

**Allopatric hybridization and introgression between *Juniperus scopulorum* Sarg. and *Juniperus blancoi* Martínez in northern Mexico: Unidirectional gene flow**

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**ABSTRACT**

Analyses of nrDNA (ITS) and petN-psbM (cpDNA) confirmed that allopatric hybridization and introgression are occurring between *J. scopulorum* and *J. blancoi* in northern Mexico. Hybrids (by ITS data) all have *J. blancoi* var. *mucronata* as the maternal parent and *J. scopulorum* as the paternal (pollen donor) parent in Sonora and Chihuahua, with two backcrossed plants in the Guadalupe Mtns., TX and two hybrids discovered in northeastern Arizona. No hybrids or introgressants were found that contained the *J. blancoi* type chloroplast, suggesting the gene flow is unidirectional, north to south, from *J. scopulorum* to *J. blancoi*. Prevailing winds (Jan, Feb, March) give support to northern pollen flow hypothesis. Isolated occurrences of hybrids in northern Arizona seem more likely to be products of Pleistocene sympatry, and not modern northern pollen flow from *J. blancoi* in Mexico to northern Arizona because no *J. blancoi* chloroplasts (i.e., ex pollen) were found in the USA. It appears that *J. b. var. mucronata* is in the midst of a chloroplast capture event. Published on-line [www.phytologia.org](http://www.phytologia.org) *Phytologia* 102(1): 14-26 (March 22, 2020). ISSN 030319430.

**KEY WORDS:** *Juniperus blancoi*, *J. scopulorum*, nrDNA, petN-psbM, hybridization, introgression, Pleistocene sympatry.

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Variation in *Juniperus scopulorum* Sarg. has been reported using terpenoids (Adams 1983, 2011a). Recently, an extensive DNA analysis confirmed allopatric hybridization and introgression between *Juniperus maritima* R.P. Adams and *J. scopulorum* (Adams et al. 2010; Adams 2015a, b). The overall trend was the presence of *J. maritima* in the northwestern US and British Columbia (BC) with intermediate trees (hybrids and backcrosses) in eastern WA and OR, eastern BC and Kalispell, MT (Fig. 1). All the intermediate trees had *J. scopulorum* as the paternal parent (via pollen), with only two intermediate trees having *J. maritima* cp DNA (Wallowa, WO, Fig. 1). *Juniperus maritima* nrDNA (ITS) was found in all trees, except for two putative hybrids at Williams Lake, BC (WL, Fig. 1) and two hybrids at Fairmont Hot Springs, BC (FH, Fig. 1).

However, it was surprising to find no typical *J. scopulorum* (by all three DNA markers) in Wallowa, eastern Washington, or Kalispell, MT (Adams 2015b). Reference *J. scopulorum* trees from Utah and New Mexico were pure *J. scopulorum* (by the three DNA markers) (Fig. 1). Evidence was found of *J. maritima* introgression eastward into *J. scopulorum* in Montana, Wyoming, Idaho and Utah.

