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## Genome-wide RAD sequencing resolves the evolutionary history of serrate leaf *Juniperus* and reveals discordance with chloroplast phylogeny

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1 **Title:** Genome-wide RAD sequencing resolves the evolutionary history of serrate leaf  
2 *Juniperus* and reveals discordance with chloroplast phylogeny

3

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21 **Abstract**

22 Juniper (*Juniperus*) is an ecologically important conifer genus of the Northern  
23 Hemisphere, the members of which are often foundational tree species of arid regions. The  
24 serrate leaf margin clade is native to topologically variable regions in North America, where  
25 hybridization has likely played a prominent role in their diversification. Here we use a reduced-  
26 representation sequencing approach (ddRADseq) to generate a phylogenomic data set for 68  
27 accessions representing all 22 species in the serrate leaf margin clade, as well as a number of  
28 close and distant relatives, to improve understanding of diversification in this group.  
29 Phylogenetic analyses using three methods (SVDquartets, maximum likelihood, and Bayesian)  
30 yielded highly congruent and well-resolved topologies. These phylogenies provided improved  
31 resolution relative to past analyses based on Sanger sequencing of nuclear and chloroplast DNA,  
32 and were largely consistent with taxonomic expectations based on geography and morphology.  
33 Calibration of a Bayesian phylogeny with fossil evidence produced divergence time estimates for  
34 the clade consistent with a late Oligocene origin in North America, followed by a period of  
35 elevated diversification between 12 and 5 Mya. Comparison of the ddRADseq phylogenies with  
36 a phylogeny based on Sanger-sequenced chloroplast DNA revealed five instances of pronounced  
37 discordance, illustrating the potential for chloroplast introgression, chloroplast transfer, or  
38 incomplete lineage sorting to influence organellar phylogeny. Our results improve  
39 understanding of the pattern and tempo of diversification in *Juniperus*, and highlight the utility  
40 of reduced-representation sequencing for resolving phylogenetic relationships in non-model  
41 organisms with reticulation and recent divergence.

42

43 **Keywords:** diversification, juniper, RADseq, reticulation, western North America

## 44 1. Introduction

45 The complex geologic and climatic history of western North America played an  
46 important role in the diversification of many plant groups throughout the Cenozoic (Axelrod,  
47 1948, 1950). Tectonic uplift, climate change, transcontinental land bridges, and glacial cycles  
48 created opportunity for range shifts, geographic barriers to admixture, and allopatric speciation  
49 (Hewitt, 1996; Calsbeek et al., 2003; Hewitt, 2004; Weir and Schluter, 2007). Hybridization has  
50 also been prominent in the evolutionary history of Nearctic plant taxa, as glacial cycles allowed  
51 periods of isolation and subsequent secondary contact (Swenson and Howard, 2005; Hewitt,  
52 2011). The interactions among topography, climate, and reticulation have shaped diversification  
53 and challenged phylogenetic analyses for many plant genera in western North America (e.g.,  
54 Rieseberg et al., 1991; Kuzoff et al., 1999; Bouillé et al., 2011; Xiang et al., 2018; Shao et al.,  
55 2019). However, improved genomic sampling enabled by high-throughput sequencing data has  
56 recently increased phylogenetic resolution for many young and reticulated groups (e.g., Stephens  
57 et al., 2015; Massatti et al., 2016; McVay et al., 2017; Moura et al., 2020) and generally stands to  
58 enhance our understanding of diversification for plant taxa in this region.

59 Junipers (*Juniperus*, Cupressaceae) are ecologically and economically important conifers  
60 of arid and semi-arid landscapes throughout the Northern Hemisphere (Farjon, 2005; Adams,  
61 2014). Unlike other genera in Cupressaceae, the juniper lineage evolved a fleshy female cone,  
62 functionally resembling a berry, which is an important food source for many birds and small  
63 mammals (Phillips, 1910; Santos et al., 1999). The serrate junipers, distinguished by the presence  
64 of microscopic serrations on their scale leaf margins, are particularly resistant to water stress  
65 compared with other juniper groups (Willson et al., 2008) and often represent the dominant trees  
66 in arid habitats of the western United States and Mexico (West et al., 1978; Romme et al., 2009).

67 A number of species in this clade are expanding their range in North America, and while the  
68 main causes of these expansions are unclear for some taxa (Miller and Wigand, 1994; Weisberg  
69 et al., 2007; Romme et al., 2009), fire suppression, over-grazing by cattle, and under-browsing  
70 by native herbivores appear to be the dominant factors underlying *J. ashei* and *J. pinchotii* range  
71 expansion in west Texas (Taylor, 2008). Despite several attempts to resolve phylogenetic  
72 relationships in this ecologically important clade (Mao et al., 2010; Adams and Schwarzbach,  
73 2013a,b), its complex evolutionary history including recent divergence, long generation times,  
74 and hybridization have likely obfuscated phylogenetic signal in previous molecular data sets.

75 The juniper lineage likely originated in Eurasia during the Eocene and subsequently split  
76 into three major monophyletic sections (Mao et al., 2010; Adams and Schwarzbach, 2013a): sect.  
77 *Caryocedrus* (1 sp., *J. drupacea*, eastern Mediterranean); sect. *Juniperus* (14 spp., Asia and the  
78 Mediterranean except *J. jackii* and *J. communis*); and the largest clade, sect. *Sabina*  
79 (approximately 62 spp., Northern Hemisphere except *J. procera*). Section *Sabina* contains three  
80 main monophyletic clades (Mao et al., 2010; Adams and Schwarzbach, 2013a): the turbinate,  
81 single-seeded, entire leaf margin junipers of the Eastern Hemisphere (16 spp.); the multi-seeded,  
82 entire leaf margin junipers of both the Eastern and Western Hemispheres (23 spp.); and the  
83 serrate leaf margin junipers (serrate junipers hereafter) of western North America (22 spp.),  
84 which are the focus of this study. The ancestral serrate juniper lineage likely arrived in North  
85 America from Eurasia via the North Atlantic Land Bridge (NALB) or Bering Land Bridge (BLB)  
86 (Mao et al., 2010). Extant serrate junipers are largely restricted to North America, inhabiting arid  
87 and semi-arid regions of the western United States, Mexico, and the high, dry mountains of  
88 Guatemala (*J. standleyi*; Adams, 2014) (Fig. 1).

89           A previous phylogenetic analysis based on Sanger sequencing data with complete  
90 species-level sampling of the serrate juniper clade was highly biased towards chloroplast DNA  
91 (cpDNA), utilizing four cpDNA regions and one nuclear DNA (nrDNA) region [full data set  
92 representing 4,411 base pairs (bp), referred to as nr-cpDNA hereafter; Adams and Schwarzbach,  
93 2013b]. Hybridization and discordance between cpDNA and nrDNA based phylogenies have  
94 been reported across *Juniperus* (Adams, 2016; Adams et al., 2016) and within the serrate  
95 junipers in particular (Adams et al., 2017) and may have contributed to unexpected topologies in  
96 the previous predominantly cpDNA based phylogeny (Adams and Schwarzbach, 2013b).  
97 Incomplete lineage sorting due to long generation times and recent divergence may have also  
98 contributed to paraphyletic and unresolved relationships in the nr-cpDNA analyses of Adams and  
99 Schwarzbach (2013b). Multi-locus data encompassing larger genealogical variation should  
100 reduce topological uncertainty in this clade, while also allowing for insight into nuclear-  
101 chloroplast discordance and its potential causes. Mao et al. (2010) estimated divergence times,  
102 diversification rates, and geographic origins of all major juniper clades; however, limited  
103 sampling of the serrate juniper clade precluded dating for many of its internal nodes. Divergence  
104 time estimation for a complete serrate juniper phylogeny stands to elucidate patterns of  
105 diversification at more recent time scales which appear to be important for diversification across  
106 the genus (Mao et al., 2010).

107           High-throughput sequencing technologies have rapidly improved our ability to apply  
108 genome-wide information to phylogenetic inference (McCormack et al., 2013; Leaché and Oaks,  
109 2017; Bravo et al., 2019). Data from whole genomes (e.g., Kimball et al., 2019; Allio et al.,  
110 2020), whole transcriptomes (e.g., Leebens-Mack et al., 2019), targeted capture (e.g., de La  
111 Harpe et al., 2019; Liu et al., 2019; Karimi et al., 2020), and genome-skimming approaches (e.g.,

112 Liu et al., 2020; Nevill et al., 2020) have resolved evolutionary relationships complicated by  
113 incomplete lineage sorting and reticulate evolution (Faircloth et al., 2013; Alexander et al., 2017;  
114 Carter et al., 2019). Methods using restriction enzyme digest to reduce genome complexity [e.g.,  
115 restriction site-associated DNA sequencing (RADseq; Miller et al., 2007; Baird et al., 2008)]  
116 have been particularly valuable for phylogenetic applications in non-model organisms due to  
117 their ability to sample large numbers of informative polymorphisms without requiring prior  
118 genomic resources (Takahashi et al., 2014; Leaché and Oaks, 2017; Near et al., 2018; Salas-  
119 Lizana and Oono, 2018; Hipp et al., 2020). RADseq data have improved the resolution of many  
120 groups that have been recalcitrant to phylogenetic analysis with small numbers of Sanger-  
121 sequenced loci due to rapid, recent, or reticulate evolution (Wagner et al., 2013; Massatti et al.,  
122 2016; Paetzold et al., 2019; Rancilhac et al., 2019; Lèveillé-Bourret et al., 2020). Although  
123 allelic dropout (i.e., the nonrandom absence of sequence data at a locus due to restriction site  
124 mutations) can result in larger amounts of missing data across more strongly diverged lineages,  
125 analyses of empirical and simulated RADseq data have illustrated its effectiveness for resolving  
126 even relatively deep divergences (e.g., up to 60 Mya, Rubin et al., 2012; Cariou et al., 2013;  
127 Eaton et al., 2017; Lecaudey et al., 2018; Du et al., 2020).

128       Here we utilized a double-digest RADseq approach (ddRADseq; Parchman et al., 2012;  
129 Peterson et al., 2012) to generate a phylogenomic data set for all extant species of serrate  
130 junipers (*Juniperus* sect. *Sabina*) as well as several close and distant relatives. As methods for  
131 phylogenetic inference utilizing multi-locus data make different assumptions about genealogical  
132 variation among lineages, we inferred phylogenetic trees using three distinct approaches  
133 (SVDquartets, maximum likelihood, and Bayesian). Our results produce consistent and highly  
134 resolved topologies, reveal discordance with phylogenies inferred with cpDNA alone, and

135 illustrate variation in diversification rates consistent with the climatic and geologic history of  
136 western North America.

137

## 138 **2. Materials & Methods**

### 139 **2.1 Taxon sampling and ddRADseq library prep**

140 We sampled leaf material from 68 individuals representing all 22 serrate juniper species  
141 and six outgroup species (Table S1). Most serrate juniper taxa and two outgroup taxa  
142 (*Hesperocyparis bakeri* and *H. arizonica*, Cupressaceae; Zhu et al., 2018) were either the same  
143 individuals or different individuals collected from the same populations as those analyzed  
144 previously by Adams and Schwarzbach (2013b). Thus, analyses of the data presented here have  
145 50 samples (73.5%) in common with Adams and Schwarzbach (2013b) and 18 samples (26.5%)  
146 which are unique to this study. Five additional outgroup taxa [*Juniperus drupacea* (*Juniperus*  
147 sect. *Caryocedrus*); *J. communis* (*Juniperus* sect. *Juniperus*); *J. virginiana*, *J. sabina* var. *sabina*,  
148 and *J. sabina* var. *balkanensis* (smooth leaf junipers of sect. *Sabina*)] were added to better  
149 understand evolutionary divergence at deeper time scales in this genus. Two additional *J.*  
150 *poblana* var. *poblana* localities (Nayarit, MX, and Amozoc de Mota, Puebla, MX), one  
151 additional *J. poblana* variety (*J. poblana* var. *decurrens*), and an additional *J. durangensis*  
152 locality (Sierra de Gamón, Durango, MX) were included to investigate the potential for recent  
153 evolutionary divergence in these taxa. Finally, we substituted *J. ashei* samples from Waco, TX,  
154 with *J. ashei* samples from nearby Tarrant County, TX, for this study.

155 DNA was extracted from dried leaf tissue with Qiagen DNeasy Plant Mini Kits and  
156 quantified with a Qiagen QIAxpert microfluidic analyzer prior to library preparation (Qiagen  
157 Inc., Valencia, CA, USA). Reduced-representation libraries for Illumina sequencing were



158 constructed using a ddRADseq method (Parchman et al., 2012; Peterson et al., 2012) in which  
159 genomic DNA was digested with two restriction enzymes, *EcoRI* and *MseI*, and custom oligos  
160 with Illumina base adaptors and unique barcodes (8, 9 or 10 bases in length) were ligated to the  
161 digested fragments. Ligated fragments were PCR amplified with a high-fidelity proofreading  
162 polymerase (Iproof polymerase, BioRad Inc., Hercules, CA, USA) and subsequently pooled into  
163 a single library. Libraries were size-selected for fragments between 350 and 450 bp in length  
164 with the Pippin Prep System (Sage Sciences, Beverly, MA) at the University of Texas Genome  
165 Sequencing and Analysis Facility. Two lanes of single-end 100-base sequencing were executed  
166 at the University of Wisconsin-Madison Biotechnology Center using an Illumina HiSeq 2500  
167 platform.

168

## 169 **2.2 Preparation, filtering, and assembly of ddRADseq data**

170 To identify and discard Illumina primer/adaptor sequences and potential biological  
171 sequence contaminants (e.g., PhiX, *E. coli*), we used the `tapioca` pipeline  
172 (<https://github.com/ncgr/tapioca>), which uses `bowtie2` (v. 2.2.5; Langmead and Salzberg, 2012)  
173 to identify reads which align to a database of known contaminant sequences. To ensure that  
174 cpDNA did not influence our analyses, we used the same approach to discard all reads which  
175 aligned to the *Juniperus squamata* chloroplast genome (GenBank Accession Number  
176 MK085509; Xie et al., 2019). To demultiplex reads to individual, we used a custom Perl script  
177 that corrects one or two base sequencing errors in barcoded regions, parses reads according to  
178 their associated barcode sequence, and trims restriction site-associated bases. Files with the read  
179 data for each individual are available at Dryad (<https://doi.org/10.5061/dryad.qbzkh18df>).

180 To process the raw data into a matrix of putatively orthologous aligned loci, we utilized  
181 `iPyRAD` (v. 0.9.16; Eaton, 2014) which was designed to process reduced-representation data for  
182 phylogenetic workflows and allows for indel variation across samples during clustering (Eaton,  
183 2014; Razkin et al., 2016). We largely used default values, as these settings produced multiple  
184 alignments of tractable size which led to highly resolved, supported, and consistent topologies  
185 across inference methods. First, nucleotide sites with phred quality scores less than 33, which  
186 represent base calls with an error probability greater than 0.0005%, were considered missing and  
187 replaced with an ambiguous nucleotide base (“N”). Next, sequences were *de novo* clustered  
188 within individuals using `vsearch` (v. 2.14.1; Rognes et al., 2016) and aligned with `muscle` (v.  
189 3.8.155; Edgar, 2004) to produce stacks of highly similar reads. A similarity clustering threshold  
190 (*clust\_threshold*) of 85% was applied during this and a later clustering step because it produced a  
191 thorough yet tractable number of loci and a highly supported topology with the `TETRAD`  
192 (SVDquartets) inference method. To ensure accurate base calls, all stacks with a read depth less  
193 than 6 were discarded. Observed base counts across all sites in all stacks informed the joint  
194 estimation of the sequencing error rate and heterozygosity, which informed statistical base calls  
195 according to a binomial model. At this step, each stack within each individual was reduced to  
196 one consensus sequence with heterozygote bases represented by IUPAC ambiguity codes, and  
197 any consensus sequences with more than 5% ambiguous bases (*max\_Ns\_consens*) or  
198 heterozygous sites (*max\_Hs\_consens*) were discarded to remove poor alignments. The remaining  
199 consensus sequences from all individuals were clustered again, this time across individuals,  
200 using the same assembly method and similarity threshold as used in the previous within-sample  
201 clustering step. The resulting clusters, which represent putative ddRADseq loci shared across  
202 individuals, were discarded if they contained more than 8 indels (*max\_Indels\_locus*) or 20%

203 variable sites (*max\_SNPs\_locus*), as an excess of either could indicate poor alignment. To detect  
204 potential paralogs, consensus sequences were removed if they contained one or more  
205 heterozygous sites shared across more than 50% of all samples (*max\_shared\_Hs\_locus*) or more  
206 than 2 haplotypes (Eaton, 2014). We retained all loci that were present in a minimum of four  
207 samples (*min\_samples\_locus*) to prevent over-filtering of missing data, which can negatively  
208 affect downstream inference (Rubin et al., 2012; Wagner et al., 2013; Huang and Knowles, 2014;  
209 Takahashi et al., 2014). Two sequence alignment formats, ipyRAD's database file and a phylip  
210 file of concatenated loci, were used as input for SVDquartets (TETRAD) and maximum likelihood  
211 (RAxML) phylogenetic analyses, respectively. The database file contains the clustered sequence  
212 data as well as linkage information for each locus. We used a python script  
213 ([http://github.com/btmartin721/raxml\\_ascbias/](http://github.com/btmartin721/raxml_ascbias/)) to remove all invariant sites from the phylip  
214 sequence alignment prior to analysis with RAxML.

