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Author(s): Dylan O. Burge, Diane M. Erwin, Melissa B. Islam, Jürgen Kellermann, Steven W. Kembel, Dieter H. Wilken, Paul S. Manos
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DIVERSIFICATION OF *CEANOTHUS* (RHAMNACEAE) IN THE CALIFORNIA FLORISTIC PROVINCE

Dylan O. Burge,[1],* Diane M. Erwin,† Melissa B. Islam,‡ Jürgen Kellermann,§ Steven W. Kembel,[2],‖
Dieter H. Wilken,# and Paul S. Manos*

*Department of Biology, Duke University, Durham, North Carolina 27708, U.S.A.; †Museum of Paleontology, 1101 Valley Life Sciences
Building, University of California, Berkeley, California 94720, U.S.A.; ‡Department of Ecology and Evolutionary Biology, University
of Colorado, Boulder, Colorado 80309, U.S.A.; and Denver Botanic Gardens, 909 York Street, Denver, Colorado 80206, U.S.A.;
§State Herbarium of South Australia, Department of Environment and Natural Resources Science Resource Centre, P.O. Box
2732, Kent Town, South Australia 5071, Australia; and Australian Centre for Evolutionary Biology and Biodiversity,
School of Earth and Environmental Science, University of Adelaide, Adelaide, South Australia 5005,
Australia; ‖Department of Integrative Biology, University of California, Berkeley, California
94720, U.S.A.; and #Santa Barbara Botanic Garden, 1212 Mission Canyon Road,
Santa Barbara, California 93105, U.S.A.

High diversity and endemism in the California Floristic Province (CFP) are an alleged response to the late Cenozoic advent of Mediterranean-type climate in this region. *Ceanothus* comprises two divergent subgenera with centers of diversity in the CFP. We reconstruct the evolution of *Ceanothus* by using DNA sequence data from the nuclear gene nitrate reductase. We find that the timing of diversification events is related to geological and climatic history. In both subgenera, diversification is characterized by recent divergence of extant taxa and geographically structured phylogenetic relationships. A strong north-south divergence of subgenus *Cerastes* across the Transverse Ranges indicates that phylogenetic relationships may be structured by climatically divergent regions of the CFP. Divergence-time estimation suggests that the age of extant diversification in both subgenera is ~6 Ma. This agrees with the fossil record but predates the hypothesized Quaternary (2-Ma) origin of Mediterranean-type climate in the region.

Keywords: *Ceanothus*, California, divergence time, fossil, nitrate reductase, *rbc*L, *trn*L-F.

Online enhancement: zip file.

Introduction

The California Floristic Province (CFP) of western North America (fig. 1) is characterized by elevated diversity and endemism, with a native flora comprising 4452 species, 2125 of which are endemic (Raven and Axelrod 1978). The origins of this high diversity and endemism have long fascinated western North American botanists (Axelrod 1958, 1975, 1977, 1989; Stebbins and Major 1965; Raven and Axelrod 1978; Stebbins 1978*a*, 1978*b*; Valiente-Banuet et al. 1998; Ackerly 2009). A large proportion of CFP endemism is contributed by in situ diversification associated with dramatic geoclimatic changes in western North America during the late Cenozoic. These changes included uplift of mountain ranges and development of a Mediterranean-type climate characterized by the alternation of hot, dry summers and

mild, wet winters (Axelrod 1966; Raven and Axelrod 1978). The combination of Mediterranean-type climate with topographic and edaphic diversity generated by mountain building created an array of new microenvironments that are thought to have facilitated divergence and persistence of endemic taxa (Stebbins and Major 1965; Raven and Axelrod 1978). Although past research has focused on the question of how the unique environmental history of the CFP contributed to the high diversity and endemism of the region, many questions remain, particularly with respect to the age and geographic origin of the endemics (Ackerly 2009). By combining phylogenetic reconstruction and divergence-time estimation (DTE) with surveys of the paleobotanical record and information on geoclimatic history, it may be possible to discern trends in the origin of CFP diversity. We focus on *Ceanothus* L., which is thought to have diversified primarily in the CFP (Mason 1942; Raven and Axelrod 1978; Ackerly et al. 2006; Fross and Wilken 2006).

Ceanothus comprises ~53 species of evergreen or deciduous shrubs native to North America, with a center of diversity in the CFP (Fross and Wilken 2006; table 1; fig. 1). Extant geographic distribution of the genus encompasses much of North America, from Panama to central British Columbia and from the eastern United States to maritime Cali-
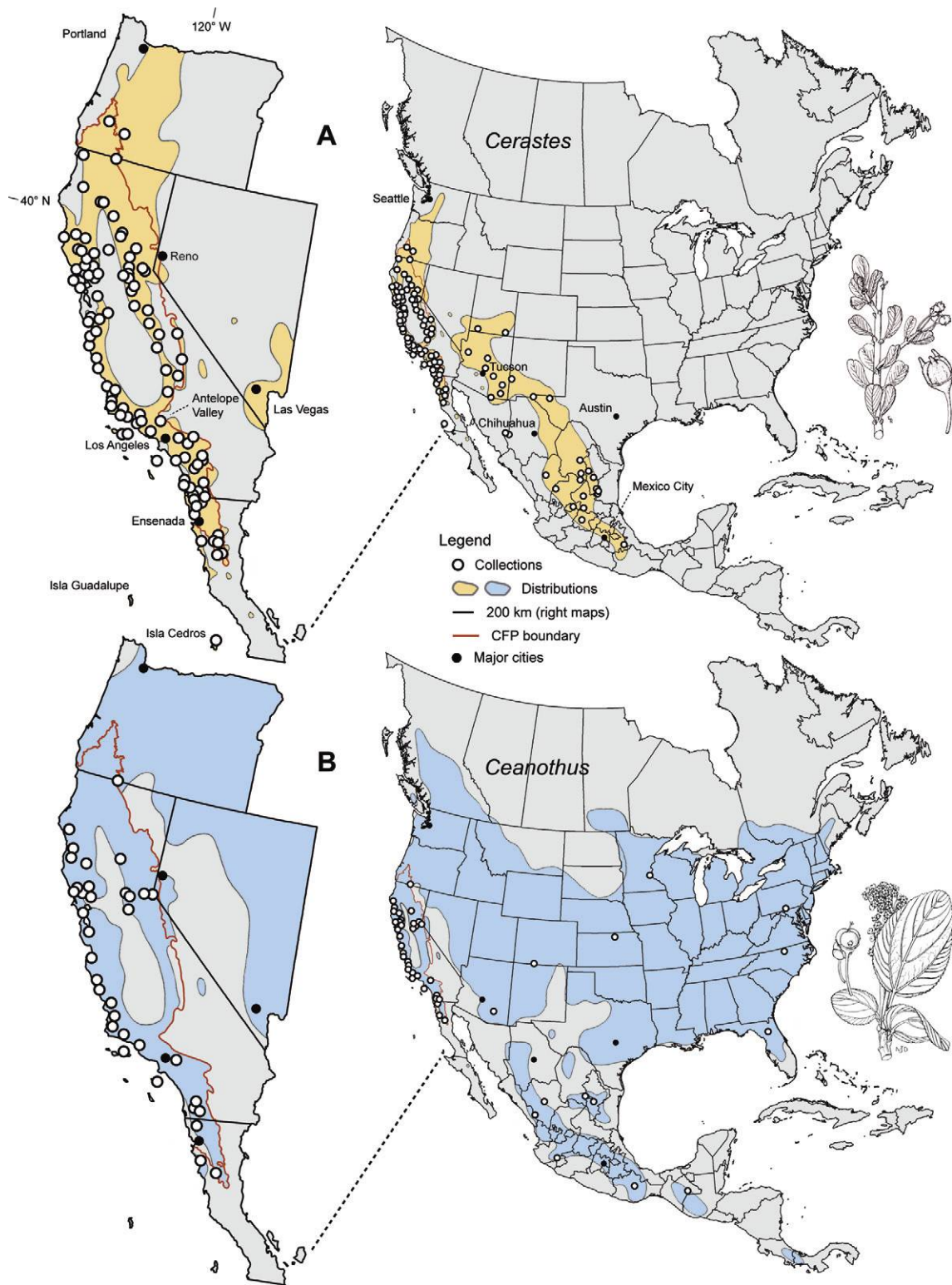
Fig. 1 Geographic distribution of *Ceanothus*, with sampling locations. *A*, Known distribution of subgenus *Cerastes* (light brown). *B*, Known distribution of subgenus *Ceanothus* (light blue). Genetic sampling locations for this study (app. A) are plotted on respective maps for each subgenus. Distributional shading is approximate (see "Material and Methods").

fornia (Fross and Wilken 2006; fig. 1). *Ceanothus* is a dominant component of many North America ecosystems, particularly in the CFP, and contributes significantly to ecosystem nitrogen budgets through its symbiotic relationship with nitrogen-fixing bacteria (McMinn 1942; Franklin et al. 1985). *Ceanothus* diversity is traditionally divided between two subgenera (or sections; see Fross and Wilken 2006), *Cerastes* Watson (24 species) and *Ceanothus* (29 species), which differ strongly in morphology, physiology, and life history (McMinn 1942; Nobs 1963; Fross and Wilken 2006; Ackerly et al. 2006). In molecular phylogenetic studies based on the chloroplast genes *rbc*L and *ndh*F (Jeong et al. 1997) as well as chloroplast *matK* (Hardig et al. 2000) and the ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA (hereafter ITS), the *Ceanothus* subgenera are strongly divergent, reciprocally monophyletic groups. Although the two subgenera of *Ceanothus* probably diverged from a common ancestor before 10 Ma (Calsbeek et al. 2003; Richardson et al. 2004), independent diversification of the subgenera did not begin until later; using ITS sequences from Hardig et al. (2000), Ackerly et al. (2006) estimated that diversification of the subgenera may have commenced as recently as 4 Ma. Diversification of both subgenera is associated with the CFP: 21 of the 24 species in subgenus *Cerastes* and 17 of the 29 species in subgenus *Ceanothus* are endemic to the region (Mason 1942; Fross and Wilken 2006; table 1). Independent and parallel diversification of *Ceanothus* subgenera in the CFP may have been spurred by dramatic geoclimatic changes that began around 5 Ma, including uplift of mountains and development of a Mediterranean-type climate (Mason 1942; Ackerly et al. 2006).

The aims of this study are (1) to reconstruct the evolutionary history of *Ceanothus* by using a rapidly evolving low-copy nuclear marker, (2) to obtain estimates for the absolute timing of diversification events by using rate-calibrated phylogenies for *Ceanothus* and Rhamnaceae based on nuclear ribosomal DNA (ITS) and two chloroplast regions (*trn*L-F and *rbc*L), and (3) to compare these data with information on the geoclimatic history of western North America so as to learn about how diversification of *Ceanothus* has contributed to hyperdiversity in the CFP.

Material and Methods

Genetic Sampling

Sampling of *Ceanothus* was conducted in Mexico, California, Oregon, Nevada, Arizona, Utah, New Mexico, Texas, North Carolina, and Florida (app. A; table A3, available in a zip file in the online edition of the *International Journal of Plant Sciences*). Field sampling was supplemented by herbarium material, particularly for subgenus *Ceanothus*. We selected samples to represent the taxonomic diversity of the genus and the geographic range of taxa (table 1; fig. 1). DNA from 200 plants was analyzed; these represented 76 of the ~78 *Ceanothus* taxa (species, subspecies, varieties) currently recognized (Fross and Wilken 2006; table 1; app. A). Sampling was more intensive in subgenus *Cerastes*, including individuals from 148 populations representing 34 of the 35 taxa currently recognized (Fross and Wilken 2006; table 1).

Higher-density sampling in *Cerastes* reflects the focus of our study on diversification of this taxonomically and genetically complex group (Nobs 1963; Fross and Wilken 2006; Burge and Manos 2011). In subgenus *Ceanothus*, we sampled 52 populations representing 42 of the 43 currently recognized taxa (Fross and Wilken 2006; table 1). In widespread taxa such as *Ceanothus cuneatus* Nutt. and *Ceanothus vestitus* Greene, we sampled many populations to represent genetic variation across the wide geographic areas inhabited by these species (fig. 1). To obtain sequence data for DTE, several non-*Ceanothus* Rhamnaceae taxa were also sampled from herbarium material (app. B; see "Results"). All *Ceanothus* specimens were identified according to Fross and Wilken (2006), with the exception of three species (table 1): (1) *Ceanothus arcuatus* McMinn (treated within *Ceanothus fresnensis* Abrams by Fross and Wilken [2006]), (2) *Ceanothus masonii* McMinn (synonymized with *Ceanothus gloriosus* J.T. Howell by Fross and Wilken [2006]); and (3) *Ceanothus perplexans* Trel. (synonymized with *Ceanothus vestitus* by Fross and Wilken [2006]).

Molecular Methods

Genomic DNA was extracted as described in Burge and Manos (2011). In the case of some herbarium specimens, DNA was extracted from embryo and endosperm tissue excised from intact seeds. For extractions of seed DNA, several seeds from a single plant were pooled before preparation (Burge and Barker 2010). Amplification conditions for PCR reactions and methods of isolation and sequencing of the third intron from the low-copy nuclear gene nitrate reductase (NIA) are described by Burge and Manos (2011). We used the primers ITS4 (White et al. 1990) and ITSA (Blattner 1999) to amplify ITS. In some cases, cloning of ITS PCR products was required because of length variation. ITS cloning methods were identical to those for NIA (Burge and Manos 2011). All other ITS sequences were obtained by direct sequencing. We used primers c and f of Taberlet et al. (1991) to amplify the *trn*L-F plastid region, which comprises the *trn*L intron and the *trn*L-F intergenic spacer. We used primers rbcl-20 and rbcl-ctl (Fay et al. 1998) to amplify the plastid *rbc*L gene. All plastid regions were sequenced directly. Methods for cleanup of PCR products and DNA sequencing are described elsewhere (Burge and Manos 2011). Genomic DNA, ITS, and *trn*L-F sequences of some Australian species (as indicated in table 2) were prepared as described in Kellermann et al. (2005) and Kellermann and Udovicic (2007); some of these sequences were first reported in Kellermann (2002).

Sequences and Alignment

Ceanothus NIA sequences were assembled and edited in Sequencher 4.1 (Gene Codes). Alignments were made in MUSCLE (Edgar 2004) under default settings. For each plant, we assessed sequence variation by using an alignment of cloned sequences (hereafter "isolates"). Fifty-seven plants yielded pools of identical isolates, 85 yielded two different types of NIA isolate (hereafter "isolate types"), 45 were represented by a single successfully cloned NIA isolate, and 13

Table 1

Ceanothus Taxa, Sampling, Geographic Distribution, and Endemic Status in the California Floristic Province (CFP)

Taxon	Sampling[a]	Distribution[b]	CFP endemic[c]
Subgenus *Cerastes*:			
Ceanothus arcuatus McMinn	2	North CA	X
C. bolensis S. Boyd & J. Keeley	1	BC	X
C. crassifolius Torr. var. *crassifolius*	4	South CA, BC	X
C. crassifolius Torr. var. *planus* Abrams	3	South CA	X
C. cuneatus Nutt. var. *cuneatus*	38	West US, BC	X
C. cuneatus Nutt. var. *dubius* J.T. Howell	3	North CA	X
C. cuneatus Nutt. var. *fascicularis* (McMinn) Hoover	3	North CA	X
C. cuneatus Nutt. var. *ramulosus* Greene	2	North CA	X
C. cuneatus Nutt. var. *rigidus* (Nutt.) Hoover	2	North CA	X
C. divergens Parry subsp. *confusus* (J.T. Howell) Abrams	2	North CA	X
C. divergens Parry subsp. *divergens*	0	North CA	X
C. divergens Parry subsp. *occidentalis* (McMinn) Abrams	1	North CA	X
C. ferrisiae McMinn	2	North CA	X
C. fresnensis Abrams	2	North CA	X
C. gloriosus J.T. Howell var. *exaltatus* J.T. Howell	1	North CA	X
C. gloriosus J.T. Howell var. *gloriosus*	2	North CA	X
C. gloriosus J.T. Howell var. *porrectus* J.T. Howell	1	North CA	X
C. jepsonii Greene var. *albiflorus* J.T. Howell	4	North CA	X
C. jepsonii Greene var. *jepsonii*	2	North CA	X
C. maritimus Hoover	1	North CA	X
C. masonii McMinn	2	North CA	X
C. megacarpus Nutt. var. *insularis* (Eastw.) Munz	3	South CA	X
C. megacarpus Nutt. var. *megacarpus*	4	South CA	X
C. ophiochilus S. Boyd, T. Ross, & L. Arnseth	1	South CA	X
C. otayensis McMinn	3	South CA, BC	X
C. pauciflorus DC.	7	Mexico	. . .
C. perplexans Trel.	7	South CA, BC	X
C. pinetorum Coville	2	North CA	X
C. prostratus Benth.	3	North CA	. . .
C. pumilus Greene	1	North CA	X
C. purpureus Jeps.	2	North CA	X
C. roderickii W. Knight	2	North CA	X
C. sonomensis J.T. Howell	2	North CA	X
C. verrucosus Nutt.	6	South CA, BC	X
C. vestitus Greene	27	West US, Mexico	. . .
Subgenus *Ceanothus*:			
C. americanus L. var. *americanus*	1	East US, Canada	. . .
C. americanus L. var. *intermedius* (Pursh) Torr. & A. Gray	1	East US	. . .
C. americanus L. var. *pitcheri* Pickering ex. Torr. & A. Gray	1	East US	. . .
C. arboreus Greene	2	South CA	X
C. buxifolius Schult.	1	Mexico	. . .
C. caeruleus Lag.	4	Mexico, Central America	. . .
C. cordulatus Kellogg	1	West US, BC	. . .
C. cyaneus Eastw.	1	South CA, BC	X
C. dentatus Torr. & A. Gray	1	North CA	X
C. diversifolius Kellogg	1	North CA	X
C. fendleri A. Gray var. *fendleri*	2	West US, Mexico	. . .
C. fendleri A. Gray var. *venosus* Trel.	1	West US, Mexico	. . .
C. foliosus Parry var. *foliosus*	1	California	X
C. foliosus Parry var. *medius* McMinn	1	North CA	X
C. foliosus Parry var. *vineatus* McMinn	1	North CA	X
C. hearstiorum Hoover & Roof	1	North CA	X
C. herbaceus Raf.	1	East US, Mexico	. . .
C. impressus Trel. var. *impressus*	1	North CA	X
C. impressus Trel. var. *nipomensis* McMinn	1	North CA	X
C. incanus Torr. & A. Gray	3	North CA	X
C. integerrimus Hook. & Arn. var. *integerrimus*	1	North CA	X
C. integerrimus Hook. & Arn. var. *macrothyrsus* (Torr.) G.T. Benson	1	West US	. . .
C. lemmonii Parry	1	North CA	X

Table 1

(*Continued*)

Taxon	Sampling[a]	Distribution[b]	CFP endemic[c]
C. leucodermis Greene	2	California, BC	X
C. martinii M.E. Jones	0	West US	...
C. microphyllus Michx.	1	East US	...
C. ochraceus Suess.	2	Mexico	...
C. oliganthus Nutt. var. *oliganthus*	1	California	X
C. oliganthus Nutt. var. *orcuttii* (Parry) Jeps.	1	South CA, BC	X
C. oliganthus Nutt. var. *sorediatus* (Hook. & Arn.) Hoover	1	California, BC	X
C. papillosus Torr. & A. Gray var. *papillosus*	1	California	X
C. papillosus Torr. & A. Gray var. *roweanus* McMinn	1	South CA, BC	X
C. parryi Trel.	1	California, Oregon	X
C. parvifolius (S. Watson) Trel.	1	North CA	X
C. sanguineus Pursh	1	West US, Canada	...
C. spinosus Nutt. var. *palmeri* (Trel.) K. Brandegee	1	California, BC	X
C. spinosus Nutt. var. *spinosus*	1	California, BC	X
C. thyrsiflorus Eschsch. var. *griseus* Trel.	1	North CA	X
C. thyrsiflorus Eschsch. var. *thyrsiflorus*	2	California, BC	X
C. tomentosus Parry var. *olivaceus* Jeps.	1	California, BC	X
C. tomentosus Parry var. *tomentosus*	1	California, BC	X
C. velutinus Hook. var. *hookeri* M. C. Johnst.	1	West US, Canada	...
C. velutinus Hook. var. *velutinus*	1	West US, Canada	...

