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Systematics of multi-seeded eastern hemisphere *Juniperus* based on leaf essential oils and RAPD DNA fingerprinting

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Abstract

The composition of the leaf essential oils of 15 taxa of the multi-seeded *Juniperus* in sect. *Sabina* from the eastern hemisphere are reported and compared (*J. chinensis*, *J. davurica*, *J. excelsa*, *J. excelsa* var. *polycarpos*, *J. foetidissima*, *J. jarkendensis*, *J. phoenicea*, *J. procera*, *J. sabina*, *J. sabina* var. *erectopatens*, *J. semiglobosa*, *J. seravschanica*, *J. talassica*, *J. thurifera*, *J. turcomanica*). The volatile leaf oil compositions for these *Juniperus* species are presented. In addition, DNA fingerprinting revealed similar patterns among these species. Based on these data, *J. seravschanica* is treated as *J. turcomanica* B. Fedtsch. var. *seravschanica* (Kom.) R.P. Adams *comb. nov.*, and *J. talassica* is recognized as *J. semiglobosa* var. talassica (Lipinsky) Silba. These data also support the continued recognition of *J. jarkendensis* Kom. as a distinct species. *Juniperus sabina* var. *erectopatens* (Cheng. and L.L. Fu) Y.F. Yu and L.K. Fu was found to be very distinct in both its terpenoids and RAPDs and is recognized as a distinct species, *J. erectopatens* (Cheng and L.K. Fu) R.P. Adams stat nov. The correct name for the Balochistan, ~~Pakistan juniper is *J. turcomanica* var. *seravschanica* (Kom.) R.P. Adams, not *J. excelsa* or *J. excelsa* ssp. *polycarpos*~~. In addition, the systematic and evolutionary relationships are discussed.

J. polycarpos (Lem. Adams, 2001)

Keywords: *Juniperus*; *Cupressaceae*; Essential oils; Terpenes; RAPD; DNA fingerprinting; Systematics

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1. Introduction

The genus *Juniperus* consists of approximately 60 species, all of which grow in the northern hemisphere, although *J. procera* Hochst. ex Endl. also grows southward along the rift mountains in east Africa into the southern hemisphere (Adams and Demeke, 1993). The genus is divided into three sections: *Caryocedrus* (one species, *J. drupacea* Labill.); *Juniperus* (= *Oxycedrus*), with 9 or 10 species) and *Sabina* (the remaining, approximately 50 species). A previous study using RAPDs (Adams and Demeke, 1993), indicated that section *Sabina* could be further divided into junipers with serrate and those with entire (smooth) leaf margins. The serrate leaf margined junipers are confined to the western hemisphere (except for *J. phoenicea*, which I would call "pseudoserrate" as its DNA clearly points to *J. phoenicea's* affinity with the smooth leaf margined junipers (Adams and Demeke, 1993).

The *Juniperus* of section *Sabina*, of the eastern hemisphere can be further divided into two groups based on the number of seeds per female cone (often called berry) and female cone shape. The single seed/cone (single seeded) *Juniperus* of the eastern hemisphere have cones that are ovoid with a noticeable pointed tip, whereas the multi-seeded *Juniperus* are generally round and often have an irregular surface.

The volatile leaf oils of several of the species in this study have been reported on and reviewed recently by the author (*J. chinensis* L., Adams et al., 1994a; *J. davurica* Pall., Adams et al., 1994b; *J. excelsa* M.-Bieb., Adams et al., 1992,1993; *J. jarkendensis* Kom., Adams et al., 1998; *J. phoenicea* L. Adams et al., 1996; *J. procera* Hochst. ex Endl., Adams et al., 1993; *J. sabina* L., Adams et al., 1998; and *J. semiglobosa* Regel, Adams et al., 1992).

The literature reports on the oils of *J. excelsa* var. *polycarpos* (K. Koch) Silba, *J. seravschanica* Kom., *J. talassica* Lipsky, and *J. turcomanica* B.A. Fedtsch. presents difficulty because of the taxonomic confusion of these taxa. There have been two recent taxonomic treatments of central Asian multi-seeded junipers (Table 1). One can see disagreements concerning *J. jarkendensis* and *J. talassica*. Farjon (1992) recognized *J. excelsa* ssp. *polycarpos* (K. Koch) Takhtajan and reduced *J. turcomanica* and *J. seravschanica* as synonyms (along with *J. macropoda* Boiss.). Farjon (1992) recognized *J. semiglobosa* and treated *J. talassica* and *J. jarkendensis* as synonyms. Silba (1986,1990) treated *J. jarkendensis* as a variety of *J. sabina*, treated both *J. seravschanica* and *J. turcomanica* as *J. excelsa* var. *polycarpos*, and treated *J. talassica* as *J. semiglobosa* var. *talassica* (Table 1).

Rafique et al. (1993) reported on the berry oil of the juniper from near Quetta, Balochistan, Pakistan (referred to as *J. excelsa* in their paper and treated as putative *J. excelsa* var. *polycarpos* in our collections). They reported the berry oil was dominated by α-pinene (64.4%) and myrcene (12.4%). Sadri and Assadi (1994) reported that the leaf oil of *J. polycarpos* (*J. excelsa* var. *polycarpos*) was dominated by α-pinene (71.4%) with a moderate amount of α-gurjunene (5.2%) and a number of cadinene isomers. Chavchanidze and Kharabava (1989) reported that the leaf oil of their sample of *J. polycarpos* (*J. excelsa* var. *polycarpos*) was dominated by α-pinene (40.2%) and limonene (8.3%) with considerable number of sesquiterpenes that ranged from 2–8%.

