TAXONOMY OF INFRASPECIFC TAXA OF *ABIES CONCOLOR* BASED ON DNA SEQUENCES FROM nrDNA AND FOUR CHLOROPLAST REGIONS

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ABSTRACT

The DNA sequences from five gene regions (nrDNA, trnS-trnG, trnL-trnF, petN-psbM, psbM-trnD) for *Abies concolor* var. *concolor* and var. *lowiana* from seven populations from throughout their range were analyzed and sequences compared. Whereas the leaf essential oil compositions from these seven populations were quite differentiated, the DNA data revealed *A. concolor* to be fairly uniform having only 1-4 mutations (from 4556 bp of data) between populations, with the exception of the Klamath Mtns. population that was highly differentiated (in its trnS-trnG and psbM-trnD regions). The current DNA data does not support recognition of these taxa as distinct species nor do they support recognition of *A. concolor* var. *lowiana*.
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KEY WORDS: *Abies concolor* var. *concolor, A. c.* var. *lowiana*, DNA sequencing, SNPs, nrDNA, trnS-trnG, trnL-trnF, petN-psbM, psbM-trnD, Klamath Mtns., taxonomy.

Abies concolor (Gord. & Glend.) Hilde. is a forest tree of western North America ranging from Oregon to northern Mexico (Zavarin et al. 1975). Hunt (1993) recognized both *A. concolor* and *A. lowiana*. Eckenwalder (2009) recognized two varieties: var. *concolor* and var. *lowiana* (Gord.) Lemm. and noted that these have been treated as species by some authors. Adams et al (2011) recently reported on the composition of the leaf essential oils and validated the chemical races reported by Zavarin et al. (1970, 1975); in general the leaf oils supported the recognition of vars. *concolor* and *lowiana* (Fig. 1).

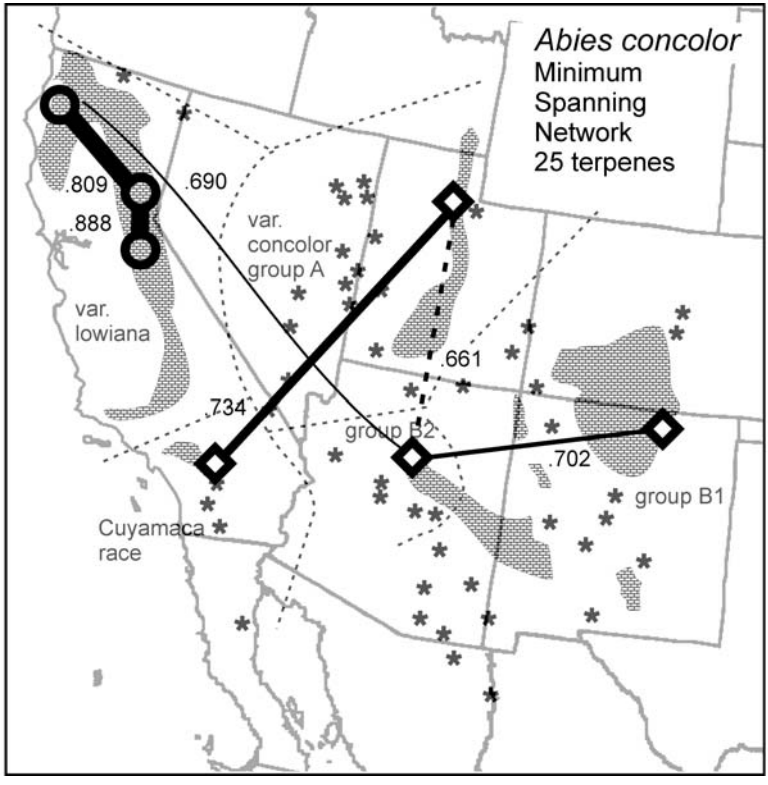

Figure 1. Minimum spanning network based on 20 terpenes of *A. concolor*. The open circles are *A. c.* var. *lowiana*, the open squares are generally treated as *A. c.* var. *concolor*. The numbers next to the lines are similarities. From Adams et al. (2011).

