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DNA sequencing and taxonomy of unusual serrate Juniperus from Mexico: Chloroplast capture and incomplete lineage sorting in J. coahuilensis and allied taxa

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DNA sequencing and taxonomy of unusual serrate *Juniperus* from Mexico: Chloroplast capture and incomplete lineage sorting in *J. coahuilensis* and allied taxa

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ABSTRACT

Analysis of nrDNA, petN-psbM, trnS-trnG, trmD-trnT, and trnF-trnL of *Juniperus coahuilensis* and allied taxa of Mexico found typical *J. coahuilensis*, as well as individuals with: *coahuilensis* cp and hybrid ITS; *coahuilensis* cp and novel ITS sequence (La Parrilla type); novel Blue Fruited cp (blue fruited taxon) and *coahuilensis* ITS; plus Blue Fruited cp and La Parrila ITS. nrDNA data was examined and found to detect hybridization, chloroplast capture and incomplete lineage sorting. In addition, a new taxon was found with Blue Fruited (Blue Fruited) cp and *J. martinezii* ITS, suggestive of chloroplast capture. New records of *J. saltillensis* were confirmed from Zacatecas. A new record of *J. martinezii* from Durango was also confirmed. Several plants affiliated with either *J. martinezii*, or *J. flaccida* were in distinct clades showing the need for additional research on their volatile leaf oils, morphology and ecology to address their taxonomic status. And lastly, a very unusual population of junipers large, single stemmed trees with aff. *J. poblana* was found in Nayarit, with long and pendulous foliage. Analysis of the leaf volatile oils, ecology and morphology of this taxon is necessary (in progress) to ascertain its taxonomic rank. Published on-line www.phytologia.org *Phytologia 99(1): 62-73 (Jan 19, 2017).* ISSN 030319430.

KEY WORDS: *Juniperus coahuilensis, J. flaccida, J. martinezii, J. poblana*, Cupressaceae, hybridization, introgression, incomplete lineage sorting, nrDNA polymorphisms, petN-psbM DNA.

As a part of on-going research on *Juniperus*, recently, Adams (2016) found (by petN-psbM sequencing) that *Juniperus arizonica*, previously known only from Arizona and New Mexico, occurs in northern Sonora and Chihuahua, trans-Pecos Texas in the Franklin Mtns., Hueco Mtns., Hueco Tanks State Park, Quitman Mtns., Eagle Mtns. and Sierra Vieja Mtns., primarily on igneous material. These trans-Pecos juniper populations have previously been identified as *J. coahuilensis*.

Additional examination of populations of *J. coahuilensis* in the Trans-Pecos, Texas region (Adams 2017) revealed that situation was more complex with a relatively sharp demarcation between *J. arizonica* and *J. coahuilensis* (Fig. 1). The zone of contact and likely hybridization is in Hueco Tanks State Park, Quitman Mtns., and Anima Mtns. and this appears to be a region of introgression northward from *J. coahuilensis* (Fig. 1).

Although it appeared that the *J. coahuilensis* at La Zarca, MX was a pure population (Adams 2017), new specimens of aff. *J. coahuilensis* with violet, reddish and blue colored fruits have been discovered in north central Mexico that do not fit the current *Juniperus* keys (Adams 2014). The present distribution map of *J. coahuilensis* is shown in Figure 2.

The purpose of this paper is to report on the results of DNA sequencing for these new, morphologically variable samples in an effort to better understand the variation in the serrate junipers of Mexico, with particular emphasis on *J. coahuilensis* and its allies.

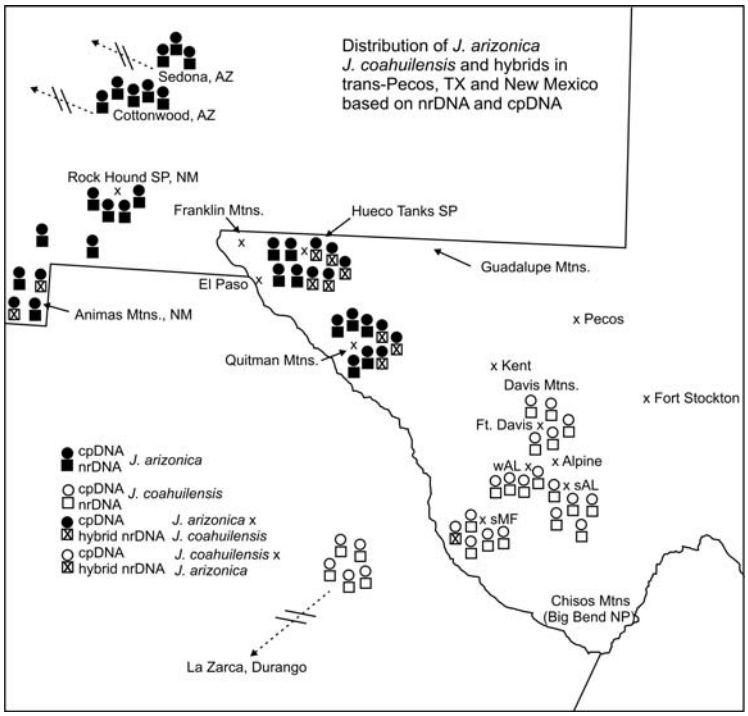

Figure 1. Plant distribution map showing their classification as *J. arizonica, J. coahuilensis*, or hybrids based on the results from both nrDNA and cpDNA analysis. From Adams (2017).

MATERIALS AND METHODS

Plant material and populations studied:

J. coahuilensis, large population with thousands of trees. Mexico, Durango, 85 km n. of La Zarca on Mex. 45, 26° 21' N, 105° 16.66’ W,1740m, 10 Dec 1991, *Robert P. Adams 6829-6831*,

J. coahuilensis, large population in *Bouteloua* grassland, multi-stemmed tree, 4 m tall, female, female cones glaucous, blue-pinkish when mature. Mexico, Durango, at km 18 on Mex. 45, north of Durango, pollen shed in fall. bark exfoliating in narrow strips. 24° 09.067’N, 104° 42.462’ W, 1938 m, 7 May 2004, Coll. *R. P. Adams 10241, 10242*.

