TAXONOMY OF *JUNIPERUS*, SECTION *JUNIPERUS*: SEQUENCE ANALYSIS OF nrDNA AND FIVE cpDNA REGIONS

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ABSTRACT

A robust analysis of *Juniperus*, sect. *Juniperus* is presented based on nrDNA and five cpDNA regions. The section is clearly divided into two groups composed of blue and red seed cone species and is composed of four major clades: *J. communis* and allies; *J. cedrus* - *oxycedrus* allies; *J. brevifolia* - *deltoides* - *navicularis;* and a loose clade of *J. formosana*, *f.* var. *mairei*, and *J. communis* var. *jackii*. *Juniperus c*. var. *jackii* was found to be the most divergent taxon in the blue seed cone group and is recognized at the specific level: ***Juniperus jackii* (Rehder) R. P. Adams, comb. nov.** *Juniperus formosana* and var. *mairei* were found to be very distinct and the DNA data supports the recognition of *J. mairei* Lemee & H. Lev. The DNA data also support the recognition of *J. lutchuensis* Koidz. and *J. communis* var. *hemisphaerica* (J. & C. Presl) Parl. The putative *J. communis* var. *saxatilis* from Kamchatka peninsula, Russia was found to be unique in its DNA sequence and was recognized as: ***Juniperus communis* var. *kamchatkensis* R. P. Adams, var. nov.** *Phytologia 94(2): 280-297 (August 1, 2012).*

KEY WORDS: Phylogeny, *Juniperus*, section *Juniperus*, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, ycf3 intron 2, trnK-matK, *J. jackii*, *J. c*. var. *kamchatkensis*.

The genus *Juniperus* consists of approximately 68 species and 37 varieties (Adams, 2011), all of which grow in the northern hemisphere, although *J. procera* Hochst. ex Endl. extends southward into the southern hemisphere along the rift mountains in east Africa (Adams and Demeke, 1993). The genus is divided into three sections: *Caryocedrus* (one species, *J. drupacea* Labill.); *Juniperus* (= *Oxycedrus*, 11 species) and *Sabina* (56 species). Section *Juniperus* is circumboreal (Fig. 1), whereas sect. *Caryocedrus* is restricted to the eastern Mediterranean region.

Mao et al. (2010) presented an abbreviated phylogeny of *Juniperus* as part of a study focused on intercontinental dispersal. The study did not include all species of *Juniperus*, as a complete phylogeny was not the goal of their study. The purpose of the current paper is to

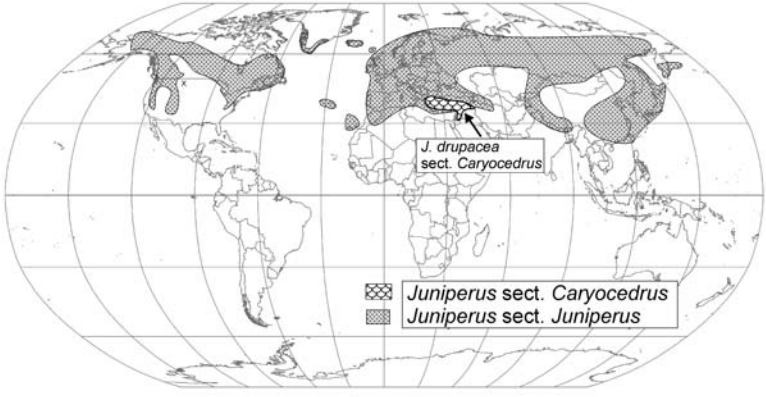

Figure 1. Distributions of *Juniperus* sect. *Caryocedrus* and sect. *Juniperus* (adapted from Adams, 2011).

present a robust analysis based on the most informative nuclear (nrDNA) and cpDNA regions (petN-psbM, trnS-trnG, trnD-trnT, ycf3 intron 2, trnK-matK) of section *Juniperus*, with particular attention to include all known species in this section plus, *J. drupacea* (section *Caryocedrus*) as an outgroup.

