Preliminary examination of hybridization and introgression between *Juniperus flaccida* and *J. poblana*: nrDNA and cpDNA sequence data

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ABSTRACT

Analyses of nrDNA and cpDNA (petN-psbM, trnSG, trnDT, trnLF) from *J. flaccida, J. poblana, J. p.* var. *decurrens*, and putative hybrids revealed some populations of pure *J. flaccida*, and a few areas of pure trees of *J. poblana*. However, most *J. poblana* or putative hybrids in the eastern part the range were found to be introgressed by chloroplast capture by *J. flaccida*. A few putative hybrids (*J. flaccida* × *J. poblana)* were confirmed by the presence of heterozygous specific indicator bases at nrDNA site 768. Introgression of *J. flaccida* into *J. poblana* appears to be widespread in eastern Mexico. *Phytologia 100(2): 145-152 (Jun 22, 2018).* ISSN 030319430.
KEY WORDS: *J. flaccida, J. poblana*, Cupressaceae, hybridization, nrDNA, trnSG DNA.

Juniperus flaccida Schltdl. and *J. poblana* (Martínez) R.P. Adams are closely related species with large, multi-seeded cones and weeping (flaccid) foliage that make them difficult to differentiate morphologically (Adams 2014). In some areas they appear to grow together and appear to hybridize.

In January 2018 Allen Coombes and Lucio Caamaño O. visited the type locality of *J. poblana* as well as the municipio of Cuautinchán in order to investigate the extent of distribution of *J. poblana*. It was observed that typical *J. poblana* appeared to be restricted to north-facing slopes close to the type locality while further south and at lower altitudes the junipers showed a great deal of variation with some appearing closer to *J. flaccida*. This work is a result of a follow-up visit to the area with Socorro González.

Recently, Adams et al. (2018) re-examined *Juniperus flaccida* and *J. poblana*. They reported that analysis of nrDNA, petN-psbM, trnS-trnG, trnD-trnT, and trnF-trnL revealed that *J. flaccida* is diverse, but distinct from *J. poblana* from Amozoc and *J. poblana* from Nayarit by at least 10 Mutational Events (MEs, SNPs + indels). Divergent *J. poblana* from Oaxaca is 2 MEs distant from the Nayarit trees. The *J. poblana* samples from the type locality (Amozoc, Pue.) vary by only 1 ME among samples. Leaf oils, on the other hand, show that the Nayarit juniper is quite differentiated from other *J. poblana*.

This paper is a preliminary report on possible hybridization and introgression between *J. flaccida* and *J. poblana* utilizing nrDNA and cp DNA (trnS-trnG) sequence data.

MATERIALS AND METHODS

Plant material and populations studied:

Putative *J. flaccida* - *J. poblana* hybrids:

1.4 km al S de San José Laguna, Mpio. Amozoc, Edo. Pue. 19° 01' 15" N, 98° 01' 57.8" W, 2317 m, 11 Sep 2017. Remanente de bosque de *Juniperus*. Arbol de 2 - 5 m de altura, Coll. Lucio Caamaño 11265, Allen Coombes, S. González; Lab Acc. *Robert P. Adams No. 15296*

1.6 km al S de Amozoc por carretera a Santa Cruz, Mpio. Amozoc, Edo. Pue. 19° 01' 42.4" N, 98° 05' 17.5" W, 2335 m, 11 Sep 2017. Bosque de encino, arbol de 4 m de altura. Coll. *Lucio Caamaño 11276-a, Allen Coombes, S. González*; Lab Acc. *Robert P. Adams 15297*

1.6 km al S de Amozoc por carretera a Santa Cruz, Mpio. Amozoc, Edo. Pue. 19° 01' 42.4" N, 98° 05' 17.5" W, 2335m, 11 Sep 2017. Bosque de encino, arbol de 5 - 6 m de altura, Coll. *Lucio Caamaño 11277, Allen Coombes, S. González*; Lab Acc. *Robert P. Adams 15298*

