

Phylogenetic Relationships of *Pinus* Subsection *Ponderosae* Inferred from Rapidly Evolving cpDNA Regions

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Abstract—*Pinus* subsection *Ponderosae* includes approximately 17 tree species distributed from western Canada to Nicaragua. We inferred phylogenetic relationships of multiple accessions for all widely recognized species from 3.7 kb of cpDNA sequence (*matK*, *trnD-trnY-trnE* spacer, *chlN-ycf1* spacer, and *ycf1*). The sister relationship between subsections *Ponderosae* and *Australes* was corroborated with high branch support, and several clades, most with lower branch support, were identified within subsection *Ponderosae*. *Pinus jeffreyi* was sister to *P. coulteri*, *P. sabiniana*, and *P. torreyana*. Californian accessions of *P. ponderosa* and *P. washoensis* occurred in a clade separate from *P. arizonica* and *P. scopulorum* from the southwestern United States. Western Mexican species *P. cooperi* and *P. durangensis* had cpDNA sequences identical to one or more accessions of *P. arizonica* and *P. scopulorum*, and together these taxa were closely related to clades of *P. engelmannii-P. devoniana* (Mexico) and *P. douglasiana-P. yecorensis-P. maximinoi* (western Mexico to Guatemala). A well supported clade of taxa from Mexico and Central America included *P. pseudostrobus*, *P. montezumae*, *P. hartwegii*, *P. maximinoi* (one of three accessions), *P. nubicola*, and *P. donnell-smithii*. Chloroplast DNA sequences were nonmonophyletic for most species, although the degree of support varied.

Keywords—introgression, phylogeny, *Pinus*, species delimitation, systematics, *ycf1*.

Phylogenetic studies of the genus *Pinus* have demonstrated congruent relationships among subsections, sections, and subgenera based on nuclear (Liston et al. 1999, 2003; Syring et al. 2005) and chloroplast (cp) DNA sequences (Gernandt et al. 2005; Eckert and Hall 2006). In contrast, phylogenetic inference among closely related species within subsections presents a challenge. Lack of species monophyly was found for the internal transcribed spacer of nuclear ribosomal DNA in *Pinus* subsection *Cembroides* (Gernandt et al. 2001) and for single- or low-copy nuclear genes in *Pinus* subgenus *Strobus* (Syring et al. 2007). Lineage sorting has been identified as one source of species nonmonophyly, but introgression and hybridization are also important (Critchfield 1966; Saylor and Smith 1966; Mirov 1967), with chloroplast introgression documented in natural populations (e.g. for North America: Hong et al. 1993; Latta and Mitton 1999; Matos and Schaal 2000; Delgado et al. 2007; Liston et al. 2007).

Species of *Pinus* subsection *Ponderosae* Loudon (section *Trifoliae* Duhamel, subgenus *Pinus*) are economically important trees distributed in temperate montane and subtropical forests of western North America and Central America (Little and Critchfield 1969; Farjon and Styles 1997; Price et al. 1998; Gernandt et al. 2005). Members possess three to five (less commonly two or eight) medium to long (6–40 cm) needle-shaped leaves arranged in fascicles with a persistent sheath, two fibrovascular bundles per needle, and medium to large (5 × 3.5–35 × 20 cm) woody ovulate cones, with scales possessing a dorsal umbo. Species circumscriptions and interrelationships are poorly understood. Price et al. (1998; Table 1) recognized seventeen species divided into four informal groups based on thin or thick, scaly or pruinose branches, the number of needles per fascicle, ovulate cone size, scale shape, whether cone scale prickles are conspicuous or inconspicuous, thin or thick, persistent or deciduous cone peduncle, and the ability to form hybrids with other species in the subsection (Haller 1962; Shaw 1914; Martínez 1945; Little and Critchfield

1969; Perry 1991). In contrast, Farjon (2005) recognized only 14 species, and did not divide them into groups.

Divergence time estimation using a molecular clock approach suggests that the last extant *Pinus* subsections to diverge were *Ponderosae* and *Australes* approximately 8–15 million years ago (Willyard et al. 2007). Interspecific divergence of cpDNA is low in subsection *Ponderosae* relative to other subsections of *Pinus*, with only eight variable characters found for 14 taxa in *matK* (four characters) and *rbcl* (four characters; Gernandt et al. 2005). The present study uses *matK* and four other highly variable cpDNA regions chosen for their ability to discriminate among species of *Pinus* subsection *Ponderosae*. We infer a cpDNA sequence phylogeny for multiple accessions per species to 1) identify the principal cpDNA lineages of the subsection, 2) test the monophyly of the groups recognized by Price et al. (1998), and 3) determine whether multiple accessions per species form monophyletic groups.

MATERIALS AND METHODS

Two to five individuals were collected for all species of *Pinus* subsection *Ponderosae* recognized by Price et al. (1998). When possible, accessions were chosen that maximized geographic distance, morphological variation, or both (Appendix 1). We follow Farjon (2005) in recognizing *P. arizonica* at the species level. One individual of *P. yecorensis* (Debrezcy and Rácz 1995) was also included; this taxon was not recognized in the classification of Price et al. (1998) or by Farjon (2005); the latter considered it a synonym of *P. pseudostrobus*. We also recognize *P. scopulorum* Lemmon (see below). The outgroup included five representatives of subsection *Australes* (the sister group of subsection *Ponderosae*), two representatives of subsection *Contortae* (the sister group of *Australes* + *Ponderosae*), and one representative of section *Pinus* (the sister group of *Australes* + *Ponderosae* + *Contortae*).

Complete cpDNA sequences of *P. thunbergii* (subgenus *Pinus*; 119,707 bp; Wakasugi et al. 1994; NC_001631.1) and *P. koraiensis* (subgenus *Strobus*; 117,190 bp NC_004677; Noh et al. unpublished) were aligned with ClustalW (Thompson et al. 1994) and refined by eye. Twenty-two variable regions were identified, and primer pairs were chosen using Primer3 (Rozen and Skaletsky 2000) to amplify fragments ranging from approximately

TABLE 1. Classification of *Pinus* subsection *Ponderosae* by Price et al. (1998). Taxa not recognized at the species level by Farjon (2005) are indicated with an asterisk. Farjon (2005) also recognized *P. arizonica* Engelm.

Taxon	Distribution
1. <i>P. cooperi</i> * Blanco	Western Mexico
2. <i>P. durangensis</i> Martínez	Western Mexico
3. <i>P. engelmannii</i> Carrière	Western Mexico to Arizona and New Mexico
4. <i>P. jeffreyi</i> Balfour	Southern Oregon to Northern Baja California
5. <i>P. ponderosa</i> Douglas ex P. Lawson & C. Lawson var. <i>arizonica</i> (Engelmann) Shaw	Northern Mexico to Arizona and New Mexico
var. <i>ponderosa</i>	Western Canada to Southern California
var. <i>scopulorum</i> Engelmann	Montana to Northern Mexico
6. <i>P. washoensis</i> * Mason & Stockwell	Eastern California to Western Nevada
'Montezumae Group'	
7. <i>P. devoniana</i> Lindley	Western and Central Mexico to Guatemala
8. <i>P. donnell-smithii</i> * Masters	Guatemala
9. <i>P. hartwegii</i> Lindley	Northern Mexico to Honduras
10. <i>P. montezumae</i> Lambert	Northern Mexico to Guatemala
'Pseudostrobus Group'	
11. <i>P. douglasiana</i> Martínez	Western to Southern Mexico
12. <i>P. maximinoi</i> Moore	Western Mexico to Nicaragua
13. <i>P. nubicola</i> * Perry	Southern Mexico to Guatemala
14. <i>P. pseudostrobus</i> Lindley var. <i>apulcensis</i> (Lindley) Shaw	Central Mexico to El Salvador
var. <i>estevezii</i> Martínez	Eastern Mexico
var. <i>pseudostrobus</i>	Northern Mexico to El Salvador
'Sabinianae Group'	
15. <i>P. coulteri</i> D. Don	California to Baja California
16. <i>P. sabiniana</i> Douglas	California
17. <i>P. torreyana</i> Perry ex Carrière subsp. <i>insularis</i> Haller	Santa Rosa Island, California
subsp. <i>torreyana</i>	Del Mar County, California

400–700 bp. The primer pairs were evaluated for their ability to obtain satisfactory quality sequence in three to five members of subsection *Ponderosae*, and to reveal variable sites within otherwise poorly resolved subclades (Hernández León 2007; detailed screening results available upon request). Four regions were selected for sequencing in all accessions; these were combined with sequences from *matK* for the complete matrix (Table 2).