MATERIALS AND METHODS

Specimens used in this study: *J. brevifolia* (Seub.) Ant., *Adams 8152,* Serra da Tronqueira, San Miguel Island, Azores Islands; *J. cedrus* Webb & Berthol., *Adams 11510*, La Palma, Canary Islands, Spain; *J. communis* L. *v*ar. *communis*, *Adams 7846*, Stockholm, Sweden; *J. c.* var. *charlottensis* R. P. Adams, *Adams 10304*, Queen Charlotte Island, BC, Canada; *J. c.* var. *depressa* Pursh, *Adams 7802*, Victor, CO, USA; *J. c.* var. *hemisphaerica* (J. & C. Presl) Parl. in Candolle, *Adams 9045,* Mt. Etna, Sicily, Italy (type loc.); *J. c.* var. *hemisphaerica*, *Adams 7194*, Sierra Nevada, Spain; *J. c.* var. *jackii* Rehder, *Adams 10287,* sw Oregon, USA; *J. c.* var. *megistocarpa*, Fernald & H. St. John, *Adams 8575*, Magdalen, Isl., Quebec, Canada; *J. c.* var. *nipponica* (Maxim.) E. H. Wilson, *Adams 8579*, Japan; *J. c.* var. *oblonga* hort. ex Loudon, *Adams 8764,* Armenia; *J. c.* var. *saxatilis* Pall., *Adams 7589*, Altai Mtns., Mongolia; *J. c.* var. *saxatilis*, *Adams 11206*, Norway; *J. c.* var. *saxatilis*, *Adams 9182*, Kamchatka, Russia; *J. c.* var. *saxatilis*, *Adams 10188*, Sakhalin Island, Russia; *J. c.* var. *saxatilis*, *Adams 10890*, Redfish Lake, ID, USA; *J. c.* var. *saxatilis*, *Adams 8686*, Japan; *J. deltoides*, *Adams 9431*, Turkey; *J. formosana* Hayata var. *formosana*, *Adams 9071*, Taiwan; *J. f.* var. *mairei* (Lemee & Lev.) R. P. Adams & C-F. Hsieh, *Adams 6772*, Gansu, China; *J. macrocarpa* Sibth. & Sm., *Adams 7205*, 15 km w Tarifa, Paloma sand dunes, Spain; *J. maderensis* (Menezes) R. P. Adams, *Adams 11497*, Madeira Island, Portugal; *J. navicularis* Gand., *Adams 8240*, Lisbon, Portugal; *J. oxycedrus* L. var. *oxycedrus*, *Adams 9039*, 4 km e Forcalquier, France; *J. o.* var.. *badia* H. Gay, *Adams 7795*, Jaen, Spain; *J. rigida* Mig. in Sieb., *Adams 8544*, Gifu Prefecture, Japan (provided by Jin Murata); *J. r*. var. *conferta* (Parl.) Patschka, *Adams 8585*, Tottori Sand Dunes, Japan (provided by Jin Murata); *J. taxifolia* Hook. & Arn. var. *taxifolia*, *Adams 8448*, Bonin Islands, Japan (provided by Jin Murata); *J. t.* var. *lutchuensis* (Koidz.) Satake, *Adams 8541*, Japan. Section *Caryocedrus*: *J. drupacea* Labill., *Adams 8795, 8796*, Achladokampos Pass, 18 km e Tripolis, Greece. Voucher specimens are deposited in the herbarium, BAYLU, Baylor University.

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 (Qiagen, Valencia, CA) 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 5'trnK-matK-3'trnK region was amplified with primers based on Johnson and Soltis (1994) and modified for use in Cupressaceae (matK1437F: TTGGAAGTTTCGTTCGCAAT; matK1291R: GTAGGGCACTCGTATATCTG; trnK3914F(2565): TGGGTTGC TAACTCAATGG; trnKRcup: AGCTCGTCGGATGGAGTGG. PCR reactions were conducted in 50 µl reactions using 40 ng of genomic DNA, containing 0.5 U Phusion polymerase (NEB, Ipswich, MA) with 1× Phusion HF Buffer (containing 1.5 mM $MgCl_2$), 0.2 mM dNTPs, 0.2 µM each primer. Since the Phusion polymerase has been designed for reduced cycling times, we used increased denaturation and annealing temperatures and shortened thermal cycling times (per the manufacturer's recommendations) as follows: an initial denaturation at 98°C for 30s; 35 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 165 s; hold at 72°C for 5 min; and an indefinite hold at 4°C.

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. 5.4 (Drummond et al. 2011), the MAFFT alignment program 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. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975). The Splitstree v.4 program was used to calculate a splits network tree (Huson and Bryant 2006).

RESULTS AND DISCUSSION

Eight gene regions were surveyed to determine applicability (Table 1). Based on these data, one nuclear region (nrDNA) and five cpDNA regions (petN-psbM, trnS-trnG, trnD-trnF, trnK-matK, ycf3-intron 2) were used in the analyses.