Table 1
Taxonomic treatment of the multi-seeded *Juniperus*, section *Sabina*, eastern hemisphere (– = not discussed)

Initial taxa	Farjon (1992)	Silba (1986, 1990)	This study results
J. chinensis	–	*J. chinensis*	*J. chinensis*
J. davurica	–	*J. davurica*	*J. davurica*
J. excelsa	*J. excelsa*	*J. excelsa*	*J. excelsa*
J. excelsa var. *polycarpos*	*J. excelsa ssp. polycarpos*	*J. excelsa* var. *polycarpos*	*J. excelsa* var. *polycarpos*
J. foetidissima	*J. foetidissima*	*J. foetidissima*	*J. foetidissima*
J. jarkendensis	= *J. semiglobosa*	*J. sabina var. jarkendensis*	*J. jarkendensis*
J. phoenicea	–	*J. phoenicea*	*J. phoenicea*
J. procera	*J. procera*	*J. procera*	*J. procera*
J. sabina	*J. sabina*	*J. sabina*	*J. sabina*
J. sabina var. *erectopatens*	–	–	*J. erectopatens*
J. semiglobosa	*J. semiglobosa*	*J. semiglobosa*	*J. semiglobosa*
J. seravschanica	= *J. excelsa* var. *polycarpos*	= *J. excelsa var. polycarpos*	*J. turcomanica* var. *seravschanica*
J. talassica	= *J. semiglobosa*	*J. semiglobosa* var. *talassica*	*J. semiglobosa* var. *talassica*
J. thurifera	–	*J. thurifera*	*J. thurifera*
J. turcomanica	= *J. excelsa* var. *polycarpos*	= *J. excelsa* var. *polycarpos*	*J. turcomanica*

The leaf oil of *J. foetidissima* is reported to be dominated by limonene (21.2%), sabinene (16.6%) and thujone (cis/trans?) (13.4%) (Akimov et al., 1976). Chavchanidze and Kharabava (1989) reported a different profile for *J. foetidissima* with the dominate compound being limonene (19.5%), followed by α-pinene (11.5%) and sabinene (6.3%). It is interesting to note that Goriaev and Ignatova (1969) reported that *J. foetidissima* oil contained major components of α-pinene and cedrol. Cedrol has not been reported in other papers for *J. foetidissima*.

The leaf oil of *J. sabina* var. *erectopatens* (Cheng and L.K. Fu) Y.F. Yu and L.K. Fu has not apparently been reported. Dembitsky (1969) analyzed the leaf oil of *J. seravschanica* and noted that it is dominated by α-pinene (46%), limonene (11.5%) and myrcene (10.5%). Akimov et al. (1976) reported that α-pinene ranged from 27 to 51%, myrcene from 4.2 to 36.5%, and limonene from 4.6 to 12.1% for *J. seravschanica*. No reports were found on the leaf oil of *J. talassica*.

de Teresa et al. (1980) found the berry oil of *J. thurifera* to be dominated by limonene (88.5%). San Feliciano et al. (1988) reported a number of sesquiterpenes and diterpenes from the leaf oil of *J. thurifera*. Akimov et al. (1976) found the leaf oil of *J. thurifera* to have small amounts of monterpenes (less than 2.6% for α-pinene, the largest monoterpene hydrocarbon), but 86.7% for the combined oxygenated mono- and sesquiterpene fraction (individual compounds not reported). The only report discovered on the leaf oil of *J. turcomanica* (Karryev, 1967) compared the biological activities of various juniper oils.

In this paper, both the volatile leaf oils and DNA fingerprints will be examined for the multi-seeded *Juniperus* of the eastern hemisphere to aid in understanding of the circumspection of the species.

2. Materials and methods

Specimens used in this study: *J. chinensis*, Adams 6765-6767-Lanzhou, Gansu, China; *J. davurica*, Adams 7252, 7253–15 km se Ulan Bator, Mongolia; *J. excelsa*, Adams 5983–5887–7 km w of Lemos, Greece; *J. excelsa* var. *polycarpos*, Adams 6139–6141–Tbilisi Botanic Garden, Georgia, CIS; *J. foetidissima*, Adams 5645–5646–Mt. Parnassus, Greece, and 5982, 5986, 7 km w. Lemos, Greece; *J. phoenicea*, Adams 5653, 5654–10 km n Nea Epoidavios, and 7077–El Penon, Spain; *J. jarkendensis*, Adams 7820-7825–Oeetak, Xinjiang, China; *J. procera*, Adams 6184, 6185–40 km w of Addis Ababa, Ethiopia, and Adams 5333–5335–38 km nw of Nairobi, Kenya; *J. sabina*, Adams 7811–7813–30 km n Jarkent, Kazakstan; *J. sabina* var. *erectopatens*, Adams 8432–8434–24 km s Songpan, Sichuan, China; *J. seminglobosa*, Adams 8210–8212–60 km sw Bishket, Kyrgystan, and 8227, 8229, 8230–2 km s Dzhabagly, Kazakstan; *J. seravschanica*, Adams 8224-8226–2 km s Dzhabagly, Kazakstan; *J. seravschanica* (collected as *J. excelsa* var. *polycarpos*), Adams 8483-8486 – Quetta, Balochistan, Pakistan; *J. talassica*, Adams 8220-8223–20 km s Talas, Kyrgystan; *J. turcomanica*, Adams 6713–6716 – Almaty Botanic Garden, Kazakstan (origin = Ashgabat, Turkmenistan); *J. thurifera*, Adams 7083–7085–2 km e Ruidera, Spain. Voucher specimens for all collections are deposited at SRCG.

Fresh leaves (200 g fresh wt.) were steam distilled for 2 h using a circulatory Cleavenger apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at $-20°C$ until analyzed. The extracted leaves were oven-dried (48h, 100°C) for determination of oil yields. After initial GCMS analyses, composite oil samples were made for each of the taxa in this study. These composite (average) oil samples were then subjected to GCMS for compound identification and quantitation by TIC.

The essential oils were analyzed on a Finnigan Ion Trap (ITD) mass spectrometer, model 800, directly coupled to a Varian 6500 gas chromatograph, using a J and W DB-5, 0.26 mm × 30 m, 0.25 μm coating thickness, fused silica capillary column (see Adams, 1995 for operating details). Identifications were made by library searches of our volatile oil library, LIBR(TP) (Adams, 1995), using the Finnigan library search routines based on fit and purity, coupled with retention time data of reference compounds.

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 the hot CTAB protocol (Doyle and Doyle, 1987) with 1% (w/v) PVP added to the extraction buffer. The RAPDs analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5′-3′): 134: AAC ACA CGA G; 153: GAG TCA CGA G; 184: CAA ACG GAC C; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 244: CAG CCA ACC

G; 249: GCA TCT ACC G; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 268: AGG CCG CTT A; 327: ATA CGG CGT C; 338: CTG TGG CGG T; 346: TAG GCG AAC G; 347: TTG CTT GGC G.

