

**RE-EXAMINATION OF THE TAXONOMY OF THE ONE-
SEEDED, SERRATE LEAF *JUNIPERUS* OF SOUTHWESTERN
UNITED STATES AND NORTHERN MEXICO
(CUPRESSACEAE)**

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ABSTRACT

The one-seeded, serrate leaf margined junipers of Southwestern United States and northern Mexico consist of *J. angosturana*, *J. californica*, *J. coahuilensis* var. *arizonica*, *J. c.* var. *coahuilensis*, *J. monosperma*, *J. pinchotii*, *J. occidentalis* var. *australis*, *J. o.* var. *occidentalis* and *J. osteosperma*. Recent nrDNA and trnC-trnD sequence data were compared with RAPDs analysis and two of the taxa (*J. c.* var. *arizonica*; *J. o.* var. *australis*) were found to be in separate clades. In addition, the two taxa were as distinct as other recognized species in the group. Because of this, *Juniperus coahuilensis* var. *arizonica* is recognized at the specific level as *J. arizonica*. Since the specific name *J. australis* is unavailable due to prior use, *J. occidentalis* var. *australis*, was given a new name, *J. grandis*, to denote the robust tree nature of the big western juniper. All the remaining taxa were quite distinct in both their sequences and RAPDs data.

KEY WORDS: *Juniperus*, *J. angosturana*, *J. californica*, *J. arizonica* (= *J. coahuilensis* var. *arizonica*), *J. coahuilensis*, *J. monosperma*, *J. pinchotii*, *J. grandis* (= *J. occidentalis* var. *australis*), *J. occidentalis*, *J. osteosperma*, RAPDs, nrDNA, trn C-trnD, Cupressaceae

The one-seeded, serrate leaf margined *Juniperus* of Southwestern United States and northern Mexico are *J. angosturana*, *J. californica*, *J. coahuilensis* var. *arizonica*, *J. c.* var. *coahuilensis*, *J. monosperma*, *J. pinchotii*, *J. occidentalis* var. *australis*, *J. o.* var. *occidentalis* and *J. osteosperma* (Adams, 2004). Previous studies using morphology and terpenoids failed to clearly arrange the taxa into groups (Zanoni and Adams, 1976, 1979). However, recently DNA sequencing of nrDNA and trnC-trnD (Schwarzbach, et al., 2007) has shed new light on the relationships within this group. Firstly, the one-seeded, serrate leaf margined junipers were found to be paraphyletic. Secondly, *J. californica* was shown to be quite distinct (Fig. 1), however, analysis of nrDNA and trnC-trnD sequence data individually gives weak support that *J. californica* is sister to the *J. occidentalis* - *J. osteosperma* clade, and addition research will be needed to resolve this issue. A third aspect is that the remaining all are divided into two large clades (Fig. 1). One clade consists of *J. angosturana*, *J. coahuilensis* var. *coahuilensis*, *J. monosperma*, and *J. pinchotii*, taxa from the Chihuahuan desert margins. The second clade is composed of *J. coahuilensis* var. *arizonica*, *J. occidentalis* var. *australis*, *J. o.* var. *occidentalis*, and *J. osteosperma* is from the Sonoran, high Utah-Nevada, Mojave deserts, and Sierra Nevada. The sequence data revealed two taxonomic problems: *Juniperus coahuilensis* var. *coahuilensis* and *J. c.* var. *arizonica* are well supported as members of different clades (Fig. 1) and *J. occidentalis* var. *australis* is 100% supported as being more closely related to *J. osteosperma* than to *J. o.* var. *occidentalis* (Fig. 1). Both of these taxa have evolved independently and are an additional case of cryptic speciation within the genus *Juniperus*. Cryptic speciation has also been found between *Juniperus deltoides* and *J. oxycedrus* in the Mediterranean region (Adams et al. 2004) and between *J. erectopatens* and *J. sabina* in China (Adams, 2004).

