

Phylogenetic and Population Genetic Studies in *Grindelia* (Asteraceae: Astereae)

By

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## Abstract

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*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. I 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 South American taxa are much more diverse in habit than the North American taxa. 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 (CA-FP) 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, I 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 my trees.

Polyploid complexes have long been a source of confusion to taxonomists due to their combination of morphological and ecological variability with a lack of obvious boundaries between putative species. *Grindelia* (Asteraceae) in the CA-FP provides a prime example of both of these attributes. Both diploid and tetraploid plants occur within the CA-FP, with tetraploids predominating along the coast and diploids in the interior. Although phylogenetic analysis shows that CA-FP *Grindelia* form a clade, relationships within the clade remain unresolved due to a lack of sequence divergence. Complex ecological and morphological variation within CA-FP *Grindelia* has been interpreted as being indicative of either extensive or no taxonomic diversity. I have chosen to follow an intermediate approach, recognizing what I consider to be the most morphological and ecologically distinctive ecotypes or clusters of ecotypes as taxa. In addition, I emphasized putative taxa that appear to maintain their morphological distinctiveness when growing sympatrically or peripatrically in the field.

Most of the morphological and ecological diversity in CA-FP *Grindelia* is present in the tetraploids, which appear to be autotetraploids based on cytogenetic data from prior studies. I used data from six nuclear microsatellites to examine 439 individuals from ten populations (nine tetraploid and one diploid) of *Grindelia* collected in and near the San Francisco Bay Area. I wanted to assess whether any genetic structure was evident across populations or taxa or both. Each of the ten populations was genetically distinct from the others and gene flow among populations appeared to be low. Although the plants grouped more strongly according to population than according to taxon, it was possible to classify > 90% of the individuals according to taxon using discriminant analysis of the microsatellite data.

I would like to dedicate my dissertation to my parents,  
Jane Ehardt Moore and William Loyd Moore,  
and to my brother, John Loyd Moore,  
for all of their love, support, and good humor  
throughout my graduate career.

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## Chapter One

### A taxonomic revision of California *Grindelia*.

#### GENERAL INTRODUCTION

*Grindelia* is a New World genus, with approximately 75 species. It has an amphitropical distribution, with the North American and South American species forming sister clades in phylogenies based on ITS and ETS sequence data (Chapter Two). Within the North American clade, the species native to the Pacific states form a well-supported clade along with *G. howellii* from Montana and Idaho. This clade (called the Pacific Clade in Chapter Two) corresponds to a group hypothesized by Steyermark (1937) to have radiated rapidly in newly-created habitats along the Pacific Coast of North America. The Pacific Clade also corresponds to a group of species found by Dunford (1964) to have the same chromosomal arm arrangement, which he called the Hallii Genome (Dunford 1970).

Most of the species in California belong to this Pacific Clade. The relationships among the plants in this clade were generally poorly resolved in the molecular phylogenies, consistent with a lack of sequence divergence among members of the group. The individuals collected in the California Floristic Province did form a subclade within the Pacific Clade that was supported in the Bayesian analyses.

There are two species of *Grindelia* in California that are not part of the Pacific Clade. Both of these species occur mainly outside of California: *Grindelia squarrosa* occurs throughout most of the western and central United States and Canada; *G. fraxinipratensis* occurs in the Mojave Desert in the vicinity of Ash Meadows in south-eastern Nevada and adjacent California.

#### TAXONOMIC OVERVIEW

The genus *Grindelia* was described in 1807 by Willdenow, based on plants of *G. inuloides* that were being grown in the Royal Botanical Garden in Berlin. These plants were grown from seed collected in Mexico by Humboldt and Bonpland (Edwards 1817). The first specimens of *Grindelia* to be described from California were collected on the Beechey Expedition and described by Hooker and Arnott (1833). Douglas also collected early specimens of *Grindelia* in California, which were described by de Candolle (1836). De Candolle recognized 11 species in his treatment of worldwide *Grindelia*. He noted that the species were difficult to delimit, stating “Genus naturalissimum etiamsi ab auctoribus etiam gravissimus variè vexatum” (1836, p. 316).

Throughout the remainder of the 1800s and early 1900s, the trend was to recognize more species of Californian *Grindelia*. Gray (1884) recognized seven species in California in his treatment of North American *Grindelia*. Jepson (1925) recognized six species of *Grindelia* in California and seven additional varieties. However, only three of Jepson’s six species were the same as those recognized by Gray. Steyermark (1934) recognized 14 species and 23 additional infra-specific taxa in his revision of North American *Grindelia*.

This trend towards recognizing more species was then reversed, and subsequent authors have tended to recognize fewer species in California. Keck (1959) recognized only eleven species with four additional infraspecific taxa. Lane (1993) recognized six species with six additional varieties. One of these was the recently described *G. fraxinipratensis*, which has been universally recognized as distinct by subsequent authors. Strother and Wetter (2006) recognized only three species in California: *G. squarrosa*, *G. fraxinipratensis*, and *G. hirsutula*, the last of which included all of the other species recognized in California by previous authors as well as plants from elsewhere in western North America.

In this chapter, I present a taxonomic treatment of California *Grindelia* based on morphological study of living plants and herbarium specimens.

## METHODS

Herbarium specimens were examined from throughout the range of the Pacific Clade of *Grindelia*. These specimens were examined from the standpoint of morphological and ecological variation without assumptions about the merits of previous taxonomies. Geographic proximity and habitat similarity were considered in assessing the potential significance of discontinuities in morphological variation. Insofar as is possible, examination of herbarium specimens was supplemented by extensive field work throughout California. The bioregional abbreviations used in the taxon ranges are those found in *The Jepson Manual* (Baldwin et al. 2011).

**Grindelia** Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 1:261. 1807. (*Grindelia inuloides* Willd.)

*Donia* R.Br., Hortus Kew. (W.T. Aiton), ed. 2. 5: 82. 1813 [Nov 1813]. (*Donia glutinosa* (Cav.) R.Br.)

*Aurelia* Cass., Dict. Sc. Nat. xxxvii. 468. 1825. (*Aurelia decurrens* Cass. or *Aurelia amplexicaulis*)

*Demetria* Lag., Gen. Sp. Pl. [Lagasca] 30. 1816. (*Demetria spathulata* Lag. or *Demetria glutinosa* (Cav.) Lag.)

*Hoorebeckia* Cornelissson, Mussche, Hort. Gand. 13. 1817. (*Hoorebeckia chiloensis*)

Perennial to subshrub [annual] from a taproot or woody caudex, glabrous or tomentose, often glandular-sticky. Leaves simple, alternate, generally not fleshy; entire, crenate, serrate, or pinnately lobed; gland-dotted. Heads generally radiate; involucre obconic to hemispheric, generally gummy; phyllaries in 4–10 graduated series; receptacle flat to convex, more or less pitted, epaleate. Ray flowers 0–60, corollas yellow. Disk flowers with yellow corollas. Anther tips lanceolate; style appendages linear to lanceolate, equal to or longer than stigmatic portion. Cypselae cylindrical or swollen-obconic, shiny-white to more or less brown, smooth or ridged, glabrous. Pappus of 1–6 narrow awns [25–40 bristles], shorter than disk corolla, generally entire, deciduous.

**Etymology**—Named for David Hieronymus Grindel (1776–1836), Latvian chemist, doctor, pharmacist, and botanist.

**Distribution and Ecology**—Approximately 60 species, native to central and western North America and southern and western South America.

1. Leaves crenate, teeth rounded, each tooth with a distinct, yellowish bump near tip.—*G. squarrosa* var. *serrulata*.
- 1' Leaves entire or serrate; if serrate, teeth pointed, yellowish bump absent.—2
2. Pappus composed of 15–40 bristles; bristles united at the base and falling as a unit.—*G. ciliata* (Nutt.) Spreng. (This species is a historical waif, not described below.)
- 2' Pappus of 2–6 awns, awns not united.—3
3. Plants of dunes, salt marshes, coastal bluffs, tidal flats, sloughs; leaves more or less fleshy; NCo to SCo, Suisun delta (deltaic GV) .—4
- 3' Plants of fields, grasslands, woodlands, serpentine soils, disturbed areas, or interior wetlands; leaves not fleshy; widespread (but absent from the Suisun delta).—8
4. Stems woody in proximal 3–15 dm, erect; phyllaries appressed to head except for short, erect tips (tip < 3 mm long); tidal wetlands; CCo (San Francisco Bay).—*G. stricta* var. *angustifolia*.
- 4' Stems herbaceous or woody in proximal 0–1 dm, erect, decumbent, or prostrate; phyllaries generally spreading, recurved, or coiled; widespread.—5
5. Stems erect, 6–20 dm; salt marshes, sloughs.—6
- 5' Stems prostrate, decumbent, or erect (if erect, stems 1–6 dm); dunes, coastal bluffs.—7
6. Leaves on flowering stems generally widest at base or of approximately equal width throughout; Deltaic GV (Suisun).—*G. xpaludosa*.
- 6' Leaves on flowering stems generally widest at rounded tip or approximately equally wide at tip and base but narrower in middle of leaf; NCo.—*G. stricta* var. *stricta*(2).
7. Plants decumbent or erect; leaves generally sessile, sometimes clasping stems, approximately the same width throughout or widest near base; NCo to SCo, ChI.—*G. stricta* var. *platyphylla*.
- 7' Plants decumbent; leaves generally tapered to petioles, widest at rounded tip; NCo.—*G. stricta* var. *stricta*(2).
8. Phyllaries flattened throughout, gradually tapered to tips; phyllary tips erect; plants generally more or less hairy.—9
- 8' Phyllaries flattened only at bases, abruptly narrowed to tips; phyllary tips rounded in cross-section, spreading, reflexed, or coiled; plants generally glabrous.—10
9. Involucres 7–10 mm in diameter; rays 8–9 mm long; PR (San Diego Co.).—*G. hallii*(2).
- 9' Involucres 7–25 mm in diameter; rays 8–20 mm long; NCoR, GV, CW.—*G. hirsutula*.
10. Heads obconic; e DMoj, wet clay of meadows, woodland borders.—*G. fraxinipratensis*.
- 10' Heads hemispheric or bell-shaped, widening abruptly at the base; widespread
11. Phyllaries appressed to head for > 3/4 of length, reflexed or coiled portion < 3 mm; PR.—*G. hallii*(2).
- 11' Phyllaries appressed to head for < 1/2 of length, spreading, reflexed, or coiled portion > 5 mm; widespread.—12
12. Outer phyllaries reflexed, curved, or coiled < 270°.—*G. camporum*(2).
- 12' > 75% of phyllaries coiled or recurved 270–360° or more.—13
13. Plants 1–5 dm; mature involucres bell-shaped, 7–12 mm in diameter; CaR, MP.—*G. nana*.
- 13' Plants 6–25 dm; mature involucres hemispheric to bell-shaped, 15–22 mm in diameter; SnJV, SCo.—*G. camporum*(2)

**Grindelia camporum** Greene, Man. Bot. San Francisco 171. 1894 [2 Feb 1894]. (*Greene s.n.*; Antioch; Contra Costa Co., California; 5 September 1881; lectotype at MO)

*G. robusta* Nutt. var. *rigida* A.Gray in W.H.Brewer & S.Watson Bot. California [W.H.Brewer] 1: 304. 1876 (probably *Brewer 850*; Monte Diablo; 1860–1862; GH, US)

*Grindelia procera* Greene, Man. Bot. San Francisco 172. 1894 [2 Feb 1894]. (*Bioletti s.n.*; Banta; San Joaquin Co., California; 9 September 1892; lectotype at UC)

*Grindelia robusta* var. *davyi* Jeps., Fl. W. Calif. [Jepson] 554. 1901. *G. camporum* Greene var. *davyi* (Jeps.) Steyerl., Ann. Missouri Bot. Gard. 21: 536. 1934. *G. hirsutula* Hook. & Arn. var. *davyi* (Jeps.) M.A.Lane, Novon 2: 216. 1992. (*Davy 1896*; Berkeley, Peralta Park; Alameda Co., California; 10 June 1896; holotype at JEPS)

*Grindelia rubricaulis* DC. var. *interioris* Jeps., Man. Fl. Pl. Calif. [Jepson] 1021. 1925 [24 Nov 1925]. *G. camporum* Greene var. *interioris* (Jeps.) Steyerl., Ann. Missouri Bot. Gard. 21: 538. 1934. (*Davy 1383*; Mokelumne Hill; Calaveras Co., California; 18–30 May 1895; holotype at UC)

*Grindelia bracteosa* J.T.Howell, Madroño 2: 22. 1931. *G. rubricaulis* var. *bracteosa* (J.T.Howell) Steyerl., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]. *G. robusta* Nutt. var. *bracteosa* (J.T.Howell) D.D.Keck, Aliso 4: 102. 1958. *G. camporum* Greene var. *bracteosa* (J.T.Howell) M.A.Lane, Novon 2: 216. 1992. (*Howell 2786*; “north side of Santa Ana Canyon,... at 600 feet”; Orange Co., California; 16 July 1927; holotype at CAS)

*Grindelia rubricaulis* var. *elata* Steyerl., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]. (*Howell 6574*; “on clay hills, 7 miles southwest of Simi”; Ventura Co., California; 29 May 1931; holotype at CAS)

*Grindelia camporum* Greene var. *australis* Steyerl., Ann. Missouri Bot. Gard. 21: 228. 1934 [12 Apr 1934]. (*Munz & Johnston 11157*; “at base of dry slope, 3 miles east of Elizabeth Lake”; Los Angeles Co., California; 4 September 1928; holotype at POM)

*Grindelia camporum* Greene var. *abbreviata* Steyerl., Ann. Missouri Bot. Gard. 21: 228. 1934 [12 Apr 1934]. (*Hoffmann s.n.*; “on alkaline flats, Lancaster”; Los Angeles Co., California; 23 September 1927; holotype at SBBG)

*Grindelia camporum* Greene var. *parviflora* Steyerl., Ann. Missouri Bot. Gard. 21: 534. 1934. (*Howell 5496*; “at Liberty School on Marsh Creek Road”; Contra Costa Co., California; 28 September 1930; holotype at CAS)

*Grindelia camporum* Greene var. *interioris* (Jeps.) Steyerl. f. *foliacea* Steyerl., Ann. Missouri Bot. Gard. 21: 538. 1934. (*Howell 5201*; “on clay hill, 1 mile from Vacaville on road to Elmira”; Solano Co., California; 30 May 1930; holotype at CAS)

Perennial 6–25 dm, erect, generally much-branched throughout. Leaves 2–15 cm; basal generally absent at anthesis, distal reduced; blades lanceolate to ovate, sessile and clasping or narrowed to petiole-like base, generally glabrous, often resinous, yellow- to gray-green, entire or serrate, teeth pointed. Involucres 10–22 mm in diameter, hemispheric to campanulate when mature, glabrous, resinous. Phyllaries in 5–7 series; bases wide, straw-colored; tips green, acuminate, more or less round in cross-section, outer spreading to reflexed, or coiled 180–360°. Ray flowers (0)25–39, rays 5–11 mm long. Cypselae 2–5 mm, white to golden-brown, top ridged; pappus awns 2–6. Diploid or tetraploid ( $2n = 12, 24$ ).

**Etymology**—*Camporum* is Latin for of the fields.

**Distribution and Ecology**—Sandy or saline bottomland, roadsides. Flowering May to November. NCoR, CaRF, SNF, n SNH, Teh, GV, SnFrB, SCoRO, SW, SNE, DMoj; below 1400 m. Extends from northern California to the portion of the California Floristic Province in Baja California.

**Discussion**—*Grindelia camporum* has both diploid and tetraploid populations. Successful crosses have been made between ploidy levels, with some of the resulting offspring being triploid and some tetraploid (Dunford 1964). *Grindelia camporum* is a widespread, variable species that should perhaps be further split. The plants that have been called *G. procera* are endemic to the marshes of the San Joaquin Valley. The plants that have been called *G. bracteosa* are from southern coastal California and have large heads with coiled phyllaries. The plants that have been called *G. camporum* var. *davyi* occur in the foothills around the Central Valley, have reddish stems, are less frequently branched than the other forms, and flower in the earlier part of the year.

**Representative Specimens from California**—ALAMEDA: Vallecitos, *Jepson s.n.* (JEPS 41867); North Berkeley, *Jepson s.n.* (JEPS 41873); Zumbach Ranch, *Moore et al.* 259 (JEPS); Lime Ridge, *Moore & Kersh* 310 (JEPS); Del Valle Reservoir, *Moore* 387 (JEPS); Springtown, *Moore & Zacharias* 433 (JEPS); Oakland, .5 mi W of Lake Temescal, *Robbins* 3805 (JEPS); San Leandro, *Robbins* 3955 (JEPS); Berkeley, *Robbins* 4113 (JEPS); Anthony Chabot Regional Park, *Stratford s.n.* (JEPS 84521). AMADOR: Ione, *Braunton* 1007 (UC); 2 mi. W of Ione, *Heller* 16116 (UC); 3 mi. NNW of West Point, *Johannsen* 1211 (UC); near Jackson, *Mulliken* 113 (UC); Creek City, *Nordstrom* 799 (UC). BUTTE: Bald Rock Dome trailhead, *Ahart* 10462 (JEPS); Oroville, *Heller* 10780 (UC); CA-70, *Moore & Welch* 558 (JEPS); 2 mi. NE of Concow, *Oswald & Ahart* 4416 (UC). CALAVERAS: Murphy's, *Brandeggee s.n.* (UC87886); 1.75 mi. N of Esmeralda, *Johannsen* 909 (UC). COLUSA: 3 mi. W of Colusa, *Baker* 11582 (UC); Princeton, *Chandler s.n.* (UC 71783); Maxwell Rd. rest area, *Moore & Moore* 266 (JEPS); confluence of North and Middle Forks of Stony Creek, *Sharsmith* 4264 (UC). CONTRA COSTA: Walnut Creek, *Jepson s.n.* (JEPS 41884); Shell Ridge, *Moore & Sandel* 265 (JEPS); Deer Flat, Mt. Diablo, *Moore et al.* 862 (JEPS); Pt. Pinole, *Moore et al.* 864 (JEPS); 1 mi. S of Pittsburg, *Rose* 54136 (JEPS); Brushy Creek, 4.5 mi SW of Byron Hot Springs, *Stone* 579 (JEPS); 3 mi. N of Altamont Pass, *Stone* 645 (JEPS); Antioch Sand Hills, *Turner s.n.* (JEPS 40435); Hampton Road Natural Reserve, *Yorks* 482 (JEPS). EL DORADO: Sweetwater Creek, *Brandeggee s.n.* (UC 133131); 0.25 mi. SW of Cool, *Dunford* 564 (UC); 0.5 mi. N of Diamond Springs, *Dunford* 565 (UC); St. Lawrence, *Jones* 3611 (UC); Bear Creek Rd., *Moore* 975 (JEPS); Eddy Arboretum, *Robbins* 1259 (UC). FRESNO: King Slough, *Bacigalupi & Heckard* 8780 (JEPS); S to Mendota and E to Friant, *Jepson* 12942 (JEPS); Auberry Road, *Kyhos* 58-202 (JEPS); Clovis, *Taylor* 745 (JEPS); 6 mi S of Mendota on Hwy 33, *Twisselmann* 9138 (JEPS). GLENN: near Norman, *Davy* 4271 (UC); 5 mi. NW of Hamilton, *Heller* 11351 (UC); btwn. Norman and Willows, *Heller* 15483 (UC); Sacramento National Wildlife Refuge, Tract C, *Moore & Silveira* 822 (JEPS); Black Diamond Rd., *Oswald & Ahart* 5438 (UC); NW of Alder Springs, *Semple* 8564 (UC). HUMBOLDT: S of Garberville, *Heller* 13764 (UC); near Garberville, *Tracy* 5038 (UC); Smith Point Bridge, *Tracy* 17941 (UC). KERN: Walker Basin, *Grinnell s.n.* (JEPS 41929); Poso and Homeland Canals, *Moore et al.* 948 (JEPS); Kern National Wildlife Refuge, *Moore & Williams* 950 (JEPS); Temblor Range, 1.5 mi. E of Lost Hills, *Twisselmann* 713 (JEPS); Monolith, Tehachapi Mts., *Twisselmann* 10167 (JEPS); W portion of Kern National Wildlife Refuge, *Twisselmann* 10252 (JEPS); Kern National Wildlife Refuge, Section 28, *Twisselmann* 11573 (JEPS); Castaic Valley, *Twisselmann* 12792 (JEPS). LAKE: Laurel Beach, *Bradshaw* 149 (UC); S face Elk Mt., *Ertter* 7661 (UC); Bartlett Mt., *Mason* 2574 (UC); location of Old Witter Springs Hotel, *Simontacchi* 183 (UC). LOS ANGELES: Mandeville Canyon, *Clokey & Templeton* 4557 (UC); N of Lancaster, *Lane* 3094 (UC); Lake Elizabeth Rd., *Moore & Moore* 957 (JEPS); Santa Monica Mts., W of U.C. campus, *Raven* 9667 (JEPS); Point Dume, Santa Monica Mts., *Raven &*

*Thompson 14344* (JEPS); Saddle Peak, Santa Monica Mts., *Thompson 2034* (JEPS); Antelope Valley, Hwy. 14 and Ave. A, *Twisselmann 17493* (JEPS). MADERA: N of Madera, *Moore & Moore 980* (JEPS); Nelder Grove Rd. (Forest Rd 6S10), *Taylor 8591* (UC). MARIN: Mt. Tamalpais, *Munz 6452* (UC). MARIPOSA: Indian Gulch, *Bolt 39* (UC); btwn. Merced Falls and Hornitos, *Ertter 5795* (UC); Triangle and Valley View Rds., *Moore & Park 781* (JEPS); Merced River, *Moore et al. 977* (JEPS); 1 mi. S of Mariposa, *Tilforth & Wisura* (UC). MERCED: Volta Wildlife Area, *Allen 1988-52* (JEPS); 10 mi. W of Merced, *Hoover 1124* (JEPS); Canal Creek Bridge, *Howell 46469* (JEPS); 5 mi. N of Merced, *Rodin 7114* (UC); Los Banos Game Refuge, *True 435* (UC); Willie Wright Rd., *Zacharias 947* (JEPS). MONTEREY: Cholame Valley, *Twisselmann 8782* (JEPS). NAPA: 2 mi. W of Chiles Valley, *Dunford 555* (UC); 0.75 mi. N of Samuel Springs, *Dunford 556* (UC); Mt. St. Helena, *Jepson 19050* (JEPS); Mt. St. Helena, *Jepson s.n.* (JEPS 41878); Upper Napa Valley, *Jepson s.n.* (JEPS 41928); Mt. St. Helena, Van Ness Ranch, *King s.n.* (JEPS 41923); between Conn Valley and Howell Mt., *Raven 2355* (JEPS); Imola to Napa Junction, *Raven 3098* (JEPS). NEVADA: Nevada City, *Eastwood & Howell 588* (UC); near Nevada City, *Heller 8117* (UC); Nevada City, *Raven 7968* (JEPS); 1 mi. NE of Wolf Mt., *Roderick s.n.* (JEPS 52285). ORANGE: Capistrano, *Abrams 3273* (UC); Newport Bay, *Booth 1092* (JEPS, UC); Huntington Beach, *Condit s.n.* (UC 455831); Dana Point, *Heckard & Bacigalupi 1836* (JEPS); W end of Newport Bay, *Wolf 8101* (UC). PLACER: near Newcastle, *French 74* (UC); Mosquito Ridge Rd., *Moore & Moore 976* (JEPS); Auburn, *Shockley s.n.* (JEPS 41903); Colfax Rd., Tahoe National Forest, *Smith 1875* (JEPS). SACRAMENTO: Delta Meadows River Park, *Bowcutt 1642* (JEPS); Sacramento, *Copeland 1650* (JEPS, UC); 4.25 mi. NW of mouth of Crevis Creek, *Nordstrom 70* (UC); East Sacramento, *Ramaley 11233* (UC). SAN BERNARDINO: summit of Carbon Canyon Rd., *Wolf 3830* (UC). SAN DIEGO: La Presa, *Abrams 3903* (UC); Ramona, *Brandegge s.n.* (UC 134823); National City, *Cleveland s.n.* (UC 134624); Oceanside, *Parish 4452* (JEPS); N of Oceanside, *Reed 3927* (UC). SAN JOAQUIN: Stockton Diverting Canal, *Bacigalupi & Constance 8720* (JEPS); Stockton, *Davy 1198* (UC); 5.5 mi. NE of Tracy, *Dunford 572* (UC); Atlanta-Stockton rd., *Jepson s.n.* (JEPS 41919); Corral Hollow Rd., *Moore 381* (JEPS); 2 mi. N of Escalon, *Strother 1278b* (UC). SAN LUIS OBISPO: 6 mi. E of Paso Robles, *Hoover 6393* (UC); N of San Luis Obispo, Fox Hollow Rd., *Moore 973* (JEPS); 2.5 mi. E of Huero Huero Creek, *Twisselmann 14930* (JEPS). SANTA BARBARA: Coal Oil Point, *Moore 954* (JEPS); Santa Cruz Island, Islay Canyon Rd., *Moore & Moore 965* (JEPS); Del Sol Vernal Pool Reserve, *Pritchett VP-15* (JEPS). SANTA CLARA: Metcalf Canyon, *Belshaw 3021* (UC); E of San Felipe, *Lane 3103* (UC); San Antonio Valley, *Sharsmith 3903* (UC); 1.5 mi. NE of Madrone, *Thomas 8111* (JEPS). SHASTA: Oak Run, *Baker 456* (UC); 1 mi. W of Cow Creek, *Johannsen 131* (UC); Hwy. 89 near Burney Creek, *Oswald & Ahart 9615* (JEPS). SOLANO: Pleasant Valley, *Heller 16312* (UC); Vacaville, *Jepson 10024* (JEPS); Jepson Prairie, *Moore 986* (JEPS); Vaca Mts., along Blue Ridge, *Willoughby 1885* (JEPS). SONOMA: Bennett Valley, *Armstrong 493* (JEPS); 1.8 mi. NE of Mark West Springs, *Dunford 559* (UC); the Geysers, *Jones 29142* (UC); Sonoma, *Rose 40364* (UC). STANISLAUS: Grayson, *Bacigalupi & Constance 8704* (JEPS); Modesto, *Hoover 1* (JEPS); N of Grayson, *Jepson 10297* (JEPS); near Westley, *Jepson 19487* (JEPS); Tuolumne River Ferry, below La Grange, *Jepson s.n.* (JEPS 41868); W of Patterson, *Semple & Heard 8584* (UC); Vernallis to Modesto Rd., *Raven & Robbins 8129* (JEPS); Vernalis-Modesto Rd., *Robbins 3647* (JEPS). SUTTER: Marysville Buttes, *Lee 2085* (JEPS). TEHAMA: 8 mi. SW of Paskenta, *Ahart 11027* (JEPS); Hog Lake Plateau, *Oswald & Ahart 7956* (JEPS). TUOLUMNE: road btwn. Twain Heart and Sonora, *Alexander & Kellogg 3712* (UC); Harden Ranch, *Hall 1577* (UC); near Sonora, *Hall 3302* (UC); 22.2 mi. W of Pine

Crest, *Hesse 2391* (JEPS); 0.25 mi. NW of Soulsbyville, *Wiggins 6873* (UC). TULARE: Ave. 54, *Moore et al. 947*. YUBA: near Toland Ranch, *Yates 3642* (UC).

***Grindelia fraxinipratensis*** Reveal & Beatley, Bull. Torrey Bot. Club 98:334. 1972. (*Reveal & Holmgren 1887*; “Amargosa Desert, Ash Meadows, in meadows along the Ash Meadows Road between Devils Hole and Ash Meadows Lodge, 1.5 airline km northwest of the Lodge, associated with *Distichlis*, at 660 m, T. 18S., R. 50 E., sec. 23 (SE1/4)”; Nye Co., Nevada; 21 August 1968; holotype at US)

Perennial 5–12 dm, erect, branched throughout. Leaves 1–8 cm; basal absent at anthesis, distal reduced; blades oblanceolate to oblong, narrowed to base, glabrous, resinous, dark green to yellow-green, entire or serrate, teeth pointed. Involucres 5–9 mm in diameter, more or less obconic, glabrous, resinous. Phyllaries in 4–7 series; bases wide, straw-colored; tips green, acuminate, more or less round in cross-section, spreading to reflexed or coiled 180°. Ray flowers 8–12, rays 4–6 mm long. Cypselae 2.5–4 mm, white to golden-brown, top generally truncate; pappus awns 2. Tetraploid ( $2n = 24$ ).

**Etymology**—*Fraxinipratensis* is Latin for of Ash Meadows, the type locality.

**Distribution and Ecology**—Wet clay of meadows, woodland edges near alkaline springs. Flowering July to October. DMOj, ca. 700 m. Endemic to Ash Meadows in Nevada and the adjacent Amargosa Basin, extending into California.

**Discussion**—*Grindelia fraxinipratensis* is federally Threatened, listed as critically endangered in Nevada, and is a CNPS list 1B.2 plant (California Native Plant Society 2010). Although its habitat in Nevada is protected, the wetlands it lives in are threatened by water diversion. This plant is sister to the Pacific Clade (Chapter Two).

**Representative Specimens from California**—One specimen from California, not seen by me, but included in the description: Ash Meadows, Carson Slough, Inyo Co., 5 May 1971, *Reveal 2295* (NTS, US).

***Grindelia hallii*** Steyerl., Ann. Missouri Bot. Gard. 21: 229. 1934 [12 Apr 1934]. *G. hirsutula* Hook. & Arn. var. *hallii* (Steyerl.) M.A.Lane, Novon 2: 216. 1992. (*Abrams 3957*; “in open meadows about Cuyamaca Lake”; San Diego Co., California; 30 June 1903; holotype at NY!)

