Plant Syst Evol
DOI 10.1007/s00606-012-0696-3

Phylogenetic relationships among the New World cypresses (*Hesperocyparis*; Cupressaceae): evidence from noncoding chloroplast DNA sequences

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Received: 18 October 2011 / Accepted: 20 August 2012

Abstract Nearly 5.6 kb of noncoding chloroplast DNA sequence was combined with 9.2 kb of previously published sequence in addressing phylogenetic relationships among *Callitropsis*, *Xanthocyparis*, and the New World cypresses (*Hesperocyparis*; Cupressaceae). Maximum likelihood and Bayesian analyses of aligned nucleotide sequence and coded length mutations provide strong support for several relationships. These include a clade in which *Xanthocyparis* and *Callitropsis* are successively nested at the base of a monophyletic *Hesperocyparis* and identification of *H. bakeri* as sister to the remaining *Hesperocyparis*. Two principal clades are recovered within *Hesperocyparis*; (1) the Arizonica group, which contains taxa sometimes recognized as varieties of *H. lusitanica*, *H. guadalupensis*, and *H. arizonica*, and (2) the Macrocarpa group, which contains *H. macrocarpa* and *H. goveniana* and its allies. Our results are equivocal with respect to placement of *H. macnabiana*, a morphologically distinct species resolved as the sister group to either the Macrocarpa or Arizonica group, depending on the data set analyzed. We discover many instances in which taxa recognized as varieties or closely related species are placed in disparate parts of the phylogeny. These include segregates of *H. lusitanica*, *H. guadalupensis*, and *H. arizonica*, all of which are included in clades with other species. Despite analyzing 14,799 bp of aligned sequence and 230 binary characters, we find poor support for several relationships, especially within the Arizonica group. These results suggest recovery of well-supported relationships among the closely related taxa of *Hesperocyparis* will require additional sources of evidence (e.g., morphological, biochemical characters). Implications for morphological evolution and taxonomic revision are discussed.

Keywords New World cypresses (NWC) · *Hesperocyparis* · Western cypress · Noncoding chloroplast DNA (cpDNA) · Phylogenetic relationships

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Introduction

Hesperocyparis Bartel & R. A. Price (Cupressaceae) is a group of 16 western-hemisphere species as defined by Bartel (Adams et al. 2009). Most species occur in chaparral or montane forests in the western US and northern Mexico and are characterized by populations limited to well-defined groves or "arboreal islands" (Bowers 1965, 1982). The only exception is *H. lusitanica*, which commonly occurred in narrow ecotonal forest "between fir forest and cloud forest at 2,600 m" prior to deforestation in central Mexico (Velázquez et al. 2000). Two California species, *H. abramsiana* and *H. goveniana*, are listed by the US Fish and Wildlife Service as endangered or threatened, respectively, under the Endangered Species Act of 1973 (see http://www.fws.gov/endangered/index.html). In his monograph of the group, Wolf (1948a) recognized the New World taxa as a segregate of *Cupressus* and noted that New

Published online: 25 September 2012

and Old World species did not appear closely related. Although cryptic in morphology, *Hesperocyparis* are distinguished from most eastern hemisphere species by the number of cotyledons (3–5 vs. 2, respectively), two orders of ultimate branching forming three-dimensional clusters (as opposed to flat sprays), a generally glaucous seed coat, and leaves of ultimate branch segments monomorphic (Adams et al. 2009). Little (2006) noted that no single morphological feature could reliably distinguish New and Old World cypresses and stressed the importance of character suites in delimiting the two groups. In contrast, molecular phylogenetic studies have found strong support for a split between New and Old World taxa traditionally assigned to *Cupressus* (Little et al. 2004; Xiang and Li 2005; Little 2006; Adams et al. 2009; Mao et al. 2010).

The taxonomic status of the New World cypresses (NWC) and related taxa has been unstable, having been particularly unsettled by a spate of studies published in the last decade. Analysis of 54 morphological features placed *Xanthocyparis vietnamensis* Farjon and T. H. Nguyen and *Chamaecyparis nootkatensis* (D. Don) Spach in a paraphyletic Cupressoideae, prompting the transfer of *C. nootkatensis* to *Xanthocyparis* (Farjon et al. 2002). *X. vietnamensis* is a recently described species from northern Vietnam (Averyanov et al. 2002; Farjon et al. 2002), while *C. nootkatensis* has been placed into one of four genera (*Chamaecyparis*, *Cupressus*, *Callitropsis*, and *Xanthocyparis*) by various authors (see Adams et al. 2009, Little et al. 2004; Debreczy et al. 2009 for discussions). Little et al. (2004) corroborated the close phylogenetic relationship between *X. vietnamensis* and *X. nootkatensis* and, citing nomenclatural priority, transferred both species to *Callitropsis*. A subsequent phylogenetic study placed *Callitropsis* in a well-supported clade with the NWC, although a sister-group relationship for *X. vietnamensis* and *C. nootkatensis* was either unresolved or poorly supported (Little 2006). Based on these findings, Little (2006) undertook what he considered the most conservative revisionary approach, combining all 16 New World *Cupressus* with *X. vietnamensis* and *C. nootkatensis* in an expanded *Callitropsis* and restricting the Old World species to *Cupressus*. Another option, recognizing both *Xanthocyparis* and *Callitropsis* as monotypic genera and creating a new genus for the New World species was not exercised, apparently in part because a polytomy between *Callitropsis* and NWC was interpreted to include the possibility that *X. vietnamensis*, *C. nootkatensis*, or both might be placed within the NWC clade in a more well-supported phylogeny (Little 2006). However, as acknowledged by Little (2006), neither *Callitropsis* nor *Xanthocyparis* ever nested within a consistently recovered and well-supported NWC clade. Collectively, these findings are consistent with placement of *X. vietnamensis* and *C. nootkatensis* in a distinct genus (as suggested by Xiang and Li 2005) or recognition of each as monotypic genera (as suggested by Debreczy et al. 2009; see Adams et al. 2009 for a review).

Adams et al. (2009) further examined relationships between *X. vietnamensis*, *C. nootkatensis*, and the Old and New World cypresses using data from three nuclear DNA gene regions (nrDNA ITS, 4-coumarate:CoA ligase, and abscisic acid-insensitive 3 or ABI3) and the *pet*N-*psb*M intergenic spacer (IGS) from the chloroplast genome. Results from neighbor-joining analysis of individual and combined data sets were consistent with previous findings in recognizing the New World and Old World cypresses as distinct groups (Adams et al. 2009). However, none of the analyses strongly supported a *Callitropsis–Xanthocyparis* clade, and the abscisic acid-insensitive 3 and combined data sets provided strong and moderate support respectively for a group containing *C. nootkatensis* and the NWC to the exclusion of *X. vietnamensis*. Based on these findings, Adams et al. (2009) placed the 16 NWC species per the monographic treatment of Wolf (1948a, b) in the new genus *Hesperocyparis* Bartel & R. A. Price (Table 1).

Until recently, concepts of relationships within NWC have been based largely on traditional taxonomic treatments, most of which differ on the number of species and infraspecific taxa recognized. The most comprehensive treatment of the group is that of Wolf (1948a), who recognized 16 species and 2 subspecies (Table 1). In his study, Wolf (1948a) also suggested a more reduced New World *Cupressus*, which treated *C. montana*, *C. nevadensis*, *C. glabra*, and *C. stephensonii* as subspecies of *C. arizonica* (i.e., referred to as the *C. arizonica* complex and treated as species of *Hesperocyparis* in this study), and *C. abramsiana*, *C. pigmaea*, and *C. sargentii* as subspecies of *C. goveniana* (i.e., the *C. goveniana* complex and treated as species of *Hesperocyparis* herein). Little (1970) recognized eight species and ten varieties, being largely consistent with Wolf's expanded concepts of *C. arizonica* and *C. goveniana*, in addition to recognizing *C. forbesii* as a variety of *C. guadalupensis* (Table 1). Most authors have followed either Little's (1970) or Wolf's (1948a) treatment, although some have recognized *C. benthamii* Endl. as a variety of *C. lusitanica* (Silba 1981, 1982; Farjon 1998, 2005; but see Martinez 1947; Wolf 1948a).

