241); Argentina, Chubut, Sarmiento; *Ratto s.n.* (BAA 25870); JQ011953, JQ011842, JQ012071. ***Grindelia chiloensis***, 4 (242); Argentina, Río Negro, Sierra Grande to Cerro Corona; *Bartoli & Tortosa 2-02* (BAA); JQ011965, JQ011854, JQ012083. ***Grindelia ciliata*** (Nutt.) Spreng., 1 (10); U.S.A., TX, Jeff Davis; *Moore & Park 365* (JEPS); JQ011940, JQ011829, JQ012052. ***Grindelia ciliata***, 2 (347); U.S.A., TX, Ector; *Moore, Moore & Moore 1008* (JEPS); JQ011997, JQ011886, JQ012050. ***Grindelia coronensis*** Adr. Bartoli & Tortosa, 1 (248); Argentina, Río Negro, Cerro Corona; *Bartoli & Tortosa 39/02* (BAA); JQ011962, JQ011851, JQ012080. ***Grindelia coronensis***, 2 (351); Argentina, Chubut, Telsen; *Bartoli & Tortosa 44/02-2* (BAA); JQ012002, JQ011892, JQ012088. ***Grindelia covasii*** Adr. Bartoli & Tortosa, 1 (229); Argentina, La Pampa, Lihue-Calel; *Moore & al. 1* (BAA); JQ011950, JQ011839, JQ012068. ***Grindelia covasii***, 2 (247); Argentina, La Pampa, Río Colorado; *Bartoli & Tortosa 59/02-1* (BAA); JQ011955, JQ011844, JQ012073. ***Grindelia decumbens*** Greene, 1 (99); U.S.A., CO, La Plata; *Moore & Moore 607* (JEPS); JQ011920, JQ011809, JQ012031. ***Grindelia decumbens***, 2 (126); U.S.A., CO, Montrose; *Moore & Moore 595* (JEPS); JQ011921, JQ011810, JQ012032. ***Grindelia fastigiata*** Greene, 1 (13); U.S.A., UT, Grand; *Moore & Park 397* (JEPS); JQ011910, JQ011799, JQ012021. ***Grindelia fastigiata***, 2 (17); U.S.A., UT, San Juan; *Moore & Park 406* (JEPS); JQ011945, JQ011834, JQ012063. ***Grindelia fastigiata***, 3 (148); U.S.A., NM, Cibola; *Moore & Moore 622* (JEPS); JQ011922, JQ011811, JQ012033. ***Grindelia fraxinipratensis*** Reveal & Beatley, 1 (253); U.S.A., NV, Nye; *Reveal & Holmgren 1887* (UC); JQ011987, JQ011876, none. ***Grindelia fraxinipratensis***, 2 (297); U.S.A., NV, Nye; *Reveal 1536* (DS); JQ011988, JQ011877, none. ***Grindelia fraxinipratensis***, 3 (298); U.S.A., NV, Nye; *Beatley s.n.* (DS 644251); JQ011898, JQ011787, JQ012009. ***Grindelia globularifolia*** Griseb. (240); Argentina, Córdoba, Pampa de Achala; *Tourn s.n.* (BAA); JQ011963, JQ011852, JQ012081. ***Grindelia glutinosa*** (Cav.) Mart. (214); Peru, Arequipa, Islay; *Eyerdam 25175* (UC); JQ011966, JQ011855, JQ012084. ***Grindelia grandiflora*** Hook. (344); U.S.A., TX, Sutton; *Moore, Moore & Moore 995* (JEPS); JQ011995, JQ011884, JQ012061. ***Grindelia greenmanii*** Steyerm. (266); Mexico, Coahuila, Coahuila; *Schoenfeld & Fairey T72M5S* (UC); JQ011932, JQ011821, JQ012043. ***Grindelia hallii*** Steyerm. (319:1); U.S.A., CA, San Diego; *Moore 967:1* (JEPS); JQ011990, JQ011879, JQ012006. ***Grindelia havardii*** Steyerm., 1 (39); U.S.A., TX, Pecos; *Moore & Park 347* (JEPS); JQ011895, JQ011784, JQ012005. ***Grindelia