Two new cases of chloroplast capture in incongruent topologies in the *Juniperus excelsa* complex: *J. excelsa* var. *turcomanica* comb. nov. and *J. excelsa* var. *seravschanica* comb. nov.

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ABSTRACT

Juniperus excelsa, J. polycarpos, J. turcomanica, J. seravschanica and *J. procera* were analyzed for incongruent topologies between their nrDNA and cp DNA (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF). Incongruent topologies suggest that there are two cases of chloroplast capture in the *J. excelsa* complex: *J. p*. var. *turcomanica* appears to have recently captured its chloroplast from *J. polycarpos* or an ancestor; and *J. seravschanica* seems to posess an anciently captured chloroplast from an ancestor of *J. foetidissima*/ *J. thurifera*. Thus, *J. p*. var. *turcomanica* and *J. seravschanica* seem to defy an uncluttered taxonomic classification. Two new varieties are recognized: *J. excelsa* var. *turcomanica* (B. Fedtsch.) R. P. Adams, **comb. nov.** and *J. excelsa* var. *seravschanica* (Kom.) R. P. Adams, **comb. nov.** This constitutes *J. excelsa* M.-Bieb. with four varieties: var. *excelsa*, var. *polycarpos* (K. Koch) Silba, var. *turcomanica* (B. Fedtsch.) R. P. Adams and var. *seravschanica* (Kom.) R. P. Adams. Due to incongruent topologies, *J. excelsa* is presently a polyphyletic species. Published on-line **www.phytologia.org** *Phytologia 98(3):219-231 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Juniperus excelsa, J. polycarpos* var. *polycarpos, J. polycarpos* var. *turcomanica, J. seravschanica, J. procera,* chloroplast capture, incongruent topologies, DNA sequencing, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF.

Recently, Adams, Schwarzbach and Tashev (2016) reported a case of putative chloroplast capture by plants of *J. sabina* in Bulgaria and northern Greece. The Balkan plants had nrDNA exactly the same as other *J. sabina* plants in other regions, but their cp DNA differed by only 6 MEs(SNPs + indels) from that of *J. thurifera*, but 36 MEs from typical *J. sabina* cp. To call attention to these unusual individuals, with a geographic range, the authors recognized the plants as *J. sabina* var. *balkanensis* R. P. Adams and A. Tashev. By naming a new variety, the authors hope this will initiate additional research on this unusual situation.

The idea of chloroplast capture is not new, even two decades ago, Rieseberg and Soltis (1991) warned of chloroplast capture (both recent or ancient via hybridization) that provides incongruent topologies in phylogenetic trees between nuclear and cp data. They found evidence of chloroplast capture in 37 cases and, of those, 28 were thought to be conclusive (Table 1, Rieseberg and Soltis, 1991). With the explosion of the use of nrDNA and cp markers, there are hundreds of examples of chloroplast capture today. A few examples of putative chloroplast capture include *Heuchera* (Soltis and Kuzoff, 1995), *Brassica napus - B. rapa* (Haider et al. 2009), and *Osmorhiza* (Yi et al. 2015).

There are fewer examples of chloroplast capture in conifers. In *Pinus* and other conifers, Hipkins et al. (1994) concluded that "past hybridization and associated 'chloroplast capture' can confuse the phylogenies of conifers." Bouille et al. (2011) found significant topological differences in phylogenetic trees based on cpDNA (vs. mtDNA sequences) in *Picea* that suggested organelle capture.

In *Juniperus*, Terry et al. (2000) suggested that chloroplast capture was involved in the distribution of cp haplotypes in *J. osteosperma* in western North America. More recently, Adams (2015a, b) found widespread hybridization and introgression between *J. maritima* and *J. scopulorum* in the Pacific northwest, with introgression from *J. maritima* into *J. scopulorum* eastward into Montana. The

disparity between cpDNA and nuclear markers (nrDNA and maldehy) suggested that cp capture had occurred. Incongruent topologies between nrDNA and cpDNA has been found for *J. horizontalis* and *J. virginiana* var. *silicicola*, that have the common cpDNA found in the Caribbean junipers, but have nrDNA like *J. blancoi* and *J. virginiana*, respectively (Adams 2014, Adams, Schwarzbach and Morris 2008).

Although chloroplast capture, on its face, seems unlikely, Tsitrone et al. (2003) proposed a model of chloroplast capture that provides some basis for the concept.

Farjon (1992), in a seminal paper on the taxonomy of multiseed junipers of Southwest Asia and east Africa examined hundreds of specimens of *Juniperus excelsa, J. polycarpos, J. turcomanica, J. seravschanica* and *J. procera.* Farjon (1992, 2005, 2010) treated *J. polycarpos, J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpos.* Thus, he recognized only *J. excelsa* and *J. excelsa* subsp. *polycarpos*. His taxonomic treatment appears to be in common usage in the mid-East at this time. Farjon (1992) noted that branch morphology of *J. excelsa* and *J. e.* subsp. *polycarpos* are, in part, clinal and the taxa overlap in areas. Farjon (1992) keyed these taxa as:

Ultimate branchlets slender to very fine, 0.7-1 mm diam., often disposed in dorsiventral sprays; scale leaves very small, on ultimate branchlets 0.6-1.1 mm long, appressed; female cones 6-11 mm diam.....*J. excelsa* subsp. *excelsa*.

Ultimate branchlets coarser, thicker, 1-1.3 mm diam., more irregularly disposed; scale leaves larger, on ultimate branchlets 1.2-1.6 mm long, appressed or sometimes free at apex; female cones 6-14 mm diam.....*J. excelsa* subsp. *polycarpos* (including *J. turcomanica* and *J. seravschanica*).