TABLE 2. Source and position of chloroplast primers used in this study. Primer name indicates the position of the 3' nucleotide in the complete cpDNA genome of *Pinus thunbergii*.

DNA region	Primer name	Primer sequence (5'-3')	Source
<i>matK</i>	1F (Pt1548F)	taa acg atc ctc tca ttc acg a	Wang et al. (1999)
	2R (Pt2567R)	gaa ctc gtc gga tgg agt g	Wang et al. (1999)
<i>trnD-trnY-trnE</i>	Pt28456F	cag ggc ggt act cta acc aa	This study
	Pt28893R	ttt gtc caa cca acc cat tt	This study
<i>chlN-ycf1</i>	Pt95235F	gat ttg cca atg cga gag at	This study
	Pt96059R	tag aat atg acc gcc caa cc	This study
<i>ycf1</i> amplicon A	Pt96499F	gga ccg aag tct cct aat att ttt	This study
	Pt97365R	cgc caa aca ctc gaa aag g	This study
<i>ycf1</i> amplicon B	Pt98232F	ctt ttc gtt tga agc ctt gg	This study
	Pt99121R	tgt ggt ttt tcg tga tcc aa	This study

Total genomic DNA was extracted from needles from single trees, except for *P. torreyana* subsp. *torreyana* (CAL2), *P. ponderosa* (MONT), and *P. yecorensis* (SON), for which DNA was extracted from megagametophyte tissue from a single seed. Extractions were carried out with either a modified CTAB protocol (Doyle and Doyle 1987) or a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Final concentrations for the polymerase chain reaction (PCR) were as follows: 1× *Taq* buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 1 μM of each primer, and 0.67–1.0 U recombinant *Taq* polymerase. All PCR reagents were from Invitrogen (Carlsbad, California). PCR reactions were performed in 30–50 μL under the following conditions: 94°C for three min, followed by 30 cycles of 94°C for 1 min, 50°C for 50 s, and 72°C for 80 s; the cycles were terminated with an extension step at 72°C for 5 min.

Initially, PCR products were purified and sequenced on an ABI 310 automated sequencer (Gernandt et al. 2008). Subsequently, PCR products were sequenced by Macrogen, Inc. (Seoul, South Korea) and the University of Washington High Throughput Genomic Unit (Seattle, Washington). Sequence reads were assembled in Sequencher 4.8 (Gene Codes, Inc., Ann Arbor, Michigan) and edited in BioEdit 7.0.5 (Hall 1999). Multiple sequence alignments were performed for each region separately using web-based MAFFT 6 (Katoh et al. 2005) with the FFT-NS-i option.

The alignment contained 1.34% missing data. The numbers of substitutions within subsection *Ponderosae* and for the entire matrix were determined in PAUP* (Swofford 2002). A heuristic search using parsimony was performed in PAUP* with identical sequences deleted. The search used default settings except that 500 replicates of random addition sequence and tree-bisection reconnection were used with the maximum number of trees saved set at 10,000. Branch support was measured from heuristic searches of 10,000 bootstrap replicates with 10 replicates of random addition sequence, and decay indices with the converse constraints command. Recombination of cpDNA is presumed to be negligible, but it has been reported in *Pinus contorta* using microsatellite markers (Marshall et al. 2001). A 500 replicate bootstrap of each of the five cpDNA regions separately found no conflicting clades supported by values > 70%.

A maximum likelihood analysis was also performed in PAUP*. Nucleotide substitution models were chosen with the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall 1998; Posada and Buckley 2004). A heuristic search with 50 random addition replicates was performed, with all other options left on default. Branch support was calculated from heuristic searches of 500 bootstrap replicates using the same model in GARLI 0.96 (Zwickl 2006), with all other options left on default. The sequence alignment and likelihood tree are deposited in TreeBASE (study number S2255).

Topological constraints consistent with the group classification of Price et al. (1998; Table 1) or with broad species concepts of *P. ponderosa* were enforced in PAUP* under both parsimony and likelihood (using the same model as for the unconstrained search). The resulting trees were compared to the best parsimony and likelihood trees using the Templeton test (Templeton 1983; p-scores /nonpara) and the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999; l-scores /shtest = rell). Because we were interested in discovering whether one or more of the trees recovered in the constrained analyses were similar to the most parsimonious trees, the one-tailed *p* value for the least significant pairwise test was used to assess significance (Goldman et al. 2000).

RESULTS

Seventy-five accessions representing the taxonomic diversity of *Pinus* subsection *Ponderosae* and eight outgroups were included (Appendix 1). The concatenated DNA matrix consisted of *matK*, *trnD-trnY-trnE*, the *chlN-ycf1* spacer, and two noncontiguous sections of *ycf1* (amplicons A and B). To eliminate missing data adjacent to primers and to reduce the quantity of missing data owing to a midstudy primer redesign, the alignment was trimmed to 3,703 bp. Based on comparison to *P. thunbergii*, the matrix represents 3.1% of the cpDNA genome.

The alignment included 105 parsimony informative sites and 180 sites that were variable but parsimony-uninformative (Table 3). The most variable region when considering all outgroups (except the *chlN-ycf1* spacer in subsection *Contortae*, for which we failed to obtain sequence) was *ycf1* amplicon A, with 43 parsimony informative sites and 86 variable but uninformative sites (Table 3). The region with the fewest parsimony informative sites was *matK*, with seven compared to a range of 14–43 in the other four regions (Table 3). Within subsection *Ponderosae*, four indels were observed with the same beginning and ending positions: two were in the *trnD-trnY-trnE* spacer and two were in the *chlN-ycf1* spacer. The *trnD-trnY-trnE* spacer also had a variable-length mononucleotide A/T run that ranged from eight to 11 bp in subsections *Ponderosae* and *Australes*, and from six to seven bp in the two accessions from subsection *Contortae* and in *P. thunbergii*. Indels in *matK*, *ycf1* amplicon A, and *ycf1* amplicon B (in multiples of 3 bp) were only observed among subsections. With the exception of the mononucleotide repeat, the four indels occurring within subsection *Ponderosae* were diagnostic of clades that were well-supported by multiple substitutions. To simplify description of the phylogenetic results and comparison of parsimony with maximum likelihood, the indels were not coded as additional characters.

A parsimony search of the 37 unique cpDNA sequences recovered 50 most-parsimonious trees (MPTs; L = 325, consistency index = 0.93, consistency index excluding uninformative characters = 0.83, retention index = 0.95) from which a strict consensus was generated (Fig. 1). For the likelihood analysis, the nucleotide substitution model chosen using AIC was K81uf + I (K81uf + G using hLRT). Base frequency parameters were A = 0.3292, C = 0.1750, G = 0.1747, T = 0.3211, and rate matrix parameters were AC = 1.0000, AG = 1.0159, AT = 0.3389, CG = 0.3389, CT = 1.0159, and GT = 1. The value for invariant sites (I) was 0.7559. A single optimal tree was recovered (Fig. 2) with a likelihood value of Ln = -7,083.72192. Parsimony and likelihood recovered the same principal lineages, but because identical sequences were eliminated from

the parsimony tree (Fig. 1), monophyletic clades of identical haplotypes are only apparent in the likelihood tree (Fig. 2).

Pinus subsection *Ponderosae* was monophyletic in a sister position to subsection *Australes* (Figs. 1 and 2). Relationships within the subsection varied in their level of support. We divided subsection *Ponderosae* into five groups (four clades and a poorly resolved grade) but caution that alternative subdivisions are valid. Poorly resolved among the first nodes of the subsection were identical sequences from a *P. washoensis* × *P. jeffreyi* artificial hybrid and an accession of *P. ponderosa* (CAL2), four identical sequences of *P. jeffreyi* (the Jeffrey grade) sister to a Sabiniana clade (corresponding to the 'Sabiniana Group' sensu Price et al. 1998), and a larger clade consisting of a trichotomy of three subclades (hereafter referred to as the Ponderosa, Devoniana, and Montezumae clades).

