TAXONOMIC STUDY OF *JUNIPERUS EXCELSA* AND *J. POLYCARPOS* USING SNPs FROM nrDNA AND cp trnC-trnD, PLUS ESSENTIAL OILS AND RAPD DATA.

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ABSTRACT

SNPs from nrDNA and cp trnC-trnD were analyzed for *J. excelsa*, *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica* and *J. p*. var. *turcomanica* and compared to terpene and RAPDs data. These data, taken together, support the continued recognition of *J. excelsa* and *J. polycarpos* as separate species as well as the three varieties of *J. polycarpos*: var. *polycarpos*, var. *seravschanica* and var. *turcomanica*.

KEY WORDS: *Juniperus excelsa, J. polycarpos, J. p.* var. *seravschanica*, *J. p.* var. *turcomanica*, Cupressaceae, nrDNA, trnC-trnD, SNPs, essential oils, terpenes, DNA fingerprinting, systematics.

The taxonomy of *J. polycarpos* K. Koch. from Armenia, Kazakhstan, Pakistan and Turkmenistan has been examined using leaf oil compositions and DNA fingerprinting (Adams, 1999; Adams, 2001). The compositions of the volatile leaf oils (Adams, 2001) are given in Table 1. Notice the large amounts of α-pinene in the plants from Armenia, Kazakhstan and Turkmenistan. Myrcene is a large component in the oils from Kazakhstan and Pakistan (*J. p.* var. *seravschanica*). Several compounds distinguish *J. excelsa*: decadienal

isomer (KI 1312), trans-cadina-1(6),4-diene, cubebol, 1-epi-cubenol, and KI 1666 (Table 1). Compounds that distinguish *J. polycarpos* (including *J. p.* var. *turcomanica* and *J. p.* var. *seravschanica* for this discussion) are: hexyl 3-methyl butanoate, δ-elemene, γ-cadinene, elemol, germacrene B, germacrene D-4-ol, α & β-eudesmols and KI 1688 (Table 1). Several diterpenes are unique to *J. procera* (Table 1) and show its separation from *J. excelsa* and the other junipers.

The trend in the volatile leaf oils is seen in figure 1. The leaf

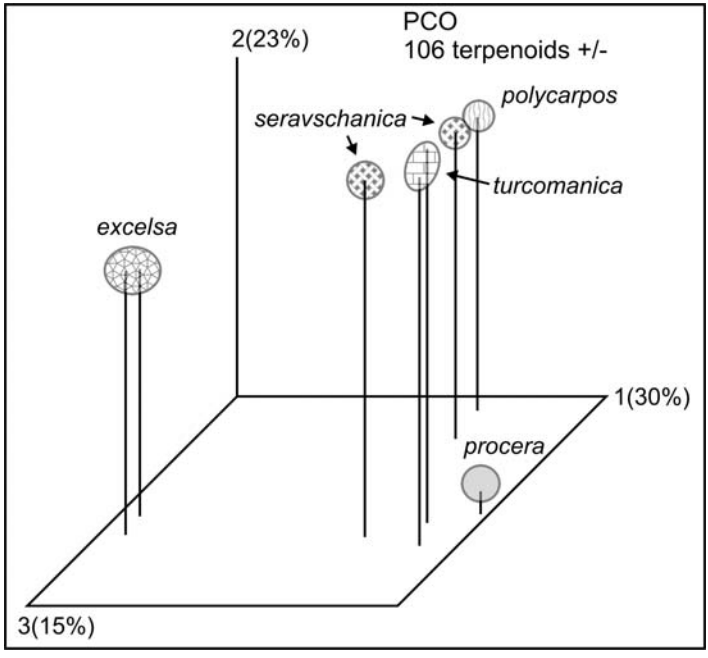

Figure 1. PCO of *J. excelsa*, *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica* and *J. procera* based on 106 leaf terpenoids scored as present (+) or absent (-). Based on Adams (2001) data.

terpenoids clearly separate *J. excelsa* and *J. procera* from *J. polycarpos*. The varieties of *J. polycarpos* are not well resolved. This is seen in the raw data in table 1.

PCO analysis based on RAPDs (Adams, 2001) shows (Fig. 2) a very similar pattern to that seen with terpenoids. Notice that again, *J. excelsa* and *J. procera* are very well resolved from *J. polycarpos* (Fig. 2). There appears to be more slightly more separation of the *J. polycarpos* varieties in the RAPDs data (Fig. 2) than in the terpenoids (Fig. 1) but the overall trend is very similar.

