JUNIPERUS RECURVA VAR. *UNCINATA*, THE HOOKED BRANCHLET JUNIPER, A NEW VARIETY FROM NEPAL

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ABSTRACT

Routine field work in Nepal led to the discovery of a new variety of *J. recurva* (*J. recurva* var. *uncinata* R. P. Adams), the hooked branchlet juniper, a shrub with short leaves and hooked or recurved branchlet tips, growing at 3900 m. Analyses of sequence data from nrDNA and cpDNA (petN-psbM) of *Juniperus indica, J. i. var. caespitosa, J. i. var. rushforthiana, J. recurva* and *J. squamata* confirmed the distinct nature of the new variety. Analyses of leaf terpenoids from the parents, and several intermediates between *J. recurva* and *J. r.* var. *uncinata* support hybridization. *Phytologia 91(3): 361-382 (December, 2009).*

KEY WORDS: *Juniperus indica, J. i.* var. *caespitosa, J. i. var. rushforthiana, J. recurva, J. recurva* var. *uncinata* R. P. Adams, **var. nov.**, *J. squamata*, SNPs, nrDNA (ITS), petN, psbM, sequences, terpenoids, taxonomy, hybridization.

The junipers of the central Himalayans consist of *J. communis* L. var. *saxatilis* Pall., *J. indica* Bertol. var. *caespitosa* Farjon, *J. i.* var.

indica Bertol., *J. i.* var. *rushforthiana* R. P. Adams, *J. recurva* Buch.-Ham. ex D. Don, and *J. squamata* Buch.-Ham. ex D. Don (Adams, 2008). While collecting specimens of *J. recurva* in Nepal, we discovered a shrub that differs from typical *J. recurva* in having branchlet tips that are hook-shaped, having shorter leaves, and branching near the base. In addition, several plants were found that appear to be intermediate between typical *J. recurva* and the newly discovered 'hooked-tip' plants.

The purpose of this paper is to report on analyses of *J. recurva,* the newly discovered hooked-tipped shrubs and compare these with closely related species (*Juniperus indica, J. i.* var. *caespitosa, J. i.* var. *rushforthiana, J. squamata*) using nrDNA (ITS) and cp petN-psbM SNPs. In addition, the volatile leaf terpenoids from the *J. recurva* plants and putative hybrids were analyzed.

MATERIALS AND METHODS

Specimens (GenBank nrDNA, petN-psbM) used in this study: *J. indica* var. *indica, Adams 8775-8777*, (GQ118641, GQ118648), L. Singh, Dumpa, Jomson, 2900 m, Nepal; *J. indica* var. *caespitosa*, *Adams 7625-7627* (GQ118642, GQ118649), R. Chaudhary, between Kyangjin Gompa and Langtang Glacier, 4000 m, Nepal; *J. indica* var. *rushforthiana, Adams 8140, 8141,* (GQ118643, GQ118650), K. Rushforth, Soe Tajitang, 3000 m and Lingshi, 3480 m, Bhutan, *J. recurva* var. *recurva, Adams 7210, 7211, 7215, 7217-7219* (GQ118644, GQ118651), between Sing Gompa and Cholan Pati lodge, 3360-3570 m, Nepal; *J. recurva var. uncinata, Adams 7212-7214* (GQ118645, GQ118652) Lauri Binayak, 3900 m, Nepal, *J. squamata* var. *squamata, Adams 6796,* (GQ118646, GQ118653) Xian Botanical Garden, China, *7012*, Kunming Botanical Garden, China, *J. squamata* f. *wilsonii, Adams 5521* (GQ118647, GQ118654), Arnold Arboretum, acc.1010-64A. Voucher specimens 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.

Amplification and sequencing

ITS (nrDNA) and petN-psbM 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) and 1.8 µM each primer.

Gene	Primers	2x buffer	annealing	program	size bp
nrDNA	ITSA-42F/ ITSB+57R	K	50ºC	(94-50x30)	1106-141
petN	petN5F/psbM111R	E	50ºC	(94-50x30)	741-825

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

additional ITS primers (based on *Juniperus* sequences):

ITSA-42F = GAT TGA ATG ATC CGG TGA AGT
ITSB+57R = ATT TTC ATG CTG GGC TCT

petN - psbM:

petN5F = AAC GAA GCG AAA ATC AAT CA
psbM111R = AAA GAG AGG GAT TCG TAT GGA

petN and psbM primers were based on conserved sequences from *Juniperus* species.

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.). In each case, the single 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 MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/), manually corrected, and re-analyzed using NJ with 1000 bootstrap replications (http://align.bmr.kyushu-u.ac.jp/mafft/).