PCR was performed in a volume of 12.5 μl containing 50 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each DNTPs, 0.36 μM primers, 0.25 ng genomic DNA, and 0.5 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94°C (1.5 min) for initial strand separation, then 40 cycles of 38°C (2 min), 72°C (2 min), 91°C (1 min). Two additional steps were used: 38°C (2 min) and 72°C (5 min) for final extension. Bands were scored in four classes: very bright (= 6); medium bright (= 5), faint (= 4) and absent (= 0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower, 1971; Adams, 1975a,1975b). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966). Program PCO3D is available for MS DOS IBM compatibles with a hard disk and math co- processor (correspond to RPA for distribution details).

3. Results and discussion

The compositions of the volatile leaf oil are given in Table 2. Several trends are apparent from the terpenoid data. Notice that several of the taxa are dominated by sabinene and most of the other taxa are dominated by either α-pinene or myrcene (although the oil of *J. thurifera* is dominated by limonene and *J. procera* is dominated by the diterpenes). Clearly combinations of various compounds easily distinguish between the taxa. The two most similar oils are *J. semiglobosa* and *J. talassica* (SM and TA in Table 2), which differ in methyl citronellate, geranial, germacrene D-4-ol, and a couple of trace components. Several of the taxa contained considerable amounts of heartwood oil components (α-cedrene, β-cedrene, cis-thujopsene, and cedrol) in the leaves (Table 2). In each case, cedrol and related heartwood compounds were found to be polymorphic in the samples. Amounts of cedrol ranged from 0 to 40% from tree to tree in a single population. Cedrol is very rare in the western hemisphere junipers, being found in a trace in only *J. barbadensis* from St. Lucia (Adams, 1995). The presence of the heartwood compounds in the leaves and their mosaic mode of expression seems to be an evolutionary adaptation found only in several taxa of *Juniperus* of the eastern hemisphere.

The leaf oil of *J. foetidissima* was also found to be very variable in the amounts of cis- and trans-thujone. In general, these high levels of chemical polymorphisms found in these *junipers* are not found in *Juniperus* of the western hemisphere.

In order to assimilate the overall trend in the volatile leaf oils, similarities were first computed using quantitative similarities. This resulted in clustering of *J. talassica* and

Table 2
Comparisons of the per cent total oil for leaf essential oils for *J. davurica* (DA), *J. sabina* (SA), *J. sabina* var. *erectopatens* (ER), *J. semiglobosa* (SM), *J. talassica* (TA), *J. jarkendensis* (JA), *J. chinensis* (CH), *J. excelsa* (EX), *J. excelsa* var. *polycarpos* (EP), *J. seravschanica* – Kazakstan (SK), *J. seravschanica* - Pakistan (SP), *J. turcomanica* (TU), *J. foetidissima* (FT), *J. thurifera* (TH), *J. phoenicea* (PH) and *J. procera* (PR). Components that tend to separate the species are highlighted in boldface

KI	Compound	DA	SA	ER	SM	TA	JA	CH	EX	EP	SK	SP	TU	FT	TH	PH	PR
926	tricyclene	–	t	–	t	t	t	t	0.1	0.1	0.3	0.1	0.2	–	t	0.3	t
931	**α-thujene**	**1.3**	**1.7**	**0.8**	**2.4**	**2.6**	**2.0**	**0.7**	**–**	**–**	**0.6**	**0.4**	**t**	**1.3**	**t**	**–**	**t**
939	**α-pinene**	**1.9**	**17.2**	**1.5**	**3.7**	**3.4**	**4.4**	**16.7**	**22.5**	**26.5**	**44.4**	**15.5**	**59.7**	**2.6**	**2.2**	**41.8**	**12.5**
953	α-fenchene	–	–	–	–	–	t	–	0.2	t	–	0.2	t	t	t	t	0.1
953	camphene	–	0.2	–	t	t	0.1	0.2	0.5	1.0	0.5	0.2	0.5	t	t	0.6	0.1
957	thuja-2,4(10)-diene	–	–	–	–	–	–	–	0.1	–	–	–	t	–	–	0.1	–
975	verbenene	–	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–
976	**sabinene**	**35.4**	**36.8**	**33.3**	**44.1**	**45.7**	**43.0**	**17.8**	**t**	**0.1**	**0.9**	**0.5**	**0.4**	**15.9**	**1.0**	**0.1**	**t**
978	1-octen-3-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	0.5	–	0.3
980	β-pinene	0.1	1.9	0.1	0.3	0.3	0.2	0.4	0.6	1.0	2.2	1.2	1.8	–	0.2	1.3	1.2
991	myrcene	2.8	4.8	1.0	4.8	4.7	3.6	3.2	1.9	2.2	19.2	20.7	3.7	2.0	3.4	4.5	1.2
996	**hexanoic acid, 4-methyl, methylester***	–	–	**0.8**	**0.3**	**0.3**	**0.3**	–	–	–	–	–	–	–	–	–	–
1001	δ-2-carene	0.3	0.2	0.2	–	–	–	–	–	–	–	–	–	–	2.2	0.1	–
1005	α-phellandrene	t	t	–	0.1	0.1	0.1	–	0.1	0.1	0.1	0.1	t	0.2	t	0.6	–
1011	δ-3-carene	–	0.2	–	t	t	t	–	2.3	0.4	–	3.5	t	t	1.1	–	6.1
1018	α-terpinene	0.8	0.9	0.4	2.1	2.2	1.3	0.4	0.1	0.1	0.1	0.1	t	4.4	0.1	t	t
1026	p-cymene	0.3	0.1	t	2.8	0.4	0.2	0.3	0.4	0.2	0.1	0.7	0.2	0.4	t	0.8	t
1028	sylvestrene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
1031	limonene	1.6	3.2	37.1	2.4	1.6	1.4	15.1	22.6	5.5	4.4	9.0	1.8	0.8	51.5	4.7	0.2
1031	β-phellandrene	t	0.9	–	0.4	0.9	0.4	t	t	–	0.5	1.0	0.1	0.6	–	3.5	0.8
1032	1,8-cineole	–	–	–	–	–	–	–	–	t	–	–	–	–	–	–	t
1050	(E)-β-ocimene	0.4	0.1	0.2	0.3	0.4	0.2	0.2	t	–	t	0.2	t	–	0.3	0.1	t
1062	γ-terpinene	1.4	1.5	0.5	3.3	3.2	2.1	0.7	0.6	0.9	1.4	1.3	0.6	6.5	0.2	0.7	t
1068	cis-sabinene hydrate	1.9	0.6	0.3	1.3	1.5	1.2	0.6	–	–	0.1	0.2	–	01.9	0.1	–	–
1074	trans-linalool oxide(furanoid)	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4	–	–
1088	terpinolene	0.7	1.0	0.6	1.5	1.5	1.0	0.6	0.9	1.1	1.5	1.7	1.3	1.9	1.0	1.3	1.1
1091	2-nonanone	0.9	0.6	–	t	t	–	–	–	–	–	–	–	–	–	–	–