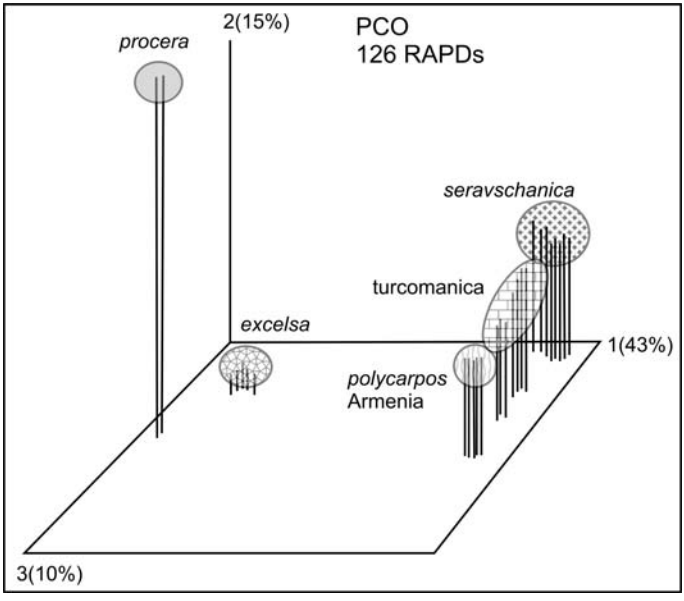

Figure 2. PCO based on 126 RAPDs. Notice that 43% of the variance separated *J. excelsa*/J. *procera* from *J. polycarpos*/*turcomanica*/*seravschanica* (axis 1) and 15% of the variance separates *J. procera* from all other taxa on axis 2. Based on Adams (2001) data.

The pattern is simplified by removing *J. procera* from the RAPDs data, as seen in figure 3. *Juniperus excelsa* is still well

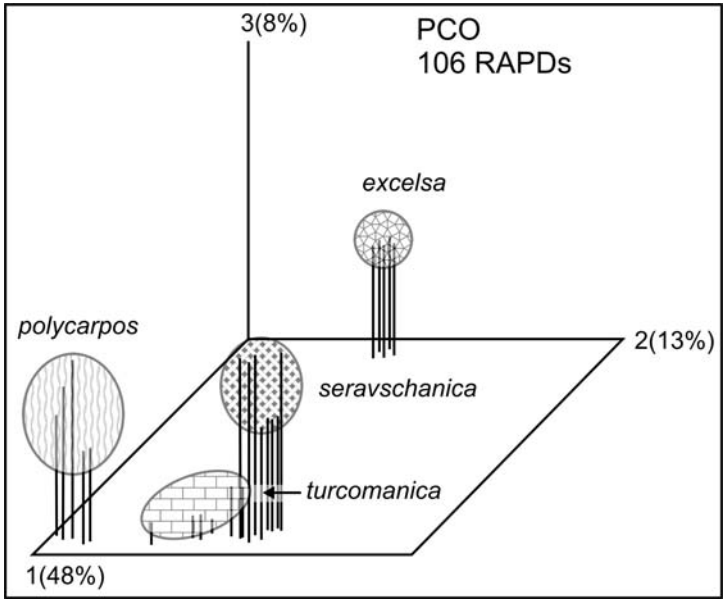

Figure 3. PCO of J. excelsa, *J. polycarpos*, *J. p*. var. *seravschanica* and *J. turcomanica* using 106 RAPDs. Based on Adams (2001) data.

resolved. However, there is now some separation between *J. polycarpos*, *J. p*. var. *seravschanica* and *J. turcomanica* (Fig. 3).

Removing *J. excelsa* from the data set and re-analyzing the RAPDs data gave a clearer picture of the pattern among the *J. polycarpos* varieties (or populations). This resulted in four groups, each well resolved. The *J. p*. var. *seravschanica* populations are resolved into the Pakistan and Kazakhstan sites.

Should these entities be recognized as varieties of *J. polycarpos* or do they merely represent geographical interspecific variation?

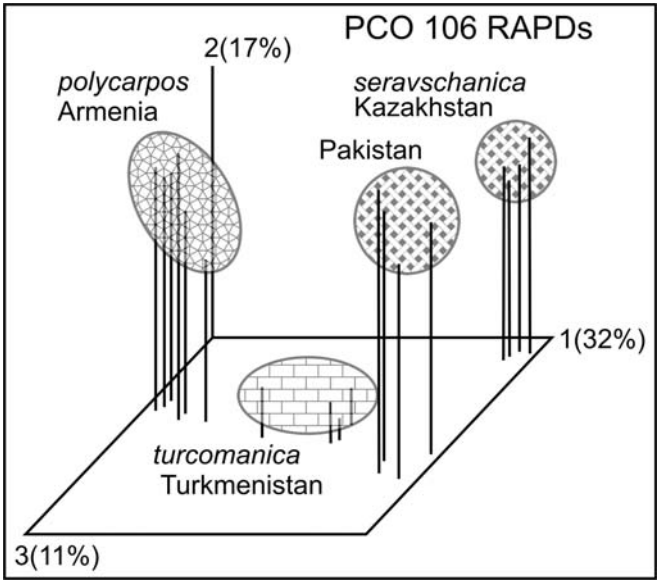

Figure 4. PCO of four taxa of *J. polycarpos* based on RAPDs. Based on Adams' (2001) data.