To further investigate this problem, DNA fingerprint analyses were performed for the same taxa. RAPDs (Random Amplified Polymorphic DNAs) is a form of DNA fingerprinting that has been used

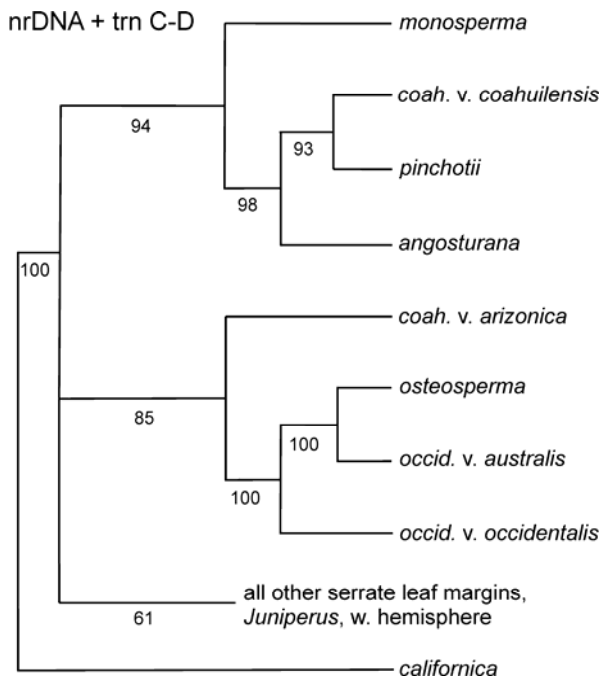


Figure 1. Partial phylogenetic tree derived from nrDNA + trnC-D sequence data (adapted from Schwarzbach et al., 2007). Values below branches are posterior probabilities.

in several *Juniperus* studies and has proved useful in systematics generally (Adams, 1999, 2000a-d; Adams and Demeke, 1993; Adams and Nguyen, 2005), when stringent laboratory procedures are followed (Adams, Flournoy and Pandey, 1998). In this study, we report on RAPDs analysis and combine these results with the DNA sequence data and morphology to evaluate the taxonomic status of *J. coahuilensis* var. *arizonica* and *J. occidentalis* var. *australis*.

MATERIALS AND METHODS

Specimens collected: *J. angosturana*, Adams 6881-25, 12.7 km e of Villa Juarez, San Luis Potosi, Mexico; *J. californica*, Adams 8695-7, 13 km n of Amboy/Kelso I40 exit on the road to Kelso, CA; *J. coahuilensis*

var. *arizonica*, Adams 10634-36, on AZ hwy 179 between I17 and Sedona, AZ; *J. c.* var. *coahuilensis*, Adams 6829-31, on Mex. hwy. 45, 85 km n of La Zarca, Durango, Mexico; *J. monosperma*, Adams 7638-40, on I40, Santa Rosa, NM; *J. pinchotii*, Adams 7483-87, 5 km w of Ozona, TX on US 290; *J. occidentalis* var. *australis*, Adams 8692-94, n side of L. Baldwin, San Bernardino Mtns., CA; *J. o.* var. *occidentalis*, Adams 8592-94, 0.2 km nw of Sisters, OR; *J. osteosperma*, Adams 6811-13, Little Cottonwood Canyon, Salt Lake City, UT. Voucher specimens are deposited at BAYLU.

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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia, CA). The RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Columbia (5'-3'): 134, AAC ACA CGA G; 153, GAG TCA CGA G; 184, CAA ACG GAC C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 268, AGG CCG CTT A; 338, CTG TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 478, CGA GCT GGT C.

PCR stock solutions (Taq, primer, and buffer) were made in bulk so that all the PCR reaction tubes for a primer were prepared using the same bulk stock. This is a critical factor for minimizing variation in band intensities from sample to sample (see Adams, Flournoy and Pandey, 1998, for protocols to minimize PCR band variation). PCR was performed in a volume of 15 µl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 µM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A negative control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). Samples were run in duplicate to insure reproducibility (Adams, Flournoy and Pandey, 1998). A temperature profile was obtained for each well of the thermocycler to be sure that no variation existed among wells in the heating/ cooling block. The thermal cycle used was: 94° C (1.5 min) for initial strand separation, then 40 cycles of 40° C (2 min), 72° C (2 min), 91° C (1 min). Two additional steps were used: 40° C (2

min) and 72° C (5 min) for final extension. The temperature inside a PCR tube containing 15 µl buffer was monitored with a temperature probe, quantitated and printed for each step for each of the 40 cycles for every PCR run (Adams, Flournoy and Pandey, 1998) to insure that each cycle met temperature specifications and that each PCR run was exactly the same. Amplification products were analyzed by electrophoresis on 1.5% agarose gels, 75V, 55 min, and detected by staining with ethidium bromide. The gels were photographed over UV light using Polaroid film 667 and scanned to digital images. The digital images were size normalized in reference to pGem® DNA size markers before band scoring. Bands were scored as present (1) and absent (0). Bands that were inconsistent in replicate analyses were not scored.

