JUNIPERUS ZANONII, A NEW SPECIES FROM CERRO POTOSI, NUEVO LEON, MEXICO

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ABSTRACT

Analyses of nrDNA and trnC-trnD sequence data revealed that the shrubby alpine juniper from Cerro Potosi is not allied with *J. monticola* f. *compacta* from Ixtaccihuatl in the trans-volcanic belt of central Mexico, but forms a distinct clade with *J. saltillensis*. Research using Single Nucleotide Polymorphisms (SNPs) with samples of *J. monticola* f. *compacta* from the near the type locality confirmed that the Cerro Potosi alpine-subalpine juniper is not related to either *J. monticola* f. *monticola* nor *J. m.* f. *compacta*. A new species is proposed, ***Juniperus zanonii*** R.P. Adams, **sp. nov.** from Cerro Potosi and nearby alpine-subalpine mountains of NE Mexico. The alpine-subalpine *J. monticola* junipers of the trans-volcanic belt in central Mexico were shown to be in a different phylogenetic group than *J. zanonii*. *Phytologia 92(1): 105-117 (April, 2010).*

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

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Analyses of nrDNA and trnC-trnD sequences (Adams et al. 2008) of the serrate leaf margined junipers of the western hemisphere has revealed some un-expected phylogenetic information. Figure 1 shows a Bayesian tree based on nrDNA and trnC-trnD sequences for this group. Notice that the alpine shrubby juniper from Cerro Potosi (3490 m) is in a well support clade with *J. saltillensis*. However, *J.*

monticola (f. *monticola* from El Chico National Park, Hidalgo, is in a clade with *J. jaliscana*.

