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Systematics of *Juniperus* section *Juniperus* based on leaf essential oils and random amplified polymorphic DNAs (RAPDs)

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Abstract

The composition of the leaf essential oils of all the species of *Juniperus* in sect. *Juniperus* (= sect. *Oxycedrus*) are reported and compared (*J. brevifolia*, *J. cedrus*, *J. communis*, *J. c.* var. *saxatilis*, *J. c.* var. *oblonga*, *J. formosana*, *J. oxycedrus*, *J. o.* subsp. *badia*, *J. o.* subsp. *macrocarpa*, *J. o.* subsp. *transtagana*, *J. rigida*, *J. r.* subsp. *conferta*, *J. sibirica*, *J. taxifolia* and *J. t.* var. *lutchuensis*). In addition, DNA fingerprinting by RAPDs was utilized. Based on these data, several taxa remained at the same taxonomic level: *J. brevifolia*, *J. cedrus*, *J. communis*, *J. c.* var. *saxatilis*, *J. formosana*, *J. oxycedrus*, *J. rigida*, *J. r.* var. *conferta*, and *J. taxifolia*. However, several taxa exhibited considerable differentiation that warranted their recognition at the specific level: *J. oblonga* M.-Bieb. (= *J. communis* var. *oblonga*), *J. badia* H. Gay (= *J. oxycedrus* subsp. *badia*), *J. macrocarpa* Sibth. and Sm. (= *J. oxycedrus* subsp. *macrocarpa*), *J. navicularis* Gand. (= *J. oxycedrus* subsp. *transtagana*), *J. sibirica* Brugsd. (= *J. communis* var. *saxatilis* in part), and *J. lutchuensis* Koidz. (= *J. taxifolia* var. *lutchuensis*). © 2000 Elsevier Science Ltd. All rights reserved.

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

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

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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*, 11–13 species) and *Sabina* (the remaining, approximately 50 species).

I recently (Adams, 1998) reviewed the literature and reported the compositions of the leaf essential oils of *Juniperus*: *J. brevifolia* (Seub.) Ant., *J. cedrus* Webb and Berth., *J. conferta* Parl., *J. communis* L., *J. formosana* Hayata, *J. rigida* Mig. in Sieb., *J. navicularis* Gand. (in the Flora Europea it was treated as *J. oxycedrus* ssp. *transtagana* Franco, Franco, 1964), *J. oblonga* M.-Bieb. (often treated as *J. communis* var. *oblonga* (M.-Bieb.) Loud.), *J. oxycedrus* L., J. *sibirica* Brugsd. (often included in *J. communis* var. *saxatilis* Pall.).

In this study, the leaf essential oils of *J. communis* var. *saxatilis* Pall. from Switzerland, *J. oxycedrus* subsp. *macrocarpa* Sibth. and Sm. from Spain, *J. oxycedrus* var. *badia* H. Gay from Spain, *J. taxifolia* Hook. et. Arn. and *J. taxifolia* var. *lutchuensis* (Koldz.) Satake from Japan are reported to complete the species oil compositions for the entire section *Juniperus* (= section *Oxycedrus*).

The leaf oil of *J. communis* var. *saxatilis* was reviewed by Adams (1998). The oils of *J. oxycedrus* subsp. *macrocarpa* and *J. oxycedrus* var. *badia* have been recently reported (Adams et al., 1999). These oil compositions are included in this report for comparative use.

Yatagai and Takahashi (1988) reported that the leaf essential oil of *J. taxifolia* var. *lutchuensis* (Koldz.) Satake is dominated by α-pinene (65.7%) with moderate amounts of 3-carene (12.2%), β-pinene (5.8%) and myrcene (4.6%), but there appears to be no report on the leaf essential oil of *J. taxifolia*

The purpose of this paper is to make extensive reports on the leaf essential oils of *J. taxifolia* and *J. taxifolia* var. *lutchuensis* and compare the oil compositions between species in section *Juniperus* with data from random amplified polymorphic DNAs (RAPDs). The synthesis of these data sets are utilized to define the taxonomy of section *Juniperus*.