However, Adams et al. (2008), Adams and Schwarzbach (2012), Adams et al. (2014, 2016a), Adams and Hojjati (2012, 2014) and Adams (2013, 2014), utilizing DNA sequence data, recognized *J. excelsa*, in addition to *J. polycarpos, J. p.* var. *turcomanica* and *J. seravschanica.* They found *J. seravschanica* to be in a separate clade with *J. foetidissima* and *J. thurifera* (Fig. 1), whereas, *J. excelsa, J. polycarpos,* and *J. p.* var. *turcomanica* were in a clade with *J. procera* (Fig. 1). Based largely on these DNA differences, they concluded *J. seravschanica* was not con-specific with *J. excelsa* subsp. *polycarpos*, but is a distinct species. They also found that *J. e.* subsp. *turcomanica* was more allied with *J. polycarpos* (i.e., as *J. p*. var. *turcomanica*).

Recently, I have been examining specimens of *J. excelsa* and *J. polycarpos* from Asia Minor. The DNA classification of these taxa (Table 1) appears to bear little correlation with the branchlet sizes, so critical for their identification. This was especially noticeable in specimens from eastern Turkey and Azerbaijan. These specimens often had

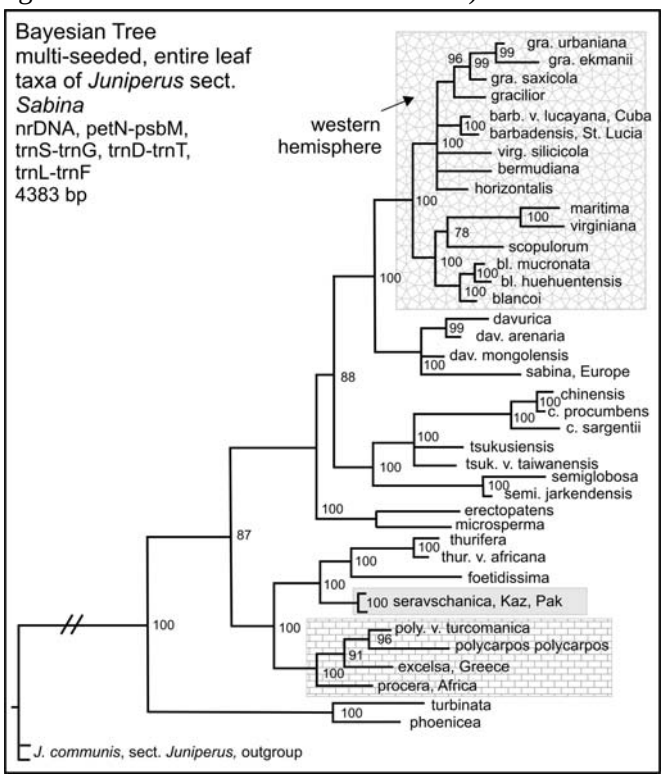

Figure 1. Bayesian analysis based on nrDNA and 4 cp genes. Numbers at the branch points are posterior probabilities. Modified from Adams et al. (2013).

very fine foliage and keyed to *J. excelsa*, yet in many cases, their DNA placed them in *J. polycarpos* (Table 1).

In Adams (2014), these taxa are keyed as follows:

24a.Ultimate branchlets 0.6 - 1 mm diam.; scale-leaves 0.5 - 1 mm; 2 - 5 seeds per cone, seed cones reddish-brown to purple-black; trees with pendulous foliage; endemic to e. Africa, Arabian Peninsula..... .....***J. procera***

24b.Ultimate branchlets 0.7 - 1 mm, scale-leaves 0.6 - 1.6 mm long, 3 - 6 (-8) seeds per cone, reddish-brown to purple black, trees and shrubs, foliage erect to pendulous

25a.Ultimate branchlets 0.7 - 1 mm diam.; scale-leaves very small, 0.6 - 1.1 mm long, appressed; seed cones 6 - 11 mm diam, globose. .....***J. excelsa***

25b.Ultimate branchlets 1 - 1.3 mm diam.; scale-leaves coarse, 1.2 - 1.6 mm long, appressed or apex free; seed cones 8 - 14 mm diam, globose

25.1a. Foliage slender, 0.7- 0.8 mm in cross section on ultimate branchlets, seed cones 7-9(-10) mm, scale leaves tightly appressed, giving a smooth branchlet, (1-)2-3(-4) seeds/cone...***J. polycarpos*** var**. *turcomanica***

25.1b. Foliage stout, 0.9-1 mm in cross section on ultimate branchlets, seed cones 9-11 mm or more, scale leaves with a beak or keel so branchlet appears as a string of beads, 3-6 seeds/cone

25.2a. Seed cones 9-11mm, at least some scale leaves with very narrow, elongated, brown glands, not ruptured. .....***J. polycarpos*** var**. *polycarpos***

25.2b. Seed cones 8-10 mm, scale leaves with clear, ellipsoid glands, often ruptured, with a clear exudate.....***J. seravschanica***

It is obvious from the above key, that these taxa are nearly impossible to distinguish by their morphology. So the question remains, Should these taxa be recognized at the specific level?

To visualize genetic variation in this region, plants were mapped with their nrDNA and cp (petN) DNA coded (Fig. 2). It is sometimes difficult to determine whether variation is due to incomplete lineage sorting or hybridization (see discussion in Naciri and Linder, 2015). The odd occurrence of *J. seravschanica* nrDNA in central-eastern Turkey plants seems more likely incomplete lineage sorting than hybridization, because no pure *J. seravschanica* grows sympatric with *J. polycarpos* in the area. Long distance cross-pollination is possible but unlikely, as the nearest known *J. seravschanica* is quite distant. In northwestern Iran, one P,P and three S,P plants were found. This may be due to either hybridization or incomplete lineage sorting. Additional samples are needed to better understand that region.

