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Systematics of the one seeded *Juniperus* of the eastern hemisphere based on leaf essential oils and random amplified polymorphic DNAs (RAPDs)

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Abstract

The compositions of the leaf essential oils of all the one seed/cone species of *Juniperus* (sect. *Sabina*) of the eastern hemisphere are reported and compared (*J. convallium*, *J. convallium* var. *microsperma*, *J. indica*, *J. komarovii*, *J. pingii*, *J. pingii* var. *carinata*, *J. prezewalskii*, *J. pseudosabina*, *J. recurva*, *J. recurva* var. *coxii*, *J. saltuaria*, *J. squamata*, *J. squamata* var. *morrisonicola*, *J. tibetica*, *J. wallachiana*). In addition, DNA fingerprinting by RAPDs was utilized. The combined terpenoid and DNA data supported the continued recognition of the aforementioned taxa as distinct species except for four varieties which were recognized at the specific level: *Juniperus carinata* (Y.K. Yu & L.K. Fu) R.P. Adams, *stat. nov.* (Syn.: *J. pingii* var. *carinata*); *J. coxii* A.B. Jacks. (Syn.: *J. recurva* var. *coxii*); *Juniperus microsperma* (Cheng & L.K. Fu) R.P. Adams, *stat. nov.* (Syn.: *J. convallium* var. *microsperma*); *J. morrisonicola* Hayata (Syn.: *J. squamata* var. *morrisonicola*).

Keywords: *Juniperus*; *Cupressaceae*; Essential oils; Terpenes; RAPDs; DNA polymorphisms; Chemosystematics

1. Introduction

The group of one seed/cone (one seeded) *Juniperus* species, section *Sabina*, of the eastern hemisphere appears to be a natural division of *Juniperus* (Adams and Demeke, 1993). These junipers are characterized by having single seeded female, black

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(occasionally brownish-black) cones that are usually pointed on the seed tip end. This is the fourth paper in the series (Adams, 1999a,b, 2000) to serve as the basis for a modern monographic treatment of the genus *Juniperus*. The purpose of this paper is to make extensive reports on the leaf essential oils of *J. komarovii* Florin (= *J. glaucescens* Florin), *J. pingii* Cheng & Ferre, *J. recurva* Buch.-Ham. ex D. Don var. *coxii* (A.B. Jacks.) Melville, *J. squamata* D. Don in Lamb., *J. s.* var. *morrisonicola* (Hayata) Li & Keng, *J. tibetica* Kom., *J. wallachiana* Hook F. & Thomson ex Brandis, plus an alpine form of *J. pingii* (*J. pingii* var. *carinata* Y.F. Yu & L.K. Fu) and a putative variety of *J. convallium* Rehder & Wilson (*J. c.* var. *microsperma* (W.C. Cheng & L.K. Fu) Silba) from Sichuan, China that the author found growing in a permanently wet, seep area at 3530 m elevation. *Juniperus pingii* var. *carinata* is common on alpine passes in western Yunnan, China. It is not conspecific with *J. pingii* var. *wilsonii* (Rehder) Silba (which I treat as *J. squamata* f. *wilsonii* Rehder).

Although I have reported on the essential leaf oils of several of these one-seeded junipers, their compositions are included in tabular form for comparison purposes: *J. convallium* Rehd. & Wils. (Adams et al., 1993a); *J. indica* Bertol. (Adams & Chaudhary, 1996); *J. przewalskii* Kom. (Adams et al., 1994); *J. pseudosabina* Fisch., May. & Ave-Lall. (Adams et al., 1998a,b); *J. saltuaria* Rehd. & Wils. (Adams et al., 1993b); *J. recurva* Buch.-Ham. ex. D. Don (Adams et al., 1998a,b).

There appear to be no reports in the open literature on the leaf essential oils for *J. convallium* var. *microsperma*, *J. komarovii*, *J. pingii*, *J. p.* var. *carinata*, *J. recurva* var. *coxii*, *J. squamata* var. *morrisonicola*, *J. tibetica*, or *J. wallachiana*. The literature has been reviewed and the oils reported for *J. squamata* from Nepal and India (Adams et al., 1998a,b) and *J. squamata* var. *fargesii* (Adams et al., 1996) from Gansu. Weyerstahl et al. (1988) reported on the leaf oil of *J. recurva* var. *squamata* (= *J. squamata*), but there is some question as to the exact species extracted. Examination of the specimen sheet (RPA observation, Srivastava 19592, Regional Research Laboratory, Jammu, India), revealed that there are leaves from two taxa on the same sheet. It seems wise to utilize fresh collections of verifiable material of *J. squamata* from Yunnan for the comparisons in this report.

The purpose of this paper is to compare the oil compositions between the one seeded species in section *Sabina*, of the eastern hemisphere with data obtained from Random Amplified Polymorphic DNAs (RAPDs) (the reader is referred to Demeke and Adams (1994) for a comprehensive review of RAPD applications, including the genetics of RAPDs). The synthesis of these data sets are utilized to define the taxonomy of these *Juniperus*.