havardii***, 2 (345); U.S.A., TX, Terrell; Moore, *Moore & Moore 1003* (JEPS); JQ011994, JQ011883, JQ012059. ***Grindelia hirsutula*** Hook. & Arn. (18); U.S.A., CA, Marin; *Moore, Byrne & al. 424* (JEPS); JQ011899, JQ011788, JQ012010. ***Grindelia howellii*** Steyerm. (255); U.S.A., ID, Clearwater; *Stillinger 246* (UC); JQ011989, JQ011878, none. ***Grindelia integrifolia*** DC. (8); U.S.A., OR, Benton; *Moore & Moore 276* (JEPS); JQ011907, JQ011796, JQ012018. ***Grindelia inuloides*** Willd. (207); Mexico, Oaxaca, Oaxaca; *King 2941* (UC); JQ011901, JQ011790, JQ012012. ***Grindelia laciniata*** Rydb. (14); U.S.A., UT, San Juan; *Moore & Park 400* (JEPS); JQ011911, JQ011800, JQ012022. ***Grindelia lanceolata*** Nutt., 1 (9); U.S.A., TX, Burnet; *Moore & Park 322* (JEPS); JQ011908, JQ011797, JQ012019. ***Grindelia lanceolata***, 2 (23); U.S.A., TX, Montgomery; *Moore & Park 333* (JEPS); JQ011914, JQ011803, JQ012025. ***Grindelia lanceolata***, 3 (28); U.S.A., TX, Kendall; *Moore & Park 342* (JEPS); JQ011915, JQ011804, JQ012026. ***Grindelia lanceolata***, 4 (355); U.S.A., TX, Hays; *Hobbs s.n.* (JEPS); JQ011991, JQ011880, JQ012057. ***Grindelia mendocina*** Adr. Bartoli & Tortosa

Appendix. Continued.

(352); Argentina, Mendoza, Potezuelo del Choique; *Bartoli & Tortosa 16b/97* (BAA); JQ012003, JQ011893, JQ012087. ***Grindelia nana*** Nutt., 1 (152); U.S.A., CA, Shasta; *Park 1944* (JEPS); JQ011894, JQ011783, JQ012004. ***Grindelia nana***, 2 (153); U.S.A., CA, Shasta; *Park 1944* (JEPS); JQ011903, JQ011792, JQ012014. ***Grindelia nana***, 3 (258); U.S.A., CA, Siskiyou; *Park & al. 2112* (JEPS); JQ011942, JQ011831, JQ012054. ***Grindelia nana***, 4 (259); U.S.A., CA, Siskiyou; *Park & al. 2112* (JEPS); JQ011933, JQ011822, JQ012044. ***Grindelia nana***, 5 (267); U.S.A., MT, Sanders; *Lessica 10076* (JEPS); JQ011926, JQ011815, JQ012037. ***Grindelia nuda*** Wood, 1 (20); U.S.A., TX, Jeff Davis; *Moore & Park 366* (JEPS); JQ011913, JQ011802, JQ012024. ***Grindelia nuda***, 2 (356); U.S.A., TX, Hays; *Hobbs s.n.* (JEPS); JQ011996, JQ011885, JQ012062. ***Grindelia oolepis*** S.F. Blake, 1 (342:1); U.S.A., TX, San Patricio; *Moore 990:1* (JEPS); JQ011993, JQ011882, JQ012060. ***Grindelia oolepis***, 2 (342:2); U.S.A., TX, San Patricio; *Moore 990* (JEPS); JQ011992, JQ011881, JQ012058. ***Grindelia orientalis*** Adr. Bartoli, Tortosa & G.H. Rua (238); Uruguay, Maldonado, Punta Colorada; *Bartoli & al. s.n.