The purpose of this paper is to examine incongruent topologies in the *J. excelsa* complex and review the taxonomic status for these taxa.

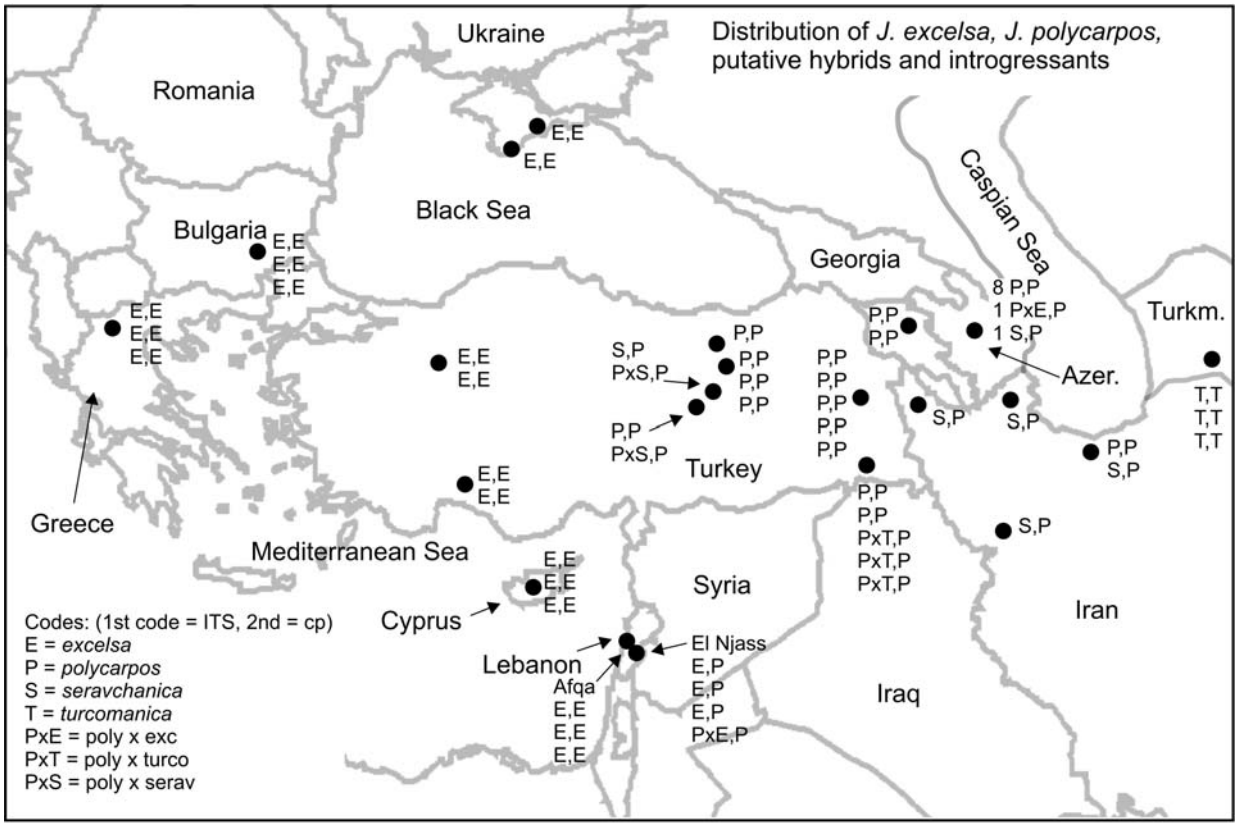

Figure 2. Distribution of *J. excelsa, J. polycarpos*, putative hybrids and introgressants based on ITS and cp sequences. The pair of capital letters (eg., E,E) gives the sample classification based on ITS (1st letter) and cp (2nd letter). Note: revised 30 May 2016, as ambiguity between ITS of *excelsa* and *turcomanica* has been resolved by the discovery of an indel (insertion) at position 526: *excelsa* = AACTCGCCCCT; *turcomanica* = AACTCG**G**CCCT. Adapted from Adams et al. (2016b).

MATERIALS AND METHODS

Plant material - See Adams et al. (2016b).

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, 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. 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.) or Sequencher v. 5 (genecodes.com). Sequence datasets

were analyzed using Geneious v. R8 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. 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. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

The overall DNA pattern among the taxa in the *J. excelsa-J. polycarpos-J. procera* and *J. seravschanica-J. foetidissima-J. thurifera* clades (Fig. 1), reveals that *excelsa - procera* are differ by 21 Mutation Events (SNPs + indels). In *Juniperus*, these five gene sequences have been used for the monograph and all species (Adams, 2014). It has been found that about 6-8 MEs differentiate varieties and species are delineated by about 9-15 MEs. Thus, Adams (2014 and refs. within) recognized *J. excelsa*, *J. procera*, *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica*.

In view of the fact that these five taxa are nearly impossible to identify from morphology alone, it seems timely to re-examine this set of DNA sequences, because the nuclear DNA data (nrDNA) does not tell the same story as the cp genome DNA (petN-psbM, trnSG, trnDT, trnLF). Figure 4 shows the Minimum Spanning Network (MSN) based on nrDNA. Notice that *J. polycarpos* and *J. seravschanica* nrDNAs differ by only 1 SNP. Also note that *J. seravschanica* differs by only 7 MEs from *J. foetidissima*. and by 8 MEs from *J. thurifera* (Fig. 4), such that *J. seravschanica* as similar to *J. foetidissima* as to *J. excelsa* and *J. p.* var. *turcomanica* (Fig. 4) in its nrDNA.

