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RELATIONSHIPS WITHIN CUPRESSACEAE SENSU LATO: A COMBINED MORPHOLOGICAL AND MOLECULAR APPROACH[1]

PAUL A. GADEK,[2] DERYN L. ALPERS, MARGARET M. HESLEWOOD, AND CHRISTOPHER J. QUINN[3]

School of Biological Science, University of New South Wales, Sydney, NSW 2052, Australia

Parsimony analysis of *matK* and *rbcL* sequence data, together with a nonmolecular database, yielded a well-resolved phylogeny of Cupressaceae sensu lato. Monophyly of Cupressaceae sensu stricto is well supported, and separate northern and southern hemisphere subclades are resolved, with *Tetraclinis* within the northern subclade; there is no support for any of the tribes sensu Li. Taxodiaceae comprise five separate lineages. *Chamaecyparis nootkatensis* falls within *Cupressus,* clustering with a robust clade of New World species. *Libocedrus* Florin is paraphyletic and should incorporate *Pilgerodendron.* Evolution of several characters of wood and leaf anatomy and chemistry is discussed in light of this estimate of the phylogeny; numerous parallelisms are apparent. A new infrafamilial classification is proposed in which seven subfamilies are recognized: Callitroideae Saxton, Athrotaxidoideae Quinn, Cunninghamioideae (Sieb. & Zucc.) Quinn, Cupressoideae Rich. ex Sweet, Sequoioideae (Luerss.) Quinn, Taiwanioideae (Hayata) Quinn, Taxodioideae Endl. ex K. Koch. The *rbcL* sequence for *Taxodium distichum* is corrected, and the implications for a previously published estimate of the minimum rate of divergence of the gene since the Miocene are highlighted.

Key words: *Chamaecyparis*; conifers; Cupressaceae; *Libocedrus*; *matK*; phylogeny; *Pilgerodendron*; *rbcL*; systematics.

The Cupressaceae sensu stricto (s.s.) were separated from Taxodiaceae by Pilger (1926), but following the phenetic analysis of Eckenwalder (1976) this distinction has been widely questioned. Recent phylogenetic analyses of molecular (Brunsfeld et al., 1994; Stefanović et al., 1998) and nonmolecular (Hart, 1987) databases have supported Eckenwalder's proposal of incorporating both Cupressaceae and Taxodiaceae sensu Pilger in a single family. Molecular data have also clearly demonstrated that *Sciadopitys* must be excluded from that family and placed in a monotypic Sciadopityaceae (Chase et al., 1993; Chaw et al., 1997).

Existing tribal and subfamilial concepts have also been challenged, but no consensus on a more appropriate treatment has emerged. None of the published arrangements within Taxodiaceae (Endlicher, 1847; Pilger, 1926; Pilger and Melchior, 1954; Eckenwalder, 1976; Liu and Su, 1983; Hart, 1987; see Table 1) has been well supported by recent molecular evidence (Price and Lowenstein, 1989; Brunsfeld et al., 1994). The widely used distinction between imbricate and valvate cone scales on which the subfamilies of Cupressaceae s.s. are based (Li, 1953) has been questioned by de Laubenfels (1965), and neither the tribal nor subfamilial groupings accord well with anatomical or flavonoid data (Gadek and Quinn, 1985, 1988; Quinn, 1989).

The analyses of Hart (1987) recognized the monophyly of Callitroideae (Li, 1953), but gave no support to Li's tribal concepts (Table 1) and showed Cupressoideae to be paraphyletic, comprising some four separate lineages. However, available flavonoid data (Gadek and Quinn, 1985) were not included in this database, and there is some confusion in the literature on wood characters.

Relationships within the family were only weakly resolved by *rbcL* sequence data (Brunsfeld et al., 1994), much of the topology collapsing when parsimony was relaxed by only one or two steps. Taxon density in this analysis was also low in Cupressaceae s.s., only 13 of the 19 genera being represented, all but one by a single species, and southern hemisphere genera were poorly represented. Despite these limitations, the topology obtained was strongly divergent from that implied by Li's subfamilies and tribes. Hence, there is obvious need for a broader database containing a larger number of informative characters in order to gain a more robust estimate of the phylogeny against which to test the existing infrafamilial taxonomy.

Because of the relatively conserved nature of the *rbcL* locus in this long-lived group of plants (Brunsfeld et al., 1994), this locus alone is unlikely to provide a robust resolution of relationships between the genera. Sequence data for *matK,* a chloroplast-encoded locus that has been shown to be much more variable than *rbcL* in several studies (Johnson and Soltis, 1995; Gadek, Wilson, and Quinn, 1996), have therefore been assembled. These are analyzed in conjunction with a revised nonmolecular database and a slightly augmented *rbcL* database.

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[2] Current address: Department of Botany and Tropical Agriculture, James Cook University of North Queensland, PO Box 6811, Cairns, Qld, 4870, Australia.

[3] Author for correspondence (e-mail: chris.quinn@unsw.edu.au).

TABLE 1. A comparison of taxonomic treatments of taxa assigned to Cupressaceae sensu Eckenwalder (1976).

Endlicher (1847)	Pilger (1926)	Li (1953)	Pilger and Melchior (1954)	Eckenwalder (1976)
CUPRESSINEAE	CUPRESSACEAE	CUPRESSACEAE s.s.	TAXODIACEAE	CUPRESSACEAE s.l.
Cupressineae verae	**Cupressoideae**	**Cupressoideae**	**Athrotaxeae**	**Sciadopitoideae**
Cupressus	*Chamaecyparis*	Cupresseae	*Athrotaxis*	*Sciadopitys*
Chamaecyparis	*Cupressus*	*Chamaecyparis*	**Cryptomerieae**	**Cupressoideae**
Junipereae	**Juniperoideae**	*Cupressus*	*Cryptomeria*	Cupresseae
Juniperus	*Arceuthos* [= *Juniperus* p.p.]	*Fokienia*	**Cunninghamieae**	Subtribe 1
Thujopsideae	*Juniperus*	Junipereae	*Cunninghamia*	*Sequoia*
Biota (= *Platycladus*)	**Thujoidae**	*Arceuthos*	**Metasequoieae**	*Sequoiadendron*
Thuja	*Actinostrobus*	(= *Juniperus* p.p.)	*Metasequoia*	Subtribe 2
Thujopsis	*Callitropsis* [= *Neocallitropsis*]	*Juniperus*	**Sequoieae**	*Metasequoia*
Actinostrobeae	*Callitris*	Thujeae	*Sequoia*	Subtribe 3
Actinostrobus	*Diselma*	*Biota* (= *Platycladus*)	*Sequoiadendron*	*Cupressus*
Callitris	*Fitzroya*	*Heyderia* (= *Calocedrus*)	**Taiwanieae**	*Chamaecyparis*
Frenela (= *Callitris* p.p.)	*Fokienia*	*Thuja*	*Taiwania*	*Juniperus*
Libocedrus sens. lat.	*Libocedrus* incl. *Austrocedrus*,	*Thujopsis*	**Taxodieae**	*Calocedrus*
Widdringtonia	*Papuacedrus* and *Calocedrus*	**Callitroideae**	*Taxodium*	*Fokienia*
Taxodineae	*Thuja*	Actinostrobeae	*Glyptostrobus*	*Microbiota*
Cryptomeria	*Thujopsis*	*Actinostrobus*		*Platycladus*
Glyptostrobus		*Callitris*		*Thuja*
Taxodium	TAXODIACEAE	*Fitzroya*		*Thujopsis*
	Taxodioideae	Libocedreae		Subtribe 4
ABIETINAE	*Athrotaxis*	*Diselma*		*Actinostrobus*
Abietinae verae	*Cryptomeria*	*Libocedrus* incl.		*Callitris*
Araucarieae	*Cunninghamia*	*Austrocedrus*		*Tetraclinis*
Cunninghamieae	*Glyptostrobus*	*Neocallitropsis*		*Austrocedrus*
Athrotaxis	*Sequoia* incl. *Sequoiadendron*	*Octoclinis* (= *Callitris* p.p.)		*Diselma*
Cunninghamia	*Taiwania*	*Papuacedrus*		*Fitzroya*
Dammara [= *Araucaria* p.p.]	*Taxodium*	*Pilgerodendron*		*Libocedrus* incl. *Papuacedrus*
Sequoia incl. *Sequoiadendron*	**Sciadopityoideae**	*Widdringtonia*		and *Pilgerodendron*
	Sciadopitys	Tetraclineae		*Neocallitropsis*
		Tetraclinis		*Widdringtonia*
				Subtribe 5
				Taxodium
				Glyptostrobus
				Cryptomerieae
				Cryptomeria
				Cunninghamieae
				Subtribe 1
				Cunninghamia
				Taiwania
				Subtribe 2
				Athrotaxis

TABLE 2. Primers used; in some instances PCR primers were used in sequencing as well.