J. aff. *coahuilensis,* shrub or tree 3-6 m, seed cones globose, fleshy, bright rose to salmon colored, sweet, 1(2)-seeded, on limestone, Mexico, Durango, Mpio. Nombre de Dios, San Jose de La Parrilla, 23° 44' 20" N, 104° 07' 20" W, 2120 m, 27 Aug 2004, Coll. *Socorro Gonzalez 6988,* Lab Acc. *Robert P. Adams 10454*,

J. aff. *coahuilensis,* Plant on limestone, with unusual seed cones: fibrous, bluish appearance because of the dense glaucous cover on a green surface, one seed [Not fleshy, nor rose or salmon, nor sweetish as in *J. coahuilensis*]; bark thin, fibrous, gray-brown, Mexico, Durango, Mpio. Nombre de Dios, San Jose de La Parrilla; on limestone, 23° 44' 20" N, 104° 7' 20" W, 2120 m, 27 Aug 2004, Coll. *Socorro Gonzalez 6989,* Lab Acc. *Robert P. Adams 10455,*

J. aff. *coahuilensis* hybrid?, Plant with unusual seed cones: fleshy as found in *J. coahuilensis* (present in the same site), but differs having dull purple to dull rose color, glaucous, seed cones in dense groups; branches firm, ascendant; bark thin, fibrous, gray-brown, Mexico, Durango, Mpio. Nombre de Dios, San Jose de La Parrilla; on limestone. 23° 44' 20" N, 104° 7' 20" W, 2120 m, 27 Aug 2004, Coll. *Socorro Gonzalez 6990;* Lab Acc. *Robert P. Adams 10456,*

J. aff. *coahuilensis,* Abundant shrubs, 2-3 m, seed cones rose-pale cherry, without glaucous cover, Mexico, Durango, Mpio. Guanaceví; SE of Guanaceví, on road to Durango, 25° 53' 14" N, 105° 50' 59"

W, 1990 m, 27 Aug 2004, Coll. *Socorro Gonzalez and M. Gonzalez-Elizondo7005;* Lab Acc. *Robert P. Adams 10459,*

J. aff. *coahuilensis,* Abundant, trees on limestone, to 3 m, seed cones fleshy, red-orange, sweet, Mexico, Durango, Mpio. Nombre de Dios, S of El Porvenir and NE of San José de La Parrilla, 23° 46' 30" N, 104° 09' 30" W, 1980 m, 4 Nov 2004, Coll. *Socorro Gonzalez 7016-1, 7016-2,* Lab Acc. *Robert P. Adams 10503, 10504,*

J. aff. *coahuilensis,* shrub-trees, on limestone, seed cones violet colored, somewhat fibrous and resinous, Mexico, Durango, Mpio. Nombre de Dios, NE of San José de La Parrilla, 23° 46' N, 104° 9' W, 1980 m, 4 Nov2004, Coll. *Socorro Gonzalez* 7017a, Lab Acc. *Robert P. Adams 10505,*

J. aff. *coahuilensis,* shrub-trees, on limestone, seed cones: densely grouped, fleshy, sweet, reddish-orange, 1(2) seeds; thin, fibrous bark; on branches pale gray to whitish, Mexico, Durango, Mpio. Nombre de Dios, 0.4 km SW of San Jose de La Parrilla; on limestone, 23° 44' 20" N, 104° 7' 20" W, 2120 m, 4 Nov2004, Coll. *Socorro Gonzalez* 7019-1, 7019-2, Lab Acc. *Robert P. Adams 10511, 10512,*

J. cf. flaccida, Short trees, 1.5-3 m tall; bark on branches papery and exfoliating, inner bark smooth, reddish; no seed cones, similar to *J. flaccida,* but in a very dry habitat in the Chihuahuan desert region, Mexico, Durango, Mpio. Lerdo, Sierra del Rosario, nearly atop the mountain, with Yucca and oak scrub; on limestone, 25° 38’ 44” N, 103° 54’ 40” W, 2700 m, 8 Apr 2008, Coll. *M. S. Gonzalez-Elizondo et al. 7375 a,b;* Lab Acc. *Robert P. Adams 14616, 14617.*

J. aff. *martinezii/ durangensis,* Shrub, seed cones orangish color and fibrous, with pinyon pine and oaks. Mexico, Durango, Mpio. Panuco, Sierra de Gamón, NW slopes, 24° 35’ N, 104° 16’ W, 2500 m, 4 June 2008, Coll. *M. S. Gonzalez-Elizondo et al. 7391 a,b;* Lab Acc. *Robert P. Adams 14618, 14619,*

J. aff. *saltillensis,* Abundant shrub 1-1.8 m, dark blue seed cones, somewhat glaucous, Mexico, Zacatecas, Sierra de Mazapil, Mpio. Concepción del Oro, 24° 37’ 21” N, 101° 28’ 05” W, 2850-2900 m, 16 Oct 2009, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 7567,7568;* Lab Acc. *Robert P. Adams 14620, 14621*

J. aff. *poblana,* uncommon young trees (saplings) 2 m, in oak woodland dominated by *Quercus resinosa*, Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz, Km 86.8; S of bridge of arroyo del Fraile, E of El Maguey, 22° 10’ 08” N, 104° 43’ 51” W, 1150 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8381 with L. López, A. Torres Soto;* Lab Acc. *Robert P. Adams 14896*

