

Phylogeny and classification of *Pinus*

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We used chloroplast DNA sequences from *matK* and *rbcL* to infer the phylogeny for 101 of the approximately 111 species of *Pinus* (Pinaceae). At the level of subsection and above, the cpDNA tree is congruent with phylogenies based on nuclear DNA with one notable exception: cpDNA sequences from subsect. *Contortae* are sister to all other North American hard pines rather than occupying a more derived position in the same clade. We used the cpDNA tree plus evidence from nuclear ribosomal DNA and morphology to propose a new classification for the genus. The molecular phylogenies are symmetrical at the deepest branches of the genus, allowing for the delineation of two subgenera, each with two sections that form sister groups. Within sections, clades were slightly asymmetric and sometimes ambiguously resolved. To accommodate ambiguity in some interrelationships, avoid the creation of new ranks, and retain traditional names, we recognised up to three monophyletic subsections per section. Subgenus *Pinus* (the diploxylon, or hard pines) is divided into the predominantly Eurasian and Mediterranean section *Pinus*, composed of subsections *Pinus* and *Pinaster*, and the strictly North American section *Trifoliae*, composed of subsections *Australes*, *Ponderosae*, and *Contortae*. Subgenus *Strobus* (the haploxylon, or soft pines) is divided into the strictly North American section *Parrya*, composed of subsections *Cembroides*, *Nelsoniae*, and *Balfourianae*, and the Eurasian and North American section *Quinquefoliae*, composed of subsections *Gerardianae*, *Krempfianae*, and *Strobus*. Mapping of ten morphological and distributional characters indicates that two were diagnostic for infrageneric taxa: the number of vascular bundles per leaf distinguishes subgenus *Pinus* from subgenus *Strobus*, and a terminal-positioned umbo on the ovulate cone scale is diagnostic of subsect. *Strobus*.

KEYWORDS: classification, *matK*, phylogeny, *Pinus*, *rbcL*.

INTRODUCTION

Pinus (Pinaceae), with over 100 widely recognised species, is the largest extant genus of conifers (Price & al., 1998; Farjon, 2001). Pines are ecologically important as a major, often dominant component of boreal, sub-alpine, temperate, and tropical forests, as well as arid woodlands (reviewed by Richardson & Rundel, 1998). Economically, pines are an important source of wood, paper, resins, charcoal, food (particularly seeds), and ornamentals (reviewed by Le Maitre, 1998). The natural distribution of the genus is confined to the Northern Hemisphere except for one population of *P. merkusii* located just south of the equator in Sumatra (Mirov, 1967). Species such as *P. caribaea*, *P. patula*, *P. pinaster*, and *P. radiata* are cultivated worldwide (Le Maitre, 1998).

The taxonomic history of pines was reviewed recently by Price & al. (1998), who discussed evidence from

morphology, anatomy, cytology, crossability, secondary metabolites, protein, and DNA comparisons. Price & al. (1998) also proposed a classification for the genus, recognizing 111 species in two subgenera, four sections, and 17 subsections. The species circumscriptions showed over 90% correspondence to a separate compilation by Farjon (2001), who recognised 109 species. New species described since 1997 are not included here, although at least two, *P. luzmariae* (Perez de la Rosa, 1998) and *P. fragilissima* (Businský, 2003) probably should be recognised. Recent results from phylogenetic analyses of pine nuclear ribosomal DNA sequences (Liston & al., 1999, 2003; Gernandt & al., 2001) and chloroplast DNA (Wang & al., 1999; Geada López & al., 2002; Gernandt & al., 2003) provided refined hypotheses of interrelationships in the genus, and suggested the need for new circumscriptions and for a reduction in the number of subsections. These studies were based on sequences from less than half of the recognised species of pines, and did not

always include representatives of all major lineages. The aim of the present study is to present a new analysis of chloroplast *matK* and *rbcL* sequences from 101 of the 111 species recognised by Price & al. (1998) representing all the subsectional diversity of the genus, and to propose a new classification of pines based on the chloroplast phylogenetic hypothesis recovered here together with results from other recent studies.

MATERIALS AND METHODS

Sampling. — Collection information for the current study is given in the Appendix. All subsections were sampled; missing species were confined to subsections. *Australes* (four), *Ponderosae* (three), and *Strobus* (three). Approximately one-half of the specimens originated from well-documented wild localities, while the origin for the remaining specimens is less certain because they were collected from botanical gardens, forest genetics institutions, or university campuses. Sequence of *matK* and *rbcL* came from different individuals for fifteen Eurasian species (Appendix). Many sequences were published previously by Wakasugi & al. (1994), Wang & al. (1999) or by other authors (Geada López & al., 2002; Gernandt & al., 2003). Pinaceae outgroup *matK* and *rbcL* sequences were downloaded from GenBank from the following accessions: *Cathaya argyrophylla* (AF143435, AF015786), *Cedrus deodara* (AF143431, X63662), *Larix decidua* (AB019863, AB019826), *Picea sitchensis* (AY035203, X63660), *Pseudolarix amabilis* (AB019866, AB019829) (Doerksen & al., unpubl.; Germano & Klein, unpubl.; Wang & al., 1998, 1999, 2000).

DNA extraction, amplification, and sequencing. — Conditions for extraction, amplification, and sequencing followed Geada López & al. (2002) and Gernandt & al. (2003). Primers used for amplification of *rbcL* were either *rbcL1a* (Olmstead & al., 1992) and *rbcL42977* (Gernandt & al., 2003) or *rbcL1F* and *rbcL3R* (Wang & al., 1999). Additional sequencing primers were *rbcL3F*, *rbcL2F*, *rbcL1R*, and *rbcL2R* (Wang & al., 1999). Primers used for amplification of the 5' portion of *matK* were *orf515-900F* (Gadek & al., 2000) and 2496R (Gernandt & al., 2003) and the 3' portion was amplified with *matK2R* and *matK1F* (Wang & al., 1999). Additional sequencing primers were *matK1R*, *matK2F* (Wang & al., 1999), and *matK2000F* (Gadek & al., 2000). PCR products were sequenced using Applied Biosystems BigDye terminator kits on either an ABI Model 310 or 373 automated sequencer (PE Applied Biosystems, Inc.).

Analysis. — Sequence reads were assembled and edited in BioEdit Sequence Alignment Editor (Hall,

1999). To investigate whether several traditional characters used in classification were diagnostic for any infrageneric group and to measure their level of homoplasy and information content, we mapped nine morphological and one distributional character onto an arbitrarily-chosen most parsimonious tree from the cpDNA analysis. Nine of ten characteristics tabulated by Farjon (1984) were examined (one ecological character was excluded) together with position of the cone scale umbo (dorsal or terminal). Alternate states for six of the morphological characters are shown in Fig. 1. Several modifications were made to the matrix relative to Farjon (1984), mainly based on information from Farjon & Styles (1997), Malusa (1992), Ortiz García (1999), Zobel (1970) and personal observations. Furthermore, the following species recognised by Price & al. (1998) but not treated by Farjon (1984) were included: *P. bhutanica*, *P. chiapensis*, *P. cooperi*, *P. densata*, *P. devoniana*, *P. discolor*, *P. douglasiana*, *P. durangensis*, *P. edulis*, *P. hartwegii*, *P. hwangshanensis*, *P. johannis*, *P. maximartinezii*, *P. maximinoi*, *P. monophylla*, *P. remota*, *P. quadrifolia*, *P. rzedowskii*, and *P. squamata*. Characters were treated as unordered and multistate, and outgroups were excluded because character states were not applicable in some cases (basal sheath of needle, number of needles per fascicle) or polymorphic in others (number of vascular bundles per needle and distributional characters). Mapping was performed with MacClade version 4.03 (Maddison & Maddison, 2001). Both molecular and morphological matrices are deposited in TreeBase (study accession number S1143; matrix accession numbers M1964 and M1965).

Phylogenetic analyses were run in PAUP* 4.0b10 for Windows (Swofford, 2003). Heuristic searches used equally weighted parsimony as the selection criterion and tree-bisection and reconnection (TBR) branch swapping. To generate a consensus tree that took into account the high number of most parsimonious trees (MPTs) found in preliminary searches and still incorporate several replicates of random addition sequence, 50,000 replicates were run with two trees saved per replicate. Branch support was measured with bootstrap values (Felsenstein, 1985) and decay indices (Bremer, 1994). Bootstraps were performed with 500 replicates using simple taxon addition and TBR swapping, with 500 trees saved per replicate. Decay indices were calculated using the CONVERSE and CONSTRAINTS commands.

