American Journal of Botany 91(11): 1872–1881. 2004.

THE CIRCUMSCRIPTION AND PHYLOGENETIC RELATIONSHIPS OF *CALLITROPSIS* AND THE NEWLY DESCRIBED GENUS *XANTHOCYPARIS* (CUPRESSACEAE)[1]

DAMON P. LITTLE,[2,6] ANDREA E. SCHWARZBACH,[3] ROBERT P. ADAMS,[4] AND CHANG-FU HSIEH[5]

[2]L. H. Bailey Hortorium, 228 Plant Science Building, Cornell University, Ithaca, New York 14853 USA; [3]Department of Biological Sciences, Kent State University, 256 Cunningham Hall, Kent, Ohio 44242 USA; [4]Biology Department, Baylor University, P.O. Box 97388, Waco, Texas 76798 USA; and [5]Department of Botany, National Taiwan University, Taipei 106, Taiwan

A new species of conifer was recently discovered in northern Vietnam. In a preliminary phylogenetic analysis of morphological data a possible sister species, *Chamaecyparis nootkatensis* (D. Don) Spach, was identified; however, because of the presumed phylogenetic remoteness of these two species to the remainder of the Cupressaceae, a new genus—*Xanthocyparis*—was described to accommodate both species. Here an analysis of ITS (nrDNA), *matK*, and *rbcL* sequence data in combination with 58 informative morphological characters was aimed at testing the monophyly of the remainder of *Chamaecyparis* and evaluating the placement and monophyly of *Xanthocyparis*. *Chamaecyparis*, minus *C. nootkatensis*, was resolved as a monophyletic group, remote from *Cupressus* and *Xanthocyparis*. *Cupressus*, *Juniperus*, and *Xanthocyparis* formed a very highly supported monophyletic group. However, *Cupressus* was not monophyletic. Instead the Old World species sampled were resolved sister to a clade containing a monophyletic *Juniperus*, a monophyletic *Xanthocyparis*, and a clade of New World *Cupressus* species. If both species of *Xanthocyparis* are to be treated as members of the same genus, then due to the principal of priority they will have to be recognized in the genus *Callitropsis*. Research is continuing to resolve the status of New World and Old World *Cupressus*.

Key words: *Callitropsis*; *Chamaecyparis*; Cupressaceae; Cupressoideae; *Cupressus*; phylogeny; taxonomy; *Xanthocyparis*.

Recently, a new species of conifer was found among the remnants of moist karst forest in northern Vietnam (Averyanov et al., 2002; Farjon et al., 2002). The morphological features of this new conifer strongly suggested affinity to Cupressaceae—in particular to the subfamily Cupressoideae—but the placement of this species within the existing genera of Cupressaceae proved to be problematic. As a result, a new genus and species were described. *Xanthocyparis vietnamensis* is characterized as having the following:

1. Dimorphic leaves: This feature is very common among members of the Cupressoideae absent only from *Juniperus*, *Microbiota decussata*, and some species of *Cupressus*.
2. Small ovulate cones consisting of either two or three whorls of opposite decussate cone scales: This configuration is also characteristic of *C. benthamii*, *C. nootkatensis*, and *M. decussata*. Additionally, several species in Cupressoideae consistently produce either two or three whorls of opposite decussate cone scales, but unlike *X. vietnamensis*, these species are not polymorphic.
3. Biennial seed maturation: Two-year seed maturation can also be found in all species of *Cupressus* and some species of *Juniperus*.
4. Flattened, winged seeds: With the exception of *Juniperus*, *M. decussata*, and *Platycladus orientalis*, this state occurs in all species of Cupressoideae.
5. All three leaf developmental phases (juvenile, transition, and adult; see de Laubenfels, 1953) are regularly found on branches of mature trees. This trait is uncommon within Cupressoideae, but does characteristically occur in some species (e.g., *Juniperus chinensis* L.). In addition, individuals belonging to species characterized as having the standard developmental sequence can, on occasion, display this trait when mechanically damaged. However, the regular appearance of all three leaf types in *X. vietnamensis* appears to be an unusual characteristic.

Farjon et al. (2002) analyzed 54 morphological characters that placed *X. vietnamensis* sister to *C. nootkatensis* within a paraphyletic Cupressoideae. The results of this analysis prompted the nomenclatural transfer of *C. nootkatensis* to *Xanthocyparis* as well as the renaming of three intergeneric hybrids. Xiang and Farjon (2003) provided a phenetic analysis of epidermal characters that placed *X. vietnamensis* in a cluster with *Chamaecyparis formosensis* and *C. obtusa* to the exclusion of *Cupressus nootkatensis*. Analysis of this data matrix with parsimony resulted in a completely unresolved consensus tree (D. P. Little, unpublished data).

Ironically, *C. nootkatensis* itself has had a troubled taxonomic history within Cupressoideae, having been placed in four different genera: *Cupressus*, *Chamaecyparis*, *Callitropsis*, and *Xanthocyparis*. The species was first described as a *Cupressus* by Don in 1824. Upon the description of *Chamaecyparis* (as a segregate of *Cupressus*) by Spach in 1842 (p. 331), *Cupressus nootkatensis* was transferred to *Chamaecyparis*. Prior to the middle of the 20th century, most works treated *Chamaecyparis* as an infrageneric taxon within *Cupressus* or as a synonym of *Cupressus*—probably because the only mor-

[1] Manuscript received 27 January 2004; revision accepted 8 July 2004.

The authors wish to thank J. J. Doyle, M. A. Luckow, K. C. Nixon, and two anonymous reviewers for their constructive comments on earlier drafts of this manuscript. B. Øllgaard kindly translated a passage from Örsted. N. Franz generously translated several German works. Thanks to M. H. Alford, J. Bartel, D. K. Harder, T. Hudson, and K. Rushforth for providing plant materials for this project. Funding from NSF grant DEB 0206092 (to D. P. L.), NSF grant DEB 0316685 (to A. E. S. and R. P. A.), the American Society of Plant Taxonomists (to D. P. L.) and the Clausen Fund (to D. P. L.; a gift to BH by T. J. Cohn) is gratefully acknowledged.

[6] E-mail: dpl10@cornell.edu.

phological characters available at the time to distinguish somewhat reliably between the genera were ovulate cone size and branching pattern.

Because of the somewhat unusual ovulate cone configuration of *C. nootkatensis*, the monotypic genus *Callitropsis* was created by Örsted in 1865 (''1864'') and promptly sank into obscurity. Örsted hypothesized that *Callitropsis* was most closely related to *Callitris* and *Fitzroya*, a hypothesis not borne out by recent analyses (Gadek et al., 2000; Farjon et al., 2002).

As anatomical, embryological, and chemical data accumulated, it became clear that *Chamaecyparis nootkatensis* (D. Don) Spach was an extraordinary member of *Chamaecyparis*, differing in the duration of seed maturation (Camus, 1914), seed wing anatomy (Camus, 1914), wood anatomy (Greguss, 1955), wood secondary chemistry (reviewed in Erdtman and Norin, 1966), fertilization (Owens and Molder, 1975), cuticular micromorphology (Alvin et al., 1980; Oladele, 1983a), leaf transfusion tracheid pitting (Gadek et al., 2000 contra Prause, 1909), and low cross-compatibility of microsatellite primers (Bérubé et al., 2003). In addition, *C. nootkatensis* was found to form spontaneous hybrids when grown in cultivation with any of several *Cupressus* species (Jackson and Dallimore, 1926; Mitchell, 1970), while at the same time, no hybrids between *Chamaecyparis nootkatensis* and other *Chamaecyparis* species have been reported. Of these hybrids, ×*Cupressocyparis leylandii* (A. B. Jacks. & Dallim.) Dallim. is the most widely cultivated. It has been formed spontaneously multiple times with both *Cupressus macrocarpa* and *Chamaecyparis nootkatensis* acting as the maternal parent. Notably, fertile offspring are produced by F_1 ×*Cupressocyparis leylandii* (Jackson and Dallimore, 1926).