[a] Number of plants sampled for genetic (nitrate reductase) analysis (app. A; table A3, in the online edition of the *International Journal of Plant Sciences*).

[b] Geographic distribution of taxon based on Fross and Wilken (2006), with minor modifications. BC = Baja California, Mexico; north CA = region of California north of 34.45°N latitude (Point Conception, Santa Barbara County); south CA = region to the south of this line; east US = distributed predominantly or entirely east of the Mississippi River; west US = distributed predominantly or entirely west of the Mississippi River.

[c] An "X" indicates that the taxon is endemic to the CFP; ellipses indicate that the taxon is found at least partly outside the CFP.

were directly sequenced. Among plants with two isolate types, we used one isolate representing each isolate type for subsequent analysis. In the case of plants with one isolate type, a single isolate was selected. Two hundred eighty-five isolates were selected, 230 from subgenus *Cerastes* and 55 from subgenus *Ceanothus*. Sequences were deposited in GenBank (app. A; table A3).

NIA was chosen because of its high rate of evolution (Howarth and Baum 2002). However, high rates of sequence evolution, particularly insertion and deletion events, may lead to alignment ambiguity, homoplasy, and ultimately reconstruction artifacts such as long-branch attraction (Bergsten 2005). Indeed, preliminary results revealed the presence of several highly variable indel-rich regions that were unalignable between the subgenera of *Ceanothus*. To attain the most well-resolved and reliable NIA gene trees for *Ceanothus*, we built three separate alignments, one for each subgenus and a third for all of *Ceanothus*. For the separate subgenus alignments, ambiguously aligned regions were completely removed (alignments A4, A6 [all numbered "alignments" are in a zip file available in the online edition of the *International Journal of Plant Sciences*]; table 3; for unaltered initial alignments, see alignments A5, A7). The alignment for all of *Ceanothus* (alignment A8) was then prepared manually in BioEdit, version 5.0.6 (Hall 1999) with the separate subgenus alignments from which the ambiguous regions had already been removed (alignments A4, A6).

The other three loci were sequenced for two DTE analyses, a rate-calibrated analysis based on ITS for *Ceanothus* only

and a fossil-calibrated analysis based on ITS combined with chloroplast *rbc*L and *trn*L-F for Rhamnaceae (table 3). For rate-calibrated DTE, ITS sequences were obtained for 77 of the 78 *Ceanothus* taxa. Individuals were selected from among previous collections supplemented by data from GenBank for *Ceanothus martinii* M.E. Jones. New sequences were assembled and edited as for NIA and deposited in GenBank (app. A). Sequences were aligned in MUSCLE with no manual adjustments (alignment A9).

For fossil-calibrated DTE, DNA sequences were obtained from Rhamnaceae. Taxa were selected to represent the diversity of the family, with an emphasis on the ziziphoid Rhamnaceae (Richardson et al. 2000*a*). The majority of data are from GenBank, supplemented with 12 newly generated sequences (table 2) and five previously reported sequences that had not yet been deposited in GenBank (Kellermann 2002; table 2). The 17 new or previously reported sequences were assembled and edited as described for NIA and deposited in GenBank (table 2; app. B). The complete data set included 37 terminal "taxa." Some taxa were represented by sequences from multiple species of the same genus and/or multiple individuals of the same species (table 2). Sequences for *rbc*L and *trn*L-F were aligned in MUSCLE (Edgar 2004) under default settings, with minor manual adjustments. For ITS, the intergenic spacer regions were strongly divergent among Rhamnaceae lineages. To attain the most informative alignment and minimize the need for exclusion of ambiguously aligned regions, we first used MAFFT, version 6 (Katoh et al. 2002; Katoh and Toh 2008), under default settings to

Table 2

Rhamnaceae Sequences for Fossil-Calibrated Divergence-Time Estimation

	GenBank (NCBI) accession numbers			
Taxa[a]	ITS	*rbc*L	*trn*L-F	References[b]
Colletieae:				
Adolphia Meisn.[c]	AF048973	AJ390055	AY460408, AY642142	3, 4, 8
Gouanieae:				
Crumenaria erecta Reissek[d]	HQ325385	AJ390042	AJ390346	12, 4, 4
Helinus integrifolius (Lam.) Kuntze[d]	HQ325386	AJ390043	AJ390347	12, 4, 4
Reissekia smilacina Endl.[d]	DQ146614	AJ390041	AJ390345	10, 4, 4
Incertae sedis:				
Alphitonia excelsa (Fenzl) Benth.[d]	HQ340157[e]	AJ390049	HQ325600[e]	12, 4, 12
Ceanothus cordulatus Kellogg[d]	HQ325315	U78904	HQ325601	12, 1, 12
Cerastes pumilus Greene[d]	HQ340158	U78905	HQ325602	12, 1, 12
Colubrina asiatica (L.) Brongn.	AF328831	AJ390047	AJ390350	5, 4, 4
Emmenosperma alphitonioides F. Muell.[d]	HQ340159[e]	AJ390048	AJ390351	12, 4, 4
Granitites intangendus (F. Muell.) Rye[d]	HQ340160[e]	AJ306539	HQ325603[e]	12, 6, 12
Lasiodiscus mildbraedii Engl.	AF328833	AJ390050	AJ390353	5, 4, 4
Schistocarpaea johnsonii F. Muell.[f]	AY911539	AJ390046	AJ390349	9, 4, 4
Paliureae:				
Hovenia dulcis Thunb.[d]	DQ146607	AJ390039	AJ390343	10, 4, 4
Paliurus spina-christi Mill.[d]	DQ146613	AJ390051	AJ390354	10, 4, 4
Ziziphus amole (Sessé & Moc.) M.C. Johnst.	DQ146579	HQ325595	DQ146535	10, 12, 10
Ziziphus calophylla Wall. ex Hook. f.	DQ146580	HQ325597	DQ146536	10, 12, 10
Ziziphus jujuba Mill.	DQ146573	HQ325594	DQ146529	10, 12, 10
Ziziphus mauritiana Lam.	DQ146588	HQ325598	DQ146544	10, 12, 10
Ziziphus rugosa Lam.	DQ146601	HQ325599	DQ146557	10, 12, 10
Ziziphus spina-christi Willd.	DQ146603	HQ325596	DQ146559	10, 12, 10
Phyliceae:				
Nesiota elliptica Hook. f.	AF328823	AJ225783	AJ225803	5, 2, 2
Noltea africana (L.) Endl.[d]	AF328822	AJ390054	AJ390357	5, 4, 4
Phylica nitida Lam.	AF328821	AJ390053	AJ390356	5, 4, 4
Phylica polifolia (Vahl) Pillans[d]	AF328805	AJ225784	AJ390373	5, 2, 4
Trichocephalus stipularis (L.) Brongn.[d]	AF328824	AM235105	AF327621	5, 11, 5
Pomaderreae:				
Pomaderris rugosa Cheeseman	DQ146615	AJ390063	AJ390363	10, 4, 4
Siegfriedia darwinioides C.A. Gardner[f]	AY911575	AJ390064	AJ390375	9, 4, 4
Spyridium globulosum (Labill.) Benth.[f]	AY911590	AJ390058	AJ390358	9, 4, 4
Stenanthemum complicatum (F. Muell.) Rye[f]	AY911599	AJ390059	AJ390359	9, 4, 4
Trymalium odoratissimum Lindl.[f]	AY911578	AJ390062	AJ390362	9, 4, 4
Rhamneae:				
Berchemia discolor Hemsl.	AY626455	AJ225786	AJ225793	7, 2, 2
Frangula alnus Mill.[d]	AY626431	AJ390026	AJ251691	7, 4, 4
Rhamnella franguloides Weberbauer.	AY626454	AJ390027	AJ390330	7, 4, 4
Rhamnidium elaeocarpum Reissek	AY626452	AJ390030	AJ390332	7, 4, 4
Rhamnus lycioides L.[d]	AY626437	AJ390070	AJ390374	7, 4, 4
Sageretia thea (Osbeck) M.C. Johnst.	AY626453	AJ225785	AJ225792	7, 2, 2
Scutia buxifolia Reissek	AY626451	AJ390033	AJ390335	7, 4, 4

[a] Subheadings for entries under column heading "Taxa" refer to tribal classification of Rhamnaceae (Richardson et al. 2000*b*).

[b] Original publications in which the ITS, *rbc*L, and *trn*L-F sequences, respectively, were cited: 1, Jeong et al. (1997); 2, Thulin et al. (1998); 3, Hardig et al. (2000); 4, Richardson et al. (2000*a*); 5, Richardson et al. (2001); 6, Fay et al. (2001); 7, Bolmgren and Oxelman (2004); 8, Aageson et al. (2005); 9, Kellermann et al. (2005); 10, Islam and Simmons (2006); 11, Forest et al. (2007); 12, present study (app. B).

[c] At least two sequences came from different species of the same genus.

[d] At least two of the sequences came from different individuals of the same species.

[e] Sequence originally reported by Kellermann (2002), edited and deposited in GenBank as part of the present study.

[f] Genomic DNA, ITS and *trn*L-F sequences were obtained using the methods described in Kellermann et al. (2005) and Kellermann and Udovicic (2007).

make a preliminary alignment. Manual adjustments were then made according to Zurawski and Clegg (1987) to minimize the number of evolutionary events. The remaining ambiguously aligned regions were coded as missing, and the alignment was analyzed with a parsimony jackknife analysis conducted in PAUP*, version 4 (Swofford 2000; 200 replicates, four iterations of TBR branch swapping per replicate). Ambiguous regions within the major clades identified from

Table 3

DNA Alignments

Name	Terminals[a]	Length[b]	G + C (%)[c]	Variable[d]	PIC[e]
Subgenus *Cerastes* NIA:					
Alignment A4	230	461	…	229	96
Alignment A5	230	1110	34.4	…	…
Subgenus *Ceanothus* NIA:					
Alignment A6	55	662	…	150	53
Alignment A7	55	1205	37.5	…	…
All *Ceanothus* NIA:					
Alignment A8	285	693	…	332	167
Rate-calibrated DTE:					
Alignment A9	53	619	60.3	87[f]	54[f]
Fossil-calibrated DTE:					
Alignment A10:					
ITS portion	37	895	59.9	386[g]	261[g]
*rbc*L portion	37	1383	44.1	237	119
*trn*L-F portion	37	864	36.9	248	102

Note. NIA = nitrate reductase.
[a] Number of terminals in the alignment.
[b] Length of the alignment (some of which may have been excluded from analysis).
[c] G + C content of the entire alignment.
[d] Number of variable characters in the portion of the alignment used for analysis.
[e] Number of parsimony-informative characters.
[f] Statistics refer to the ITS1 and ITS2 regions of the alignment (excluding 5.8S).
[g] Statistics refer to the region of ITS (the ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA) that was not excluded from analysis (801 total characters); ellipses indicate that the statistic was not relevant to this alignment or portion of alignment.

this preliminary analysis were then manually aligned, allowing for retention of the characters within these areas. The remaining ambiguously aligned regions were coded as missing data for one or both clades to generate the final alignment (alignment A10).

Justification of Analysis Methods

Preliminary tree-building exercises for *Ceanothus* using NIA and ITS indicated a lack of monophyly for most *Ceanothus* species as well as a widespread lack of cohesion between NIA isolates obtained from the same plant. As discussed below, this is consistent with past and ongoing gene flow among species within each subgenus, as hypothesized by past researchers (Nobs 1963; Hardig et al. 2000; Fross and Wilken 2006; see below). Alternatively, the lack of species monophyly may indicate a lack of speciation, with potential consequences for the evolutionary models used here. For the purposes of this work, we have assumed that nonmonophyly reflects recent divergence combined with gene flow rather than a lack of speciation.

Tree Reconstruction

For the NIA alignments corresponding to the *Ceanothus* subgenera (table 3; alignments A4, A6), tree reconstruction was carried out under the Bayesian criterion only. Analyses were conducted with the best-fit model of evolution from Akaike Information Criterion (AIC) output of MrModeltest (Nylander 2004; subgenus *Cerastes*: K80+G; subgenus *Ceanothus*: GTR+G). Tree sampling was performed with MrBayes 3.0 (Ronquist and Huelsenbeck 2003). For each

subgenus alignment, we performed three separate runs of 5×10^6 Markov chain Monte Carlo (MCMC) generations, using one heated and three cold chains and sampling every 1000 generations. Chains were examined for convergence (standard deviation of split frequencies approaching 0.001). Log likelihoods were inspected to identify the burn-in period (Ronquist and Huelsenbeck 2003). For each analysis, the majority-rule consensus tree was generated with MrBayes on the basis of the post-burn-in sample of trees. Trees from each of the three independent runs per alignment were compared to verify similarity of the results.

Using the NIA alignment for all of *Ceanothus* (table 3; alignment A8), tree reconstruction was carried out with both Bayesian and maximum likelihood (ML) methods. The Bayesian methods were identical to those used for the subgenus alignments. ML analyses were conducted using the best-fit model of evolution from AIC output of Modeltest, version 3.6 (Posada and Crandall 1998; GTR+G). Searches were performed in GARLI, version 1.0 (Zwickl 2006). Two search replicates of 10^6 generations were performed in a single execution using a random starting tree. Other parameters were kept at default values. Statistical support was inferred with 100 replicates of bootstrap reweighting (Felsenstein 1985) that used 5×10^5 generations per replicate. The majority-rule consensus tree was calculated using the 100 best bootstrap trees.

To root *Ceanothus* trees, we used a method based on the reciprocal monophyly of the subgenera, which was established by previous molecular phylogenetic work (Jeong et al. 1997; Hardig et al. 2000). In the consensus tree for all of *Ceanothus*, we rooted the subgenera on each other. Trees based on the separate alignments for each subgenus were then

rooted using the earliest-diverging terminals from the tree for all of *Ceanothus*; in subgenus *Cerastes*, this was DOB 1195a-c1 (*Cerastes pauciflorus* DC.); in subgenus *Ceanothus*, this was DOB 930a (*Ceanothus oliganthus* Nutt. var. *orcuttii* [Parry] Jeps.). We chose this method because NIA sequences obtained from other Rhamnaceae proved unalignable to *Ceanothus*.

Network Reconstruction

Although the evolutionary history of a group of organisms is usually represented by a phylogenetic tree, this model of evolution does not always adequately describe more complex evolutionary scenarios, such as those resulting from hybrid speciation or recombination (Huson and Bryant 2006). Hybridization among species within subgenera of *Ceanothus* has long been noted anecdotally (Parry 1889; Nobs 1963; Fross and Wilken 2006) and is assumed to have played a role in *Ceanothus* evolution (Nobs 1963; Hardig et al. 2002). Furthermore, even in systems where relationships may be realistically represented by a tree, reconstruction of phylogenetic networks has the potential to enhance interpretation of relationships (Clement et al. 2000; Huson and Bryant 2006). Here we use methods of phylogenetic network reconstruction to estimate relationships among NIA isolates within each of the *Ceanothus* subgenera.

Two methods of network reconstruction were applied to the subgenus *Ceanothus* and subgenus *Cerastes* alignments used for tree reconstruction (*Cerastes*: alignment A4; *Ceanothus*: alignment A6): (1) neighbor-net splits network (NSN; Bryant and Moulton 2004), as implemented in SplitsTree4 (Huson and Bryant 2006), based on Kimura two-parameter genetic distances, with 1000 bootstrap replicates to estimate statistical support, and (2) statistical-parsimony gene genealogy (Templeton et al. 1992), as implemented in TCS (Clement et al. 2000) under default settings, with gaps treated as missing data.

Testing for Recombination

The fundamental biological process of recombination leads to the merger of historically distinct genotypes. Recombination is considered a reticulate event in the reconstruction of networks (Hein 1990), with assumptions concerning the underlying population dynamics (Huson and Bryant 2006). Because all *Ceanothus* species examined to date are strongly outcrossing, with no known intrinsic barriers to gene flow with species of the same subgenus (McMinn 1942; Nobs 1963), we tested the potential contribution of recombination to the patterns of sequence variation seen in the sampled NIA sequences for each subgenus. Two statistical methods were used to test for possible recombination among NIA isolates within each of the two *Ceanothus* subgenera: (1) the pairwise homoplasy, or Φ_w (PHI), statistic of Bruen et al. (2006), calculated in SplitsTree4 (Huson and Bryant 2006) using permutation to test for significantly small Φ_w (Huson and Bryant 2006), and (2) the Dss statistic of McGuire et al. (1997), as modified and refined by McGuire and Wright (2000) and implemented in TOPALi, version 2 (Milne et al. 2009), using flexible window

size and 100 rounds of bootstrapping to test for significantly large Dss.