2. Materials and methods

Specimens used in this study: *J. brevifolia*, Adams 8152–8154, Serra da Tronqueira, San Miguel Island, Azores Islands; *J. cedrus*, Adams 8127, 8146, 8140, S. of La Orotava, Tenerife, Canary Islands; *J. communis* var. *communis*, Adams 7846–7848, Stockholm, Sweden; *J. communis* var. *saxatilis*, Adams 7618–7621, Switzerland, *J. conferta*, Adams 4925, Kew Gardens (origin = Japan), London; Adams 5413, 5414, Strybing Arboretum (origin = Japan), San Francisco, Adams 5625, Royal Botanic Garden (origin = Japan), Edinburgh; Adams 8585–8589, Tottori Sand Dunes, Japan (provided by Jin Murata); *J. formosana*, Adams 6772, 6774, 6792, Gansu, China; *J. navicularis*, Adams 8239–8243, Lisbon, Portugal; *J. oblonga*, Adams 5509–5510, Arnold Arboretum (origin = Stavropol Bot. Gard.), Adams 5640, Berlin Bot. Garden (origin = Starvopol Province, Russia), Adams 6144, Tbilisi Botanic Garden (origin = Caucasus Mts.); *J. oxycedrus* subsp. *oxycedrus*, Adams 7080–7082, El Penon,

Spain, 720 m; *J. o.* subsp. *badia*, Adams 7795–7800 (provided by Joaquin Altarejos, University of Jaen, Jaen, Spain); *J. o.* subspecies *macrocarpa*, Adams 7205–7207, Tarifa, Spain; *J. rigida*, Adams 6797–6799, Beijing Bot. Garden (origin = ne China) and Adams 8544–8546, Gifu Prefecture, Japan (provided by Jin Murata); *J. sibirica*, Adams 7589–7591, Altai Mts., 2550 m, Mongolia, *J. taxifolia*, Adams 8448, 8449, Bonin Islands, Japan (provided by Jin Murata); *J. taxifolia* var. *lutchuensis*, Adams 8538–8543, Japan. 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). 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 & 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 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 0.2 to 0.5%. The oils were clear to very light yellow in color. Table 1 shows the tabulated results. All the unknown compounds have been previously discussed (Adams, 1998; Adams et al., 1999).

Morphologically, *J. oxycedrus* is one of the most distinct taxa in the section *Juniperus*. The essential oil is dominated by α-pinene (41.3%), with moderate amounts of α-phellandrene, p-cymene, β-phellandrene, limonene, myrcene, α-terpineol, (E)-nerolidol and manoyl oxide (Table 1). In contrast, *J. oxycedrus* subsp. *macrocarpa* has large amounts of sabinene (26.5%) and α-pinene (22.6%). The oil of *J. oxycedrus* subsp. *badia* has a large amount of α-pinene (20.7%), very little sabinene (0.1%) and considerable amount of manoyl oxide (10.9%) along with several, apparently unique, unknown sesquiterpenes (see Adams et al., 1999, for discussion).

The oils of *J. taxifolia* and *J. taxifolia* var. *lutchuensis* were dominated by α-pinene (47.5, 46.6%) with moderate amount of myrcene, and β-pinene (Table 1). They differ in their amounts of limonene, bornyl acetate and other components (Table 1).

The composition of leaf essential oils of section *Juniperus* is generally much simpler and dominated by simple monoterpenes, in contrast to the essential oils of section *Sabina*, where oxygenated monoterpenes (e.g. camphor) and sesquiterpenes (e.g. cadinols, cedrol) are the major constituents (Adams, 1991b). This may reflect the evolutionary history of the genus, as it appears (Adams and Demeke, 1993) that section *Sabina* is the derived (advanced) group relative to section *Juniperus* (= *Oxycedrus*). It may be that the radiation of the approximately 50 species in section *Sabina* around the northern hemisphere has led to increased selection in various habitats and, thus, to the increased diversity found in the leaf essential oils of section *Sabina*.

In order to get a picture of the overall similarities of the oils, similarities were computed using presence/ absence matching. The minimum spanning network (Fig. 1) reveals that *J. taxifolia* and *J. t.* var. *lutchuensis* were the most similar oils. *Juniperus oxycedrus* clusters loosely with *J. o.* subsp. *macrocarpa* and J. *communis* clusters loosely with *J. navicularis* (Fig. 1). However, the overall pattern is for each taxon to be rather loosely clustered, suggesting that the taxa are distinct species.