215         To understand the timing and tempo of diversification within the serrate juniper clade, we  
216 utilized fossil evidence to inform divergence time estimates in a Bayesian phylogenetic inference  
217 framework. For this analysis, we included one sample per serrate juniper species, including three  
218 outgroup samples from the closely related smooth leaf juniper clade (*J. virginiana*, *J. sabina* var.  
219 *sabina*, and *J. sabina* var. *balkanensis*), with priority given to juniper samples with higher  
220 sequencing coverage depth. Sequencing reads for this subset of 25 samples were *de novo*  
221 assembled with default ipyRAD parameter values except for the *min\_samples\_locus* parameter,  
222 which was increased from 4 to 20, and the *clust\_threshold* parameter, which was increased from  
223 85% to 90%. Increasing these parameters effectively reduced both the proportion of missing data  
224 and the size of the sequence alignment to ensure tractable computation time with Bayesian  
225 inference methods. However, one caveat of excluding missing data in RADseq data sets is that it

226 can bias the distribution of mutation rates represented across loci and lower the accuracy of  
227 downstream phylogenetic inference (Huang and Knowles, 2014). The resulting `nexus` sequence  
228 alignment of concatenated loci was utilized as input for Bayesian analysis (`RevBayes`).  
229 Complete information on parameter settings for this and the aforementioned assembly, as well as  
230 the sequence alignment files, are archived at Dryad (<https://doi.org/10.5061/dryad.qbzkh18df>).

231

### 232 **2.3 Phylogenetic analyses**

233 After removing invariant sites, the `phylip` formatted sequence alignment for all taxa,  
234 including outgroups, was analyzed with maximum likelihood as implemented by `RAxML` (v.  
235 8.2.12; Stamatakis, 2014) under the GTR+ $\Gamma$  model of nucleotide substitution corrected for  
236 ascertainment bias (`-m ASC_GTRGAMMA`). Support was assessed with 100 rapid bootstrap  
237 replicates (`-N 100`), followed by a thorough maximum likelihood search for the best-scoring  
238 tree (`-f a`). Although `RAxML` is fast and often used for analysis of concatenated RADseq loci  
239 (Lemmon and Lemmon, 2013), phylogenetic inference with concatenated data necessarily  
240 ignores genealogical variation among loci and is statistically inconsistent as the number of genes  
241 increases (Kubatko and Degnan, 2007; Roch and Steel, 2015).

242 To account for genealogical variation among sampled loci and to incorporate coalescent  
243 stochasticity into analyses, we also conducted species tree inference using a site-based approach,  
244 SVDquartets (Chifman and Kubatko, 2014), as implemented by `TETRAD` (Eaton et al., 2017).  
245 `TETRAD` is included with `ipyRAD` and implements the SVDquartets algorithm, using information  
246 on genotype calls and linkage to sample unlinked SNPs. Briefly, SVDquartets uses the multi-  
247 species coalescent model to generate a probability distribution on the data patterns at the tips of a  
248 species tree which can be used to compute a score on a quartet of taxa and infer the true quartet

249 relationship (Chifman and Kubatko, 2014, 2015). These quartet relationships can be inferred for  
250 all or a subset of all possible quartets, and a quartet amalgamation software (in this case, QMC v.  
251 2.10; Snir and Rao, 2012) joins the inferred quartets into the species tree. Here, we used  
252 TETRAD's default number of quartets, which is the number of samples to the power of 2.8, which  
253 yielded 135,215 quartets (16.6% of total possible). To quantify support for the nodes of the  
254 species tree, we implemented a standard nonparametric bootstrapping procedure for 100  
255 replicates. The inferred tree was manually rooted with the clade containing *Hesperocyparis*  
256 *bakeri* and *H. arizonica*.

257 To enable comparison of topologies produced with ddRADseq and cpDNA Sanger  
258 sequencing data, we repeated the methods of Adams and Schwarzbach (2013b) on the same  
259 individuals or different individuals collected from the same populations as those analyzed in the  
260 ddRADseq analysis for a total of 66 individual samples. Thus, the cpDNA analysis presented  
261 here has 59 samples (89.4%) in common with the aforementioned ddRADseq analyses and 7  
262 substitutional samples (10.6%). DNA extractions, PCR amplifications, and Sanger sequencing of  
263 the four chloroplast loci (petN-psbM, trnS-trnG, trnD-trnT, and trnL-trnF) were conducted using  
264 the methods described in Adams and Schwarzbach (2013b). The GTR+ $\Gamma$ +I nucleotide  
265 substitution model provided the best fit to the cpDNA data according to Akaike's information  
266 criterion in Modeltest (v.3.7; Posada and Crandall, 1998), and analysis was conducted with  
267 Mr. Bayes (v.3.1; Ronquist and Huelsenbeck, 2003). Two rounds of four chains were run for a  
268 total of 10 million generations, sampling every 1000 generations after an initial burn in of 25%  
269 of generations.

270 To understand diversification rate variation and the timing of divergence events across  
271 the serrate juniper clade, we inferred a time-calibrated phylogeny for a subset of individuals

272 representing all serrate juniper taxa and three closely related outgroup samples from the smooth  
273 leaf juniper clade (*J. virginiana*, *J. sabina* var. *sabina*, and *J. sabina* var. *balkanensis*) with a  
274 Bayesian method (RevBayes v. 1.0.12; Höhna et al., 2017). First, we implemented a model-  
275 selection procedure to compare the relative fits with Bayes factors of the JC, HKY, GTR,  
276 GTR+ $\Gamma$ , and GTR+ $\Gamma$ +I models of nucleotide substitution. Second, the `nexus` sequence  
277 alignment of concatenated loci generated with `ipyRAD` was modeled under the best fit  
278 substitution model given a topology modeled with a constant-rate birth-death process, which was  
279 parameterized with a sampling fraction of 0.39 due to incomplete sampling of the smooth leaf  
280 juniper clade (Kendall, 1948; Nee et al., 1994; Höhna, 2015). We relaxed the assumption of a  
281 global molecular clock by allowing each branch-rate variable to be drawn from a lognormal  
282 distribution. Eight independent MCMC chains were run for 400,000 generations with a burn-in  
283 of 10,000 generations and sampled every 10 generations. Chains were visually assessed for  
284 convergence with `Tracer` (v. 1.7.1; Rambaut et al., 2018) and quantitatively assessed with  
285 effective sample sizes (ESS) and the Gelman-Rubin convergence diagnostic (Gelman and Rubin,  
286 1992) using the `gelman.diag` function in R (`CODA` package; Plummer et al., 2006).

287 Fossil calibration points and node age prior distributions can influence estimates of  
288 divergence times (Graur and Martin, 2004; Sauquet et al., 2012; Wang and Mao, 2016). We used  
289 three fossil calibration points: one at the root node for the serrate juniper clade (not shown in Fig.  
290 4A) and two at internal nodes (asterisks, Fig. 4A) representing the MRCA (Most Recent  
291 Common Ancestor) of all extant serrate leaf junipers and the MRCA of the western U.S. clade  
292 (*J. californica*, *J. osteosperma*, *J. occidentalis*, and *J. grandis*). Fossil assignments were based on  
293 morphology and coincided with those made by a previous phylogenetic analysis of *Juniperus*  
294 (Mao et al., 2010). Justifications for these assignments can be found in Table S2. A fossil

295 specimen of *J. creedensis* (23 Mya; Axelrod, 1987), representing the first appearance of a serrate  
296 juniper in the fossil record, provided the minimum age constraints for both the root node  
297 (representing the MRCA of the serrate leaf juniper clade and the smooth leaf juniper outgroup  
298 taxa) and the internal node representing the MRCA of the serrate junipers. The maximum age  
299 constraint for the root node, specified with a uniform prior distribution, was the estimated age of  
300 the crown lineage of Cupressoideae (134 Mya; Mao et al., 2012), a subfamily of Cupressaceae  
301 which contains *Thuja*, *Cupressus*, *Juniperus*, and other genera. A fossil specimen of *J.*  
302 *desatoyana* (16 Mya; Axelrod, 1991), representing a stem ancestor of a subclade containing *J.*  
303 *osteosperma*, *J. occidentalis*, and *J. grandis*, provided the minimum age constraint of 16 Mya for  
304 the divergence of this subclade from *J. californica* (i.e., the MRCA of the western U.S. clade).  
305 For the internal nodes representing the MRCA of the serrate leaf junipers and the MRCA of the  
306 western U.S. clade, the ages of the fossil specimens were modelled as exponential distributions  
307 with means of 23 Mya + 1 and 16 Mya + 1, respectively, divided by  $\lambda$ , the parameter of the  
308 exponential distribution. The maximum clade credibility tree was inferred from the burned  
309 distribution of posterior trees, and the smooth leaf juniper outgroup samples were pruned in R  
310 with the *drop.tip* function (*ape* package; Paradis and Schliep, 2019) prior to subsequent  
311 visualization and analyses.

312 The inferred Bayesian chronogram was used to generate a lineage through time plot with  
313 the *ltt.plot* function in R (*ape* package; Paradis and Schliep, 2019). To determine whether the  
314 rate of lineage diversification was constant through time, we used the *diversi.gof* function in R  
315 (*ape* package; Paradis and Schliep, 2019) to compute the Cramér-von Mises and Anderson-  
316 Darling goodness-of-fit tests (Stephens, 1974; Paradis, 1998).

317 To estimate the probability of all possible ancestral ranges at each ancestral node, we  
318 utilized the `BiOGeOBEARS` package (v. 1.1.2; Matzke, 2013a,b) and its dependencies, `rexpokit`  
319 (Matzke et al., 2019) and `cladoRcpp` (Matzke, 2018), in R. This package permits statistical  
320 selection of six competing historical biogeographical models (DEC, DEC+J, DIVALIKE,  
321 DIVALIKE+J, BAYAREALIKE, and BAYAREALIKE+J) and includes an additional  
322 cladogenetic event, founder-event speciation, represented by the +J notation in DEC+J,  
323 DIVALIKE+J, and BAYAREALIKE+J models (Matzke, 2014). While these six methods  
324 similarly assume that anagenetic dispersal and extinction occur along branches, they allow for  
325 different subsets of cladogenetic range-changing processes. The `BiOGeOBEARS` supermodel  
326 incorporates all of these different processes, treating them as free parameters which can be  
327 excluded or estimated from the data.

328 Five operational geographic areas (A, western U.S.; B, central U.S.; C, eastern U.S.; D,  
329 northern/central MX; E, southern MX; Fig. 5) were defined by both geopolitical and  
330 ecologically-relevant boundaries (Level I Ecoregions of North America; see  
331 <https://www.epa.gov/eco-research/ecoregions>). To determine the contemporary geographic range  
332 of each species, we referenced U.S. tree species range maps when available (Little, 1971) and  
333 juniper range maps otherwise (Adams, 2014) (Table S3). This matrix of distribution information  
334 for each species, as well as the maximum clade credibility tree inferred with `RevBayes`, was  
335 used as input for ancestral range estimation. We used plotting functions provided by  
336 `BiOGeOBEARS` to visualize estimates of ancestral range for the model with the lowest AIC.

337

### 338 3. RESULTS

#### 339 3.1 Assembly of ddRADseq data for phylogenetic inference



340 Two Illumina HiSeq lanes generated approximately 460 million reads, of which  
341 373,596,722 remained after quality and contaminant filtering. `Bowtie2` aligned 4,007,039 reads  
342 (1.07%) to the *J. squamata* chloroplast genome, which we subsequently removed prior to read  
343 assembly and SNP calling. Three samples were removed prior to assembly due to low read count  
344 relative to other samples, providing 68 samples for `ipyRAD` input. The full data set of 68 samples  
345 was initially assembled into 307,146 loci, of which 130,581 remained after filtering, providing  
346 929,267 SNPs (344,189 parsimony informative) for phylogenetic inference with `RAxML` and  
347 `TETRAD`. Each individual possessed, on average, approximately five million raw reads which  
348 were assembled, on average, into 19,417 loci (14.9% of total loci). Similar to other RADseq  
349 phylogenetic data sets (Cariou et al., 2013; Eaton et al., 2017), the resulting sequence alignments  
350 provided as input for `RAxML` and `TETRAD` exhibited a large proportion of missing data (84.69%  
351 and 83.51% of sites contained missing values, respectively). 10,461,968 invariant sites were  
352 removed from the `phylip` formatted sequence alignment prior to analysis with `RAxML`. `TETRAD`  
353 sampled 124,530 unlinked SNPs for its analysis.

354 For the Bayesian analysis, increasing the *min\_samples\_locus* and *clust\_threshold*  
355 parameters for assembly of the 22 serrate juniper and 3 outgroup samples effectively diminished  
356 the effect of allelic dropout and reduced the proportion of missing data at the expense of  
357 incorporating fewer loci for phylogenetic inference. An initial set of 479,143 loci were reduced  
358 to 2,390 after filtering steps, providing 18,436 SNPs (7,894 parsimony informative) for  
359 phylogenetic inference. On average, each individual possessed 5.7 million raw reads which were  
360 assembled into 2,078 loci (86.9% of total loci). Only 14.72% of sites contained missing values in  
361 the resulting `nexus` sequence alignment.

362

### 363 3.2 Phylogenetic analyses

364 The maximum likelihood and SVDquartets analyses of ddRADseq data (hereafter  
365 referred to as the ddRADseq phylogenies) recovered high support (>95%) for most nodes in the  
366 phylogeny, with few exceptions (Fig. 2). The maximum likelihood phylogeny identified nine  
367 monophyletic clades within the serrate junipers (Fig. 2 left), which are colored accordingly in  
368 Figs. 2-4. The SVDquartets phylogeny resolved the same nine clades (Fig. 2 right), although two  
369 were less supported: 1) the Cerro Petosí clade (*J. zanonii* and *J. saltillensis*, which are sympatric  
370 on Cerro Petosí, MX) and 2) the subalpine-alpine clade (*J. jaliscana*, *J. standleyi*, and *J.*  
371 *monticola*, which are collectively found in subalpine/alpine environments). The ddRADseq  
372 phylogenies consistently recovered deeper relationships among three main monophyletic clades:  
373 1) the western U.S. clade (*J. californica*, *J. osteosperma*, *J. occidentalis*, and *J. grandis*); 2) the  
374 *ashei* clade (*J. comitana*, *J. ovata*, and *J. ashei*), the *J. deppeana* species complex, the one-  
375 seeded serrate junipers (*J. arizonica*, *J. monosperma*, *J. coahuilensis*, *J. pinchotii*, and *J.*  
376 *angosturana*, which largely exhibit 1 seed per cone); and 3) the Cerro Petosí clade, the *J.*  
377 *durangensis* clade (*J. martinezii* and *J. durangensis* subsp.), the subalpine-alpine clade, *J.*  
378 *flaccida*, and the *J. poblana* species complex. The ddRADseq phylogenies were consistent in  
379 their relationships among the three high-level clades, including the placement of the western U.S.  
380 clade as basal to the other serrate juniper clades (Fig. 2). Although nearly all relationships were  
381 strongly supported and consistent across both phylogenies (Fig. 2), three were inconsistently  
382 resolved. In the maximum likelihood phylogeny, the outgroup taxa *J. drupacea* and *J. communis*  
383 are in distinct clades, whereas they are sister to one another in the SVDquartets phylogeny (Fig.  
384 2). In the maximum likelihood phylogeny, the *ashei* clade is basal to the *J. deppeana* species  
385 complex and the one-seeded group with high support; whereas, in the SVDquartets phylogeny,

386 the *J. deppeana* species complex is basal, with high support (Fig. 2). Finally, although both  
387 placements had low support, the maximum likelihood phylogeny placed *J. flaccida* as sister to  
388 the *J. poblana* complex, whereas the SVDquartets phylogeny placed *J. flaccida* as basal to the  
389 subalpine-alpine clade (Fig. 2).