Several recent studies have examined relationships among NWC using molecular data. Based on results from distance analysis of randomly amplified polymorphic DNAs (RAPDs), Bartel et al. (2003) suggested taxa recognized as subspecies or varieties sensu Little (1970) be treated as distinct species. These included varieties *glabra*, *montana*, and *stephensonii* of the *C. arizonica* complex, *C. guadalupensis* var. *forbesii*, *C. lusitanica* var. *benthamii*, and varieties *pigmaea* and *abramsiana* of the *C. goveniana* complex. Moreover, an unexpectedly close relationship

Table 1 Species and varietal epithets used in taxonomic treatments of New World cypresses referenced in the text

(Wolf 1948a, b)	Little (1970)	Bartel in Adams et al. (2009)
Abramsiana	*Goveniana* var. *abramsiana*	*Abramsiana*
Arizonica	*Arizonica* var. *arizonica*	*Arizonica*
Bakeri var. *bakeri* (*typica*)	*Bakeri*	*Bakeri*
Bakeri var. *matthewsii*	(Included in species)	(Included in species)
Benthamii[a]	(= *Lusitanica*)	*Benthamii*
Forbesii	*Guadalupensis* var. *forbesii*	*Forbesii*
Glabra	*Arizonica* var. *glabra*	*Glabra*
Goveniana	*Goveniana* var. *goveniana*	*Goveniana*
Guadalupensis	*Guadalupensis* var. *guadalupensis*	*Guadalupensis*
Lindleyi[a]	*Lusitanica* (includes *benthamii*)	*Lusitanica*
Macnabiana	*Macnabiana*	*Macnabiana*
Macrocarpa	*Macrocarpa*	*Macrocarpa*
Montana	*Arizonica* var. *montana*	*Montana*
Nevadensis	*Arizonica* var. *nevadensis*	*Nevadensis*
Sygmaea	*Goveniana* var. *pigmaea*	*Pygmaea*
Sargentii	*Sargentii*	*Sargentii*
Stephensonii	*Arizonica* var. *stephensonii*	*Stephensonii*

[a] Although throughout most of Wolf's (1948a) treatment he used *lusitanica* for a broadly delineated Mexican cypress, Wolf (1948b) concedes in an epilogue to his monograph to accept Martinez' (1947) recognition of *benthamii* and *lindleyi* in lieu of *lusitanica*

between *C. goveniana* var. *pigmaea* and *C. sargentii* was recovered, as well as relationships confirming varieties *nevadensis* and *montana* as members of the *C. arizonica* complex. Little et al. (2004) used molecular, morphological, and biochemical data to examine phylogenetic relationships among Cupressoideae. Six NWC species and both species of *Xanthocyparis* (*X. vietnamensis* and *X. nootkatensis* sensu Farjon et al. 2002) were sampled as part of this study. Analysis of nuclear ribosomal ITS data provided strong support for *Xanthocyparis*, NWC, and *Xanthocyparis* + NWC, although *Xanthocyparis* collapsed to a polytomy in the combined analysis. Branch support was weak for relationships within the NWC for both the molecular and combined data (Little et al. 2004). Little (2006) expanded Little et al. (2004) by sampling additional molecular and organismic characters and by including all NWC. Maximum parsimony (MP) analysis of chloroplast DNA (cpDNA) sequences, nuclear DNA sequences (nrITS and *NEEDLY* intron 2), and combined molecular and organismic data provided strong support for a *Xanthocyparis–Callitropsis*-NWC clade and a monophyletic NWC. Only nrITS identified a *Xanthocyparis* + *Callitropsis* clade as sister to NWC, but with weak branch support. The cpDNA and combined data provided strong support for NWC sensu stricto (NWC excluding *H. bakeri*), while the nrITS and *NEEDLY* data provided weak support for or did not resolve this clade, respectively. Only in a very few instances were strongly supported relationships within NWC recovered (see Little 2006). Collectively, these findings place *Xanthocyparis* and *Callitropsis*, either as a clade (rarely) or as successively diverging taxa (usually), at the base of a well supported NWC, and suggest that *H. bakeri* may be sister to the remaining NWC, but provide little resolution of relationships among most of the NWC. Finally, in a study of phylogenetic relationships within *Juniperus*, Mao et al. (2010) included 12 NWC plus *Xanthocyparis* and *Callitropsis*. Bayesian and MP analysis of nine cpDNA regions successively nested *X. vietnamensis* and *C. nootkatensis* at the base of a well-supported NWC clade and identified *H. bakeri* as sister to a well-supported *Hesperocyparis* sensu stricto. Two well-supported clades were identified within NWC sensu stricto: one containing *H. lusitanica*, *H. forbesii*, and *H. arizonica* plus all of its sampled varieties, and the other containing *H. macnabiana*, *H. macrocarpa*, and *H. goveniana* plus sampled varieties sensu Little (1970).

In this study new data from seven noncoding chloroplast DNA regions was combined with published sequences of nuclear and other chloroplast (coding and non-coding) DNA regions to: (1) obtain well-supported relationships among NWC, (2) test the monophyly of existing taxonomic groupings (e.g., the *C. arizonica* and *C. goveniana* complexes (Little 1970; Wolf 1948a), (3) compare morphologically based concepts of relationships (sensu Wolf 1948a) with the molecular phylogeny in exploring implications for taxonomic revision of *Hesperocyparis*, and (4) identify the sister group to NWC, i.e., is *C. nootkatensis* or a *C. nootkatensis* + *X. vietnamensis* clade sister to NWC?

Materials and methods

Plant material

Single accessions of all 16 NWC species (*Hesperocyparis* sensu Adams et al. 2009), 3 species of *Cupressus* (Old World cypresses or OWC), 3 species of *Juniperus*, and the monotypic *Xanthocyparis* and *Callitropsis* were included in the present study (Table 2). For nucleic acid extraction, approximately 1 g (fresh weight) of leaf tissue was placed in 20 g of activated silica gel in the field and subsequently stored at −20 °C in the laboratory. Voucher specimens are deposited at BAYLU and LAMU, respectively.

DNA extraction, PCR amplification, and DNA sequencing

Total genomic DNA was extracted from 0.020 g of silica dried leaf tissue using a DNeasy Plant Mini Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). The chloroplast regions *trn*S–*trn*G IGS and the *trn*G intron were amplified as a contiguous fragment using terminal primers $trnS^{GCU}$ and $3'trnG^{UUC}$ of Shaw et al. (2005). The *trn*C–*trn*D IGS was amplified using primers CD10F and CD3R of Adams (2007). The *psb*D–*trn*T IGS, *trn*T–*trn*D IGS, *ycf*3–*psa*A IGS, and the second intron of *ycf*3 were amplified as contiguous fragments using primers designed for this study. Sequences for terminal primers used in amplification and sequencing as well as internal primers used in sequencing larger templates (i.e., the *trn*S–*trn*G and *ycf*3-*psa*A spacers) are given in Table 3. PCR was performed in 50-μl volumes containing 1 μM of each primer, 0.2 mM of dNTP mix, and 1.25 U of TAQ polymerase. Magnesium chloride concentrations and annealing temperatures were optimized for each PCR primer pair (Table 3). Thermal cycling protocols for all amplifications excluding *trn*S–*trn*G were as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 2 min at the optimized annealing temperature, and 72 °C for 2 min, followed by 72 °C for 7 min. Thermal cycling conditions for the *trn*S–*trn*G IGS were according to protocol 1 of Shaw et al. (2005). All PCR was performed using GoTAQ Core System I polymerase and reagents (Promega Corp., Madison, WI, USA).

PCR products were microconcentrated, electrophoresed in 1 % agarose gels containing 1 μg/ml ethidium bromide, and visualized under UV illumination. Sequencing templates were excised in agarose, column purified according to the manufacturer's protocol (Wizard SV Gel and PCR Clean-Up System, Promega Corp., Madison, WI, USA), and sequenced using v.3.1 Big Dye Terminators (Applied Biosystems, Foster City, CA, USA) on an ABI 310 Genetic Analyzer or an ABI 3730 DNA Sequencer (MCLAB Inc., San Francisco, CA, USA).