2.4 km por camino de terraceria Av. Gonzalo Bautista Pte. al W de Cuautinchán, Mpio. Cuautinchán, Edo. Pue. 18° 57' 29.3' N, 98° 1' 54' W, 2170 m, 11 Sep 2017. Vegetación secundaria a orilla de río, arbol de 8 m de altura, Coll. *Lucio Caamaño 11286, Allen Coombes, S. González*; Lab Acc. *R Adams 15299*

Al W de Alpatlahuac, Mpio. Cuautinchán, Edo. Puebla, 18° 55' 50" N, 98° 2' 24.1" W, 2115 m, 11 Sep 2017. Vegetación secundaria asociada a *Juniperus*, arbol de 8 - 9 m de altura, Coll. *Lucio Caamaño 11287, Allen Coombes, S. González,* Lab Acc. *R Adams 15300*

Al W de Alpatlahuac, Mpio. Cuautinchán, Edo. Puebla, 18° 55' 50" N, 98° 2' 24.1" W, 2115m, 11 Sep 2017. Vegetación secundaria asociada a *Juniperus*, arbol de 8 m de altura, Coll. *Lucio Caamaño 11290, Allen Coombes, S. González,* Lab Acc. *R Adams 15301*

Reference specific samples:

J. flaccida

J. flaccida, Adams 6892-6896, 24° 41' 54"N, 100° 04' 59" E, 5800 ft., 23 km E of San Roberto Junction on Mex. 58, Nuevo Leon, Mexico;

J. flaccida, Reserva Ecologica Municipal de Sierra y Cañon de Jimulco, 25° 07' 38" N, 103° 16' 15" W, 2118 m, 17 Jan 2017, Torreon, Coahuila, Mexico, Coll. *Manuel Rodríguez Munoz et al. #1,2,3,4,5,* Lab Acc. *Adams 15203 - 15207*;

J. flaccida, Sierra del Rosario, Durango, Mexico, 25° 38' 44" N, 103° 54' 40" W, 2700 m, with *Yucca carnerosana*, *Agave gentryi*, and *Quercus* scrub, 27 Apr 2008, Coll. *M. S. Gonzalez-Elizondo et al. 7375a, b*, Lab Acc. *Adams 14616 – 14617*;

J. poblana

J. poblana, scattered, at Km 62, 62 km S of Oaxaca on Mex 190. 16° 41' 36" N, 96° 19' 41" W, 1710m, 19 Dec 1991, Oaxaca, Mexico, Coll: *Robert P. Adams 6868-6872*

J. 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. 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. poblana, scattered in a *J. poblana* - oak forest. Amozoc de Mota, just s of town. lvs for spec, oil, DNA. 19° 01' N, 98° 01' W., 2300 m. Date 16 Dec 2016. Mpio. Amozoc, Type locality of *J. poblana*. Puebla, Mexico. Coll. *L. Caamaño* and *Allen Coombes 10172,10173,10174,10180,10181*. 2344-2358 m, 16 Dec 2016. Det. Socorro Gonzalez, Lab Acc. *Robert P. Adams 15208 - 15212.*

J. poblana var. *decurrens,* scattered

J. poblana var. *decurrens* R. P. Adams, small tree, 5m tall, with strong central axis, foliage very, very, weeping, female, common, about 2 km s of Valle de Topia. All leaves decurrent, and prickly. Not juvenile leaves. 25° 14' 11" N, 106° 26' 55.7" W, 1818 m, 30 June 30, 2009, Durango, Mexico. Coll. *Robert P. Adams 11926* with *Socorro and Martha Gonzalez.*

Voucher specimens are deposited at BAYLU, CIIDIR and HUAP when applicable.