The recognition that *Chamaecyparis nootkatensis* would best be accommodated within *Cupressus*, rather than in *Chamaecyparis*, came with the realization that the similarities in gross morphology between *Cupressus* and *Chamaecyparis* were largely from a combination of plesiomorphic characters and parallelism rather than common ancestry (Gadek et al., 2000). Interestingly, the notion that *Cupressus* and *Chamaecyparis* were associated on the basis of primitive, rather than derived, features was first put forth by Masters (1895, p. 313) and later reiterated by Li (1953).

Recent analyses of chloroplast DNA sequences and morphological data suggest that *Cupressus* and *Chamaecyparis* are rather distantly related within the Cupressoideae and that *Chamaecyparis* as commonly circumscribed (including *C. nootkatensis*) was not monophyletic (Gadek et al., 2000). Previous phylogenetic studies of the Cupressaceae (Hart, 1987; Gadek and Quinn, 1993; Brunsfeld et al., 1994) had failed to discover this because either composite terminals were constructed (Hart, 1987) or *C. nootkatensis* was not sampled (Gadek and Quinn, 1993; Brunsfeld et al., 1994).

Cupressus and *Chamaecyparis* were placed by Li (1953) in the subfamily Cupressoideae (Appendix 1; see Supplemental Data accompanying the online version of this article). In the cladograms presented by Hart (1987) and Farjon et al. (2002), Cupressoideae sensu Li is paraphyletic. However, reanalysis of the matrix of Hart (1987) without compartmentalization or synthetic outgroups results in a monophyletic Cupressoideae sensu Li (D. P. Little, unpublished data). Recent family-level molecular analyses (Gadek and Quinn, 1993; Brunsfeld et al., 1994; Gadek et al., 2000) also support a monophyletic Cupressoideae, but indicate that the monotypic genus *Tetraclinis*, which was placed in Callitroideae by Li (and Hart, 1987), should be included within Cupressoideae sensu Li.

Li (1953) arranged the genera of Cupressoideae in an almost linear series (there is a single branching point; plate II in Li, 1953) featuring reduction in both the number of cone scales and the number of seeds. *Cupressus* was considered the most primitive and *Juniperus* the most derived. This tribal classification (Appendix 1; see Supplemental Data accompanying the online version of this article) was largely overturned by the results of Gadek et al. (2000), but no revised classification has been put forth.

This study was designed to (1) fully test the monophyly of *Chamaecyparis* by sampling all *Chamaecyparis* species, (2) evaluate the placement of *Xanthocyparis* within the Cupressoideae, and (3) test the monophyly of *Xanthocyparis* as circumscribed by Farjon et al. (2002).

METHODS AND MATERIALS

Taxon sampling—The sampling scheme for the Cupressoideae used by Gadek et al. (2000) was replicated with four changes: (1) *Calocedrus macrolepis* var. *formosana* was used in the current study in place of *C. macrolepis* Kurz. var. *macrolepis*; (2) *Cupressus arizonica* var. *arizonica* was used in place of *C. arizonica* var. *glabra* (Sudw.) Little; (3) *C. lusitanica* var. *lusitanica* was used in place of *C. lusitanica* var. *benthamii* (Endl.) Carrière; and (4) *Thuja standishii* (Gordon) Carrière was omitted. In addition, seven taxa—*Chamaecyparis formosensis*, *C. obtusa* var. *formosana*, *C. pisifera*, *C. thyoides* var. *henryae*, *C. thyoides* var. *thyoides*, *Cupressus macrocarpa*, and *Xanthocyparis vietnamensis*—were added to the sample. Complete sampling and voucher information can be found in Appendix 1 (see Supplemental Data accompanying the online version of this article).

Molecular techniques—DNA was isolated from 0.015–0.025 g of silica dried tissue using the DNeasy Plant Mini kit (Qiagen, Valencia, California, USA) according to the supplier's instructions. The polymerase chain reaction (PCR) was used to amplify ITS (nrDNA), *matK* (cpDNA), and *rbcL* (cpDNA).

The ITS was amplified in a volume of 50 μL in PCR buffer (at the concentration recommended by the supplier; Promega, Madison, Wisconsin, USA) with 0.23 μmol/mL of each amplification primer (5′ GGAAGGAGAAGTCGTAACAAGG 3′; 5′ CTTTTCCTCCGCTTATTGATATG 3′), 1.5 units *Taq* polymerase, 2 mmol/L $MgCl_2$, 0.4 mmol/L dNTPs, and ca. 20–50 ng genomic DNA. The reaction mixture was incubated for 60 s at 94°C and then cycled 38 times (60 s at 94°C, 60 s at 50°C, 60 s at 72°C) with a final step of 300 s at 72°C.

A 100-μL PCR reaction was used to amplify *matK* in modified Pääbo (1990) buffer (67 mmol/L tris-HCl pH 8.8, 4 μg/mL [m/v] bovine serum albumin, 0.2 mmol/L dNTPs) with 2 μmol/mL of each amplification primer (Table 2 in Kusumi et al., 2000), 0.25 units *Taq* polymerase, 1.5 mmol/L $MgCl_2$, and ca. 25 ng genomic DNA. The reaction mixture was incubated for 180 s at 95°C and then cycled 35 times (30 s at 95°C, 30 s at 55°C, 120 s at 72°C).

The PCR amplification of *rbcL* was similar to that of *matK* except different primers were used (5′ ATGTCACCACAAACAGAAACTAAAGCAAGT 3′; 5′ TCACAAGCAGCAGCTAGTTCAGGACTC 3′), the concentration of primers was reduced to 0.7 μmol/mL, and the $MgCl_2$ concentration was increased to 2.5 mmol/L.

Unincorporated dNTPs and primers were removed from the PCR products with the QIAquick PCR Purification kit (Qiagen) following the manufacturer's instructions. The ITS sequencing reactions were performed in a 10-μL volume: 2 μL BigDye Terminator version 3.1 sequencing solution (Applied Biosystems, Foster City, California, USA), 2 μL Half Term dye terminator (Genpak, St. James, New York, USA), 3 μL (ca. 45 ng) PCR product, 1 μL primer (0.29 μmols/mL; 5′ TCGCATTTCGCTACATTCTTC 3′; 5′ TGTGTTGGG TGTTGACACATC 3′; 5′ GAAGAATGTAGCGAAATGCGA 3′), and 2 μL of water. The cycling program was 25 cycles of 30 s at 96°C, 15 s at 50°C,

240 s at 60°C. Sequencing of *matK* (primers from Table 2 in Kusumi et al., 2000) and *rbcL* (5′ TCGCATGTACCTGCAGTAGC 3′; 5′ GCGTTGGAGAGAYCGTTTCT 3′) followed the recommendations accompanying the BigDye Terminator cycle sequencing kit. Sequencing reactions were run on an ABI 3700 sequencer (Applied Biosystems).

Sequence alignment and manipulation—Sequences were assembled using Sequencher 4.1 (Gene Codes, Ann Arbor, Michigan, USA). Sequences were aligned with either ClustalX 1.81 (Thompson et al., 1997) or Sequencher and then adjusted manually. Inferred insertion/deletion events (indel) in ITS and *matK* were coded using the ''simple gap coding'' method of Simmons and Ochoterena (2000) as implemented in GapCoder (Young and Healy, 2003). The *rbcL* sequence alignment did not imply any indel events, so no gap coding was necessary. Sequence alignments can be found in Appendices 2–4 (see Supplemental Data accompanying the online version of this article).