Farjon (2005, p. 291) treated *J. polycarpos* as a variety of *J. excelsa* (*J. excelsa* var. *polycarpos* (K. Koch) Takht.) and treated *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as synonyms of *J. excelsa* var. *polycarpos*. Clearly, neither the terpenoids nor RAPDs support Farjon's merging of *J. polycarpos* and *J. excelsa*.

Farjon (2005, p. 343) states his philosophy as "I consider species based on the chemistry of terpenes and/or RAPD analysis as based on inconclusive evidence" although he allows that "DNA sequence data certainly can (which is the main reason for their 'superiority')" (Farjon, 2005, p. 232).

The goal of sequencing a single gene is now easily accomplished but these data are proving to be more difficult to interpret than perhaps imagined. For example, Syring et al. (2007) examined *Pinus* species utilizing sequences from three nuclear genes (AGP6, cesA1, LEA-like). They found that none of the three genes,

analyzed separately, placed the multiple accessions of *P. strobus*, *P. monticola* and *P. chiapensis* into monophyletic clades. We suggest that additional data from multiple genome sites such as RAPDs as well as phenotypic data such as leaf terpenoids can help to complement single gene sequences. In cases where sequence data alone results in multiple alternative scenarios, those additional data might be able to help discriminate between them.

Recent DNA sequence phylogenetic research of *Juniperus* (Schwarzbach et al., in prep.) has shown (Fig. 5) that *J. polycarpos* (Armenia, cf. *J. p.* var. *polycarpos*, above) is 100% supported as being separate from *J. procera* and *J. excelsa* and not forming a monophyletic group. The thesis of Farjon (2005) that *J. polycarpos* is a variety of *J. excelsa* is not supported by these data. In fact, the sequencing data is in full agreement with both the terpenoid and RAPD

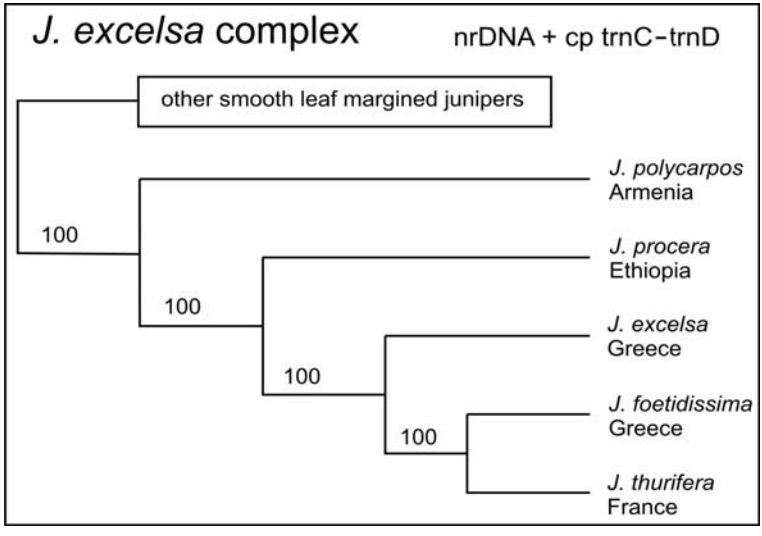

Figure 5. Bayesian tree based on nrDNA and cp trnC-trnD sequences. J*uniperus polycarpos* is resolved from *J. procera*, *J. excelsa*, *J. foetidissima* and *J. thurifera*. Numbers above the branch points are posterior probabilities on a percent basis.

data (above) that show *J. excelsa*, *J. polycarpos*, and *J. procera* being well differentiated. In fairness to Farjon (2005), it should be conceded that if only morphological data are utilized, then one could readily make a case for the merging of *J. excelsa* and *J. polycarpos* (and perhaps *J. procera*). However, morphology can be misleading when used solely for species circumscriptions, as has been shown in several other studies. Cryptic speciation has been found in *J. deltoides* R. P. Adams (Adams et al. 2005) and *J. maritima* R. P. Adams (Adams 2007), to name but two cryptic juniper species.

Schwarzbach et al. (in prep) have analyzed one accession per species for *J. excelsa*, *J. polycarpos*, and *J. procera*. As a result, the relationships of the species were established in a basic framework. However, the sampling in this previous study did not allow any assessments of intraspecific variation or the monophyly of the taxa involved. The purpose of the present paper is to re-examine the taxonomy of *J. excelsa*, *J. polycarpos* and its varieties using SNPs from sequence data (nrDNA and cpDNA trnC-trnD) by adding multiple accessions for each taxon.

MATERIALS AND METHODS

Specimens used in this study: *J. excelsa*, Adams , 8785-8786 - 7 km w of Lemos, Greece; Adams 9433-9435, 40 km n of Eskisehir, Turkey; *J. p.* var. *seravschanica*, Adams 8224-8226, 2 km s Dzhabagly, Kazakhstan (not Kyrgystan as previously reported, Adams, 1999); *J. p.* var. *seravschanica*, Adams 8483-8486, Quetta, Balochistan, Pakistan; *J. turcomanica*, Adams 8757-8760, Kopet Mts., Turkmenistan. Voucher specimens for all collections 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^{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.