J. aff. *poblana,* large, single stemmed trees, foliage long and pendulous, abundant trees, up to 25 m high, on strongly rocky slope, forest of *Juniperus-Clusia* with elements of mesophytic forest (Magnolia) and tropical forest (*Bursera*, *Opuntia*, *Pilosocereus purpusii*) as well as *Agave attenuata* and *Yucca jaliscensis,* Mexico, Nayarit, Mpio. El Nayar, SW of Mesa del Nayar on road to Ruiz; NE of El Maguey, 22° 07’40” N, 104° 47’ 47” W, 1430 m, 19 Jan. 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo 8379a,b,c,d, with L. López, A. Torres Soto;* Lab Acc. *Robert P. Adams14897-14900,*

J. martinezii, new record for Durango, Abundant tree with drooping branchlets, pale grayish-green foliage with white resin marks, Mexico, Durango, Mpio. Vicente Guerrero, Sierra de Órganos, near the border of state of Zacatecas, northernmost known population of *J. martinezii*. The closest population is about 220 km to the SE [Aguascalientes, San José de Gracia (acc. Pérez de la Rosa 1985) 23° 47’ 28” N, 103° 49’ 44” W, 2225 m, 21 Jan 2016, Coll. *M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo) 8384;* Lab Acc. *Robert P. Adams 14901,*

J. aff. *coahuilensis,* Shrub, blue seed cones, Mexico, Durango, Mpio. Nombre de Dios, 4 km w of San José de La Parrilla, 23° 43' N, 104° 08' W, 2150 m, 25 Oct 1983, Coll. *M. S. Gonzalez-Elizondo et al. 2776;* Lab Acc. *Robert P. Adams 14902,*

J. aff. *coahuilensis,* Shrub, blue seed cones, Mexico, Durango, Mpio. Tepehuanes, SE edge of town, 25° 20' N, 105° 43' W, 1800 m, 10 Sep 1989, *O. Bravo 288*; Lab Acc. *Robert P. Adams 14903,*

J. aff. *coahuilensis,* Shrub, blue seed cones, Mexico, Durango, Mpio. Santiago Papasquiaro, 9 km por el camino a Los Altares, 25° 06' N, 105° 27' W, 1940 m, 30 July 1990, Coll. *A. Benitez P. 1646;* Lab Acc. *Robert P. Adams 14904,*

Voucher specimens for new collections are deposited in the Herbarium, Baylor University (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 from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

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), D (maldehy) 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 primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R7 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

Sequencing nrDNA, petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF resulted in 4,351 bp of concatenated sequence data. A Bayesian tree shows the placement of most of the samples collected as *J.* aff. *coahuilensis* (*10241, 10242, 10503, 10504, 10505*) are in the clade with typical *J. coahuilensis* (shaded box, Fig. 2). However, an adjacent clade (cross-hatched box, Fig. 2) contains two sub-clades: blue seed cones plants (*14902, 14903, 14904*) and La Parrilla plants, with very variable seed cone colors from violet to bluish to orange (*14055, 10454, 10456, 10459, 10511*).

Plants *14620, 14621, J.* aff. *saltillensis* from Zacatecas, Sierra de Mazapil, Mpio. Concepción del Oro, are nested, loosely in a clade with *J. saltillensis* (Fig. 2). Additional research on the leaf volatile oils, ecology and morphology (in progress) may prove these to be a new variety of *J. saltillensis*.

Sample *14901*, collected as *J. martinezii* from Durango, Mpio. Vicente Guerrero, Sierra de Órganos, near the border of state of Zacatecas, is in a clade with *J. martinezii* (Fig. 2). This is the first report of *J. martinezii* from Durango and is the northernmost known population of *J. martinezii*. The closest population is about 220 km to the SE (Aguascalientes, San José de Gracia, Perez de la Rosa, 1985).

Two other collections (*14618, 14619*, shrubs, seed cones orangish color and fibrous, with pinyon pine and oaks. Mexico, Durango, Mpio. Panuco, Sierra de Gamón) with affinities to both *J. martinezii* and *J. durangensis*, were placed in a clade with *J. martinezii* and *J. durangensis* (Fig. 2). There is some support for it being in a distinct clade (51%, Fig. 2), but additional research is needed on the leaf volatile oils, ecology and morphology (in progress) to determine if this taxon is a new variety of *J. martinezii* or perhaps a new species.

Plants *14616, 14617*, collected as *J.* aff. *flaccida*, were short trees, 1.5-3 m tall with the bark on branches papery and exfoliating, and inner bark smooth, reddish. These samples were in a well supported