The published *matK* sequences, deposited in GenBank by Gadek et al. (2000), were edited in the following manner: (1) bases archived as ''N'' were changed to indels (''−'') if the position(s) corresponded to one reported as an indel in the original publication (Table 4 in Gadek et al., 2000), and (2) three bases archived as ''NNN'' that were not reported as indels (Table 4 in Gadek et al., 2000) in the sequences of *Thuja plicata* and *Fokienia hodginsii* were deleted to allow the sequences to properly align to the remaining sequences and not count as a potentially synapomorphic indel.

The fuse taxon option of WinClada 0.9.99.88 (Nixon, 2003) was used to merge (i.e., create the mathematical union) sequences into single terminals representing individual taxa when multiple accessions were used (Appendix 1; see Supplemental Data accompanying the online version of this article). In all cases the individual sequences that were later fused were either sister or paraphyletic to each another in preliminary analyses.

Morphological data—The morphological matrix used in this study (Appendix 5; see Supplemental Data accompanying the online version of this article) was based in part on that of Gadek et al. (2000) which was in turn based on the matrix of Hart (1987). In this case, the terminals used are species or varieties rather than genera (cf. Hart, 1987) or a mixture of genera and species (cf. Gadek et al., 2000). The morphology of *Xanthocyparis vietnamensis* was scored from Farjon et al. (2002) and Xiang and Farjon (2003). Characters are nonadditive (unordered) unless otherwise noted.

Character 0: growth form—This character reflects the natural growth form of the plant excluding environmental effects: (0) no dominant central stem, specimens either sprawling and prostrate or a caespitose shrub; (1) single stemmed (monopodial) tree.

Characters 1–5—Conifers generally have three distinct ontogenetic phases—seedling, sapling, and adult—that are usually correlated with the occurrence of different phyllotaxy and leaf types (de Laubenfels, 1953). Because branching pattern is in part dependent upon phyllotaxy, characteristics of branching pattern were coded only for those species that produce the adult (scale-like) leaf type (see characters 7 and 8). In Cupressoideae, adult leaves are arranged in an opposite or whorled manner.

Character 1: arrangement of ultimate branchlets at the node—The ultimate branch segments (i.e., branch segments that do not bear any branches) are arranged on the stem such that either (0) one, or occasionally two, ultimate segments occur at a given node or (1) never more than one ultimate segment occurs at a given node.

Character 2: arrangement of ultimate branchlets—Ultimate branchlets can be arranged on the stem such that (0) the segments more or less alternate on the stem or (1) the vast majority of the segments occur on one side of the stem (usually directed towards the apex of the next highest branching order).

Character 3: arrangement of the ultimate segments—During any one season of growth, the ultimate branch segments can be arranged either (0) all on one plane or (1) on two planes. In some species (e.g., *Cupressus macnabiana*), the branching plane occasionally shifts (by 90°) between growing seasons.

Character 4: arrangement of the penultimate segments—Like the ultimate segments, the penultimate branch segments can be arranged either (0) all on one plane or (1) on two planes.

Character 5: arrangement of the antepenultimate segments—(0) All on one plane or (1) on two or more planes.

Character 6: number of cotyledons (additive)—Very rare (<5%) counts were considered anomalies and therefore excluded from consideration. This character was scored from new observations, Camus (1914), Butts and Buchholz (1940), Wolf (1948), and Li (1975).

Character 7: needle-like leaves—In Cupressaceae, the transition from seedling to sapling is usually marked by a change in leaf type, but the transition from sapling to adult—as measured by organism size and reproductive ability—is not marked in many species. The number of steps in this ontogenetic series that are realized in each species is recorded in two characters: the retention of needle-like (character 7) leaves in reproductively mature adults (the result of skipping the final two steps of the series); and the occurrence of a distinctive ''transitional'' (i.e., sapling) leaf type (character 8).

Needle-like leaves (0) produced only in young individuals (not reproductively mature) or (1) produced as the only leaf type in reproductively mature individuals.

Character 8: transition leaf type—(0) Not produced or scale-like, indistinguishable from mature leaves or (1) lanceolate, different from the mature leaf type.

Characters 9–20—These characters refer to adult type (scale-like) leaves. Species that do not produce adult scale-like leaves were scored as inapplicable.

Character 9: mature phyllotaxy—(2) Opposite decussate or (4) in whorls of four.

Character 10: internode length—(1) Of approximately uniform lengths or (2) of two different lengths (alternating long and short).

Character 11: leaves born on adjacent nodes—(0) Leaves of two different sizes, orientated such that the apices of the leaves at the given nodes are concurrent, or (1) leaves, of one or two sizes, that do not have concurrent apices.

Character 12: externally dimorphic mature leaves—Leaves were considered dimorphic if alternating whorls differed significantly in size and/or shape. The occurrence of resin glands was not considered, nor were differences in leaf shape caused by bending around a nonradially symmetrical stem (note: *Chamaecyparis lawsoniana* and *X. nootkatensis* have externally dimorphic leaves, but the dimorphism is extremely subtle). Leaves (0) monomorphic or (1) dimorphic.

Character 13: symmetry of the lamina leaves around the midrib—(0) Symmetric or (1) asymmetric. In species with dimorphic leaves, the lateral leaves were scored.

Character 14: leaf transfusion tracheid pitting type (additive)—The pitting of the leaf transfusion tissue can be either (0) simple, circular bordered pits; (1) slightly thickened with small, non-vermiform bars; or (2) vermiform thickenings (i.e., trabeculate). This character was scored from new observations, Prause (1909), Camus (1914), Gadek and Quinn (1988), and Gadek et al. (2000).

Character 15: anastomosis of vermiform thickenings—Generally, the large vermiform pits on the leaf transfusion tracheids do not fuse to one another

inside the tracheids (0), but in some cases, the pits are large enough to fuse to one another (1). This character was coded from original observations and Gadek and Quinn (1988).

Character 16: leaf epicuticular wax—(0) Straight tubules or (1) curly tubules. This character was scored from Wilhelmi and Barthlott (1997).

Character 17: leaf cuticular crystals—Calcium oxalate crystal tubercles in the adaxial leaf cuticle (0) absent or (1) present. Coded from data presented by Oladele (1983a) and Xiang and Farjon (2003).

Character 18: stomatal papillae on leaf adaxial surface—(0) absent or (1) present. Scored from Oladele (1983b) and Xiang and Farjon (2003).

Character 19: stomatal papillae on abaxial leaf surface—(0) absent or (1) present. Recorded from data presented by Oladele (1983b).

Character 20: florin ring—The (0) presence or (1) absence of interruptions in the Florin ring was coded from Oladele (1983b) and Xiang and Farjon (2003).

Characters 21–32—Characteristics of *Microbiota decussata* were gleaned from Jagel and Stützel (2001).

Character 21: ovulate cone scale shape—The shape of the most proximal fully developed ovulate cone scale can be either (0) apically flattened (length $\leq$ width) or (1) elongate (length $\gg$ width).

Character 22: ovulate cone scale phyllotaxy—(2) Whorls of two or multiples of two or (3) whorls of 3.

Character 23: ovulate cone scale whorls (additive)—The number of fully developed whorls of ovulate cone scales.

Character 24: ovulate cone retention—Ovulate cones are either (0) abscised upon maturity (deciduous) or (1) remain attached to the tree for an extended period (persistent). Abscission of mature cones was scored from field observations where possible. In some instances, it had to be inferred from the presence of large quantities of secondary growth in the cone pedicle.

Character 25: seed cones—Ovulate cones (0) wither and open upon seed maturation or (1) remain alive and closed after seed maturation (serotinous).