The MSN using four cp genes (3113 bp), shows several incongruent topologies: *J. polycarpos* and *J. seravschanica* are quite differentiated by 23 MEs (Fig. 5). In fact, the cp genes of *J. seravschanica* differ by 10 and 12 MEs from *J. foetidissima* and *J. thurifera*, respectively.

The nrDNAs of *J. polycarpos* and *J. p.* var. *turcomanica* differ by 8 MEs (6 SNPs + 2 indels), which is larger between many Juniper species. Yet, they have no differences in these four cp genes (3113 bp) (Fig. 5). This suggests chloroplast capture by *J. p.* var. *turcomanica* from *J. polycarpos*. resulting in var. *turcomanica* cpDNA being identical to that of *J. polycarpos* (Fig. 5). The fact that var. *turcomanica* cpDNA is identical to *J. polycarpos*, supports the concept that this cp capture is of recent origin.

The nrDNAs of *J. polycarpos* and *J. seravschanica* differ by only 1 SNP, yet their cpDNA differ by 23 MEs Fig. 4). *Juniperus seravschanica* differs by only 10 MEs from *J. foetidissima* and 12 MEs from *J. thurifera* (Fig. 5), but by 7 and 8 MEs in its nrDNA from *J. foetidissima* and *J. thurifera*, respectively. This suggests ancestral chloroplast capture by *J. seravschanica* from an ancestor of *J. foetidissima*/ *J. thurifera*.

It is useful to consider the overall genome organization. The major 'storehouse' of genes is in the nucleus. Sterck et al. (2007) reported over 26,500 genes in *Arabidopsis*, 41,000 in rice (*Oryza sativa*), 45,000 in popular (*Populus*), and 40,000 in *Medicago* and *Lotus*. For Norway spruce, *Picea abies*), Nystedt, et al. (2013) reported 28,354 well-supported genes.

The mitochondrion is another 'storehouse' of genes and a just-published study (Jackman, et al., 2016) on the *Picea glauca* mt genome, reported its size as 5.94 Mb (Mbases), containing only 106 protein-coding, 8 rRNA, and 29 tRNA genes (total 143 genes). Jackman, et al. (2016) also reported the size of the *P. glauca* cp genome as 123,266 bp, with 74 protein-coding, 4 rRNA, and 36 tRNA genes (114 genes).

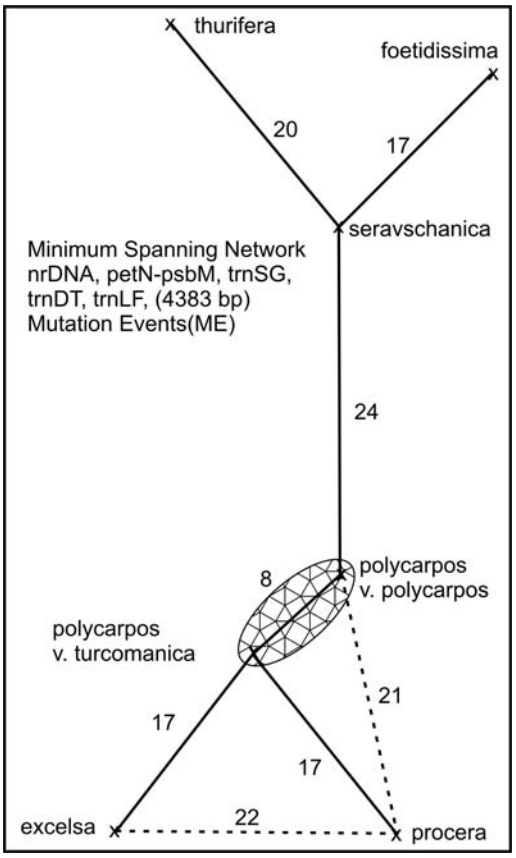

Figure 3. Minimum spanning network of the *excelsa* group. Numbers next to links are the number of Mutation Events (MEs). Dashed lines are second nearest links. Modified from Adams and Schwarzbach (2012).

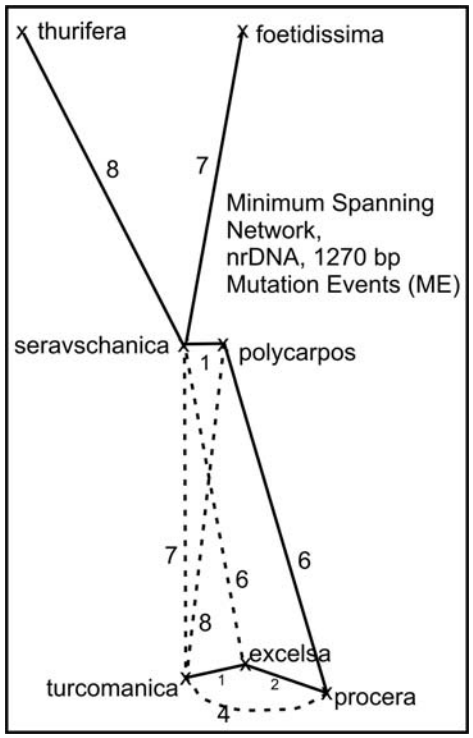

Fig. 4. MSN based on nrDNA. Dashed lines are second nearest links. Notice that *polycarpos* and *seravschanica* differ by one SNP in their nrDNA sequences.

