

**IDENTIFICATION OF THE ELBURZ MOUNTAINS, IRAN  
JUNIPER AS *JUNIPERUS POLYCARPOS* VAR. *POLYCARPOS***

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**ABSTRACT**

The utilization of 3,714 bp from four gene regions (nrDNA, petN-psbM, trnD-trnT, trnS-trnG) was sufficient to accurately identify an unknown juniper taxon from the Elburz Mtns., Iran as *Juniperus polycarpus* var. *polycarpus*, not *J. excelsa*. The combined NJ tree (3,714 bp) showed *J. polycarpus* var. *turcomanica* to be more closely related to *J. excelsa*, than to *J. p.* var. *polycarpus*. *Phytologia* 93(3): 316-321 (December 1, 2011).

**KEY WORDS:** *Juniperus polycarpus* var. *polycarpus*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica*, *J. excelsa*, Cupressaceae, Iran, nrDNA, petN-psbM, trnD-trnT, trnS-trnG.

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Recently, Shanjani et al. (2010) reported on the composition of the leaf essential oil from a putative *Juniperus excelsa* M.-Bieb. from the Elburz Mtns., Iran. However, the oil did not seem typical of *J. excelsa* since it was high in  $\alpha$ -pinene and missing trans-cadina-1(6),4-diene, cubebol, 1-epi-cubenol, cedrol and abietadiene. In addition, the oil contained  $\gamma$ -cadinene, elemol, and germacrene B, these not reported in *J. excelsa* by Adams (2000).

The distribution of *J. excelsa* and *J. polycarpus* is not well understood. Adams (2011) noted the occurrence of *J. excelsa* in Turkey and thence eastward into Armenia (Fig. 1.). Based on the

location of the Iranian juniper in the Elburz Mtns. (S in Fig. 1), it could be *J. p. var. turcomanica*, *J. excelsa* or *J. p. var. polycarpus*.

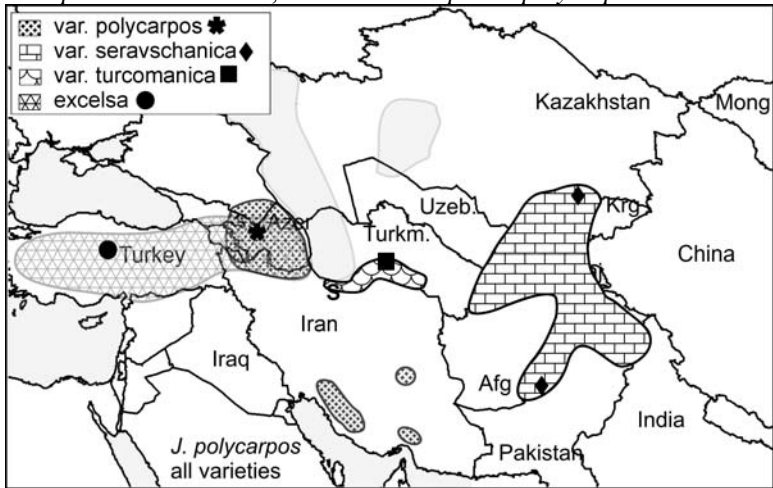


Figure 1. Distributions of *J. excelsa* (Greece not shown), *J. polycarpus* var. *polycarpus*, *J. p. var. seravschanica*, *J. p. var. turcomanica*. (adapted from Adams, 2011). Symbols indicate the populations sampled for each taxon. S is the site of the Iranian juniper sample in the Elburz Mtns., Iran.

The purpose of this study was to utilize DNA sequence data from nrDNA, petN-psbM, trnD-trnT, trnS-trnG regions to identify the Iranian juniper from the Elburz Mtns., Iran.

## MATERIALS AND METHODS

Plant material: *J. excelsa*, n of Eskisehir, Turkey, Adams 9433-9435, *J. polycarpus* var. *polycarpus*, Lake Sevan, Armenia, Adams 8761-8763, *J. p. var. seravschanica*, Quetta, Pakistan, Adams 8483-8485, Dzhabagly, Kazakhstan, Adams 8224-8226, Iranian juniper, Elburz Mtns., *Shanjani s. n.*, [=Adams 12603, 12604]. Voucher specimens are deposited at Baylor University (BAYLU).

DNA Analysis - One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at  $-20^{\circ}$  C until the DNA was extracted. DNA was extracted using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30  $\mu$ l reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15  $\mu$ l 2x buffer E (petN-psbM, trnDT, trnSG) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200  $\mu$ M each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM  $MgCl_2$  according to the buffer used) 1.8  $\mu$ M each primer. See Adams et al. (2011) for the ITS, petN-psbM, trn D-trnT and trnS-trnG 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. (South 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

The NJ tree based on nrDNA sequences shows (Fig. 2) the Iranian juniper grouping with *J. polycarpus* var. *polycarpus* from nearby Armenia. Interestingly, *J. excelsa* and *J. p.* var. *turcomanica* show no differences in their nrDNA sequences (Fig. 2).

Analysis based on petN-psbM (cp DNA) gave a different perspective (Fig. 3). The Iranian juniper is again clearly associated with *J. p.* var. *polycarpus*, Armenia, but *J. excelsa* is in a well supported distinct clade (Fig. 3). *Juniperus p.* var. *turcomanica* is loosely associated with *J. p.* var. *polycarpus*, Armenia.