SNPs obtained from DNA sequencing

ITS (nrDNA) and 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 or K (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. All 12 (A-L) of the Epi-Centre's buffers were screened and buffer K gave the cleanest, most-abundant amplification for both ITSA/ITSB and buffer E was best for trnC-trnD (CD10F/CD3R) primers. However, buffers D, F, G, H, and J were nearly as good as buffer E or K.

Primers (5'-3'): for nrDNA: ITSA and ITSB primers from Blattner (1999), for trnC-trnD: CD10F and CD3R, see Adams et al. (2008). The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. The internal trnC-trnD primers, CD10F-CD3R produced a band of approx. 850 bp. 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. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS region) resulted in 1210 bp of data. Aligning sequences for *J. excelsa* (5 individuals, Greece, Turkey), *J. polycarpos* var. *polycarpos* (4 individuals, Armenia); *J. p*. var. *seravschanica* (4 individuals, Kazakhstan, 4 individuals, Pakistan) and *J. p*. var. *turcomanica* (4 individuals, Turkmenistan) revealed 14 SNPs among these individuals. PCO of these individuals gave three significant eigenroots accounting for 74.4%, 9.1% and 6.7% of the variance among individuals. PCO ordination shows (Fig. 6) two major groups to be present: *J. excelsa* - *J. p*. var. *turcomanica* and *J. p*. var. *polycarpos* and var. *seravschanica*.

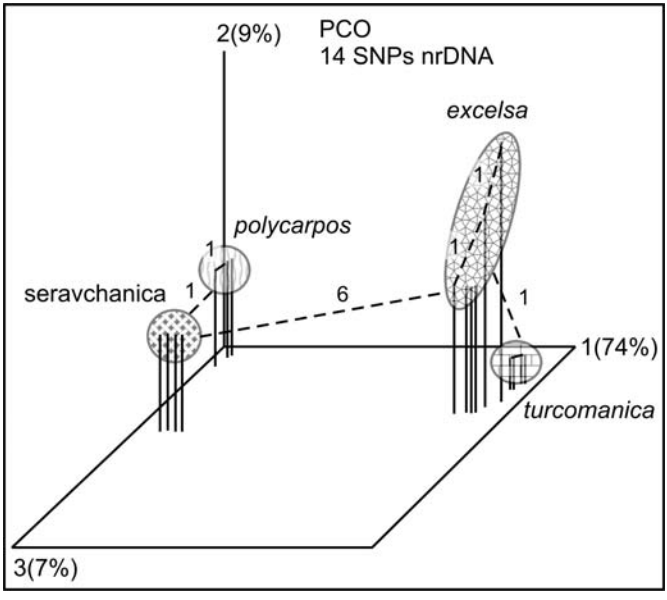

Figure 6. PCO based on 14 nrDNA SNPs. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Equally spaced lines denote identical DNA sequences.

Sequencing the partial trnC-trnD sequence resulted in 877 bp of data when utilizing the same genomic DNA as above. Aligning these 21 sequences revealed 14 SNPs. Factoring the association matrix resulted in 3 eigenroots that accounted for 49.1%, 43.9% and 3.3% of the variance among these individuals in their partial trnC-trnD SNPs. Ordination of these individuals reveals (Fig. 7) three groups: *J. excelsa*; *J. polycarpos* - *J. p*. var. *turcomanica*; and *J. seravschanica*. These groups are separated by 7 and 8 SNPs. Notice (Fig. 7) that there was no variation among individuals of any taxon except *J. excelsa*.

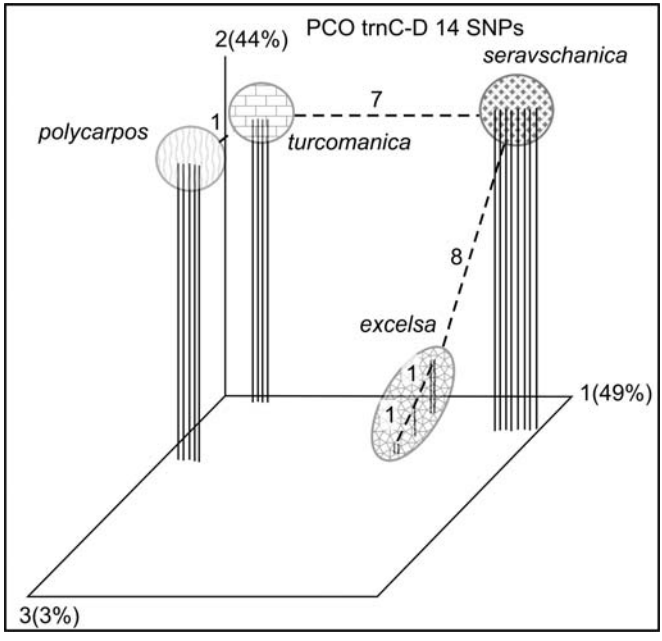

Figure 7. PCO ordination based on 14 SNPs from trnC-trnD sequences. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Equally spaced lines denote identical DNA sequences.