Divergence-Time Estimation

Divergence-time estimates indicate a Paleocene origin of stem Rhamnaceae (62–64 Ma; Wikström et al. 2001), followed by diversification of the crown clade beginning in the late Paleocene to early Eocene (Wikström et al. 2001; Richardson et al. 2004). The idea of early Eocene diversification in Rhamnaceae is supported by the fossil record, where reliably identifiable members of the crown clade are known as early as 55 Ma (Burge and Manchester 2008). However, a fossil fruit and leaves with strong affinities to crown-clade Rhamnaceae recently described from the late Cretaceous (~68 Ma; Correa et al. 2010) suggest that the origin of Rhamnaceae and diversification of the crown clade may have occurred earlier than recent divergence-time estimates indicate. Earlier dates are also consistent with recent work on angiosperms as a whole, which pushes the origin of flowering plants 25–75 Myr earlier than most recent estimates (Smith et al. 2010; but see Bell et al. 2010).

Previous research into molecular phylogenetic relationships in *Ceanothus* used an independently estimated rate of substitution for the *rbc*L gene to infer early divergence between the subgenera (18–39 Ma; Jeong et al. 1997). After publication of an ITS-based *Ceanothus* phylogeny by Hardig et al. (2000), Calsbeek et al. (2003) used an estimate for ITS substitution rate to obtain a date of 13.9 Ma for subgeneric divergence. Richardson et al. (2004) used an external fossil calibration applied to *rbc*L and *trn*L-F to infer a similar age for the subgeneric split (10.7–11.1 ± 4.2 Ma). Later research used the divergence-time estimate of Jeong et al. (1997) to calibrate the ITS phylogeny and to infer that diversification of the two subgenera began simultaneously at 4–5 Ma (Ackerly et al. 2006). Here we aim to test previous estimates for major events in *Ceanothus* diversification and to provide new estimates for events within the *Ceanothus* subgenera, using recently developed methods (Drummond et al. 2006) applied to two data sets.

Rate-calibrated DTE. This analysis relied on the ITS alignment for *Ceanothus* (table 3; alignment A9). A relaxed clock with an uncorrelated lognormal model of rate variation among branches was used to obtain estimates of divergence time. We implemented this model in BEAST, version 1.5.3 (Drummond et al. 2006; Drummond and Rambaut 2007). Two topological constraints were implemented, defining each of the *Ceanothus* subgenera as monophyletic. We selected a substitution model from AIC output of MrModeltest (Nylander 2004). We used flat priors for molecular evolution but modeled the tree prior according to a Yule speciation process. The substitution pattern was selected on the basis of AIC output from MrModeltest (GTR+G). To obtain estimates of absolute divergence times, we placed a prior on the overall absolute rate of nucleotide substitution (the meanRate parameter in BEAST). We selected a substitution rate, based on the work of Kay et al. (2006), in which the rate of evolution at the ITS1 and ITS2 intergenic spacers was found to vary between 0.38×10^{-9} and 7.83×10^{-9} substitutions/site/Myr (mean 2.15×10^{-9}) in woody perennials. To account for this variation, we set the meanRate parameter to 2.15×10^{-9}, with

a lognormal distribution and standard deviation that placed the minimum and maximum rates of Kay et al. (2006) within the 95% confidence interval of the prior (Log(Stdev) parameter set to 0.84). We ran MCMC chains for 3.0×10^7 generations, sampling parameters every 1000 generations. Results were inspected in Tracer, version 1.5.3 (Rambaut and Drummond 2007), to confirm stationarity and acceptable mixing; the effective sample size (ESS) for all statistics was above 200; divergence times and confidence intervals were noted after 5% of samples were removed as burn-in.

Fossil-calibrated DTE. This analysis relied on the alignment for Rhamnaceae (table 3; alignment A10). The incongruence length difference test (Farris et al. 1995) indicated significant disagreement between chloroplast (*rbc*L and *trn*L-F) and nuclear (ITS) data ($P = 0.025$). To determine the potential influence of conflict, we conducted separate maximum parsimony analyses of the chloroplast and nuclear data. These analyses were conducted in PAUP*. Heuristic searches were performed using 1000 random-addition replicates and TBR branch swapping, holding 10 trees per replicate. Conflict between data sets may derive from differences in position for a handful of taxa (treefiles A11, A12 [all numbered "treefiles" are in a zip file available in the online edition of the *International Journal of Plant Sciences*]). To ascertain the effect of combined analysis on DTE, the analyses described below for combined data were carried out separately for chloroplast and nuclear data.

We used relaxed-clock methods implemented in BEAST to obtain divergence-time estimates. Two topological constraints were implemented to define the following monophyletic groups: (1) *Ceanothus* and (2) Old World *Ziziphus* and *Paliurus* Mill. (table 2; Islam and Simmons 2006). Substitution patterns were selected on the basis of AIC output from MrModeltest (ITS and *rbc*L: GTR+I+G; *trn*L-F: GTR+G). Priors for molecular evolution and the tree were identical to those used for rate-calibrated DTE. To obtain absolute estimates of divergence time, we placed two priors on the tree, the first an internal calibration based on a fossil and the second an external calibration based on the age of Rhamnaceae. For fossil calibration, we used the early Eocene (55 Ma) *Paliurus clarnensis* Burge and Manchester, which represents the earliest reliably identified material for *Paliurus* or Old World *Ziziphus* (Burge and Manchester 2008; but see Correa et al. 2010 for older material with possible affinities to *Paliurus*). To place this prior, we used an exponential distribution on the time to most recent common ancestor (tMRCA) for Old World *Ziziphus* and *Paliurus* with an offset of 55 Ma and a standard deviation of 4.4 Ma (Ho and Phillips 2009). The external calibration was based on Wikström et al. (2001), in which the estimated age for crown clade Rhamnaceae was 56.5 Ma. To place this prior, we set the treeModel.RootHeight parameter to 56.5 Ma with a normal distribution and a standard deviation of 1.7 Ma. MCMC sampling and postprocessing of data were as for rate-calibrated DTE; the ESS for all statistics was above 200.

Mapping and Bioclimate

Geographic locations of *Ceanothus* sampling were plotted with ArcGIS, version 9.3 (ESRI, Redlands, CA; fig. 1). For collections by D. O. Burge (app. A), georeference data for individual localities were obtained with a handheld Garmin GPS 12 Personal Navigator GPS unit (Garmin International, Olathe, KS). For collections by others (app. A), georeference data were obtained from specimen labels. In cases where georeference data were not provided, the latitude and longitude of localities were inferred from maps supplemented with information from geographic databases. The distribution of *Ceanothus* subgenera was plotted on the basis of data from the Consortium of California Herbaria (http://ucjeps.berkeley .edu/consortium) and the Global Biodiversity Information Facility (http://www.gbif.org) that we interpreted using written summaries of geographic distribution from Fross and Wilken (2006). Locations were plotted in ArcGIS, version 9.1 (ESRI); data for the CFP boundary are from Myers et al. (2000).

As an aid to interpretation of geographic distribution patterns among genetic groups in subgenus *Cerastes*, geographic locations were plotted against bioclimatic data. We focused on average annual minimum and maximum temperature (hereafter AAMin and AAMax, respectively) and average annual precipitation (hereafter AAP). Raster digital-data layers for these variables were obtained from PRISM Climate Group (2006) at Oregon State University and from WorldClim, version 1.4 (Hijmans et al. 2005; http://www.worldclim.org). PRISM layers cover the coterminous United States and are interpolated from weather station data collected between 1971 and 2000 (PRISM Climate Group 2006). WorldClim layers cover most land areas of the globe and are interpolated from weather station data collected between 1950 and 2000 (Hijmans et al. 2005). We obtained both the PRISM and the WorldClim layers at 30-arcsec (1-km^2) resolution. Layers and locations were plotted with ArcGIS, version 9.3. Estimates for AAMin, AAMax, and AAP at each sampling location were obtained with the *maptools* (Lewin-Koh and Bivand 2010) and *spatstat* (Baddeley and Turner 2005) packages implemented in R (R Development Core Team 2010). As an aid to interpretation of the bioclimatic data, each of the variables was plotted against latitude. Summary statistics were also calculated for bioclimatic variation among genetic groups.

Results

Subgenus Cerastes *Phylogeny*

The three independent replicates of Bayesian analysis for subgenus *Cerastes* phylogeny yielded trees with nearly identical topology. Only one run was used for final tree building, with 5% of sampled trees removed as burn-in. The resulting tree (fig. 2; treefile A13) contained 53 nodes resolved with posterior probability (PP) above 0.50, including 17 with PP of 1.0 (fig. 2). None of the 19 species represented by more than one sampled plant are monophyletic (table 1). Instead, most taxa are polyphyletic, recovered in disparate clades, or simply unresolved. It is important to note, however, that some species and lower-level taxa have a tendency to group together, though often as members of clades containing isolates from other taxa (e.g., *Cerastes jepsonii* Greene). Most resolved nodes support relationships near the tips of the tree, with less overall support at internal nodes (fig. 2). However, several major clades are recovered, including one made up of
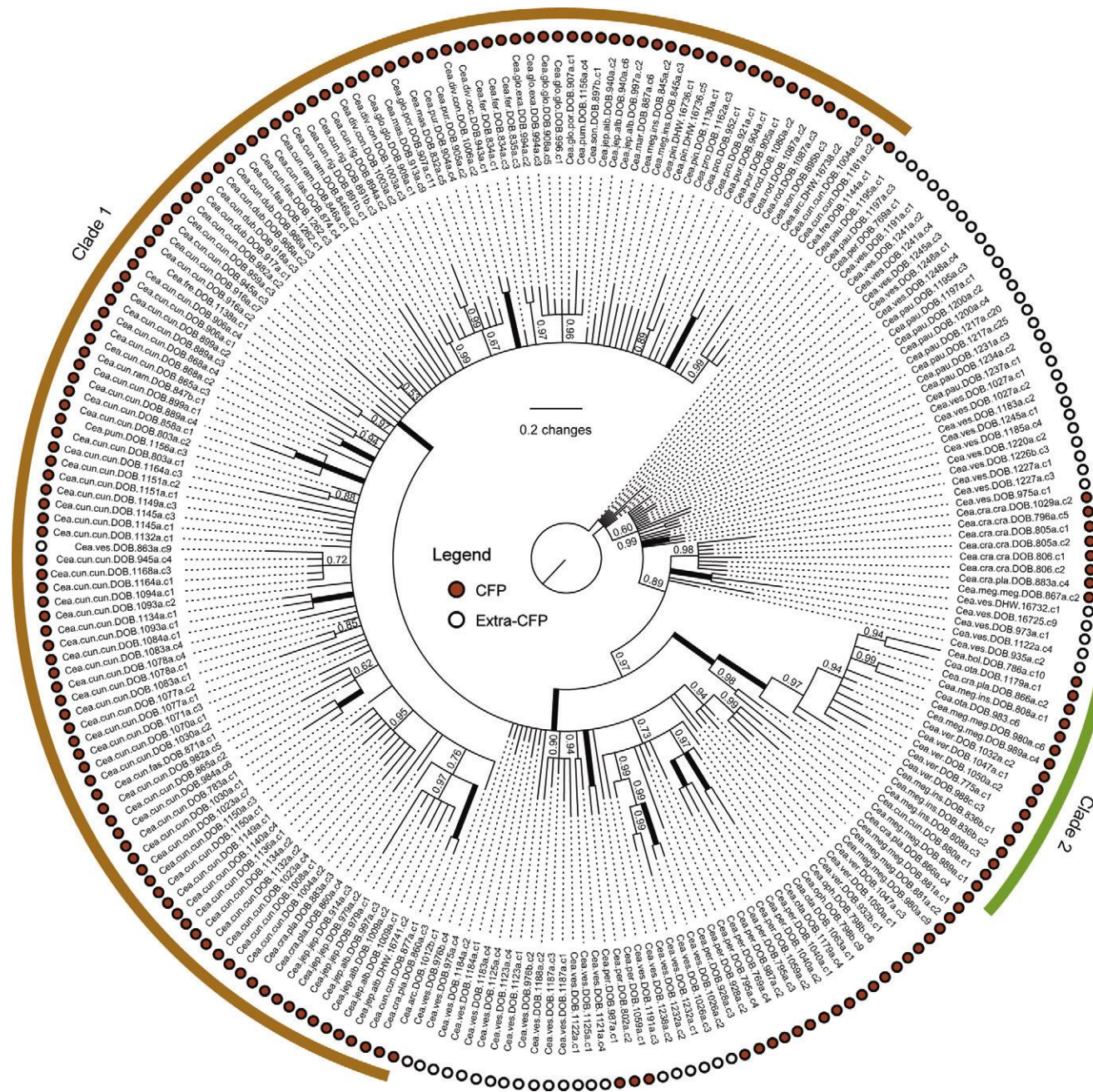
Fig. 2 Bayesian consensus phylogram for subgenus *Cerastes*, based on alignment of nitrate reductase (NIA) DNA sequences from subgenus *Cerastes* (alignment A4 in the online edition of the *International Journal of Plant Sciences*). The tree represents majority-rule consensus of post-burn-in trees with posterior probabilities (PP) above 0.5 plotted; thick branches indicate PP of 1.0. Terminal labels are abbreviated as taxon names in three-letter code followed by collector initials (DOB = D. O. Burge; DHW = D. H. Wilken), plant collection number, and NIA isolate number (cx), all separated by periods (app. A; table A3, in the online edition of the *International Journal of Plant Sciences*).

isolates from seven taxa (fig. 2, Clade 2; PP = 1.0). The sister group to Clade 2 contains many subclades or individual isolates in unresolved positions, including a large clade made up of 128 isolates obtained from 19 *Cerastes* species (fig. 2, Clade 1; PP = 1.0). Only 6 of the 82 plants from which two NIA sequence types were recovered have these isolates as each other's closest relatives (supported as sister with PP > 0.95). A large number of *Cerastes* isolates are not resolved in one

of the major clades. This group is hereafter referred to as "Unresolved *Cerastes*," although the group may represent a basal grade (fig. 2).

The NSN for the subgenus *Cerastes* NIA alignment contains 5489 edges connecting the 230 included sequences (fig. 3*A*; treefile A14). Of the 541 inferred splits within the network, 172 receive more than 50% bootstrap support, including 20 with 95% support or greater. Two groups identified

by these well-supported splits correspond to Clades 1 and 2 from Bayesian phylogenetic analysis (fig. 2). The split separating members of Clade 1 from remaining *Cerastes* receives bootstrap support of 51.4% (fig. 3*A*). However, the splits tree contains no single split corresponding to the divergence between Clade 2 and remaining *Cerastes*, as in the Bayesian tree (fig. 2). Among the 230 isolates, TCS identified 181 unique sequences types, 25 of which are represented by more than one sequence.

The statistical-parsimony gene genealogy inferred by TCS for subgenus *Ceanothus* is highly reticulate (treefile A15). Major clades identified in Bayesian trees (fig. 2) are supported; Clade 1 is connected to remaining *Cerastes* by three substitutions, at positions 76, 110, and 204; Clade 2 is connected by five substitutions, at positions 69, 98, 182, 289, and 437.

Subgenus Ceanothus *Phylogeny*

The three independent replicates of Bayesian analysis for subgenus *Ceanothus* phylogeny yielded trees with nearly identical topology. Only one run was used for final tree building, with 5% of sampled trees removed as burn-in. The resulting tree (fig. 4; treefile A16) contained 22 nodes resolved with PP above 0.50, including three nodes with PP of 1.0 (fig. 4). In this tree only one of the seven taxa represented by more than a single plant is monophyletic (*Ceanothus leucodermis* Greene). Instead, most taxa are polyphyletic, recovered in disparate clades, or unresolved. As in *Cerastes*, however, some species have a tendency to group together, though often as members of clades containing isolates from other taxa (e.g., *Ceanothus caeruleus* Lag.). Although only a handful of nodes are well resolved in the tree, one large clade is recovered (fig. 4, Clade 3; PP = 1.0). The bulk of diversity, however, is poorly resolved. Furthermore, none of the three individuals from which two NIA sequence types were recovered have isolates that are each other's closest relatives, although two pairs of isolates (*Ceanothus fendleri* A. Gray var. *fendleri*, E. Lyonnet 3593 and *Ceanothus incanus*, D.O. Burge 1273) are members of Clade 3 (fig. 4). As in *Cerastes*, a large number of isolates are not resolved as members of the major clade; this group is hereafter referred to as "Unresolved subgenus *Ceanothus*," although some members may have a closer relationship to Clade 3 than do others (fig. 4).

The NSN for the subgenus *Ceanothus* NIA alignment contains 1141 edges connecting the 55 included sequences (fig. 5*A*; treefile A17). Of the 159 inferred splits within the network, 58 receive more than 50% bootstrap support, including 21 with 95% support or better. The split separating members of Clade 3 from remaining subgenus *Ceanothus* has bootstrap support of 97.5%. Among the 55 isolates, TCS identified 54 unique sequence types; the sequence obtained from *Ceanothus buxifolius* Schult. DOB 759a was identical to an isolate obtained from *C. fendleri* A. Gray var. *venosus* Trel. EL 3593.

The statistical-parsimony gene genealogy inferred by TCS is highly reticulate (treefile A18). The major clade identified in Bayesian tree building (fig. 4, Clade 3) is also recovered in this analysis; Clade 3 is connected to remaining subgenus *Ceanothus* by eight substitutions, at positions 190, 195, 357, 447, 453, 512, 517, and 521.