390         Aside from the few conflicts above, the topologies inferred across multiple approaches  
391 (maximum likelihood, SVDquartets, and Bayesian) were consistent, highly supported, and  
392 congruent with established taxonomy based on morphological and chemical characters (Figs. 2,  
393 4A). Whereas Adams and Schwarzbach (2013b) inferred a paraphyletic relationship for *J. sabina*  
394 in which *J. virginiana* was sister to *J. sabina* var. *sabina* (Fig. 1 from Adams and Schwarzbach,  
395 2013b), the ddRADseq phylogenies recovered a monophyletic relationship for the two *J. sabina*  
396 varieties (Fig. 2). In addition, three of the nine monophyletic clades recovered with generally  
397 high support in the ddRADseq phylogenies (Fig. 2) were paraphyletic in the nr-cpDNA  
398 phylogeny of Adams and Schwarzbach (2013b): 1) the western U.S. clade; 2) the *J. ashei* clade;  
399 and 3) the subalpine-alpine clade. First, the western U.S. clade was paraphyletic in the nr-  
400 cpDNA tree of Adams and Schwarzbach (2013b) and is not basal to the other serrate juniper  
401 clades, except for *J. californica*. Second, the *J. ashei* clade was paraphyletic in the nr-cpDNA  
402 tree, with *J. comitana* basal to the western U.S. clade, *J. ovata* basal to the Cerro Petosí clade,  
403 and *J. ashei* sister to *J. deppeana* (Fig. 1 from Adams and Schwarzbach, 2013b). Third, the nr-  
404 cpDNA tree of Adams and Schwarzbach (2013b) placed *J. flaccida* and *J. poblana* in the  
405 subalpine-alpine clade, causing the subalpine-alpine clade to be paraphyletic.

406         Sanger-sequenced data spanning four cpDNA regions (petN-psbM, trnS-trnG, trnL-trnF,  
407 trnD-trnT), originally generated by Adams and Schwarzbach (2013b), was reanalyzed here with  
408 additional samples to produce a phylogeny for detection of cyto-nuclear discordance when

409 compared with ddRADseq phylogenies (both analyses were largely based on the same sets of  
410 individuals, or individuals from the same populations). The cpDNA phylogeny inferred here had  
411 less resolution and a distinctly different topology than that of the combined nr-cpDNA analysis  
412 of Adams and Schwarzbach (2013b). Figure 3 illustrates five areas of discordance between the  
413 maximum likelihood ddRADseq and Bayesian cpDNA phylogenies. First, the cpDNA phylogeny  
414 inferred a sister relationship between *J. sabina* var. *balkinensis* and *J. virginiana* (Fig. 3 right),  
415 whereas the maximum likelihood phylogeny inferred a sister relationship between *J. sabina* var.  
416 *balkinensis* and *J. sabina* var. *sabina* (Fig. 3 left), consistent with taxonomic expectations.  
417 Second, the western U.S. clade is paraphyletic in the cpDNA tree, and *J. californica* is sister to *J.*  
418 *comitana* rather than grouped with the other western U.S. serrate junipers (Fig. 3 right). Third,  
419 the cpDNA tree placed *J. zanonii* sister to *J. ovata* and nested within a clade with *J. ashei* (Fig. 3  
420 right), rather than sister to *J. saltillensis* as it is in the maximum likelihood tree (Fig. 3). Fourth,  
421 the cpDNA tree also included *J. arizonica* in this highly supported clade, making the one-seeded  
422 group (*J. arizonica*, *J. monosperma*, *J. coahuilensis*, *J. pinchotii*, and *J. angosturana*)  
423 paraphyletic (Fig. 3 right). Finally, in the cpDNA tree, *J. flaccida* is nested within *J. poblana*,  
424 which causes this complex to be paraphyletic (Fig. 3 right).

425

### 426 3.3 Diversification history of the serrate junipers

427 The GTR+ $\Gamma$  model of nucleotide substitution provided the best fit to the sequence  
428 alignment generated for the subset of serrate juniper samples, including three outgroup samples  
429 (*J. virginiana*, *J. sabina* var. *sabina*, and *J. sabina* var. *balkanensis*). The Bayesian topology was  
430 largely consistent with the maximum likelihood and SVDquartets phylogenies, with an exception  
431 being the paraphyletic relationship among the one-seeded junipers (Fig. 4A). The other eight of

432 the nine monophyletic clades and all three high-level clades recovered by the ddRADseq  
433 phylogenies (Fig. 2) were likewise recovered by the Bayesian phylogeny (Fig. 4A) with high  
434 support (>99% posterior support for all nodes; Figure 4A). Our Bayesian calibration suggests  
435 that the serrate juniper clade arose during the late Oligocene (crown age 23.73 Mya, 95% highest  
436 posterior density [HPD]: 23 – 25.15 Mya), which is slightly younger but not inconsistent with  
437 previous estimates of 25.82 (23.00 – 31.20) and 29.43 Mya (23.25 – 41.72) inferred from  
438 cpDNA data with BEAST and MULTIDIVTIME, respectively (Mao et al. 2010). According to  
439 our analysis, the western U.S. clade (*J. californica*, *J. osteosperma*, *J. occidentalis*, and *J.*  
440 *grandis*) arose in the early Miocene (crown age 17.20 Mya, HPD: 16.00 – 19.32 Mya), which is  
441 slightly younger but not inconsistent with previous estimates of 19.16 (16.00 – 25.44) and 24.39  
442 (15.88 – 36.64) Mya inferred from cpDNA with BEAST and MULTIDIVTIME, respectively  
443 (Mao et al., 2010).

444 The Bayesian phylogenetic model estimated a mean speciation rate for the serrate juniper  
445 and closely related smooth leaf juniper clades of 0.14 sp/Ma (HPD: 2.46E-5 – 0.21 sp/Ma), and  
446 an extinction rate of 0.03 sp/Ma (HPD: 7.18E-8 – 0.11 sp/Ma), resulting in a mean net  
447 diversification rate (speciation rate – extinction rate) of 0.11 sp/Ma (HPD: -0.07 – 0.20 sp/Ma).  
448 A lineage through time plot (Fig. 4B) suggests deviations from a constant rate of diversification  
449 over time, which was confirmed quantitatively with the Cramér-von Mises and Anderson-  
450 Darling goodness-of-fit tests, both of which rejected the null model of constant diversification  
451 rate and exponentially distributed branching times (Cramér-von Mises:  $W_2 = 2.326$ ,  $p < 0.01$ ;  
452 Anderson-Darling GOF:  $A_2 = 3.189$ ,  $p < 0.01$ ). Comparing lineage origination over time with a  
453 constant rate of diversification reveals a period of notably elevated diversification from ~12-5  
454 Mya (Fig. 4B).

455 Comparison of AIC and AICc values for each of the six historical biogeographical  
456 models with BiOGeOBEARS suggested that the DIVALIKE model provided the best fit to the  
457 data (AICc weight = 0.62). According to this model, the most probable ancestral range for the  
458 serrate juniper clade is a combined range of the Western U.S. and northern/central MX (Fig. 5).  
459 The ancestral range of the western U.S. serrate junipers was estimated as the western U.S., but  
460 the ancestral range of the remaining serrate junipers was estimated as northern/central MX (Fig.  
461 5).

462

#### 463 **4. Discussion**

464 Junipers are considered foundational plants throughout arid regions of North America,  
465 where they provide habitat and food resources for numerous animal species (Poddar and Lederer,  
466 1982; Gottfried, 1992; Adams, 2014). The serrate juniper clade is endemic and adapted to arid  
467 environments of North America, yet lack of phylogenetic resolution has precluded thorough  
468 understanding of how geography and climate may have influenced diversification in this  
469 relatively young group. Compared with previous work on limited numbers of serrate juniper taxa  
470 and Sanger-sequenced cp and nr loci (Mao et al., 2010; Adams and Schwarzbach, 2013b), the  
471 phylogenies inferred here with ddRADseq data offer greater resolution and support, and are  
472 largely consistent with longstanding taxonomy. Our results provide insight into the evolutionary  
473 history of the serrate junipers, including variation in the tempo of diversification, and reveal  
474 notable instances of discordance among phylogenies inferred from nuclear and chloroplast  
475 variation.

476

#### 477 **4.1 Diversification history of the serrate leaf margin junipers**

478 Our results are consistent with the hypothesis (Mao et al. 2010) that the ancestral serrate  
479 juniper lineage originated during the Oligocene epoch in North America (Fig. 4). During the  
480 Eocene-Oligocene transition (~33.9 Mya), decreasing temperatures and increasing seasonality  
481 occurred in many regions globally, potentially favoring the expansion of arid-adapted juniper  
482 populations (Kennett, 1977; Buchardt, 1978; Wolfe, 1978). As suggested by Mao et al. (2010),  
483 the serrate juniper ancestor may have first reached North America via the North Atlantic Land  
484 Bridge (NALB) or the Bering Land Bridge (BLB). The NALB, which provided an Atlantic  
485 connection through Greenland, was beginning to fragment during the Eocene, but fossil evidence  
486 suggests that it continued to facilitate the transatlantic migration of tree species well into the  
487 Miocene (Donoghue et al., 2001; Grímsson and Denk, 2005; Denk et al., 2010; Helmstetter et al.,  
488 2019). The BLB, which provided a Pacific connection across the Bering Strait, likely facilitated  
489 numerous transcontinental migrations during the Cenozoic (Hopkins, 1959, 1967; Donoghue et  
490 al., 2001; Wang and Ran, 2014), including other North American tree genera (e.g., *Fagus* and  
491 *Quercus*, Manos and Stanford, 2001; *Hesperocyparis* + *Callitropsis*, Terry et al., 2016; *Pinus*,  
492 Badik et al., 2018; *Picea*, Shao et al., 2019).

493 We inferred a combined ancestral range for the serrate juniper clade which included the  
494 western U.S. and northern/central MX (Fig. 5). Two lines of evidence suggest that the common  
495 ancestor of the serrate junipers established in the western United States after migrating from  
496 Eurasia and potentially before expanding into northern and central Mexico. First, our results  
497 generally suggest that the western U.S. clade is basal to all other serrate juniper clades (Figs. 2).  
498 Second, the earliest appearances of serrate junipers in the fossil record date to the late Oligocene  
499 and early Miocene in the western United States, and feature characteristics similar to extant  
500 western U.S. junipers (Axelrod, 1956, 1987, 1991; Wolfe, 1964). During the Oligocene, the



501 western United States was characterized by drier climates, expanding sclerophyll vegetation, and  
502 the origin of many contemporary tree species (Axelrod, 1976; Reveal, 1980). Moderate  
503 temperatures during this time shifted mixed conifer and subalpine forests coastward (Axelrod,  
504 1976), which, alongside increasingly xeric conditions throughout the region, may have provided  
505 ecological opportunity for serrate juniper establishment.

506 Divergence time estimates suggest that approximately one-third of all divergence events  
507 occurred relatively recently in the serrate juniper clade. Elevated diversification rates occurred  
508 from approximately 12 to 5 Mya during the late Miocene and early Pliocene (Fig. 4B). Notably,  
509 this period coincided with enhanced diversification rates across juniper generally, which was  
510 attributed by Mao et al. (2010) to global cooling and uplift of the Qinghai-Tibetan plateau,  
511 though the latter is not relevant for North America. In western North America, uplift of the  
512 American Cordillera during the late Miocene (~12-5 Mya) induced a rain shadow effect and the  
513 expansion of arid habitats (Axelrod, 1950, 1985; Leopold and Denton, 1987; Wilson and Pitts,  
514 2010), causing population extirpation and the evolution of drought adapted flora (Reveal, 1980).  
515 The serrate junipers are particularly tolerant to water stress (Willson et al., 2008) and may have  
516 persisted or expanded into newly vacant habitats during this period. Furthermore, increased fire  
517 and the expansion of grassland habitat at lower elevations may have restricted junipers to higher  
518 elevations, causing range disjunctions between mountain chains and allopatric divergence across  
519 altitudinal zones (Retallack, 1997; Wilson and Pitts, 2010). Indeed, some extant sister species  
520 exhibit geographical associations with adjacent mountain ranges, with one example being *J.*  
521 *occidentalis* and *J. grandis*, which diverged around the Miocene-Pliocene boundary: *Juniperus*  
522 *occidentalis* inhabits low to intermediate elevations associated with the Cascade range and *J.*  
523 *grandis* occupies mid to high elevation alpine environments associated with the Sierra Nevada



524 range (Terry et al., 2000). Miocene diversification has also been observed in other temperate  
525 trees (*Pinus*; Willyard et al., 2007; *Cupressus*; Xu et al., 2010; *Abies*; Aguirre-Planter et al.,  
526 2012; *Quercus* section *Lobatae*, series *Agrifoliae*; Hauser et al., 2017) and has been similarly  
527 attributed to falling global temperatures and mountain uplift.

528

#### 529 **4.2 Utilizing ddRADseq data to resolve relationships among the serrate junipers**

530 Our analyses were highly consistent across different inference approaches and  
531 recapitulated many of the general patterns suggested by previous analyses, including the  
532 monophyly of the “one-seeded”, “Cerro Potosí”, and “*durangensis*” clades (Adams and  
533 Schwarzbach, 2013b) and recognition of two *J. deppeana* varieties, var. *gamboana* and var.  
534 *deppeana* (Mao et al., 2010; Adams and Schwarzbach, 2013b). However, ddRADseq analyses,  
535 based on more extensive genomic sampling, provided enhanced resolution of early divergences  
536 in the serrate juniper clade by consistently recovering three major groups with high support: 1)  
537 the western U.S. clade; 2) the *J. ashei* clade, *J. deppeana* species complex, and one-seeded clade  
538 [also suggested by Mao et al. (2010)]; and 3) the Cerro Potosí clade, *J. durangensis* clade, the  
539 subalpine-alpine clade, *J. flaccida*, and *J. poblana* species complex. Our analyses additionally  
540 recovered some relationships which were previously unresolved due to incomplete sampling,  
541 predominantly cpDNA-based inference, or analyses being based on limited genomic sampling  
542 (e.g., Mao et al., 2010; Adams and Schwarzbach, 2013b). We highlight noteworthy examples of  
543 these results below.

544 Members of the western U.S. clade (*J. occidentalis*, *J. grandis*, *J. osteosperma*, and *J.*  
545 *californica*) are morphologically cohesive (see Vasek, 1966) and occur along a north-south  
546 moisture gradient from the montane zone of the eastern Cascade and Sierra Nevada ranges (*J.*

547 *occidentalis* and *J. grandis*, respectively), through the pinyon-juniper woodlands of the Great  
548 Basin and Colorado Plateau (*J. osteosperma*), to the Mojave Desert (*J. californica*). Nonetheless,  
549 both Mao et al. (2010) and Adams and Schwarzbach (2013b) inferred paraphyletic placements of  
550 *J. californica* relative to other members of the group. In contrast, our analyses inferred *J.*  
551 *californica* as the most basal member of a monophyletic western U.S. clade (Figs. 2, 4A),  
552 consistent with previous taxonomic classification. Our analyses additionally resolved  
553 relationships among *J. osteosperma*, *J. occidentalis*, and *J. grandis*, which hybridize in western  
554 Nevada (Terry et al., 2000; Terry, 2010; Adams, 2013a,b). *Juniperus grandis* and *J. occidentalis*  
555 were previously classified as *J. occidentalis* varieties based on morphological similarities which  
556 exhibit clinal variation (Vasek, 1966); however, they were not sister to one another in the  
557 analysis of Adams and Schwarzbach (2013b). Our analyses assigned them as sister taxa and  
558 placed *J. osteosperma* basal to them (Figs. 2, 4A), consistent with expectations based on  
559 morphology and geography.