The Sabiniana clade included four unique haplotypes representing six individuals from *P. torreyana* (mainland and island), *P. coulteri*, and *P. sabiniana*. Relationships among these three species were unresolved, with only the two identical *P. torreyana* forming a poorly supported subclade (Fig. 2). The Ponderosa clade consisted of two unique haplotypes representing six individuals from *P. ponderosa* (from California, Montana, and Oregon) and all three nonhybrid accessions of *P. washoensis*. The Devoniana clade included 14 unique haplotypes representing 26 individuals from *P. arizonica*, *P. cooperi*, *P. durangensis*, and *P. scopulorum* (*P. ponderosa* var. *scopulorum*, sensu Price et al. 1998), *P. douglasiana*, *P. yecorensis*, *P. maximinoi* (two of three accessions), *P. devoniana*, and *P. engelmannii*. A well supported subclade was recovered for some but not all accessions of *P. arizonica*, *P. cooperi*, *P. durangensis*, and *P. scopulorum*. A moderately supported subclade included *P. douglasiana*, *P. yecorensis*, and *P. maximinoi*. The Montezumae clade included seven unique haplotypes representing 23 individuals of *P. montezumae*, *P. pseudostrobus* (including vars. *apulcensis*, *estevezii*, and *pseudostrobus*), *P. nubicola*, *P. hartwegii*, *P. donnell-smithii*, and one of three *P. maximinoi* accessions. Support for the Montezumae clade was high, but internal relationships were unresolved or received low branch support.

Topological constraints were enforced to evaluate whether the cpDNA data could be used to reject the four group subdivision of *Pinus* subsection *Ponderosae* sensu Price et al. (1998; Table 1). Compared to the best trees (L = 325; Ln = -7,083.72192; Figs. 1 and 2), simultaneously constraining monophyly on the 'Ponderosae,' 'Montezumae,' 'Pseudostrobus,' and 'Sabiniana' Groups (L = 337; Ln = -7,174.31492) was rejected (Templeton $p < 0.01$; SH $p < 0.01$). Constraining only the 29 accessions representing the six species of the 'Ponderosae Group' to monophyly (L = 330, Ln = -7,123.04078) was rejected (Templeton $p < 0.05$; SH $p < 0.05$). Constraining all 13 accessions representing the four species of the 'Montezumae Group' to monophyly (L = 333; Ln = -7,139.55555) was rejected (Templeton $p = 0.01$; SH $p < 0.01$). Constraining all 19 accessions representing the four species of the 'Pseudostrobus Group' (including *P. yecorensis*) to monophyly (L = 332; Ln = -7,129.76827) also was rejected (Templeton $p < 0.05$; SH $p < 0.05$).

We also performed constraints based on the observed separation in the cpDNA trees of taxa thought to be closely related to *P. ponderosa* (Figs. 1. and 2). Constraining *P. ponderosa*, *P. arizonica*, and *P. scopulorum* to monophyly (*P. ponderosa* sensu Price et al. 1998; L = 336; Ln = -7,168.09176) was rejected (Templeton $p < 0.01$; SH $p < 0.001$). Constraining

TABLE 3. Molecular variation of the five cpDNA regions selected for subsection *Ponderosae*. *We were unable to obtain *chlN-ycf1* spacer sequence for the two species of subsection *Contortae*.

DNA region	No. seqs	Aligned Length	Variable-Uninformative		Informative	
			Total	<i>Ponderosae</i>	Total	<i>Ponderosae</i>
<i>matK</i>	75	975	16	4	7	2
<i>trnD-trnY-trnE</i>	75	859	24	2	14	4
<i>chlN-ycf1</i> spacer	73*	483	34	1	14	7
<i>ycf1</i> amplicon A	75	534	86	3	43	6
<i>ycf1</i> amplicon B	75	852	20	3	27	5
TOTAL	75	3703	180	13	105	24

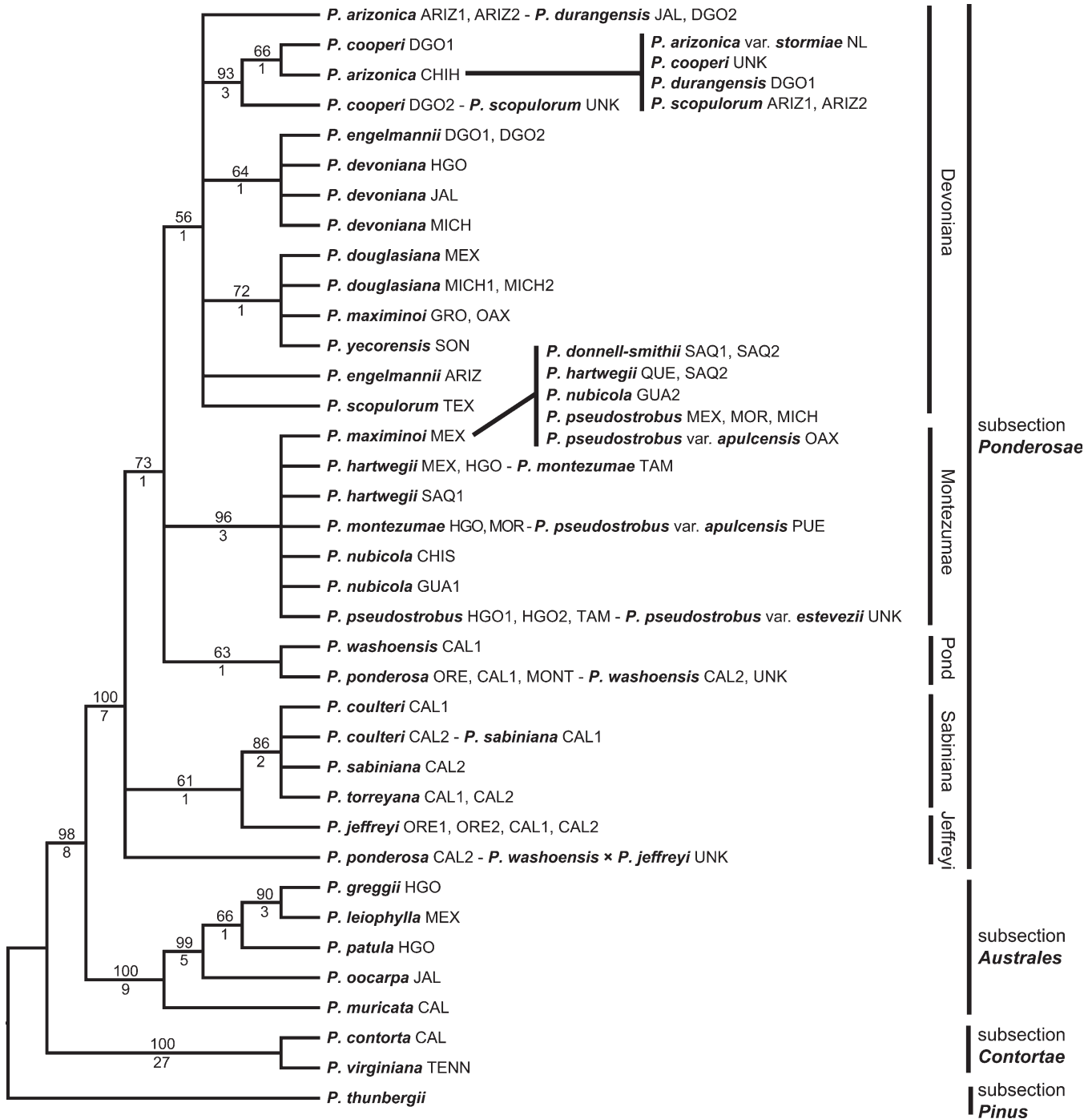


FIG. 1. Chloroplast DNA relationships for subsection *Ponderosae* inferred from parsimony. Strict consensus of 50 MPTs. Bootstrap values > 50% are shown above branches and decay values are shown below branches. Haplotype provenance, identical cpDNA haplotypes, clade names and subsectional names are indicated on the right side of tree. See text for further explanation of species and clade names.