Ceanothus *Phylogeny*

In ML tree building, the best *Ceanothus* tree had a log likelihood (GarliScore; Zwickl 2006) of −4965.67 (treefile A19). ML support values from 100 replicates of resampling were summarized in a 50% majority-rule consensus tree (treefile A20). The three independent replicates of Bayesian analysis for *Ceanothus* phylogeny yielded trees with nearly identical topology. Only one run was used for final tree building, with 5% of sampled trees removed as burn-in (treefile A21). Overall, the *Ceanothus* trees resulting from Bayesian and ML analysis support similar relationships. Subsequent discussion is based on the ML tree with reference to Bayesian PP for critical nodes. The ML tree comprises 88 nodes with bootstrap support above 50% (not counting basal divergence between subgenera), including six with PP of 1.0 (fig. 6). The ML tree supports relationships similar to those recovered in trees based on separate alignments for the two subgenera, although many of these groups receive lower levels of support (figs. 2, 4 vs. treefile A20). Major clades from the subgenus tree are also recovered in the *Ceanothus* tree, though with lower levels of statistical support in some cases; Clade 1 has a PP of 1.0 in the *Cerastes* tree (fig. 2) but a PP of 0.50 and ML bootstrap support of less than 50% in the *Ceanothus* tree (fig. 6). Clade 2 trees both yield a PP of 1.0 (figs. 2, 6), and Clade 3 has a PP of 1.0 in both the subgenus *Ceanothus* tree (fig. 4) and the *Ceanothus* tree (fig. 6); ML bootstrap support for this clade is correspondingly high (93%; fig. 6).

Recombination

The pairwise homoplasy PHI (Φ_w) statistic was 0.066 for the subgenus *Cerastes* NIA alignment (alignment A4) and 0.098 for the subgenus *Ceanothus* NIA alignment (alignment A6). Permutation tests did not detect statistically significant evidence for recombination on the basis of PHI ($P = 0.42$ for both subgenera). In subgenus *Cerastes*, no statistically significant peaks in Dss were detected across the alignment with either a 95% or a 99% significance point, indicating a lack of recombination break points. In subgenus *Ceanothus*, a single statistically significant peak in Dss was detected when a 95% significance point was used, but it was not detected at the more conservative 99% threshold. The significant break point occurred at base 555 in the subgenus *Ceanothus* alignment (alignment A6).

Divergence-Time Estimates

Mean divergence time between subgenus *Cerastes* and subgenus *Ceanothus* estimated by BEAST for rate-calibrated DTE (based on ITS for *Ceanothus* only) was 9.2 Ma (95% HPD [highest posterior density interval] = 0.3–26.0 Ma; table 4). Timing for onset of diversification in the two subgenera was offset, with a mean tMRCA of 5.3 Ma (95% HPD = 0.2–15.2) for subgenus *Cerastes* and 6.3 Ma (95% HPD = 0.3–17.7) for subgenus *Ceanothus* (table 4). Mean divergence time between subgenus *Cerastes* and subgenus *Ceanothus* estimated by BEAST for fossil-calibrated DTE (based on ITS, *trn*L-F and *rbc*L for Rhamnaceae) was 12.9 Ma (95% HPD = 4.2–22.1; table 4). For fossil-calibrated DTE, divergence time between
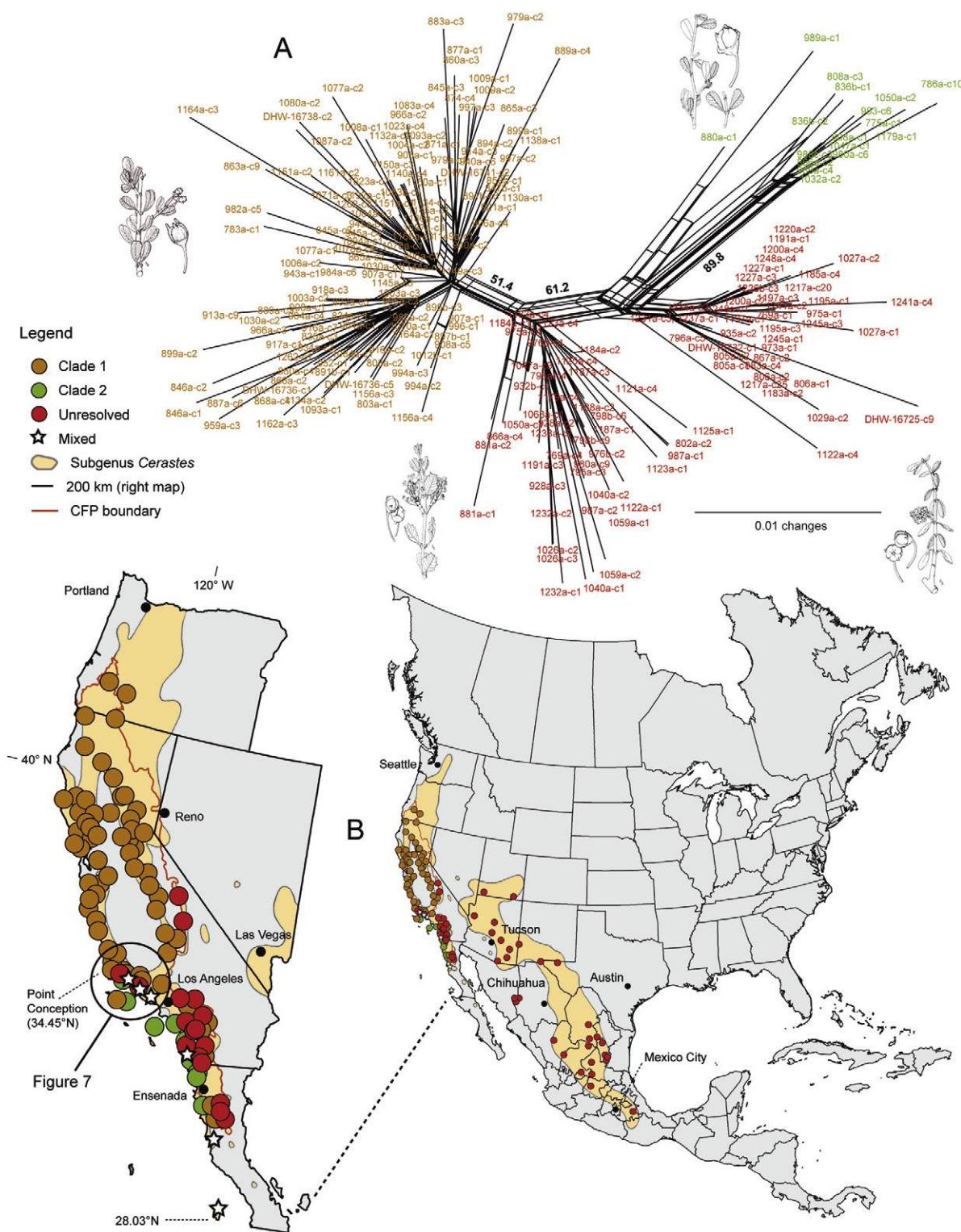
Fig. 3 Neighbor-net splits network and geographic map for subgenus *Cerastes*. *A*, Neighbor-net splits network colored according to major clade (Clades 1, 2) or "Unresolved" status; boostrap support values (%) cited in text are plotted adjacent to applicable splits. *B*, Distribution of subgenus *Cerastes*, with genetic sampling locations colored to match groups from *A*. "Mixed" individuals are those with with one nitrate reductase (NIA) isolate in Clade 1 or 2 and a second among the Unresolved group. Terminal labels are abbreviated as collector initials, plant collection number, and NIA isolate number (abbreviated cx), all separated by periods (app. A; table A3, in the online edition of the *International Journal of Plant Sciences*); terminals with collector initials missing were collected by D. O. Burge. See also treefile A14 in the online edition of the *International Journal of Plant Sciences*. CFP = California Floristic Province.
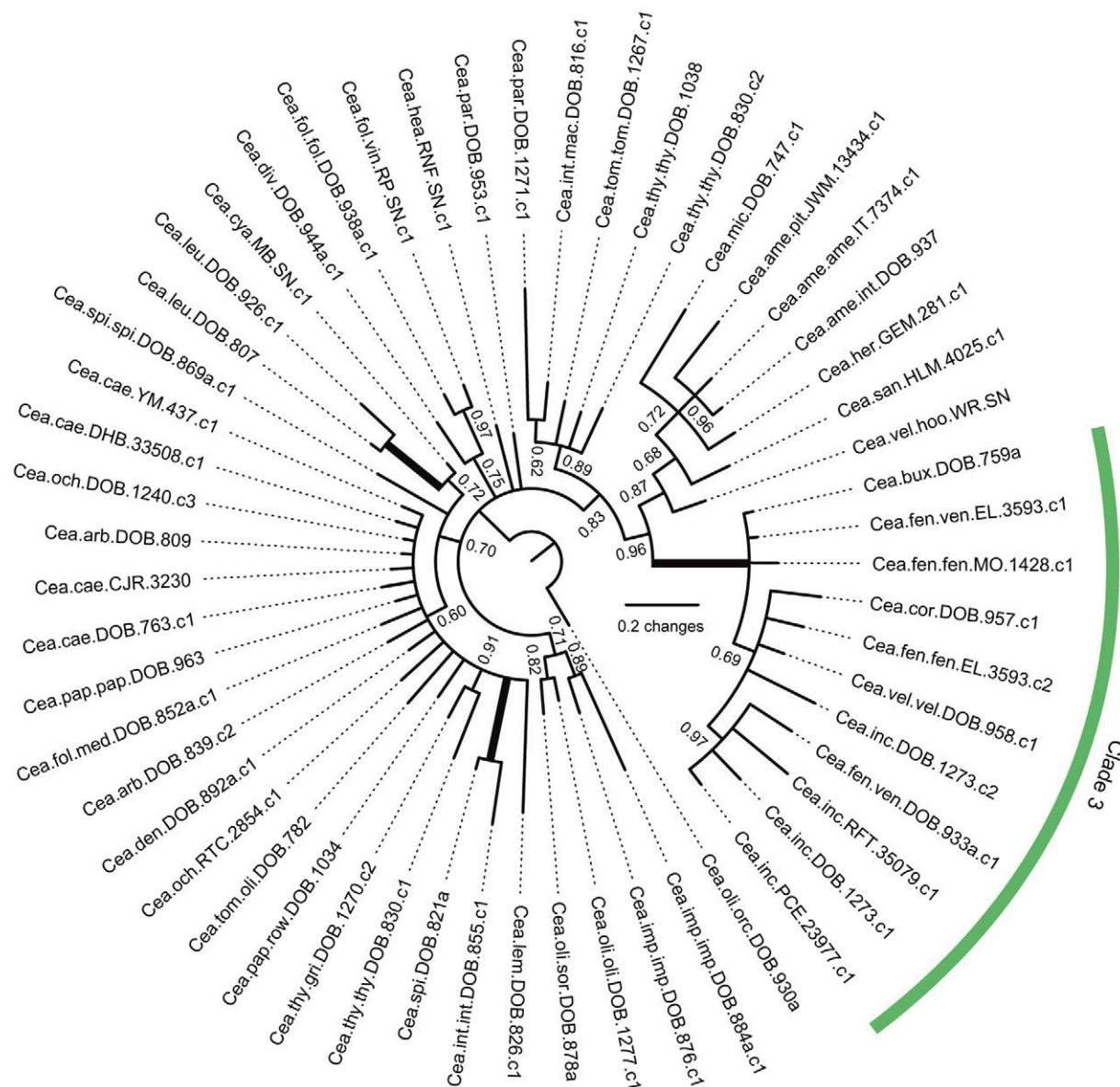
Fig. 4 Bayesian consensus phylogram for subgenus *Ceanothus*, based on alignment of nitrate reductase DNA sequences from subgenus *Ceanothus* (alignment A6 in the online edition of the *International Journal of Plant Sciences*). The tree represents majority-rule consensus of post-burn-in trees with posterior probabilities (PP) above 0.5 plotted; thick branches indicate PP of 1.0. Terminal labels are as in figure 2.

the subgenera inferred from nuclear (ITS) data alone (mean = 13.2 Ma; 95% HPD = 3.7–24.2) was similar to that inferred from the combined chloroplast and nuclear data. However, the divergence time from chloroplast data was younger than that from the combined data (mean = 8.0 Ma; 95% HPD = 1.0–17.8; table 4).

Bioclimate

Although elevation, latitude, and interpolated estimates for bioclimatic parameters vary widely in *Cerastes*, identifiable geographic trends exist (fig. 7). With respect to temperature, AAMin decreases with decreasing latitude (fig. 7*A*), although

the same pattern does not appear to hold for AAMax (fig. 7*B*). With respect to precipitation, a pattern of decreasing rainfall with decreasing latitude is found (fig. 7*C*). The climatic variables, however, must be examined in light of the relationship between elevation and latitude: there is a trend for plants found at lower latitudes to occur at higher elevations (fig. 7*D*).

A comparison of these variables with those for genetic groups in *Cerastes* reveals several trends (table 5). Clade 2 has the lowest average elevation, with successively higher average elevations in Clade 1 and Unresolved *Cerastes* (table 5; fig. 7*D*). For latitude, Clade 1 tends to occur farther north than Clade 2, which in turn occurs farther north than Unre-
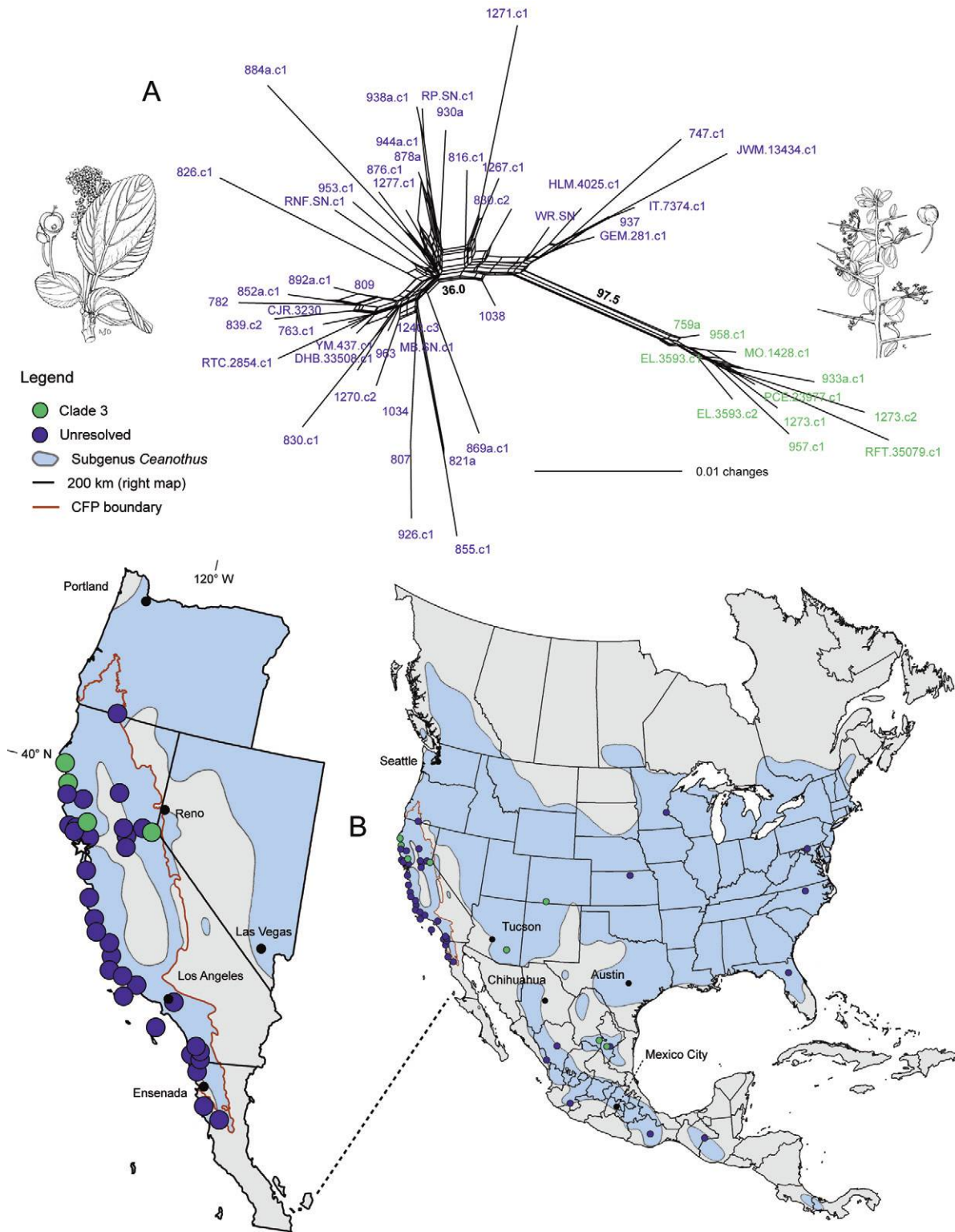
Fig. 5 Neighbor-net splits network and geographic map for subgenus *Ceanothus*. *A*, Neighbor-net splits network colored according to Clade 3 membership or "Unresolved" status; bootstrap support values (%) cited in text are plotted adjacent to applicable splits. *B*, Distribution of subgenus *Ceanothus*, with genetic sampling locations colored to match groups from *A*. Terminal labels are as in figure 3. See also treefile A18 in the online edition of the *International Journal of Plant Sciences*. CFP = California Floristic Province.
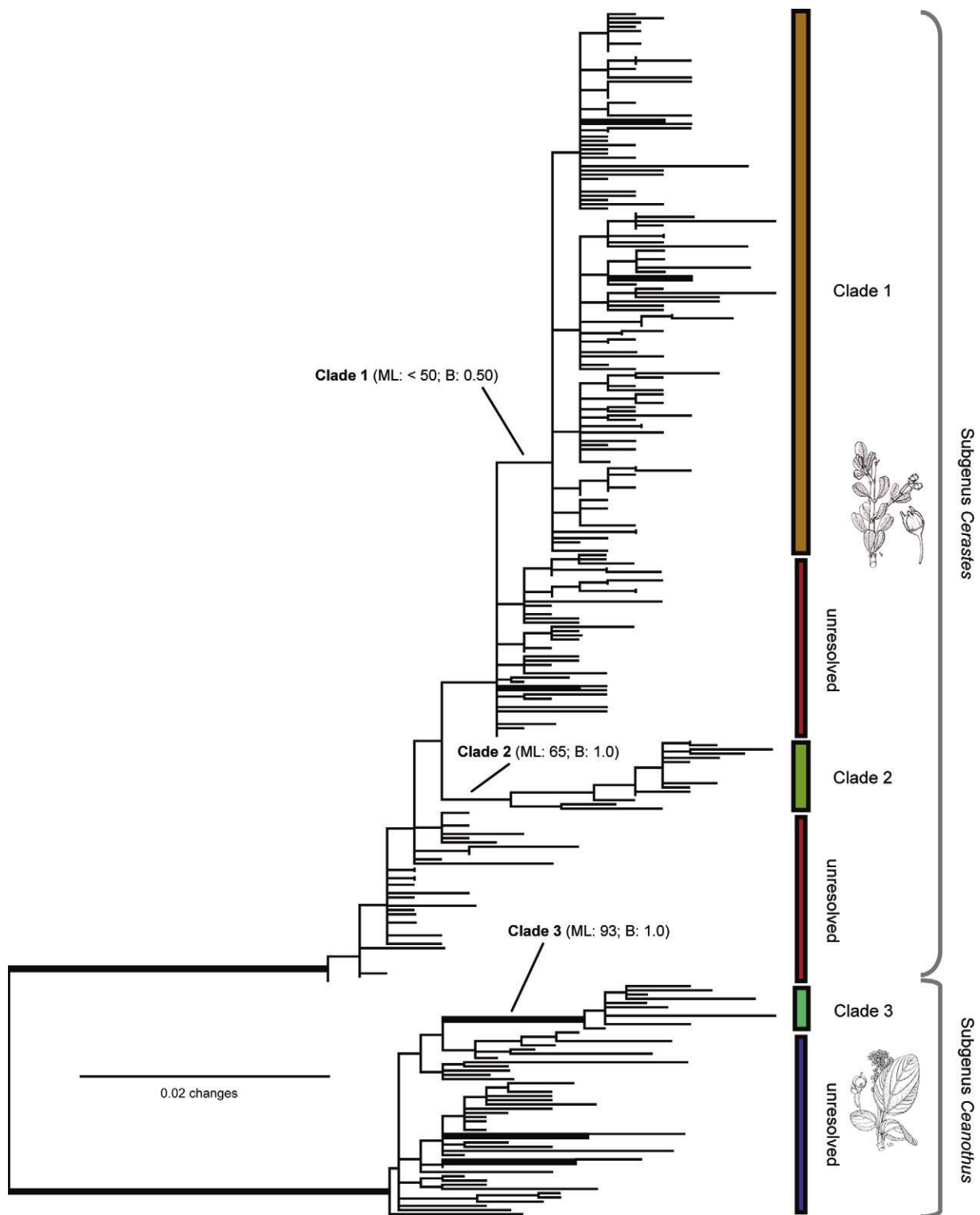
Fig. 6 Best maximum likelihood (ML) tree for *Ceanothus*, presented as a phylogram, based on alignment of nitrate reductase DNA sequences for all of *Ceanothus* (alignment A8 in the online edition of the *International Journal of Plant Sciences*). Thick branches indicate ML bootstrap support above 90% and Bayesian posterior probability (PP) of 1.0. The tree is manually rooted on the basis of reciprocal monophyly of *Ceanothus* subgenera; terminal names are omitted for clarity (see treefiles A19–A21 in the online edition of the *International Journal of Plant Sciences*). Major clades and unresolved regions cited in text (Clades 1–3; unresolved *Cerastes* and *Ceanothus*) are indicated with labeled lines and colored bars. Arrows are labeled with ML bootstrap support values followed by Bayesian (B) PP values.