Table 1. Comparison of gene regions and variation in section *Juniperus* (and *J. drupacea*, section *Caryocedrus*). ycf2-IGS-psbA and trnL-trnF were not included in the final analysis. subs = nucleotide substitutions, % inf = % potentially informative = total / length (bp) as percent.

gene region	length(bp)	subs	indels	total	% inf
nrDNA	1278	80	16	96	7.51
petN-psbM	850	21	22	43	5.06
trnS-trnG	830	16	23	39	4.70
trnD-trnF	676	16	10	26	3.84
trnK-MatK	2354	27	3	30	1.27
trnL-trnF	679	8	9	17	2.50
ycf3 intron 2	877	6	6	12	1.37
ycf3 - IGS - psbA	558	2	4	6	1.07

The aligned concatenated data set was composed of 6862 bp from nrDNA, and five cpDNA regions (petN-psbM, trnS-trnG, trnD-trnF, trnK-matK, ycf3-intron 2) sequences. Bayesian analysis shows section *Juniperus* to be clearly divided (Fig. 2, 100% support) into the blue and red seed cone groups (as previously found, Mao et al. 2010). However, in the red seed cone group, there are two sub-clades of *oxycedrus-cedrus* allies and *deltoides-brevifolia-navicularis* (Fig. 2, 100% support). The *J. communis* taxa from North America, form two

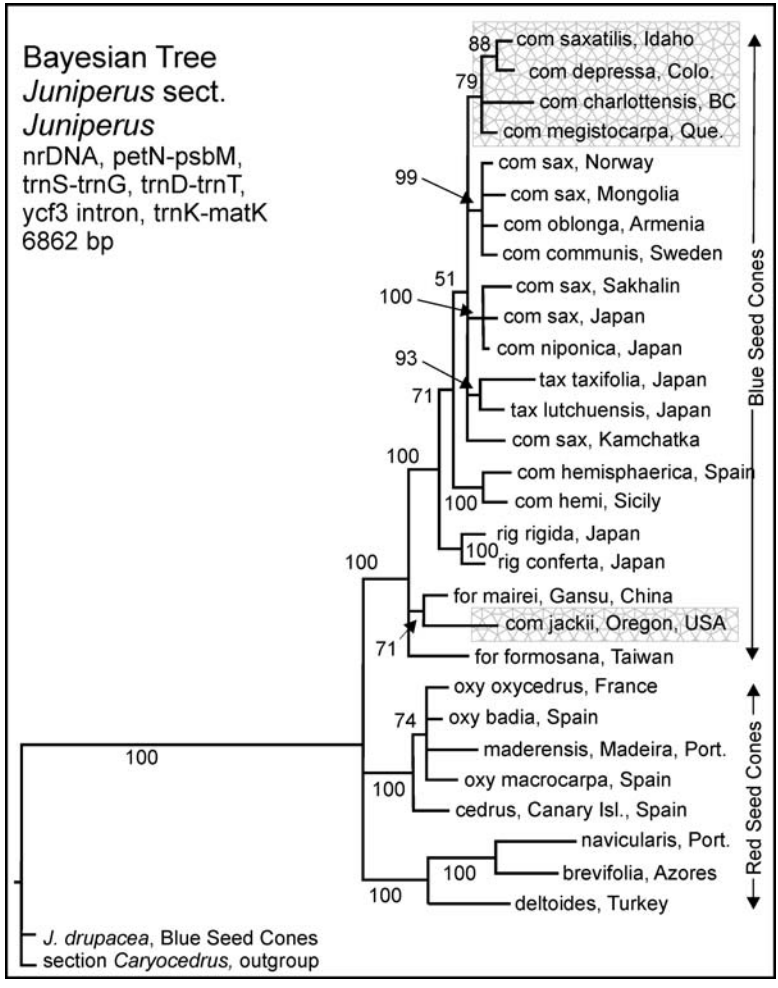

Figure 2. Bayesian tree for all proposed *Juniperus* sect. *Juniperus* taxa. Numbers at the branch points are posterior probabilities (as percent).

clades: var. *jackii*, Oregon and var. *saxatilis*, Idaho; var. *depressa* Colorado, var. *charlottensis*, BC, Canada and var. *megistocarpa*, Quebec, Canada (Fig. 2). *Juniperus taxifolia* and *J. t.* var. *lutchuensis* (Japan) are nested within the main *J. communis* clade. *Juniperus c.* var. *hemisphaerica* (treated as *J. c.* var. *saxatilis* by Adams 2011), has 100% support as a distinct clade (Fig. 2). The two varieties of *J. formosana* (v. *formosana*, Taiwan, v. *mairei*, Gansu, China) are in separate clades (Fig. 2.).

