

Taxonomy of *Hesperocyparis montana*, *H. revealiana* and *H. stephensonii*: Evidence from leaf essential oils analyses and DNA sequences

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ABSTRACT

DNA sequences and leaf essential oils were analyzed from *Hesperocyparis montana*, *H. revealiana* and *H. stephensonii*. The cpDNA sequences placed *H. stephensonii* in a clade with *H. arizonica*, *H. forbesii*, and *H. guadalupensis*, separate from the clade for *H. montana* and *H. revealiana*. The generally considered close relationship between *H. montana* and *H. revealiana*, and *H. stephensonii*, was not supported by DNA sequencing. The cpDNA sequences differ by 3 mutations between *H. montana* and *H. revealiana*. Bayesian analysis provide strong support for an *H. montana* + *H. revealiana* clade, as well as for a *H. montana* clade to the exclusion of *H. revealiana*. Support for *H. revealiana* as separate from *H. montana* is less defined. Two chemotypes were found in *H. montana*: high cedrol (28.2 - 33.7%) and low cedrol (0.02 - 0.5%). The oil of *H. montana* differs from *H. revealiana* by the presence of 2-nonanone, borneol, linalool, carvone, α -copaene, γ -muurolene, epi-cubebol, α -muurolene, γ -cadinene, endo-1-bourbonanol, α -cadinene, germacrene D-4-ol, 1-epi-cubebol, amorpho-4,9-dien-2-ol, oplophenone, oplopanonyl acetate, manoyl oxide and nezukol. The leaf oil of *H. revealiana* differs from *H. montana* by the presence of cis- and trans-p-menth-2-en-1-ols, karahanaenone, terpinen-4-yl acetate, α -terpinyl acetate, epi-zonarene, cis-muurola-4,5-diene, cis-muurola-5-en-4- α and β -ols, and α -acorenol. The leaf oil of *H. stephensonii* was high in sabinene (9.9%) camphor (9.1%) and terpinen-4-ol (8.9%), with moderate amounts of α -pinene, limonene, β -phellandrene, α -cadinol and 2.6% iso-abienol, not found in the other taxa. The leaf oils of each of the three taxa are quite differentiated and are as dissimilar as many *Hesperocyparis* species. The differences in leaf oil compositions and DNA among *H. montana*, *H. revealiana* and *H. stephensonii* support the recognition of these species. Published on-line www.phytologia.org *Phytologia* 96(2): 71-83 (April 1, 2014). ISSN 030319430

KEY WORDS: *Hesperocyparis* (=Cupressus) *montana*, *H. revealiana*, *H. stephensonii*, volatile leaf terpenoids, cp DNA sequences, taxonomy, cedrol chemotypes.

The taxonomic position of the western hemisphere cypresses have been the source of considerable flux in the past. Commencing with a study by Little et al. (2004), utilizing DNA sequencing data, demonstrated the eastern hemisphere and western hemisphere cypresses in a clade that contained *Juniperus*, *Xanthocyparis*, *Chamaecyparis nootkatensis*. The authors noted that the western hemisphere cypresses were in need recognition as a separate genus. Little (2006) recognized the new world cypresses (*Cupressus*) as *Callitropsis*, along with the inclusion of *Chamaecyparis nootkatensis* as *Callitropsis*

nootkatensis and maintained *Cupressus* for the old world cypresses. However, using additional DNA sequence data, Adams, Bartel and Price (2009) placed the new world cypresses in a clade, distinct from *Callitropsis nootkatensis*, and a new genus was recognized for the new world cypresses, *Hesperocyparis*. Mao et al. (2010), although focusing on *Juniperus* biogeography, included several cypresses from the old and new worlds, which they treated as *Cupressus* and *Hesperocyparis* (see Fig. 2, in Mao et al., 2010). In addition, they included *Callitropsis nootkatensis* and *Xanthocyparis vietnamensis* as monotypic genera.

The most robust study to date of the phylogeny of *Hesperocyparis* (Terry, Bartel and Adams, 2012) used 12.8 kb of nrDNA and cpDNA sequence data (Fig. 1). High (1.0) posterior probabilities support were found in support for the recognition of five genera: *Hesperocyparis* (western hemisphere cypresses), *Callitropsis* (monotypic *C. nootkatensis*), *Xanthocyparis* (monotypic *X. vietnamensis*), *Cupressus* (eastern hemisphere cypresses) and *Juniperus* (used as an outgroup). Unfortunately, *H. revealiana* was not included in their study.