Table 4

Divergence-Time Estimates in *Ceanothus*

			Time to most recent common ancestor (Myr)[a]		
Study	Data	Calibration[b]	*Ceanothus*	Subgenus *Ceanothus*	Subgenus *Cerastes*
Jeong et al. 1997	*rbc*L	Substitution	18–39	...	...
Calsbeek et al. 2003	ITS	Substitution	13.9	...	...
Richardson et al. 2004	*rbc*L, *trn*L-F	Fossil	10.7–11.1	...	...
Ackerly et al. 2006	ITS	Substitution	...	<4–5	<4–5
Present study	ITS	Substitution	9.2 (.3–26.0)[c]	6.3 (.2–17.7)[c]	5.3 (.2–15.2)[c]
Present study	ITS, *rbc*L, *trn*L-F	Fossil	12.9 (4.2–22.1)[c]	...	...
Present study	Nuclear[d]	Fossil	13.2 (3.7–24.2)[c]	...	...
Present study	Chloroplast[d]	Fossil	8.0 (1.0–17.8)[c]	...	...

[a] Ellipses indicate that node age was not inferred by relevant study.

[b] Data used to calibrate divergence-time estimation: "substitution" refers to methods that used an estimate for rate of nucleotide substitution; "fossil" refers to methods that used fossil material.

[c] Intervals following date indicate 95% highest posterior density interval from BEAST (Drummond et al. 2006; Drummond and Rambaut 2007) for that date.

[d] Divergence-time estimates using separate nuclear (ITS, the ITS1-5.8S-ITS2 region of the nuclear ribosomal DNA) and chloroplast (*rbc*L, *trn*L-F) portions of the three-region data set.

solved *Cerastes* (table 5). The lowest average AAMin is found among Unresolved *Cerastes*; AAmin is successively higher in Clades 1 and 2 (table 5; fig. 7*A*). Average AAMax has the opposite pattern: highest among Unresolved *Cerastes* and successively lower in Clades 1 and 2 (table 5; fig. 7*B*). Finally, average AAP is slightly higher in Clade 1 than in the other groups (table 5; fig. 7*C*).

Discussion

Subgeneric Divergence

We obtained additional support for deep phylogenetic divergence between the *Cerastes* and *Ceanothus* subgenera (fig. 6). This divergence is fundamental to understanding the systematics, biology, and ecology of *Ceanothus*, because it contrasts with the more recent diversification that has taken place within each subgenus (Ackerly et al. 2006; fig. 6). Morphology, physiology, and life history, which are strongly divergent between subgenera, support the genetic divergence (Nobs 1963; Keeley 1975, 1987; Fross and Wilken 2006). Ackerly et al. (2006) suggest that these very different adaptive modes have played an important role in the codiversification of the *Ceanothus* subgenera by facilitating coexistence of species from both groups in the same habitats. Most research concerning *Ceanothus* will benefit from appreciation of the strong divergence between the subgenera as well as the close relationship among species belonging to the same subgenus. A lack of appreciation for this evolutionary contrast may lead to confusion, as in the work of Calsbeek et al. (2003), where timing of divergence between subgenera was confounded with timing of diversification within subgenera.

Diversification versus Gene Flow in the Ceanothus Subgenera

Ceanothus has been cited as an example of a plant group in which diversification, and presumably speciation, has taken place in the absence of intrinsic barriers to gene flow (Fross and Wilken 2006). This idea derives from the long-noted propensity for hybridization among species within subgenera of *Ceanothus* (Parry 1889; Nobs 1963; Fross and Wilken 2006) as well as from taxonomic, biosystematic, and molecular genetic research that directly addressed the problem of how hybridization and gene flow might have influenced diversification of *Ceanothus* (McMinn 1942; Nobs 1963; Hardig et al. 2002). Research conducted by McMinn (1944) and Nobs (1963) showed that no intrinsic pre- or postzygotic isolating mechanisms exist among members of the same subgenus. These results indicate that long-observed patterns of morphological overlap among taxa within subgenera might be explained by hybridization and gene flow (McMinn 1944; Nobs 1963). Later phylogenetic research discovered a lack of congruence between chloroplast and nuclear gene trees for each subgenus (Hardig et al. 2000), as well as strong geographic, rather than taxonomic, signal, both of which are consistent with hybridization and/or gene flow. Here we report related patterns, including a lack of phylogenetic cohesion among NIA isolates obtained from the same taxon or plant (figs. 2, 4). Similar patterns, however, are also expected to result from incomplete lineage sorting (Pamilo and Nei 1988), a phenomenon that often occurs during rapid diversification (Maddison and Knowles 2006).

Subgenus Cerastes Phylogeny

Our work confirms some previously identified relationships among taxa within subgenus *Cerastes* and supports new groupings. The first new group is Clade 1 (fig. 2), which is the subject of a separate, more focused study (Burge and Manos 2011). Clade 1 encompasses all NIA isolates from populations of *Cerastes* occurring within the CFP north of the latitude of Point Conception, Santa Barbara County, California (34.45°N; fig. 8*B*), as well as those found north of the CFP in Oregon (fig. 3*A*). Clade 1 contains the bulk of taxonomic diversity for subgenus *Cerastes* (24 of 35 sampled
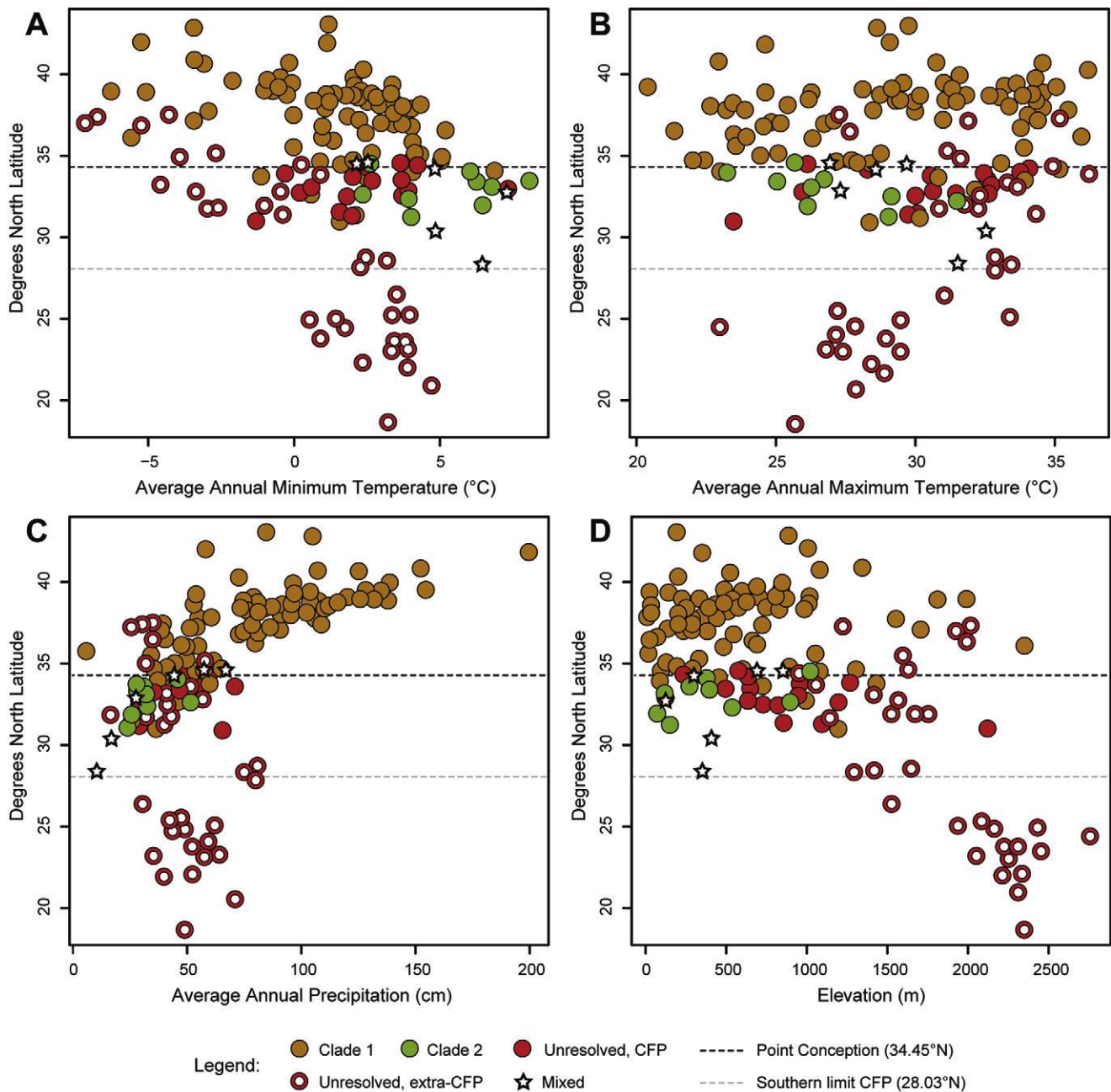
Fig. 7 Biplots of climatic and distributional data for subgenus *Cerastes*. *A*, Average annual minimum temperature versus latitude. *B*, Average annual maximum temperature versus latitude. *C*, Average annual precipitation versus latitude. *D*, Elevation versus latitude. All points are colored according to groups discussed in text. The latitudes of Point Conception, Santa Barbara County, California (34.45°N), and the southern tip of Isla Cedros, Baja California, Mexico (28.03°N; southern point of the California Floristic Province [CFP]), are plotted as dashed lines.

taxa) and is almost entirely restricted to the CFP. Clade 1 may partly correspond to the "North Coast Clade" noted by Hardig et al. (2000) as a result of their work using ITS. A second unusual new grouping is Clade 2 (fig. 2), which is entirely restricted to coastal portions of the southern CFP (fig. 3*B*) and includes isolates from many *Cerastes* taxa characteristic of this region, particularly the coastal species *Cerastes megacarpus* Nutt. and *Cerastes verrucosus* Nutt. This highly divergent clade may be indicated by a weakly supported

grouping between *C. megacarpus* and *C. verrucosus* in the work of Hardig et al. (2000).

Clades 1 and 2 are nested within a poorly resolved basal grade of isolates that we have termed "Unresolved *Cerastes*" (see "Results," fig. 2, and Unresolved in fig. 3*A*). This portion of the tree is overwhelmingly made up of isolates from plants collected in the southern CFP, the southwestern United States, and Mexico. It includes all isolates from non-CFP areas outside of Oregon (fig. 3*B*). The basalmost portion of

Table 5

Distributional and Bioclimatic Summary Statistics for Subgenus *Cerastes*

Group	Elevation (m)	Latitude (°N)	AAMin (°C)	AAMax (°C)	AAP (cm)
Clade 1	610 ± 475	37.5068 ± 2.3785	1.5 ± 4.1	29.3 ± 2.4	81.3 ± 34.9
Clade 2	429 ± 337	32.9520 ± 1.0345	5.2 ± 2.5	27.0 ± 1.0	38.9 ± 14.1
Unresolved	1515 ± 662	30.1906 ± 4.9413	1.0 ± 3.1	30.4 ± 4.9	48.0 ± 14.6
Mixed	463 ± 269	32.4741 ± 2.5710	4.7 ± 2.3	29.5 ± 2.6	38.5 ± 22.7

Note. Group means genetic groups of subgenus *Cerastes* mentioned in text. AAMin = average annual minimum temperature; AAMax = average annual maximum temperature; AAP = average annual precipitation (see "Material and Methods" for more information). All statistics are given as mean ± standard deviation.

this grade contains all isolates from the Mexican endemic *Cerastes pauciflorus* and most isolates from Mexican collections of *Cerastes vestitus* (fig. 3*A*). Interestingly, Hardig et al. (2000) recovered a basal placement for the sample of Mexican *Cerastes* included in their ITS-based work, *Cerastes lanuginosus* (Jones) Rose, a synonym of *C. pauciflorus* (Fross and Wilken 2006). Higher portions of the basal *Cerastes* grade contain isolates from *C. vestitus* as well as several species endemic to the southern CFP (fig. 3). Interpretation of relationships in Unresolved *Cerastes* should be treated with caution, because these relationships are based on ad hoc manual rooting using the basalmost terminal in the tree containing all of *Ceanothus* (fig. 6).

In our phylogenies, most *Cerastes* taxa are polyphyletic, with few cases of strong genetic cohesion among sampled individuals at the level of species, variety, or subspecies (fig. 2). This result agrees with the ITS-based work of Hardig et al. (2000, 2002). In our work, several taxa are shared between major genetic groups. In *Cerastes cuneatus*, for example, a single isolate is recovered as part of Clade 2; all other isolates from this species are in Clade 1 (fig. 2). The isolate recovered in Clade 2 was obtained from a plant collected in the Santa Ynez Mountains, an area of overlap and potential hybridization between the taxa characteristic of Clades 1 and 2 (fig. 2, DOB 880a; figs. 3, 8; but see above).

Subgenus Ceanothus *Phylogeny*

As in subgenus *Cerastes*, phylogenetic patterns in subgenus *Ceanothus* are dominated by a handful of genetic groups. Clade 3 is the most obvious grouping in subgenus *Ceanothus* (fig. 4). Clade 3 includes isolates from populations of several taxa collected over a very wide region in western North America (figs. 4, 5). With the exception of *Ceanothus velutinus* Hook., this group is unified by the trait of spine-tipped twigs (fig. 4), which are found in just two other taxa (Fross and Wilken 2006). A group comparable to Clade 3 was not identified by the work of Hardig et al. (2000). However, Jeong et al. (1997) recovered a close relationship between two spinescent species, albeit with a much smaller overall sampling of taxa. A second, weakly supported (PP = 0.87) clade of isolates closely allied to Clade 3 (fig. 4) is made up of eastern and western North American taxa (table 1). While the work of Hardig et al. (2000) did not recover this grouping, Jeong et al. (1997) found a close relationship between *Ceanothus americanus* L. and *Ceanothus sanguineus* Pursh. In our tree, the remaining terminals, which come almost en-

tirely from plants collected in the CFP and Mexico, are not well resolved (fig. 4; Unresolved in fig. 5). This group represents the bulk of taxonomic diversity in subgenus *Ceanothus* (Fross and Wilken 2006).

As in subgenus *Cerastes*, many taxa from subgenus *Ceanothus* are polyphyletic (fig. 4). Hardig et al. (2000) discovered similar patterns in subgenus *Ceanothus*. Of the seven taxa represented in our analysis by more than one plant (table 1), only one (*Ceanothus leucodermis*) is monophyletic. In *C. velutinus*, for example, one population is recovered in Clade 3 and the other in Unresolved subgenus *Ceanothus* (fig. 4, DOB 958 and WR SN). *Ceanothus velutinus* is the only nonspinescent member of Clade 3. The isolate recovered in Clade 3 is from a plant collected in the central Sierra Nevada, an area of overlap and potential hybridization between *C. velutinus* and *Ceanothus cordulatus* Kellogg (Fross and Wilken 2006), which may explain the presence of isolates from the former species in otherwise entirely spinescent Clade 3 (figs. 4, 5).