An alternative portrayal of the phylogeny is by a split tree diagram (Fig. 3); in this, the division between the blue and red seed cone junipers is very clear. Four major groups are evident in the split tree as shown by circles (Fig. 3). Two groups are of particular interest: the very closely related *J. communis* and allies form a group and the diverse collection of *J. formosana, J. f.* var. *mairei* and *J. communis* var. *jackii* (Fig. 3) form another. The divergence of the *oxycedrus-cedrus* and *brevifolia-deltoides-navicularis* clade is very pronounced.

Taxonomic Considerations

Aside from elucidating phyletic relationships, DNA sequence data is useful for clarification of taxonomic decisions. For example, the taxonomy of sect. *Juniperus* has been controversial for decades. Many of the taxa are highly variable (particularly in *J. communis* and allies), and taxa are defined on the basis of a single, often quantitative, morphological character (such as width of stomatal band).

In order to examine the divergence among taxa in sect. *Juniperus*, the entire data set of mutations were utilized. This included 221 nucleotide substitutions and 67 indels. All indels were treated as present/ absent regardless of their length. This set of 288 mutational events (ME) were utilized to construct minimum spanning networks (MSN). MSN are particularly useful to ascertain the magnitude of genetic mutations that have accumulated between the lowest levels of classification such as varieties, subspecies and forms. These data are important because such categories are the most controversial and are often based on only one or two morphological characters. The MSN of the red seed cone taxa (Fig. 4) shows most of the recognized species separated by 10- 30 MEs (mutations).

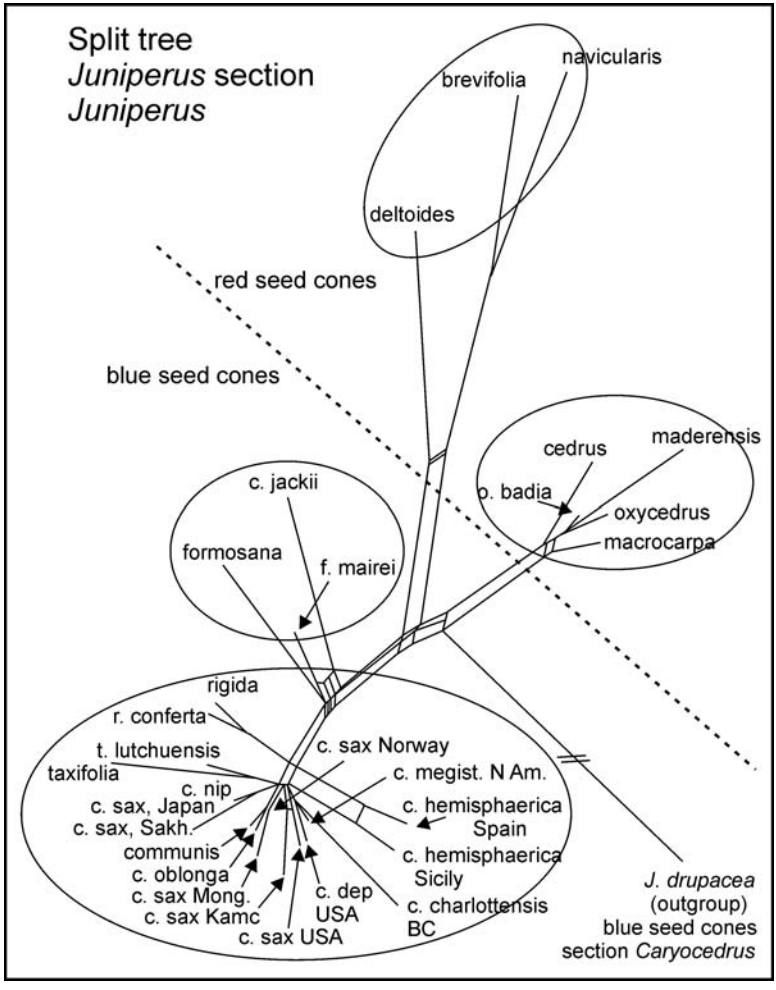

Figure 3. Splits tree analysis of *Juniperus* sect. *Juniperus* evolution.

