JUNIPERUS COMPACTA (CUPRESSACEAE) A NEW SPECIES FROM MEXICO

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ABSTRACT

Recent nrDNA and trnC-trnD sequence data revealed that *J. monticola* and *J. m*. f. *compacta* are not monophyletic, and this prompted additional research using Single Nucleotide Polymorphisms (SNPs). The SNPs data clearly show that *J. monticola* f. *compacta* is not conspecific with *J. monticola* f. *monticola*. *Juniperus monticola* f. *compacta* Mart. is raised to the specific level as: ***Juniperus compacta*** (Mart.) R. P. Adams**, comb. et. stat. nov.**

KEY WORDS: *Juniperus jaliscana*, *Juniperus monticola, J. compacta, J. saltillensis,* nrDNA, trn C-trnD, SNPs, Cupressaceae

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Adams (2004), in his monograph of *Juniperus*, followed traditional classifications in recognizing *J. monticola* Mart. f. *monticola*, *J. m*. f. *compacta* Mart. and *J. m.* f. *orizabensis* Mart. However, DNA sequencing of nrDNA and trnC-trnD for *Juniperus* (Schwarzbach, et al., 2008) has shed new light on the relationships within this group. One surprising finding was that *J. m.* f. *monticola* formed a clade with *J. jaliscana* whereas *J. m*. f. *compacta* formed a clade with *J. saltillensis* M. T. Hall. These clades were well separated.

To further investigate this problem, additional sequencings of nrDNA and the trnC-trnD cp DNA region were performed to obtain SNPs to reexamine the relationship of *J. monticola* to *J. m.* f. *compacta*.

MATERIALS AND METHODS

Specimens collected: *J. jaliscana*, *Adams 6846-6848*, 12/12/1991, 940 m, 19 km E of Mex. 200 on the road to Cuale, Jalisco, Mexico; *J. monticola* f. *compacta*, *Adams 6898-6902*, 12/21/1991, 3490 m, Cerro Potosi, Nuevo Leon, Mexico; putative *J. m*. f. *compacta*, *S. Gonzalez et al. 7169a,b 6/17/2006,* (=*Adams 11221, 11222*), 4000 m, Nevado de Colima, Jalisco, Mexico; *J. monticola* f. *monticola*, *Adams 6874-6878*, 12/20/1991, 2750 m, El Chico National Park, Hidalgo, Mexico; *J. saltillensis*, *Adams 6886-6890*, 12/21/1991, 2090m, on Mex. 60, 14 km E. of San Roberto Junction, Nuevo Leon, Mexico. 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^{o} C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer (final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM $MgCl_2$, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4%(by vol.) DMSO.

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

trnC-trnD: CDFor: CCA GTT CAA ATC TGG GTG TC
CDRev: GGG ATT GTA GTT CAA TTG GT

CDFor, CDRev primers from Demesure et al. (1995).

CD10F: AAA GAG AGG GAT TCG TAT GGA
CD3R: AAC GAA GCG AAA ATC AAT CA
CD10F and CD3R primers from Andrea Schwarzbach (per. comm.).

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 45 cycles, 94°C (1 min.), 50°C (1 min.), 72°C (1 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. The internal trnC-trnD primers, CD10F-CD3R produced a band of approx. 850 bp. In each case the band was excised and purified by use of a Qiagen QIAquick gel extraction kit.

The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., 2008).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams et al., 2003).

RESULTS AND DISCUSSION

Analyses of the nrDNA sequences revealed 13 SNPs among the taxa. PCO of the SNPs resulted in 3 eigenroots that accounted for 42, 22 and 19 % of the variation among the OTUs. Ordination (Fig. 1) shows 4

groups as *J. jaliscana*, *J. m.* f. *monticola*, *J. m.* f. *compacta* and *J. saltillensis*. Notice that the two alpine plants (NC1, NC2) from Nevado de Colima (4000 m) appear as somewhat intermediate between taxa.