Code		Sequence 5′-3′	
PCR primers			
909	trnK3914F	GGGGTTGCTAACTCAACGG	Gadek et al 1996
1359	orf515-900F	TACGCAATTTCTCATGATCA	
1366	515-2150R	CGTATCGTACTTTTATGTTT	
1368	515-2550R	AGCTCGTCGGATGGAGTGG	
Sequencing primers			
1367	515-2000F	TCAGGGCGGCCAATTAGTAA	
1565	orf-352F	AAGGAATGGATGGATGGAATAG	
1566	orf-352R	CTATTCCATCCATCCATTCCTT	
1567	orf-1000R	ACCACGAGAGGTCTCATTT	

MATERIALS AND METHODS

Total DNA was extracted from fresh leaves or leaves dried in silica gel crystals using either the CTAB (cetyldimethylammonium bromide) method (Doyle and Doyle, 1990) or the DNeasy Plant Minikit (QIAGEN, Clifton Hill, Victoria, Australia). Double-stranded templates were amplified using the primers listed in Table 2 and sequenced on an ABI Prism Automated Sequencer (Perkin Elmer, Norwalk, Connecticut). Sequences were assembled and checked using ABI Prism software (Factura and Autoassembler), manually aligned and stored in a DNA and Protein Sequence Alignment program (DAPSA; Dr E. Harley, University of Cape Town), and translated in MacClade Version 3.05 (Maddison and Maddison, 1992) to assist with the positioning on segments affected by insertion/deletion mutations (indels) and to check for stop codons. Deleted segments were treated as missing data in the analyses and potentially informative indels scored as additional characters (present/absent) that were added to the sequence database.

A database incorporating available morphological, anatomical, and chemical characters was assembled. Characters obtained from the literature were checked against original sources. Several characters used by Hart (1987) that are poorly documented within the ingroup were excluded. Because of some conflict in the literature on wood anatomy, those characters were reinvestigated by light microscopy of semithin sections of plastic-embedded material and scanning electron microscopy of wood blocks (De Nardi and Quinn, unpublished data).

Characters were polarized by the outgroup method, using a representative of *Picea* to root the analyses and including *Amentotaxus argotaenia* as a closer outgroup taxon, these choices being made in light of recent broad estimates of conifer phylogeny based on *rbcL* (Dr. R. Price, personal communication, University of Georgia) and 18S (Chaw et al., 1997) sequence data. Heuristic searches were conducted in PAUP Version 4.0b2a (Swofford, 1999) using TBR (tree bisection reconnection) branch-swapping and the MULPARS option. Replicate analyses involving random-taxon addition were employed to search for multiple islands of trees. Branch lengths for trees were calculated using the ACCTRAN (accelerated transformation optimisation) option in PAUP. Relative support for the clades identified by parsimony analysis was estimated by bootstrap (Felsenstein, 1985) in PAUP, and decay analyses (Donoghue et al., 1992) using PAUP and AutoDecay version 4.0.1 (Eriksson, 1998) with a simple heuristic search on each constraint tree. Output trees were imported into MacClade in order to explore evolution of nonmolecular characters and to construct constraint trees in order to test alternative hypotheses against the data. Analyses were then performed in PAUP using the option ''topological constraint enforced.''

RESULTS

The matK database—The *matK* locus was sequenced for 44 ingroup species (Table 3) drawn from all currently recognized genera and subgenera, and from New and Old World species of *Cupressus* and *Calocedrus.* Sequences for *Juniperus drupacea* and *Cupressus goveniana* were incomplete, the respective numbers of positions determined being 946 and 781. When aligned, considerable variation in the position of the stop codons was evident. In *Microbiota* and *Taiwania* there is a TAA stop at codon 508; in most taxa there is a TGA stop at codon 510 or 512; in *Papuacedrus* there is a TGA stop at codon 542. Thirteen potentially informative indels were recognized (Table 4) and their presence/absence scored and added to the database. All but two consisted of 1–11 entire codons; the other two involved four base pairs (bp). As a result, the length of the gene varied from 1515 bp in *Fitzroya* to 1620 bp in *Papuacedrus.* A total of 1530 aligned positions was included in the analyses. There were 735 (48%) variable positions among ingroup taxa, 401 (26.2%) being potentially informative. The codon position ratio was calculated as 1.21: 1: 1.59.

Heuristic analysis of this database gave 48 equally parsimonious trees of 1427 steps, the strict consensus of which is shown in Fig. 1 (RC [rescaled consistency index] = 0.52). The results of bootstrap and decay analyses are shown on the branches. Cupressaceae s.s. constitute a very strongly supported clade (97% bootstrap; +12 decay), whereas Taxodiaceae constitute five separate lineages which associate sequentially with the Cupressaceae clade. Two major clades are identifiable within Cupressaceae s.s. The cupressoid clade, comprising all the northern hemisphere genera including *Tetraclinis,* decays at +4 and is included in 90% of bootstrap trees; four subclades may be identified within it (I–IV, Fig. 1). The callitroid clade, which comprises all the southern hemisphere genera and is strongly supported (100% bootstrap, +25 decay), includes two robust subclades labelled V and VI in Fig. 1, as well as two smaller robust clusters: *Actinostrobus, Callitris,* and *Neocallitropsis* (100%, +28); *Libocedrus bidwillii, L. yateensis,* and *Pilgerodendron* (100%, +6).

The representatives of the three subgenera of *Juniperus,* and the three species of *Thuja* each cluster strongly (98%, +6 and 100%, +11, respectively), as also do the New and Old World species of *Calocedrus* (97%, +4). Neither *Chamaecyparis* nor *Cupressus,* however, are monophyletic. New and Old World species of *Cupressus* form separate robust groups (100%, +6 and 100%, +9, respectively), with *Chamaecyparis nootkatensis* as the weakly supported sister of the New World group (71%, +1). *Chamaecyparis lawsoniana* and *C. obtusa* cluster with *Fokienia* in subclade III. There is strong support for

sister relationships between *Thuja* and *Thujopsis* (100%, +8), *Chamaecyparis* p.p. (pro parte) and *Fokienia* (99%, +9), and *Microbiota* and *Platycladus* (100%, +7). *Tetraclinis* clusters strongly (92%, +3) with the last two genera within subclade II.

The potentially informative indels (Table 4) are mapped on Fig. 1 and can be seen to provide support for several clades.

Congruence with rbcL analysis—There is apparent incongruence between the above analysis and that based on *rbcL* data (Brunsfeld et al., 1994), particularly with respect to the placement of *Taxodium.* In order to check this, two accessions of *Taxodium distichum* (Table 3) were resequenced for *rbcL.* These yielded two identical sequences that showed considerable divergence from the original (29 differences in 1400 bp). A partial sequence (~950 bp) derived from a third accession originating from Strybing Arboretum (Dr. R. Price, personal communication, University of Georgia), diverged at only one position from our new sequences. A new database was constructed with the consensus of these three sequences, all other available ingroup sequences, mostly drawn from Brunsfeld et al. (1994), and those of *Amentotaxus argotaenia* and *Picea sitchensis* as outgroups (Table 3). Heuristic analysis yielded 42 equally parsimonious trees, the strict consensus of which is shown in Fig. 2 (RC = 0.49). This is highly congruent with the topology in Fig. 1; it differs from the topology obtained by Brunsfeld et al. (1994) chiefly in the placement of all *Taxodium* sequences in a clade with *Cryptomeria* and *Glyptostrobus.* The sequences derived from living and fossil material used in Brunsfeld et al. (1994) form a highly robust cluster (bootstrap 100%, decay +17) that is only weakly clustered with the new extant sequence (60%, +2).

Given the more congruent topology obtained in the revised *rbcL* analysis, we feel justified in combining the two sets of sequence data for all those taxa common to the *matK* and revised *rbcL* databases, i.e., representatives of all taxodiaceous genera and a subset of cupressaceous genera (Table 3). Heuristic search, again rooted on *Picea,* yielded two equally parsimonious trees (Fig. 3). Once more, Cupressaceae s.s. and the callitroid clade are highly robust monophyletic groups (100%, +20; 100%, +35). Support for the cupressoid clade is increased (100%, +7; cf. 90%, +4 in Fig. 1). The Taxodiaceae are arranged in the same five lineages as in Fig. 1. Support for the order of divergence of the sequoioid clade, *Athrotaxis* and *Taiwania* is still weak, especially for the relative positions of the first two (71%, +2).