Phylogenetic analysis

A total of 5,598 bp of unambiguously aligned sequence from *Xanthocyparis*, *Callitropsis*, all species of *Hesperocyparis*, and six outgroups were newly obtained in this study. All sequences are from chloroplast noncoding regions, including 4,091 bp from five IGSs and 1,507 bp from two introns. Sequences were obtained for all taxa by gene region combinations targeted in this study, except for the *trn*S–*trn*G IGS and *trn*G intron for *H. macrocarpa* and the *trn*D–*trn*T IGS for *C. atlantica*, which did not amplify successfully using the primer combinations and amplification conditions described. A summary of results from the seven chloroplast noncoding regions is provided in Table 4. Uncorrected pair-wise distances between taxa were calculated using PAUP*v.4.0b10 (Swofford 2002).

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 (Müller 2005, 2006). Sequences were readily aligned by inserting gaps usually of a few nucleotides in length. Some of the larger length mutations included an 80-bp indel in the *psb*D-*trn*T IGS distinguishing *Juniperus* from all other taxa, a 24-bp indel in the *psb*D-*trn*T IGS distinguishing *J. grandis* and *J. osteosperma* from all other taxa, a 24-bp indel in the *trn*T–*trn*D IGS distinguishing species of *Hesperocyparis* sensu stricto (*Hesperocyparis* excluding *H. bakeri*) from all other taxa, a 23-bp indel in the *trn*T–*trn*D IGS distinguishing species of the Macrocarpa group of *Hesperocyparis* from all other taxa, and indels of 63 and 31 bp in the *ycf*3–*psa*A IGS distinguishing *Cupressus* and *Juniperus* respectively from all other taxa. All nucleotides were included in the final alignment excluding 101 positions within the *trn*S–*trn*G IGS that could not be aligned unambiguously.

Combining data from this study with chloroplast and nuclear sequences from GenBank produced 14,799 bp of aligned sequence and 230 binary characters. The matrix included sequences from 12 noncoding chloroplast regions (9 IGSs and 3 introns), 2 chloroplast genes (*rbc*L and *psb*B), and 2 nuclear genes (nrITS and *NEEDLY* intron 2). Sequences not available in GenBank for taxa included here were scored as missing. These included *trn*K–*mat*K, *rbc*L, *trn*L–*trn*F, nrITS, and *NEEDLY* sequences for *J. grandis*, and the *rps*4-*trn*S, *psb*B, *pet*B-*pet*D, and *trn*V intron sequences for *J. grandis*, *C. dupreziana*, *H. benthamii*, *H. guadalupensis*, *H. nevadensis*, and *H. pigmaea* (Table 2).

Table 2 Taxa included in the present study, with collection number and locality or source data, and GenBank accession

Taxon[a]	Voucher information/source	GenBank accession (this study)						
		*psb*A–*ycf*3	*psb*D–*trn*T	*trn*C–*trn*D	*trn*D–*trn*T	*trn*S–*trn*G	*trn*G intron	*ycf*3 intron 2
Callitropsis nootkatensis	Adams 9086/WA, USA	JQ740466	JQ740514	JQ740490	JQ740419	JQ740538	JQ740396	JQ740442
Xanthocyparis vietnamensis	Adams 10142/Vietnam	JQ740467	JQ740515	JQ740491	JQ740420	JQ740539	JQ740397	JQ740443
Cupressus atlantica	Adams 8429/Morocco	JQ740487	JQ740535	JQ740511	NA	JQ740558	JQ740416	JQ740463
Cupressus dupreziana	Adams 8432/Algeria (ex Hillier Gardens)	JQ740488	JQ740536	JQ740512	JQ740440	JQ740559	JQ740417	JQ740464
Cupressus sempervirens	Adams 8434/ Elburz Mtns., Iran	JQ740489	JQ740537	JQ740513	JQ740441	JQ740560	JQ740418	JQ740465
Hesperocyparis abramsiana	Adams 11464/CA; USA	JQ740477	JQ740525	JQ740501	JQ740430	JQ740548	JQ740406	JQ740453
Hesperocyparis arizonica	Adams 9378/Pima Co., AZ; USA	JQ740481	JQ740529	JQ740505	JQ740434	JQ740552	JQ740410	JQ740457
Hesperocyparis bakeri	Adams 9362/CA; USA	JQ740468	JQ740516	JQ740492	JQ740421	JQ740540	JQ740398	JQ740444
Hesperocyparis benthamii	Adams 8712/Pachuca, Mexico	JQ740474	JQ740522	JQ740498	JQ740427	JQ740545	JQ740404	JQ740450
Hesperocyparis forbesii	Adams 9370/San Diego Co., CA; USA	JQ740486	JQ740534	JQ740510	JQ740439	JQ740557	JQ740415	JQ740462
Hesperocyparis glabra	Adams 9389/Gila, Co., AZ; USA	JQ740473	JQ740521	JQ740497	JQ740426	JQ740544	JQ740402	JQ740449
Hesperocyparis goveniana	Adams 11449/Monterey Co., CA; USA	JQ740482	JQ740530	JQ740506	JQ740435	JQ740553	JQ740411	JQ740458
Hesperocyparis guadalupensis	Adams 8417/Guadalupe Island, Mexico (ex Berkeley Botanical Garden)	JQ740483	JQ740531	JQ740507	JQ740436	JQ740554	JQ740412	JQ740459
Hesperocyparis lusitanica	Adams 7072/Bussaco, Portugal (cultivated)	JQ740475	JQ740523	JQ740499	JQ740428	JQ740546	JQ740404	JQ740451
Hesperocyparis macnabiana	Adams 9359/Napa Co., CA; USA	JQ740480	JQ740528	JQ740504	JQ740433	JQ740551	JQ740409	JQ740456
Hesperocyparis macrocarpa	Adams 11460/Crocker Grove, CA; USA	JQ740472	JQ740520	JQ740496	JQ740425	NA	NA	JQ740448
Hesperocyparis montana	Adams 9660/Baja, CA; USA	JQ740476	JQ740524	JQ740500	JQ740429	JQ740547	JQ740405	JQ740452
Hesperocyparis nevadensis	Adams 9367/Kern Co., CA; USA	JQ740478	JQ740526	JQ740502	JQ740431	JQ740549	JQ740407	JQ740454
Hesperocyparis pigmaea	Adams 11489/CA; USA	JQ740484	JQ740532	JQ740508	JQ740437	JQ740555	JQ740413	JQ740460
Hesperocyparis sargentii	Adams 9348/San Luis Obispo Co., CA; USA	JQ740479	JQ740527	JQ740503	JQ740432	JQ740550	JQ740408	JQ740455
Hesperocyparis stephensonii	Adams 9376/San Diego Co., CA; USA	JQ740485	JQ740533	JQ740509	JQ740438	JQ740556	JQ740414	JQ740461
Juniperus grandis	Terry 115/Mono Co., CA; USA	JQ740469	JQ740517	JQ740493	JQ740422	JQ740541	JQ740399	JQ740445
Juniperus occidentalis	Terry 128/Baker Co., OR; USA	JQ740470	JQ740518	JQ740494	JQ740423	JQ740542	JQ740400	JQ740446
Juniperis osteosperma	Terry 058/Garfield Co., UT; USA	JQ740471	JQ740519	JQ740495	JQ740424	JQ740543	JQ740401	JQ740447

Taxon[a]	GenBank accession (other studies)								
	*rps*4–*trn*S	*psb*B	*pet*B–*pet*D	*trn* V intron	*trn* L–*trn* F	*trn* K–*mat* K	*rbc* L	nrITS	*NEEDLY* intron 2
Callitropsis nootkatensis	HM024353	HM024173	HM024171	HM023885	AY988207	FJ475239	AF127431	AY380858	AY988304
Xanthocyparis vietnamensis	HM024447	HM024267	HM024170	HM023979	HM008378	AY380850	AY380895	AY380877	AY988329
Cupressus atlantica	HM024360	HM024180	HM024083	HM023892	AY988182	AY988335	AY988235	AY988367	AY988280
Cupressus dupreziana	NA	NA	NA	NA	AY988191	AY988342	AY988243	AY988375	AY988290
Cupressus sempervirens	HM024367	HM024187	HM024090	HM023899	AY988212	AF152187	L12571	FJ705221	AY988306
Hesperocyparis abramsiana	HM024370	HM024190	HM024093	HM023902	AY988179	AY988333	AY988234	FJ705220	AY988277