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 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 revealed that *J. flaccida* and *J. poblana* growing in uniform populations (assumed pure, as they are isolated from other areas, and their nrDNA is not polymorphic at any base sites) differed consistently by only 1 SNP at site 768. This SNP is G in *J. flaccida*, and A in *J. poblana* (Table 1). Comparison of sequencing for the reference populations of *J. flaccida* and *J. poblana* reveal few informative SNPs: trnSG 1 SNP (site 96), 1 indel; petN-psbM, 0 SNPs; trnLF: 1 SNP, 1 indel; trnDT: 2 SNPs, 1 indel. Because the classifications based on the cp DNA were consistent, only trnSG, site 96, was utilized to survey additional samples.

All the samples from NL (Nuevo Leon, 23 km east of San Roberto Junction) and from near Torreon, Coahuila were found to be uniform by having clean (homozygous) peaks of *J. flaccida* nrDNA (ITS) and also having *J. flaccida* cp DNA (trnSG). However, 'pure' *J. poblana* was found in the Nayarit population, 2 trees from Amozoc (15208, 15209), and 2 trees of *J. poblana* var. *decurrens* (11927, 11928, Table 1). Other *J. poblana* trees from Amozoc and Oaxaca had clean, *J. flaccida* nrDNA, but *J. poblana* cp DNA, suggesting chloroplast capture by hybridization and introgression (Table 1).

Several putative hybrids were sampled (Table 1). Sample 15296, Amozoc, had heterozygous nrDNA (G/A ~2:1), with *J. poblana c*pDNA. Recently, the inheritance of nrDNA polymorphisms has been reported (Adams and Matsumoto, 2016, Adams et al. 2016) in two Cupressaceae genera. In cultivars of *Cryptomeria japonica*, Adams and Matsumoto (2016) found that 'Haara' and 'Kumo' differ at three sites in their nrDNA. However, 'Kumo' was polymorphic at each of the three sites (indicating that 'Kumo' was a cross of 'Haara' with an unknown plant). Thus, the seven artificial crosses between 'Haara' and 'Kumo' are actually backcrosses to 'Haara'. They reported that three of the backcrosses were polymorphic at the three nrDNA sites, but four (of the seven crosses) were monomorphic (homozygous) at the three nrDNA sites! Thus, it appears that in this instance, some backcrosses may rather quickly eliminate heterozygous sites in favor of the recurring parent.

Adams et al. (2016) analyzed crosses between *Hesperocyparis* (= *Cupressus*) *arizonica* and *H. macrocarpa* that included the parents and 18 artificial hybrids. In this case, 8 nrDNA sites differed between *H. arizonica* and *H. macrocarpa*, and all of these sites were homozygous in each parent. Adams et al. (2016) reported the peak ratios in the sequencing chromatograms were not always 1:1 as expected, but varied from 1:1 to 3:1 (3:1 being more like *H. arizonica* nrDNA). They concluded that due to concerted evolution, analysis of heterozygous sites in nrDNA may underestimate the degree of hybridization and introgression. Nevertheless, all of the 18 artificial hybrids were polymorphic at each of the 8 sites, and this clearly revealed that they were hybrids (although, from the data, one might presume some plants were backcrosses or introgressants). Only 3 of the 18 hybrids had nearly 1:1 ratios of the bases of the parents at all 8 sites. This paper (Adams et al. 2016) is very relevant to the present (and future) studies in *Juniperus*, as *Hesperocyparis* is very closely related to *Cupressus* and *Juniperus* (Adams, Bartel and Price, 2009; Terry et al. 2016), so it seems likely that the mode of inheritance in *Juniperus* nrDNA is similar to that found in *Hesperocyparis*.

Examination of the six putative hybrids (ex per field observations) shows (Table 1) that two (15296, 15300) were heterozygous at site 768 indicating they are hybrids (or backcrosses). The remaining four samples, each had *J. flaccida* nrDNA and *J. poblana* cpDNA, implying ancient chloroplast capture by introgression.

To visualize the distribution of putative hybridization and introgression, samples were mapped. Their nrDNA and cpDNA were denoted by icons (Fig. 1). The northernmost populations of *J. flaccida* appear to be 'genetically pure' (for nrDNA and cpDNA) and they are geographically isolated from *J. poblana*. The only 'genetically pure' *J. poblana* plants were in Nayarit, two plants from Amozoc, and two plants of *J. poblana* var. *decurrens*.