* (BAA 22840); JQ011958, JQ011847, JQ012076. ***Grindelia oxylepis*** Greene (205); Mexico, Chihuahua,; *Stuessy 1041* (UC); JQ011900, JQ011789, JQ012011. ***Grindelia patagonica*** Adr. Bartoli & Tortosa (243); Argentina, Chubut, Camarones; *Bartoli & Tortosa 19/99* (BAA); JQ011969, JQ011858, JQ012090. ***Grindelia procera*** Greene (284); U.S.A., CA, Tulare; *Moore, Laymon & Williams 947* (JEPS); JQ011944, JQ011833, JQ012056. ***Grindelia prunelloides*** (Less.) Adr. Bartoli & Tortosa (234); Argentina, Neuquén, Aluminé; *Moore & al. 36* (BAA); JQ011952, JQ011841, JQ012070. ***Grindelia pulchella*** Dunal, 1 (215); Argentina, Mendoza,; *Ruiz Leal 7957* (UC); JQ011964, JQ011853, JQ012082. ***Grindelia pulchella***, 2 (239); Argentina, Buenos Aires, San Antonio de Areco; *Ratto s.n.* (BAA 26018); JQ011960, JQ011849, JQ012078. ***Grindelia pusilla*** (Steyerm.) G.L. Nesom (261); U.S.A., TX, Medina; *Moore & Steyermark 3003* (UC); JQ011938, JQ011827, JQ012049. ***Grindelia pygmaea*** Cabrera (249); Argentina, Río Negro, Meseta de Somuncura; *Bartoli & Tortosa 54/02* (BAA); JQ011956, JQ011845, JQ012074. ***Grindelia revoluta*** Steyerm., 1 (125); U.S.A., CO, Larimer; *Moore & Moore 573* (JEPS); JQ011902, JQ011791, JQ012013. ***Grindelia revoluta***, 2 (149); U.S.A., CO, Jefferson; *Moore & Moore 577* (JEPS); JQ011923, JQ011812, JQ012034. ***Grindelia robinsonii*** Steyerm. (206); Mexico, San Luis Potosi,; *Purpus 5151* (UC); JQ011924, JQ011813, JQ012035. ***Grindelia scabra*** Greene var. ***scabra*** (101); U.S.A., NM, Otero; *Moore & Moore 616* (JEPS); JQ011929, JQ011818, JQ012040. ***Grindelia scorzonerifolia*** Hook. & Arn. (211); Argentina, Corrientes, San Roque; *Schinini & Ahumada 16002* (UC); JQ011970, JQ011859, JQ012091. ***Grindelia squarrosa*** (Pursh) Dunal, 1 (7); U.S.A., ID, Ada; *Moore & Moore 291* (JEPS); JQ011906, JQ011795, JQ012017. ***Grindelia squarrosa***, 2 (12); U.S.A., NV, White Pine; *Moore & Park 394* (JEPS); JQ011937, JQ011826, JQ012048. ***Grindelia squarrosa***, 3 (16); U.S.A., UT, San Juan; *Moore & Park 402* (JEPS); JQ011912, JQ011801, JQ012023. ***Grindelia squarrosa***, 4 (33); U.S.A., OR, Grant; *Moore & Moore 289* (JEPS); JQ011904, JQ011793, JQ012015. ***Grindelia squarrosa***, 5 (97); U.S.A., CO, El Paso; *Moore & Moore 582* (JEPS); JQ011905, JQ011794, JQ012016. ***Grindelia squarrosa***, 6 (103); U.S.A., CA, Nevada; *Moore 638* (JEPS); JQ011936, JQ011825, JQ012047. ***Grindelia squarrosa***, 7 (114); U.S.A., CA, Nevada; *Moore & Moore 306* (JEPS); JQ011935, JQ011824, JQ012046. ***Grindelia squarrosa***, 8 (124); U.S.A., WY, Carbon; *Moore & Moore 565* (JEPS); JQ011919, JQ011808, JQ012030. ***Grindelia squarrosa***, 9 (146); U.S.A., AZ, Coconino; *Moore & Moore 631* (JEPS); JQ011934, JQ011823, JQ012045. ***Grindelia stricta*** DC. var. ***platyphylla*** (Greene) M.A. Lane, 1 (251); U.S.A., CA, Santa Barbara; *Moore 847* (JEPS); JQ011941, JQ011830, JQ012053. ***Grindelia stricta*** var. ***platyphylla***, 2 (282); U.S.A., CA, San Luis Obispo; *Moore 