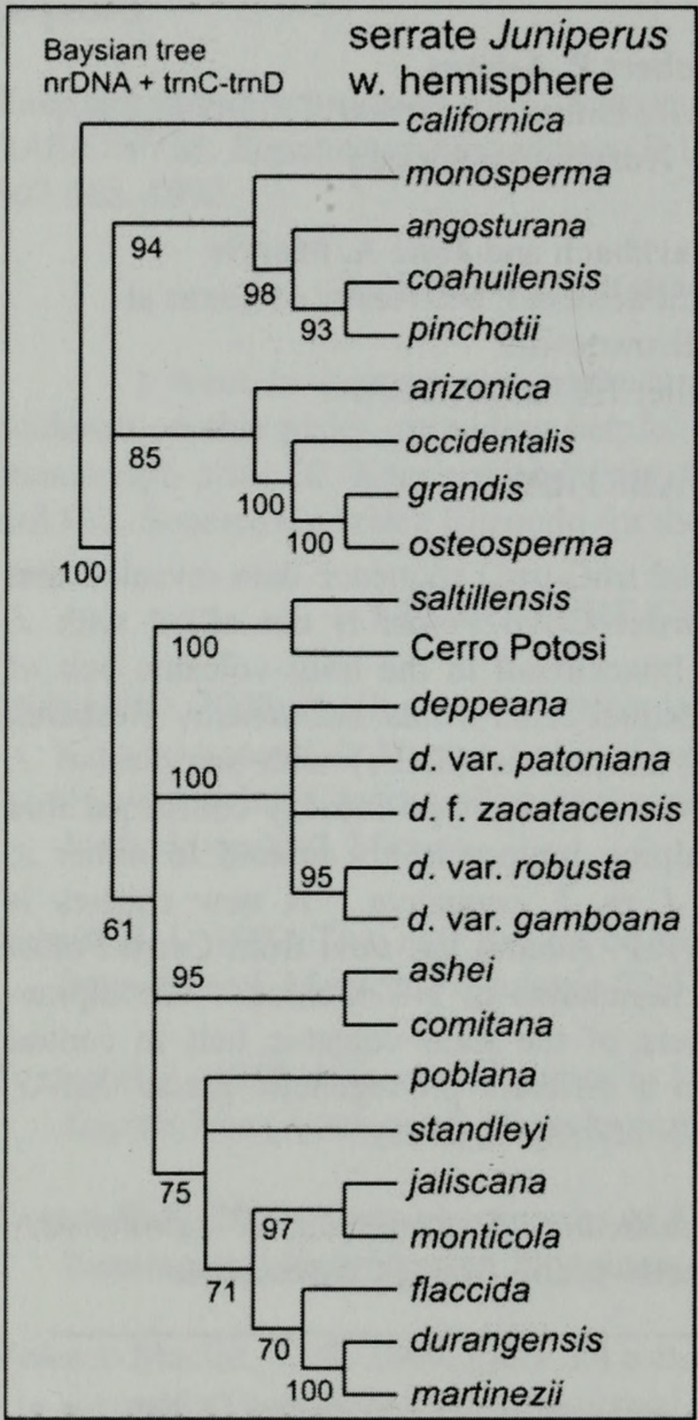

Figure 1. Bayesian tree for the serrate-leave *Juniperus* of the western hemisphere (modified from Adams, 2008).

Notice that the Cerro Potosi juniper is in a well support clade with *J. saltillensis*, whereas *J. monticola* (f. *monticola*) from El Chico National Park, Hidalgo, is in a clade with *J. jaliscana*. But, *J. m.* f. *compacta* from Popocatepetl (nor nearby Ixtaccihuatl) was not included in this analyses.

The alpine juniper on Cerro Potosi has been treated as *J. monticola* f. *compacta* by many authors (Adams, 2008; Farjon, 2005; Martinez, 1693; McDonald, 1990, 1993; Zanoni and Adams, 1975, 1976, 1979). So it is not surprising that Adams et al. (2007) treated the Cerro Potosi juniper as a disjunct population of *J. monticola* f.

compacta. As previously mentioned, the alpine-subalpine juniper shrub from Cerro Potosi, Nuevo Leon was found to be so distinct in its nrDNA and cp trnC-trnD that it was recognized at the specific level as *J. compacta* (Mart.) R. P. Adams (=*J. monticola* f. *compacta* Mart.) (Adams et al., 2007). However, because the holotype for *J. monticola* f. *compacta* is from Volcan Popocatepetl (*Martinez 7003*) and the habitats there differ from Cerro Potosi (where it grows as an understory plant beneath *Pinus culminicola* and *P. hartwegii* on limestone) and Popocatepetl (where it is found above timberline, on volcanic material, in full sunlight), it seemed of interest to compare these populations with putative *J. monticola* f. *compacta*. In addition, Zanoni and Adams (1976) examined the leaf oils of plants from Cerro Potosi and Ixtaccihuatl (their *J. m.