Isolation and analysis of Oils - see Adams et al. (2009). *Data Analysis* - Terpenoids (as per cent total oil) were coded and compared among the taxa by the Gower metric (Gower, 1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The hooked branchlet junipers were found at 3900 m on a south facing slope at timberline at Lauri Binayak (Fig. 1). A large population of *J. recurva* grew just west of Sing Gompa (Fig. 1) and other individual *J. recurva* plants were found between Sing Gompa and Cholan Pati lodge (Fig. 1). Plants morphologically intermediate between *J. recurva* and the hooked branchlets junipers were found around Cholan Pati between 3400 and 3600 m (Fig. 1). The small elevation change between Sing Gompa (3400 m) and Lauri Binayak (3800 m) appears sufficient to cause habitat differences that support typical *J. recurva* and the hooked-tip juniper, as well as intermediate habitats around Cholan Pati.

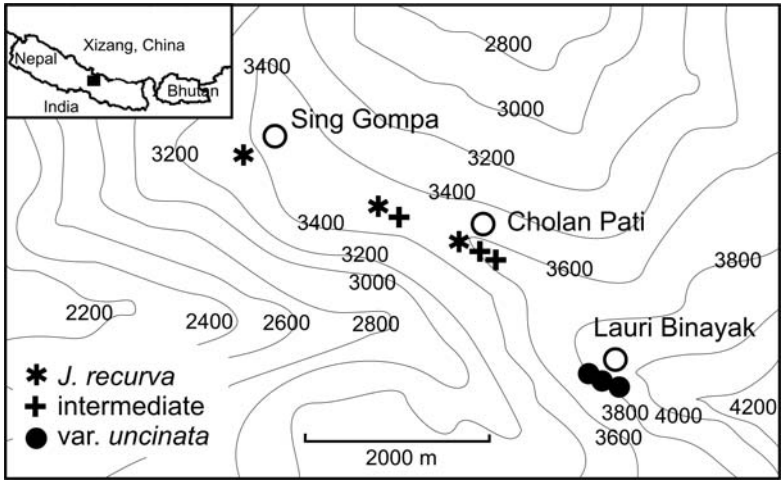

Figure 1. Site map of juniper collections between Sing Gompa and Lauri Binayak, Nepal.

The hooked branchlet junipers near Lauri Binayak are recognized as a new variety of *J. recurva:*

Juniperus recurva var. **uncinata** R. P. Adams, **var. nov.** TYPE: Nepal, 100 m s of Lauri Binayak, N 28 05.537', E 85 22.879', 3900 m, on south facing slope, 0.5 m x 0.5 m shrub, 1 Nov 1993, *Adams 7212* (HOLOTYPE: BAYLU, TOPOTYPES: *Adams 7213, 7214*, BAYLU), Fig. 2).

Junipero recurvae similis sed differt habitu fruticoso, apicibus ramulorum unciformibus vel recurvatis, et foliis brevibus (20–28 mm).

Similar to *Juniperus recurva*, but differing in that the branchlet tips are hooked or recurved, the leaves are short (20 - 28 mm) and it grows as a shrub.

The terminal branchlet tips of var. *uncinata* are striking as they form a hook at their tips (Fig. 2), in contrast to var. *recurva* that has lax tips that are not hooked (Fig. 3). The leaves of *uncinata* are 20 - 28 mm long, compared to 35-50 mm in var. *recurva* (Figs. 2, 3). In addition, var. *uncinata* is multi-stemmed at the base whereas var. *recurva* has a strong central axis, even when appearing as a shrub.

It appears that the two varieties hybridize in the area around Cholan Pati. A putative hybrid (*Adams 7211*) is shown in Fig. 4. Note that the hooked branchlets are like those of var. *uncinata*, but the longer leaves are like var. *recurva*; this plant was a shrub, branched at the base, 1 m wide x 1 m tall. Due to the cutting of limbs for use as incense, many of the trees in this area appear as shrub-like.

In addition to hybrids, individuals were found that appear to be back-crossed, or of a F_2 generation. *Adams 7210* is from a shrub, 4 m wide x 0.7 m tall, with very small leaves, but the branchlet tips are scarcely hooked (Fig. 5), suggesting the presence of some genes from *J. recurva* var. *recurva* in an otherwise typical *J. r.* var. *uncinata* individual.

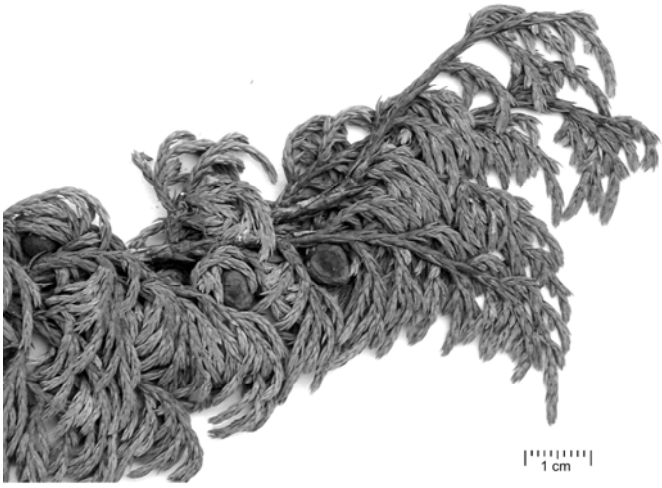

Fig. 2. *Juniperus recurva* var. *uncinata*, Holotype, *Adams 7212*.