It is interesting that the nearest link to a non-red seed cone taxon is that of *J. formosana* var. *mairei*, whose mature seed cones are reddish-blue under a bright blue glaucous coating. It should be noted that the Bayesian tree did not include data from indels, so that may, in part, account for this oddity. *Juniperus deltoides*, *J. brevifolia* and *J. navicularis* are especially distinct in the Bayesian tree (Fig. 2), and they are separated by 40 MEs (Fig. 4), and from each other by 20 and 32 MEs. The *cedrus* - *oxycedrus* group is much less diverse (Fig. 4). It is well accepted that *J. oxycedrus* and *J. cedrus* are distinct species (Adams, 2011, Eckenwalder, 2009, Farjon, 2005, 2012) and these differ by 10 MEs (Fig. 4). Note particularly that *J. maderensis* (not accepted

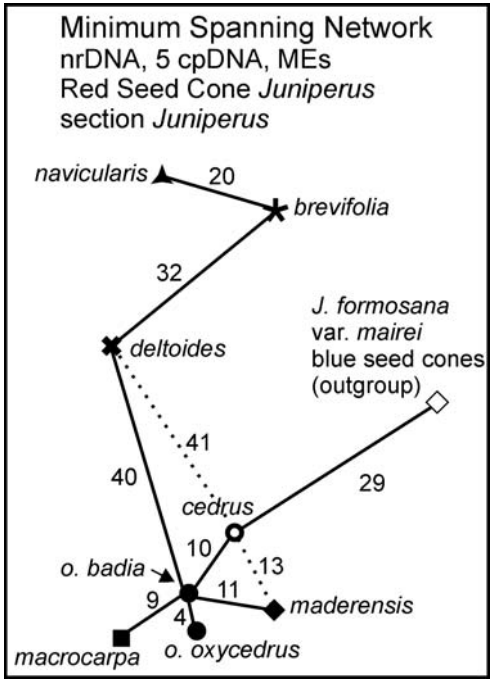

Figure 4. MSN (Minimum spanning network) for the red seed cone junipers, based on 221 nucleotide substitutions and 67 indels. Numbers next to the links are the number of Mutational Events (MEs). *J. formosana* var. *mairei* is the nearest blue seed cone species. Dotted lines show the second nearest links.

by Farjon, 2005, 2012, see Table 2) differs by 11 MEs, giving support for the continued recognition of this species, endemic and threatened on Madeira Island. It is important to note that *J. maderensis* is about equally removed from *J. cedrus* (13 MEs) and *J. oxycedrus* var. *badia* (11 MEs), further supporting their recognition as separate species. The reader is referred to Adams, et al. (2010) for a more detailed analysis of *J. cedrus* and *J. maderensis* from Canary and Madeira Islands.

Juniperus macrocarpa is often treated as *J. oxycedrus* var. or subsp. *macrocarpa* (Table 2), because it differs by its very large seed cones with three raised cone scales and its unusual sea-coast habitat. It is surprising that it is not universally accepted as a species based solely on its morphology. The present study found *J. macrocarpa* to be separated by 9 MEs from *J. oxycedrus* var. *badia* (Fig. 4). Considering morphological and ecological differences as well as the 9 MEs, the continued recognition of *J. macrocarpa* is supported (Table 2).

Juniperus oxycedrus var. *badia* is recognized by its smaller seed cones and shorter leaves than found in *J. oxycedrus* var. *oxycedrus* (Adams 2011). However, in practice, these characters tend to overlap and var. *badia* is scarcely distinct. This study confirms the very close relationship of these being separated by 4 MEs (Fig. 4) out of 6862 bp. Recognition of var. *badia* is only weakly supported by the sequence data (Table 2).

The blue seed cone junipers in sect. *Juniperus* consist of a diverse assemblage. The *J. communis* and allies group are especially difficult and subject to endless nomenclatural changes due to the fact that the habit and leaf characters of varieties (in North America) or subspecies (in Europe) are very variable. In many populations of *J. c.* var. *communis* (typically a small, upright tree) and *J. saxatilis* (a shrub), one finds forms ranging from trees with a strong central axis to decumbent or trailing shrubs. Even in 'pure' var. *communis* populations, the tree habit is often not completely expressed. It seems likely that this 'key' character is controlled by only a few genes or by gene-environmental interactions.