2. Materials and methods

Specimens used in this study: *J. convallium*, Adams 6781–6783, 6785, 6786 Gansu, China and 8525–8527, Sichuan, China; *J. convallium* var. *microsperma*, Adams 8522–8524, Sichuan, China; *J. indica*, Adams 7025, 7625, 7626, Nepal; *J. komarovii*, Adams 8518–8520, Sichuan, China; *J. pingii*, Adams 8506, 8507, Yunnan, China; *J. pingii* var. *carinata*, Adams 8497–8499, 8501–8504; Sichuan, China; *J. prezewalskii*,

Adams 6775–6777, Gansu, China; *J. pseudosabina*, Adams 7592–7595, Altai Mts., Mongolia, 7808–7810, Jarkent, Kazakstan, 7833–7835, Xinjiang, China; *J. recurva*, 7209–7214, 7217–7219, Nepal; *J. recurva* var. *coxii*, Adams 8137, clone from the type tree (R. Farrer 1407, upper Burma), cultivated by Keith Rushforth, UK, Adams 8508–8510, Yunnan, China; *J. saltuaria*, Adams 6788–6790, Gansu, China, 8494–8496; 8505, Yunnan, China; *J. squamata*, Adams 8491–8493, Yunnan, China, 8521, Sichuan, China; *J. squamata* var. *morrisonicola*, Adams 5639, 8681, 8682, Younger Botanic Garden, Scotland, ex Taiwan and Adams 8750–8753, Taiwan; *J. tibetica*, Adams 8512–8517, Sichuan, China; *J. wallachiana* Adams 8140, 8141, Devon, England, cultivated by Keith Rushforth, ex Bhutan. Voucher specimens are deposited at SRCG (Science Research Center-Gruver) herbarium, Baylor University.

Fresh leaves (200 g fresh wt.) were steam distilled for 2 h using a circulatory Cleavenger apparatus (Adams, 1991a, b). 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 (48 h, 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&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, then 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 RAPD analyses follow that of Adams and Demeke (1993). Ten-mer primers were purchased from the University of British Colombia (5′–3′): 153: GAG TCA CGA G; 184: CAA ACG GAC C; 204: TTC GGG CCG T; 212: GCT GCG TGA C; 218: CTC AGC CCA G; 239: CTG AAG CGG A; 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: CTA GAG GTC C; 338 CTG TGG CGG T; 346: TAG GCG AAC G.

PCR was performed in a volume of 15 μl containing 50 mM KCl, 10 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, 0.3 ng genomic DNA, 15 ng BSA and 0.6 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 that occurred once or did not show fidelity within the two replicated samples of each taxon were eliminated. It should be noted that these bands contain very useful

information for the study of genetic variance and individual variation, but are merely "noise" in the present taxonomic study. Bands were scored in 4 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, b). For the terpenoid data, similarities were computed as quantitative matches as well as simple presence/absence matches. The presence/absence (±) matching was found to be more similar to the DNA data. Principal coordinate analysis (PCO) of the similarity matrices follows Gower (1966). Program PCO3D is available for MS DOS IBM compatible computers with a math co-processor (correspond to RPA for distribution details).

3. Results and discussion

Oil yields (calculated as oil wt./wt. of oven-dried, extracted leaves) varied from 0.4 to 1.5%. The oils were clear to yellow in color. Several components previously unidentified have now been identified (Table 1) (Note: RT in the following revised compound identities refers back to the original publication): *Juniperus convallium* (Adams et al., 1993a): RT 1519, *trans*-murrola-3,5-diene; RT 1577 tentatively β-cadinene is actually cadina-1(6), 4-diene; RT 1622, *trans*-murrola-4(14), 5-diene; RT 1720, should be *trans*-cadina-1,4-diene; RT 2660 (epi-13-manool) is sandaracopimara-8(14), 15-diene (our previous reports of epi-13-manool were incorrect, these reports of epi-13-manool should be changed to sandaracopimara-8,(14), 15-diene in our *Juniperus* papers); RT 2972, nezukol is now used instead of 8-β-hydroxyisopimarene in this and all subsequent papers (Adams, 1995). *Juniperus saltuaria* (Adams et al., 1993b): RT 2034 (elemol acetate) has now been identified as bulnesol in this and all juniper papers; RT 2556 tentatively pimara-9(11), 15-diene. *Juniperus przewalskii* (Adams et al., 1994): RT 1984 torreyol is now called α-muurolol, RT 2070, eudesma-4(15), 7-dien-1-β-ol. *Juniperus indica* (Adams & Chaudhary, 1996): KI 1473, tentatively identified as β-cadinene is actually *trans*-muurola-4(14), 5-diene, KI 1491 is *trans*-muurola-4(14), 5-diene, KI 1524 is zonarene, KI 1576 is germacrene D-4-ol, KI 1908 is tentatively pimara-9(11), 15-diene, KI 1930 ent-rosadiene is rosa-5, 15-diene. *Juniperus recurva* (Adams et al., 1998a,b), RI 1473, tentatively identified as β-cadiene is actually *cis*-muurola-4(14), 5-diene.

The overall pattern of similarities in the terpenoids is depicted in Fig. 1. *Juniperus indica* and *J. wallachiana* have the most similar oils, followed by *J. komarovii* and *J. tibetica* (Fig. 1). Farjon (1998) treated *J. wallachiana* as a synonym of *J. indica* and the terpenoids support that treatment, however, the DNA results (Fig. 2) suggest that these taxa are about as similar as other recognized species (cf. *J. saltuaria* – *J. tibetica*; *J. komarovii* – *J. convallium*). Morphologically, *J. indica* and *J. wallachiana* are quite similar. *Juniperus wallachiana* could be treated as variety of *J. indica*, but to be consistent with the nomenclature of the other species in this study, it seems wise to recognize *J. wallachiana* at the specific level until additional data is brought forth.