Biogeographic Boundaries

In the *Cerastes* subgenus of *Ceanothus*, a strong north-south geographic break between major genetic groups across the Transverse Ranges near the latitude of Point Conception (35.45°N; figs. 6, 8) is consistent with separate histories of diversification for *Cerastes* in climatically divergent regions of the CFP. Point Conception is in a region of climatic shift (Russell 1926; Kesseli 1942) that has long been recognized on the basis of latitudinal range studies in benthic marine fauna (Valentine 1966) and strand flora (Breckon and Barbour 1974; Barbour and Johnson 1988). The climatic boundary near Point Conception appears to be driven by shifts in ocean currents at this latitude (Hickey 1979; Huyer 1983; Münchow 2000; fig. 8). The ecofloristic boundary at the latitude of Point Conception also roughly corresponds to the boundary between two large areas of Coast Range endemism defined by Stebbins and Major (1965). Furthermore, recent phytogeographical classification schemes based on floristic analysis of vascular plant distributions on the North American Pacific Coast place the boundary between two coastal Mediterranean-climate zones at the latitude of Point Conception (Peinado et al. 2009).

The latitude of Point Conception represents the approximate northern limit of CFP distribution for Clade 2 and Unresolved *Cerastes* and the southern limit for all but one member of Clade 1 (fig. 8). However, the zone of geographic overlap
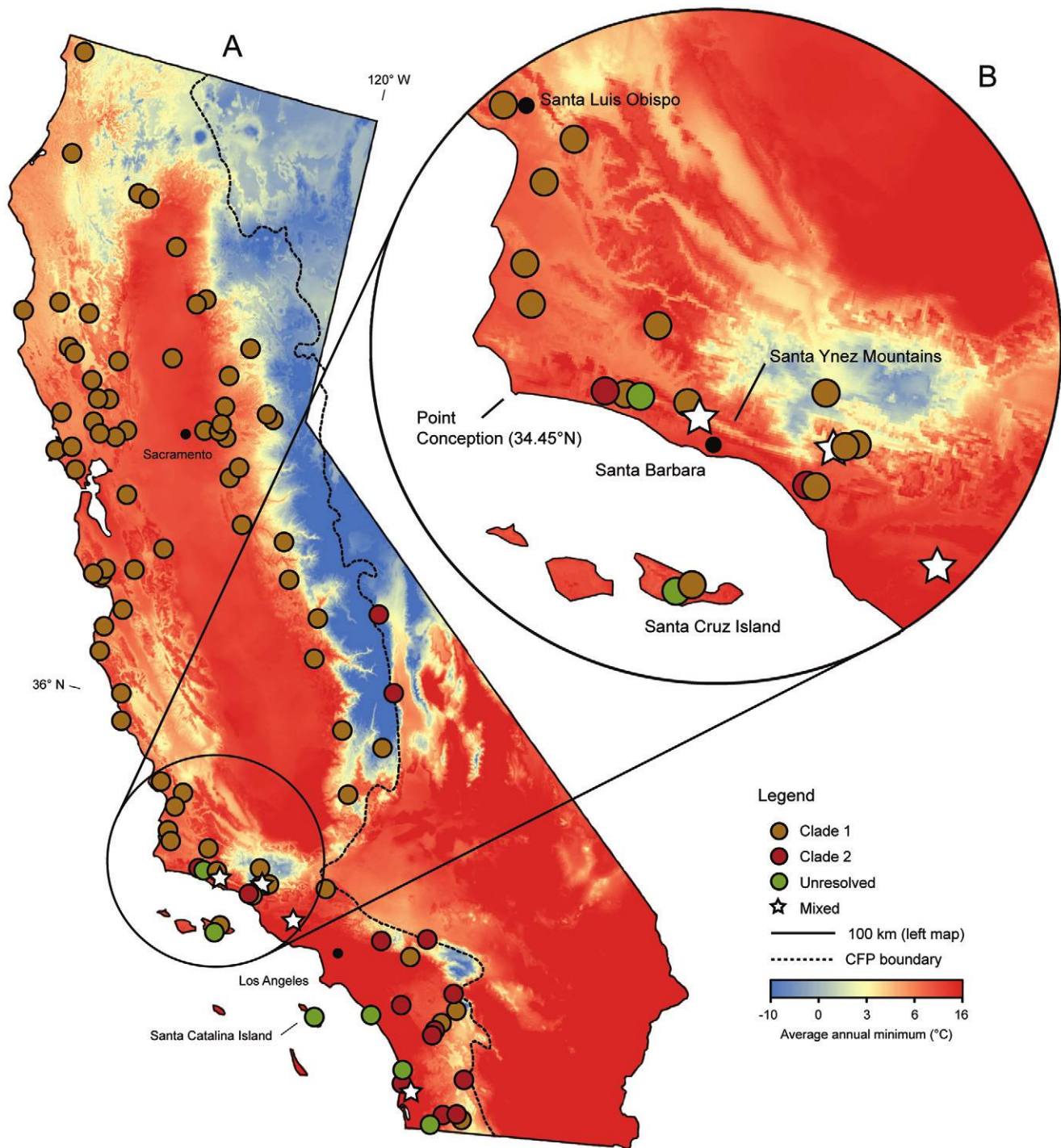
Fig. 8 Map of average annual minimum temperature (AAMin) for California, with overlay of subgenus *Cerastes* genetic sampling; temperature data layer from PRISM Climate Group (2006). Inset shows northern part of geographic overlap among major genetic groups of *Cerastes* discussed in text. Genetic sampling locations are colored according to major genetic group (see fig. 3). Color shading represents approximate AAMin. CFP = California Floristic Province.

between these groups extends as far south as northern Baja California, Mexico, along the arc of the Transverse and Peninsular ranges (figs. 3, 8). Within this latitudinal band of the CFP (28.0°–34.5°N; fig. 7), members of Clade 1 (*C. cuneatus* var. *cuneatus*) and Unresolved *Cerastes* tend to occupy higher elevations than members of Clade 2 (fig. 7D), with correspondingly lower AAmin (fig. 7A). The apparent higher tolerance for colder temperatures in Clade 1 and Unresolved
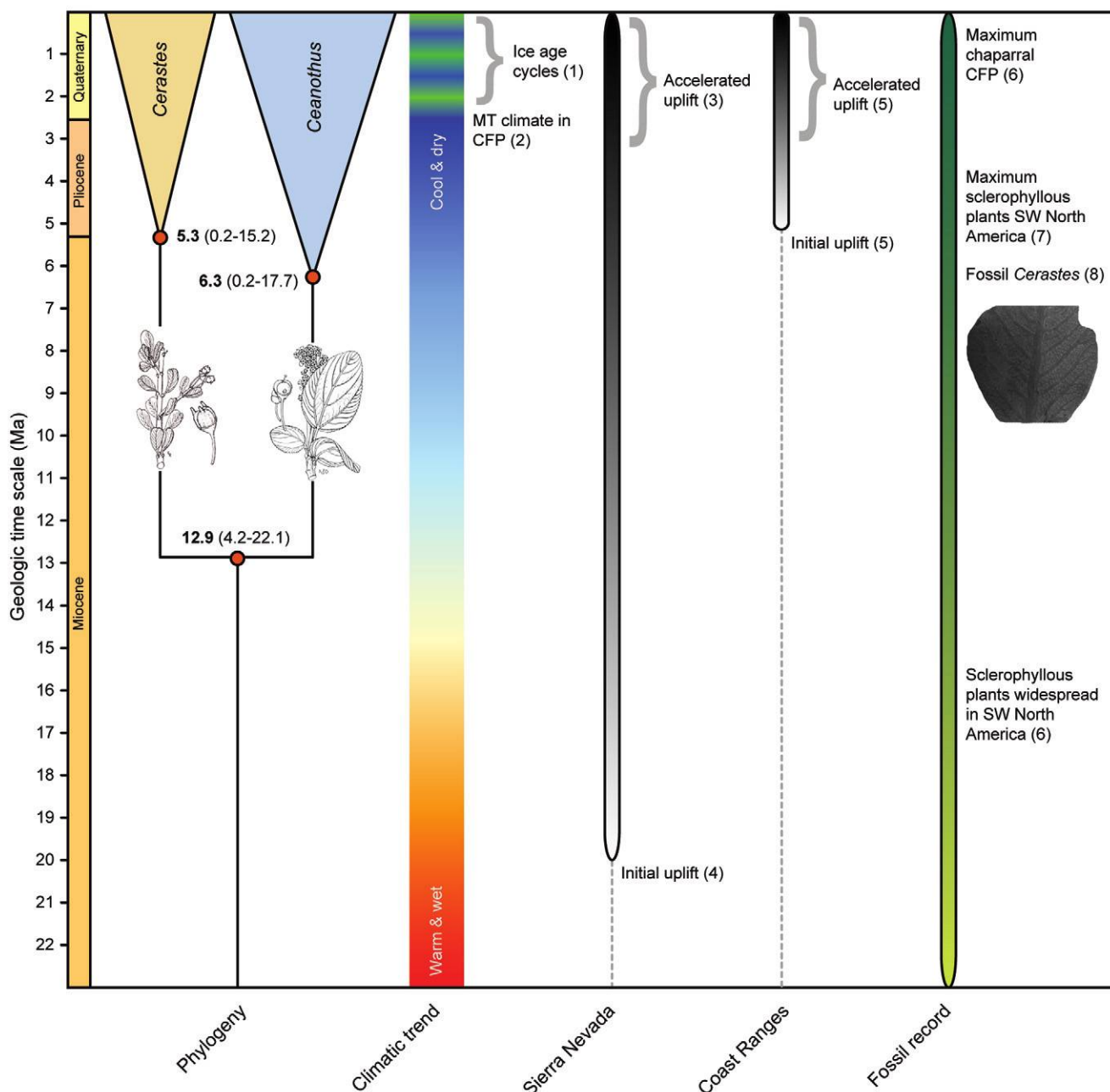
Fig. 9 Lineage-through-time schematic for diversification of *Ceanothus*. Chronology reflects the summary of Schorn et al. (2007) for the Neogene. Numbers adjacent to nodes on tree schematic represent mean time to most recent common ancestor (Ma), followed by 95% highest posterior density interval from BEAST (table 4). Numbers in parentheses refer to the following source: 1, Chappell (1978); 2, Raven (1973); 3, Wakabayashi and Sawyer (2001); 4, Mix et al. (2011); 5, Argus and Gordon (2001); 6, Axelrod (1973); 7, Axelrod (1948); 8, Axelrod (1950).

Cerastes, compared to that in Clade 2, is corroborated by studies of chaparral zonation in the Santa Monica Mountains (Davis et al. 2007) as well as by experimental studies in which taxa characteristic of Clade 1 and Unresolved *Cerastes* (*C. cuneatus* and *Cerastes crassifolius* Torr., respectively) were found to have a much higher resistance to xylem embolism under freeze/thaw stress than a species characteristic of Clade 2 (*C. megacarpus*; Ewers et al. 2003; Davis et al. 2007). In the southern CFP zone of geographic overlap

among major genetic groups of *Cerastes*, these physiological differences appear to drive zonation of species (Davis et al. 2007), with *C. megacarpus* occupying areas less prone to freezing, such as lowland areas close to the coast and very steep upland areas, while both *C. cuneatus* and *C. crassifolius* occur in areas at greater risk of very cold conditions, including swales and ravine bottoms (Davis et al. 2007). It is possible that these physiological differences are also at work in driving the large-scale distribution of *Cerastes*

across the climatic boundary represented by the northern Transverse Ranges.

Diversification of Ceanothus in the CFP

Our results indicate that the initial divergence of the *Ceanothus* subgenera occurred in the late Miocene (~13 Ma; fig. 9). The late Miocene was a time of unusually cool and dry climatic conditions worldwide (Graham 1999) as well as widespread mountain building in western North America (Mix et al. 2011; fig. 9). Mountain uplift, particularly of the Sierra Nevada, cast a rain shadow over parts of interior western North America that led to development of arid conditions (Wernicke et al. 1996; Mulch et al. 2008; Mix et al. 2011). The initial divergence of the *Ceanothus* subgenera may have taken place in response to such ecological opportunities.

Results presented here indicate that diversification of the two *Ceanothus* subgenera commenced nearly simultaneously near the beginning of the Pliocene (~6 Ma; fig. 9). The early Pliocene was the coolest, driest part of the Cenozoic worldwide (reviewed in Graham 1999), and it corresponds to the onset of uplift for the North American Coast Ranges, including the Transverse and Peninsular ranges (Argus and Gordon 2001; J. Wakabayashi personal communication, December 2010; but see Page 1981; Namson and Davis 1988; Page et al. 1998). Uplift of the Coast Ranges combined with faulting and tectonic movement to expose diverse geology and create new, discontinuously arrayed habitats (Kruckeberg 1984). These conditions are thought to have driven rapid plant speciation (Stebbins and Major 1965; Raven 1973; Raven and Axelrod 1978; Stebbins 1978*a*, 1978*b*; Peinado et al. 2009) and may have contributed to diversification of the *Ceanothus* subgenera, both of which have centers of diversity in the Coast Ranges (table 1). The beginning of the Pliocene is also when reliably identifiable *Ceanothus* fossils are first recorded (Axelrod 1950; Fisk and Myers 2008; D. O. Burge and D. M. Erwin, unpublished manuscript; fig. 9). These leaf impressions were recovered from the late Miocene Anaverde Formation of Antelope Valley, California (Axelrod 1950; figs. 1, 9). The plants were part of a community that experienced year-round rainfall (Axelrod 1950), which is consistent with the idea that morphological characteristics of *Cerastes*, once supposed to represent de novo adaptations to Mediterranean-type climate (e.g., sclerophylly and stomatal crypts), are in fact preadaptations that evolved before such a climate developed in western North America around 2 Ma (fig. 9; Axelrod 1958; Ackerly 2004).

Although our results indicate that diversification of the *Ceanothus* subgenera began several million years before the advent of a Mediterranean-type climate in California, it is possible that more recent diversification has taken place in response to this unusual climatic regime (fig. 9). During the Quaternary, glacial cycles probably drove the alternation of Mediterranean-type climatic conditions with cooler, wetter conditions (Herbert et al. 2001), leading to cycles of geographic range expansion and contraction in many groups of plants. During the same time period, uplift was ongoing in the Coast Ranges (Page 1981; Wakabayashi 1999) and possibly portions of the Sierra Nevada (3.2 Ma–present; fig. 9; Huber 1981; Wakabayashi and Sawyer 2001; but see Henry 2009

and references therein; Mix et al. 2011), which resulted in the extreme topographic and edaphic complexity that is seen in the region today. As previous authors have suggested, recent diversification of the two *Ceanothus* subgenera, as well as other CFP diversifications, may have resulted from the combined action of population fragmentation and local adaptation taking place in the milieu of climatic cycles and recent geological upheaval during the Quaternary (Wells 1969; Raven 1973; Stebbins 1978*a*; Raven and Axelrod 1978).

Summary

Ceanothus diversity reaches its peak in the CFP, with 72% of described species endemic to the region, including 21 species from subgenus *Cerastes* and 17 from subgenus *Ceanothus* (table 1). Our results indicate that nearly simultaneous diversification of the two *Ceanothus* subgenera began in the early Pliocene (~6 Ma; fig. 9), with regional patterns of differentiation that are particular to each group, though focused in the CFP. This timing agrees with the fossil record for *Ceanothus* but predates the hypothesized Quaternary (2-Ma) origin of Mediterranean-type climate in the region. Furthermore, a strong north-south divergence of subgenus *Cerastes* across the Transverse Ranges in southern California indicates that phylogenetic relationships in this subgenus may be structured by climatically divergent regions of the CFP.

Acknowledgments

Appendix A

Sampled *Ceanothus* Populations

For each sampled population (see table 1), the format is as follows: collector name and number (herbarium of voucher specimen deposition), description of locality, county (if applicable), state, country (if other than the United States); GenBank accession number for first NIA sequence, GenBank accession number for second NIA sequence (if applicable); GenBank accession number for ITS (if applicable); GenBank accession number for *trn*L-F (if applicable); clade membership according to major clades discussed in text. Separate sections are presented for subgenus *Cerastes* and subgenus *Ceanothus*. Within subgenus, order is by taxon; within taxa, populations are in order by collection number. Data summarized here may also be found in table A3. Please see the collection notes of Dylan O. Burge (http://purl.oclc.org/net/dylan-o-burge-field-notes) for details on collection localities, ecology, additional herbarium depositions, maps, and photographs.