Character 26: resin secretion—Active resin secretion on the outer surface of the ovulate cone scales (0) absent or (1) present.

Character 27: seeds per scale—The maximum number of seeds per ovuliferous scale was recorded as (0) more than one or (1) only one.

Character 28: fertile ovulate cone scales—(0) More than one per cone or (1) one per cone.

Character 29: terminal ovulate cone scales—The ultimate whorl of ovulate cone scales (1) may or (0) may not bear seeds. This character was scored as inapplicable for taxa with only one whorl of cone scales.

Character 30: edges of ovulate cone scales—After pollination but before seed dispersal the ovulate cone is sealed either by (0) the confluence of elongate cells that interlock when inflated or (1) the irreversible fusion to the edges of neighboring scales.

Character 31: interlocking cells—The location of the elongate inflated cells on the fertile scales was recorded as either (0) at edge of the ovulate cone scale or (1) on the outer face of ovulate cone scales. This character provides an unambiguous way to distinguish between the concept of ''valvate'' and ''imbricate'' ovulate cones put forth by Li (1953)—a concept that has been criticized as being poorly defined by de Laubenfels (1965) and Gadek et al. (2000) among others. In several cases, taxa are assigned states that differ from those implied by Li (1953).

Character 32: seed maturation—Number of years (growing seasons) required for seeds to mature.

Character 33: seed shape—The cross-sectional seed shape was recorded as either (0) round or (1) flattened.

Character 34: seed symmetry—The symmetry of the seed along the transverse plane was scored as either (0) symmetric or (1) asymmetric.

Character 35: seed uniformity—Mature seeds can be described as either (0) more or less uniform in shape or (1) irregular.

Character 36: seed wings—Lateral seed wings were scored as (0) absent or (1) present. Highly reduced (but still observable) wings in some species (e.g., *Cupressus pigmaea*) were recorded as present, even though these species are frequently described as lacking seed wings.

Character 37: seed wing symmetry—The relative size and shape of the seed wings was recoded as either (0) equal or (1) unequal.

Character 38: seed coat—Resin pustules in the seed coat (0) absent or (1) present.

Character 39: pollen at pollination—At the time of pollination, pollen can be either (0) binucleate (occasionally multinucleate) or (1) uninucleate. This character was scored from Doak (1937), Owens and Molder (1975), Gadek et al. (2000), and the literature reviewed in Mehra and Malhotra (1947).

Characters 40–49—Characteristics of stem wood anatomy were obtained from Peirce (1937), Bannan (1941, 1944), Kaeiser (1953), Greguss (1955), and Crespo (1976).

Character 40: rays—Rays can be either (0) strictly uniseriate or (1) a mixture of uniseriate and occasionally partially biseriate or even multiseriate.

Character 41: ray cell walls—The tangential (end) walls of the ray cells can be either (0) smooth or (1) nodular.

Character 42: indentures—Indentations at the transverse/tangential wall union in the walls of the ray parenchyma are either (0) absent or (1) present.

Character 43: ray composition—Rays composed of (0) tracheids and parenchyma or (1) some, but not all, rays composed of tracheids only.

Character 44: xylem parenchyma—The transverse (end) walls of the xylem parenchyma can be (0) smooth or (1) nodular.

Character 45: cross field pits—(0) distinctly bordered or (1) borderless.

Characters 46–56—Tropolone characters (46: tropolone backbone, 47: α-thujaplicin, 48: α-thujaplicinol, 49: α-dolabrinol, 50: pygmaein, 51: β-thujaplicin, 52: β-dolabrin, 53: β-thujaplicinol, 54: nootkatin, 55: nootkatinol, and 56: γ-thujaplicin) were coded from data summarized in Zavarin and Anderson (1956), Zavarin et al. (1959), Hegnauer (1962), Enzell and Krolikowska (1963), Erdtman and Norin (1966), and Zavarin et al. (1967).

Because the actual biosynthetic pathway for tropolones in Cupressaceae is largely unknown (Fujita et al., 2000), a character state tree (Fig. 1) was constructed in such a way as to minimize the number of inferred steps in the biochemical pathway. See Barkman (2001) for an explanation as to why it is desirable to use a character state tree. With few exceptions, the binary decomposition of the character state tree was equivalent to simply coding each of the compounds as (0) absent or (1) present.

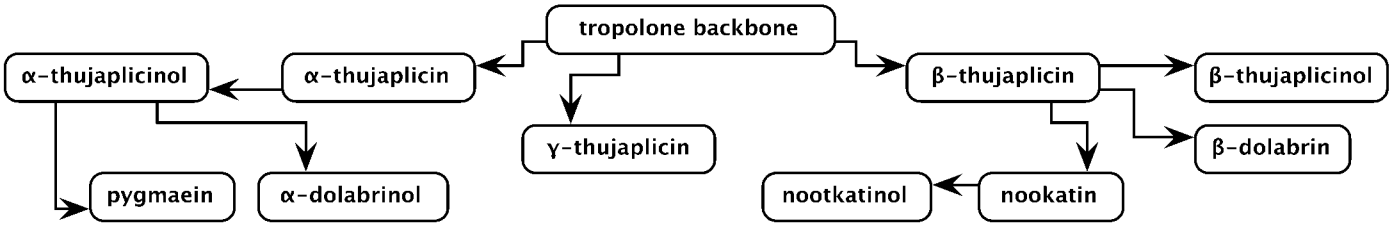

Fig. 1. Assumed biosynthetic pathway used to code tropolone characters (46–56). See Erdtman and Norin (1966) for chemical structures.

Cupressus goveniana and *C. pigmaea* were scored as ''?'' for α-thujaplicin because, given the assumed biosynthetic pathway, it would have to be produced as a precursor to α-thujaplicinol despite the fact that α-thujaplicin was reported to be absent from these taxa—compare Zavarin and Anderson (1956) to Zavarin et al. (1967). For similar reasons, *X. nootkatensis* was scored as ''?'' for β-thujaplicin (see Erdtman and Norin, 1966).

Characters 57 and 58—Biflavones from leaf tissue (57, cupressuflavone; 58, robustaflavone) were scored as (0) absent or (1) present from the data presented in Erdtman and Norin (1966), Natarajan et al. (1970), Pelter et al. (1970), Gadek and Quinn (1983, 1985), Qasim et al. (1985), John et al. (1989), and Gadek et al. (2000). Although Gadek and Quinn (1985) reported the presence of cupressuflavone in *Platycladus orientalis*, it was not detected by Natarajan et al. (1970), Pelter et al. (1970), and John et al. (1989). Therefore, *Platycladus orientalis* was scored as polymorphic.

Because there are only two potentially cladistically informative biflavones, a character state tree could not be constructed for these characters.

Phylogenetic analysis—All analyses were conducted with parsimony uninformative characters removed. Individual matrices were combined using WinClada. NONA 2.0 (Goloboff, 1993) was used to conduct ''heuristic'' searches of the individual and combined data sets: all sequence characters were treated as non-additive (unordered). Indels were treated as missing data. All characters were equally weighted. Ambiguously supported nodes were collapsed (''amb-''). Up to 20 trees per random addition replicate were held (''h/20''). One thousand random addition sequence replicates, using simple pruning regrafting (SPR) followed by tree bisection-reconnection (TBR) swapping (''rs0; mult*1000''), were conducted for each data set.

WinClada was used to generate a bootstrap (Felsenstein, 1985) procedure file. Each resampled matrix was searched with NONA using 100 random addition replicates, up to 20 trees per replicate were held, using SPR and TBR swapping (''rs0; amb-; h/20; mult*100;''). The strict consensus of the shortest trees, found for each iteration, was saved. The frequency of each clade was mapped onto the strict consensus tree using WinClada.