560 *Juniperus ashei* and *J. ovata* (previously *J. ashei* var. *ovata*; Adams and Baker, 2007)  
561 hybridize extensively where they occur parapatrically in the trans-Pecos region of Texas, and  
562 were considered subspecies until recent phylogenetic analysis merited the recognition of *J. ovata*  
563 at the specific level (Adams and Schwarzbach, 2013b). In contrast to Adams and Schwarzbach  
564 (2013b), our analyses indicate a sister relationship for *J. ashei* and *J. ovata*, which is supported  
565 by morphology and the geographical proximity of these taxa (Figs. 2, 4A). The inference of *J.*  
566 *comitana* as the basal member of this clade (Figs. 2, 4A), however, is not supported by  
567 morphology and chemistry (Adams, 2000) and merits additional research.

568 We included new collections of *J. durangensis* from Sierra Gamon, Durango, in our  
569 analyses due to their atypical morphology relative to type localities of *J. durangensis* (Socorro

570 Gonzales, pers., comm.). Our analyses suggest phylogenetic distinctness of *J. durangensis* from  
571 Sierra Gamon, despite growing only 150 km northeast of the type locality near El Salto, Durango  
572 (Fig. 2). Ongoing morphological and phytochemical analyses may help determine whether *J.*  
573 *durangensis* from Sierra Gamon merits recognition as a new variety. Similarly, new *J. poblana*  
574 accessions were analyzed from Nayarit, Oaxaca, and Puebla, as potential cases of intraspecific  
575 divergence. The only additional variety suggested by our analyses besides the previously  
576 recognized *J. poblana* var. *decurrens* is represented by samples from Oaxaca, which formed a  
577 monophyletic group in both the maximum likelihood and SVDquartet analyses (Fig. 2).

578 In contrast to Adams and Schwarzbach (2013b), the ddRADseq maximum likelihood  
579 analysis placed *J. jaliscana*, *J. monticola*, and *J. standleyi* in a highly-supported monophyletic  
580 clade, and *J. flaccida* and *J. poblana* in a distinct sister clade with low support (Fig. 2 left). The  
581 SVDquartets and Bayesian trees likewise indicate monophyly of *J. jaliscana*, *J. monticola*, and *J.*  
582 *standleyi*, but with lower support (Figs. 2 right, 4A). We refer to this group as the “subalpine-  
583 alpine clade” because they occur at mid-high elevations. *Juniperus monticola* is widespread in  
584 Mexico and occupies subalpine and alpine habitats at elevations of 2400-4500 m (Adams, 2014),  
585 while *J. jaliscana* occupies pine-oak forests at elevations of 1335-2670 in southern Durango and  
586 northwest Jalisco (Zanoni and Adams, 1979). *Juniperus standleyi* is found in extreme southeast  
587 Mexico and Guatemala at elevations of 3000-4250 m (Adams, 2014). Phylogenetically adjacent  
588 taxa, *J. flaccida* and *J. poblana*, likewise occur in subalpine habitats but are distinguished  
589 morphologically from the subalpine-alpine clade by branches which are flaccid at the tips so that  
590 their foliage appears to be drooping (Adams, 2014).

591 The relationship between *J. flaccida* and *J. poblana* (previously *J. flaccida* var. *poblana*)  
592 has been taxonomically challenging due to the paucity of distinguishing morphological features

593 and their ability to hybridize (Zanoni and Adams, 1976; Adams et al., 2018c). Our analyses  
594 suggest a distinct taxonomic status for *J. poblana*, but disagree on the relationship between *J.*  
595 *poblana* and *J. flaccida*. Consistent with taxonomic expectations, maximum likelihood and  
596 Bayesian phylogenies support a sister relationship between *J. flaccida* and *J. poblana* (although  
597 poorly supported in the former) (Figs. 2 left, 4A); however, the SVDquartets tree suggests a  
598 more distant placement of *J. flaccida* basal to the subalpine-alpine clade (Fig. 2 right). An  
599 affinity of *J. flaccida* towards the subalpine-alpine clade was suggested by the Adams and  
600 Schwarzbach (2013b) phylogeny, which recovered a sister relationship between *J. flaccida* and  
601 *J. standleyi*. A potential explanation for this, and for conflicting phylogenetic signal in the  
602 ddRADseq data, could be introgression from *J. standleyi* into *J. flaccida*.

603         While the maximum likelihood and SVDquartets analyses produced predominantly  
604 consistent results, there were three instances of discordance which highlight areas where gene  
605 tree variation may have influenced inference (Maddison, 1997; Huang et al., 2010; Tonini et al.,  
606 2015). As incomplete lineage sorting (ILS) is a major source of gene tree-species tree  
607 discordance, phylogenetic inference under the multi-species coalescent (e.g., SVDquartets) may  
608 perform more accurately under high ILS conditions compared with concatenation approaches  
609 (e.g., RAxML) (Chou et al., 2015). Shallow divergences may be especially prone to ILS, which  
610 may explain the discordance between the *J. ashei* clade, the *J. deppeana* complex, and the one-  
611 seeded clade (Figs. 2, 4A). Alternatively, hybridization is widely reported throughout *Juniperus*  
612 (e.g., Adams, 1994; Terry et al. 2000; Adams et al., 2020) and may have contributed to  
613 topological discordance in areas of low support, e.g., the relationship of *J. flaccida* (Fig. 2).  
614 Finally, allelic dropout in reduced-representation data may complicate the resolution of older  
615 splits, and may have played a role in the discordance observed among two outgroup samples, *J.*

616 *communis* and *J. drupacea* (Fig. 2). Overall, differences in model assumptions and conflicting  
617 phylogenetic signal likely influenced the few points of discordance observed among our different  
618 inference methods.

619

#### 620 **4.3 Discordance between phylogenies inferred with nuclear and chloroplast DNA**

621       Discordance among nr and cpDNA is common, and can arise from processes including  
622 incomplete lineage sorting (Degnan and Rosenberg, 2009), hybridization (Rieseberg and Soltis,  
623 1991; Rieseberg et al., 1996), and lateral transfer of organellar genomes (Stegemann et al.,  
624 2012). In angiosperms prone to hybridization, discordance among nr and cpDNA gene trees has  
625 often been attributed to introgression and chloroplast capture (e.g., Acosta and Premoli, 2010;  
626 Lee-Yaw et al., 2019; Liu et al., 2020). When maternally inherited in angiosperms, cpDNA  
627 exhibits more intraspecific population divergence and higher introgression across species  
628 boundaries than nrDNA (Petit and Excoffier, 2009; Du et al., 2009). However, conifer cpDNA is  
629 usually paternally inherited through pollen (Neale and Sederoff, 1989; Mogensen, 1996),  
630 typically exhibits weaker population differentiation than nr or mtDNA, and is expected to move  
631 less readily across species boundaries (e.g., Petit et al., 2005; Gerardi et al., 2010; Godbout et al.,  
632 2010). Thus, chloroplast introgression should generally be less likely in conifers, although  
633 potential examples of chloroplast introgression and capture have been described (e.g., Liston et  
634 al., 2007; Gernandt et al., 2018). Interestingly, theoretical work suggests cp capture may be  
635 driven by mitochondrial based cytoplasmic male sterility (Frank, 1989) in hybridizing  
636 angiosperms with maternal co-inheritance of mt and cp genomes (Tsitrone et al., 2003). This  
637 mechanism couldn't operate in most conifers (e.g., *Picea* and *Pinus*) which inherit mt (maternal)  
638 and cp (paternal) genomes separately. However, Cupressaceae (including *Juniperus*) have

639 paternal inheritance of both mt and cp genomes (Mogensen, 1996; Adams, 2019), which could  
640 increase the probability of chloroplast capture via cytoplasmic interactions (Tsitrone et al.,  
641 2003). Alternatively, lateral transfer of chloroplast through natural grafting during periods of  
642 sympatry could lead to apparent chloroplast capture in the absence of hybridization (Stegeman et  
643 al., 2012).

644 As in other conifers (Petit and Hampe, 2006), reproductive isolation is often weak among  
645 *Juniperus*, and hybridization has been documented among serrate juniper species including *J.*  
646 *occidentalis* and *J. osteosperma* (Terry et al. 2000; Terry 2010), *J. ashei* and *J. ovata* (Adams et  
647 al., 2020), and *J. angosturana* and *J. coahuilensis* (Adams, 1994). Potential cases of  
648 introgression or horizontal transfer of cpDNA have also been noted in the group (Adams, 2016;  
649 Adams et al., 2016, 2017). For example, *J. occidentalis* and *J. osteosperma* hybridize extensively  
650 in northwestern Nevada, and a cpDNA haplotype fixed in *J. occidentalis* appears to have  
651 introgressed through the western range of *J. osteosperma* (Terry et al., 2000, Terry, 2010). A  
652 potential case of chloroplast capture occurred in the closely related smooth leaf juniper clade  
653 (Fig. 3 right) between *J. thurifera* (chloroplast donor, not shown) and *J. sabina* var. *sabina*  
654 (chloroplast recipient), giving rise to the allotetraploid *J. sabina* var. *balkanensis* (Adams et al.,  
655 2016, 2018a,b; Farhat et al., 2019). The cpDNA tree indicates notable discordance consistent  
656 with this idea, placing *J. sabina* var. *balkanensis* in a clade with *J. virginiana* (Fig. 3 right),  
657 while ddRADseq analyses inferred the expected monophyletic relationship for the *J. sabina*  
658 varieties (Figs. 2, 3 left). As the ddRADseq phylogenies are congruent with taxonomic  
659 expectations based on morphology and geography, several strong instances of discordance in the  
660 cpDNA phylogeny suggest the potential for chloroplast introgression or transfer, although  
661 incomplete lineage sorting remains plausible for several of these cases.

662 Clear instances of discordance involve species from diverged lineages inferred with  
663 nuclear data that unexpectedly share cpDNA variation (Fig. 3). ddRADseq data inferred a  
664 western U.S. clade containing *J. californica* (Fig. 3 left), as expected based on morphology and  
665 geography; however, the cpDNA tree placed *J. californica* in a well-supported clade with *J.*  
666 *comitana*, which is restricted to southern Mexico/northern Guatemala (Fig. 3 right).  
667 Introgression or transfer of a *J. comitana*-type chloroplast from an ancestral *J. comitana* lineage  
668 into *J. californica* could underly such discordance (Fig. 3 right). Second, cpDNA placed *J.*  
669 *zanonii*, a sub-alpine plant that grows at the 3550 m summit of Cerro Potosí, NL, Mexico, within  
670 a clade with *J. ashei* and *J. ovata*, sibling species that grow on limestone in Central Texas  
671 (Adams, 2008) (Fig. 3 right). The *ashei* clade is substantially diverged from *J. zanonii* in  
672 ddRADseq analyses, which placed *J. zanonii* with *J. saltillensis* (Fig. 3 left), consistent with *J.*  
673 *zanonii* and *J. saltillensis* exhibiting altitudinal zonation at Cerro Petosí, Mexico. This  
674 discordance could have arisen from chloroplast introgression or transfer from an ancestral *J.*  
675 *ovata*/*J. ashei* into ancestral *J. zanonii*, as these lineages likely experienced sympatry during the  
676 Pleistocene (Adams and Baker, 2007). Third, *J. arizonica* and *J. coahuilensis* occur  
677 parapatrically, but the two taxa are highly similar morphologically and hybridize in the Trans-  
678 Pecos, Texas region (Adams, 2014, 2017). ddRADseq analyses placed *J. arizonica* in the one-  
679 seeded group with *J. coahuilensis* (Fig. 3 left), as expected, while cpDNA placed *J. arizonica*  
680 within the *J. ashei* clade (Fig. 3 right). Chloroplast introgression or transfer from *J. ashei* to *J.*  
681 *arizonica* could underly such discordance (Fig. 3 right), although incomplete lineage sorting is  
682 also possible for these closely related clades. These discordances suggest that nr and cpDNA  
683 histories can vary prominently in *Juniperus*, and while evidence for chloroplast capture or  
684 horizontal transfer is scarce in conifers, these processes may deserve further study in  
685 Cupressaceae.



686

687 **5. Conclusion**

688 Our analyses of ddRADseq data produced highly resolved and largely consistent  
689 phylogenies depicting the evolutionary history of the serrate junipers of western North America.  
690 While these phylogenies were strongly consistent with taxonomic expectations based on  
691 morphology and ecology, cpDNA phylogenies illustrated several pronounced cases of  
692 discordance, suggesting the potential for processes to differentially influence the evolutionary  
693 history of the chloroplast genome. An improved understanding of the timing and tempo of  
694 diversification, including the age of origin of the serrate juniper clade and its elevated rate of  
695 diversification during the late Miocene, illustrates how the interaction between geologic,  
696 geographic, and climatic processes may have influenced patterns of diversification in this group.  
697 This study contributes to a growing body of research demonstrating the effectiveness of reduced-  
698 representation sequencing data for resolving the phylogenies of non-model organisms (e.g.,  
699 Eaton and Ree, 2013; Herrera and Shank, 2016; Massatti et al., 2016; Eaton et al., 2017; Near et  
700 al., 2018; Paetzold et al., 2019) and the complex evolutionary histories of western North  
701 American taxa characterized by reticulate evolution and recent divergence.

702

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713

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714 **Literature Cited**

- 715 Acosta, M.C., Premoli, A.C., 2010. Evidence of chloroplast capture in South American  
716 *Nothofagus* (subgenus *Nothofagus*, Nothofagaceae). Mol. Phylogenet. Evol. 54 (1), 235-242,  
717 <https://doi.org/10.1016/j.ympev.2009.08.008>
- 718 Adams, R.P., 1994. Geographic variation and systematics of monospermous *Juniperus*  
719 (Cupressaceae) from the Chihuahua Desert based on RAPDs and terpenes. Biochem. Syst.  
720 Ecol. 22 (7), 699-710, [https://doi.org/10.1016/0305-1978\(94\)90056-6](https://doi.org/10.1016/0305-1978(94)90056-6)
- 721 Adams, R.P., 2000. The serrate leaf margined *Juniperus* (Section Sabina) of the western  
722 hemisphere: systematics and evolution based on leaf essential oils and Random Amplified  
723 Polymorphic DNAs (RAPDs). Biochem. Syst. Ecol. 28 (10), 975-989,  
724 [https://doi.org/10.1016/S0305-1978\(00\)00022-3](https://doi.org/10.1016/S0305-1978(00)00022-3)
- 725 Adams, R.P., 2008. Distribution of *Juniperus ashei* var. *ashei* and var. *ovata* around New  
726 Braunfels, Texas. Phytologia. 90 (1), 97-102.
- 727 Adams, R.P., 2013a. Hybridization between *Juniperus grandis*, *J. occidentalis* and *J.*  
728 *osteosperma* in northwest Nevada I: Terpenes, Leviathan mine, Nevada. Phytologia. 95 (1),  
729 58-69.
- 730 Adams, R.P., 2013b. Hybridization between *Juniperus grandis*, *J. occidentalis* and *J.*  
731 *osteosperma* in northwest Nevada II: Terpenes, Buffalo Hills, Northwestern Nevada.  
732 Phytologia. 95 (1), 107-114.
- 733 Adams, R.P., 2014. Junipers of the World: The genus *Juniperus*. Trafford Publishing,  
734 Bloomington.
- 735 Adams, R.P., 2016. Two new cases of chloroplast capture in incongruent topologies in the  
736 *Juniperus excelsa* complex: *J. excelsa* var. *turcomanica* comb. nov. and *J. excelsa* var.  
737 *seravschanica* comb. nov. Phytologia. 98 (3), 219-231.