Combining the 14 nrDNA SNPs and 14 trnC-trnD SNPs for a PCO analysis resulted in 3 eigenroots of 52%, 30% and 12%. Ordination (Fig. 8) shows 4 well-defined groups, each separated by 7 to 9 SNPs. It is interesting that the 8 samples of *J. p*. var.

seravschanica (4 from Kazakhstan and 4 from Pakistan) had identical sequences for both nrDNA (1210 bp) and cp trnC-trnD (877 bp). *Juniperus p.* var. *polycarpos* and *J. p*. var. *turcomanica* both had a single SNP within their samples. In contrast, *J. excelsa* had 2 SNPs within its samples.

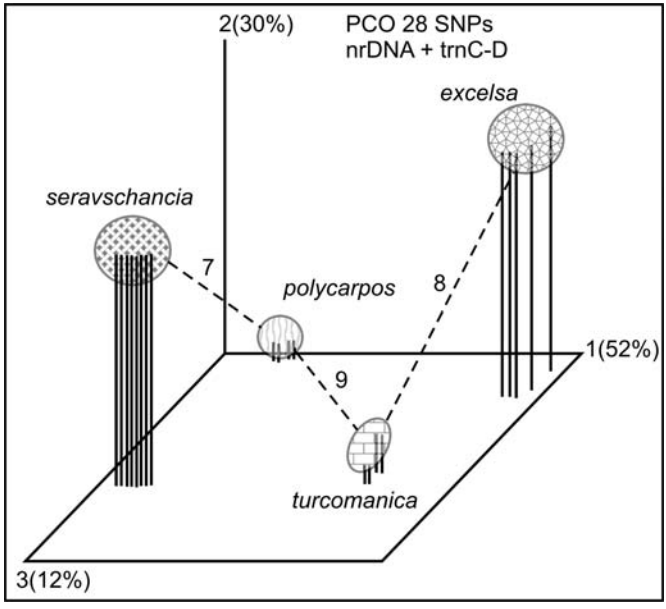

Figure 8. PCO using SNPs from both nrDNA and trnC-trnD. Note the four well defined groups. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Equally spaced lines denote identical DNA sequences.

Graphic summaries of morphology, terpenes (+/- basis), RAPDs, nrDNA, trnC-trnD and combined nrDNA + trnC-trnD reveal (Fig. 9) general agreement between morphology, terpenes, RAPDs and combined nrDNA + trnC-trnD classifications. The combined nrDNA + trnC-trnD SNPs showed the largest differences between *J. polycarpos* var. *polycarpos*, *J. p*. var. *seravschanica* and *J. p*. var. *turcomanica* of any data set. Using only nrDNA or trnC-trnD SNPs would lead to very

different taxonomies in this study. The concordance of terpenes, RAPDs and combined nrDNA + trnC-trnD classifications seems to

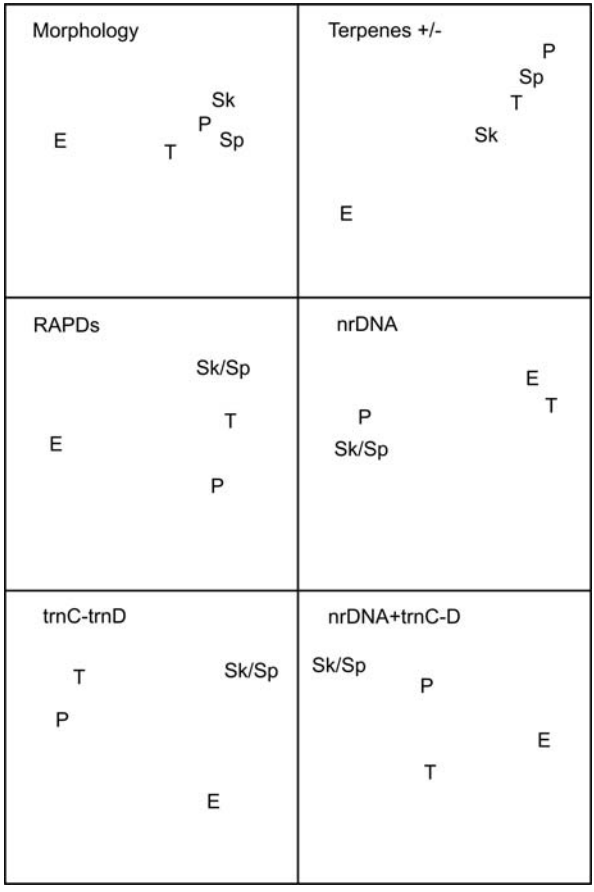

Figure 9. Graphic summaries of morphology, terpenes, RAPDs, nrDNA, trnC-trnD, and combined nrDNA + trnC-trnD data. E = *J. excelsa*, P = *J. p*. var. *polycarpos*, T = *J. p*. var. *turcomanica*, Sk = *J. p*. var. *seravschanica*, Kazakhstan, Sp = *J. p*. var. *seravschanica*, Pakistan.

provide the strongest evidence that *J. polycarpos* is composed of three genetically distinct (but scarcely distinct in morphology) taxa, supporting the continued recognition of these taxa at the variety level.