* f. *compacta* population was only about 16 km from the Popocatepetl type locality). They reported that the Cerro Potosi plant oils were very different from *J. m.* f. *compacta*, *J. m.* f. *monticola* and *J. m.* f. *orizabensis* populations from the central Mexico volcanoes, but they did not follow up on these data.

The purpose of this study was to compare materials of *J. monticola* f. *compacta* from near the type locality with materials from the shrubby alpine-subalpine juniper from Cerro Potosi using sequencing of nrDNA and the petN-psbM cp DNA. *Juniperus jaliscana*, *J. saltillensis* and *J. virginiana* were included as outgroups.

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*: *Alicia Mastretta, 1-4*, I1-I4, (= *Adams 11738-11740*), 19° 10' N, 98° 38' W, 4270 m, Pico Ixtaccihuatl, Mexico; putative *J. m. f. compacta*, *Adams 6898-6902*, 12/21/1991, 3490 m, Cerro Potosi, Nuevo Leon, Mexico; *J. monticola* f. *monticola*, *Adams 6874-6878*, 12/20/1991, 2750 m, El Chico National Park, Hidalgo, Mexico; *J. monticola* f. *orizabensis*: *S. Gonzalez 7243a,b, 7244a,b,* O1-O4 (=*Adams 11267-11270*), Pico de Orizaba, Vera Cruz, Mexico; *J. saltillensis*, *Adams 6886-6890*, 12/21/1991, 2090 m, on Mex. 60, 14 km E. of San Roberto Junction, Nuevo Leon, Mexico; *J. virginiana*, *Adams 10230-10232*, Knoxville, TN. 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). ITS (nrDNA), petN-psbM 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 (petN-psbM) 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. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. 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