Table 1
Comparisons of the percent total oil for leaf essential oils for *J. brevifolia* (BR), *J. cedrus* (CE), *J. communis* (CO), *J. communis* var. *saxatilis* (CS), *J. sibirica* (SB), *J. oblonga* (OL), *J. oxycedrus* (OX), *J. oxycedrus* subsp. *macrocarpa* (OM), *J. oxycedrus* var. *badia* (OB), *J. navicularis* (NA), *J. formosana* (FR), *J. rigida* (RG), *J. conferta* (CF), *J. taxifolia* (TX) and *J. taxifolia* var. *lutchuensis* (TL). Components that tend to separate the species are highlighted in boldface

KI	Compound	BR	CE	CO	CS	SB	OL	OX	OM	OB	NA	FR	RG	CF	TX	TL
854	(E)-2-hexenal	0.2	0.2	0.7	1.2	0.0	0.8	0.1	—	t	0.2	0.2	2.4	0.3	—	—
856	Ethyl isovalerate	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—
926	Tricyclene	t	0.3	0.3	t	0.2	0.1	0.1	0.1	t	0.1	0.1	0.2	0.1	0.8	0.2
931	α-Thujene	0.1	0.1	0.1	4.1	t	1.9	0.1	2.8	t	2.1	—	t	t	t	t
939	**α-Pinene**	**6.1**	**70.7**	**56.8**	**14.1**	**58.2**	**21.7**	**41.3**	**22.6**	**20.7**	**22.9**	**47.7**	**39.7**	**53.2**	**47.5**	**46.6**
953	α-Fenchene	t	—	0.4	0.1	—	0.2	—	0.1	—	—	—	0.6	0.2	—	—
953	Camphene	0.1	0.6	0.6	0.2	0.6	0.4	0.2	0.1	0.2	0.2	0.6	1.0	0.8	1.6	0.8
957	Thuja-2,4 (10)-diene	—	—	—	—	—	0.1	0.1	0.1	0.1	—	0.1	t	—	t	t
967	Verbenene	—	—	—	0.1	0.3	0.2	—	—	—	—	1.5	0.1	—	—	—
976	**Sabinene**	**2.5**	**1.0**	**0.7**	**32.8**	**1.0**	**13.4**	**0.6**	**26.5**	**0.1**	**8.2**	**0.2**	**t**	**0.4**	**0.2**	**0.5**
978	1-Octen-3-ol	1.4	1.0	—	—	—	t	—	—	0.6	—	—	—	1.0	—	—
980	**β-Pinene**	**0.2**	**4.1**	**4.4**	**1.9**	**4.7**	**2.2**	**1.7**	**0.8**	**0.6**	**3.5**	**2.9**	**1.9**	**8.0**	**8.8**	**8.3**
991	**Myrcene**	**6.3**	**6.3**	**5.2**	**5.0**	**4.5**	**3.8**	**4.7**	**2.9**	**0.6**	**8.6**	**7.2**	**11.2**	**10.4**	**11.2**	**10.0**
1001	δ-2-Carene	—	t	0.2	0.4	0.2	0.3	0.3	0.4	t	1.2	0.8	0.8	t	—	—
1005	**α-Phellandrene**	—	**0.5**	**2.1**	**0.5**	**0.1**	**0.8**	**8.2**	**0.4**	**0.3**	**8.0**	**1.2**	**1.0**	**0.3**	**t**	**t**
1011	**δ-3-Carene**	—	—	**4.7**	**0.5**	—	**2.4**	**t**	**0.5**	**t**	—	—	**t**	**3.2**	**3.8**	**t**
1018	α-Terpinene	0.2	t	—	1.9	—	1.1	0.2	1.8	—	0.9	t	t	—	t	0.1
1026	***P*-Cymene**	**0.1**	**0.2**	**0.3**	**0.3**	—	**1.2**	**6.2**	**3.4**	**0.5**	**2.6**	**0.9**	**0.6**	**0.2**	**t**	**t**
1031	**Limonene**	**43.4**	**4.5**	**6.9**	**6.7**	**1.3**	**2.0**	**4.5**	**2.5**	**0.1**	**14.3**	**4.0**	**4.2**	**1.9**	**3.2**	**17.5**
1031	**β-Phellandrene**	—	**4.6**	**6.9**	**0.6**	**1.2**	**1.9**	**5.0**	**2.5**	**1.0**	**3.5**	**1.4**	**2.1**	**4.0**	**3.1**	**0.1**
1040	(Z)-β-Ocimene	t	—	0.2	—	—	t	—	—	—	—	—	t	—	t	t
1050	**(E)-β-Ocimene**	**3.8**	—	—	**0.1**	—	**0.5**	—	—	—	**0.4**	—	**t**	—	**t**	**t**
1057	Pentyl isobutyrate	—	—	0.2	—	—	0.4	—	—	—	0.1	0.2	0.6	0.2	—	0.3
1062	γ-Terpinene	0.3	0.1	t	3.4	—	2.0	0.4	3.0	—	1.6	0.1	0.2	0.1	t	0.1
1065	3-Methyl-2-buten-1-yl, acetate[a]	—	—	—	—	—	—	—	—	—	—	0.6	—	—	—	—
1068	*cis*-Sabinene hydrate	t	—	—	1.8	—	2.3	—	1.7	—	0.1	—	t	—	—	0.1
1087	Fenchone	—	—	—	—	—	—	—	—	—	—	—	1.6	—	—	—
1088	Terpinolene	4.4	0.6	1.1	3.0	0.7	2.2	2.9	1.2	0.8	2.9	1.0	0.8	0.5	0.8	0.8