Associational measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). It should be noted that problems of homology of RAPD DNA bands on agarose gels can be significant (Rieseberg, 1996), but these errors can be accounted for using multivariate statistical methods (PCO) (see Adams and Rieseberg, 1998). A minimum spanning diagram was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in a network (Adams, et al. 2003).

RESULTS AND DISCUSSION

The minimum spanning network based on RAPDs data (Fig. 2) is very similar to the nrDNA + trnC-trnD sequence tree (Fig. 1). As with the sequence data, *J. californica* is shown to be very distinct. However, *J. monosperma* is depicted as more distinct (Fig. 2) than with the sequence data (Fig. 1). *Juniperus occidentalis* var. *australis* links with *J. osteosperma* rather than *J. o.* var. *occidentalis* (Fig. 2) just as seen in the sequence data (Fig. 1). These three taxa are difficult to distinguish. *Juniperus osteosperma* is the most xeric of the three, has the largest branchlets and the least apparent oil glands that seldom rupture, whereas *J. occidentalis* var. *occidentalis* is the most mesic, has the smallest branchlets and very noticeable, ruptured leaf oil glands. *Juniperus occidentalis* var. *australis* is more or less intermediate in its

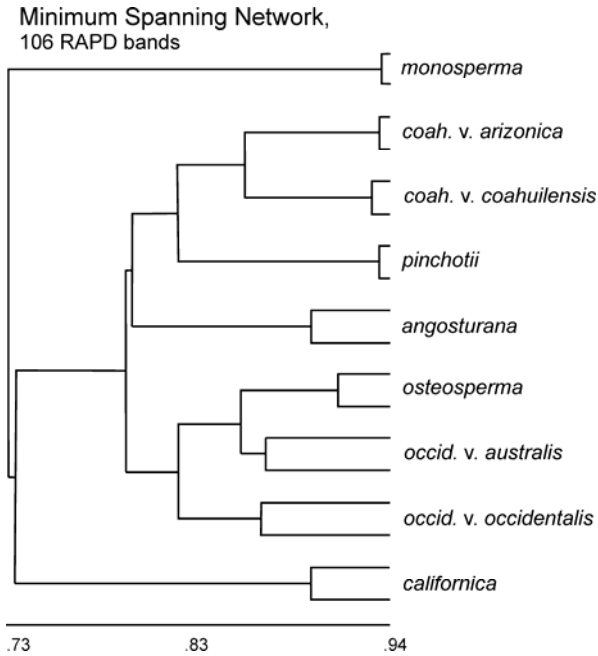


Figure 2. Minimum spanning network based on 106 RAPD bands. Notice that *J. coahuilensis* var. *arizonica* links loosely with *J. c.* var. *coahuilensis*, while *J. occidentalis* var. *occidentalis* is linked with *J. osteosperma*, rather than with *J. o.* var. *occidentalis*.

morphology and habitat. Vasek (1966) concluded that hybridization was occurring in northwestern Nevada between *J. osteosperma* and both *J. occidentalis* varieties. Terry et al. (2000), using nrDNA and cpDNA data, came to the same conclusion.

Multivariate ordination of RAPD data is valuable to complement minimum spanning networks. In order to concentrate on the two clades with taxonomic problems, *J. californica* and *J. monosperma* were removed from the RAPD data set and a PCO analysis was conducted. Factoring the similarity matrix yielded 9 eigenroots that appeared to asymptote after the sixth eigenroot. These six eigenroots accounted for 80.06% of the variance among the taxa (30.0, 12.0, 11.7, 11.3, 8.6 and

6.5%). Ordination using the first 3 axes (Fig. 3) separates the clades on axis 1 (30%). Axes 2 and 3 separate *J. occidentalis* var. *occidentalis*,