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LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 1999. Systematics of multi-seeded eastern hemisphere Juniperus based on leaf essential oils and RAPD DNA fingerprinting. Biochem. Syst. Ecol. 27: 709-725.

Adams, R. P. 2001. Geographic variation in leaf essential oils and RAPDs of *Juniperus polycarpos* K. Koch in central Asia. Biochem. Syst. Ecol. 29: 609 - 619.

Adams, R. P. 2004. Junipers of the World: The genus *Juniperus*. Trafford Publ., Vancouver, B. C.

Adams, R. P. 2007. *Juniperus maritima*, the seaside juniper, a new species from Puget Sound, North America. Phytologia 89(3): 263-283.

Adams, R. P., J. A. Morris, R. N. Pandey and A. E. Schwarzbach. 2005. Cryptic speciation between *Juniperus deltoides* and *J. oxycedrus* (Cupressaceae) in the Mediterranean. Biochem. Syst. Ecol. 33: 771-787.

Adams, R., P., A. E. Schwarzbach and J. A. Morris. 2008. The evolution of Caribbean *Juniperus* (Cupressaceae): Terpenoids, RAPDs, and DNA SNPs data Phytologia 90(1): 103-119.

Farjon, A. 1992. The taxonomy of the multiseed junipers (*Juniperus* sect *Sabina*) in southwest Asia and east Africa. Edinb. J. Bot. 49: 251-283.

Farjon, A. 2005. A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew. Richmond, Surrey, UK.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Syring, J., R. F. del Castillo, R. Cronn and A. Liston. 2007. Multiple nuclear loci reveal the distinctiveness of the threatened, neotropical *Pinus chiapensis*. Syst. Bot. 32: 703-717.

Table 1. Comparisons of the percent total oil for leaf essential oils for *J. excelsa* - Greece (EG), *J. excelsa*, Tbilisi Botanic Garden (ET), *J. polycarpos* var. *polycarpos*: Armenia, L. Sevan (AS); *J. p*. var. *turcomanica*, Turkmenistan, Kopet Mts. (TK), Alma Ata Botanic Garden (ex. Ashgabad, Turkmenistan, TA); *J. p*. var. *seravschanica*: Kazakhstan, Talasskiy Mtns. (KT), Pakistan, Quetta (PQ), and *J. procera*, east Africa (PR). Components that tend to separate the species are highlighted in boldface. From Adams (2001).

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
926	tricyclene	0.1	0.1	0.1	0.1	0.2	0.3	0.1	t
931	**α-thujene**	**-**	**-**	**t**	**t**	**t**	**0.6**	**0.4**	**t**
939	**α-pinene**	**22.5**	**26.5**	**68.4**	**68.8**	**59.7**	**44.4**	**15.5**	**12.5**
953	α-fenchene	0.2	t	t	t	t	-	0.2	0.1
953	camphene	0.5	1.0	0.2	0.1	0.5	0.5	0.2	0.1
957	thuja-2,4(10)-diene	0.1	-	t	t	t	-	-	-
975	verbenene	t	-	-	-	-	-	-	-
976	sabinene	t	0.1	0.2	0.1	0.4	0.9	0.5	t
978	1-octen-3-ol	-	-	-	-	-	-	-	0.3
980	β-pinene	0.6	1.0	0.5	0.6	1.8	2.2	1.2	1.2
991	**myrcene**	**1.9**	**2.2**	**1.2**	**1.5**	**3.7**	**19.2**	**20.7**	**1.2**
1005	α-phellandrene	0.1	0.1	-	-	t	0.1	0.1	-
1011	δ-3-carene	2.3	0.4	t	t	t	-	3.5	6.1
1018	α-terpinene	0.1	0.1	t	t	t	0.1	0.1	t
1026	p-cymene	0.4	0.2	0.1	0.1	0.2	0.1	0.7	t
1028	sylvestrene	-	-	-	-	-	-	-	0.1
1031	**limonene**	**22.6**	**5.5**	**1.2**	**1.5**	**1.8**	**4.4**	**9.0**	**0.2**
1031	β-phellandrene	t	-	-	-	0.1	0.5	1.0	0.8
1032	1,8-cineole	-	t	-	-	-	-	-	t
1050	(E)-β-ocimene	t	-	t	t	t	t	0.2	t
1062	γ-terpinene	0.6	0.9	0.2	0.3	0.6	1.4	1.3	t
1068	cis-sabinene hydrate	-	-	-	-	-	0.1	0.2	-
1068	fenchone	-	-	t	t	t	t	t	-
1088	terpinolene	0.9	1.1	0.4	0.5	1.3	1.5	1.7	1.1
1097	trans-sabinene hydrate	-	-		-	t	-	-	-
1098	linalool	-	0.1	0.1	t	t	0.5	0.7	0.5
1103	isopentyl-isovalerate	-	-	t	t	t	-	-	-
1110	1,3,8-p-menthatriene	-	-	-	-	-	-	-	t
1112	endo-fenchol	0.2	-	-	-	t	-	-	-
1114	trans-thujone	-	-	t	t	0.2	0.1	-	-
1121	cis-p-menth-2-en-1-ol	0.1	-	-	-	-	t	-	t
1125	chrysanthenone	-	-	t	t	t	-	-	-
1125	α-campholenal	0.1	0.2	0.2	0.1	0.2	t	0.1	t