The easternmost distributed *J. poblana* - hybrids generally display (Fig. 1) a pattern of chloroplast capture with introgression from *J. flaccida* (Fig. 1). Only two 'genetically pure' *J. poblana* plants were found in the eastern region (near Amozoc).

Finally, it is interesting to analyze the volatile oils of samples of *J. poblana* var. *decurrens*: Adams 11926 (flac, pob), Adams 11927 (pob, pob), Adams 11928 (pob, pob). The leaf volatile oils differ little between *J. flaccida* and *J. poblana* (Adams and Zanoni, 2015; Adams and Schwarzbach, 2015; Adams, Gonzalez-Elizondo and Gonzalez-Elizondo, 2017b). However, *J. flaccida* and *J. poblana* differ in their concentrations of linalool, δ-2-carene, δ-3-carene, naphthalene, methyl chavicol, cubebol and (E)-nerolidol. In 11926, the concentrations of linalool, δ-3-carene, naphthalene, and cubebol are transgressive (i.e., Table 2, outside the range of the putative parents). For δ-2-carene and (E)-nerolidol, 11926 is very similar to *J. flaccida*. For methyl chavicol, it is very similar to *J. poblana*.

Interestingly, analyses of these seven compounds for plants 11927 and 11928 showed some of the same transgressive patterns (Table 2). This is not surprising, because studies on the inheritance of terpenoids in *Cryptomeria japonica* (Adams and Tsumura, 2012) and *Pseudotsuga menziesii* (Adams and Stoehr, 2013) artificial hybrids revealed that many compounds are transgressive in the hybrids and some are intermediate in concentration between the parents' values.

Classification of the seven oil constituents by parent types (Table 4) revealed that 11926 and 11928 have the same patterns except for δ-2-carene. In contrast, 11927 displays a different patterns (from that of 11926, 11928) for δ-3-carene, cubebol and (E)-nerolidol. This seems to indicate that there is more genetic mixing in the *J. poblana* var. *decurrens* population than as revealed in the nrDNA data.

Although we have sampled from much of the range of *J. poblana* (Figs.1, 2, 3), there is a large portion of the range of *J. flaccida* that was not been sampled in this study (Figs. 1,2,3). Additional sampling is needed from central Mexico and more extensive sampling is needed in the *J. poblana* var. *decurrens* population, as well as in the Amozoc - Cuautinchán area.

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LITERATURE CITED

Adams, R. P. 2014. The junipers of the world: The genus *Juniperus*. 4th ed. Trafford Publ., Victoria, BC.

Adams, R. P. and M. E. Kauffmann. 2010. Geographic variation in the leaf essential oils of *Juniperus grandis* and comparison with *J. occidentalis* and *J. osteosperma*. Phytologia 92: 167-185.

Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the new world. Phytologia 91: 160-185.

Adams, R. P. and Y. Tsumura. 2012. Multivariate detection of hybridization using conifer terpenes I: Analysis of terpene inheritance patterns in *Cryptomeria japonica* F_1 hybrids Phytologia 94: 253-275.

Adams, R. P. and M. Stoehr. 2013. Multivariate detection of hybridization using conifer terpenes II: Analyses of terpene inheritance patterns in *Pseudotsuga menziesii* F_1 hybrids. Phytologia 95: 42-57.

Adams, R. P. and A. E. Schwarzbach. 2015. A new, flaccid, decurrent leaf variety of *Juniperus poblana* from Mexico: *J. poblana* var. *decurrens* R. P. Adams. Phytologia 97: 152-163.

Adams, R. P. and T. A. Zanoni. 2015. The volatile leaf oils of *Juniperus flaccida* Schltdl., *J. martinezii* Perez de la Rosa and *J. poblana* (Mart.) R. P. Adams, re-examined. Phytologia 97: 145-151.