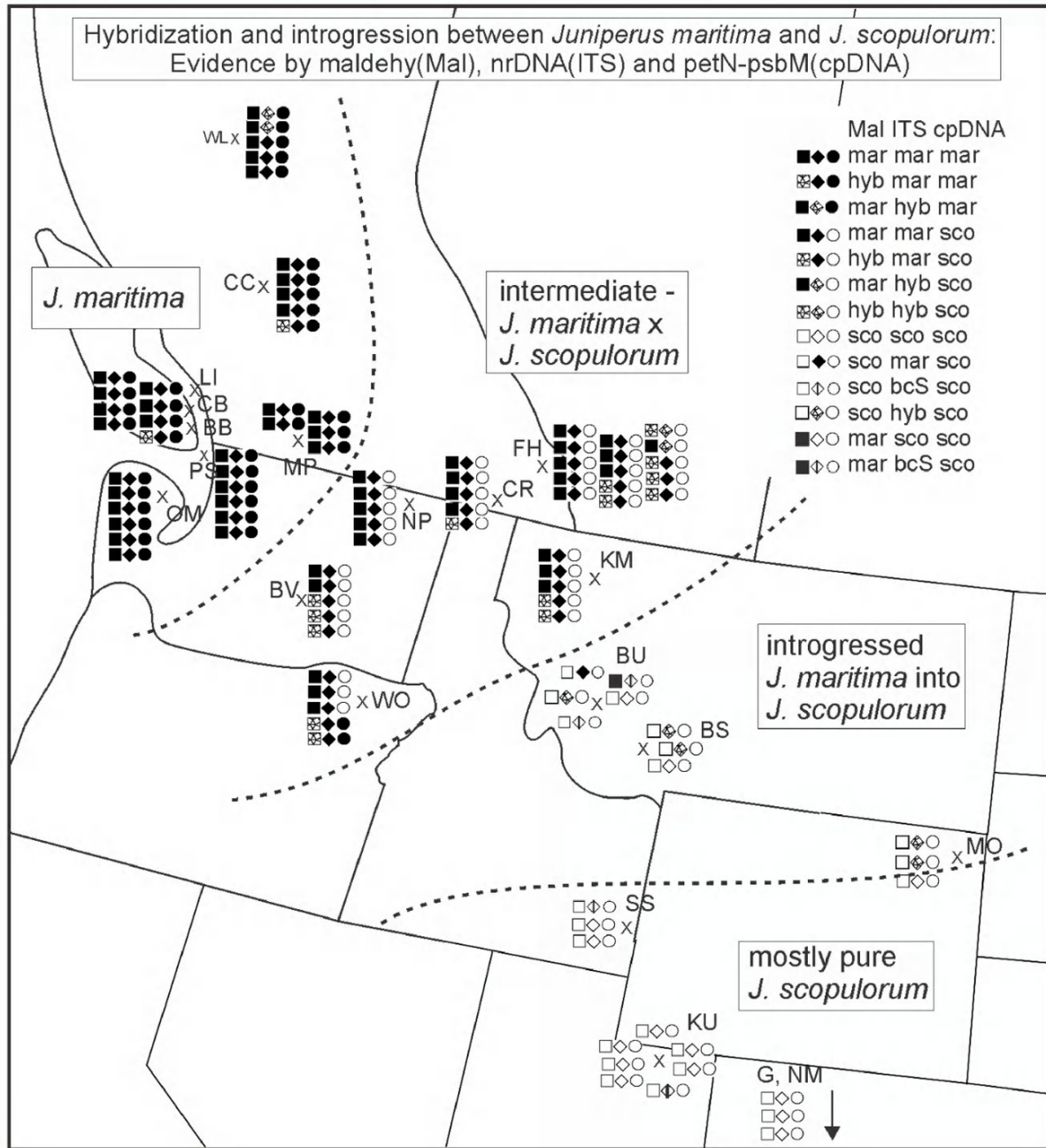


Figure 1. Combined mapping using three gene classifications. The study area can be divided into roughly four zones: 1. typical *J. maritima*: Puget Sound, Vancouver Island, islands in the Strait of Georgia, and western BC; 2. intermediate trees: eastern WA, Wallowa, OR, se BC, and western MT; 3. trees introgressed from *J. maritima* into *J. scopulorum*: Montana and ne Wyoming; 4. mostly typical *J. scopulorum*: se ID, Utah and south in the Rocky Mtns. Adapted from Adams 2015b.

Hybridization was discovered between *J. arizonica* (R.P. Adams) R.P. Adams and *J. coahuilensis* (Martínez) Gaussen ex R.P. Adams in southern New Mexico and trans-Pecos Texas using nrDNA and cp markers (Adams 2017). Introgression was found in the Hueco Tanks and Quitman Mtns. by pollen from *J. arizonica* (from the northwest) producing hybrids that were all paternal *J. arizonica* and maternal *J. coahuilensis*. Only one hybrid individual was found in the trans-Pecos (Alpine-Marfa-Ft. Davis area) and it had *J. coahuilensis* as its paternal parent. Considerable heterozygosity (in the nrDNA) appeared to be relictual from previous hybridization with unknown taxa.



The range of *Juniperus scopulorum* extends nearly to northern Mexico (Adams 2011b; 2014) and *J. blancoi* var. *mucronata* (R.P. Adams) Farjon ranges into northern Sonora and Chihuahua (Adams 2000, 2014). Individuals in Sonora and western Chihuahua are thought to be intermediate between *J. scopulorum* and *J. b.* var. *mucronata* (Adams 2014). Therefore, it seemed timely to apply molecular methods to determine the extent (if any) of gene exchange between these taxa. The purpose of the present paper is to report on extensive analyses of nrDNA (ITS) and cp DNA (petN-psbM) of populations of *J. blancoi* Martínez (and its varieties) in northern Mexico and *J. scopulorum* in the southwestern USA with emphasis on determining hybridization and introgression.

It should be noted that analysis of the distribution of cp types is very useful in the analysis of hybridization and introgression due to the uniparental inheritance of chloroplasts in conifers (see Adams 2019 for a recent review). In the Cupressaceae, chloroplasts have been reported to be paternally (pollen) inherited in all species, except *Cunninghamia konshii* and 2 out of 6 plants examined in the intergeneric cross (*Callitropsis nootkatensis* x *Hesperocyparis macrocarpa*) that were maternally inherited (Adams 2019). Although, at present, no study has documented the inheritance of cp in *Juniperus*, Adams et al. (2016) found cp are paternally (via pollen) inherited in a cross between *Hesperocyparis* (= *Cupressus*) *arizonica* x *H. macrocarpa*. Because *Hesperocyparis* is a sister genus to *Juniperus* (Adams 2014), it seems likely that inheritance of cp via pollen (e.g., paternal) in *Juniperus* is the same as for *Hesperocyparis* and other Cupressaceae species.

## MATERIALS AND METHODS

Plant material: (species, population acronym, location, vouchers):

*Juniperus blancoi* var. *blancoi*:

Carmona - El Oro area, central México

**Adams 6849, 6850, 6851**, stream-side, 7-8 km south of Carmona, 19° 45'N, 100° 07' W, 2580 m, 15 Dec 1991, Mexico, MX.

El Salto, Durango

**Adams 10257, 10258**, with S. Gonzalez and M. Gonzalez-Elizondo, dioecious, tree-shrub, 1.5 m tall, with 1 main stem, but damaged by flood water so it is branched, foliage lax or pendulous, ultimate branchlets generally planate; bark brown, exfoliating in thin plates, reddish beneath, branchlets ~1cm dia. scaly with bronze color beneath scales; female, past pollination. mature fruit with 2 lobes or globose (if one seeded), dark blue upon maturity. On the banks of a running stream approx. 7 km from El Salto town square on road to Pueblo Nuevo, 23° 45.241'N, 105° 22.851' W, 2580 m, 9 May 2004, Durango, MX

*J. b.* var. *huehuentensis* R.P. Adams, S. González & M. González:

Cerro Huehuento, Durango

**Adams 10247-10249**, with S. Gonzalez and M. Gonzalez Elizondo, dioecious, shrub, 0.5 m x 2 m wide, foliage yellowish green, common on rock at top of Cerro Huehuento. brown bark exfoliating in thin plates. most female cones bi-lobed, dark blue when ripe (1 yr.), female cones on the underside of planate branches. pollen shedding now (just finishing), (mid-March - mid-May). 24° 04.587'N, 105° 44.463' W, 3227 m, 8 May 8 2004, Durango, MX

Cerro Mohinora, Chihuahua

**Lab acc. Adams 11436**, ex Socorro Gonzalez 7348a, prostrate shrub 0.3 m tall, strong lime aroma, on rock almost atop Cerro Mohinora, 25° 57' 22" N, 107° 02' 51" W, 3303 m, 2 Nov 2007, Chihuahua, MX.

**Lab acc. Adams 11916, 11917, 11918**, ex Socorro Gonzalez 7531a, b, c with D. Ramírez, A. Mastretta and S. Tena, shrub 0.5-1 m, decumbent, branches olive green color, on rock almost atop Cerro Mohinora, 25° 57' 34" N, 107° 02' 58" W, 3160 m, 2 June 2009, Chihuahua, MX.