—continued

Table 1—*continued*

KI	Compound	BR	CE	CO	CS	SB	OL	OX	OM	OB	NA	FR	RG	CF	TX	TL
1091	2-Nonanone	—	—	—	—	—	—	—	—	—	—	0.1	t	—	—	—
1095	α-Pinene oxide	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	—
1097	Ipsenol	—	—	—	—	—	—	—	—	—	—	0.7	—	—	—	—
1097	*trans*-Sabinene hydrate	—	—	—	1.3	—	—	—	1.7	—	—	—	—	—	0.7	0.1
1098	Linalool	0.9	—	—	t	—	—	t	—	0.2	0.2	—	0.6	—	0.8	0.2
1102	*n*-Nonanal	t	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—
1103	Isopentyl-isovalerate	—	—	0.1	t	0.1	0.3	—	—	—	—	—	—	—	—	0.1
1110	1-Octen-3-yl acetate	—	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—
1112	**endo-Fenchol**	—	—	—	—	—	—	—	—	—	—	**0.5**	**0.8**	**0.5**	—	—
1114	*trans*-Thujone	—	—	—	0.6	—	0.5	—	0.1	—	—	—	—	—	—	—
1116	3-Methyl butanoate, 3-methyl-3-butenyl	—	—	0.1	—	0.1	—	—	—	—	—	—	—	—	t	t
1121	*cis-p*-Menth-2-en-1-ol	—	—	—	—	—	0.6	0.1	0.7	—	0.1	0.2	—	—	t	t
1125	α-Campholenal	—	—	—	—	t	0.3	0.5	0.7	2.9	—	0.3	0.2	—	0.2	0.3
1139	***trans*-Pinocarveol**	—	—	—	—	—	—	**0.5**	**1.2**	**1.3**	—	**0.3**	**0.2**	**t**	**t**	**0.3**
1140	*trans-p*-Menth-2-en-1-ol	—	—	—	0.3	—	—	0.5	—	—	—	t	—	—	—	—
1140	*trans*-Sabinol	—	—	—	—	—	0.1	—	—	0.4	—	—	—	—	—	—
1140	*cis*-Verbenol	—	—	—	—	—	—	t	0.2	—	—	0.1	t	—	—	—
1143	Camphor	—	—	—	—	—	—	—	—	—	—	—	0.6	t	0.4	t
1143	*cis*-Sabinol[a]	—	—	—	t	0.1	0.8	—	—	1.1	—	—	—	t	t	0.4
1143	*trans*-Verbenol	—	—	—	—	—	—	0.5	1.3	—	—	0.5	t	—	—	—
1148	**Camphene hydrate**	—	—	—	—	—	—	—	—	—	—	**0.3**	**0.2**	**0.2**	**t**	**0.1**
1153	Citronellal	—	—	—	—	—	0.3	—	—	—	—	—	—	—	—	—
1156	Sabina ketone	—	—	—	—	—	—	—	0.5	—	—	—	—	—	—	—
1159	*p*-Mentha-1,5-dien-8-ol	—	—	—	—	—	—	0.1	t	—	0.3	—	—	—	—	—
1160	*trans*-Pinocamphone	—	—	—	—	—	—	—	—	0.3	—	t	—	—	t	t
1162	Pinocarvone	—	—	—	—	—	—	—	0.1	0.4	—	t	—	—	—	—
1165	Borneol	0.1	0.1	0.2	t	t	0.3	—	—	1.0	—	0.4	0.5	0.4	0.5	0.1
1166	δ-Terpineol	—	—	—	—	—	—	—	0.3	0.6	—	—	—	—	—	—
1173	*cis*-Pinocamphone	—	—	—	—	—	—	—	—	t	—	t	t	t	t	t
1177	**Terpinen-4-ol**	**0.8**	**t**	**0.2**	**7.3**	**0.2**	**6.4**	**1.5**	**7.3**	**0.3**	**2.8**	**0.5**	**0.6**	**0.2**	**0.2**	**0.3**
1179	Naphthalene	0.2	0.1	t	0.3	—	—	—	—	—	0.1	—	t	—	1.8	0.2