Subgenus *Cerastes*

C. arcuatus McMinn—D.H. Wilken 16738 (DUKE), Titlow Road, SW of Horse Mountain, Humboldt Co., CA; NIA-c2: HQ325387; ITS: HQ325351; Clade 1. D.O. Burge 1012b (DUKE), Robbs Peak, El Dorado Co., CA; NIA-c1: HQ325388; Clade 1. *C. bolensis* **S. Boyd & J. Keeley**—D.O. Burge 786a (DUKE), Cerro Bola, Baja California, Mexico; NIA-c10: HQ325389; ITS: HQ325352; Clade 2. *C. crassifolius* **Torr. var. *crassifolius***—D.O. Burge 796a (DUKE), SW of Vail Lake, Riverside Co., CA; NIA-c5: HQ325391. D.O. Burge 805a (DUKE), Santa Ana Mountains, USFS Road 3S04, Orange Co., CA; NIA-c1: HQ325392, NIA-c2: HQ325393. D.O. Burge 806a (DUKE), San Gabriel Mountains, between Horse Canyon and Oak Canyon, Los Angeles Co., CA; NIA-c1: HQ325394, NIA-c2: HQ325395. D.O. Burge 1029a (DUKE), Sierra San Pedro Mártir, Baja California, Mexico; NIA-c2: HQ325390; ITS: HQ325353. *C. crassifolius* **Torr. var. *planus* Abrams**—D.O. Burge 860a (DUKE), Hwy 33 and Rose Valley Road, Ventura Co., CA; NIA-c3: HQ325396, NIA-c4: HQ325397; ITS: HQ325354; Clade 1. D.O. Burge 866 (DUKE), North Fork Matilija Creek, roadside on Hwy 33, Ventura Co., CA; NIA-c2: HQ325398, NIA-c4: HQ325399; Mixed (Clade 2 and Unresolved *Cerastes*). D.O. Burge 883a (DUKE), Santa Ynez Mountains, East Camino Cielo Road, Santa Barbara Co., CA; NIA-c3: HQ325400, NIA-c4: HQ325401; Mixed (Clade 1 and Unresolved *Cerastes*). *C. cuneatus* **Nutt. var. *cuneatus***—D.O. Burge 783a (DUKE), Sierra San Pedro Mártir, Baja California, Mexico; NIA-c1: HM240337; Clade 1. D.O. Burge 803a (DUKE), San Jacinto Mountains, Chimney Flats Road, Riverside Co., CA; NIA-c1: HQ325404, NIA-c2: HQ325405; Clade 1. D.O. Burge 858a (DUKE), Santa Lucia Range, Nacimiento-Fergusson Road, Monterey Co., CA; NIA-c1: HM240338; Clade 1. D.O. Burge 865a (DUKE), North Fork Matilija Creek drainage, roadside on Hwy 33, Ventura Co., CA; NIA-c2: HQ325406, NIA-c3: HQ325407; Clade 1. D.O. Burge 868a (DUKE), W shore of Lake Casitas, Ventura Co., CA; NIA-c2: HQ325408, NIA-c4: HQ325409; Clade 1. D.O. Burge 877a (DUKE), Santa Ynez River watershed, roadside on Paradise Road, San Luis Obispo Co., CA; NIA-c1: HQ325410; Clade 1. D.O. Burge 880a (DUKE), Santa Ynez Mountains, West Camino Cielo Road, Santa Barbara Co., CA; NIA-c1: HQ325411; Clade 2. D.O. Burge 889a (DUKE), Botchers Gap, Monterey Co., CA; NIA-c3: HQ325412, NIA-c4: HQ325413; Clade 1. D.O. Burge 899a (DUKE), Vaca Mountains, S of East Mitchel Canyon, Napa Co., CA; NIA-c1: HM240339, NIA-c2: HM240340; Clade 1. D.O. Burge 906a (DUKE), Vaca Mountains, Blue Ridge, Solano Co., CA; NIA-c1: HQ325414, NIA-c4: HQ325415; Clade 1. D.O. Burge 916a (DUKE), Mount Diablo, Contra Costa Co., CA; NIA-c2: HM240341, NIA-c7: HM240342; Clade 1. D.O. Burge 945a (DUKE), Elk Mountain Road, Lake Co., CA; NIA-c3: HQ325416, NIA-c4: HQ325417; Clade 1. D.O. Burge 959a (DUKE), Santa Lucia Mountains, Arroyo Grande Creek watershed, San Luis Obispo Co., CA; NIA-c3: HM240343; Clade 1. D.O. Burge 982a (DUKE), Tucalota Creek watershed, roadside on Sage Road, Riverside Co., CA; NIA-c2: HM240344, NIA-c5: HM240345; Clade 1. D.O. Burge 984a (DUKE), Morena Valley, San Diego Co., CA; NIA-c6: HM240346; Clade 1. D.O. Burge 1004a (DUKE), Del Puerto Canyon, Stanislaus Co., CA; NIA-c2: HQ325402, NIA-c3: HQ325403; ITS: HQ325355; Clade 1. D.O. Burge 1008a (DUKE), Fourmile Glade, Lake Co., CA; NIA-c1: HM240295; Clade 1. D.O. Burge 1023a (DUKE), Durock Road, El Dorado Co., CA; NIA-c4: HM240297, NIA-c7: HM240296; Clade 1. D.O. Burge 1030a (DUKE), Sierra San Pedro Mártir, Los Llanitos, Baja California, Mexico; NIA-c1: HM240298, NIA-c2: HM240299; Clade 1. D.O. Burge 1070a (DUKE), Rialto Municipal Airport, San Bernardino Co., CA; NIA-c1: HM240300; Clade 1. D.O. Burge 1071a (DUKE), Sierra Pelona Mountians, Ruby Canyon, Los Angeles Co., CA; NIA-c3: HM240301; Clade 1. D.O. Burge 1077a (DUKE), Forest Hill Divide, Placer Co., CA; NIA-c1: HM240305, NIA-c2: HM240304; Clade 1. D.O. Burge 1078a (DUKE), Magalia Reservoir, Butte Co., CA; NIA-c1: HM240306, NIA-c4: HM240307; Clade 1. D.O. Burge 1083a (DUKE), Goodyears Bar, Sierra Co., CA; NIA-c1: HM240308, NIA-c4: HM240309; Clade 1. D.O. Burge 1084a (DUKE), Community of Hills Flat, Nevada Co., CA; NIA-c1: HM240310; Clade 1. D.O. Burge 1093a (DUKE), Sutter Buttes, Peace Valley, Sutter Co., CA; NIA-c1: HM240312, NIA-c2: HM240311; Clade 1. D.O. Burge 1094a (DUKE), Lake Natoma, Sacramento Co., CA; NIA-c1: HM240313; Clade 1. D.O. Burge 1132a (DUKE), Clear Creek watershed, S of Ball Mountain, Kern Co., CA; NIA-c1: HM240319, NIA-c2: HM240320; Clade 1. D.O. Burge 1134a (DUKE), Middle Fork Tule River, roadside on SR 190, Tulare Co., CA; NIA-c2: HM240322, NIA-c1: HM240321; Clade 1. D.O. Burge 1136a (DUKE), Dalton Mountain, Fresno Co., CA;

NIA-c1: HM240323; Clade 1. D.O. Burge 1140a (DUKE), Chowchilla River watershed, N of Miami Mountain, Mariposa Co., CA; NIA-c4: HM240324; Clade 1. D.O. Burge 1145a (DUKE), Red Hills, Tuolumne Co., CA; NIA-c1: HM240326, NIA-c3: HM240325; Clade 1. D.O. Burge 1149a (DUKE), North Fork Calaveras River watershed, NE of Golden Gate Hill, Calaveras Co., CA; NIA-c1: HM240327, NIA-c3: HM240328; Clade 1. D.O. Burge 1150a (DUKE), Grass Valley Creek watershed, NE of Mount Zion, Amador Co., CA; NIA-c1: HM240330, NIA-c3: HM240329; Clade 1. D.O. Burge 1151a (DUKE), Crystal Creek watershed, N of Crystal Creek Road, Shasta Co., CA; NIA-c1: HM240331, NIA-c2: HM240332; Clade 1. D.O. Burge 1161a (DUKE), South Umpqua River watershed, roadside on Dole Drive, Douglas Co., OR; NIA-c2: HM240333; Clade 1. D.O. Burge 1164a (DUKE), Cottonwood Creek watershed, roadside on US Hwy 5, Jackson Co., OR; NIA-c1: HM240334, NIA-c3: HM240335; Clade 1. D.O. Burge 1168a (DUKE), Paynes Creek watershed, immediately W of Palmer Gulch, Tehama Co., CA; NIA-c3: HM240336; Clade 1. *C. cuneatus* Nutt. var. *dubius* J.T. Howell—D.O. Burge 917a (DUKE), Santa Cruz Mountains, S of Laurel Road, Santa Cruz Co., CA; NIA-c1: HQ325418; ITS: HQ325356; Clade 1. D.O. Burge 918a (DUKE), Henry Cowell Redwoods State Park, Santa Cruz Co., CA; NIA-c3: HM240347; Clade 1. D.O. Burge 966a (DUKE), Bean Creek Watershed, Mount Hermon Road, Santa Cruz Co., CA; NIA-c2: HQ325419, NIA-c3: HQ325420; Clade 1. *C. cuneatus* Nutt. var. *fascicularis* (McMinn) Hoover—D.O. Burge 871a (DUKE), Vandenberg Village, Santa Barbara Co., CA; NIA-c1: HM240348; Clade 1. D.O. Burge 874 (DUKE), Lompoc Casmalia Road, Santa Barbara Co., CA; NIA-c4: HQ325423; Clade 1. D.O. Burge 1262 (DUKE), Nipomo Mesa, San Luis Obispo Co., CA; NIA-c1: HQ325421, NIA-c3: HQ325422; ITS: HQ325357; Clade 1. *C. cuneatus* Nutt. var. *ramulosus* Greene—D.O. Burge 846a (DUKE), Birabent Canyon, Santa Barbara Co., CA; NIA-c1: HQ325424, NIA-c2: HQ325425; ITS: HQ325358; Clade 1. D.O. Burge 847b (DUKE), Prefumo Canyon, San Luis Obispo Co., CA; NIA-c1: HM240349; Clade 1. *C. cuneatus* Nutt. var. *rigidus* (Nutt.) Hoover—D.O. Burge 891b (DUKE), South Boundary Road, Monterey Co., CA; NIA-c1: HM240351, NIA-c3: HM240350; ITS: HQ325359; Clade 1. D.O. Burge 894a (DUKE), Pesante Canyon, Monterey Co., CA; NIA-c2: HQ325426; Clade 1. *C. divergens* Parry ssp. *confusus* (J.T. Howell) Abrams—D.O. Burge 1003a (DUKE), Mount Hood, Sonoma Co., CA; NIA-c2: HM240352, NIA-c3: HM240353; ITS: HQ325360; Clade 1. D.O. Burge 1006a (DUKE), Mount Saint Helena, Napa Co., CA; NIA-c2: HQ325427; Clade 1. *C. divergens* Parry subsp. *occidentalis* (McMinn) Abrams—D.O. Burge 943a (DUKE), Boggs Mountain, Lake Co., CA; NIA-c1: HM240354; ITS: HQ325361; Clade 1. *C. ferrisiae* McMinn—D.O. Burge 834a (DUKE), Pigeon Point, Santa Clara Co., CA; NIA-c1: HM240356, NIA-c3: HM240355; ITS: HQ325362; Clade 1. D.O. Burge 835a (DUKE), Leroy Anderson Dam, Santa Clara Co., CA; NIA-c3: HQ325428; Clade 1. *C. fresnensis* Abrams—D.O. Burge 1138a (DUKE), Big Creek Watershed, Fresno Co., CA; NIA-c1: HM240357; ITS: HQ325363; Clade 1. D.O. Burge 1144a (DUKE), Crocker Ridge, Tuolumne Co., CA; NIA-c1: HQ325429; Clade 1. *C. gloriosus* J.T. Howell var. *exaltatus* J.T. Howell—D.O. Burge 994a (DUKE), Oilwell Hill, Mendocino Co., CA; NIA-c2: HM240358, NIA-c3: HM240359; ITS: HQ325364; Clade 1. *C. gloriosus* J.T. Howell var. *gloriosus*—D.O. Burge 908a (DUKE), Point Reyes National Seashore, Marin Co., CA; NIA-c1: HM240361, NIA-c5: HM240360; ITS: HQ325365; Clade 1. D.O. Burge 996 (DUKE), Mendocino Bay, Mendocino Co., CA; NIA-c1: HQ325430; Clade 1. *C. gloriosus* J.T. Howell var. *porrectus* J.T. Howell—D.O. Burge 907a (DUKE), Inverness Ridge, Marin Co., CA; NIA-c1: HM240362, NIA-c11: HM240363; ITS: HQ325366; Clade 1. *C. jepsonii* Greene var. *albiflorus* J.T. Howell—D.H. Wilken 16741 (DUKE), Butts Canyon Road, Napa Co., CA; NIA-c2: HQ325431; ITS: HQ325367; Clade 1. D.O. Burge 940a (DUKE), Rattlesnake Spring, Napa Co., CA; NIA-c2: HQ325434, NIA-c6: HQ325435; Clade 1. D.O. Burge 997a (DUKE), Rathburn-Petray Mine, Colusa Co., CA; NIA-c2: HM240364, NIA-c3: HM240365; Clade 1. D.O. Burge 1009a (DUKE), Red Mountain, Mendocino Co., CA; NIA-c1: HQ325432, NIA-c2: HQ325433; Clade 1. *C. jepsonii* Greene var. *jepsonii*—D.O. Burge 914a (DUKE), Alpine Lake, Marin Co., CA; NIA-c3: HM240366; ITS: HQ325368; Clade 1. D.O. Burge 979a (DUKE), Duvoul Creek, near confluence with Dutch Bill Creek, Sonoma Co., CA; NIA-c1: HQ325436, NIA-c2: HQ325437; Clade 1. *C. maritimus* Hoover—D.O. Burge 887a (DUKE), Arroyo de los Chinos, San Luis Obispo Co., CA; NIA-c6: HM240367; ITS: HQ325369; Clade 1. *C. masonii* McMinn—D.O. Burge 832a (DUKE), Salmon Creek, Marin Co., CA; NIA-c5: HQ325438; ITS: HQ325370; Clade 1. D.O. Burge 913a (DUKE), Bolinas Ridge, Marin Co., CA; NIA-c9: HM240368; Clade 1. *C. megacarpus* Nutt. var. *insularis* (Eastw.) Munz—D.O. Burge 808a (DUKE), Santa Catalina Island, Los Angeles Co., CA; NIA-c1: HQ325439, NIA-c3: HQ325440; ITS: HQ325371; Clade 2. D.O. Burge 836b (DUKE), Santa Cruz Island, Center 2 Peak, Santa Barbara Co., CA; NIA-c1: HQ325443, NIA-c2: HQ325444; Clade 2. D.O. Burge 845a (DUKE), Santa Cruz Island, Pelican Bay Trail, Santa Barbara Co., CA; NIA-c2: HQ325441, NIA-c3: HQ325442; Clade 1. *C. megacarpus* Nutt. var. *megacarpus*—D.O. Burge 867a (DUKE), W shore of Lake Casitas, Ventura Co., CA; NIA-c2: HQ325445; ITS: HQ325372. D.O. Burge 881a (DUKE), Refugio Pass Road, Santa Barbara Co., CA; NIA-c1: HQ325446, NIA-c2: HQ325447. D.O. Burge 980a (DUKE), Camarillo, Ventura Co., CA; NIA-c6: HQ325448, NIA-c9: HQ325449; Mixed (Clade 2 and Unresolved *Cerastes*). D.O. Burge 989a (DUKE), Niguel Hill, Orange Co., CA; NIA-c1: HQ325450, NIA-c4: HQ325451; Clade 2. *C. ophiochilus* S. Boyd, T. Ross, & L. Arnseth—D.O. Burge 798b (DUKE), Woodchuck Road, Riverside Co., CA; NIA-c6: HQ325452, NIA-c9: HQ325453; ITS: HQ325373. *C. otayensis* McMinn—D.O. Burge 983 (DUKE), Otay Mountain, San Diego Co., CA; NIA-c6: HQ325457; Clade 2. D.O. Burge 1063a (DUKE), Cerro Jesús María (Cerro San Ysidro), Baja California, Mexico; NIA-c1: HQ325454; ITS: HQ325374. D.O. Burge 1179a (DUKE), Kearny Mesa, San Diego Co., CA; NIA-c1: HQ325455, NIA-c4: HQ325456; Mixed (Clade 2 and Unresolved *Cerastes*). *C. pauciflorus* DC.—D.O. Burge 1195a (DUKE), Sierra La Concordia, Coahuila, Mexico; NIA-c1: HQ325460, NIA-c3: HQ325461; ITS: HQ325375. D.O. Burge 1197a (DUKE), Sierra Potrero de Abrego (Sierra de Zapalinamé), Coahuila, Mexico; NIA-c1: HQ325462, NIA-c3: HQ325463. D.O. Burge 1200a (DUKE), Sierra Las Bocas, Zacatecas, Mexico; NIA-c2: HQ325464, NIA-c4: HQ325465. D.O. Burge 1217a (DUKE), Cerro El Potosí, Nuevo León, Mexico; NIA-c20: HQ325458, NIA-c25: HQ325459. D.O. Burge 1231a (DUKE), Sierra El Borrego, San Luis