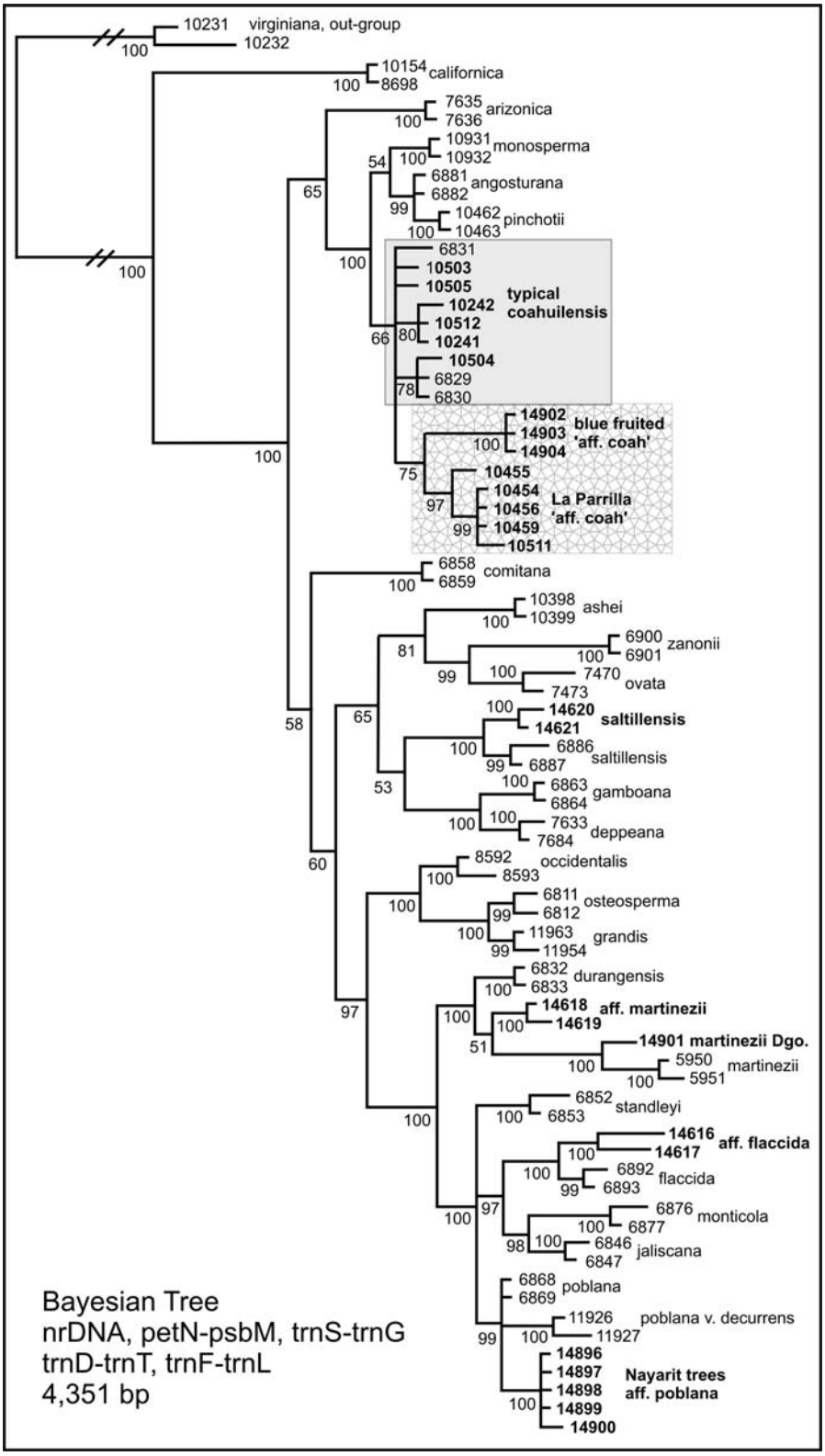

Figure 2. Bayesian tree of serrate leaved *Juniperus* of North America. Numbers next to branch points are posterior probabilities as percents. Note the typical *J. coahuilensis* (shaded box) and the adjacent clade (cross-hatched box). See text for discussion. Samples in boldface print are new collections. Samples in regular font are the reference set of serrate junipers.

clade with *J. flaccida*, but yet, quite distinct (Fig. 2). The site is in a very dry habitat in the Chihuahuan desert region, Mexico, Durango, Mpio. Lerdo, Sierra del Rosario. No seed cones were found (April, 2008), so new collections with seed cones are needed. Clearly, additional research is needed on the leaf

volatile oils, ecology and morphology (in progress) to determine if this taxon might be a new variety of *J. flaccida*.

And lastly, a very unusual population with aff. *poblana*, was found with large, single stemmed trees, and foliage long and pendulous in Nayarit. Analysis of their DNA did place them (*14986, 14897, 14898, 14899, 14900*) in a large clade with *J. poblana* and *J. p*. var. *decurrens* (Fig. 2). However, they are quite distinct and well supported as a separate clade. Analysis of the leaf volatile oils, ecology and morphology (in progress) should be sufficient to determine if this taxon is a new species, or perhaps another (new) variety of *J. poblana*.

A detailed examination of variable nrDNA (ITS) sites of *J. coahuilensis* aff. samples, as well as *J. coahuilensis* from the Trans-Pecos, Texas region is shown in Table 1. Overall, *J. coahuilensis* and the aff. samples from Mexico do not have as many variable sites as found in the Trans-Pecos region (see also Adams, 2017).

Mapping the classification of individuals based on ITS and cp (petN) data shows (Fig. 3) only four samples in Durango that have both ITS and cpDNA of *J. coahuilensis* (as found in the Trans-Pecos, Texas area).

The cpDNA of the blue fruited taxon (black filled circles, Fig. 3) was found in combination with various types of ITS DNA in central and southern (La Parrilla area) Durango. The cpDNA of typical *J. coahuilensis* was found in both northern and southern Durango (Fig. 3). Two of the blue fruited samples (black filled circle, open diamond, Fig. 3) were found in central Durango, and the third sample was found in the La Parrilla area. Two samples with La Parrilla type ITS (LaPar, Table 1; black square, Fig. 3) were found in the La Parrilla area and are in northwestern Durango.