- 738 Adams, R.P., 2017. Multiple evidences of past evolution are hidden in nrDNA of *Juniperus*  
739 *arizonica* and *J. coahuilensis* populations in the trans-Pecos, Texas region. *Phytologia*. 99  
740 (1), 38-47.
- 741 Adams, R.P., 2019. Inheritance of chloroplasts and mitochondria in Conifers: A review of  
742 paternal, maternal, leakage and facultative inheritance. *Phytologia*. 101 (2), 134-138.
- 743 Adams, R.P., Baker, L.E., 2007. Pleistocene infraspecific evolution in *Juniperus ashei* Buch.  
744 *Phytologia*. 89 (1), 8-23.
- 745 Adams, R.P., Johnson, S.T., Schwarzbach, A.E., 2020. Long distance gene flow facilitated by  
746 bird-dispersed seeds in wind-pollinated species: A story of hybridization and introgression  
747 between *Juniperus ashei* and *J. ovata* told by nrDNA and cpDNA. *Phytologia*. 102 (2), 55-  
748 74.
- 749 Adams, R.P., Boratynski, A., Marcysiak, K., Roma-Marzio, F., Peruzzi, L., Bartolucci, F.,  
750 Conti, F., Mataraci, T., Tashev, A.N., Siljak-Yakovlev, S., 2018a. Discovery of *Juniperus*  
751 *sabina* var. *balkanensis* R. P. Adams & Tashev in Macedonia, Bosnia-Herzegovina, Croatia  
752 and southern Italy and relictual polymorphisms found in nrDNA. *Phytologia*. 100 (2),  
753 117-127.
- 754 Adams, R.P., Farhat, P., Shuka, L., Silak-Yakovlev, S., 2018b. Discovery of *Juniperus sabina*  
755 var. *balkanensis* R. P. Adams and A. N. Tashev in Albania and relictual polymorphisms  
756 found in nrDNA. *Phytologia*. 100 (3), 187-194.
- 757 Adams, R.P., Johnson, S., Coombes, A.J., Caamaño, L., González-Elizondo, M.S., 2018c.  
758 Preliminary examination of hybridization and introgression between *Juniperus flaccida* and  
759 *J. poblana*: nrDNA and cpDNA sequence data. *Phytologia*. 100 (2), 145-152.

- 760 Adams, R.P., Schwarzbach A.E., 2013a. Phylogeny of *Juniperus* using nrDNA and four cpDNA  
761 regions. *Phytologia*. 95 (2), 179-187.
- 762 Adams, R. P., Schwarzbach, A.E., 2013b. Taxonomy of the serrate leaf *Juniperus* of North  
763 America: Phylogenetic analyses using nrDNA and four cpDNA regions. *Phytologia*. 95 (2),  
764 172-178.
- 765 Adams, R.P., Schwarzbach, A.E., Tashev, A.N., 2016. Chloroplast capture by a new variety,  
766 *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev, from the Balkan  
767 peninsula: A putative stabilized relictual hybrid between *J. sabina* and ancestral *J. thurifera*.  
768 *Phytologia*. 98 (2), 100-111.
- 769 Adams, R.P., Socorro González-Elizondo M., González-Elizondo M., Ramirez Noy D.,  
770 Schwarzbach A.E., 2017. DNA sequencing and taxonomy of unusual serrate *Juniperus* from  
771 Mexico: Chloroplast capture and incomplete lineage sorting in *J. coahuilensis* and allied  
772 taxa. *Phytologia*. 99 (1), 62-73.
- 773 Aguirre-Planter, É., Jaramillo-Correa, J.P., Gómez-Acevedo, S., Khasa, D.P., Bousquet, J.,  
774 Eguiarte, L.E., 2012. Phylogeny, diversification rates and species boundaries of  
775 Mesoamerican firs (*Abies*, Pinaceae) in a genus-wide context. *Mol. Phylogenet. Evol.* 62  
776 (1), 263-274, <https://doi.org/10.1016/j.ympev.2011.09.021>
- 777 Alexander, A.M., Su, Y.C., Oliveros, C.H., Olson, K.V., Travers, S.L., Brown, R.M., 2017.  
778 Genomic data reveals potential for hybridization, introgression, and incomplete lineage  
779 sorting to confound phylogenetic relationships in an adaptive radiation of narrow-mouth  
780 frogs. *Evolution*. 71 (2), 475-488, <https://doi.org/10.1111/evo.13133>
- 781 Allio, R., Scornavacca, C., Nabholz, B., Clamens, A.L., Sperling, F.A., Condamine, F.L., 2020.  
782 Whole genome shotgun phylogenomics resolves the pattern and timing of swallowtail  
783 butterfly evolution. *Syst. Biol.* 69 (1), 38-60, <https://doi.org/10.1093/sysbio/syz030>

- 784 Axelrod, D.I., 1948. Climate and evolution in western North America during middle Pliocene  
785 time. *Evolution*. 2, 127-144, <https://doi.org/10.2307/2405373>
- 786 Axelrod, D.I., 1950. Evolution of desert vegetation in western North America. Carnegie Inst.  
787 Wash. Publ. 590, 215-306.
- 788 Axelrod, D.I., 1956. Mio-Pliocene floras from west-central Nevada. Vol. 33. University of  
789 California Press.
- 790 Axelrod, D.I., 1976. History of the coniferous forests, California and Nevada. University of  
791 California Press, Berkeley.
- 792 Axelrod, D.I., 1985. Rise of the grassland biome, central North America. *Bot. Rev.* 51 (2), 163-  
793 201.
- 794 Axelrod, D.I., 1987. The late Oligocene Creede flora, Colorado. Vol. 130. University of  
795 California Press.
- 796 Axelrod, D.I., 1991. The Early Miocene buffalo canyon flora of western Nevada. Vol. 135.  
797 University of California Press, Berkeley.
- 798 Badik, K.J., Jahner, J.P., Wilson, J.S., 2018. A biogeographic perspective on the evolution of  
799 fire syndromes in pine trees (*Pinus*: Pinaceae). *R. Soc. Open Sci.* 5 (3), 172412,  
800 <http://dx.doi.org/10.1098/rsos.172412>
- 801 Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U.,  
802 Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using  
803 sequenced RAD markers. *PLoS One*. 3 (10), e3376,  
804 <http://doi.org/10.1371/journal.pone.0003376>
- 805 Bouillé, M., Senneville, S., Bousquet, J., 2011. Discordant mtDNA and cpDNA phylogenies  
806 indicate geographic speciation and reticulation as driving factors for the diversification of

- 807 the genus *Picea*. *Tree Genet. Genomes*. 7 (3), 469-484, <http://doi.org/10.1007/s11295-010->  
808 [0349-z](http://doi.org/10.1007/s11295-010-0349-z)
- 809 Bravo, G.A., Antonelli, A., Bacon, C.D., Bartoszek, K., Blom, M.P., Huynh, S., Jones, G.,  
810 Knowles, L.L., Lamichhaney, S., Marcussen, T. Morlon, H., 2019. Embracing  
811 heterogeneity: coalescing the Tree of Life and the future of phylogenomics. *PeerJ*. 7, e6399.
- 812 Buchardt, B., 1978. Oxygen isotope palaeotemperatures from the Tertiary period in the North  
813 Sea area. *Nature*. 275 (5676), 121-123.
- 814 Calsbeek, R., Thompson, J.N., Richardson, J.E., 2003. Patterns of molecular evolution and  
815 diversification in a biodiversity hotspot: the California Floristic Province. *Mol. Ecol.* 12 (4),  
816 1021-1029, <https://doi.org/10.1046/j.1365-294X.2003.01794.x>
- 817 Cariou, M., Duret, L., Charlat, S., 2013. Is RAD-seq suitable for phylogenetic inference? An in  
818 silico assessment and optimization. *Ecol. Evol.* 3 (4), 846-852,  
819 <https://doi.org/10.1002/ece3.512>
- 820 Carter, K.A., Liston, A., Bassil, N.V., Alice, L.A., Bushakra, J.M., Sutherland, B.L., Mockler,  
821 T.C., Bryant, D.W., Hummer, K.E., 2019. Target capture sequencing unravels *Rubus*  
822 evolution. *Front. Plant Sci.* 10, 1615, <https://doi.org/10.3389/fpls.2019.01615>
- 823 Chifman, J., Kubatko, L., 2014. Quartet inference from SNP data under the coalescent model.  
824 *Bioinformatics*. 30 (23), 3317-3324, <https://doi.org/10.1093/bioinformatics/btu530>
- 825 Chifman, J., Kubatko, L., 2015. Identifiability of the unrooted species tree topology under the  
826 coalescent model with time-reversible substitution processes, site-specific rate variation, and  
827 invariable sites. *J. Theor. Biol.* 374, 35-47, <https://doi.org/10.1016/j.jtbi.2015.03.006>
- 828 Chou, J., Gupta, A., Yaduvanshi, S., Davidson, R., Nute, M., Mirarab, S., Warnow, T., 2015. A  
829 comparative study of SVDquartets and other coalescent-based species tree estimation  
830 methods. *BMC Genomics*, 16 (10), 1-11

- 831 Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the  
832 multispecies coalescent. *Trends Ecol. Evol.* 24 (6), 332-340,  
833 <https://doi.org/10.1016/j.tree.2009.01.009>
- 834 de La Harpe, M., Hess, J., Loiseau, O., Salamin, N., Lexer, C., Paris, M., 2019. A dedicated  
835 target capture approach reveals variable genetic markers across micro- and macro-  
836 evolutionary time scales in palms. *Mol. Ecol. Resour.* 19 (1), 221-234,  
837 <https://doi.org/10.1111/1755-0998.12945>
- 838 Denk, T., Grímsson F., Zetter, R., 2010. Episodic migration of oaks to Iceland: Evidence for a  
839 North Atlantic “land bridge” in the latest Miocene. *Am. J. Bot.* 97 (2), 276-287,  
840 <https://doi.org/10.3732/ajb.0900195>
- 841 Donoghue, M.J., Bell, C.D., Li, J., 2001. Phylogenetic patterns in Northern Hemisphere plant  
842 geography. *Int. J. Plant Sci.* 162 (S6), S41-S52, <https://doi.org/10.1086/323278>
- 843 Du, F.K., Petit, R.J., Liu, J.Q., 2009. More introgression with less gene flow: chloroplast vs.  
844 mitochondrial DNA in the *Picea asperata* complex in China, and comparison with other  
845 conifers. *Mol. Ecol.* 18 (7), 1396-1407, <https://doi.org/10.1111/j.1365-294X.2009.04107.x>
- 846 Du, Z.Y., Harris, A.J., Xiang, Q.Y.J., 2020. Phylogenomics, co-evolution of ecological niche  
847 and morphology, and historical biogeography of buckeyes, horsechestnuts, and their  
848 relatives (Hippocastaneae, Sapindaceae) and the value of RAD-seq for deep evolutionary  
849 inferences back to the Late Cretaceous. *Mol. Phylogenet. Evol.* 145, 106726,  
850 <https://doi.org/10.1016/j.ympev.2019.106726>
- 851 Eaton, D.A.R., 2014. PyRAD: assembly of de novo RADseq loci for phylogenetic analyses.  
852 *Bioinformatics.* 30 (13), 1844-1849, <https://doi.org/10.1093/bioinformatics/btu121>

- 853 Eaton, D.A.R., Ree, R.H., 2013. Inferring phylogeny and introgression using RADseq data: an  
854 example from flowering plants (*Pedicularis*: Orobanchaceae). *Syst. Bio.* 62 (5), 689-706,  
855 <https://doi.org/10.1093/sysbio/syt032>
- 856 Eaton, D.A., Spriggs, E.L., Park, B., Donoghue, M.J., 2017. Misconceptions on missing data in  
857 RAD-seq phylogenetics with a deep-scale example from flowering plants. *Syst. Bio.* 66 (3),  
858 399-412, <https://doi.org/10.1093/sysbio/syw092>
- 859 Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high  
860 throughput. *Nucleic Acids Res.* 32 (5), 1792-1797, <https://doi.org/10.1093/nar/gkh340>
- 861 Faircloth, B.C., Sorenson, L., Santini, F., Alfaro, M.E., 2013. A phylogenomic perspective on  
862 the radiation of ray-finned fishes based upon targeted sequencing of ultraconserved  
863 elements (UCEs). *PloS One.* 8 (6), e65923,  
864 <https://dx.doi.org/10.1371/journal.pone.0065923>
- 865 Farhat, P., Siljak-Yakovlev, S., Adams, R.P., Robert, T., Dagher-Kharrat, M.B., 2019. Genome  
866 size variation and polyploidy in the geographical range of *Juniperus sabina* L.  
867 (Cupressaceae). *Bot. Lett.* (in press), <https://doi.org/10.1080/23818107.2019.1613262>
- 868 Farjon, A., 2005. Monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew,  
869 Richmond.
- 870 Frank, S.A., 1989. The evolutionary dynamics of cytoplasmic male sterility. *Am. Nat.* 133 (3),  
871 345-376, <https://doi.org/10.1086/284923>
- 872 Gelman, A., Rubin, D.B., 1992. Inference from iterative simulation using multiple sequences.  
873 *Stat. Sci.* 7 (4), 457-472, <https://www-jstor-org.unr.idm.oclc.org/stable/2246093>
- 874 Gerardi, S., Jaramillo-Correa, J.P., Beaulieu, J. and Bousquet, J., 2010. From glacial refugia to  
875 modern populations: new assemblages of organelle genomes generated by differential



- 876 cytoplasmic gene flow in transcontinental black spruce. *Mol. Ecol.* 19 (23), 5265-5280,  
877 <https://doi.org/10.1111/j.1365-294X.2010.04881.x>
- 878 Gernandt, D.S., Aguirre Dugua, X., Vázquez-Lobo, A., Willyard, A., Moreno Letelier, A.,  
879 Pérez de la Rosa, J.A., Piñero, D., Liston, A., 2018. Multi-locus phylogenetics, lineage  
880 sorting, and reticulation in *Pinus* subsection *Australes*. *Am. J. Bot.* 105(4), 711-725,  
881 <https://doi.org/10.1002/ajb2.1052>
- 882 Gernandt, D.S., López, G.G., García, S.O., Liston, A., 2005. Phylogeny and classification of  
883 *Pinus*. *Taxon.* 54 (1), 29-42, <https://doi.org/10.2307/25065300>
- 884 Godbout, J., Beaulieu, J. and Bousquet, J., 2010. Phylogeographic structure of jack pine (*Pinus*  
885 *banksiana*; Pinaceae) supports the existence of a coastal glacial refugium in northeastern  
886 North America. *Am. J. Bot.* 97 (11), 1903-1912, <https://doi.org/10.3732/ajb.1000148>
- 887 Gottfried, G.J., 1992. Ecology and management of the southwestern pinyon-juniper woodlands.  
888 In: Ffolliott, P.F., Gottfried, G.J., Bennett, D.A., Hernandez, V.M., Ortega-Rubio, C.A.,  
889 Hamre, R.H. [Tech Coords.], Ecology and management of oak and associated woodlands:  
890 perspectives in the southwestern United States and northern Mexico. United States  
891 Department of Agriculture, Forest Service, Washington, D.C., pp. 78-85.
- 892 Graur, D., Martin, W., 2004. Reading the entrails of chickens: molecular timescales of evolution  
893 and the illusion of precision. *Trends Genet.* 20 (2), 80-86.
- 894 Grímsson, F., Denk, T., 2005. *Fagus* from the Miocene of Iceland: systematics and  
895 biogeographical considerations. *Rev. Palaeobot. Palynol.* 134 (1-2), 27-54,  
896 <https://doi.org/10.1016/j.revpalbo.2004.11.002>
- 897 Hauser, D.A., Keuter, A., McVay, J.D., Hipp, A.L., Manos, P.S., 2017. The evolution and  
898 diversification of the red oaks of the California Floristic Province (*Quercus* section *Lobatae*,  
899 series *Agrifoliae*). *Am. J. Bot.* 104 (10), 1581-1595, <https://doi.org/10.3732/ajb.1700291>

- 900 Helmstetter, A.J., Buggs, R.J., Lucas, S.J., 2019. Repeated long-distance dispersal and  
901 convergent evolution in hazel. *Sci. Rep.* 9 (1), 1-12, [https://doi.org/10.1038/s41598-019-](https://doi.org/10.1038/s41598-019-52403-2)  
902 [52403-2](https://doi.org/10.1038/s41598-019-52403-2)
- 903 Herrera, S., Shank, T.M., 2016. RAD sequencing enables unprecedented phylogenetic  
904 resolution and objective species delimitation in recalcitrant divergent taxa. *Mol. Phylogenet.*  
905 *Evol.* 100, 70-79, <https://doi.org/10.1007/s10709-011-9547-3>
- 906 Hewitt, G.M., 1996. Some genetic consequences of ice ages, and their role in divergence and  
907 speciation. *Biol. J. Linn. Soc.* 58 (3), 247-276, [https://doi.org/10.1111/j.1095-](https://doi.org/10.1111/j.1095-8312.1996.tb01434.x)  
908 [8312.1996.tb01434.x](https://doi.org/10.1111/j.1095-8312.1996.tb01434.x)
- 909 Hewitt, G.M., 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philos.*  
910 *Trans. R. Soc. Lond., B, Biol. Sci.* 359 (1442), 183-195,  
911 <https://doi.org/10.1098/rstb.2003.1388>
- 912 Hewitt, G.M., 2011. Quaternary phylogeography: the roots of hybrid zones. *Genetica.* 139 (5),  
913 617-638.
- 914 Hipp, A.L., Manos, P.S., Hahn, M., Avishai, M., Bodénès, C., Cavender-Bares, J., Crowl, A.A.,  
915 Deng, M., Denk, T., Fitz-Gibbon, S., Gailing, O., 2020. Genomic landscape of the global  
916 oak phylogeny. *New Phytol.* 226 (4), 1198-1212, <https://doi.org/10.1111/nph.16162>
- 917 Höhna, S., 2015. The time-dependent reconstructed evolutionary process with a key-role for  
918 mass-extinction events. *J. Theor. Biol.* 380, 321-331,  
919 <https://doi.org/10.1016/j.jtbi.2015.06.005>
- 920 Höhna, S., Landis, M.J., Heath, T.A., 2017. Phylogenetic inference using RevBayes. *Curr.*  
921 *Protoc. Bioinform.* 57 (6.16), 1-34, <https://doi.org/10.1002/cpbi.22>
- 922 Hopkins, D.M., 1967. *The Bering land bridge*. Vol. 3. Stanford University Press, Palo Alto.