*J. b. var. mucronata:*

Yepachic area, Chihuahua/ Sonora

**Adams 8701-8702**, trees to 8 m, with strong central axis, foliage lax at tips, bark exfoliating in strips, fruits reniform (bilobed) and globose, with oaks, *Juniperus deppeana*, *Pinus*, on Mex. Highway 16 between km 336-337, 10 km east of Yepáchi, Chih., 14 km west of Sipachic, Chia., on road to Yecora, Sonora, 28° 26' 32" N, 108° 28' 39" W, 1530 m, 20 Dec 1998, Chihuahua, MX.

**Adams 8703**, trees to 8m, with strong central axis, wood bright purple, foliage weeping at tips, leaf tips mucronate, bark exfoliating in strips, with Oaks. common along the south banks of the Puente El Talayote, 19 km west of Maicoba on Mex. Highway 16 at km 307, on road to Yecora, Son. 28° 22' 29" N 108° 45' 46" W, 1180m, 20 Dec.1998, Sonora, Mexico

Mesa Tres Ríos - Río Gavilán area, Sonora

**Lab acc. Adams 15548**, ex *George M. Ferguson 4390*, N-facing slope, female tree 8.5 m tall, no cones, bright green 29° 48' 51.8" N, 108° 44' 7.8" W, 1745 m, 7 Aug 2018, Arroyo El Palmilloso (=Rio La Cueva), Nácori Chico, Sierra Madre Occidental, Sonora, MX.

Colonia Pacheco, Chihuahua (near Mesa Tres Rios, so included in that group in text, table and figures)

**Adams 2512**, growing next to flowing stream on rocks, with 2-lobed fruits as in *J. blancoi*, with pines and oaks on flatlands, lots of little junipers under oaks with *J. deppeana*, 5 km E of Colonia Pacheco, on Rio Piedras Verde, 30° 05' 23.8" N, 108° 20' 19" W, 1980 m, 20 Feb 1978, Colonia Pacheco, Chihuahua, MX.

**Lab acc. Adams 15549**, ex *George M. Ferguson 4401*, N-facing slope, female tree 20 cm dbh, 7 m tall; numerous cones dark blue, Arroyo San Antonio, 4.8 km (by road) N Mesa Tres Rios, 29° 52' 1.9" N, 108° 42' 41.0" W, 1705 m, 8 Aug 2018, Nácori Chico, Sierra Madre Occidental, Sonora, MX.

**Lab Acc. Adams 15550**, ex *George M. Ferguson, 4404*, male tree 62 cm dbh, 12 m tall, pollen cones dry (shedding completed), bark longitudinally furrowed, Arroyo San Antonio, 6.4 km (by road) N Mesa Tres Rios, 29° 52' 35.4" N, 108° 43' 15.2" W, 1570 m, 8 Aug 2018, Nácori Chico, Sierra Madre Occidental, Sonora, MX.

**Lab Acc. Adams 15551, 15552**, ex *George M. Ferguson 4434, 4435*, trees, 5-8 m tall; bark longitudinally plated, confluence Rio Gavilán and Rio La Cueva, 29° 52' 58.4" N, 108° 37' 36.5" W, 1400 m, 10 Aug 2018. Nácori Chico, Sierra Madre Occidental, at Sonora, MX.

Los Ajos, Sonora

**Adams 14424, 14425, 14426**, ex *George M. Ferguson 3651, 3652, 3653*, trees to 8 m, bark plated dark brown. Riparian in pine-oak-juniper woodland w/ *Juniperus deppeana*, *Acer grandidentatum*, *Alnus oblongifolia*, *Quercus arizonica*, *Pinus chihuahuana*, *Pinus engelmannii*, *Platanus wrightii*. Municipio Cananea, Sierra Los Ajos, Canon Evans, 1.5 mi NW (by road) Los Ajos Nuevos CONANP headquarters. 30° 59' 14.8" N, 109° 58' 22.8" W, 1800 m, 26 Aug 2014, Sonora, MX.

*J. scopulorum:*

Guadalupe Mtns., NM

**Lab acc. Adams 15602**, ex *Richard Worthington 28617*, UTEP Herbarium accession 58749, Devil's Den Spring, Guadalupe Mtns. 32° 02' 3.12" N, 104° 16' 0.12" W. 2103 m (6000ft), 5 Sept. 1999, Eddy Co., NM.

**Lab Acc. Adams 15603**,

ex *Richard Worthington 28673*, UTEP Herbarium accession 58750, North Fork, Big Canyon, Guadalupe Mtns. 32° 02' 3.12" N, 104° 45' 0.12" W. 1828m (6000ft), 6 Sept. 1999, Eddy Co., NM.

**Lab acc. Adams 15783, 15784, 15785**, ex *George M. Ferguson 4624, 4625, 4626* with J. Ferguson, 3-9 m tall; bark longitudinally plated, pollen cones forming, riparian woodland, limestone, associated species: *Pinus ponderosa*, *Pinus edulis*, *Juniperus deppeana*, *Quercus muehlenbergii*, *Quercus grisea*, *Acer grandidentatum*, *Berberis haematocarpa*, *Arbutus xalapensis*, *Dasyllirion leiophyllum*, *Agave parryi*, Dark Canyon, S of Klondike Gap near confluence Hooper Canyon, 0.2 mi (by FR 307) E jct County Road 412 (FR 69), just inside USFS boundary. Lincoln National Forest, Guadalupe Mountains, 32° 6' 0" N, 104° 46' 15.6" W, 1920 m (6300 ft.), 3 Nov 2019 Eddy County, NM.