Fig. 3. *Juniperus recurva* var. *recurva*. *Adams 7219.*

Fig. 4. Putative hybrid between *Juniperus recurva* var. *recurva* and *J. r*. var. *uncinata, Adams 7211.*

Fig. 5. Putative backcross of *J. recurva* var. *recurva* to *J. r.* var. *uncinata*, *Adams 7210.*

To gather additional information on the relationships within the *J. recurva* complex and other related taxa, the nrDNA (ITS region) was sequenced. This resulted in 1106 to 1141 bp of sequence data, with 19 mutational events that included 5 indels. A 29 bp deletion was found in all samples of *J. recurva* vars. *recurva* and *uncinata*. A 4 bp deletion was found in all var. *uncinata* and *J. squamata* samples. Eight of the mutational events were single occurrences and not deemed of taxonomic interest. This resulted in 11 events (simply noted as SNPs for discussion) that were used for analysis. An associational matrix was constructed using these 11 SNPs; the resulting minimum spanning network is shown in figure 6 (left). No variation was found among *J. recurva* var. *recurva* and only one SNP was found in *J. r.* var. *uncinata*

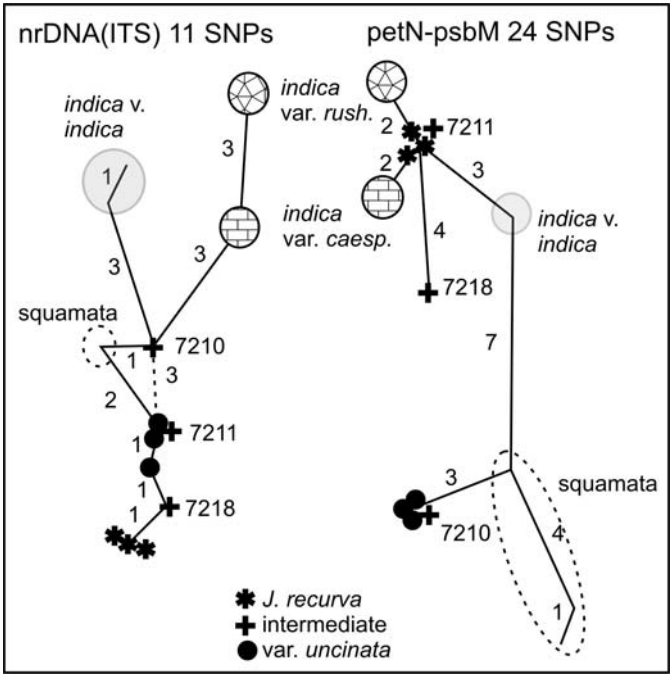

Figure 6. Left: Minimum spanning network based on 11 nrDNA SNPs. Right: Minimum spanning network based on 24 petN-psbM SNPs. The numbers next to the links are the number of SNPs. Dashed line (7210-7211) is the second nearest link.

(Fig. 6, left). No variation was found among the three *J. squamata* samples. However, the three varieties of *J. indica* showed considerable differences.

Sequencing the cp petN-spacer-psbM region resulted in 741-825 bp with 35 mutational events that included 9 indels. Five of the indels and 6 of the nucleotide mutations were single occurrence events (autapomorphies) and eliminated, leaving 24 informative mutational events that were utilized (herein called SNPs). An associational matrix was constructed using these 24 SNPs, and the resulting minimum spanning network is shown in figure 6 (right). Interestingly, again, no variation was found within *J. recurva* var. *uncinata* or among *J. r.* var. *recurva* (Fig. 6, right). However, the three *J. squamata* samples showed considerable variation. The three varieties of *J. indica*, as before, showed considerable differences, with *J. recurva* var. *recurva* being placed in the midst of the three *J. indica* varieties (Fig. 6, right).

The intermediate plants (7210, 7211, 7218) are plotted differently in each data set. Plant 7210 has 3 nrDNA mutations that separate it from all *J. recurva* plants (Fig. 6, left), but its cp petN-psbM sequences are identical to *J. recurva* var. *uncinata* (Fig. 6, right) suggesting that the male (pollen and cp transmitting) parent was *J. r.* var. *uncinata*. Plant 7211 is identical in its nrDNA to two of the *J. recurva* var. *uncinata* plants (Fig. 6, left), whereas, its cp petN-psbM sequence is identical to *J. recurva* var. *recurva* plants (Fig. 6, right), suggesting that the male parent was *J. r.* var. *recurva*. If this is correct, then gene flow seems to be bi-directional. Plant 7218 is intermediate in its nrDNA between *J. r.* var. *recurva* and var. *uncinata* (Fig. 6, left) and has 4 SNPs separating it from *J. r.* var. *recurva* (Fig. 6, right).

A minimum spanning network based on combining the 11 nrDNA and 24 petN-psbM SNPs shows that *J. recurva* var. *recurva* is well separated from *J. r.* var. *uncinata* (Fig. 7). The varieties of *J. indica* are nearly equidistant. The accessions of *J. squamata* show considerable differences.