all accessions of *Pinus ponderosa* (including CAL2) and *P. washoensis* to monophyly with the Devoniana clade (including *P. arizonica* and *P. scopulorum*; $L = 327$; $L_n = -7,130.71858$) was not rejected by the Templeton test ($p = 0.2071$) but was by the SH test ($p < 0.01$). Constraining *P. jeffreyi* to monophyly with *P. ponderosa* and *P. washoensis* ($L = 328$; $L_n = -7,105.05211$) was not rejected (Templeton $p = 0.1284$; SH $p = 0.053$). Constraining *P. ponderosa*, *P. washoensis*, and *P. jeffreyi* to the Devoniana clade ($L = 328$; $L_n = -7,103.14136$) was not rejected (Templeton $p = 0.129$; SH $p = 0.051$). Constraining *P. ponderosa*, *P. washoensis*, *P. scopulorum*, *P. arizonica*, and

P. cooperi to monophyly ($L = 326$; $L_n = -7,090.59331$) was not rejected (Templeton $p = 0.3273$; SH $p = 0.094$).

DISCUSSION

Gene Flow and Retention of Ancestral Polymorphism—Lineage sorting and hybridization or introgression can cause conflicts between gene trees and species trees (Maddison 1997), and there is a growing awareness of the importance of inferring relationships among closely-related species using multiple individuals and loci (e.g. Rosenberg 2003; Knowles

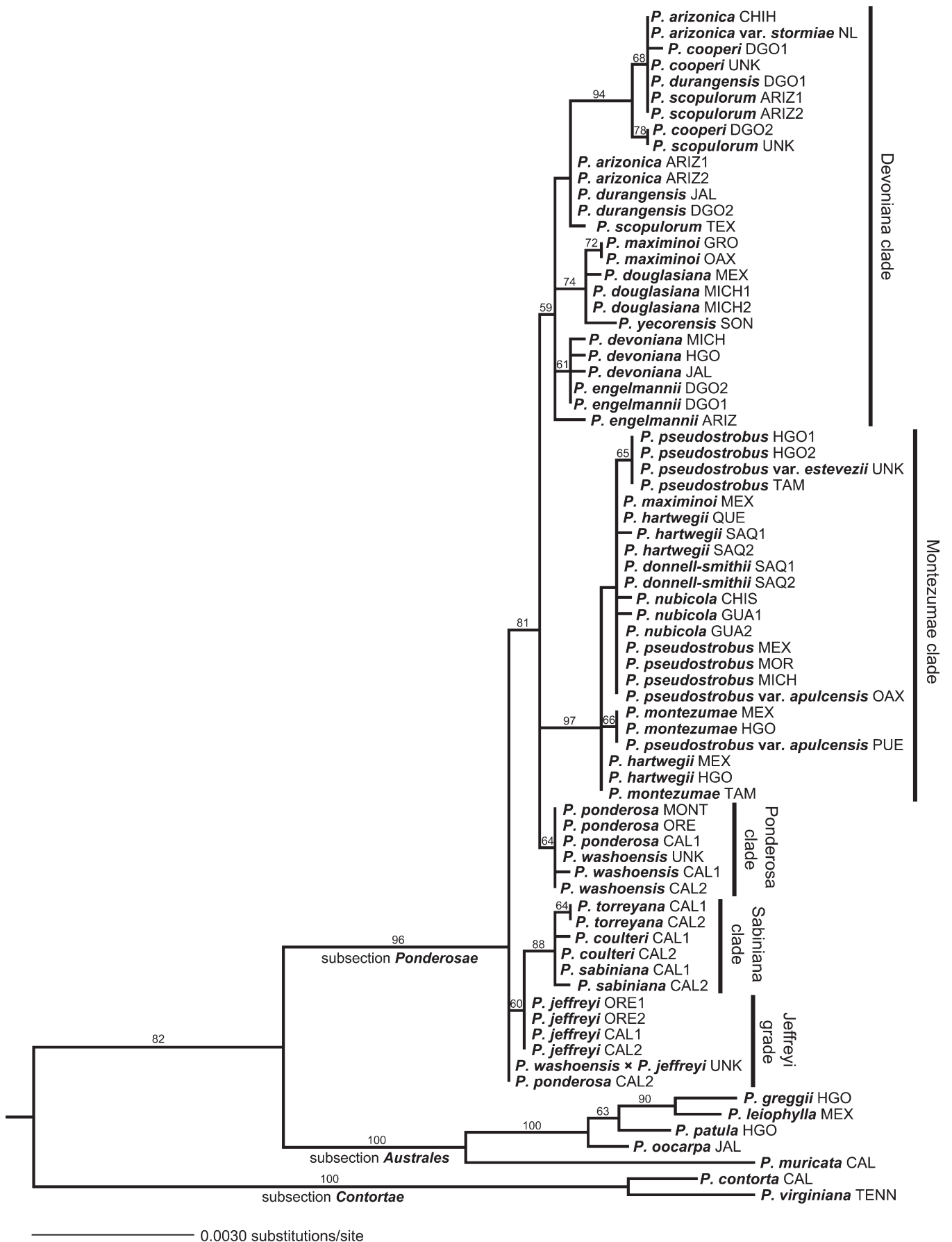


FIG. 2. Maximum likelihood tree of the cpDNA data set. The *Pinus thunbergii* outgroup was pruned after the analysis to amplify the branch lengths for subsection *Ponderosae*. Bootstrap values > 50% are shown above branches.

and Carstens 2007; Syring et al. 2007). Furthermore, coalescent approaches have been proposed to address species delimitation (Rosenberg 2003; Knowles and Carstens 2007). A study of *Pinus* subsection *Ponderosae* based primarily on nuclear loci (Willyard et al. 2009) found that species nonmonophyly by locus was common, as was incongruence among loci. The larger effective population size of nuclear compared to chloroplast markers apparently results in a relatively slower coalescence rate and consequently lineage sorting. An issue of fundamental importance to the aims of this study is whether introgression or retention of ancestral polymorphism has substantially affected our ability to use cpDNA to circumscribe subsection *Ponderosae*, divide the subsection into phylogenetic subgroups, or delimit species. Our approach is to compare the cpDNA phylogeny to previous classifications, and to the morphological, biochemical, and genetic evidence on which they were based.

Hybridization and introgression are well documented in subsection *Ponderosae*. The best studied species in respect to controlled crosses is *P. ponderosa*, which is compatible with species of the *Ponderosa* clade (*P. ponderosa* and *P. washoensis*; Critchfield 1984), with *P. jeffreyi* (Critchfield 1966), with species of the *Devoniana* clade (*P. arizonica*, *P. scopulorum*, *P. engelmannii*, and *P. durangensis*), and has also yielded low numbers of sound seeds when pollinated by species of the *Montezumae* clade (*P. hartwegii*, *P. montezumae*, and *P. pseudostrobus*; Conkle and Critchfield 1988). Hybridization is thought to have played an important role in the genetic makeup of wild populations throughout the range of subsection *Ponderosae* (Haller 1962; Mirov 1967; Perry 1991; Matos and Schaal 2000; Delgado et al. 2007; Willyard et al. 2009). A *P. washoensis* × *P. jeffreyi* artificial hybrid from the Institute of Forest Genetics, California, was included in this study and was sister to the clade of its pollen donor, *P. jeffreyi*. Several other cases of species nonmonophyly may be due to natural hybridization or introgression: the cpDNA sequence of *P. ponderosa* (CAL2) has the same haplotype as the *P. washoensis* × *P. jeffreyi* hybrid, *P. maximinoi* (MEX) occurs in the *Montezumae* clade, and *P. pseudostrobus* var. *apulcensis* (PUE) has the same haplotype as two accessions of *P. montezumae*. Another possible hybrid is *P. pseudostrobus* (TAM), which has foliage similar to *P. arizonica* var. *stormiae* (four needles per fascicle > 1 mm thick). Additional morphological markers for detecting hybrids (e.g. Delgado et al. 2007) would be useful, but morphological intermediacy may not always mark historical introgression events (Liston et al. 2007).