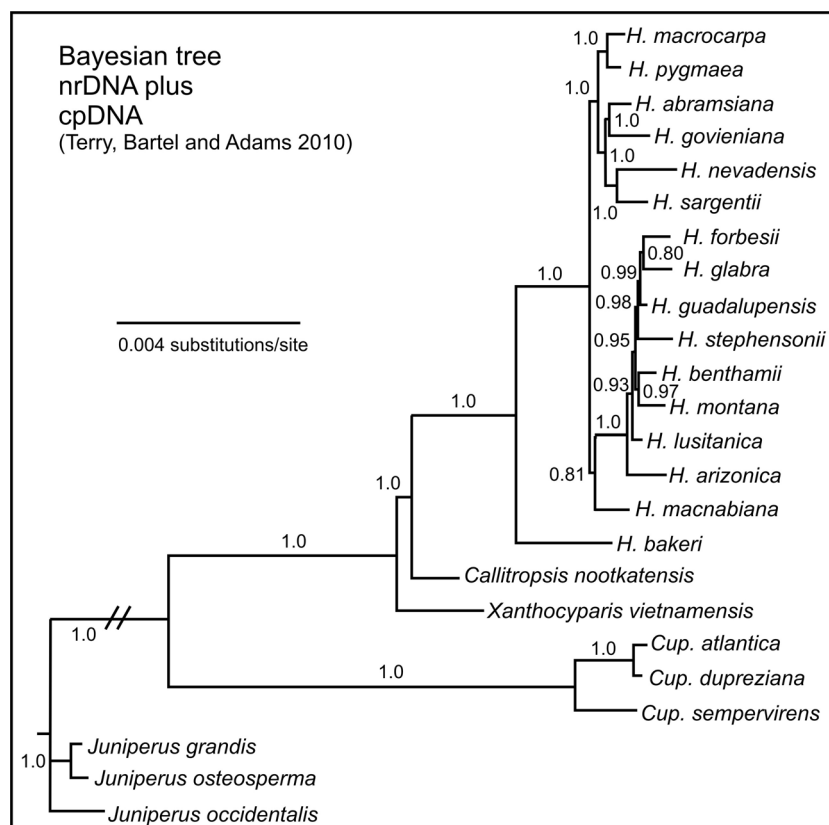


Figure 1. Bayesian tree of *Hesperocyparis*, *Callitropsis*, *Xanthocyparis*, *Cupressus* and *Juniperus*. Numbers at nodes are posterior probabilities.

Suggesting a relationship with *Cupressus* (*Hesperocyparis*) *nevadensis* and *arizonica*, Wiggins described *C. montana* in 1933, which is endemic to the Sierra de San Pedro Mártir of central Baja California of Mexico (Figure 2). Wolf also treated *C. montana* as a species in his 1948 monograph on western cypress. Although Elbert Little (1966) reduced *C. (H.) montana* to a variety of *C. arizonica*, recent authors treat *H. montana* as a species (e.g., Little 2006, Adams et al. 2009). Wolf (1948) described *C. stephensonii* from the King Creek watershed in the Cuyamaca Mountains of San Diego County (Figure 2), which he differentiated from *H. montana* by its “smooth polished, cherry-red or mahogany brown bark” versus the Mexican species and its “rough fissured, fibrous, gray or dark brown, non-exfoliating

bark.” Wolf (1948) also noted that the seed cones of *H. montana* open soon after maturity rather than retaining its seeds for years in closed (serotinous) cones.

In 1981, Silba described a new cypress variety from near El Rincón in the Sierra Juarez of northern Baja, Mexico (Figure 2) as *Cupressus arizonica* var. *revealiana*. The taxon had been previously noted by Broder (1963) and thought to be *Cupressus arizonica* var. *stephensonii* by Moran (1977). Silba (1998) differentiated the new variety by its “very thin cone scales” and smaller “distinctly dark” seeds. While Silba later elevated the variety to a subspecies rank in 2005 (*C. arizonica* subsp. *revealiana*), he contrasted it to the multi-colored bark and much larger, lighter-colored, and distinctly elongated winged seed of *C. stephensonii*. Silba (2009) eventually elevated the Sierra Juarez cypress to the specific level, *Hesperocyparis revealiana*. Farjon (2005), Eckenwalder (2009), and Debreczy and Racz (2011) treated *Cupressus* (*Hesperocyparis*) *revealiana* as conspecific with *C. (H.) stephensonii*. In addition, Damon Little (2005) in his dissertation on *Cupressus* sensu lato and *Callitropsis* did not recognize *H. revealiana* because he could find no morphological characteristics to distinguish it from *H. stephensonii*.

Bartel et al. (2003) used RAPDs to analyze various *Cupressus* (*Hesperocyparis*) species, however, they did not include *H. revealiana*. Bisbee and Maerki (2012) compared the morphology, phenology and physiology of *H. revealiana* to *H. stephensonii* and concluded that both taxa should be recognized as species. In addition, the authors included a table comparing the morphology and phenology of *H. montana*, *revealiana*, and *stephensonii*.

The purposes of this paper are to compare DNA sequences of *H. revealiana* with other *Hesperocyparis* species and to present the compositions of the leaf essential oils of *H. montana* and *H. revealiana* that, to date, have not been reported. Using these data, we address the affinity and taxonomy status of *H. revealiana*.

MATERIALS AND METHODS

Callitropsis nootkatensis, Adams 9086, Washington Park Arb., U. of Washington, Seattle, WA
Xanthocyparis vietnamensis, Adams 10142 (K. Rushforth 7745) UK, ex Vietnam
Cupressus atlantica, Adams 8429, Morocco
Cupressus dupreziana, Adams 8432, Algeria (ex Hillier Gardens)
Cupressus sempervirens, Adams 8434, Elburz Mtns., Iran (ex Hillier Gardens)
H. abramsiana, Adams 11464 (Bartel 1598a), Bonny Doon, sw of CDF fire station, CA
H. arizonica, Adams 9378 (Bartel 1580a) upper Bear Canyon, Pima Co., AZ
H. bakeri, Adams 9362, (Bartel 1572a) nw of Thousand Lake Wilderness, Shasta Co., CA

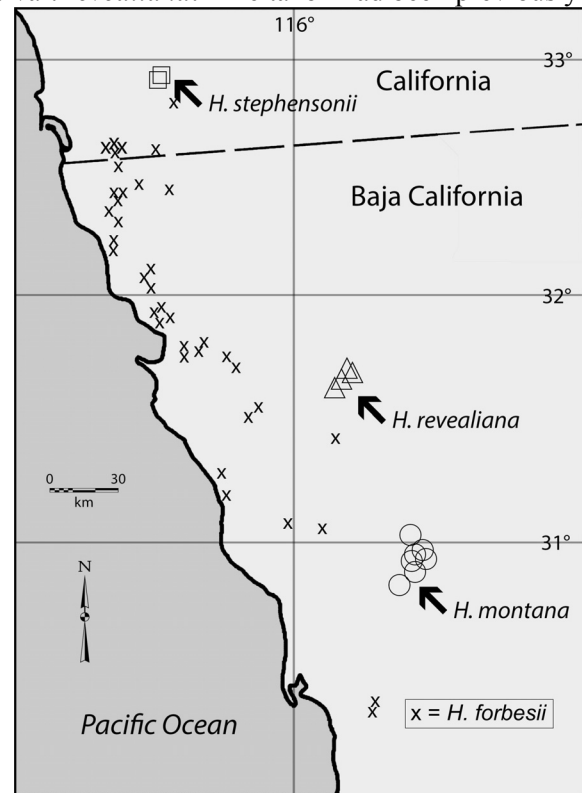


Fig. 2. General distributions of *Hesperocyparis* in Baja, MX and s CA. The Baja map portion based on Minnich (1987) and Southwestern Environmental Information Network. The California map portion based on Consortium of California herbaria records.