The intermediate individuals largely follow the cp petN-psbM differences in that each are associated with either *J. r.* var. *recurva* (7211, 7218) or *J. r.* var. *uncinata* (7210) (Fig. 7). Although *J. r.* var.

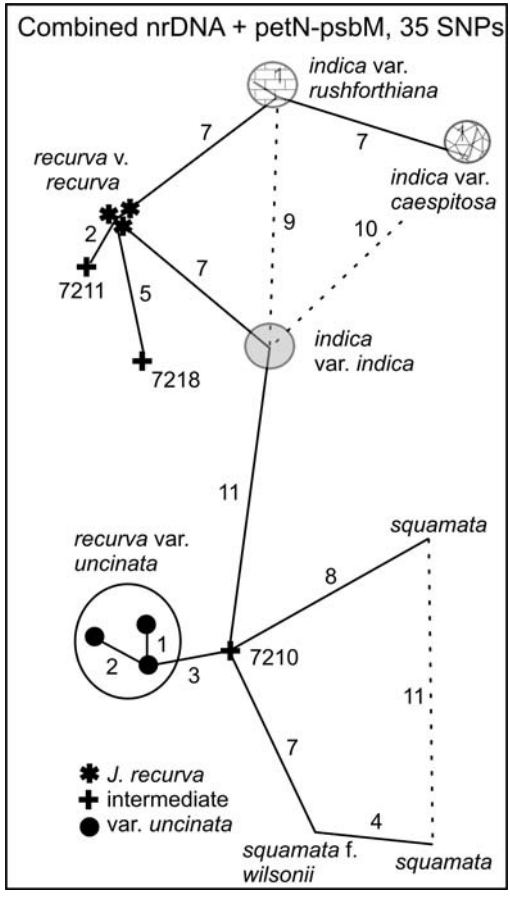

Figure 7. Minimum spanning network based on 11 nrDNA and 24 cp petN-psbM SNPs. The dotted lines show the 2nd nearest link. Numbers next to the links are the number of SNP differences.

uncinata appears to be as distinct from *J. r.* var. *recurva* as other species. However, due to the likely hybridization and intergradation, it seems unwise to recognize it at the specific level at this time.

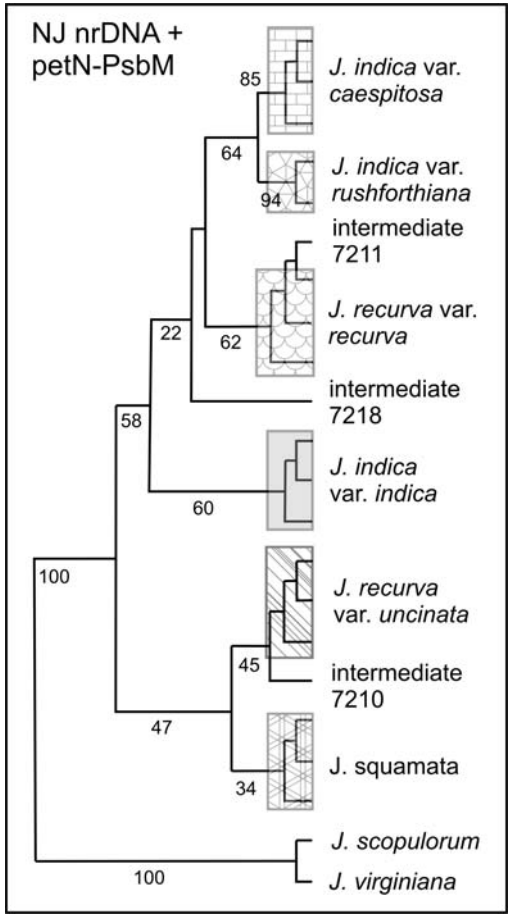

Figure 8. NJ tree based on nrDNA and cp petN-psbM sequences. The numbers on the branches are bootstrap probabilities (1000 reps.).

Another method to examine the sequence differences is by adding an outgroup (*J. scopulorum, J. virginiana*) to the data set and computing a NJ tree. Figure 8 shows a NJ tree based on combined nrDNA and petN-psbM sequences. Notice, although the species group

into clades, these clades are generally not well supported. The intermediate plants grouped with *J. r.* var. *recurva* (7211) or *J. r.* var. *uncinata* (7210), reflecting the male chloroplast donor. Intermediate plant 7218 is not closely associated with any clade.

There is weak support for *J. recurva* var. *uncinata* and *J. squamata* as a clade (Fig. 8, 47). Examination of the leaves of the taxa concerned, show (Fig. 9) that the leaves of *J. recurva* var. *uncinata* are very similar to those of *J. recurva* var. *recurva* and not like those of *J. squamata*. In addition, the gene-flow between var. *recurva* and var. *uncinata* suggest a very close relationship. It is also noteworthy that the leaves of *J. indica* and its varieties are scale-like and differ from the decurrent leaves of *J. recurva* var. *recurva* (Fig. 9) such that the placement of var. *recurva* within the *J. indica* clade is inconsistent with leaf morphology. Perhaps there are ancestral gene-patterns of *J. indica* that are still expressed in *J. recurva* var. *recurva* for the two gene regions sequenced.