Phylogenetic analyses, based on combined nrDNA plus petN-psbM (cp DNA) sequences, revealed that the alpine-subalpine juniper shrubs from Cerro Potosi are in a clade with *J. saltillensis* and not with *J. monticola* f. *compacta* (Fig. 2). Morphologically, it is difficult to distinguish the Cerro Potosi juniper shrubs from *J. m.* f. *monticola* at Popocatepetl (and Ixtaccihuatl) as both taxa have compact foliage, with reduced leaves at the high elevation sites. It is easy to understand why Adams et al. (2007) failed to include *J. m.* f. *compacta* from Popocatepetl (or Ixtaccihuatl) in their DNA analyses. This led them to assume that the Cerro Potosi alpine-subalpine juniper was in fact *J. m.* f. *compacta* and led to the elevation of *J. m.* f. *compacta* to *J. compacta*. The results from the Bayesian tree (Fig. 2) clearly show that both *J. m.* f. *monticola* (Hidalgo, El Chico NP) and *J. m.* f. *compacta* (Ixtaccihuatl) are not phylogenetically closely related to the alpine-subalpine juniper of Cerro Potosi.

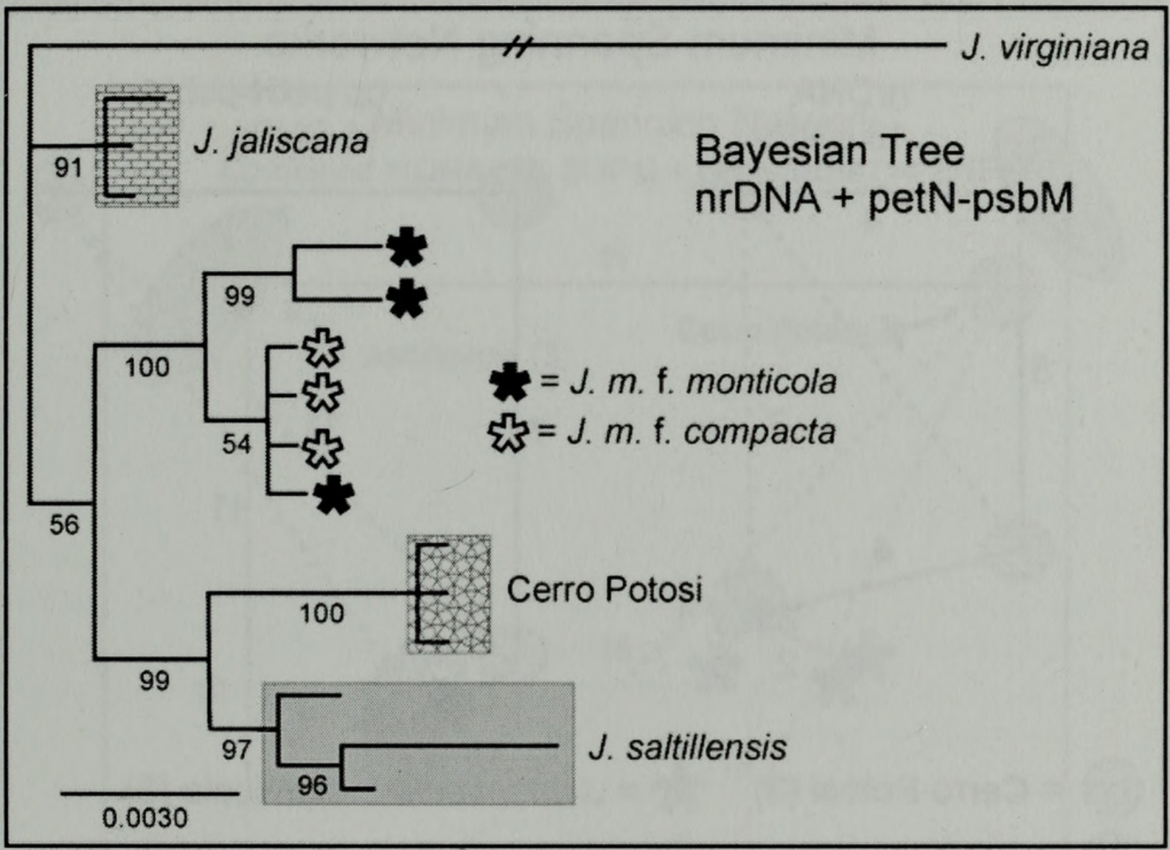

Figure 2. Bayesian tree based on combined sequences from nrDNA and petN-psbM (cp DNA). Numbers at the branch points are posterior probabilities.