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
1134	cis-limonene oxide	-	-	-	-	-	-	-	0.1
1139	trans-pinocarveol	0.2	0.3	0.1	0.1	t	-	t	t
1143	camphor	0.5	0.2	t	0.3	1.7	t	t	t
1143	cis-sabinol*	-	-	0.4	0.3	-	-	0.2	0.2
1143	trans-verbenol	-	0.5	-	-	t	t	-	t
1159	p-mentha-1,5-dien-8-ol	-	-	-	-	-	-	-	0.1
1160	trans-pinocamphone	-	-	t	-	-	-	-	-
1163	pinocarvone	-	0.1	t	-	-	-	-	-
1165	borneol	-	t	-	t	t	t	t	0.2
1167	δ-terpineol	-	-	t	t	-	t	t	t
1173	cis-pinocamphone	-	-	t	-	-	-	-	-
1177	terpinen-4-ol	0.2	0.2	0.1	t	0.2	0.4	0.3	0.1
1178	naphthalene	t	t	0.1	0.4	t	t	t	-
1180	m-cymen-8-ol	-	-	-	-	-	-	-	0.1
1183	p-cymen-8-ol	-	-	t	-	-	-	-	t
1185	trans-p-mentha-1(7), 8-dien-2-ol	0.1	-	-	-	-	-	-	-
1189	α-terpineol	t	0.1	t	t	0.2	0.1	t	0.5
1191	hexyl butyrate	-	-	-	-	0.1	-	-	-
1204	verbenone	0.1	0.1	0.1	0.1	t	-	-	-
1217	trans-carveol	0.1	0.1	t	t	-	-	-	-
1220	endo-fenchyl acetate	0.3	0.1	-	-	-	-	-	-
1242	**hexyl 3-methyl butanoate**	**-**	**-**	**0.1**	**0.2**	**0.4**	**-**	**t**	**-**
1257	4Z-decen-1-ol	-	-	-	-	0.2	-	-	-
1274	unknown,79,91,105, 147,FW162	-	-	-	-	-	-	-	0.3
1285	bornyl acetate	0.4	0.9	0.2	0.2	0.7	1.0	0.6	0.4
1286	linalool oxide acetate (pyranoid)	0.2	0.1	-	-	-	-	-	-
1290	trans-sabinyl acetate	-	-	-	-	-	-	0.1	-
1312	**decadienal isomer?**	**3.3**	**5.6**	**-**	**-**	**-**	**-**	**-**	**-**
1319	2E,4E-decadienal	-	-	-	-	t	t	t	-
1339	**δ-elemene**	**-**	**-**	**t**	**0.1**	**t**	**t**	**t**	**-**
1376	α-copaene	-	0.2	-	t	-	-	-	-
1383	β-bourbonene	0.1	-	-	-	-	-	-	-
1381	hexyl n-hexanoate	-	-	-	0.1	0.7	-	-	-
1389	β-cubebene	0.1	0.1	-	0.1	-	-	-	-
1409	α-cedrene	-	t	-	-	-	-	-	-
1409	1,7-di-epi-β-cedrene	1.6	0.7	1.3	-	-	0.2	1.4	-
1418	(E)-caryophyllene	-	0.1	0.3	0.4	-	0.1	0.2	0.5

