Edaphic Ecology and Genetics of the Gabbro-Endemic Shrub *Ceanothus roderickii* (Rhamnaceae)

Author(s) :Dylan O. Burge and Paul S. Manos
Source: Madroñño, 58(1):1-21. 2011.
Published By: California Botanical Society
DOI: 10.3120/0024-9637-58.1.1
URL: http://www.bioone.org/doi/full/10.3120/0024-9637-58.1.1

MADROÑO, Vol. 58, No. 1, pp. 1–21, 2011

EDAPHIC ECOLOGY AND GENETICS OF THE GABBRO-ENDEMIC SHRUB *CEANOTHUS RODERICKII* (RHAMNACEAE)

DYLAN O. BURGE AND PAUL S. MANOS
Duke University Department of Biology, Box 90338, Durham, NC 27705
dylan.o.burge@gmail.com

ABSTRACT

Edaphic-endemic plant taxa are often interpreted as recently derived entities that evolved in situ, with genetic divergence driven by substrate specialization. However, little is known about the evolution of specific edaphic-endemic taxa, particularly the role that soil conditions may play in their initial divergence and continued persistence. Our study focuses on *Ceanothus roderickii*, a strict specialist on soils derived from a single outcrop of the geological material gabbro located in southwestern El Dorado County, California. In order to elucidate the evolutionary history of *C. roderickii* we sequenced the third intron of the low-copy nuclear gene nitrate reductase (NIA) for individuals representing four populations of *C. roderickii* and a wide taxonomic and geographic sampling of closely related plants, including 37 populations of *Ceanothus cuneatus* and a single representative from 16 other taxa. Analysis of NIA shows that *C. roderickii* is closely related to *C. cuneatus* var. *cuneatus*, a widely distributed taxon found on a diversity of soils. *Ceanothus cuneatus* var. *cuneatus* is paraphyletic and comprises two major geographic groups, one coastal and one interior, the latter containing *C. roderickii*. Thirteen soil chemistry variables were assayed in 42 populations of *C. cuneatus* representing the wide geographic range of this species, and in 10 populations of *C. roderickii*. Analysis of these data indicates that evolution of *C. roderickii* was associated with specialization to nutrient-deficient forms of gabbro-derived soil. Soil chemistry associations of *C. cuneatus* var. *cuneatus* and *C. roderickii* are most divergent where the species come into close contact on gabbro, with *C. cuneatus* var. *cuneatus* occupying comparatively nutrient-rich forms of gabbro-derived soils, a result that is consistent with reinforcement.

Key Words: *Ceanothus*, edaphic, evolution, gabbro, NIA, Pine Hill intrusive complex.

Edaphic factors—those pertaining to the substrate or soil—have long been interpreted as potential drivers of plant diversification (Stebbins 1942; Kruckeberg 1986; Rajakaruna 2004). This idea derives from the strong association of many so-called 'edaphic endemic' taxa with particular soil or substrate conditions (Mason 1946; Gankin and Major 1964; Kruckeberg 1986, 2002). In California, for example, approximately 10% of native vascular plants at the level of species and below are endemic to soils derived from serpentinite parent material (Kruckeberg 1986; Hickman 1993). Edaphic endemics are often classified either as relicts (paleoendemics) or as recently derived entities (neoendemics) that evolved in situ, with substrate specialization accompanying genetic divergence (Raven and Axelrod 1978). Recent work by Baldwin (2005) provided the first phylogenetic evidence for recent divergence of an edaphic endemic taxon, discovering that the serpentinite-endemic herb *Layia discoidea* D. D. Keck "budded off" from within a less specialized species less than 1 mya. It is not clear, however, whether this pattern is common to the large number of other edaphic endemics in California and elsewhere. By combining detailed genetic surveys with analyses of edaphic conditions experienced by edaphic endemics and their close relatives, it may be possible to discern general trends in the evolution of edaphic endemics, and how these trends relate to soil conditions. Here we focus on the *Cerastes* subgenus of *Ceanothus*, which contains 10 edaphic-endemic taxa restricted to California and Baja California, Mexico (Table 1). Our goal is to discern the evolutionary history of a single species of edaphic endemic *Cerastes*, and relate this history to the substrate conditions experienced by this taxon and its closest relatives.

In the Sierra Nevada foothills of western El Dorado County, California, soils weathered from mafic rocks of the Pine Hill intrusive complex (~100 km^2, Fig. 1; Springer 1971) support approximately 600 vascular plant species (Wilson et al. 2009, Appendix 1), representing more than 10% of the California flora (5867 species; Hickman 1993), including several endemics and taxa of limited or disjunct distribution (Wilson 1986; Hunter and Horenstein 1991; Wilson et al. 2009). Endemics of the Pine Hill intrusive complex include *Ceanothus roderickii* W. Knight, *Fremontodendron californicum* (Torr.) Coville subsp. *decumbens* (R. M. Lloyd) Munz, *Galium californicum* Hook. & Arn. subsp. *sierrae* Dempster & Stebbins, and *Wyethia reticulata* Greene. The first three of these plants are federally-listed Endangered Species (USFWS 1996).

The Pine Hill intrusive complex is composed primarily of the rock gabbro, with minor

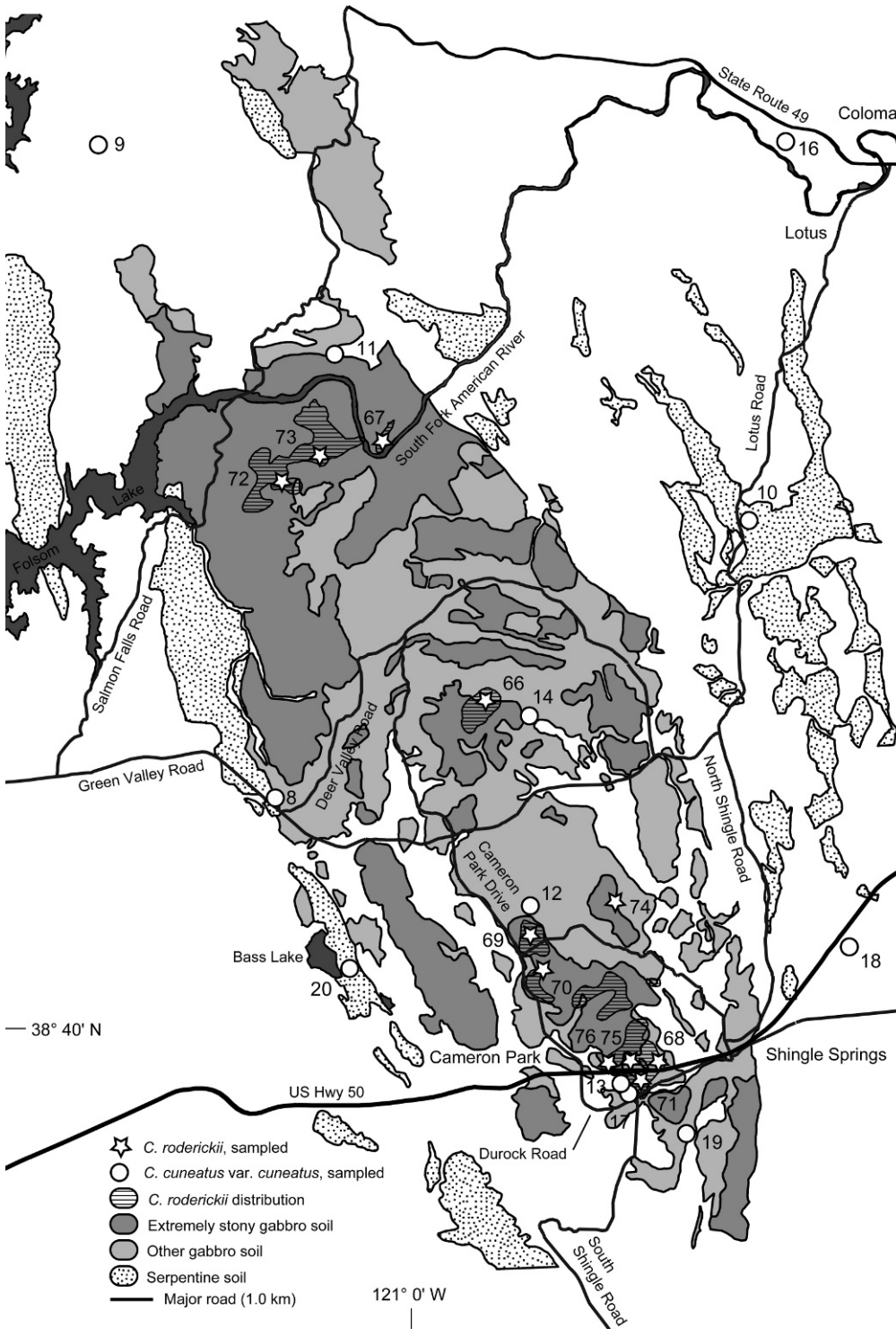
TABLE 1. *CEANOTHUS*, SUBGENUS *CERASTES* TAXA. Taxon = taxa from Fross and Wilken (2006). Sampled = populations sampled for genetic and/or soil analyses (Appendix 1). Geographic distribution = distribution of taxa in North America (North CA: region of CA from the latitude of Point Conception, north; South CA: region of CA from the latitude of Point Conception, south; BC, Mexico: Mexican state of Baja California). Soil = geological parent material(s) for soils on which taxon occurs (Fross & Wilken 2006). [a] 25 populations of *C. cuneatus* var. *cuneatus* sampled for genetics and soil, 8 for genetics only, and 13 for soil only. [b] Three populations of *C. roderickii* sampled for genetics and soil, 1 for genetics only, and 7 for soil only. [c] Also found on gabbro-derived soils. [d] Parent material classified as metavolcanic ("Mzv") by Jennings (1977).

Taxon	Sampled	Geographic distribution	Soil
Ceanothus arcuatus McMinn	0	North CA	various
C. bolensis S. Boyd & J.E. Keeley	0	BC, Mexico	basalt
C. crassifolius Torr. var. *crassifolius*	0	South CA; BC, Mexico	various
C. crassifolius Torr. var. *planus* Abrams	0	South CA	various
C. cuneatus Nutt. var. *cuneatus*	46[a]	West US; BC, Mexico	various
C. cuneatus Nutt. var. *dubius* J.T. Howell	1	North CA	various
C. cuneatus Nutt. var. *fascicularis* (McMinn) Hoover	1	North CA	various
C. cuneatus Nutt. var. *ramulosus* Greene	1	North CA	various
C. cuneatus Nutt. var. *rigidus* (Nutt.) Hoover	1	North CA	various
C. divergens Parry subsp. *confusus* (J.T. Howell) Abrams	1	North CA	various
C. divergens Parry subsp. *divergens*	0	North CA	various
C. divergens Parry subsp. *occidentalis* (McMinn) Abrams	1	North CA	various
C. ferrisiae McMinn	1	North CA	serpentinite
C. fresnensis Abrams	1	North CA	various
C. gloriosus J.T. Howell var. *exaltatus* J.T. Howell	1	North CA	various
C. gloriosus J.T. Howell var. *gloriosus*	1	North CA	various
C. gloriosus J.T. Howell var. *porrectus* J.T Howell	1	North CA	granite
C. jepsonii Greene var. *albiflorus* J.T. Howell	1	North CA	serpentinite
C. jepsonii Greene var. *jepsonii*	1	North CA	serpentinite
C. maritimus Hoover	1	North CA	various
C. masonii McMinn	1	North CA	various
C. megacarpus Nutt. var. *insularis* (Eastw.) Munz	0	South CA	various
C. megacarpus Nutt. var. *megacarpus*	0	South CA	various
C. ophiochilus S. Boyd, T.S. Ross & Arnseth	0	South CA	pyroxenite[c]
C. otayensis McMinn	0	South CA; BC, Mexico	basalt[d]
C. pauciflorus DC.	0	Mexico	various
C. perplexans Trel.	0	South CA; BC, Mexico	various
C. pinetorum Coville	1	North CA	various
C. prostratus Benth.	1	North CA	various
Ceanothus pumilus Greene	1	North CA	serpentinite
C. purpureus Jeps.	1	North CA	volcanic
C. roderickii W. Knight	11[b]	North CA	gabbro
C. sonomensis J.T. Howell	1	North CA	various
C. verrucosus Nutt.	0	South CA; BC, Mexico	various
C. vestitus Greene	0	West US; Mexico	various

amounts of pyroxenite and diorite (Springer 1980; hereafter referred to collectively as "gabbro"), which weather to form reddish-brown sandy loams with very stony to clayey variants (the Rescue Series; Rogers 1974). Gabbro contains less iron, Mg, and potentially plant-toxic transition elements (e.g., Cr, Co, Ni) than are found in ultramafic rocks such as serpentinite

→

FIG. 1. Sampling and soil map for the Pine Hill region, El Dorado Co., California. Polygons for gabbro or serpentinite derived soils adapted from GIS data layers in Soil Survey Geographic (SSURGO) database for El Dorado Area, California (U.S. Department of Agriculture, Natural Resources Conservation Service 2007). *Extremely stony gabbro soil*: shallow soils derived from gabbro parent material, corresponding to "Rescue extremely stony sandy loam" (Rogers 1974, RgE2). *Other gabbro soil*: deeper soils derived from gabbro parent



material, corresponding to ''Rescue sandy loam'' (Rogers 1974, ReC, ReB, & ReD) and ''Rescue very stony sandy loam'' (Rogers 1974, RfC, RfD, & RfE). *Serpentine soil*: very shallow, rocky soils derived from serpentinite parent material, corresponding to 'Serpentine rock land' (Rogers 1974, SaF). *Ceanothus roderickii* distribution adapted from Hinshaw (2008) in consultation with G. Hinshaw and L. Fety (March, 2010). Sampling locations indicated by stars or open circles (Table 2).