Analyses of the nrDNA sequences revealed 20 mutations (nucleotide differences plus indels), of which 7 occurred only once. The remaining 13 mutations were treated at SNPs for analysis. A minimum spanning network (Figure 3, left) shows the Cerro Potosi junipers to be well resolved from both *J. monticola* f. *compacta* (Ixtaccihuatl) and *J. m.* f. *monticola* (El Chico Natl. Park, Hidalgo).

Analysis of the petN-psbM sequences revealed 17 mutations (nucleotide differences plus indels), of which 3 occurred only once. The remaining 14 mutations were treated at SNPs for analysis. A minimum spanning network (Figure 3, right) revealed the Cerro Potosi junipers to be well resolved from both *J. monticola* f. *compacta* (Ixtaccihuatl) and *J. m.* f. *monticola* (El Chico Natl. Park, Hidalgo). The petN-psbM SNPs did not separate *J. monticola* f. *compacta* (Ixtaccihuatl) from *J. m.* f. *monticola* (Figure 3, right).

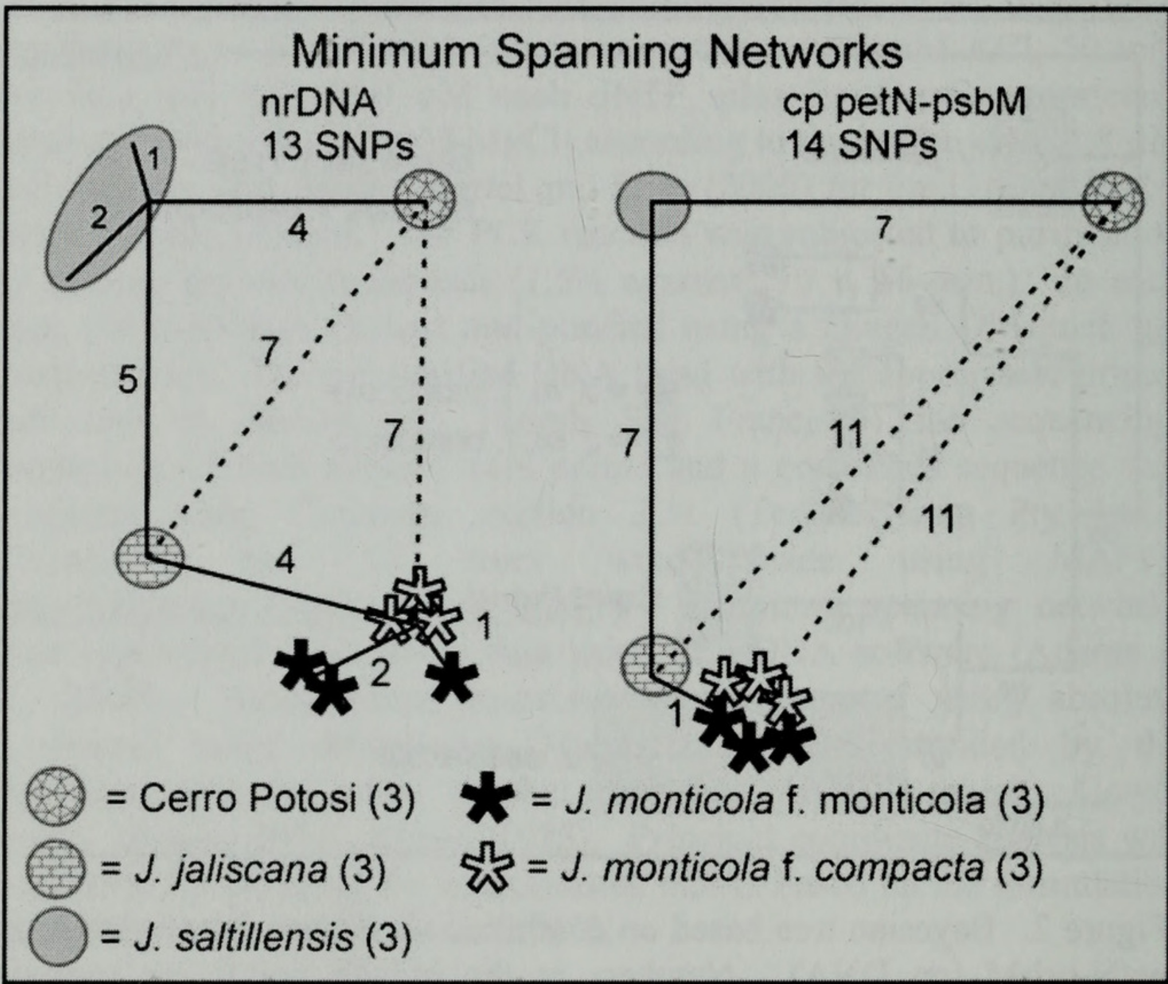

Figure 3. Minimum spanning networks based on nrDNA (left) and petN-psbM (right). The numbers next to the lines (links) are the number of SNPs. The dashed lines are the next shortest link from the Cerro Potosi junipers to other taxa. The numbers in parenthesis after the names are the number of samples analyzed per taxon.

Combining the 13 SNPs from nrDNA and 14 SNPs from cp petN-psbM sequences shows the additive nature of these two gene regions (Figure 4). The Cerro Potosi junipers are clearly very differentiated from *J. monticola* f. *compacta* (Ixtaccihuatl) and *J. m.* f. *monticola*; in short, the common application of *J. m.* f. *monticola* to the alpine junipers of Cerro Potosi is not supported. The recognition (Adams et al., 2007) of the Cerro Potosi juniper as a part of *J. m.* f. *compacta* and the elevation of *J. m.* f. *compacta* to *J. compacta* (Mart.) R. P. Adams is not supported. The juniper populations at alpine-subalpine habitats on Cerro Potosi and adjacent areas are not closely related to *J. monticola* f. *compacta*, but represent a new species, more related to *J. saltillensis*.