**Lab Acc. Adams 15786**, ex George M. Ferguson 4627 with J. Ferguson, male tree, ca. 15 cm dbh, 4 m tall; bark longitudinally plated, associated species: *Pinus ponderosa*, *Pinus edulis*, *Juniperus deppeana*, *Quercus muehlenbergii*, *Quercus grisea*, *Acer grandidentatum*, *Berberis haematocarpa*, *Arbutus xalapensis*, *Dasyllirion leiophyllum*, *Agave parryi*, riparian woodland, limestone, Dark Canyon, S of Klondike Gap near confluence Hooper Canyon, 0.2 mi (by FR 307) E jct County Road 412 (FR 69), just inside USFS boundary, Lincoln National Forest, Guadalupe Mountains, 32° 6' 0" N, 104° 46' 15.6" W, 1920 m (6300 ft.), 3 Nov 2019 Eddy County, NM. *Juniperus scopulorum* hybrid ITS x *J. blancoi* Black Canyon, Gila NF, NM

**Lab acc. Adams 15562, 15563**, ex George M. Ferguson 4279, 4280, 8 m tall, foliage weeping, Gila National Forest, East Fork Gila River, 0.5 mi below confluence with Black Canyon, 33° 10' 45.1" N, 108° 9' 32.0" W, 1743 m (5720 ft.), 28 July 2018, Grant Co., NM.

Sapillo Creek, Gila National Forest, NM

**Lab acc. Adams 15714, 15715, 15716, 15717**, ex George M. Ferguson 4549, 4550, 4551, 4552, male tree, 4 m tall, bark longitudinally plated, Pine forest, associated species: *Pinus ponderosa* var. *brachyptera*, *Pinus edulis*, *Juniperus deppeana*, Gila National Forest, Sapillo Creek (Gila River drainage), 1.0 mi (by NM hwy 35) E Upper End Campground of Lake Roberts, 33° 01' 34.3" N, 108° 08' 8.6" W, 1875 m (6150 ft), 4 July 2019. Grant Co., NM.

Gila Hot Springs, Gila National Forest, NM

**Lab acc. Adams 15718, 15719, 15720** ex George M. Ferguson 4553, 4554, 4555 trees to 10 m tall, Pine forest, bark longitudinally plated, with: *Pinus ponderosa* var. *brachyptera*, *Pinus edulis*, *Juniperus deppeana*, *Juniperus monosperma*, Gila Hot Springs, on private land along W Fork Gila River, 33° 11' 44.5" N, 108° 12' 25.9" W, 1707 m (5600 ft), 5 July 2019. Grant Co. NM

**Lab acc. Adams 15722, 15723**, ex George M. Ferguson 4557, 4558, Pine forest; trees, 8 m tall, bark longitudinally plated, with: *Pinus ponderosa* var. *brachyptera*, *Pinus edulis*, *Juniperus monosperma*. Gila National Forest, near Woody's Corral, 0.5 mi (by NM hwy 15) E Gila Cliff Dwellings National Monument, along West Fork of Gila River. 33° 13' 30.2" N, 108° 15' 05.2" W, 1707 m, (5650 ft), 5 July 2019, Catron Co., NM

Sedona, AZ

**Adams 10637-10639**, Common at top of canyon, trees to 6 m, with *J. deppeana*. On AZ highway 89A, between Sedona and Flagstaff in Oak Creek Canyon. 34° 57' 19" N 111° 45' 17" W, 1521 m, 13 March 2005, Yavapai Co., AZ

Brian Head, UT

**Adams 10908-10910**, female, 6 m tree with strong central axis, common with pinyon, sage and oak on gravelly soil, approx. 10 km SE of Parowan, near Brian Head on UT hwy 148, 37° 45' 09" N, 112° 50' 19" W, 2414 m, 6 Aug 2005, Iron Co., UT.

Lukachukai, AZ

**Adams 10915-10917**, 4-6 m tall, strong central axis, very glaucous foliage, with Ponderosa pine and oaks on rocky soil. Chuska Mts., 14 km NE of Lukachukai, AZ on AZ hwy. 13. 36° 27' 14" N, 109° 09' 48" W, 2409 m, 7 Aug 2005, Apache Co., AZ

Eagar, AZ

**Adams 10928-10930**, 10-12 m trees, strong central axis, foliage very glaucous and weeping, on rocky soil, scattered with Ponderosa pine, spruce and *J. deppeana*, *J. monosperma* and *J. communis* on Water Canyon Rd., 6 km S. of Eagar, AZ., White Pine Mts., 34° 02' 41" N, 109° 17' 52" W, 2441 m, Aug 8, 2005, Apache Co., AZ.

Glorieta, NM

**Adams 10933-10935**, 5-7 m trees, strong central axis, very glaucous foliage, abundant with *J. monosperma*, Glorieta Mesa, on I25, E of Santa Fe, Exit 297 (Valencia), 35° 34' 44" N, 105° 47' 35" W, 2256 m, 9 Aug 2005 m, Santa Fe Co., NM.

Voucher specimens 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), (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<sub>2</sub> 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. 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.).

## RESULTS AND DISCUSSION

Sequencing of petN-psbM (cp DNA) proved be useful in distinguishing *J. blancoi* from *J. scopulorum* by the presence of a 5 bp indel at site<sup>1</sup> 703 (TTTTA insert) in *J. blancoi*. The presence of the insert was found in *J. blancoi* from Carmona, and El Salto, as well as all *J. b. var. huehuentensis* from Cerro Huehuento (Table 1). However, in the Cerro Mohinora population of *J. b. var. huehuentensis*, one plant (11917) contained the *J. scopulorum* cp type (Table 1). Further north, all the *J. b. var. mucronata* populations (Yepachic, Colonia Pacheco, Mesa Tres Rios, Los Ajos) contained only the *J. scopulorum* cp type. This supports the hybrid origin of *J. b. var. mucronata* from *J. blancoi* and *J. scopulorum* hybridization in the past. In contrast, no plants collected as *J. scopulorum* contained the *blancoi* cp type (Table 1).

DNA sequencing of nrDNA (ITS) yielded 1270bp, with three informative sites (284<sup>2</sup>, 346<sup>3</sup>, 348<sup>4</sup>) that distinguished *J. scopulorum* and *J. blancoi* (all varieties) (Table 1). Several *J. b. var. mucronata* trees in northern Mexico had polymorphic sites indicative of present or past hybridization with unknown taxa (Table 1). Four *J. scopulorum* trees appear to be of hybrid origin (15786, 15803, Guadalupe Mtns., 19015, 10916, Lukachukai, AZ), although all had the *J. scopulorum* cp type.