Perennial 2–6 dm, erect, openly branched in distal half. Leaves 1–12 cm; basal generally present at anthesis, distal reduced; blades of proximal leaves narrowed to petioles; distal ovate-lanceolate, sessile; glabrous, resinous, yellow-green, serrate, teeth pointed. Involucres 7–10 mm in diameter, hemispheric, glabrous, more or less resinous. Phyllaries in 4–6 series, tapered to acute tips, erect or tips recurved. Ray flowers 12–20, rays 8–9 mm long. Cypselae 4–5 mm, tan to brown, top truncate with triangular projections; pappus awns 2. Exclusively diploid ( $2n = 12$ ).

**Etymology**—Named for Harvey Monroe Hall (1874–1932), American botanist and founder of Clausen, Keck, and Hiesey’s biosystematic studies.

**Distribution and Ecology**—Meadows, dry slopes, open pine/oak woodland. Flowering July to October. PR; 800–1700 m. Endemic to the vicinity of the Cuyamaca and Laguna Mountains in central San Diego County.

**Discussion**—*Grindelia hallii* is a CNPS list 1B.2 plant (California Native Plant Society 2010), due to its restricted distribution and the threat of habitat destruction from development, although much of its range is on Forest Service or California State Park lands. It resembles a



miniature *G. camporum*, but is found on its own, isolated from other species of *Grindelia*. Steyermark (1937) considered it to be ancestral to the remaining species from the Pacific States (the Pacific Clade of Chapter Two).

**Representative Specimens from California**—SAN DIEGO: Cuyamaca, *Brandegeae s.n.* (UC 87917); Lake Cuyamaca, *Dunford 595* (UC); Kessler Flat, *Moore 967* (JEPS); Julian, *Moore 970* (JEPS); Lucky 5 Ranch, Laguna Mts., *Rebman 9405* (UC); S of Santa Ysabel, *Solbrig 2764* (UC); Julian, *Woodcock 63* (UC).

***Grindelia hirsutula*** Hook. & Arn., Bot. Beechey Voy. 147. 1833 [pp.145–192 (Oct 1833)]. (*Beechey s.n.*; California; holotype at E)

*Grindelia humilis* Hook. & Arn. Bot. Beechey Voy. 147. 1833 [pp.145–192 (Oct 1833)]. (*Beechey s.n.*; holotype at E)

*Grindelia rubricaulis* DC., Prodr. (DC.) 5: 316. 1836 [1–10 Oct 1836]. *G. hirsutula* Hook. & Arn. var. *rubricaulis* (DC.) D.D.Keck, Aliso 4: 102. 1958. (*Douglas 55*; California; 1833; holotype G-DC)

*Grindelia rubricaulis* DC. var. *maritima* Greene, Pittonia 2: 289. 1892. *G. robusta* Nutt. var. *maritima* (Greene) Jeps., Fl. W. Calif. [Jepson] 554. 1901. *G. maritima* (Greene) Steyermark., Ann. Missouri Bot. Gard. 21: 576. 1934. *G. hirsutula* Hook. & Arn. var. *maritima* (Greene) M.A.Lane, Novon 2: 216. 1992. (*Greene s.n.*; Pt. Lobos; Monterey Co., California; 12 August 1892; holotype at ND-G)

*Grindelia patens* Greene, Pittonia 2:290. 1892. *G. robusta* var. *patens* (Greene) Jeps., Fl. W. Calif. [Jepson] 554. 1901. *G. hirsutula* Hook. & Arn. var. *hirsutula* f. *patens* (Greene) Steyermark., Ann. Missouri Bot. Gard. 21: 570. 1934. (*Greene s.n.*; Berkeley; Alameda Co., California; July 1881; types at F, ND-G)

*Grindelia hirsutula* Hook. & Arn. var. *subintegra* Steyermark., Ann. Missouri Bot. Gard. 21: 229. 1934 [12 Apr 1934]. (*Howell 11414*; “on grassy hillside, 2 miles east of Ojai”; Ventura Co., California; 1 July 1933; holotype at MO)

*Grindelia hirsutula* Hook. & Arn. var. *hirsutula* f. *cacumena* Steyermark., Ann. Missouri Bot. Gard. 21: 571. 1934. (*Bolander 389*; “on Oakland Hills,... alt. 2000 ft.”; Alameda Co., California; 1866; holotype at US)

*Grindelia hirsutula* Hook. & Arn. var. *brevisquama* Steyermark., Ann. Missouri Bot. Gard. 21: 572. 1934. (*Howell 5338*; “in shallow clay soil of rocky hill, Black Point Road”; Sonoma Co., California; 13 July 1930; holotype at CAS)

*Grindelia hirsutula* Hook. & Arn. var. *brevisquama* Steyermark. f. *glabrata* Steyermark., Ann. Missouri Bot. Gard. 21: 573. 1934. (*Howell 5221 in part*; “in clay soil, 8 miles west of Petaluma, on Bodega Road”; Sonoma Co., California; 8 June 1930; holotype at MO)

*Grindelia hirsutula* Hook. & Arn. var. *brevisquama* Steyermark. f. *tomentulosa* Steyermark., Ann. Missouri Bot. Gard. 21: 574. 1934. (*Suksdorf 279*; Crystal Springs Lake; San Mateo Co., California; 23 June 1913; holotype at MO)

*Grindelia hirsutula* Hook. & Arn. var. *brevisquama* Steyermark. f. *pedunculoides* Steyermark., Ann. Missouri Bot. Gard. 21: 574. 1934. (*Eastwood s.n.*; Calistoga; Napa Co., California; 7 May 1900; holotype at UC)

*Grindelia hirsutula* Hook. & Arn. var. *calva* Steyermark., Ann. Missouri Bot. Gard. 21: 575. 1934. (*Roadhouse 65*; San Luis Obispo; San Luis Obispo Co., California; spring 1905; holotype at UC)

*Grindelia maritima* (Greene) Steyererm. f. *anomala* Steyererm., Ann. Missouri Bot. Gard. 21: 578. 1934. (Howell 11658; Laguna Honda; San Francisco Co., California; 15 September 1933; holotype at MO)

*Grindelia rubricaulis* DC. var. *permixta* Steyererm., Ann. Missouri Bot. Gard. 21: 582. 1934. (Howell 4352; “on rocky bluff, San Leandro”; Alameda Co., California; 22 July 1929; holotype at CAS)

Perennial 2–15 dm, erect, few-branched, side branches generally not branched. Leaves 1–10 cm; basal generally present at anthesis, distal not much reduced; blades oblong to lanceolate, basal sometimes lobed, glabrous or tomentose, generally not resinous, yellow-, red-, or gray-green, base narrowed to more or less sessile, margin entire or serrate, with teeth pointed. Involucres 7–25 mm in diameter, hemispheric to campanulate, glabrous or more often tomentose, resinous or not. Phyllaries in 4–5 series; gradually tapered to acute tips; tips flat in cross-section, erect; outer generally green throughout. Ray flowers 10–60; rays 8–20 mm long. Cypselae 2.5–5.5 mm, golden- to red-brown, top truncate to knobby; pappus awns 2–4. Exclusively tetraploid ( $2n = 24$ ).

**Etymology**—*Hirsutula* is Latin for sparingly hairy.

**Distribution and Ecology**—Sandy, clay, or serpentine slopes or roadsides. Flowering from April to June. NCoR, GV, CW; below 1700 m. Endemic to California.

**Discussion**—The distributions of *G. hirsutula* and *G. camporum* overlap broadly. However, when they occur together, *G. hirsutula* flowers earlier than does *G. camporum*. In addition, *G. hirsutula* commonly occurs on serpentine-derived soils, while *G. camporum* does not. The name *G. hirsutula* has priority over all other names in the Pacific Clade (of Chapter Two) and some authors have recommended that many of the species in that clade be subsumed into *G. hirsutula* (Strother and Wetter 2006).

**Representative Specimens from California**—ALAMEDA: Berkeley Hills behind Clark Kerr campus, *Ertter 10671* (UC); Redwood Rd., Oakland, *Jarecki 55* (UC); Berkeley Hills, *Jepson s.n.* (JEPS 41872); Berkeley, *Klee s.n.* (UC 32119); Miller-Knox Park, *Moore et al. 216* (JEPS); Zumbach Ranch, *Moore et al. 258* (JEPS); Joaquin Miller Park, *Moore & Park 964* (JEPS); Oakland, along Joaquin Miller Blvd., *Robbins 3876* (JEPS); Cragmont, *Rose 34013* (UC). CONTRA COSTA: Richmond Field Station, *Echols s.n.* (JEPS 111071); Browns Island, *Ertter 10716* (UC); Point Richmond, *Hall 1659* (UC); Mangini Property, *Moore et al. 163* (JEPS); Lime Ridge, *Moore & Kersh 180* (JEPS); Tilden, *Moore et al. 218* (JEPS); Mt. Diablo, *Moore et al. 261* (JEPS); Wildcat Canyon, *Moore et al. 861* (JEPS); Richmond Field Station, *Powell 1647* (UC). MARIN: btwn. Fairfax and Woodacre, *Dunford 578* (UC); Sausalito, *Eastwood s.n.* (UC 410735); Bootjack Camp, Mt. Tamalpais, *Jepson 9511* (JEPS); Mt. Tamalpais, *Jepson s.n.* (JEPS 41887); Shaver Grade, *Lee & Joseph 2241* (JEPS); Mt. Tamalpais, near Pantoll Ranger Station, *Moore 818* (JEPS). MONTEREY: btwn. Lucia and Little’s Hot Springs, *Brandeggee s.n.* (UC 468770); near Pacific Grove, *Hoover 5245* (UC); Plaskett Ridge, Santa Lucia Range, *Twisselmann 16672* (JEPS). NAPA: Howell Mt., *Jepson s.n.* (UC 32141); near Lokoya Lodge, *True 732* (UC). SAN FRANCISCO: btwn. Lobos Creek and Fort Point, *Raven 7931* (JEPS); Point Lobos, *Raven 8189* (JEPS); N summit of Twin Peaks, *Raven 11312* (JEPS); above Bakers Beach, *Raven & Snow 13699* (JEPS); Presidio, *Rose 38234* (UC). SAN LUIS OBISPO: School Canyon, *Condit s.n.* (UC 455830); summit of Cuesta Pass, *Hoover 11395* (UC); Rancho Marino Reserve, *Moore 944* (JEPS); West Cuesta Ridge Rd., *Moore & Moore 956* (JEPS); 1 mi. N of San Luis Obispo, *Rodin 5966* (UC); Poly Canyon, *Rodin 7076* (UC); mouth

of Toro Creek *Twisselmann 5259* (JEPS). SAN MATEO: Pilarcitos Lake, Pilarcitos Canyon, *Davy s.n.* (UC 32136); along Alpine Rd., *Bacigalupi & Robbins 4550* (JEPS); 4 mi. N of Saratoga Summit, *Hesse 2690a* (JEPS); Fifield Ridge, *Moore et al. 225* (JEPS); San Andreas Lake, *Rose 33221* (UC); San Bruno Mt., *Taylor 9480* (JEPS). SANTA BARBARA: Santa Barbara, *Elmer s.n.* (UC 184297). SANTA CLARA: Black Mt., *Elmer 4584* (UC); Page Mill Rd., *Hesse 1146* (JEPS); Tulare Hill, *Jepson 12704* (JEPS); Coyote Creek, *Jepson s.n.* (JEPS 41874); outside Henry Coe State Park, *Moore et al. 486* (JEPS); Alum Rock, *Smith s.n.* (UC 154592). SANTA CRUZ: West Marshall Field, U.C., Santa Cruz campus, *Buck 805* (JEPS); Summit Rd., *Hesse 1096* (JEPS). SOLANO: Vacaville, *Bacigalupi 4848* (JEPS). SONOMA: Glenn Ellen, *Bioletti s.n.* (JEPS 41926); 2.5 mi. NW of camp Meeker, *Guggolz & Guggolz 1210* (JEPS); 1 mi. E of Bodega, *Howell 5256* (UC); Occidental, *Hoover 5306* (JEPS); Sonoma Valley, *Torrey 221* (UC).

***Grindelia nana*** Nutt. Trans. Amer. Philos. Soc. ser. 2, 7: 314. 1840 [Oct–Dec 1840]. (*Nuttall s.n.*; near Fort Vancouver (although more likely in Oregon; Cronquist 1955); types at GH, PH)

*Grindelia nana* Nutt. var. *altissima* Steyerem., Ann. Missouri Bot. Gard. 21: 544. 1934. (*Tracy 4669*; “on gravel bar, Eel River, at mouth of Larabee Creek,...alt. 200 ft.”; Humboldt Co., California; 12 September 1915; holotype at UC)

*Grindelia nana* Nutt. var. *altissima* Steyerem. f. *puberula* Steyerem., Ann. Missouri Bot. Gard. 21: 545. 1934. (*Blankinship s.n.*; “on dry roadsides, Kelseyville; Lake Co., California; 12 June 1924; holotype at CAS)

*Grindelia nana* Nutt. var. *turbinella* Steyerem., Ann. Missouri Bot. Gard. 21: 545. 1934. (*Heller 12579*; “in gravelly soil at base of hill, Shasta Valley, 10 miles south of Gazelle,...alt. about 3000 ft.”; Siskiyou Co., California; 7 September 1917; holotype at GH)

Perennial 1–5 dm, decumbent to erect, branched throughout. Leaves 3–9 cm; basal generally absent at anthesis, distal not much reduced; blades oblanceolate, glabrous, resinous, yellow- to gray-green, bases generally tapered, margins entire or serrate, with teeth pointed. Involucres 7–12 mm in diameter, campanulate when mature, glabrous, resinous. Phyllaries in 5–7 series; bases wide, straw-colored; tips green, acuminate, more or less round in cross-section, coiled 270–360°. Ray flowers 11–28; rays 5–11 mm long. Cypselae 3.5–4 mm, light brown, top ridged; pappus awns 2. Exclusively diploid ( $2n = 12$ ).

**Etymology**—*Nana* is Latin for dwarf.

**Distribution and Ecology**—Dry, sandy hills; roadsides. Flowering from June to September. CaR, MP; 100–1800 m. Extending north to Washington and east to Montana.

**Discussion**—*Grindelia nana* resembles *G. squarrosa* with its tightly coiled phyllaries, but differs from *G. squarrosa* in having sharply-pointed teeth on its leaves and in tending to have only one or a few upright stems from the base, instead of being much-branched from the base and presenting a generally rounded appearance. The two taxa appear to be hybridizing, as *G. squarrosa*-like Internal Transcribed Spacer and External Transcribed Spacer sequences have been recovered from plants with the morphology of *G. nana*. If *G. squarrosa* is indeed invasive in the western part of its range, it may be displacing *G. nana*.

**Representative Specimens from California**—LASSEN: Susanville, *Brandegge s.n.* (UC 87888). MODOC: Warner Mts., along rd. to Alturas, *Alexander & Kellog 4998* (UC); Goose Lake Valley, *Austin s.n.* (UC 87884); Forestdale, *Baker s.n.* (UC 76029). SHASTA: W of Burney Mt., *Park 1944*. SISKIYOU: Hornbrook, *Brandegge s.n.* (UC 87887); Ager, *Brandegge s.n.* (UC

87889); near Montague, *Butler 499* (JEPS, UC); Sisson, *Copeland 3884* (UC); 10 mi. S of Gazelle, *Heller 12979* (UC).

***Grindelia* ×*paludosa*** Greene (pro sp.), Man. Bot. San Francisco 172. 1894 [2 Feb 1894]. *G. cuneifolia* Nutt. var. *paludosa* (Greene) Jeps., Fl. W. Calif. [Jepson] 554. 1901. (*Greene s.n.*; Suisun marsh; Solano Co., California; 29 September 1889 and 30 October 1892; holotype at ND-G)

Perennial 8–20 dm, erect, branched throughout. Leaves 1–17 cm; basal generally absent at anthesis, distal smaller; blades ovate-lanceolate, more or less fleshy, sessile or tapered to a more or less petiole-like base; glabrous, green to reddish-green, margin entire or serrate, teeth pointed. Involucres 10–20 mm in diameter, hemispheric, glabrous, generally resinous. Phyllaries in 4–5 series; bases wide, straw-colored; tips acute to acuminate, flat to more or less round in cross-section, the outer spreading, reflexed, or coiled 270–360°. Ray flowers 20–30, rays 10–17 mm long. Cypselae approximately 4 mm, tan, top truncate; awns 2–5. Apparently exclusively tetraploid ( $2n = 24$ ).

**Etymology**—*Paludosa* is Latin for marsh-dwelling.

**Distribution and Ecology**—Salt marshes, banks of sloughs. Flowering from July to November. Deltaic GV; below 30 m. Endemic to the Suisun Marsh.

**Discussion**—*Grindelia* ×*paludosa* is a putative hybrid between *G. stricta* var. *angustifolia* and *G. camporum* (Steyermark 1934). It can be distinguished from *G. stricta* var. *angustifolia* by being wholly herbaceous (a character often not apparent on herbarium specimens or the accompanying notes) and from *G. camporum* by its nearly unbranched, upright habit and fleshy leaves. In some places, the plants referred to as *G. ×paludosa* appear to be self-perpetuating, stabilized hybrids, while in other places, they appear to be newly formed from their parental taxa. However, the two putative parents are also capable of growing together without hybridizing (Chapter Three).

**Representative Specimens from California**—SOLANO: Grizzly Island, *Alexander & Kellogg 1869* (JEPS, UC); Suisun Marsh, *Heller 7542* (UC); Suisun Marshes, *Jepson 10230* (JEPS); Benecia, *Jepson s.n.* (JEPS 41869); Grizzly Island, *Mason 12654* (UC); Hill Slough, *Moore & Park 819*; Tubbs Island, *Parks & Parks 411* (UC); Suisun Marsh, *Rose 38242* (UC).

***Grindelia squarrosa*** (Pursh) Dunal var. ***serrulata*** (Rydb.) Steyermark., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]. *Grindelia serrulata* Rydb., Bull. Torrey Bot. Club 31: 646. 1904 [1905]. (*Cowe s.n.*; Fort Collins; Larimer Co., Colorado; 10 August 1891; holotype at NY)

Biennial 1–6 dm, decumbent to erect, much branched throughout. Leaves 1.5–7 cm, basal leaves generally absent at anthesis, distal leaves not much reduced compared to basal leaves; blades oblong to ovate, sessile or narrowed at base, glabrous, resinous, gray-green; margins crenate, with teeth rounded, each tooth with a yellowish bump near tip. Involucres 10–17 mm in diameter, campanulate, glabrous, resinous. Phyllaries in 5–6 series; bases wide, straw-colored; tips green, acuminate, more or less round in cross-section, coiled 360°. Ray flowers absent or 24–36; rays 8–10 mm long. Cypselae 2.3–3 mm, light brown to yellowish, top truncate; pappus awns 2–3(6). Exclusively diploid ( $2n = 12$ ).

**Etymology**—*Squarrosa* is Latin for roughened from spreading or recurved tips. *Serrulata* is Latin for minutely serrate.

**Distribution and Ecology**—Disturbed roadsides, stream sides. Flowering from July to September. CaRH, SNH, TR, GB, DMOj; 700–2300 m. From Wyoming and New Mexico west to the Cascade-Sierra axis.

**Discussion**—*Grindelia squarrosa* var. *serrulata* may be introduced in the western part of its range, including California and the Great Basin. It does appear to be spreading and apparently displacing other species of *Grindelia* outside of California. It is the most distinctive species of *Grindelia* in California due to the teeth on the leaves, which are always rounded, with a yellowish bump near the tip. This is in contrast to the other species, which have teeth with sharply pointed or awned tips. It is a member of the Eastern *Grindelia* Clade (Chapter Two).

**Taxonomic Note**—The citation for the species is as follows: *Grindelia squarrosa* (Pursh) Dunal, Mém. Mus. Hist. Nat. 5:50 1819. *Donia squarrosa* Pursh, Fl. Amer. Sept. 2:559. 1814. (*Lewis 40*; “banks of the Missouri, near the Old Maha village”; holotype at PH)

**Representative Specimens from California**—KERN: E of Lake Isabella, *Twisselmann 5542* (CAS). LASSEN: Hwy. 395, 4 mi. N of Hwy. 70, *Taylor 5024* (CAS). LOS ANGELES: Rock Creek, San Gabriel Mts., *Abrams & McGregor 626* (DS); Antelope Valley, *Eastwood s.n.* (CAS 7765). NEVADA: Florestan, *Moore & Moore 306* (JEPS); Donner Summit, *Moore 638* (JEPS). PLUMAS: Chilcoot, *Howell 50907* (CAS); 2.8 mi. E of Crystal Springs, *Janeway 4286* (CAS).

***Grindelia stricta*** DC., Prodr. (DC.) 7(1): 278. 1838 [late Apr 1838]. (*Haenke s.n.*; “in America boreali-occid. ad portum Mulgrave”; Alaska; holotype at G-DC)

Perennial or subshrub, 2–20 dm, decumbent to erect, branched throughout. Leaves 1–15 cm; basal present at anthesis or not, distal not much reduced; blade oblong to lanceolate, more or less fleshy, sessile or narrowed at base, glabrous or sparsely tomentose, green or red-veined, margin serrate, teeth pointed. Involucres 10–45 mm in diameter, hemispheric, glabrous or tomentose, resinous. Phyllaries in 4–6 series; bases wide, straw-colored; tips green, erect, reflexed, spreading, or coiled 270–360°. Ray flowers 16–60; rays 12–25 mm long. Cypselae 3.5–7 mm, whitish or gray- to red-brown, top knobby; pappus awns 2–6. Almost exclusively tetraploid ( $2n = 24$ ).

**Etymology**—*Stricta* is Latin for upright.

**Distribution and Ecology**—Sloughs, salt marshes, coastal bluffs, dunes. NCo, CCo, SCo, ChI; below 300 m. Flowering all year. From California north to southern Alaska.

**Discussion**—*Grindelia stricta* as here circumscribed is united more by habitat than by morphology. More research may show that the three varieties each merit treatment as species.

***Grindelia stricta*** DC. var. ***angustifolia*** (A.Gray) M.A.Lane, Novon 2: 217. 1992. *G. robusta* Nutt. var. *angustifolia* A.Gray in W.H.Brewer & S.Watson, Bot. California [W.H.Brewer] 1: 304. 1876. Nomenclatural synonym of *G. cuneifolia* Nutt., Trans. Amer. Philos. Soc. ser. 2, 7: 315. 1840 [Oct–Dec 1840]. (*Nuttall s.n.*; Santa Barbara; Santa Barbara Co., California; 1841; holotype at PH)

*Grindelia humilis* Hook. & Arn. f. *reflexa* Steyerl., Ann. Missouri Bot. Gard. 21: 526. 1934. (*Greene s.n.*; “in Oakland salt marsh”; Alameda Co., California; 15 November 1883; holotype at GH)

*Grindelia humilis* Hook. & Arn. f. *pubescens* Steyerl., Ann. Missouri Bot. Gard. 21: 527. 1934. (Howell 10805; “upper edge of tidal flat, Cuttings Warf, on Napa River”; Napa Co., California; 8 October 1932; holotype at CAS)

Subshrub 10–20 dm, erect; stems woody in proximal 3–15 dm. Leaves generally tapered to base, glabrous, tips generally acute. Heads not subtended by leaf-like bracts. Phyllary tips acute, flat in cross-section, erect. Ray flowers 16–56; rays 12–17 mm. Cypselae 5–7 mm. Almost entirely tetraploid ( $2n = 24$ ), but some diploid ( $2n = 12$ ) counts have been reported.

**Etymology**—*Angustifolia* is Latin for narrow-leaved.

**Distribution and Ecology**—Tidal wetlands. Flowering from May to December. CCo; below 10 m. Endemic to the salt marshes surrounding San Francisco Bay.

**Discussion**—*Grindelia stricta* var. *angustifolia* is among the most distinctive of the California Floristic Province taxa in the field due to its morphological uniformity and restricted range. However, there is much confusion about the proper name of this taxon, due to the poor quality of early herbarium specimens and their lack of locality data. The type specimen of *G. humilis* Hook. & Arn. was likely collected in the vicinity of either San Francisco Bay or Monterey Bay. The specimen has unusual morphology, including the clustering of many leaves below the head, which is typical of shoots that flower outside of their normal season. The name *G. humilis* has priority over all names of California *Grindelia* with the exception of *G. hirsutula*. Steyerl. (1934) hypothesized that this specimen belongs to the taxon here referred to as *G. s.* var. *angustifolia* and, if so, it would have priority over the name *G. stricta*. However, I here followed Lane (1992), in considering the type of *G. humilis* to be conspecific with *G. hirsutula*. The type specimen of *G. cuneifolia* resembles the plant called here *G. stricta* var. *angustifolia*. However, the location of the collection is listed as Santa Barbara. It is possible this locality is in error. It is also possible that the specimen represents instead an anomalous branch from a plant of a different taxon that is native to the Santa Barbara area (Steyerl. 1934). *Grindelia robusta* var. *angustifolia* is a nomenclatural synonym of *G. cuneifolia*, and the epithet *angustifolia* has priority at the varietal rank.

**Representative Specimens from California**—ALAMEDA: Alameda marshes, *Hall 5720* (UC); Bay Farm Island, Alameda, *Raven 5188* (JEPS); 0.5 mi E of Bay Bridge toll plaza, *Robbins 3938* (JEPS); San Leandro, *Robbins 3945* (JEPS); Berkeley, *Walker 436* (UC). CONTRA COSTA: Browns Island, *Knight et al. 3297* (JEPS); Pt. Pinole, *Moore et al. 865* (JEPS); Richmond, just W of Carlson Blvd., *Robbins 3941* (JEPS). MARIN: near San Rafael, *Davy 4066* (UC); Corte Madera, *Howell 15330* (UC); Inverness, *Howell 20739* (UC); China Camp, *Moore et al. 866* (JEPS); 1 mi. NE of San Rafael, *Rose 69101* (JEPS). SAN MATEO: La Riviere Marsh, *Moore & Park 867* (JEPS); Brisbane, *Rose 38229* (UC); SANTA CLARA: Palo Alto marshes, *Baker 47* (UC); Palo Alto salt marshes, *Stinchfield 252* (UC); Shoreline Park, Mountain View, *Taylor 116188* (UC); Alviso Slough, *Thomas 5307* (JEPS).

***Grindelia stricta* DC. var. *platyphylla*** (Greene) M.A.Lane, Novon 2: 217. 1992. *G. robusta* var. *platyphylla* Greene, Pittonia 2: 289. 1892. *G. rubricaulis* var. *platyphylla* (Greene) Steyerl., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]; *G. latifolia* Kellogg var. *platyphylla* (Greene) D.D.Keck, Aliso 4: 102. 1958. (*Howe s.n.*; “Monterey and Pacific Grove”; Monterey Co., California; July 1892; holotype at UC)

*Grindelia latifolia* Kellogg, Proc. Calif. Acad. Sci. 5: 36. 1873. *G. rubricaulis* var. *latifolia* (Kellogg) Steyererm., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]. (*Harford*; Santa Rosa Island; Santa Barbara Co., California; 1872–1873; holotype at GH)

*Grindelia rubricaulis* DC. var. *latifolia* (Kellogg) Steyererm. f. *minor* Steyererm., Ann. Missouri Bot. Gard. 21: 589. 1934. (*Eastwood 794*; “on Casmalia [sic] sands”; Santa Barbara Co., California; 13 June–3 July 1906; holotype at GH)

*Grindelia rubricaulis* DC. var. *latifolia* (Kellogg) Steyererm. f. *pubescens* Steyererm., Ann. Missouri Bot. Gard. 21: 589. 1934. (*Brandegee s.n.*; “between Lucia’s and Little’s Hot Springs”; Monterey Co., California; 14 June 1909; holotype at UC)

*Grindelia rubricaulis* DC. var. *platyphylla* (Greene) Steyererm. f. *villosa* Steyererm., Ann. Missouri Bot. Gard. 21: 590. 1934. (*Smith 1016*; “Pacific Grove, coast above Chinatown Point”; Monterey Co., California; 10 July 1905; holotype at GH)

Perennial 1–10 dm, decumbent to erect, herbaceous or stems proximally woody up to 1 dm above ground-level. Leaves generally sessile, sometimes clasping, glabrous or sparsely tomentose, tips acute or rounded. Heads often subtended by leaf-like bracts. Phyllary tips acuminate, more or less round in cross-section, spreading, reflexed, or coiled 270–360°. Ray flowers generally 20–60; rays 12–20 mm long. Cypselae 3.5–5 mm. Exclusively tetraploid ( $2n = 24$ ).

**Etymology**—*Platyphylla* is Greek for wide-leaved.

**Distribution and Ecology**—Coastal bluffs, dunes. Flowering all year. NCo, CCo, SCo, ChI; below 300 m. Endemic to California.

**Discussion**—*Grindelia stricta* var. *platyphylla* resembles the decumbent form of *G. s.* var. *stricta*, but *G. s.* var. *platyphylla* is generally larger and more robust, has horizontal branches that are not resting on the ground, and has ovate or obovate, instead of spatulate leaves. It occurs only along the immediate coast in northern California, but extends into coastal grasslands in southern California.

**Representative Specimens from California**—MARIN: Pt. Reyes Lighthouse, *Dunford 579* (UC); N shore Pt. Reyes Peninsula, *Ewan 8093* (UC); Pt. Reyes, *Hoover 4749* (JEPS); Pt. Reyes, *Lloyd 2102* (JEPS); Pt. Reyes, *Ray 1971B* (JEPS); Pt. Reyes, *Rosbach 543* (JEPS). MONTEREY: btwn. Pt. Pinos and Pacific Grove, *Heller 6843* (UC); Pacific Grove, *Jepson s.n.* (UC 32171); Pacific Grove, *Rose 33349* (UC); Elkhorn Slough, *Taylor 10531* (JEPS). SAN LUIS OBISPO: N of Peidras Blancas Pt., *Bacigalupi 9297* (JEPS); Rancho Marino Reserve, *Moore 945* (JEPS); 2.3 mi. N of Arroyo de la Cruz Creek, *Raven 11059a* (JEPS). SAN MATEO: Moss Beach, *Brandegee s.n.* (UC 472386); Montara State Beach, *Moore et al. 863* (JEPS); 2 mi. S of Pescadero, *True 480* (UC). SANTA BARBARA: Santa Rosa Island, *Brandegee s.n.* (JEPS 41902); W of mouth of Cuyama River, *Lee 296* (UC); intersection of Lompoc-Casmalia and Bishop Rds., *Pritchett CSM-50* (JEPS); Santa Rosa Island, *Raven 15009* (JEPS). SANTA CRUZ: Davenport, *Hesse 1150* (JEPS); mouth of Waddell Creek, *Hesse 2548* (JEPS); 5 mi. W of Watsonville, *Wright s.n.* (UC 118554). SONOMA: N of Salmon Creek, *Jepson 15949* (JEPS); Bodega Marine Lab, *Moore 987* (JEPS). VENTURA: Anacapa Island, *Blakley 5737* (JEPS); Anacapa Island, *Muller 1180* (JEPS).