- 923 Hopkins, D.M., 1959. Cenozoic history of the Bering land bridge. *Science*. 129 (3362), 1519-  
924 1528, <https://www.jstor.org/stable/1757656>
- 925 Huang, H., He, Q., Kubatko, L.S., Knowles, L.L., 2010. Sources of error inherent in species-  
926 tree estimation: impact of mutational and coalescent effects on accuracy and implications  
927 for choosing among different methods. *Syst. Biol.* 59 (5), 573-583,  
928 <https://doi.org/10.1093/sysbio/syq047>
- 929 Huang, H., Knowles L.L., 2014. Unforeseen consequences of excluding missing data from next-  
930 generation sequences: simulation study of RAD sequences. *Syst. Biol.* 65 (3), 357-365,  
931 <https://doi.org/10.1093/sysbio/syu046>
- 932 [Karimi, N., Grover, C.E., Gallagher, J.P., Wendel, J.F., Ané, C., Baum, D.A., 2020. Reticulate](#)  
933 [evolution helps explain apparent homoplasy in floral biology and pollination in baobabs](#)  
934 [\(\*Adansonia\*; Bombacoideae; Malvaceae\). \*Syst. Biol.\* 69 \(3\), 462-478,](#)  
935 <https://doi.org/10.1093/sysbio/syz073>
- 936 Kendall, D.G., 1948. On the generalized "birth-and-death" process. *Ann. Math. Stat.* 19 (1), 1-  
937 15, <https://www.jstor.org/stable/2236051>
- 938 Kennett, J.P., 1977. Cenozoic evolution of Antarctic glaciation, the circum-Antarctic Ocean,  
939 and their impact on global paleoceanography. *J. Geophys. Res.* 82 (27), 3843-3860,  
940 <https://doi.org/10.1029/JC082i027p03843>
- 941 Kimball, R.T., Oliveros, C.H., Wang, N., White, N.D., Barker, F.K., Field, D.J., Ksepka, D.T.,  
942 Chesser, R.T., Moyle, R.G., Braun, M.J., Brumfield, R.T., 2019. A phylogenomic supertree  
943 of birds. *Diversity*, 11 (7), 109, <https://doi.org/10.3390/d11070109>
- 944 Kubatko, L.S., Degnan, J.H., 2007. Inconsistency of phylogenetic estimates from concatenated  
945 data under coalescence. *Syst. Biol.* 56 (1), 17-24,  
946 <https://doi.org/10.1080/10635150601146041>

- 947 Kuzoff, R.K., Soltis, D.E., Hufford, L., Soltis, P.S., 1999. Phylogenetic relationships within  
948 *Lithophragma* (Saxifragaceae): hybridization, allopolyploidy, and ovary diversification.  
949 Syst. Bot. 24 (4), 598-615, <https://doi.org/10.2307/2419645>
- 950 Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods.  
951 9 (4), 357, <http://www.nature.com/doifinder/10.1038/nmeth.1923>
- 952 Leaché, A.D., Oaks, J.R., 2017. The utility of single nucleotide polymorphism (SNP) data in  
953 phylogenetics. Annu. Rev. Ecol. Evol. Syst. 48, 69-84, <https://doi.org/10.1146/annurev-ecolsys-110316-022645>
- 954
- 955 Lecaudey, L.A., Schlieven, U.K., Osinov, A.G., Taylor, E.B., Bernatchez, L., Weiss, S.J., 2018.  
956 Inferring phylogenetic structure, hybridization and divergence times within Salmoninae  
957 (Teleostei: Salmonidae) using RAD-sequencing. Mol. Phylogenet. Evol. 124, 82-99,  
958 <https://doi.org/10.1016/j.ympev.2018.02.022>
- 959 Leebens-Mack, J.H., Barker, M.S., Carpenter, E.J., et al., 2019. One thousand plant  
960 transcriptomes and the phylogenomics of green plants. Nature. 574, 679-685,  
961 <https://doi.org/10.1038/s41586-019-1693-2>
- 962 Lee-Yaw, J.A., Grassa, C.J., Joly, S., Andrew, R.L., Rieseberg, L.H., 2019. An evaluation of  
963 alternative explanations for widespread cytonuclear discordance in annual sunflowers  
964 (*Helianthus*). New Phytol. 221 (1), 515-526, <https://doi.org/10.1111/nph.15386>
- 965 Lemmon, E.M., Lemmon A.R., 2013. High-throughput genomic data in systematics and  
966 phylogenetics. Annu. Rev. Ecol. Evol. Syst. 44, 99-121, <https://doi.org/10.1146/annurev-ecolsys-110512-135822>
- 967
- 968 Leopold, E.B., Denton, M.F., 1987. Comparative age of grassland and steppe east and west of  
969 the northern Rocky Mountains. Ann. Mo. Bot. Gard. 74, 841-867,  
970 <https://doi.org/10.2307/2399452>

- 971 Lèveillé-Bourret, É., Chen, B.H., Garon-Labrecque, M.É., Ford, B.A., Starr, J.R., 2020. RAD  
972 sequencing resolves the phylogeny, taxonomy and biogeography of Trichophoreae despite a  
973 recent rapid radiation (Cyperaceae). *Mol. Phylogenet. Evol.* 145, 106727,  
974 <https://doi.org/10.1016/j.ympev.2019.106727>
- 975 Liston, A., Parker-Defeniks, M., Syring, J.V., Willyard, A. and Cronn, R., 2007. Interspecific  
976 phylogenetic analysis enhances intraspecific phylogeographical inference: a case study in  
977 *Pinus lambertiana*. *Mol. Ecol.* 16 (18), 3926-3937, [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-294X.2007.03461.x)  
978 [294X.2007.03461.x](https://doi.org/10.1111/j.1365-294X.2007.03461.x)
- 979 Little Jr, E.L., 1971. Atlas of United States trees. Volume 1. Conifers and important hardwoods.  
980 Miscellaneous publication 1146. US Department of Agriculture, Forest Service,  
981 Washington, DC.
- 982 Liu, B.B., Campbell, C.S., Hong, D.Y., Wen, J., 2020. Phylogenetic relationships and  
983 chloroplast capture in the *Amelanchier-Malacomeles-Peraphyllum* clade (Maleae,  
984 Rosaceae): evidence from chloroplast genome and nuclear ribosomal DNA data using  
985 genome skimming. *Mol. Phylogenet. Evol.* 147, 106784,  
986 <https://doi.org/10.1016/j.ympev.2020.106784>
- 987 Liu, Y., Johnson, M.G., Cox, C.J., Medina, R., Devos, N., Vanderpoorten, A., Hedenäs, L.,  
988 Bell, N.E., Shevock, J.R., Agüero, B., Quandt, D., 2019. Resolution of the ordinal  
989 phylogeny of mosses using targeted exons from organellar and nuclear genomes. *Nat.*  
990 *Commun.* 10 (1), 1-11, <https://doi.org/10.1038/s41467-019-09454-w>
- 991 Maddison, W.P., 1997. Gene trees in species trees. *Syst. Biol.* 46 (3), 523-536,  
992 <https://doi.org/10.1093/sysbio/46.3.523>

- 993 Manos, P.S., Stanford, A.M., 2001. The historical biogeography of Fagaceae: tracking the  
994 tertiary history of temperate and subtropical forests of the Northern Hemisphere. *Int. J. Plant*  
995 *Sci.* 162 (S6), S77-S93, <https://doi.org/10.1086/323280>
- 996 Mao, K., Hao, G., Liu, J., Adams, R.P., Milne, R.I., 2010. Diversification and biogeography of  
997 *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental  
998 dispersals. *New Phytol.* 188 (1), 254-272, <https://doi.org/10.1111/j.1469-8137.2010.03351.x>
- 999 Mao, K., Milne, R.I., Zhang, L., Peng, Y., Liu, J., Thomas, P., Mill, R.R., Renner, S.S., 2012.  
1000 Distribution of living Cupressaceae reflects the breakup of Pangea. *Proc. Natl. Acad. Sci.*  
1001 *U.S.A.* 109 (20), 7793-7798.
- 1002 Massatti, R., Reznicek, A.A., Knowles, L.L., 2016. Utilizing RADseq data for phylogenetic  
1003 analysis of challenging taxonomic groups: A case study in *Carex* sect. *Racemosae*. *Am. J.*  
1004 *Bot.* 103 (2), 337-347, <https://doi.org/10.3732/ajb.1500315>
- 1005 Matzke, N.J., 2013a. BioGeoBEARS: BioGeography with Bayesian (and likelihood)  
1006 evolutionary analysis in R Scripts. University of California, Berkeley, Berkeley, CA.
- 1007 Matzke, N.J., 2013b. Probabilistic historical biogeography: new models for founder-event  
1008 speciation, imperfect detection, and fossils allow improved accuracy and model-testing.  
1009 *Front. Biogeogr.* 5 (4), 242-248.
- 1010 Matzke, N.J., 2014. Model selection in historical biogeography reveals that founder-event  
1011 speciation is a crucial process in island clades. *Syst. Biol.* 63 (6), 951-970.
- 1012 Matzke, N.J., 2018. cladoRcpp v0.15.1: C++ Implementations of Phylogenetic Cladogenesis  
1013 Calculations. University of Auckland, New Zealand.
- 1014 Matzke, N.J., Sidje, R., Schmidt, D., 2019. rexpokit v0.26.6.6: R wrappers for EXPOKIT; other  
1015 matrix functions. School of Biological Sciences, University of Auckland, New Zealand.

- 1016 McCormack, J.E., Hird, S.M., Zellmer, A.J., Carstens, B.C., Brumfield, R.T., 2013.  
1017 Applications of next-generation sequencing to phylogeography and phylogenetics. *Mol.*  
1018 *Phylogenet. Evol.* 66 (2), 526-538, <https://doi.org/10.1016/j.ympev.2011.12.007>
- 1019 McVay, J.D., Hauser, D., Hipp, A.L., Manos, P.S., 2017. Phylogenomics reveals a complex  
1020 evolutionary history of lobed-leaf white oaks in western North America. *Genome.* 60 (9),  
1021 733-742, <https://doi.org/10.1139/gen-2016-0206>
- 1022 Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A., Johnson, E.A., 2007. Rapid and cost-  
1023 effective polymorphism identification and genotyping using restriction site associated DNA  
1024 (RAD) markers. *Genome Res.* 17 (2), 240-248, <https://doi.org/10.1101/gr.5681207>
- 1025 Miller, R.F., Wigand, P.E., 1994. Holocene changes in semiarid pinyon-juniper woodlands:  
1026 response to climate, fire, and human activities in the US Great Basin. *BioScience.* 44 (7),  
1027 465-474, <https://doi.org/10.2307/1312298>
- 1028 Mogensen, H.L., 1996. Invited special paper: the hows and whys of cytoplasmic inheritance in  
1029 seed plants. *Am. J. Bot.* 83 (3), 383-404, [https://doi.org/10.1002/j.1537-](https://doi.org/10.1002/j.1537-2197.1996.tb12718.x)  
1030 [2197.1996.tb12718.x](https://doi.org/10.1002/j.1537-2197.1996.tb12718.x)
- 1031 Moura, A.E., Shreves, K., Pilot, M., Andrews, K.R., Moore, D.M., Kishida, T., Möller, L.,  
1032 Natoli, A., Gaspari, S., McGowen, M., Gray, H., 2020. Phylogenomics of the genus  
1033 *Tursiops* and closely related Delphininae reveals extensive reticulation among lineages and  
1034 provides inference about eco-evolutionary drivers. *Mol. Phylogenet. Evol.* 146, 106756.
- 1035 Neale, D.B., Sederoff, R.R., 1989. Paternal inheritance of chloroplast DNA and maternal  
1036 inheritance of mitochondrial DNA in loblolly pine. *Theor. Appl. Genet.* 77 (2), 212-216.
- 1037 Near, T.J., MacGuigan, D.J., Parker, E., Struthers, C.D., Jones, C.D., Dornburg, A., 2018.  
1038 Phylogenetic analysis of Antarctic notothenioids illuminates the utility of RADseq for



- 1039 resolving Cenozoic adaptive radiations. *Mol. Phylogenet. Evol.* 129, 268-279,  
1040 <https://doi.org/10.1016/j.ympev.2018.09.001>
- 1041 Nee, S., May, R.M., Harvey, P.H., 1994. The reconstructed evolutionary process. *Philos. Trans.*  
1042 *R. Soc. Lond., B, Biol. Sci.* 344 (1309), 305-311, <https://doi.org/10.1098/rstb.1994.0068>
- 1043 Nevill, P.G., Zhong, X., Tonti-Filippini, J., Byrne, M., Hislop, M., Thiele, K., van Leeuwen, S.,  
1044 Boykin, L.M., Small, I., 2020. Large scale genome skimming from herbarium material for  
1045 accurate plant identification and phylogenomics. *Plant Methods.* 16 (1), 1-8,  
1046 <https://doi.org/10.1186/s13007-019-0534-5>
- 1047 Paetzold, C., Wood, K.R., Eaton, D., Wagner, W.L., Appelhans, M.S., 2019. Phylogeny of  
1048 Hawaiian *Melicope* (Rutaceae): RAD-Seq resolves species relationships and reveals ancient  
1049 introgression. *Front. Plant Sci.* 10, 1074, <https://doi.org/10.3389/fpls.2019.01074>
- 1050 Paradis, E., 1998. Testing for constant diversification rates using molecular phylogenies: a  
1051 general approach based on statistical tests for goodness of fit. *Mol. Biol. Evol.* 15 (4), 476-  
1052 479, <https://doi.org/10.1093/oxfordjournals.molbev.a025946>
- 1053 Paradis, E., Schliep, K., 2019. ape 5.0: an environment for modern phylogenetics and  
1054 evolutionary analyses in R. *Bioinformatics.* 35 (3), 526-528,  
1055 <https://doi.org/10.1093/bioinformatics/bty633>
- 1056 Parchman, T.L., Gompert, Z., Mudge, J., Schilkey, F.D., Benkman, C.W., Buerkle, C.A., 2012.  
1057 Genome-wide association genetics of an adaptive trait in lodgepole pine. *Mol. Ecol.* 21 (12),  
1058 2991-3005, <https://doi.org/10.1111/j.1365-294X.2012.05513.x>
- 1059 Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest  
1060 RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and  
1061 non-model species. *PloS One.* 7 (5), e37135,  
1062 <https://dx.doi.org/10.1371/journal.pone.0037135>