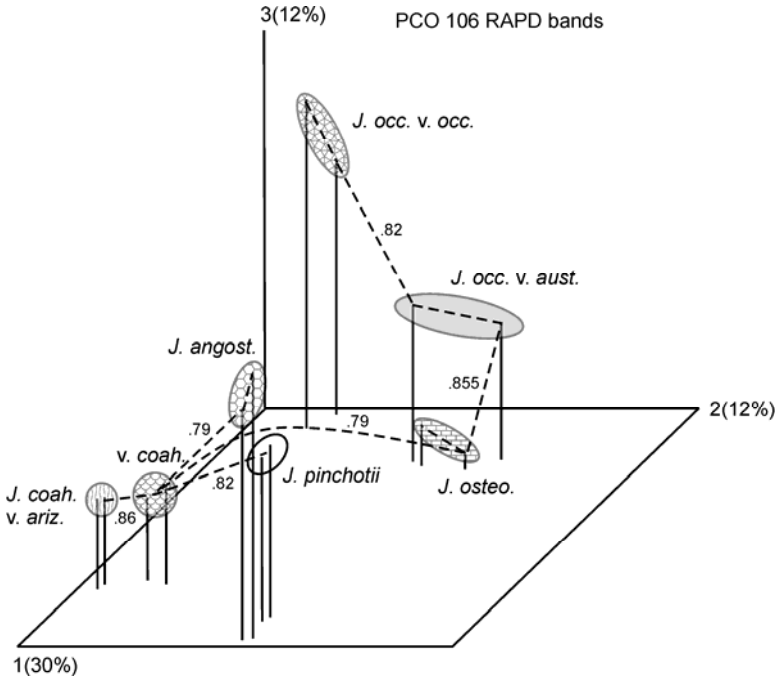


Figure 3. PCO of taxa based on 106 RAPD bands. Note the intermediate, but distinct position of *J. o.* var. *australis* between *J. o.* var. *occidentalis* and *J. osteosperma*.

J. o. var. *australis* and *J. osteosperma*, and to a lesser extent *J. coahuilensis* var. *coahuilensis* from *J. c.* var. *arizonica*. It should be noted that axes 4 and 5 separate *J. angosturana* from *J. pinchotii* and *J. c.* var. *coahuilensis* from *J. c.* var. *arizonica*.

Neither the sequence nor the RAPD data show that *J. occidentalis* var. *australis* and *J. o.* var. *occidentalis* form a monophyletic group. Vasek (1966) struggled with the appropriate taxonomic level for *J. o.* var. *australis*. Morphologically, *J. californica*, *J. o.* var. *occidentalis*, *J. o.* var. *australis*, and *J. osteosperma* are very similar. They do,

however, differ in their essential oils (Adams, 2004). Based on these new DNA data, it is deemed appropriate to recognize *J. occidentalis* var. *australis* at the specific level. Unfortunately, there exists an earlier *Juniperus australis* (Endl.) Pilg., in Urban, Aymb. Antill. 7: 479. 1913, which according to Farjon (2005) is synonymous with *Juniperus barbadensis* var. *lucayana* (Britton) R. P. Adams. Farjon (2005) designated a neotype for *J. australis* as *H. F. A. von Eggers 3586* (E), from Jamaica. Adams (2004) lists *J. australis* as a synonym under *J. lucayana* Britton. Regardless, *J. australis* is unavailable due to prior use, consequently new name is proposed:

***Juniperus grandis* R. P. Adams, nom. nov.**

Basionym: *Juniperus occidentalis* W. J. Hooker subsp. *australis* Vasek, Brittonia 18: 352 (1966), big western juniper, Type: United States, California, San Bernardino Mtns., CA, *Vasek 610929* (HOLOTYPE: RSA).

Distribution: California, w Nevada. See map, p. 140, Adams (2004).

Synonyms: *Juniperus occidentalis* var. *australis* (Vasek) A. & N. Holmgren., Intermountain Fl. 1: 239 (1972).