RESULTS

Individual datasets. — Alignment was achieved manually by inserting the following gaps, all in *matK*: a six bp autapomorphic deletion in *P. parviflora*, an

autapomorphic six bp deletion in *P. nigra*, an autapomorphic six bp insertion for *P. canariensis*, a phylogenetically informative (synapomorphic) six bp insertion for 16 section *Pinus* species (see below), and a two, a six, and a five bp autapomorphic insertion for outgroup sequence *Cathaya argyrophylla*. The *rbcL* dataset included 1262 bp of the *rbcL* gene (88.4% complete, missing 67 bp from the 5' end and 99 bp from the 3' end) and had 279 cells (273 from *P. bhutanica*) scored as missing (0.21%). As a result of difficulty in amplification, only the *rbcL* was sequenced for *P. krempfii*; a shorter *matK* sequence was taken from Wang & al. (1999), resulting in 568 positions missing for this species. The *matK* dataset was 1555 bp long, including 1431 bp of the *matK* gene (92.4% complete, missing approximately 117 bp from the 5' end) plus 107 bp of the 3' flanking region, and had 1737 cells scored as missing (1.1%; all from *P. krempfii*, *Cedrus* and *Cathaya*).

The G+C content was 45.1% in *rbcL* and 36.5% in *matK*. The *rbcL* matrix had 78 informative sites (plus 56 variable but non-informative), and the *matK* matrix had

157 informative sites (plus 182 variable). Heuristic searches of individual datasets were not run to completion, but the shortest *rbcL* trees found had a length of 215 steps, consistency index (CI) of 0.66, CI excluding uninformative characters (CI_{exc}) of 0.54, and a retention index of 0.94. The shortest *matK* trees found had a length of 469 steps (CI = 0.795, CI_{exc} = 0.656, RI = 0.958). Fast bootstrap trees (mulpars off, 500 replicates) did not reveal any conflicting clades supported by values greater than 70% so we proceeded to analyze the *matK* and *rbcL* matrices together.

Combined cpDNA matrix. — The combined *matK* and *rbcL* matrices had 237 informative sites (plus 235 variable). A heuristic search recovered over 55,536 MPTs of length = 696 steps (Fig. 2). Despite the large number of trees found, 28 branches were supported by bootstrap values of 70% or greater. The following infrageneric taxa recognised by Price & al. (1998) were recovered as monophyletic in the strict consensus tree: subgenera *Pinus* and *Strobus*, sections *Pinus*, the “New World Diploxylon Pines”, and *Strobus*; and subsections

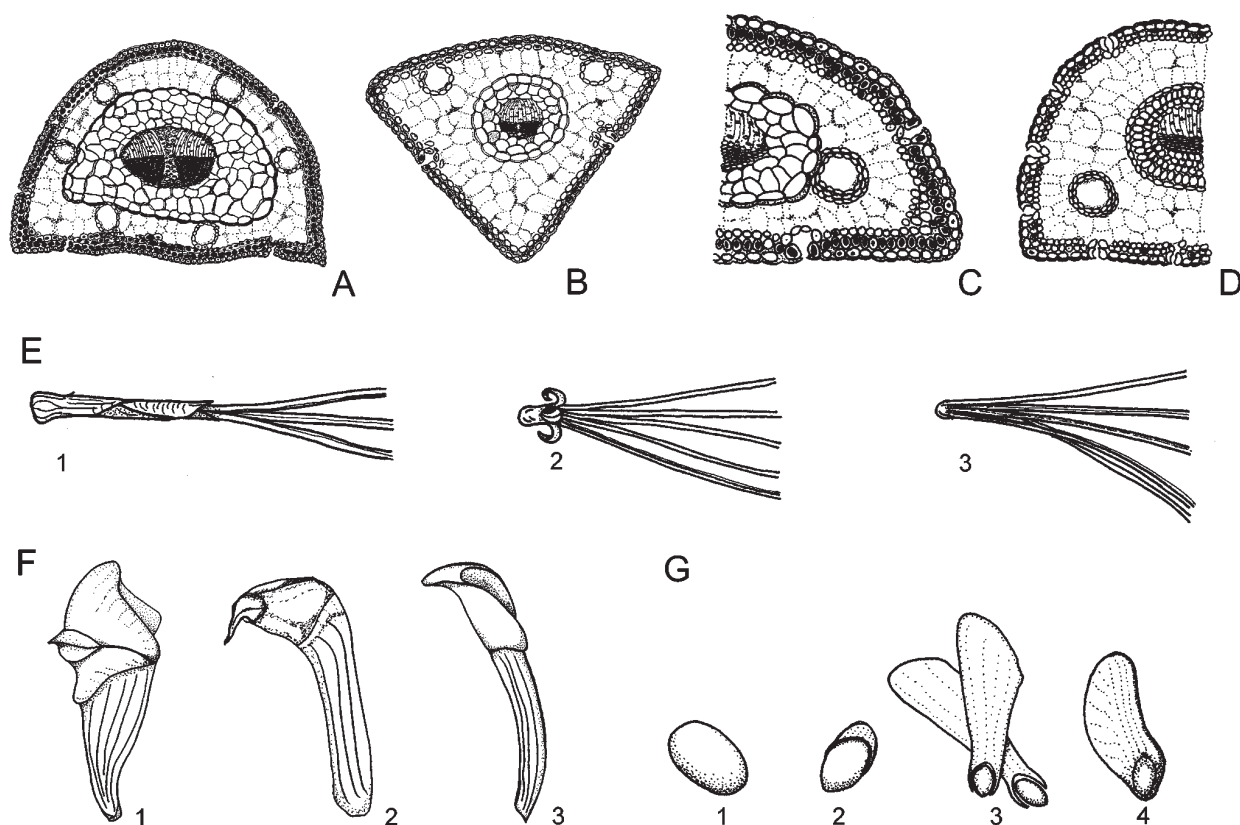


Fig. 1. Several characters used in pine classification. A, two fibrovascular bundles per needle with septal resin ducts (*P. tropicalis*); B, one fibrovascular bundle per needle with external resin ducts (*P. ayacahuite*); C, internal resin ducts (*P. cubensis*); D, medial resin ducts (*P. contorta*); E, fascicle sheaths 1-persistent (*P. teocote*); 2-curling back (*P. cembroides*); and 3-deciduous (*P. ayacahuite*); F, cone scale with umbo; 1-dorsal (*P. pinceana*); 2-dorsal with prickle (*P. jeffreyi*), and 3-terminal (*P. ayacahuite*); G, seed wings, 1-absent (*P. pinceana*), 2-rudimentary (*P. ayacahuite* var. *strobiformis*), 3-articulate (*P. engelmannii*), and 4-adnate (*P. roxburghii*).

Attenuatae, *Contortae*, *Ponderosae*, *Halepenses*, *Balfourianae*, *Gerardianae*, and *Krempfianae*. For the purposes of classification, recognition of three of these taxa would render other groups paraphyletic: (1) section *Parrya* was recovered as paraphyletic to sect. *Strobus* (the correct name is *Quinquefoliae*, see below); (2) subsect. *Attenuatae* was unresolved with respect to two species classified by Price & al. (1998) in subsects. *Australes*, *Leiophyllae*, and *Oocarpae*; and (3) subsect. *Halepenses* was unresolved with respect to species classified by Price & al. (1998) in subsects. *Canarienses*, *Pineae*, and *Pinus*. We propose a change in circumscriptions for sections *Parrya* and *Quinquefoliae*, and have not recognised *Attenuatae*, *Halepenses*, *Canarienses*, or *Pineae* at the level of subsection (Fig. 2 and Table 2).

Subsections that were not recovered as monophyletic as circumscribed by Price & al. (1998) included *Pinus*, *Canarienses*, *Australes*, *Oocarpae*, *Leiophyllae*, *Cembroides*, *Rzedowskianae*, *Strobus*, and *Cembrae*. Rather than occur in subsect. *Pinus*, *P. pinaster* occurred within a clade we are calling subsect. *Pinaster*. Likewise, *P. heldreichii* was sister to this clade, and not a member of subsect. *Pinus*. Members of subsects. *Australes*, *Attenuatae*, *Oocarpae*, and *Leiophyllae* occurred in a single clade. Within this clade, the three members of subsect. *Attenuatae sensu* Price & al. (*P. attenuata*, *P. radiata* and *P. muricata*) were monophyletic, as were four species from eastern North America: *P. pungens*, *P. rigida*, *P. serotina*, and *P. taeda*. A sister relationship between *P. cubensis* and *P. occidentalis* was also recovered in the strict consensus tree, but these clades formed a large polytomy with the remaining members of the group. Subsect. *Leiophyllae sensu* Price & al. (*P. leiophylla* and *P. lumholtzii*) was monophyletic in a subset of the MPTs (not shown).