Mapping samples using nrDNA types (Fig. 2) reveals that hybrids (by nrDNA) are found in northern Mexico in *J. b. var. mucronata*; and two hybrids at Lukachukai, AZ, (Fig. 2). Two backcrossed plants were found in the Mesa Tres Rios population and two in the *J. scopulorum* site in the Guadalupe Mtns. (Fig. 2).

Overall, the variation can be divided into three zones (Fig. 2): northern zone - mostly pure *J. scopulorum*; central zone that contains hybrids and introgressed *J. b. var. mucronata* that have only the *J. scopulorum* cp; and zone 3: mostly pure *J. blancoi* and *J. b. var. huehuentensis*. An interesting trend is that in the central (red) zone, all samples have the *J. scopulorum* cp (Fig. 2). However, the nrDNA varies from 'pure' *J. blancoi*, to hybrids (*J. scopulorum* x *J. b. var. mucronata*), to 2 backcrossed plants with 2 nrDNA homozygous *J. blancoi* sites and one site each, heterozygous *J. blancoi*, *J. scopulorum*. (Table 1, Fig. 2). Two plants from Guadalupe Mtns., NM have nrDNA suggesting they are backcrosses (Table 1).

<sup>1</sup>Informative intel in petN-psbM at site 703: xTTTTATAGTAA, where x = TTTTA  
Informative nrDNA (ITS) sites: <sup>2</sup>284 xCCCGCGGTGC; <sup>3</sup>346 GAAACGACx; <sup>4</sup>348 xTGTGCGGA.

A detailed examination of the cp types, shows (Fig. 2) all the var. *mucronata*, and one var. *huehuentensis* had *J. scopulorum* cp (via a paternal, pollen parent), but no *J. scopulorum* contained the *J. blancoi* cp DNA (i.e., via pollen from *J. blancoi*). Gene flow via pollen is decidedly unidirectional from *J. scopulorum* to *J. blancoi*, north to south. Unidirectional hybridization has been reported when pollen of *Pinus brutia* was placed on ovulate cones of *P. halepensis*, no viable seeds were found, but the reciprocal cross (pollen, *P. halepensis* x ovulate cones, *P. brutia*) yielded viable seeds (Conkle et al. 1988; Moulalis et al. 1976). The occurrence of two nrDNA hybrids in the Lukachukai population is anomalous. There is no indication of pollen flow northward into *J. scopulorum* in this region.

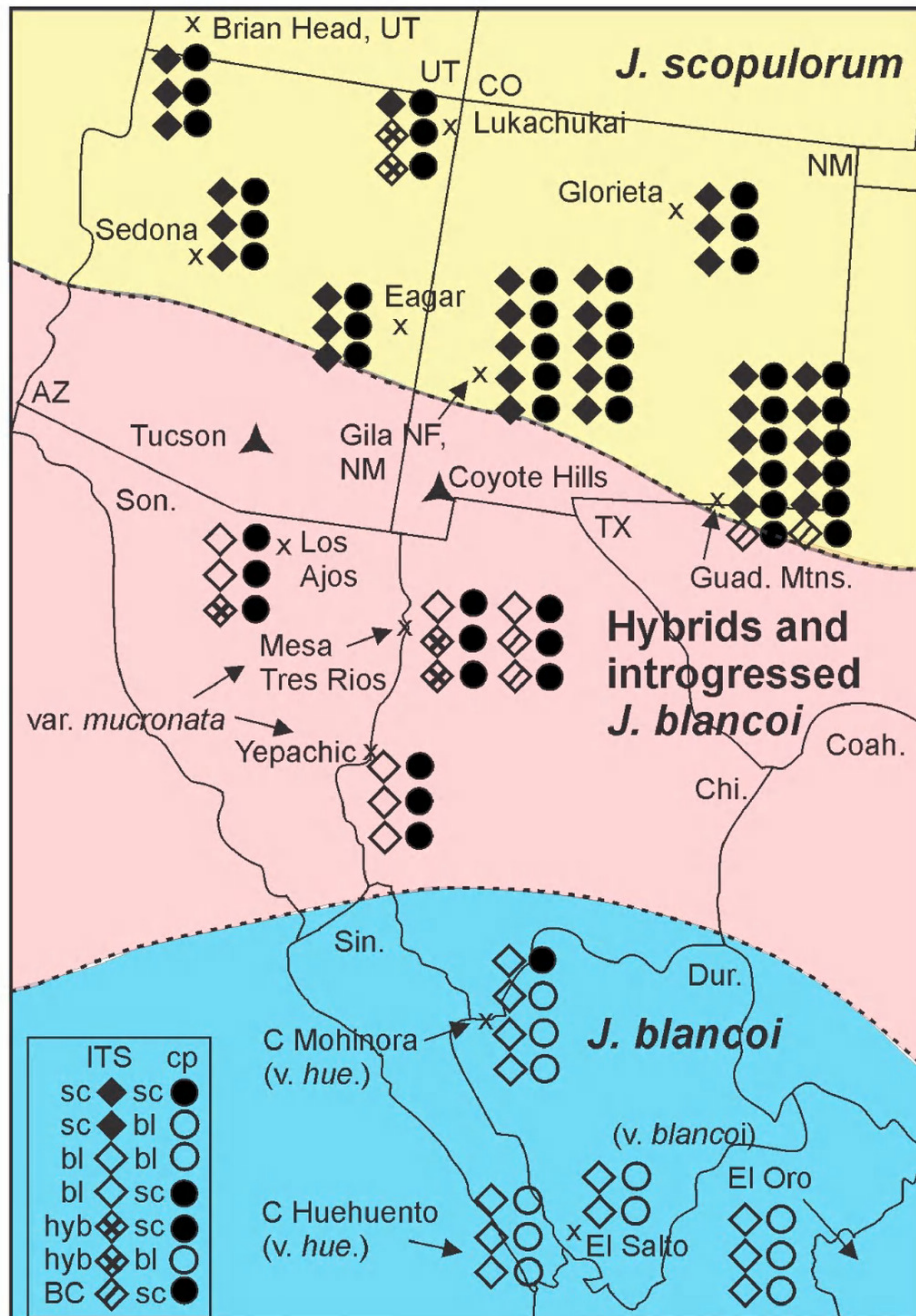


Figure 2. Distribution of cp and nrDNA types in three zones: mostly pure *J. blancoi* (blue), mostly pure *J. scopulorum* (yellow) and a zone of hybrids and introgressants (red). Combinations of cp and nrDNA shown in box, lower left. See text for discussion.