(Alexander 1993, unpublished). As a result, soils derived from gabbro usually contain elevated levels of Mg relative to soils derived from less mafic rocks such as diorite, but have lower Ca to Mg ratios than soils weathered from ultramafic rocks such as serpentinite (Goldhaber et al. 2009).

Endemism on the Pine Hill intrusive complex, as well as the presence of species normally restricted to serpentinite-derived soils, has been attributed to the similar properties of gabbro and serpentinite rock (Wilson 1986). However, analyses of soils from the Pine Hill intrusive complex have not identified soil parameters that predict plant distributions (Hunter and Horenstein 1991; Alexander, unpublished), leading Hunter and Horenstein (1991) to conclude that endemism on these soils may be attributed to the island-like topographic position of the complex in the otherwise low-lying foothills of the central Sierra Nevada. However, these results may be confounded by plant demography, especially in the case of *C. roderickii*, which is dependant on fire for significant recruitment (Boyd 2007).

Ceanothus roderickii is a member of the *Cerastes* subgenus of *Ceanothus*, a group of 24 species that is almost entirely restricted to the California Floristic Province (CFP) of western North America (Fross and Wilken 2006). Members of *Cerastes* possess a suite of morphological and physiological adaptations for drought resistance (Ackerly et al. 2006) and are associated with chaparral vegetation. However, the group is both morphologically and ecologically diverse, with an array of growth forms and a broad spectrum of habitat associations, sometimes including specialized edaphic ecology (McMinn 1942; Nobs 1963; Fross and Wilken 2006). *Ceanothus roderickii* is a decumbent shrub spreading horizontally via arching branches that usually root adventitiously when nodes contact soil (Knight 1968), a trait that allows this species to reproduce clonally during fire-free intervals when recruitment from the seed-bank is limited (Boyd 2007). A close relationship between *C. roderickii* and the widespread *Ceanothus cuneatus* Nutt. was proposed by Knight (1968). However, this author also speculated on the possibility of a close relationship between *C. roderickii* and *Ceanothus fresnensis* Abrams or *Ceanothus prostratus* Benth., the only other decumbent members of *Cerastes* known from the central Sierra Nevada.

Sequence data from nuclear ribosomal DNA suggest that *C. roderickii* is closely related to *C. cuneatus* var. *cuneatus* (Hardig et al. 2000). *Ceanothus cuneatus* is among the most widely distributed members of *Ceanothus*, occupying forest, woodland, and chaparral habitats at low to moderate elevations in far western North America from Baja California, Mexico to northwestern Oregon (Fig. 2), almost entirely within the CFP (Fig. 2). *Ceanothus cuneatus* comprises five varieties (Table 1), four of which are narrowly distributed (Fross and Wilken 2006). The most widely distributed variety, *C. cuneatus* var. *cuneatus*, is a characteristic component of chaparral and woodland communities in the foothills and mountains of the CFP and is known to grow on soils derived from a variety of geological parent materials (Fross and Wilken 2006; Table 1). *Ceanothus cuneatus* var. *cuneatus* is the only member of *Cerastes* other than *C. roderickii* known to occur in the Pine Hill region of El Dorado Co., California.

This study was designed to elucidate the evolutionary history of *C. roderickii* and relate this history to the substrate specificity of the taxon and its closest relatives. Specifically, this study aimed to 1) test the hypothesis that *C. roderickii* is most closely related to and possibly derived from within *C. cuneatus* var. *cuneatus*, 2) characterize the soil chemistry associations of *C. roderickii* relative to those of *C. cuneatus* var. *cuneatus*, and 3) identify specific chemical properties of *C. roderickii* soils that may have provided selective pressure during evolution of the species.

Materials and Methods

Genetic Sampling

Genetic sampling of *Ceanothus* populations was designed to represent the geographic range and edaphic tolerances of the focal taxa *C. roderickii* and *C. cuneatus* var. *cuneatus*, and to encompass related plants (Tables 1 and 2, Fig. 2, Appendix 1). DNA from 57 plants was studied, representing 22 of the approximately 35 *Cerastes* taxa (species, subspecies, and varieties) currently recognized (Table 1; Fross and Wilken 2006). All individuals sampled for the present work were collected by D. Burge, with the exception of a sample of *Ceanothus pinetorum* Coville obtained by D. Wilken (DHW 16736, Table 2, Appendix 1). Individuals from four populations of *C. roderickii* were sampled to represent the geographic distribution of this species (Table 2, Fig. 1, Appendix 1). Individuals from 33 populations of *C. cuneatus* var. *cuneatus* were sampled to represent the extensive geographic range of this taxon and the variety of edaphic conditions that it experiences over this area (Table 2, Fig. 2). Individuals representing populations of 20 additional *Cerastes* taxa were sampled for genetic analysis based on a large-scale phylogenetic study of *Ceanothus* (Burge et al. in press). In this large-scale study, which is based on more than 140 *Cerastes* populations from across the geographic range of the group, individuals included in the present study (Table 2, Appendix 1) form a

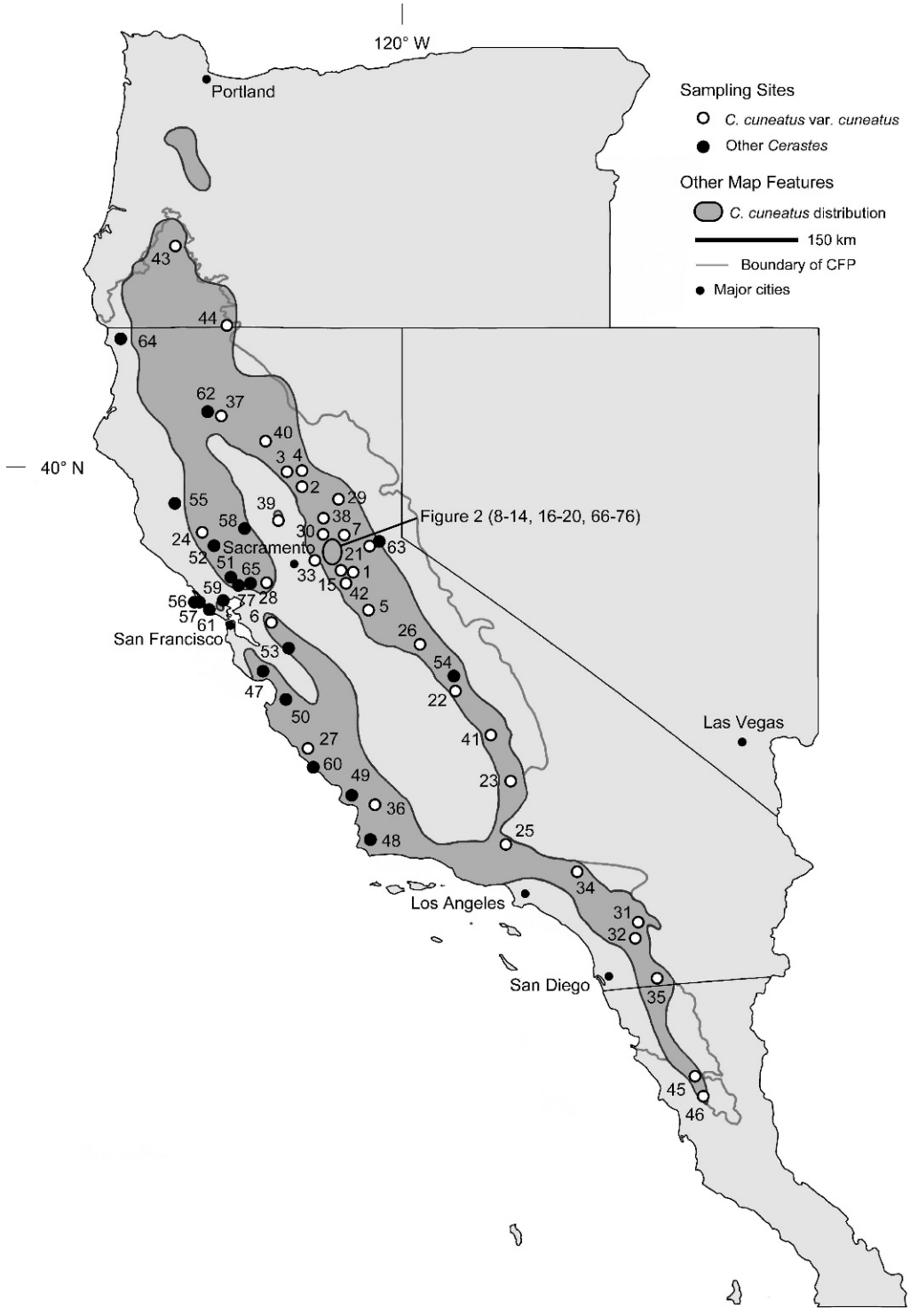


FIG. 2. Sampling map for western North America. Soil and/or genetic sampling locations indicated by open circles (Table 2). Global distribution of *C. cuneatus* indicated by gray shading (data provided by the participants of the Consortium of California Herbaria; March, 2010). Boundary of the California Floristic Province (CFP) adapted from Myers et al. (2000).

TABLE 2. GENETIC AND SOIL SAMPLING. Taxon = taxa from Fross and Wilken (2006). Map = numbering corresponds to Figs. 1 and 2. State, county & voucher = state and county for plant and/or soil sampling, and collection number for corresponding voucher specimen (Appendix 1); all collections by D. O. Burge (except DHW 16736, see Appendix 1), all voucher specimens deposited at DUKE. Soil = generalized geological parent material from which local soil is derived; interpreted based on Jennings (1977). GenBank = GenBank accession number(s) for NIA; –, no sequence data.[a]Soil chemistry data obtained. See Appendix 1 for additional data.

Taxon	Map	State, county & voucher	Soil	GenBank
C. cuneatus Nutt. var. *cuneatus*	1	CA; Amador; 1150a	metamorphic[a]	HM240329; HM240330
	2	CA; Butte; 1109a	granite[a]	—
	3	CA; Butte; 815a	volcanic[a]	—
	4	CA; Butte; 1078a	serpentinite[a]	HM240306; HM240307
	5	CA; Calaveras; 1149a	sedimentary[a]	HM240327; HM240328
	6	CA; Contra Costa; 916a	sedimentary[a]	HM240341; HM240342
	7	CA; El Dorado; 1011a	serpentinite[a]	—
	8	CA; El Dorado; 1024a	serpentinite[a]	—
	9	CA; El Dorado; 1074a	volcanic[a]	—
	10	CA; El Dorado; 1076a	serpentinite[a]	—
	11	CA; El Dorado; 1088a	gabbro[a]	—
	12	CA; El Dorado; 1089a	gabbro[a]	—
	13	CA; El Dorado; 1101a	gabbro[a]	—
	14	CA; El Dorado; 1116a	gabbro[a]	—
	15	CA; El Dorado; 1174a	sedimentary[a]	—
	16	CA; El Dorado; 1175a	granite[a]	—
	17	CA; El Dorado; 1023a	gabbro[a]	HM240296; HM240297
	18	CA; El Dorado; 1075a	metamorphic[a]	HM240302; HM240303
	19	CA; El Dorado; 1095a	gabbro[a]	HM240314; HM240315
	20	CA; El Dorado; 1110a	serpentinite[a]	HM240316
	21	CA; El Dorado; 1117a	volcanic[a]	HM240317; HM240318
	22	CA; Fresno; 1136a	metamorphic[a]	HM240323
	23	CA; Kern; 1132a	granite[a]	HM240319; HM240320
	24	CA; Lake; 1008a	sedimentary	HM240295
	25	CA; Los Angeles; 1071a	granite[a]	HM240301
	26	CA; Mariposa; 1140a	granite[a]	HM240324
	27	CA; Monterey; 858a	sedimentary	HM240338
	28	CA; Napa; 899a	sandstone	HM240339; HM240340
	29	CA; Nevada; 1084a	serpentinite[a]	HM240310
	30	CA; Placer; 1077a	metamorphic[a]	HM240304; HM240305
	31	CA; Riverside; 803a	granite[a]	—
	32	CA; Riverside; 982a	granite[a]	HM240344; HM240345
	33	CA; Sacramento; 1094a	sedimentary[a]	HM240313
	34	CA; San Bernardino; 1070a	sedimentary[a]	HM240300
	35	CA; San Diego; 984a	granite[a]	HM240346
	36	CA; San Luis Obispo; 959a	sedimentary	HM240343
	37	CA; Shasta; 1151a	metamorphic	HM240331; HM240332
	38	CA; Sierra; 1083a	serpentinite[a]	HM240308; HM240309
	39	CA; Sutter; 1093a	volcanic	HM240311; HM240312
	40	CA; Tehama; 1168a	volcanic[a]	HM240336
	41	CA; Tulare; 1134a	granite[a]	HM240321; HM240322

TABLE 2. Continued.