Table 2 continued

Taxon[a]	GenBank accession (other studies)								
	*rps4–trn*S	*psb*B	*pet*B–*pet*D	*trn* V intron	*trn* L–*trn* F	*trn* K–*mat* K	*rbc* L	nrITS	*NEEDLY* intron 2
Hesperocyparis arizonica	HM024371	HM024191	HM024094	HM023903	AY988181	AY380845	AY380886	CAU77961	AY988278
Hesperocyparis bakeri	HM024372	HM024192	HM024095	HM023904	AY988184	AY988337	AY988237	AY988369	AY988282
Hesperocyparis benthamii	NA	NA	NA	NA	AY988185	AY988338	AY988238	AY988370	AY988284
Hesperocyparis forbesii	HM024373	HM024193	HM024096	HM023905	AY988192	AY988343	AY988244	CFU60752	AY988291
Hesperocyparis glabra	HM024374	HM024194	HM024097	HM023906	AY988196	AY988347	AY988247	CGU60748	AY988295
Hesperocyparis goveniana	HM024375	HM024195	HM024098	HM023907	AY988197	AY380846	AY380888	AY380865	AY988296
Hesperocyparis guadalupensis	NA	NA	NA	NA	AY988198	AY988348	AY988248	AY988381	AY988297
Hesperocyparis lusitanica	HM024376	HM024196	HM024099	HM023908	AY988200	AY988351	AY988250	AY988383	AY988300
Hesperocyparis macnabiana	HM024377	HM024197	HM024100	HM023909	AY988203	AY380848	AY380890	AY380867	AY497212
Hesperocyparis macrocarpa	HM024378	HM024198	HM024101	HM023910	AY988204	AY380849	AY380891	AY380868	AY988301
Hesperocyparis montana	HM024379	HM024199	HM024102	HM023911	AY988205	AY988352	AY988252	CMU60753	AY988302
Hesperocyparis nevadensis	NA	NA	NA	NA	AY988206	AY988353	AY988253	CNU60750	AY988303
Hesperocyparis pigmaea	NA	NA	NA	NA	AY988209	AF152192	AY380892	FJ705219	AY988305
Hesperocyparis sargentii	HM024380	HM024200	HM024103	HM023912	AY988211	AY497215	AY988254	CSU60749	AY497211
Hesperocyparis stephensonii	HM024381	HM024201	HM024104	HM023913	AY988213	AY988354	AY988255	CSU60751	AY988308
Juniperus grandis	NA	NA	NA	NA	NA	NA	NA	NA	NA
Juniperus occidentalis	HM024412	HM024232	HM024135	HM023944	AF211517	AY988362	AY988263	EU277695	AY988319
Juniperis osteosperma	HM024413	HM024233	HM024136	HM023945	AF211509	AY988363	AY988264	EU277693	AY988320

[a] All Hesperocyparis are according to Bartel (see Adams et al. 2009)

Table 3 Primers used for amplification and sequencing in this study

Region	Reference	Primer	Sequence (5′-3′)	Annealing temp (°C)[a]	$[MgCl_2]$[b]
*trn*S–*trn*G	Shaw et al. (2005)	*trn*S(F)	AGATAGGGATTCGAACCCTCGGT	66	1.5
	Shaw et al. (2005)	3′*trn*G(R)	GTAGCGGGAATCGAACCCGCATC	66	
	This study	*trn*S1(F)	TCTGTCATAAAGAAAAACTAATTCCAA		
	Shaw et al. (2005)	5′*trn*G2G(F)	GCGGGTATAGTTTAGTGGTAAAA		
*ycf*3–psaA	This study	*ycf*3-903F	CCATGCGACCGGAAATTGACCCCT	53	2.0
	This Study	*psa*A(R)	ATGATCTTTACTTCTGGTTCCGGTGA	53	
	This study	*ycf*3-1843F	GCTCCAAGCAATTATATCGAAGCACA		
	This study	*ycf*3-1843R	TGTGCTTCGATATAATTGCTTGGAGC		
*psb*D–*trn*T	This study	*psb*D(F)	GCAAAATAAGCACAAGGAAAAA	47.5	3.0
	This study	*trn*T(R)	GTAAGGCGTAAGTCATCGGTTC	47.5	
*trn*T–*trn*D	This study	*trn*T(F)	GAACCGATGACTTACGCCTTAC	50	1.5
	This study	*trn*D(R)	CTTGACAGGGCGGTACTCTAAC	50	
*trn*C–*trn*D	Adams (2007)	CD10F	AAAGAGAGGGATTCGTATGGA	50	3.5
	Adams (2007)	CD3R	AACGAAGCGAAAATCAATCA	50	

[a] Annealing temperatures are given for terminal primer pairs used in PCR

[b] Concentrations are in mM

Table 4 Summary of results from seven noncoding plastid regions sequenced in this study

Data summary	*trn*S–*trn*G	*trn*G Intron	*trn*C–*trn*D	*psb*D–*trn*T	*trn*T–*trn*D	*ycf*3 Intron 2	*ycf*3–*psa*A
Unaligned length (bp)	785–943	631–641	786–844	914–975	634–688	856–864	482–544
Aligned length (bp)	869	641	856	995	800	866	571
Excluded sites (bp)	101	0	0	0	0	0	0
Uncorrected pairwise distances[a]	0–0.012	0–0.013	0–0.016	0–0.023	0–0.011	0–0.005	0–0.017
No. gaps scored	23	5	20	24	20	6	20
No. accessions not sequenced (of 24)	1	1	0	0	1	0	0

[a] Distances are for ingroup taxa only

All together, about 9.4 % of the aligned sequence was scored as missing. Of the missing binary data, about 4.9 % was due to missing sequence in the alignment, with the remainder attributable to indels completely overlapped by longer length mutations and scored as inapplicable in simple indel coding (Simmons and Ochoterena 2000). Of the sequences from GenBank, 13 were from taxa of the same collection as presented here (5 from *X. vietnamensis*, 5 from *H. lusitanica*, and 3 from *C. sempervirens)*. All other sequences published here are from collection numbers unique to this study. All sequences from this study have been deposited in GenBank (Table 2). A data matrix of aligned nucleotides and binary characters and associated trees is available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S12614).

Phylogenetic incongruence between the chloroplast and nuclear data sets was assessed by performing individual Bayesian analyses on aligned sequences without binary characters. Trees resulting from analyses of the "all-chloroplast" versus "all-nuclear" data sets were compared for differences in clades having posterior probabilities (*PP*) greater than 0.90. Because well-supported ($PP > 0.90$) differences were not observed in trees resulting from the two individual analyses, we combined the nuclear and chloroplast data sets into a concatenated matrix, which was then analyzed under maximum likelihood and Bayesian methods, both with and without gaps.

Maximum likelihood (ML) analyses were performed with raxmlGUI (Stamatakis 2006; Silvestro and Michalak 2011) under the GTR + G (general time reversible with gamma distributed rates) model. Trees were generated in each run by swapping on parsimony-generated starting trees randomized by stepwise addition of taxa with the data partitioned by gene region and branch lengths on the best tree optimized on a per partition basis. In an initial analysis, 500 replicates of ML analysis were performed using the "ML search" command in RAxML. This analysis produced a single best tree as well as 500 trees from which

a majority-rule consensus tree was constructed. Support for clades was assessed by non-parametric bootstrap (Felsenstein 1985) in RAxML using the ML + thorough bootstrap option and 1,000 bootstrap replicates.

Bayesian analyses were performed using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003). Best-fit evolutionary models were estimated for individual gene regions using the Akaike information criterion (AIC) implemented in the software jModelTest v.0.0.1 (Posada 2008; Guindon and Gascuel 2003) using the default settings for likelihood calculations and the uncorrected AIC. Based on results from jModelTest, the 16 gene regions included here were assigned to one of five models in the Bayesian analyses; JC + G (*trn*G intron), K80 (*psb*B), K80 + G (*rbc*L), GTR (*trn*L–*trn*F, *psb*D–*trn*T, *NEEDLY* intron 2, *trn*V intron, and *ycf*3 intron 2), and GTR + G (nrITS, *trn*K–*mat*K, *trn*S–*trn*G, *pet*B–*pet*D, *trn*C–*trn*D, *rps*4–*trn*S, *trn*T–*trn*D, and *psa*A–*ycf*3). We performed a heterogeneous Bayesian analysis in which each gene was allowed to evolve under its own substitution model by partitioning the data set by gene region, and unlinking the model parameter for each partition. The overall rate of substitution was also allowed to vary among partitions by using the rate multiplier option "prset rate = variable" in MrBayes. For the gap partition, we used the restriction site model with gamma variation in rates and the ascertainment coding bias set to variable, as recommended in the MrBayes manual (http://mrbayes.sourceforge.net/mb3.2manual.pdf).