- 1063 Petit, R.J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D., Vendramin, G.G., 2005. Invited  
1064 review: comparative organization of chloroplast, mitochondrial and nuclear diversity in  
1065 plant populations. *Mol. Ecol.* 14(3), 689-701, [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-294X.2004.02410.x)  
1066 294X.2004.02410.x
- 1067 Petit, R.J., Excoffier, L., 2009. Gene flow and species delimitation. *Trends Ecol. Evol.* 24 (7),  
1068 386-393, <https://doi.org/10.1016/j.tree.2009.02.011>
- 1069 Petit, R.J., Hampe, A., 2006. Some evolutionary consequences of being a tree. *Annu. Rev. Ecol.*  
1070 *Evol. Syst.* 37, 187-214, <https://doi.org/10.1146/annurev.ecolsys.37.091305.110215>
- 1071 Phillips, F.J., 1910. The dissemination of junipers by birds. *J. For.* 8 (1), 60-73.
- 1072 Plummer, M., Best, N., Cowles, K., Vines, K., 2006. CODA: convergence diagnosis and output  
1073 analysis for MCMC. *R news*, 6 (1), 7-11.
- 1074 Poddar, S., Lederer, R.J., 1982. Juniper berries as an exclusive winter forage for Townsend's  
1075 Solitaires. *Am. Midl. Nat.* 108 (1), 34-40, <https://doi.org/10.2307/2425289>
- 1076 Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA  
1077 substitution. *Bioinformatics*, 14 (9), 817-818,  
1078 <https://doi.org/10.1093/bioinformatics/14.9.817>
- 1079 Rancilhac, L., Goudarzi, F., Gehara, M., Hemami, M.R., Elmer, K.R., Vences, M., Steinfarz, S.,  
1080 2019. Phylogeny and species delimitation of near Eastern *Neurergus* newts (Salamandridae)  
1081 based on genome-wide RADseq data analysis. *Mol. Phylogenet. Evol.* 133, 189-197,  
1082 <https://doi.org/10.1016/j.ympev.2019.01.003>
- 1083 Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior  
1084 summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* 67 (5), 901,  
1085 <https://dx.doi.org/10.1093/sysbio/syy032>

- 1086 Razkin, O., Sonet, G., Breugelmanns, K., Madeira, M.J., Gómez-Moliner, B.J., Backeljau, T.,  
1087 2016. Species limits, interspecific hybridization and phylogeny in the cryptic land snail  
1088 complex *Pyramidula*: the power of RADseq data. *Mol. Phylogenet. Evol.* 101, 267-278,  
1089 <https://doi.org/10.1016/j.ympev.2016.05.002>
- 1090 Retallack, G.J., 1997. Neogene expansion of the North American prairie. *Palaios*. 12 (4), 380-  
1091 390, <https://doi.org/10.2307/3515337>
- 1092 Reveal, J.L., 1980. Intermountain biogeography—a speculative appraisal. *Mentzelia*. 4, 1-92.
- 1093 Rieseberg, L.H., Beckstrom-Sternberg, S.M., Liston, A., Arias, D.M., 1991. Phylogenetic and  
1094 systematic inferences from chloroplast DNA and isozyme variation in *Helianthus* sect.  
1095 *Helianthus* (Asteraceae). *Syst. Bot.* 50-76, <https://doi.org/10.2307/2418973>
- 1096 Rieseberg, L.H., Soltis, D. E., 1991. Phylogenetic consequences of cytoplasmic gene flow in  
1097 plants. *Evol. Trends Plants*. 5, 65-84.
- 1098 Rieseberg, L.H., Whitton, J., Linder, C.R., 1996. Molecular marker incongruence in plant  
1099 hybrid zones and phylogenetic trees. *Acta Bot. Neerl.* 45 (3), 243-262.
- 1100 Roch, S., Steel, M., 2015. Likelihood-based tree reconstruction on a concatenation of aligned  
1101 sequence data sets can be statistically inconsistent. *Theor. Popul. Biol.* 100, 56-62,  
1102 <https://doi.org/10.1016/j.tpb.2014.12.005>
- 1103 Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open  
1104 source tool for metagenomics. *PeerJ*. 4, e2584, <https://dx.doi.org/10.7717/peerj.2584>
- 1105 Romme, W.H., Allen, C.D., Bailey, J.D., Baker, W.L., Bestelmeyer, B.T., Brown, P.M.,  
1106 Eisenhart, K.S., Floyd, M.L., Huffman, D.W., Jacobs, B.F., Miller, R.F., Muldavin, E.H.,  
1107 Swetnam, T.W., Tausch, R.J., Weisberg, P.J., 2009. Historical and modern disturbance  
1108 regimes, stand structures, and landscape dynamics in pinon–juniper vegetation of the

- 1109 western United States. *Rangeland Ecol. Manag.* 62 (3), 203-222, [https://doi.org/10.2111/08-](https://doi.org/10.2111/08-1110)  
1110 [188R1.1](https://doi.org/10.2111/08-1110)
- 1111 Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under  
1112 mixed models. *Bioinformatics.* 19 (12), 1572-1574,  
1113 <https://doi.org/10.1093/bioinformatics/btg180>
- 1114 Rubin, B.E.R., Ree, R.H., Moreau, C.S., 2012. Inferring phylogenies from RAD sequence data.  
1115 *PloS One.* 7 (4), e33394, [https://dx.doi.org/10.1371%2Fjournal.pone.0033394](https://dx.doi.org/10.1371/journal.pone.0033394)
- 1116 Salas-Lizana, R., Oono, R., 2018. Double-digest RAD seq loci using standard Illumina indexes  
1117 improve deep and shallow phylogenetic resolution of *Lophodermium*, a widespread fungal  
1118 endophyte of pine needles. *Ecol. Evol.* 8 (13), 6638-6651, <https://doi.org/10.1002/ece3.4147>
- 1119 Santos, T., Tellería, J.L., Virgós, E., 1999. Dispersal of Spanish juniper *Juniperus thurifera* by  
1120 birds and mammals in a fragmented landscape. *Ecography.* 22 (2), 193-204,  
1121 <https://doi.org/10.1111/j.1600-0587.1999.tb00468.x>
- 1122 Sauquet, H., Ho, S.Y., Gandolfo, M.A., Jordan, G.J., Wilf, P., Cantrill, D.J., Bayly, M.J.,  
1123 Bromham, L., Brown, G.K., Carpenter, R.J. and Lee, D.M., 2012. Testing the impact of  
1124 calibration on molecular divergence times using a fossil-rich group: the case of *Nothofagus*  
1125 (Fagales). *Syst. Biol.* 61 (2), 289-313.
- 1126 Shao, C.C., Shen, T.T., Jin, W.T., Mao, H.J., Ran, J.H., Wang, X.Q., 2019.  
1127 Phylotranscriptomics resolves interspecific relationships and indicates multiple historical  
1128 out-of-North America dispersals through the Bering Land Bridge for the genus *Picea*  
1129 (Pinaceae). *Mol. Phylogenet. Evol.* 141, 106610,  
1130 <https://doi.org/10.1016/j.ympev.2019.106610>
- 1131 Snir, S., Rao, S., 2012. Quartet MaxCut: a fast algorithm for amalgamating quartet trees. *Mol.*  
1132 *Phylogenet. Evol.* 62 (1), 1-8, <https://doi.org/10.1016/j.ympev.2011.06.021>

- 1133 Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of  
1134 large phylogenies. *Bioinformatics*. 30 (9), 1312-1313,  
1135 <https://doi.org/10.1093/bioinformatics/btu033>
- 1136 Stegemann, S., Keuthe, M., Greiner, S., Bock, R., 2012. Horizontal transfer of chloroplast  
1137 genomes between plant species. *Proc. Natl. Acad. Sci. U.S.A.* 109 (7), 2434-2438,  
1138 <https://doi.org/10.1073/pnas.1114076109>
- 1139 Stephens, J.D., Rogers, W.L., Mason, C.M., Donovan, L.A., Malmberg, R.L., 2015. Species  
1140 tree estimation of diploid *Helianthus* (Asteraceae) using target enrichment. *Am. J. Bot.* 102  
1141 (6), 910-920, <https://doi.org/10.3732/ajb.1500031>
- 1142 Stephens, M.A., 1974. EDF statistics for goodness of fit and some comparisons. *J. Am. Stat.*  
1143 *Assoc.* 69 (347), 730-737, <https://doi.org/10.2307/2286009>
- 1144 Swenson, N.G., Howard, D.J., (2005). Clustering of contact zones, hybrid zones, and  
1145 phylogeographic breaks in North America. *Am. Nat.* 166 (5), 581-591,  
1146 <https://doi.org/10.1086/491688>
- 1147 Takahashi, T., Nagata, N., Sota, T., 2014. Application of RAD-based phylogenetics to complex  
1148 relationships among variously related taxa in a species flock. *Mol. Phylogenet. Evol.* 80,  
1149 137-144, <https://doi.org/10.1016/j.ympev.2014.07.016>
- 1150 Taylor, C.A., 2008. Ecological consequences of using prescribed fire and herbivory to manage  
1151 *Juniperus* encroachment. In: Van Auken, O.W. (Ed.), *Western North American Juniperus*  
1152 *Communities*. Springer, New York, pp. 239-252.
- 1153 Terry, R.G., 2010. Re-evaluation of morphological and chloroplast DNA variation in *Juniperus*  
1154 *osteosperma* Hook and *Juniperus occidentalis* Torr. Little (Cupressaceae) and their putative  
1155 hybrids. *Biochem. Syst. Ecol.* 38 (3), 349-360, <https://doi.org/10.1016/j.bse.2010.03.001>

- 1156 Terry, R.G., Nowak, R.S., Tausch, R.J., 2000. Genetic variation in chloroplast and nuclear  
1157 ribosomal DNA in Utah juniper (*Juniperus osteosperma*, Cupressaceae): evidence for  
1158 interspecific gene flow. *Am. J. Bot.* 87 (2), 250-258, <https://doi.org/10.2307/2656913>
- 1159 Terry, R.G., Pyne, M.I., Bartel, J.A., Adams, R.P., 2016. A molecular biogeography of the New  
1160 World cypresses (*Callitropsis*, *Hesperocyparis*; Cupressaceae). *Plant Syst. Evol.* 302 (7),  
1161 921-942.
- 1162 Tonini, J., Moore, A., Stern, D., Shcheglovitova, M., Ortí, G., 2015. Concatenation and species  
1163 tree methods exhibit statistically indistinguishable accuracy under a range of simulated  
1164 conditions. *PLoS Curr.* 7.
- 1165 Tsitrone, A., Kirkpatrick, M., Levin, D.A., 2003. A model for chloroplast capture. *Evolution.* 57  
1166 (8), 1776-1782, <https://doi.org/10.1111/j.0014-3820.2003.tb00585.x>
- 1167 Vasek, F.C., 1966. The distribution and taxonomy of three western junipers. *Brittonia.* 18 (4),  
1168 350-372, <https://doi.org/10.2307/2805152>
- 1169 Wagner, C.E., Keller, I., Wittwer, S., Selz, O.M., Mwaiko, S., Greuter, L., Sivasundar, A.,  
1170 Seehausen, O., 2013. Genome-wide RAD sequence data provide unprecedented resolution  
1171 of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Mol.*  
1172 *Ecol.* 22 (3), 787-798, <https://doi.org/10.1111/mec.12023>
- 1173 Wang, Q., Mao, K.S., 2016. Puzzling rocks and complicated clocks: how to optimize molecular  
1174 dating approaches in historical phylogeography. *New Phytol.* 209 (4), 1353-1358.
- 1175 Wang, X.Q., Ran, J.H., 2014. Evolution and biogeography of gymnosperms. *Mol. Phylogenet.*  
1176 *Evol.* 75, 24-40, <https://doi.org/10.1016/j.ympev.2014.02.005>
- 1177 Weir, J.T., Schluter, D., 2007. The latitudinal gradient in recent speciation and extinction rates  
1178 of birds and mammals. *Science.* 315 (5818), 1574-1576,  
1179 <https://doi.org/10.1126/science.1135590>

- 1180 Weisberg, P.J., Lingua, E., Pillai, R.B., 2007. Spatial patterns of pinyon–juniper woodland  
1181 expansion in central Nevada. *Rangeland Ecol. Manag.* 60 (2), 115-124,  
1182 <https://doi.org/10.2111/05-224R2.1>
- 1183 West, N.E., Tausch, R.J., Rea, K.H., Tueller, P.T., 1978. Phylogeographical variation within  
1184 juniper-pinyon woodlands of the Great Basin. *Great Basin Naturalist Memoirs.* 8 (2), 119-  
1185 136, <https://www.jstor.org/stable/23376562>
- 1186 Willson, C.J., Manos, P.S., Jackson, R.B., 2008. Hydraulic traits are influenced by phylogenetic  
1187 history in the drought-resistant, invasive genus *Juniperus* (Cupressaceae). *Am. J. Bot.* 95  
1188 (3), 299-314, <https://doi.org/10.3732/ajb.95.3.299>
- 1189 Willyard, A., Syring, J., Gernandt, D.S., Liston, A., Cronn, R., 2007. Fossil calibration of  
1190 molecular divergence infers a moderate mutation rate and recent radiations for *Pinus*. *Mol.*  
1191 *Biol. Evol.* 24 (1), 90-101, <https://doi.org/10.1093/molbev/msl131>
- 1192 Wilson, J.S., Pitts, J.P., 2010. Illuminating the lack of consensus among descriptions of earth  
1193 history data in the North American deserts: a resource for biologists. *Prog. Phys. Geogr.* 34  
1194 (4), 419-441, <https://doi.org/10.1177%2F0309133310363991>
- 1195 Wolfe, J.A., 1964. Miocene floras from Fingerrock wash, southwestern Nevada. *US Geological*  
1196 *Survey Professional Paper.* 454-N, 1-36.
- 1197 Wolfe, J.A., 1978. A paleobotanical interpretation of Tertiary climates in the Northern  
1198 Hemisphere: Data from fossil plants make it possible to reconstruct Tertiary climatic  
1199 changes, which may be correlated with changes in the inclination of the earth's rotational  
1200 axis. *Am. Sci.* 66 (6), 694-703, <https://www.jstor.org/stable/27848958>
- 1201 Xiang, Q.P., Wei, R., Zhu, Y.M., Harris, A.J., Zhang, X.C., 2018. New infrageneric  
1202 classification of *Abies* in light of molecular phylogeny and high diversity in western North  
1203 America. *J. Syst. Evol.* 56 (6), 562-572, <https://doi.org/10.1111/jse.12458>

- 1204 Xie, S., Jialiang, L., Jibin, M., Jingjing, X., Kangshan, M., 2019. The complete chloroplast  
1205 genome of *Juniperus squamata* (Cupressaceae), a shrubby conifer from Asian Mountains.  
1206 Mitochondrial DNA Part B. 4 (2), 2137-2139.
- 1207 Xu, T., Abbott, R.J., Milne, R.I., Mao, K., Du, F.K., Wu, G., Zhaxi, C., Liu, J., 2010.  
1208 Phylogeography and allopatric divergence of cypress species (*Cupressus* L.) in the Qinghai-  
1209 Tibetan Plateau and adjacent regions. BMC Evol. Biol. 10 (1), 194.
- 1210 Zanoni, T.A., Adams, R.P., 1976. The genus *Juniperus* in Mexico and Guatemala: Numerical  
1211 and chemosystematic analysis. Biochem. Syst. 4 (3), 147-158.
- 1212 Zhu, A., Fan, W., Adams, R.P., Mower, J.P., 2018. Phylogenomic evidence for ancient  
1213 recombination between plastid genomes of the *Cupressus-Juniperus-Xanthocyparis*  
1214 complex (Cupressaceae). BMC Evol. Biol. 18 (1), 137.
- 1215

1216 **Figure Legends**

1217

1218 **Figure 1:** The serrate leaf junipers are distributed across arid and semi-arid regions of the  
1219 western United States, Mexico, and Guatemala. Colors representing sampling localities  
1220 correspond with those designating serrate juniper clades in the phylogenies of Figures 2-4.  
1221 Outgroup specimens are not shown in map. Map created with ArcGIS Pro 2.4.0

1222 (<http://www.esri.com>).

1223

1224 **Figure 2:** Phylogenetic analyses of ddRADseq data with maximum likelihood (left) and  
1225 SVDquartets (right) provide largely consistent topologies for the serrate juniper clade and its  
1226 relatives. Nine monophyletic clades resolved by both methods are indicated by colored boxes.  
1227 Bootstrap support values are reported for all nodes. Branch lengths are not meaningful for the  
1228 SVDquartets tree.

1229

1230 **Figure 3:** Comparison of the maximum likelihood ddRADseq tree (left) to a Bayesian cpDNA  
1231 tree (right) reveals five clear instances of discordance, indicated by dashed arrows. Nine low-  
1232 level clades resolved with ddRADseq data (Fig. 2) are indicated by colored boxes.