Taxon	Map	State, county & voucher	Soil	GenBank
	42	CA; Tuolumne; 1145a	serpentinite[a]	HM240325; HM240326
	43	OR; Douglas; 1161a	serpentinite	HM240333
	44	OR; Jackson; 1164a	volcanic	HM240334; HM240335
	45	Baja CA; N/A; 1030a	granite[a]	HM240298; HM240299
	46	Baja CA; N/A; 783a	metamorphic[a]	HM240337
C. cuneatus Nutt. var. *dubius* J.T. Howell	47	CA; Santa Cruz; 918a	sedimentary[a]	HM240347
C. cuneatus Nutt. var. *fascicularis* (McMinn) Hoover	48	CA; Santa Barbara; 871a	sand[a]	HM240348
C. cuneatus Nutt. var. *ramulosus* Greene	49	CA; San Luis Obispo; 847b	serpentinite[a]	HM240349
C. cuneatus Nutt. var. *rigidus* (Nutt.) Hoover	50	CA; Monterey; 891b	sedimentary[a]	HM240350; HM240351
C. divergens Parry subsp. *confusus* (J.T. Howell) Abrams	51	CA; Sonoma; 1003a	serpentinite	HM240352; HM240353
C. divergens Parry subsp. *occidentalis* (McMinn) Abrams	52	CA; Lake; 943a	volcanic	HM240354
C. ferrisiae McMinn	53	CA; Santa Clara; 834a	serpentinite	HM240355; HM240356
C. fresnensis Abrams	54	CA; Fresno; 1138a	granite	HM240357
C. gloriosus J.T. Howell var. *exaltatus* J.T. Howell	55	CA; Mendocino; 994a	sediment	HM240358; HM240359
C. gloriosus J.T. Howell var. *gloriosus*	56	CA; Marin; 908a	sand	HM240360; HM240361
C. gloriosus J.T. Howell var. *porrectus* J.T. Howell	57	CA; Marin; 907a	granite	HM240362; HM240363
C. jepsonii Greene var. *albiflorus* J.T. Howell	58	CA; Colusa; 997a	serpentinite	HM240364; HM240365
C. jepsonii Greene var. *jepsonii*	59	CA; Marin; 914a	serpentinite	HM240366
C. maritimus Hoover	60	CA; San Luis Obispo; 887a	sediment	HM240367
C. masonii McMinn	61	CA; Marin; 913a	sediment	HM240368
C. pinetorum Coville	62	CA; Trinity; DHW 16736	granite	HM240369; HM240370
C. prostratus Benth.	63	CA; El Dorado; 952a	metamorphic	HM240371
C. pumilus Greene	64	CA; Del Norte; 1156a	serpentinite	HM240372; HM240373
C. purpureus Jeps.	65	CA; Napa; 904a	volcanic	HM240374; HM240375
C. roderickii W. Knight	66	CA; El Dorado; 1080a	gabbro[a]	HM240376
	67	CA; El Dorado; 1087a	gabbro[a]	HM240377; HM240378
	68	CA; El Dorado; 1090a	gabbro[a]	—
	69	CA; El Dorado; 1096a	gabbro[a]	—
	70	CA; El Dorado; 1100a	gabbro[a]	—
	71	CA; El Dorado; 1102a	gabbro[a]	—
	72	CA; El Dorado; 1104a	gabbro[a]	—
	73	CA; El Dorado; 1105a	gabbro[a]	—
	74	CA; El Dorado; 1111	gabbro[a]	HM240379
	75	CA; El Dorado; 1171a	gabbro[a]	—
	76	CA; El Dorado; 824b	gabbro	HM240380
C. sonomensis J.T. Howell	77	CA; Sonoma; 895b	volcanic	HM240381

monophyletic group nested within *Cerastes* as a whole (Burge et al. in press). Voucher specimens were identified based on Fross and Wilken (2006). However, *Ceanothus masonii* McMinn, treated as part of *Ceanothus gloriosus* J. T. Howell by Fross and Wilken (2006) is recognized here.

Molecular Methods

Genomic DNA was extracted from fresh or silica-dried leaf and/or flower-bud tissue using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. Polymerase chain reactions were performed using Qiagen *Taq* DNA Polymerase. Amplification was performed using an initial incubation at 94C for 10 min and 30 cycles of three-step PCR (1 min at 94C, 30 s at 55C, and 2 min at 72C), followed by final extension at 72C for 7 min. Primers NIA-3F and NIA-3R (Howarth and Baum 2002) were initially used to amplify the third intron of the low-copy nuclear gene nitrate reductase (NIA). Subsequent to amplification from representative *Ceanothus* species, NIA PCR products were cloned using the TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Non-degenerate primers (NIARHA-3F, AGGTGGAGGTTCTTAACCTTCTC; NIARHA-3R, GAACCAGCAATTGTTCATCATTCC) were designed based on the alignment of these cloned sequences. These primers have been used to amplify NIA from all members of *Cerastes* as well as members of the *Ceanothus* subgenus of *Ceanothus*. Analysis of NIA sequences from representative *Ceanothus* individuals (Burge et al. in press) demonstrated that these primers always amplify a single orthologous copy of NIA, which is frequently represented by two sequence types (putative alleles) that vary in length and base composition. As a result of this heterogeneity, cloning of NIA was required for most plants. Subsequent to initial primer design, cloning was carried out using the pGEM-T Easy Vector kit (ProMega, Madison, WI) according to the manufacturer's instructions. NIA inserts were amplified directly from 4–10 large positive colonies using the non-degenerate primers and PCR protocol described above. Excess primer and dNTPs were removed using exonuclease I (New England Biolabs, Ipswich, MA [NEB]; 0.2 units/µl PCR product) and antarctic phosphatase (NEB; 1.0 unit/µl PCR product) incubated for 15 min at 37C followed by 15 min at 80C. For sequencing, Big Dye chemistry (Applied Biosystems, Foster City, CA) was utilized according to manufacturer instructions. Sequences were determined on an Applied Biosystems 3100 Genetic Analyzer at the Duke University Institute for Genome Science and Policy Sequencing and Genetic Analysis Facility.

Phylogenetic Analysis

DNA sequences were assembled and edited using the program Sequencher 4.1 (Gene Codes Corporation). Sequences were aligned using MUSCLE (Edgar 2004) under the default settings, with minor adjustments made manually. For each individual plant (Table 2), sequence variation was assessed based on an alignment of cloned NIA sequences (hereafter "isolates") obtained from that plant. Twenty-four plants yielded pools of identical isolates, 30 contained two different types of NIA sequence (hereafter "sequence types"), and three were represented by a single successfully cloned isolate (Table 2). For plants with two sequence types, a single isolate representing each type was selected randomly for subsequent analysis (Table 2). For plants with one sequence type, a single isolate was selected at random from the pool of isolates for that plant (Table 2). A total of 87 isolates were selected for analysis and aligned and edited as described above. Two ambiguously-aligned regions were excluded from analysis (see below). Edited sequences were deposited in GenBank (Table 2, Appendix 1).

Phylogenetic analyses under the Bayesian criterion were conducted using the best-fit model of evolution from Akaike information criterion (AIC) output of the program MrModeltest v2 (Nylander 2004). Sampling of trees was performed using the program MrBayes 3 (Ronquist and Huelsenbeck 2003). Three separate runs of 5,000,000 MCMC generations were performed using one heated and three cold chains, sampling every 1000 generations. Independent chains were inspected for convergence (standard deviation of split frequencies nearing 0.001). Log-likelihood for the sampled tree was plotted and examined in Microsoft Excel. Sampled trees from the burn-in period (Ronquist and Huelsenbeck 2003) were identified and eliminated. A consensus phylogram for each independent run was constructed based on the post-burnin sample of trees using MrBayes 3.0 (Ronquist and Huelsenbeck 2003). Trees from each of the three independent runs were compared to verify the similarity of the results. The final Bayesian consensus tree was manually rooted based on results from an expanded phylogenetic study of *Ceanothus* (Burge et al. in press).

Statistical parsimony (Templeton et al. 1992), as implemented in the program TCS (Clement et al. 2000), was used to reconstruct a gene genealogy for NIA based on the alignment described above. Statistical parsimony is a network-based method that accommodates reticulate relationships such as those that result from recombination, and therefore has advantages over traditional tree building methods such as parsimony, neighbor-joining, and maximum like-

lihood when considering population-level relationships (Clement et al. 2000). Analyses were conducted under default settings of the TCS program. The network output by TCS was adjusted manually in order to facilitate interpretation. The network was examined visually for loops (ambiguities) representing potential reticulate relationships among NIA isolates (Clement et al. 2000).

Utilization of Highly-Variable Regions

Initial inspection of NIA alignments revealed the presence of two highly-variable AT-rich regions. In the initial alignment of NIA isolates (see Results) the first variable region begins at position 136 (5′) and ends at position 152 (3′), with a maximum un-aligned length of 16 bases. The second region begins at position 401 (5′) and ends at position 448 (3′), with a maximum un-aligned length of 45 bases. Due to ambiguity inherent in aligning such regions, they were excluded from phylogenetic analysis, as described above. Initial inspection, however, showed that sequence variation in these regions corresponds with relationships implied by phylogenetic analysis of the remaining sequence data. Due to its less ambiguous alignment, the first variable region was focused upon for subsequent work. This highly-variable region was treated as a single character and unique "motif types" identified based on the exact sequence of the region. Each of the 87 NIA isolates was binned according to motif type. In a hypothetical example of this process, isolates from a four-base-pair-long highly-variable region with sequences of ATTT, AATT, and AAAT would represent three separate motif types. Following reconstruction of phylogenetic trees based on an alignment that excluded both of the highly-variable regions, motif type was mapped onto trees and used to help identify natural groups.

Soil Sampling

Fifty-two soils samples, representing 42 populations of *C. cuneatus* (including all five varieties of this species) and 10 populations of *C. roderickii*, were subjected to chemical analysis (Tables 1 and 2). Thirty-two of the 54 samples correspond to populations included in the genetic analysis (Tables 1 and 2). Sampling of soil was carried out in April and May, 2009. At each site, one liter of soil was collected by consolidation of sub-samples taken within the rooting zone of three plants growing in a 5 m^2 area. Sub-samples were collected using a garden trowel with a steel blade, excavating to a depth of at most 10 cm, depending on the depth of the soil profile. Soils were air-dried and returned to Duke University for storage and preparation.

Soil Chemistry Assays

Soil chemistry analyses were carried out by the Texas A&M University Soil, Water, and Forage Testing Laboratory. Samples were passed through a 2 mm sieve prior to analysis. Major nutrients (P, K, Ca, Mg, S) and sodium were extracted using the Mehlich III extractant (Mehlich 1978, 1984) and determined by inductively coupled plasma mass-spectroscopy (ICP). Micronutrients (Cu, Fe, Mn, and Zn) were extracted using a modified DPTA solution (Lindsay and Norvell 1978), and determined by ICP. Soil pH was determined in a 1:2 soil:deionized water extract (Schofield and Taylor 1955). Electrical conductivity (a proxy for soluble salts) was determined in a 1:2 soil:deionized water extract using a soil conductivity probe (Rhoades 1982). Finally, nitrate (NO_3^-) was extracted in 1 M KCl solution, reduced to nitrite (NO_2^-) using a cadmium column, and determined by spectrophotometer (Keeney and Nelson 1982). In total, thirteen soil chemistry properties were assayed (Table 3).

Statistical Analysis of Soil Chemistry

Soil chemistry data for the 52 sampled *Ceanothus* populations were treated using univariate and multivariate statistical methods. First, differences among pre-defined groups were tested for each of the 13 soil chemistry variables using Student's paired t-tests (Student 1908). Second, differences among groups were summarized using principal component analysis (PCA; Pearson 1901), which simultaneously accounted for variation in all 13 soil chemistry variables. Principal component analysis was carried out in the program R, version 2.10.1 (R Development Core Team 2009), using the "ecodist" package of Goslee and Urban (2007). The soil chemistry variables were transformed into Z-scores prior to analysis. The first two principal components were visualized in bivariate space and the relative contribution of the soil chemistry variables to the components was assessed based on vector loadings. Based on PCA scores, differences among pre-defined groups were tested using a combination of 1) Student's paired t-test, 2) analysis of variance (ANOVA; Fisher 1918), and 3) Tukey's HSD test (Zar 1999). Comparisons involving just two groups were carried out using Student's paired t-test (Student 1908) on scores from the first two principal components. To test for overall differences among three or more groups, one-way ANOVA was carried out on scores from the first two principal components. For analyses yielding a significant ANOVA result, Tukey's HSD (Honestly Significant Difference) test was used to determine which groups were significantly different from one another. Tukey's HSD test

TABLE 3. SUMMARY STATISTICS FOR SOIL CHEMISTRY VARIABLES. All values given as group mean ± standard deviation. Analysis group = groups of soil samples treated in text; "*C. cuneatus* all samples" refers to all soil samples analyzed for *C. cuneatus* (Table 2); "*C. cuneatus* El Dorado non-gabbro" and "*C. cuneatus* El Dorado gabbro" refer to *C. cuneatus* populations collected in El Dorado Co., CA on non-gabbro and gabbro-derived soils, respectively. *C. roderickii*, n = 10; *C. cuneatus* all samples, n = 42; *C. cuneatus* El Dorado non-gabbro, n = 9; *C. cuneatus* El Dorado gabbro, n = 6. Con. = electrical conductivity of soil, reported as umho/cm; nitrate and all other nutrient levels reported as PPM.