Table 1
Comparisons of the percent total oil for leaf essential oils for *J. convallium* (CV), *J. c.* var. *microsperma* (CM), *J. indica* (IN), *J. komarovii* (KM), *J. pingii* (PG), *J. pingii* var. *carinata* (PC), *J. prezewalskii* (PZ), *J. pseudosabina* (PS), *J. recurva* (RC), *J. r.* var. *coxii* (CX), *J. saltuaria* (SA), *J. squamata* (SQ), *J. s.* var. *morrisonicola* (MO), *J. tibetica* (TB), *J. wallachiana* (WA)

KI	Compound	CV	CM	KM	TB	SA	PZ	WA	IN	PS	PG	PC	RC	CX	SQ	MO
926	Tricyclene	0.1	—	t	t	—	t	t	t	0.1	t	0.1	t	0.1	0.1	t
931	α-Thujene	0.2	2.3	2.4	1.7	1.3	0.3	1.7	1.4	0.2	0.9	2.2	0.5	t	1.0	t
939	α-Pinene	47.6	4.6	3.4	9.5	2.2	9.4	9.4	2.8	52.1	19.5	20.9	6.9	18.4	26.0	0.8
953	α-Fenchene	t	t	t	t	—	t	t	—	t	0.4	0.1	1.0	0.4	t	t
953	Camphene	0.4	t	t	t	—	0.1	t	t	0.5	t	0.2	—	0.1	0.1	t
957	Thuja-2,4(10)-diene	t	—	—	—	—	—	—	—	—	—	t	—	—	—	—
976	Sabinene	1.5	40.2	32.5	23.0	38.2	7.2	31.8	26.1	5.8	19.2	23.7	13.4	0.5	15.5	0.3
978	1-Octen-3-ol	—	—	—	—	—	—	t	0.2	—	—	—	t	—	—	—
980	β-Pinene	1.1	0.2	0.2	0.3	0.2	0.2	0.3	t	4.5	1.4	0.6	0.2	0.4	0.6	t
991	Myrcene	8.4	2.9	2.6	2.4	2.1	1.2	3.7	3.3	3.8	8.7	3.4	2.4	1.9	1.3	0.4
1001	δ-2-Carene	t	t	—	3.8	—	4.1	t	t	—	0.1	0.2	—	t	0.6	0.6
1005	α-Phellandrene	0.1	t	t	0.1	0.1	0.1	0.3	0.1	t	—	0.1	t	t	0.1	t
1011	δ-3-Carene	t	0.1	t	0.1	—	0.2	t	t	0.3	8.3	0.1	23.7	7.4	—	t
1018	α-Terpinene	t	1.8	2.2	1.8	1.2	0.4	1.6	1.7	0.2	0.7	2.3	0.8	t	0.7	t
1022	*o*-Cymene	—	—	—	—	—	—	—	—	—	—	—	t	t	—	—
1026	*p*-Cymene	0.8	0.2	0.3	0.3	0.9	0.6	0.2	0.2	t	t	1.1	0.2	t	0.6	0.5
1027	Sylvestrene	—	—	—	—	—	—	—	—	—	—	—	0.2	t	—	—
1031	Limonene	5.1	2.5	9.4	8.5	2.0	11.6	1.7	0.4	0.9	8.2	2.1	18.4	15.7	3.3	27.3
1031	β-Phellandrene	1.7	0.2	—	0.2	0.1	—	1.6	1.6	1.8	0.4	0.2	t	—	13.6	—
1032	1,8-Cineole	t	—	—	—	—	—	0.2	1.3	t	—	—	t	—	—	—
1050	(E)-β-Ocimene	—	—	—	—	—	—	t	—	—	—	—	—	0.3	—	0.1
1062	γ-Terpinene	0.2	2.8	3.4	2.8	1.9	0.5	2.6	2.7	0.3	1.3	4.2	1.3	0.1	1.2	0.2
1068	*cis*-Sabinene hydrate	0.1	2.0	2.3	1.3	1.6	0.2	1.2	1.6	0.1	0.3	1.8	0.6	—	0.5	t
1074	*trans*-Linalool oxide(furanoid)	t	—	—	—	—	t	—	—	—	—	—	—	—	—	—
1086	*p*-Mentha-2,4(8)-diene	—	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—
1088	Terpinolene	0.7	0.9	1.1	1.2	0.7	0.6	1.0	0.9	0.3	1.0	1.5	1.8	0.9	0.7	0.2
1089	*p*-Cymenene	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1091	2-Nonanone	—	—	—	—	0.8	—	—	—	3.4	—	—	—	—	—	—