For the Bayesian analysis, two independent runs of four Metropolis coupled chains each were run from different random trees for 5 million generations, sampling every 1,000th generation. In each run, three chains were heated using a temperature of 0.2 with one swap between chains every generation. The burnin fraction was enforced to 0.2 using the "relburnin" command, resulting in the first 1,000 of 5,000 trees being discarded, and the remaining trees (4,000) 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 and mixing were assessed by examining plots of likelihood values against chain generation over the course of the run and by monitoring the standard deviation of split frequencies among runs in MrBayes.

Previous studies are equivocal with respect to the sister group of the *Xanthocyparis* + *Callitropsis* + NWC clade. *Juniperus* is most often resolved as the sister group (Little et al. 2004; Little 2006; Adams et al. 2009), but sometimes a *Juniperus*-Old World cypress clade (Little 2006) or less often the OWC alone (Adams et al. 2009), depending on the data set and method of analysis. In this study, ML analyses were rooted using as outgroup three species each of *Juniperus* and *Cupressus*, while Bayesian trees were rooted with *J. occidentalis*.

Results

No significant phylogenetic incongruence was found between the individual Bayesian analyses of the nuclear and chloroplast data sets (results not shown). The only difference between the two consensus trees was found in the position of *H. goveniana*, *H. nevadensis*, *H. sargentii*, and *H. macnabiana*, which were included in the Arizonica group in the nuclear data set and in the Macrocarpa group in the chloroplast and combined (nuclear + chloroplast) data sets. However, none of the clades in the nuclear consensus tree have $PP > 0.81$, and many relationships were unresolved. The consensus tree of the chloroplast data set was topologically very similar to that of the combined data set with respect to clades with $PP > 0.90$, but in general the combined data set showed better resolution and higher *PP* values (>0.98).

For the combined data set, maximum likelihood and Bayesian analyses excluding gaps produced topologically identical trees. Results from the ML analysis of combined data (excluding gaps) are presented in Fig. 1. The best tree from 500 ML replicates is presented, which has the same topology as the 50 % majority-rule tree of the individual replicates. The maximum likelihood tree provides strong support for several clades including the ingroup (*Xanthocyparis*, *Callitropsis*, and *Hesperocyparis*), *Hesperocyparis*, and *Hesperocyparis* sensu stricto (Fig. 1). *Xanthocyparis* and *Callitropsis* are successively nested at the base of *Hesperocyparis*, and *H. bakeri* is recovered as the first lineage within the genus. Two main groups within *Hesperocyparis* are recovered; one (the Arizonica group) with strong support (bootstrap = 99), and the other (the Macrocarpa group) with moderate support (bootstrap = 84). Several relationships within these two clades are weakly supported (bootstrap value <70 %) or unresolved in the ML tree. Exceptions include the *H. nevadensis–H. sargentii* and *H. macrocarpa–H. pigmaea* clades, which are moderately supported with bootstrap values of 83 and 79, respectively.

The 50 % majority-rule consensus tree from Bayesian analysis of the combined chloroplast and nuclear data including gaps is presented in Fig. 2. The topology of the Bayesian tree is similar to that of the ML tree with most clades supported by $PP \geq 0.95$ (Figs. 1, 2). Relationships unique to the Bayesian tree include recovery of *H. macnabiana* as sister to the Arizonica group ($PP = 0.81$), and a sister group relationship between the *H. abramsiana–H. goveniana* and *H. nevadensis–H. sargentii* clades within the Macrocarpa group ($PP = 1.0$; Fig. 2). The Bayesian tree also resolves a three-species polytomy (includes *H. glabra*, *H. guadalupensis*, and *H. forbesii*) present in the ML tree, but provides weak support ($PP = 0.80$) for a *H. forbesii–H. glabra* clade within this group (Fig. 2).

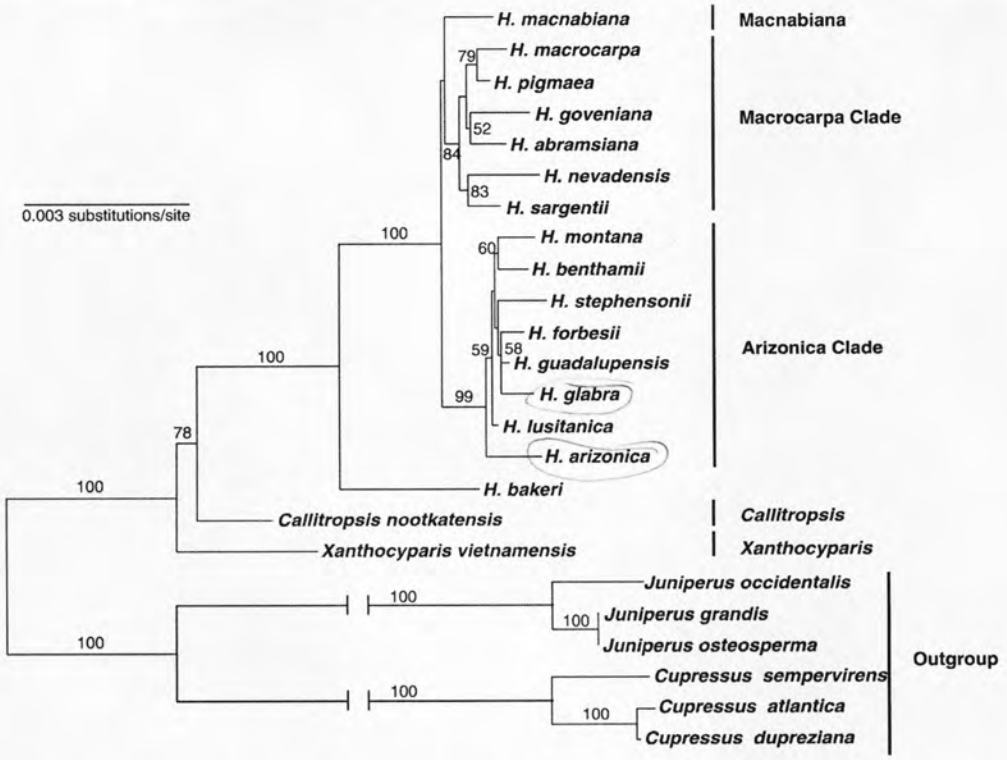
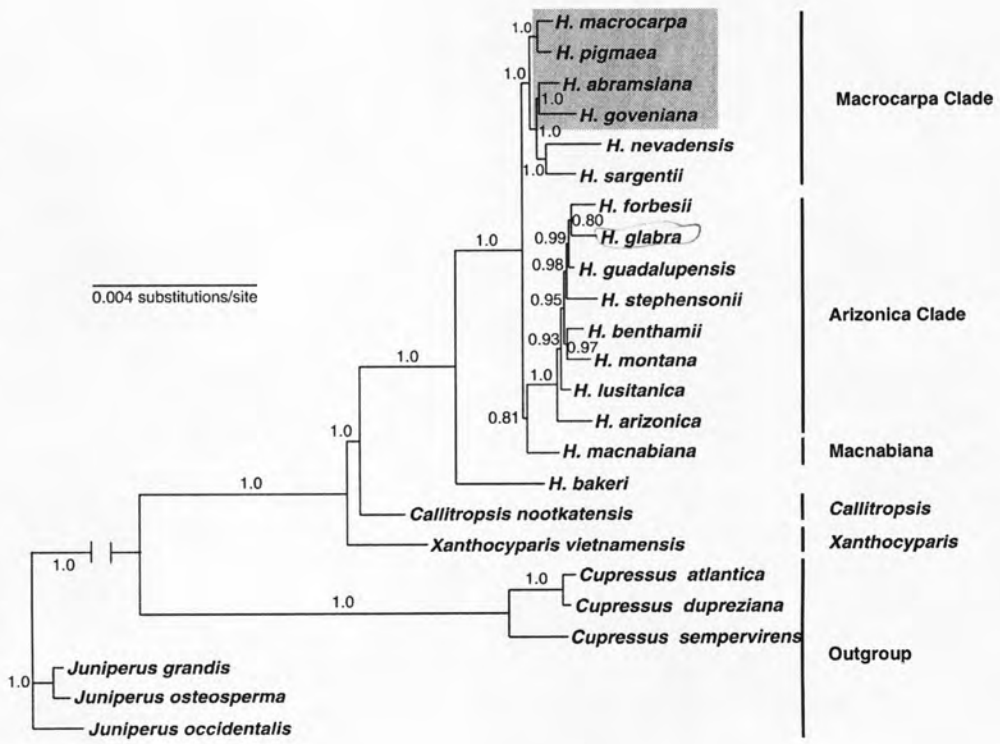