1233

1234 **Figure 4:** (A) Maximum clade credibility tree (MCC) from analyses in *RevBayes* of the serrate  
1235 leaf juniper clade calibrated with fossil evidence. Smooth leaf juniper outgroup taxa were  
1236 excluded from the figure for clarity. Asterisks identify two of the three calibration nodes (the  
1237 calibrated root node is not shown because it was pruned prior to visualization; see Methods and  
1238 Table S2 for details). All nodes received greater than 99% Bayesian posterior support. The nine  
1239 low-level clades resolved in RAXML and SVDquartets phylogenetic analyses of the full set of



1240 ddRADseq data (Fig. 2) are indicated by colored boxes. (B) Lineage through time plot for the  
1241 serrate juniper clade generated with the Bayesian MCC tree in panel A. Grey dashed line  
1242 represents linear diversification rate through time given the estimated crown age of the serrate  
1243 clade and the extant number of species.

1244  
1245 **Figure 5:** Ancestral ranges for the serrate junipers based on a dated phylogeny produced with  
1246 *RevBayes* and the *DIVALIKE* model in *BioGeoBEARS*. The map inset shows the delineation  
1247 of five operational areas (A, western U.S.; B, central U.S.; C, eastern U.S.; D, northern/central  
1248 MX; E, southern MX), which, along with information of species distributions, informed the  
1249 geographic ranges assigned to each species and model-based estimates of ancestral ranges. Pie  
1250 charts at each node represent the marginal probabilities for each range estimated with maximum  
1251 likelihood, where the colors of the pie sectors either represent single ancestral ranges indicated  
1252 within the map inset or a possible combination of two ancestral ranges, in which case a novel  
1253 color was chosen.

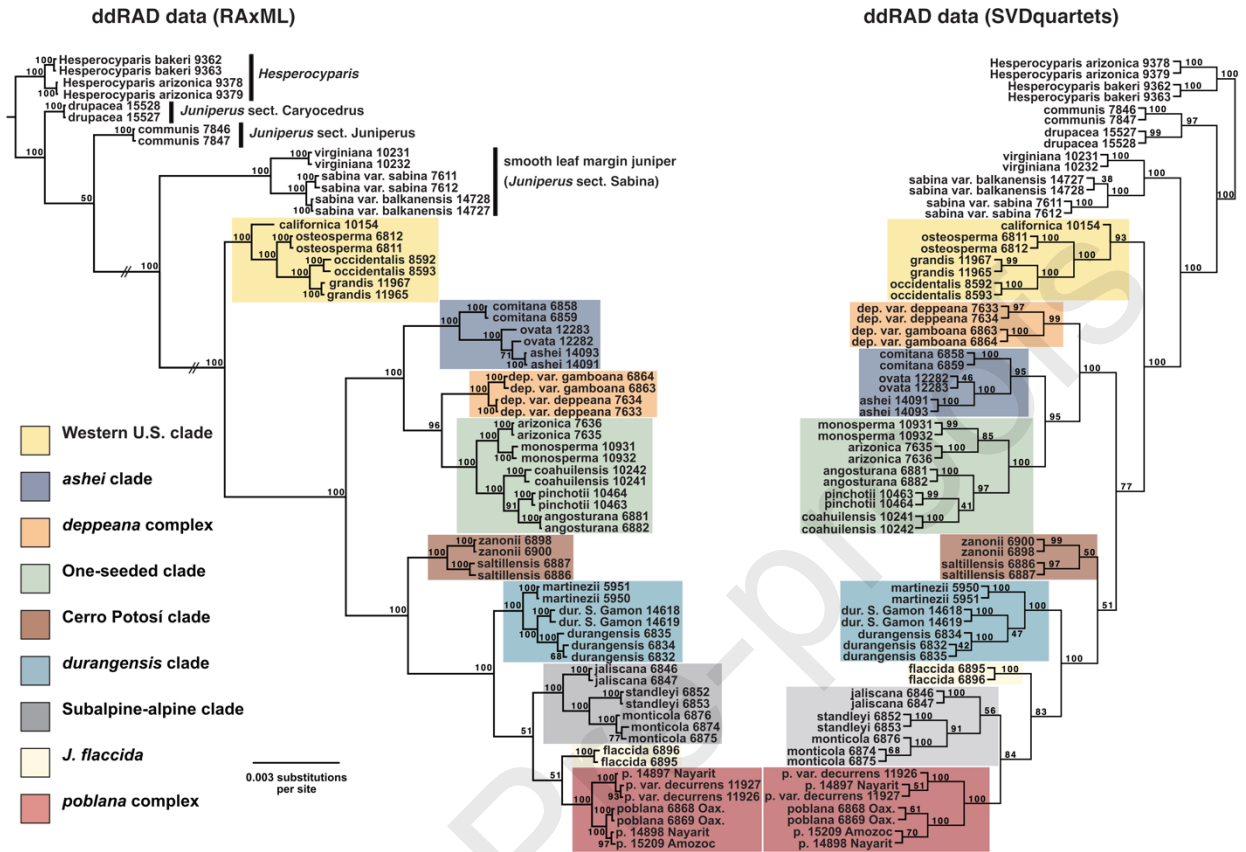
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1255 **Figures**  
 1256  
 1257 **Figure 1**



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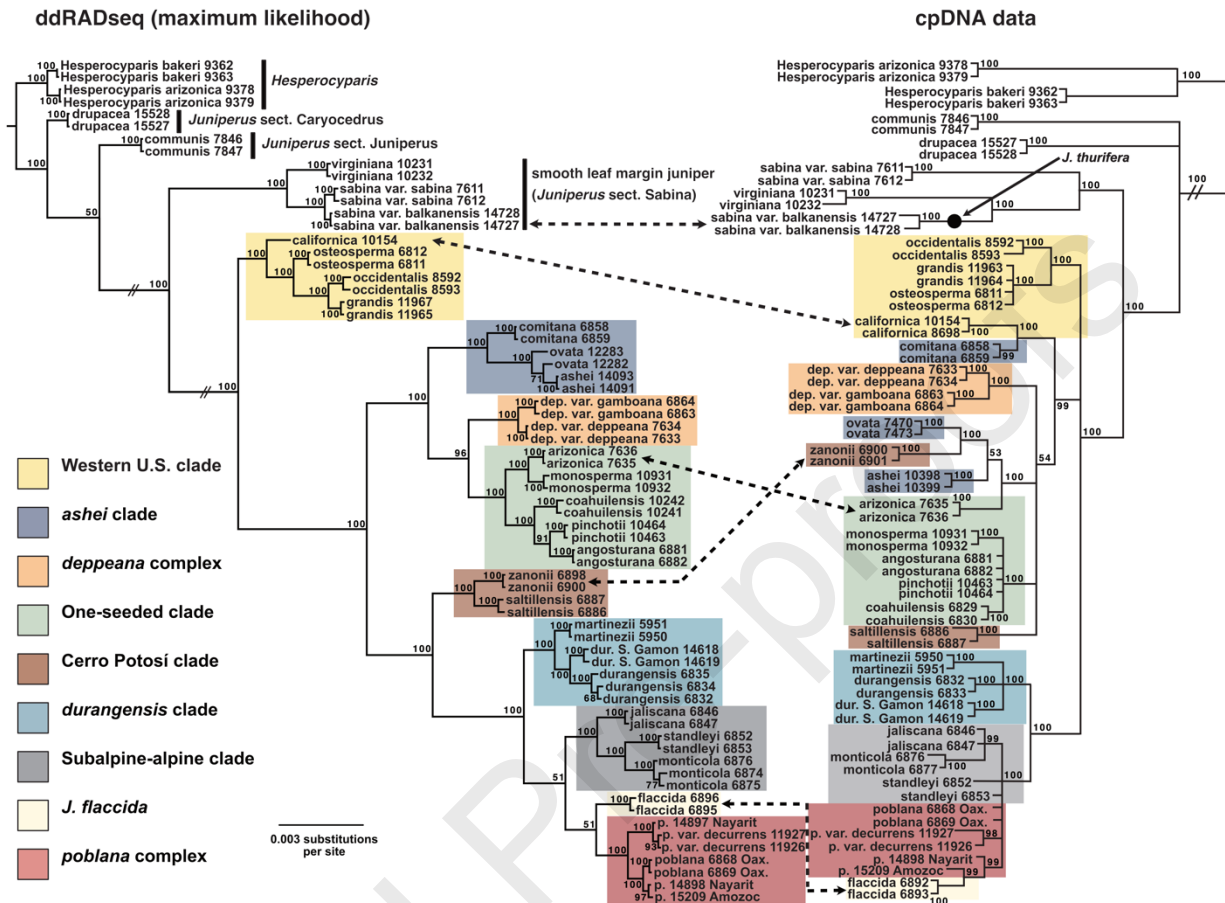
1259 **Figure 2**



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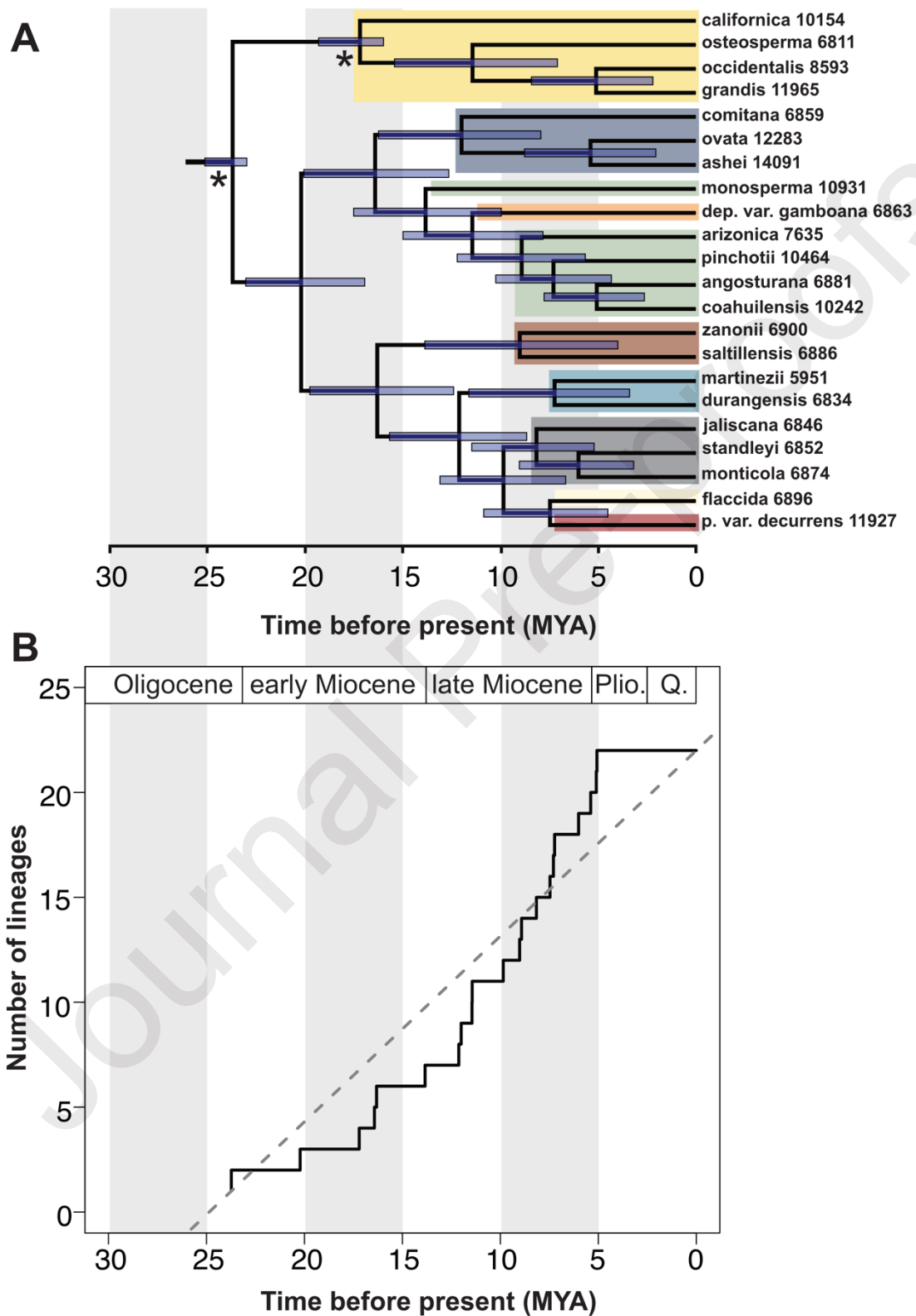
1262 **Figure 3**



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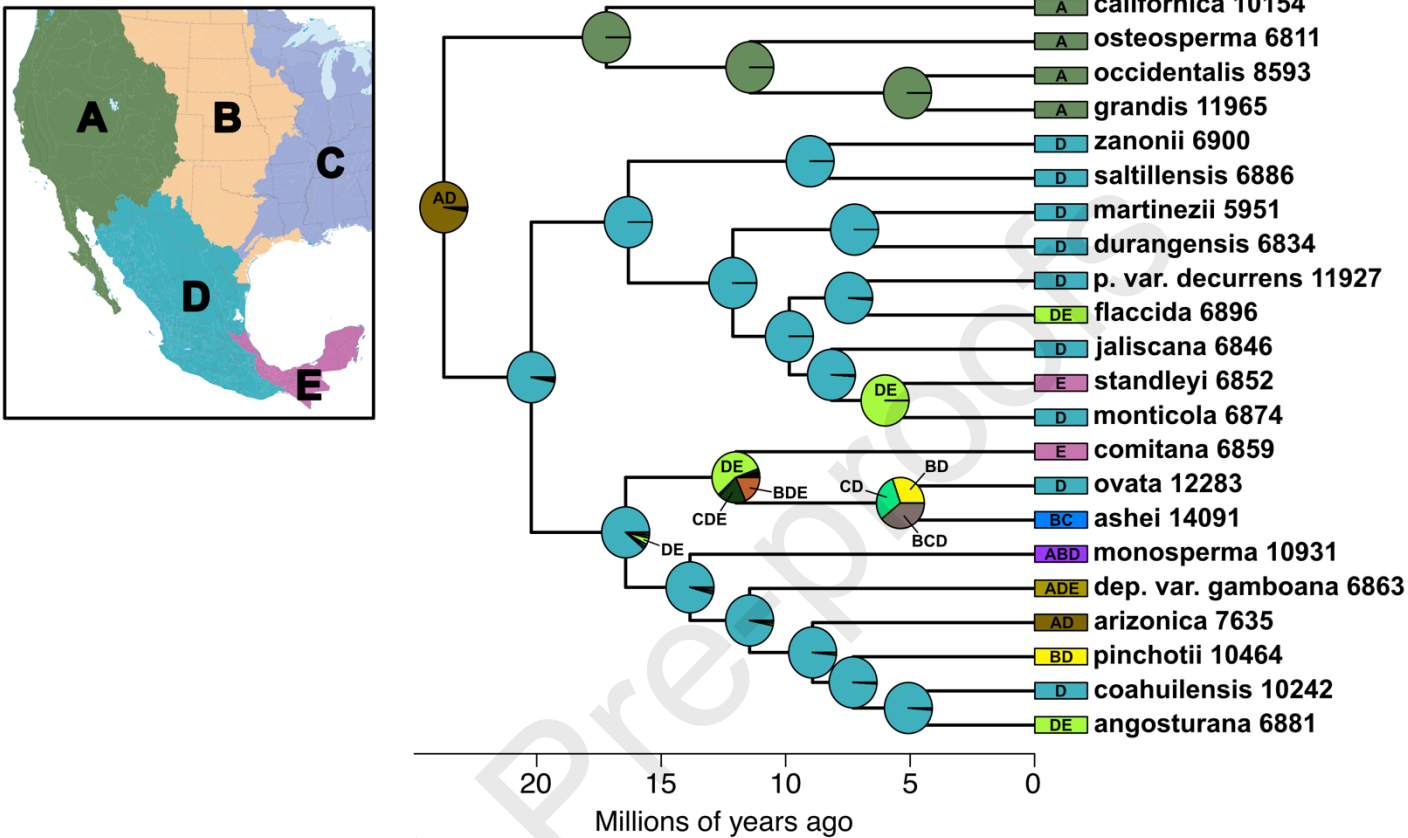
1265 **Figure 4**



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1268 **Figure 5**  
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1270 **Highlights**  
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- 1272
- 1273 • Serrate junipers are ecologically significant trees of western North America  
1274 (76 characters)
  - 1275 • RADseq data produced strongly resolved phylogeny for North American serrate junipers  
1276 (84 characters)
  - 1277 • Comparison of RADseq and cp phylogenies revealed cases of strong discordance (76)
  - 1278 • Serrate junipers originated in Oligocene and diversified rapidly in the late Miocene  
1279 (84 characters)

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Journal Pre-proofs