Revised nonmolecular database—The following characters and states were included in the nonmolecular database:

1. Phyllotaxis: helical [0]; opposite and decussate [1]; ternate [2]; whorls of 4 [3].
2. Branching pattern: axillary branchlets arising on all sides of the stem [0]; branchlets restricted to one plane [1].
3. Determinate short shoots seasonally deciduous: absent [0]; present [1].
4. Vertical parenchyma in the wood: absent [0]; present [1].
5. Transverse walls of vertical parenchyma: smooth [0]; with small nodules [1]; with large nodules [2].
6. Arrangement of bordered pits in the early wood tracheids: alternate and multiseriate [0]; uniseriate [1].
7. Torus on the membrane of intertracheal pits: present [0]; absent [1].
8. Pitting of the tangential walls of ray parenchyma: one large pit occupying most or all of the wall [0]; several small pits separated by thick wall, giving appearance of distinct ''nodules'' of thickening [1]. Taxa with thinwalled ray parenchyma are scored inapplicable [-].
9. Ray tracheids: absent [0]; present [1].
10. Form of adult leaves: falcate in profile and tetragonal in cross section [0]; linear to lanceolate and bifacially flattened [1]; scale-like [2].
11. Seedling phyllotaxis: whorled [0]; opposite [1]; helical [2].
12. Mature foliage leaves: monomorphic [0]; dimorphic [1].
13. Stomatal distribution on adult leaves: amphistomatic [0]; hypostomatic [1]; epistomatic [2].
14. Distribution of transfusion tracheids in leaves: separate strands lateral to vascular bundle [0]; continuous band across the adaxial side of the xylem strand [1]; surrounding the vascular bundle [2].
15. Form of transfusion tracheids: large cells with many pits [0]; small cells with few pits [1]; large cells with few pits [2].
16. Thickening of walls of transfusion tracheids: evenly thickened [0]; walls bearing prominent ribs of lignification [1].
17. Pits on transfusion tracheids (Gadek and Quinn, 1988): circular bordered [0]; barred [1]; trabeculate [2]; large irregular pits with narrow border [3].
18. Development of xylem in the leaf trace (Quinn and Gadek, 1988): acropetal [0]; basipetal [1].
19. Tropolones in wood extractives: absent [0]; present [1].
20. Accumulation of 2,3 dihydroamentoflavone in adult leaves: absent [0]; present [1].
21. Accumulation of cupressuflavone derivatives in adult leaves: absent [0]; present [1].
22. Accumulation of hinokiflavone derivatives in adult leaves: absent [0]; present [1].
23. Accumulation of 2,3 dihydrohinokiflavone in adult leaves: absent [0]; present [1].
24. Accumulation of taiwaniaflavone derivatives in adult leaves: absent [0]; present [1].
25. Accumulation of robustaflavone derivatives in adult leaves: absent [0]; present [1].
26. Methylation pattern in leaf biflavone fraction (Gadek and Quinn, 1985): di- and trimethyl ethers conspicuous [0]; monomethyl ethers and parental compounds as major constituents, dimethyl ethers as minor constituents only [1]; parental compounds as major constituents, methyl ethers present as minor constituents or absent [2]. Scored inapplicable in *Picea,* where biflavonoids are absent.
27. Accumulation of nootkatin in the heart wood: absent [0]; present [1].
28. Germination of pollen grains: without papilla [0]; with papilla [1].

TABLE 3. Details of sequence data; taxa arranged according to new classification. P indicates sequence published but not lodged in GenBank.[a]

	GenBank accessions[b]		
	*mat*K	*rbc*L	Voucher and/or source
CUPRESSACEAE			
Athrotaxidoideae			
Athrotaxis laxifolia Hook.	GBAN-AF152176	GBAN-L25754	Brunsfeld et al., 1994
Callitroideae			
Actinostrobus acuminatus Parl.	GBAN-AF152175	—	Crayn 7, UNSW
Austrocedrus chiliensis (D. Don) Florin	GBAN-AF152177	—	Syd Ac 863527
Callitris rhomboidea R. Br. ex L. C. Rich.	GBAN-AF152180	GBAN-L12537	UNSW21734
Diselma archeri Hook. f.	GBAN-AF152193	GBAN-L12572	UNSW21742
Fitzroya cupressoides (Mol.) Johnston	GBAN-AF152194	—	Syd Ac 851215
Libocedrus bidwillii Hook. f.	GBAN-AF152202	—	UNSW23286, Syd
L. plumosa (D. Don) Sarg.	GBAN-AF152200	GBAN-L12574	UNSW21741
L. yateensis Guillaumin	GBAN-AF152201	—	Grossbechler 108, E; UNSW23310
Neocallitropsis araucarioides	—	GBAN-AF127426	J. Read s.n., UNSW
	GBAN-AF152205	—	Syd Ac 871405
Papuacedrus papuana (F. Muell.) Li	GBAN-AF152206	—	Syd Ac 901639
Pilgerodendron uviferum (D. Don) Florin	GBAN-AF152207	—	UNSW23247, Syd; UNSW23402, Ed
Widdringtonia schwartzii Marsh	GBAN-AF152218	—	Tomlinson s.n., UNSW
W. cedarbergensis Marsh	—	GBAN-L12538	UNSW17574
Cunninghamioideae			
Cunninghamia lanceolata (Lamb.) Hook.	GBAN-AF152185	GBAN-L25757	Brunsfeld et al., 1994
Cupressoideae			
Calocedrus decurrens (Torrey) Florin	GBAN-AF152178	GBAN-L12569	UNSW22326
C. macrolepis Kurz.	GBAN-AF152179	—	Syd. Ac. 697111
Chamaecyparis lawsoniana (Murray) Parl.	GBAN-AF152181	—	UNSW14297
C. nootkatensis (D. Don) Spach	GBAN-AF152182	—	Kew Ac. 142-50-14202
	—	GBAN-AF127431	UNSW23790
C. obtusa (Sieb. & Zucc.) Endl.	GBAN-AF152183	GBAN-L12570	UNSW21735
Cupressus arizonica var. *glabra* (Sudw.) Little	GBAN-AF152188	GBAN-AF127430	UNSW23793, Syd
C. duclouxiana Hickel ex Camus	GBAN-AF152186	—	Rushforth 361, K
C. goveniana Gordon	GBAN-AF152191	—	UNSW24025, Syd
C. pygmaea Sargent	GBAN-AF152192	—	UNSW24052, Syd
C. lusitanica var. *benthamii* (Endl.) Carr.	GBAN-AF152189	—	UNSW24026, Mel
C. macnabiana Murr.	GBAN-AF152190	—	UNSW24023, Syd
C. sempervirens L.	GBAN-AF152187	GBAN-L12571	UNSW14297
Fokienia hodginsii (Dunn.) Henry & Thomas	GBAN-AF152195	GBAN-AF127429	Syd. Ac. 812716
Juniperus conferta Parl.	GBAN-AF152197	GBAN-L12573	UNSW14290
J. drupacaea Labill.	GBAN-AF152198	—	Kew Ac. 056-81-0032
J. procera Hochst.	GBAN-AF152199	—	Kew Ac. 720-85-72002
Microbiota decussata Komar.	GBAN-AF152204	GBAN-L12575	Gadek s.n. UNSW, Mis
Platycladus orientalis (L.) Franco	GBAN-AF152208	GBAN-L13172	UNSW21737
Tetraclinis articulata (Vahl) Mast.	GBAN-AF152213	GBAN-L12576	UNSW21730
Thuja occidentalis L.	GBAN-AF152214	GBAN-L12578	UNSW21732
T. plicata D. Don	GBAN-AF152216	—	UNSW21736
	—	GBAN-L25758	Brunsfeld et al., 1994
T. standishii (Gordon) Carr.	GBAN-AF152215	GBAN-AF127428	Kew Ac 000-69-15537
Thujopsis dolobrata (L.f.) Sieb. & Zucc.	GBAN-AF152217	GBAN-L12577	UNSW21736
Sequoioideae			
Metasequoia glyptostroboides Hu & Cheng	GBAN-AF152203	P	Soltis, Soltis, and Smiley, 1992
Sequoia sempervirens (D. Don) Endl.	GBAN-AF152209	GBAN-L25755	Brunsfeld et al., 1994
Sequoiadendron giganteum (Lindl.) Buchholz	GBAN-AF152210	P	Brunsfeld et al., 1994
Taiwanioideae			
Taiwania cryptomerioides Hayata	GBAN-AF152211	GBAN-L25756	Brunsfeld et al., 1994
Taxodioideae			
Cryptomeria japonica D. Don	GBAN-AF152184	GBAN-L25751	Brunsfeld et al., 1994
Glyptostrobus pensilis (Staunton ex D. Don) K. Koch	GBAN-AF152196	GBAN-L25750	Brunsfeld et al., 1994
Taxodium distichum (L.) Rich.	—	GBAN-S75127	Soltis, Soltis, and Smiley, 1992
	GBAN-AF152212	GBAN-AF127427	UNSW23327; Quinn s.n., UNSW; R. Price s.n., Stry
Taxodium sp. (Miocene fossil)	—	P	Soltis, Soltis, and Smiley, 1992
TAXACEAE			
Amentotaxus argotaenia (Hance) Pilg.	—	GBAN-L-12580	R. Price (unpublished)
	GBAN-AF152219	—	Page 10330, E, Ed
PINACEAE			
Picea mariana (Mill.) B.S.P.	GBAN-AF059343	—	Germano and Klein (unpublished)
P. sitchensis (Bong.) Carr.	—	GBAN-X63660	Doerksen, Strauss, and Price (unpublished)

29. Number of nuclei within pollen grain at pollination: binucleate or multinucleate [0]; uninucleate [1].
30. Layer of peripheral cells in megagametophyte: absent [0]; present [1].
31. Thickness of megaspore membrane: prominently thickened [0]; not distinctly thickened [1].
32. Complexing of archegonia: archegonia separated by at least one layer of sterile tissue of the prothallus [0]; two or more archegonia in direct contact, usually enclosed within a common jacket layer [1].
33. Tiered arrangement of proembryo cells: proembryo differentiated into three or four distinct tiers of cells, including upper, suspensor and embryo tiers [0]; proembryo nontiered [1].
34. Fusion between seed scale complex and subtending bract: free [0]; partially fused [1]; completely fused [2].
35. Orientation of ovules: inverted [0]; erect [1].
36. Number of ovules per cone scale: one [0]; two [1]; more than two [2].
37. Base chromosome number: 11 [0]; 12 [1].
38. Archegonial position within female gametophyte: all apical [0]; apical and/or lateral [1].
39. Archegonial jacket layer: clearly differentiated [0]; not clearly differentiated [1].
40. Number of cells in the initial cellular proembryo, reflecting the number of free-nuclear divisions: eight [0]; four [1]; two [2].
41. Prosuspensors: absent [0]; present [1].
42. Size of proembryo relative to archegonium: basal in archegonium [0]; filling archegonium [1].
43. Cleavage polyembryony: absent [0]; present [1].
44. Seed maturation: in the first year [0]; second year or later [1].
45. Number of fertile scales in the female cone: 1–3 [0]; 4 [1]; up to 6 [2]; >6 [3].