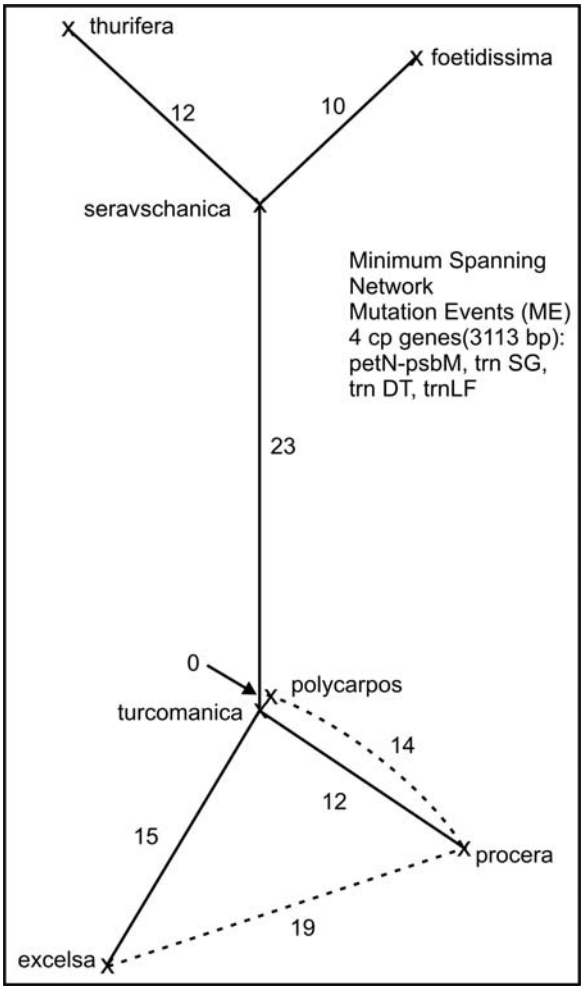

Fig. 5. MSN based on 4 cp gene regions. Note these 4 cp gene regions are identical for *J. polycarpos* and *J. p.* var. *turcomanica*. (i,e, 0 differences in 3113 bp).

A recent study (Guo et al. 2014) of cp genomes of four *Juniperus* species reported the sizes as: *J. bermudiana*, 127,659 bp, *J. monosperma*, 127,744 bp, *J. scopulorum*, 127,774 bp and *J. virginiana*, 127,770 bp. Each of the species had 82 protein-coding, 4 rRNA, and 33 tRNA genes (total 119 genes).

It is instructive to examine the percentage of the genomes that have been studied in the *J. excelsa* - *polycarpos* complex:

Genome	genes utilized	# genes used	#genes/ genome	% genes used
nuclear	nrDNA (1270bp)	1	ca. 28,000	0.0036%
mt genome	none	0	ca. 143 genes	0.0
cp genome	petN-psbM, trnSG, trnDT, trnLF(3113bp)	4	ca. 119 genes	3.36%

It is quite apparent that the sampling of the nuclear genes (0.0036%) is vastly under-represented, compared to the sampling of the cp genes (3.36%). Does the nrDNA sequence data well-represent variation among taxa in their 28,000 nuclear genes? It is thought that nrDNA is subject to concerted evolution (Liao, 1999) such that point mutations are harmonized to conserve the structure of ribosomes. It seems unlikely that protein-coding nuclear genes are subjected to concerted evolution. So, it is possible that nrDNA (RNA structural genes) might not show the same phylogenetic patterns as protein-coding nuclear genes. Due to the lack of single copy genes (SCNG) in conifers, few SCNG are currently utilized in evolutionary studies in conifers.

Adams (2009) examined variation among smooth-leaf margined *Juniperus* in Mexico using nrDNA, two SCNG (4CL, ABI3), and petN-psbM. Figure 6 show MSNs based on nrDNA (left), and two SCNGs (4CL and ABI3, right). Figure 7 shows MSN based on cp petN-psbM sequences. There is considerable agreement between the MSNs using nrDNA and petN-psbM. Both show *J. virginiana* as the most distinct taxon and both show *J. scopulorum*, distinct, but closely related to *J. blancoi* varieties. 4CL generated only 5 SNPs, so it may be unfair to compare with the other sequences that generated 9 to 17 SNPs. Nevertheless, 4CL does show *J. virginiana* distinct (Fig. 6, right), but fails to resolve *J. scopulorum* and *J. blancoi*. ABI3 gave a MSN that is different from any of the other MSNs (Figs. 6, 7). So, this limited comparison of nuclear and cp data gives some support that nrDNA may represent genomic DNA, but not unequivocal support.

Graphic comparison (Fig. 8) shows *excelsa* (E) distinct in terpenes and RAPDs but only somewhat distinct in morphology. nrDNA presents another pattern with two groups: (*excelsa-turcomanica*), (*polycarpos-seravschanica*).

petN-psbM depicts three groups: (*excelsa*), (*polycarpos=turcomanica*), (*seravschanica*) (Kazakhstan = Pakistan). Finally, the lower right (Fig. 8) presents the grouping based on all DNA data.

Incongruent topologies suggest that there are two cases of chloroplast capture in the *J. excelsa* complex: *J. p*. var. *turcomanica* has recently captured its chloroplast from *J. polycarpos* or an extinct ancestor; and *J. seravschanica* has an anciently captured chloroplast from an ancestor of *J. foetidissima*/ *J. thurifera*. Thus, *J. p*. var. *turcomanica* and *J. seravschanica* seem to defy an uncluttered taxonomic classification. Not only does incongruent topologies disrupt a linear, taxonomic classification, but the situation is further confused by wide-spread hybridization, incomplete linage sorting and reticulate evolution in the *J. excelsa* complex. The morphology is reticulated in a manner that defines a multi-dimensional set of relationships among different character sets.