—continued

Table 1—*continued*

KI	Compound	CV	CM	KM	TB	SA	PZ	WA	IN	PS	PG	PC	RC	CX	SQ	MO
1097	*trans*-Sabinene hydrate	t	2.0	2.3	1.3	2.0	0.2	1.0	1.0	t	0.2	1.8	0.3	—	0.3	t
1098	Linalool	0.5	—	1.1	t	0.7	0.3	t	—	2.1	t	t	—	t	—	0.4
1102	*cis*-Thujone (= α-thujone)	—	0.3	0.3	t	0.3	0.7	0.1	2.3	—	—	—	—	—	—	—
1103	Isopentyl-isovalerate	—	—	—	—	—	—	—	—	—	0.4	0.3	0.1	0.1	3.2	0.1
1110	1,3,8-*p*-Menthatriene	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—
1114	*trans*-Thujone(= β-thujone)	—	2.0	1.4	t	1.3	3.0	0.1	16.0	—	—	—	—	—	—	t
1116	3-Methyl butanoate, 3-methyl-3-butenyl-	—	—	—	0.3	—	—	—	—	0.6	0.6	0.5	0.2	0.2	2.9	—
1121	*cis*-*p*-Menth-2-en-1-ol	0.1	0.4	0.5	0.6	0.2	0.5	0.3	0.6	t	0.1	0.5	0.2	—	0.2	—
1125	α-Campholenal	t	—	—	—	—	t	—	—	t	t	0.1	—	—	t	—
1134	*cis*-*p*-Mentha-2,8-dien-1-ol	—	t	—	—	—	—	—	—	—	—	—	—	—	—	t
1134	*cis*-Limonene oxide	—	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—
1139	*trans*-Limonene oxide	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t
1140	*trans*-Sabinol	—	0.4	—	—	0.2	—	—	1.4	—	—	t	—	—	—	—
1140	*trans*-*p*-Menth-2-en-1-ol	0.2	—	0.2	0.4	—	—	0.2	0.7	0.1	0.1	0.4	t	—	0.1	—
1149	neo-3-Thujanol	—	—	—	—	—	—	—	0.1	—	—	—	—	—	—	—
1143	*cis*-Sabinol[a]	—	—	—	—	—	0.7	0.1	—	—	—	t	—	—	0.2	—
1143	*trans*-Verbenol	0.2	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—
1148	Camphene hydrate	—	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—
1156	Sabina ketone	—	—	t	—	—	—	t	t	—	—	—	—	—	—	—
1159	*p*-Mentha-1,5-dien-8-ol	—	—	—	—	—	0.2	—	—	—	t	—	0.5	t	—	—
1165	Borneol	t	—	t	—	—	—	—	—	0.1	—	0.1	—	—	—	—
1167	δ-Terpineol	—	—	—	t	—	—	—	—	—	—	—	0.3	—	—	—
1171	Umbellulone	—	t	—	—	—	—	0.1	0.2	—	—	—	—	—	—	t
1177	Terpinen-4-ol	0.2	6.6	8.1	6.2	3.9	1.4	4.5	7.2	0.8	2.9	8.8	3.7	0.2	3.3	0.1
1179	Naphthalene	—	—	—	t	—	—	t	—	—	—	—	—	—	—	t
1180	*m*-Cymen-8-ol	—	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—
1183	*p*-Cymen-8-ol	—	t	t	t	0.1	0.2	—	—	—	—	—	0.1	—	—	—
1189	α-Terpineol	t	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.3	0.3	0.3	t	t

KI	Compound	CV	SC	KM	TB	SA	PZ	WA	IN	PS	PG	PP	RC	CX	SQ	MO
1191	Myrtenol	—	—	—	—	—	—	t	—	—	—	—	—	—	—	t
1193	Myrtenal	—	—	—	—	—	—	—	—	t	—	—	—	—	—	—
1193	*cis*-Piperitol	—	—	—	—	t	0.1	—	—	—	—	—	t	—	—	—
1204	Verbenone	0.1	—	—	—	—	—	—	—	t	—	—	—	—	—	—
1205	*trans*-Piperitol	—	—	—	—	—	0.1	—	—	—	—	—	0.1	—	—	—
1217	*trans*-Carveol	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—	t
1228	Citronellol	—	—	—	—	—	0.6	—	t	0.2	—	—	—	—	—	—
1229	*cis*-Carveol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t
1235	Thymol, methyl ether	—	t	—	—	—	—	—	—	—	—	—	—	—	—	t
1237	(Z)-3-Hexenyl 3-methylbutyrate	—	—	—	—	—	—	—	—	0.1	—	—	—	—	—	—
1242	Carvone	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t
1243	Hexyl 3-methylbutanoate	—	—	—	—	—	—	—	—	—	t	t	0.1	0.1	—	—
1244	Carvacrol, methyl ether	—	—	—	—	t	—	—	t	—	—	—	—	—	—	t
1252	Piperitone	t	—	—	4.2	—	6.8	—	0.1	t	—	—	—	—	0.2	t
1261	Methyl citronellate	—	t	—	—	—	—	0.1	0.1	—	—	—	0.1	—	—	t
1274	Unknown, 79,91,105,147,FW162	—	4.3	4.5	5.6	0.9	3.7	t	—	—	2.9	4.9	2.2	—	5.5	1.3
1285	Bornyl acetate	0.9	t	t	0.1	0.1	0.2	0.2	0.2	0.8	0.2	0.7	0.1	0.5	0.2	t
1286	*trans*-Linalool oxide acetate (pyranoid)	t	—	—	—	—	0.1	t	0.1	—	0.4	—	—	t	—	t
1290	*trans*-Sabinyl acetate	—	—	—	—	t	0.7	0.1	15.7	0.2	—	—	—	—	0.4	t
1291	2-Undecanone	0.2	0.1	t	t	0.2	—	—	—	t	—	—	—	—	—	—
1297	*trans*-Pinocarvyl acetate	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1321	Aromatic, 149, FW 164	0.8	0.2	—	—	0.8	—	4.0	—	0.9	—	0.1	—	—	—	—
1322	Methyl geranate	—	—	—	—	—	—	—	—	—	—	—	t	—	—	—
1351	α-Cubebene	0.4	—	—	—	—	—	0.4	t	—	—	—	—	0.5	—	—
1376	α-Copaene	t	—	—	t	—	—	0.2	t	—	—	—	—	0.1	—	—
1390	β-Cubebene	0.8	—	—	—	—	—	0.8	t	—	t	—	—	0.9	—	—
1391	β-Elemene	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—
1409	α-Cedrene	—	—	—	t	—	—	—	—	0.1	—	—	—	—	—	—
1409	1,7-di-epi-β-Cedrene	—	—	t	t	—	—	—	—	—	t	—	—	—	—	—
1418	β-Cedrene	—	—	—	—	—	—	—	—	t	—	—	—	—	—	—
1418	(E)-Caryophyllene	0.1	t	t	0.1	t	t	0.3	t	—	0.1	0.9	t	0.4	0.6	t