In subgenus *Strobus*, subsect. *Cembroides* included *P. rzedowskii* (subsect. *Rzedowskianae sensu* Price & al., 1998) but did not include *P. nelsonii*, which formed a unique lineage. In sect. *Quinquefoliae*, no separation was seen between subsects. *Strobus* and *Cembrae*, rather four members of *Cembrae* occupied derived positions in the tree indistinguishable from other subsect. *Strobus* sequences, and *P. albicaulis* occurred on a separate branch together with other subsect. *Strobus* sequences. Proposed changes in the circumscription and nomenclature of these groups are indicated in Fig. 2, Table 1 and the Appendix. The proposed names will be used in the following descriptions of results.

Morphological versus cpDNA phylogenies. — A heuristic search of the ten morphological characters (5,000 random addition sequence replicates) and scoring polymorphic characters as ambiguous recovered 84 trees of 50 steps (CI = 0.36, RI = 0.91). The strict consensus tree was poorly resolved (not shown); thirteen branches were found in all trees but the only infrageneric taxa resolved as monophyletic were the two subgenera (*Pinus* and *Strobus*) and subsect. *Strobus*. Treating polymorphic characters as polymorphic rather than ambiguous found 76 trees of 162 steps (CI = 0.80, RI = 0.91). Eleven branches were recovered in the strict consensus tree with the two *Pinus* subgenera and subsect. *Strobus* again appearing as monophyletic.

Mapping ten morphological and distributional characters onto the cpDNA tree revealed that two characters are non-homoplasious: number of vascular bundles in the leaves, delineating subgenus *Pinus* (two) from subgenus *Strobus* (one); and umbo position, delineating subsect. *Strobus* (terminal umbo) from all other pines (dorsal). A synopsis of the ten characters by subsection together with consistency and retention indices is given in Table

Table 1. Classification of *Pinus*. Species not sampled in the present study are marked with an asterisk.

Subgenus *Pinus* (Diploxylon or hard pines)

Section *Pinus*, Subsection *Pinus* (Eurasia, Mediterranean, E North America, Cuba): *P. densata*, *densiflora*, *hwangshanensis*, *kesiya*, *luchuensis*, *massoniana*, *merkusii*, *mugo*, *nigra*, *resinosa*, *sylvestris*, *tabuliformis*, *taiwanensis*, *thunbergii*, *tropicalis*, *uncinata*, *yunnanensis*; **Subsection *Pinaster*** (Canary Islands, Mediterranean, Himalayas): *P. brutia*, *canariensis*, *halepensis*, *heldreichii*, *pinaster*, *pineae*, *roxburghii*.

Section *Trifoliae*, Subsection *Contortae* (North America): *P. banksiana*, *clausa*, *contorta*, *virginiana*; **Subsection *Australes*** (North America, Mexico, Central America, Caribbean): *P. attenuata*, *caribaea*, *cubensis*, *echinata*, *elliottii*, *glabra**, *greggii*, *herrerae*, *jaliscana**, *lawsonii*, *leiophylla*, *lumholtzii*, *muricata*, *occidentalis*, *oocarpa*, *palustris*, *patula*, *praetermissa**, *pringlei*, *pungens*, *radiata*, *rigida*, *serotina*, *taeda*, *tecunumanii**, *teocote*; **Subsection *Ponderosae*** (W U.S.A., Mexico, Central America): *P. cooperi*, *coulteri*, *donnell-smithii**, *devoniana*, *douglasiana*, *durangensis*, *engelmannii*, *hartwegii*, *jeffreyi*, *maximinoi*, *montezumae*, *nubicola**, *ponderosa*, *pseudostrobus*, *sabineana*, *torreyana*, *washoensis**.

Subgenus *Strobus* (Haploxylon or soft pines)

Section *Parrya*, Subsection *Balfourianae* (W U.S.A.): *P. aristata*, *balfouriana*, *longaeva*; **Subsection *Cembroides*** (SW North America, Mexico): *P. cembroides*, *culminicola*, *discolor*, *edulis*, *johannis*, *maximartinezii*, *monophylla*, *pinceana*, *quadrifolia*, *remota*, *rzedowskii*; **Subsection *Nelsoniae*** (Mexico): *P. nelsonii*.

Section *Quinquefoliae*, Subsection *Gerardianae* (E Asia, Himalayas): *P. bungeana*, *gerardiana*, *squamata*; **Subsection *Krempfianae*** (Vietnam): *P. krempfii*; **Subsection *Strobus*** (North America, Eurasia): *P. albicaulis*, *armandii*, *ayacahuite*, *bhutanica*, *cembra*, *chiapensis*, *dabeshanensis**, *dalatisensis**, *fenzeliana*, *flexilis*, *koraiensis*, *lambertiana*, *monticola*, *morrisonicola*, *parviflora*, *peuce*, *pumila*, *sibirica*, *strobus*, *wallichiana*, *wangii**.

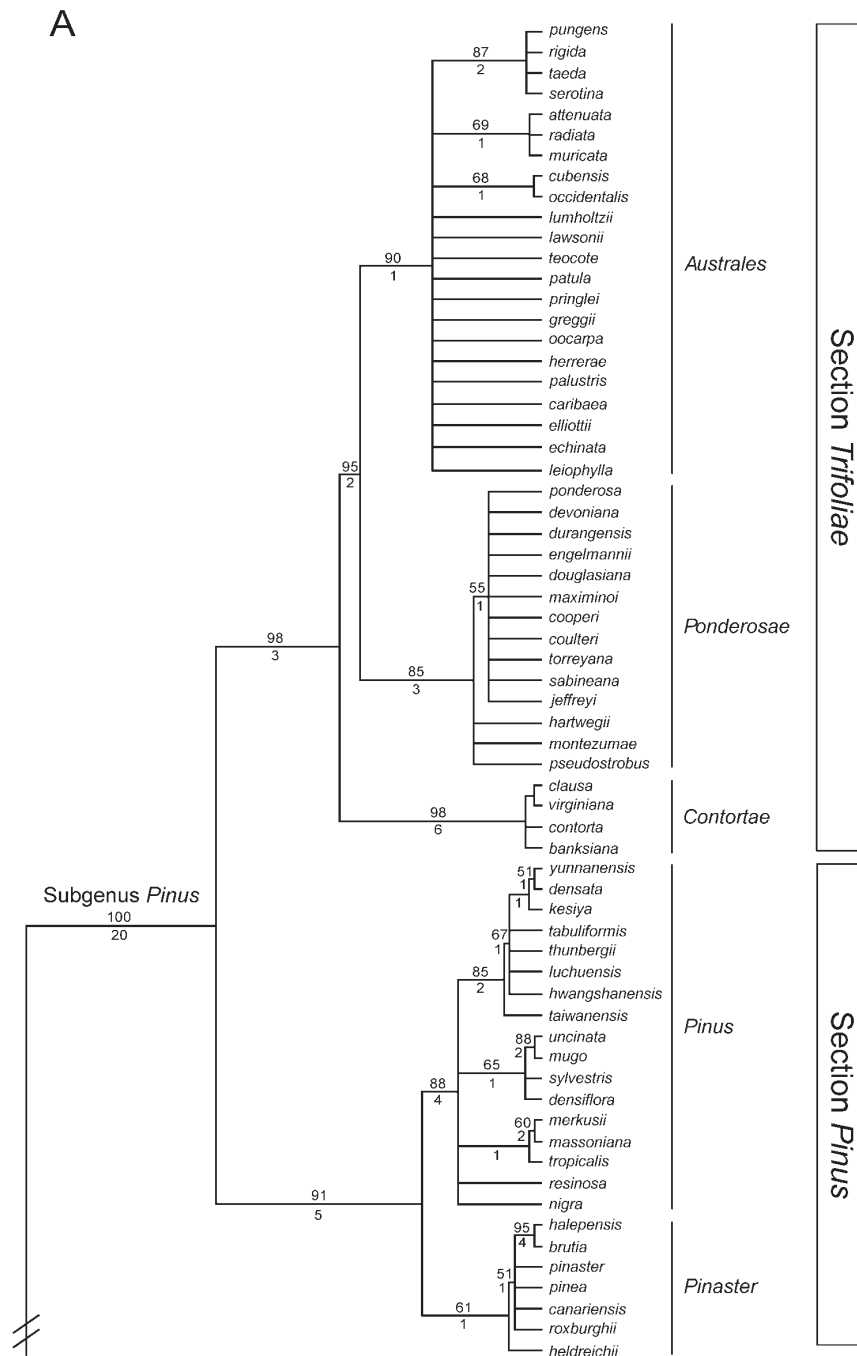


Fig. 2. Strict consensus of 55,536 trees for the combined *rbcl* and *matK* matrix (2817 bp, 235 informative characters, L = 696 steps, CI = 0.740, Cl_{exc} = 0.598, RI = 0.947). Branch lengths do not reflect sequence divergence. Many terminal polytomies are due to identical sequences. Bootstrap values over 50% are shown above branches, and decay indices are shown below branches. Infrageneric groups as modified in this study are shown on the right. A, subgenus *Pinus*. B, subgenus *Strobos* and outgroups.