Circumscription of *Pinus* Subsection *Ponderosae*—Both *Pinus* subsection *Australes* and *Pinus* subsection *Ponderosae* are New World hard pines characterized by relatively dense wood with dentate ray tracheids and pinoid cross-field pits, medium to long needles in fascicles of three to five (occasionally two, six, or eight), medial or internal leaf resin ducts, and medium to large cones that usually dehisce at maturity. Crossing experiments demonstrated that species of the eastern and southern United States are highly incompatible with species from the western United States (Duffield 1952; Critchfield 1963), prompting the division of species from these regions into separate groups despite ambiguous morphological evidence (Lauria 1991). The North American pines south of the U. S. A. were incompletely represented in crossing studies, and for this reason, together with their high morphological variability, their classification has been a source of disagreement. The species makeup of the subsection *Ponderosae* clade

(Figs. 1 and 2) is consistent with previous molecular studies of *Pinus* (Krupkin et al. 1996; Liston et al. 2003; Gernandt et al. 2005; Eckert and Hall 2006). Many past circumscriptions of subsection *Ponderosae* based principally on morphology, mixed species of subsections *Ponderosae* and *Australes* (Shaw 1914; Pilger 1926; Duffield 1952; Little and Critchfield 1969; Perry 1991; Farjon and Styles 1997). Martínez (1945) and Van der Burgh (1973) avoided mixing subsections *Ponderosae* and *Australes*, but each recognized many sections, and their hierarchies did not reflect the mutual monophyly of subsections *Ponderosae* and *Australes*.

Group Subdivisions—*Pinus* subsection *Ponderosae* has often been divided into three to four informally named groups (Martínez 1945; Duffield 1952; Little and Critchfield 1969; Van der Burgh 1973; Perry 1991; Price et al. 1998). Lauria (1991) argued that proposed group subdivisions of subsection *Ponderosae* were artificial. Price et al. (1998) used a group classification for subsection *Ponderosae* (Table 1). An alternative division of the neotropical members of subsection *Ponderosae* by Farjon and Styles (1997) was subsequently abandoned (Farjon 2005). The cpDNA topology includes a *Sabiniana* clade but is inconsistent with other proposed subdivisions.

Loudon (1838) proposed subsection *Sabinianae* for *P. roxburghii* (as *P. longifolia*) from the Himalayas and two of the three California big-cone pines, *P. coulteri*, and *P. sabiniana*. These species have long, thick needles in fascicles of three and large ovulate cones with enlarged scale umbos terminating in a spine or hook. *Pinus torreyana*, with needles in fascicles of five, was not described until 1855. Shaw (1914) classified *P. coulteri*, *P. sabiniana*, and *P. torreyana* in group *Macrocarpae* (the California big-cone pines). Little and Critchfield (1969) classified the three California species in subsection *Sabinianae*, but Price et al. (1998) included them in subsection *Ponderosae* as an informal group. Monophyly of the ‘*Sabinianae* group’ with other members of subsection *Ponderosae* has been corroborated in molecular phylogenetic studies of the internal transcribed spacer region of nuclear ribosomal DNA (Liston et al. 1999, six species) and chloroplast DNA (Krupkin et al. 1996, five species; Gernandt et al. 2005, 14 species; Eckert and Hall 2006, 10 species).

Four of five identical *Pinus jeffreyi* sequences were in a sister position to the *Sabiniana* clade (Figs. 1 and 2: bootstrap values < 70%), consistent with the cpDNA tree of Krupkin et al. (1996). The fifth sequence, from *P. washoensis* × *P. jeffreyi* of unknown origin, was identical to a *P. ponderosa* accession from northern California, and poorly resolved. Based on its ability to cross both artificially and naturally with *P. ponderosa* and *P. coulteri*, *P. jeffreyi* was hypothesized to form a genetic link between *P. ponderosa* and the three species of the *Sabiniana* clade (Duffield 1952; Haller 1962; Critchfield 1966). *Pinus jeffreyi* and *P. ponderosa* are morphologically similar: both have straight trunks, rigid, medium length needles (15–25 cm) in fascicles of three, and ovulate cone scales with persistent prickles. *Pinus jeffreyi* lacks probable morphological apomorphies of the *Sabiniana* clade which include an irregular trunk with spreading branches, slightly longer (typically 20–25 cm), often spreading needles, and ovulate cone scales with well developed apophyses, and enlarged seeds with highly reduced wings. Like *P. coulteri*, *P. sabiniana*, and *P. torreyana*, n-heptane is an important component of *P. jeffreyi* oleoresin (Mirov 1929, 1967). The sister relationship between *P. jeffreyi* and the *Sabiniana* clade (Figs. 1 and 2) renders the ‘*Ponderosae* group’ sensu Price et al. (1998) paraphyletic, consistent with

the cpDNA restriction site study of Krupkin et al. (1996) that included four species of subsection *Ponderosae* (*P. ponderosa*, *P. jeffreyi*, *P. torreyana*, and *P. coulteri*).

The *Ponderosa* and *Devoniana* Clades—One of the more surprising aspects of the cpDNA tree (Figs. 1 and 2) was the separation of *P. ponderosa* var. *ponderosa* and *P. washoensis* from *P. arizonica* and *P. scopulorum* (*P. ponderosa* var. *scopulorum* sensu Price et al. 1998). Short branch lengths and low bootstrap values separate many of these taxa, but the *P. scopulorum*, *P. cooperi*, *P. arizonica* (CHIH and NL only), and *P. durangensis* (DGO1) clade received high branch support. Overall morphological similarity, particularly between *P. ponderosa* and *P. scopulorum*, contrasts with clear genetic differences. This has generated taxonomic controversies around both 'Ponderosae Group' (sensu Price et al. 1998) and the "*P. ponderosa* species complex" (Price et al. 1998; Farjon 2005). The separation of *P. arizonica*, *P. scopulorum*, and *P. ponderosa* var. *ponderosa* (together with *P. washoensis*) in the cpDNA tree (Figs. 1 and 2) is further evidence that *P. arizonica* and *P. scopulorum* should be recognized at the species rank.

Lemmon (1897) proposed the combination *P. scopulorum* for *P. ponderosa* var. *scopulorum*, differentiating it from *P. ponderosa* based on its smaller overall size, thinner and harder bark, thinner needles tending to grow in scopulate tufts, commonly three but sometimes two needles per fascicle, and smaller ovulate cones with darker scales. Other characters that distinguish *P. scopulorum* from *P. ponderosa* include fewer cotyledons, sunken stomata, smaller seeds, higher levels of α -pinene and lower levels of β -pinene, and different isozyme allele frequencies (reviewed by Conkle and Critchfield 1988). Both chloroplast and mitochondrial haplotype frequencies are differentiated between *P. ponderosa* and *P. scopulorum*, with a narrow zone of secondary contact in west-central Montana (Latta and Mitton 1999). The two taxa apparently exhibit low levels of reproductive compatibility; controlled crosses between *P. scopulorum* and *P. ponderosa* from California result in lower frequencies of viable seed (Conkle and Critchfield 1988). Despite these differences, the prevailing opinion is to treat *P. scopulorum* as *P. ponderosa* var. *scopulorum* (Kral 1993; Price et al. 1998; Farjon 2005). Broader sampling of this taxon is needed throughout its range in the Rocky Mountains, but the cpDNA topology is consistent with known genetic and morphological differences with *P. ponderosa* var. *ponderosa* and indicates a closer relationship to species from Mexico. For these reasons we have chosen to use the name *P. scopulorum*.