***Grindelia stricta* DC. var. *stricta***

*Grindelia robusta* Nutt., Trans. Amer. Philos. Soc. ser. 2, 7: 314. 1840 [Oct–Dec 1840]. *G. rubricaulis* var. *robusta* (Nutt.) Steyererm., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]. (Nuttall; St. Pedro; California; isotypes at GH, PH)

*Grindelia pacifica* M.E.Jones, Bull. Torrey Bot. Club ix. (1882) 81. 1882. (Jones 2750; “on the hills at Santa Cruz”; Santa Cruz Co., California; holotype at POM)

*Grindelia venulosa* Jeps., Man. Fl. Pl. Calif. [Jepson] 1021. 1925 [24 Nov 1925]. *Grindelia stricta* DC. var. *stricta* f. *venulosa* Steyererm., Ann. Missouri Bot. Gard. 21: 558. 1934. *Grindelia stricta* DC. subsp. *venulosa* (Jeps.) D.D.Keck, Aliso 4: 102. 1958. (Bolander 6493; “at quicksand on sea-shore near Big Flat”; Humboldt Co., California; 1867; holotype at JEPS)

*Grindelia arenicola* Steyererm., Ann. Missouri Bot. Gard. 21: 227. 1934 [12 Apr 1934]. (Howell 5473; “in swale-like hollows in sand dunes, Mendocino Coastal plain, 4.5 mi N of Ft. Bragg”; Mendocino Co., California; 14 September 1930; holotype at CAS)

*Grindelia stricta* DC. var. *procumbens* Steyererm., Ann. Missouri Bot. Gard. 21: 559. 1934. (Moore & Steyermark 3687; “on sandy seashore at base of cliffs, 10 miles north of Trinidad,... alt. 1 m.”; Humboldt Co., California; 9 August 1931; holotype at MO)

*Grindelia blakei* Steyererm., Ann. Missouri Bot. Gard. 21: 567. 1934. *G. stricta* DC. subsp. *blakei* (Steyererm.) D.D.Keck, Aliso 4: 102. 1958. (Moore & Steyermark 3686; “in salt meadows along canal, Eureka,... alt. ½ m”; Humboldt Co., California; 9 August 1931; holotype at MO)

*Grindelia arenicola* Steyererm. var. *pachyphylla* Steyererm., Ann. Missouri Bot. Gard. 21: 596. 1934. (Howell 8106; “on open coastal plain, 6 mi. south of Point Arena”; Mendocino Co., California; 27 September 1931; holotype at CAS)

*Grindelia arenicola* Steyererm. var. *pachyphylla* Steyererm. f. *trichophora* Steyererm., Ann. Missouri Bot. Gard. 21: 596. 1934. (Schmitt s.n.; Halfmoon Bay; San Mateo Co., California; 1913; holotype at US)

Perennial 1–10 dm, decumbent to erect, herbaceous or stems proximally woody up to 0.5 dm. Leaves generally long-tapered to base; glabrous or sparsely tomentose, especially near head; tips rounded to acute. Heads not subtended by leaf-like bracts. Phyllary tips acuminate, more or less round in cross-section, spreading, reflexed, or coiled 270–360°. Ray flowers 30–60, rays 13–25 mm long. Cypselae 3.5–7 mm.  $2n = 24$ .

**Distribution and Ecology**—Sloughs, salt marshes, coastal bluffs, dunes. Flowering from June to November. NCo; below 60 m. From approximately the San Francisco Bay area north to Alaska.

**Discussion**—*Grindelia stricta* var. *stricta* appears to have two growth forms. One is an upright form with stems up to 1 m in length that grows in salt marshes and sloughs. The other is a form with decumbent stems that grows on sand dunes and coastal bluffs. Unlike *G. stricta* var. *platyphylla* in the same sand dune or coastal bluff habitat, plants of *G. s.* var. *stricta* are generally smaller, grow flat along the ground (instead of having horizontal branches that are not resting on the ground), and have spatulate instead of ovate or obovate leaves.

**Representative Specimens from California**—DEL NORTE: Point St. George, Heckard & Chuang 1977 (JEPS); mouth of Smith River, Nobs & Smith 1288 (UC); W of Lake Talawa, Thorne 35570 (UC). HUMBOLDT: Dry Lagoon Beach, Heckard 1220 (JEPS); 2.5 mi S of Fields Landing, Jepson 17924 (JEPS); Arcata Bay, Moore et al. 247 (JEPS); Lanphere Dunes, Moore & Pickart 1019 (JEPS); Arcata Marsh, Moore & Pickart 1021 (JEPS); Stone Lagoon, Moore & Pickart 1022 (JEPS); Eureka, Moore & Steyermark 3686 (UC); SW edge of Big Lagoon, Oswald & Ahart 8845 (JEPS); W of Honeydew along Mattole Rd., Raiche 30540 (JEPS); W of Arcata,



*Strother 1355* (UC); Eureka Slough, Humboldt Bay, *Wetherwax & Downing 2458* (JEPS).  
MENDOCINO: mouth of Big River, *Ertter & Sholars 8048* (UC); 1 mi. N of Fort Bragg, *Heller 15331* (UC); 4.5 mi. N of Fort Bragg, *Howell 5473* (UC); Newport, *Jepson s.n.* (JEPS 41876); .25 mi. N of Manchester, *Kamb & Chisaki 2309* (UC); Fort Bragg, *Moore 1017* (JEPS); Westport Union State Beach, *Semple & Heard 8534* (UC).

## Chapter Two

A phylogeny of *Grindelia* reconstructed from nuclear ribosomal and chloroplast sequence data.

### INTRODUCTION

In plant biogeography, certain disjunction patterns appear to occur more often than would be expected based on chance or the occurrence of suitable habitats (Thorne 1972). Among these are the New World amphitropical patterns, with groups that occur in the temperate areas of North America and South America, but are absent from the intervening tropics (Raven 1963, 1972, Wen and Ickert-Bond 2009). There are three major amphitropical disjunction patterns (Raven 1963, Wen and Ickert-Bond 2009): temperate, often (but not always) between the west coast of North America and the west coast of South America (e.g., *Blennosperma* Less. and *Lasthenia* Cass., Ornduff 1963; *Sanicula* L., Vargas et al. 1998); desert, between the deserts of North America and South America (e.g., *Larrea* Cav., Lia et al. 2001; *Tiquilia* Pers., Moore et al. 2006); and bipolar, between far-northern North America and far-southern South America (e.g., *Deschampsia* P.Beauv., Parodi 1949; *Primula* L., Guggisberg et al. 2009). In addition, amphitropical disjuncts may differ in the closeness of relationship of the disjunct plants (Wen and 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 et al. 2002; *Sanicula crassicaulis* Poepp. and *S. graveolens* Poepp., Vargas et al. 1998; *Tiquilia nuttallii* (Benth.) A.T.Richardson, Moore et al. 2006). In other cases, the plants have undergone substantial independent evolution, and sometimes diversification, on each continent (e.g., *Astragalus* L., Scherson et al. 2008; *Gentianella* Moench, Hagen and Kadereit 2001; *Hoffmannseggia* Cav., Simpson et al. 2005).

Both vicariance and dispersal hypotheses have been advanced for such amphitropical disjunctions. Given the great distance between temperate regions of North America and South America, hypotheses of vicariance during a period when the climate was cooler and suitable habitat may have occurred throughout the tropics (e.g., Solbrig 1972) or of shorter-distance dispersal between suitable, mountain-top habitats (Cruden 1966) have been invoked. While some so-called amphitropical disjuncts have limited diversity in mountainous areas throughout part of the tropics (e.g., *Epilobium* L., Seavey and Raven 1977; *Phacelia* Juss., Heckard 1963; *Trifolium amabile* Kunth, Ellison et al. 2006), many would unlikely have been able to find suitable habitats in the necessary time range (e.g., Carlquist 1983, Morrell et al. 2000, Moore et al. 2006). In addition, paucity or, for desert regions, absence of disjunct animals between temperate North America and South America (Simpson and Neff 1985) suggests that the 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 with an high diversity of taxa in temperate regions of both North America and South America. 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, Texas, and north-eastern Mexico (Steyermark 1934; Nesom 1990, 1992; Strother and Wetter 2006). South America has 26 species, with the center of diversity in Argentina, but with some species that occur west of the Andes (Bartoli and Tortosa 1999b, 2003b). Steyermark (1937) inferred a single disjunction between North American 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.

It is unclear what type of disjunction (or disjunctions) arose in *Grindelia* given the distribution of its taxa. *Grindelia* has species that are native to both Mediterranean-climate regions of the New World, so a temperate disjunction (dispersal between the CA-FP and Chile) is possible. *Zeltnera* G.Mans., which has a distribution similar to that of *Grindelia* in North America (Mansion and Zeltner 2004), appears to have dispersed from California to Texas and Mexico, as would have been required of *Grindelia* if it represents a temperate disjunction.

A desert disjunction pattern is also possible for *Grindelia*, given that the genus has centers of diversity in the dry-land areas of Argentina and in Texas and north-eastern Mexico. A pattern of dispersal from the North American deserts into the CA-FP is found in other CA-FP clades (e.g., *Lessingia* Cham., Markos and Baldwin 2001; various genera of the tribe Cichorieae in Asteraceae, Lee et al. 2002). In this study, I attempt to determine the type(s) and direction(s) of amphitropical dispersal event(s) in *Grindelia*.

Differences in ploidy between plants of North America 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 and Steyermark 1935, Dunford 1964, Bartoli 1993, Bartoli and Tortosa 1998b). No aneuploidy 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 and 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* 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 the genus *Grindelia* ambiguous.

Understanding relationships within *Grindelia* will be critical to resolving the biogeographic and ecological history of the group. There has been general agreement about the circumscription of *Grindelia* as a whole. All members of *Grindelia* have yellow ray and disc corollas, although ray florets are sometimes absent. As *Grindelia* was originally circumscribed, it 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 et al. 1993), *G. anethifolia* (formerly *Haplopappus pectinatus* Phil., Bartoli and Tortosa 1998a), and *G. prunelloides* (formerly *H. prunelloides* (Less.) DC., Bartoli and Tortosa 1999a).

Delimitation of the species of *Grindelia* and understanding their ranges of morphological and ecological variation have proven to be more controversial. 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. More recent authors have tended to recognize fewer and fewer of Steyermark's taxa (e.g., Keck 1959, Lane 1993). This trend culminated in Strother and 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*.

Habit is widely variable across *Grindelia* (Bartoli and Tortosa 2003a), which 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. In many cases, variation in habit is correlated with variation in habitat. *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* S.F.Blake, *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).

Most previous phylogenetic hypotheses involving *Grindelia* concern the North American taxa. Within North America, Steyermark (1937) considered the most ancient 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, nor has any other taxonomist. Steyermark (1937) considered the North American species to be too closely related and to show too much intergradation for useful sections to be formed, 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)

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 California species, Dunford 1964),

(2) the Havardii Genome (present in various species from Texas as well as the widespread *G. aphanactis*; Dunford 1970a,b, 1971), (3) the Oxylepis Genome (present in various species from Colorado and Mexico as well as the widespread *G. squarrosa*; Dunford 1970a, 1986), and (4) the Subalpina Genome (detected only in *G. subalpina* from Colorado and Wyoming, Dunford 1986). The tetraploids that Dunford examined from California (with the Hallii Genome) behave as autotetraploids in both their chromosome pairing during meiosis and the fact that both of their sets of chromosomes belong to the same structural genome (Dunford 1964, 1983).

Although Dunford (1970a, 1986) found several different chromosomal arrangements, with associated barriers to interfertility, he did not propose a sectional classification based on them and did not investigate enough of the species for the production of such a classification to be possible from his work. Similarly, no sectional classifications have been proposed for the South American species (Cabrera 1932, Bartoli and Tortosa 1999b).

I 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 and Markos 1998; Shaw et al. 2007). My goals in this study were (1) to examine the biogeographical history of the genus, (2) to examine the evolution of morphology and habitat in a phylogenetic context, (3) to re-examine previous hypotheses of the evolution of *Grindelia*, (4) to examine Dunford's genomic data in a phylogenetic context, and (5) to provide a phylogenetic context for a forthcoming sectional classification of the genus.

## MATERIALS AND METHODS

A total of 118 plants of 73 taxa were sampled. In total, 27 of the approximately 45 species of North American *Grindelia* (with many Mexican species unfortunately absent from the phylogeny) and 16 of the 26 species of South American *Grindelia* were included. When possible, wide-ranging or morphologically variable species were sampled multiple times. Outgroup taxa were chosen according to the phylogenies of Markos and Baldwin (2001) and Morgan (2003).

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 (Table 1). DNA was extracted using the Qiagen Plant Mini Kit (Qiagen Inc., Valencia, CA, 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, CA, U.S.A.) using 0.375  $\mu$ M concentration of each primer and 17  $\mu$ l of genomic DNA that was diluted 1:50 from the original concentration upon extraction. The remaining samples were amplified with component-based PCR with 1 $\times$  ThermoPol reaction buffer (New England Biolabs, Ipswich, MA, U.S.A.), 1.5 units of *Taq* polymerase (New England Biolabs), 0.4  $\mu$ M each primer, 0.6mM DNTPs, 0.5  $\mu$ g BSA, and 3  $\mu$ l genomic DNA at 1:10 dilution. The ITS region was amplified using the primers ITS4 (White et al. 1990) and either ITS-I (Urbatsch et al. 2000) or ITS5 (White et al. 1990) and sequenced using the primers ITS5 and ITS4. 411 base pairs of the 3' end of the ETS region were amplified and sequenced using the primers Ast-1 (Markos and Baldwin 2001) and 18S-ETS (Baldwin and Markos 1998). The *psaI-accD* spacer was amplified as a whole using the primers *psaI-72R* and *accD* (Shaw et al. 2007) or in two pieces using the internal primers *Rforpsal* (GCC

TAG TGA ATG AAA TTC GAA GAC) and FforaccD (GTG AGT ATA TAA TGT AGT TTT TCA TC). 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 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, OH, U.S.A.) and were cycle-sequenced using Big Dye v. 3.1 (Applied Biosystems Inc., Foster City, CA, U.S.A.). Sequencing products were resolved on ABI 377, ABI 3730, or ABI 3730xl automated sequencers (Applied Biosystems). Sequences were corrected using ChromasPro Version 1.5 and earlier versions (Technelysium Pty. Ltd., Tewantin, QLD, Australia) and aligned by eye in SeaView (Galtier et al. 1996, Gouy et al. 2010). Positions 1193-1215, 1247-1257, 1473-1510, and 1546-1613 were removed from the original *psaI-accD* alignment prior to analysis due to the difficulty of assessing sequence homology 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 analyzed separately and together. The nrDNA data set was slightly larger than the cpDNA data set (119 sequences instead of 94 sequences), because cpDNA sequences could not be obtained from some herbarium specimens and some outgroup taxa were sampled from GenBank, from which only ITS and ETS sequences were available.

Parsimony heuristic searches were performed in PAUP\* v. 4.0 b10 (Swofford 2002) with random taxon addition (5,000 replicates for cpDNA and 20,000 replicates for nrDNA and combined data), 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 1,000 bootstrap replicates, simple taxon addition, and rearrangements limited to 10,000,000 per replicate for nrDNA and combined data and 200,000 per replicate for cpDNA analyses.

Maximum likelihood heuristic searches were performed using RAxML version 7.2.5: HPC2 on Abe (Stamatakis 2006, Stamatakis et al. 2008) in the Cipres Portal (Miller et al. 2009). The searches were run with the GTRCAT model with 25 rate categories for the bootstrap search and the GTRGAMMA model for the final tree and 10,000 rapid bootstrap replicates. Bootstrap values were obtained by constructing majority-rule consensus trees in PAUP\*.

Bayesian analyses were run using Mr. Bayes version 3.1.2 (Huelsenbeck and Ronquist 2001) on Abe in the Cipres Portal (Miller et al. 2009). Two runs were performed with four chains each; the chains were run for 5,000,000 generations for cpDNA data, 15,000,000 generations for nrDNA data, and 26,417,000 generations for combined data and sampled every 1,000 generations. The sequence evolution model was GTR plus invgamma. For the analysis with combined data, the data were partitioned so that nrDNA and cpDNA could have separate models of sequence evolution. Posterior probabilities were derived from the set of post-burnin trees found after the standard deviation of the split frequencies dropped below 0.01 for the nrDNA and cpDNA trees (generations 1,860,000–5,000,000 for cpDNA and 3,093,000–15,000,000 for nrDNA). The standard deviation of the split frequencies did not drop below 0.024 in 26,417,000 generations (a 70 hour run, which appears to be the maximum for Mr. Bayes on Cipres) for the combined data, so trees from generations 15,000,000–26,417,000 were used to calculate estimated posterior probabilities. However, the results for the combined analysis could be unreliable given that the run ended prematurely.

Reconstructions of the ancestral character states for ploidy were performed in Mesquite version 2.5 (Maddison and Maddison 2008). The ploidy of the individuals in question was not

always known, as some individuals were sampled from herbarium specimens. This led some terminals to have polymorphic character states when their taxon could have multiple different ploidies. Only parsimony reconstructions were performed due to this polymorphism.

Parsimony analyses were performed in PAUP\* to examine the evolution of Dunford's (1986) genomic characters. Analysis parameters were identical to those used in the other parsimony analyses but with 10,000 random addition replicates. 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 separate them. Analyses were performed with the genome character weighted 1, 5, 7, 10, 15, and 20 times as heavily than an individual position in the sequence alignment.

To illustrate biogeography, the states of the tips are shown (Fig. 9). This figure is merely an illustration, not the result of rigorous analyses. Proper biogeographical analyses will be conducted prior to publication.

## 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 2). 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 did not have any strongly supported incongruences. There was only one clade that was strongly supported in one of the analyses (parsimony or likelihood bootstrap > 75% or Bayesian posterior probability > 0.95) and not present in the maximum likelihood trees (and thus not shown in the figures): the grouping of (*G. inuloides* 180 plus *G. greenmanii*) + (*G. ciliata* plus both accessions of *G. adenodonta*) in the Bayesian analysis of the nrDNA (0.97 posterior probability).

*Grindelia* was well-supported as monophyletic in trees from nrDNA, cpDNA, and combined data (Figs. 1–6). The remaining members of Morgan et al.'s (Morgan and Simpson 1992; Morgan 1997, 2003)  $x = 6$  clade were strongly supported as monophyletic in the nrDNA tree (Figs. 1–2); the clade was represented only by one sample of *Isocoma menziesii* in the cpDNA (Figs. 3–4) and combined (Figs. 5–6) trees. This clade was moderately supported as sister to *Grindelia* in all trees.

*Haplopappus* was resolved as monophyletic in the nrDNA and combined trees, but polyphyletic in the cpDNA tree. *Haplopappus* was part of a polytomy with the  $x = 6$  clade, *Pyrrocoma*, and a clade composed of *Lessingia*, *Hazardia*, and relatives in the nrDNA tree. The three *Haplopappus* clades alone formed a polytomy with the  $x = 6$  clade in the cpDNA tree, where many of these other genera were not included. In the tree from the combined data, which included the same taxa as in the cpDNA tree, *Haplopappus* was sister to the clade formed by *Grindelia* plus *Isocoma*.

Within *Grindelia*, the North American and South American taxa were sister clades in the nrDNA and combined trees. 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: *G. grandiflora* on a branch by itself, plus two major clades. The first major clade (the Eastern Clade; see Fig. 1 for clade labels) contained taxa from the eastern part of the range of *Grindelia*, east of the Continental Divide, as well as the more widespread species *G. aphanactis*, *G. arizonica*, and *G. squarrosa*. Within the Eastern Clade, there were several smaller clades containing 1–3 species as well as one large clade (the *G. squarrosa* 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 clade.

None of these clades within North American *Grindelia* were present in the cpDNA tree. Chloroplast data resolved only a few relationships, due in part to low sequence divergence, but mainly due to a high level of autapomorphic changes. Only one of these relationships was also found in the nrDNA tree, namely the sister relationship between *G. ciliata* and *G. adenodonta* (only one accession of which was present in the cpDNA data set).

The topology of the tree from analysis of combined data was generally similar to that of the nrDNA tree for North American *Grindelia*, with the exception that some of the relationships that were found only in the cpDNA tree were also present. For example, the two accessions of *Grindelia* from Marin County, California were sister in both combined and cpDNA trees, but not in the nrDNA tree.

In the nrDNA tree, the South American clade was divided at the base into two well-supported clades. One (the *G. brachystephana* Clade) consisted of *G. brachystephana*, one accession of *G. pulchella*, and *G. scorzonerifolia*. The other (the *G. chiloensis* Clade) contained all of the remaining species of South American *Grindelia*. Within the *G. chiloensis* Clade, the two accessions of *G. buphthalmoides* constituted a clade, while all of the accessions of *G. chiloensis* and *G. anethifolia* formed a clade together with *G. coronensis*.

Resolution and sequence divergence in the cpDNA tree are 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 is the clade uniting the two accessions of *G. buphthalmoides*, although the *G. covasii*-*G. patagonica* clade found in the cpDNA tree was not contradicted in the nrDNA tree.

Relationships among South American *Grindelia* were generally more poorly supported in the tree from the combined data than they were in the trees from nrDNA or cpDNA analyzed separately. This is likely due to the large amount of conflict between the two data sets. Both the clade formed by *G. covasii* and *G. patagonica*, as found in the cpDNA tree, and the *G. brachystephana* Clade, as found in the nrDNA tree, were recovered in the tree from the combined data.

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*, *H. uncinatus*, and *H. velutinus*. The



third clade was composed of *H. foliosus*, *H. marginalis*, a paraphyletic *H. multifolius*, and *H. undulatus*.

Although *Haploppapus* was also composed of three lineages in the cpDNA tree, they did not correspond to the clades found in the nrDNA tree. The one congruent finding was the clade formed of *H. undulatus* and the two accessions of *H. multifolius*, which was identical to their clade in the nrDNA tree, with the removal of two taxa for which cpDNA data were not available.

Relationships among the *Haploppapus* accessions in the tree from the combined data were congruent with those found in the nrDNA tree.

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. Tetraploidy appears to have arisen at least twice in North American *Grindelia*, once in the Eastern Clade and once in the Western Clade (Fig. 7). 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.

In the analyses in which Dunford's (1986) genomic data were combined with the nrDNA data, the tree topologies differed based on the weighting of the genomic character (Fig. 8). The tree became less resolved as the weight of the genomic character was increased. However, the reconstructed history of the genomes remained the same. The Hallii and Subalpina genomes each appeared to have arisen only once; however, the Subalpina Genome is only present in a single species. The Havardii Genome is confined to the Eastern Clade and the isolated *G. grandiflora*. The Oxylepis Genome was present in both Eastern and Western clades.

## DISCUSSION

All analyses of all types of data support the monophyly of *Grindelia* as a whole and the monophyly of North American *Grindelia*. Analyses of nrDNA and combined nrDNA and cpDNA 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 some more widely distributed taxa). Within the Western Clade, species from the Pacific states form a well-supported subclade.

### Dunford's Crossing 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 North American *Grindelia*. 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 the Pacific clade (Fig. 8); all members of that 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).

Dunford's (1970a) Havardii Genome is shared by many of the early-diverging members of the Eastern Clade as well as *G. grandiflora*, which is not a member of either of the two major North American clades (Dunford 1970a, 1971, 1986).

The Oxylepis Genome (Dunford 1970a, 1986) occurs in two places on the tree: in the *G. squarrosa* Clade and in *G. fastigiata*, which is part of the sister group of the Pacific Clade. Dunford (1970a) considered the Oxylepis Genome to possibly be ancestral among North American *Grindelia*. It is intermediate in structure between the Hallii and Havardii genomes and is separated from each of them by a single arm-interchange event.

Most members of the *G. squarrosa* Clade have the Oxylepis Genome, but four species in that clade have different genomes. Two of those species, *G. lanceolata* (Havardii Genome) and *G. nana* (likely the Hallii Genome), were represented by sequences from different populations, with some of those sequences placed in the *G. squarrosa* Clade and some placed in other clades. The individuals of *G. lanceolata* and *G. nana* in the *G. squarrosa* Clade quite likely possess *G. squarrosa* nrDNA as a result of secondary hybridization, with other genes most closely related to their presumed conspecifics that were placed outside the *G. squarrosa* Clade. There is some range overlap between *G. nana* and *G. squarrosa* in north-western California, and putative hybrid individuals have been found in the field and herbarium (pers. obs.). The third member of the *G. squarrosa* Clade that appears to lack the Oxylepis Genome is the tetraploid *G. aphanactis*. Although Dunford (1970b) considered *G. aphanactis* to have the Havardii Genome, his data are somewhat equivocal on this point. The possibility also remains that *G. aphanactis* may be an allotetraploid instead of an autotetraploid, with both the Oxylepis and the Havardii genomes.

The fourth species that was placed in the *G. squarrosa* Clade based on molecular data but that does not have the Oxylepis Genome is *G. subalpina*. It is the only species known to possess the Subalpina Genome (Dunford 1986). This genome is separated from the Oxylepis Genome 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 determine its structure relative to the remaining genomes (Dunford 1971, 1986). These two species are known to have genomes that differ by one rearrangement each from the Havardii and the Oxylepis genomes. Because only *G. scabra* is represented in the trees, it cannot be determined whether its genomic arrangement is diagnostic of a clade or whether it may have evolved more than once.

#### Ploidy:

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. 7).

Tetraploidy appears to have arisen three times in North American *Grindelia* based on the molecular data: in *G. aphanactis* (see Raven et al. 1960, Dunford 1970b for chromosome numbers), in the Pacific Clade (see Dunford 1964 for chromosome numbers), and in *G. fraxinipratensis* (Strother and Wetter 2006). 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. *Grindelia 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 and Tortosa 1998b). Tetraploidy appears to have arisen several times, once in the *G. brachystephana* Clade and at least twice in the *G. chiloensis* Clade. Although I was 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.

#### Steiermark's Lineages:

Steiermark (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 (Steiermark 1937, fig. 3).

Although the molecular trees do not correspond closely to Steiermark'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*, 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.

Steiermark (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 Steiermark did not discuss in detail the relationships of the other species resolved here within the *G. squarrosa* Clade, he did place them near *G. squarrosa* in his tree.

The only one of Steiermark's (1937) lineages that is found intact in the molecular tree corresponds to the Pacific Clade resolved here. Steiermark 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 taxa of the Pacific Clade from one another and from their most recent common ancestor. Steiermark 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" (Steiermark, 1937, p. 246).

#### Other Treatments:

All three species that were once included in other genera based on their pappus morphology—*G. ciliata* (formerly *Prionopsis ciliata*, Nesom et al. 1993), *G. anethifolia* (formerly *Haplopappus pectinatus*, Bartoli and Tortosa 1998a), and *G. prunelloides* (formerly *H. prunelloides*, Bartoli and Tortosa 1999a)—are nested well within *Grindelia*. This is congruent with the close relationship of *Prionopsis ciliata* and the single representative of *Grindelia*, *G. lanceolata*, in the higher-level trees of Morgan and 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*. 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 and Wetter (2006) took a much broader view of the circumscription of many *Grindelia* species than had previous authors. Most of the plants that Strother and 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 by molecular data. However, other members of the Western Clade (*G. fraxinipratensis*, *G. laciniata*) were not considered by Strother and Wetter to be allied with *G. hirsutula*. One member of Strother and Wetter's *G. hirsutula* was resolved in the Eastern Clade, *G. revoluta*. It appears to be closely related to *G. squarrosa*, based on molecular results.

In addition, Strother and Wetter (2006) expanded *G. arizonica* to include *G. laciniata*. Molecular data indicate that the similarity of those two species may be caused by convergence, because *G. arizonica* was resolved in the Eastern Clade, whereas *G. laciniata* was resolved in the Western Clade. *Grindelia aphanactis*, treated by Strother and 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 and Wetter's (2006) treatment can be fully tested and a revised classification of North American *Grindelia* constructed based in part on molecular data.

#### Biogeography and Ecology: North America:

The two major clades of North American *Grindelia* have overlapping but distinctive distributions (Fig. 9). 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 the somewhat more widespread *G. arizonica* and the very widespread *G. squarrosa* and *G. aphanactis*. Given that *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 kept approximately their current elevation since the end of the Laramide Orogeny in the Eocene (Dickinson et al. 1988, McMillan et al. 2006), 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 California Floristic Province Clade is part of the Pacific Clade, which otherwise consists mainly of taxa long considered to be closely related to, or even conspecific with, the plants in the California Floristic Province. The Pacific Clade in turn appears to have descended from species native to the desert southwest. This interpretation is congruent with Raven and Axelrod's (1978) hypothesis that *Grindelia* is a desert element in the California flora.

It has been hypothesized that desert plants would be pre-adapted to the summer drought of the CA-FP, given their ability to survive without rain for multiple hot months (Axelrod 1975, Raven and Axelrod 1978, Ackerly 2009). *Grindelia* belongs to a subtribe (Machaeranthrinae) of the Astereae that mainly occurs in desert or other dry-land habitats in western North America (e.g., Morgan 2003). Other clades within the Machaeranthrinae have either radiated in the CA-FP (*Lessingia/Corethrogyne* DC., Markos and Baldwin 2001), or have CA-FP endemic taxa (e.g., *Hazardia*, *Isocoma*, Markos and 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 salt- and brackish-water wetland habitats. Thus, some members of the Pacific Clade are able to tolerate physiological drought at the same time as their roots are continually moist. One of these taxa, *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 2 meters in length.