1097	**trans-sabinene hydrate**	–	–	**0.1**	**0.9**	**0.8**	**0.8**	**0.4**	–	–	–	t	t	**1.9**	–	–	–
1098	linalool	0.9	0.8	–	1.1	2.1	0.9	1.0	–	0.1	0.5	0.7	t	t	7.1	0.7	0.5
1102	n-nonanal	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1102	**cis-thujone (=α-thujone)**	–	–	–	–	–	**0.3**	–	–	–	–	–	–	**18.6**	–	–	–
1103	isopentyl-isovalerate	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1110	1,3,8-p-menthatriene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	t
1111	cis-rose oxide	0.2	–	–	t	t	–	–	–	–	–	–	–	–	–	–	–
1112	endo-fenchol	–	–	–	–	–	–	–	0.2	–	–	–	t	–	–	–	–
1114	trans-thujone(= β-thujone)	–	0.4	–	0.1	0.1	3.6	–	–	–	0.1	–	0.2	3.5	–	–	–
1116	3-methyl butanoate,	–	0.3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1121	3-methyl-3-butenyl-cis-p-menth-2-en-1-ol	0.4	–	0.1	0.4	0.4	0.4	0.2	0.1	–	t	–	–	1.2	0.4	0.1	t
1125	α-campholenal	–	–	–	–	–	–	–	0.1	0.2	–	0.1	0.2	–	–	0.6	t
1127	trans-rose oxide	0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	t
1133	iso-3-thujanol	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–
1134	cis-limonene oxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
1139	trans-pinocarveol	–	–	–	–	–	–	–	0.2	0.3	–	t	t	–	–	0.6	t
1140	trans-sabinol	–	–	–	–	–	0.3	–	–	–	–	–	–	1.1	–	–	–
1140	trans-p-menth-2-en-1-ol	0.2	0.1	–	0.2	0.2	0.2	–	–	–	–	–	–	–	0.3	–	–
1143	camphor	–	–	–	–	–	–	–	0.5	0.2	t	–	1.7	–	–	0.4	t
1143	cis-sabinol[a]	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	0.2
1143	trans-verbenol	–	–	–	–	–	–	–	–	5.5	–	–	–	–	–	–	–
1148	camphene hydrate	–	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1149	neo-3-thujanol	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–
1153	citronellal	0.6	–	–	0.4	0.2	0.1	–	–	–	–	–	–	–	–	–	–
1156	sabina ketone	–	–	–	t	0.1	t	–	–	–	–	–	–	0.2	–	–	–
1159	p-mentha-1,5-dien-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
1163	pinocarvone	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–
1165	borneol	–	–	–	–	–	–	–	–	t	t	t	t	t	–	–	0.2
1166	3-thujanol	–	–	–	–	–	–	–	–	–	–	–	–	t	–	–	–
1167	δ-terpineol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6	–
1170	2-(2-propenyl)-phenol[a]	–	–	–	0.1	t	–	–	–	–	–	–	–	–	0.2	–	–
1177	**terpinen-4-ol**	**5.6**	**4.1**	**0.9**	**6.8**	**6.4**	**6.0**	**2.1**	**0.2**	**0.2**	**0.4**	**0.3**	**0.2**	**17.6**	**0.6**	**0.2**	**0.1**
1180	m-cymen-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1

—continued

Table 2—*continued*

KI	Compound	DA	SA	ER	SM	TA	JA	CH	EX	EP	SK	SP	TU	FT	TH	PH	PR
1181	thuj-3-en-10-al	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–
1183	p-cymen-8-ol	–	–	–	–	–	–	–	–	–	–	–	–	0.1	t	0.2	t
1185	trans-p-mentha-1(7),8-dien-2-ol	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
1189	α-terpineol	0.2	0.2	–	2.2	0.2	0.2	0.1	t	0.1	0.1	t	0.2	0.7	0.7	0.8	0.5
1191	myrtenol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–
1191	hexyl butyrate	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–
1193	4Z-decenal	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1193	cis-piperitol	–	t	–	0.2	t	t	–	–	–	–	–	–	0.3	0.1	–	–
1195	methyl chavicol(= estragole)	–	–	–	–	–	–	0.3	–	–	–	–	–	–	–	–	–
1204	verbenone	–	–	–	–	–	–	–	0.1	0.1	–	–	t	–	–	–	–
1205	trans-piperitol	t	t	–	t	t	–	–	–	–	–	–	–	0.3	–	–	–
1217	trans-carveol	–	–	–	–	–	–	–	0.1	0.1	–	–	–	–	–	t	–
1220	endo-fenchyl acetate	–	–	–	–	–	–	–	0.3	0.1	–	–	–	–	–	–	–
1228	citronellol	4.8	0.3	–	0.9	–	0.2	0.3	–	–	–	–	–	–	0.4	0.6	–
1237	butanoic acid, 3-methyl-3Z-hexen-1-yl ester	–	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1240	neral	–	–	–	–	0.1	t	–	–	–	–	–	–	–	–	–	–
1242	hexyl 3-methyl butanoate	–	–	–	–	–	–	–	–	–	–	t	0.4	–	–	–	–
1252	piperitone	t	0.1	–	–	–	–	–	–	–	–	–	–	–	3.2	–	–
1252	trans-sabinene hydrate acetate	t	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1257	linalyl acetate	t	0.2	–	–	–	–	–	–	–	–	–	–	–	7.6	2.0	–
1257	4Z-decen-1-ol	1.4	–	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–
1261	methyl citronellate	5.4	t	–	2.1	–	1.5	t	–	–	t	–	–	–	–	–	–
1270	geranial	–	–	–	–	0.2	0.1	–	–	–	–	–	–	–	–	–	–
1273	isopulegol acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.3	–
1274	unknown,79,91,105,147,FW162	–	–	13.2	–	–	–	–	–	–	–	–	–	–	–	–	0.3
1279	methyl nerolate	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–	–
1281	iso-isopulegol acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–
1284	E-anethole	–	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–