2. In general, much homoplasy was observed, although the consistency index (which measures lack of homoplasy) increased markedly when polymorphic characters were not scored as ambiguous. The retention index,

which has been described as a measure of information content (Savolainen & al., 2000), tended to be somewhat high except when mapping whether mature cones open or remain closed.

Inference of the ancestral state could not be inferred in five of ten characters because not only were states coded as missing in the outgroup genera, but the state varied on the first branch within *Pinus* dividing the two subgenera. For that reason, we emphasise transformations in branches leading to sections and subsections in the following description of results. Persistent fascicle sheaths, though showing three changes on the arbitrarily-chosen MPT and consequently high homoplasy, had a high information content as measured by the retention index. The distribution of the states of this character supported the delineation between subgenus *Pinus* (persistent, with subsequent transformations to deciduous in the branch leading to *P. lumholtzii* and *P. leiophylla*) and subgenus *Strobis* (deciduous, with a transformation to persistent in *P. nelsonii*).

The number of needles per fascicle was also highly homoplasious when polymorphic characters were treated as ambiguous (Table 2). The character was particularly polymorphic in sect. *Parrya* and subsect. *Ponderosae*. The number of needles per fascicle sheath in subsection *Australes* showed an intermediate level of polymorphism, with species tending to have three. Section *Pinus*

tended to have two needles per fascicle, but *P. roxburghii* and *P. canariensis* of subsect. *Pinaster* showed a transformation to three and *P. kesiya*, *P. yunnanensis*, *P. densata*, and *P. tabuliformis* of subsect. *Pinus* were polymorphic for two to three. All members of subsect. *Contortae* had two needles per fascicle, and all members of subsect. *Strobis* had five.

The position of resin ducts was also highly homoplasious when polymorphic characters were treated as ambiguous (Table 2). Resin ducts were mapped as external in subgenus *Strobis* but showed transformation to medial in three species of subsect. *Strobis*. They tend to be medial in subsections *Ponderosae* and *Contortae*, although two species in the former subsection were polymorphic. The character showed more polymorphism in subsect. *Australes*, whose members tend to have internal or medial resin ducts, and in sect. *Pinus*, whose members tend to have medial or external resin ducts.

Cone scales are thick in most pine species. On the chloroplast tree, thin cone scales appeared derived in the branch leading to subsect. *Strobis*, and the character was polymorphic in subsect. *Cembroides*. Scales remain closed in mature female cones for species in several

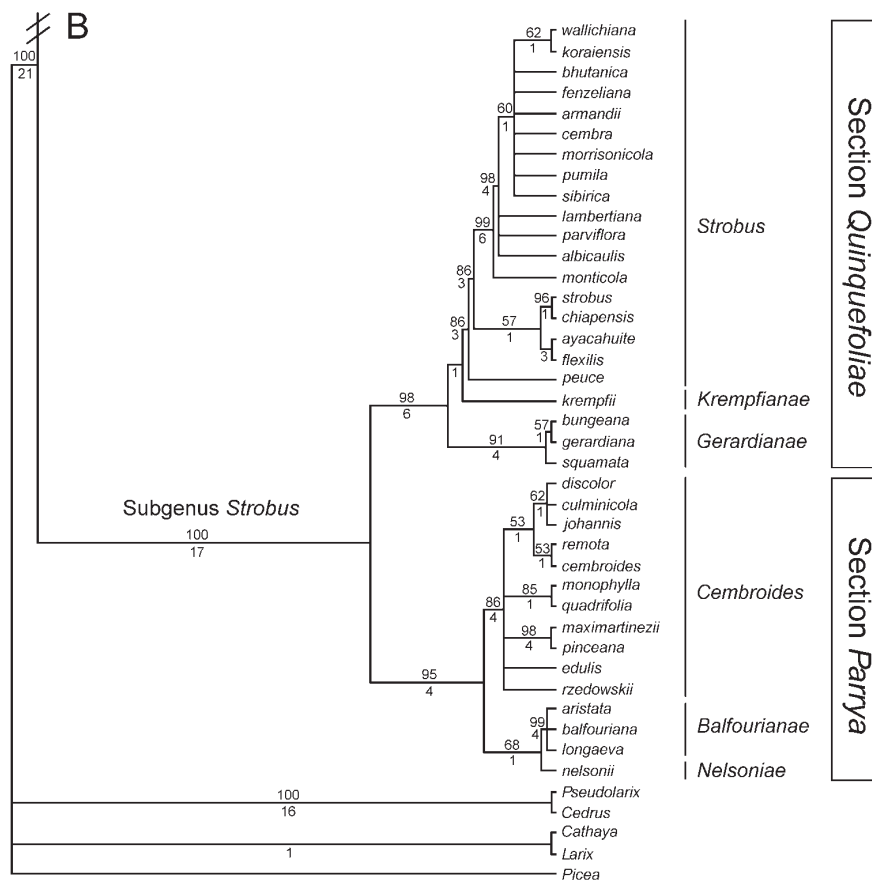


Fig. 2 (continued).

Table 2. Synopsis of character variation in subsections of *Pinus* (modified from Farjon, 1984).

	leaf vascular bundles	fascicle sheaths	needles per fascicle	needle resin ducts	cone scale	mature cones	umbo prickle	seed wing	umbo position	distribution
Section <i>Pinus</i>										
subsect. <i>Pinus</i>	two	persistent	2 (3)	medial, external (septal)	thick	open	variable	articulate	dorsal	Eurasia (E NA)
subsect. <i>Pinaster</i>	two	persistent	2 (3)	medial, external	thick	open (closed)	absent	adnate, articulate	dorsal	Mediterranean (Himalayas)
Section <i>Trifoliae</i>										
subsect. <i>Contortae</i>	two	persistent	2	medial	thick	open, closed	variable	articulate	dorsal	W & E NA & Mex
subsect. <i>Austroales</i>	two	persistent	2–5	septal, internal, medial	thick	open, closed	variable	articulate	dorsal	NA & Mex
subsect. <i>Ponderosae</i>	two	persistent	(2) 3–5 (8)	internal, medial	thick	open	variable	articulate	dorsal	W NA & Mex
Section <i>Quinquefoliae</i>										
subsect. <i>Strobus</i>	one	deciduous	5	medial, external	thin	open, closed	absent	absent, rudimentary, adnate	terminal	NA, Mex, Eurasia
subsect. <i>Krempfianae</i>	one	deciduous	2	external	thick	open	absent	articulate	dorsal	SE Asia
subsect. <i>Gerardianae</i>	one	deciduous	3, 5	external	thick	open	variable	articulate	dorsal	SE Asia
Section <i>Parrya</i>										
subsect. <i>Cembroides</i>	one	deciduous	1–5 (6)	external	thick	open	absent	absent (articulate)	dorsal	W NA & Mex
subsect. <i>Nelsoniae</i>	one	persistent	3	external	thick	open	absent	absent	dorsal	Mex
subsect. <i>Balfourianae</i>	one	deciduous	5	external	thick	open	variable	articulate	dorsal	W NA
Consistency Index	1.0	0.25	0.18	0.23	0.25	0.09	0.06	0.20	1.0	0.19
CI (polymorphic)	1.0	0.25	0.85	0.74	0.25	0.17	0.30	0.39	1.0	0.26
Retention Index	1.0	0.92	0.72	0.77	0.88	0.29	0.57	0.72	1.0	0.65

derived parts of the tree: in *P. albicaulis*, *P. cembra*, *P. koraiensis*, *P. pumila* and *P. sibirica* (subsect. *Strobus*), in *P. torreyana* and *P. coulteri* (subsect. *Ponderosae*), in *P. attenuata*, *P. muricata*, *P. patula* and *P. greggii* (subsect. *Austroales*), in *P. contorta* and *P. banksiana* (subsect. *Contortae*), and in *P. pinea* (subsect. *Pinaster*). The presence of a prickle on the female cone scale was highly homoplasious regardless of whether or not the ambiguous characters were treated as polymorphic, plus the retention index of this character was the second lowest (open or closed mature cones had the lowest). Prickles tend to be present in sect. *Trifoliae*, and the character appears sporadically in the other three sections as well. Within sect. *Parrya*, all three species of subsect. *Balfourianae* have a prickle, but the character also occurs independently in *P. rzedowskii* of subsect. *Cembroides*.