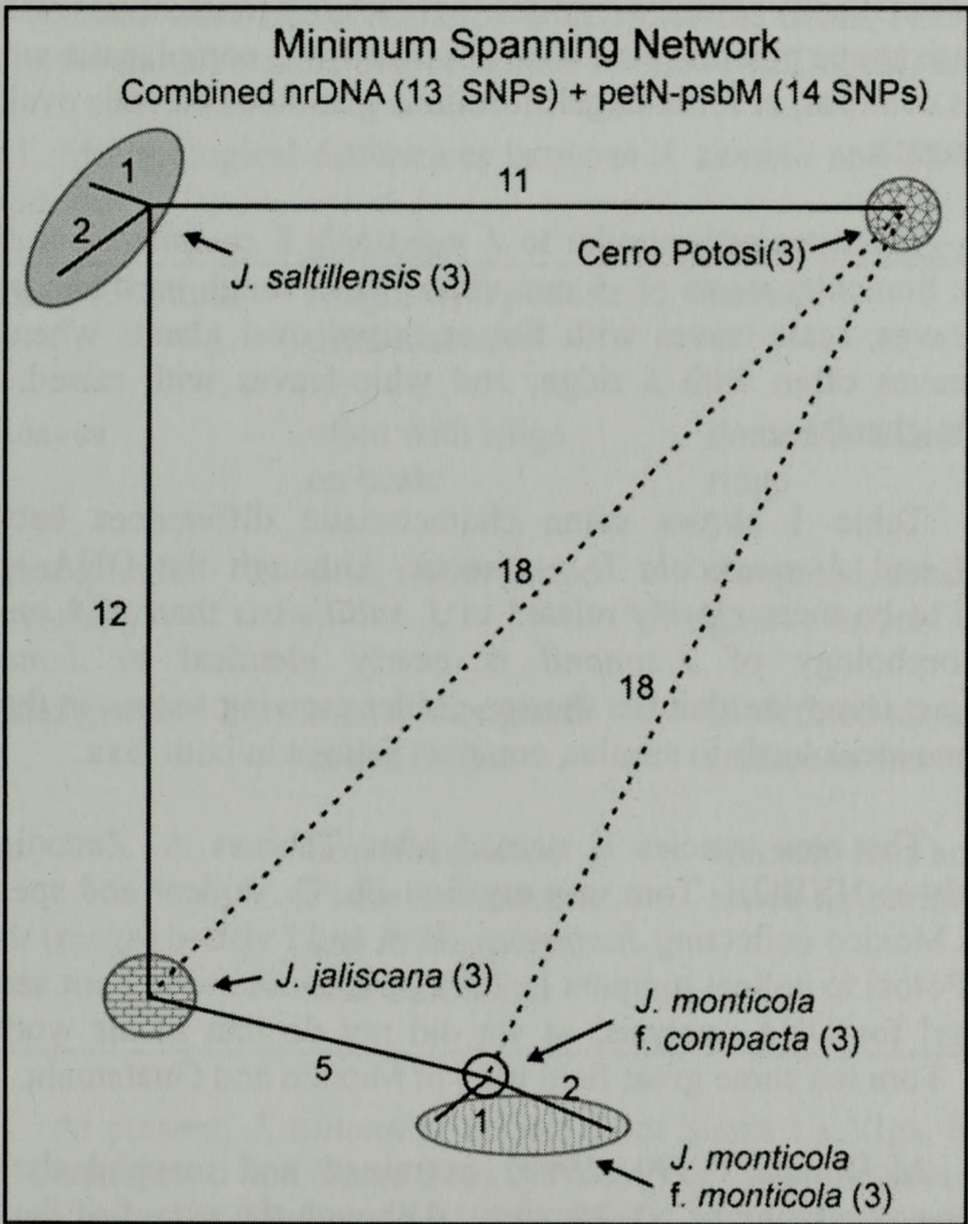

Figure 4. Minimum spanning network using 27 SNPs combined from nrDNA and petN-psbM. See figure 2 for details.

A new alpine-subalpine species in NE Mexico is recognized as:

Juniperus zanonii R.P. Adams, **sp. nov.** Type: Mexico, Nuevo Leon, Cerro Potosi, 3550 m, *J. A. McDonald 1820*, 26 Jul 1985 (HOLOTYPE: TEX).

J. monticolae forma *compactae* similis sed differt caulibus ramulorum (3-5 mm in diam.) cortice asperi foliis emortuis persistentibus, foliis squamatis saepe porcatis glandibus ubi manifestis complanatis vel elevatis ovalibus, et foliis flagelliformibus glandibus elevatis ovalibus vel elongatis.

This species is similar to *J. monticola* f. *compacta* but differs in that the branchlet stems (3 -5 mm diam.) have rough bark and persistent dead leaves, scale-leaves with flat or raised oval glands when visible, scale-leaves often with a ridge, and whip-leaves with raised, oval to elongate glands.

Table 1 shows some characteristic differences between *J. zanonii* and *J. monticola* f. *compacta.* Although the DNA shows *J. zanonii* to be more closely related to *J. saltillensis* than to *J. monticola*, the morphology of *J. zanonii* is nearly identical to *J. monticola.* Of course, it may be that the shorter, colder growing season in the alpine-subalpine areas leads to similar, compact foliage in both taxa.

The new species is named after Thomas A. Zanoni (1949-, presently at NYBG). Tom was my first Ph. D. student and spent much time in Mexico collecting *Juniperus*. Tom and I visited (again) the top of Cerro Potosi to collect junipers in 1991 and, fortunately, I put samples in silica gel for DNA analysis, as we did not do that in his work in the 1970s. Tom led some great field trips in Mexico and Guatemala.

McDonald (1990, 1993) examined and mapped the alpine-subalpine vegetation of NE Mexico. Although the extent of the areas is limited (Fig. 5), floristic affinities were shown (McDonald, 1990) to be strong among these areas and not similar to that of the trans-volcanic belt of central Mexico. McDonald (1993) used the Sorenson index of genetic similarity between alpine-subalpine vegetations and showed that the NE Mexico alpine-subalpine flora was more similar to that of the White

Mtns., NM, U. S. A., than to that of the trans-volcanic belt of central Mexico.

McDonald (1993) argues that the alpine zones extended downward about 1000 m during the Wisconsin glacial maximum, leading to much larger alpine-subalpine areas in NE Mexico. However, even if the alpine-subalpine zone descended 1000 m, the alpine-subalpine areas of NE Mexico were not continuous with either the Trans-Pecos, Texas Mtns, or the trans-volcanic belt of central Mexico (McDonald, 1993).