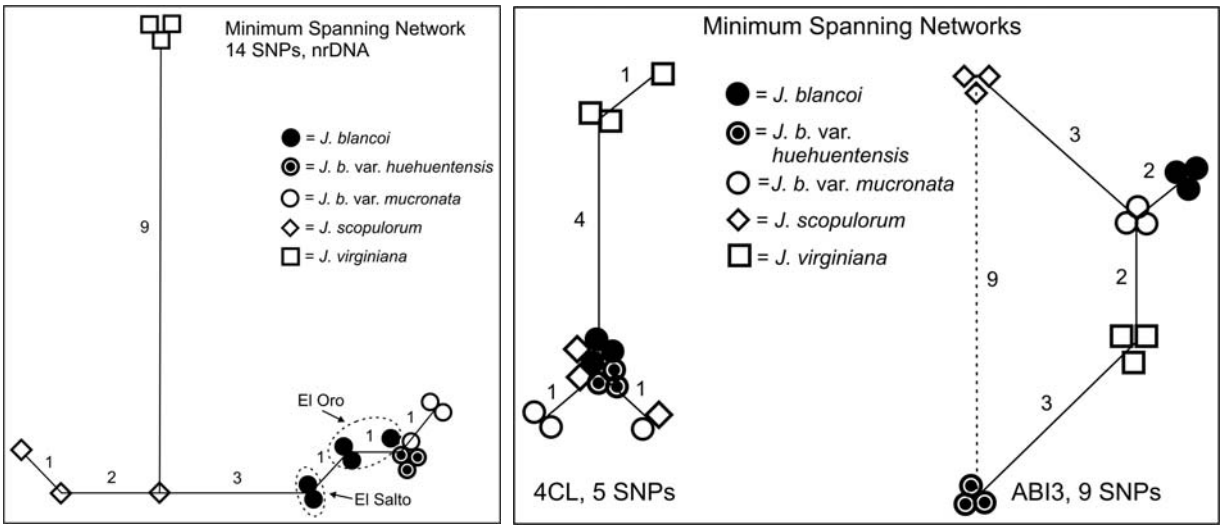

Fig. 6. Comparison of MSN based on nrDNA (left) and two SCNGs (4CL, ABI3, right). Modified from Adams (2009).

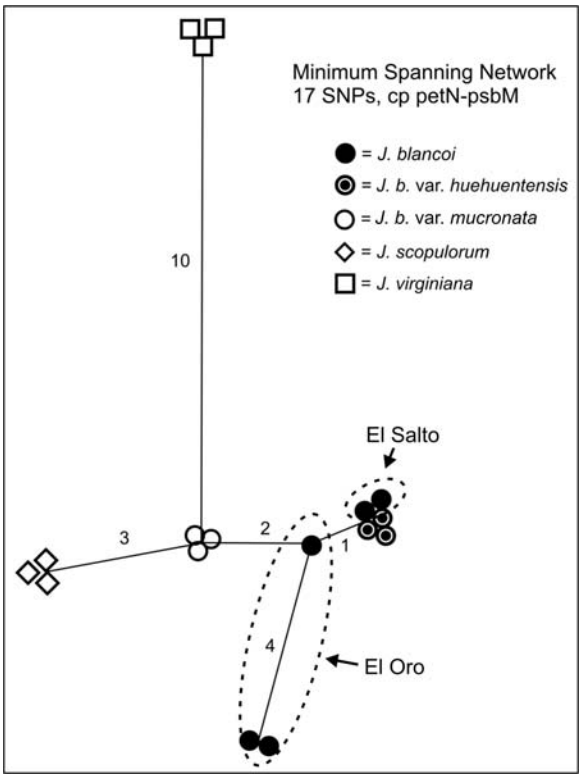

Figure 7. MSN based on petN-psbM. Modified from Adams (2009).

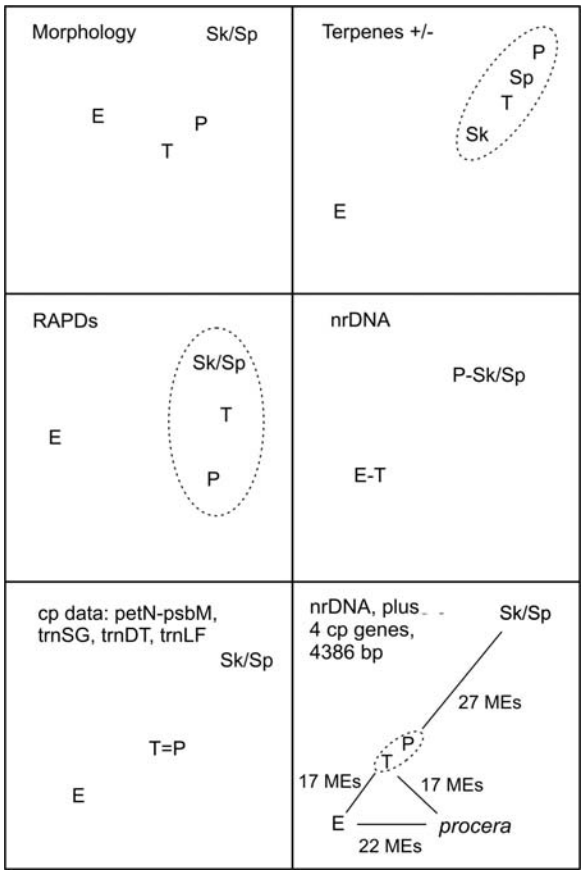

Figure 8. Graphic grouping of *excelsa* (E), *polycarpos* (P), *turcomanica* (T), and *seravschanica* (Sk, Kazak., Sp, Pakistan). Modified from Adams (2014).

Because these taxa are reproducing themselves in nature, occupy distinct geographical regions, and contain unique combinations of genetic material, it is important to recognize two new varieties in order to call attention to chloroplast capture and the unique evolutionary nature of these taxa. These taxa are not merely isolated hybrids (Table 1).