Mapping the condition of the seed wing onto the chloroplast tree suggested that an articulate (deciduous) state was plesiomorphic for the genus, as basal members of both subgenera possess this character state. In subgenus *Strobus*, seed wings are absent in *P. nelsonii* (subsect. *Nelsoniae*) and in all members of subsect. *Cembroides* except *P. rzedowskii*. The character transformed to adnate (persistent) in subsect. *Strobus*, with later losses of the wing in *P. cembra* and *P. pumila*, *P. koraiensis*, *P. sibirica*, and *P. albicaulis*. The number of losses is uncertain, although a minimum of two are inferred because *P. albicaulis* is resolved apart from the former four species, which in turn form a polytomy with other species that possess an adnate seed wing. In subgenus *Pinus*, seed wings are deciduous in all species

except two members of subsect. *Pinaster*: *P. canariensis*, and *P. roxburghii*, in which it is adnate.

The inferred ancestral distribution of pines was ambiguous. All members of sects. *Parrya* (subgenus *Strobus*) and *Trifoliae* (subgenus *Pinus*) occur in North America, Mexico, Central America, and the Caribbean. Two subsections of sect. *Trifoliae*, *Austroales* and *Contortae*, have some members in eastern North America, while the other four subsections of *Parrya* and *Trifoliae* are confined to western North America, Mexico, and Central America. The ancestral state of *Trifoliae* was inferred to be eastern North America. Section *Pinus* is predominantly a Eurasian genus (including the Mediterranean region), although it includes two eastern American species, *P. tropicalis* and *P. resinosa*, the latter of which is the sister group to the remaining members of subsect. *Pinus* in a subset of the MPTs (not shown). Subsection *Pinaster* is a Mediterranean taxon that also includes *P. roxburghii* from the western Himalayas. The first taxa that branch off within section *Quinquefoliae* are from Asia and then the Balkans, but more derived species occur throughout the Northern Hemisphere. Distribution was also explored by using a simpler, binary recoding of the data. Eurasian and Mediterranean species were coded as Old World and the species of North and Central America and the Caribbean were coded as New World. When the outgroup genera were also included, with genera present in both regions coded as polymorphic, the ancestral distribution of extant pines was inferred to be Old World, with single North American derivations for sect. *Parrya* and sect. *Trifoliae*.

The ancestral state is ambiguous when outgroups are excluded. In sect. *Quinquefoliae*, the ancestral state was Old World. A single derivation of all New World species followed by a reversion to Old World species was inferred, with both transformations occurring in subsect. *Strobis*. In sect. *Pinus*, two separate derivations of New World species (*P. resinosa* and *P. tropicalis*) were inferred on the arbitrarily-chosen tree.

DISCUSSION

Comparison of cpDNA tree with phylogenies from other recent studies. — The cpDNA strict consensus tree has several differences with phylogenies based on partial ITS region sequences (Liston & al., 1999, 2003). Compared to the 47 pine species phylogeny of Liston & al. (1999), a subsequent work (Liston & al., 2003) included higher-quality sequences; we will refer exclusively to the more recent phylogeny. The ITS and cpDNA phylogenies have a conflict relative to the subsectional relationships of sect. *Trifoliae*. In the ITS region tree, and in an earlier three genome restriction fragment tree based on 18 species (Strauss & Doerksen, 1990), subsect. *Contortae* occurred together with subsect. *Australes*, and together these subsections were the sister group of subsect. *Ponderosae*. Branch support as measured by bootstrap values for the monophyly of subsections *Australes* and *Contortae* was 90% for Strauss & Doerksen (1990) and 77% for Liston & al. (2003). In the cpDNA tree reported here (Fig. 2), as well as in previously reported cpDNA phylogenies (Krupkin & al., 1996; Geada López & al., 2002), subsect. *Contortae* was the sister group to a clade that included subsections *Ponderosae* and *Australes*, a relationship also supported by high bootstrap values (Fig. 2). The shallow fossil record of subsect. *Contortae*, together with morphological evidence and artificial hybridization studies suggest that its placement as an early diverging lineage in the cpDNA trees does not reflect its species phylogeny, which is more consistent with a close relationship to subsect. *Australes* (Saylor & Koenig, 1967; Price, 1989; Krupkin & al., 1996; Liston & al., 1999). Thus the placement of subsect. *Contortae* in cpDNA trees may either be a result of long branch attraction (Felsenstein, 1978) or chloroplast capture resulting from introgression (Rieseberg & Soltis, 1991). Geada López & al. (2002) argued that the placement of subsect. *Contortae* in cpDNA phylogenies is robust whether parsimony is used or whether other methods are used that are thought to be less sensitive to long branch attraction, such as neighbor-joining and maximum likelihood. The possibility exists that the position of subsect. *Contortae* in cpDNA trees better reflects its evolutionary history than the earlier

works that were based wholly or in part on nuclear data. For the purposes of classification, this uncertainty suggests caution when relying on a single genomic source for proposing a new infrageneric arrangement. Fortunately in the present case, the placement and circumscription of the subsections in question is not affected by the conflicting results because subsections *Contortae*, *Ponderosae* and *Australes* are each recognised as members of sect. *Trifoliae*; the classification is neutral with respect to their interrelationships.

Other conflicts between the ITS region tree and the cpDNA tree at the level of subsection and above were not supported by robust bootstrap values. For instance, in the strict consensus ITS region tree, species indicated in the present study to represent subsections *Australes* and *Contortae* are poorly resolved, with subsect. *Australes* actually paraphyletic with respect to subsect. *Contortae*. Additional examples of poorly supported conflicts in the ITS region phylogenetic hypothesis include sect. *Pinus* paraphyletic to sect. *Trifoliae*, subsect. *Pinaster* paraphyletic to subsect. *Pinus*, and subsect. *Gerardianae* as the basal lineage of subgenus *Strobis* rather than the basal lineage of sect. *Quinquefoliae* as indicated from cpDNA sequences (Fig. 2). Regarding the position of subsect. *Gerardianae*, a subsequent ITS region study using a 2424 bp alignment (Gernandt & al., 2001) compared to the 651 bp alignment of Liston & al. (1999, 2003) recovered the same position for the subsection as indicated by cpDNA results, suggesting that these ITS results might change if more data were used.

The subgenus *Pinus* cpDNA phylogeny of Geada López & al. (2002) was based on four cpDNA regions from 35 hard pine species (as recognised by Price & al., 1998) compared to the two cpDNA regions from 64 hard pines presented here. Several of the same *matK* and *rbcL* sequences were used in both studies. The latter study was better resolved in two parts of the phylogeny, which we attribute either to the use of additional sequences, fewer taxa, or a combination of both factors. First, the strict consensus parsimony tree from Geada López & al. (2002), resolved the three species often classified in subsect. *Attenuatae* (*P. attenuata*, *P. muricata* and *P. radiata*) as the sister group to subsect. *Australes*. In the present study, this clade occurs in a polytomy with subsect. *Australes* (Fig. 2). The separation of these three species from subsect. *Australes*, together with partial resolution of subsect. *Oocarpae* (also not recognised here), was also recovered by Dvorak & al. (2000) using the random amplified polymorphic DNA (RAPD) method on 16 species of subsect. *Australes* (as circumscribed in the present study). We have included all these species in subsect. *Australes* (Loudon, 1838). This name has priority over *Attenuatae* (van der Burgh, 1973). *Pinus patula*

(which incidentally also has attenuate cones) was sister to subsect. *Attenuatae* in the latter study, but bootstrap support was less than 50% for the position, as well as for the monophyly of the remaining members of subsect. *Australes*. Additional data are needed to determine the position of the three species of subsect. *Attenuatae* with respect to *P. patula* and other species of subsect. *Australes*. We have conservatively decided not to recognise subsection *Attenuatae* at present because sampling the four missing species in this clade and including more sequences may end up rendering subsect. *Australes* paraphyletic. If further study resolves these groups more clearly, a solution might be to recognise the names *Attenuatae*, and perhaps even *Oocarpae* and *Leiophyllae*, at the rank of series rather than subsection.

A second difference found in the study of Geada López & al. (2002) is the resolution of *P. brutia* and *P. halepensis* (subsect. *Halepenses sensu* Price & al., 1998) as a sister group to *P. canariensis*, *P. roxburghii*, and *P. pinaster*. The greater resolution of these clades in the study of Geada López & al. (2002) opens the possibility of the recognition of subsect. *Halepenses* for these two taxa, but a monotypic subsection may be needed for *P. heldreichii*. Again, an alternative would be to name these groupings as series rather than subsections.