Partial and full constraints were utilized to evaluate the strength of unexpected groupings. Constraints were implemented with weighted group membership variables (Farris, 1974)—full constraints coded all taxa as either ''0'' or ''1,'' while partial constraints coded taxa as either ''0,'' ''1,'' or ''?.'' Constrained matrices were analyzed as described earlier. The statistical significance of differences in tree topology between the unconstrained and constrained analyses were tested with the Wilcoxon (1945) signed rank test as described by Templeton (1983). Critical values for the test were obtained from Zar (1984, p. 563).

All trees were rooted between the *Thuja-Thujopsis* clade and the remaining Cupressoideae as suggested by the results of Gadek et al. (Figs. 1, 3, and 5 in 2000).

RESULTS

Matrix properties—Among the three sources of DNA sequence data, ITS manifested the greatest quantity in both percentage and absolute terms of potentially parsimony informative variation (41.0%), while *rbcL* produced the least (3.7%; Table 1). In addition, the ITS alignment produced the greatest number of indel characters—more than half of which were not potentially parsimony informative. Interestingly, of the molecular data sets, trees derived from ITS sequence had the highest resolution coupled with the lowest consistency and retention indices (CI, RI; Table 1).

The partition of homoplasy between indel and sequence characters varied greatly between ITS and *matK*. For ITS, the 120 potentially informative indel characters had a lower CI (0.50) than the 522 potentially informative sequence characters (0.63), whereas the 10 potentially informative *matK* indel characters had a higher CI (0.90) than the 141 potentially informative sequence characters (0.79).

Differences among data sets—The individual data sets differed greatly in the amount of resolution (Table 1). The ITS data set differed from the cpDNA data set in the following ways (Fig. 2): (1) The placement of *Fokienia hodginsii* varied. The ITS matrix embedded *F. hodginsii* within *Chamaecyparis*, while the cpDNA data set placed *F. hodginsii* unresolved in relation to *Chamaecyparis*. (2) The relationships of *Tetraclinis articulata* differed. The ITS data set resolved *T. articulata* sister to a clade containing *Calocedrus*, *Cupressus*, *Juniperus*, *Microbiota*, *Platycladus*, and *Xanthocyparis*, whereas the chloroplast matrix put *T. articulata* within a clade containing *Calocedrus*, *Microbiota*, and *Platycladus*. (3) The topology within the New World *Cupressus* clade changed. Although the monophyly of New World *Cupressus* species was not impugned by any of the data sets, the topology within this clade differed markedly between the different data sets. This is reflected in the low bootstrap frequencies for the combined data set (53–79%) within the clade.

The morphological data set differed in many ways from both the ITS and cpDNA data set as well as from the combined molecular data set. Notably, *Juniperus* was very remote from *Cupressus*. In addition, *Chamaecyparis* was paraphyletic to *Cupressus*, *Microbiota*, and *Xanthocyparis*.

Simultaneous analysis—The combined data produced a single most parsimonious tree, 2069 steps long (Fig. 2; Table 1).

In the combined analysis, *Chamaecyparis* was resolved, albeit with low bootstrap support (49%), as a monophyletic group, distantly removed from *Cupressus*. Among other characters, the monophyly of *Chamaecyparis* is supported by the presence of curly epicuticular wax tubules (character 16) and two ITS indel characters. Within *Chamaecyparis*, there does not appear to be a clear division associated with geographic distribution as can be seen in other genera of Cupressaceae (e.g., *Cupressus*, described later). The New World species (*Chamaecyparis thyoides* and *C. lawsoniana*) were not resolved as monophyletic.

TABLE 1. Tree statistics for the different data sets and some of their combinations.

Data set	Total		Informative			Tree length[b]	No. trees	No. collapsed nodes[c]	CI[a]	RI	Missing data[b] (%)
	No. characters[a]	No. indels	No. characters[a]	No. indels	Percentage[a]						
ITS 1, 5.8S, and ITS 2	1272	224	522	120	41.0	1566	1	0	0.61	0.81	10.33
matK	1710	14	141	10	8.2	219	3	5 (1)	0.79	0.93	9.3
rbcL	1419		53		3.7	78	3	10 (1)	0.73	0.88	4.65
cpDNA	3129	14	193	10	6.2	304	18	8 (5)	0.76	0.91	8.11
DNA	4401	238	715	130	16.2	1885	3	1 (1)	0.63	0.83	9.79
Morphology	59		58			160	8	4 (4)	0.40	0.68	19.09
DNA + morphology	4698		903			2069	1	0	0.60	0.81	10.54

[a] Excluding indel characters.
[b] Informative characters only.
[c] For 29 terminals, a fully resolved tree has 27 nodes. Numbers in parentheses represent nodes collapsed due to conflict rather than lack of character support.

Cupressus, *Juniperus*, and *Xanthocyparis* formed a very highly supported monophyletic group (bootstrap = 100%). Among other characters, they share the same penultimate branching pattern (character 4; there are some reversals), uniformly sized internodes (character 10), two-year seed maturation (character 32; there are some reversals in species of *Juniperus* not sampled in the present study), two *matK* indel characters, and five ITS indel characters.

In the combined analysis, *Cupressus* was not resolved as a monophyletic group. Instead, the two Old World species sampled were resolved sister to a clade containing a monophyletic *Juniperus*, a monophyletic *Xanthocyparis*, and a clade composed of the six species of New World *Cupressus* sampled.

Support for the monophyly of *Juniperus*, *Xanthocyparis*, and New World *Cupressus* comes from the non-planar arrangement of the ultimate segments (character 3) and one ITS indel character. This clade received moderate bootstrap support (51%). A well-supported, monophyletic *Xanthocyparis* (bootstrap = 93%) was placed in a clade intercalated between a monophyletic *Juniperus* and a clade of New World *Cupressus*. The monophyly of *Xanthocyparis* is supported by three ITS indel characters, the inequilateral arrangement of the ultimate segments (character 2; a character state also found in *Thuja*, *Thujopsis*, and *Chamaecyparis*), and the presence of externally dimorphic mature leaves (character 12). Among other characters, the monophyly of the New World *Cupressus* is supported by the presence of multiple branches per node (character 1), large numbers of cotyledons (character 6), and four ITS indel characters. This clade received a bootstrap value of 100%.

DISCUSSION

Morphological character evolution—As measured by the increase in resolution (Table 1), the addition of the morphology matrix to the sequence data seems to have had a synergistic effect—producing more resolution than either data type did individually. The character state tree used for the tropolone data (Fig. 1) was not invalidated by the combined analysis: When the tropolone characters were optimized onto the tree produced by the total evidence analysis none of the hypothetical ancestors had an ''impossible'' combination of states (i.e., a set of states that indicated that a particular compound was produced without the production of all the assumed chemical precursors in the biosynthetic pathway, such as producing nootkatinol without first producing β-thujaplicin; see Fig. 1).

Li (1953) arranged the genera of Cupressoideae based on the number of ovulate cone scale whorls (character 23), the number of ovules per scale (character 27), the number of scales that bear ovules (character 28), whether or not the terminal whorl is fertile (character 29), the aestivation of the ovulate cone scales (character 31), winged seeds (character 36), seed wing symmetry (character 37), the ovulate cone scale shape (this character could not be properly defined for analysis), and the ovulate cone scale texture (this character could not be properly defined for analysis). When compared to the other morphological characters in the combined analysis, these characters have, on average, a higher CI (0.43 vs. 0.35).