Juniperus excelsa M.-Bieb. var. ***turcomanica*** (B. Fedtsch.) R. P. Adams, **comb. nov.**

BASIONYM: *Juniperus turcomanica* B. Fedtsch. in Fedtschenko et al. Fl. Turkmenii 1:14. 1932. TYPE: Lost or destroyed (Imkhaniskaya, 1990). (LECTOTYPE: *D. P. Gedevanov & D. A. Dranitsyn 148, 3 v 1912*, Turkmenia, Kopet Dag. Dschalilu (chosen by Imkhaniskaya, 1990, LE!)

J. turcomanica B. Fedtsch. in Fedtschenko & al., Fl. Turkmenii 1:14 (1932)
Sabina turcomanica B. Fedtsch). Nevski, Trudy Bot. Inst. Akad. Nauk S.S.S.R. ser. 1, Fl. Sist. Vyss Rast. 4:218 (1937)
J. excelsa M.-Bieb. subsp. *turcomanica* (B. Fedtsch.) Imkhan., Bot. Zurn. 75 (3):408 (1990)

Distribution: Elburz and Kopet Mtns., of Iran and Turkmenistan.

Juniperus excelsa M.-Bieb. var. ***seravschanica*** (Kom.) R. P. Adams, **comb. nov.**

BASIONYM: *Juniperus seravschanica* Kom. Bot. Zurn. (Moscow & Leningrad) 17: 481. 1932. TYPE: Tadjikistan. Zaravshan Range: Zaravshan Valley, Darch, V. L. Komarov s.n. (LECTOTYPE: (chosen by Imkhaniskaya, 1990, LE!)

J. excelsa M.-Bieb. subsp. *seravschanica* (Kom.) Kitam., (Fl. Pl. W. Pakist. Afghan. 7. 1964)
J. polycarpos var. *seravschanica* (Komarov) Kitamura, Fl. Pl. W. Pakist. Afghan Add. & Corr. Fl. Afghan.: 68 (1966).
J. polycarpos K. Koch var. *seravschanica* (Kom.) Kitam., Add. & Corr. Fl. Afghan.: 68 (1966)
J. excelsa M.-Bieb. subsp. *seravschanica* (Kom.) Imkhan., Bot. Zurn. 75 (3): 407 (1990)
Sabina seravschanica (Kom.) Nevski, Trudy Bot. Inst. Akad. Nauk S.S.S.R., ser. 1, Fl. Sist. Vyss. Rast. 4:245 (1937)

Distribution: Central Asia to Iran and Oman.

It should be noted that this creates a polyphyletic species, *J. excelsa*, with varieties in two distinct clades (Fig. 1) when both nrDNA and cpDNA are utilized. So, one should view this work as interim and as a practical matter. It calls attention to this situation, that at present time, appears to escape traditional taxonomic classification.

This treatment gives a variable, polyphyletic species, *J. excelsa*, with three varieties (or subspecies if one prefers, but I prefer to use variety as that is used throughout the genus *Juniperus* and most other Cupressaceae). For those who can not accept polyphyletic species, they are free to use *J. seravschanica*, instead of *J. e.* var. *seravschanica*, and then both it and *J. excelsa* would be monophyletic.

Juniperus excelsa M.-Bieb. var. *excelsa* or subsp. *excelsa*
var. *polycarpos* (K. Koch) Silba (Phytologia Mem. 7: 34. 1984)
(or subsp. *polycarpos* (K. Koch) Tahkt. (Fl. Yerev. 53. 1972.))
var. *turcomanica* (B. Fedtsch.) R. P. Adams, **comb. nov.** this issue.
var. *seravschanica* (Kom.) R. P. Adams, **comb. nov.** this issue.
(or subsp. *seravschanica* (Kom.) Kitam., Fl. Pl. W. Pakist. Afghan. 7. 1964)

It is likely as NexGen sequencing develops, single copy nuclear genes will become widely identified and applied so the concept of *J. excelsa* will also change. As for *J. procera*, it appears to have such a strong geographic integrity, that it seems best to continue its recognition.

The currently understood distributions of *J. excelsa*, var. *polycarpos*, var. *seravschanica* and var. *turcomanica* are depicted in Figure 9. The dashed line in central Turkey indicates the boundary between *J. excelsa* and var. *polycarpos*.