1285	bornyl acetate	t	0.5	0.2	0.4	0.1	0.1	0.1	0.4	0.9	1.0	0.6	0.7	0.1	0.3	–	0.4
1285	safrole	–	–	–	–	–	–	5.1	–	–	–	–	–	–	–	–	–
1286	linalool oxide acetate(pyranoid)	–	–	–	t	t	–	–	0.2	0.1	–	–	–	–	0.3	0.5	–
1290	trans-sabinyl acetate	–	–	–	t	t	6.2	–	–	–	–	0.1	–	0.9	–	–	–
1291	2-undecanone	4.4	0.1	–	0.2	0.4	–	–	–	–	–	–	–	–	–	–	–
1312	decadienal isomer?	–	–	–	–	–	–	–	3.3	5.6	–	–	–	–	1.2	–	–
1319	2E,4E-decadienal	–	–	–	0.1	t	1.5	–	–	–	t	t	t	–	–	–	–
1321	aromatic, 149, FW 164	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	0.3	–
1323	methyl geranate	–	0.6	–	0.6	1.8	–	–	–	–	–	–	–	–	–	–	–
1350	**α-terpinyl acetate**	–	**0.2**	–	–	–	–	–	–	–	–	–	–	–	**3.2**	**4.6**	–
1365	neryl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2	–	–
1376	α-copaene	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–
1383	geranyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	t	–
1383	β-bourbonene	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	–	–
1381	hexyl n-hexanoate	–	–	–	–	–	–	–	–	–	–	–	0.7	–	–	–	–
1389	β-cubebene	–	–	–	–	–	–	–	0.1	0.1	–	–	–	–	–	t	–
1401	methyl eugenol	–	–	–	–	–	–	3.4	–	–	–	–	–	–	–	–	–
1409	α-cedrene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1409	1,7-di-epi-β-cedrene	–	0.2	–	–	–	0.1	t	–	t	–	–	–	–	–	–	–
1418	(E)-caryophyllene	–	–	–	0.2	0.2	t	0.2	0.9	0.5	0.1	0.2	–	–	–	–	–
1418	β-cedrene	–	–	–	0.2	0.2	t	0.2	0.9	0.5	0.1	0.2	–	–	–	–	–
1429	cis-thujopsene	–	0.3	–	0.3	0.3	0.1	0.3	0.3	0.2	0.2	0.4	–	–	–	–	–
1446	cis-muurola-3,5-diene	t	–	–	–	–	–	–	0.2	0.6	t	–	–	–	–	–	–
1454	α-humulene	t	–	–	–	–	–	–	0.2	0.2	–	–	–	0.1	–	0.3	0.7
1458	E-β-farnesene	–	–	–	–	–	–	–	0.2	0.1	–	0.1	–	–	–	–	–
1461	cis-muurola-4(14),5-diene	0.2	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–
1466	β-acoradiene	–	–	–	–	–	–	–	0.1	t	–	t	–	–	–	–	–
1473	trans-cadina-1(6),4-diene	–	–	–	–	–	–	–	0.4	0.8	–	–	–	–	–	–	–
1477	γ-muurolene	0.1	–	–	–	–	–	0.1	–	t	t	–	0.1	–	–	–	–
1480	**germacrene D**	**0.2**	–	**0.2**	–	–	–	**0.1**	**0.9**	**1.7**	**0.1**	**0.2**	**0.8**	–	–	**0.8**	**0.3**
1490	cis-β-guaiene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.9	–
1491	trans-murrola-4(14),5-diene	0.1	–	–	–	–	–	–	0.4	1.4	t	–	0.1	–	–	–	–
1493	epi-cubebol	0.2	–	–	–	–	–	t	–	1.3	t	–	0.2	–	–	0.6	–