The sister group of the Pacific Clade is *Grindelia fraxinipratensis*, which is endemic to alkaline meadows in the Amargosa Valley in south-western 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 et 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 namophilum* (Reveal, Broome, & Beatley) G. Mans. is nested well within the California Clade of *Zeltnera* G. Mans. (Mansion and 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 and Olmstead 2008).

#### Biogeography: Amphitropical Disjunction:

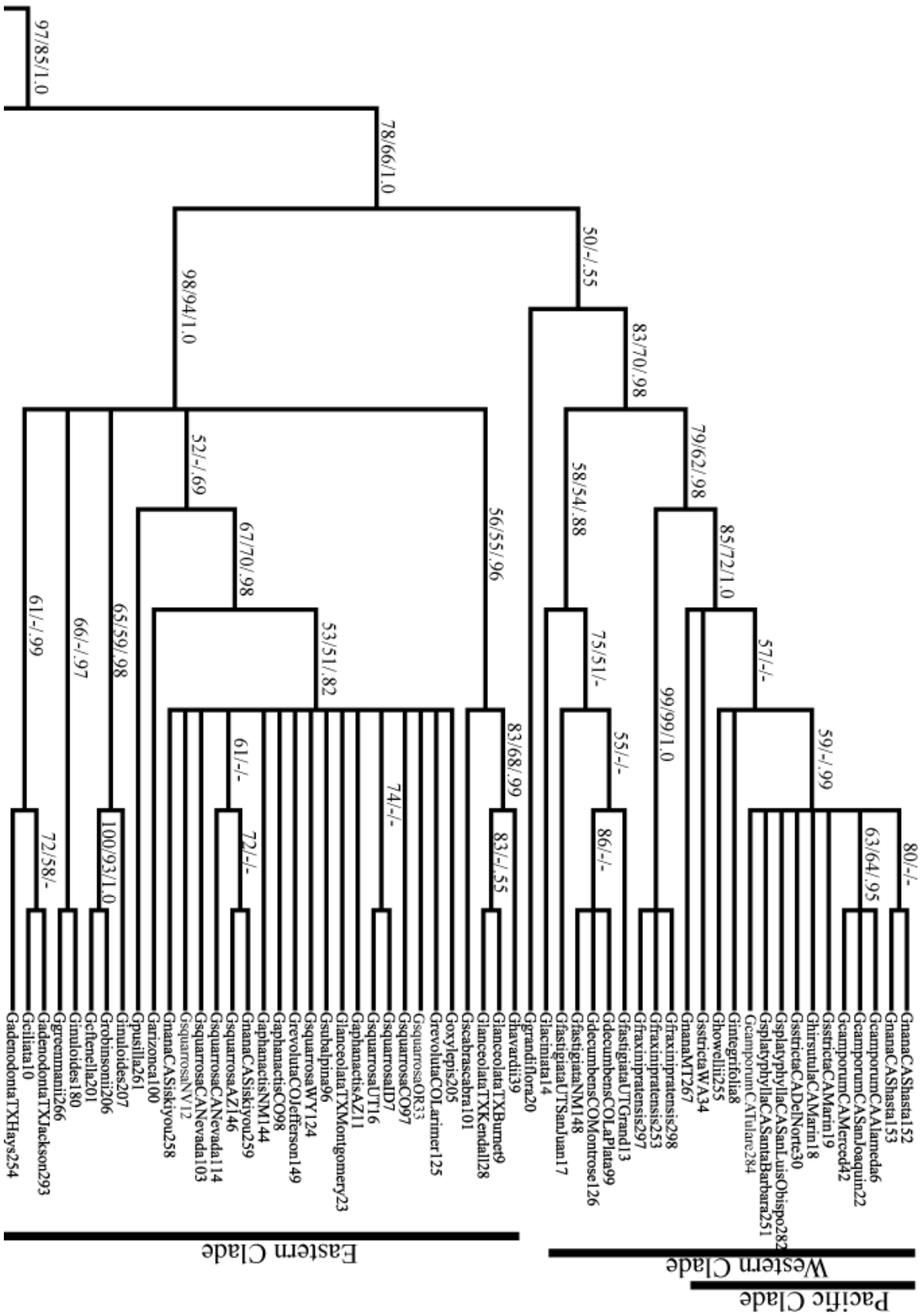
*Grindelia* appears to fit the pattern of a desert amphitropical disjunct. Within North America, species from Texas, Mexico, and the dry-land areas of the southwestern United States diverge basally in both clades. The taxa that grow along the Pacific coasts of North America and South America are well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

*Grindelia* does not appear to have any adaptations for long-distance dispersal. Its fruits (cypsela) do have a pappus, but the pappus falls off so readily that removing a cypselae from a head with the pappus still attached is difficult (pers. obs.). Birds readily consume the thin-walled fruits (pers. obs.), but the seed is the main nutritive component and endozoochory therefore 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 (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 occurred in *Grindelia*. Although the pattern of relationships between North American and South American *Grindelia* by itself cannot rule out the possibility of vicariance instead of dispersal as an explanation for the intercontinental disjunction, vicariance appears to be less likely given a lack of suitable habitat in the intervening regions throughout the conceivable timeframe for divergence between the clades and a lack of animals shared between the two areas, as would be expected under a vicariance scenario (Carlquist 1983, Simpson and Neff 1985, Morrell et al. 2000, Moore et al. 2006).

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 western North American endemism of most of close relatives of *Grindelia* (Nesom and Robinson 2007). If this hypothesis is correct, the ancestors of the South American genus *Haplopappus* would have undergone an independent dispersal from North America to South America.

Figure 1. Maximum likelihood bootstrap consensus tree from the nrDNA data. Support values are above the branches (maximum likelihood bootstrap values, parsimony bootstrap values, Bayesian posterior probabilities). The Western Clade and Eastern Clade together comprise North American *Grindelia*, shown in the first part of the figure. The *G. chiloensis* Clade and *G. brachystephana* Clade together comprise South American *Grindelia*, shown in the second part of the figure along with the non-*Grindelia* taxa. *Grindelia* accessions are indicated by a G followed by the specific epithet. *Haplopappus* accessions are indicated by an H followed by the specific epithet. Duplicate taxa are differentiated by state and (where appropriate) county or collecting locality.





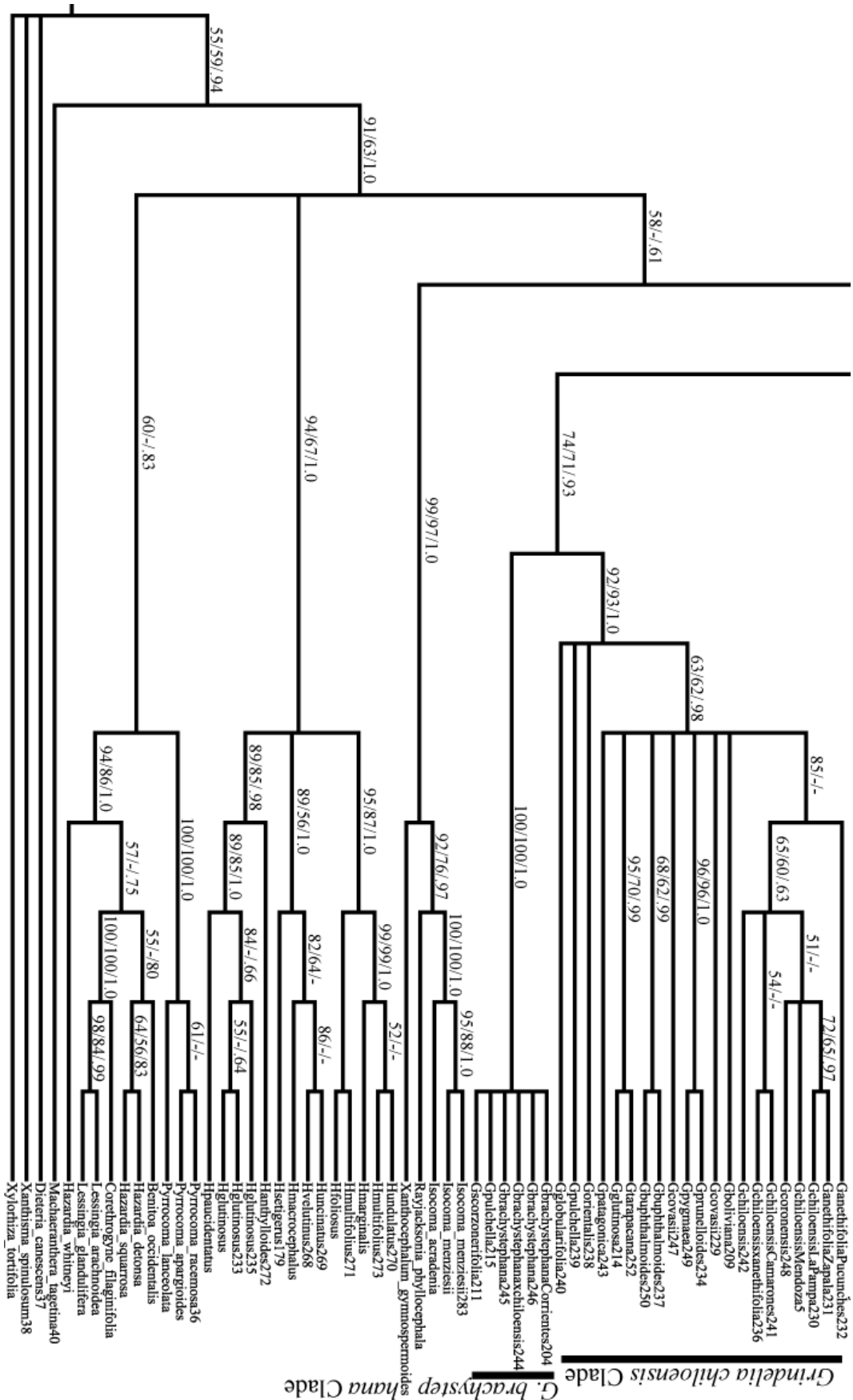
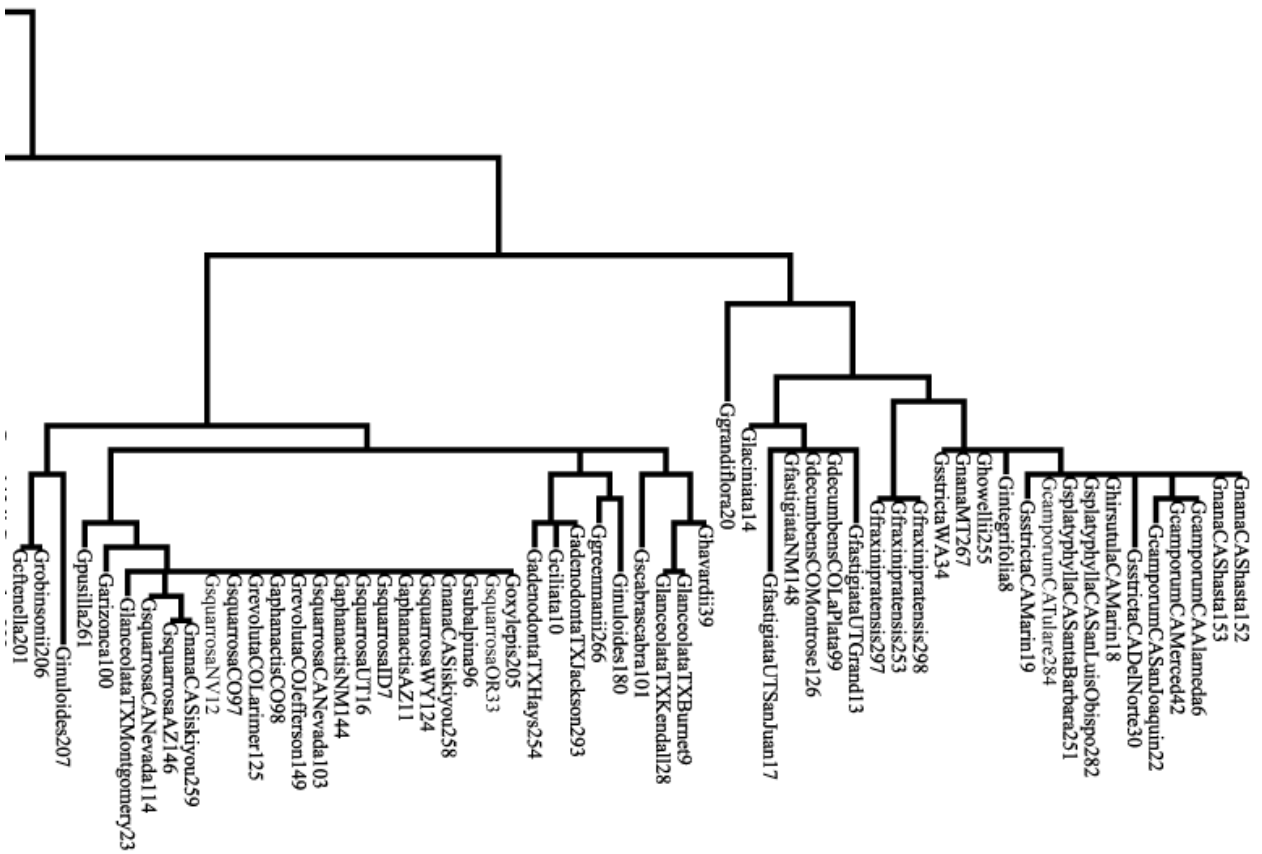


Figure 2. Maximum likelihood tree from the nrDNA data (lnL= -4359.06). Taxon labeling follows Figure 1. North American *Grindelia* in the first part of the figure, South American *Grindelia* and non-*Grindelia* taxa in the second part of the figure.



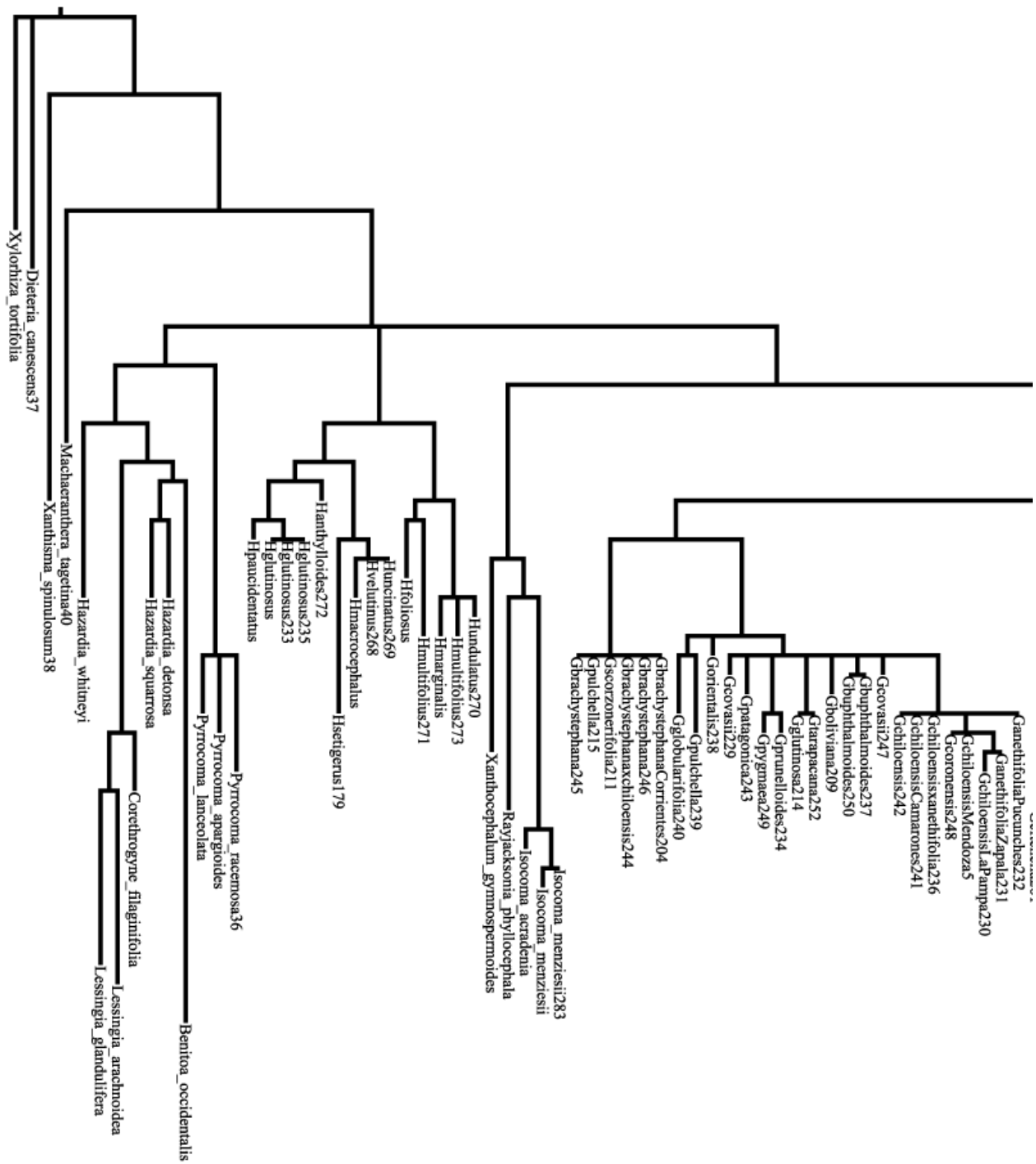
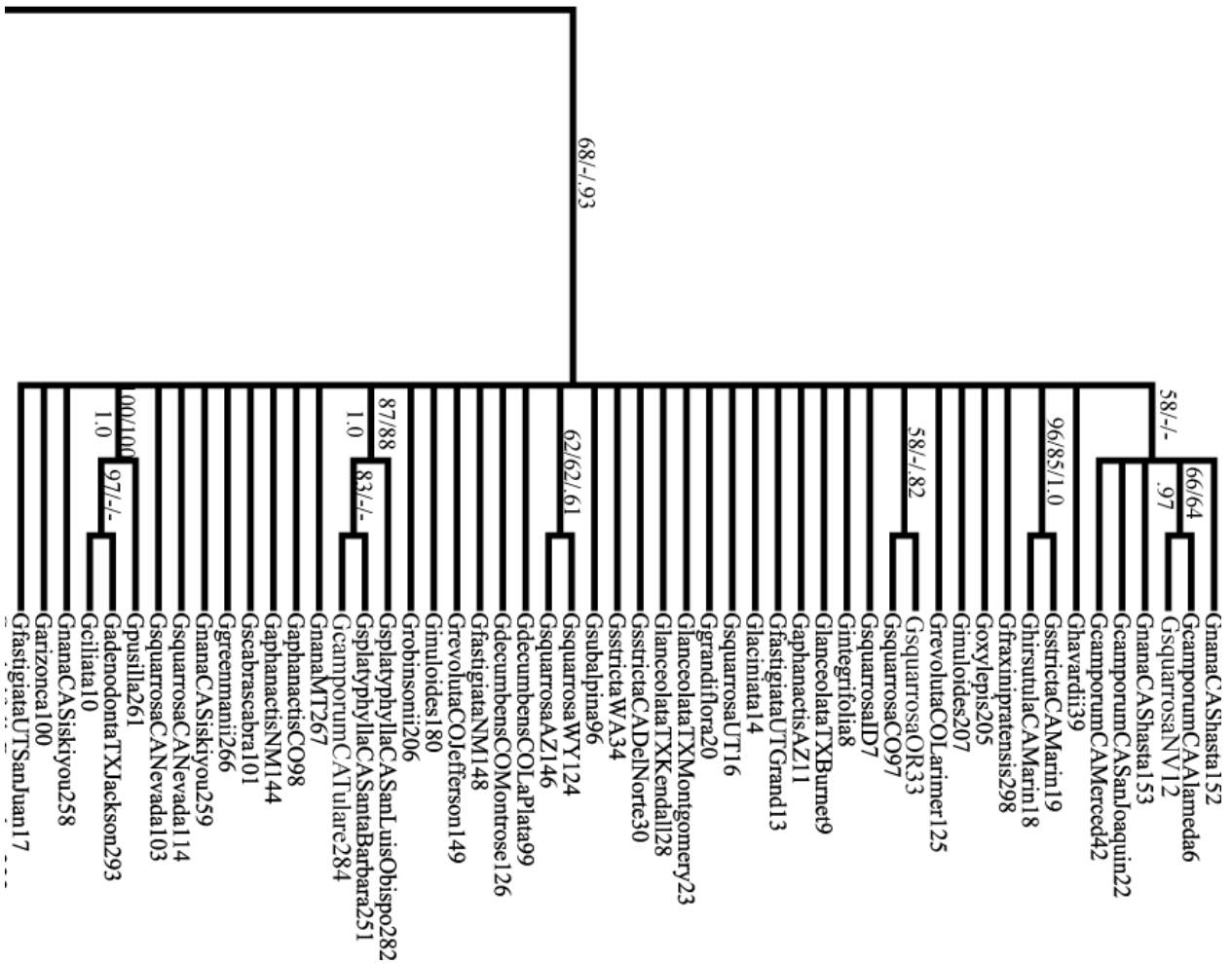


Figure 3. Maximum likelihood bootstrap consensus tree from the cpDNA data. Support values are above the branches (maximum likelihood bootstrap values, parsimony bootstrap values, Bayesian posterior probabilities). Taxon labeling follows Figure 1. North American *Grindelia* in the first part of the figure, South American *Grindelia* and non-*Grindelia* taxa in the second part of the figure.



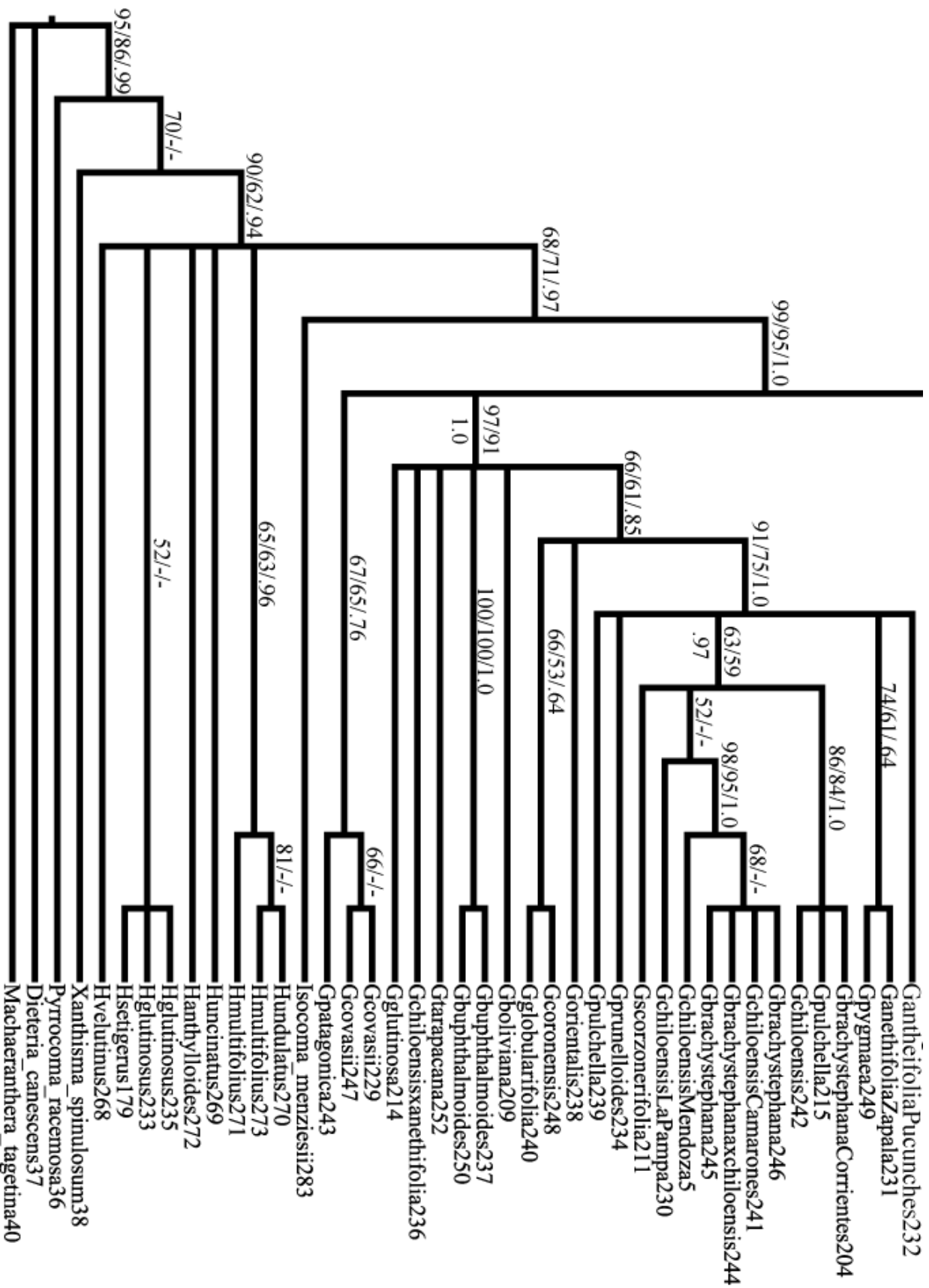
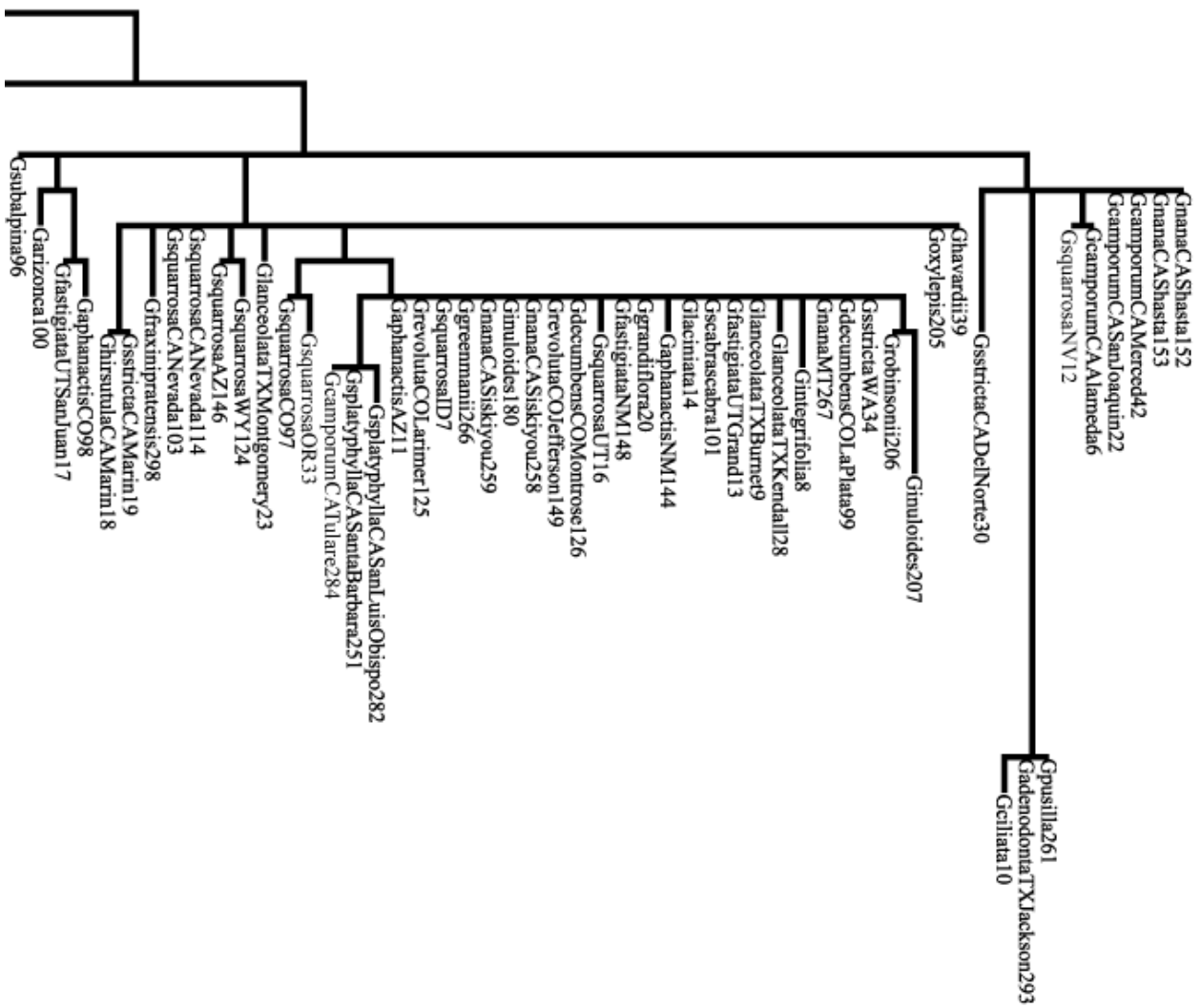


Figure 4. Maximum likelihood tree from the cpDNA data (lnL= -3350.88). Taxon labeling follows Figure 1. North American *Grindelia* in the first part of the figure, South American *Grindelia* and non-*Grindelia* taxa in the second part of the figure.