945* (JEPS); JQ011925, JQ011814, JQ012036. ***Grindelia stricta*** var. ***stricta***, 1 (19); U.S.A., CA, Marin; *Moore, Byrne & al. 425* (JEPS); JQ011897, JQ011786, JQ012008. ***Grindelia stricta*** var. ***stricta***, 2 (30); U.S.A., CA, Del Norte; *Moore, Lutzy & Welch 249* (JEPS); JQ011916, JQ011805, JQ012027. ***Grindelia stricta*** var. ***stricta***, 3 (34); U.S.A., WA, King; *Moore, Moore & Moore 313* (JEPS); JQ011917, JQ011806, JQ012028. ***Grindelia subalpina*** Greene (96); U.S.A., WY, Albany; *Moore & Moore 566* (JEPS); JQ011918, JQ011807, JQ012029. ***Grindelia tarapacana*** Phil. (252); Peru, Arequipa, near Arequipa; *di Vittorio s.n.* (UC); JQ011961, JQ011850, JQ012079. ***Grindelia*** cf. ***tenella*** Steyerm. (201); Mexico, San Luis Potosi, San Luis Potosi; *Roe & Roe 2211* (UC); JQ011986, JQ011875, none. ***Benitoa occidentalis*** (H.M. Hall) D.D. Keck; U.S.A., CA, Fresno; *Markos 122* (JEPS); AF251586, AF251644, none. ***Corethrogyne filaginifolia*** Nutt., 1 (350); U.S.A., CA, Mendocino; *Moore 1018* (JEPS); JQ011998, JQ011888, JQ012107. ***Corethrogyne filaginifolia***, 2; U.S.A., CA, Santa Cruz; *Markos 116* (JEPS); AF251593, AF251651, none. ***Dieteria canescens*** (Pursh) Nutt. var. ***aristata*** (Eastw.) D.L. Morgan & R.L. Hartm. (37); U.S.A., UT, Grand; *Moore & Park 398* (JEPS); JQ011980, JQ011869, JQ012101. ***Haplopappus anthylloides*** Meyen & Walp. (272); Chile, Región Metropolitana, Lagunillas; *Teillier 5619* (BAA); JQ011975, JQ011864, JQ012096. ***Haplopappus foliosus*** DC.; Chile; *Rundel s.n.* (UC); AF251577, AF251635, none. ***Haplopappus glutinosus*** Cass., 1 (233); Argentina, Neuquén, Aluminé; *Moore & al. 12* (BAA); JQ011978, JQ011867, JQ012099. ***Haplopappus glutinosus***, 2 (235); Argentina, Neuquén, Aluminé; *Moore & al. 41* (BAA); JQ011976, JQ011865, JQ012097. ***Haplopappus glutinosus***, 3; Chile, Araucanía, Cautin; *Sparre & Constance 17927* (UC); AF251578, AF251636, none. ***Haplopappus macrocephalus*** DC.; Chile, Maule, Talca; *Mahú & Stebbins 8846* (UC); AF251579, AF251637, none. ***Haplopappus marginalis*** Phil.; Chile; *DeVore 1326* (UC); AF251580, AF251638, none. ***Haplopappus multifolius*** Phil., 1 (271); Chile, Región Metropolitana, Lagunillas; *Teillier 5594* (BAA); JQ011974, JQ011863, JQ012095. ***Haplopappus multifolius***, 2 (273); Chile, Región Metropolitana, Valle Río Colorado; *Teillier 5814* (BAA); JQ011973, JQ011862, JQ012094. ***Haplopappus paucidentatus*** Phil.; Chile; *DeVore 1261* (UC); AF251581, AF251639, none. ***Haplopappus setigerus*** (Phil.) F. Meigen (179); Chile, Región Metropolitana, Near Santiago; *Kelch 06.002* (CDF); JQ011979, JQ011868, JQ012100. ***Haplopappus uncinatus*** Phil. (269); Chile, Valparaíso, Las Chilcas; *Teillier 