Table 1. Morphological differences between *J. zanonii* and *J. monticola* f. *compacta.*

	J. zanonii	*J. m.* f. *compacta*
Branchlets (3-5 mm diam.)	rough, with persistent dead leaves.	smooth
Scale leaves	often with ridge on beak	domed beak, no ridge
Scale leaf glands (when visible)	oval, flat or raised	elongated, sunken groove on leaf
Whip leaf glands	oval to elongated raised or flat	ill-defined elongated, sunken to flat
Habitat	limestone, under *Pinus culminicola* and *P. hartwegii* near timberline	volcanic lava and rocks above timberline

At present, *J. zanonii* is known from Sierra La Viga, Sierra La Marta, Cerro Potosi and Sierra Pena Nevada (Fig. 5) of the seven alpine-subalpine areas of McDonald (1990, 1993). It seems likely that *J. zanonii* occurs on the three other alpine-subalpine areas (S. Potrero de Abrego, S. Coahuilon, and S. Borrado, Fig. 5).

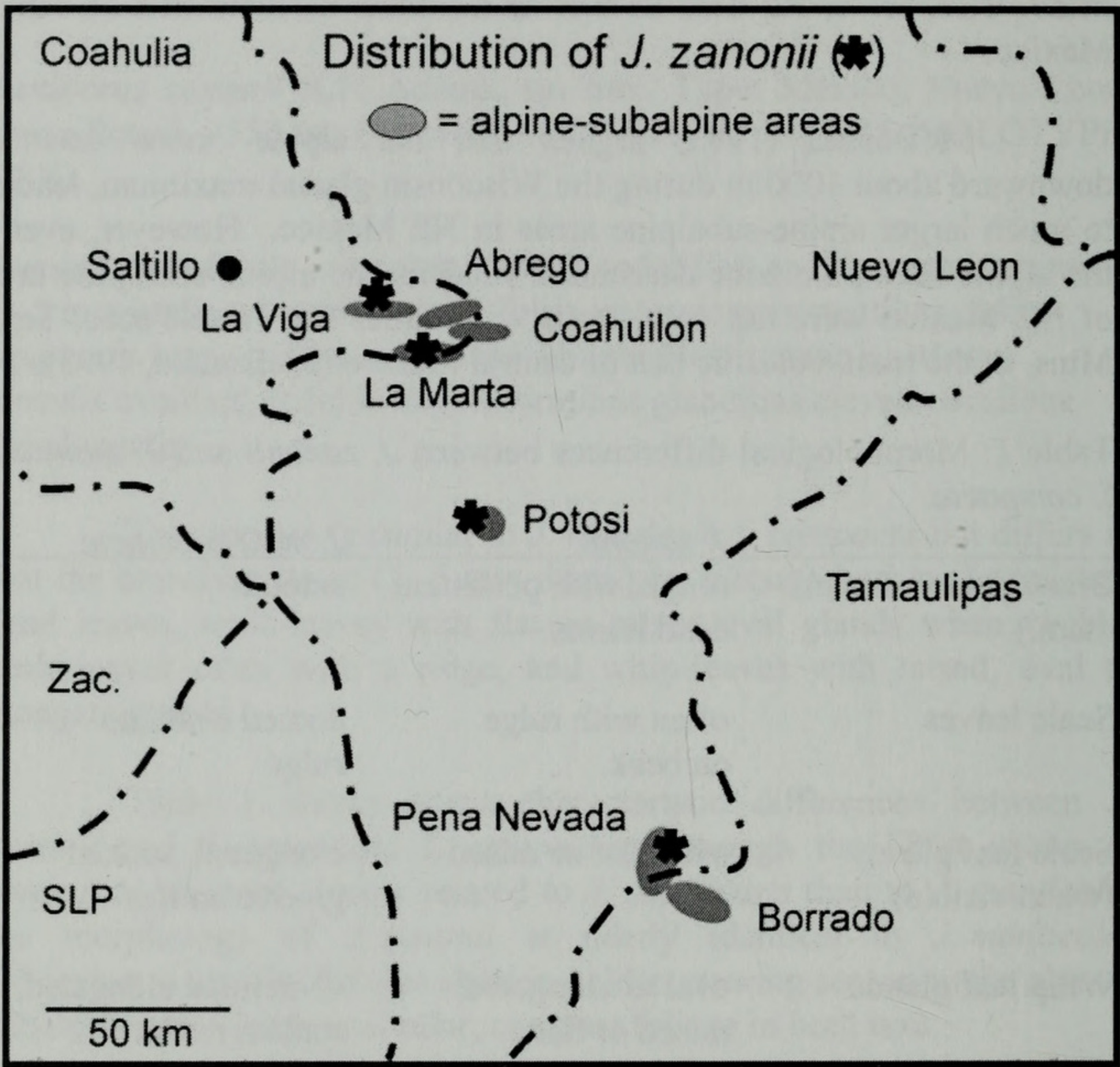

Figure 5. Distribution of *J. zanonii* overlaid onto a map of alpine-subalpine areas in NE Mexico based on McDonald, 1990, 1993. Alpine areas: La Viga (Sierra La Viga); Abrego (Sierra Potrero de Abrego); Coahuilon) Sierra Coahuilon); La Marta (Sierra La Marta); Potosi (Cerro Potosi); Pena Nevada (Sierra Pena Nevada) and Borrado (Sierra Borrado).

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Appendix I. Specimens examined.

J. zanonii

Coahuila: Cerro de Viga, *Henrickson, et al. 16130b, 16143* (TEX); *Hinton et al. 20201* (TEX); Sierra La Marta *McDonald 2140* (TEX).

Nuevo Leon: Cerro Potosi, *Adams 6898-6902* (BAYLU); *Gilbert 13* (TEX); *Marroquin 346* (TEX); *McDonald 1820* (TEX); *Mueller 2262* (TEX); *Schneider 963* (A, ARIZ, F, MICH, MO, NA); *Zanoni 2588, 2589, 2590, 2591, 2592* (BAYLU); *Zanoni 2589, 2594, 2597, 2598* (TEX).