Analysis group	pH	Con.	Nitrate	Mg	Ca	K	P	S	Na	Fe	Zn	Mn	Cu
C. roderickii	6.0 ± 0.1	78 ± 16	5.0 ± 1.8	633 ± 179	1744 ± 208	60.7 ± 11.9	2.2 ± 0.8	9.6 ± 1.2	65.8 ± 6.8	7.0 ± 1.8	0.4 ± 0.3	13.1 ± 3.3	1.6 ± 0.8
C. cuneatus all samples	6.1 ± 0.5	83 ± 53	8.5 ± 7.5	573 ± 573	1513 ± 862	129.3 ± 7.5	19.2 ± 21.2	10.9 ± 3.0	84.7 ± 36.4	16.6 ± 10.7	1.1 ± 1.3	14.9 ± 10.8	1.0 ± 1.6
C. cuneatus El Dorado non-gabbro	6.2 ± 0.4	75 ± 16	6.5 ± 3.9	972 ± 564	1355 ± 653	94.5 ± 3.9	8.5 ± 8.9	9.1 ± 1.7	63.1 ± 5.0	17.2 ± 13.2	0.8 ± 0.4	16.0 ± 15.4	0.7 ± 0.3
C. cuneatus El Dorado gabbro	6.1 ± 0.3	97 ± 20	8.4 ± 5.0	474 ± 132	2450 ± 333	117.0 ± 5.0	15.8 ± 21.1	13.1 ± 1.4	71.8 ± 9.8	12.5 ± 4.5	2.3 ± 2.5	26.9 ± 8.9	4.2 ± 2.3

compensates for false positives (type I error) in multiple comparisons and therefore reveals which differences among group means are "honestly" significant (Zar 1999). Student's paired t-tests, One-way ANOVA, and Tukey's HSD tests were carried out in R (R Development Core Team 2009). In all statistical tests the threshold of significance was $\alpha = 0.05$.

RESULTS

DNA Sequences

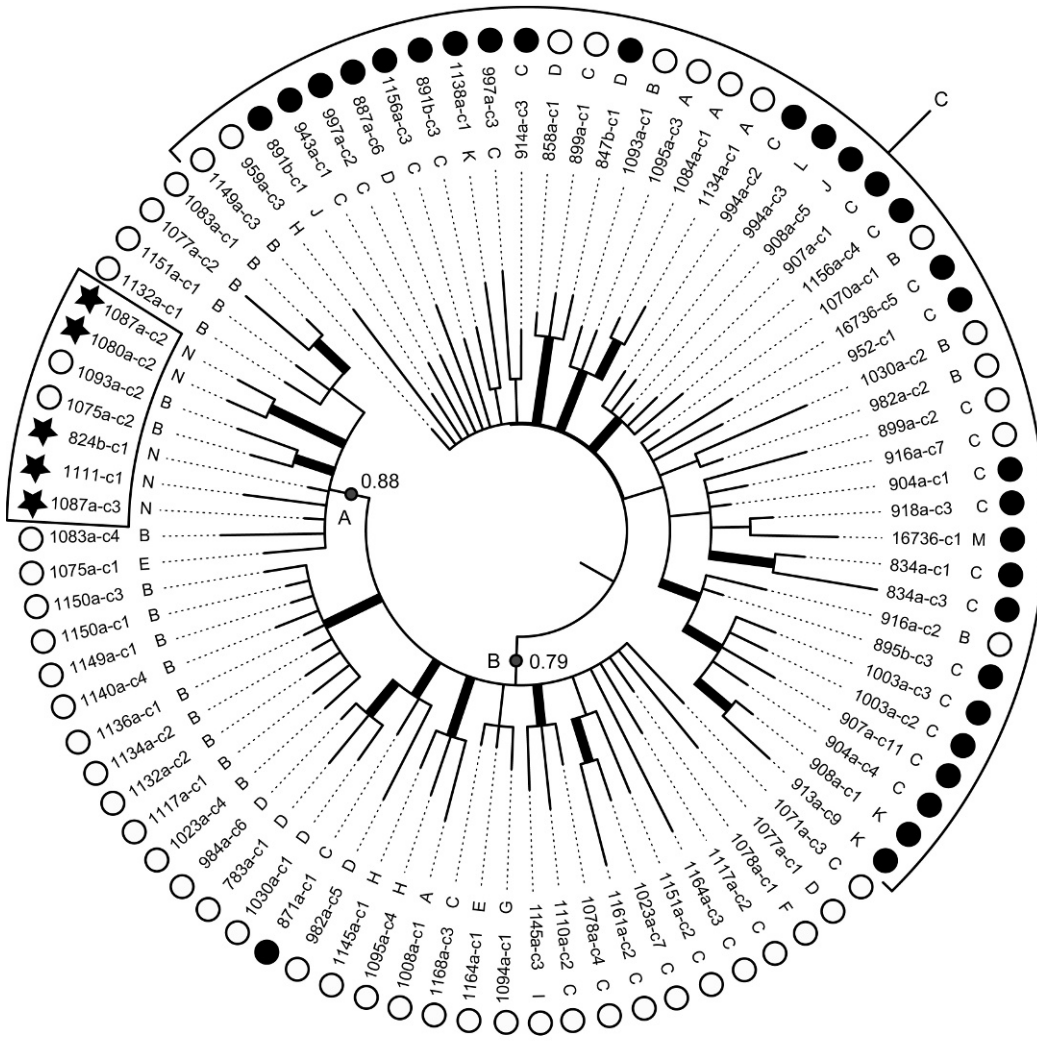
The portion of the third intron of nitrate reductase selected for analysis varied in length from 558 bp (*C. cuneatus* var. *cuneatus* isolates DOB 1136a-c1, 1140a-c4, 1149a-c1, 1023a-c4, 1117a-c1, 1134a-c2, and 1150a-c1; Table 2, Appendix 1) to 670 bp (*C. cuneatus* var. *cuneatus* isolates DOB 1084a-c1 and 1134a-c1). The initial alignment of the 87 NIA isolates selected for analysis (Table 2, Appendix 1) contained 694 characters and had an average G + C content of 37.1% (TreeBase Study S10898, Matrix M6862). Following exclusion of two ambiguously aligned regions (base positions 136–152 and 401–448; see Materials and Methods), the alignment contained 618 characters, 149 of which (24.1%) were variable. The ambiguously-aligned regions were excluded from all subsequent analyses.

Phylogeny

Bayesian analysis replicates had a burn-in period of 500,000 generations (500 sampled trees), leaving 4,500,000 generations (4500 sampled trees) of explored tree space for computing branch lengths and posterior probabilities (PP) of clades. The three Bayesian replicates yielded trees with nearly identical topology. Only one run was used for final tree building.

In the Bayesian consensus tree neither *C. roderickii* nor *C. cuneatus* var. *cuneatus* are recovered as monophyletic (Fig. 3). Instead, *C. roderickii* and *Ceanothus cuneatus* var. *cuneatus* are polyphyletic. All five NIA isolates from the four *C. roderickii* individuals included in this study (Table 2, Appendix 1) are recovered as members of a clade of NIA isolates that are otherwise from individuals of *C. cuneatus* var. *cuneatus* (Fig. 3, Clade A; PP 0.88). Clade A is in turn nested within a larger clade made up almost entirely of isolates from individuals of *C. cuneatus* var. *cuneatus* (Fig. 3, Clade B; PP 0.79). Considering *C. cuneatus* var. *cuneatus* as a whole, seven isolates are recovered in strongly supported (PP > 0.95) relationships with isolates from other taxa, including other varieties of *C. cuneatus* and other species of *Cerastes* (Fig. 3).

Out of the 30 *Ceanothus* plants from which two NIA sequence types were recovered (Table 2,



Type	Variable region motif
A	TTTTAAACAAAA-TTA
B	TTTTTAAAAAAA-TTA
C	TTTTAAAAAAAA-TTA
D	TTTAAAAAAAAA-TTA
E	TTTTTAAAAAAAA-TA
F	TTTTAAAAAGAA-TTA
G	TTTTTTAAAAAAA-TA
H	TTTTAAAAAAA--TTA
I	TTTTAAAATAAAATTA
J	TTTTAACAAAAA-TTA
K	TTTTTAAAAAAAATTA
L	TTTTAAAAAAA--TTG
M	TTTTTAAAAGAAATTA
N	TTTTTTAAA-AAATTA

Figure key

C. cuneatus var. *cuneatus*

C. roderickii

Other *Cerastes*

0.09 Substitutions/site

1101-c1 A Isolate code and motif type

FIG. 3. Bayesian 50% majority-rule consensus phylogram for nitrate reductase. Heavy bars indicate posterior probability >0.95. Phylogram is manually rooted based on root position inferred from expanded *Ceanothus* phylogeny (see Materials and Methods). Highly-variable region motifs from NIA (see Materials and Methods) shown below phylogram; motif types mapped on phylogram using letter codes. ''A, B, C'': groups and clades discussed in text; numbers on branches indicate posterior probabilities. All NIA isolates from DOB collections, with exception of 16736-c1 from D.H. Wilken 16736 (Table 2; Appendix 1).

Appendix 1), 21 have these isolates in conflicting positions on the phylogeny (Fig. 3; PP $>$ 0.95). Of the remaining nine plants from which two NIA sequence types were recovered, five have both isolates as members of a single well-supported clade (PP $>$ 0.95), and four have isolates that are neither strongly supported as members of the same clade, nor in conflicting phylogenetic positions (Fig. 3).

Gene Genealogy

Among the 87 NIA isolates included in the analysis, TCS identified 82 unique sequences. Three of these are represented by more than one NIA isolate (Fig. 4), one comprising four isolates from individuals of *C. cuneatus* var. *cuneatus* collected in the southern Sierra Nevada (1150a-c1, 1149a-c1, 1136a-c1, and 1134a-c2), a second comprising two isolates from individuals of *C. cuneatus* var. *cuneatus* collected in the northern Sierra Nevada of California and Cascade Ranges of Oregon (1164a-c1 and 1168a-c3), and a third represented by two isolates from individuals of *C. roderickii* collected in different populations (1087a-c3 and 824b-c1). All remaining sequence types are unique. The gene genealogy inferred by TCS is reticulate, with 22 loops (ambiguities) as reconstructed (Fig. 4).

Highly-Variable Region Motifs

Among the 87 NIA isolates utilized in the study, a total of 14 motif types were identified for the first highly-variable region (Materials and Methods; Fig. 3). The "N" motif (Fig. 3) is unique to *C. roderickii* and was present in all 16 isolates (four per individual plant) obtained from individuals of this species, as well as 16 isolates obtained from 4 additional individuals of the species collected in different populations or subpopulations (unpublished data). Nine motif types are found in *Ceanothus cuneatus* var. *cuneatus* (Fig. 3). Seven of these types are unique to *C. cuneatus* var. *cuneatus*, including three known from just a single NIA isolate each (F, G, and I). The remaining two motifs recovered in *C. cuneatus* var. *cuneatus* (C and D) are shared with other varieties of *C. cuneatus* or other *Cerastes* species (Fig. 3). None of the motifs from *C. cuneatus* var. *cuneatus* is unique to a well-supported group of *C. cuneatus* var. *cuneatus* isolates in the NIA tree (Fig. 3), although the "B" motif is found predominantly in Clade B (Fig. 3; PP 0.79) and is found in all but one of the *C. cuneatus* var. *cuneatus* isolates that group with *C. roderickii* in Clade A (Fig. 3; PP 0.88). Four additional motifs (J, K, L, and M; Fig. 3) are found only in taxa other than *C. cuneatus* var. *cuneatus* and *C. roderickii*. Two of these are found in more than one taxon (J and K; Fig. 3), and two are unique to a particular isolate (L and M; Fig. 3).

Soil Analyses

At a large geographic scale (Fig. 2), considering all 52 soil samples collected within populations of *C. cuneatus* (all five varieties) and *C. roderickii* (Tables 1 and 2), the soils of *C. roderickii* have, on average, lower pH, lower electrical conductivity, and lower concentrations of nitrate, K, P, S, Na, Fe, Zn, and Mn (Table 3, *C. roderickii* vs. *C. cuneatus* all samples). Concentrations of Mg, Ca, and Cu, on the other hand, are on average higher in the soils of *C. roderickii* than in those of *C. cuneatus* (Table 3). Differences are significant in the case of K, P, S, Fe, and Zn (Student's paired t-tests, P $<$ 0.03). Principal component analysis summarizes these results for the 13 soil chemistry variables. In PCA the first two principal components account for 39% of total variance, with 21% on the first principal component and 19% on the second. The first principal component is strongly positively correlated with Mg (vector loading = 0.48) and electrical conductivity (vector loading = 0.34), and strongly negatively correlated with P (vector loading = 0.46) and K (vector loading = 0.41). These results are summarized in a biplot of the first two principal components (Fig. 5A). Student's paired t-tests allow for rejection of the null hypothesis of no difference between the mean PCA scores for *C. roderickii* and *C. cuneatus* on the second principal component (P $<$ 0.001; Fig. 5B) but not the first (P = 0.052).

