DNA BARCODING A JUNIPER: THE CASE OF THE SOUTH TEXAS DUVAL COUNTY JUNIPER AND SERRATE JUNIPERS OF NORTH AMERICA

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ABSTRACT

The utilization of 4351 bp from five gene regions (nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG) was sufficient to accurately identify an unknown juniper species from Duval County, TX as *J. pinchotii.* Bayesian, Maximum Likelihood, Parsimony, and NJ analyses were equally adept in identifying the juniper, but UPGMA was barely able to identify it and Minimum Linkage was equivocal. A robust phylogeny of the serrate-leaf junipers of North America is presented as a consequence of the study. *Phytologia 93(2): 146-154 (August 1, 2011).*

KEY WORDS: *Juniperus pinchotii,* serrate-leaf junipers, North America, barcode, phylogeny, Cupressaceae, nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG.

Recently, a colleague, William Carr, while doing routine collecting in Duval County, Texas, obtained samples from a small juniper shrub growing on Cedro Hill on white claystone or caliche of the Fant Tuff member of the Catahoula and Frio formations. There are about 15 juniper plants in a 15 x 20 m area, growing generally with or under the shrubs *Gochnatia hypoleuca* and *Leucophyllum frutescens* in very xeric conditions. The juniper specimen had ball-like foliage without terminal whips (due to drought) and one very small, immature

seed cone that had (apparently) turned blue during drying. Without terminal whip leaves and mature seed cones, most *Juniperus* are impossible to identify to the species level. The unknown juniper had serrate-leaf margins, which put it in the serrate *Juniperus* group of North America; but that group contains 21 species (Adams, 2011) and since the Duval Co. site is several hundred miles from the nearest known, natural population of serrate *Juniperus* routine identification proved to be impossible.

There has recently been considerable discussion about using DNA barcoding to identify plants (Chase and Fay, 2009; Seberg and Petersen, 2009; Chase et al. 2007; Cowan et al. 2006; Newmaster et al. 2006); Kress et al. 2005). However, Seberg and Petersen (2009) found that even using 6 average sized barcode regions would not identify all of the 86 known *Crocus* species.

Because several recent DNA sequencing studies of serrate-leaf *Juniperus* have been published (Mao et al. 2010; Willson et al. 2008; Adams and Kauffmann, 2010; Adams, 2009; Adams, Schwarzbach and Morris, 2010), it seemed an opportune time to complete sequences for all the serrate junipers of North America for nrDNA, petN-psbM, trnD-trnT, trnL-trnF and trnS-trnG and test if these five regions would be sufficient to identify the unknown Duval County juniper.

MATERIALS AND METHODS

Plant material: *J. pinchotii, W. Carr 28809* (*Adams 12248* in lab), 27º 45' 30.8"N, 98º 41' 58.5"W, 666ft., Cedro Hill, Duval County, Texas 26 Apr, 2010 and *Adams 12534-12538*, same location, 13 Nov. 2010. Voucher specimens are deposited at Baylor University (BAYLU) and the University of Texas (TEX).

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 as per manufacturer's instructions.

PCR amplification 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, trnD-T, trnL-F, trnS-G) 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 (1.5% agarose, 70 v, 55 min.). 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 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.). Sequence datasets were analyzed using Geneious v. 5.1 (Drummond et al. 2010), the MAFFT alignment program (Katoh et al. 2005), and the PAUP* program, version 4.0b10 (Swofford 2003) for neighbor joining, parsimony, and maximum likelihood tree searches. 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. Sequences were aligned by use of MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009, Adams, 1975). GenBank sequences were downloaded as available to complement the sequences for all five gene regions for all of the 23 taxa (2 accessions each) of serrate junipers present in N. America, plus the unknown Duval County juniper and two accessions of *J. virginiana*, Tennessee (as an entire-leaf juniper outgroup).

RESULTS AND DISCUSSION

The concatenated data set was composed of 4,351 bp from nrDNA, petN-psbM, trnD-trnT, trnL-trnF and trnS-trnG sequences. Bayesian analysis (Fig. 1) placed the Duval juniper in a clade with *J. pinchotii*. All of the serrate junipers are well resolved. However, the position of *J. californica* seems odd as it is normally considered a sibling species to the western junipers (*J. grandis, J. occidentalis* and *J.*

osteosperma). It appears that some major mutations have occurred in the nrDNA of *J. californica* and this perhaps explains its unusual position. Analyses of the data set without nrDNA resulted in an unresolved clade containing *J. angosturana*, *J. pinchotii* and the Duval juniper as well as several other unresolved taxa (data not shown).