H. benthamii, Adams 8712, Pachuca, Mexico

H. forbesii, Adams 9370 (Bartel 1576a) s of O'Neal Canyon, San Diego Co., CA

H. glabra, Adams 9389 (Bartel 1585b), nw of East Verde River, Gila Co., AZ; USA

H. goveniana, Adams 11449 (Bartel 1595a), Point Lobos Ranch, Monterey Co., CA; USA

H. guadalupensis, Adams 8417, Guadalupe Island, Mexico (ex Berkeley Botanical Garden)

H. lusitanica, Adams 7072, Bussaco, Portugal (cultivated, ex Mexico)

H. macnabiana, Adams 9359 (Bartel 1569b), n of Knoxville, Napa Co., CA

H. macrocarpa, Adams 11460 (Bartel 1597b), Crocker Grove, Monterrey Co., CA

H. montana

Adams 9660-9661 (Bartel 1590a,b) collected by David R. Johnson of the Forest Service Institute of Genetics (IFG) in Placerville, California. Branches collected at IFG (Pedigree #16 Row 25 Line 12 Eddy West) from a living specimen grown from seed collected by Richard A. Minnich (UCR) on 21 July 1978,

Adams 11640(=13913) (R F Thorne et al 63552), 20 July 1988, near the end of upper Vallecito Rd., Sierra San Pedro Mártir, 2300m, Baja, Mexico, seeds sent to Huntington Botanical Gardens, 15 Feb 1990, acc 65891, Kathy Musial,

Adams 11661 (=8421), ex Inst. of Forest Genetics, Placerville, CA, ex Baja, MX, San Pedro Mártir, La Encantada meadow, 7,000', ex Berkeley Botanic Garden acc. 77.0521, Holly Forbes,

Adams 13833-13836 (Bisbee mon-1-4) cult. at Colfax, CA by Jeff Bisbee s. n., July 2001, near Botella Azul, Sierra San Pedro Mártir. Note: 13836 had unusual foliage with very short branchlets,

Adams 13840 (F. Callahan, s. n.), ex Merlin May Arb., OR, ex seed from Sierra San Pedro, Baja, CA, Mexico,

Adams 13899, (Medbury s.n.25 Feb 1996) ex. Arnold Arboretum acc. 165-96*A, wild coll. Mexico, Baja, Sierra San Pedro Mártir, 3 km from Los Llanitos Rd. at stream crossing. ca 2460m,

Adams 13901-13902, Bartel 1614, 1615, collected by R. Mitchel Beauchamp, ex Tree of Life Nursery, Orange Co., CA.

H. nevadensis, Adams 9367 (Bartel 1574b), Greenhorn Mountains, Kern Co., CA,

H. pygmaea, Adams 11489 (Bartel 1693a), Casper Little Lake, Mendocino Co., CA,

H. revealiana,

Adams 13837-13839 (Bisbee rev-1-3) cult. at Colfax, CA, Jeff Bisbee s. n., July 2004, El Rincon, Baja, Mexico. Note: 13838 had only juvenile leaves (neoteny),

Adams 13848 Bartel 1613, ex Greg Abbot s. n. 1992, foothills of Sierra de Juarez near village of Santa Catarina, Baja CA, MX, ex San Diego Zoo Safari Park, Escondido, CA,

H. sargentii, Adams 9348 (Bartel 1564c), Cuesta Ridge, San Luis Obispo Co., CA,

H. stephensonii, Adams 9376 (Bartel 1579a), Cuyamaca Rancho State Park, San Diego Co., CA,

H. stephensonii, Adams 12623-12628 (Callahan 1-6), King Creek, San Diego Co., CA

Juniperus grandis, Terry 115, Mono Co., CA,

Juniperus occidentalis, Terry 128, Baker Co., OR,

Juniperus osteosperma, Terry 058, Garfield Co., UT.

Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields. Oils from each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007, for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

DNA extraction, PCR amplification, sequencing and data analyses

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. For primers and PCR amplifications for nrDNA (ITS) primers see Adams, Bartel and Price (2009). The trnS-trnG, trnC-trnD, trnD-trnT, and psbD/trnT intergenic spacers and the trnG intron were amplified according to Terry, Bartel and Adams, 2012.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com).

Phylogenetic analysis - A total of 4,130 bp of unambiguously aligned sequence from all species of *Hesperocyparis*, including four accessions of *H. montana* var. *montana* and two accessions of *H. montana* var. *revealiana*, was included in this study. All sequences are from chloroplast noncoding regions, including 3489 bp from four intergenic spacers (*trnS-trnG*, *trnC-trnD*, *trnD-trnT*, and *psbD-trnT*) and 641 bp from one intron (*trnG* intron). Sequence alignments were performed using ClustalW (Thompson et al. 1994; Kyoto University Bioinformatics Center, Kyoto, Japan) and refined manually using Seq-AL v.2.0a9 (Rambaut 2002). Gaps shared by two or more taxa were scored as binary characters using simple indel coding (Simmons and Ochoterena 2000) implemented in SeqState v.1.4.1 (Muller 2005, 2006). All nucleotides were included in the final alignment excluding 101 positions within the *trnS-trnG* IGS that could not be aligned unambiguously.