Adams, R. P. and Asako Matsumoto. 2016. Inheritance of nrDNA in artificial hybrids of *Cryptomeria japonica* cv. *Haara* and *C. japonica* cv. *Kumotoshi.* Phytologia 98: 37-41.

Adams, R. P., M. Miller and C. Low. 2016. Inheritance of nrDNA in artificial hybrids of *Hesperocyparis arizonica* x *H. macrocarpa*. Phytologia 98: 277-283.

Adams, R. P., M. S. Gonzalez-Elizondo, M. Gonzalez-Elizondo, D. Ramirez Noya and A. E. Schwarzbach. 2017a. DNA sequencing and taxonomy of unusual serrate *Juniperus* from Mexico: Chloroplast capture and incomplete lineage sorting in *J. coahuilensis* and allied taxa. Phytologia 99: 62-73.

Adams, R. P., M. S. Gonzalez-Elizondo and M. Gonzalez-Elizondo. 2017b. Re-examination of the volatile leaf oils of *Juniperus flaccida, J. martinezii*, and *J. poblana.* Phytologia 99: 191-199.

Adams, R. P., M. Socorro González-Elizondo, Martha González-Elizondo and Andrea E. Schwarzbach. 2018..The *Juniperus flaccida-J. poblana* complex revisited: insights from molecular and oil analysis. Phytologia 100: 19-26.

Adams, R. P. and A. E. Schwarzbach. 2015. A new, flaccid, decurrent leaf variety of *Juniperus poblana* from Mexico: *J. poblana* var. *decurrens* R. P. Adams. Phytologia 97: 152-163.

Terry, R. G., M. I. Pyne, J. A. Bartel and R. P. Adams. 2016. A molecular biogeography of the New World cypresses (Callitropsis, Hesperocyparis; Cupressaceae). Plant Syst. Evol. DOI 10.1007/s00606-016-1308-4.

Table 1. Analyses of putative hybrids of *J. flaccida* x *J. poblana*, utilizing nrDNA and trnSG sequences. *J. flaccida* and *J. poblana* nrDNA differs at only 1 SNP at site768 and their trnS-G differs by only 1 SNP at site 96.

Reference Samples Collected as:	nrDNA, site 768/ classification (A/G)	trnS-G, site 96 / classification	combined classification
6892 flaccida, San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6893 flaccida, San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6894 flaccida, San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6895 flaccida , San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
6896 flaccida , San Roberto Jct. NL	G clean, flaccida	A/ flaccida	flaccida
15203 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15204 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15205 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15206 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
15207 flaccida, Torreon, Coah.	G clean, flaccida	A/ flaccida	flaccida
14616 flaccida, S. del Rosario, Dur.	G clean, flaccida	A/ flaccida	flaccida
14617 flaccida, S. del Rosario, Dur.	G clean, flaccida	A/ flaccida	flaccida
14896 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14897 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14898 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14899 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
14900 poblana, Nayarit	A clean, poblana	G/ poblana	poblana
15208 poblana, Amozoc	A clean, poblana	G/ poblana	poblana
15209 poblana, Amozoc	A clean, poblana	G/ poblana	poblana
15210 poblana, Amozoc	G clean, flaccida	G/ poblana	introgressant?
15211 poblana, Amozoc	G clean, flaccida	G/ poblana	introgressant?
15212 poblana, Amozoc	G clean, flaccida	G/ poblana	introgressant?
6868 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6869 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6870 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6871 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
6872 poblana, Oaxaca	G clean, flaccida	G/ poblana	introgressant?
11926 poblana v. *decurrens*	G clean, flaccida	G/ poblana	introgressant?
11927 poblana v. *decurrens*	A clean/ poblana	G/ poblana	poblana
11928 poblana v. *decurrens*	A clean/ poblana	G/ poblana	poblana
Collected as putative hybrids:			
15296, Amozoc	G/A (2:1)	G/ poblana	hybrid/backcross
15297, Amozoc	G, flaccida	G/ poblana	introgressant?
15298, Amozoc	G, flaccida	G/ poblana	introgressant?
15299, Cuautinchán	G, flaccida	G/ poblana	introgressant?
15300, Cuautinchán	G/A (4:1)	G/ poblana	backcross
15301, Cuautinchán	G, flaccida	G/ poblana	introgressant?