Pinus arizonica is sometimes considered a four to five (rarely three) needled per fascicle variety of *P. ponderosa* smaller in height and with smaller cones (Shaw 1909, 1914; Little and Critchfield 1969; Price et al. 1998; Kral 1993). It has also been recognized as a separate species (Farjon and Styles 1997; Farjon 2005). Species concepts for *Pinus* subsection *Ponderosae* in southeastern Arizona have been much studied but remain unclear (Peloquin 1984; Rehfeldt et al. 1996; Rehfeldt 1999; Epperson et al. 2001). Two of our collections of *P. arizonica* (ARIZ1 and ARIZ2) and one collection of *P. scopulorum* (ARIZ1) are from the Santa Catalina Mountains, where hybrids with high needle variability (three to five needles per fascicle) may exist between high elevation *P. scopulorum* and lower elevation *P. arizonica*. The individuals *P. arizonica* ARIZ1 and ARIZ2 had five or occasionally four relatively short (14–19 cm) and thin needles per fascicle. In the cpDNA trees (Figs. 1 and 2) they occurred with two of three *P. durangensis* accessions and near *P. scopulorum* from Texas, but sepa-

rate from *P. scopulorum* from the Catalina Mountains and the Chiricauhuas (ARIZ1 and ARIZ2, respectively), suggesting that despite some needle number variability, their cpDNA was representative of *P. arizonica* and not *P. scopulorum*. No material of *P. arizonica* was included from the type locality in the Santa Rita Mountains.

Other taxa of questionable species status had low or null sequence divergence from other species (Fig. 2). Two northern Mexico taxa, *P. cooperi* and *P. durangensis*, have been hypothesized to be closely related to *P. arizonica* (Martínez 1945; Farjon 2005). The three accessions of each of these species were mixed among accessions of *P. arizonica* and *P. scopulorum*. *Pinus washoensis* has been separated from the wider ranging *P. ponderosa* based mainly on its shorter needles and smaller cones with persistent, reflexed prickles. It is most similar morphologically and genetically to the North Plateau race of *P. ponderosa* (Critchfield 1984; Niebling and Conkle 1990) and several authors have treated *P. washoensis* as a synonym of *P. ponderosa* (Mirov 1961; Farjon 2005). *Pinus washoensis* grouped with *P. ponderosa* (Figs. 1 and 2).

The occurrence of *P. engelmannii* in the Devoniana clade with *P. arizonica* and *P. scopulorum* is consistent with previous classifications (Shaw 1909, 1914; Martínez 1945; Price et al. 1998; Table 1). Species of the Devoniana clade that have not been proposed as closely related to this group are *P. devoniana*, *P. douglasiana*, and *P. maximinoi*. These species occur in the southern part of the Mexican Sierra Madre Occidental and they have ranges that extend farther south than other species in this clade: *P. devoniana* to Guatemala, *P. douglasiana* to Oaxaca, Mexico, and *P. maximinoi* to Nicaragua. Similarities between *P. devoniana* and *P. engelmannii* suggest that its position in this clade does not present a great problem. Both have thick scaly branches, long fascicle sheaths (up to 4 cm), and long needles (up to 40 cm), among the longest of subsection *Ponderosae* (Farjon and Styles 1997). *Pinus devoniana* usually has five needles per fascicle (rarely four or six) and long ovulate cones (15–35 cm) with flat scale umbos with a small, deciduous prickle, while *P. engelmannii* usually has three needles per fascicle (rarely four or five) and relatively shorter ovulate cones (8–15 cm), the scale umbo raised with a stout prickle. Matos (1998) found 25 polymorphic restriction sites in 175 individuals of *P. devoniana*, which amounts to much more variation than the three nucleotide substitutions found in the present study.

Pinus douglasiana and *P. maximinoi* are strikingly different morphologically from other members of subsection *Ponderosae*: their leaf hypodermal cells intrude into the mesoderm, often touching the endodermis, and the cones have a persistent peduncle and fewer, relatively thin scales. *Pinus maximinoi* tends to have thinner needles and cone scales (Stead 1983; Stead and Styles 1984), but the two are notoriously difficult to tell apart (Farjon and Styles 1997). Two of the three *P. maximinoi* accessions (from Guerrero and Oaxaca) group with *P. douglasiana*, but the third (from the state of Mexico) occurs in the Montezumae clade. We confirmed the identification of the accessions and suggest that the placement of one *P. maximinoi* in the Montezumae clade could be due to introgression. The sequence of *P. yecorensis* is from a population near Yécora, Sonora. In its original description, the species was compared to *P. devoniana*, *P. pseudostrobus* var. *estevezii*, *P. nubicola*, *P. durangensis*, *P. engelmannii*, and *P. maximinoi* (Debreczy and Rácz 1995). The taxon lacks hypodermal intrusions but otherwise resembles *P. douglasiana*.

The Montezumae Clade—The Montezumae clade, represented by 23 accessions from Mexico and Central America, shows low levels of sequence divergence and minimal phylogenetic structure. Few artificial crossings have been attempted between these species (Critchfield 1966; Saylor and Smith 1966), but natural hybridization has been postulated on morphological evidence (Mirov 1967; Perry 1991) and studied with cpDNA markers between *P. hartwegii* and *P. montezumae* (Matos and Schaal 2000) and *P. montezumae* and *P. pseudostrobus* (Delgado et al. 2007). Separation of species into the 'Montezumae' and 'Pseudostrobus' groups was based principally on whether branches are rough and scaly or pruinose, and secondarily on needle thickness. The two species of the 'Pseudostrobus' group with characteristically pruinose branches and thin needles are *P. pseudostrobus* and *P. maximinoi*. The third species of the 'Pseudostrobus' group, *P. douglasiana*, can have scaly or pruinose branches and has thicker needles (Farjon and Styles 1997). The cpDNA topology contradicts group delimitation based on branch morphology and needle thickness.

Haplotypes in the Montezumae clade are similar despite striking diversity in branch, leaf and cone morphology. Six species are represented here sensu Price et al. (1998), but only four sensu Farjon and Styles (1997) and Farjon (2005). Farjon and Styles (1997) considered *P. donnell-smithii* as a synonym of *P. hartwegii* and *P. nubicola* as a synonym of *P. pseudostrobus*. Low resolution in this part of the cpDNA phylogeny can be considered as lack of support for recognition of these two species, but it is important to recognize the limited scope of our cpDNA characterization. An earlier study of 351 accessions of *P. montezumae* and *P. hartwegii* was able to distinguish 51 different cpDNA restriction site haplotypes with relatively low nucleotide diversity (Matos and Schaal 2000). In comparison, the present study includes eight accessions of *P. montezumae* and *P. hartwegii* (10 if *P. donnell-smithii* is considered a synonym of *P. hartwegii*) and distinguishes four haplotypes.

Slightly more cpDNA variation was found within *P. pseudostrobus* than between species such as *P. montezumae* and *P. hartwegii*, but the cpDNA clades did not coincide with the two varieties of *P. pseudostrobus* as delimited by Farjon and Styles (1997) based on differences in cone shape and degree of apophysis elongation. A poorly supported subclade (Fig. 2) includes four identical haplotypes: three accessions of *P. pseudostrobus* var. *pseudostrobus*, and a *P. pseudostrobus* var. *estevezii* accession of unknown provenance. These comprise all of the *P. pseudostrobus* collections from the Sierra Madre Oriental. One accession (HGO1) is from Apulco, Hidalgo, the type locality of another variety, *P. pseudostrobus* var. *apulcensis*. Ovulate cones of our accession do not have the elongated apophyses of *P. pseudostrobus* var. *apulcensis* and therefore correspond better to *P. pseudostrobus* var. *pseudostrobus*, which is more abundant at the locality (Farjon 1995; Farjon and Styles 1997). Ovulate cones in this clade of Sierra Madre Oriental collections are more ovoid and have wider scales compared to the more elongated cones with narrower scales typical of *P. pseudostrobus* var. *pseudostrobus* from the Eje Neovolcanico, which includes the type locality.