Mapping of morphological and distributional characters. — Two of the ten traditional characters had a single transformation on the cpDNA tree. The number of vascular bundles in the leaf could be used to unequivocally separate subgenera, and a terminal cone scale umbo is diagnostic for subsect. *Strobis* (Table 2). Low homoplasy was also found in the condition of the fascicle sheaths. Fascicle sheaths are deciduous in all species of subgenus *Strobis* except *P. nelsonii*, and persistent in all species of subgenus *Pinus* except *P. leiophylla* and *P. lumholtzii*. No non-homoplasious character was found to delimit a section.

Biogeographic inferences for *Pinus* are ambiguous in many respects, but some generalizations can be made. There is a preliminary indication that the ancestral state of the genus is Old World, but this must take into account the distribution of other genera in Pinaceae because excluding outgroups results in an ambiguous coding for the ancestral condition of *Pinus*. The sister genus of *Pinus* is uncertain, but *Picea* (with a distribution in both the Old and New World) and *Cathaya* (China), are the preferred candidates based on recent phylogenies from all three genomes (Wang & al., 2000). With respect to sections, all four include New World members: sects. *Parrya* and *Trifoliae* are confined to the New World while sects. *Quinquefoliae* and *Pinus* appear to be ancestrally Old World sections with New World species derived from them.

Nine of the eleven subsections recognised here are

confined either to the New World (six) or the Old World (three). The greater diversity of New World subsections suggests that regardless of its continental origin, lineages underwent more diversification and survived better in the New World than in the Old. Apparently vicariance predominantly defined subsections, while cases of intercontinental dispersal are confined to subsections. *Strobis* and *Pinus*. With respect to subsect. *Strobis*, an American ancestry for the Asian closed cone white pines (subsect. *Cembrae sensu* Price & al., 1998), as first hypothesised by Liston & al. (1999), still holds, although the details of the white pine phylogeny are different. The cpDNA hypothesis suggests that subsect. *Strobis* has a Eurasian origin. Subsequently, a lineage dispersed to North America, giving rise to the open-coned species *P. strobus*, *P. chiapensis*, *P. ayacahuite*, *P. flexilis*, *P. monticola*, and *P. lambertiana*, as well as the closed-coned species *P. albicaulis*. This derived lineage then apparently dispersed back to Eurasia, giving rise to both open-(plesiomorphic) and closed-cone (derived) species. With respect to subsect. *Pinus*, either one or two dispersal events to eastern North America are needed to explain the incompletely-resolved positions of *P. resinosa* and *P. tropicalis*.

The intention of mapping traditional characters onto the cpDNA tree was to determine whether they were diagnostic of major clades. It is highly desirable that the infrageneric taxa recognised here be defined by morphological characters. Preferably these easily-observable characters would show a single transformation on the tree (no homoplasy) so that species could be unambiguously classified. Nine of the ten characters used for mapping were selected because of their availability; Farjon (1984) tabulated the characters and their states for most pine species, but cautioned that wood characters were not included although they were more important in the recognition of many infrageneric groups. The fact that most of the monophyletic groups identified in the chloroplast tree could not be diagnosed by any of these characters further emphasises the need for a detailed, genus-wide morphological analysis. A morphological matrix of 70 characters for all species of pines is being refined (Ortiz García, 1999), and we hope that a comparison of that matrix with molecular data will allow combinations of characters to be identified for more of the sections and subsections recognised here.

Proposed classification of *Pinus*. — *Pinus* is differentiated from other genera of Pinaceae by possession of a shoot dimorphism that includes short shoots (fascicles) that bear one to eight narrow leaves (needles) surrounded by bud scales at their base, and woody cone scales with a specialised, raised apical structure representing the part of the cone scale left exposed after the first growing season (the umbo) and in the mature cone

(the apophysis; see Price & al., 1998). The genus has been recovered as monophyletic in numerous studies (Prager & al., 1976; Strauss & Doerksen, 1990; Liston & al., 1999, 2002; Wang & al., 1999, 2000).

Subgenus *Pinus*, the diploxylon or hard pines, is characterised by two fibrovascular bundles per needle, decurrent pulvini at the cataphyl bases (“fascicle-bracts”), and usually with persistent fascicle sheaths (Shaw, 1914; Little & Critchfield, 1969; Farjon & Styles, 1997), although the latter character has been lost in two species, *P. leiophylla* and *P. lumholtzii*. Cone scales in subgenus *Pinus* tend to be thicker and more rigid than in subgenus *Strobus*, although some species in the latter subgenus, for example *P. maximartinezii*, also have thick and rigid cone scales. Other characters tabulated by Farjon (1984) are highly polymorphic or at least provide little diagnostic value for the subgenus: needles per fascicles vary from two up to eight and the position of resin ducts is highly polymorphic (septal, internal, medial external), seed wings are articulate or adnate, mature cones either open soon or are serotinous, and the subgenus is distributed throughout the Northern Hemisphere in a wide range of habitat types.

Subgenus *Strobus*, the haploxylon or soft pines, is characterised by a single fibrovascular bundle per needle, non-decurrent pulvini at the cataphyl bases, and with deciduous fascicle sheaths except for *P. nelsonii*, for which persistent fascicle sheaths apparently were independently derived. Cone scales tend to be thinner and more flexible (but see above), needles per fascicle vary from one to five (uncommonly six), resin ducts are either medial or external, seed wings are highly polymorphic (absent, rudimentary, adnate, and articulate), mature cones usually open soon, and the subgenus is distributed throughout the Northern Hemisphere in a wide range of habitat types, with the exception of subtropical lowlands.

Section *Trifoliae* is characterised by persistent fascicle sheaths, but it also includes the two exceptional species in subgenus *Pinus* with deciduous fascicle sheaths, *P. leiophylla* and *P. lumholtzii*. Needle number ranges from two to five (rarely up to eight). Resin ducts are internal or medial in most species, but *P. leiophylla* has medial to external resin ducts and *P. oocarpa* and *P. pringlei* have internal or septal resin ducts (Farjon & Styles, 1997). The majority of the species have cones with thick woody scales that open at maturity, but serotiny occurs in several species. Seed wings are deciduous; they are articulate but effective, the wing being attached to the seed by two claws (Farjon & Styles, 1997). The section contains all North American hard pines except *P. tropicalis* and *P. resinosa*.

Section *Trifoliae* is referred to informally as the “New World Diploxylon Pines” by Price & al. (1998). Members of the section were divided between sect.

Pinea (subsect. *Leiophyllae*) and sect. *Pinus* (subsects. *Australes*, *Ponderosae*, *Sabinianae*, *Contortae*, and *Oocarpae*) in the classification of Little & Critchfield (1969). Van der Burgh (1973) separated *P. leiophylla* and *P. lumholtzii* into two sections apart from other members of his sect. *Pinaster*. Farjon & Styles (1997) moved *P. lumholtzii* to sect. *Pinaster* but retained *P. leiophylla* as a separate monotypic section. Duhamel (1755) proposed section *Trifoliis* to accommodate the southeastern American species with three needles per fascicle, including *P. palustris*. This name has priority over the names sect. *Taeda* Spach 1842 and sect. *Ternatae* Loudon 1838.

Section *Pinus* is characterised by persistent fascicle sheaths, needle numbers from two to three, usually external or medial resin ducts, but sometimes internal (*P. merkusii*) or septal (*P. tropicalis*). Mature cones open at maturity (except *P. pinea*) and have thick scales. Seed wings are articulate in most species, but adnate in *P. canariensis* and *P. roxburghii*. The section is distributed throughout Eurasia and the Mediterranean and also includes two species from the Americas, *P. resinosa* from eastern North America and *P. tropicalis* from western Cuba. Analysis of the morphological character matrix did not recover any character that clearly distinguished sect. *Pinus* from those species in sect. *Trifoliae* that have two to three needles per fascicle. However, the two species can be differentiated from all other North American hard pines by their wood anatomy (large pits occurring together with dentate tracheids) and partly external resin ducts (Shaw, 1914), although the ducts of *P. tropicalis* were scored as (mostly) septal by Farjon (1984).

Section *Parrya* is characterised by deciduous fascicle sheaths (except *P. nelsonii*), needles in fascicles from one to five (rarely six), external resin ducts, mature cones with thick scales that open to expose seeds, and seed wings that are articulate or absent. Gernandt & al. (2003) compared a cpDNA phylogeny with a morphological phylogeny (Malusa, 1992) and identified an additional character that characterises the group: except for *P. nelsonii*, the fascicle sheaths curl back to form a rosette before abscising. *Pinus nelsonii* is the only species in the genus that combines a single vascular bundle per fascicle (diagnostic for subgenus *Strobus*) with persistent fascicle sheaths. It also can be distinguished from other species by its combination of connate needles, cones on stout recurved peduncles, and functionally wingless seeds. The natural distribution of sect. *Parrya* is confined entirely to southwestern North America and Mexico.