As far as possible, these characters were scored (Table 5) for the same set of terminal taxa as for the molecular data. However, because of the limited availability of data, the states of some characters have been extrapolated from other species in the genus, subgenus, or group where there is evidence of uniformity within the group (e.g., wood and chemical characters).

Several previous authors have recorded nodulation of the transverse walls of vertical and ray parenchyma. Characters 9 and 10 of Hart (1987) score nodulation of the same walls as seen in radial or tangential section by different authors, and some taxa are scored differently. Close examination of both cell types under light microscopy (LM) and scanning electron microscopy (SEM) has revealed that differences in wall thickness and number and size of pits contribute to the variation observed. In taxa where these cells are thinwalled, the characters are scored inapplicable [-]; in others the end wall is occupied by a single very large pit, so that the end wall appears thin and is certainly free from any nodules. The scoring of characters 5 and 8 is based on our own data.

TABLE 4. Potentially informative insertion/deletion events (indels) recognized in the aligned *matK* database, showing affected base positions in the aligned database. Distribution of indels is shown in Fig. 1.

Indel	Affected positions
a	215–220
b	250–252
c	250–258
d	271–273
e	549–554
f	568–576
g	610–612
h	769–771
i	1391–1423
j	1399–1401
k	1411–1422
I	1486–1490
m	1529–1532

Our character 6 is invariable in Hart (1987) among the taxa scored here, but our own observations show the pits to be alternate, multiseriate in *Cunninghamia* and *Neocallitropsis.* We have found a pronounced torus to be regularly present in the intervascular pits (character 7) of *Sequoia,* as well as *Thuja* and *Thujopsis.* Similarly, we have observed the tangential walls of the ray parenchyma (character 8) to be thickened and nodulated in *Microbiota* and *Platycladus,* as well as in those taxa scored by Hart. Ray tracheids (character 9) were also found by us to be common in *Cunninghamia, Metasequoia,* and *Taiwania.* Hart recorded the genus *Cunninghamia* with amphistomatic leaves (character 13), which is true for *C. konishii,* but *C. lanceolata,* the taxon we have sequenced and are scoring here, is hypostomatic. He also recorded inverted ovules in all Taxodiaceae, but those of *Cryptomeria, Glyptostrobus,* and *Taxodium* are erect (Sporne, 1965; Krüssmann, 1985).

The tropolones in the heart wood were scored by Hart as absent in *Austrocedrus* (his character 46) despite the taxon being recorded as rich in tropolones by Erdtman and Norin (1966).

There are conflicting reports on the chromosome number of *Fokienia hodginsii* ($n = 12$, ten large metacentrics and two small submetacentrics, [Chen, 1983]; $n = 11$, all metacentric, [Li and Hsu, 1984]). This may be due to the presence of different cytotypes, but $n = 12$ would clearly be a derived number. The taxon is scored as $n = 11$ for this analysis. References cited by Hart (1987) do not substantiate his scoring of some characters (e.g., 29, 43), and we are unable to find adequate descriptions of some aspects in several genera (viz., *Austrocedrus, Diselma, Fok-*

←

[a] Location of vouchers: E, Royal Botanic Gardens, Edinburgh; K, Royal Botanic Gardens, Kew; UNSW, John T Waterhouse Herbarium, Sydney. Specimens recorded by collector's number or UNSW collection number. Living collection accessions indicated as follows: Ac, accession number; Ed, Royal Botanic Gardens, Edinburgh; Kew, Royal Botanic Gardens, Kew; Mel, Royal Botanic Gardens, Melbourne; Mis, Missouri Botanical Garden, St Louis; Stry, Strybing Arboretum, San Francisco; Syd, Royal Botanic Gardens, Sydney.

[b] The prefix GBAN-has been added to link the online version of *American Journal of Botany* to GenBank but is not part of the actual accession number.

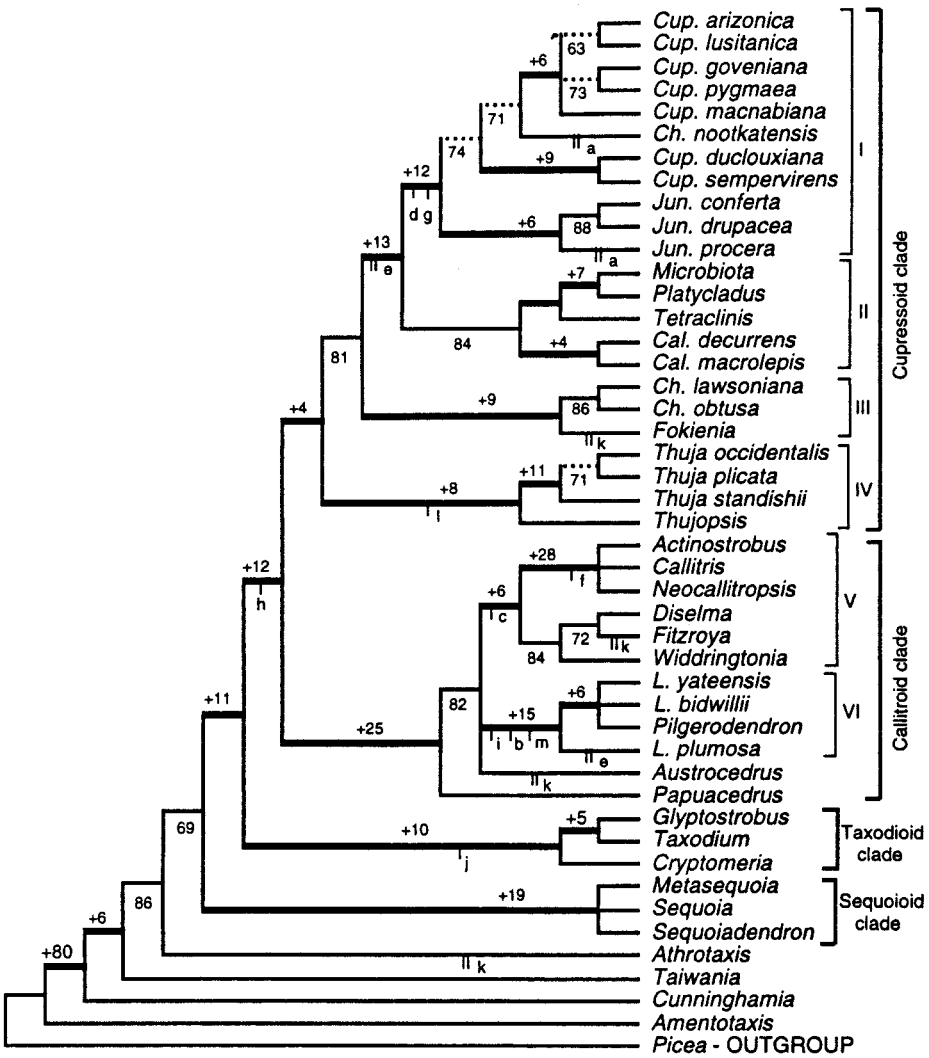

Fig. 1. Strict consensus of 48 equally parsimonious trees of 1427 steps found from a heuristic search of the *matK* data; CI = 0.56; RI = 0.78; RC = 0.52. Thick branches received at least 90% bootstrap support (500 replicates); dotted branches decayed at +1; bootstrap values <90% are shown below branches, decay values >3 are shown above. Informative indels *a–m* (see Table 4) are mapped on the tree; double line indicates parallelism. *Figure Abbreviations*: Cal., *Calocedrus*; Ch., *Chamaecyparis*; Cup., *Cupressus*; Jun., *Juniperus*; L., *Libocedrus.* CI, consistency index excluding uninformative characters; RC, rescaled consistency index; RI, retention index.

ienia, Neocallitropsis, Papuacedrus, Pilgerodendron). Cleavage polyembryony is recorded as present in *Thujopsis* and of variable occurrence and form in *Thuja occidentalis* (Chowdhury, 1962).