Xiang et al. (2009) recently published a phylogeny of *Abies* based on nrDNA sequences. They found *A. concolor* to form an unresolved clade with *A. grandis, A. religiosa* and *A. durangensis.* However, it is unlikely that nrDNA data alone is sufficient to portray phylogenetic relationships. In this study, we present sequence data for nrDNA, petN-psbM, psbM-trnD, trnL-trnF and trnS-trnD of *A. concolor* from throughout its range including the chemical races found by Zavarin et al.(1975) and Adams et al. (2011).

MATERIALS AND METHODS

Plant specimens: *Abies concolor* var. *concolor*: *Adams 12405-12407*, Mill B trailhead, Big Cottonwood Canyon, Salt Lake City, UT, 40º 37.996' N, 111º 43.418' W, 6242 ft., *Adams 12481-12485*, (by D. Thornburg) 7 mi. nw of Pine, AZ along Rim Rd., 34º 26.844'N, 111º 21.520'W, 7597 ft., *Adams 12679-12683*, 13 mi. w of Cimarron, NM on US 64, 36º 31.509' N, 105º 10.932' W, 7872 ft.
Abies concolor var. *lowiana*: *Adams 12427-12431* (by R. Lanner) 2 mi. n of jct. US50 on White Meadows Rd., ca. 22 mi e of Placerville, CA, 38º 47' 00" N, 120º 29' 20" W, 3450 ft., *Adams 12432-12436* (by R. Lanner) Mormon Emigrant Trail at jct. with Park Creek Rd., ca. 24 mi ese of Placerville, CA, 38º 43' 30" N, 120º 28' 20" W, 4000 ft., *Adams 12438-12442* (by M. Kauffmann) Klamath Mtns., CA, 40º 50' 21.4" N, 123º 43' 11.09" W, 4820 ft., *Adams 12464-12468* (by B. Miller) Lee Summit, CA on Hwy 70/89, 39º 52.674' N, 120º 45.736' W, 4414 ft., *Abies concolor* var. *concolor / lowiana*: *Adams 12522-12526*, on CA Hwy 38 north side of Onyx Summit, CA, 34º 12.037' N, 116º 43.520' W, 8490 ft. Outgroup: *A. lasiocarpa* var. *bifolia*: *Adams 12400-12404*, Brighton Ski lodge parking lot. 40° 35' 48.76" N; 111° 35' 09.18" W, 2682m, Sept. 4, 2010, Salt Lake Co., UT. All specimens are deposited in the BAYLU herbarium.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20º C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

The nrDNA region of *Abies concolor* proved to be too large (~2000 bp) to sequence by use of ITSA and ITSB (Fig. 2).

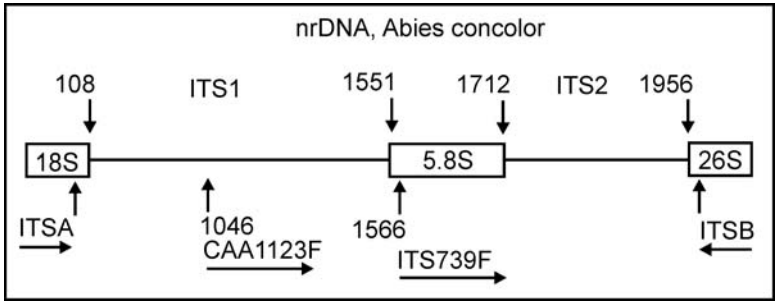

Figure 2. Diagram of the nrDNA region for *Abies lasiocarpa*.

Two addition primers were utilized:
CAA1123F: AC CTC CTA TGT CGG TTG TGC (Xiang et al. 2009)
ITS739F: AAC GGA TAT CTC GGC TCT, based on conserved sequences in the 5.8S region.

The trnC-trnD region of *Abies concolor* also proved to be large (~2400, Fig. 3). Due to the small area from trnC to petN, that region was skipped. Two regions were sequenced: petN-psbM and psbM-trnD using four primers (Fig. 2) based on sequences of *Abies* from GenBank:
petNAc373F: TGG TAG TTT TTA CAT TTT CC,
psbMAc1294R: TTA TCC CTT ACG TCA AAA CG
and
psbMAc1382F: AGA TCC ATG AAA TAG ATG TG
trnDrev: GGG ATT GTA GTT CAA TTG GT