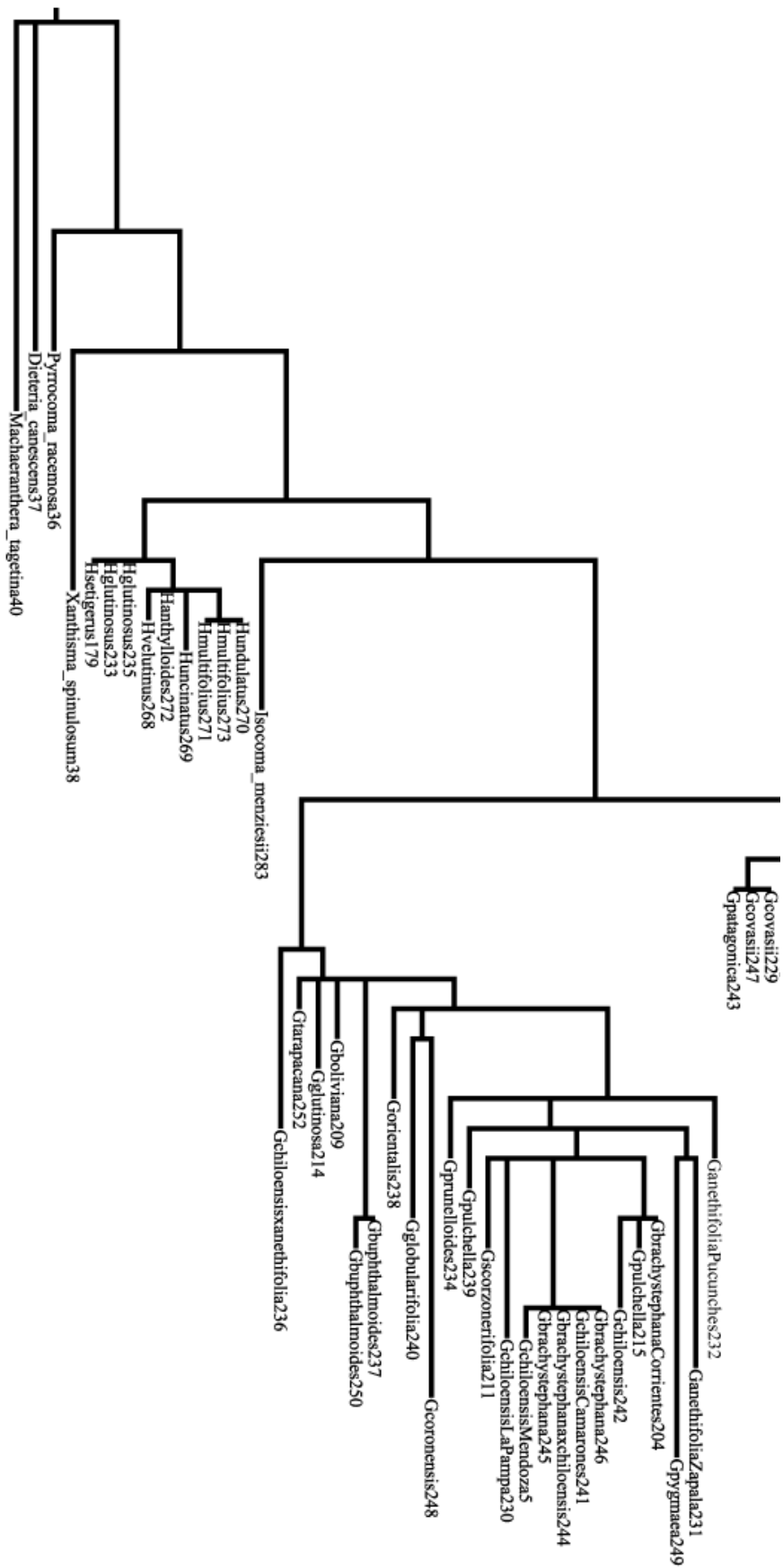
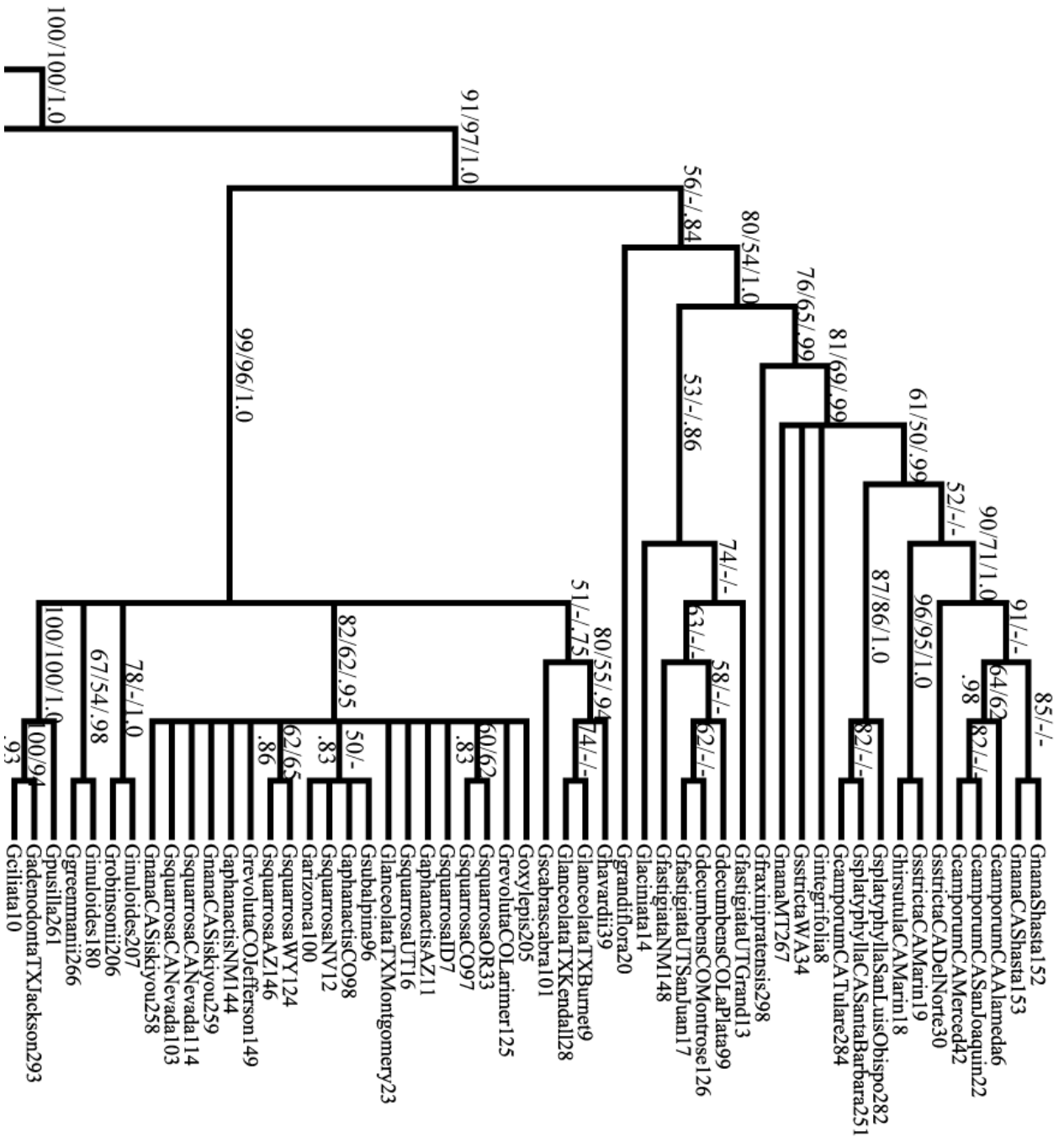


Figure 5. Maximum likelihood bootstrap consensus tree from the combined data. Support values are above the branches (maximum likelihood bootstrap values, parsimony bootstrap values, Bayesian posterior probabilities). Taxon labeling follows Figure 1. North American *Grindelia* in the first part of the figure, South American *Grindelia* and non-*Grindelia* taxa in the second part of the figure.



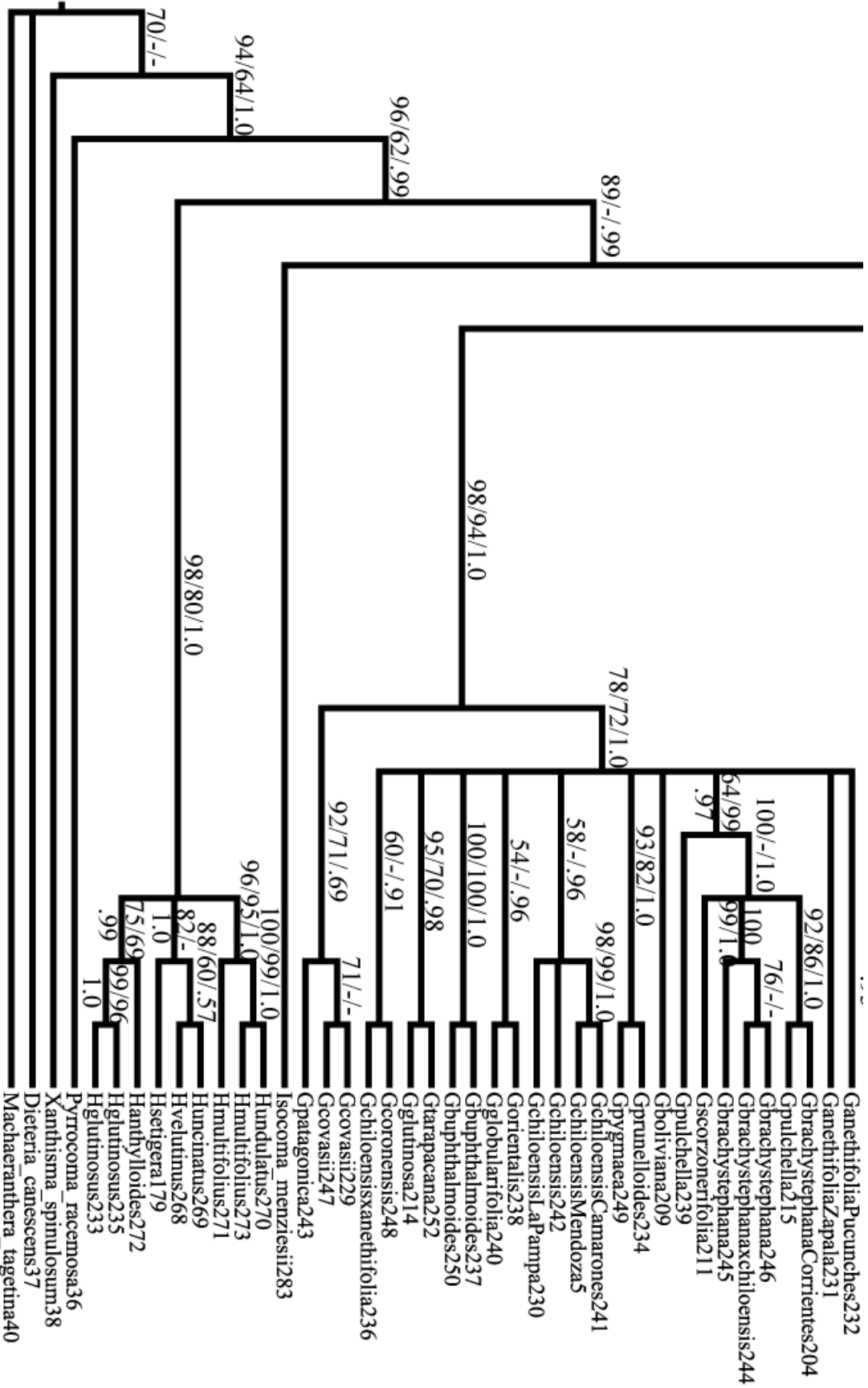
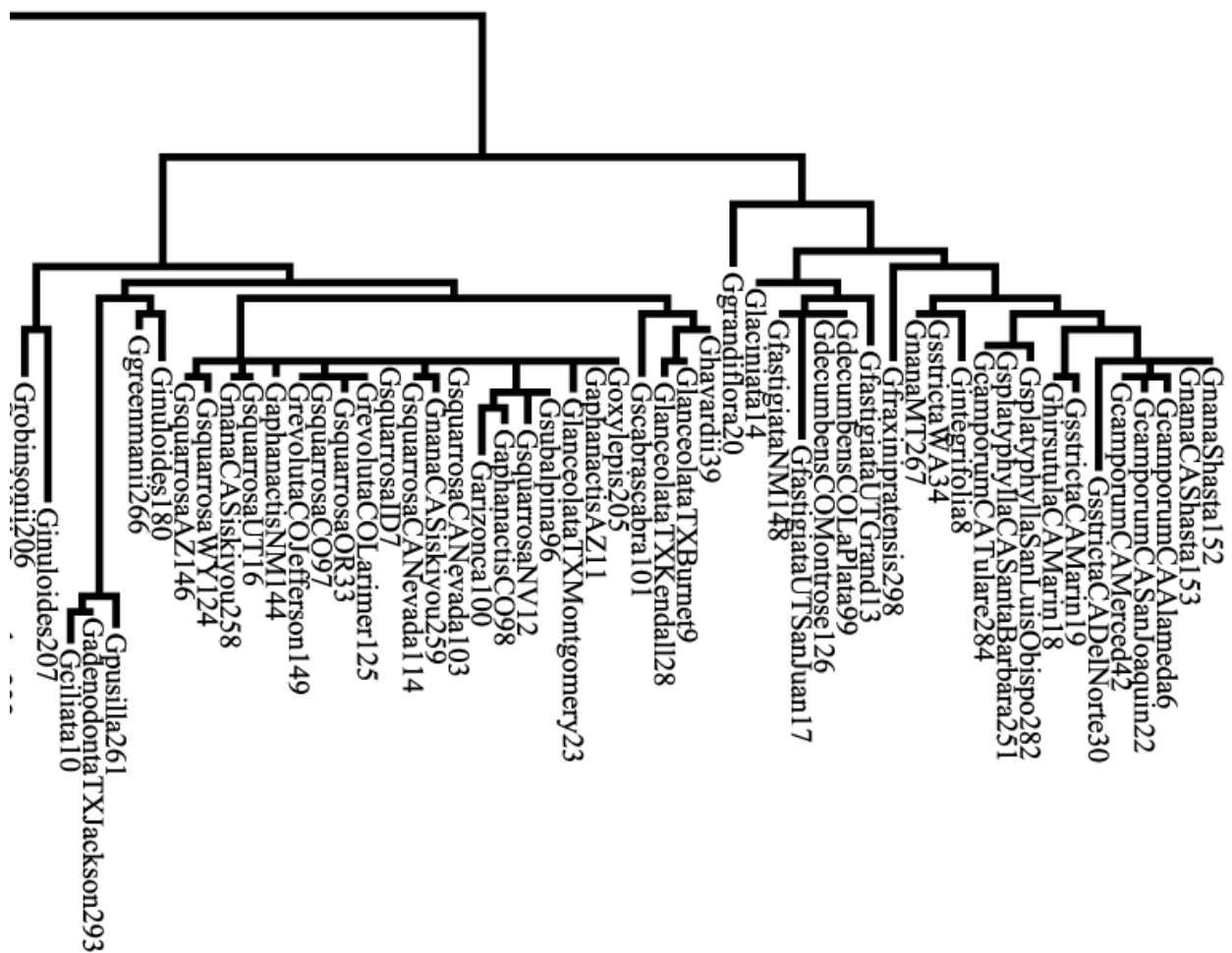


Figure 6. Maximum likelihood tree from the combined data (lnL= -7256.65). Taxon labeling follows Figure 1. North American *Grindelia* in the first part of the figure, South American *Grindelia* and non-*Grindelia* taxa in the second part of the figure.



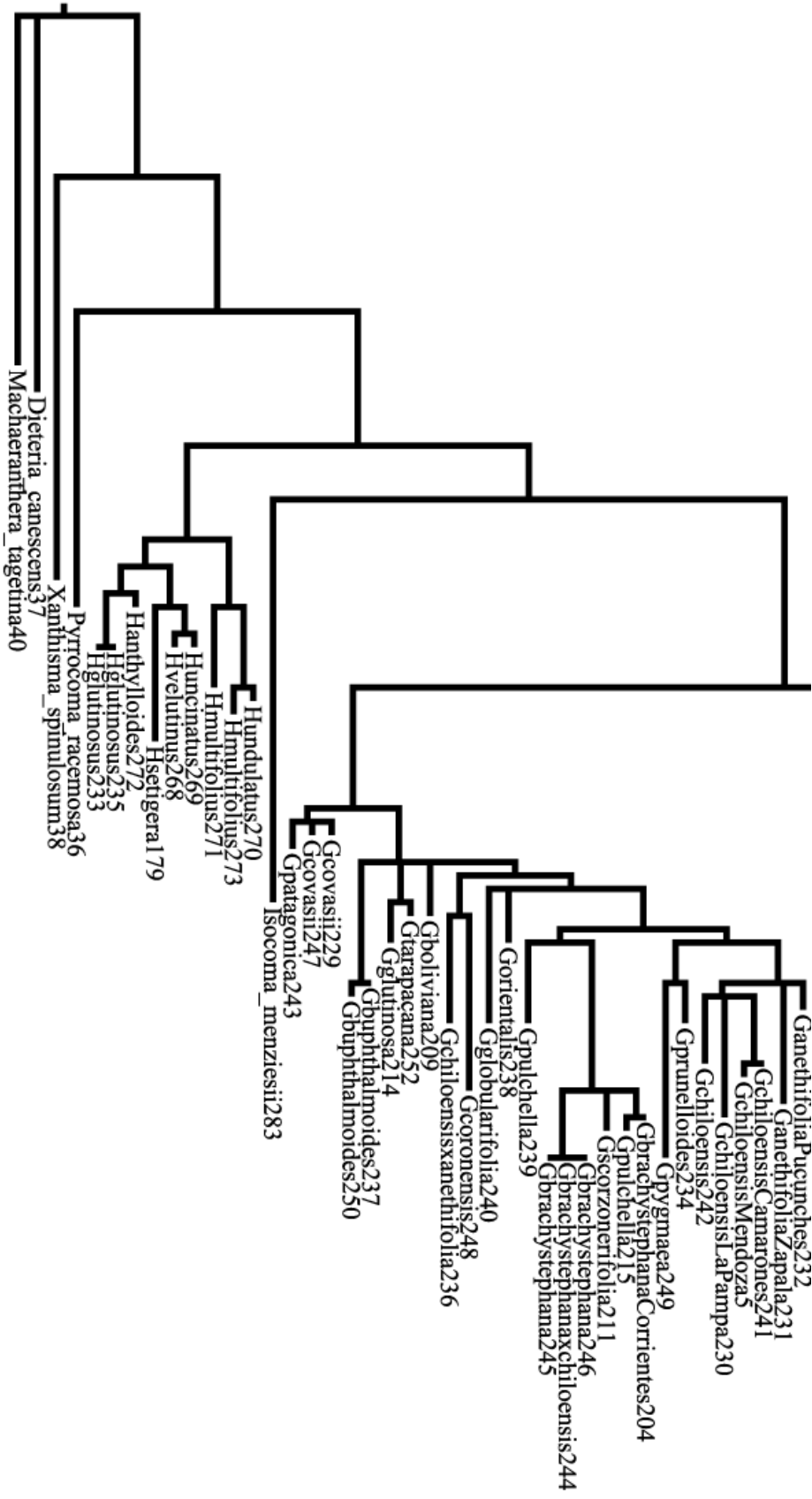




Figure 7. Parsimony reconstruction of ancestral ploidy on the maximum likelihood nrDNA tree. Taxon labeling follows Figure 1.

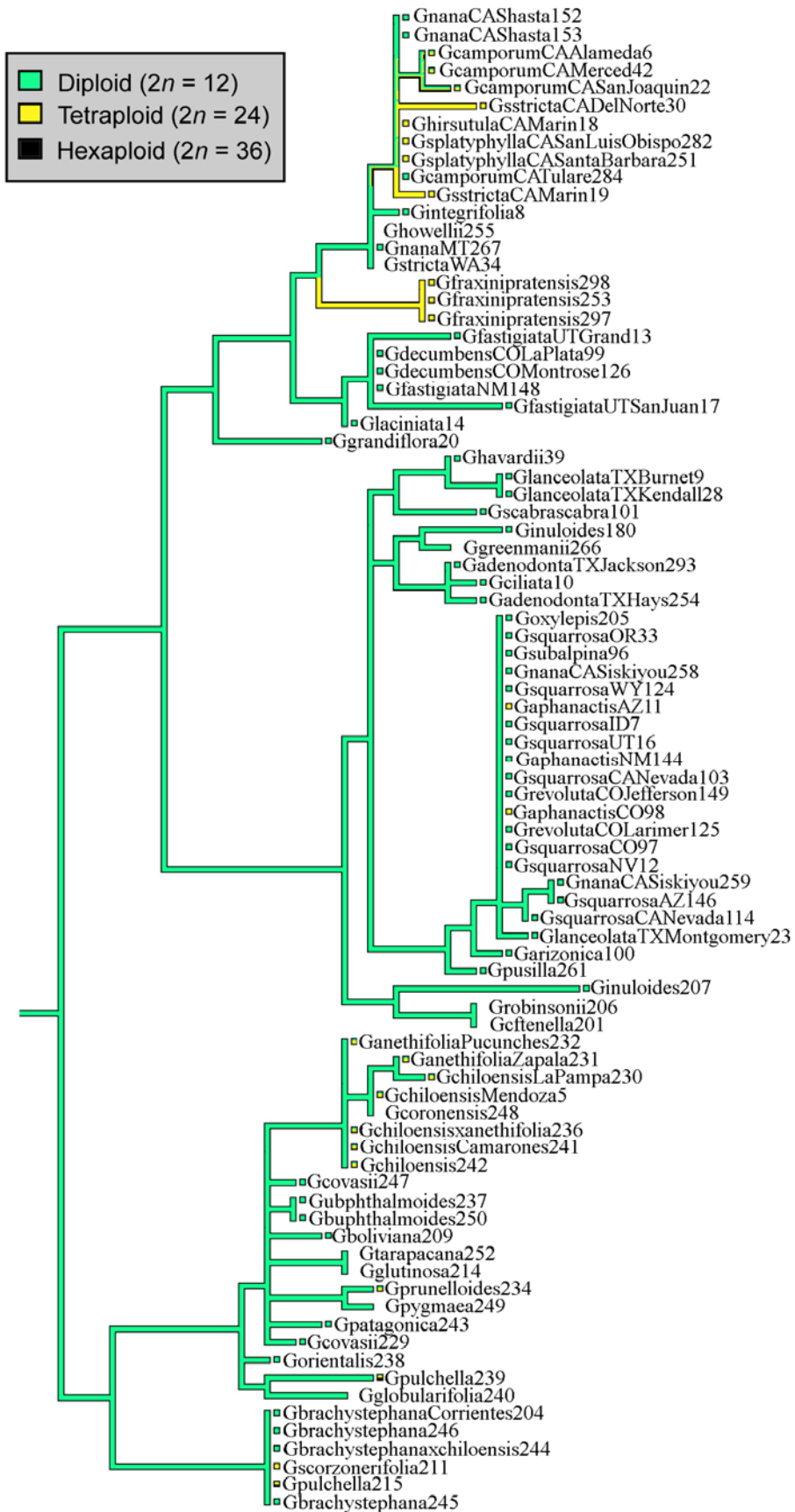


Figure 8. One of 2464 equally parsimonious tree from an analysis of the nrDNA data combined with Dunford's (1986) genomes (456 steps, CI = 0.68, RI = 0.91). The genome character was weighted five times more heavily than an individual position in the sequence alignment. Branches are colored according to the parsimony reconstruction of the genome character (see legend), with black branches indicating an undetermined genome for that species. Taxon labeling follows Figure 1.

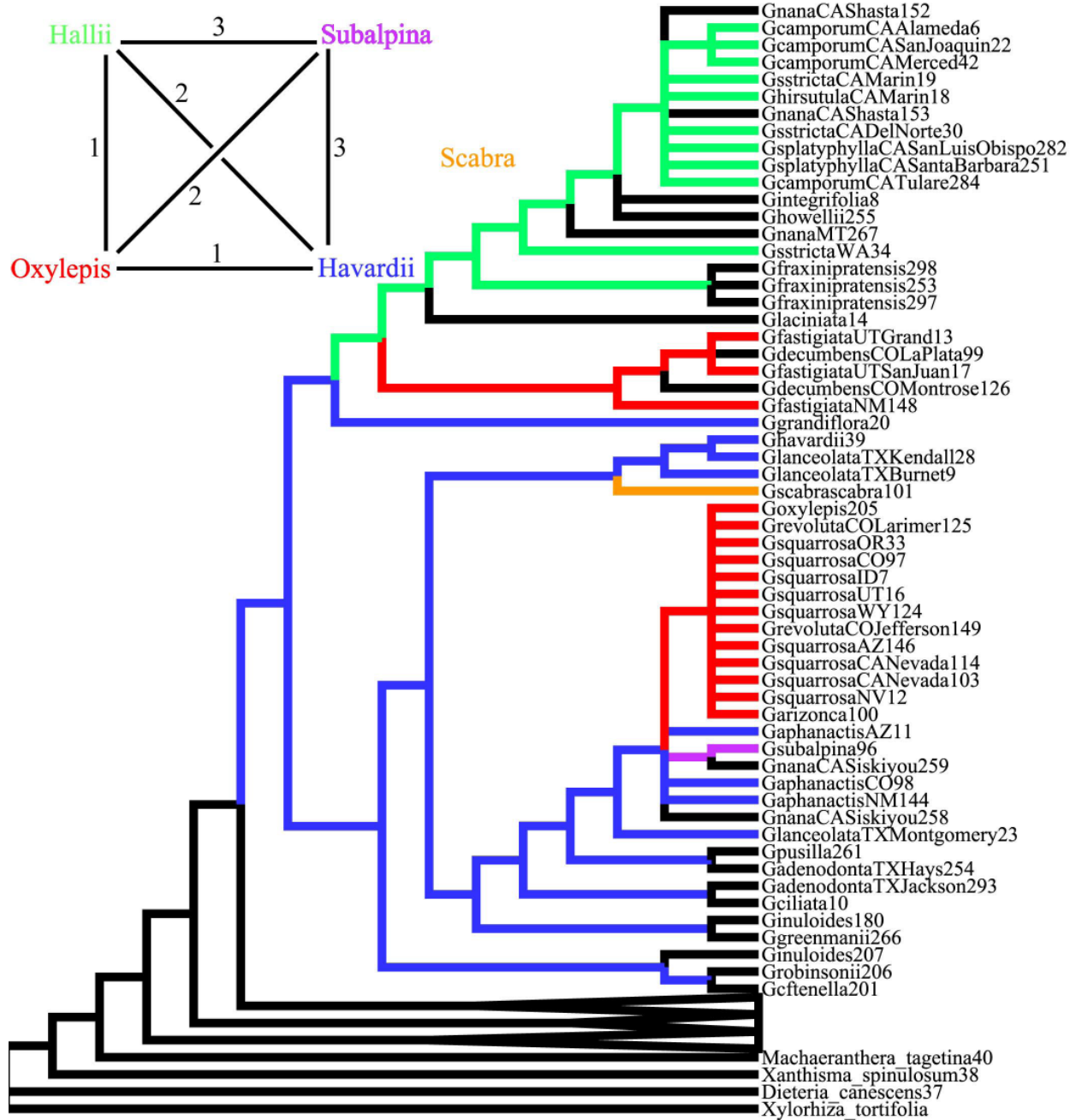
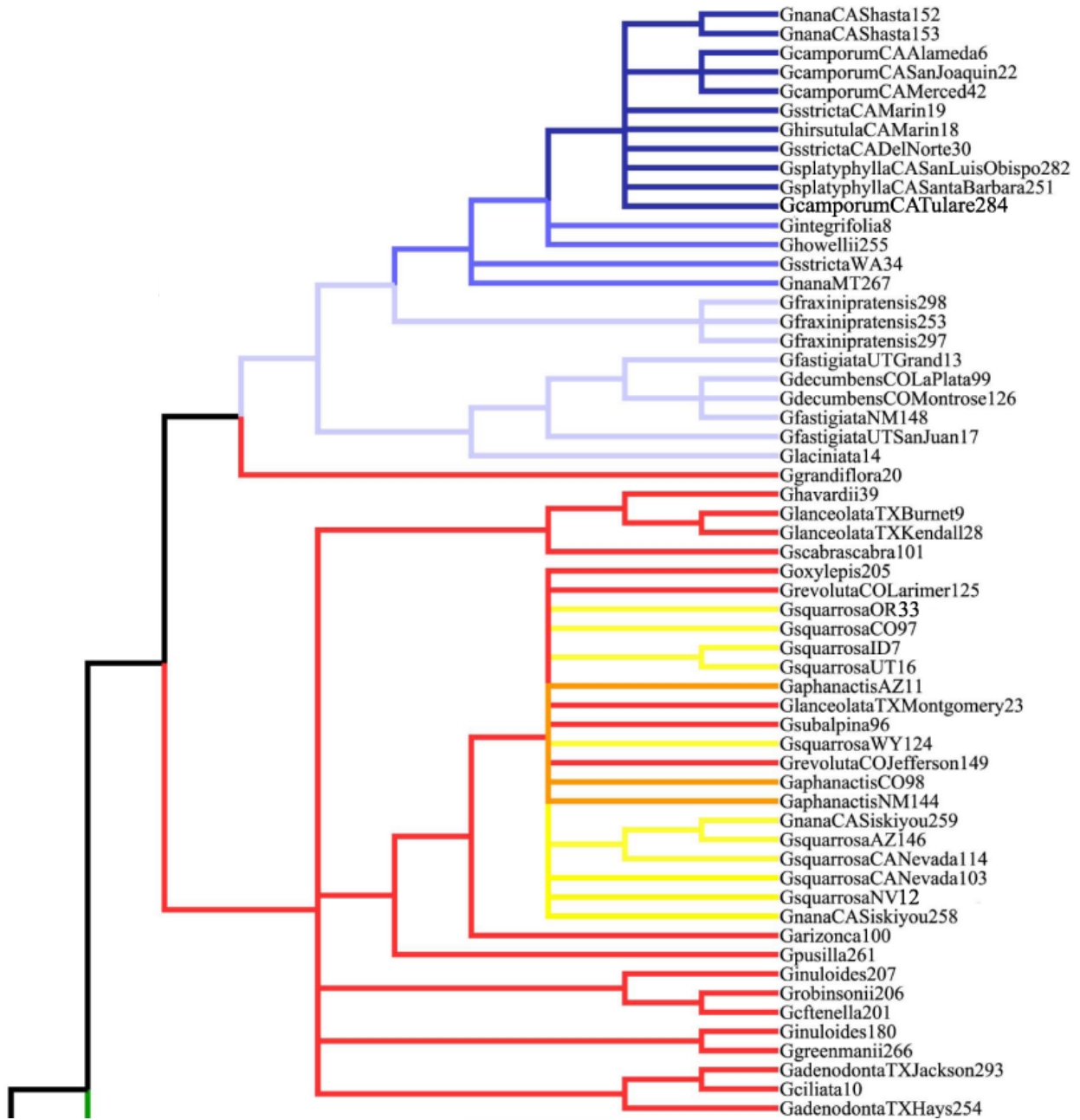
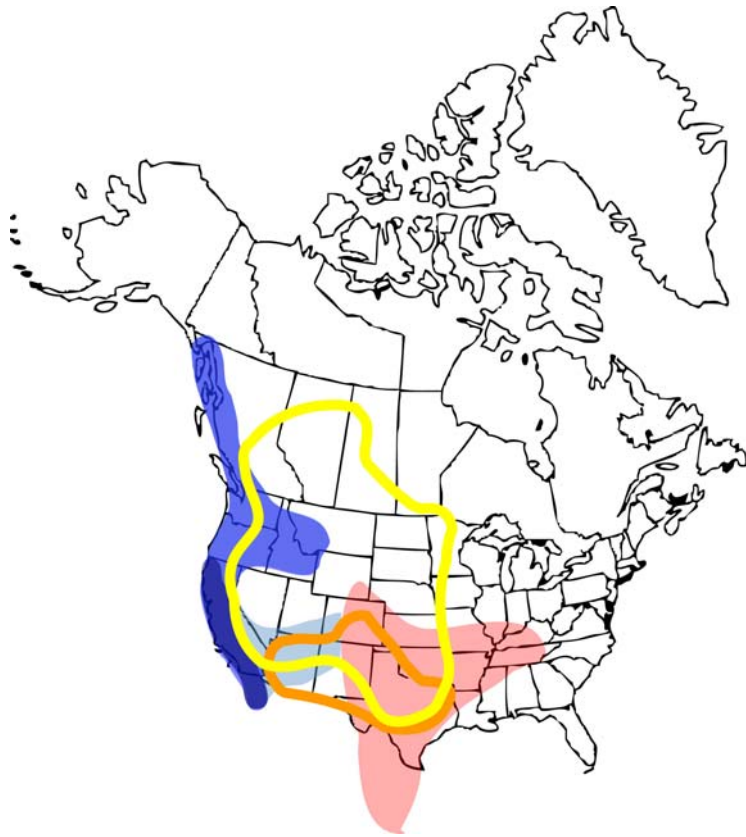


Figure 9. Maximum likelihood nrDNA bootstrap consensus tree with branches of the North American accessions colored according to the distribution of the clades on the corresponding map. For the South American accessions, dark green branches correspond to plants collected east of the crest of the Andes, while light green branches correspond to plants collected west of the crest of the Andes. Taxon labeling follows Figure 1. North American *Grindelia* in the first part of the figure, map with a key to the colors of North American *Grindelia* in the second part of the figure, and South American *Grindelia* and non-*Grindelia* taxa in the last part of the figure. This figure is presented by way of illustration only, not as the result of rigorous analysis. Such analyses will be performed prior to publication.