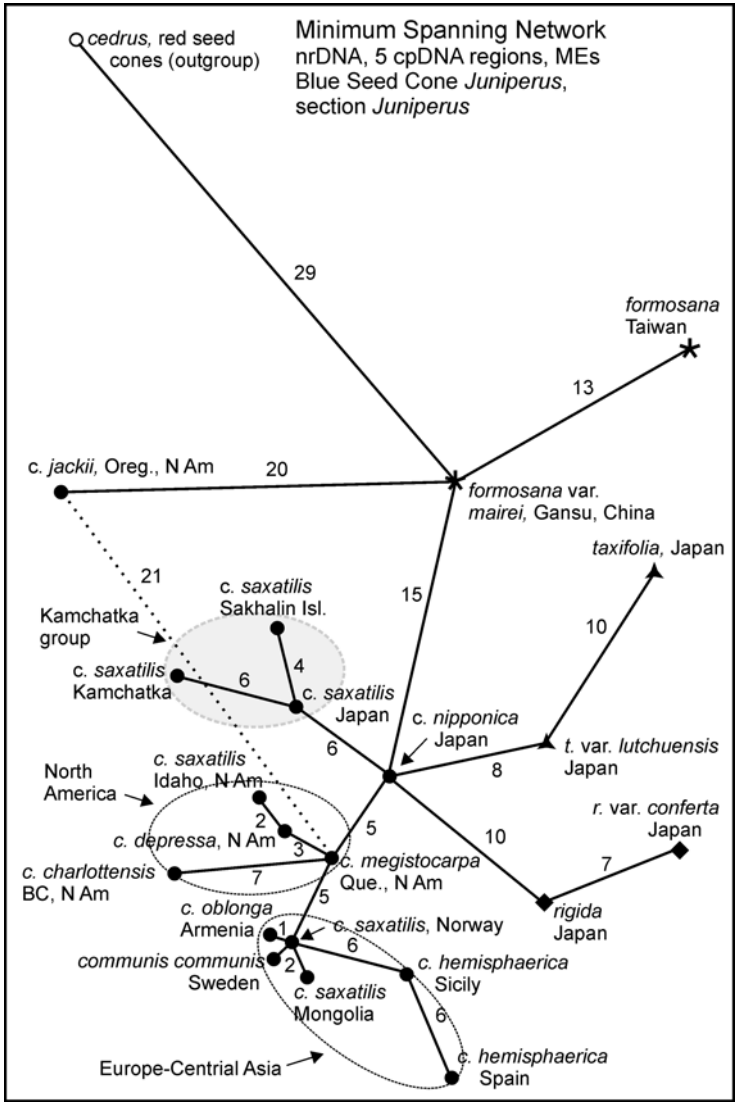

Figure 5. MSN of the blue seed cone junipers (see notes Fig. 4). *J. cedrus* is the nearest of the red seed cone species.

Table 2. Taxonomy of taxa in sect. *Juniperus* by Adams (2011) and Farjon (2010). See also Farjon (2005) for detailed comments. New taxa and taxa newly supported by this study.

Adams(2011)	Farjon (2005, 2010)	Supported, this study
J. brevifolia	*J. brevifolia*	*J. brevifolia*
J. cedrus	*J. cedrus*	*J. cedrus*
J. communis	*J. communis*	*J. communis*
v. *charlottensis*	v. *saxatilis*	v. *charlottensis*
v. *depressa*	v. *depressa*	v. *depressa*
v. *hemisphaerica*	v. *communis*	v. *hemisphaerica*
v. *megistocarpa*	v. *megistocarpa*	v. *megistocarpa*
v. *nipponica*	v. *nipponica*	v. *nipponica*
v. *oblonga*	v. *communis*	**v. *communis***
v. *saxatilis* (Europe,	v. *saxatilis*	**v. *communis*** (Europe, central Asia)
v. *saxatilis* (Japan, Sakhalin Isl.)	v. *saxatilis*	**v. *nipponica*? or v. *kamchatkensis***
v. *saxatilis*(Kamchatka)	v. *saxatilis*	**v. *kamchatkensis***
v. *saxatilis* (N Am.)	v. *saxatilis*	***v. depressa***
v. *jackii*	v. *saxatilis*	***J. jackii***
J. deltoides	*J. oxycedrus*	*J. deltoides*
J. formosana	*J. formosana*	*J. formosana*
v. *mairei*	*J. formosana*	***J. mairei***
J. macrocarpa	*J. oxycedrus. subsp. macrocarpa*	*J. macrocarpa*
J. navicularis	*J. oxycedrus. subsp. transtagana*	*J. navicularis*
J. oxycedrus	*J. oxycedrus*	*J. oxycedrus*
v. *badia*	*subsp. badia*	***J. oxycedrus***
J. rigida	*J. rigida*	*J. rigida*
v. *conferta*	subsp. *conferta*	v. *conferta*
J. taxifolia	*J. taxifolia*	*J. taxifolia*
v. *lutchuensis*	*J. taxifolia*	*J. lutchuensis* or *J. t.* v. *lutchuensis*

The MSN for the blue seed cone sect. *Juniperus* shows considerable differentiation (20 MEs) for *J. communis* var. *jackii*, endemic to serpentine and ultra-mafic rocks in Oregon and northern California (Fig. 5). Its nearest link is with *J. formosana* var. *mairei*, Gansu, China (with a link of 20 MEs differences), but note (Fig. 5) that it is 21 MEs from *J. c.* var. *megistocarpa* N. America, and 22 MEs removed (data not shown) from *J. c.* var. *communis*, Sweden. It is apparently not closely related to any extant juniper. The sequence data strongly support the recognition of var. *jackii* at the specific level (Table 2), ecology and morphology support the recognition of the taxon as:

***Juniperus jackii* (Rehder) R. P. Adams, comb. nov.**

Basionym: *Juniperus communis* var. *jackii* Rehder Mitt. Deutsch. Dendrol. Ges. 1907 (16): 70 (1907). Type: Siskiyou Mtns., on the road from Waldo, Oregon to Crescent City, CA, 3000 ft., 25 Aug., 1904, *J. G. Jack (A. Rehder) s. n.*, (lectotype: A!, designated by Farjon (2005). Named in honor of J. G. Jack.

The next largest link shown in figure 4 is between *J. formosana* var. *mairei* and *J. communis* var. *nipponica* (15 MEs) followed by the gap between *J. f.* var. *mairei* and *J. f.* var. *formosana* (13 MEs). These data indicate that *J. f.* var. *mairei* is not conspecific with *J. formosana* and supports the recognition of this taxon as distinct species:

***Juniperus mairei* Lemee & H. Leveille**, Monde Pl. 2(16): 20 (1914). Maire's juniper, Type: Yunnan, Jong-tohouan, *J. mairei* Lemee & H. Lev., *E. E. Maire s. n.*, (holotype A! barcode #*38339*, isotype E?)

J. chekiangensis Nakai

J. formosana f. *tenella* Handel-Mazzetti

J. formosana var. *mairei* (Lemee & Lev.) R. P. Adams & C-F. Hsieh

Juniperus rigida and *J. r.* var. *conferta* differ by 10 MEs from *J. communis* var. *nipponica*, Japan (Fig. 5). The sequencing data supports the recognition of *J. rigida* (Table 2). *Juniperus r.* var. *conferta* has also been treated as a *J. conferta* Parl. The taxa differ in several

morphological and ecological characters (Adams, 2011) as well as by 7 MEs in this data set. However, it seems prudent to recognize *J. r.* var. *conferta* at this time (Table 2).

Juniperus taxifolia and *J. t.* var. *lutchuensis* present a more difficult taxonomic problem. They were found to form a clade within the *J. communis* clade (Fig. 2) and MSM analysis reveals *J. t.* var. *lutchuensis* is a little closer to *J. c.* var. *nipponica* (8 MEs, Fig. 5) than to *J. taxifolia* (10 MEs, Fig. 5). The divergence of *J. taxifolia* from *J. t.* var. *lutchuensis* (10 MEs) is similar to that found in other closely related species pairs (*macrocarpa* - *oxycedrus*, 9; *maderensis* - *oxycedrus* - 11; *cedrus* - *oxycedrus* 10, *rigida* - *communis* 10). The taxa appear to occupy similar habitats and are very similar in their morphology. At present, it seems prudent to maintain *J. t.* var. *lutchuensis* (Table 2).

The *J. communis nipponica* - *saxatilis* complex from Japan (and nearby Sakhalin Island and the Kamchatka peninsula) is closely allied with North America (5 MEs, Fig. 5), while differing by 4-6 MEs between taxa. The putative *J. c.* var. *saxatilis*, Japan is 6 MEs from *J. c.* var. *nipponica*, compared to 8 MEs (data not shown) to *J. c.* var. *saxatilis*, Norway and 8 MEs (data not shown) to *J. c.* var. *depressa*, N. America. The putative var. *saxatilis* from Japan, Kamchatka and Sakhalin does not appear to be part of the traditionally recognized *J. c.* var. *saxatilis* from Europe and central Asia. It appears that the key character defining var. *saxatilis*, stomatal band twice as wide as each green leaf margin (Adams, 2011), may have evolved independently several times. There is, at present, no easy solution to this taxonomy. Comparison of their leaf morphology (Table 3) shows differences in the stomatal band width (relative to the green leaf margins), leaf cross sections and overall leaf shape. The leaves of var. *saxatilis* from mainland Japan are quite similar to var. *saxatilis* from Europe, including having keeled leaves the latter was thought to be a useful character to separate var. *saxatilis* from var. *nipponica* (Adams, 2011). Morphology and DNA sequence data separate the taxon on Kamchatka from *J. c.* var. *saxatilis*, Japan and *J. c.* var. *nipponica* Europe, including having keeled leaves the latter was thought to be a useful

Table 3. Comparison of the leaf morphology of *J. c.* var. *saxatilis*, Japan and Kamchatka, and *J. c.* var. *nipponica*, Japan.