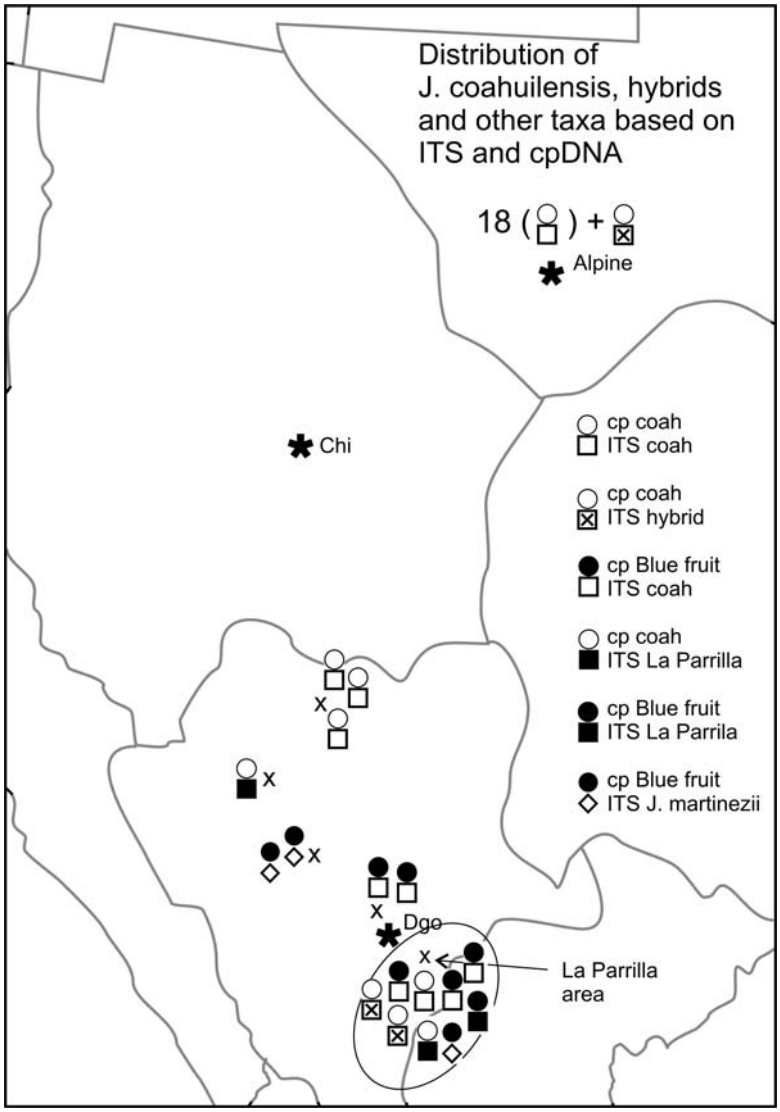

Figure 3. Map of *J. coahuilensis* and aff. samples by their cpDNA (petN) (circles) and ITS DNA (squares). Data in the Trans-Pecos, Texas area from Adams (2017.

Two samples, putatively hybrids based on their ITS, were found in the La Parrilla area (crossed squares, Fig. 3). All six of the cpDNA/ ITS types were found in the La Parrilla area (Fig. 3). It may be that other areas are equally as diverse, but additional sampling is needed to address this question.

Several of the nrDNA (ITS) sites display interesting geographic patterns. ITS site 191 (A,G, A/G) has considerable variation in the Trans-Pecos, Texas region (Fig. 4) and continues into northern Durango. However, no other A/G sites were found in central and southern (La Parrilla) Durango. This may be the result of hybridization/ introgression from some juniper in the Alpine area.

One individual with site 191 (A) was found south of Alpine and another found in the La Parrilla area of southern Durango.

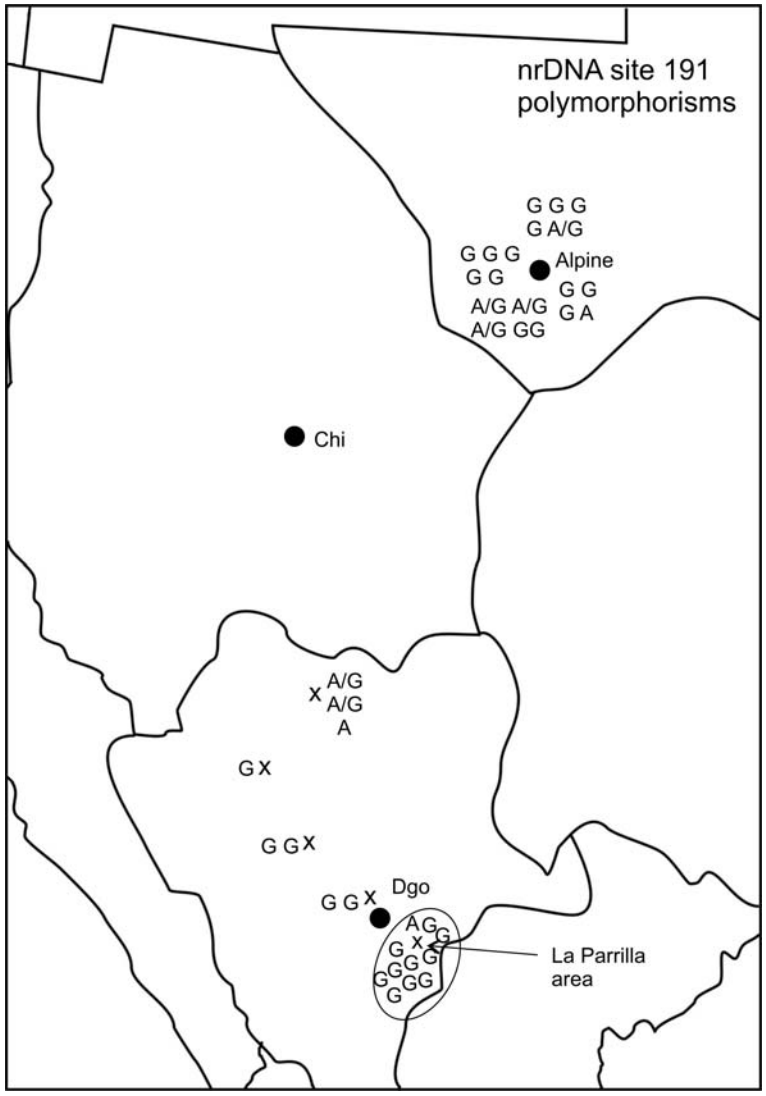

Figure 4. Geographic variation in ITS site 191. See text for discussion.