—continued

Table 1—*continued*

KI	Compound	CV	CM	KM	TB	SA	PZ	WA	IN	PS	PG	PC	RC	CX	SQ	MO
1423	2,5-Dimethoxy-p-cymene	—	—	—	—	—	—	—	t	—	t	—	—	—	—	—
1429	*cis*-Thujopsene	—	—	t	—	—	0.8	—	—	0.3	0.2	t	—	—	—	—
1450	*trans*-Muurola-3,5-diene	0.9	—	—	—	—	0.1	1.6	0.3	—	0.1	—	—	3.6	—	—
1454	α-Humulene	0.2	t	—	—	—	—	0.2	t	—	—	—	—	0.2	—	—
1461	*cis*-Muurola-4(14),5-diene	—	t	t	t	—	0.2	—	—	—	—	—	t	0.1	—	—
1473	*trans*-Cadina-1(6),4-diene	0.6	t	—	—	—	t	1.2	0.2	—	t	—	—	3.2	—	—
1476	γ-Himachalene	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—
1477	γ-Muurolene	—	—	t	t	—	0.2	0.1	t	0.1	—	—	t	0.1	—	t
1480	γ-Curcumene	—	—	—	—	—	—	—	—	—	—	—	—	—		0.1
1480	Germacrene D	0.4	—	t	t	—	0.3	t	—	—	0.2	—	t	0.1	—	—
1483	ar-Curcumene	—	—	—	—	—	—	—	—	—	—	0.3	—	—	—	—
1499	β-Himachalene	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.1
1490	Phenyl ethyl 3-methyl-butanoate	—	t	—	—	—	—	—	—	t	—	—	0.1	—	—	—
1491	*trans*-Murrola-4(14),5-diene	2.4	t	t	t	—	0.1	3.9	0.9	—	0.1	—	—	8.6	—	—
1493	*cis*-cadina-1,4-diene	—	—	t	—	—	—	—	—	t	—	—	—	2.2	—	—
1493	4-epi-Cubebol	0.9	—	—	—	—	0.1	0.8	—	—	—	—	t	—	—	—
1499	α-Muurolene	0.2	0.2	0.1	0.2	—	0.3	0.2	0.1	0.4	t	—	0.2	0.4	—	0.3
1503	Germacrene A	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—
1509	β-Bisabolene	—	—	—	—	—	—	—	—	—	t	—	—	—	—	t
1512	β-Curcumene	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—	—
1513	γ-Cadinene	—	0.4	0.3	0.3	0.2	0.7	3.8	0.7	0.4	0.1	—	0.4	6.0	t	—
1513	Cubebol	3.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1521	*cis*-Calamenene	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1524	δ-Cadinene	1.4	0.9	0.7	0.8	0.3	1.3	1.6	0.8	1.4	0.1	t	0.8	3.6	0.1	—
1526	Zonarene	0.4	—	—	—	—	—	0.8	t	—	—	—	—	2.3	—	—
1532	*trans*-Cadina-1,4-diene	0.3	—	—	—	—	—	0.3	t	—	—	—		0.4	—	—
1538	α-Cadinene	—	t	t	t	—	t	—	—	t	—	—	t	—	—	—
1549	Elemol	—	2.6	2.5	3.9	7.6	2.0	1.3	0.6	t	3.0	2.5	3.9	0.3	2.9	3.8
1556	Germacrene B	—	t	t	t	—	—	—	—	—	t	0.4	t	—	0.4	t
1564	(E)-Nerolidol	—	—	—	—	—	—	—	—	t	—	—	t	—	—	—
1574	Germacrene D-4-ol	0.3	2.2	1.2	1.2	0.6	1.2	0.7	0.4	2.0	0.1	t	1.0	0.3	0.1	—
1581	Caryophyllene oxide	—	—	—	—	—	—	—	—	—	—	0.1	—	—	t	t