At a smaller geographic scale (Fig. 1), considering only those 28 soil samples collected in El Dorado Co., California (Tables 1 and 2), there are differences in chemistry between soils of *C. roderickii* and *C. cuneatus* var. *cuneatus* that are partitioned with respect to both taxon and geological parent material (Table 3). Within this geographic region *C. cuneatus* var. *cuneatus* grows on soils derived from a variety of geological parent materials, including gabbro (Tables 1 and 2). In comparison to *C. roderickii*, soils of *C. cuneatus* var. *cuneatus* that are derived from non-gabbro parent material (including serpentinite; Table 2; see below) have, on average, higher pH and higher concentrations of nitrate, Mg, K, P, Fe, Zn, and Mn (Table 3, *C. roderickii* vs. C. *cuneatus* El Dorado non-gabbro). Electrical conductivity and concentrations of Ca, S, Na, and Cu, on the other hand, are lower in soils of *C. cuneatus* var. *cuneatus* derived from non-gabbro parent material than in the soils of *C. roderickii* (Table 3). Differences are significant in the case of Fe, Zn, and Cu (Student's paired t-tests, P $<$ 0.04). Comparing the exclusively gabbro-derived soils of *C. roderickii* to the soils of *C. cuneatus* var. *cuneatus* that are also derived

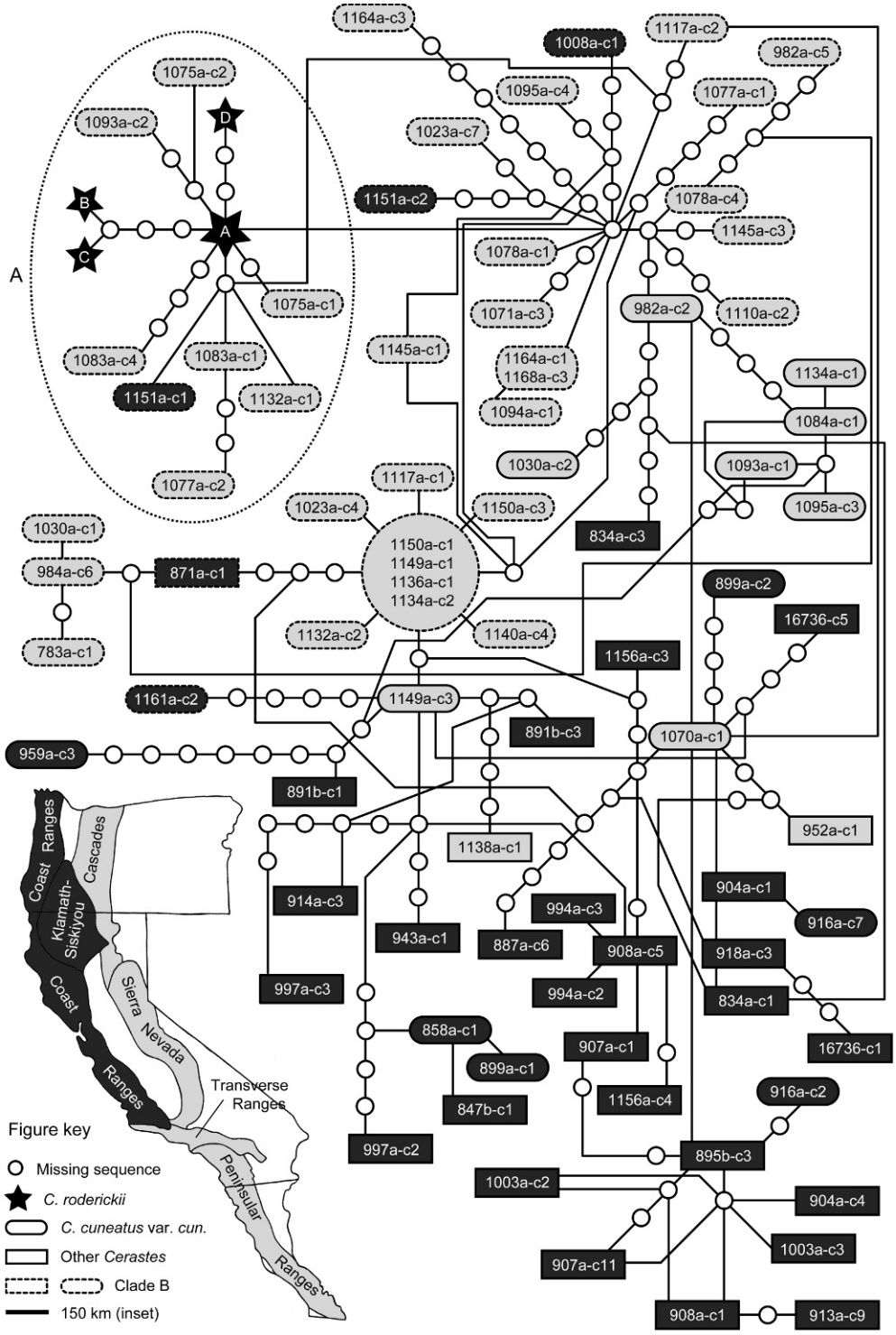


FIG. 4. Gene genealogy of NIA isolates generated under the statistical parsimony criterion in the program TCS (Clement et al. 2000). Open circles represent un-sampled (missing) sequences, as inferred by TCS. Some branch lengths not proportional to number of substitutions. *Ceanothus roderickii* (solid black stars): A, 1087a-c3 & 824b-c1; B, 1080a-c2; C, 1087a-c2; D, 1111-c1. ''Clade A'': group discussed in text. Sequences color-coded according to geography (see inset map).

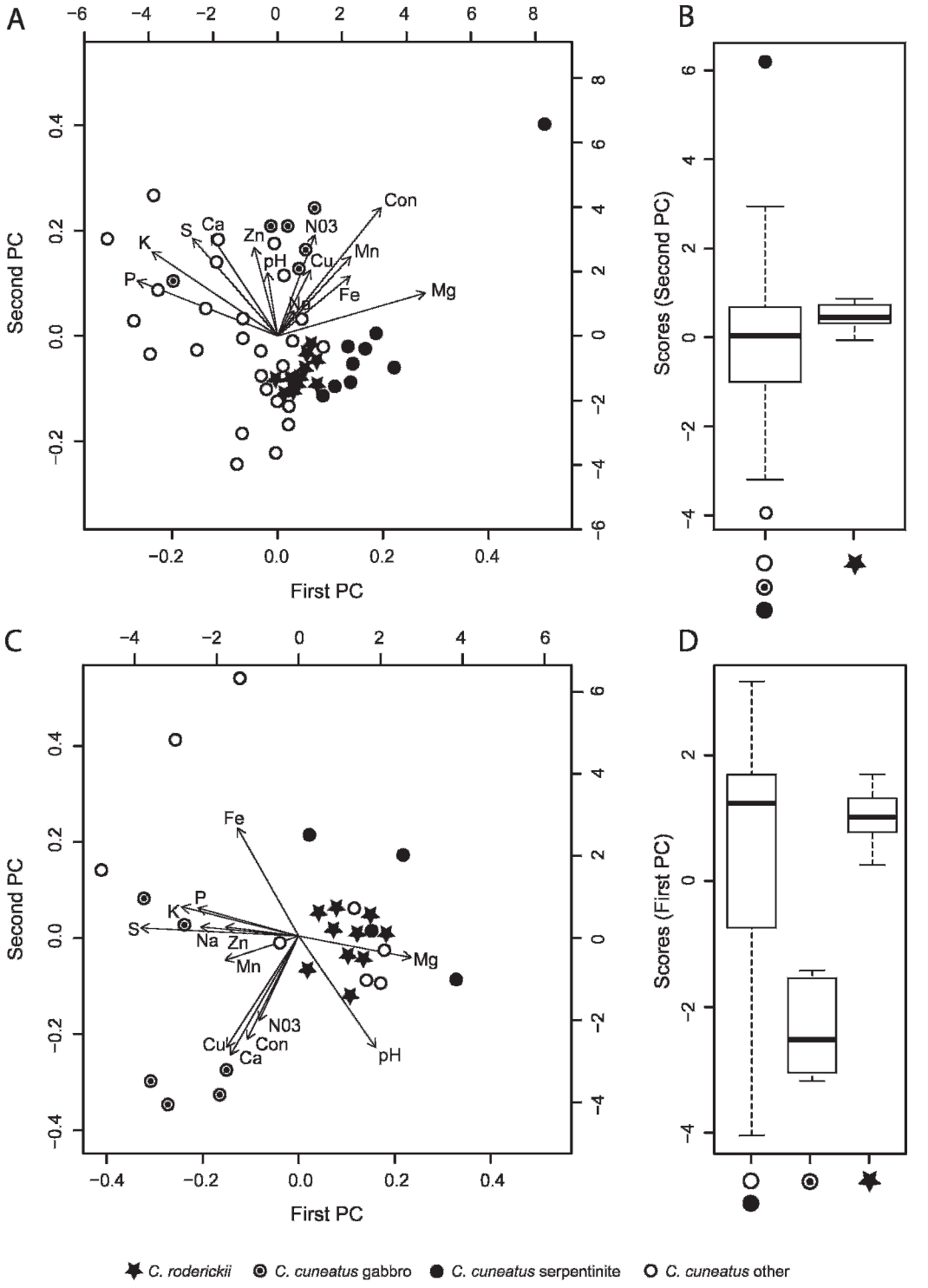


FIG. 5. Plots from principal component analysis (PCA) of soil chemistry data. A, biplot for first two principal components of PCA on soil chemistry for 52 assayed soil samples; arrows represent direction and magnitude of loading on the principal component axes; bottom and left axes apply to loading; top and right axes apply to PCA scores. B, boxplot of PCA scores from the second principal component of PCA on all 52 assayed soil samples, partitioned by species. C, biplot for first two principal components of PCA on soil chemistry for all soils collected in El Dorado County, CA; axes as in A. D, boxplot of PCA scores from the first principal component of PCA on El

from gabbro parent material, the gabbro-derived soils of *C. cuneatus* var. *cuneatus* have, on average, higher pH, higher electrical conductivity, and higher concentrations of nitrate, Ca, K, P, S, Na, Fe, Zn, Mn, and Cu (Table 3). Mg is the only element that is present in lower concentrations in soils of *C. roderickii* than in gabbro-derived soils of *C. cuneatus* var. *cuneatus*. Differences are significant for K, Ca, F, S, Fe, Mn, and Cu (Student's paired t-tests, $P < 0.04$). Principal component analysis summarizes these results for the 13 soil chemistry variables. In PCA the first two principal components account for 45% of total variance, with 27% on the first principal component and 18% on the second. The first principal component is strongly positively correlated with Mg (vector loading = 0.35) and pH (vector loading = 0.24), and strongly negatively correlated with S (vector loading = 0.49) and K (vector loading = 0.36). These results are summarized in a biplot of the first two principal components (Fig. 5C). Differences among the exclusively gabbro-derived soils of *C. roderickii*, the gabbro-derived soils of *C. cuneatus* var. *cuneatus*, and the non-gabbro derived soils of *C. cuneatus* var. *cuneatus* were tested using ANOVA on PCA scores (Fig. 5C). ANOVA allowed for rejection of the null hypothesis of no difference among the three group means on the basis of the first principal component ($F = 10.96$; $P < 0.001$; Fig. 5D) as well as the second ($F = 6.34$; $P = 0.006$). Tukey's HSD test allowed for rejection of the null hypothesis of no difference between the gabbro-derived and non-gabbro derived soils of *C. cuneatus* var. *cuneatus* ($P = 0.002$), as well as between the gabbro-derived soils of *C. cuneatus* var. *cuneatus* and those of *C. roderickii* ($P < 0.001$). The mean PCA scores for the non-gabbro derived soils of *C. cuneatus* var. *cuneatus* was not significantly different from those of *C. roderickii* ($P = 0.611$).

Comparing the gabbro-derived soils of *C. roderickii* and *C. cuneatus* var. *cuneatus* to serpentinite-derived soils of *C. cuneatus* (including *C. cuneatus* var. *cuneatus* and *C. cuneatus* Nutt. var. *ramulosus* Greene), there are strong differences among groups. Average Ca:Mg for serpentinite-derived soils of *C. cuneatus* ($n = 8$; Table 2) was 0.6 (standard deviation = 0.3), the average for soils of *C. roderickii* ($n = 10$) was 2.9 (± 0.6), the average for the gabbro-derived soils of *C. cuneatus* var. *cuneatus* ($n = 6$) was 5.5 (± 1.5), and the average for all "other" (non-gabbro and non-serpentinite derived) soils occupied by *C. cuneatus* ($n = 27$) was 7.2 (± 4.1). The difference in Ca:Mg is significant for all three contrasts among 1) the exclusively gabbro-derived soils of *C. roderickii,* 2) the gabbro-derived soils of *C. cuneatus* var. *cuneatus* and 3) the serpentinite-derived soils of *C. cuneatus* (Student's paired t-tests, $P < 0.01$). Overall differences in soil chemistry among these groups are summarized in a biplot of the first two principal components from the PCA described above (Fig. 5A). The differences in soil chemistry among the three groups listed above, as well as the "other" group (non-gabbro and non-serpentinite derived soils occupied by *C. cuneatus*) were tested using ANOVA in terms of scores on the second principal component of the PCA (Fig. 5A). ANOVA allowed for rejection of the null hypothesis of no difference among group means ($F = 5.01$; $P = 0.004$). Furthermore, Tukey's HSD test allowed for rejection of the null hypothesis of no difference between means for two contrasts among the four groups listed above: a) gabbro-derived soils of *C. cuneatus* var. *cuneatus* versus "other" (non-gabbro & non-serpentinite derived) soils of *C. cuneatus* ($P = 0.014$), and b) gabbro-derived soils of *C. cuneatus* var. *cuneatus* versus those of *C. roderickii* ($P = 0.002$). The remaining three contrasts among the four groups were not significant.