Perry (1987) described *Pinus nubicola*, differentiating it from *P. pseudostrobus* in having longer needles (25–43 cm) in fascicles of five, six, or more, and large ovoid cones with relatively thicker scales with "unequal apical projections." Price et al. (1998) accepted *P. nubicola* as a separate species ("needing further study"), but Farjon and Styles (1997) treated *P. nubicola* as

a synonym of *P. pseudostrobus* var. *pseudostrobus* f. *pseudostrobus*, making mention of the striking variation in leaf and cone morphology of *P. pseudostrobus* in Central America. Although cones of *P. nubicola* are similar in shape to those of *P. pseudostrobus* var. *pseudostrobus* from Apulco, Hidalgo (Farjon and Styles 1997), the oleoresin profile of *P. nubicola* is more similar to the profile of *P. pseudostrobus* var. *estevezii* and *P. pseudostrobus* var. *apulcensis* in having higher levels of heptane, nonane, and limonene, and lower levels of α -pinene relative to *P. pseudostrobus* (Perry 1987). The cones of our two accessions of *P. pseudostrobus* from Hidalgo (including Apulco), are morphologically similar to *P. nubicola* cones but their haplotypes were identical to two other Sierra Madre Oriental accessions of *P. pseudostrobus* (Figs. 1 and 2). Perry (1987) suggested that *P. nubicola* and *P. pseudostrobus* may form natural hybrids. We observed trees with foliage and cone characters intermediate between *P. pseudostrobus* var. *apulcensis* and *P. nubicola* south of San Cristóbal de Las Casas, Chiapas (Mexico), but the individuals we collected from the population at the type locality near San José Pinula, Jalapa (Guatemala) conformed more closely to *P. nubicola*. We tentatively support the recognition of this species, based on its biochemical and morphological differences from *P. pseudostrobus* var. *pseudostrobus*.

Masters (1891) described *P. donnell-smithii* for a taxon with short needles (approximately 13 cm long), with "few" resin canals from the summit of Volcán de Agua in Guatemala. The species was considered a synonym of *P. montezumae* var. *hartwegii* by Shaw (1914) and of *P. hartwegii* by Farjon and Styles (1997) but was recognized by Perry (1991) and by Price et al. (1998; "needing further study"). Perry (1991) provided several distinguishing characters for the taxon, including a needle length of 15–22 (–25) cm, which exceeds the length in the type description of 13 cm (Masters 1891). Farjon and Styles (1997), in treating *P. donnell-smithii* as a synonym of *P. hartwegii*, wrote "there is only one species of *Pinus* present around the crater rim of this stratovolcano [Volcán de Agua]; the correct name for it is *P. hartwegii*."

One of us (DSG) made collections on Volcán de Agua along the trail to the summit between 3,170 and 3,720 m. Trees at lower altitudes had both short and long needles (12–20 cm; here designated *P. donnell-smithii* SAQ1) and long needles (17.5–20.5 cm; *P. hartwegii* SAQ1 and SAQ2) growing together, but at higher altitudes all trees had short needles. Trees near the crater are subject to high winds and are small, but a dense forest of taller trees is found inside the rim of the crater near the summit, where a single collection was made (needle length = 9.5–11.6 cm; *P. donnell-smithii* SAQ2). The short-needled individuals conform to the written description of Masters (the cone illustration is difficult to interpret) but also fit comfortably within the known morphological and genetic variation of *P. hartwegii*. Of the four individuals sequenced from Volcán de Agua, three were identical to haplotypes found in *P. pseudostrobus*, *P. montezumae*, *P. nubicola*, and *P. maximinoi* and the fourth (SAQ1) was unique by a single substitution.

The variation in foliar and cone characters on Volcán de Agua resembles that found for high altitude pine forests in Mexico. Perry (1991) described these forests as including *P. montezumae* at lower elevations and *P. hartwegii* and *P. rudis* Endlicher at higher elevations. Trees of these forests vary in needle length and number per fascicle, resin ducts per needle, cone color and scale thickness. Matos (1995) analyzed variation in 25 morphological characters on two mountains in Mexico and could only discriminate two taxa, *P. montezumae*

and *P. hartwegii*. Gene flow between the two species was later confirmed with cpDNA (Matos and Schaal 2000). A combined morphological and molecular study of the pines of Volcán de Agua may also turn up hybridization between *P. montezumae* and *P. hartwegii*. Nevertheless, we agree with Farjon and Styles (1997) that the taxon described from the summit, *P. donnell-smithii*, should be treated as a synonym of *P. hartwegii*.

Future Directions—The cpDNA phylogeny reported here corroborates the monophyly of *Pinus* subsection *Ponderosae* and arguably provides a better framework for future systematic and evolutionary studies than previous group classifications. Of the 17 species recognized by Price et al. (1998), the only one recovered as monophyletic was *P. torreyana*, which occurs in two small allopatric populations reported to display extremely low cpDNA divergence (Waters and Schaal 1991). Divergence with our data set was low and only took into account 3.1% of the cpDNA genome; monophyly of other species in the Sabiniana clade (except hybrids) might be found if more cpDNA sequence is included. Monophyly cannot be expected for other species in *Pinus* subsection *Ponderosae*, as delimited in recent works (Farjon and Styles 1997; Price et al. 1998; Farjon 2005), or in the six species classification of Shaw (1914).

Relatively high sequence divergence and the cpDNA tree topologies are consistent with the separation of *P. arizonica* and *P. scopulorum* from *P. ponderosa*, but low to null divergence was found for other occasionally recognized species such as *P. donnell-smithii*, *P. nubicola*, and *P. washoensis*. Hybridization, and possibly lineage sorting mean that haplotype monophyly cannot be applied as a strict criterion for delimiting species in this group, but examples of haplotype polyphyly for several taxa support alternate species delimitations that need to be corroborated with additional molecular and morphological data, greater taxonomic sampling, and the use of coalescent approaches.

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APPENDIX 1. Taxa, collection number, herbarium abbreviation, locality information, geographic abbreviation, and GenBank numbers (*matK*, *trnD-trnY-trnE*, and three segments of *ycf1*). Herbarium abbreviations follow Index Herbariorum.

P. arizonica var. *arizonica*, Gernandt 781 (OSC) Pima Co., Arizona USA (ARIZ1), FJ580053, FJ580114, FJ580188; Gernandt 784 (OSC) Pima Co., Arizona USA (ARIZ2), FJ580054, FJ580115, FJ580189; Gernandt s.n. (K) Cerro Calvo Mohinora, Chihuahua MEX (CHIH1), FJ580055, FJ580116, FJ580190; *P. arizonica* var. *storniaea*, Ortiz-García s.n. (MEXU) Cerro Potosí, Nuevo León MEX (NL), FJ580056, FJ580117, FJ580191; *P. cooperi*, Gwiazdowski D1765 (MEXU) El Salto, Durango MEX (DGO1), FJ580057, FJ580118, FJ580192; Gernandt 929 (MEXU) El Salto, Durango MEX (DGO2), FJ580058, FJ580119, FJ580193; Ortiz-García 121coop03 (MEXU) Pinetum Maximino Martínez, México MEX (UNK), DQ353708, FJ580120, FJ580194; *P. coulteri*, Gernandt s.n. (K) San Bernardino Co., California USA (CAL2), AY724751, FJ580169, FJ580243; Gernandt 406 (OSC) Riverside Co., California USA (CAL1), FJ580103, FJ580170, FJ580244; *P. devoniana*, Gernandt 6099 (MEXU) Pátzcuaro, Michoacán MEX (MICH), AY497277, FJ580121, FJ580195; Gernandt 677 (MEXU) Ocotillo, Hidalgo MEX (HGO), FJ580059, FJ580122, FJ580196; Gernandt 727 (MEXU) Bolaños, Jalisco MEX (JAL), FJ580060, FJ580123, FJ580197; *P. donnell-smithii*, Gernandt 905 (USCG) Volcán de Agua, Sacatepéquez GUA (SAQ1), FJ580087, FJ580152, FJ580226; Gernandt 907 (USCG) Volcán de Agua, Sacatepéquez GUA (SAQ2), FJ580088, FJ580153, FJ580227; *P. douglasiana*, Gernandt 434 (MEXU) Temascaltepec, México MEX (MEX), FJ580061, FJ580124, FJ580198; Gernandt 643 (MEXU) Dos Aguas, Michoacán MEX (MICH1), FJ580062, FJ580125, FJ580199; Gernandt 798 (MEXU) Uruapan, Michoacán MEX (MICH2), FJ580063, FJ580126, FJ580200; *P. durangensis*, Gernandt 733 (MEXU) Bolaños, Jalisco MEX (JAL), FJ580065, FJ580128, FJ580202; Gernandt 931 (MEXU) El Salto, Durango MEX (DGO2), FJ580066, FJ580129, FJ580203; Gwiazdowski D1770 (MEXU) El Salto, Durango MEX