ITS site 196 featured the deletion of T in many samples ranging from the Alpine to central and southern Durango (Fig. 5). All three BF (blue fruited) and the 'LaPar' ITS type samples had the 196 deletion (Table 1). Plants *10454*, *10456*, and *14903* appear to be hybrids. The deletion caused slippage during sequencing, so all the sites downstream from 196 were polymorphic. To remedy this problem, a new internal reverse primer was synthesized and used to reverse-sequence the immediate 700 bp past site 196 to obtain clean sequences from some plants. It is not known if this deletion is of contemporary or ancient origin.

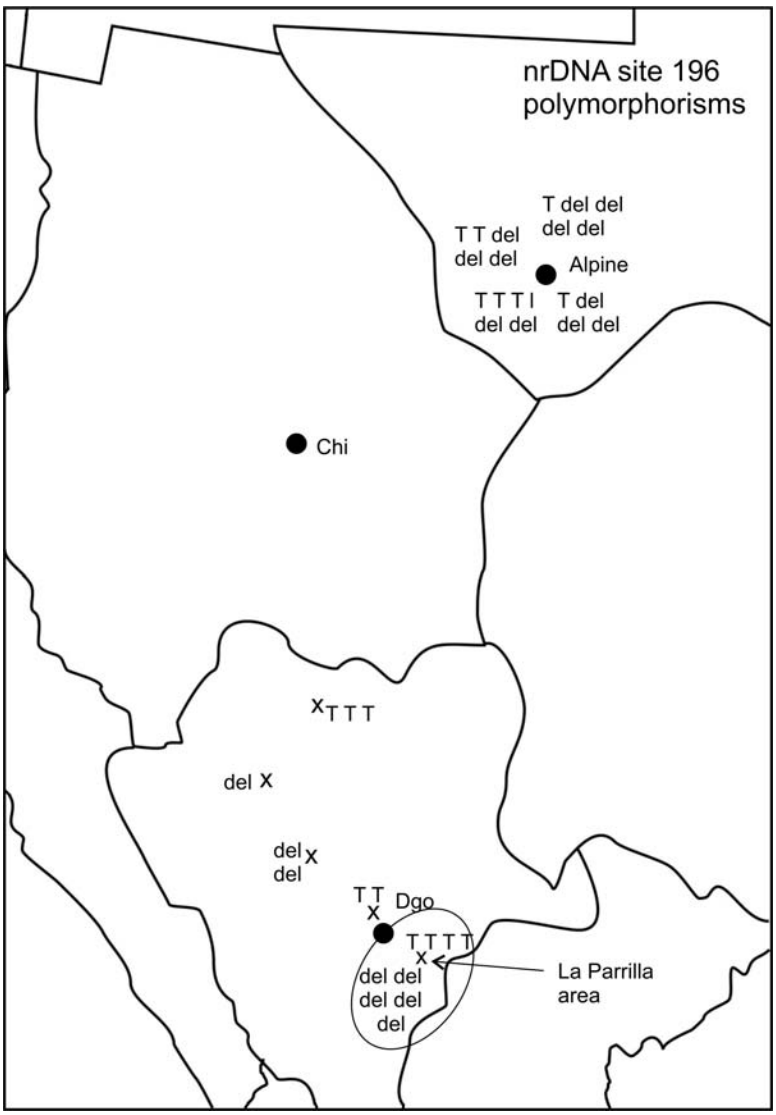

Figure 5. Geographic variation in ITS site 196. See text for discussion.

Mapping ITS site 303 provided a novel pattern not seen in other ITS sites. The presence of C/T polymorphisms for site 303 in the Trans Pecos area (Fig. 6) was not found in Mexico (nor in AZ, NM, see Adams, 2017). This seems to imply that the event was modern and due to hybridization with some unknown extant or extinct juniper in the Trans-Pecos area. Of interest to this study was the finding many plants with either C or T, but no plants with C/T in Durango.

In addition, the three BF (blue fruited) plants each contained G at site 303 (Table 1) and are shown (Fig. 6) with two in central Durango and one in the La Parrilla area. In addition, G (site 303) is also found in *J. martinezii* (Table 1). This site, no doubt, supported the placing of the BF junipers in a clade with *J. martinezii* in a NJ tree based on ITS sequences (data not shown), suggesting the BF taxon has a nuclear affinity to *J. martinezii*. However, sequences from the four cp gene regions was concatenated to nrDNA data in the construction of the Bayesian tree (Fig. 2), and this led to the positioning of the BF taxon loosely in the *J. coahuilensis* clade (Fig. 2).