Bayesian analyses were performed using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Best-fit evolutionary models and analysis settings are described in Terry et al. (2012), except a burnin fraction of 0.25 was enforced, resulting in the first 1250 of 5000 trees being discarded, and the remaining trees (3750) pooled to construct the posterior distribution of the phylogeny. A 50% majority-rule consensus tree was generated from the pooled trees using the “contype=halfcompat” command. Convergence was confirmed and effective sample sizes monitored using Tracer 1.5 (Rambaut and Drummond 2007).

RESULTS AND DISCUSSION

There are no reports on the volatile leaf oils of *H. montana* or *H. revealiana*. No detailed report on the volatile leaf oil of *H. stephensonii* was found in the literature, except Cool, Jiang and Zavarin (1994) reported that *C. (H.) stephensonii* contained karahanaenone in the leaf oil. Senter, Zavarin and Zedler (1975) reported carvacrol (78%) was the major component of the heartwood oil of *C.(H.) stephensonii*.

The yields of volatile leaf oils of *H. montana* varied considerably (0.5, 0.06, 0.7, 1.2, 1.5, 0.9, 1.2, 1.1%). The *H. montana* 13834 had almost no oil (0.06%), but its oil composition was not unusual. That individual appears to have a gene almost completely blocking the mono-, sesqui- and di-terpenoids pathway. The eight *H. montana* individuals displayed two chemotypes: high cedrol and low cedrol (Table 1). Four samples (11640, 11661, 13840, 13899) were high in cedrol (28.2 to 33.7%). Six samples (13833-13836, 13901-13902) had only trace amounts (0.02, 0.05, 0.1, 0.05, 0.05, 0.5%) of cedrol. Cedrol and related compounds (Table 1, italics) are characteristic components (Adams 1991) of cedarwood oils

(eg. heartwood oils of *Juniperus* and *Cupressus*, but not *Hesperocyparis* heartwood oil, as far as known). However, occasionally in *Juniperus* leaf oils, one finds trees with considerable amounts of cedrol and associated heartwood oil components (cf. α -cedrene β -cedrene, thujopsene, cuparene, cedrol, widdrol, etc., see Adams et al., 2013). Cedrol is a major component in the leaf oils of *J. excelsa*, 25.4 - 29.3% (Adams et al., 2013), *J. polycarpus*, 30.3% and *J. seravschanica*, 13.8 - 22.7% (Adams and Hojjati, 2013). In the Cupressaceae, the leaf and wood essential oils also appear to be generally distinct. However, Adams, et al. (1997) reported cedrol in several *Cupressus* (*Hesperocyparis*) leaf oils: 0.5 - 0.7%, *C. lusitanica*; 0.2% *C. lindleyi*; and 0.1% *C. glabra* (mis-reported as *C. arizonica*). Adams and Bartel (2009) found 0.1 - 0.2% cedrol in *H. goveniana*. Adams et al. (2010) reported 1.2% cedrol in *H. glabra*, but no cedrol in any population of *H. arizonica*. If one corrects for the 'cedarwood oil' components in the high cedrol *H. montana*, then the oil is similar to the low cedrol type (Table 1). The oil of *H. montana* differs from *H. revealiana* by the presence of 2-nonanone, borneol, linalool, carvone, α -copaene, γ -muurolene, epi-cubebol, α -muurolene, γ -cadinene, endo-1-bourbonanol, α -cadinene, germacrene D-4-ol, 1-epi-cubenol, amorpho-4,9-dien-2-ol, oplophenone, oplopanonyl acetate, manoyl oxide and nezukol (Table 1).

The leaf oil of *H. revealiana* differs from *H. montana* by the presence of cis- and trans-p-menth-2-en-1-ols, karahanaenone, terpinen-4-yl acetate, α -terpinyl acetate, epi-zonarene, cis-muurola-4,5-diene, cis-muurola-5-en-4- α and β -ols, and α -acorenol (Table 1). In addition, the oils differently quantitatively for many compounds (sabinene, γ -terpinene, β -phellandrene, terpinolene, camphor, umbellulone, terpinen-4-ol and trans-muurola-3,5-diene, Table 1). Overall, the number of differences between *H. montana* and *H. revealiana* is comparable to differences between recognized *Hesperocyparis* species.

The leaf oil of *H. stephensonii* was high in sabinene (9.9%) camphor (9.1%) and terpinen-4-ol (8.9%), with moderate amounts of α -pinene, limonene, β -phellandrene, α -cadinol and 2.6% iso-abienol, not found in the other taxa.

The putative hybrids (see nrDNA below), *H. montana* 11661 and 13899 did not contain compounds typical of *H. revealiana* (Table 1). Thus, the volatile leaf oils offer no support that *H. montana* 11661 and 13899 are hybrids as suggested by nrDNA.

Sequencing revealed that *H. montana* and *H. revealiana* are extremely closely related (Table 2). Eight potentially informative nrDNA (ITS) substitutions were found, but upon closer inspection it appears more likely that this is a case of incomplete lineage sorting (Syring et al., 2007) in *Hesperocyparis*. Two of the *H. montana* samples (Table 2) contained 7 complementary bases found in both *montana* and *revealiana* suggesting that these individuals are hybrids. However, the terpenoids clearly indicate that they are not hybrids. In addition, a search of GenBank revealed that, for nrDNA data, in nearly every instance where there are 2 or more accessions of a *Hesperocyparis* species, the nrDNA sequences differ by from 4 to 12 bases within the species. This well illustrates the variability of nrDNA sequences in *Hesperocyparis* and may be a consequence of incomplete lineage sorting. Thus, nrDNA data were not further utilized in this study.