Heuristic analysis yielded two separate islands totalling 120 equally parsimonious trees, the strict consensus of which is shown in Fig. 4 (RC = 0.27). There is only limited resolution of relationships. The paraphyly of Taxodiaceae is clearly apparent, but Cupressaceae, both sensu lato and sensu stricto also appear to be paraphyletic, in the first case due to the placement of *Amentotaxus* with *Cunninghamia* and *Metasequoia,* and in the second case because of the placement of *Neocallitropsis* with *Taiwania* among the early diverging lineages that constitute Taxodiaceae. There is no support in these data for the present subfamilies or tribes, nor for the monophyly of *Calocedrus, Chamaecyparis,* or *Thuja.* Almost all the topology collapses at +1 step, so apart from the monophyly of *Cupressus* and *Libocedrus* (+2), the clustering of *Actinostrobus* with *Callitris* (+2), J*uniperus conferta* and *J. drupacea* (+3), and of Cupressaceae s.s. minus *Neocallitropsis* (+3), there is no support in the data for any of the groups in Fig. 4.

The matK plus nonmolecular data—Heuristic search of the combined *matK* and nonmolecular data produced four equally parsimonious trees, the strict consensus of which is shown in Fig. 5 (RC = 0.45). The topology resembles that in Fig. 1 in all major respects. The trichotomy that existed in subclade V is resolved, with *Neocallitropsis* diverging first (91%, +4). *Chamaecyparis nootkatensis* is now the first lineage to diverge within subclade I (73%, +3), and *Sequoiadendron* diverges first within the sequoioid clade, although there is little support for the latter arrangement (61%, +1).

DISCUSSION

The frequency of variable and informative positions in *matK* compares favorably with that for the *rbcL* locus:

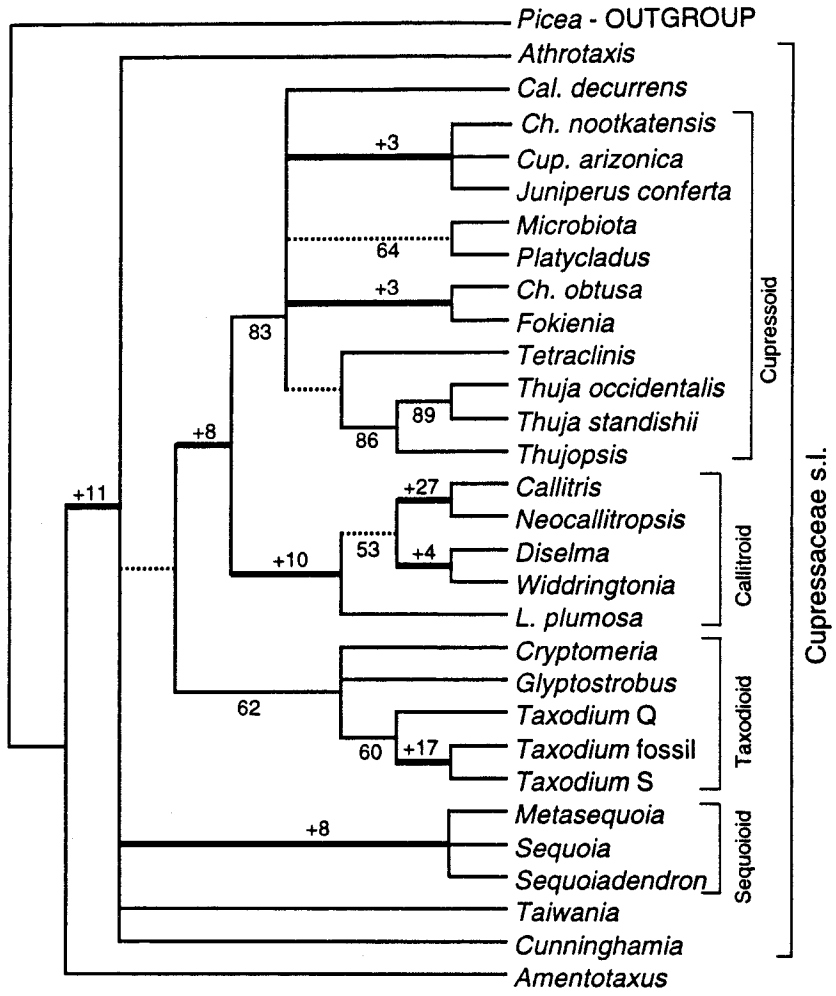

Fig. 2. Strict consensus of 42 equally parsimonious trees of 469 steps found from a heuristic search of *rbcL* sequence data; positions 1–18 and 1411–1428 excluded; CI = 0.55, RI = 0.70, RC = 0.49. Sequences for *Taxodium distichum* derived from this study and from Soltis, Soltis, and Smiley (1992) indicated by Q and S, respectively. Decay values >2 are shown above branches; dotted branches collapse at +1 step. Thick branches received ≥90% bootstrap support; values between 50% and 90% are shown below branches.

32.7 and 17% for *mat*K and 14.7 and 7.5% for *rbcL*, respectively, when measured across the range of Cupressaceae s.l. for which both loci have been sequenced. Hence *matK* has considerably more potential to be informative of relationships within Cupressaceae. The observation that *matK* is evolving at more than twice the rate of *rbcL* agrees closely with other comparative studies. It was found to be twice as variable in Polemoniaceae (Steele and Vilgalys, 1994) and Epacridaceae (Crayn, 1998), and three times in Saxifragaceae (Johnson and Soltis, 1994).

Variation in the position of the stop codon and the tolerance of frame-shift indels in several taxa suggest that the downstream end of the locus, at least, is under very little functional constraint. The codon position ratio of 1.21:1:1.59 displays very little bias toward the third position; this is much less pronounced than has been recorded in Myrtaceae (1.56:1:3.06; Gadek, Wilson, and Quinn, 1996), but is within the range reported by Steele and Vilgalys (1994). This contrasts markedly with the codon ratio of 1.4:1:6.2 in the *rbcL* data for the same ingroup taxa and confirms the relative absence of functional constraint on the *matK* locus as a whole that has been commented on by Liang and Hilu (1996). However, the fact that all indels but the two situated at the extreme 5′ end of the locus conform with the reading frame provides strong evidence of selection against frame-shift mutations.

The variations in position of the stop codon provide some interesting insight into the evolution of the group. Stop codons are present in most taxa at codons 510, 512 and, 542 in the aligned database. Both *Microbiota* and *Taiwania* have an additional stop (TAA) at codon 508, which would require two separate origins on this estimate of the phylogeny. The most parsimonious explanation for the distribution of the 510 (TGA) stop in Cupressaceae s.l. is that it is a plesiomorph, being also present in *Amentotaxus*, although absent in *Picea*, and that it has been lost in the callitroid clade and in *Athrotaxis* and *Fokienia*. In the last case this codon is beyond the end of the gene, but in the other two taxa the 512 codon acts as the stop. The 512 stop codon (TGA) is almost universal in the aligned database, despite the fact that it is mostly beyond the coding region. The frame-shift indels in the *Liboced-*

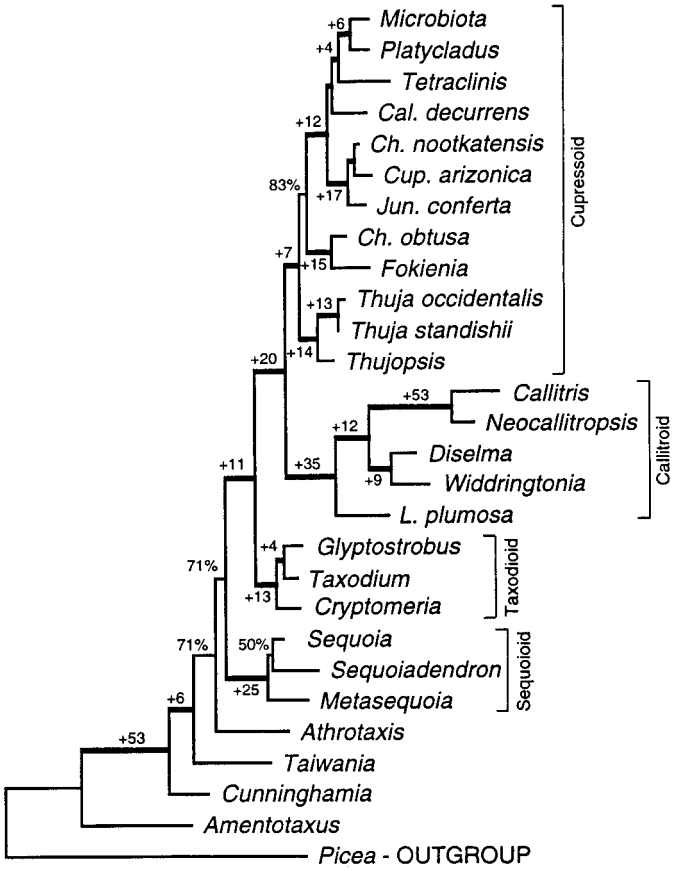

Fig. 3. One of the two most parsimonious trees found from a heuristic search of the combined *matK* plus *rbcL* database, with branches proportional to the amount of change. Positions 1–18 and 1411–1428 excluded from the *rbcL* data. The sequoioid clade collapses into a trichotomy in the strict consensus. Tree length 1601 steps; CI = 0.59; RI = 0.72; RC = 0.53. Thick branches received ≥90% bootstrap support; bootstrap values <90% and decay values >3 are shown on branches.

rus and *Thuja* clades (indels *j* and *k*) mean that neither the 510 nor 512 stop codon, both of which are still present in all members of both clades in the aligned database, are functional; a potential stop codon at positions 1541–1543 that is universal in the aligned database becomes the active stop in both these clades. Hence, the active stop codon falls within a region of 22 bp in the aligned database in all but *Papuacedrus,* where both the 510 and 512 stop codons have been lost. The end of the gene in this case is at codon 542, which was found to be universal in the database. The existence of such widely distributed stop codons beyond the end of the *matK* gene suggests that there has been considerable change in the length of the gene during the evolution of this group. The distribution of the stop codons throughout the conifers and even the gymnosperms may reveal something of the changes that have occurred.