Potosí, Mexico; NIA-c3: HQ325466. D.O. Burge 1234a (DUKE), Cerro Las Manzanillas, Aguascalientes, Mexico; NIA-c2: HQ325467. D.O. Burge 1237a (DUKE), Sierra de Los Organos, Zacatecas, Mexico; NIA-c1: HQ325468. *C. perplexans* Trel.—D.O. Burge 769 (DUKE), Sierra Juarez, Mexico Hwy 3, Baja California, Mexico; NIA-c1: HQ325473, NIA-c4: HQ325474; ITS: HQ325376. D.O. Burge 795a (DUKE), Chariot Canyon, San Diego Co., CA; NIA-c3: HQ325475, NIA-c4: HQ325476. D.O. Burge 802a (DUKE), San Jacinto Mountains, Poppet Flat Road, Riverside Co., CA; NIA-c2: HQ325477. D.O. Burge 928a (DUKE), Wilson Creek Watershed, Lyons Valley Road, San Diego Co., CA; NIA-c2: HQ325478, NIA-c3: HQ325479. D.O. Burge 987a (DUKE), Los Pinos Mountain, San Diego Co., CA; NIA-c1: HQ325480, NIA-c2: HQ325481. D.O. Burge 1040a (DUKE), Sierra San Pedro Mártir, Baja California, Mexico; NIA-c1: HQ325469, NIA-c2: HQ325470. D.O. Burge 1059a (DUKE), Cerro Dieciseis, Baja California, Mexico; NIA-c1: HQ325471, NIA-c2: HQ325472. *C. pinetorum* Coville—D.H. Wilken 16736 (DUKE), Trinity-Shasta County line, near Buckhorn Summit, Trinity Co., CA; NIA-c1: HM240369, NIA-c5: HM240370; ITS: HQ325377; Clade 1. D.O. Burge 1130a (DUKE), Jackass Creek watershed, S of Jackass Meadows, Tulare Co., CA; NIA-c1: HQ325482; Clade 1. *C. prostratus* Benth.—D.O. Burge 921c (DUKE), Lake de Sabla, Butte Co., CA; NIA-c1: HQ325484; Clade 1. D.O. Burge 952a (DUKE), Wentworth Road, El Dorado Co., CA; NIA-c1: HM240371; Clade 1. D.O. Burge 1162a (DUKE), Rogue River watershed, roadside on FR 6215, Jackson Co., OR; NIA-c3: HQ325483; ITS: HQ325378; Clade 1. *C. pumilus* Greene—D.O. Burge 993a (CAS), Confluence of Measly Creek with Rattlesnake Creek, Mendocino Co., CA; ITS: HQ340158; *trn*L-F: HQ325602; Clade 1. D.O. Burge 1156a (DUKE), Smith River watershed, Elk Camp Ridge, Del Norte Co., CA; NIA-c3: HM240372, NIA-c4: HM240373; ITS: HQ325379; Clade 1. *C. purpureus* Jeps.—D.O. Burge 904a (DUKE), Wooden Grade, Napa Co., CA; NIA-c1: HM240374, NIA-c4: HM240375; ITS: HQ325380; Clade 1. D.O. Burge 905a (DUKE), Atlas Road, Napa Co., CA; NIA-c1: HQ325485, NIA-c2: HQ325486; Clade 1. *C. roderickii* W. Knight—D.O. Burge 1080a (DUKE), Pine Hill, El Dorado Co., CA; NIA-c2: HM240376; ITS: HQ325381; Clade 1. D.O. Burge 1087a (DUKE), South Fork American river, near confluence with Weber Creek, El Dorado Co., CA; NIA-c2: HM240377, NIA-c3: HM240378; Clade 1. *C. sonomensis* J.T. Howell—D.O. Burge 895b (DUKE), Mayacamas Mountains, head of Hooker Canyon, Sonoma Co., CA; NIA-c3: HM240381; ITS: HQ325382; Clade 1. D.O. Burge 897b (DUKE), Mayacamas Mountains, W flank of Mount Hood, Sonoma Co., CA; NIA-c1: HQ325487; Clade 1. *C. verrucosus* Nutt.—D.O. Burge 775a (DUKE), Arroyo immediately S of Cerro las Pinitas, Baja California, Mexico; NIA-c1: HQ325490; Clade 2. D.O. Burge 932b (DUKE), Torrey Pines State Preserve, San Diego Co., CA; NIA-c1: HQ325491. D.O. Burge 988c (DUKE), City of Encinitas, San Diego Co., CA; NIA-c3: HQ325492; Clade 2. D.O. Burge 1032a (DUKE), NE of El Sauzal, Baja California, Mexico; NIA-c2: HQ325493; ITS: HQ325383; Clade 2. D.O. Burge 1047a (DUKE), Isla Cedros, Baja California, Mexico; NIA-c1: HQ325488, NIA-c3: HQ325489; Mixed (Clade 2 and Unresolved *Cerastes*). D.O. Burge 1050a (DUKE), Sierra San Miguel, Baja California, Mexico; NIA-c1: HQ325494, NIA-c2: HQ325495; Mixed (Clade 2 and Unresolved *Cerastes*). *C. vestitus* Greene—D.H. Wilken 16725a (DUKE), Hogback Road, Inyo Co., CA; NIA-c9: HQ325496. D.H. Wilken 16732a (DUKE), Buttermilk Road, Inyo Co., CA; NIA-c1: HQ325497. D.O. Burge 863a (DUKE), Hwy 33, S of intersection with Lockwood Valley Road, Ventura Co., CA; NIA-c9: HQ325532; Clade 1. D.O. Burge 935a (DUKE), Dragoon Mountains, Cochise Co., AZ; NIA-c2: HQ325533. D.O. Burge 973a (DUKE), Mojave River Forks Reservoir, San Bernardino Co., CA; NIA-c1: HQ325534. D.O. Burge 975a (DUKE), Pinal Mountains, Gila Co., AZ; NIA-c1: HQ325535, NIA-c4: HQ325536. D.O. Burge 976b (DUKE), Cave Creek Road, Maricopa Co., AZ; NIA-c2: HQ325537, NIA-c4: HQ325538. D.O. Burge 1026a (DUKE), Guanajuato area, roadside on Mexico Hwy 110, Guanajuato, Mexico; NIA-c2: HQ325498, NIA-c3: HQ325499; ITS: HQ325384. D.O. Burge 1027a (DUKE), Chapulco area, roadside on Mexico Hwy 28, Puebla, Mexico; NIA-c1: HQ325500, NIA-c2: HQ325501. D.O. Burge 1121a (DUKE), Ash Creek watershed, roadside on US Hwy 15, Washington Co., UT; NIA-c4: HQ325502. D.O. Burge 1122a (DUKE), Cedar Mesa, Muley Point, San Juan Co., UT; NIA-c1: HQ325503, NIA-c4: HQ325504. D.O. Burge 1123a (DUKE), Wilson Mountain, Coconino Co., AZ; NIA-c1: HQ325505, NIA-c4: HQ325506. D.O. Burge 1125a (DUKE), Hualapai Mountains, Mohave Co., AZ; NIA-c1: HQ325507, NIA-c4: HQ325508. D.O. Burge 1183a (DUKE), Pinaleno Mountains, Graham Co., AZ; NIA-c2: HQ325509, NIA-c4: HQ325510. D.O. Burge 1184a (DUKE), San Francisco River Watershed, Sundial Springs Road, Catron Co., NM; NIA-c1: HQ325511, NIA-c2: HQ325512. D.O. Burge 1185a (DUKE), Sonoita Creek watershed, SW of Patagonia Lake, Santa Cruz Co., AZ; NIA-c4: HQ325513. D.O. Burge 1187a (DUKE), South Franklin Mountain, El Paso Co., TX; NIA-c1: HQ325514, NIA-c3: HQ325515. D.O. Burge 1188a (DUKE), Guadalupe Mountains, Culberson Co., TX; NIA-c2: HQ325516. D.O. Burge 1191a (DUKE), Sierra San Marcos y Pinos, Coahuila, Mexico; NIA-c1: HQ325517, NIA-c3: HQ325518. D.O. Burge 1220a (DUKE), Sierra Los Soldados, Nuevo León, Mexico; NIA-c2: HQ325519. D.O. Burge 1226a (DUKE), Sierra El Pedregoso, Tamaulipas, Mexico; NIA-c3: HQ325520. D.O. Burge 1227a (DUKE), Sierra El Pinal, Tamaulipas, Mexico; NIA-c1: HQ325521, NIA-c3: HQ325522. D.O. Burge 1232a (DUKE), Sierra San Miguelito, San Luis Potosí, Mexico; NIA-c1: HQ325523, NIA-c2: HQ325524. D.O. Burge 1238a (DUKE), Sierra de Coneto, Durango, Mexico; NIA-c2: HQ325525. D.O. Burge 1241a (DUKE), Arroyo Los Pilares, Sonora, Mexico; NIA-c2: HQ325526, NIA-c4: HQ325527. D.O. Burge 1245a (DUKE), Arroyo de La Soledad, Sonora, Mexico; NIA-c1: HQ325528, NIA-c3: HQ325529. D.O. Burge 1248a (DUKE), Roadside on Mexico Hwy 16, Chihuahua, Mexico; NIA-c1: HQ325530, NIA-c4: HQ325531.

Subgenus *Ceanothus*

C. americanus L. var. *americanus*—I. Tidestrom 7374 (UC), Mont Alto, Franklin Co., PA; NIA-c1: HQ325539; ITS: HQ325309. *C. americanus* L. var. *intermedius* (Pursh) Torr. & A. Gray—D.O. Burge 937 (DUKE), North Carolina State Uni-

versity Forest, Durham Co., NC; NIA-c1: HQ325540; ITS: HQ325310. *C. americanus* L. var. *pitcheri* Pickering ex. Torr. & A. Gray—J.W. Moore 13434 (UC), S of Inver Grove, Dakota Co., MN; NIA-c1: HQ325541; ITS: HQ325311. *C. arboreus* Greene—D.O. Burge 809 (DUKE), Santa Catalina Island, Los Angeles Co., CA; NIA: HQ325542; ITS: HQ325312. D.O. Burge 839 (DUKE), Santa Cruz Island, ridge S of Cañada Cervada, Santa Barbara Co., CA; NIA-c2: HQ325543. *C. buxifolius* Schult.—D.O. Burge 759a (DUKE), Cerro el Potosí, Nuevo León, Mexico; NIA: HQ325544; ITS: HQ325313; Clade 3. *C. caeruleus* Lag.—C.J. Rothfels 3230 (DUKE), Nevado de Colima, Jalisco, Mexico; NIA-c1: HQ325545; ITS: HQ325314. D.E. Breedlove 33508 (DH), Road from Las Margaritas to Campo Alegre, Chiapas, Mexico; NIA-c1: HQ325546. D.O. Burge 763 (DUKE), Cerro el Potosí, Nuevo León, Mexico; NIA-c1: HQ325547. Y. Mexia 437 (UC), Pico del Aquila, near El Batel, Sinaloa, Mexico; NIA-c1: HQ325548. *C. cordulatus* Kellogg—D.O. Burge 957 (DUKE), Ridge between Cascade Lake and Emerald Bay, El Dorado Co., CA; NIA-c1: HQ325549; ITS: HQ325315; *trn*L-F: HQ325601; Clade 3. *C. cyaneus* Eastw.—M. Beauchamp, s.n. (RSA), Montana Serena Road, San Diego Co., CA; NIA-c1: HQ325550; ITS: HQ325316. *C. dentatus* Torr. & A. Gray—D.O. Burge 892a (DUKE), South Boundary Road, Monterey Co., CA; NIA-c1: HQ325551; ITS: HQ325317. *C. diversifolius* Kellogg—D.O. Burge 944a (DUKE), Elk Mountain Road, Lake Co., CA; NIA-c1: HQ325552; ITS: HQ325318. *C. fendleri* A. Gray var. *fendleri*—E. Lyonnet 3593 (CAS), El Recreo, Coahuila, Mexico; NIA-c1: HQ325553, NIA-c2: HQ325554; ITS: HQ325319; Clade 3. M. Ownbey 1428 (UC), Hills near Chromo, Archuleta Co., CO; NIA-c1: HQ325555; Clade 3. *C. fendleri* A. Gray var. *venosus* Trel.—D.O. Burge 933a (DUKE), Santa Catalina Mountains, Pima Co., AZ; NIA-c1: HQ325556; ITS: HQ325320; Clade 3. *C. foliosus* Parry var. *foliosus*—D.O. Burge 938a (DUKE), Mayacamas Mountains, head of Hooker Canyon, Sonoma Co., CA; NIA-c1: HQ325557; ITS: HQ325321. *C. foliosus* Parry var. *medius* McMinn—D.O. Burge 852a (DUKE), Cuesta Ridge, San Luis Obispo Co., CA; NIA-c1: HQ325558; ITS: HQ325322. *C. foliosus* Parry var. *vineatus* McMinn—Ray Prag, s.n. (DAV), Vine Hill School Road, Sonoma Co., CA; NIA-c1: HQ325559; ITS: HQ325323. *C. hearstiorum* Hoover & Roof—R.N. Philbrick, s.n. (SBBG), Arroyo de la Cruz, San Luis Obispo Co., CA; NIA-c1: HQ325560; ITS: HQ325324. *C. herbaceus* Raf.—G.E. Morley 281 (UC), Sections 33 and 34 T4S R3W, Republic Co., KS; NIA-c1: HQ325561; ITS: HQ325325. *C. impressus* Trel. var. *impressus*—D.O. Burge 884a (DUKE), Vandenberg Village, Santa Barbara Co., CA; NIA-c1: HQ325562; ITS: HQ325326. *C. impressus* Trel. var. *nipomensis* McMinn—D.O. Burge 876 (DUKE), Nipomo Mesa, San Luis Obispo Co., CA; NIA-c1: HQ325563; ITS: HQ325327. *C. incanus* Torr. & A. Gray—P.C. Everett 23977 (UC), Old Coast Road to Ettersburg, SE of Honeydew, Humboldt Co., CA; NIA-c1: HQ325564; Clade 3. R.F. Thorne 35079 (UC), Wilderness Lodge (Angelo Coast Range Reserve), Mendocino Co., CA; NIA-c1: HQ325565; Clade 3. D.O. Burge 1273 (DUKE), Mayacamas Mountains, Ida Clayton Road, Sonoma Co., CA; NIA-c1: HQ325566, NIA-c2: HQ325567; ITS: HQ325328; Clade 3. *C. integerrimus* Hook. & Arn. var. *integerrimus*—D.O. Burge 855 (DUKE), Santa Lucia Range, Nacimiento-Fergusson Road, Monterey Co., CA; NIA-c1: HQ325568; ITS: HQ325329. *C. integerrimus* Hook. & Arn. var. *macrothyrsus* (Torr.) G.T. Benson—D.O. Burge 816 (DUKE), Butte Creek watershed, below Doe Mill Ridge, Butte Co., CA; NIA-c1: HQ325569; ITS: HQ325330. *C. lemmonii* Parry—D.O. Burge 826 (DUKE), Durock Road, El Dorado Co., CA; NIA-c1: HQ325570; ITS: HQ325331. *C. leucodermis* Greene—D.O. Burge 807 (DUKE), San Gabriel Mountains, Morris Reservoir, Los Angeles Co., CA; NIA: HQ325571; ITS: HQ325332. D.O. Burge 926 (DUKE), Wisecarver Truck Trail, San Diego Co., CA; NIA-c1: HQ325572. *C. microphyllus* Michx.—D.O. Burge 747 (DUKE), Gainesville, East University Avenue, Alachua Co., FL; NIA-c1: HQ325573; ITS: HQ325333. *C. ochraceus* Suess.—D.O. Burge 1240 (DUKE), Sierra de Coneto, Durango, Mexico; NIA-c3: HQ325574; ITS: HQ325334. R. Torres C. 2854 (CAS), N of Díaz Ordáz, Oaxaca, Mexico; NIA-c1: HQ325575. *C. oliganthus* Nutt. var. *oliganthus*—D.O. Burge 1277 (DUKE), Santa Cruz Mountains, Hamms Gulch, San Mateo Co., CA; NIA-c1: HQ325576; ITS: HQ325335. *C. oliganthus* Nutt. var. *orcuttii* (Parry) Jeps.—D.O. Burge 930a (DUKE), Wildcat Canyon, San Diego Co., CA; NIA-c1: HQ325577; ITS: HQ325336. *C. oliganthus* Nutt. var. *sorediatus* (Hook. & Arn.) Hoover—D.O. Burge 878a (DUKE), Santa Ynez Mountains, West Camino Cielo Road, Santa Barbara Co., CA; NIA-c1: HQ325578; ITS: HQ325337. *C. papillosus* Torr. & A. Gray var. *papillosus*—D.O. Burge 963 (DUKE), Santa Lucia Mountains, S of Chalk Peak, Monterey Co., CA; NIA-c1: HQ325579; ITS: HQ325338. *C. papillosus* Torr. & A. Gray var. *roweanus* McMinn—D.O. Burge 1034 (DUKE), Cerro Bola, Baja California, Mexico; NIA-c1: HQ325580; ITS: HQ325339. *C. parryi* Trel.—D.O. Burge 1271 (DUKE), Austin Creek watershed, S of Cazadero, Sonoma Co., CA; NIA-c1: HQ325581; ITS: HQ325340. *C. parvifolius* (S. Watson) Trel.—D.O. Burge 953 (DUKE), Wentworth Road, El Dorado Co., CA; NIA-c1: HQ325582; ITS: HQ325341. *C. sanguineus* Pursh—H.L. Mason 4025 (UC), Siskiyou Summit, Josephine Co., OR; NIA-c1: HQ325583; ITS: HQ325342. *C. spinosus* Nutt. var. *palmeri* (Trel.) K. Brandegee—D.O. Burge 821a (DUKE), Rattlesnake Bar Road, El Dorado Co., CA; NIA: HQ325584; ITS: HQ325343. *C. spinosus* Nutt. var. *spinosus*—D.O. Burge 869a (DUKE), W shore of Lake Casitas, Ventura Co., CA; NIA-c1: HQ325585; ITS: HQ325344. *C. thyrsiflorus* Eschsch. var. *griseus* Trel.—D.O. Burge 1270 (DUKE), Russian Gulch, Sonoma Co., CA; NIA-c2: HQ325586; ITS: HQ325345. *C. thyrsiflorus* Eschsch. var. *thyrsiflorus*—D.O. Burge 830 (DUKE), Bolinas Ridge, Marin Co., CA; NIA-c1: HQ325588, NIA-c2: HQ325589. D.O. Burge 1038 (DUKE), Arroyo immediately S of Cerro las Pinitas, Baja California, Mexico; NIA-c1: HQ325587; ITS: HQ325346. *C. tomentosus* Parry var. *olivaceus* Jeps.—D.O. Burge 782 (DUKE), Sierra San Pedro Mártir, Baja California, Mexico; NIA: HQ325590; ITS: HQ325347. *C. tomentosus* Parry var. *tomentosus*—D.O. Burge 1267 (DUKE), Dry Creek watershed, S of Charles Howard Park, Amador Co., CA; NIA-c1: HQ325591; ITS: HQ325348. *C. velutinus* Hook. var. *hookeri* M.C. Johnst.—W. Roberts, s.n. (DAV), Jackson State Forest, Mendocino Co., CA; NIA-c1: HQ325592; ITS: HQ325349. *C. velutinus* Hook. var. *velutinus*—D.O. Burge 958 (DUKE), Ridge between Cascade Lake and Emerald Bay, El Dorado Co., CA; NIA-c1: HQ325593; ITS: HQ325350; Clade 3.

Appendix B

New Rhamnaceae Sequences Other than *Ceanothus*

For each sampled plant (see table 2), the format is as follows: collector name and number (herbarium of voucher deposition, if applicable), description of sample origin. Order is by species.

Alphitonia excelsa (**Fenzl**) **Benth.**—J. Kellermann 103 (MEL); cultivated, Royal Botanic Gardens, Melbourne. *Crumenaria erecta* **Reissek**—E.M. Zardini & S.R. Benítez 51053 (US); Paraguay. *Emmenosperma alphitonioides* **F. Muell.**—L. Bird 91 (CBG at CANB); cultivated, Australian National Botanic Gardens. *Granitites intangendus* (**F. Muell.**) **Rye**—B.J. Mole 417 (MEL); Australia: Western Australia. *Helinus integrifolius* (**Lam.**) **Kuntze**—A. Balsinhas 3112 (US); South Africa. *Ziziphus amole* (**Sessé & Moc.**) **M.C. Johnst.**—O. Dorado et al. 1585 (NY); Mexico. *Ziziphus calophylla* **Wall. ex Hook. f.**—S. Lee 04 (CS); cultivated, Singapore Botanical Garden. *Ziziphus jujuba* **Mill.**—C. Bordelon, s.n.; cultivated, United States National Arboretum. *Ziziphus mauritiana* **Lam.**—W.J. Kress 03–7317 (US); Burma. *Ziziphus rugosa* **Lam.**—W.J. Kress 03–7355 (US); Burma. *Ziziphus spina-christi* **Willd.**—K.I. Christensen, s.n. (DUKE); cultivated, University of Copenhagen Botanic Garden.

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