Our data suggest that the ancestral cone of the Cupressoideae had three or four whorls of ovulate cone scales, multiple seeds per scale, multiple fertile scales, a sterile terminal whorl of scales, ''valvate'' cone scales (elongate cells positioned on the scale edge), and bore seeds with symmetrical wings. This

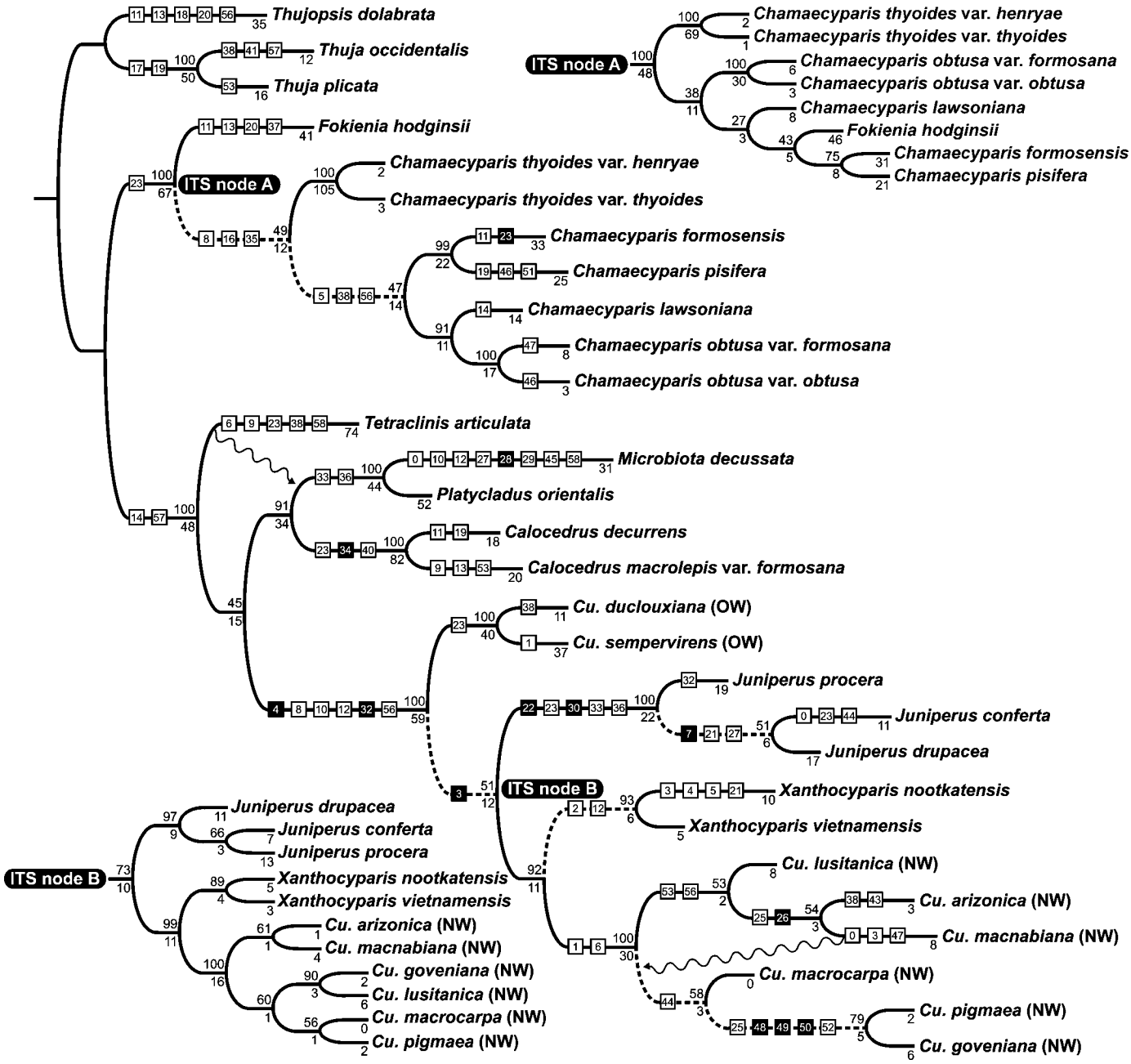

Fig. 2. Single most parsimonious tree for the combined data set (see Table 1 for tree statistics). The generic name of *Cupressus* species has been abbreviated and the generalized geographic range (OW = Old World; NW = New World) is indicated. The unambiguous optimization (the intersection of ACCTRAN and DELTRAN) of the informative morphological characters is shown. Open squares represent homoplasious character state changes; filled squares represent non-homoplasious state changes. Unambiguously optimized branch lengths for all parsimony-informative characters are given below each branch. Strict consensus bootstrap frequencies, where applicable, are indicated above the branches. Cases in which the strict consensus of the cpDNA data conflicted with the combined data are indicated by arrows with wavy tails showing the placement of taxa in the cpDNA tree. Branches drawn with dashed lines were not resolved in the strict consensus of the cpDNA data. The ITS subtrees (ITS nodes A and B) indicate portions of the ITS tree that conflict with the combined data. Bootstrap and branch length values on the ITS subtrees reflect only the ITS data.

contrasts with the extant species of *Cupressus*—described as the ''ancestral type'' by Li (1953)—in fertility of the terminal whorl of cone scales.

As previously indicated by Farjon and Ortiz Garcia (2002), the highly reduced configuration found in *Juniperus* and *Microbiota* arose independently in these two lineages.

Previous studies—An earlier study of ITS variation within *Chamaecyparis* (Li et al., 2003) produced the same results as the present ITS data set. The concordance of independently derived ITS sequence data may indicate that taxon misidentification (as suggested by an anonymous reviewer) is not the cause of discordance between the cpDNA and ITS data sets.

It may, however, suggest that incomplete homogenization of nrDNA repeats has resulted in the sampling of paralogous loci. Alternatively, the discordance may be the result of introgression. No evidence of sequence heterogeneity was detected in the process of generating the sequence data, but that does not eliminate paralogy as a potential source of error. No evidence for, or against, introgression was collected.

The simultaneous analysis of Gadek et al. (Fig. 5 in 2000) was based on a combination of chloroplast sequence data and morphology. That analysis placed *Tetraclinis articulata* within a clade containing *Calocedrus*, *Microbiota*, and *Platycladus*, as does the cpDNA data used in the present study. Because the ITS sequence data provided the largest number of informative characters and those data argued for the placement of *T. articulata* in a different position (Fig. 2), that placement is reflected in the simultaneous analysis of this study.

The only previous studies to sample both Old and New World species of *Cupressus* showed that *Cupressus* was either monophyletic (Figs. 4 and 5 in Gadek et al., 2000), paraphyletic (Fig. 1 in Gadek et al., 2000), or polyphyletic (Farjon et al., 2002). The present combined analysis (Fig. 2) indicates that *Cupressus* is polyphyletic (sensu Farris, 1974). When *Cupressus* (sensu Farjon, 1998) was constrained to be monophyletic using the combined data set, the New World clade and the Old World clade remained monophyletic, but the tree length increased by six steps (ca. 0.29% of overall length; $P > 0.10$). The length increased by one step (ca. 0.05% of overall length; $P > 0.25$), when *Cupressus* was constrained to be either paraphyletic to *Xanthocyparis* or monophyletic; again the New and Old World clades remained monophyletic, but *Cupressus* was paraphyletic to *Xanthocyparis*. Though the combined analysis suggests that *Cupressus* may be polyphyletic, it does not provide strong evidence for polyphyly.

The Old and New World species of *Cupressus* share a common ovulate cone and leaf morphology that is apparently due to the retention of ancestral features rather than to convergent evolution. The polyphyly of *Cupressus* is due to the recognition of two distinctive groups: *Juniperus* has long been recognized as a distinctive group because of its unusual cone morphology that resembles—in both form and function—an angiospermous drupe. More recently, *Xanthocyparis* has been recognized because its morphology is similar to, but not the same as, that of *Cupressus*. Many of the morphological differences between *Cupressus* and *Xanthocyparis* can possibly be attributed to adaptations to moist vs. arid habitats (e.g., dimorphic vs. monomorphic leaves).