Section *Quinquifoliae* is characterised by deciduous fascicle sheaths, needles in fascicles of five except *P. krempfii* (two), *P. gerardiana* (three), and *P. bungeana* (three), external or medial resin ducts, thin or thick cone scales that either open at maturity or are indehiscent (as an adaptation for seed dispersal by birds) but not seroti-

nous. Seed wings are highly polymorphic, being absent, rudimentary, adnate, or articulate. Species in this section are distributed widely throughout Eurasia and North America, with one species, *P. chiapensis*, extending into southern Mexico.

Based on the autonym rule (*ICBN* art. 22), Little & Critchfield (1969) used the name sect. *Strobis* for most members of this group, but the *St. Louis Code* (Greuter & al., 2000) only requires the use of autonyms for the type species of the genus. Britton (1908) chose *P. sylvestris* as the lectotype of the genus, which is in subgenus *Pinus* rather than subgenus *Strobis*. Use of the type species for the name of a subgenus is recommended “where there is no obstacle under the rules” (Recommendation 22A). We have considered priority as a valid obstacle, and under this circumstance, the sections of Duhamel (1755), although published in the ablative form (*Bifoliis*, *Trifoliis*, *Quinquefoliis*) have priority (Price & al., 1998; Gernandt & al., 2003). Furthermore, incorrect declensions for Duhamel’s sections were applied in a recent publication by two of the present coauthors (Gernandt & al., 2003), and the widely-used name, subsect. *Strobi*, needs to be corrected to subsect. *Strobis* (W. Greuter, pers. comm.).

The delineation between the two main groups of subgenus *Strobis* has historically been based on whether the position of the umbo on the ovulate cone is dorsal or terminal (Shaw, 1914; Little & Critchfield, 1969). Although this distinction is very practical, the dorsal umbo is a symplesiomorphic character shared among all hard pines and presumably basal soft pines. Molecular phylogenetic evidence from cpDNA and the ITS region concurs in identifying the transformation from dorsal to terminal umbo not at the first divergence of extant lineages of subgenus *Strobis*, but nested within the differentiation of sect. *Quinquefoliae* (Liston & al., 1999, 2003; Wang & al., 1999; Gernandt & al., 2001, 2003). As a result, no single morphological character differentiates the two sections into mutually monophyletic groups.

CONCLUSION

The classification proposed here is a modification of the influential classification of Little & Critchfield (1969), which was based primarily on morphology and data from interspecific crosses, and of the classification of Price & al. (1998), which incorporated more recently described species as well as several early molecular phylogenetic studies. Our proposed changes to these earlier classifications amount to recircumscription of the four sections recognised by Price & al. and reduction of the number of subsections to eleven (with several recircumscriptions). These proposals are based on advances not

only in cpDNA sequence phylogenies (Fig. 2), but also on concordance with nuclear ITS region results (Liston & al., 1999, 2003). We have applied the criterion of monophyly to groups that are emerging from molecular studies. Despite these advances, several of the groups are not clearly delineated based on our current knowledge of informative morphological characters in the genus. We hope that the current contribution represents an improved, more stable, hypothesis of relations within the genus *Pinus*. In addition to further testing of this hypothesis, much work is needed in delineating species, which has not been addressed here.

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Appendix. Collection data for *Pinus* species. GenBank numbers marked with an asterisk (*) are from Wang & al. (1999).**Proposed classification, collection locality and voucher data, GenBank No. (matK, rbcL).**

Subgenus *Pinus*, section *Pinus*, subsection *Pinus* (17/17): *P. densata* Masters, Japan, Kamigamo Experimental Stn, Kyoto, *EUS188*, AB097779, AB097770; *P. densiflora* Siebold & Zucc., Japan, Kamigamo Experimental Stn, Kyoto, *EUS183*, AB084497, AB019814*; *P. hwangshanensis* W. Y. Hsia, Japan, Kamigamo Experimental Stn, Kyoto, *EUS179*, AB161007, AB019812*; *P. kesiya* Royle ex Gordon, Thailand, *Liston 961*, AY497289, AY497253; *P. luchuensis* Mayr, Japan, Kamigamo Experimental Stn, Kyoto, *EUS187*, AB097780, AB097772; *P. massoniana* D. Don, Japan, Kamigamo Experimental Stn, Kyoto, AB081088, AB019815*; *P. merkusii* Jungh. & de Vriese, Thailand, *Liston 95*, AY497287, AY497251; *P. mugo* Turra, Japan, Kamigamo Experimental Stn, Kyoto, *EUS153*, AB081087, AB063372; *P. nigra* J.F. Arnold, Japan, Kamigamo Experimental Stn, Kyoto, *EUS154*, AB084498, AB019817*; *P. resinosa* Ait., U.S.A., *Michigan Oxender s.n.* (OSC), AY497288, AY497252; *P. sylvestris* L., Japan, Kamigamo Experimental Stn, Kyoto, *EUS171*, AB084492, AB019809*; *P. tabuliformis* Carrière, Japan, Kamigamo Experimental Stn, Kyoto, *EUS158*, AB161015, AB019810*; *P. taiwanensis* Hayata, Japan, Kamigamo Experimental Stn, Kyoto, *EUS153*, AB161016, AB161045; *P. thunbergii* Parl., Wakasugi & al., 1994, NC001631.1, NC001631.1; *P. tropicalis* Morelet, Cuba, Pinar del Río Univ., *HAB343*, AB063510, AB063378; *P. uncinata* Ram. ex DC., Spain, Experimental Stn Polytech Univ. Madrid, AB097778, AB097774; *P. yunnanensis* Franch., Japan, Kamigamo Experimental Stn, Kyoto, *EUS185*, AB161017, AB019816*. **Subsection *Pinaster Loudon* (7/7):** *P. brutia* Ten., Spain, Experimental Stn Polytech Univ. Madrid, AB161018, AB019820*; *P. canariensis* C. Sm., Cuba, Pinar del Río Univ., AB084494, AB019823*; *P. halepensis* Mill., Cuba, Pinar del Río Univ., AB081089, AB019819*; *P. heldreichii* Christ, Japan, Kamigamo Experimental Stn, Kyoto, *EUS153*, AB161006, AB019821*; *P. pinaster* Ait., Spain, Experimental Stn Polytech Univ. Madrid, AB084493, AB019818*; *P. pinea* L., Cuba, Pinar del Río Univ., AB084496, AB019822*; *P. roxburghii* Sarg., Nepal, Kewakot, Royal Botanic Gardens, Kew, 1979.06113 (K), AY724752, AY724760.