- (DGO1), FJ580067, FJ580130, FJ580204; *P. engelmannii*, Gernandt 851 (OSC) Chiricahuas, Arizona USA (ARIZ), FJ580068, FJ580131, FJ580205; Gernandt 927 (MEXU) El Salto, Durango MEX (DGO2), FJ580069, FJ580132, FJ580206; Gwiazdowski wpt546 (MEXU) El Salto, Durango MEX (DGO1), FJ580070, FJ580133, FJ580207; *P. hartwegii*, Liston HART04 (OSC) Quetzaltenango, Quetzaltenango GUA (QUE), FJ580083, FJ580147, FJ580221; Gernandt 903 (USCG) Volcán de Agua, Sacatepéquez GUA (SAQ1), FJ580084, FJ580148, FJ580222; Gernandt 906 (USCG) Volcán de Agua, Sacatepéquez GUA (SAQ2), FJ580085, FJ580149, FJ580223; Gernandt 6199 (MEXU) Paso de Cortes, México MEX (MEX), AY497267, FJ580150, FJ580224; Gernandt 616 (MEXU) Zinguilucan, Hidalgo MEX (HGO), FJ580086, FJ580151, FJ580225; *P. jeffreyi*, Gernandt 300 (OSC) Iron Mountain, Oregon USA (ORE1), AY497271, FJ580171, FJ580245; Gernandt 656 (OSC) Iron Mountain, Oregon USA (ORE2), FJ580104, FJ580172, FJ580246; Gernandt 668 (OSC) Siskiyou Co., California USA (CAL1), FJ580105, FJ580173, FJ580247; Gernandt 701 (OSC) Plumas Co., California USA (CAL2), FJ580106, FJ580174, FJ580248; *P. maximinoi*, Gernandt 446 (MEXU) Temascaltepec, México MEX (MEX), FJ580071, FJ580134, FJ580208; Gernandt 742 (MEXU) San Vicente, Guerrero MEX (GRO), FJ580072, FJ580135, FJ580209; Pérez de la Rosa 1970 (IBUG) Candelario Loxica, Oaxaca MEX (OAX), FJ580073, FJ580136, FJ580210; *P. montezumae*, Gernandt 101 (MEXU) Lagunas de Zempoala, Morelos MEX (MOR), FJ580089, FJ580154, FJ580228; Gernandt 416 (MEXU) Real del Monte, Hidalgo MEX (HGO), AY497269, FJ580155, FJ580229; Gernandt 538 (MEXU) Miquihuana, Tamaulipas MEX (TAM), FJ580090, FJ580156, FJ580230; *P. nubicola*, Gernandt 893 (MEXU) Teopisca, Chiapas MEX (CHIS), FJ580091, FJ580157, FJ580231; Gernandt 898 (USCG) San José Pinula, Guatemala GUA (GUA1), FJ580092, FJ580158, FJ580232; Gernandt 900 (USCG) San José Pinula, Guatemala GUA (GUA2), FJ580093, FJ580159, FJ580233; *P. ponderosa*, Liston POND06s1 (OSC) East fork of Elk, Montana USA (MONT), FJ580074, FJ580137, FJ580211; Gernandt 975 (OSC) Deschutes Co., Oregon USA (ORE), FJ580075, FJ580138, FJ580212; Gernandt 665 (OSC) Trinity Co., California USA (CAL), FJ580076, FJ580139, FJ580213; Gernandt 698 (OSC) Butte Co., California USA (CAL2), FJ580108, FJ580176, FJ580250; *P. pseudo-strobus* var. *pseudostrobus*, Gernandt 411 (MEXU) Apulco, Hidalgo MEX (HGO1), FJ580094, FJ580160, FJ580234; Gernandt 631 (MEXU) Maguey Verde, Hidalgo MEX (HGO2), FJ580095, FJ580161, FJ580235; Gernandt 769 (MEXU) Picacho, Mexico MEX (MEX), FJ580096, FJ580162, FJ580236; Gernandt 822 (MEXU) Tres Marias, Morelos MEX (MOR), FJ580097, FJ580163, FJ580237; Gernandt 815 (MEXU) Angangueo, Michoacán MEX (MICH), FJ580098, FJ580164, FJ580238; *P. pseudo-strobus* var. *apulcensis*, Gernandt 7499 (MEXU) Soltepec, Puebla MEX (PUE), FJ580099, FJ580165, FJ580239; Gernandt 531 (MEXU) Santa Catarina Ixtapeli, Oaxaca MEX (OAX), FJ580100, FJ580166, FJ580240; *P. pseudo-strobus* var. *estevezii*, Gernandt 681 (MEXU) Institute of Forest Genetics, California USA (UNK), FJ580101, FJ580167, FJ580241; *P. pseudo-strobus*, Gernandt 543 (MEXU) Miquihuana, Tamaulipas MEX (TAM), FJ580102, FJ580168, FJ580242; *P. sabiniana*, Gernandt s.n. (K) Shasta Co., California USA (CAL1), AY497272, FJ580177, FJ580251; Gernandt 696 (OSC) Butte Co., California USA (CAL2), FJ580109, FJ580178, FJ580252; *P. scopulorum*, Gernandt 601 (OSC) Jeff Davis Co., Texas USA (TEX), FJ580077, FJ580140, FJ580214; Gernandt 780 (OSC) Pimas Co., Arizona USA (ARIZ1), FJ580078, FJ580141, FJ580215; Gernandt 679 (OSC) Institute of Forest Genetics, California USA (UNK), FJ580079, FJ580142, FJ580216; Gernandt 858 (OSC) Chiricahuas, Arizona USA (ARIZ2), FJ580080, FJ580143, FJ580217; *P. torreyana* subsp. *insularis*, Gernandt 407 (OSC) San Diego Co., California USA (CAL1), AY497273, FJ580179, FJ580253; *P. torreyana* subsp. *torreyana*, Liston TORR01 (OSC) Santa Barbara Co., California USA (CAL2), FJ580110, FJ580180, FJ580254; *P. washoensis*, Liston WASH01 (OSC) Modoc Co., California USA (CAL2), DQ353706, FJ580144, FJ580218; Gernandt 708 (OSC) Modoc Co., California USA (CAL1), FJ580081, FJ580145, FJ580219; Gernandt 672 (OSC) Institute of Forest Genetics, California USA (UNK), FJ580082, FJ580146, FJ580220; *P. washoensis* × *P. jeffreyi*, Gernandt 707 (OSC) Modoc Co., California USA (UNK), FJ580107, FJ580175, FJ580249; *P. yecorensis*, Ferguson 2422 (OSC) Yécora, Sonora MEX (SON), FJ580064, FJ580127, FJ580201; *P. greggii*, Gernandt 426 (MEXU) Maguey Verde, Hidalgo USA (HGO), AY497282, FJ580181, FJ580255; *P. leiophylla*, Gernandt 433 (MEXU) Sultepec, México MEX (MEX), AY497279, FJ580182, FJ580256; *P. muricata*, Liston MURI01 (OSC) Santa Barbara Co., California USA (CAL), FJ580111, FJ580183, FJ580257; *P. oocarpa*, Gernandt 459 (MEXU) El Tuito, Jalisco USA (JAL), DQ353710, FJ580184, FJ580258; *P. patula*, Gernandt 408 (MEXU) Rancho Santa Elena, Hidalgo USA (HGO), AY497284, FJ580185, FJ580259; *P. con-torta*, Gernandt 710 (OSC) Modoc Co., California USA (CAL), FJ580112, FJ580186, FJ580260; *P. virginiana*, Liston VIRG02 (OSC) Monroe Co., Tennessee USA (TENN), FJ580113, FJ580187, FJ580261