Incomplete lineage sorting and gene coalescence

Degnan and Rosenberg (2009) define incomplete lineage sorting as "the failure of two or more lineages in a population to coalesce, leading to the possibility that at least one of the lineages first coalesces with a lineage from a less closely related population." Syring et al. (2007, Table 6) suggest that the number of years for monophyly to be more likely than paraphyly in *Pinus* may be from 1.7 to 24 Myr and it may take from 5.4 to 76 Myr for a species to attain a complete genome-wide coalescence. The oldest known fossil of *Juniperus* is from Europe (dated at 35 m yr bp, Kvacek, 2002). The Himalayas began to form with the collision of the Indian and Eurasian plates about 40-50 m yr bp (USGS, 2009). It may be that *J. indica* and *J. recurva* have had insufficient evolutionary time to fully complete lineage sorting (at least for the two gene regions sequenced in this study).

Leaf terpenoids and analysis of hybridization

A more detailed examination of *J. r.* var. *recurva, J. r.* var. *uncinata* and putative hybrids was undertaken by examining the volatile leaf terpenoids. Table 1 shows the compositions of the oils of *J. recurva* from the study area (Fig. 1). The oils of var. *recurva* are high in α-pinene, sabinene, δ-3-carene, limonene, β-phellandrene and elemol

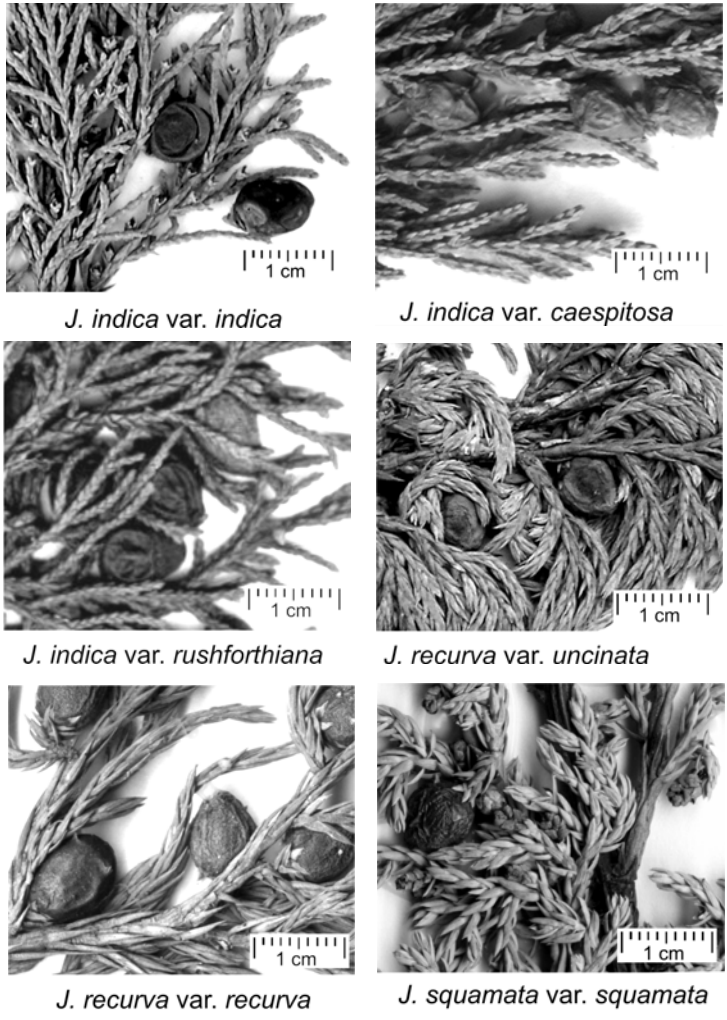

Figure 9. Leaves and female cones of taxa in this study.

with moderate amounts of myrcene, terpinolene, terpinen-4-ol, pregeijerene B, γ-eudesmol and abietadiene (Table 1).

The leaf oils of var. *uncinata* are very high in δ-3-carene (27.0 - 37.0%) and high in terpinolene, trans-muurola-4(14),5-diene, cubebol, δ-cadinene, with moderate amounts of α-pinene, sabinene, limonene, β-phellandrene, cis-muurola-3,5-diene, and trans-cadina-1(6),4-diene (Table 1). Several compounds were present in var. *recurva*, but absent in var. *uncinata*: α-thujene, cis-sabinene hydrate, trans-sabinene hydrate, hexenyl isovalerate, linalyl acetate, germacrene B, humulene epoxide II, and γ-eudesmol. Two compounds were found in all var. *uncinata* oils but were absent in var. *recurva* oils: cis-limonene oxide and β-oplopenone (Table 1).