1587	Sesquiterpene,161,41,105,204	0.4	—	—	—	—	—	0.7	—	—	—	—	—	0.5	—	—
1592	Longiborneol(= juniperol)	—	—	—	—	—	0.1	—	—	—	—	—	—	0.5	—	t
1596	Cedrol	t	1.3	7.7	1.0	3.2	0.2	—	t	10.7	5.4	t	—	0.4	0.4	0.1
1606	β-Oplopenone	t	—	0.1	t	—	t	0.1	—	t	—	—	0.1	t	—	—
1627	1-epi-Cubenol	1.9	—	—	—	—	0.2	2.4	0.3	—	—	—	—	4.8	—	—
1630	Acorenol	—	—	t	—	—	—	—	—	0.3	—	—	—	—	t	—
1630	γ-Eudesmol	—	0.1	0.2	0.5	0.5	0.3	0.3	t	—	0.5	0.6	0.1	—	0.7	0.6
1640	epi-α-Cadinol	t	0.4	0.4	0.2	t	1.2	0.2	0.2	0.4	—	—	0.3	1.0	0.4	—
1640	epi-α-Muurolol	—	0.4	0.5	0.7	—	1.2	0.6	t	0.6	—	—	0.5	0.1	—	—
1642	Cubenol	0.4	—	—	—	—	—	—	0.2	—	—	—	—	—	—	—
1643	Sesquiterpene,41,119,204,222	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4
1644	Sesquiterpene,41,105,119,204	—	—	—	—	—	—	—	—	—	0.7	—	—	—	—	—
1645	α-Muurolol (= torreyol)	t	t	t	t	—	0.6	0.1	t	0.1	—	—	0.1	0.3	—	—
1649	β-Eudesmol	—	0.3	0.7	1.0	1.8	0.8	0.3	0.1	—	0.7	1.1	1.5	t	1.5	1.2
1652	α-Eudesmol	—	0.4	0.5	1.0	4.3	t	0.5	0.2	—	0.9	1.1	1.0	0.1	2.2	1.1
1653	α-Cadinol	0.4	1.0	1.3	1.3	—	3.2	0.7	0.2	1.3	—	—	0.8	0.5	—	—
1666	Bulnesol	—	0.5	0.5	0.9	1.8	0.4	0.4	0.1	—	0.5	0.5	0.8	t	0.5	0.7
1685	Eudesma-4(15),7-dien-1-β-ol	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—
1733	Oplopenone	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—	—
1789	8-α-Acetoxyelemol	—	1.6	2.0	3.6	0.3	3.9	0.8	—	—	1.4	2.0	1.9	—	4.7	4.4
1894	Rimuene	—	t	—	—	0.2	0.1	—	—	—	t	t	—	0.1	—	0.2
1898	Isopimara-9(11),5-diene	—	t	—	—	0.2	0.4	—	—	—	t	t	—	0.2	—	0.3
1909	Pimara-9(11),5-diene[a]	—	0.6	—	t	1.0	0.3	0.2	0.2	—	0.4	—	—	0.6	—	3.7
1923	Isohibaene	—	—	—	—	—	—	—	—	—	—	—	—	—	t	—
1924	Beyerene	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—
1930	Rosa-5,15-diene	t	0.3	—	t	t	0.5	0.2	t	—	0.2	t	—	3.0	—	—
1961	Sandaracopimara-8(14),15-diene	0.2	1.5	t	0.3	2.5	0.8	0.8	0.3	t	0.4	t	—	1.9	t	2.3
1963	Isophyllocladene	—	—	—	—	—	—	—	—	—	—	0.1	—	—	t	—
1989	Manoyl oxide	—	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—
1991	Diterpene, 41,109,257,FW272.	1.6	t	1.1	0.1	0.5	1.2	—	—	—	0.2	0.6	—	0.1	0.3	0.5
2011	Phyllocladene	—	—	—	0.3	—	0.6	—	—	—	—	0.5	—	0.3	0.3	0.4
2011	Abieta-8,12-diene[a]	—	—	—	0.1	—	—	—	—	—	—	—	—	—	—	—
2054	Abietatriene	4.7	t	0.3	1.0	0.3	6.3	t	t	t	t	0.5	0.5	0.4	t	0.4

—continued

Table 1—*continued*

KI	Compound	CV	CM	KM	TB	SA	PZ	WA	IN	PS	PG	PC	RC	CX	SQ	MO
2056	Manool	—	0.9	—	0.9	—	—	t	0.8	t	1.8	2.0	t	t	—	—
2080	Abietadiene	4.7	—	0.3	4.5	—	5.1	0.5	0.3	0.2	0.3	0.6	1.3	t	t	t
2126	Nezukol	0.4	6.0	—	0.5	5.3	4.0	4.0	—	t	3.5	—	—	3.3	—	42.8
2140	Diterpene,41,257, FW272	—	0.2	—	—	—	—	—	—	—	—	—	—	t	—	2.0
2147	Abieta-8(14),13(15)-diene[a]	—	—	—	0.1	—	0.4	—	—	—	—	—	—	—	—	—
2181	Diterpene,91,257,271,FW286	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.2
2200	Diterpene,41,91,120,257,FW272	—	—	—	0.1	—	—	—	—	—	—	0.8	—	t	1.6	—
2200	Phyllocladanol	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—
2208	Diterpene,257,105,273, FW288	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.5
2266	Diterpene,257,41,273,FW288	—	—	—	—	—	—	—	—	—	—	—	—	—		0.4
2278	*cis*-totarol	0.3	—	—	—	—	0.2	—	—	—	—	—	—	—	—	t
2288	4-epi-Abietal	—	—	—	—	—	—	0.4	t	—	—	—	t	—	—	—
2302	*trans*-Totarol	0.7	t	t	t	0.7	1.6	0.2	t	—	—	t	0.2	0.1	—	t
2325	*trans*-Ferruginol	t	—	—	—	—	0.3	—	—	—	—	—	—	—	—	—

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

[a]Tentatively identified.

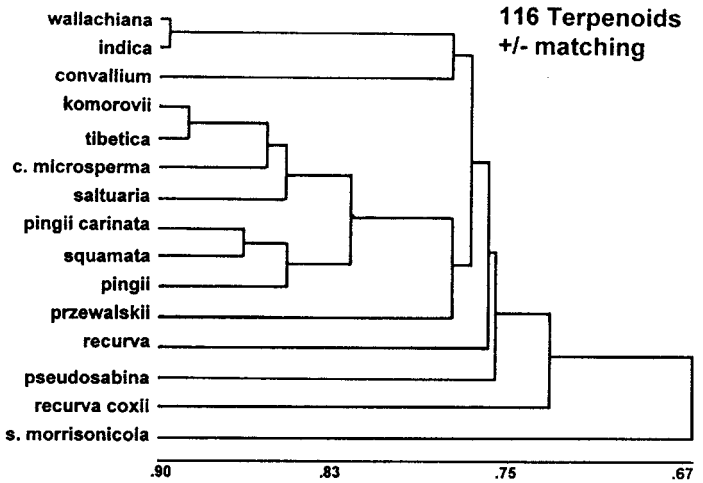

Fig. 1. Minimum spanning network based on 116 terpenoids, with similarities computed as presence/absence data. Note the very close similarity of *J. indica* and *J. wallachiana*, and the differentiation of *J. recurva* var. *coxii* and *J. squamata* var. *morrisonicola*. See text for discussion.