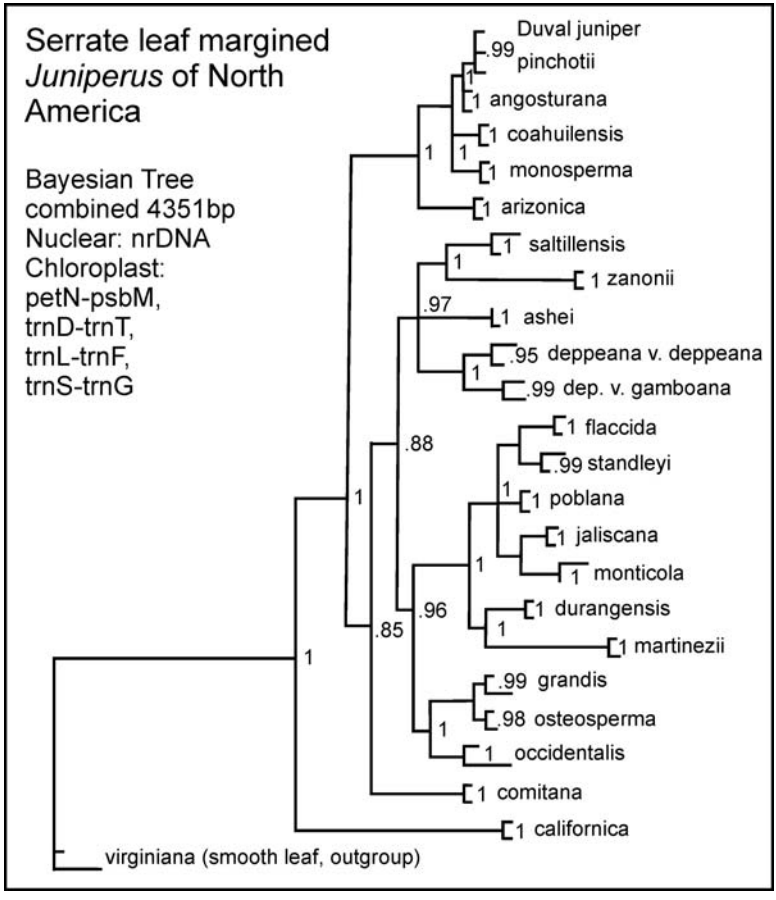


Figure 1. Bayesian tree of the serrate *Juniperus* of North America with the Duval juniper in a clade with *J. pinchotii*. Numbers at the branch points are posterior probabilities (0 - 1).

A NJ tree was very similar to the Bayesian tree. The Duval juniper was placed in a clade with *J. pinchotii* (Fig. 2). In fact, except for differences in the relationships of the four major clades, Bayesian and NJ