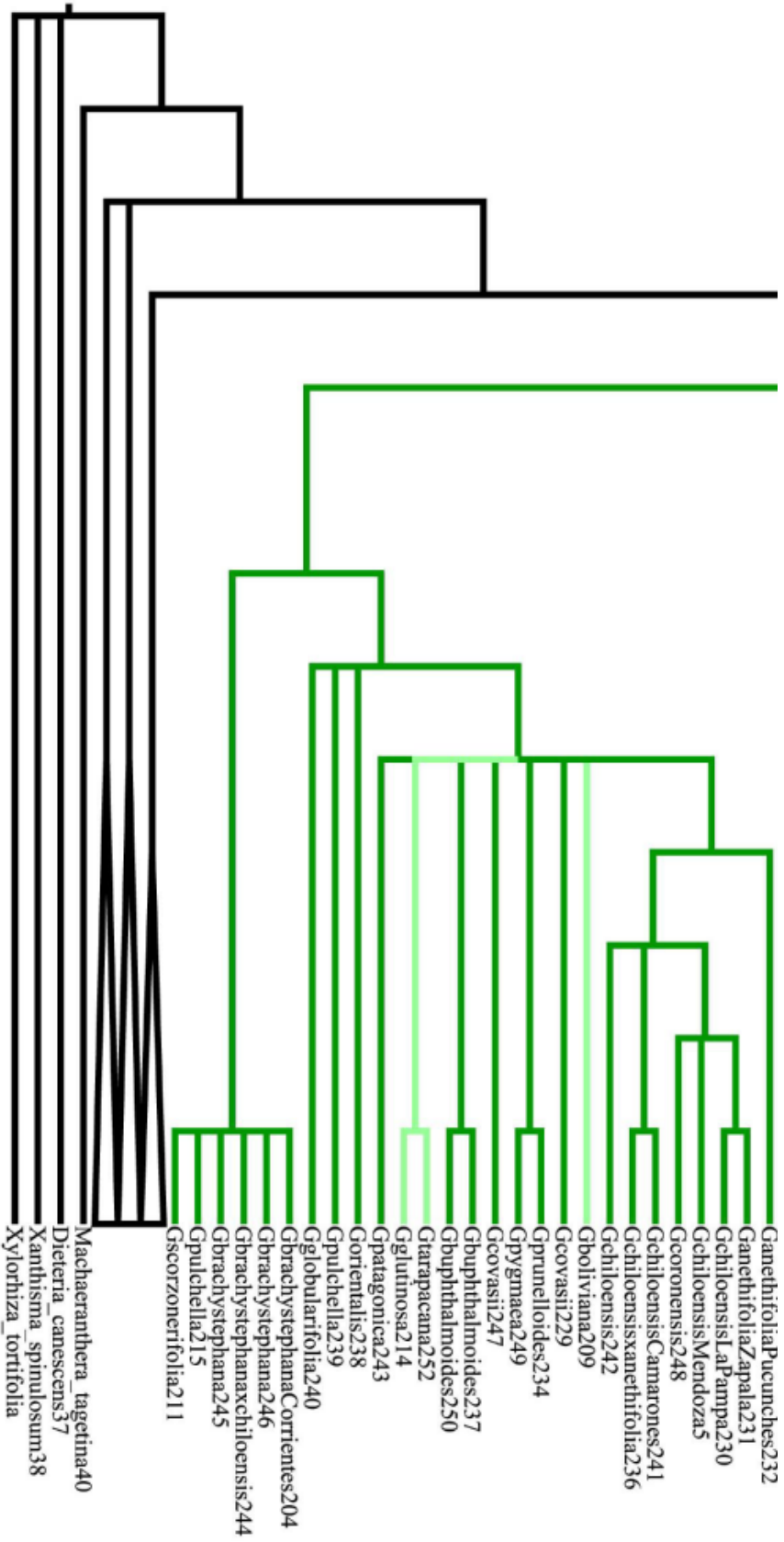




Table 1. Specimens used in the phylogeny. AJM number is my extraction number, which follows the taxon name in the phylogeny, when applicable. Herbarium abbreviations follow Thiers, B. [continuously updated]. Original sequences do not yet have GenBank numbers. GenBank numbers are given for ITS and ETS sequences when those sequences were obtained from GenBank. No *psaI-accD* sequences were obtained from GenBank.

AJM #	taxon name	collection locality	collection	GenBank #s ITS, ETS
254	<i>Grindelia adenodonta</i> (Steerm.) G.L.Nesom	Hays Co., Texas	Moore & Steyermark 3000 (UC)	
293	<i>Grindelia adenodonta</i> (Steerm.) G.L.Nesom	Jackson Co., Texas	Wetter 605 (CAS)	
231	<i>Grindelia anethifolia</i> (Phil.) A. Bartoli & Tortosa	Prov. Neuquén, Argentina	Argentina 7 (BAA)	
232	<i>Grindelia anethifolia</i> (Phil.) A. Bartoli & Tortosa	Prov. Neuquén, Argentina	Argentina 8 (BAA)	
11	<i>Grindelia aphanactis</i> Rydb.	Cochise Co., Arizona	Moore & Park 367 (JEPS)	
98	<i>Grindelia aphanactis</i> Rydb.	Las Animas Co., Colorado	Moore & Moore 589 (JEPS)	
144	<i>Grindelia aphanactis</i> Rydb.	Santa Fe Co., New Mexico	Moore & Moore 614 (JEPS)	
100	<i>Grindelia arizonica</i> A.Gray	Archuleta Co., Colorado	Moore & Moore 609 (JEPS)	
209	<i>Grindelia boliviana</i> Rusby	Dpto. Puno, Peru	Soukup 56 (UC)	
244	<i>Grindelia brachystephana</i> Griseb. x <i>G. chiloensis</i> (Cornel.) Cabrera	Argentina	14-Jan-09 (BAA)	
204	<i>Grindelia brachystephana</i> Griseb.	Prov. Corrientes, Argentina	Schinini 16051 (UC)	
245	<i>Grindelia brachystephana</i> Griseb.	Argentina	14-Jan-09 (BAA)	
246	<i>Grindelia brachystephana</i> Griseb.	Argentina	16-Jan-02 (BAA)	
237	<i>Grindelia buphthalmoides</i> DC.	Prov. Buenos Aires, Argentina	Argentina 72 (BAA)	
250	<i>Grindelia buphthalmoides</i> DC.	Prov. Buenos Aires, Argentina	Marzal & Rattosn 7/Jan/2009 (BAA)	
6	<i>Grindelia camporum</i> Greene	Alameda Co., California	Moore & Zacharias 433 (JEPS)	
22	<i>Grindelia camporum</i> Greene	San Joaquin Co., California	Moore 381 (JEPS)	
42	<i>Grindelia camporum</i> Greene	Merced Co., California	Zacharias 947 (JEPS)	
284	<i>Grindelia camporum</i> Greene	Tulare Co., California	Moore, Laymon, and Williams 947 (JEPS)	
236	<i>Grindelia chiloensis</i> (Cornel.) Cabrera x <i>G. anethifolia</i> (Phil.)	Prov. Neuquén, Argentina	Argentina 62 (BAA)	

	A. Bartoli & Tortosa			
5	<i>Grindelia chiloensis</i> (Cornel.) Cabrera	Prov. Mendoza, Argentina	UCBG 95.0506 (UC)	
230	<i>Grindelia chiloensis</i> (Cornel.) Cabrera	Prov. La Pampa, Argentina	Argentina 5 (BAA)	
241	<i>Grindelia chiloensis</i> (Cornel.) Cabrera	Camarones, Argentina	Bartoli & Tortosa 14/I 2009 (BAA)	
242	<i>Grindelia chiloensis</i> (Cornel.) Cabrera	Argentina	Bartoli & Tortosa 7 Jan 2002 (BAA)	
10	<i>Grindelia ciliata</i> (Nutt.) Spreng.	Jeff Davis Co., Texas	Moore & Park 365 (JEPS)	
248	<i>Grindelia coronensis</i> A. Bartoli & Tortosa	Prov. Rio Negro, Argentina	Bartoli & Tortosa 39/02 (BAA)	
229	<i>Grindelia covasii</i> A. Bartoli & Tortosa	Prov. La Pampa, Argentina	Argentina 1 (BAA)	
247	<i>Grindelia covasii</i> A. Bartoli & Tortosa	Dpto. Caleu- calegu, Argentina	16-Jan-09 (BAA)	
99	<i>Grindelia decumbens</i> Greene	La Plata Co., Colorado	Moore & Moore 607 (JEPS)	
126	<i>Grindelia decumbens</i> Greene	Montrose Co., Colorado	Moore & Moore 595 (JEPS)	
13	<i>Grindelia fastigiata</i> Greene	Grand Co., Utah	Moore & Park 397 (JEPS)	
17	<i>Grindelia fastigiata</i> Greene	San Juan Co., Utah	Moore & Park 406 (JEPS)	
148	<i>Grindelia fastigiata</i> Greene	Cibola Co., New Mexico	Moore & Moore 622 (JEPS)	
253	<i>Grindelia fraxinipratensis</i> Reveal & Beatley	Nye Co., Nevada	Reveal & Holmgren 1887 (UC)	
297	<i>Grindelia fraxinipratensis</i> Reveal & Beatley	Nye Co., Nevada	Reveal 1536 (DS)	
298	<i>Grindelia fraxinipratensis</i> Reveal & Beatley	Nye Co., Nevada	Beatley sn (DS)	
240	<i>Grindelia globularifolia</i> Griseb.	Prov. Córdoba, Argentina	(BAA)	
214	<i>Grindelia glutinosa</i> (Cav.) Mart.	Dpto. Arequipa, Peru	Eyerdam 25175 (UC)	
20	<i>Grindelia grandiflora</i> Hook.	Jeff Davis Co., Texas	Moore & Park 366 (JEPS)	
266	<i>Grindelia greenmanii</i> Steyerm.	Coahuila, Mexico	Schoenfeld & Fairey T72M5S (UC)	
39	<i>Grindelia havardii</i> Steyerm.	Pecos Co., Texas	Moore & Park 347 (JEPS)	
18	<i>Grindelia hirsutula</i> Hook. & Arn.	Marin Co., California	Moore, Byrne, et al. 424 (JEPS)	
255	<i>Grindelia howellii</i> Steyerm.	Clearwater Co.,	Stillinger 246 (UC)	

		Idaho		
8	<i>Grindelia integrifolia</i> DC.	Benton Co., Oregon	Moore & Moore 276 (JEPS)	
180	<i>Grindelia inuloides</i> Willd.	Coahuila, Mexico	Schoenfeld & Fairey T72M5S (UC)	
207	<i>Grindelia inuloides</i> Willd.	Oaxaca, Mexico	King 2941 (UC)	
14	<i>Grindelia laciniata</i> Rydb.	San Juan Co., Utah	Moore & Park 400 (JEPS)	
9	<i>Grindelia lanceolata</i> Nutt.	Burnet Co., Texas	Moore & Park 322 (JEPS)	
23	<i>Grindelia lanceolata</i> Nutt.	Montgomery Co., Texas	Moore & Park 333 (JEPS)	
28	<i>Grindelia lanceolata</i> Nutt.	Kendall Co., Texas	Moore & Park 342 (JEPS)	
258	<i>Grindelia nana</i> Nutt.	Siskiyou Co., California	Park et al. 2112 (JEPS)	
259	<i>Grindelia nana</i> Nutt.	Siskiyou Co., California	Park et al. 2112 (JEPS)	
267	<i>Grindelia nana</i> Nutt.	Sanders Co., Montana	Lessica 10076 (JEPS)	
152	<i>Grindelia nana</i> Nutt.	Shasta Co., California	Park 1944 (JEPS)	
153	<i>Grindelia nana</i> Nutt.	Shasta Co., California	Park 1944 (JEPS)	
238	<i>Grindelia orientalis</i> A. Bartoli, Tortosa, & G.H.Rua		(BAA)	
205	<i>Grindelia oxylepis</i> Greene	Chihuahua, Mexico	Stuessy 1041 (UC)	
243	<i>Grindelia patagonica</i> A. Bartoli & Tortosa	Argentina	14-Jan-09 (BAA)	
234	<i>Grindelia prunelloides</i> (Less.) A. Bartoli & Tortosa	Prov. Neuquén, Argentina	(BAA)	
215	<i>Grindelia pulchella</i> Dunal		Leal 7957/201 (UC)	
239	<i>Grindelia pulchella</i> Dunal		(BAA)	
261	<i>Grindelia pusilla</i> (Steyerm.) G.L.Nesom	Medina Co., Texas	Moore & Steyermark 3003 (UC)	
249	<i>Grindelia pygmaea</i> Cabrera	Prov. Río Negro, Argentina	Projecto Somuncura 54 (BAA)	
125	<i>Grindelia revoluta</i> Steyerm.	Larimer Co., Colorado	Moore & Moore 573 (JEPS)	
149	<i>Grindelia revoluta</i> Steyerm.	Jefferson Co., Colorado	Moore & Moore 577 (JEPS)	
206	<i>Grindelia robinsonii</i> Steyerm.	San Luis Potosi, Mexico	Purpus 5151 (UC)	
101	<i>Grindelia scabra</i> Greene	Otero Co., New Mexico	Moore & Moore 616 (JEPS)	

211	<i>Grindelia scorzonerifolia</i> Hook. & Arn.	Prov. Corrientes, Argentina	Schinini & Ahumada 16002 (UC)	
7	<i>Grindelia squarrosa</i> (Pursh) Dunal	Ada Co., Idaho	Moore & Moore 291 (JEPS)	
12	<i>Grindelia squarrosa</i> (Pursh) Dunal	White Pine Co., Nevada	Moore & Park 394 (JEPS)	
16	<i>Grindelia squarrosa</i> (Pursh) Dunal	San Juan Co., Utah	Moore & Park 402 (JEPS)	
33	<i>Grindelia squarrosa</i> (Pursh) Dunal	Grant Co., Oregon	Moore & Moore 289 (JEPS)	
97	<i>Grindelia squarrosa</i> (Pursh) Dunal	El Paso Co., Colorado	Moore & Moore 582 (JEPS)	
103	<i>Grindelia squarrosa</i> (Pursh) Dunal	Nevada Co., California	Moore 638 (JEPS)	
114	<i>Grindelia squarrosa</i> (Pursh) Dunal	Nevada Co., California	Moore & Moore 306 (JEPS)	
124	<i>Grindelia squarrosa</i> (Pursh) Dunal	Carbon Co., Wyoming	Moore & Moore 565 (JEPS)	
146	<i>Grindelia squarrosa</i> (Pursh) Dunal	Coconino Co., Arizona	Moore & Moore 631 (JEPS)	
251	<i>Grindelia stricta</i> DC. var. <i>platyphylla</i> (Greene) M.A.Lane	Santa Barbara Co., California	Moore 847 (JEPS)	
282	<i>Grindelia stricta</i> DC. var. <i>platyphylla</i> (Greene) M.A.Lane	San Luis Obispo Co., California	Moore 945 (JEPS)	
19	<i>Grindelia stricta</i> DC. var. <i>stricta</i>	Marin Co., California	Moore, Byrne, et al. 425 (JEPS)	
30	<i>Grindelia stricta</i> DC. var. <i>stricta</i>	Del Norte Co., California	Moore, Lutzy, & Welch 249 (JEPS)	
34	<i>Grindelia stricta</i> DC. var. <i>stricta</i>	King Co., Washington	Moore, Moore, & Moore 313 (JEPS)	
96	<i>Grindelia subalpina</i> Greene	Albany Co., Wyoming	Moore & Moore 566 (JEPS)	
252	<i>Grindelia tarapacana</i> Phil.	near Arequipa, Peru	di Vittorio s.n. (JEPS)	
201	<i>Grindelia</i> cf. <i>tenella</i> Steyerm.	San Luis Potosi, Mexico	Roe & Roe 2211 (UC)	
	<i>Benitoa occidentalis</i> (H.M.Hall) D.D.Keck	Fresno Co., California	Markos 122 (JEPS)	AF251586, AF251644
	<i>Corethrogyne filaginifolia</i> Nutt.	Santa Cruz Co., California	Markos 116 (JEPS)	AF251593, AF251651
37	<i>Dieteria canescens</i> (Pursh) Nutt.	Grand Co., Utah	Moore & Park 398 (JEPS)	
272	<i>Haplopappus anthylloides</i> Meyen & Walp.		Teillier 5619	

	<i>Haplopappus foliosus</i> DC.	Chile	Rundel s.n., UCBG 80.0298 (UC)	AF251577, AF251635
233	<i>Haplopappus glutinosus</i> Cass.	Prov. Neuquén, Argentina	Argentina 12 (BAA)	
235	<i>Haplopappus glutinosus</i> Cass.	Prov. Neuquén, Argentina	Argentina 41 (BAA)	
	<i>Haplopappus glutinosus</i> Cass.	Cautin, Chile	Sparre and Constance 17927 (UC)	AF251578, AF251636
	<i>Haplopappus macrocephalus</i> DC.	Prov. Talca, Chile	Mahú and Stebbins 8846 (UC)	AF251579, AF251637
	<i>Haplopappus marginalis</i> Phil.	Chile	DeVore 1326 (UC)	AF251580, AF251638
271	<i>Haplopappus multifolius</i> Phil.		Teillier 5594	
273	<i>Haplopappus multifolius</i> Phil.		Teillier 5814	
	<i>Haplopappus paucidentatus</i> Phil.	Chile	DeVore 1261 (UC)	AF251581, AF251639
179	<i>Haplopappus setigerus</i> (Phil.) F.Meigen	near Santiago, Chile	Kelch 06.002 (CDF)	
269	<i>Haplopappus uncinatus</i> Phil.		Teillier 5832	
270	<i>Haplopappus undulatus</i> Klingenb.		Teillier 5682	
268	<i>Haplopappus velutinus</i> J.Rémy		Teillier 5663	
	<i>Hazardia detonsa</i> (Greene) Greene	Santa Barbara Co., California	UCBG 95.0527	AF251582, AF251640
	<i>Hazardia squarrosa</i> (Hook. & Arn.) Greene	Los Angeles Co., California	Ross 5908 (UC)	AF251583, AF251641
	<i>Hazardia whitneyi</i> (A.Gray) Greene	Tuolumne Co., California	Erter 7918 (UC)	AF251584, AF251642
	<i>Isocoma acradenia</i> (Greene) Greene	Riverside Co., California	Thorne 55404 (UC)	AF251572, AF251630
	<i>Isocoma menziesii</i> (Hook. & Arn.) G.L.Nesom	Los Angeles Co., California	Bartholomew 535, UCBG 78.0157	AF251571, AF251629
283	<i>Isocoma menziesii</i> (Hook. & Arn.) G.L.Nesom	San Luis Obispo Co., California	Moore 946 (JEPS)	
	<i>Lessingia arachnoidea</i> Greene	San Mateo Co., California	Markos 126 (JEPS)	AF251588, AF251646
	<i>Lessingia glandulifera</i> A.Gray	Madera Co., California	Markos 163 (JEPS)	AF251602, AF251660
40	<i>Machaeranthera tagetina</i> Greene	Cochise Co., Arizona	Moore & Park 368 (JEPS)	
	<i>Pyrocoma apargioides</i> (A.Gray) Greene	Plumas Co., California	Schoolcraft 2072 (UC)	AF251573, AF251631
	<i>Pyrocoma lanceolata</i> (Hook.) Greene	Piute Co., Utah	Neese 17626 (UC)	AF251574, AF251632
36	<i>Pyrocoma racemosa</i> Torr. &	Mono Co.,	Park 1338 (JEPS)	

	A.Gray	California		
	<i>Rayjacksonia phyllocephala</i> (DC.) R.L.Hartm. & M.A.Lane	Chambers Co., Texas	Morgan 2032 (TEX)	U97645, AF516074
38	<i>Xanthisma spinulosum</i> (Pursh) D.R.Morgan & R.L.Hartm.	Pecos Co., Texas	Moore & Park 346 (JEPS)	
	<i>Xanthocephalum</i> <i>gymnospermoides</i> Benth. & Hook.	Jeff Davis Co., Texas	Morgan 2200 (WWB)	U97650, AF516075
	<i>Xylorhiza tortifolia</i> (Torr. & A.Gray) Greene	Inyo Co., California	Wisura 4770 (UC)	AF251570, AF251628

Table 2. Characteristics of the regions sequenced in this study. ITS and ETS are the internal transcribed spacer and external transcribed spacer regions, respectively, of the nuclear ribosomal DNA, while *psaI-accD* is a chloroplast spacer region. The number of sequences for each region (# seqs.), aligned sequence length (# chars.), number and proportion of variable characters, and number and proportion of parsimony informative characters are shown.



region	# seqs.	# chars.	# variable characters	prop. variable	# pars. inform. characters	prop. pars. inform.
<i>psaI-accD</i>	94	1420	208	0.146	81	0.057
ITS	94	641	99	0.154	41	0.064
ITS w/o 5.8S	94	478	94	0.197	41	0.086
ETS	94	411	89	0.217	51	0.124
ITS	119	644	133	0.207	73	0.113
ITS w/o 5.8S	119	480	126	0.263	72	0.150
ETS	119	412	108	0.262	72	0.175
combined	119	1058	241	0.228	145	0.137

## Chapter Three

### Population structure in autotetraploid *Grindelia* of the California Floristic Province.

#### INTRODUCTION

Polyploidy is one of the most important processes in plant evolution. It has been implicated in 15% of speciation events in angiosperms and 31% of speciation events in ferns (Wood et al. 2009). Polyploidy is also present among non-plant eukaryotes (Aury et al. 2006), including animals (Jaillon et al. 2004) and fungi (Kellis et al. 2004). In the wake of a polyploidization event, genetic diversity is increased. In allopolyploids, genes with differing expression patterns that evolved in different genetic backgrounds and with different selective pressures come together in the same individual. In autopolyploids, the expression patterns and selective environments of genes in the two genomes are likely to have been the same, but the number of alleles at each locus is suddenly increased. In addition, the number of ways these alleles can combine into dimeric or multimeric proteins is increased even more than the number of alleles (Barber 1970).

A longer-term consequence of polyploidy may be increased adaptive potential. The duplicated genes provide back-up copies that could allow one copy to evolve a new function while the other continues to perform its original function (Adams 2007, Flagel and Wendel 2009). This greater evolutionary potential of recent polyploids has been implicated in rapid radiations of diverse plant families such as Brassicaceae, Fabaceae, Poaceae, and Solanaceae (Soltis et al. 2009); of animals such as the ray-finned fishes (Le Comber and Smith 2004); and in the recent adaptive radiations of some island groups (e.g., Hawaiian silverswords, Barrier et al. 1999; Hawaiian mints, Lindqvist et al. 2003).

Different autopolyploids or allopolyploids derived from closely related parental taxa can form complexes of related species, which often show much greater morphological and ecological variation than their closest diploid relatives (e.g., *Phacelia* Juss., Heckard 1960; *Antennaria* Gaertn., Bayer 1990). In many cases, much more gene exchange is possible between polyploids than between their diploid parental taxa, due weaker crossing barriers between the polyploids (e.g., Ehrendorfer 1959) or sometimes to a greater degree of sympatry between the polyploids (e.g., Guo et al. 2008).

Given their rapid evolution, microsatellite markers are useful for examining gene flow among members of recently derived polyploid complexes and potentially for obtaining evidence on their relationships. However, there are two complications with using microsatellites in polyploids. Unlike the case in diploids, in polyploids the allelic phenotype of a locus (i.e., the different alleles that are present at that locus), sometimes simply called the phenotype, cannot be directly translated into that locus' genotype (i.e., the number of copies of each allele). For example, a diploid can have two types of allelic phenotypes: one allele of a single length (for example, an allele that is 214 base pairs long) or two alleles of different lengths (for example, 214 and 216 base pairs). Each of these corresponds unambiguously to a single genotype: a phenotype of 214 would be a genotype of [214, 214], and a phenotype of 214, 216 would be a genotype of [214, 216]. The situation is more complicated in polyploids; in tetraploids, for

example, only phenotypes with one band or four bands correspond unambiguously to a genotype, while phenotypes with two or three bands each correspond to multiple genotypes. A tetraploid phenotype of 214, 216 could correspond to [214, 214, 214, 216], [214, 214, 216, 216], or [214, 216, 216, 216].

The second complication with analyzing microsatellite data from polyploids is the potential for double reduction in autopolyploids, which complicates the inference of population genetic parameters even when genotypes are known. If an organism is a true autotetraploid, it has four homologous copies of each chromosome (instead of two as in a diploid), any two of which could end up in a given gamete. Each chromosome in diploids has a single homolog with which it pairs in meiosis, forming a bivalent. In autotetraploids, on the other hand, each chromosome has three homologs and is capable of pairing with two of them at once, forming a multivalent. If a multivalent is formed during meiosis, after the first meiotic division, at least one of the daughter cells will contain two chromosomes that paired with each other (and that exchanged genetic material through crossing-over). In some of these cases, after the second meiotic division, the “original” of a given allele as well as the copy of that allele that arose through crossing-over will end up in the same gamete and the individual formed from that gamete will have inherited two copies of the same allele from one of its parents. Those two copies would represent only one independent draw from the gene pool instead of two, as they would if double reduction had not taken place.

The amount of double reduction depends on the frequency of formation of multivalents at meiosis as well as the distance from the locus in question to the centromere (as double reduction depends on crossing-over occurring between the locus and the centromere; Mather 1936). The frequency of double reduction in autotetraploids should range from 0 (when the locus is adjacent to the centromere) to a theoretical maximum of 1/6 (Mather 1935, Stift et al. 2008) and has been shown to vary between loci in a genome (Stift et al. 2008). Double reduction causes the frequency of rare alleles to be overestimated (since it would be more likely for them to be present twice in the same gamete through double reduction than through two independent draws from the gene pool). Methods have been developed to estimate the frequency of double reduction and correct for it in analyses of microsatellite data (e.g., Luo et al. 2006, Stift et al. 2008).

### *Grindelia*:

The genus *Grindelia* Willd. (Asteraceae) contains diploid, tetraploid, and hexaploid members, all with a chromosome base number of  $x = 6$ . The plants I am examining in this study are members of a species complex with its center of diversity in the California Floristic Province (CA-FP), a floristically diverse region on the west coast of North America that is characterized by its Mediterranean climate. Members of this complex formed a monophyletic group, the Pacific Clade, in molecular phylogenies (Chapter Two). Within the Pacific Clade, plants from the CA-FP grouped together into a moderately supported clade; however, the relationships among the members of that clade remained unresolved. This CA-FP clade contained both diploid and tetraploid members, with the tetraploids showing much greater ecological variability (Chapter One). Studies of crossing and chromosome pairing indicate that the tetraploids were derived from the diploids via autotetraploidy (Dunford 1964) an unknown number of times. Tetraploids tend to have between 0 and 2 tetravalents (multivalents containing all four homologous chromosomes) per sporocyte during meiosis (Dunford 1964).