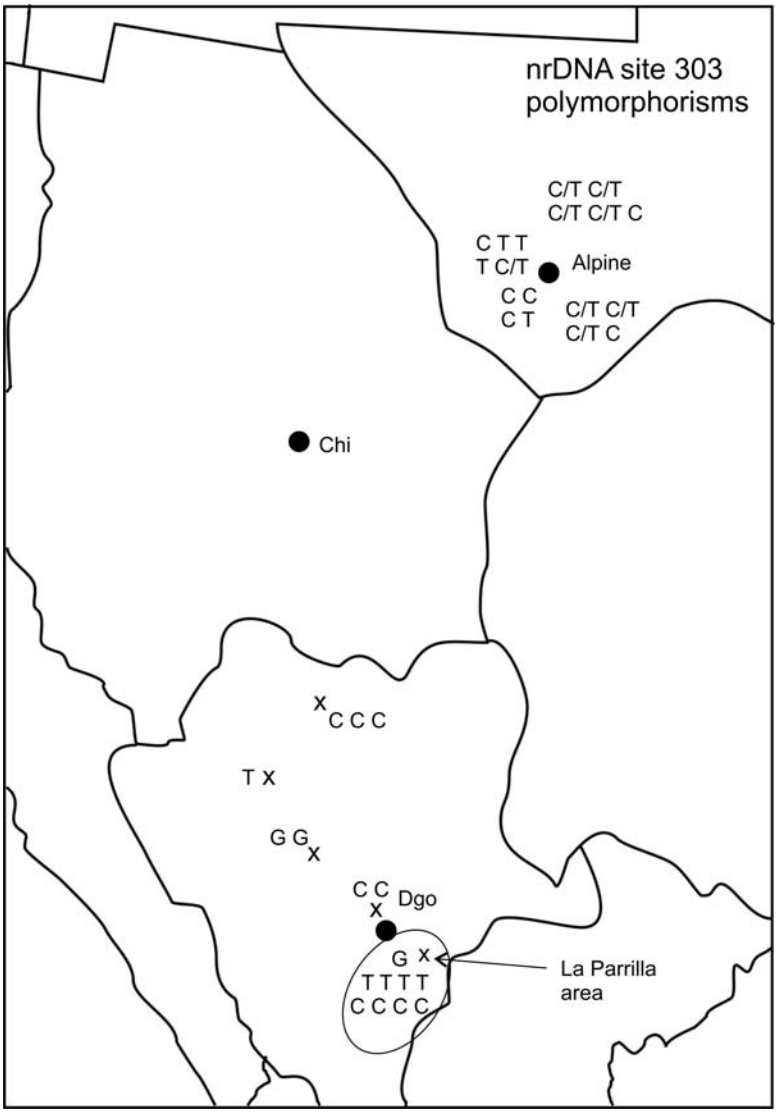

Figure 6. Geographic variation in ITS site 303. See text for discussion.

Finally, examination of ITS site 1116 presents an interesting situation in that every case with C/T at site 1116 (Table 1) has a deletion (del) at 196 (Table 1). Re-examination of the nrDNA sequence for 14814 revealed that the site 196 contains mostly T, there is a small (ca. 20% C peak). From 196 onward, small peaks (ca. 20% high) are present in the sequence. The del at 196, the slippage of the sequence for ca. 20% of the DNA strains perfectly explains the minor bases from 196 onward. This suggests that the plant is of backcross origin and that incomplete lineage sorting has not yet removed the minority copies that contain a del in 196). It should be noted that several samples (Table 1) have a del at 196 but have either a clean C or T at 1116.

The pattern seen for site 1116 (Fig. 7) suggests (as seen in Fig. 5) hybridization throughout the range of *J. coahuilensis* from Alpine to southern Durango, with the presence of numerous plants with C or T at site 1116.

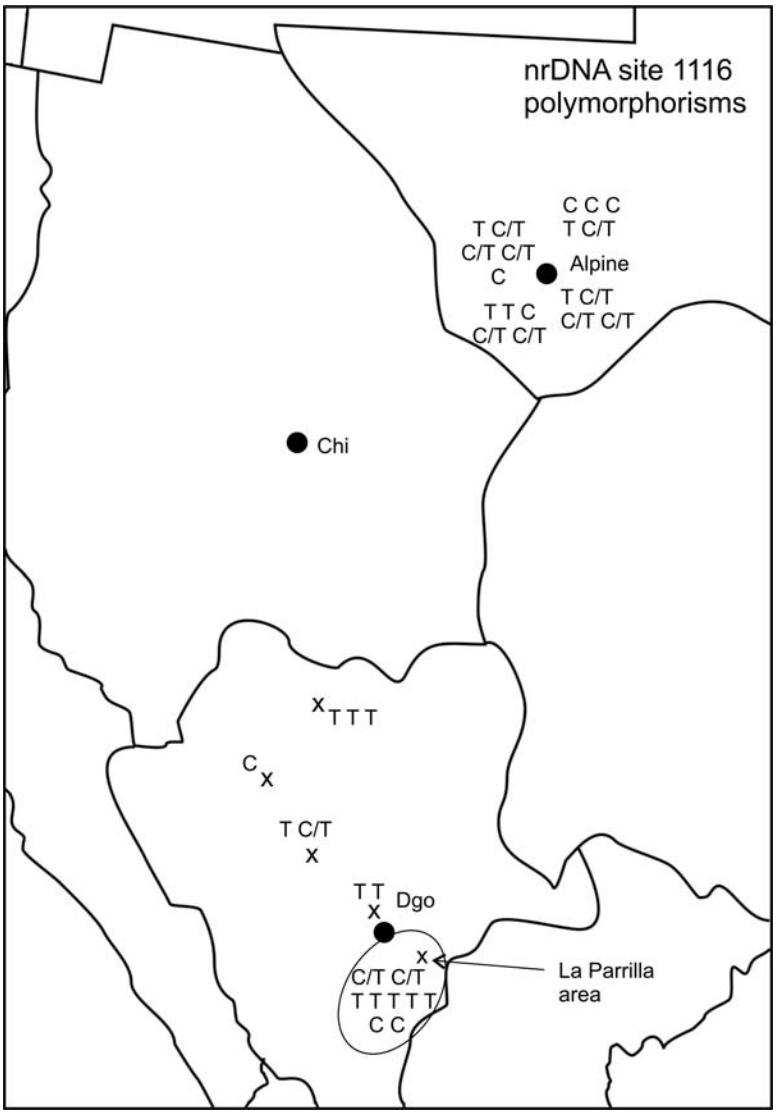

Figure 7. Geographic variation in ITS site 1116. See text for discussion.