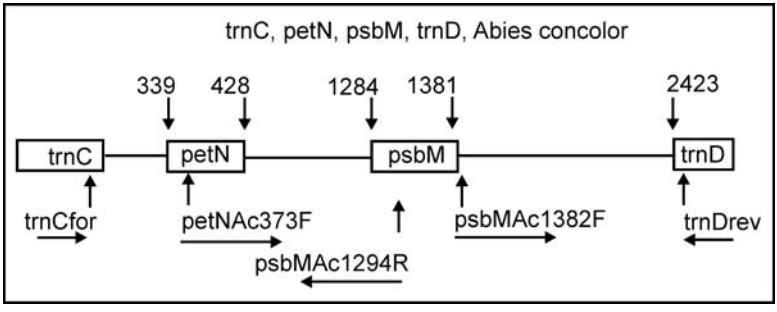

Figure 3. Diagram of the trnC-trnD region of *Abies concolor*.

Primers for trnL-trnF and trnS-trnG have been previously reported (Adams and Kauffmann, 2010).

PCR 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 (cpDNA regions) or K (nrDNA) (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 $MgCl_2$ according to the buffer used) 1.8 µM each primer. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/).

Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Considering only the *Abies concolor* samples, different DNA regions gave variable numbers of taxonomically informative characters (Table 1). The nrDNA region gave no informative characters. The cpDNA regions proved the most useful, with psbM-trnD and trnS-trnG yielding 11 and 5 useful characters, respectively (Table 1).

Table 1. Summary of variation discovered in nrDNA, trnS-trnG, trnL-trnF, petN-*psbM and psbM - trnD regions.* The variation is based solely on *A. concolor* samples. #subs = # nucleotide substitutions.

region	bp	# subs	# indels	# single events	# informative characters (SNPs)
nrDNA	951	0	0	1	0
trnS-trnG	908	3	2	0	5
trnL-trnF	920	1	1	2	2
petN-psbM	824	2	0	1	2
psbM-trnD	953	5	6	2	11
totals	4556	11	9	6	20

Analysis of the concatenated five-gene sequences revealed unresolved clades of the Sierra and San Bernardino Mtns. populations (Figure 4). The major facet found was the uniqueness of the Klamath Mtns. population (Fig. 4) with a support of 97%. There is some support (69%) for the Arizona clade.

Examination of the 20 informative characters (Table 1), 11 are substitutions and 9 are indels. To utilize the information in the indels, these were coded as match/ mis-match data and a minimum spanning network was constructed. The minimum spanning network (Fig. 5) shows clearly the closeness of nearly all the *A. concolor* populations, except the Klamath Mtns. population, that differs by 16 mutational events from the Utah population.

To visualize the geographical variation among populations, a minimum spanning was plotted (Fig. 6) onto the chemical races map of Zavarin et al. (1975). Notice the broad links (highly related groups with few differences) linking var. *concolor* (open squares) and var. *lowiana* (open circles). The Klamath Mtns. individuals, although in relative close proximity to the Sierras populations, appear quite differentiated (Fig. 6).

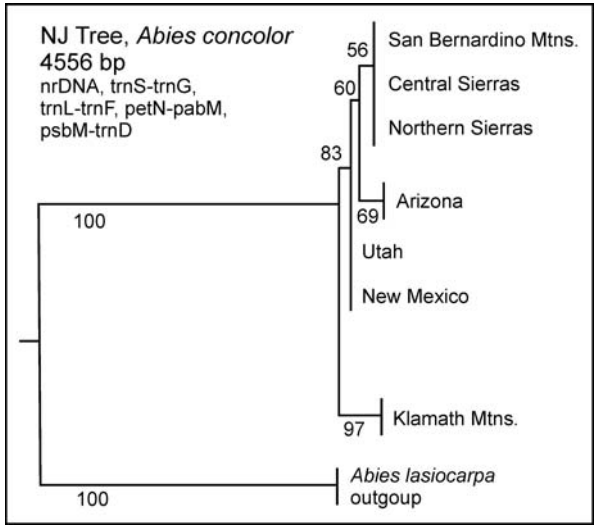

Figure 4. NT tree based on 4556 bp of sequence data. The numbers at the branch points are bootstrap values as percent.