The distributions of ten of the 13 potentially informative indels identified (Table 4) are consistent with a single origin when mapped on the tree (Fig. 1) and provide support for subclades I, IV, V, and VI. Indel *e* has separate origins within the cupressoid and callitroid clades, the former being a synapomorph for the sister relationship between subclades I and II. The other two indels are uninformative of relationships: *a* arises twice in subclade I (*Juniperus procera* and *Chamaecyparis nootkatensis*); *k,* a four-codon deletion, appears to have four separate origins, being found in *Athrotaxis, Austrocedrus, Fitzroya,* and *Fokienia.* Obvious parallelisms in the origin of structurally identical indels have been widely observed in noncoding regions (e.g., Golenberg et al., 1993), but indel distribution in *matK* has been found to generally support the topology generated from data on base substitutions: e.g., the distributions of all five indels detected within Saxifragaceae were consistent with single origins (Johnson and Soltis, 1994). By contrast, the distribution of indel *k* provides an extreme case of homoplasy for a coding region.

The high degree of noise relative to phylogenetic signal in the nonmolecular data is apparent in the low rescaled consistency index (0.28), as well as the lack of character support for the topology in Fig. 4. This is also apparent from the reduction in RC in the analysis of the combined *matK* and nonmolecular data (0.45) compared with the *matK* data alone (0.52). The signal in the *matK* data dominated in the former analysis, so that the result was roughly equivalent to superimposing the nonmolecular data on the *matK* gene tree (cf. Figs 1 and 5). Despite the pronounced homoplasy in the nonmolecular data, there is support for this topology in some characters (Fig. 5). Basipetalous development of xylem within the leaf trace [18/1], small transfusion tracheids with few pits [15/1], pollen germinating without a papilla [28/0], and adult phyllotaxis opposite and decussate [1/1] (despite the reversal within *Widdringtonia* and parallelism in *Metasequoia*) are all synapomorphies for Cupressaceae s.s., the first two being unique synapomorphies in this data base. The placement of the taxodioid clade sister to Cupressaceae s.s. is supported by the distribution of the archegonial jacket clearly defined [39/0], as well as by the immunological data (Price and Lowenstein, 1989). Nootkatin [19/1] is a unique synapomorphy for subclade I, and subclade IV is supported by the synapomorphy 7/1 (intertracheal pits with torus). The sister relationship between *Actinostrobus* and *Callitris* is supported by 30/1 (megagametophyte with peripheral cells) and 41/1 (prosuspensors not developed), although there are many taxa for which the latter character is missing. Available data for characters 38 and 42 provide support for subclade V, but again many taxa are unknown. Furthermore, some of the clearly homoplastic characters still provide support for parts of the topology obtained from the molecular data (Fig. 5). Ray parenchyma with several separate pits on thickened tangential walls [8/1] support the sister relationship between subclades I and II amongst the northern taxa, and also the sister relationship between *Fitzroya* and *Diselma* within the southern clade. Constraint analysis revealed that an extra 62 steps (on a tree length of 1601) are required in the combined *matK* and nonmolecular database to achieve a single origin of the apomorph. The distribution of cupressuflavone [21/1] requires two separate origins and a loss in the cupressoid clade, and a third origin in the callitroid clade. In this case an extra 89 steps is required for a single origin. The accumulation of this unusual class of biflavonoid has been developed separately in Podocarpaceae and Araucariaceae, as well as in angiosperms (Geiger and Quinn, 1982). Hence, it

TABLE 5. Nonmolecular data set. Characters and states numbered as in text. Taxon abbreviations as in Fig. 1; full details given in Table 3; -, inapplicable character; ?, state unknown; polymorphisms within parentheses.

Character number	1 23456789	1 1111111112222222	2 333333	3 333	4 44	4 44
	0 12345678	9 012345	6 789	0 12	3 45	
Actinostrobus	2 00101000	2 0000102?0001000200	1 111111	2 110	1 11	1 01
Amentotaxus	0 101?1000	1 0010???000100??01	0 0100?1	? ?00	0 00	0 0-
Athrotaxis	0 00111000	(02)0021000?0100000000	0 011010	2 111	1 00	0 00
Austrocedrus	1 001110-0	2 0110100?0001000110	? 011011	1 1?0	? ??	? 03
Cal. muelleri	2 00101000	2 0000102100010000200	1 111111	2 110	1 11	1 01
Cal. decurrens	1 10121010	2 0100101?0010011110	0 011011	1 100	0 00	1 03
Cal. macrolepis	1 10121010	2 0100101?0000000110	? 011011	0 1??	? ??	? 03
Ch. lawsoniana	1 10111000	2 0100100100000001110	? 011011	2 100	? 00	1 00
Ch. nootkatensis	1 101210-0	2 0100101?1010001110	? 011011	2 1??	? ??	? 11
Ch. obtusa	1 10111000	2 0100100?0000001110	0 011011	2 100	0 00	1 00
Crypt. japonica	0 00121000	0 0001000000000001001(01)	011011	2 100	0 00	1 00
Cun. lanceolata	0 00100001	1 0010000000000001101	0 011010	2 100	0 00	1 00
Cup. arizonica	1 00101010	2 ?000102?1011000210	1 011011	2 10?	? ??	? 10
Cup. sempervirens	1 00101010	2 0000102110110002l0	1 011011	2 101	1 01	1 10
Diselma	1 00121010	2 0020100?0010001000	? 011011	1 1?0	? ??	? 03
Fitzroya	2 00121010	2 1000100?0011000100	1 011011(12)	1?0	1 ?1	1 01
Fokienia	1 10121000	2 0100100?0000001100	? 011011	1 1??	? ??	? 00
Glyptostrobus	0 01111000	(02)010001300000000101	0 011011	1 100	0 00	1 00
Jun. conferta	2 001?1010	1 0020102110110012l0	1 011011	0 100	0 00	1 13
Jun. drupacea	2 001?1010	1 0020102?1010001210	1 011011	0 1??	? ??	? 13
Jun. procera	1 00121010	2 0000102?1011001210	? 011011(01)	100	? 00	1 13
L. plumosa	1 10111000	2 0100100?0001000200	? 011011	0 1??	? ??	? 03
L. yateensis	1 10111000	2 0100100?0001000200	? 011011	0 1??	? ??	? 03
Metasequoia	1 01121001	1 0010013?0100101001	0 011010	2 100	? 00	1 00
Microbiota	1 10121010	2 0000101?001?0002?0	? 011011	0 1??	? ??	? 03
Neocallitropsis	3 00000000	0 0002200?0000010100	? 011011	2 1??	? ??	? 00
Papuacedrus	1 10101000	2 0100100?0001000110	? 011011	1 1??	? ??	? 03
Picea	0 001?1011	3 0001100000010000-00	0 000000	1 000	1 00	0 00
Pilgerodendron	1 00111000	2 1020100?0000000100	? 011011(01)	1??	? ??	? 03
Platycladus	1 10121010	(02)0100102?0010001210	0 011011(012)	100	0 00	1 02
Sequoia	0 0012100?	(12)000001300000000101	0 011110	2 111	2 00	1 00
Sequoiadendron	0 00111100	2 0000000?0000000101	0 001011	2 111	0 00	1 10
Taiw. cryptomerioides	0 00101001	2 0001000?0100011101	0 011011	1 101	0 00	1 00
Taxodium distichum	0 01121000	1 0000013000000000001(01)	011011	1 100	0 00	1 00
Tetraclinis	3 00101000	2 0100102?0011000210	1 011011(12)	110	0 00	1 03
Th. occidentalis	1 10121100	2 0100100?0010001110	0 011011(01)	1??	0 ??(01)	00
Th. standishii	1 10121100	2 0100100?0001001110	? 011011(01)	100	? 00	0 00
Th. plicata	1 10121100	2 0100100?0001000110	? 011011(01)	1??	? ??	? 00
Thujopsis	1 1012110?	2 0100100?0000001010	0 011011	2 100	0 00	1 12
Widdringtonia	(01)00101000	2 0000101100100000000	1 011111	2 110(01)	?1 1	02

appears to be a relatively poor indicator of affinity. Barred and trabeculate pitting on the transfusion tracheids [17/1, 17/2] are unique to Cupressaceae s.s. and are linked in a developmental sequence suggestive of a transformation series (Quinn and Gadek, 1988); their distribution in Fig. 5 provides some support for this hypothesis. The occurrence of the apomorphs of these last two characters mainly in the same taxa led Quinn (1989) to consider a possible relationship between some members of the cupressoid and callitroid clades. Constraint analyses revealed a single origin of this novel type of pitting, but with unrestricted reversal between barred and trabeculate again requires a minimum of 89 additional steps. Hence, there is strong support in the combined database for separate northern and southern origins of the apomorph in all three of these characters. Tropolone distribution [27/1] requires four evolutionary events on Fig. 5: either a single origin and three separate losses, or three origins and a single loss in *Fokienia*. Constraint analyses revealed that only one additional step was required to place *Austrocedrus* below *Pilgerodendron*, thus reducing the number of necessary events to three. A single origin without reversal requires at least 47 extra steps, so again there is considerable support for homoplasy in this character.