Our results agree with those of Gadek et al. (2000), who found that *X. nootkatensis* has close affinity with *Cupressus*. The topology presented here (Fig. 2) contrasts strongly with that of Farjon et al. (2002), where both species of *Xanthocyparis* were placed, distant from *Cupressus*, in an unresolved clade of Callitroideae (sensu Li, 1953). Because the morphological matrix of Farjon et al. (2002) was not published, the source of this discordance cannot be ascertained.

Taxonomic implications: Chamaecyparis—The type species of *Chamaecyparis* is *C. thyoides*. Therefore, the only change implied by the current data to the commonly used circumscription of *Chamaecyparis*, is to exclude *C. nootkatensis*, as previously indicated by several authors (Gadek et al., 2000; Farjon et al., 2002). Because *F. hodginsii* is included within *Chamaecyparis* in only one partition of the data, it seems unwise to add this species to *Chamaecyparis*. Should additional data suggest that *Fokienia* and *Chamaecyparis* be merged, the correct name for the genus would be *Chamaecyparis*.

Taxonomic implications: Cupressus—It appears that *Cupressus* is not monophyletic. If this pattern persists as new data are added, *Cupressus* may have to be divided into two genera—with the Old World species of *Cupressus* retaining their current names, while a new generic name will be needed for the New World species. Currently, this is being further investigated with more exhaustive sampling of *Juniperus* and *Cupressus* species.

Taxonomic implications: Xanthocyparis—As circumscribed by Farjon et al. (2002), the genus *Xanthocyparis* appears to be monophyletic. However, based on the established rules for botanical nomenclature, this circumscription unfortunately cannot stand: The genus *Callitropsis* non *Callitropsis* sensu Compton (1922), with *C. nootkatensis* (D. Don) Örest. designated as its type, was described in 1865. Because *Callitropsis* is the oldest name, the principle of priority (Greuter et al., 2000: article 11.3), in combination with the phylogenetic evidence presented here, dictates that *Xanthocyparis* cannot include *C. nootkatensis*. Either *Xanthocyparis vietnamensis* must be transferred to the genus *Callitropsis* or *Xanthocyparis* must remain a monotypic genus.

Because *X. vietnamensis* and *C. nootkatensis* are sister taxa and appear to be relatively closely allied, it is more informative to recognize this relationship in a single genus rather than two monotypic sister genera. We therefore transfer *X. vietnamensis* to *Callitropsis*.

Callitropsis vietnamensis (Farjon and Hiep) D. P. Little comb. nov. Basionym: *Xanthocyparis vietnamensis* Farjon and Hiep in Farjon, Hiep, Harder, Loc, and Averyanov Novon 12: 180. 2002.

As now circumscribed, *Callitropsis* contains two species with disjunct distribution: *C. nootkatensis* is native to western North America from 41° N to 60° N. It is primarily found near the coast (in the moist fog belt), but more inland populations are known from the southern portion of the range (e.g., Frog Lake, Siskiyou County, California). *Callitropsis vietnamensis* is native to moist karst forest in northern Vietnam (23° N; see Averyanov et al., 2002).

Callitropsis is diagnosed with the combination of primarily apically distributed ultimate branchlets (character 2) and externally dimorphic mature leaves (character 12; see also Farjon et al., 2002).

LITERATURE CITED

ALVIN, K. L., D. H. DABY, AND F. A. OLADELE. 1980. Numerical analysis of cuticular characters in Cupressaceae. *In* D. F. Cutler, K. L. Alvin, and C. E. Price [eds.], The plant cuticle, 379–396. Academic Press, London, UK.

AVERYANOV, L. V., N. T. HIEP, D. K. HARDER, AND P. K. LOC. 2002. The history of discovery and natural habitats of *Xanthocyparis vietnamensis* (Cupressaceae). *Turczaninowia* 5: 31–39.

BANNAN, M. W. 1941. Wood structure of *Thuja occidentalis*. *Botanical Gazette* 103: 295–309.

BANNAN, M. W. 1944. Wood structure of *Libocedrus decurrens*. *American Journal of Botany* 31: 346–351.

BARKMAN, T. J. 2001. Character coding of secondary chemical variation for use in phylogenetic analyses. *Biochemical Systematics and Ecology* 29: 1–20.

BÉRUBÉ, Y., C. RITLAND, AND K. RITLAND. 2003. Isolation, characterization, and cross-species utility of microsatellites in yellow cedar (*Chamaecyparis nootkatensis*). *Genome* 46: 353–361.

BRUNSFELD, S. J., P. S. SOLTIS, D. E. SOLTIS, P. A. GADEK, C. J. QUINN, D. D. STRENGE, AND T. A. RANKER. 1994. Phylogenetic relationships among the genera of Taxodiaceae and Cupressaceae: evidence from *rbcL* sequences. *Systematic Botany* 19: 253–262.

BUTTS, D., AND J. T. BUCHHOLZ. 1940. Cotyledon numbers in conifers. *Illinois State Academy of Science Transactions* 33: 58–62.

CAMUS, A. 1914. Les Cyprés. Académie des Sciences, Paris, France.

COMPTON, R. H. 1922. A systematic account of the plants collected in New Caledonia and the Isle of Pines by Mr. R. H. Conmpton, M. A. in 1914, part II. Gymnosperms and cryptograms. *Journal of the Linnean Society of London, Botany* 45: 421–466.

CRESPO, J. H. 1976. Anatomía de la madera de 12 especies de coniferas Mexicanas. *Boletín Técnico Instituto Nacional de Investigaciones Forestales Mexico* 51: 1–56.

DE LAUBENFELS, D. J. 1953. The external morphology of coniferous leaves. *Phytomorphology* 3: 1–20.

DE LAUBENFELS, D. J. 1965. The relationships of *Fitzroya cupressoides* (Molina) Johnston and *Diselma archeri* J. D. Hooker based on morphological considerations. *Phytomorphology* 15: 414–419.

DOAK, C. C. 1937. Morphology of *Cupressus arizonica*: gametophytes and embryogeny. *Botanical Gazette* 98: 808–815.

DON, D. 1824. A description of the genus *Pinus*. Messrs. Wendell, London, UK.

ENZELL, C., AND M. KROLIKOWSKA. 1963. The chemistry of the natural order Cupressales 48: heartwood constituents of *Cupressus arizonica* Green. *Arkiv För Kemi* 20: 157–167.

ERDTMAN, H., AND T. NORIN. 1966. The chemistry of the order Cupressales. *In* L. Zechmeister [ed.], Progress in the chemistry of organic natural products, 206–287. Springer Verlag, New York, New York, USA.

FARJON, A. 1998. World checklist and bibliography of conifers. Royal Botanic Gardens, Kew, UK.

FARJON, A., N. T. HIEP, D. K. HARDER, P. K. LOC, AND L. AVERYANOV. 2002. A new genus and species in the Cupressaceae (Coniferales) from northern Vietnam, *Xanthocyparis vietnamensis*. *Novon* 12: 179–189.

FARJON, A., AND S. ORTIZ GARCIA. 2002. Towards the minimal conifer cone: ontogeny and trends in *Cupressus*, *Juniperus* and *Microbiota* (Cupressaceae s. str.). *Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie* 124: 129–147.

FARRIS, J. S. 1974. Formal definitions of paraphyly and polyphyly. *Systematic Zoology* 23: 548–554.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FUJITA, K., T. YAMAGUCHI, R. ITOSE, AND K. SAKAI. 2000. Biosynthetic pathway of β-thujaplicin in the *Cupressus lusitanica* cell culture. *Journal of Plant Physiology* 156: 462–467.