Discussion

Phylogenetic Relationships

Our results indicate a very close relationship between the gabbro-endemic *C. roderickii* and the less soil-specialized *C. cuneatus* var. *cuneatus*. Nevertheless, relationships among the 87 NIA isolates included in this study are poorly resolved, with few nodes receiving high levels of support (Fig. 3). This result is consistent with past genetic work on *Cerastes* as a whole, in which nuclear and chloroplast DNA sequence data failed to resolve species-level relationships (Hardig et al. 2000; 2002). Nevertheless, a lack of phylogenetic signal is consistent with the hypothesis that *Cerastes* diversified recently, perhaps as late as 5 mya (Ackerly et al. 2006; Burge et al. in press). If the diversification of *Cerastes* took place during so short a time interval, then a lack of phylogenetic resolution is not unexpected. In addition, genetic divergence among taxa might be further eroded by hybridization, which is common among *Cerastes* taxa and has long been

←

Dorado County soil samples, partitioned by species-soil group (see Results). "*C. cuneatus* gabbro" corresponds to soil samples obtained from *C. cuneatus* populations growing on soils derived from gabbro parent material; "*C. cuneatus* serpentinite" corresponds to serpentinite parent material, and "*C. cuneatus* other" to non-gabbro and non-serpentinite parent materials. Symbols: Con = electrical conductivity; N03 = nitrate.

thought to play an important role in *Cerastes* evolution (McMinn 1942; Nobs 1963).

In spite of the low phylogenetic resolution achieved in the present study using NIA, comparison of phylogenetic results with the gene genealogy and information from highly-variable region motifs (Figs. 3, 4) allows for interpretation of the relationship of *C. roderickii* to remaining *Cerastes*. All of the NIA isolates obtained from *C. roderickii*, representing four populations, are nested within a small clade made up of NIA isolates from *C. cuneatus* var. *cuneatus* populations sampled in the Sierra Nevada and Cascade mountains of California (Fig. 3, Clade A). This group is also present in the gene genealogy for NIA, in which only two potential connections were reconstructed between this group and remaining isolates (Fig. 4 ''Clade A''). The close relationship between *C. roderickii* and *C. cuneatus* var. *cuneatus* is further emphasized by the nested position of Clade A within Clade B, which is made up almost entirely of isolates from *cuneatus* var. *cuneatus* (Fig. 3). However, it is important to note that members of Clade B are more strongly connected to the remaining NIA isolates in the gene genealogy than are members of Clade A (Fig. 4). Finally, all NIA isolates from *C. roderickii* contained an identical highly-variable region motif that has proven unique to *C. roderickii* (Fig. 3, type N). The type N motif was present in all 20 isolates obtained from *C. roderickii*. Four *C. roderickii* individuals representing additional populations were also found to share the type N highly-variable region motif (unpublished data). The presence of a unique highly-variable region motif in all sampled *C. roderickii* individuals indicates that *C. roderickii* populations are genetically cohesive, in spite of the fact that they do not form a clade in the phylogeny reconstructed using complete NIA sequences (Fig. 3). Thus, the type N highly-variable region motif may be taken as a genetic autapomorphy of *C. roderickii*.

Genetic evidence for the cohesiveness of *C. roderickii* with respect to *C. cuneatus* is supported by the morphology of *C. roderickii*, which differs from that of *C. cuneatus* in several significant ways. First, the habit of *C. roderickii* is always prostrate to decumbent, with shrubs rarely attaining more than a meter in height (Knight 1968; James 1996), whereas *C. cuneatus* is invariably erect and ascending, frequently reaching more than three meters is height (Fross and Wilken 2006). However, some populations of *C. cuneatus* in the Sierra Nevada and Klamath-Siskiyou region of California and Oregon are much lower-growing (Fross and Wilken 2006). *Ceanothus roderickii* also differs from *C. cuneatus* with respect to mode of reproduction. Individuals of *C. roderickii* spread laterally via arching or creeping branches that root adventitiously when they contact soil. This mode of reproduction allows *C. roderickii* to reproduce clonally during fire-free intervals when seedling recruitment is limited (James 1996; Boyd 2007). Clonal reproduction is not known in *C. cuneatus*. Finally, the leaves of *C. roderickii* are usually strongly ascending (Knight 1968), such that the leaf surface is typically held perpendicular to the soil surface. Few other *Cerastes* species are known to possess this trait (Knight 1968), which may represent an adaptation to the very high light levels that are typical of the open habitats favored by *C. roderickii* (James 1996).

Overall, phylogenetic findings of the present study agree with previous systematic work on *C. roderickii*. Citing general similarities in habit, ecology, and geographic distribution, Knight (1968) argued that *C. roderickii* is probably most closely related to *C. cuneatus*, although he did not rule-out the possibility of a relationship with several other *Cerastes* species from the Sierra Nevada. The results of the present study also agree with those of Hardig et al. (2000), in which an individual of *C. roderickii* grouped with an individual of *C. cuneatus* var. *cuneatus* in phylogenies based on sequences from ITS and *matK*.

In addition to the relationship between *C. roderickii* and *C. cuneatus*, results presented here bear on relationships among other *Cerastes* included in the taxonomically diverse clade that is the focus of the present study (Table 1). The Bayesian consensus tree contains a moderately supported clade comprising 38 out of the 52 NIA isolates from *C. cuneatus* var. *cuneatus,* all of the isolates for *C. roderickii*, and a single isolate from *Ceanothus cuneatus* Nutt. var. *fascicularis* (McMinn) Hoover (Fig. 3, Clade B, PP 0.79). Clade B is made up almost entirely of NIA isolates from *C. cuneatus* var. *cuneatus* individuals collected in the mountains of Baja California, Mexico, southern California, eastern California, and eastern Oregon, which includes the Sierra Nevada, Cascade Ranges, Peninsular Ranges, and Transverse Ranges (Fig. 4). Although the relationship is less obvious than in the Bayesian consensus tree (Fig. 3), Clade B and its unusual geography is recognizable in the gene genealogy, which contains few connections between members of Clade B and remaining isolates (Fig. 4). The relationship between Clade B and remaining NIA isolates is not resolved in the Bayesian consensus tree (Fig. 3); several small clades of isolates, as well as some individual isolates, form a large polytomy with Clade B (Fig. 3, Group C). In an expanded analysis of *Ceanothus* phylogeny (Burge et al. in press) the root of our tree (Fig. 3) falls within this polytomy, indicating that a lack of resolution here is not an artifact of sampling.

All but 10 of the plants represented by the Group C isolates were collected in the Klamath-Siskiyou and Coast Ranges of California, the

exceptions being seven isolates from individuals of *C. cuneatus* var. *cuneatus* collected in the Sierra Nevada, Peninsular Ranges, and Transverse Ranges (1149a-c3, 1095a-c3, 1084a-c1, 1134a-c1, 1070a-c1, 1030a-2, and 982a-c2), one isolate of *C. cuneatus* var. *cuneatus* collected in the Sutter Buttes (1093a-c1), and one isolate each from individuals of *C. fresnensis* (1138a-c1) and *C. prostratus* (952a-c1) collected in the Sierra Nevada (Fig. 4).

The genetic break between the Klamath-Siskiyou/Coast Ranges and the remaining CFP mountains (Sierra Nevada, Peninsular Ranges, and Transverse Ranges; Fig. 4) appears to represent a biogeographic split between *Cerastes* inhabiting these regions, although the presence of isolates from individuals collected in the Klamath-Siskiyou/Coast Ranges within Clade B, and the presence of individuals collected in other mountain ranges of the CFP in Group C, suggests that opportunities for migration and/or gene-flow between the regions have been available (Figs. 3, 4). In addition, the frequent lack of monophyly between NIA isolates from the same individual (Fig. 3), including 6 cases in which isolates from a single individual are found in both Clade B and Group C (1093a, 1149a, 1134a, 1030a, 982a, 1095a), suggests the operation of gene-flow or hybridization. Hybridization and gene flow are thought to be common in *Cerastes* (McMinn 1942; Nobs 1963; Fross and Wilken 2006), and so it is not unexpected to find evidence consistent with these phenomena.

Edaphic Ecology

At a large geographic scale, considering all sampled populations of *C. cuneatus* and *C. roderickii* (Fig. 2), results of our study show that edaphic conditions experienced by the narrowly distributed gabbro-endemic *C. roderickii* represent a small, highly cohesive subset of the range of conditions experienced by the widespread soil-generalist *C. cuneatus* (Fig. 5A, B). Soils of *C. roderickii* are characterized by low concentrations of available K, P, S, Fe, and Zn, all of which are necessary plant nutrients (Brady and Weil 2002). For many plants, low availability of these elements results in disorders affecting growth and reproduction (Brady and Weil 2002).

At the scale of the Pine Hill intrusive complex in western El Dorado Co., California (Fig. 1), our study shows that *C. roderickii* is specialized to nutrient-deficient forms of gabbro-derived soil (Fig. 5C, D). On the Pine Hill intrusive complex *C. cuneatus* var. *cuneatus* and *C. roderickii* both occur on soils that are considered gabbro-derived (Fig. 1). However, the gabbro-derived soils of *C. roderickii* sampled in our study, which are classified as "Rescue extremely stony sandy loam" (Rogers 1974), contain significantly lower levels of K, Ca, P, S, Fe, Mn, and Cu than gabbro-derived soils of *C. cuneatus* var. *cuneatus* ($P < 0.04$; Table 3), which are classified as "Rescue sandy loam" or "Rescue very stony sandy loam" (Fig. 1; Rogers 1974). Although these elements are necessary plant nutrients, high levels of some, such as Mn, Fe, and Cu, are known to induce growth and reproductive disorders in plants (Brady and Weil 2002). Our work is the first to report this strong soil-chemistry divergence between *C. cuneatus* var. *cuneatus* and *C. roderickii* on the Pine Hill intrusive complex.

The relatively higher fertility of gabbro-derived soils occupied by *C. cuneatus* var. *cuneatus* compared to those occupied by *C. roderickii* may result from the greater development of the former, which are typically found in swales and at the bases of steep slopes, where they receive run-off from the Rescue extremely stony sandy loams that are found on the steeper slopes, hills, and ridge crests of the Pine Hill intrusive complex (Rogers 1974; D.O. Burge, personal observation). While our study is the first to report significantly divergent chemistry between groups of gabbro-derived soils on the Pine Hill intrusive complex, similar phenomena are known from other soils; on some serpentinite outcrops, soils at the base of steep slopes have strongly divergent chemistry from the soils closer to the top of the slope, despite their common geological parent material (Rajakaruna and Bohm 1999).

Endemism on gabbro-derived soils of the Pine Hill intrusive complex, as well as the presence on these soils of many taxa normally restricted to serpentinite-derived substrates, have been attributed to similar properties in gabbro-derived as compared to serpentinite-derived soils (Wilson 1986). Soils derived from serpentinite contain little Ca relative to Mg, and are rich in heavy metals such as Ni, Cr, and Co (Kruckeberg 2002). Gabbro rock itself is usually rich in heavy metals and tends to contain little Ca relative to Mg, although these parameters are not as extreme in gabbro as in serpentinite (Alexander 1993, unpublished). Research on the Pine Hill intrusive complex, however, found that the gabbro-derived soils from this area do not contain unusually low levels of Ca relative to Mg, or elevated heavy metals (Hunter and Horenstein 1991), results that are corroborated by regional geochemical studies (Goldhaber et al. 2009; Morrison et al. 2009). A later study focused on the gabbro-endemic plants of the Pine Hill intrusive complex asked whether soils from locations harboring endemics had low Ca to Mg ratios, or differences in a suite of other chemical and physical parameters, compared to areas without these plants (Alexander, unpublished). This study did not detect significant differences in Ca to Mg ratio between sites harboring rare plants versus those without, and

failed to identify other parameters that might explain the differences in plant distribution. However, it is possible that the results of this study were confounded by plant demography. This may be especially true of *C. roderickii*, which depends on fire for recruitment (Boyd 2007).