The numerous differences (29 in 1400 bp or 2.07%) detected between the original *rbcL* sequence for *Taxodium distichum* (Soltis, Soltis, and Smiley, 1992) and those we have determined raises a question about the accuracy of the estimate of divergence between the Miocene fossil sequence and extant *Taxodium* made in that paper. Given that the fossil and extant sequences were only found to diverge at 11 out of 1320 sites (0.83%), some caution clearly needs to be exercised regarding the accuracy of their estimate of the minimum rate of sequence divergence of $4.2–4.9 \times 10^{-4}$ substitutions per site per million years for the *rbcL* gene in that lineage. The observed divergence between our own sequence and that determined for the fossil is 26 out of 1320 sites (1.97%), or more than twice the previous estimate. Nevertheless, that comparison is based on the assumption that

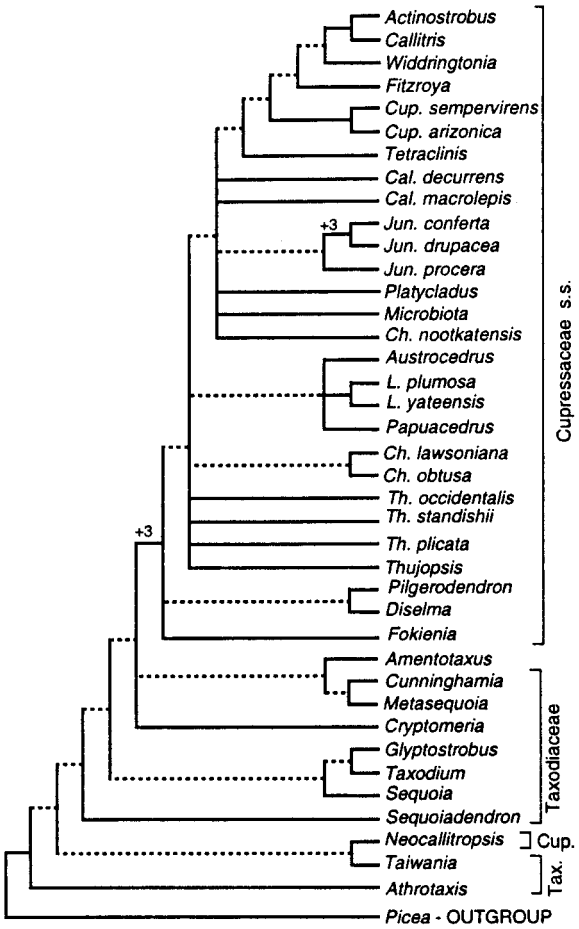

Fig. 4. Strict consensus of two islands totalling 120 equally parsimonious trees of 180 steps found from a heuristic search of the nonmolecular database; CI = 0.41; RI = 0.67; RC = 0.28. Dotted branches collapse at +1 step; continuous branches collapse at +2; decay values >2 are shown on branches.

the divergence is not affected by inaccuracies in the sequencing of the fossil DNA.

Systematic implications—The separation of Cupressaceae s.s. into the cupressoid and callitroid clades differs from the subfamilial distinction of Li (1953) only in the placement of *Tetraclinis* in the former. The placement of *Tetraclinis* with *Calocedrus, Microbiota,* and *Platycladus* in the sister clade to *Cupressus* and *Juniperus* is supported by the distinctive barred and trabeculate pitting [17/1, 17/2], and the derived biflavonoid patterns [21/1, 26/2] that characterize the taxa in subclades I and II. *Tetraclinis,* which occurs in north Africa and the Mediterranean, was the sole exception to a northern/southern hemisphere split in Li's arrangement, having been included in his otherwise totally southern Callitroideae. This basal dichotomy between the hemispheres is not surprising in such an ancient group, which evolved during a period of cooling and drying of the world's climate. Most of the taxa that are today found in lower latitudes (particularly species of *Juniperus, Cupressus,* and *Callitris*) are demonstrably derived in this phylogeny; a notable exception is *Papuacedrus,* which is an early-diverging lineage in the callitroid clade, but this taxon inhabits a much more mesic niche than the others. Florin (1963) expressed dissatisfaction with the placement of *Tetraclinis* in the ''southern'' subfamily, and de Laubenfels (1965) labeled Li's distinction between valvate and imbricate scales as ''nebulous'' pointing out the contradictory scoring of the character in several genera by different authors. *Pilgerodendron, Libocedrus* s.s., *Diselma,* and *Fitzroya* have all been scored as either imbricate (Buchholz, 1948; Janchen, 1950) or valvate (Li, 1953); *Cupressus* and *Chamaecyparis* are scored valvate (Buchholz, 1948) or imbricate (Li, 1953); all three authors agree that the scales are imbricate in *Calocedrus, Thuja,* and *Thujopsis.*

The paraphyly of *Chamaecyparis* finds considerable support: monophyly requires at least 38 extra steps (on 1601) in the *matK* plus nonmolecular database. *Chamaecyparis nootkatensis* is distinguished from other species of the genus in its wood [5/2, 9/1] and leaf anatomy [17/1], flavonoid pattern [21/1], and wood extractives [27/1] and was placed in *Cupressus* when first described (Lambert, 1824). Its placement within subclade I is supported by the presence of nootkatin-type tropolones in the heartwood, and indels *d* and *g* in *matK,* all unique synapomorphies for the subclade, and also by the ripening of the cones in the second year [44/1]. While the *rbcL* data placed *Chamaecyparis nootkatensis* in a polytomy with *Juniperus* and *Cupressus,* the *matK* data placed it inside *Cupressus* as the weakly supported sister group (71%, +1) of the New World clade. The former analysis was clearly affected by the lower sequence divergences and lower taxon density. On the basis of the combined *matK* and nonmolecular data, however, *Chamaecyparis nootkatensis* was placed outside the reduced *Cupressus* + *Juniperus* clade, although support for this position is only moderate (73%, +3). The morphological distinctiveness of this species, which has been responsible for the difficulty in placing it satisfactorily in a taxonomy, has again been influential here. Support for a separate genus is certainly lacking in the molecular data, and despite the differences in its morphology, it appears that *Chamaecyparis nootkatensis* should be returned to *Cupressus.* Hybrids are recorded to have arisen in cultivation between *Chamaecyparis nootkatensis* and *Cupressus lusitanica, C. arizonica* var. *arizonica* and *C. arizonica* var. *glabra* (Krüssmann, 1985), and this fact has been used by some authors to submerge the genera (e.g., Bartel, 1993). Since it is, however, the only species of *Chamaecyparis* to hybridize with *Cupressus,* the occurrence of these so-called intergeneric hybrids is in full accord with our conclusion that *Chamaecyparis nootkatensis* is in fact a member of the genus *Cupressus.* Another line of evidence that has often been cited as linking these two genera is the supposed similarity between *C. funebris* and *Chamaecyparis*: small cones and flattened branchlets (e.g., Bartel, 1993). This idea should have been thoroughly disposed of by our earlier study of leaf anatomy and biflavones (Gadek and Quinn, 1987) and the placement of the other species of *Chamaecyparis* in subclade III, which is so strongly separated from subclade I (Figs. 1 and 5), emphasizes the distinction that is to be made between the two generic concepts as redefined here.