—continued

Table 2—*continued*

KI	Compound	DA	SA	ER	SM	TA	JA	CH	EX	EP	SK	SP	TU	FT	TH	PH	PR
1499	γ-amorphene	–	–	–	–	–	–	–	–	–	–	t	–	–	–	–	–
1499	α-muurolene	0.3	0.2	–	–	–	–	0.1	0.2	0.1	0.2	0.2	0.2	t	0.1	0.2	–
1499	bicyclogermacrene	–	–	–	–	–	–	–	–	–	–	t	–	–	–	–	–
1502	cuparene	–	–	–	–	–	–	–	–	–	–	t	–	–	–	–	–
1503	germacrene A	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–	–
1508	E,E-α-farnesene	–	–	–	t	t	t	–	–	–	–	–	–	–	–	–	–
1509	β-bisabolene	–	–	–	–	–	–	–	–	–	–	t	–	–	–	–	–
1512	α-alaskene	–	–	–	–	t	–	t	0.3	t	–	0.4	–	–	–	–	–
1513	γ-cadinene	1.1	0.3	–	t	–	–	0.2	–	–	0.4	0.4	0.9	t	0.1	–	–
1513	cubebol	–	–	–	–	–	–	–	0.8	2.6	–	–	–	–	–	0.8	–
1521	cis-calamenene	–	–	–	–	–	–	–	t	–	–	–	–	–	–	t	–
1524	δ-cadinene	1.5	0.8	t	0.2	t	0.1	0.5	0.7	1.5	1.0	0.7	1.2	t	0.4	1.3	–
1532	trans-γ-bisabolene	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–
1532	trans-cadina-1,4-diene	–	–	–	–	–	–	–	t	0.2	t	–	0.2	–	–	t	–
1538	α-cadinene	0.2	–	–	–	–	–	t	–	–	0.1	0.1	0.2	–	t	–	–
1549	elemol	–	–	3.7	t	t	0.1	–	–	–	0.5	0.5	1.9	t	1.2	–	4.3
1554	elemicin	–	–	–	–	–	–	0.5	–	–	–	–	–	–	–	–	–
1556	germacrene B	t	–	–	t	t	t	–	–	–	0.4	0.6	1.8	–	0.2	t	–
1570	Z-hexenyl benzoate	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–
1574	germacrene D-4-ol	5.7	0.8	0.1	0.2	–	t	0.6	–	t	0.5	2.0	1.5	–	0.6	–	0.1
1581	caryophyllene oxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.3	0.5
1587	**sesquiterpene, FW220?**	**–**	**–**	**–**	**1.1**	**0.8**	**0.4**	**1.1**	**1.9**	**1.7**	**1.0**	**1.7**	**–**	**t**	**–**	**–**	**–**
1596	**cedrol**	**t**	**15.2**	**–**	**14.8**	**15.2**	**14.5**	**20.1**	**28.1**	**30.8**	**14.6**	**26.4**	**–**	**3.2**	**t**	**–**	**–**
1606	humulene epoxide II	–	–	–	–	–	–	–	t	–	–	–	–	–	–	t	0.5
1606	β-oplopenone	0.5	–	–	0.1	–	t	0.9	t	–	–	–	0.2	0.5	0.2	–	–
1607	4E-tridec-6-yne[a]	–	–	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–
1611	epi-cedrol	–	–	–	–	–	t	–	–	–	–	–	–	–	–	–	–
1627	1-epi-cubenol	–	–	–	–	–	–	–	1.6	2.2	–	–	–	–	0.2	2.3	–
1630	α-acorenol	–	0.2	–	–	–	t	–	–	–	–	–	–	–	–	–	–
1630	γ-eudesmol	–	–	–	–	–	–	–	–	–	–	–	0.5	–	0.3	–	1.4

1640	epi-α-cadinol	0.7	0.3	–	t	–	t	0.3	t	0.3	0.5	0.4	0.9	0.3	0.3	t	–
1640	epi-α-muurolol	0.7	0.3	–	t	–	–	0.4	t	0.5	0.1	0.1	0.9	0.3	0.3	–	–
1642	cubenol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7	–
1645	α-muurolol	0.2	t	–	–	–	–	0.1	t	0.2	t	t	0.4	t	t	0.2	–
1649	β-eudesmol	–	–	0.4	–	–	–	–	–	–	t	0.1	0.8	–	0.6	–	2.3
1652	α-eudesmol	–	–	0.9	–	–	–	–	–	–	0.1	t	0.5	–	0.7	–	3.8
1653	α-cadinol	–	0.8	–	0.2	–	t	1.3	t	0.2	0.9	0.8	3.2	1.0	0.8	0.7	–
1666	bunesol	–	–	0.4	–	–	–	–	–	–	–	0.1	0.4	–	0.3	–	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	–	–	–	–	–	–	–	–	–	0.4	–	3.6	–	0.5	0.7	–
1688	cadinol isomer	–	–	–	–	–	–	–	–	–	–	1.2	–	–	–	–	–
1733	oplopenone	–	–	–	–	–	–	0.6	–	–	–	–	–	–	–	–	–
1789	8-α-acetoxyelemol	–	–	1.5	–	–	–	–	–	–	–	–	–	–	0.3	–	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	t	–	–	–	–	t	–	–	0.3
1989	manoyl oxide	–	–	–	–	–	t	–	t	0.2	–	0.3	–	0.2	0.3	–	0.5
2010	epi-13-manoyl oxide	–	–	–	–	–	–	–	–	–	–	–	–	0.4	t	–	–
2054	abietatriene	t	–	–	–	–	–	t	t	0.4	–	t	0.3	0.2	t	0.5	1.3
2080	abietadiene	0.9	–	–	–	t	t	t	0.3	2.2	–	0.2	–	0.4	0.2	0.6	15.4
2103	diterpene, 41,79,191,257,FW286?	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.6
2147	**abieta-8(14),13(15)-diene**[a]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**0.3**
2181	diterpene,41,91, 271,257,FW286	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8
2278	cis-totarol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6
2288	4-epi-abietal	0.9	–	–	t	t	t	0.2	0.1	1.2	–	1.0	1.7	–	–	t	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	–	–	–	–	–	–	t	–	–	–	–	–	0.4	–	1.9	21.4
2325	trans-ferruginol	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–	0.3	3.4

KI = Kovat's Index on DB-5(= SE54) column.