Although the present study did not focus on the contrast between serpentinite and gabbro, our results show that Ca to Mg ratios in serpentinite-derived soils of *C. cuneatus* (average 0.6 ± 0.3) are closest to those in the exclusively gabbro-derived soils of *C. roderickii* (average 2.9 ± 0.6). Values become successively higher in gabbro-derived soils of *C. cuneatus* var. *cuneatus* (5.5 ± 1.5), and "other" (non-gabbro and non-serpentinite derived) soils of *C. cuneatus* (7.2 ± 4.1). Although soils of *C. roderickii* have Ca to Mg ratios that are closest to those in serpentinite-derived soils, ratios in serpentinite-derived soils are still significantly lower (Student's paired t-test, $P < 0.001$). Nevertheless, serpentinite-derived soils associate closely with the exclusively gabbro-derived soils of *C. roderickii* in PCA (Fig. 5A, C). Furthermore, the two groups are not significantly different in terms of their scores on these axes (Tukey's HSD test, $P = 0.489$), indicating that the serpentinite-derived soils are similarly nutrient deficient. Overall, nutrient deficiency and low Ca to Mg ratios may provide an explanation for the evolution of endemics on some gabbro-derived soils of the Pine Hill intrusive complex, and the presence on these soils of plants that are usually restricted to serpentinite-derived substrates (Wilson 1986).

Evolution of Edaphic Ecology

Evolution of the gabbro-endemic *C. roderickii* appears to have been associated with specialization to strongly nutrient-deficient forms of gabbro-derived soil. The closest relative of *C. roderickii*, *C. cuneatus* var. *cuneatus*, has a very wide distribution in the California Floristic Province (Fig. 2), and is a common component of chaparral habitats in the Sierra Nevada. On the Pine Hill intrusive complex of western El Dorado Co., California, *C. cuneatus* var. *cuneatus* occupies nutrient-rich forms of gabbro-derived soils in close geographic proximity to the poorer forms favored by *C. roderickii*, sometimes no more than 100 m distant from the latter species (Fig. 1).

Although there is not a well-supported "progenitor-derivative" relationship (Gottlieb 2003; Baldwin 2005) between *C. cuneatus* var. *cuneatus* and *C. roderickii*, the nested position of *C. roderickii* within a large group of *C. cuneatus* var. *cuneatus* individuals collected predominantly in the Sierra Nevada, Transverse Ranges, and Peninsular Ranges is suggestive of this pattern (Figs. 3, 4). Rocks of the Pine Hill intrusive complex have probably been exposed since Eocene time (J. Wakabayashi, personal communication). Thus, it is possible that during the diversification of *Ceanothus* in western North America, which began approximately 5 mya (Ackerly et al. 2006; Burge et al. in press), *C. cuneatus* var. *cuneatus* colonized the Pine Hill region and gave rise to *C. roderickii* through specialization to the nutrient-poor forms of gabbro-derived soil.

Because intrinsic (pre-zygotic) barriers to gene flow are not known in *Cerastes* (Nobs 1963), it is expected that hybridization will occur when different species come into contact with one another (Fross and Wilken 2006), potentially leading to gene flow and introgression. However, *C. roderickii* persists as a relatively genetically isolated, morphologically divergent entity in spite of its close proximity to *C. cuneatus* var. *cuneatus* on the Pine Hill intrusive complex (Fig. 1). One possible explanation for the lack of introgression is the action of environmental isolating factors. The fact that soil chemistry associations of *C. cuneatus* var. *cuneatus* and *C. roderickii* are most divergent where the taxa come into close contact on gabbro outcrops, with *C. cuneatus* var. *cuneatus* occupying comparatively nutrient-rich forms of gabbro-derived soil, is suggestive of character displacement and possibly reinforcement based on soil-chemistry (Levin 1970). Overall, edaphically-based barriers to gene-flow might provide an explanation for the initial divergence and continued persistence of *C. roderickii*, as well as other edaphic-endemic *Cerastes* taxa.

Acknowledgments

The authors thank Lauren Fety, Albert Franklin, Graciela Hinshaw, Sandra Namoff, and Dieter Wilken for providing constructive criticism of drafts. The authors also would like to thank Bonnie McGill, Kaila Davis, Sang-Hun Oh, and Sandy Bowles for assistance with lab work and development of methods. Assistance with field logistics was provided by Lauren Fety, Graciela Hinshaw, Sandra Namoff, and Dieter Wilken. Funding was provided by the American Society of Plant Taxonomists, The Hunt Institute for Botanical Documentation, Duke University, and a National Science Foundation grant to DOB and PSM (DEB 0808427).

Literature Cited

Ackerly, D. D., D. W. Schwilk, and C. O. Webb. 2006. Niche evolution and adaptive radiation: testing the order of trait divergence. Ecology 87:S50–61.

Alexander, E. B. 1993. Gabbro and its soils. Fremontia 21:8–10.

Baldwin, B. G. 2005. Origin of the serpentine-endemic *Layia discoidea* from the widespread *L. glandulosa* (Compositae). Evolution 59:2473–2479.

Boyd, R. S. 2007. Response to fire of *Ceanothus roderickii* (Rhamnaceae), a federally endangered California endemic shrub. Madroño 54:13–21.

BRADY, N. C. AND R. R. WEIL. 2002. The nature and properties of soils. Thirteenth Ed. Pearson Education, Upper Saddle River, NJ.

BURGE, D. O., D. M. ERWIN, M. B. ISLAM, J. KELLERMANN, S. S. KEMBEL, P. S. MANOS, AND D. H. WILKEN. In Press. Diversification of the genus *Ceanothus* (Rhamnaceae) in the California Floristic Province. International Journal of Plant Sciences.

CLEMENT, M., D. POSADA, AND K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

EDGAR, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

FISHER, R. A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Philosophical Transactions of the Royal Society of Edinburgh 52:399–433.

FROSS, D. AND D. WILKEN. 2006. *Ceanothus*. Timber Press, Portland, OR.

GANKIN, R. AND J. MAJOR. 1964. *Arctostaphylos myrtifolia*, its biology and relationship to the problem of endemism. Ecology 45:792–808.

GOLDHABER, M. B., J. M. MORRISON, J. M. HOLLOWAY, R. B. WANTY, D. R. HELSEL, AND D. B. SMITH. 2009. A regional soil and sediment geochemical study in northern California. Applied Geochemistry 24:1482–1499.

GOSLEE, S. C. AND D. L. URBAN. 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software 22:1–19.

GOTTLIEB, L. D. 2004. Rethinking classic examples of recent speciation in plants. New Phytologist 161:71–82.

HARDIG, T. M., P. S. SOLTIS, AND D. E. SOLTIS. 2000. Diversification of the North American shrub genus *Ceanothus* (Rhamnaceae): conflicting phylogenies from nuclear ribosomal DNA and chloroplast DNA. American Journal of Botany 87:108–123.

———, ———, ———, AND R. B. HUDSON. 2002. Morphological and molecular analysis of putative hybrid speciation in *Ceanothus* (Rhamnaceae). Systematic Botany 27:734–746.

HICKMAN, J. C. (ed.). 1993. The Jepson Manual: higher plants of California. University of California Press, Berkeley, CA.

HINSHAW, G. 2008. Pine Hill Preserve management plan. U.S. Department of the Interior. Bureau of Land Management, Mother Lode Field Office, El Dorado Hills CA. Website: http://www.blm.gov/pgdata/etc/medialib/blm/ca/pdf/folsom/plans.Par.44114.File.dat/FINAL_PHPmgmtPlan0708.pdf [accessed 10 April 2011].

HOWARTH, D. G. AND D. A. BAUM. 2002. Phylogenetic utility of a nuclear intron from nitrate reductase for the study of closely related plant species. Molecular Phylogenetics and Evolution 23:525–528.

HUNTER, J. C. AND J. E. HORENSTEIN. 1991. The vegetation of the Pine Hill area (California) and its relation to substratum. Pp. 197–206 *in* A. J. M. Baker, J. Proctor, and R. D. Reeves, (eds.), The vegetation of ultramafic (serpentine) soils. Intercept, Andover, U.K.

JAMES, S. C. 1996. A demographic study of *Ceanothus roderickii* (the Pine Hill ceanothus) El Dorado County, California. M.S. Thesis. California State University, Sacramento, CA.

JENNINGS, C. W. 1977. Geologic map of California: California Division of Mines and Geology Geologic Data Map No. 2, scale 1:750,000. Website http://www.conservation.ca.gov/cgs/cgs_history/Pages/geologicmaps.aspx [accessed 11 March 2011].

KEENEY, D. R. AND D. W. NELSON. 1982. Nitrogen - inorganic forms. Pp. 643–687 *in* A. L. Page, T. H. Miller, and D. R. Keeney, (eds.), Methods of soil analysis, part 2 (second ed.). American Society of Agronomy, Madison, WI.

KNIGHT, W. 1968. A new California *Ceanothus*. The Four Seasons 2:23–24.

KRUCKEBERG, A. R. 1986. An essay: the stimulus of unusual geologies for plant speciation. New Phytologist 11:455–463.

———. 2002. Geology and plant life. University of Washington Press, Seattle, WA.

LEVIN, D. A. 1970. Reinforcement of reproductive isolation: plants versus animals. The American Naturalist 940:571–581.

LINDSAY, W. L. AND W. A. NORVELL. 1978. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sciences Society of America Journal 42:421–428.

MASON, H. L. 1946. The edaphic factor in narrow endemism. I. The nature of environmental influences. Madroño 8:209–240.

MCMINN, H. E. 1942. A systematic study of the genus *Ceanothus*. Pp. 131–279 *in Ceanothus* Part II. Santa Barbara Botanical Gardens, Santa Barbara, CA.

MEHLICH, A. 1978. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. Communications in Soil Science and Plant Analysis 9:477–492.

———. 1984. Mehlich-3 soil test extractant: a modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis 15:1409–1416.

MORRISON, J. M., M. B. GOLDHABER, L. LEE, J. M. HOLLOWAY, R. B. WANTY, R. E. WOLF, AND J. F. RANVILLE. 2009. A regional-scale study of chromium and nickel in soils of northern California, USA. Applied Geochemistry 24:1500–1511.

MYERS, N., R. A. MITTERMEIER, C. G. MITTERMEIER, G. A. B. DA FONSECA, AND J. KENT. 2000. Biodiversity hotspots for conservation priorities. Nature 403:853–858.

NOBS, M. A. 1963. Experimental studies on species relationships in *Ceanothus*. Carnegie Institute of Washington, Washington, D.C.

NYLANDER, J. A. A. 2004. MrModeltest, v2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Sweden.

PEARSON, K. 1901. On lines and planes of closest fit to systems of points in space. Philosophical Magazine 2:559–572.

R DEVELOPMENT CORE TEAM. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://cran.r-project.org [accessed 11 March 2011].

RAJAKARUNA, N. 2004. The edaphic factor in the origin of plant species. International Geology Review 46:471–478.

——— AND B. A. BOHM. 1999. The edaphic factor and patterns of variation in *Lasthenia californica* (Asteraceae). American Journal of Botany 86:1576–1596.

RAVEN, P. H. AND D. I. AXELROD. 1978. Origin and Relationships of the California Flora. University of California Press, Berkeley, CA.

RHOADES, J. D. 1982. Soluble salts. Pp. 167–178 *in* A. L. Page, T. H. Miller, and D. R. Keeney, (eds.), Methods of soil analysis, part 2 (second ed.). American Society of Agronomy, Madison, WI.

ROGERS, J. H. 1974. Soil survey of El Dorado area, California. U.S. Government Printing Office, Washington, D.C.

RONQUIST, F. AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

SCHOFIELD, R. K. AND A. W. TAYLOR. 1955. The measurement of soil pH. Soil Science Society of America Proceeding 19:164–167.

SPRINGER, R. K. 1971. Geology of the Pine Hill intrusive complex, El Dorado County, California. Ph.D. dissertation. University of California at Davis, Davis, CA.

———. 1980. Geology of the Pine Hill intrusive complex, a layered gabbroic body in the western Sierra Nevada foothills, California: summary. Geological Society of America Bulletin, Part I 91:381–385.

STEBBINS, G. L. 1942. The genetic approach to problems of rare and endemic taxa. Madroño 6:241–258.

STUDENT. 1908. The probable error of a mean. Biometrika 6:1–25.

TEMPLETON, A. R., K. A. CRANDALL, AND C. F. SING. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

UNITED STATES DEPARTMENT OF AGRICULTURE, NATURAL RESOURCE CONSERVATION SERVICE. 2007. Soil Survey Geographic (SSURGO) database for El Dorado Area, California. U.S. Department of Agriculture, Natural Resources Conservation Service, Fort Worth, TX.

UNITED STATES FISH AND WILDLIFE SERVICE (USFWS). 1996. Endangered and threatened wildlife and plants; determination of endangered status for four plants and threatened status for one plant from the central Sierran foothills of California. Federal Register 61:54346–54358.

WILSON, J. L. 1986. A study of plant species diversity and vegetation patterns associated with the Pine Hill gabbro formation and adjacent substrata, El Dorado County, California. M.S. thesis. California State University, Sacramento, Sacramento, CA.

———, D. R. AYRES, S. STEINMAUS, AND M. BAAD. 2009. Vegetation and flora of a biodiversity hotspot: Pine Hill, El Dorado County, California, USA. Madroño 56:246–278.

ZAR, J. H. 1999. Biostatistical analysis. Prentice Hall, Upper Saddle River, NJ.