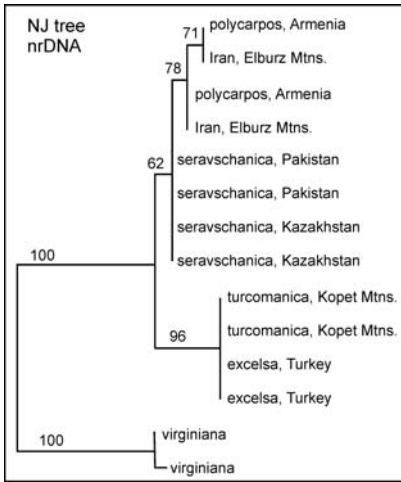


Fig. 2. NJ tree, nrDNA

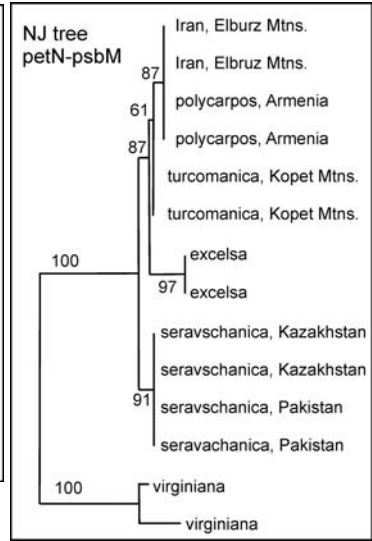


Fig. 3. NJ tree, petN-psbM.

Sequences from trnS-trnG also show the Iranian juniper in a clade with *J. p. var. polycarpus*, along with *Juniperus p. var. turcomanica* (Fig. 4). *Juniperus excelsa* is in a well-supported distinct clade.

The NJ tree based on trnD-trnT (Fig. 5) is similar to that for petN-psbM (Fig. 3) and trnS-trnG (Fig. 4) in showing the Iranian juniper in a clade with *J. p. var. polycarpus* and *J. p. var. turcomanica*. *Juniperus excelsa* is, again, in a well-supported clade (Fig. 5).

The sequences for nrDNA, petN-psbM, trnD-trnT, and trnS-trnG were concatenated to give a 3,714 bp data set. The NJ tree shows (Fig. 6) 100% support for the clade containing the Iranian juniper and *J. p. var. polycarpus*, Armenia. It is interesting to the note the support for the clade containing *J. excelsa* and *J. turcomanica* (Fig. 6). Adams et al. (2008) noted the non-concordance of morphology, terpenes, RAPDs and DNA sequence data among these taxa.

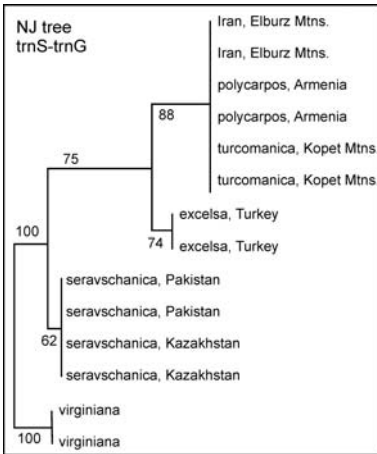


Fig. 4. NJ tree, trnS-trnG.

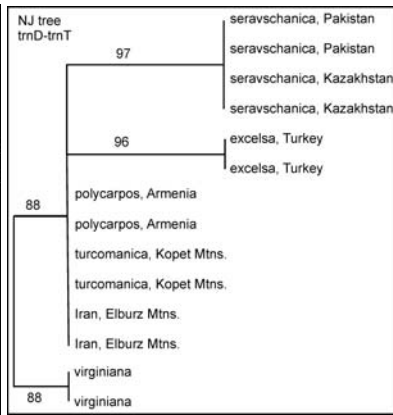
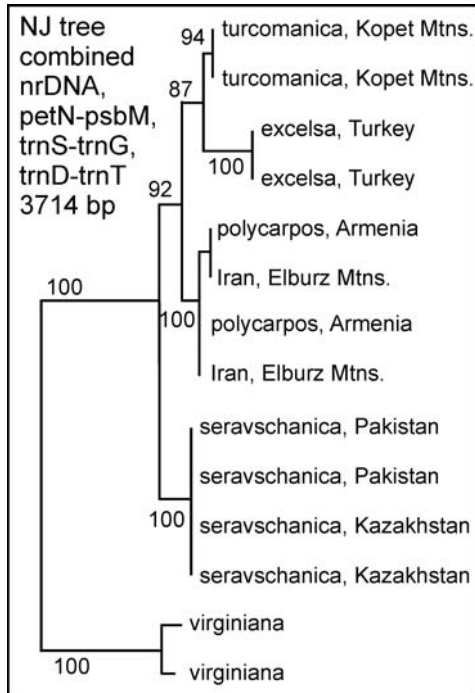


Fig. 5. NJ tree, trnD-trnT.

Figure 6. NJ tree based on 3,714 bp of sequences from nrDNA, petN-psbM, trnS-trnG, trnD-trnT, and trnS-trnG. The numbers at that branch points are bootstrap probabilities (1000 reps.).



## CONCLUSION

The Iranian juniper from the Elburz Mtns. was found to be *J. polycarpos* var. *polycarpos* not *J. excelsa*.

## ACKNOWLEDGEMENTS

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