Despite the separation of the American from the Asian

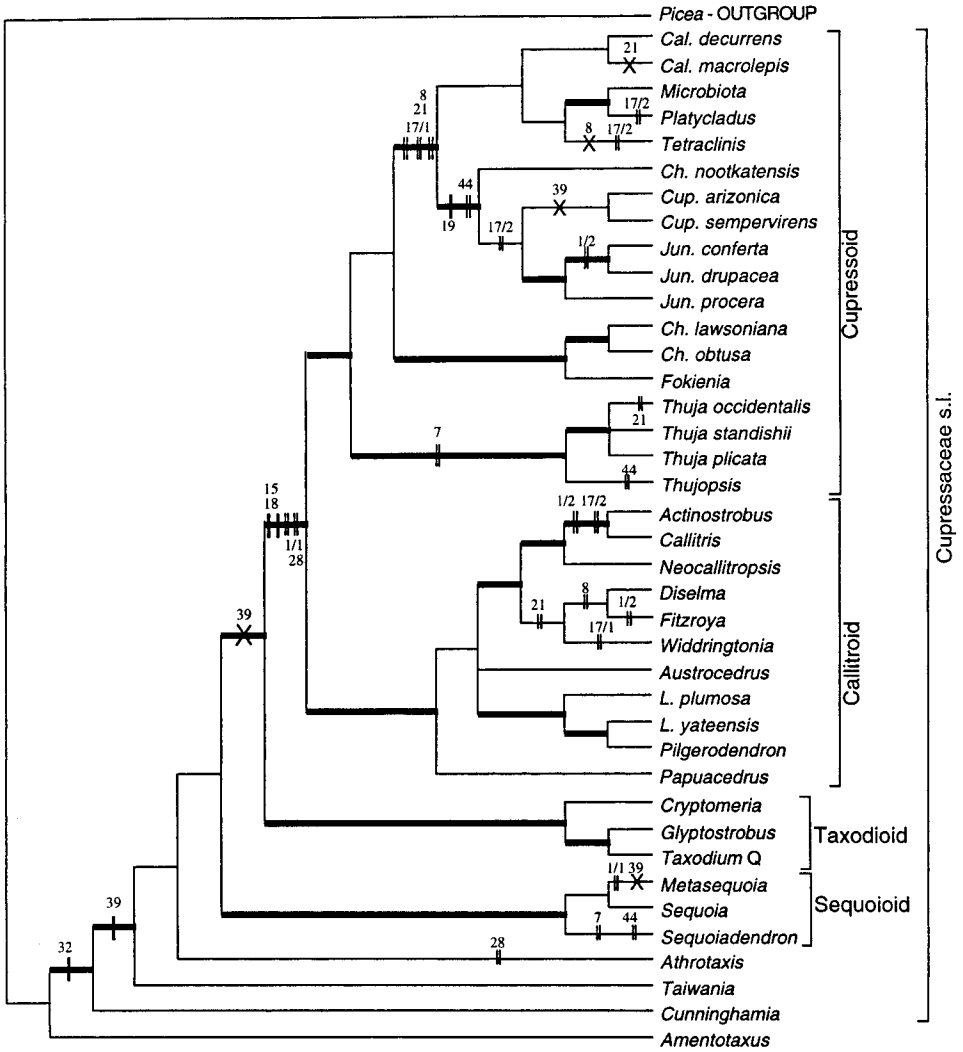

Fig. 5. Strict consensus of four equally parsimonious trees of 1601 steps found from a heuristic search of the combined *matK* plus nonmolecular data; CI = 0.51; RI = 0.72; RC = 0.45. Thick branches received >90% bootstrap support. Abbreviations are as in Fig. 1. State changes in selected nonmolecular characters are shown as: single line, unique synapomorphy; double line, parallelism; cross, reversal. Characters and states are numbered as per text.

species of *Calocedrus* in the nonmolecular analysis, the monophyly of the genus is strongly supported by the molecular data.

Neocallitropsis is a rather unusual member of Cupressaceae s.s., as evidenced by its position in the nonmolecular analysis. It is distinctive in its phyllotaxis, leaf flavonoids (Gadek and Quinn, 1985), and transfusion tissue (Gadek and Quinn, 1988). Its strong association with *Actinostrobus* and *Callitris*, which is hardly surprising given its geographical proximity to the Australian continent and the occurrence of *Callitris* spp. in New Caledonia, is highly novel.

Diselma has previously been linked to *Fitzroya* (Hart, 1987), with which it shares the heavy lignification of wood ray parenchyma and numerous small intraray pits that give the prominent nodules on the tangential walls seen in radial longitudinal section (Greguss, 1955). There is weak support (72%, +2) in the *matK* analysis for a sister relationship between the two, and somewhat stronger support (84%, +3) for the clustering of both with *Widdringtonia*. This constitutes one of the most striking Gondwanan patterns of relationship within the family. The other is the placement of *Pilgerodendron* within subclade VI, as close to *Libocedrus bidwillii* as to the other New Zealand species, *L. plumosa*. Given the dry and relatively heavy seeds in the group, this poses an interesting problem of how such a close relationship has been established across the South Pacific. All species are diploids (de Azkue, 1982). In order to confirm this relationship and to discount the possibility of hybridization and chloroplast capture, sequences for the nuclear-encoded ribosomal internal transcribed spacer region were assembled for a subset of taxa in the callitroid clade. Sequences for *Austrocedrus, Libocedrus bidwillii, L. plumosa, Papuacedrus, Pilgerodendron,* and *Widdringtonia* could be aligned with some confidence. Pairwise divergences between the aligned sequences calculated in PAUP showed *Pilgerodendron* to be less divergent from the *Libocedrus* species (2%) than any of the other taxa (8–10%). These preliminary data for a nuclear-encoded region are consis-

tent with the pattern of relationship determined on chloroplast-encoded sequences. Hence, there is no support in the sequence data for the distinction between *Pilgerodendron* and *Libocedrus* s.s. made by Florin (1930).

Two very strong clades have been identified within taxodiaceous taxa, neither of which was retrieved by Hart (1987). The sequoioid clade was identified by Brunsfeld et al. (1994) and is in agreement with cytological and immunological data (Schlarbaum and Tsuchiya, 1984; Price and Lowenstein, 1989). The well-supported relationship among *Taxodium, Cryptomeria,* and *Glyptostrobus* identified in these data was first suggested by Endlicher (1847) and was also recognized by Liu and Su (1983). This grouping and its placement as sister to the Cupressaceae s.s. clade agree well with the immunological data presented by Price and Lowenstein (1989). The remaining genera represent individual lineages that diverged early in the evolution of the family. There is strong molecular support (94%, +6; Fig. 3) for *Cunninghamia* being the first to separate, but the order of divergence of *Taiwania, Athrotaxis,* and the sequoioid clade, although resolved (cf. Brunsfeld et al., 1994), receives less support (71%, +2; 71%, +2).

Classification—On the basis of these analyses a more informative infrafamilial classification can be constructed. Seven subfamilies are recognized; these are listed in the order of the divergence of lineages in Fig. 5.

CUPRESSACEAE

Cunninghamioideae (Hayata) Quinn stat. nov.

Cunninghamiaceae Hayata, *Botanical Magazine (Tokyo)* 46: 26. 1932

Trees with adult leaves helically arranged, two-ranked, leathery, stiff, sharply acuminate; ovules more than 2 per scale, inverted; cotyledons 2.

Type: *Cunninghamia* R. Br.

Monogeneric.

Taiwanioideae (Hayata) Quinn stat. nov.

Taiwaniaceae Hayata, *Botanical Magazine (Tokyo)* 46: 26. 1932

Trees with adult leaves helically arranged, accumulating taiwaniaflavone; ovules 2 per scale, erect; cotyledons 2.

Type: *Taiwania* Hayata

Monogeneric.

Athrotaxidoideae Quinn subfam. nov.

Arbores; folia monomorpha in ramulis omnibus spiraliter disposita, amphistomatica; strobili solitarii, terminales; ovula 3–6, inversa; cotyledones duae.

Type: *Athrotaxis* D. Don

Monogeneric.

Sequoioideae (Luerss.) Quinn stat. nov.

Sequoiaceae Luerss., *Gründzuge der Botanik*: 265. 1877.

Metasequoiaceae H. H. Hu and W. C. Cheng, *Bulletin of the Fan Memorial Institute of Biology n.s.* 1(2): 154. 1948.

Trees with leaves opposite or helically arranged; ovules 2–12 per scale, erect or inverted; cotyledons 2–5.

Type: *Sequoia* Endl.

Other included genera: *Metasequoia, Sequoiadendron.*

Taxodioideae Endl. ex K. Koch, *Dendrologie* 2(2): 186. 1873.

Limnopityaceae Hayata, *Botanical Magazine (Tokyo)* 46: 25. 1932.

Cryptomeriaceae Hayata, *Botanical Magazine (Tokyo)* 46: 26. 1932.

Trees with adult leaves helically arranged, not accumulating taiwaniaflavone; ovules erect.

Type: *Taxodium* Rich.

Other included genera: *Cryptomeria, Glyptostrobus.*

Callitroideae Saxton, *New Phytologist* 12: 253. 1913.

Trees with adult phyllotaxis opposite or whorled; mostly with adult leaves reduced to appressed scales; with southern hemisphere distribution.

Type: *Callitris* Vent.

Other included genera: *Austrocedrus, Callitris, Diselma, Fitzroya, Libocedrus* (including *Pilgerodendron*), *Neocallitropsis, Papuacedrus, Widdringtonia.*

Cupressoideae Rich. ex Sweet, *Hortus Britanica*: 372. 1826.

Trees with adult phyllotaxis opposite or whorled; mostly with adult leaves reduced to appressed scales; mainly distributed in the northern hemisphere.

Type: *Cupressus* L.

Other included genera: *Calocedrus, Chamaecyparis, Fokienia, Juniperus, Microbiota, Platycladus, Tetraclinis, Thuja, Thujopsis.*

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