[a]Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

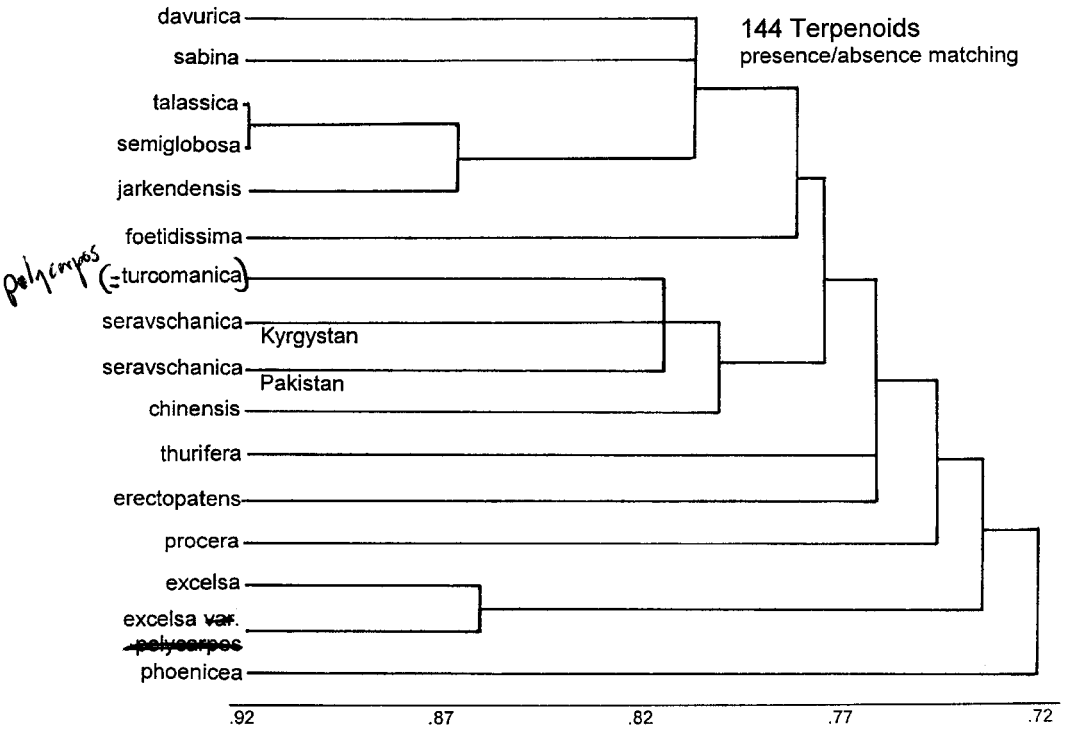

Fig. 1. Minimum spanning network based on 144 terpenoids, with similarities computed as presence/absence data. Notice the very close similarity of *J. talassica* and *J. semiglobosa*, the clustering of *J. turcomanica* with *J. seravschanica* and the clustering of *J. excelsa* and *J. e var. polycarpos*.

J. semiglobosa. But a sub-group was formed containing *J. sabina* and *J. seravschanica* (Kyrgystan and Pakistan). That combination does not seem likely based on morphology. In addition, the morphologically very similar taxa, *J. excelsa* and *J. excelsa* var. *polycarpos*, did not link together using quantitative matching. It seems that at the species level, quantitative matching of leaf terpenoid data may not always be useful.

The taxa were then analyzed by the computation of similarities based on simple presence/absence matching. This diagram (Fig. 1) shows that *J. jarkendensis* (treated as conspecific with *J. semiglobosa* by Farjon (1992)), clusters with *J. semiglobosa*/*J. talassica*. In addition, the two *J. seravschanica* samples (Kyrgystan, Pakistan) cluster together (Fig. 1) as do *J. excelsa* and *J. excelsa* var. polycarpos. Note that *J. sabina* var. *erectopatens* is very distinct from *J. sabina* (as distinct as several other species, Fig. 1). In this analysis, *J. phoenicea* is the most distinct taxon, followed by *J. excelsa*, *J. procera*, *J. erectopatens*, and *J. thurifera*.

The initial analysis of the RAPD bands resulted in 231 bands that were generated by 14 random primers and yielded an average of 16.5 bands/primer. A preliminary inspection of the bands revealed that several bands were merely local polymorphisms (i.e., present in only one individual), several bands were present in several individuals

but not in two individuals of the same taxon, some bands showed fidelity (present in both samples of a taxon) in some taxa but not in others and some bands showed both fidelity and discrimination characteristics. DNA fingerprinting can be used at several taxonomic levels, depending on the primer used; ranging from intra-generic levels (Adams and Demeke, 1993) to distinguishing between individuals (Demeke et al., 1992). It is important to realize that the presence of a band could be very significant when searching for a marker for disease resistance (for example), but in the present instance, fidelity is needed to screen out this "individual variation". Thus, 40 bands were removed leaving 191 bands for taxonomic analysis.

This analysis shows a close parallel to the terpene analysis (Fig. 2). Both data sets reveal the *talassica-semiglobosa-jarkendensis*, *excelsa-excelsa* var. *polycarpos*, and *turcomanica-seravschanica* groups (Fig. 1 and Fig. 2). In addition, *J. davurica*, *J. sabina*, *J. foetidissima*, *J. thurifera*, *J. phoenicea*, *J. sabina* var. *erectopatens*, and *J. procera* are very distinct entities based on the RAPD data (Fig. 2). *Juniperus jarkendensis* weakly clusters with *J. semiglobosa-talassica*, followed closely by *J. sabina*. There is some support for Farjon (1992) inclusion of *J. jarkendensis* under *J. semiglobosa*, but con-specific status does not seem appropriate (even while admitting that the two taxa are morphologically almost indistinguishable).

The data sets support the recognition of *J. polycarpos* as a variety of *J. excelsa*. Both Farjon (1992) and Silba (1986) Silba (1990) considered *J. seravschanica* and *J. turcomanica* to be synonyms of *J. excelsa* var. *polycarpos* (Table 1). However, both data sets indicate that these taxa are quite distinct from *J. excelsa* var. *polycarpos* (Fig. 1 and Fig. 2).

The Balochistan, Pakistan juniper is still called *J. excelsa* (Ciesla et al., 1998). So it was of interest to analyze samples from near Quetta, Ziarat District, Balochistan. It seemed these might be *J. excelsa* var. *polycarpos*, but during analyses they were clearly most associated with *J. seravschanica* from Kyrgystan (Fig. 1 and Fig. 2). It should be noted that the separation of *J. seravschanica* from *J. turcomanica* is just about the same as between *J. excelsa* and *J. excelsa* var. *polycarpos* (Fig. 2). It would seem prudent to recognize this relationship. Because *J. turcomanica* was published before *J. seravschanica* (26 March, 1932 vs. 14 October 1932, A. Farjon, pers. communication), then these taxa are now: *Juniperus turcomanica* B. Fedtsch. and *Juniperus turcomanica* B. Fedtsch. var. *seravschanica* (Kom.) R.P. Adams, *comb. nov.* So the Balochistan, Ziarat juniper should be referred to as *J. turcomanica* var. seravschanica not *J. excelsa* or *J. macropoda*.

Juniperus talassica has been separated on the basis of "fruits with a high sugar content..., foliage conspicuously weeping.." (Komarov, 1934). I was unable to verify the high sugar content or conspicuously weeping in the field in Kazakstan and Kyrgystan, and the terpene data showed essentially no differences between *J. talassica* and *J. semiglobosa*. However, the RAPD data seems to indicate that there is some differentiation (Fig. 2). It seems reasonable to recognize *J. talassica* as a variety of *J. semiglobosa*, pending additional information (*J. semiglobosa* var. *talassica* (Lipsky) Silba).