Pollen is shed by *J. scopulorum* in March-April and *J. blancoi* in January- mid-May (Adams 2014), although local differences and yearly differences in pollen shedding times are well known (Levetin 1998). Analysis of wind directions and velocities show that the predominant directions in January through April are from the southeast, but 6 to 10% of the winds are from the northwest (Fig. 3). The winds from the northwest are mostly 7.4 - 11.2 mph and 11.2 - 19.9 mph (Fig. 3). The distance from Sedona to Los Ajos is 290 miles and from Eager to Mesa Tres Rios, 290 miles. If northwest *J. scopulorum* pollen was carried by winds of 10 mph, it would take only 29 hrs. to reach Los Ajos or Mesa Tres Rios.

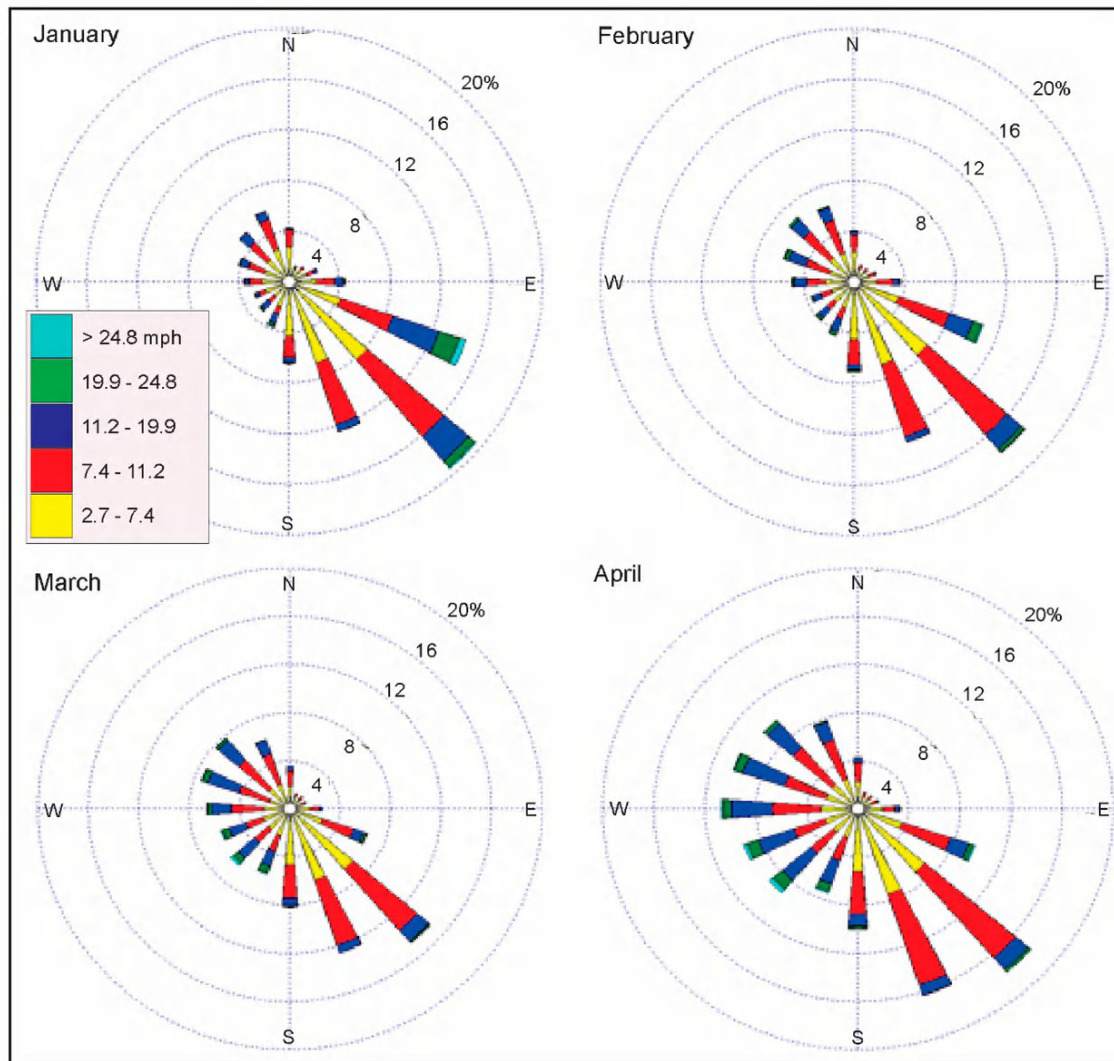


Figure 3. Frequencies of wind directions and velocities in January - April, in Tucson, AZ. Data from Wind Rose Resources, NRCS, <https://www.wcc.nrcs.usda.gov/climate/windrose.html>

Several other studies in conifers have reported long distance transport (LDT) of pollen from a few km to several hundred km (Szczepanek et al. 2017; Neale and Wheeler 2019; Stewart et al. 2012; Sarvas 1962; Koski 1970; Nichols et al. 1978; Campbell et al. 1999). Importantly, several studies have reported that LDT pollen has maintained its viability (Lindgren et al. 1995; Varis et al. 2009; Williams 2010). Pollen from *Juniperus communis*, in the western Alps, was stored at ambient conditions and found to be 40-90% viable for fresh pollen, 20-40% viable after two weeks and 0-10% viable after two months storage (Carmeliello et al. 1990). Finally, it should be mentioned that in a preliminary study on LDT pollen viability, Levetin (per. comm.) found viable *Juniperus* (*J. ashei*) LDT airborne pollen in Tulsa, OK, after having traveled at least 200 mi. from southern Oklahoma/Texas.