The cpDNA markers exhibited very little variation between *H. montana* and *H. revealiana* (Table 2). No variation was found in psaA/ycf3 and trnT-trnTD. Only 2 SNPs were found in psbD/trnT and trnC-trnD, and these are single nucleotide differences. The trnS-trnG plus trnG intron (1661 bp) proved to be of use with 3 SNPs (1 informative) and 2 indels (both informative).

Bayesian tree analysis based on cpDNA revealed that *H. montana* and *H. revealiana* are in a well-supported clade sister to a clade containing *H. glabra*, *H. forbesii*, *H. arizonica*, *H. guadalupensis* and *H. stephensonii* (Fig. 3). The *H. montana* samples form a well-supported clade, but the two accessions of

H. revealiana are not supported as monophyletic. In contrast, this was not seen in the leaf essential oils data, where the oils were found to be relatively uniform for the four accessions of *H. revealiana*.

Table 2. Comparison of information content for primers used in this study among the 4 *H. montana* and 2 *H. revealiana* accessions examined. MEs = mutational events = # SNPs + # indels. * nrDNA was not useful due to incomplete lineage sorting.

gene region	length	# SNPs	infor. SNPs	# indels	infor. indels	infor. MEs
nrDNA (ITS)	1271	12	8	0	0	8*
<i>montana</i> 11661	YSYMKYYG					
<i>montana</i> 13899	YSYMKYYC					
<i>montana</i> 13840	CCTCGCTC					
<i>montana</i> 13838	CCTCGCTG					
<i>revealiana</i> 13836	TGCATTCTG					
<i>revealiana</i> 13837	TGCATTCTG					
cpDNA						
trnS-trnG + trnG intron	1661	3	1	2	2	3
psaA/ycf3	818	0	0	0	0	0
psbD/trnT	927	2	0	0	0	0
trnC-trnD	787	2	0	0	0	0
trnT-trnTD	721	0	0	0	0	0

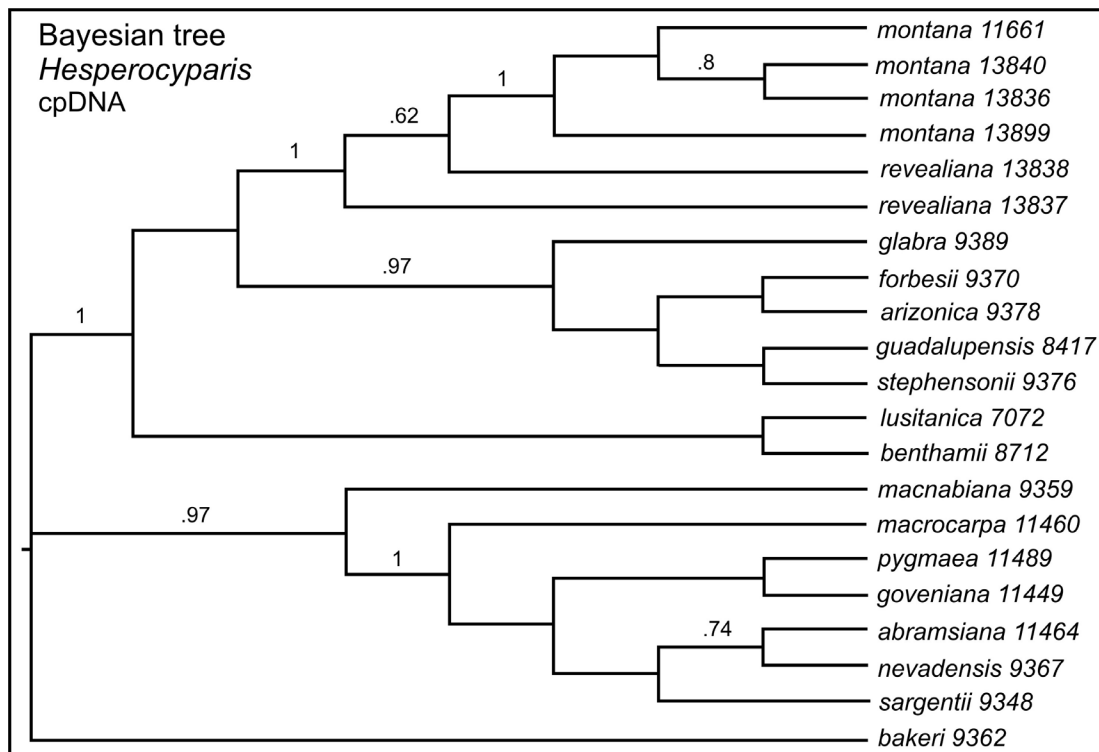


Figure 3. Bayesian tree of *Hesperocyparis* using cpDNA with *H. bakeri* as an outgroup. Numbers at nodes are posterior probabilities (PP). PP less than 0.6 are not shown.