Juniperus saltuaria and *J. przewalskii* are not very similar in their oils (Fig. 1) but link at the highest similarity (0.92) of any taxa by their DNA (Fig. 2). Of all the taxa examined by use of the DNA, these taxa might be considered as varieties. Morphologically, these taxa are distinct, with *J. saltuaria* having dark green, 4-sided branchlets (leaves in pairs), smaller greenish black, female cones (7–8 mm), monecious vs. *J. prezewalskii* with yellowish-green, terete (and occasionally paired leaves) but not 4-sided branchlets, larger dark black, female cones (9–11 mm), dioecious. So it seems wise to maintain these taxa as distinct species. If taxa of this level of DNA similarity are recognized as species, then one must reconsider the taxonomic levels of the traditional varieties of this study: *J. convallium* var. *microsperma*, *J. pingii* var. *carinata*, *J. recurva* var. *coxii*, and *J. squamata* var. *morrisonicola*, because each is quite dissimilar to its type variety (Fig. 2).

The morphology of *J. convallium* var. *microsperma* is distinct. The glands on the scale leaves are scarcely visible and if so, are basal, round and level with the leaf surface. In contrast, in *J. convallium*, the glands on the scale leaves are very visible, forming a depression in the leaf that extends from near the leaf tip to the base. The scale leaves of *J. convallium* var. *microsperma* have a pronounced keel which is lacking in *J. convallium*. The taxa also differ in female cone size (5 mm, *J. c.* var. *microsperma* vs. 7–9 mm, *J. convallium*) and in habitat as the var. *microsperma* is found in mesic areas and *J. convallium* is in dryer hillsides.

Both the oils and DNA of *J. convallium* var. *microsperma* are very different from *J. convallium* (Table 1, Figs. 1 and 2). The DNA shows *J. convallium* and *J. c.* var.

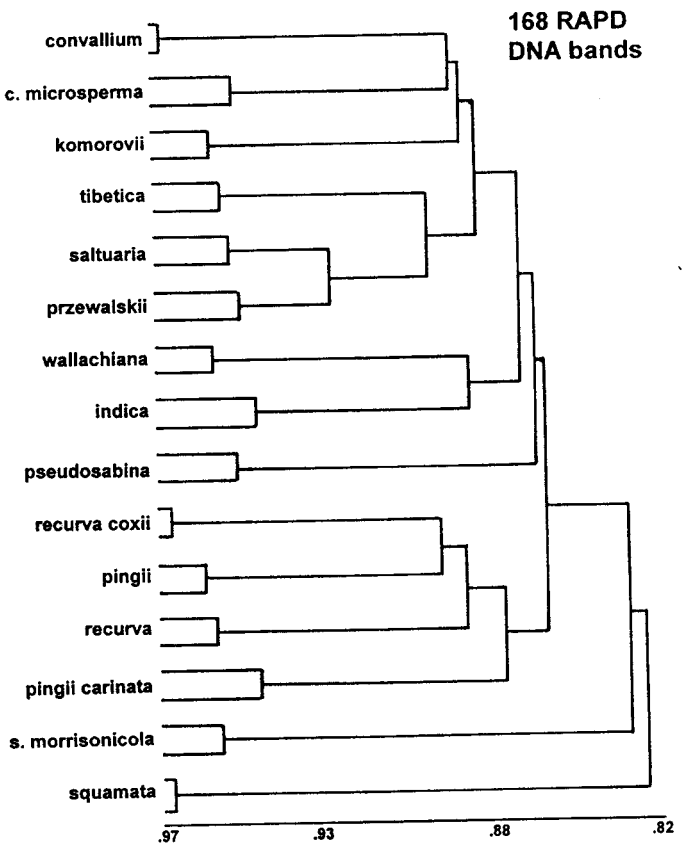

Fig. 2. Minimum spanning network based on 168 RAPD DNA bands. Each OTU is represented by two individuals. Two obvious groups are *prezewalskii-saltuaria-tibetica* and *recurva-pingii*. Several varieties fail to cluster at high levels (*J. convallium* and var. *microsperma*, *J. pingii* and var. *carinata*, *J. recurva* and var. *coxii*, *J. squamata* and var. *morrisonicola*). See text for discussion.

microsperma to cluster very loosely (almost as dissimilar as *J. komarovii*). The level of differences is comparable to other species in the study (*J. tibetica* – *J. prezewalskii*; *J. wallachiana* – *J. indica*). Based on the difference in morphology, ecology, terpenes and RAPDs, it seems appropriate to recognize *J. convallium* var. *microsperma* at the specific level:

Juniperus microsperma (Cheng & L.K. Fu) R.P. Adams, *stat. nov.*

Basionym: *Sabina convallium* (Rehd. & Wils.) Cheng & W.T. Wang var. *microsperma* Cheng & L.K. Fu. Acta Phytotax. Sin 13:86, 1975. TYPE: China, e Xizang, 4000m,

Forest Team 10019 (holotype, PE). Syn.: *Sabina microsperma* (W.C. Cheng & L.K. Fu) W.C. Cheng & L.K. Fu; *Juniperus convallium* Rehd. & Wils. var. *microsperma* (W.C. Cheng & L.K. Fu) Silba.