Thus, because the *J. scopulorum* pollen from southern Arizona and New Mexico can easily reach Los Ajos, Mesa Tres Rios and beyond, in 1 to 2 days, it seems likely the pollen would be viable to affect fertilization. The predominant southeast winds (Fig. 3), blowing pollen from northern Mexico towards the northwest favor the movement of pollen in March-April towards Sedona, Edgar and Gila National Forest. Yet, we have discovered no trees containing the *J. blancoi* cp type from there (Table 1, Fig. 2). It appears that *J. scopulorum*, in present times, has an efficient sterility barrier against fertilization by *J. blancoi* pollen. However, *J. b. var. mucronata* seems to have an ineffective barrier against *J. scopulorum* pollen fertilization as indicated by the fact that all the var. *mucronata* plants sampled have the *J. scopulorum* cp type (Table 1, Fig. 2).

The hybrids near Lukachukai (2400 m) and in the Guadalupe Mtns. (1900-2200 m) have maternal *J. blancoi* parents, but are far from any known *J. blancoi* trees (Fig. 2). Martin and Mehringer (1965) and Wells (1966) suggested woodland and forest species descended as much as 800m throughout the southwest from 13,500 to 10,000 ybp. A review of late Pleistocene- Holocene climate in Mexico (Metcalf, et al. 2000) noted that during the late Pleistocene and early Holocene, northern Mexico was much wetter and cooler than today, with Pinyon-Juniper woodland covering extensive areas which are, today, desertshrub.

*Juniperus scopulorum* dated in the late Pleistocene (13,830 ybp), has been found in packrat middens at ~1500m at Coyote Hills (Fig. 2), southwest New Mexico (Holmgren et al. 2003) in an area that is, presently, a Chihuahuan desert grassland with scattered *J. arizonica* trees (GMF, pers. obs.).

Holmgren et al. (2003) noted that *J. scopulorum* was common in Pleistocene middens at ~1500m, on limestone at Guadalupe and Sacramento Mtns. sites (Van Devender et al. 1984). If *J. scopulorum* grew at 1555m (Sacramento Mtns midden) and 1500m (Guadalupe Mtns. midden) that would be about 500m lower than present *J. scopulorum* plants in those areas. In addition, an isolated occurrence of *J. scopulorum* has been reported at 975m in Pleistocene middens from Organ Pipe National Monument on the Arizona - Mexico border (Van Devender, 1990). Van Devender (1990), noted that the nearest present-day population of *J. scopulorum* is 270 km northeast, below the Mogollon Rim, AZ. During the late Pleistocene, *J. scopulorum* and *J. blancoi* var. *mucronata*, both of which favor riparian or streamside habitats today, could have expanded their ranges such that the taxa overlapped (sympatric) in many areas, giving opportunities to hybridize. A northern origin of *J. blancoi* was hypothesized by Mastretta-Yanez et al. (2012), who found the most ancestral haplotype for the species in the Yecora population of *J. blancoi* var. *mucronata*. Their ancestral cp haplotype is surely the *J. scopulorum* cp we have found in **every** *J. b. var. mucronata* plant analyzed in this study (Fig. 2). And, this seems to agree with the present phylogeny of the smooth leaf junipers of North America (Fig. 1.16.5, p. 22, Adams 2014) showing *J. blancoi* var. *mucronata* linked to *J. scopulorum*.

nrDNA is well known to maintain heterozygosity acquired millions of years ago (see Syring et al. 2007). In fact, Syring et al. (2007) estimated that in *Pinus*, it may take up to 76 myr to achieve complete genome wide coalescence by concerted evolution (Aguilar et al. 1999; Liao 1999; Moreno-Letelier et al.

2014). So, it shouldn't be surprising to find ancestral polymorphisms in extant populations today. With the retreat of the Wisconsin glacial ice, and the subsequent warming (Crandell 1971; Flint 1971), *Juniperus* expanded its range into the higher elevation habitats that it occupies today. The expanded and overlapping *Juniperus* ranges help explain the relictual hybridization between *J. blancoi* and *J. scopulorum* that we found in northern Mexico in this study. Alternatively, pollen of *J. scopulorum* carried south into the range of *J. b. var. mucronata*, and affecting pollination may have produced the introgressed taxon.

Of course, it is possible that *J. blancoi* var. *mucronata* (in the red, central zone) has acquired the *J. scopulorum* cp by chloroplast capture as an ancient event. If so, this study may have captured a time interval catching a snapshot of *J. b. var. mucronata* in the midst of a chloroplast capture event. Chloroplast capture in *Juniperus* has been reported from an ancestor of *J. sabina* var. *balkanensis* from *J. thurifera* (Adams et al. 2017, Adams et al. 2018). Presumably, the nrDNA has become homogenous for the *J. sabina* type due to concerted evolution, however, the *J. thurifera* cp has been retained. Examination of Fig. 2 shows that indeed, all plants of *J. b. var. mucronata* contain the *J. scopulorum* cp, and about half the samples have homozygous *blancoi* nrDNA, and the rest have hybrid, heterozygous nrDNA. Without additional gene sequences, it does not seem possible to make a definitive decision. Analyses of more genes (NextGen sequencing) will undoubtedly reveal a more complete picture of the evolutionary chloroplast capture events highlighted in the present report.

#### ACKNOWLEDGEMENTS

This research was supported in part with funds from project 0324512, Baylor University. Thanks to Sam Johnson for lab assistance. Thanks to Richard Worthington and Vicky (Mingna) Zhuang at UTEP herbarium, University of Texas at El Paso, for the gift leaf fragments from specimens for DNA analyses. Collecting trips to Sonora, Mexico were facilitated by Tom Van Devender (Madrean Discovery Expeditions) sponsored by GreaterGood.org, and the Madrean Archipelago Biodiversity Assessment program of Sky Island Alliance, Tucson, AZ. Thanks to Tom Van Devender for constructive comments on the manuscript.