CONCLUSIONS

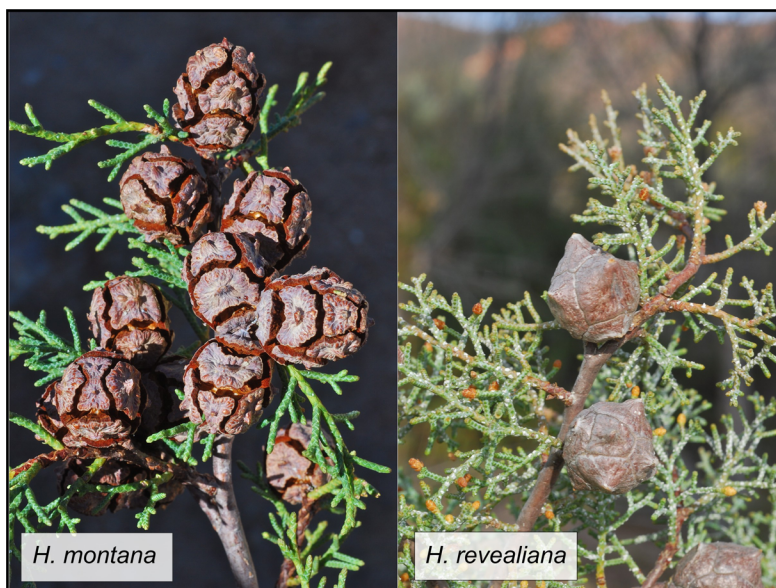
The branchlets of the samples of *H. montana* and *H. revealiana* were quite variable in terms of branching angle, color, branchlet width and especially whether the cones open or remain closed on the trees (Fig. 4). The leaf oils of each of the three taxa are quite differentiated and are as dissimilar as many *Hesperocyparis* species. The differences in leaf oil compositions and DNA among *H. montana*, *H. revealiana* and *H. stephensonii* support the recognition of these species.

The cpDNA sequencing placed *H. stephensonii* in a clade with *H. arizonica*, *H. forbesii*, and *H. guadalupensis*, separate from the clade for *H. montana* and *H. revealiana*. The generally considered close relationship between *H. montana* and *H. revealiana*, and *H. stephensonii*, was not supported by DNA sequencing. The cpDNA differs by 3 mutations, and the Bayesian analysis provide strong support for an *H. montana* + *H. revealiana* clade, as well as for a *H. montana* clade to the exclusion of *H. revealiana*. Support for *H. revealiana* separate from *H. montana* is less defined. Overall, it appears reasonable to continue the recognition of *H. montana*, *H. revealiana* and *H. stephensonii*.

Figure 4. (left) *H. montana*, with cones opened on tree.

Figure 4. (right) *H. revealiana*, with cones closed on tree.

Photos by Jeff Bisbee.



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LITERATURE CITED

- Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springer-Verlag, Berlin.
- Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 2nd ed. Allured Publ., Carol Stream, IL.

- Adams, R. P. and J. A. Bartel. 2009. Geographic variation in the leaf essential oils of *Hesperocyparis* (*Cupressus*) *abramsiana*, *H. goveniana* and *H. macrocarpa*: Systematic Implications. *Phytologia* 91(2): 226-243.
- Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the western hemisphere. *Phytologia* 91(1): 160-185.
- Adams, R. P., J. A. Bartel, D. Thornburg and A. Allgood. 2010. Geographic variation in the leaf essential oils of *Hesperocyparis arizonica* and *H. glabra*. *Phytologia* 92(3): 366-387.
- Adams, R. P. and F. Hojjati. 2013. Leaf essential oils of *Juniperus* in central and southern Iran. *Phytologia* 95(4): 288-295.
- Adams, R. P., A. N. Tashev, K. H. C. Baser and A. K. Christou. 2013. Geographic variation in the volatile leaf oils of *Juniperus excelsa* M.-Bieb. *Phytologia* 95(4): 279-285.
- Adams, R. P., T. A. Zaroni, A. L. Cambil, A. F. Barrero and L. G. Cool. 1997. Comparisons among *Cupressus arizonica* Greens, *C. benthamii* Endl., *C. lindleyi* Klotz. ex Endl. and *C. lusitanica* Mill. using leaf essential oils and DNA fingerprinting. *J. Essential Oil Res.* 9: 303-310.
- Bartel, J. A. 1993. Cupressaceae: Cypress family. *J. Ariz.-Nev. Acad. Sci.* 27:195-200.
- Bartel, J. A., R. P. Adams, S. A. James, L. E. Mumba and R. N. Pandey. 2003. Variation among *Cupressus* species from the western hemisphere based on Random Amplified Polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 31: 693-702.
- Bisbee, J. and D. Maerki. 2012. *Cupressus revealiana* (Silba) Bisbee, *comb nova* validation as a new *Cupressus* species, with notes on identification and distribution of nearby cypress species. *Bull. Cupressus Conservation Project* 1(1): 3-15.
- Cool, L. G., K. Jiang and E. Zavarin. 1994. Karahanaenone in coastal Californian *Cupressus* species. *Biochem. Syst. Ecol.* 22: 857.
- Farjon, A. 2005. A monograph of Cupressaceae and Sciadopitys. Royal Botanic Gardens, Kew Press, London.
- Little, D. P. 2005. Evolution and circumscription of the true cypresses (Cupressaceae: *Cupressus* and *Callitropsis*): a combined molecular and morphological approach. PhD. dissertation, Cornell University, Ithaca, NY. 317 pp
- Little, D. P. 2006. Evolution and circumscription of the true cypresses (Cupressaceae: *Cupressus*) *Syst. Bot.* 31: 461-480.
- Little, D. P., A. E. Schwarzbach, R. P. Adams and C-F. Hsieh. 2004. The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocyparis* (Cupressaceae). *Am. J. Bot.* 91: 1872-1881.
- Little, E.L., Jr. 1966. Varietal transfers in *Cupressus* and *Chamaecyparis*. *Madroño* 18:161-167.
- Mao, K, G. Hao, J-Q Liu, R. P. Adams and R. I. Milne. 2010. Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. *New Phytologist* 188: 254-272.
- Moran, R. 1977. Plant notes from the Sierra Juarez of Baja California, Mexico. *Phytologia* 35(3):205-227.
- Minnich, R. A. 1987. The distribution of forest trees in northern Baja California, Mexico. *Madrone* 34: 98-127.
- Müller, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Mol Phylogenet Evol* 38:667-676
- Müller K. 2005. SeqState - primer design and sequence statistics for phylogenetic DNA data sets. *Appl Bioinform* 4:65-69
- Rambaut, A. 2002. Se-Al: Sequence Alignment Editor. Available from <<http://tree.bio.ed.ac.uk/people/arambaut/>>, University of Oxford
- Rambaut, A. and A. J. Drummond. 2007. Tracer v1.4, Available from <http://beast.bio.ed.ac.uk/Tracer>
- Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574