Fig. 1 The best tree from 500 maximum likelihood replicates for the combined chloroplast and nuclear sequences excluding indels. This tree has the same topology as the 50 % majority-rule consensus of the individual replicates. Bootstrap values of at least 50 % are provided along branches. Interrupted branches are not drawn to scale

Fig. 2 The 50 % majority-rule consensus tree resulting from Bayesian analysis of combined chloroplast and nuclear sequences, including 230 binary characters resulting from simple indel coding of length mutations. Posterior probabilities are displayed along branches. The shaded box indicates clades that are sister groups in Fig. 1. Interrupted branches are not drawn to scale



Discussion

Congruence with previous studies and unique findings

We report findings from an integrative analysis of nearly 14.8 kb of aligned DNA sequence and coded length mutations in examining phylogenetic relationships among *Xanthocyparis*, *Callitropsis*, and the NWC. Results presented here corroborate many of those from previous phylogenetic studies of the group, including strong support for a *Xanthocyparis–Callitropsis–Hesperocyparis* clade, strong support for a monophyletic *Hesperocyparis*, and identification of *H. bakeri* as the first lineage in the genus. However, previous studies found little support for relationships within Hesperocyparis sensu stricto (Little 2006). Here, we find moderate to strong support for division of *Hesperocyparis* into two major clades (the Arizonica and Macrocarpa groups), resolution of a clade of four species (*H. glabra*, *H. guadalupensis*, *H. forbesii*, and *H. stephensonii*) within the Arizonica group, recovery of *H. benthamii* and *H. montana* as sister taxa, as well as recovery of *H. macrocarpa–H. pigmaea* and *H. nevadensis–H. sargentii* clades within the Macrocarpa group (Figs. 1, 2).

Placement of *Xanthocyparis* and *Callitropsis*

Like previous studies, this study finds strong support for a clade containing *Xanthocyparis*, *Callitropsis* and *Hesperocyparis* (Adams et al. 2009; Little 2006; Little et al. 2004; Figs. 1, 2). However, and like nearly all previous phylogenetic studies based on molecular data alone (Adams et al. 2009; Little 2006), we find no support for the monophyly of *Xanthocyparis* + *Callitropsis*. The only exceptions are the nrITS and 4-coumarate CoA ligase data of Adams et al. (2009), both of which supported the monophyly of *Xanthocyparis* + *Callitropsis* with bootstrap values of 75 and 31, respectively. In an analysis combining molecular and organismal data, Little et al. (2004) identified two morphological features that potentially support the relationship; primarily apically distributed ultimate branches and externally dimorphic mature leaves. However, using combined molecular and organismal data and increased sampling of both types of characters, Little (2006) found no support for the clade in the strict consensus of 12 most-parsimonious trees. Unlike molecular data, analyses of morphological data alone have sometimes supported *Callitropsis* and *Xanthocyparis* as sister taxa, depending on the data set and method of analysis. Farjon et al. (2002) analyzed 54 morphological characters in placing *C. nootkatensis* with *X. vietnamensis* in *Xanthocyparis*, a revision that was not supported by phenetic analysis of epidermal features (Xiang and Farjon 2003). Parsimony analysis of the epidermal features of Xiang and Farjon (2003) resulted in a completely unresolved consensus tree (unpublished data of Little et al. 2004). Both species were later transferred to *Callitropsis* based on nomenclatural priority and results from analysis of combined molecular and organismal data (Little et al. 2004). Considering habitat preferences and the disjunct geographic distributions of these species (i.e., *C. nootkatensis* occupies coastal environments in western North America, and *X. vietnamensis* is found on limestone substrates in northern Vietnam), the distinct leaf and ovulate cone scale characteristics of each (Little 2006), and the lack of support for a sister relationship by molecular data (Figs. 1, 2), we concur with Mill and Farjon (2006) that *Xanthocyparis* be conserved against *Callitropsis*. However, we distinguish between the distinctiveness of *Xanthocyparis* and *Callitropsis* and clear identification of the sister group of *Hesperocyparis*. Thus, although we find strong support for the monophyly of *Xanthocyparis* + *Callitropsis* + *Hesperocyparis*, support for *Callitropsis* + *Hesperocyparis* is weak in the ML tree (bootstrap value of 78; Fig. 1), but more strongly supported by Bayesian analysis ($PP = 1.0$; Fig. 2). Similarly, Adams et al. (2009) recovered the same topology as presented here with respect to placements of *Xanthocyparis*, *Callitropsis*, and *Hesperocyparis*, with a bootstrap support value of 80 for the *Callitropsis* + *Hesperocyparis* clade. Long branches support *Xanthocyparis*, *Callitropsis*, and *Hesperocyparis* (Figs. 1, 2; also see Little 2006), and examining the possible effects of long branch attraction on the inferred relationships among these genera may be informative.

H. bakeri is sister to the remainder of *Hesperocyparis*

Like all previous phylogenetic studies including NWC (Mao et al. 2010; Adams et al. 2009; Little 2006; Little et al. 2004), results presented here strongly support a monophyletic *Hesperocyparis* and, for those studies in which the species was included (Mao et al. 2010; Little 2006), identify *H. bakeri* as the sister group to the remaining *Hesperocyparis*. *H. bakeri* was first described by Jepson (1910) as a species of *Cupressus*, but several authors subsequently placed it in synonymy with *C. macnabiana* (Sudworth 1927; Abrams 1923; Jepson 1923; Sargent 1922). Later treatments followed Jepson (1910) in recognizing the distinctiveness of the species (Little 1953, 1966, 1970; Wolf 1948a). Distinguishing features of *H. bakeri* include slender (<1.3 mm in diameter) branches, narrow, open crowns, and small (10–20 mm in diameter) ovulate cones (Wolf 1948a). Wolf (1948a) suggested a close relationship between what is now *H. bakeri* and species of the *C. arizonica* species complex (*H. arizonica*, *H. glabra*, *H. montana*, *H. stephensonii*, and particularly,

H. nevadensis sensu Bartel in Adams et al. 2009). Features uniting these species in the treatment of Wolf (1948a) include active adaxial leaf glands, branchlets more or less evenly or irregularly disposed around branches, and exfoliating brown to cherry-red bark. Little (2006) discovered three morphological characters autapomorphic for *H. bakeri*: non-fibrous bark, bark exfoliating in irregular plates, and marginal leaf band constricted at the apex. The bark characters were found homoplasious and apomorphic for three species of the *C. arizonica complex* (*C. montana*, *C. glabra*, and *C. stephensonii*). The occurrence of species traditionally assigned to the *C. arizonica complex* in widely divergent clades (Figs. 1, 2) in this study is consistent with the findings of Little (2006) in supporting homoplasy in many characters shared between *H. bakeri* and species of the *C. arizonica complex*. In addition, the distribution of character states in outgroup taxa (e.g., *Juniperus*) suggests taxonomically important characters linking *H. bakeri* and the *C. arizonica complex*, as well as many of those used in *Hesperocyparis* taxonomy in general, may be symplesiomorphic.