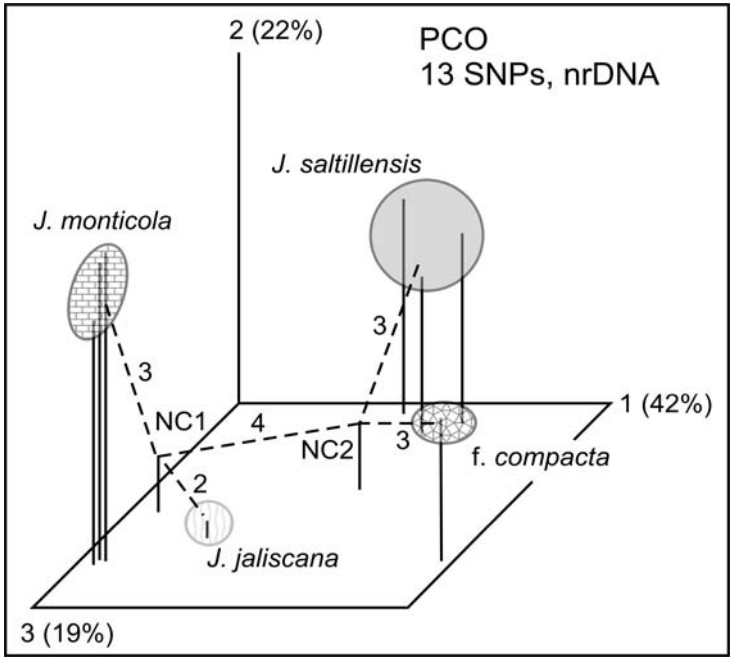

Figure 1. PCO ordination based on 13 SNPs from nrDNA. Dashed lines are the minimum spanning network with the number of nucleotide differences noted on the dashed line.

Clearly, *J. m.* f. *compacta* (Cerro Potosi) is quite different from *J. m.* f. *monticola*. No variation was found among the 3 individuals of *J. m.* f. *compacta* (Cerro Potosi), or among the 3 individuals of *J. jaliscana*. (a single stick is used in Fig. 1 to represent 3 individuals for these taxa).

Analyses of a portion of trnC-trnD revealed several indels, with a total of 15 SNPs. PCO ordination extracted 3 eigenroots that accounted for 66, 25 and 5% of the variation, implying that 4 groups were present

(Fig. 2). These four groups are the same groups as with the nrDNA: *J. jaliscana*, *J. m.* f. *compacta*, *J. m.* f. *monticola*, and *J. saltillensis*. However, the two alpine plants from Nevado de Colima (NC1, NC2) had no differences from *J. m.* f. *monticola* (- NC1) or from *J. jaliscana* (- NC2). It is possible that NC1 is of hybrid origin with pollen and cp DNA from *J. m.* f. *monticola* and that NC2 is of hybrid origin with pollen and cp DNA from *J. jaliscana*.

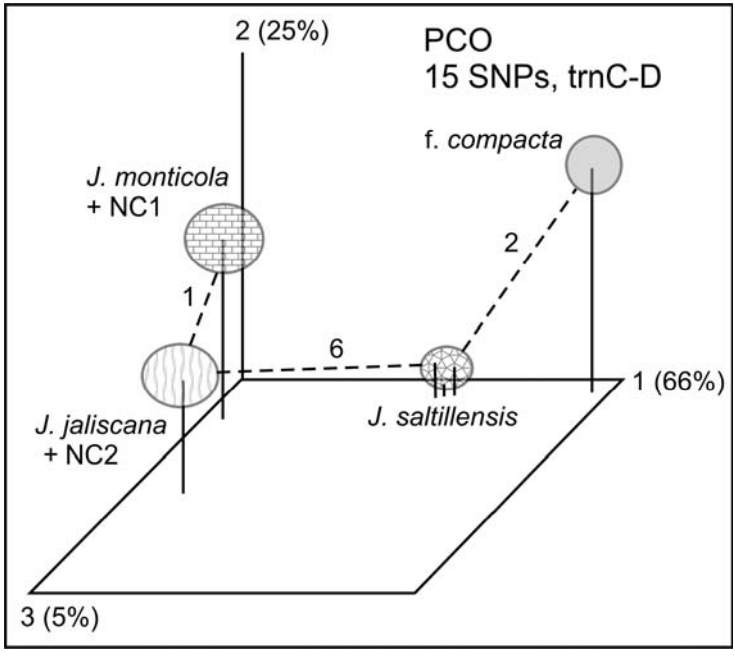

Figure 2. PCO ordination based on 15 SNPs from trnC-trnD. Dashed lines are the minimum spanning network with the number of nucleotide differences noted on the dashed line.