Tamaulipas: Cerro Pena Nevada summit. *Stanford, et al. 2571* (DS, RSA).

J. monticola* f. *compacta (=J. compacta)

Coahuila: Sierra Mojada, *M. E. Jones 191* (MO, POM, RSA, US). Location may be mis-labeled?

District Federal: La Cima station, Serjana de Ajusco, *Beaman 2807* (GH, MSC).

Jalisco: Nevada de Colima, *Brizuela* 27 Oct 1967 (ENCB).

Mexico: Tlaloc, *Beaman 2316* (GH, MSC); Ixtaccihuatl, Mexico, *Alicia Mastretta, 1-4,* L1-L4, (= *Adams 11906-11909*, BAYLU); Volcan Popocatepetl, *Martinez 7003* (MEXU), the recent eruption of Popocatepetl may have destroyed this population. *Koeppen. & Iltis 1030* (MICH, MSC, TEX, UC); Nevada de Toluca, *Zanoni 2199, 2208* (TEX), *Beaman 1721* (GH, MSC)

Tlaxacala: La Malinche, *Beaman 2248* (MICH).

Vera Cruz: Cofre de Perote, *Martinez 10525* (BH, F) and *10524b* (MO, UC); *S. Gonzalez 7240a, b, c* (BAYLU);

J. monticola* f. *monticola

District Federal: La Cima station, Serjana de Ajusco, *Koeppen. & Iltis* 12-14 Jul 1960 (TEX).

Guerrero: Cerro Teotepec, Mpio. Tlacotepec, *Rzedowski 18574* (MICH, TEX).
Hidalgo: Real del Monte, *Martinez 10523* (F, RSA); El Chico National Park, *Adams 6874-6878* (BAYLU).
Jalisco: Nevado de Colima, *Gregory & Eiten 300* (MICH, MO, SMU).
Michoacan: Pico Tancitaro, *Leavenworth & Hoogstral 1163A* (F, MO).
Morelos: km. 42 on Mexico - Cuernavaca road, *Reko & Halbinger 65* (A).
Mexico: Monte de las Cruces, *Martinez 10523A* (DS), *Martinez 10524* (UC).
San Luis Potosi: Puerto de Cedros, 3 km e of Mineral de Catorce, *Rzedowski 7270* (ENCB).

J. monticola* f. *orizabensis
Vera Cruz: Pico de Orizaba, *Beaman 1756* (GH, MSC, US); *S. Gonzalez 7243a,b, 7244a,b* (BAYLU); *Martinez 10526* (MO, RSA, UC); *Smith 488* (MO); *Balls 4394* (A, UC, US); *Balls 4619* (A, US); Sierra Nigra (sw of Pico de Orizaba), *Beaman 2519* (GH, MSC, UC).