APPENDIX 1

SAMPLED *CEANOTHUS* POPULATIONS

GenBank accession numbers for the first and second NIA sequence (where available) are in brackets []. See Table 2 for additional population information.

Ceanothus cuneatus* Nutt. var. *cuneatus—USA. CALIFORNIA. **Amador Co.**: Grass Valley Creek watershed, NE of Mount Zion, *D.O. Burge 1150a* (DUKE) [NIA: HM240330; HM240329]. **Butte Co.**: Feather Falls, *D.O. Burge 1109a* (DUKE); Doe Mill Ridge, *D.O. Burge 815a* (DUKE); Magalia Reservoir, *D.O. Burge 1078a* (DUKE) [NIA: HM240306; HM240307]. **Calaveras Co.**: North Fork Calaveras River watershed, NE of Golden Gate Hill (VABM 2064), *D.O. Burge 1149a* (DUKE) [NIA: HM240327; HM240328]. **Contra Costa Co.**: Mount Diablo State Park, roadside on South Gate Rd, *D.O. Burge 916a* (DUKE) [NIA: HM240341; HM240342]. **El Dorado Co.**: Roadside on Wentworth Springs Rd, 1.9 road mi (3.0 km) from intersection with SR 193, *D.O. Burge 1011a* (DUKE); Green Valley Rd, *D.O. Burge 1024a* (DUKE); Folsom Lake Watershed, W side of North Fork American River arm, S slope of Kelly Ravine, *D.O. Burge 1074a* (DUKE); Martinez Creek watershed, roadside on Pleasant Valley Rd, *D.O. Burge 1174a* (DUKE); Weber Creek watershed, roadside on Lotus Rd, *D.O. Burge 1076a* (DUKE); South Fork American River watershed, *D.O. Burge 1088a* (DUKE); City of Cameron Park, *D.O. Burge 1089a* (DUKE); S side of U.S. Hwy 50, between Durock Rd and U.S. Hwy 50, *D.O. Burge 1101a* (DUKE); Pine Hill, eastern slope, *D.O. Burge 1116a* (DUKE); South Fork American River watershed, Dave Moore Nature Area, *D.O. Burge 1175a* (DUKE); S side of U.S. Hwy 50, between Durock Rd and Hwy 50, *D.O. Burge 1023a* (DUKE) [NIA: HM240297; HM240296]; Tennessee Creek watershed, roadside on Shingle Springs Rd, *D.O. Burge 1075a* (DUKE) [NIA: HM240303; HM240302]; Shingle Creek watershed, S of the city of Cameron Park, *D.O. Burge 1095a* (DUKE) [NIA: HM240314; HM240315]; S shore of Bass Lake, *D.O. Burge 1110a* (DUKE) [NIA: HM240316]; South Fork American River watershed, Icehouse Rd, *D.O. Burge 1117a* (DUKE) [NIA: HM240318; HM240317]. **Fresno Co.**: Dalton Mountain, south-eastern slope, head of Tretten Canyon, *D.O. Burge 1136a* (DUKE) [NIA: HM240323]. **Kern Co.**: Clear Creek watershed, S of Ball Mountain and SE of Hooper Hill, *D.O. Burge 1132a* (DUKE) [NIA: HM240319; HM240320]. **Lake Co.**: Mayacmas Mountains, Cow Mountain Recreation Area, Fourmile Glade, *D.O. Burge 1008a* (DUKE) [NIA: HM240295]. **Los Angeles Co.**: Sierra Pelona Mountians, Ruby Canyon, *D.O. Burge 1071a* (DUKE) [NIA: HM240301]. **Mariposa Co.**: Chowchilla River watershed, East Fork, N of Miami Mountain and E of Paloni Mountain, *D.O. Burge 1140a* (DUKE) [NIA: HM240324]. **Monterey Co.**: Nacimiento-Fergusson Rd, *D.O. Burge 858a* (DUKE) [NIA: HM240338]. **Napa Co.**: Vaca Mountains, on east-west trending ridge S of East Mitchel Canyon, *D.O. Burge 899a* (DUKE) [NIA: HM240339; HM240340]. **Nevada Co.**: Community of Hills Flat, near the City of Grass Valley, *D.O. Burge 1084a* (DUKE) [NIA: HM240310]. **Placer Co.**: North Fork American River Watershed, Forest Hill Divide, *D.O. Burge 1077a* (DUKE) [NIA: HM240305; HM240304]. **Riverside Co.**: San Jacinto Mountains, at intersection of Chimney Flats Rd and USFS Rd 5S13, *D.O. Burge 803a* (DUKE); Tucalota Creek watershed, roadside on Sage Rd (County Rd R3), *D.O. Burge 982a* (DUKE) [NIA: HM240344; HM240345]. **Sacramento Co.**: American River watershed, near outlet of Willow Creek into Lake Natoma, *D.O. Burge 1094a* (DUKE) [NIA: HM240313]. **San Bernardino Co.**: Rialto Municipal Airport (Miro Field), *D.O. Burge 1070a* (DUKE)

[NIA: HM240300]. **San Diego Co.**: Morena Valley, roadside on Buckman Springs Rd, *D.O. Burge 984a* (DUKE) [NIA: HM240346]. **San Luis Obispo Co.**: Santa Lucia Mountains, Arroyo Grande Creek watershed, NW of Arroyo Grande, *D.O. Burge 959a* (DUKE) [NIA: HM240343]. **Shasta Co.**: Crystal Creek watershed, N of Crystal Creek Rd, *D.O. Burge 1151a* (DUKE) [NIA: HM240331; HM240332]. **Sierra Co.**: Goodyears Bar, near confluence of Goodyears Creek and North Yuba River, *D.O. Burge 1083a* (DUKE) [NIA: HM240308; HM240309]. **Sutter Co.**: Sutter Buttes, Peace Valley, *D.O. Burge 1093a* (DUKE) [NIA: HM240312; HM240311]. **Tehama Co.**: Paynes Creek watershed, immediately W of Palmer Gulch, *D.O. Burge 1168a* (DUKE) [NIA: HM240336]. **Tulare Co.**: Middle Fork Tule River, roadside on SR 190, *D.O. Burge 1134a* (DUKE) [NIA: HM240322; HM240321]. **Tuolumne Co.**: Red Hills, SW of Taylor Hill, *D.O. Burge 1145a* (DUKE) [NIA: HM240326; HM240325]. OREGON. **Douglas Co.**: South Umpqua River watershed, roadside on Dole Drive, *D.O. Burge 1161a* (DUKE) [NIA: HM240333]. **Jackson Co.**: Cottonwood Creek watershed, *D.O. Burge 1164a* (DUKE) [NIA: HM240334; HM240335]. MEXICO. **Baja CA**: Sierra San Pedro Martir, Los Llanitos, *D.O. Burge 1030a* (DUKE) [NIA: HM240298; HM240299]; Sierra San Pedro Mártir, 40.4 road mi (64.6 km) E of Mexico Hwy 1, *D.O. Burge 783a* (DUKE) [NIA: HM240337].

***Ceanothus cuneatus* Nutt. var. *dubius* J.T. Howell**—USA. CALIFORNIA. **Santa Cruz Co.**: Henry Cowell Redwoods State Park, *D.O. Burge 918a* (DUKE) [NIA: HM240347].

***Ceanothus cuneatus* Nutt. var. *fascicularis* (McMinn) Hoover**—USA. CALIFORNIA. **Santa Barbara Co.**: Vandenberg Village, *D.O. Burge 871a* (DUKE) [NIA: HM240348].

***Ceanothus cuneatus* Nutt. var. *ramulosus* Greene**—USA. CALIFORNIA. **San Luis Obispo Co.**: Prefumo Canyon, *D.O. Burge 847b* (DUKE) [NIA: HM240349].

***Ceanothus cuneatus* Nutt. var. *rigidus* (Nutt.) Hoover**—USA. CALIFORNIA. **Monterey Co.**: Fort Ord Military Reservation, on hillside W of South Boundary Rd, *D.O. Burge 891b* (DUKE) [NIA: HM240351; HM240350].

***Ceanothus divergens* Parry subsp. *confusus* (J.T. Howell) Abrams**—USA. CALIFORNIA. **Sonoma Co.**: Mayacmas Mountains, western slope of Mount Hood, *D.O. Burge 1003a* (DUKE) [NIA: HM240352; HM240353].

***Ceanothus divergens* Parry subsp. *occidentalis* (McMinn) Abrams**—USA. CALIFORNIA. **Lake Co.**: Boggs Mountain Demonstration State Forest, *D.O. Burge 943a* (DUKE) [NIA: HM240354].

***Ceanothus ferrisiae* McMinn**—USA. CALIFORNIA. **Santa Clara Co.**: Pigeon Point, *D.O. Burge 834a* (DUKE) [NIA: HM240356; HM240355].

***Ceanothus fresnensis* Abrams**—USA. CALIFORNIA. **Fresno Co.**: Big Creek Watershed, E flank of north-south trending ridge W of Ely Mountain, *D.O. Burge 1138a* (DUKE) [NIA: HM240357].

***Ceanothus gloriosus* J.T. Howell var. *exaltatus* J.T. Howell**—USA. CALIFORNIA. **Mendocino Co.**: Oilwell Hill, near the N end of Little Lake Valley, *D.O. Burge 994a* (DUKE) [NIA: HM240358; HM240359].

Ceanothus gloriosus* J.T. Howell var. *gloriosus—USA. CALIFORNIA. **Marin Co.**: Point Reyes National Seashore, *D.O. Burge 908a* (DUKE) [NIA: HM240361; HM240360].

Ceanothus gloriosus J.T. Howell var. *porrectus* J.T. Howell—USA. CALIFORNIA. **Marin Co.**: Point Reyes National Seashore, Inverness Ridge, *D.O. Burge 907a* (DUKE) [NIA: HM240362; HM240363].

***Ceanothus jepsonii* Greene var. *albiflorus* J.T. Howell**—USA. CALIFORNIA. **Colusa Co.**: Rathburn-Petray Mine, *D.O. Burge 997a* (DUKE) [NIA: HM240364; HM240365].

Ceanothus jepsonii Greene var. *jepsonii*—USA. CALIFORNIA. **Marin Co.**: Alpine Lake, *D.O. Burge 914a* (DUKE) [NIA: HM240366].

***Ceanothus maritimus* Hoover**—USA. CALIFORNIA. **San Luis Obispo Co.**: Roadside on Hwy 1, 0.5 road mi (0.8 km) N of bridge over Arroyo de los Chinos, *D.O. Burge 887a* (DUKE) [NIA: HM240367].

***Ceanothus masonii* McMinn**—USA. CALIFORNIA. **Marin Co.**: Golden Gate National Recreation Area, Bolinas Ridge, *D.O. Burge 913a* (DUKE) [NIA: HM240368].

***Ceanothus pinetorum* Coville**—USA. CALIFORNIA. **Trinity Co.**: Un-named rd along ridge, Trinity-Shasta County line, ca. 2.2 linear km SSE of Hoadley Peaks, *D.H. Wilken 16736* (DUKE) [NIA: HM240369; HM240370].

***Ceanothus prostratus* Benth.**—USA. CALIFORNIA. **El Dorado Co.**: El Dorado National Forest, roadside on Wentworth Road, *D.O. Burge 952a* (DUKE) [NIA: HM240371].

Ceanothus pumilus Greene—USA. CALIFORNIA. **Del Norte Co.**: Smith River watershed, near the confluence of Middle Fork Smith River and North Fork Smith River, *D.O. Burge 1156a* (DUKE) [NIA: HM240372; HM240373].

***Ceanothus purpureus* Jeps.**—USA. CALIFORNIA. **Napa Co.**: Wooden Grade, NE of Mount George, *D.O. Burge 904a* (DUKE) [NIA: HM240374; HM240375].

***Ceanothus roderickii* W. Knight**—USA. CALIFORNIA. **El Dorado Co.**: Pine Hill, just E of summit, *D.O. Burge 1080a* (DUKE) [NIA: HM240376]; South Fork American river canyon, near confluence with Weber Creek, *D.O. Burge 1087a* (DUKE) [NIA: HM240377; HM240378]; City of Cameron Park, N side of U.S. Hwy 50, *D.O. Burge 1090a* (DUKE); City of Cameron Park, E of Cameron Airpark, *D.O. Burge 1096a* (DUKE); City of Cameron Park, *D.O. Burge 1100a* (DUKE); S side of U.S. Hwy 50, between Durock Rd and U.S. Hwy 50, *D.O. Burge 1102a* (DUKE); South Fork American River watershed, NW of Mormon Hill, *D.O. Burge 1104a* (DUKE); South Fork American River watershed, NW of Mormon Hill, *D.O. Burge 1105a* (DUKE); Kelley Creek watershed, roadside on Sierrama Rd, *D.O. Burge 1111* (DUKE) [NIA: HM240379]; City of Cameron Park, N side of U.S. Hwy 50, Bureau of Land Management Pine Hill Preserve, *D.O. Burge 1171a* (DUKE); Cameron Park, *D.O. Burge 824b* (DUKE) [NIA: HM240380].

***Ceanothus sonomensis* J.T. Howell**—USA. CALIFORNIA. **Sonoma Co.**: Mayacmas Mountains, head of Hooker Canyon, *D.O. Burge 895b* (DUKE) [NIA: HM240381].