The intermediate plants (7210, 7211, 7218) often had intermediate values in their composition. Note α-thujene, α-pinene, δ-3-carene, terpinen-4-ol, cis-muurola-3,5-diene, trans-cadina-1(6),4-diene, trans-muurola-4(14),5-diene, δ-cadinene, elemol, γ-eudesmol, and abietadiene (Table 1). Two of the compounds exhibited a 'gigantism' in being markedly higher concentration in some of the intermediate plants: myrcene and β-phellandrene (Table 1).

Thirty eight of the terpenoids that were larger than trace amounts, and displayed fidelity within the taxa (boldface in Table 1), were used to compute an association matrix. Factoring the matrix resulted in two eigenroots that were larger than the average diagonal value, and likely biologically significant.

The first three eigenroots (47.3, 16.0, 10.3 % of the variance) were used to plot the individuals (Fig. 10). *Juniperus recurva* var. *recurva* individuals formed a looser cluster than those of var. *uncinata*. Two of the intermediate plants (7211, 7218) form the classical U triangle between parents and hybrids, exactly the ordination position that is expected for hybrids (see Adams, 1982, for a detailed examination of multivariate analysis of both synthetic and putative hybrids). The third intermediate plant (7210) is much closer to *J. r.* var. *uncinata* and behaves as a back-cross or F_2 generation plant in this ordination (Adams, 1982). It is interesting to note that none of the analyses of sequence data had the sensitivity to detect these hybrids, and/or back-crossed, individuals.

Pleines, Jakob and Blattner (2008) state "As tree-based methods are mostly insufficient to depict relationships within species, network approaches are better suitable to infer gene or locus genealogies. Problematic for phylogeographic studies are alleles shared among multiple species, which could result from either hybridization of incomplete lineage sorting." The results from the present study show that analysis of hybridization and introgression using multivariate statistical methods of quantitative data is more informative than the tree-based method utilized.

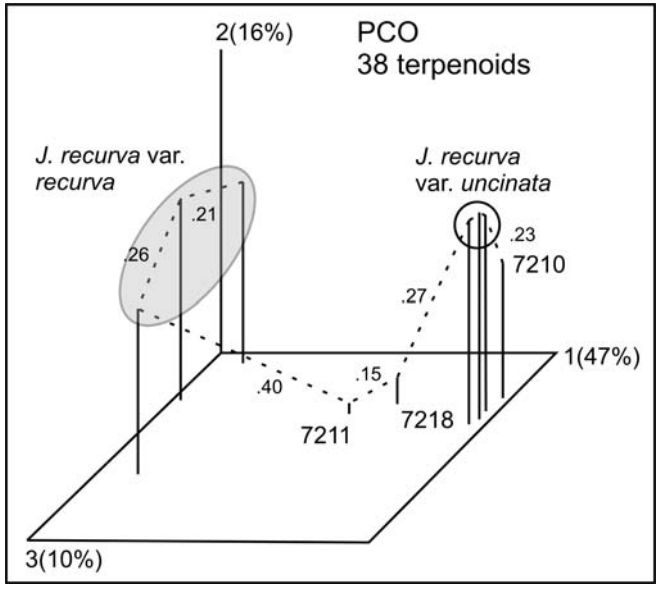

Figure 10. PCO of *J. recurva* individuals based on 38 terpenoids. The dashed line is the minimum spanning network and the values next to the links are the distance values between OTUs.

In summary, the recognition of the new variety, *J. recurva* var. *uncinata*, is supported by morphology, terpenoids and DNA sequence data. Additional research is needed to clarify the variation found in *J. squamata*.

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Table 1. Comparison of volatile leaf oils of *J. recurva* var. *recurva*, *J. r.* var. *uncinata* and intermediates.

		var. recurva			intermediate plants			var. *uncinata*		
AI	Compound	7219	7217	7215	7211	7218	7210	7212	7213	7214
921	tricyclene	t	t	-	-	t	0.1	0.1	0.1	0.1
924	**α-thujene**	**0.2**	**0.6**	**0.4**	**0.2**	**0.3**	**t**	**-**	**-**	**-**
932	**α-pinene**	**7.0**	**4.1**	**7.2**	**4.2**	**4.7**	**5.2**	**2.8**	**3.3**	**7.9**
945	**α-fenchene**	**1.2**	**1.2**	**0.4**	**0.4**	**0.8**	**1.2**	**1.8**	**2.3**	**1.9**
961	verbenene	-	-	-	-	-	0.1	-	0.1	-
969	**sabinene**	**5.6**	**13.1**	**13.4**	**0.9**	**2.2**	**0.6**	**0.4**	**0.4**	**0.3**
974	β-pinene	0.3	0.2	t	0.1	t	0.3	0.2	0.3	0.3
988	**myrcene**	**3.7**	**2.0**	**1.7**	**8.7**	**14.2**	**2.2**	**1.2**	**1.4**	**1.1**
1008	**δ-3-carene**	**18.1**	**16.4**	**8.4**	**6.8**	**10.8**	**21.1**	**32.1**	**37.0**	**27.0**
1014	**α-terpinene**	**0.3**	**0.7**	**0.6**	**0.1**	**0.1**	**0.1**	**0.1**	**t**	**t**
1020	p-cymene	t	0.3	0.1	t	t	0.1	0.5	0.6	0.4
1024	**limonene**	**7.2**	**6.2**	**12.0**	**7.0**	**6.0**	**6.0**	**3.0**	**3.6**	**0.3**
1025	**β-phellandrene**	**10.3**	**6.2**	**8.7**	**14.2**	**11.9**	**6.1**	**3.1**	**3.6**	**0.6**
1054	**γ-terpinene**	**0.4**	**1.1**	**1.0**	**0.2**	**0.2**	**0.2**	**t**	**t**	**t**
1065	**cis-sabinene hydrate**	**0.2**	**0.5**	**0.3**	**0.1**	**t**	**t**	**-**	**-**	**-**
1067	cis-linalool oxide(furanoid)	-	-	-	0.1	-	t	-	-	-
1086	**terpinolene**	**2.6**	**2.6**	**1.2**	**1.7**	**2.3**	**3.7**	**4.8**	**5.3**	**4.6**
1090	6,7-epoxy myrcene	t	-	-	t	-	-	-	-	-
1096	trans-sabinene hydrate	0.1	0.2	t	t	t	-	-	-	-
1096	linalool	0.1	0.3	0.3	0.3	t	0.2	0.3	t	t