There is abundant morphological variation within the CA-FP *Grindelia* clade, including variation in habit, shape of heads and involucre bracts, type of indumentum, and flowering time. Associated with this morphological variation is great ecological variation. Major habitat types occupied by CA-FP *Grindelia* include grasslands, salt- to fresh-water marshes, serpentine soils, and coastal bluffs and sand dunes. Judging from distinctive morphological features associated with each of these habitats, CA-FP *Grindelia* appear to be in the process of ecotypic diversification. Each of the putative ecotypes has at one time been recognized taxonomically (e.g., Steyermark 1934, who recognized a total of 14 species and 23 additional infra-specific taxa at various ranks). They have also all been combined into a single species, *G. hirsutula* DC., with no infra-specific taxa (Strother and Wetter 2006). In this study, I am following the taxonomy outlined in Chapter One, in which the taxa are delimited based on a combination of morphology and ecology. This taxonomy falls between those of Steyermark (1934) and Strother and Wetter (2006) in the number of taxa it recognizes. However, it is certainly possible that some or all of the taxa I recognized have multiple origins. In this study, I am examining five of those taxa, which each grow in different habitats.

In addition to being of evolutionary interest, *Grindelia* is widely used for restoration in the CA-FP, particularly *G. stricta* var. *angustifolia* in the salt marshes around the San Francisco Bay (I. Vogel, Invasive Spartina Project, pers. comm.) and *G. camporum* in the Central Valley (J. Silveira, Sacramento National Wildlife Refuge; J. Anderson, Hedgerow Farms; pers. comm.). *Grindelia* grows reliably from seed, flowers every year even with low precipitation, and is an important source of food for both pollinators and seed-eating animals. It is important to know how much genetic variability is present across species of CA-FP *Grindelia* in order to be able to choose the proper genotypes for restoration.

*Grindelia camporum* Greene occurs throughout the dry grasslands of California, including the Central Valley and the foothills of the Coast Ranges and the Sierra Nevada. It includes both diploids and tetraploids, which have been hypothesized to represent different taxonomic varieties (Dunford 1964). However, further investigation did not support the proposed morphological distinctions between plants of different ploidy (pers. obs.). Different populations of *G. camporum* are often morphologically differentiated, varying in average height from 0.5 m to 2 m at flowering. Differences have also been observed in head morphology, leaf shape, and the amount of resin produced. Common garden experiments have shown that the variation among populations persists in a common environment (McLaughlin 1986).

*Grindelia hirsutula sensu* Chapter One is restricted to the Coast Ranges and generally grows in areas that are influenced by fog. Like *G. camporum*, *G. hirsutula* grows in grasslands, but its peak flowering is one to three months earlier than that of *G. camporum*. Also, unlike *G. camporum*, *G. hirsutula* often grows in grasslands that are on serpentine-derived soils. It appears to be exclusively tetraploid.

*Grindelia stricta* DC. var. *angustifolia* (A.Gray) M.A.Lane is restricted to the salt marshes around San Francisco Bay. It grows along the banks of sloughs and its roots and lower stems are often inundated at high tide. Herbarium records show it to be among the most morphologically uniform of the CA-FP taxa (Chapter One). Its morphological uniformity has been enhanced by its wide use in salt marsh restoration; it is an important cover plant for the endangered California clapper rail (*Rallus longirostris obsoletus* Ridgway, 1874; De Groot 1927). The plants used in restoration come from seed collected at the Don Edwards San Francisco Bay National Wildlife Refuge at the south end of the Bay (I. Vogel, Invasive Spartina Project, pers. comm.). The three populations sampled in this study were all natural (non-

restored) populations. Almost all of the chromosome counts for *G. stricta* var. *angustifolia* are tetraploid (Whitaker and Steyermark 1935, Lane and Li 1993), although a single diploid count has been reported (Raven et al. 1960).

*Grindelia stricta* var. *platyphylla* (Greene) M.A.Lane grows on coastal bluffs, from approximately the latitude of San Francisco Bay southwards. It tends to have prostrate or ascending stems. This taxon appears to be exclusively tetraploid.

*Grindelia* ×*paludosa* Greene *pro sp.* occurs in the Suisun Delta, the brackish-water marsh at the head of the Carquinez Strait where the Sacramento and San Joaquin Rivers, which drain the Central Valley of California, drain into San Francisco Bay. Steyermark (1934) hypothesized that *G. ×paludosa* was a hybrid between *G. camporum* and *G. stricta* var. *angustifolia*. Both the habitat and the morphology of *G. ×paludosa* are intermediate between those of its putative parents; its tall stature, slightly succulent leaves, and phyllary shape resemble *G. stricta* var. *angustifolia*, while it resembles *G. camporum* in its often more serrate leaves and herbaceous habit (Steyermark 1934). More recent authors have continued to hypothesize that *G. ×paludosa* is of hybrid origin (Keck 1959, Lane 1993). *Grindelia* ×*paludosa* is exclusively tetraploid.

In this paper, I am using microsatellite data to examine genetic differentiation among nine populations of tetraploid CA-FP *Grindelia* and one population of diploid CA-FP *Grindelia*. My goals are (1) to determine whether local populations are genetically distinct, (2) to examine whether these distinctions support assignment to the taxa proposed in Chapter One, (3) to compare several techniques for analyzing microsatellite data from autotetraploids, and (4) to interpret the results in relation to the evolutionary history and current restoration uses of *Grindelia* in the San Francisco Bay region.

## MATERIALS AND METHODS

### Sampling:

A total of 439 individuals from 10 populations were sampled, with 29–50 individuals collected per population (Table 1, Fig. 1). Plants were collected from populations of five different taxa: *G. camporum* (1 diploid, 2 tetraploid populations; summer-flowering, generally interior grassland), *G. hirsutula* (2 tetraploid populations; spring flowering, one serpentine and one coastal grassland), *G. ×paludosa* (1 tetraploid population; brackish marsh), *G. stricta* var. *angustifolia* (3 tetraploid populations; salt marsh), and *G. stricta* var. *platyphylla* (1 tetraploid population; coastal bluffs). All of the populations except one (159, *G. camporum*) were collected in the San Francisco Bay area, where most of the different taxa of CA-FP *Grindelia* come into contact.

As I am using it here, the term population merely refers to a group of plants of a single species that are growing in a given area. In the three taxa that do not grow in marshes (*G. camporum*, *G. hirsutula*, and *G. stricta* var. *platyphylla*), the boundaries of the putative populations were quite distinct, and samples were obtained from the entire area where the plants occurred at a collection locality. The two marsh taxa (*G. ×paludosa* and *G. stricta* var. *angustifolia*) occurred in much more extensive stands, and I was only able to sample plants from a subset of the area occupied by a taxon at a given site. In all cases, the localities at which I collected a given taxon were separated from each other by large areas of unsuitable habitat. In one locality (Point Pinole, populations 220 and 221), *G. camporum* and *G. stricta* var. *angustifolia* approached within ca. 100 yards of each other because their habitats are adjacent,

which is likely within the flight distance of their bee pollinators (Arias and Rieseberg 1994). No morphological intermediates were observed at this location. It is worth noting that, although the taxa generally do not come into contact at present, contact between them could potentially have been much more extensive in the past before disturbance, habitat conversion, and the introduction of non-native grasses, which often out-compete *Grindelia*, especially in the absence of grazing.

#### Microsatellite Amplification and Scoring:

Sequences of microsatellite-containing loci were obtained using the protocol of Glenn and Schable (2005) using DNA from *G. camporum* (from the specimen *Moore, Silviera, and Anderson 551*, JEPS). Primer sequences for the variable loci are described in Molecular Ecology Resources Primer Development Consortium et al. (2009). In this study, I am using the six primer pairs GRIN024, GRIN026, GRIN035, GRIN045, GRIN068, and GRIN113. The remaining five primer pairs described in Molecular Ecology Resources Primer Development Consortium et al. (2009) had results that were difficult to interpret, due to length of the PCR product or number of bands produced.

DNA was extracted from fresh or silica-dried material using the Qiagen Plant Mini Kit (Qiagen, Inc., Valencia, CA, U.S.A.). The samples were ground directly in AP1 extraction buffer using a mortar and pestle or ground dry using glass beads in a Mini-Bead-Beater-16 (BioSpec Products, Inc., Bartlesville, OK, U.S.A.).

Most loci were amplified with component-based PCR with 1× ThermoPol reaction buffer (New England Biolabs, Inc., Ipswich, MA, U.S.A.), 1.5 units of *Taq* polymerase (New England Biolabs), 0.4 μM each primer, 0.6mM dNTPs, 0.5 μg BSA, and 3 μl DNA that was diluted 1:10 from the concentration of the originally extracted DNA. Loci 045 and 113 were amplified using AccuPower PCR PreMix (Bioneer Inc., Alameda, CA, U.S.A.) using 0.375 μM concentration of each primer and 3μl of DNA at 1:10 dilution. The touchdown PCR program of Glenn (2006) was used, with annealing temperatures of 55–45°C.

Samples were run on the ABI 3730xl capillary sequencing machines (Applied Biosystems, Inc., Foster City, CA, U.S.A.) at the U.C. Berkeley DNA Sequencing Facility using the GeneScan 500 ROX size standard (Applied Biosystems). Samples were scored using the Peak Scanner v.1.0 software (Applied Biosystems). A subset of individuals (ca. 5%) was run twice to ensure that amplification and scoring of alleles were consistent across runs.

All individuals produced between 1 and 4 scorable bands at all loci, with the exception of individuals 9 and 23 from population 223 (*G. stricta* var. *angustifolia*), which did not amplify for locus 024, and individuals 4, 7, and 14 from population 222 (also *G. stricta* var. *angustifolia*), which produced 5 bands at locus 026.

The microsatellites were scored by recording the presence or absence of the alleles (phenotypic scoring), instead of by attempting to determine how many copies of each allele were present in a given individual (genotypic scoring). I chose to score the phenotypes because at least some individuals at each of the different loci had stutter bands (smaller peaks that were slightly longer or slightly shorter than the main peak, which do not cause problems for scoring the main peaks, but make it more difficult to determine the areas of the peaks) or had some individuals with peaks that were overloaded (so the full area of the peak could not be determined).

## Data Analysis:

Allele frequencies, observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities, and frequencies of double reduction were calculated for each locus in each tetraploid population using the method of Luo et al. (2006) as implemented in GAUSS (Aptech Systems, Inc., Black Diamond, WA, U.S.A.). Individuals with zero or five alleles at a given locus were not analyzed for that locus, but they were analyzed for the remaining loci. The method of Luo et al. (2006) infers the maximum likelihood population allele frequencies from phenotypic data. It uses these frequencies to calculate population statistics, but does not infer maximum likelihood genotypes for individuals. The method of Luo et al. (2006) was also used to calculate  $F_{ST}$  values between all populations for each locus. In this case, it compares the maximum likelihood allele frequencies and values of  $\alpha$  for the two populations individually with those calculated for the two populations put together.

The frequency of double reduction ( $\alpha$ ) was limited to its theoretical maximum of 1/6 (Stift et al. 2008). In order to do this, allele frequencies were first estimated with  $\alpha$  allowed to assume any value. When  $\alpha$  was estimated to be greater than 1/6 in the original run, it was constrained to be less than or equal to 1/6 and the allele frequencies were re-estimated. These new values were then used to calculate the remaining population statistics. In two cases where two populations were analyzed together as a single population to calculate  $F_{ST}$  (the combined populations 156 and 217 and the combined populations 156 and 220, both for locus 024), the unconstrained value of  $\alpha$  was greater than 1/6 and the maximum likelihood function failed to converge when  $\alpha$  was set at 1/6. In these cases, increasingly large values of  $\alpha$  were tried, starting with  $\alpha = 0$ , and the largest one for which the likelihood function converged was used. Differences in population size were taken into account when calculating  $F_{ST}$ .

Allele frequencies, observed and expected heterozygosities, and departures from Hardy-Weinberg equilibrium were calculated for all loci for the diploid population 218 using Arlequin (Excoffier and Lischer 2010).

SPAGeDi v. 1.3a (Hardy and Vekemans 2002) was used to calculate  $\rho$  (Ronfort et al. 1998), an analog of  $F_{ST}$  that is calculated using allelic phenotypes.  $\rho$  is independent of both  $\alpha$  and the degree of inbreeding and can be used to compare diploids and tetraploids (Ronfort et al. 1998). The degree of inbreeding is likely the same throughout the genome, but  $\alpha$  varies from locus to locus depending on the distance of each locus from the centromere (Stift et al. 2008). Thus,  $\rho$  should be more comparable across loci in the genome, while  $F_{ST}$  is expected to vary in a systematic way.

$\rho$  was calculated for each locus separately and for all loci combined. All individuals were used. The two individuals lacking locus 024 were coded as having missing data at that locus, while only the four shortest alleles were used for the three individuals with five alleles at locus 026. Each of the five alleles present in those three individuals was also found in other plants, so it was not clear which allele was the extra one and the choice of not using the longest allele was arbitrary.

ALSCAL (SPSS, Inc., Chicago, IL, U.S.A.) was used to visualize the pattern of variation in both  $\rho$  and  $F_{ST}$  among populations. In each case, ALSCAL derived a map of the populations that best reproduces the distances between each of the population pairs. This was done for distances measured in terms of both  $F_{ST}$  and  $\rho$ . For the analyses of  $F_{ST}$ , each locus was treated as a different observation and a map was constructed that best fit the distances from each of the loci, because there was no overall measure of  $F_{ST}$  across all six loci. In contrast, SPAGeDi

calculated an overall  $\rho$  for each pair of populations across all loci; ALSCAL created a map that best reproduced those pair-wise distances.

A Mantel test was performed in AnoSim (Clarke 1993) to examine the relationships between both  $F_{ST}$  and  $\rho$  and the taxonomic division of the populations. This test examined whether there was a difference between pairs of populations that were putatively members of the same species and pairs that were putatively members of different species. Each of the five taxa was considered to be a different category and significance was tested with 10,000 permutations.

Principal Coordinates Analysis (PCO) was performed for all loci using R (R Development Core Team 2010). PCO was performed on distances between individuals that were calculated using Dice's similarity coefficient (Dice 1945). As Dice's similarity coefficient does not differentiate between loci (and thus allows an arbitrarily large number of alleles per locus), all alleles were included for the individuals with five alleles at locus 026.

Discriminant analyses were performed using SPSS to determine whether the populations differed significantly in terms of the principal coordinates that had corresponding eigenvalues greater than one. The PCO dimensions that are associated with eigenvalues greater than one explain a greater amount of the variance in inter-individual similarity than would be expected by chance. Discriminant analyses were performed with the plants grouped two different ways: into populations (10 total groups) and into putative taxa (5 total groups, with 3 of the groups made up of more than one population).

*Structure* (Pritchard et al. 2000) was run with a data set in which 4-allele genotypes were created by replacing the unknown alleles with one of the known alleles with equal probability. For example, if an individual had a phenotype of 214, 217, 221 at a given locus, the fourth allele had a one-third probability of being a repeat of any of the other three alleles. Two different analyses were performed with these data sets. First it was assumed that these were the correct genotypes and there were no recessive alleles and no ambiguity (RECESSIVEALLELES=0). The number of populations ( $K$ ) was allowed to vary from 2–15; 20 replicate runs were performed for each value of  $K$ . Each replicate was run for 60,000 generations preceded by a burn-in period of 10,000 generations. Admixture was allowed, and allele frequencies were independent in the different populations. These runs were used to calculate the optimum number of populations according to the method of Evanno et al. (2005), as the number of populations cannot be calculated with high precision when there is ambiguity in the number of alleles (Pritchard et al. 2009). This analysis was performed twice in order to take into account any differences arising from random creation of genotypes. Results were the same in the two different runs.

This same data set was run again in such a way as to take ambiguity in allele copy number into account (Falush et al. 2007). The recessive alleles were considered to be present (RECESSIVEALLELES=1) and the ambiguous allele code (the allele that would normally be recessive) was set to -9, the value for a missing allele, for each of the six loci as recommended by Pritchard et al. (2009). For this data set, the number of inferred populations ( $K$ ) was allowed to vary between 2 and 10, with 5 replicate runs for each value of  $K$ . Each replicate was run for 100,000 generations preceded by a burn-in period of 10,000 generations. Admixture was allowed, and allele frequencies were independent in the different populations.

## RESULTS

A total of 134 alleles across six loci were found, with the number of alleles per locus ranging from 7 to 31 overall and 3 to 19 within a population (Tables 2 and 3). For all of the loci,



at least one individual had only one allele and at least one individual had four different alleles, except for 068, which did not have any individuals with four alleles in this data set, likely due to the small number of alleles at that locus (although some individuals from other locations were found that had four alleles, unpublished data).

Average values of observed heterozygosity,  $H_O$ , ranged from 0.3813 (locus 068) to 0.7827 (locus 026). Population values of  $H_O$  ranged from 0.1339 (population 217 for locus 068) to 0.9204 (population 221 for locus 026). Average values of expected heterozygosity,  $H_E$ , ranged from 0.3680 (locus 068) to 0.8246 (locus 026). Population values of  $H_E$  ranged from 0.1332 (population 217 for locus 068) to 0.9178 (population 221 for locus 026). The reconstructed frequencies of double reduction ( $\alpha$ ) ranged from 0.0000 to the theoretical maximum of 0.1667 for all loci except 113, for which the lowest value of  $\alpha$  was 0.0248.

$F_{ST}$  varied widely across population pairs and loci (Tables 2 and 3). For locus 113, none of the pairs of populations showed significant differentiation. The remaining loci had a wider range of  $F_{ST}$  values, with some pairs of populations showing significant differentiation and some pairs of populations showing a lack of differentiation at each locus. All but five population comparisons had at least one  $F_{ST}$  value greater than 0.05, and only 17 of 45 population comparisons had all  $F_{ST}$  values less than 0.10.

$\rho$  also varied across loci and was generally larger than  $F_{ST}$ . However, all loci had some pairs of populations with  $\rho$  values less than 0.10 and some pairs of populations with  $\rho$  values greater than 0.20. Only one pair of populations had all  $\rho$  values less than 0.20, but ten pairs of populations had all  $\rho$  values greater than 0.10.

Locus 024 showed greater differentiation for pairs of populations that were members of different taxa than for pairs of populations that were members of the same taxon (Mantel test:  $p = 0.011$   $F_{ST}$ ,  $p = 0.032$   $\rho$ ). None of the other loci had significantly greater differentiation between than within taxa.

The first two principal coordinates explained 9.69 % and 6.04 % of the variance in the data, respectively. In the plot for those two coordinates (Fig. 2), individuals from the same population grouped together. The populations of the taxa for which more than one population was sampled also generally grouped together. The first dimension reflected primarily a separation of *Grindelia stricta* and *G. camporum* with *G. hirsutula* and *G. ×paludosa* close to the centroid. The second dimension primarily reflected a separation of *G. hirsutula* and *G. ×paludosa* as well as of populations of *G. stricta* and *G. camporum*. However, there was a large amount of overlap between some populations and taxa. *Grindelia stricta* var. *platyphylla* fell among the individuals of *G. stricta* var. *angustifolia*, as did one of the populations of *G. hirsutula*.

There were 41 principal coordinates that had corresponding eigenvalues greater than one. The discriminant analysis found a significant difference among the ten populations based on these 41 principal coordinates ( $\chi^2$  test,  $p < 0.001$ ). Furthermore, the discriminant functions were able to classify 95.9% of individuals to the correct population. The misclassified individuals were fairly evenly distributed across populations, with the exception of population 221 (*G. stricta* var. *angustifolia*), which had five of 50 individuals misclassified to population 219 (*G. stricta* var. *platyphylla*).

As found from grouping the plants according to population, grouping the individuals by taxon explained the data significantly better than leaving them ungrouped ( $\chi^2$  test,  $p < 0.001$ ). In this analysis, 92.7% of individuals were correctly classified. The misclassified individuals were again fairly evenly distributed across those taxa, with the exception of *G. stricta* var. *platyphylla*,

which had seven of its 50 individuals misclassified as *G. stricta* var. *angustifolia*. In contrast, *G. stricta* var. *angustifolia* only had five of 150 individuals misclassified as *G. stricta* var. *platyphylla*.

Plots of the centroids of each population from PCO (Fig. 3a) showed a similar pattern to those from ALSCAL analyses of  $F_{ST}$  (based on reconstructed genotypes; Fig. 3b) and  $\rho$  (based on allelic phenotypes; Fig. 3c). *Grindelia*  $\times$  *paludosa* was always somewhat intermediate in position between *G. camporum* and *G. stricta* var. *angustifolia*, while the populations of the same species generally grouped together. Plots of  $\rho$  showed the most consistent separation of populations, while PCO plots showed the least, possibly because they were only based on data from the first two principal coordinates.

When ambiguity in allele copy number was not taken into consideration, *structure* divided the plants into nine populations using the method of Evanno et al. (2005), or ten populations when the number of populations with the highest likelihood was considered to be the best (Figs. 4, 5).

For both types of *structure* analysis (with or without correcting for ambiguity in allele copy number), the sampled populations corresponded almost exactly to the *structure* populations (Fig. 5). When the plants were divided into nine populations, populations 151 (*G. hirsutula*) and 219 (*G. stricta* var. *platyphylla*) were grouped together. When the plants were divided into two populations, the distribution of individuals across populations corresponded closely to the division of individuals along the first principal coordinates axis. One group contained one population of *G. hirsutula* (151) and all populations of both varieties of *G. stricta* (219, 221-223). The second group contained two of the populations of *G. camporum* (218 and 220). The third group contained the population of *G.  $\times$ paludosa* (154) as well as the remaining populations of *G. camporum* (156) and *G. hirsutula* (217). When the plants were divided into five populations, none of the taxa were recovered as their own *structure* population.

## DISCUSSION

There appears to be only limited gene flow among the sampled populations of CA-FP *Grindelia*, as indicated by the generally high levels of  $F_{ST}$  and  $\rho$  and the fact that *structure* analyses recovered the original sampled populations. Although the PCO chart (Fig. 2) showed some overlap in populations, the first two principal coordinates only explained 15.7% of the variation in the data. A discriminant analysis, using data from the first 41 principal coordinates (which together explained 90.0% of the data) was able to correctly classify > 95% of individuals to the correct population. The general distinctiveness of all of the populations is similar to that found in tetraploid *Lotus corniculatus* L. (Savo Sardaro et al. 2008), in contrast to tetraploid *Betula pubescens* Ehrh., where genetic differentiation of populations was relatively low (Truong et al. 2007).

In contrast to the general differentiation among populations, I did not find greater differentiation between populations of different taxa than between populations of the same taxon except at a single locus, 024. In addition, when asked to divide the plants into 5 populations, the divisions that *structure* found did not correspond to taxonomy for any of the taxa. Although neither *structure* nor the PCO plots showed clean separation along taxonomic lines, there was some taxonomic signal in the data, as the individuals could be separated according to taxa using discriminant analysis of the PCO data. Better separation with discriminant analysis than with the PCO plots themselves was also found in *Cardamine* L. by Jørgensen et al. (2008).

The diploid population of *G. camporum* (218) was well separated from the tetraploid populations in the PCO. Kloda et al. (2008), in their analysis of diploids and tetraploids in the genus *Ononis* L., also found clear separation between groups of different ploidy. It is unclear to what extent this distinction was due to the inherent genetic differences between diploids and tetraploids and how much was due to lower levels of gene exchange between individuals of different ploidy than between individuals of the same ploidy.

#### Implications for the Evolution of CA-FP *Grindelia*:

Much of the evolutionary history of CA-FP *Grindelia* took place in a landscape much different from that of the present. The locations where the plants are currently found in part reflect available habitat given development, invasive species, and other anthropogenic effects. The localities I sampled were generally distant from other places where *Grindelia* can be currently found, but herbarium specimens show that even fifty years ago *Grindelia* was much more widespread in the Bay Area on lands that are now developed or otherwise unsuitable. The microsatellite data tell the story, in part, of a landscape that no longer exists.

Natural changes have also influenced the evolution of CA-FP *Grindelia*. San Francisco Bay has repeatedly filled and emptied as sea levels changed due to growth and melting of glaciers. It was empty most recently approximately 10,000 years ago, when the estuary of the Sacramento-San Joaquin River was west of the Golden Gate (Atwater et al. 1977). For example *Grindelia stricta* var. *angustifolia*, which is endemic to the San Francisco Bay marshes, could well have evolved after the most recent filling of the bay. A recent origin of *G. stricta* var. *angustifolia* would be a potential explanation for its general morphological uniformity when compared to other CA-FP *Grindelia* taxa (Chapter One), notwithstanding its lack of genetic uniformity.

Steyermark (1934) hypothesized that *G. ×paludosa* was a hybrid between *G. stricta* var. *angustifolia* and *G. camporum*. As predicted, the single population of *G. ×paludosa* was approximately intermediate between its two putative parents. In the PCO plot of all individuals, the *G. ×paludosa* individuals occupied the space in-between *G. stricta* var. *angustifolia* and *G. camporum*, while in the *structure* analyses, *G. ×paludosa* grouped more strongly with *G. stricta* var. *angustifolia* than it did with *G. camporum*.

#### Population Differentiation of CA-FP *Grindelia*:

As discussed above, the microsatellite data support the hypothesis of only minor gene flow between populations, both within and between taxa. This differentiation in neutral markers is congruent with the morphological differentiation found between populations of *G. camporum* by McLaughlin (1986). Those morphological differences had a genetic basis, as they persisted in the common garden.

Morphological and ecological differences were correlated with genetic differences even in the pair of parapatric populations (220, *G. camporum*, and 221, *G. stricta* var. *angustifolia*). These populations did not show evidence of greater gene flow than allopatric pairs of populations. Indeed, populations 220 and 221 were never grouped together in *structure*, even when the number of populations was very low (2 or 3), and they were among the most divergent populations in PCO.

Other studies have also found correlations between neutral genetic differentiation and ecology. Sork et al. (2010) found a strong association between variation in presumably neutral microsatellite loci and climatic factors in *Quercus lobata* Née. Freedman et al. (2010) compared genetic differentiation between AFLPs (amplified fragment length polymorphisms) that were under selection and AFLPs that were evolving neutrally and found significant ecological signal in the neutral as well as the selected AFLPs in the lizard *Trachylepis affinis* (Gray, 1838).

Neutral genetic variation has been used to identify populations for conservation or for seed sources in restoration or forestry in various plant species. Sutherland et al. (2010) found that populations of *Fraxinus excelsior* L. from throughout most of the species' range in Britain were genetically uniform and concluded that the widespread genotypes could likely be planted in forests throughout the country. In contrast, Honjo et al. (2009) found that *Primula sieboldii* E.Morren populations in Japan were divided into at least four distinct groups, each one of which merited protection.

The findings of strong genetic differentiation between populations of the same species support the idea that local genotypes in *Grindelia* would be better suited for restoration purposes than would genotypes from other locations. At present, the best strategy for restoration would likely be to collect seed from as close to the restoration site as possible. However, it must be noted that neutral genetic variation in itself does not demonstrate local adaptation in CA-FP *Grindelia*. Studies of loci that are under selection as well as reciprocal transplantation experiments are needed to give unequivocal evidence of local adaptation.

In addition, although different populations of the same taxon did not group together using the (presumably) neutral loci sampled here, it is certainly possible that loci that are under selection could unite each taxon. Both simulation and empirical studies show that ecological speciation cannot always be detected with neutral loci (Morjan and Rieseberg 2004, Thibert-Plante and Hendry 2010). In some cases, gene flow homogenizes the neutral loci, while the ecologically important loci are prevented from intergrading by selection (Thibert-Plante and Hendry 2010). In other cases, levels of gene flow are low enough that neutral loci travel more slowly through the ecotype or taxon, while loci that are under selection can travel much more rapidly (Morjan and Rieseberg 2004) and have much sharper boundaries at ecological transitions (Freedman et al. 2010).

#### Implications for studies of autopolyploidy:

In general, the method of Luo et al. (2006) for reconstructing population genotypes has not been widely used in autotetraploids since its original publication. However, the method is quite useful for determining statistics such as  $H_O$ ,  $H_E$ , and  $F_{ST}$  at the population level when the genotypes of individuals cannot be directly determined. The major difficulty with the method is that it requires fairly large sample sizes (at least 50 individuals per population are suggested), which limits the number of populations that can be sampled. In the future, it would be useful to compare the statistics calculated using the method of Luo et al. (2006) to those calculated by using genotypes that are inferred directly from the peak heights on the same data set to determine the comparability of the two approaches.

The calculation of the frequency of double reduction,  $\alpha$ , does appear to present some difficulties for using the method of Luo et al. (2006) and my data. The maximum likelihood estimate of  $\alpha$  was often higher than its theoretical maximum, possibly due to the presence of null alleles, which are difficult to test for in tetraploids when there are many loci and genotypes must

be estimated (cf. Luo et al. 2006). In addition, when a combined  $\alpha$  and combined allele frequencies for two populations are being estimated in order to calculate  $F_{ST}$ , the difference in allele frequencies between the two populations can cause  $\alpha$  to be larger than it would be in either population analyzed individually. For these reasons, it would be better to calculate  $\alpha$  independently from parent-offspring allele frequency data using the method of Stift et al. (2008) and to use this value in subsequent analyses.

#### Conclusions:

Genetic structure appears to be more strongly evident among populations than among the taxa recognized in Chapter One. This fact might be interpreted as supporting Strother and Wetter's (2006) view of the putative taxa within CA-FP *Grindelia*, all of which they subsumed, along with taxa from outside the CA-FP, into an expanded *G. hirsutula*. Alternatively, these findings may be explained by ongoing divergence of taxa of CA-FP *Grindelia*, with insufficient time for the neutral loci examined in this study to have diverged at the level that may well be found in loci governing morphology and ecological preference. It will be necessary to sample more loci and more individuals before the patterns of divergence in CA-FP *Grindelia* can be fully understood.

Figure 1. The locations of the ten sampled populations in Northern California.