1183	*p*-Cymen-8-ol	t	—	t	t	—	0.3	0.4	0.7	0.2	t	0.1	t	t	—	—
1189	**α -Terpineol**	**0.3**	**0.3**	**0.2**	**0.4**	**0.3**	**2.3**	**5.0**	**1.4**	**1.0**	**1.1**	**0.6**	**1.1**	**0.5**	**0.4**	**0.7**
1193	Myrtenal	—	—	—	—	—	—	—	0.4	0.5	—	—	—	—	—	—
1194	Myrtenol	—	—	—	—	t	0.2	—	—	0.3	—	0.2	t	—	—	—
1204	Verbenone	—	—	—	—	—	0.3	t	0.4	0.3	—	0.1	t	t	t	t
1217	*trans*-Carveol	—	—	—	—	—	0.1	0.2	0.3	0.5	—	0.1	t	—	t	0.1
1220	**endo-Fenchyl acetate**	—	—	—	—	—	—	—	—	—	—	**0.2**	**0.6**	**0.6**	—	—
1228	Ci tronellol	—	—	t	—	—	0.6	—	0.2	—	—	0.4	0.2	0.2	—	0.4
1235	Thymol, methyl ether	—	—	—	0.1	—	—	—	t	0.1	—	—	—	—	—	—
1235	*trans*-Chrysanthenyl acetate	—	—	—	—	0.2	0.2	—	—	—	—	0.3	—	—	—	—
1239	Cumin aldehyde	—	—	—	—	—	—	—	t	t	—	—	—	—	—	—
1242	Carvone	—	—	—	—	t	—	—	t	t	—	—	—	—	—	0.1
1244	Methyl carvacrol	0.2	—	—	—	—	—	—	—	t	—	—	—	—	—	—
1252	Piperitone	—	—	—	—	0.2	—	t	0.2	—	—	0.6	t	—	—	—
1255	Geraniol	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—	—
1261	Methyl citronellate	—	—	—	—	0.4	1.9	—	—	—	—	—	—	0.1	—	—
1262	Sesquiterpene	—	—	—	—	—	—	—	—	—	—	1.2	0.8	—	—	—
1273	*p*-Menth-1-en-7-al	—	—	—	—	—	—	t	0.1	t	—	—	—	—	—	—
1283	(E)-anethole	0.1	—	—	—	—	—	—	—	—	—	—	t	—	—	—
1285	Bornyl acetate	—	0.7	0.9	0.2	1.1	1.6	—	—	1.4	t	1.6	1.3	2.0	9.4	1.1
1291	2-Undecanone	—	—	—	—	—	1.7	—	—	—	—	—	4.8	t	—	—
1292	Sesquiterpene	—	—	—	—	—	—	—	—	—	—	1.4	—	—	—	—
1297	*trans*-Pinocarvyl acetate	—	—	—	—	—	t	—	—	t	—	—	—	—	—	—
1298	Carvacrol	0.1	—	—	—	—	—	t	—	—	—	—	—	—	—	—
1324	Terpene alcohol	—	—	—	—	0.5	0.9	—	—	—	—	0.1	—	t	—	—
1350	α-Terpinyl acetate	—	1.0	—	0.5	0.7	—	—	—	3.6	—	0.2	—	0.6	0.2	0.6
1351	α-Cubebene	0.1	—	—	—	—	—	t	—	—	—	—	—	—	—	—
1354	Citronellyl acetate	—	—	—	0.1	—	0.3	—	—	—	—	—	—	0.1	t	0.3
1376	α-Copaene	—	—	—	—	t	—	—	—	—	t	0.2	—	—	—	—
1381	*trans*-Myrtanyl acetate	—	—	—	t	—	0.2	—	—	—	—	—	—	—	—	—
1383	Geranyl acetate	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—	—
1384	β-Bourbenene	—	—	—	—	—	t	0.2	t	2.0	—	—	0.2	—	—	—
1390	β-Cubebene	0.1	—	—	—	t	—	—	—	—	—	—	—	—	—	0.1
1391	β-Elemene	—	—	0.2	t	0.2	0.2	—	—	—	—	—	—	—	t	t