Table 1. Continued		7219	7217	7215	7211	7218	7210	7212	7213	7214
1102	isopentyl-isovalerate	-	0.2	0.2	0.0	-	0.1	-	-	0.2
1112	3-methyl-3-buten-methyl butanoate	0.3	0.5	0.2	0.1	t	t	0.1	0.2	0.2
1119	trans-p-menth-2-en-1-ol	t	0.2	0.1	t	t	t	-	-	-
1132	cis-limonene oxide	-	-	-	-	-	0.2	t	t	0.2
1133	**cis-p-menth-2-en-1-ol**	**0.2**	**0.2**	**t**	**t**	**t**	**-**	**-**	**-**	**-**
1137	trans-limonene oxide	t	0.1	0.1	0.1	t	0.1	t	t	0.2
1166	p-mentha-1,5-dien-8-ol	0.4	0.1	t	t	t	0.2	t	t	t
1174	**terpinen-4-ol**	**1.1**	**2.5**	**2.4**	**0.4**	**0.3**	**0.4**	**0.2**	**t**	**t**
1179	p-cymen-8-ol	0.3	t	t	t	-	t	-	-	-
1204	p-cymen-9-ol	0.2	0.2	t	t	-	t	-	-	-
1186	**α-terpineol**	**0.2**	**0.4**	**0.8**	**0.2**	**0.1**	**0.2**	**0.5**	**0.6**	**0.6**
1204	verbenone	-	-	-	-	-	t	-	-	-
1223	citronellol	-	-	-	-	-	t	-	-	-
1235	trans-chrysanthenyl acetate	-	-	-	-	-	0.2	-	-	-
1241	**hexenyl isovalerate**	**0.3**	**0.3**	**0.2**	**0.3**	**0.1**	**-**	**-**	**-**	**-**
1241	carvacrol, methyl ether	-	-	t	t	-	t	-	-	0.1
1249	piperitone	-	-	-	t	-	0.1	t	-	-
1254	**linalyl acetate**	**0.2**	**0.3**	**0.5**	**0.2**	**-**	**-**	**-**	**-**	**-**
1257	**methyl citronellate**	**-**	**-**	**-**	**-**	**-**	**t**	**0.4**	**0.4**	**-**
1274	**pregeijerene B**	**3.5**	**4.0**	**4.9**	**t**	**t**	**0.5**	**-**	**0.3**	**0.3**
1287	**bornyl acetate**	**0.3**	**0.2**	**0.2**	**0.2**	**0.2**	**0.4**	**0.2**	**0.1**	**t**