Table 2. Comparison of selected terpenes in *J. flaccida*, *J. poblana*, and individuals of *J. poblana* var. *decurrens* (* = 11926 (ITS flacc., cp pob), # = 11927 (ITS pob, cp pob), $ = 11928 (ITS pob, cp pob).

Cpd.	*J. flaccida* (avg). .... *J. poblana* (avg)
linalool	2.9F.......................................1.6P.........1.0#....................0.3$.....0.1*
δ-2-carene	0.0F....0.05*.......................................1.6$ 1.8P 1.9#
δ-3-carene	0.0F 0.1#.......................................1.4P................3.7*..................6.9$
naphthalene	0.0F.......................................0.05P..0.3$........0.3#....................0.5*
methyl chavicol	0.0F.......................................0.5$..0.6*..0.7P..0.8#
cubebol	0.0F.......................................0.0P 0.1#........0..4$....................1.1*
(E)-nerolidol	0.0F 0.1# 0.3$...............1.2#.........................2.5P

Table 3. Classification of *J. p*oblana var. *decurrens* individuals by 7 distinctive components. Parent types: F = flaccida, P = poblana; I = intermediate. >P = cpd > conc. in poblana; >F = cpd > conc. in flaccida.

Component conc. like: F, *flaccida*, P, *poblana* or I, intermediate, <, >.			
Cpd.	11926 *	11927 #	11928 $
linalool	<P	<P	<P
δ-2-carene	F	P	P
δ-3-carene	>P	F	>P
naphthalene	>F>P	>F>P	>F>P
methyl chavicol	P	P	P
cubebol	>F>P	F,P	>F>P
(E)-nerolidol	F	intermediate	F

Table 3. Classification of components by parent types: F = *flaccida*, P = *poblana*; I = intermediate. >P = cpd > conc. in *poblana*; >F = cpd > conc. in *flaccida*.

J. p. var. *decurrens*	linalool	δ-2-carene	δ-3-carene	naphthalene	methyl chavicol	cubebol	(E)-nerolidol
11926 *	<P	F	>P	>F>P	P	>F>P	F
11927 #	<P	P	F	>F>P	P	F,P	intermediate
11928 $	<P	P	>P	>F>P	P	>F>P	F