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
1418	β-cedrene	0.9	0.5	-	-	-	0.1	0.2	-
1429	cis-thujopsene	0.3	0.2	0.2	-	-	0.2	0.4	-
1446	cis-muurola-3,5-diene	0.2	0.6	-	0.2	-	t	-	-
1454	α-humulene	0.2	0.2	-	0.1	-	-	-	0.7
1458	E-β-farnesene	0.2	0.1	0.1	-	-	-	0.1	-
1461	cis-muurola-4(14),5-diene	-	-	0.1	0.2	-	-	0.1	-
1466	β-acoradiene	0.1	t	0.1	-	-	-	t	-
1473	**trans-cadina-1(6),4-diene**	**0.4**	**0.8**	**-**	**-**	**-**	**-**	**-**	**-**
1477	γ-muurolene	-	t	-	0.2	0.1	t	-	-
1480	germacrene D	0.9	1.7	0.2	0.8	0.8	0.1	0.2	0.3
1491	trans-murrola-4(14),5-diene	0.4	1.4	-	0.1	0.1	t	-	-
1493	epi-cubebol	-	1.3	-	t	0.2	t	t	-
1499	γ-amorphene	-	-	-	-	-	-	t	-
1499	α-muurolene	0.2	0.1	-	0.3	0.2	0.2	0.2	-
1499	bicyclogermacrene	-	-	t	-	-	-	t	-
1502	cuparene	-	-	-	-	-	-	t	-
1503	germacrene A	-	-	-	0.1	0.1	-	-	-
1509	β-bisabolene	-	-	t	-	-	-	t	-
1512	α-alaskene	0.3	t	0.1	-	-	-	0.4	-
1513	**γ-cadinene**	**-**	**-**	**0.2**	**1.1**	**0.9**	**0.4**	**0.4**	**-**
1513	**cubebol**	**0.8**	**2.6**	**-**	**-**	**-**	**-**	**-**	**-**
1521	cis-calamenene	t	-	-	-	-	-	-	-
1524	δ-cadinene	0.7	1.5	0.3	1.4	1.2	1.0	0.7	-
1532	E-γ-bisabolene	0.2	-	0.1	-	-	-	-	-
1532	trans-cadina-1(2),4-diene	t	0.2	-	t	-	t	-	-
1538	α-cadinene	-	-	-	0.2	0.2	0.1	0.1	-
1549	**elemol**	**-**	**-**	**t**	**0.2**	**1.9**	**0.5**	**0.5**	**4.3**
1556	**germacrene B**	**-**	**-**	**0.7**	**1.6**	**1.8**	**0.4**	**0.6**	**-**
1574	**germacrene D-4-ol**	**-**	**t**	**0.4**	**3.0**	**1.5**	**0.5**	**2.0**	**0.1**
1581	caryophyllene oxide	-	-	-	-	-	-	-	0.5
1587	sesquiterpene, FW220?	1.9	1.7	0.8	-	-	1.0	1.7	-
1596	**cedrol**	**28.1**	**30.8**	**19.0**	**t**	**-**	**14.6**	**26.4**	**-**
1606	humulene epoxide II	t	-	-	-	-	-	-	0.5
1606	β-oplopenone	t	-	t	0.2	0.2	-	-	-
1607	4E-tridec-6-yne*	-	0.6	-	-	-	-	-	-
1627	**1-epi-cubenol**	**1.6**	**2.2**	**t**	**-**	**-**	**-**	**-**	**-**
1630	γ-eudesmol	-	-	-	t	0.5	-	-	1.4

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
1640	epi-α-cadinol	t	0.3	0.1	0.7	0.9	0.5	0.4	-
1640	epi-α-muurolol	t	0.5	t	0.7	0.9	0.1	0.1	-
1645	α-muurolol	t	0.2	-	0.2	0.4	t	t	-
1649	**β-eudesmol**	**-**	**-**	**t**	**t**	**0.8**	**t**	**0.1**	**2.3**
1652	**α-eudesmol**	**-**	**-**	**t**	**t**	**0.5**	**0.1**	**t**	**3.8**
1653	α-cadinol	t	0.2	0.2	1.6	3.2	0.9	0.8	-
1666	bunesol	-	-	t	t	0.4	-	0.1	1.3
1666	**unknown(57,41,85, 79,136)**	**0.6**	**2.4**	**-**	**-**	**-**	**-**	**-**	**-**
1685	eudesma-4(15),7-dien-1-β-ol	-	-	-	-	-	-	-	0.2
1688	**sesquiterpene alcohol, FW 222**	**-**	**-**	**0.3**	**0.9**	**3.6**	**0.4**	**-**	**-**
1688	cadinol isomer	-	-	-	t	1.2	-	-	-
1789	8-α-acetoxyelemol	-	-	-	-	-	-	-	3.5
1809	unknown(43,79,71,99, 136,252)	-	0.6	-	-	t	-	-	-
1930	rosa-5,15-diene	-	-	-	-	-	-	-	0.4
1961	sandaracopimara-8(14),15-diene	t	-	-	-	-	-	-	0.3
1989	manoyl oxide	t	0.2	0.1	0.1	-	-	0.3	0.5
2054	abietatriene	t	0.4	t	t	0.3	-	t	1.3
2080	**abietadiene**	**0.3**	**2.2**	**0.3**	**0.7**	**-**	**-**	**0.2**	**15.4**
2103	diterpene, 41,79,191, 257,FW286?	-	-	-	-	-	-	-	2.6
2147	abieta-8(14),13(15)-diene*	-	-	-	-	-	-	-	0.3
2181	diterpene,41,91, 271, 257,FW286	-	-	-	-	-	-	-	0.8
2278	sempervirol	-	-	-	-	-	-	-	0.6
2288	4-epi-abietal	0.1	1.2	0.6	1.1	1.7	-	1.0	1.8
2293	diterpene,41,55,255, 269,FW284?	-	-	-	-	-	-	-	1.0
2302	abieta-7,13-dien-3-one	-	0.1	-	-	0.2	-	-	-
2302	**trans-totarol**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**21.4**
2325	trans-ferruginol	-	0.3	-	-	-	-	-	3.4

KI = Kovat's Index on DB-5(=SE54) column. *Tentatively identified.
Compositional values less than 0.1% are denoted as traces (t).
Unidentified components less than 0.5% are not reported.