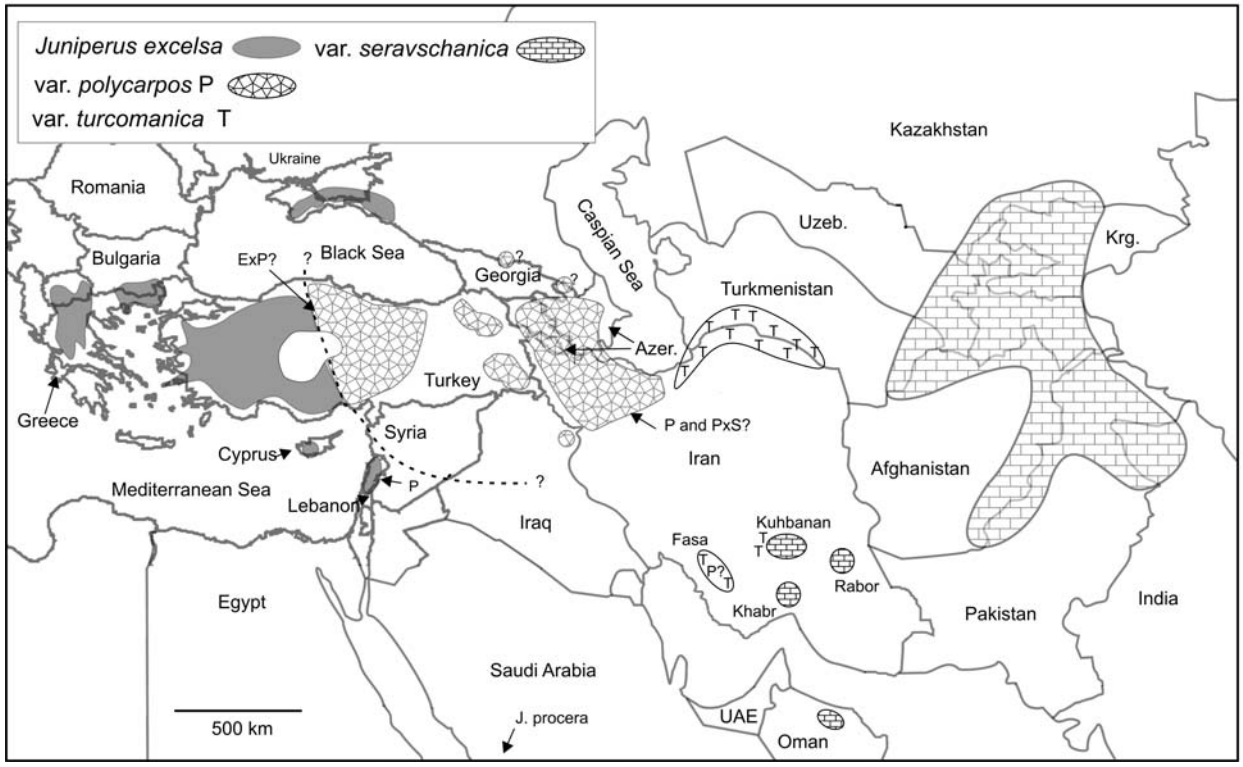

Figure 9. Distributions of *J. excelsa*, var. *polycarpos*, var. *turcomanica and* var. *seravschanica* as understood at present. The dashed line indicates the uncertain limits of *J. excelsa* and var. *polycarpos* in central Turkey. See text for discussion. From Adams et al. (2016b).

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Table 1. Classification based on ITS and cp (petN). exe = *excelsa*, pol = *polycarpos*, tur = *turcomanica*, ser = *seravschanica*. PxE = hybrid pol x exc; PxS = hybrid pol x ser; PxT = pol x tur, PxE = pol x exc. NB: indel at 527 separates exc from tur!

acc #	230	232	238	354	427	732	8952	ITS	cp
8785	A	G	C	C	C	T	A	exc	exc
8786	A	G	C	C	C	T	A	exc	exc
14742	A	G	C	C	C	T	A	exc	exc
13720	A	G	C	C	C	T	A	exc	exc
13721	A	G	C	C	C	T	A	exc	exc
13722	A	G	C	C	C	T	A	exc	exc
14570	A	G	C	C	C	T	A	exc	exc
14571	A	G	C	C	C	T	A	exc	exc
14572	A	G	C	C	C	T	A	exc	exc
14906	A	G	C	C	C	T	A	exc	exc
14907	A	G	C	C	C	T	A	exc	exc
14569	A	G	C	C	C	T	A	exc	exc
14596	A	G	C	C	C	T	A	exc	exc
9433	A	G	C	C	C	T	A	exc	exc
9434	A	G	C	C	C	T	A	exc	exc
14155	A	G	C	C	C	T	A	exc	exc
14156	A	G	C	C	C	T	A	exc	exc
14157	A	G	C	C	C	T	A	exc	exc
14158	A	G	C	C	C	T	A	exc	poly
14159	A	G	C	C	C	T	A	exc	poly
14160	A	G	C	C	C	T	A	exc	poly
14161	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxE**	poly
14750	na	na	na	na	T	C	T	poly	poly
14751	C	G	T	T	T	C	T	poly	poly
14752	C	G	T	T	T	C	T	poly	poly
14753	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
14754	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
14755	C	A	T	T	T	C	T	poly	poly
14756	C	G	T	T	T	C	T	poly	poly
14757	C	G	T	C	T	C	T	**serav**	poly
14758	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
14759	C	G	T	T	T	C	T	poly	poly
14760	C	G	T	T	T	C	T	poly	poly
14713	C	G	T	T	T	C	T	poly	poly
14714	C	**R-A/G**	T	**Y-C/T**	T	C	T	**PxS**[a]	poly
14715	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT**	poly
14709	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT**	poly
14710	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT**	poly
14711	C	G	T	T	T	C	T	poly	poly
14712	C	G	T	T	T	C	T	poly	poly
14162	C	G	T	T	T	C	T	poly	poly
14163	C	**R-A/G**	T	T	T	C	T	poly[a]	poly
14164	C	G	T	T	T	C	T	poly	poly
14165	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxE**	poly
14166	C	G	T	T	T	C	T	poly	poly
14167	C	G	T	T	T	C	T	poly	poly
14168	C	G	T	T	T	C	T	poly	poly
14169	C	G	T	T	T	C	T	poly	poly
14170	C	G	T	T	T	C	T	poly	poly
14171	C	G	T	**C**	T	C	T	**serav**	poly
8761	C	G	T	T	T	C	T	poly	poly
8762	C	**A**	T	T	T	C	T	poly	poly
12603	C	G	T	T	T	C	T	poly	poly
12604	C	G	T	**C**	T	C	T	**serav**	poly
12789	C	G	T	**C**	T	C	T	**serav**	poly
12795	C	G	T	**C**	T	C	T	**serav**	poly
12798	C	G	T	**C**	T	C	T	**serav**	poly
8483	C	G	T	**C**	T	C	T	**serav**	serav
8484	C	G	T	**C**	T	C	T	**serav**	serav
8224	C	G	T	**C**	T	C	T	**serav**	serav
8225	C	G	T	**C**	T	C	T	**serav**	serav
8757	A	G	C	C	C	T	A	turco	turco
8758	A	G	C	C	C	T	A	turco	turco