No variation was found within *J. jaliscana, J. monticola*, or *J. m.* f. *compacta*. However, the 3 individuals of *J. saltillensis* differed among themselves by a single nucleotide. This differs a little from the nrDNA where more variation within taxa was detected. It appears that in

this instance, the cp DNA has not accumulated mutations as quickly as nr DNA.

Zanoni and Adams (1976) analyzed leaf volatile oils from several locations of *J. monticola*, *J. m.* f. *compacta* and *J. m.* f. *orizabensis*. They reported that the oils from these taxa were rather uniform, except for the oil of *J. m.* f. *compacta* from Cerro Potosi.

Adams et al. (1980) compared the leaf terpenoids of *J. monticola* (El Chico), *J. m.* f. *compacta* (Nevada de Toluca) and *J. m.* f. *orizabensis* (Pico Orizaba). Table 1 shows an abbreviated summary of their results. Several compounds appear to discriminate between the three formas. These compounds include tricyclene, α-pinene, sabinene, α-terpinene, 4-terpineol, bornyl acetate, γ-terpinene, the eudesmols and 8-α-acetoyxelemol. It should be noted that the sample (average of 5 plants) of *J. m.* f. *compacta* was from Nevada de Toluca not Cerro Potosi (as used for the SNPs in this paper). Zanoni and Adams (1976) reported that the leaf oil from Cerro Potosi was quite different from *J. m.* f. *compacta* from Nevada de Toluca and Popocatepetl.

Table 1. Comparison of volatile leaf oils of *J. monticola* (El Chico), *J. m.* f. *compacta* (Nevada de Toluca) and *J. m.* f. *orizabensis* (Pico Orizaba). Several compounds that appear to separate the taxa are indicated in boldface. t = trace (<0.05%).

Compound	mont.	comp.	oriz.
tricyclene	**0.6**	**t**	**0.9**
α-pinene	**25.8**	**8.8**	**6.0**
camphene	0.8	t	1.2
verbenene	0.5	-	-
sabinene	**t**	**26.9**	**t**
β-pinene	0.8	t	t
myrcene	2.1	2.1	2.8
4-carene	3.3	0.9	2.1
α-phellandrene	t	t	t
3-carene	-	-	t
α-terpinene	**-**	**1.8**	**t**

p-cymene	t	0.5	t
camphene hydrate	0.5	t	1.3
borneol	4.0	2.5	1.7
4-terpineol	**t**	**10.1**	**0.7**
α-terpineol	t	t	t
piperitone	0.9	t	t
bornyl acetate	**25.6**	**12.8**	**48.6**
α-terpinyl acetate	t	t	t
thymol	t	t	t
(E)-caryophyllene	-	-	t
germacrene D	-	t	-
β-phellandrene	2.2	0.6	1.5
limonene	12.4	8.0	13.2
γ-terpinene	**t**	**3.3**	**0.6**
p-menth-1(7),3-diene	-	0.5	-
terpinolene	t	-	0.5
linalool	t	t	1.5
cis-sabinene hydrate	t	0.6	1.4
camphor	3.3	1.0	4.2
trans-sabinene hydrate	t	t	0.7
elemol	2.5	2.3	1.4
γ-eudesmol	**1.0**	**0.6**	**t**
β-eudesmol	**3.3**	**1.4**	**t**
α-eudesmol	**1.6**	**0.5**	**t**
8-α-acetoxyelemol	**1.4**	**0.8**	**t**
manoyl oxide	t	3.0	t
manool	-	0.6	-