Juniperus recurva var. *coxii* grows as a very pendulous tree in the sub-tropical mountains of sw Yunnan and n Myramar (Burma) in the mesic, cloud cloaked mountains (my collections from 3050 m). Jackson (1932), described the species (*J. coxii* A.B. Jackson) based on a tree cultivated at Exbury, Hants, UK, that was raised from seed collected by E.H.M. Cox and R. Farrer (Farrer 1407) in upper Burma. Keith Rushforth has a clone of the type (Farrer 1407) tree in cultivation and I obtained sample (Adams 8137) from that tree. Jackson (1932) noted for *J. coxii* "It nearest ally seemed to be the Himalayan *J. recurva*...". He differentiated the two taxa by *J. coxii* being more pendulous, having longer tapering leaves which have two greenish-white bands on the ventral surface (in contrast to a single white stomatal band for *J. recurva*) and the strong central axis of *coxii* (vs. occasionally multiple stems in *J. recurva*). Specimens of the two taxa are difficult to distinguish except for the one white vs. two green-whitish stomatal bands. The *J. r. coxii* does seem to grow in more mesic sites than *J. recurva* of the Himalayas as far as the author's experience indicates.

The oil of *J. r.* var. *coxii* was found to contain a number of compounds not found in *J. recurva* (Table 1). Overall, the oil of *J. r.* var. *coxii* is very distinct (Fig. 1). The RAPDs of the clone of the type tree and a native *J. recurva* var. *coxii* from sw Yunnan are very similar (0.97, Fig. 2), but this taxon is not very similar to *J. recurva* (Fig. 2), In fact, it is slightly more similar to *J. pingii* (Fig. 2). Although the morphology is very similar for *J. recurva* and *J. r.* var. *coxii*, both the terpenoid and DNA data show that *J. recurva* var. *coxii* is very distinct. It appears that *J. r. coxii* does represent a taxon that is reproducing itself under natural conditions and based on the current data, it should be restored to the specific rank: *Juniperus coxii* A.B. Jackson, New Flora & Silva 5, 33 (1932), Syn: *J. recurva* var. *coxii* (A.B. Jacks.) Melville.

The prostrate shrub, *J. pingii* var. *carinata* is very different from *J. pingii* in both its morphology, oils and DNA. I found this depressed shrub at timberline (4380 m) areas of w Yunnan, whereas *J. pingii* was found at a lower elevation (3560 m) in a coniferous forest. The oil of *J. p.* var. *carinata* is most similar to *J. squamata* (Table 1, Fig. 1). The DNA of *J. p.* var. *carinata* is very distinct and it forms a loose association with the *coxii-pingii-recurva* species. Because it is so distinct in its morphology, terpenes and RAPDs, *J. pingii* var. *carinata* merits recognition at the specific level:

Juniperus carinata (Y.K. Yu & L.K. Fu) R.P. Adams, *stat. nov.*

Basionym: *Juniperus pingii* Cheng & Ferre var. *carinata* Y.K. Yu & L.K. Fu, Novon 7: 443, 1997. TYPE: China, w Sichuan, Yajiang. 4460 m, T.S. Ying 3140 (holotype, PE).

Juniperus squamata var. *morrisonicola* is endemic to the high mountains of Taiwan. It has longer, narrower and more appressed leaves than *J. squamata* (from Yunnan). The female cones of *J. s. morrisonicola* are black and 4–5 mm in length vs. dark brown and 8–9 mm long for *J. squamata*. It should be noted that *J. squamata* is, morphologically, a quite variable species and an analysis throughout its range has yet to be

accomplished. The oil of *J. s. morrisonicola* was found to be most unique taxon in this study (Fig. 1). The oil is dominated by the diterpene alcohol, nezukol (= 8-β-hydroxyisopimarene) (42.8%, Table 1). It also contained several unique, unknown diterpenoids (Table 1). The RAPDs DNA also shows this taxon to be very distinct (Fig. 2). Thus, the morphology, terpenoids and DNA are concurrent in showing that this taxon is quite differentiated from the other *Juniperus* species and support its recognition as a distinct species: *J. morrisonicola* Hayata, Gard. Chron. ser. 3, 43, 194 (1908). Syn: *J. squamata* var. *morrisonicola* (Hayata) H.L. Li & H. Keng. endemic to Taiwan.

In summary, these one seeded *Juniperus* divide into three groups: large trees in the high mountains of western-central China: *convallium-micorsperma-komarovii-tibetica-saltuaria-prezewalskii*; 2 tree species (sometimes *indica* is a shrub) in the central Himalayan Mts.: *indica-wallachiana*; and the *pingii-recurva* complex in w. Yunnan and n. Myramar: *coxii-pingii-recurva-carinata*. In addition, several species are not closely associated with any other species: *J. pseudosabina* from central Asia; *J. morrisonicola* from Taiwan and *J. squamata* from central China.

Several other taxa have recently been reported from western China (Yu and Fu, 1997) but I was not able to locate these on a recent field trip. These taxa include: *J. chengii* L.K. Fu & Y.F. Yu, observation: isotype (A!) looks like a larger leafed *J. pingii*, unable to find these plants at the type locality (imprecisely specified as: Zhongdian, 3150 m, nw Yunnan); *J. baimashaneusis* Y.F. Yu & L.K. Fu, observation: similar to *J. pingii*, I could not see the ridges on the leaves of the isotype (KUN!) that are supposed to distinguish it from *J. pingii*, unable to find this taxon at the type locality (imprecisely specified as: nw Yunnan, Deqin, 3400 m). *Juniperus squamata* var. *parvifolia* Y.F. Yu & L.K. Fu, this taxon is based on having strongly curved, smaller leaves than var. *squamata*. I had no opportunity to visit sw Sichuan. *Juniperus squamata* var. *hongxiensis* Y.F. Yu & L.K. Fu., this taxon is based on its conspicuously exposed internodes and smaller, curved loosely arranged leaves (than var. *squamata*). I had no opportunity to visit sw Sichuan. It is clear from my field work in sw Yunnan that additional field and lab work will be needed to fully understand the variation in the *pingii-squamata* complex in this region.

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