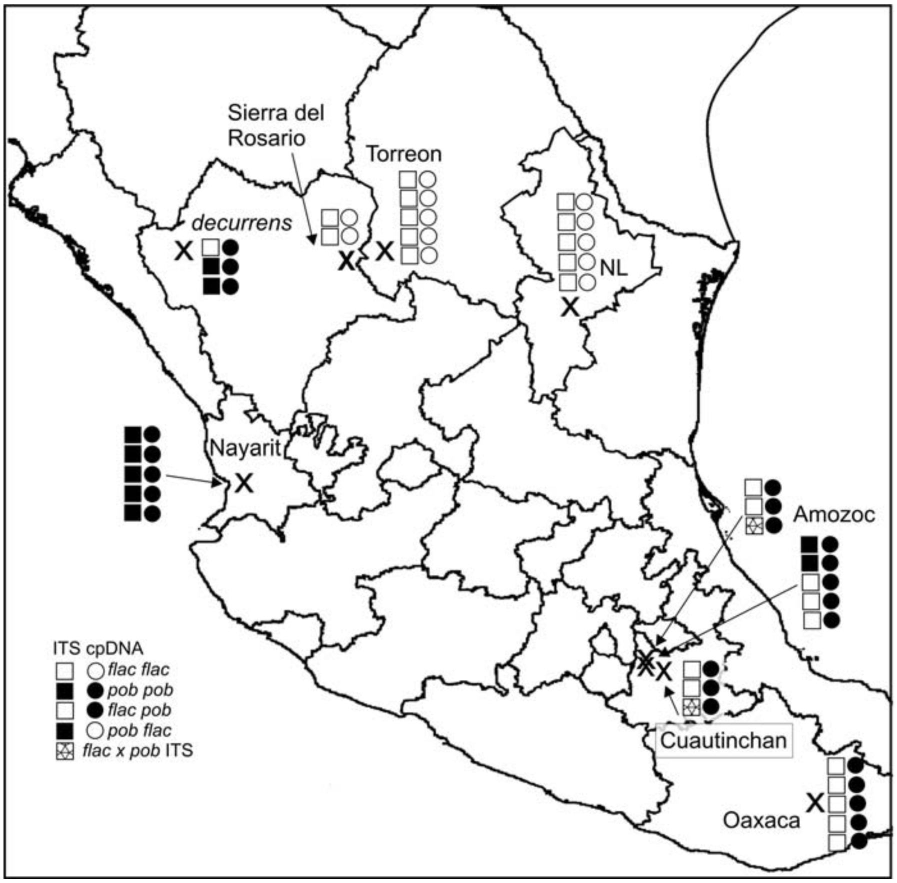
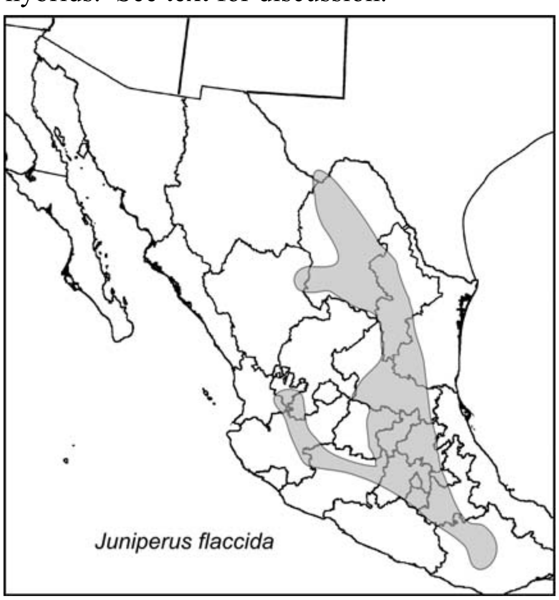
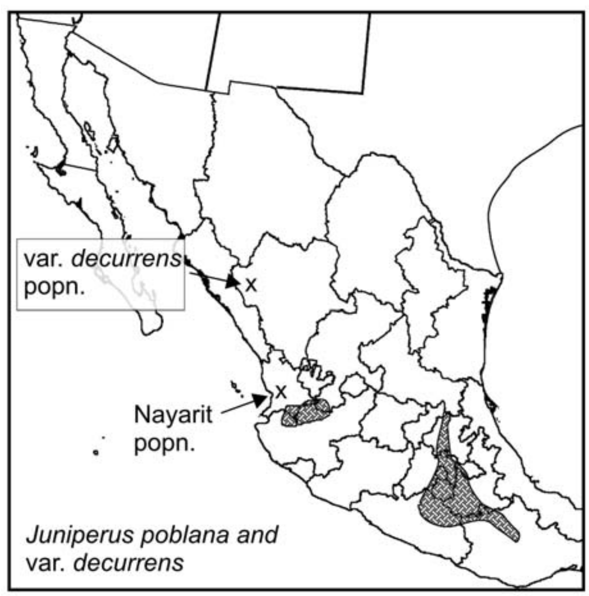


Figure 1. Distribution of ITS (nrDNA), cpDNA classifications of *J. flaccida* and *J. poblana*, and putative hybrids. See text for discussion.





Figures 2 (left) and 3 (right). Distributions of *J. flaccida* and *J. poblana* (modified from Adams, 2014).