Subgenus *Pinus*, section *Trifoliae* Duhamel, subsection *Cortortae* Little & Critchfield (4/4): *P. banksiana* Lamb., Japan, Kamigamo Experimental Stn, Kyoto, *EUS166*, AB063499, AB063367; *P. clausa* (Chapman ex Engelm.) Sarg., U.S.A., Georgia, Royal Botanic Gardens, Kew, 1988.330 (K), AY497265, AY497229; *P. contorta* Dougl. ex Loudon, U.S.A., Oregon, *RMP0410* (OSC), AY497266, AY497230; *P. virginiana* Mill., Japan, Kamigamo Experimental Stn, Kyoto, *EUS152*, AB063511, AB063379. **Subsection *Australis Loudon* (22/26):** *P. attenuata* Lemmon, Japan, Forest Product Research Institute, AB080933, AB063365; *P. caribaea* Morelet, U.S.A., Southern Inst. Forest Genetics, *RMP0405* (OSC), AY497280, AY497244; *P. cubensis* Griseb., Cuba, Pinar del Río Univ., *HAB411*, AB063502, AB063370; *P. echinata* Mill., U.S.A., Arkansas, *Rowland s.n.* (OSC), AY724746, AY724754; *P. elliotii* Engelm., U.S.A., Mississippi, *McGregor s.n.* (OSC), AY724747, AY724755; *P. greggii* Engelm. ex Parl., Mexico, Hidalgo, *DSG426* (MEXU), AY497282, AY497246; *P. herrerae* Martínez, Japan, Kamigamo Experimental Stn, Kyoto, *EUS197*, AB063518, AB063386; *P. lawsonii* Roelz ex Gordon, Japan, Kamigamo Experimental Stn, Kyoto, *EUS163*, AB097784, AB097771; *P. leiophylla* Schiede ex Schltdl. & Cham., Mexico, Mexico, *DSG433* (MEXU), AY497279, AY497243; *P. lumholtzii* Robinson & Fernald, Mexico, Chihuahua, *Ortiz García s.n.* (MEXU), AY497278, AY497242; *P. muricata* D. Don, Japan, Kamigamo Experimental Stn, Kyoto, *EUS170*, AB080935, AB063387; *P. occidentalis* Sw., U.S.A., Southern Inst. Forest Genetics, *RMP0405* (OSC), AY497281, AY497245; *P. oocarpa* Schiede ex Schltdl., Japan, Kamigamo Experimental Stn, Kyoto, *EUS184*, AB081084, AB063382; *P. palustris* Mill., U.S.A., Mississippi, *McGregor s.n.* (OSC), AY724748, AY724756; *P. patula* Schiede ex Schltdl. & Cham., Mexico, Hidalgo, *DSG408* (MEXU), AY497284, AY497248; *P. pringlei* Shaw, Mexico, Oaxaca, *Jardón Barbolla s.n.* (MEXU), AY497283, AY497247; *P. pungens* Lamb., Japan, Kamigamo Experimental Stn, Kyoto, *EUS190*, AB080932, AB063375; *P. radiata* D. Don, U.S.A., California, *DSG3799* (OSC), AY497286, AY497250; *P. rigida* Mill., U.S.A., North Carolina, *Taylor s.n.* (OSC), AY724749, AY724757; *P. serotina* Michx., U.S.A., Tennessee, *RMP0408* (OSC), AY724753, AY724761; *P. taeda* L., U.S.A., Mississippi, *McGregor s.n.* (OSC), AY724750, AY724758; *P. teocote* Schiede ex Schltdl. & Cham., Mexico, Hidalgo, *DSG415* (MEXU), AY497285, AY497249. **Subsection *Ponderosae Loudon* (14/17):** *P. cooperi* C. E. Blanco, Japan, Kamigamo Experimental Stn, Kyoto, *EUS177*, AB161004, AB161024; *P. coulteri* D. Don, U.S.A., California, Royal Botanic Gardens, Kew, 1992.723 (K), AY724751, AY724759; *P. devoniana* Lindl., Mexico, Michoacán, *DSG6099* (MEXU), AY497277, AY497241; *P. douglasiana* Martínez, Mexico, Pinetum M. Martínez, Texcoco, *Alvarez-Buylla 7*, AY497274, AY497238; *P. durangensis* Martínez, Mexico, Pinetum M. Martínez, Texcoco, *Alvarez-Buylla 4*, AY497276, AY497240; *P. engelmannii* Carrière, Mexico, Pinetum M. Martínez, Texcoco, *Ortiz García 12EN/4*, AY497275, AY497239; *P. hartwegii* Lindl., Mexico, Mexico, *DSG6199* (MEXU), AY497267, AY497231; *P. jeffreyi* Balf., U.S.A., Oregon, *DSG300* (OSC), AY497271, AY497235; *P. maximinoi* H. E. Moore, Japan, Kamigamo Experimental Stn, Kyoto, AB161010, AB161040; *P. montezumae* Lamb., Mexico, Hidalgo, *DSG416* (MEXU), AY497269, AY497233; *P. ponderosa* Dougl. ex C. Lawson, U.S.A., Oregon, *RMP0415* (OSC), AY497270, AY497234; *P. pseudostrobus* Lindl., Mexico, Hidalgo, *DSG409* (MEXU), AY497268, AY497232; *P. sabineana* Dougl. ex D. Don, U.S.A., California, Kew, 1984.2824 (K), AY497272, AY497236; *P. torreyana* Parry ex Carrière, U.S.A., California, *DSG407* (OSC), AY497273, AY497237.

Subgenus *Strobus*, section *Parrya*, subsection *Cembroides* Engelm. (11/11): *P. cembroides* Zucc., Mexico, Hidalgo, *DSG00398* (MEXU), AY115782, AY115751; *P. culminicola* Andresen & Beaman, Mexico, Nuevo León, *DSG24298* (MEXU), AY115776, AY115748; *P. discolor* D. K. Bailey & F.G. Hawksworth, Mexico, Pinetum M. Martínez, Texcoco, *DSG6699* (MEXU), AY115777, AY115745; *P. edulis* Engelm., U.S.A., Colorado, *DSG03399* (OSC), AY115765, AY115738; *P. johannis* M. -F. Robert, Mexico, Zacatecas, *DSG08199* (MEXU), AY115778, AY115746; *P. maximartinezii* Rzedowski, Mexico, Zacatecas, *DSG07699* (MEXU), AY115790, AY115755; *P. monophylla* Torr. & Frém., U.S.A., California, *DSG02399* (OSC), AY115768, AY115740; *P. pinceana* Gord., Mexico, Coahuila, *DSG08899* (MEXU), AY115788, AY115754; *P. quadrifolia* Parl. ex G. B. Sudworth, U.S.A., California, *DSG01999* (OSC), AY115771, AY115744; *P. remota* (Little) D. K. Bailey & F. G. Hawksworth, Mexico, Coahuila, *DSG19298* (MEXU), AY115775, AY115750; *P. rzedowskii* Madrigal & M. Caballero, Mexico, Michoacán, *Quijada 9008*, AY115791, AY115756. **Subsection *Nelsoniae van der Burgh* (1/1):** *P. nelsonii* Shaw, Mexico, Tamaulipas, *DSG30798* (MEXU), AY115793, AY115757. **Subsection *Balfourianae* Engelm. (3/3):** *P. aristata* Engelm., U.S.A., Colorado, *DSG03299* (OSC), AY115795, AY115758; *P. balfouriana* Balf., U.S.A., California, *Oline C111* (OSC), AY115799, AY115760; *P. longaeva* D. K. Bailey, U.S.A., California, *DSG03099* (OSC), AY115796, AY115759. **Subsection *Gerardianae* (3/3):** *P. bungeana* Zucc. ex Endl., Royal Botanic Gardens, Kew, 1999.226 (K), AY115800, AY115761; *P. gerardiana* Wall. ex D. Don, Royal Botanic Gardens, Kew, 1991.1245 (K), AY115801, AY115762; *P. squamata* X. W. Li, China, Yunan Province, *RMP0412* (OSC), AY115802, AY115763. **Subsection *Krempfianae* Little & Critchfield (1/1):** *P. krempfii* Lecomte, Scotland, Royal Botanic Gardens, Edinburgh, *RMP0411* (E), AB019831*, AY115764. **Subsection *Strobus Loudon* (18/21):** *P. albicaulis* Engelm., U.S.A., Oregon, *Liston 1120* (OSC), AY497261, AY497225; *P. armandii* Franch., Japan, Kamigamo Experimental Stn, Kyoto, *EUS193*, AB161002, AB161021; *P. ayacahuite* Ehrenb. ex Schltdl., Mexico, Hidalgo, *DSG413* (MEXU), AY497257, AY497221; *P. bhutanica* A. J. C. Grierson, D. G. Long & C. N. Page, Bhutan, Punakha District, Royal Botanic Gardens, Edinburgh, 1994.1998 (E), AY497262, AY497226; *P. cembra* L., Swiss Federal Research Institute WSL, Gugerli CH6.275, AB160985, AB161022; *P. chiapensis* (Martínez) Andresen, Mexico, Oaxaca, *DSG999* (MEXU), AY497256, AY497220; *P. fenzeliana* Hand.-Mazz., Japan, Kamigamo Experimental Stn, Kyoto, *EUS181*, AB161005, AB161025; *P. flexilis* E. James, U.S.A., California, *DSG2099* (OSC), AY497258, AY497222; *P. koraiensis* Siebold & Zucc., Japan, Kamigamo Experimental Stn, Kyoto, *EUS162*, AB161009, AB161026; *P. lambertiana* Dougl., U.S.A., Oregon, *DSG400* (OSC), AY497260, AY497224; *P. monticola* Dougl. ex D. Don, U.S.A., Oregon, *DSG200* (OSC), AY497259, AY497223; *P. morrisonicola* Hayata, Taiwan, Taoyuan Co., Royal Botanic Gardens, Kew, 1996.1179 (K), AY497263, AY497227; *P. parviflora* Siebold & Zucc., Japan, Kamigamo Experimental Stn, Kyoto, AB081086, AB019800*; *P. peuce* Griseb., Macedonia, Peristeri, Royal Botanic Gardens, Edinburgh, 1977.0427 (E), AY497254, AY497218; *P. pumila* (Pall.) Regel, Swiss Federal Research Institute WSL, Gugerli RU3.126, AB161013, AB161042; *P. sibirica* Du Tour, Russia, Irkutsk, Royal Botanic Gardens, Edinburgh, 1991.1208 (E), AY497264, AY497228; *P. strobus* L., U.S.A., New Jersey, *DSG500* (OSC), AY497255, AY497219; *P. wallichiana* A. B. Jackson, India, Himachal Pradesh, Royal Botanic Gardens, Kew, 1997.4901 (K), AY734482, AY734483.