—*continued*

Table 1—*continued*

KI	Compound	BR	CE	CO	CS	SB	OL	OX	OM	OB	NA	FR	RG	CF	TX	TL
1418	(E)-Caryophyllene	0.3	0.4	0.7	t	t	0.2	0.2	0.1	0.9	0.7	1.0	1.6	0.5	1.0	0.4
1428	β-Copaene	—	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—
1454	α-Humulene	0.4	t	0.5	t	t	0.1	t	t	1.0	0.3	0.6	1.3	0.4	0.6	0.4
1458	(E)-β-Farnesene	—	—	—	—	—	—	—	—	—	t	0.2	0.4	t	t	t
1471	Sesquiterpene	—	—	—	—	—	—	—	—	—	—	—	1.3	—	—	—
1477	γ-Muurolene	—	—	—	t	0.3	—	—	—	0.8	0.1	t	t	—	—	—
1480	Germacrene D	0.2	—	0.7	0.4	0.3	1.2	1.0	0.5	8.5	0.2	2.3	2.3	—	t	0.4
1493	epi-Cubebol	—	—	—	—	—	t	—	—	—	—	t	—	—	—	—
1494	2-Tridecanone	—	—	—	—	—	—	—	0.1	1.0	—	—	—	—	—	—
1494	Bicyclogermacrene	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—	0.1
1495	(E) —methyl isoeugenol	—	—	—	0.2	—	—	—	—	—	—	0.2	0.7	—	—	—
1499	**α-Muurolene**	**t**	—	**0.2**	**0.2**	**1.3**	**t**	—	**t**	**0.4**	**0.3**	**0.2**	**t**	—	**t**	**t**
1503	Germacrene A	—	—	0.1	0.2	0.4	0.2	—	—	—	—	—	t	—	—	0.1
1507	Sesquiterpene	—	—	—	—	—	—	—	—	—	—	0.5	—	—	—	—
1508	α-Farnesene	—	—	—	—	—	—	—	—	—	—	—	0.7	—	—	—
1513	**γ-Cadinene**	**0.3**	—	**0.2**	**0.4**	**1.7**	**0.6**	**0.5**	**t**	**1.3**	**0.6**	**2.4**	**0.6**	—	**t**	**0.7**
1517	sesquiterpene	—	—	—	—	—	—	—	—	—	—	0.5	—	—	—	—
1524	**δ-Cadinene**	**0.3**	—	**0.5**	**0.8**	**2.6**	**0.5**	**t**	**0.3**	**0.8**	**1.9**	**0.9**	**0.1**	**0.1**	**t**	**0.4**
1529	Citronellyl butyrate	—	—	—	—	—	—	—	—	—	—	0.1	—	—	—	—
1538	α-cadinene	—	—	—	t	0.3	—	—	—	—	—	0.1	—	—	—	—
1549	Elemol		—	—	—	—	0.1		—	—	—	—	—	—	—	0.1
1553	Sesquiterpene	—	—	—	—	—	—	—	—	1.7	—	—	—	—	—	—
1556	Germacrene B	0.1	—	0.3	0.3	0.2	0.7	—	—	0.3	—	—	0.2	0.1	—	—
1562	Geranyl butyrate	—	—	—	—	—	—	—	—	—	—	0.5	0.3	—	—	—
1564	**(E)-nerolidol**	—	—	—	—	—	**t**	**3.3**	—	—	**4.2**	**0.3**	**1.0**	**0.2**	**t**	**t**
1574	**Germacrene D-4-ol**	—	—	**0.8**	**1.8**	**6.8**	**1.3**	—	**0.1**	—	**0.1**	**0.9**	—	—	—	**0.4**
1576	Spathulenol	—	—	—	—	—	0.8	—	—	—	—	—	0.3	—	—	0.3
1581	Caryophyllene oxide	—	—	—	—	—	t	0.2	0.4	1.5	—	0.3	0.6	t	0.4	0.1
1588	Sesquiterpene alcohol	—	—	—	—	—	—	—	—	0.8	—	—	—	—	—	—
1591	Sesquiterpene alcohol	—	—	—	—	—	—	—	—	0.9	—	—	—	—	—	—
1596	Cedrol	—	—	—	—	—	—	—	—	—	—	—	0.3	—	—	—
1606	Humulene epoxide II	0.1	—	—	—	—	0.2	—	0.2	0.4	—	0.2	0.5	—	—	—