	var. *saxatilis*		var. *nipponica*
	Japan	Kamchatka	Japan
Stomatal band width vs. green leaf margin	2x	1-1.5x	0.5-0.25x
Leaf cross-section	flat to slightly concave with keel	concave with keel	very concave, sunken stomatal band, with keel
Leaf shape	straight to curved	straight to slightly curved	curved, boat shaped

character to separate var. *saxatilis* from var. *nipponica* (Adams, 2011). Morphology and DNA sequence data separate the taxon on Kamchatka from *J. c.* var. *saxatilis*, Japan and *J. c.* var. *nipponica* (Table 3). These differences warrant the recognition of the taxon on Kamchatka as a new variety:

***Juniperus communis* var. *kamchatkensis* R. P. Adams, var. nov.**
Type: Russia, near Esso, Kamchatka peninsula, 56º N, 159º E, 550-700 m., *J. W. Leverenz 5* (= *Adams 9164*) (HOLOTYPE: BAYLU).

Shrubs, similar to *J. communis* var. *saxatilis*, but differing in having straight to slightly curved leaves, with cross section concave with keel and stomatal band 1 - 1.5 x width of green leaf margins. Seed cones purple-blue when mature.

Other specimens studied: TOPOTYPES: *J. W. Leverenz 6* (*Adams 9181*), *J. W. Leverenz 7* (*Adams 9182*), *J. W. Leverenz 8* (*Adams 9183*).

Juniperus communis var. *kamchatkensis* is known only from the type locality and vicinity in Kamchatka; usually beneath *Betula platyphylla*, *Populus tremula* and *Salix* sp. in ravines with large rocks.

In spite of the fact that DNA sequence data (Fig. 5) shows var. *saxatilis*, Japan, not to be closely related to var. *saxatilis* of Europe and central Asia (Fig. 5), no reliable morphological character was found to separate the Europe - central Asia plants from those on Japan and Sakhalin Island.

Figure 6. Holotype of *Juniperus communis* var. *kamchatkensis*.

The North American *J. communis* taxa (vars. *charlottensis*, *depressa*, *megistocarpa* and *saxatilis*, Fig. 5) are shown to differ by at least 5 MEs from Japan and Europe taxa. The var. *charlottensis* is the most distinct (7 MEs), whereas vars. *depressa*, *megistocarpa* and *saxatilis* differ by only 2 to 3 MEs; var. *megistocarpa* is very distinct in having very large seed cones (9-13 mm and larger than leaf length, Adams, 2011) and grows on coastal sand dunes. Although the DNA differences are not large (3 MEs) the continued recognition of this variety is warranted. In North America, var. *depressa* and var. *saxatilis* differ principally by the width of stomatal bands (compared to the green leaf margin). The var. *saxatilis*, Idaho, USA, differs by only 2 MEs from var. *depressa*, USA, but by 6 MEs to var. *nipponica*, Japan and var. *saxatilis*, Norway (data not shown). Clearly it is part of var. *depressa* and not var. *saxatilis* (*sensu stricto*).

Juniperus c. var. *communis*, trees in Sweden, var. *saxatilis*, Norway and Mongolia, and var. *oblonga*, Armenia form a very tight group differing by 1 to 2 MEs (Fig. 5). Clearly there is no support for the recognition of var. *oblonga* (Fig. 5, Table 2). The separation of var. *communis* from var. *saxatilis* (in Europe and central Asia) is largely based on the tree vs. shrub habits. However, as previously mentioned,

many populations of *J. c*. var. *communis* have individuals that are semi-trees and/or both large and small shrubs. It appears that, in spite of strong DNA evidence, the shrubs in Europe and central Asia will continue to be called var. *saxatilis*.

Of special interest is the var. *hemisphaerica* from the type locality (Mt. Etna, Sicily) and Sierra Nevada, Spain. These plants differ by 6 MEs from var. *saxatilis* (Fig. 5) and between each other. The DNA data support the recognition of var. *hemisphaerica* (Table 2) in Sicily and perhaps a new variety in Sierra Nevada, Spain.

One is always looking for more data to resolve difficult problems. However, it may be that these unresolved taxonomic problems in *Juniperus* section *Juniperus* are actually evolutionary branches in the midst of complex speciation processes.

ACKNOWLEDGEMENTS

Thanks to Jin Murata, Japan for providing specimens from Japan. Thanks to Tonya Yanke and Julie Morris for lab assistance, and Baylor for funds. This research was supported in part by funds from NSF projects DEB-0316685 to RPA and AES as well as DEB-0629402 to AES.

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