- Senter, P., E. Zavarin and P. H. Zedler. 1975. Volatile constituents of *Cupressus stephensonii* heartwood. *Phytochemistry* 14: 2233-2235.
- Silba, J. 1981. Revised generic concepts of *Cupressus* L. (Cupressaceae). *Phytologia* 49: 390-399.
- Silba, J. 1998. A monograph of the genus *Cupressus* L. *J. Int. Conifer Preserv. Soc.* 5(2): 1-98.
- Silba, J. 2005. A monograph of the genus *Cupressus* L. in the twenty-first century. *J. Intl. Conifer Preservation Soc.* 12(2): 31-103.
- Silba, J. 2009. The taxonomy of the genus *Hesperocyparis* Bartel et Price in Mexico and Honduras (Cupressaceae). *J. Intl. Conifer Preservation Soc.* 16(2): 65-67.
- Simmons, M. P. and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst Biol* 49:369-381
- Syring, J., K. Farrell, R. Businsky, R. Cronn and A. Liston. 2007. Widespread genealogical nonmonophyly in species of *Pinus* subgenus *Strobus*. *Syst. Biol.* 56: 163-181.
- Terry, R.G., J. A. Bartel and R. P. Adams. 2012. Phylogenetic relationships among the New World cypresses (*Hesperocyparis*; Cupressaceae): evidence from noncoding chloroplast DNA sequences. *Plant Syst. Evol.* 298:1987-2000
- Thompson, J. D., D. G. Higgins and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.
- Wiggins, I.R. 1933. New plants from Baja California. *Contrib. Dudl. Herb.* 1:161-187.
- Wolf, C. B. 1948. Taxonomic and distributional studies of the New World cypresses. *Aliso* 1: 1-250.

Table 1. Leaf essential oil compositions for *H. stephensonii* (*stephen*), *H. revealiana* (*reveal*) and putative hybrids of *H. montana* 11661 and 13899 that show no complementation of compounds typical of *H. revealiana*. Also shown are composite oils of *H. montana*: (*mont*) hi cedrol(11640, 11661, 13840, 13899), lo-cedrol (13833-36, 13901-2). Components typical of wood oil (in *Juniperus* and *Cupressus*) are in italics. Components that separate the taxa are in boldface. .

KI	compound	<i>stephen</i>	<i>reveal</i>	<i>mont</i> 11661	<i>mont</i> 13899	<i>mont</i> hi-cedrol	<i>mont</i> lo-cedrol
846	(E)-2-hexenal	0.3	0.9	0.4	0.2	0.3	0.3
921	tricyclene	0.2	0.5	0.4	0.1	0.5	0.9
924	α -thujene	0.6	0.5	t	-	t	0.1
932	α-pinene	6.0	12.4	5.8	3.4	3.7	13.4
945	α -fenchene	-	t	-	-	-	0.3
946	camphene	0.5	1.1	0.5	0.1	0.5	1.1
953	thuja-2,4-diene	-	t	-	-	t	0.1
969	sabinene	9.9	4.9	0.1	t	0.1	0.4
974	β -pinene	0.2	0.4	0.5	0.3	0.3	0.9
988	myrcene	2.5	3.2	0.5	0.2	0.4	1.2
1002	α -phellandrene	0.1	0.3	t	t	t	t
1008	δ -3-carene	0.7	0.4	t	t	t	3.3
1014	α-terpinene	2.0	1.7	t	t	t	t
1020	p-cymene	0.6	0.9	t	t	t	0.5
1024	limonene	4.8	12.1	3.9	2.3	4.4	8.1
1025	β-phellandrene	4.8	12.1	2.6	1.5	2.9	5.4
1038	2-heptyl acetate	-	-	t	t	0.1	0.5
1054	γ-terpinene	3.5	2.5	0.1	t	0.1	0.3
1065	cis-sabinene hydrate	0.8	0.3	t	t	t	t
1086	terpinolene	1.9	2.5	0.3	0.1	0.3	0.8
1095	trans-sabinene hydrate	0.6	-	-	-	-	-
1087	2-nonanone	-	-	-	t	0.3	0.4
1099	linalool	0.5	-	-	-	-	0.3
1100	n-nonanal	0.2	0.1	t	t	t	0.4
1118	cis-p-menth-2-en-1-ol	0.6	0.5	-	t	-	-
1122	α -campholenal	-	-	t	0.1	t	0.4
1135	trans-pinocarveol	-	-	0.1	t	t	0.5
1136	trans-p-menth-2-en-1-ol	t	0.4	-	t	-	-
1141	camphor	9.1	11.2	0.9	0.8	3.0	7.3
1145	camphene hydrate	0.5	0.5	0.2	t	0.2	0.4
1148	citronellal	0.2	0.2	-	-	-	-
1154	karahanaenone	1.6	1.7	-	-	-	-
1165	borneol	-	-	0.3	0.1	0.2	0.9
1167	umbellulone	2.8	7.0	0.1	0.2	0.2	1.2
1174	terpinen-4-ol	8.9	6.0	0.2	0.1	0.2	0.9
1179	p-cymen-8-ol	0.2	0.4	-	t	-	0.3
1186	α -terpineol	0.7	0.6	0.1	0.1	0.1	0.3
1193	(4Z)-decenal	-	0.2	-	-	-	-
1194	myrtenol	0.1	-	-	-	-	0.3
1195	cis-piperitol	0.1	0.2	-	t	-	-
1204	verbenone	-	-	t	-	-	0.3
1207	trans-piperitol	0.3	0.2	-	t	-	-
1215	trans-carveol	-	0.2	t	0.1	-	0.3