The second taxonomic problem is presented by the placement of *J. coahuilensis* var. *coahuilensis* and *J. c.* var. *arizonica* in different clades (Fig. 1), coupled with the RAPDs data showing these taxa to be about as dissimilar as *J. osteosperma* and *J. occidentalis* var. *australis* (Fig. 2). Although the sequence and RAPDs data are not in complete agreement on this issue, taken together, they argue that the cryptic taxon *J. c.* var. *arizonica* is actually quite distinct and is an independent lineage that deserves recognition at the specific level as:

***Juniperus arizonica* (R. P. Adams) R. P. Adams, stat. nov.**

Basionym: *Juniperus coahuilensis* Martinez var. *arizonica* R. P. Adams, Biochem. Syst. Ecol. 22 (7): 708 (1994), Arizona juniper, Type: United States, Arizona, Yavapai Co.; 72 km s of Flagstaff, 1160 m, *R. P. Adams 2132* (HOLOTYPE: BAYLU!).

Distribution: Arizona, sw New Mexico, ne Sonora, MX, nw Chihuahua, MX. See map, p. 81, Adams (2004).

Synonyms: *J. erythrocarpa* Cory (in part: New Mexico, Arizona)

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LITERATURE CITED

- Adams, R. P. 1975. Statistical character weighting and similarity stability. *Brittonia* 27: 305-316.
- Adams, R. P. 1999. Systematics of multi-seeded eastern hemisphere based on leaf essential oils and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 27: 709-725.
- Adams, R. P. 2000a. Systematics of *Juniperus* section *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 28: 515-528.
- Adams, R. P. 2000b. Systematics of smooth leaf margin *Juniperus* of the western hemisphere based on leaf essential oils and RAPD DNA fingerprinting. *Biochem. Syst. Ecol.* 28: 149-162.
- Adams, R. P. 2000c. Systematics of the one seeded *Juniperus* of the eastern hemisphere based on leaf essential oils and random amplified polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 28: 529-543.
- Adams, R. P. 2000d. The serrate leaf margined *Juniperus* (Section *Sabina*) of the western hemisphere: Systematics and evolution based on leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 28: 975-989.
- Adams, R. P. 2004. The junipers of the world: The genus *Juniperus*. Trafford Publ., Victoria, BC.
- Adams, R. P. and T. Demeke, 1993. Systematic relationships in *Juniperus* based on random amplified polymorphic DNAs (RAPDs). *Taxon* 42: 553-571.
- Adams, R. P., L. E. Flournoy and R. N. Pandey. 1998. Obtaining reproducible patterns from random polymorphic DNA amplification

- (RAPDs). in Conservation of Plant Genes III: Conservation and Utilization of African Plants. R. P. and J. E. Adams, eds., Missouri Botanical Garden, St. Louis.
- Adams, R. P. and S. Nguyen. 2005. Infra-specific variation in *Juniperus deppeana* and f. *sperryi* in the Davis Mountains, Texas: Variation in leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs). *Phytologia* 87(2):96-108.
- Adams, R. P. and L. H. Rieseberg. 1998. The effects of non-homology in RAPD bands on similarity and multivariate statistical ordination in *Brassica* and *Helianthus*. *Theoret. Appl. Genet.* 97: 323-326.
- Adams, R. P., J. A. Morris, R. N. Pandey and A. E. Schwarzbach. 2005. Cryptic speciation between *Juniperus deltoides* and *J. oxycedrus* (*Cupressaceae*) in the Mediterranean. *Biochem. Syst. Ecol.* 33: 771-787.
- Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 326-338.
- Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874
- Rieseberg, L. H. 1996. Homology among RAPD fragments in interspecific comparisons. *Mol. Ecol.* 5: 99-105.
- Schwarzbach, A. E., R. P. Adams and J. A. Morris. 2007. Phylogeny of *Juniperus* based on nrDNA and trnC-trnD sequences. *Plant Syst. Evol.* (in prep.)
- Terry, R. G., R. S. Novak and R. J. Tausch. 2000. Genetic variation in chloroplast and nuclear ribosomal DNA in Utah juniper (*Juniperus osteosperma*, *Cupressaceae*): Evidence for interspecific gene flow. *Amer. J. Bot.* 87: 250-258.
- Zanoni, T. A. and R. P. Adams. 1976. The genus *Juniperus* (*Cupressaceae*) in Mexico and Guatemala: Numerical and

chemosystematic analysis. *Biochem. Syst. Ecol.* 4: 147-158.

Zanoni, T. A. and Adams, R. P. 1979. The genus *Juniperus* (*Cupressaceae*) in Mexico and Guatemala: Synonymy, Key, and distributions of the taxa. *Bol. Soc. Bot. Mexico* 39: 83 - 121.

Veldman D. J., 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.