DISCOVERY OF A NEW POPULATION OF JUNIPERUS GRACILIOR VAR. URBANIANA FROM THE DOMINICAN REPUBLIC: ANALYSES OF LEAF TERPENOIDS AND SNPS FROM nrDNA AND trnC-trnD

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ABSTRACT

A new population of Juniperus gracilior var. urbaniana is reported from the Dominican Republic (DR). Previously, the taxon has been known from only one location on the slopes of Pic La Selle, Haiti. The new DR population is not typical of the variety and differs in a few SNPs as well as some differences in its volatile leaf oil. The new population of J. g. var. urbaniana may be of hybrid origin with J. g. var. ekmanii. Phytologia 92(3): 413-423 (December 1, 2010).

KEY WORDS: Juniperus gracilior var. urbaniana, J. g. var. gracilior, J. g. var. ekmanii, Cupressaceae, leaf terpenoids, nrDNA, trnC-D, taxonomy.

Juniperus gracilior var. urbaniana (Pilger & Ekman) R. P. Adams is known from only a single population on Pic La Selle, Haiti, where it grows as a prostrate plant in the pine forest on an unusual
white, chalky soil (Adams, 2008). Recently, shrubby junipers were discovered in the Parque Nacional Siera de Bahoruco (Baoruco), Dominican Republic (DR), near the Haitian border. Because the determination of these juniper varieties is very difficult based only on morphology, sequencing of nrDNA and trnC-trnD was performed along with analyses of the volatile leaf oils to more precisely determine the relationship of the shrubby junipers to *J. gracilior var. urbaniana* from Haiti, *J. g. var. ekmanii* (Florin) R. P. Adams, Haiti and *J. g. var. gracilior* Pilger, Dominican Republic.

**MATERIALS AND METHODS**

Specimens collected: taxon, acronym, collector number, location: *J. barbadensis* (BA), Adams 5367-5371; Petit Piton, St. Lucia, BWI; *J. bermudiana* (BM), Adams 11080-11082, Bermuda; *J. gracilior var. ekmanii* (EK), Adams 7653-7654, 3-4 km ne Mare Rouge, Pic la Selle, Haiti; *J. gracilior var. gracilior* (GR), Adams 7664-7667, w of Constanza, Dominican Republic, Adams 3097-3105, Pedernales, DR; *J. gracilior var. urbaniana* (UR) Adams 7656-7658, 4-5 km ne Mare Rouge, Pic la Selle, Haiti, Jimenez 4160(3), (= Adams 12005, 12006, 12314 at BAYLU), Parque Nacional Siera de Bahoruco (Baoruco), N 18° 14' 57", W 71° 37' 53", 2100 m; *J. lucayana*: Adams 5259-5280, Havana Botanical Garden (seed from Sierra de Nipe), Cuba; Adams 5281-5282, Havana Botanical Garden (seed from Isle de Pinos), Cuba; *J. saxicola* (SX) Adams 5284-5285, w slope of Pico Turquino, Prov. Granma/ Santiago de Cuba boundary, Cuba; *J. virginiana var. virginiana* (VG) Adams 6753-6755; on hwy. I35, Hewitt, TX; *J. virginiana var. silicicola* (SI) Adams 9186-9188, Ft. Desoto Park, Mullet Key, Florida. Herbarium vouchers are deposited at BAYLU.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit as per manufacturer's instructions.

*PCR amplification ITS* (nrDNA), *trnC-trnD* 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 (trnC-trnD) 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 MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Schwarzbach and Morris (2008) for the nrDNA and trnC-trnD primers utilized.

The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (S. San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009).

Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Compositional analyses of the volatile leaf oils of all the taxa in Hispanola are shown in Table 1. Notice that the oil of the shrub from DR is dominated by bornyl acetate as are all the taxa from DR. The oils of *J. g. var. urbaniana* appear to share only a few unique compounds: elemol, unknown 1611, β-eudesmol and α-eudesmol. The oils of *J. g. var. ekmanii* and var. *urbaniana* also share some unique compounds: δ-2-carene, isoborneol, germacrene D, piperitone and methyl eugenol. Although some of these are found in trace amounts in the *J. g. var. gracilior* plants from Pedernales (gracP in Table 2). The oils of *J. g. var. urbaniana* from DR and Haiti also have some quantitative differences: sabinene (4.5, 12.8), borneol (10.7, 1.6) and bornyl acetate (38.1, 26.2). But overall, it appears that the leaf oil of the DR shrubs are most similar to var. *urbaniana* from Haiti. The oil
from putative J. g. var. gracilior from Pedernales (near the shrubby junipers) is similar to both J. g. var. gracilior, Constanza and J. g. var. ekmanii (Table 1) in sharing several unique compounds: unknowns 900 and 907 and linalool. But the oil also has some compounds in common with J. g. var. urbaniana and J. g. var. ekmanii (but not J. g. var. gracilior): trans-sabinene hydrate, citronellol, methyl eugenol, epi-cubebol and trans-cadina-1,4-diene, suggesting the Pedernales population is intermediate between J. g. var. gracilior and J. g. var. ekmanii.

Sequencing nrDNA revealed 23 SNPs (Single Nucleotide Polymorphisms) among the Caribbean taxa. A minimum spanning network shows that the DR shrub (Ud, Fig. 1) is separated from J. g. var. urbaniana, Haiti (Uh, Fig. 1) by 2 SNP differences. Notice that J.
saxicola is linked by 2 SNPs (Fig. 1) to J. g. var. urbaniana, Haiti (Uh, Fig. 1). It is interesting that Ud is intermediate to J. ekmanii (Ek1, Ek2).

Analyses of trnC-trnD sequences revealed no differences between the DR shrub and J. g. var. gracilior, and one difference between it and J. g. var. urbaniana (Haiti) and J. g. ekmanii (Fig. 2). Integrating these data with previous data (Adams, et al. 2008) shows (Fig. 2) that the 'urbaniana' shrubs from Dominican Republic are in the group with J. g. var. gracilior, whereas J. g. var. urbaniana (Haiti) is in a group with J. g. var. ekmanii and J. saxicola. However, these groups are separated by only one SNP.

Figure 2. Minimum Spanning Network based on 6 SNPs from trnC-trnD 798 bp sequence.
CONCLUSIONS

It is noteworthy that a new population of *J. g.* var. *urbaniana* has been discovered. This new site is in the same mountain range as Pic La Selle and represents an eastern extension of the range (Fig. 3). The new population differs somewhat from the Haiti plants in that these shrubs are about 0.3 m tall x 1-2 m wide, whereas the plants in Haiti are prostrate (5-10 cm tall x 3-5 m wide). The DR shrubs appear to grow on a hard-pan type of soil. The DR plants differ somewhat in their DNA sequences and volatile leaf oils that suggests possible hybridization with *J. g.* var. *ekmanii*. Nevertheless, it is still useful for conservation purposes to have this new population, because the Haitian population is very small and certainly threatened.

Figure 3. Distribution map for *Juniperus gracilior* var. *urbaniana*.

ACKNOWLEDGMENTS

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LITERATURE CITED


Table 1. Comparisons of the volatile leaf oils of *J. gracilior* var. *urbaniana*, shrub from Dominican Republic (urbD), *J. g.* var. *urbaniana*, Haiti (urbH), *J. g.* var. *ekmanii* (ekman), *J. g.* var. *gracilior*, Constanza, DR (gracC) and *J. g.* var. *gracilior*, Pedernales, DR (gracP). Compounds in bold appear to separate the taxa. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. RI is the Kovat's Index using a linear approximation on DB-5 column.

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