The Arizonica and Macrocarpa groups

Two major clades were recovered within *Hesperocyparis* sensu stricto, the Arizonica and Macrocarpa groups (Figs. 1, 2). The Arizonica group contains taxa sometimes recognized as varieties of *H. lusitanica* (Farjon 1998; Silba 1981, 1982), *H. guadalupensis* (Little 1953, 1970; Sudworth 1927; Sargent 1922) and *H. arizonica* (i.e., the *C. arizonica* complex of Little 1970; Table 1). The Macrocarpa group contains *H. macrocarpa* and *H. goveniana* and its allies (i.e., the *C. goveniana* complex of Little 1970; see Wolf 1948a for discussion; Figs. 1, 2). Although we have sampled more taxa here, this finding is congruent with that of Mao et al. (2010), who recovered taxonomically less inclusive versions of both clades with moderate support (i.e., MP bootstrap values of 87 and 86). No single character important in *Hesperocyparis* taxonomy is diagnostic for the Arizonica group. All except for *H. lusitanica* and *H. benthamii* have cherry red or mahogany brown exfoliating bark, and all the *C. arizonica complex* members have exudating adaxial leaf glands (Wolf 1948a). In contrast, all members of the Macrocarpa group are characterized by fibrous gray bark, and all but *H. nevadensis* have inactive adaxial leaf glands and coastal distributions (Griffin and Critchfield 1972; Wolf 1948a).

Our results are equivocal with respect to placement of *H. macnabiana*, which is resolved with weak support as sister to either the Macrocarpa or Arizonica groups in ML and Bayesian analyses, respectively, depending on the data set analyzed (see "Results"; Figs. 1, 2). *H. macnabiana* has branchlets arranged in planar sprays, a distinctive feature apparently originating in New World (*H. macnabiana* and *H. benthamii*) and Old World (*C. funebris*) taxa convergently. In addition, *H. macnabiana* "has foliage [that] is very fragrant, perhaps more so than any other species of North America" (Wolf 1948a). Wolf (1948a) noted characters suggesting affiliation with both the *C. goveniana* (gray bark of fibrous texture that is non-exfoliating) and *C. arizonica* (dorsal leaf glands that actively secrete) complexes in suggesting *H. macnabiana* was not closely related to any other North American cypress.

None of the relationships recovered from within the Arizonica group are completely consistent with any of the traditionally held infraspecific concepts of *H. lusitanica*, *H. guadalupensis*, and *H. arizonica*, with clades often containing one or more members of the *C. arizonica* complex and varieties of either *H. lusitanica* or *H. guadalupensis* (Figs. 1, 2). For example, we identify a clade of four species (*H. stephensonii, H. guadalupensis, H. glabra*, and *H. forbesii*) recognized as varieties of *C. arizonica* and *C. guadalupensis* (Figs. 1, 2). Support for this lineage is weak (bootstrap value <50 %; Fig. 1) in the ML tree but stronger in the Bayesian tree ($PP = 0.98$; Fig. 2). Little (2006) recovered the same clade in an analysis of combined molecular and organismal data and identified two synapomorphic characters for the group; orange-red inner bark and the presence of nootkatinol, a secondary metabolite derived from tropone (Fujita et al. 2000). In addition, all species of the clade have smooth exfoliating bark, and all except *H. forbesii* have conspicuous adaxial leaf glands (Wolf 1948a). Similarly, we recovered a clade containing two species (*H. benthamii* and H. *montana*) recognized as varieties of *C. arizonica* and *C. lusitanica* (Figs. 1, 2). Both *H. benthamii* and *H. montana* have ovulate cones that open and release seed immediately upon maturation, a character rare in the NWC (Wolf 1948a).

We recovered moderate to strong support for a group of six species (*H. macrocarpa, H. abramsiana, H. nevadensis, H. sargentii, H. goveniana*, and *H. pigmaea*) herein called the Macrocarpa group (Figs. 1, 2). Five of these six species (excluding *H. nevadensis*) form a morphologically coherent group in Wolf's treatment (Wolf 1948a; see pgs. 50–51), and four (*H. abramsiana, H. sargentii, H. goveniana*, and *H. pigmaea*) comprise Wolf's (1948a) *C. goveniana* complex. Members of the group share a number of distinctive features including gray fibrous bark that is non-foliating, as well as the absence of active dorsal leaf glands (*H. sargentii* has dorsal glands that are infrequently active per Wolf 1948a). Morphologically, the discordant member of the clade is *H. nevadensis*, a species traditionally included in the *C. arizonica complex* (Little 1966, 1970; Silba 1981). Wolf (1948a) noted *H. nevadensis* as "interesting" in citing characters that linked it to the *C. arizonica* (active dorsal leaf glands) and *C. goveniana* (bark of main axis gray and non-exfoliating) complexes. Although

distance analysis of RAPDs included *H. nevadensis* with other members of the *C. arizonica complex*, this group was resolved as sister to a clade consisting exclusively of *C. goveniana complex* species (Bartel et al. 2003).

Our findings resolve the six species of the Macrocarpa group into three clades of two species each (Figs. 1, 2). Support for these clades is weak to moderate in the ML tree, with bootstrap values of 52 (*H. goveniana–H. abramsiana*), 79 (*H. macrocarpa–H. pigmaea*), and 83 (*H. nevadensis–H. sargentii*), but each is strongly supported by Bayesian analyses ($PP = 1.0$). Relationships among the three clades are equivocal, with the *H. goveniana–H. abramsiana* clade being more closely related to each of the other two clades depending on the data set analyzed (Figs. 1, 2). Some authors (Silba 1981; Little 1970) recognize *H. abramsiana* and *H. pigmaea* as varieties of *H. goveniana*, and maximum likelihood analysis of nucleotides sequences alone places these three taxa in a clade with *H. macrocarpa* (Fig. 1). Wolf (1948a) conceded that few if any features warrant species recognition of *H. abramsiana*, and stated that this species along with *H. pigmaea* and *H. sargentii* could be recognized as subspecies of *H. goveniana* in broader concepts of the group. Bayesian analysis of combined nucleotides sequences and binary data group these three taxa with *H. nevadensis* (Fig. 2). The morphological intermediacy of *H. abramsiana* to that of *H. sargentii* and *H. goveniana* has been marshaled in support of interspecific hybridization in the group (Zavarin et al. 1971; McMillan 1952; Wolf 1948a) and, if corroborated, would further substantiate the apparent close relationship of these species.

With respect to the Macrocarpa group, perhaps the most noteworthy finding is the recovery of a *H. macrocarpa–H. pigmaea* as sister taxa (Figs. 1, 2). Wolf (1948a) described *C. macrocarpa* as "the outstanding large-sized member of a group of species including *C. abramsiana*, *C. goveniana*, and *C. pigmaea*" and noted similarities in growth habit in support of a close relationship between *H. macrocarpa* and the larger specimens of *H. pigmaea*. Other than general growth habit (not including crown architecture; see Wolf 1948a), there are few if any morphological features putatively synapomorphic for the *H. macrocarpa–H. pigmaea* clade. Little (2006) obtained a sister-group relationship for *H. macrocarpa* and *H. pigmaea* is his analysis of sequences from three chloroplast regions, but did not recover this association in analyses of the combined molecular-organismic data.