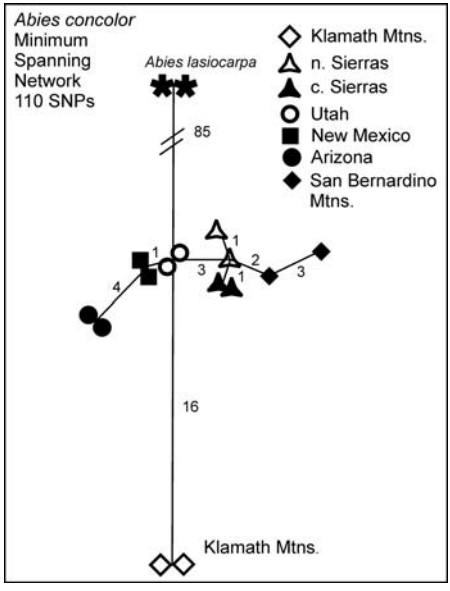

Figure 5. Minimum spanning network based on 110 SNPs. Numbers next to the line are the numbers of mutational events (substitutions + indels).

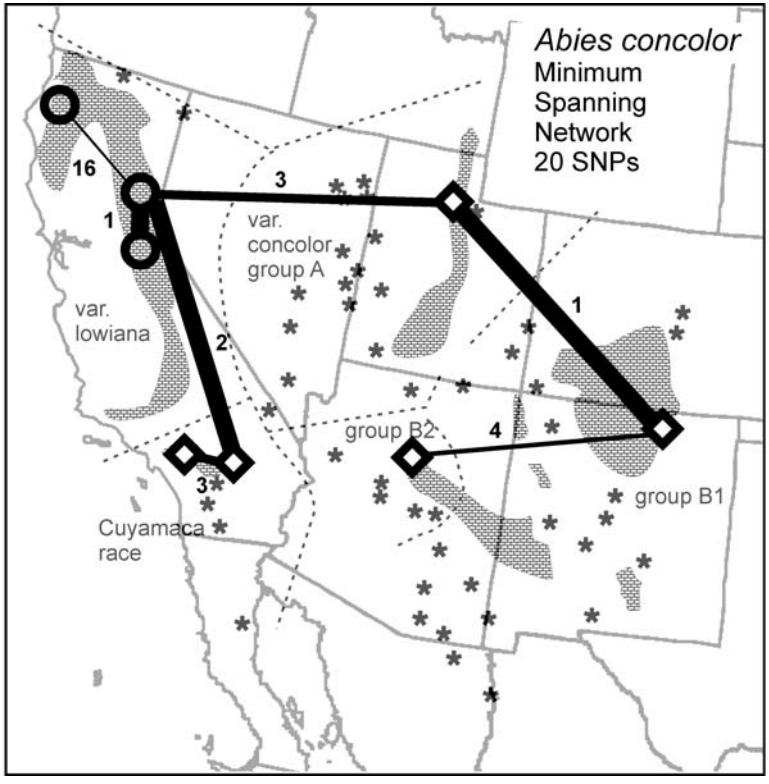

Figure 6. Minimum spanning network based on 20 SNPs, superimposed onto the chemical races map of Zavarin et al. (1975). The numbers next the links are the number of mutational events. The width of the links are inversely proportional to the relatedness.

It is interesting to compare the terpenoid differentiation (Fig. 1) with the DNA differentiation (Fig. 6). The DNA data shows basically one group for *A. concolor*, with an outlier from the Klamath Mtns. (Fig. 6) and no correlation with the chemical races of Zavarin et al. (1975) or Adams et al. (2011).

CONCLUSIONS

Whereas the leaf essential oil compositions from seven populations were quite differentiated, the DNA data revealed *A. concolor* to be fairly uniform, having only 1-4 mutations (from 4556 bp of data) between populations, with the exception of the Klamath Mtns. population that was highly differentiated (for its trnS-trnG and psbM-trnD regions). It seems odd that only these two gene regions showed the differentiation of the Klamath Mtns. plants.

Hunt (1993) recognized both *A. concolor* and *A. lowiana*. The current DNA data gives no support for the recognition of these taxa as distinct species, nor support for the recognition of *A. concolor* var. *lowiana*.

Eckenwalder (p. 92, 2009) in writing about *A. concolor*, states that "It does, however hybridize and intergrade with the closely related grand fir (*A. grandis*) in northwestern California and southwestern Oregon". It may be that our samples of *A. concolor* from the Klamath Mtns. contain germplasm of *A. grandis*. This is currently being investigated.

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