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Table 1. Analyses of *J. blancoi* and *J. scopulorum* by cpDNA (petN-psbM): indel site 703<sup>1</sup>, and nrDNA: informative sites 284, 346 and 348. BI intr Sc cp = blancoi, introgressed by scop cp; BxS, BC-BI = blancoi x scop, backcrossed to blancoi. SxB, BC-Sc = scop x blancoi, backcrossed to scopulorum.

collection number, taxon (field identification), population	id by cpDNA	id by nrDNA	284(204) T/C(Y)	346(266) T/G(K)	348(268) T/C(Y)
10908 scopulorum Brian Head, UT	scop	scop	T	T	T
10909 scopulorum Brian Head, UT	scop	scop	T	T	T
10910 scopulorum Brian Head, UT	scop	scop	T	T	T
10933 scopulorum Glorieta NM	scop	scop	T	T	T
10934 scopulorum Glorieta NM	scop	scop	T	T	T
10935 scopulorum Glorieta NM	scop	scop	T	T	T
10917 scopulorum Lukachukai, AZ	scop	scop	T	T	T
10928 scopulorum Eagar, AZ	scop	scop	T	T	T
10929 scopulorum Eagar, AZ	scop	scop	T	T	T
10930 scopulorum Eagar, AZ	scop	scop	T	T	T
10637 scopulorum Sedona, AZ	scop	scop	T	T	T
10638 scopulorum Sedona, AZ	scop	scop	T	T	T
10639 scopulorum Sedona, AZ	scop	scop	T	T	T
15562 scopulorum Black Canyon, Gila NF NM	scop	scop	T	T	T
15563 scopulorum Black Canyon, Gila NF NM	scop	scop	T	T	T
15714 scopulorum Sapillo Ck., Gila NF, NM	scop	scop	T	T	T
15715 scopulorum Sapillo Ck., Gila NF, NM	scop	scop	T	T	T
15716 scopulorum Sapillo Ck., Gila NF, NM	scop	scop	T	T	T
15718 scopulorum Gila Hot Sprs, Gila NF, NM	scop	scop	T	T	T
15719 scopulorum Gila Hot Sprs, Gila NF, NM	scop	scop	T	T	T
15720 scopulorum Gila Hot Sprs, Gila NF, NM	scop	scop	T	T	T
15722 scopulorum Woodys Corral, Gila NF NM	scop	scop	T	T	T
15723 scopulorum Woodys Corral, Gila NF NM	scop	scop	T	T	T
15602 scopulorum, Guadalupe Mtns, NM	scop	scop	T	T	T
15603 scopulorum, Guadalupe Mtns, NM	scop	scop	T	T	T
15783 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15784 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15785 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15798 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15799 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15800 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15801 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15802 scopulorum Guadalupe Mtns, NM	scop	scop	T	T	T
15786 scopulorum, Guadalupe Mtns, NM	scop	SxB, BC-Sc	T	T/G (K)	T/C (Y)
15803 scopulorum, Guadalupe Mtns, NM	scop	SxB, BC-Sc	T	T/G (K)	T/C (Y)
10915 scopulorum Lukachukai, AZ	scop	hybrid	T/C (Y)	T/G (K)	T/C (Y)
10916 scopulorum Lukachukai, AZ	scop	hybrid	T/C (Y)	T/G (K)	T/C (Y)
15552 blancoi var. mucronata, Mesa Tres Rios	scop	hybrid	T/C (Y)	T/G (K)	T/C (Y)
15548 blancoi var. mucronata, Mesa Tres Rios	scop	hybrid	T/C (Y)	T/G (K)	T/C (Y)
14425 blancoi var. mucronata, Los Ajos	scop	hybrid	T/C (Y)	T/G (K)	T/C (Y)
15551 blancoi var. mucronata, Mesa Tres Rios	scop	BxS, BC-BI	C	G	T/C (Y)
15549 blancoi var. mucronata, Mesa Tres Rios	scop	BxS, BC-BI	T/C (Y)	G	C
11917 blancoi var. huehuentensis, Cerro Mohinora	scop	blancoi	C	G	C
8701 blancoi var. mucronata, Yepachic	scop	blancoi	C	G	C
8702 blancoi var. mucronata, Yepachic	scop	blancoi	C	G	C
8703 blancoi var. mucronata, Yepachic	scop	blancoi	C	G	C
14424 blancoi var. mucronata, Los Ajos	scop	blancoi	C	G	C
14426 blancoi var. mucronata, Los Ajos	scop	blancoi	C	G	C
2512 blancoi var. mucronata, Col. Pacheco (MTRios)	scop	blancoi	C	G	C
15550 blancoi var. mucronata, Mesa Tres Rios	scop	blancoi	C	G	C
11436 blancoi var. huehuentensis, Cerro Mohinora	blancoi	blancoi	C	G	C
11916 blancoi var. huehuentensis, Cerro Mohinora	blancoi	blancoi	C	G	C
11918 blancoi var. huehuentensis, Cerro Mohinora	blancoi	blancoi	C	G	C
10247 blancoi var. huehuentensis, Cerro Huehuento	blancoi	blancoi	C	G	C
10248 blancoi var. huehuentensis, Cerro Huehuento	blancoi	blancoi	C	G	C
10249 blancoi var. huehuentensis, Cerro Huehuento	blancoi	blancoi	C	G	C
10257 blancoi, El Salto	blancoi	blancoi	C	G	C
10258 blancoi, El Salto	blancoi	blancoi	C	G	C
6849 blancoi, Carmona - El Oro	blancoi	blancoi	C	G	C
6850 blancoi, Carmona - El Oro	blancoi	blancoi	C	G	C
6851 blancoi, Carmona - El Oro	blancoi	blancoi	C	G	C





Adams, R P M Socorro González-Elizondo and Ferguson, George M. 2020.  
"Allopatric hybridization and introgression between *Juniperus scopulorum*  
Sarg. and *Juniperus blancoi* Mart. in northern Mexico: Unidirectional gene  
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