GADEK, P. A., D. L. ALPERS, M. M. HESLEWOOD, AND C. J. QUINN. 2000. Relationships within Cupressaceae sensu lato: a combined morphological and molecular approach. *American Journal of Botany* 87: 1044–1057.

GADEK, P. A., AND C. J. QUINN. 1983. Biflavones of the subfamily Callitroideae, Cupressaceae. *Phytochemistry* 22: 969–972.

GADEK, P. A., AND C. J. QUINN. 1985. Biflavones of the subfamily Cupressoideae Cupressaceae. *Phytochemistry* 24: 267–272.

GADEK, P. A., AND C. J. QUINN. 1988. Pitting of transfusion tracheids in Cupressaceae. *Australian Journal of Botany* 36: 81–92.

GADEK, P. A., AND C. J. QUINN. 1993. An analysis of relationships within the Cupressaceae sensu stricto based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 80: 581–586.

GOLOBOFF, P. A. 1993. NONA. Computer program distributed by the author, website: www.cladistics.com.

GREGUSS, P. 1955. Identification of living gymnosperms on the basis of xylotomy. Akadémiai Kiadó, Budapest, Hungary.

GREUTER, W., ET AL. 2000. International Code of Botanical Nomenclature (St. Louis code). Koeltz Scientific Books, Königstein, Germany.

HART, J. A. 1987. A cladistic analysis of conifers: preliminary results. *Journal of the Arnold Arboretum* 68: 269–307.

HEGNAUER, R. 1962. Chemotaxonomie der Pflanzen. Birkhäuser Verlag, Basel, Switzerland.

JACKSON, A. B., AND W. DALLIMORE. 1926. A new hybrid conifer. *Kew Bulletin of Miscellaneous Information* 3: 113–115.

JAGEL, A., AND T. STÜTZEL. 2001. Untersuchungen zur Morphologie und Morphogenese der Samenzapfen von *Platycladus orientalis* (L.) Franco (= *Thuja orientalis* L.) und *Microbiota decussata* Kom. (Cupressaceae). *Botanische Jahrbücher für Systematik Pflanzengeschichte und Pflanzengeographie* 123: 377–404.

JOHN, S. M., M. DANIEL, AND S. D. SABNIS. 1989. Chemosystematics of some conifers of India. *Proceedings of the Indian Academy of Sciences (Plant Sciences)* 99: 253–258.

KAEISER, M. 1953. Microstructure of the wood of *Juniperus*. *Botanical Gazette* 115: 155–162.

KUSUMI, J., Y. TSUMURA, H. YOSHIMARU, AND H. TACHIDA. 2000. Phylogenetic relationships in Taxodiaceae and Cupressaceae sensu stricto based on *matK* gene, *chlL* gene, *trnL-trnF* IGS region, and *trnL* intron sequences. *American Journal of Botany* 87: 1480–1488.

LI, H. L. 1953. A reclassification of *Libocedrus* and Cupressaceae. *Journal of the Arnold Arboretum* 34: 17–36.

LI, H. L. 1975. Cupressaceae. *In* H.-L. Li, T.-S. Liu, T.-C. Huang, T. Koyama, and C. E. DeVol [eds.], Flora of Taiwan, 534–544. Epoch, Taipei, Taiwan.

LI, J., D. ZHANG, AND M. J. DONOGHUE. 2003. Phylogeny and biogeography of *Chamaecyparis* (Cupressaceae) inferred from DNA sequences of the nuclear ribosomal ITS region. *Rhodora* 105: 106–117.

MASTERS, M. T. 1895. A general view of the genus *Cupressus*. *Journal of the Linnean Society, Botany* 31: 312–363.

MEHRA, P. N., AND R. K. MALHOTRA. 1947. Stages in the embryology of *Cupressus sempervirens* Linn with particular reference to the occurrence of multiple male cells in the male gametophyte. *Proceedings of the National Academy of Sciences, India* 17: 129–153.

MITCHELL, A. F. 1970. A note on two new hybrid cypresses. *Journal of the Royal Horticultural Society* 94: 453–454.

NATARAJAN, S., V. V. S. MURTI, AND T. R. SESHADRI. 1970. Biflavones of some Cupressaceae plants. *Phytochemistry* 9: 575–579.

NIXON, K. C. 2003. WinClada version 0.9. Computer program distributed by the author, website: www.cladistics.com.

OLADELE, F. A. 1983a. Inner surface sculpture patterns of cuticles in Cupressaceae. *Canadian Journal of Botany* 61: 1222–1231.

OLADELE, F. A. 1983b. Scanning electron microscope study of stomatal-complex configuration in Cupressaceae. *Canadian Journal of Botany* 61: 1232–1240.

ÖRSTED, A. S. 1864. Bidrag til Naaletræernes Morphologi. *Videnskabelige Meddelelser fra den naturhistorisk Forening i Kjöbenhavn series 2* 6: 1–36.

OWENS, J. N., AND M. MOLDER. 1975. Pollination female gametophyte and embryo and seed development in yellow cedar (*Chamaecyparis nootkatensis*). *Canadian Journal of Botany* 53: 186–199.

PÄÄBO, S. 1990. Amplifying ancient DNA. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and applications, 159–166. Academic Press, San Diego, California, USA.

PEIRCE, A. S. 1937. Systematic anatomy of the woods of the Cupressaceae. *Tropical Woods* 49: 5–21.

PELTER, A., R. WARREN, N. HAMEED, N. U. KHAN, M. ILYAS, AND W. RAHMAN. 1970. Biflavonyl pigments from *Thuja orientalis* (Cupressaceae). *Phytochemistry* 9: 1897–1898.

PRAUSE, A. 1909. Beiträge zur Blattanatomie der Cupressineen. University of Wroclaw, Breslau, Poland.

QASIM, M. A., S. K. ROY, M. KAMIL, AND M. ILYAS. 1985. Biflavones from leaves of *Cupressus gracilis* and *Cupressus macrocarpa*. *Journal of the Indian Chemistry Society* 62: 170.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analysis. *Systematic Biology* 49: 369–381.

SPACH, É. 1842. Histoire naturelle des végétaux: phanerogames, vol. 11. Librairie encyclopédique de Roret, Paris, France.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* 37: 221–244.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The Clustal_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882.

WILCOXON, F. 1945. Individual comparisons by ranking methods. *Biometrics Bulletin* 1: 80–83.

WILHELMI, H., AND W. BARTHLOTT. 1997. Mikromorphologie der Epicuticularwachse und die Systematik der Gymnospermen. *Tropische und subtropishe Pflanzenwelt* 97: 1–49.

WOLF, C. B. 1948. Taxonomic and distributional studies of the New World cypresses. *El Aliso* 1: 1–250.

XIANG, Q., AND A. FARJON. 2003. Cuticle morphology of a newly discovered

conifer, *Xanthocyparis vietnamensis* (Cupressaceae), and a comparison with some of its nearest relatives. *Botanical Journal of the Linnean Society* 143: 315–322.

YOUNG, N. D., AND J. HEALY. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4: 6.

ZAR, J. H. 1984. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

ZAVARIN, E., AND A. B. ANDERSON. 1956. Paper chromatography of the tropolones of Cupressaceae. *Journal of Organic Chemistry* 21: 332–335.

ZAVARIN, E., R. M. SMITH, AND A. B. ANDERSON. 1959. Paper chromatography of the tropolones of Cupressaceae II. *Journal of Organic Chemistry* 24: 1318–1321.

ZAVARIN, E., L. V. SMITH, AND J. G. BICHO. 1967. Tropolones of Cupressaceae—III. *Phytochemistry* 6: 1387–1394.