Several investigators considered *J. procera* to be conspecific with *J. excelsa* (Kerfoot, 1966; Kerfoot and Lavranos, 1984; Hall, 1984). However, both the terpenoids

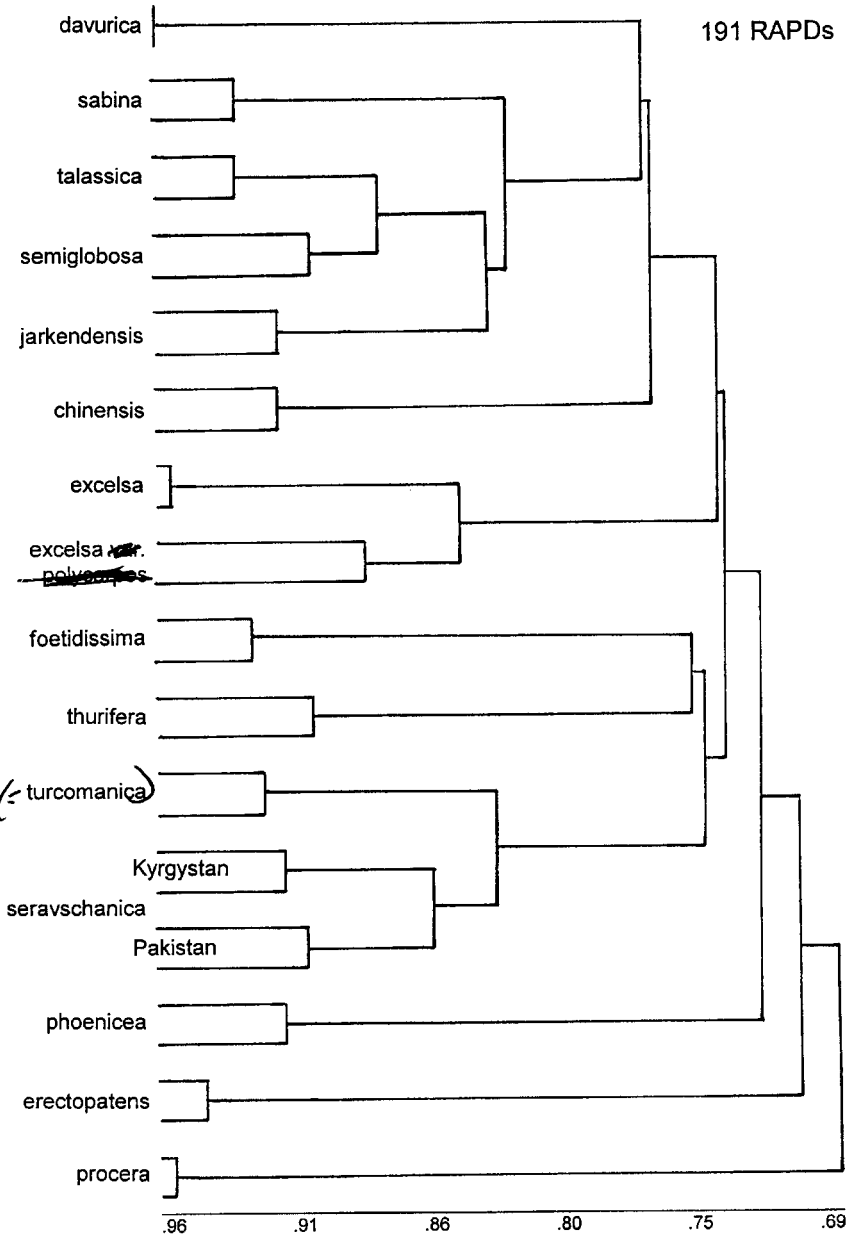

Fig. 2. Minimum spanning network based on 191 RAPD bands. Each OTU is represented by two individuals. Notice the very close similarity of *J. talassica* and *J. semiglobosa*, the clustering of *J. turcomanica* with *J. seravschanica* and the clustering of *J. excelsa* and *J. e.* var. *polycarpos*. which is similar to the pattern presented by the terpenoid data (Fig. 1). Juniperus sabina var. erectopatens and *J. procera* are the two most distinct taxa in this analysis.

and RAPDs show that *J. procera* is very distinct from *J. excelsa* (and all the other junipers in this study, Fig. 1 and Fig. 2). Yet, it is with difficulty that *J. excelsa* and *J. procera* can be distinguished, morphologically. Clearly, the evolution of morphology, chemistry and DNA mutations are not always concurrent.

Finally, we have the case of *J. sabina* var. *erectopatens*, an upright tree found in the mountains of Sichuan, China. Yu and Fu (1997) recently recognized this small tree as a variety. The author found a few of these trees near the type locality (Songpan, 2700 m, Sichuan). Morphologically, they are similar to *J. sabina*. However, both the terpenoids (Table 1, Fig. 1) and RAPDs (Fig. 2) reveal numerous differences between *J. sabina* and *J. sabina* var. erectopatens. In fact, this taxon is one of the most distinct in this group. It is unfortunate that very little is known about the biology of this taxon. But in view of its obvious divergence in both terpenoids and DNA, it deserves recognition at the specific level:

J. erectopatens (Cheng and L. K. Fu) R. P. Adams, *stat nov.*

Basionym: *Sabina vulgaris* Antoine var. *erectopatens* Cheng and L. K. Fu, Acta Phytotax. Sin. 13: 86, 1975. TYPE: China, n Sichuan, Songpan, 2700 m, T.T. Yu 2702 (holotype, PE). Syn.: *Juniperus sabina* L. var. *erectopatens* (Cheng and L.K. Fu) Y.F. Yu and L.K. Fu, Novon 7: 444, 1997.

This study has resolved several of the difficult taxonomic problems in this section of *Juniperus*. Perhaps the most surprising aspect of this research has been the divergence of morphologically near-identical taxa in their terpenoids and DNA fingerprints. Species that are separated by even minute morphological character differences (*J. semiglobosa-talassica*; *J. excelsa-J. procera*; *J. sabina – sabina* var. *erectopatens*; *J. turcomanica-seravschanica – excelsa* var. *polycarpos*; and *J. semiglobosa – jarkendensis*) were found to to possess considerable terpenoid and DNA differences. It seems apparent that evolution proceeds at different rates for different character sets. The use of multiple character sets seems prudent in *Juniperus* taxonomy and evolutionary studies.

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