5832* (BAA); JQ011972, JQ011861, JQ012093. ***Haplopappus undulatus*** Klingenb. (270); Chile, Región Metropolitana, Valle Río Maipo; *Teillier 5682* (BAA); JQ011971, JQ011860, JQ012092. ***Haplopappus velutinus*** J. Rémy (268); Chile, Región Metropolitana, Valle del Yeso; *Teillier 5663* (BAA); JQ011977, JQ011866, JQ012098. ***Hazardia detonsa*** (Greene) Greene; U.S.A., CA, Santa Barbara; *UCBG 95.0527* (UC); AF251582, AF251640, none. ***Hazardia squarrosa*** (Hook. & Arn.) Greene var. ***grindelioides*** (DC.) W.D. Clark, 1 (341); U.S.A., CA, Santa Barbara; *Moore 972* (JEPS); JQ012001, JQ011891, JQ012109. ***Hazardia squarrosa*** var. ***grindelioides***, 2; U.S.A., CA, Los Angeles; *Ross 5908* (UC); AF251583, AF251641, none. ***Hazardia whitneyi*** (A. Gray) Greene; U.S.A., CA, Tuolumne; *Ertter 7918* (UC); AF251584, AF251642, none. ***Isocoma acradenia*** (Greene) Greene subsp. ***eremophila*** (Greene) G.L. Nesom; U.S.A., CA, Riverside; *Thorne 55404* (UC); AF251572, AF251630, none. ***Isocoma menziesii*** (Hook. & Arn.) G.L. Nesom, 1 (283); U.S.A., CA, San Luis Obispo; *Moore 946* (JEPS); JQ011983, JQ011872, JQ012104. ***Isocoma menziesii***, 2; U.S.A., CA, Los Angeles; *Bartholomew 535* (UC); AF251571, AF251629, none. ***Lessingia arachnoidea*** Greene; U.S.A., CA, San Mateo; *Markos 126* (JEPS); AF251588, AF251646, none. ***Lessingia glandulifera*** A. Gray var. ***glandulifera***; U.S.A., CA, Madera; *Markos 163* (JEPS); AF251602, AF251660, none. ***Machaeranthera tagetina*** Greene (40); U.S.A., AZ, Cochise; *Moore & Park 368* (JEPS); JQ011982, JQ011871, JQ012103. ***Pyrrocoma apargioides*** (A. Gray) Greene; U.S.A., CA, Plumas; *Schoolcraft 2072* (UC); AF251573, AF251631, none. ***Pyrrocoma lanceolata*** (Hook.) Greene; U.S.A., UT, Piute; *Neese 17626* (UC); AF251574, AF251632, none. ***Pyrrocoma racemosa*** Torr. & A. Gray var. ***sessiliflora*** (Greene) G.K. Br. & D.J. Keil (36); U.S.A., CA, Mono; *Park 1338* (JEPS); JQ011981, JQ011870, JQ012102. ***Rayjacksonia phyllocephala*** (DC.) R.L.Hartm. & M.A. Lane, 1 (343); U.S.A., TX, San Patricio; *Moore 992* (JEPS); JQ012000, JQ011890, JQ012108. ***Rayjacksonia phyllocephala***, 2; U.S.A., TX, Chambers; *Morgan 2032* (TEX); U97645, AF516074, none. ***Xanthisma spinulosum*** (Pursh) D.R. Morgan & R.L. Hartm., 1 (38); U.S.A., TX, Pecos; *Moore & Park 346* (JEPS); JQ011984, JQ011873, JQ012106. ***Xanthisma spinulosum***, 2 (346); U.S.A., TX, Terrell; *Moore, Moore & Moore 1005* (JEPS); JQ011999, JQ011889, JQ012105. ***Xanthocephalum gymnospermoides*** Benth. & Hook.; U.S.A., TX, Jeff Davis; *Morgan 2200* (WWB); U97650, AF516075, none. ***Xylorhiza tortifolia*** (Torr. & A. Gray) Greene; U.S.A., CA, Inyo; *Wisura 4770* (UC); AF251570, AF251628, none.