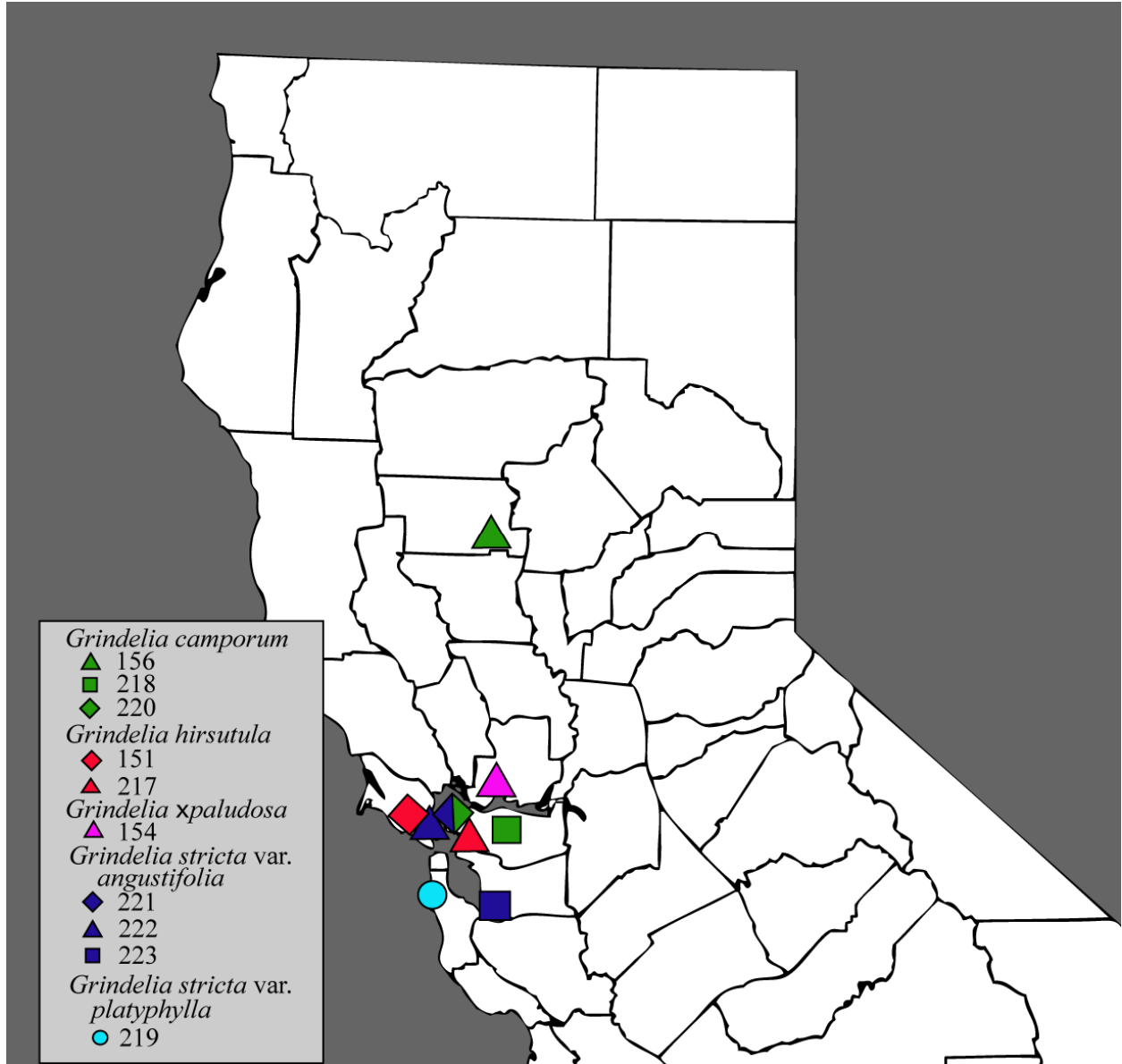


Figure 2. Principal Coordinates Analysis of all 439 individuals using Dice's similarity index. Symbol colors follow Figure 1.



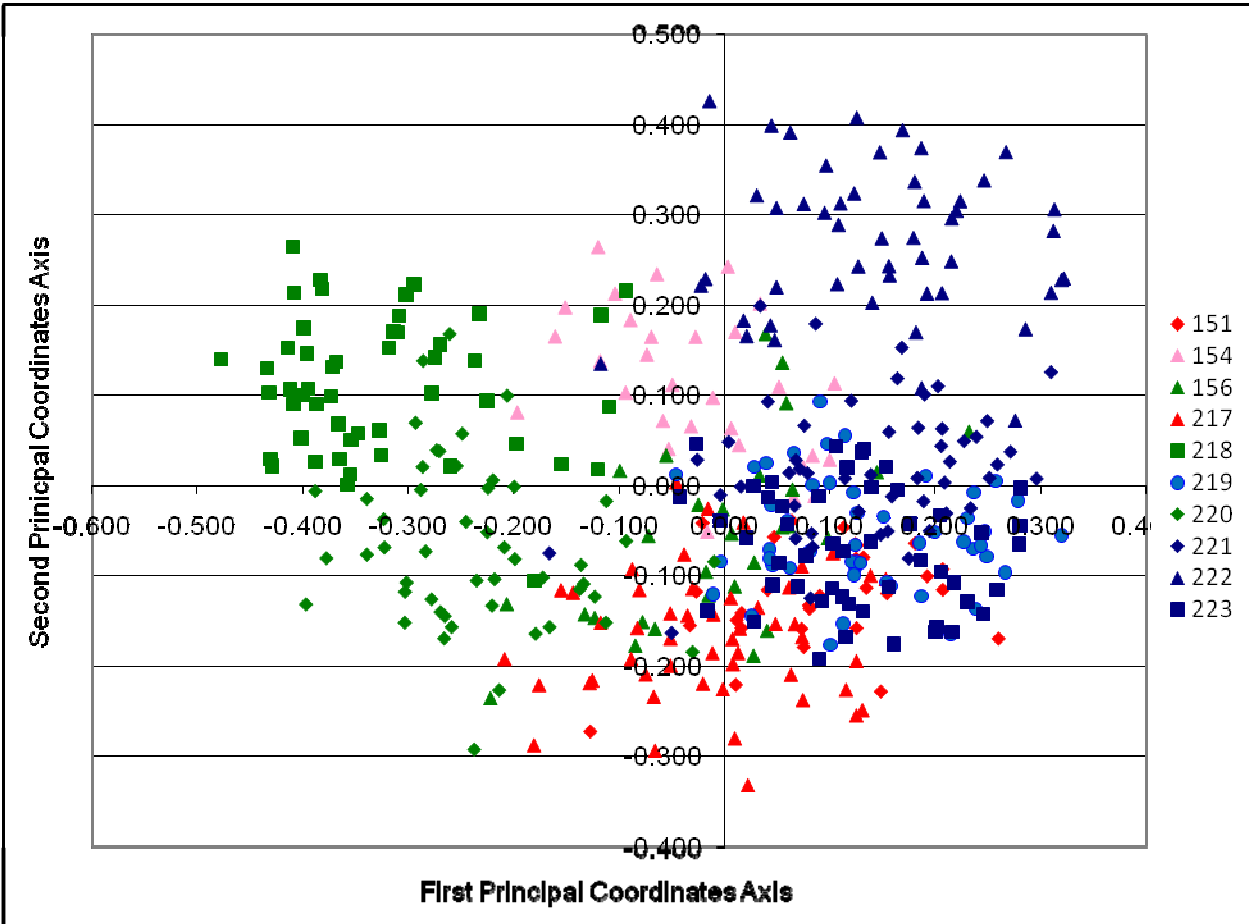


Figure 3. Population centroids from the Principal Coordinates Analysis (a); populations from an ALSCAL analysis of  $F_{ST}$  data (calculated using Luo et al.'s 2006 method) (b); and populations from an ALSCAL analysis of  $\rho$  data (c). Symbol colors follow Figure 1.

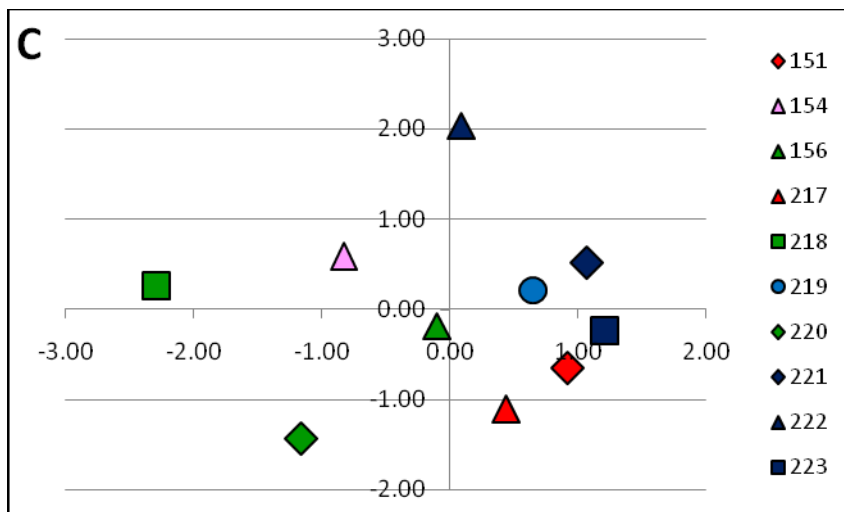
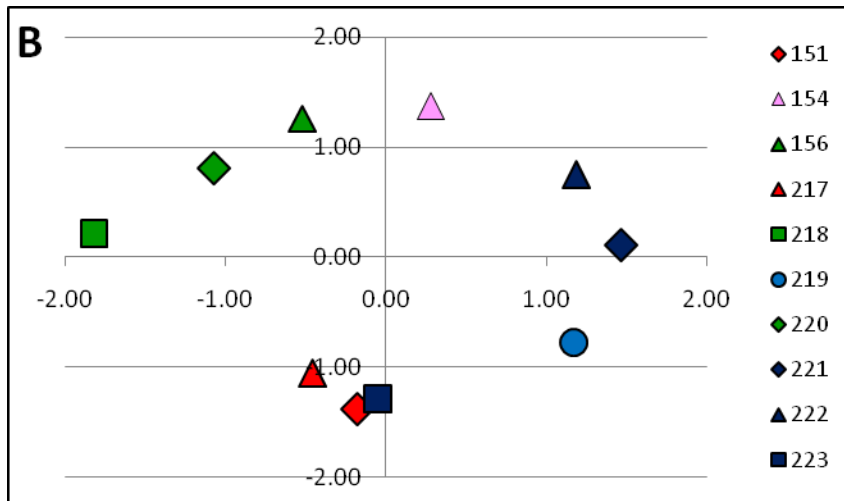
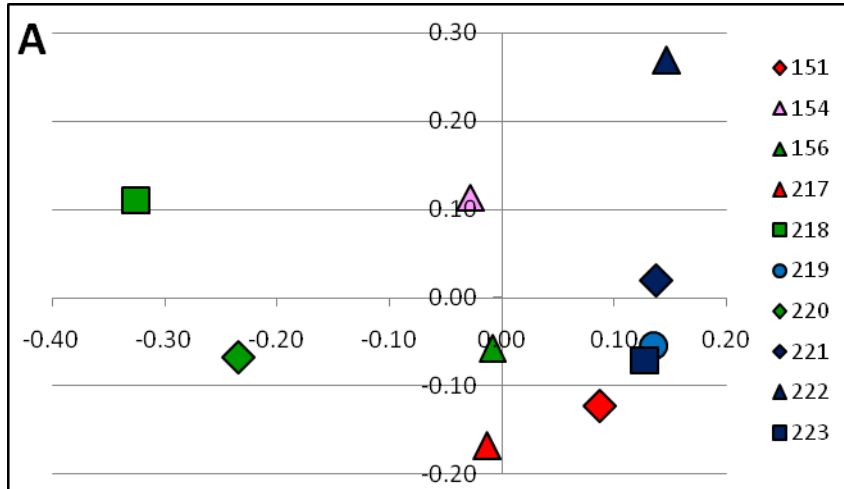


Figure 4. Two different methods for determining how many populations ( $K$ ) the individuals should be divided into using *structure*. The plot of  $\Delta K$  values (following the method of Evanno et al. 2005) averaged over 20 different runs for each value of  $K$  for the Structure analysis showing a peak at  $K = 9$  (a); the plot of mean likelihood values averaged over 20 different runs for each value of  $K$  (b).

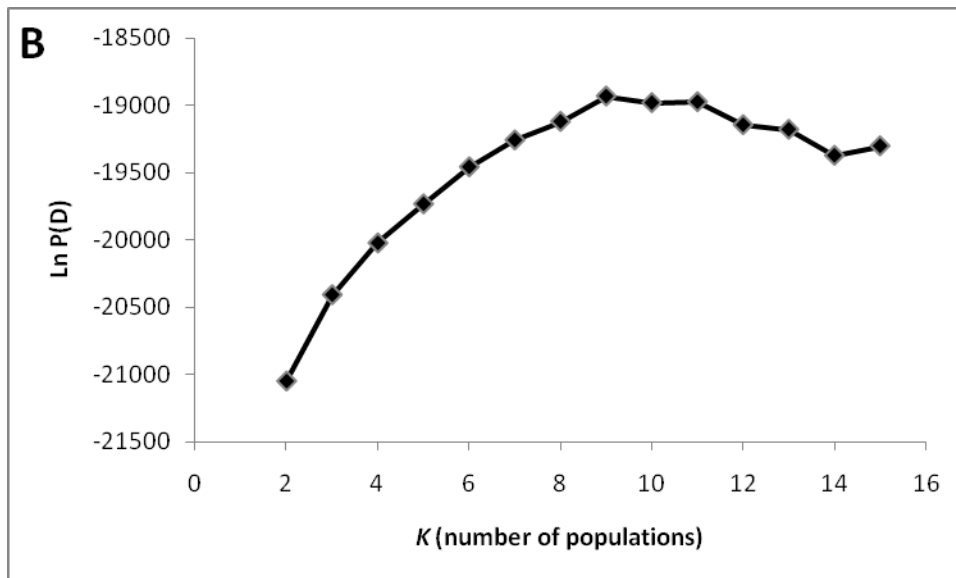
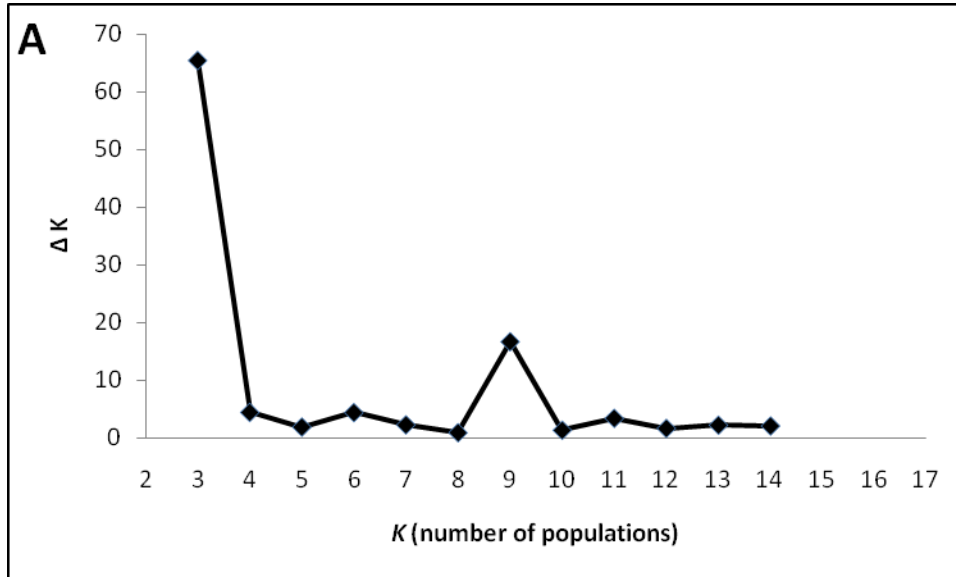
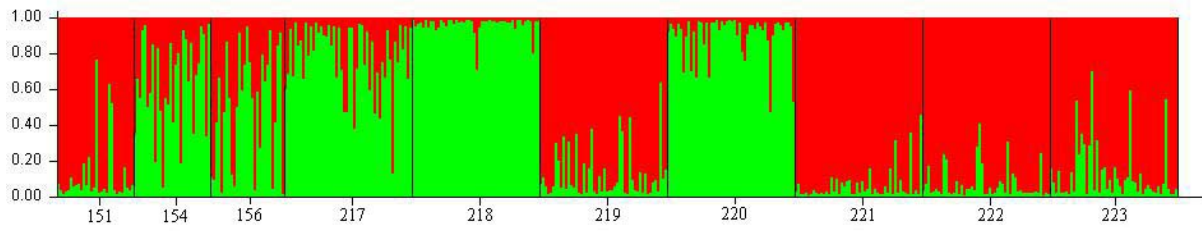
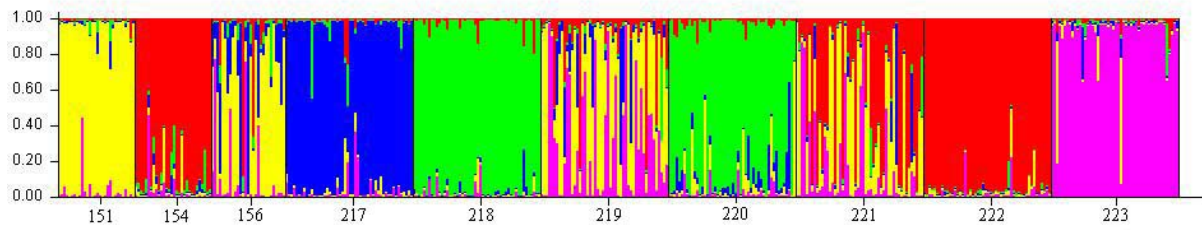


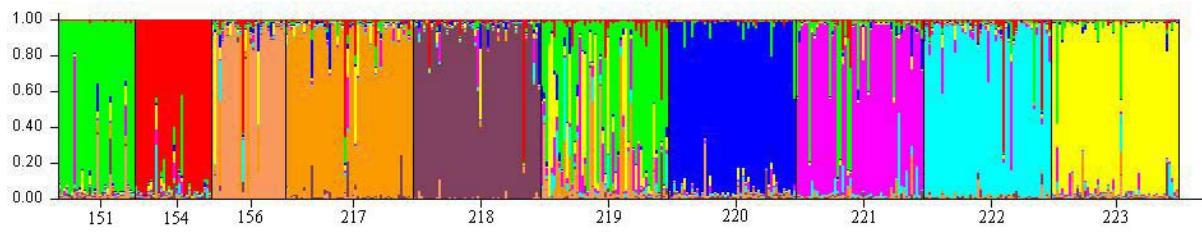
Figure 5. Plots from *structure*, with the individual genotypes ambiguous (but the individual allelic phenotypes not ambiguous).



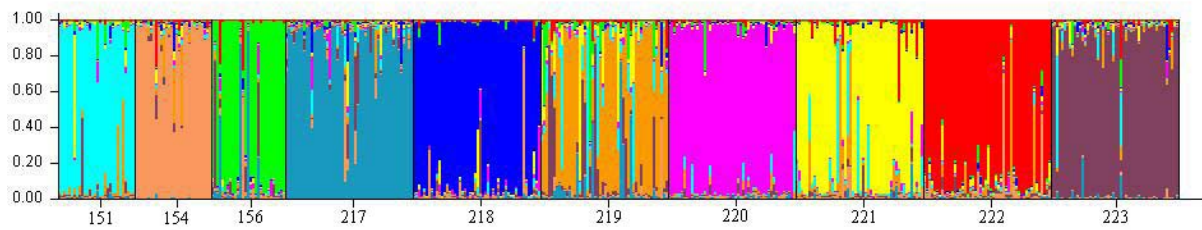
$K = 2$



$K = 5$



$K = 9$



$K = 10$

Table 1. Sampled populations.  $N$  is the number of individuals sampled.



pop. #	taxon	ploidy	county	location	lat/long	voucher	year	<i>N</i>	habitat
159	<i>G. camporum</i>	4x	Glenn	Sacramento NWR	N 39.44669 ° W 112.14539 °	Moore et al. 822	2007	29	alkaline grassland
218	<i>G. camporum</i>	2x	Contra Costa	Mount Diablo State Park	N 37.88295 ° W 121.93930 °	Moore et al. 862	2008	50	grassland
220	<i>G. camporum</i>	4x	Contra Costa	Point Pinole Regional Park	N 38.00553 ° W 122.34908°	Moore et al. 864	2008	50	grassland
151	<i>G. hirsutula</i>	4x	Marin	Mount Tamalpais State Park	N 37.90384° W 122.60544°	Moore 818	2007	30	serpentine grassland
217	<i>G. hirsutula</i>	4x	Contra Costa	Wildcat Canyon Regional Park	N 37.95162 ° W 122.30158°	Moore et al. 861	2008	50	grassland
154	<i>G. ×paludosa</i>	4x	Solano	Hill Slough Wildlife Area	N 38.24603° W 121.99523°	Moore & Park 819	2007	30	brackish marsh
221	<i>G. stricta</i> var. <i>angustifolia</i>	4x	Contra Costa	Point Pinole Regional Park	N 38.00665 ° W 122.35092°	Moore et al. 865	2008	50	salt marsh
222	<i>G. stricta</i> var. <i>angustifolia</i>	4x	Marin	China Camp State Park	N 38.0626 ° W 122.48715 °	Moore et al. 866	2008	50	salt marsh
223	<i>G. stricta</i> var. <i>angustifolia</i>	4x	Alameda	Don Edwards S.F. Bay NWR	N 37.53152 ° W 122.06911 °	Moore & Park 870	2008	50	salt marsh
219	<i>G. stricta</i> var. <i>platyphylla</i>	4x	San Mateo	Montara State Beach	N 37.55709 ° W 122.51218°	Moore et al. 863	2008	50	coastal bluffs

Table 2. Summary statistics for each locus. Observed heterozygosity,  $H_O$ ; expected heterozygosity,  $H_E$ ; frequency of double reduction,  $\alpha$ .

Locus	size range	# alleles total	# alleles per pop	mean $H_O$	mean $H_E$	range in $\alpha$	range in $F_{ST}$	mean $F_{ST}$	range in $\rho$	mean $\rho$
G024	200-276	21	5-13	0.513	0.597	0-0.1667	-0.011-0.254	0.090	-0.010-0.423	0.220
G026	211-304	31	10-19	0.783	0.825	0-0.1667	-0.021-0.101	0.020	0.027-0.231	0.128
G035	187-235	17	5-11	0.516	0.514	0-0.1667	-0.027-0.452	0.086	0.027-0.588	0.260
G045	387-431	30	9-17	0.651	0.684	0-0.1667	-0.011-0.250	0.059	0.046-0.497	0.271
G068	350-378	7	3-4	0.381	0.368	0-0.1667	-0.036-0.160	-0.014	-0.026-0.473	0.168
G113	461-535	28	8-15	0.760	0.822	0.0247-0.1667	-0.033-0.049	0.004	0.008-0.246	0.116

Table 3. Statistics for each locus with populations shown individually. Observed heterozygosity,  $H_O$ ; expected heterozygosity,  $H_E$ ; frequency of double reduction,  $\alpha$ .

	Locus	pop	size range	allele numbers	# alleles	unique alleles	most abundant allele	$H_O$	$H_E$	$\alpha$	range in $F_{ST}$	range in $p$	mean $p$
1	G024	151	214-230	6-14	7		222	0.61	0.63	0.00	-0.0018-0.1470	-0.0104-0.4123	0.22
2	G024	154	214-255	6-22	7	255	226	0.46	0.58	0.17	0.0001-0.1558	0.0957-0.3705	0.22
3	G024	156	221-246	9-21	11	233, 237, 242	226	0.47	0.57	0.17	0.0001-0.1837	0.0909-0.3046	0.19
4	G024	217	200-246	1-21	13	200, 213	222, 226	0.73	0.76	0.17	0.0184-0.0880	0.0909-0.2106	0.16
5	G024	218	205-230	3-14	7	205	226	0.58	0.61	0.17	-0.0109-0.2539	0.0957-0.4234	0.25
6	G024	219	214-238	6-18	10		222	0.59	0.57	n/a	-0.0018-0.1904	-0.0104-0.3378	0.18
7	G024	220	214-230	6-14	5		226	0.46	0.43	0.17	-0.0109-0.2222	0.1385-0.4123	0.29
8	G024	221	214-276	6-24	7	276	218, 222	0.55	0.70	0.17	0.0093-0.1837	0.0018-0.3804	0.21
9	G024	222	214-256	6-23	7		218	0.57	0.67	0.17	0.0093-0.1779	0.0018-0.3741	0.21
10	G024	223	214-234	6-16	8		222	0.36	0.61	0.17	0.0147-0.2539	0.1519-0.4234	0.27
1	G026	151	215-279	2-24	14		223	0.69	0.72	0.17	0.0148-0.1010	0.0643-0.2184	0.16
2	G026	154	227-267	7-22	13	246, 265	235 (239 close)	0.67	0.76	0.17	-0.0100-0.1010	0.0644-0.2184	0.16
3	G026	156	211-279	1-25	14		251 (259 close)	0.78	0.79	0.17	-0.0076-0.0811	0.0265-0.1925	0.14
4	G026	217	211-259	1-19	10		211 (243, 251 close-ish)	0.74	0.80	0.17	-0.0171-0.0586	0.0633-0.1694	0.12
5	G026	218	219-263	3-20	13	222	235 (239 close)	0.66	0.87	n/a	-0.0075-0.0646	0.056-0.1389	0.10
6	G026	219	211-263	1-30	18		243 (251, 255, 259 close)	0.82	0.85	0.17	-0.0213-0.0527	0.0265-0.1424	0.08
7	G026	220	211-263	1-30	16	245	235	0.85	0.85	0.03	-0.0171-0.0225	0.0687-0.2308	0.17
8	G026	221	211-263	1-30	17		247 (227 close)	0.92	0.92	0.00	-0.0190-0.0148	0.0522-0.1584	0.10
9	G026	222	219-300	3-31	17	300	219 (239 close)	0.89	0.89	0.01	-0.0213-0.0152	0.056-0.2133	0.12
10	G026	223	211-304	1-32	19	254, 281, 283, 286, 304	219, 259	0.81	0.81	0.12	0.0015-0.0530	0.0821-0.2308	0.15
1	G035	151	213-229	4-15	8	220	213	0.47	0.47	0.17	-0.0211-0.4019	0.1541-0.5849	0.27
2	G035	154	213-229	4-15	6		213	0.55	0.52	0.00	-0.0265-0.2502	0.1012-0.2892	0.20
3	G035	156	211-225	3-13	7		213	0.36	0.35	0.00	-0.0198-0.4523	0.1321-0.5165	0.24
4	G035	217	209-223	2-12	5		213	0.46	0.44	0.17	-0.0265-0.4174	0.1414-0.5884	0.28
5	G035	218	211-225	3-13	7		217	0.48	0.47	n/a	0.0085-0.4523	0.2797-0.5884	0.49

6	G035	219	213-235	4-17	11	235	213	0.60	0.63	0.12	-0.0094-0.3192	0.0383-0.56	0.20
7	G035	220	209-227	2-14	8		213	0.80	0.78	0.00	-0.0126-0.1505	0.1012-0.4841	0.25
8	G035	221	213-229	4-15	7	222	213	0.39	0.39	0.16	-0.0132-0.4372	0.0273-0.5717	0.20
9	G035	222	187-231	1-16	11	187, 216	217	0.66	0.69	0.17	-0.0126-0.1465	0.1425-0.407	0.28
10	G035	223	213-231	4-16	7		213	0.39	0.40	0.00	-0.0200-0.4098	0.0273-0.5117	0.20
1	G045	151	404-418	18-32	12		407, 414	0.72	0.74	0.17	0.0074-0.715	0.1459-0.4232	0.27
2	G045	154	397-421	11-35	9		407	0.64	0.71	0.17	-0.0082-0.1230	0.1779-0.3931	0.27
3	G045	156	395-431	9-45	17	395	406	0.79	0.78	0.17	-0.0081-0.0855	0.046-0.292	0.19
4	G045	217	387-427	1-41	11	387	407	0.73	0.74	0.17	-0.0067-0.1009	0.0522-0.3848	0.23
5	G045	218	397-411	11-25	10	398	408	0.70	0.77	n/a	0.0087-0.2502	0.2405-0.4136	0.31
6	G045	219	399-423	13-37	9		407	0.37	0.53	0.17	-0.0096-0.2069	0.0714-0.362	0.22
7	G045	220	399-427	13-41	13	401	408	0.83	0.81	0.00	0.0037-0.1413	0.2778-0.497	0.36
8	G045	221	399-418	13-32	9		407	0.36	0.37	0.17	-0.0096-0.2502	0.0714-0.419	0.29
9	G045	222	397-421	11-35	14		407, 412	0.58	0.58	0.17	0.0163-0.1909	0.1885-0.497	0.35
10	G045	223	402-431	16-45	16	430	407	0.81	0.80	0.09	-0.0108-0.0864	0.046-0.3861	0.21
1	G068	151	353-359	2-4	3		356	0.50	0.50	0.00	-0.0245-0.0071	-0.0255-0.3805	0.13
2	G068	154	353-359	2-4	3		356	0.40	0.39	0.00	-0.0359-0.0274	-0.0059-0.2187	0.10
3	G068	156	350-359	1-4	4	350	356	0.39	0.39	0.00	-0.0141-0.0475	-0.0059-0.219	0.09
4	G068	217	353-378	2-7	3	378	356	0.13	0.13	0.00	-0.0146-0.1406	-0.0085-0.4725	0.28
5	G068	218	353-376	2-5	4	376	356	0.16	0.15	n/a	-0.0308-0.1595	-0.0085-0.4397	0.24
6	G068	219	353-359	2-4	3		356	0.54	0.51	0.00	-0.0232-0.0349	-0.0255-0.4276	0.16
7	G068	220	353-359	2-4	3		356	0.19	0.19	0.00	-0.0359-0.1188	0.0223-0.2066	0.13
8	G068	221	353-377	2-6	4		356	0.42	0.42	0.17	-0.0245-0.0523	0.0436-0.3227	0.15
9	G068	222	353-359	2-4	3		353, 356	0.58	0.54	0.16	-0.0107-0.1595	0.0436-0.4725	0.23
10	G068	223	353-377	2-6	3		356	0.49	0.45	0.11	-0.0084-0.0363	-0.0055-0.3352	0.15

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