Table 1. Continued		7219	7217	7215	7211	7218	7210	7212	7213	7214
1287	**trans-linalool oxide acetate (pyranoid)**	**-**	**-**	**-**	**t**	**-**	**0.5**	**0.2**	**0.2**	**t**
1298	carvacrol	0.2	0.1	0.2	t	-	0.2	-	0.3	0.3
1339	trans-carvyl acetate	-	-	-	-	-	t	0.1	-	-
1345	**α-cubebene**	**-**	**-**	**0.2**	**0.4**	**0.4**	**0.2**	**0.4**	**0.4**	**0.4**
1346	α-terpinyl acetate	-	-	-	0.2	-	-	-	-	-
1374	α-copaene	-	-	0.1	0.2	0.1	0.2	0.2	-	0.2
1387	**β-cubebene**	**-**	**-**	**0.3**	**0.6**	**0.5**	**0.4**	**0.5**	**0.5**	**0.6**
1403	methyl eugenol	t	-	0.2	-	-	-	-	-	-
1417	(E)-caryophyllene	t	-	0.3	0.2	0.1	0.3	0.3	t	0.2
1448	**cis-muurola-3,5-diene**	**0.2**	**0.2**	**1.4**	**3.1**	**3.3**	**2.3**	**3.6**	**3.7**	**4.1**
1452	α-humulene	-	t	0.2	0.3	t	0.2	0.3	t	0.3
1461	cis-cadina-1(6),4-diene	-	-	-	-	-	0.1	t	-	t
1475	**trans-cadina-1(6),4-diene**	**t**	**0.1**	**1.1**	**2.8**	**2.8**	**2.0**	**3.5**	**3.3**	**4.3**
1478	γ-muurolene	t	t	t	0.1	t	t	t	t	t
1480	germacrene D	-	-	-	0.1	-	0.2	t	-	t
1493	**trans-muurola-4(14),5-diene**	**0.1**	**-**	**2.8**	**5.8**	**8.9**	**6.5**	**9.3**	**9.6**	**10.9**
1493	epi-cubebol	-	-	0.8	3.0	-	-	-	-	-
1500	**α-muurolene**	**0.3**	**0.3**	**0.3**	**0.5**	**0.5**	**0.8**	**0.7**	**0.6**	**1.0**
1513	**γ-cadinene**	**0.6**	**0.3**	**0.9**	**3.0**	**3.0**	**0.6**	**0.7**	**0.5**	**0.6**
1513	**cubebol**	**-**	**0.3**	**1.8**	**6.8**	**5.9**	**5.4**	**6.1**	**4.9**	**5.5**
1522	**δ-cadinene**	**1.3**	**1.3**	**1.7**	**3.7**	**3.5**	**5.2**	**6.4**	**4.4**	**6.3**

Table 1. Continued		7219	7217	7215	7211	7218	7210	7212	7213	7214
1528	zonarene	-	-	0.5	1.3	1.2	0.9	0.8	1.4	1.5
1533	trans-cadina-1,4-diene	-	0.2	0.2	0.6	0.6	0.5	0.6	0.6	0.7
1537	α-cadinene	-	-	0.1	0.2	-	t	t	-	0.1
1548	**elemol**	**6.7**	**6.5**	**5.4**	**1.7**	**0.5**	**1.2**	**t**	**0.5**	**1.5**
1559	cis-murol-5-en-5-α-ol	-	-	-	-	-	t	0.1	t	t
1559	**germacrene B**	**0.3**	**0.3**	**0.2**	**0.1**	**-**	**-**	**-**	**-**	**-**
1574	germacrene-D-4-ol	1.5	3.1	0.8	1.6	1.1	3.1	1.8	0.8	1.2
1582	caryophyllene oxide	t	-	t	0.1	t	t	t	-	-
1587	trans-muurol-5-en-4-α-ol	t	-	0.3	3.3	2.2	1.1	1.3	0.5	0.7
1607	**β -oplopenone**	**-**	**-**	**-**	**-**	**-**	**0.3**	**t**	**0.2**	**0.1**
1608	**humulene epoxide II**	**0.1**	**0.3**	**t**	**0.3**	**0.2**	**-**	**-**	**-**	**-**
1618	1,10-di-epi-cubenol	-	-	-	-	-	t	-	-	-
1627	1-epi-cubenol	-	-	1.9	5.8	4.5	3.2	3.6	3.2	3.9
1630	**γ-eudesmol**	**1.5**	**1.0**	**1.2**	**0.5**	**t**	**0.1**	**-**	**-**	**-**
1638	epi-α-cadinol	0.8	0.5	0.6	0.7	0.6	1.1	0.8	0.6	1.0
1638	epi-α-muurolol	0.8	0.5	0.5	0.8	0.5	1.0	0.8	0.6	1.0
1644	α-muurolol	t	t	t	0.3	t	0.3	0.2	t	0.2
1649	β -eudesmol	1.5	1.2	1.3	0.5	t	0.1	-	t	0.3
1652	α-eudesmol	1.0	0.7	0.9	0.7	0.3	1.2	0.6	0.4	0.9
1652	α -cadinol	1.0	0.7	1.0	0.7	0.4	1.1	0.6	0.5	0.9
1670	**bulnesol**	**0.6**	**0.6**	**0.4**	**0.3**	**-**	**t**	**t**	**-**	**-**

Table 1. Continued		7219	7217	7215	7211	7218	7210	7212	7213	7214
1688	shyobunol	t	-	-	0.2	-	-	-	-	-
1792	8-α-acetoxyelemol	2.9	2.5	2.3	t	-	0.4	-	t	0.2
2055	abietatriene	0.3	0.3	0.1	0.2	0.3	0.4	0.1	t	0.2
2056	**manool**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**	**3.9**	**1.2**	**0.1**	**2.0**
2087	**abietadiene**	**2.3**	**1.0**	**1.8**	**0.5**	**0.3**	**1.4**	**0.2**	**t**	**t**
2282	sempervirol	t	-	-	0.1	-	-	-	-	-
2298	4-epi-abietal	0.1	0.1	-	0.8	0.3	0.3	0.2	t	0.2
2314	trans-totarol	0.5	0.8	0.3	0.9	0.3	0.2	0.2	t	0.5
2331	trans-ferruginol	-	-	-	t	-	-	-	-	-