Taxonomic implications

Most NWC species consist of a few scattered, relictual, localized populations (Bartel, pers. observ.; Barbour 2007; Rehfeldt 1997; Brown 1982). This appears to be a consequence of adaptation to local conditions (e.g., fire frequency, edaphic characteristics) and long-term selective forces (e.g., decreasing minimum temperatures and increasing aridity: Barbour 2007; Brown 1982) and has resulted in varying degrees of population differentiation over evolutionary time (Rehfeldt 1997; Wolf 1948a, b). One consequence has been differences in opinion with respect to whether particular variants should be recognized taxonomically, and if so, the rank at which they should recognized. Indeed, for the same number of NWC entities, 5 species and no varieties (Little 1953), 8 species and 10 varieties (Little 1970), and 16 species, 2 subspecies, and no varieties (Wolf 1948a, b) have been recognized. Despite analyzing 14,799 bp of aligned sequence and 230 binary characters in the combined data set, we find poor support for several relationships, especially within the Arizonica group of *Hesperocyparis* (Figs. 1, 2). These results suggest recovery of well-supported relationships among the closely related taxa of *Hesperocyparis* will require a great deal of comparative data. Thus, divergence in chloroplast sequences appears to parallel the limited morphological divergence that characterizes *Hesperocyparis*. Although we are hesitant to cite lack of evidence in support of any particular contention, these findings substantiate recognition of fewer species and perhaps more infraspecific taxa within the genus, an approach many students of the group have adopted (Farjon 2005; Silba 1981; Little 1953, 1970). Although several relationships presented here are well supported, especially in the Bayesian tree (Fig. 2), and many others are consistent with those of previous studies, many infrageneric relationships are previously unreported, supported by molecular data only, and are partly or entirely inconsistent with traditional taxonomic treatments. This observation appears attributable to the complex interplay of several factors including poor genetic differentiation of species, the use of symplesiomorphic characters in taxonomic treatments, lack of synapomorphic morphological characters for clades well supported in the molecular phylogeny, lack of resolution in the molecular phylogeny, homoplasy in the molecular and morphological data, and actual discordance among phylogenetic and taxonomic groupings. To the extent that the goal of taxonomy is the description and identification of taxa with minimal effort, results presented here have little implication for revision of taxonomic treatments created with that goal in mind, especially given our current understanding of morphological variation in the genus. However, this study is an important addition to the growing cypress systematics literature in that it (1) evidences heretofore unsuspected relationships in *Hesperocyparis*, (2) provides the most robust framework to date for interpreting evolutionary trends in taxonomically important characters, and (3) suggests areas for additional study (e.g., examinations of

micromorphological or biochemical variation) that, when interpreted in light of phylogenetic relationships, could bring about useful and meaningful taxonomic and nomenclatural change in the future.

Acknowledgments We thank Jeff Pittman for assistance with DNA extractions and for writing Python programs useful in sequence management and analysis. We acknowledge the constructive comments of two anonymous reviewers and Isabel San Martin and their role in the improvement of an earlier draft of the manuscript. Support from the Biology Department and the College of Natural Sciences, Lamar University, is gratefully acknowledged.

References

Abrams L (1923) An illustrated flora of the Pacific States, Washington, Oregon, and California. Vol. 1: Ophioglossaceae to Aristolochiaceae. Stanford University, Palo Alto

Adams RP (2007) *Juniperus maritima*, the seaside juniper, a new species from Puget Sound, North America. Phytologia 89:263–283

Adams RP, Bartel JA, Price RA (2009) A new genus, *Hesperocyparis*, for the cypresses of the western hemisphere (Cupressaceae). Phytologia 91:160–185

Averyanov LV, Hiep NT, Harder DK, Loc PK (2002) The history of discovery and natural habitats of *Xanthocyparis vietnamensis* (Cupressaceae). Turczaninowia 5:31–39

Barbour MG (2007) Closed-cone pine and cypress forest. In: Barbour MG, Keeler-Wolf T, Schoenherr AA (eds) Terrestrial vegetation of California, 3e. University of California Press, Berkeley, pp 296–312

Bartel JA, Adams RP, James SA, Mumba LE, Pandey RN (2003) Variation among *Cupressus* species from the western hemisphere based on randomly amplified polymorphic DNAs. Biochem Syst Ecol 31:693–702

Bowers NA (1965) Cone-bearing trees of the Pacific Coast. Pacific Books, Palo Alto

Brown DE (1982) Relict conifer forests and woodlands. In: Brown DE (ed) Biotic communities of the American Southwest-United States and Mexico. Desert Plants 4(1–4):70–71

Debreczy ZK, Musial K, Price RA, Racz I (2009) Relationships and nomenclatural status of the Nootka cypress (*Callitropsis nootkatensis*, Cupressaceae). Phytologia 91:140–159

Farjon A (1998) World checklist and bibliography of conifers. Royal Botanic Gardens, Kew

Farjon A (2005) A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew

Farjon A, Hiep NT, Harder DK, Loc PK, Averyanov L (2002) A new genus and species in the Cupressaceae (Coniferales) from northern Vietnam, *Xanthocyparis vietnamensis*. Novon 12:179–189

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fujita K, Yamaguchi T, Itose R, Sakai K (2000) Biosynthetic pathway of B-thujaplicin in the *Cupressus lusitanica* cell culture. J Plant Physiol 156:462–467

Griffin JR, Critchfield WB (1972) The distribution of forest trees in California. U.S.D.A Forest Service, Berkeley

Guindon S, Gascuel O (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst Biol 52:696–704

Jepson WL (1910) The silva of California, vol 2. The University Press, Berkeley

Jepson WL (1923) The trees of California, 2e. Cunningham, Curtis and Welch, San Francisco

Little EL (1953) Check list of native and naturalized trees of the United States (including Alaska). U. S. Dept. Agr. Handb. 41. U. S. D. A Forest Service, Washington, DC

Little EL (1966) Varietal transfers in *Cupressus* and *Chamaecyparis*. Madroño 18:161–167

Little EL (1970) Names of the New World cypresses (*Cupressus*). Phytologia 20:429–445

Little DP (2006) Evolution and circumscription of the true cypresses (Cupressaceae: *Cupressus*). Syst Bot 31:461–480

Little DP, Schwarzbach AE, Adams RP, Hseih CF (2004) The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocyparis* (Cupressaceae). Am J Bot 91:1871–1880

Mao K, Hao G, Liu J, Adams RP, Milne RI (2010) Diversification and biogeography of *Juniperus* (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. New Phytol 188:254–272

Martinez M (1947) Los *Cupressus* de Mexico. An Inst Biol 18:71–149

McMillan C (1952) The third locality of *Cupressus abramsiana* Wolf. Madroño 11:189–194

Mill RR, Farjon A (2006) Proposal to conserve the name *Xanthocyparis* against *Callitropsis* Oerst. (Cupressaceae). Taxon 55:229–231

Müller K (2005) SeqState: primer design and sequence statistics for phylogenetic DNA data sets. Appl Bioinform 4:65–69

Müller K (2006) Incorporating information from length-mutational events into phylogenetic analysis. Mol Phylogenet Evol 38:667–676

Posada D (2008) jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253–1256

Rambaut A (2002) Se-Al: Sequence Alignment Editor. Available from <http://tree.bio.ed.ac.uk/people/arambaut/>, University of Oxford

Rehfeldt GE (1997) Quantitative analysis of the genetic structure of closely related conifers with disparate distributions and demographics: the *Cupressus arizonica* (Cupressaceae) complex. Amer J Bot 84(2):190–200

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Sargent CS (1922) Manual of the trees of North America (Exclusive of Mexico), 2e. The Riverside Press, Cambridge

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE, Small RL (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Am J Bot 92:142–166

Silba J (1981) Revised generic concepts of *Cupressus* L. (Cupressaceae). Phytologia 49:390–399

Silba J (1982) Addendum to revised generic concepts of *Cupressus* L. (Cupressaceae). Phytologia 52:349–361

Silvestro D, Michalak I (2011) Organisms diversity and evolution. doi:10.1007/s13127-011- 0056-0

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Sudworth GB (1927) Checklist of the forest trees of the United States, their names and ranges. U.S. Department of Agriculture, Washington, DC

Swofford DL (2002) PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4b10. Sinauer Associates, Sunderland, Mass

Thompson JD, Higgins DG, Gibson TJ (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

Velázquez A, Toledo VM, Luna I (2000) Mexican temperate vegetation. In: Barbour MG, Billings WD (eds) North American terrestrial vegetation, 2e. Cambridge University Press, New York, pp 571–592

Wolf CB (1948a) The New World cypresses. Aliso 1:1–250

Wolf CB (1948b) Note on two papers on Mexican cypresses. Aliso 1:437–438

Xiang Q, Farjon A (2003) Cuticle morphology of a newly discovered conifer, *Xanthocyparis vietnamensis* (Cupressaceae), and a comparison with some of its nearest relatives. Bot J Linn Soc 143:315–322

Xiang Q, Li J (2005) Derivation of *Xanthocyparis* and *Juniperus* from within *Cupressus*: evidence from sequences of the nrDNA internal transcribed spacer region. Harvard Pap Bot 9:375–382

Zavarin E, Lawrence L, Thomas MC (1971) Compositional variations of leaf monoterpenes in *Cupressus macrocarpa*, *C. pygmaea*, *C. goveniana*, *C. abramsiana* and *C. sargentii*. Phytochemistry 10:379–393