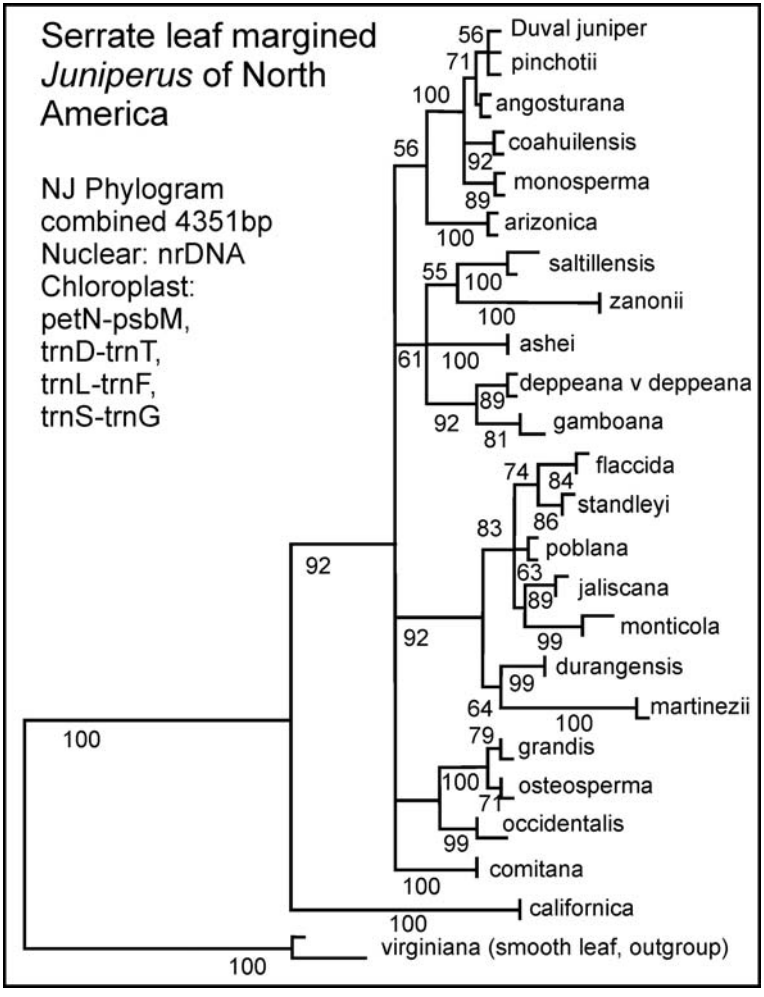


Figure 2. NJ tree of the serrate *Juniperus* of North America with the Duval juniper in a clade with *J. pinchotii*. Numbers at the branch points are bootstrap values as percent (1000 reps.).

trees were nearly identical (Fig. 1 vs. 2). In both cases, the Duval juniper was placed in a clade with *J. pinchotii*.

In addition to Bayesian and NJ analyses, Maximum Likelihood, Parsimony, UPGMA and Minimum Linkage analyses were computed. Interestingly, Bayesian, Maximum Likelihood and Parsimony gave exactly the same clade topography for the *J. pinchotii, J. angosturana, J. coahuilensis, J. monosperma* clade (Figure 3). The UPGMA diagram was intermediate between Bayesian and NJ and barely placed the Duval juniper with *J. pinchotii* (Figure 3). The Minimum Linkage diagram was slightly different and was inconclusive in classifying the Duval juniper (Figure 3).

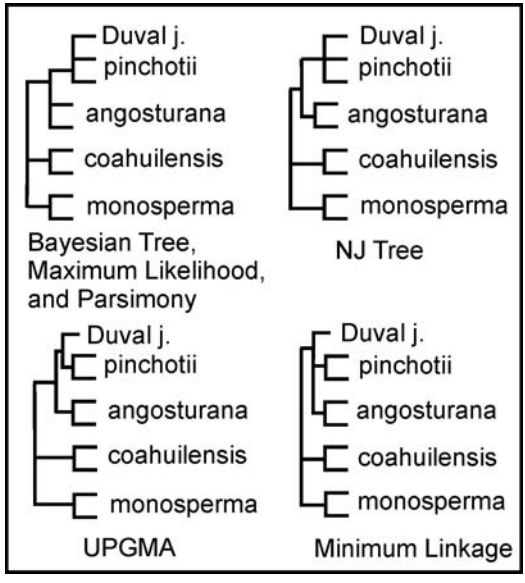
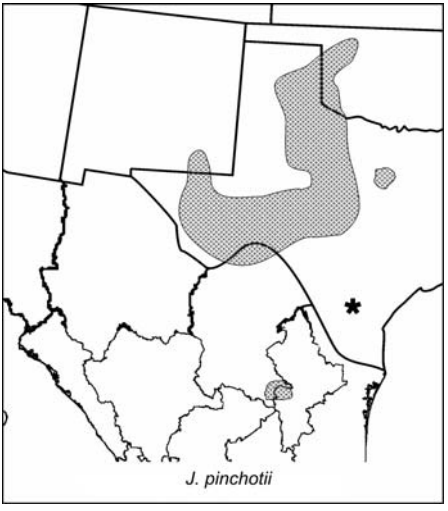


Figure 3. Comparison of six methods of data analyses.

The rest of the story - On 13 Nov. 2010, the senior author visited Cedro Hill and found approximately 15 juniper shrubs, of which one had 7 mature seed cones and long terminal whips (due to copious rainfall in the spring and summer, 2010. The seed cones were copper-red, as found only in *J. pinchotii* in the western hemisphere. The terminal whip leaves

had ruptured glands with a white exudate, typical of *J. pinchotii*. The plant was easy to identify as *J. pinchotii*. So after spending months of lab work and thousands of dollars, the mystery was easy to solve by examining the plant at the proper time. Perhaps we should continue to teach students classical taxonomy!



The disjunct Duval Co. population is shown on the distribution map for *J. pinchotii* (Fig. 4) and appears to be about equidistant from the central populations in Texas and the disjunct population in Mexico on the Coahuila - Nuevo Leon border.

Figure 4. Distribution of *J. pinchotii*. The star represents the Duval Co. population.

Figure 5. *Juniperus pinchotii* on Cedro Hill, Duval Co., Texas.

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