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Table 1. Variable sites in nrDNA for *J. arizonica* (ariz), *J. coahuilensis* (coah) and 'blue, violet, bluish' fruited (BF), and La Parrilla type nrDNA (LaPar). del = deletion, mart = *J. martinezii* nrDNA.

sample	petN	ITS	191	196	302	**303**	304	318	**533**	543	1116	1148	#poly
az10634Sedona 181A/C	ariz	ariz	G	T	A	C	T	T	A	**C/G**	T	C	2
az10635Sedona 681A/C	ariz	ariz	G	T	A	C	T	T	A	**C/G**	T	C	2
az10636Sedona	ariz	ariz	G	T	A	C	T	T	A	**C/G**	T	C	1
az14908Cottonwood	ariz	ariz	G	T	A	C	T	T	A	G	T	C	0
az14909Cottonwood	ariz	ariz	G	T	A	C	T	T	A	**C/G**	T	C	1
az14910Cottonwood	ariz	ariz	G	T	A	C	T	T	A	C	T	C	0
az14912Cottonwood	ariz	ariz	G	T	A	C	T	T	A	C	T	C	0
az14913Cottonwood 121C/T	ariz	ariz	G	T	A	C	T	T	A	**C/G**	T	C	2
az7635RockHoundSP	ariz	ariz	G	T	A	C	T	T	A	C	T	C	0
az7636RockHoundSP	ariz	ariz	G	T	A	C	T	T	A	C	T	C	0
az7637RockHoundSP	ariz	ariz	G	T	A	C	T	T	A	**C/G**	T	C	1
az10630RockHSP	ariz	ariz	G	T	A	C	T	T	A	C	T	C	0
coa14807sofAlpine	coah	coah	G	**del**	**A/G**	**C/T**	**C/T**	**C/T**	T	C	**C/T**	**C/T**	6
coa14808sofAlpine	coah	coah	G	T	A	C	T	**C/T**	T	C	T	**C/T**	2
coa14810sofAlpine	coah	coah	G	**del**	A	**C/T**	**C/T**	T	T	C	**C/T**	C	4
coa14811sofAlpine	coah	coah	A	**del**	**A/G**	**C/T**	T	T	T	C	**C/T**	C	3
coa14812wofAlpine	coah	coah	G	**del**	**A/G**	**C/T**	T	**C/T**	T	C	C	C	4
coa14813wofAlpine 313A/G	coah	coah	G	T	A	**C/T**	T	**C/T**	T	C	T	**C/T**	4
coa14814wofAlpine	coah	coah	G	**del**[6]	A	C	T	T	T	C	**C/T**	C	1
coa14815wofAlpine	coah	coah	G	**del**	**A/G**	**C/T**	T	**C/T**	T	C	**C/T**	C	5
coa14816wofAlpine	coah	coah	G	**del**	A	**C/T**	T	**C/T**	T	C	**C/T**	**C/T**	5
coa14817FtDavis	coah	coah	G	T	A	C	T	**C/T**	T	C	T	**C/T**	2
coa14818FtDavis	coah	coah	G	**del**	A	T	T	T	T	C	C	C	1
coa14819FtDavis	coah	coah	G	**del**	G	T	T	T	T	C	C	C	1
coa14820FtDavis 689G/T	coah	coah	**A/G**	**del**	**A/G**	**C/T**	T	T	T	C	**C/T**	C	6
coa14821FtDavis	coah	coah	G	**del**	**A/G**	T	T	T	T	C	C	C	2
coa14822sofMarfa	coah	coah	**A/G**	T	A	C	T	T	T	C	T	C	1
coa14823sofMarfa	coah	coah	G	T	A	C	T	T	T	C	**C/T**	C	1
coa14824sofMarfa	coah	coah	G	**del**	**A/G**	T	T	T	T	C	C	C	2
coa14825sofMarfa	coah	**AxC**	**A/G**	T	A	C	T	T	**A/T**	C	T	C	2
coa14826sofMarfa	coah	coah	**A/G**	del	na	na	na	na	T	C	**C/T**	C	3
coa6829km85, nLaZarca, rose	coah	coah	**A/G**	T	A	**C**	T	T	T	C	T	C	1
coa6830km85, nLaZarca, rose	coah	coah	A	T	A	**C**	T	T	T	C	T	C	0
coa6831km85, nLaZarca, rose [1]	coah	coah	**A/G**	T	A	**C**	T	T	T	C	T	C	2
coa10241k18 nDgo blue-pink [2]	BF	coah	G	T	A	**C**	T	T	T	**C/T**	T	C	2
coa10242k18 nDgo blue-pink [3]	BF	coah	G	T	A	**C**	T	T	T	**C/T**	T	C	2
coa10503LaParr red,sweet Fr	BF	coah	G	T	A	**C**	T	**C/T**	T	C	T	**C/T**	2
coa10504LaParr red,sweet	coah	coah	A	T	A	**C**	T	T	T	C	T	C	0
coa10505LaParr violet Fr	BF	coah	G	T	A	**C**	T	T	T	C	T	C	0
coa10512LaParr red-orange Fr	BF	coah	G	T	A	**C**	T	T	T	C	T	C	0
coa10454LaParr, rose Fr	coah	hyb?	G	**del**	A	**T**	T	T	T	C	**C/T**	C	2
coa10455LaParr bluish Fr [5]	BF	LaPar	G	**del**	**A/G**	**T**	T	T	T	C	C	C	4
coa10456LaParr rose-purple [4]	coah	hyb?	G	**del**	A	**T**	T	T	T	C	**C/T**	C	3
coa10459Guan rose-red,no blo	coah	LaPar	G	**del**	A	**T**	T	T	T	C	C	C	1
coa10511LaParr red-orange Fr	coah	LaPar	G	**del**	A	**T**	T	T	T	C	C	C	1
coaBF14902LaParr blueFr	BF	mart	G	**del**	A	**G**	T	T	T	C	T	C	1
coaBF14903Tepeh blueFr	BF	mart	G	**del**	A	**G**	T	T	T	C	**C/T**	C	2
coaBF14904SPapa blueFr	BF	mart	G	**del**	A	**G**	T	T	T	C	T	C	1
mart5950 J. martinezii	mart	mart	G	T	A	**G**	T	T	T	C	T	C	0
mart5950 J. martinezii	mart	mart	G	T	A	**G**	T	T	T	C	T	C	0

[1] 240A/G; [2]603A/G; [3]503C/T; [4]731A/G; [5]308A/G, 665C/T; [6]T with ca. 20% C at site 196