KI	compound	<i>stephen</i>	<i>reveal</i>	<i>mont</i> <i>11661</i>	<i>mont</i> <i>13899</i>	<i>mont</i> hi-cedrol	<i>mont</i> lo-cedrol
1223	citronellol	2.1	1.5	t	0.1	0.2	0.7
1232	thymol, methyl ether	-	0.2	-	-	-	-
1239	carvone	-	-	t	0.2	0.1	0.4
1241	carvacrol, methyl ether	-	0.1	-	-	-	-
1249	piperitone	0.1	0.1	-	-	-	-
1255	(4Z)-decen-1-ol	-	0.3	-	-	-	-
1284	bornyl acetate	0.2	0.7	3.3	t	1.6	4.3
1299	terpinen-4-yl acetate	-	0.6	-	-	-	-
1325	p-mentha-1,4-dien-7-ol	0.2	-	-	-	-	-
1346	α-terpinyl acetate	0.3	1.6	-	t	-	-
1345	α -cubebene	-	-	-	-	-	0.3
1374	α-copaene	0.2	-	0.3	0.9	0.8	1.2
1410	α -cedrene	-	-	0.8	0.8	0.8	-
1413	β -funebrene	-	-	1.5	1.5	1.7	-
1419	β -cedrene	-	-	0.9	1.0	1.1	-
1451	trans-muurolo-3,5-diene	-	1.8	-	0.2	-	0.2
1452	α -humulene	0.3	-	0.3	0.4	0.3	0.2
1461	cis-cadina-1(6),4-diene	-	-	0.3	0.5	0.3	0.2
1465	cis-muurolo-4,5-diene	-	4.1	-	-	-	-
1471	dauca-3,5-diene	-	-	-	-	-	0.2
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	0.3
1478	γ-muurolene	0.4	-	0.4	0.8	0.7	1.1
1493	trans-muurolo-4(14),5-diene	-	-	-	-	-	0.3
1493	epi-cubebol	0.5	-	0.5	1.1	1.0	0.7
1500	α-muurolene	0.8	-	0.7	1.5	1.3	1.9
1501	epi-zonarene	-	1.7	-	-	-	-
1513	γ-cadinene	2.0	-	2.3	5.6	5.1	6.2
1518	endo-1-bourbonanol	1.2	-	1.3	1.3	0.8	1.3
1521	trans-calamenene	-	0.2	-	-	-	-
1522	δ-cadinene	2.9	0.3	2.9	4.5	3.7	4.6
1537	trans-cadina-1,4-diene	-	-	-	-	-	0.4
1537	α-cadinene	0.2	-	0.2	0.5	0.5	0.7
1544	α -calacorene	-	-	t	0.3	0.3	0.5
1550	cis-muurolo-5-en-4-β-ol	-	0.3	-	-	-	-
1559	cis-muurolo-5-en-4-α-ol	-	0.3	-	-	-	-
1561	(E)-nerolidol	-	0.1	-	-	-	-
1561	germacrene D-4-ol	2.4	-	2.8	2.3	2.7	1.4
1564	β -calacorene	-	-	-	-	-	0.3
1589	allo-cedrol	-	-	1.1	0.8	0.9	-
1600	cedrol	-	-	33.7	29.8	31.4	0.1
1607	β -oplophenone	0.5	-	0.3	0.6	0.9	-
1618	epi-cedrol	-	-	-	-	0.5	-
1618	1,10-di-epi-cubenol	-	-	-	-	-	0.3
1627	1-epi-cubenol	0.4	-	0.4	1.2	1.2	1.2
1632	α-acorenenol	-	0.2	-	-	-	-
1638	epi-α-cadinol	1.2	0.1	1.3	2.6	1.9	2.0
1638	epi-α-muurolol	1.2	0.1	1.2	2.6	1.8	2.0
1644	α -muurolol	0.5	-	0.4	0.8	0.7	1.0

KI	compound	<i>stephen</i>	<i>reveal</i>	<i>mont</i> <i>11661</i>	<i>mont</i> <i>13899</i>	<i>mont</i> hi-cedrol	<i>mont</i> lo-cedrol
1652	α-cadinol	3.5	0.7	2.8	5.5	3.6	3.8
1675	cadalene	-	-	-	-	-	0.5
1688	cis-14-nor-muurol-5-en-4-one	-	0.3	-	-	-	-
1689	terpenoid, <u>43</u> ,167,218,236	0.5	-	-	-	-	0.9
1699	epi-nootkatol	0.1	-	-	-	-	-
1700	amorpha-4,9-dien-2-ol	0.1	-	0.4	0.6	0.5	0.4
1724	(Z)-nuciferol	-	-	-	-	0.3	-
1739	oplopenone	0.1	-	0.3	0.8	0.4	0.4
1767	<i>cedryl acetate</i>	-	-	<i>0.1</i>	<i>0.4</i>	<i>0.1</i>	-
1887	oplopanonyl acetate	0.3	-	0.1	-	0.6	0.4
1966	isophyllocladene	-	0.1	-	-	-	-
1987	manoyl oxide	0.4	-	1.2	0.3	0.6	0.3
2009	13-epi-manoyl oxide	-	-	0.3	t	0.1	-
2055	abietatriene	0.2	t	-	-	-	-
2105	iso-abienol	2.6	-	-	-	-	-
2132	nezukol	-	-	10.8	12.0	9.7	2.6
2209	phyllocladanol	1.8	0.1	0.1	0.2	0.9	0.2
2282	sempervirol	1.1	0.1	1.1	0.2	0.5	t
2314	trans-totarol	0.8	0.1	0.9	0.1	0.6	t
2331	trans-ferruginol	0.5	0.1	0.2	0.3	0.1	t

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.