It is clear from SNPs of both nrDNA and trnC-trnD cp DNA that *Juniperus monticola* f. *compacta* is not allied with *J. monticola*. In fact, it is as different from *J. monticola* as several other recognized species (*J. jaliscana*, *J. saltillensis*, Figs. 1, 2). It is also different in its volatile leaf oils (Table 1) and its morphology (Adams, 2004; Zanoni and Adams, 1976, 1979), having tightly compacted foliage and being prostrate shrubs. Silba (2006) recognized it as a subspecies (*J. m*. subsp. *compacta* (Mart) Silba) but did not discern its affinity to *J. saltillensis* (due to cryptic variation in the morphology).

Based on the data presented in this paper, it is appropriate to recognize *Juniperus monticola* f. *compacta* as a distinct species:

Juniperus compacta (Mart.) R. P. Adams, **comb. et. stat. nov.**

Basionym: *Juniperus monticola* Martinez f. *compacta* Martinez, Bol. Soc. Bot. Mexico 7: 19 (1948). Compact mountain juniper. Type: Mexico, Volcan Popocatepetl, *Martinez 7003* (HOLOTYPE: MEXU!).

Distribution: 3000-4300 (-4500) m Sierra Mojada, Coahuila; Cerro Pelado and Ajusco, Distrito Federal; Nevado de Colima, Jalisco; Popocatepetl, Iztaccihuatl, Tlaloc and Nevado de Toluca, Mexico; Cerro Potosi, Nuevo Leon; Malinche, Tlaxcala; and Cofre de Perote, Vera Cruz, Mexico.

Synonyms: *Cupressus sabinoides* H.B.K., Nova Gen. et Sp. Pl. 2:3. 1817.

J. mexicana Sprengel, Syst. Veg. 3: #909 (1826), *nom. superfl. illeg.*

J. sabinoides (Kunth) Nees. Linnaea 19: 706 (1847), *non* Griseb., Spec. Fl. Rumel. 2: 352 (1846).

J. sabinoides Humb. (erroneously attributed) in Lindley and Gordon, J. Hort. Soc. 5: 202 (1850).

J. monticola Martinez var. *monticola* f. *compacta* Martinez, Bol. Soc. Bot. Mexico 7:19 (1948).

J. monticola Martinez subsp. *compacta* (Martinez) J. Silba, J. Int. conifer Preserv. Soc. 13(1): 12 (2006).

Several questions remain unanswered concerning the alpine junipers of Mexico. What is the biological status of *J. monticola* f. *orizabensis*? Might all the disjunct alpine populations be variants of *J. compacta*? Do the large leaf oil differences correlate with more wide based genetic differences? Additional collections and analyses are being conducted to address these questions.

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LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 2004. Junipers of the World: The genus *Juniperus*. Trafford Publ., Vancouver, B. C.

Adams, R. P., A. E. Schwarzbach, and R. N. Pandey. 2003. The Concordance of Terpenoid, ISSR and RAPD markers, and ITS sequence data sets among genotypes: An example from *Juniperus*. Biochem. Syst. Ecol. 31: 375-387.

Adams, R. P., E. von Rudloff, L. Hogge and T. A. Zanoni. 1980. The volatile terpenoids of *Juniperus monticola* f. *monticola*, f. *compacta* and f. *orizabensis*. J. Nat. Prods. 43: 417 - 419.

Blattner, F. R. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. BioTechniques 27: 1180-1186.

Demesure, B., N. Sodzi and R. J. Petit. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mito-chondrial and chloroplast DNA in plants. Mol. Ecol. 4:129-131.

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.

Schwarzbach, A. E., R. P. Adams and J. A. Morris. 2008. Phylogeny of *Juniperus* based on nrDNA and trnC-trnD sequences. (in prep).

Silba, J. 2006. An International census of the coniferae, III, a checklist of the Cupressaceae, Part one (*Actinostrobus*, *Callitris*, *Diselma*, *Fitzroya*, *Libocedrus*, *Juniperus* and *Widdringtonia* only. .J. Int. Conifer Preserv. Soc. 13: 1-20.

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 R. P. Adams. 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.