1606	β-Oplopenone	—	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—
1609	Sesquiterpene alcohol	—	—	—	—	—	—	—	—	1.7	—	—	—	—	—	—
1616	Sesquiterpene alcohol	—	—	—	—	—	0.9	—	—	0.8	—	—	—	—	—	—
1627	1-epi-Cubenol	0.4	—	—	t	—	0.2	—	—	—	—	t	—	—	—	0.3
1640	epi-α-Cadinol	—	—	t	0.5	0.7	0.1	t	t	0.9	1.6	1.6	0.6	—	—	0.3
1640	epi-α-Muurolol	—	—	0.4	0.5	0.9	0.1	—	—	0.4	—	—	—	—	—	0.1
1642	Cubenol	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1645	α-Muurolol	—	—	—	0.1	0.2	t	—	—	—	0.3	0.2	—	—	—	—
1651	Sesquiterpene alcohol	—	—	—	—	—	—	—	—	2.0	—	—	—	—	—	—
1652	α-Eudesmol	—	—	—	—	t	—	—	—	—	—	t	—	—	—	—
1653	**α-Cadinol**	—	—	**0.5**	**1.3**	**2.3**	**0.6**	**t**	**0.3**	**0.2**	**2.2**	**1.1**	**0.3**	—	—	**0.4**
1670	Sesquiterpene alcohol	—	—	—	—	—	—	—	6.4	—	—	—	—	—	—	—
1685	Eudesma-4(15),7-dien-1-β-ol	—	—	—	—	—	—	—	1.2	—	—	—	—	—	—	—
1686	epi-α-Bisabolol	—	—	—	—	—	—	—	—	—	—	—	0.7	2.0	0.4	1.2
1689	Sesquiterpene alcohol	—	—	—	—	—	0.9	—	—	—	—	—	—	—	—	—
1698	2-Pentadecanone	—	—	—	—	—	—	—	—	—	—	0.2	—	—	—	—
1700	Heptadecane	—	—	—	—	—	—	—	—	—	—	0.1	—	—	—	—
1713	(Z,Z)-Farnesol	—	—	—	—	—	0.2	0.3	—	0.4	—	—	t	—	—	—
1722	**(E,E)-Farnesol**	—	—	—	—	—	**3.3**	**0.9**	**0.2**	**0.7**	—	**0.7**	**1.4**	**0.2**	—	**0.9**
1773	(E)-α -Atlantone	—	—	0.3	—	0.4	—	—	—	—	—	—	—	—	—	—
1742	(E,Z)-Farnesol	—	—	—	—	—	0.5	0.4	—	0.5	—	—	t	—	—	—
1843	(E,E)-Farnesyl acetate	—	—	—	—	—	0.2	—	—	—		—	—	—	—	—
1941	Pimaradiene	0.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1961	**Sandaracopimara-8(14),15-diene**	**19.5**	—	—	—	**0.2**	—	—	**t**	**0.1**	—	—	—	—	—	—
1989	**Manoyl oxide**	**1.2**	—	—	**0.2**	—	**0.2**	**5.3**	**2.6**	**10.9**	**0.1**	**t**	—	**0.2**	**t**	**t**
2000	Eicosane	—	—	—	—	—	—	—	—	—	—	—	—	—	1.7	t
2010	epi-13-Manoyl oxide	—	—	—	—	—	—	t	—	0.5	—	—	—	—	—	—
2054	Abietatriene	1.3	0.1	—	0.2	0.2	t	t	0.3	2.4	—	t	0.5	0.3	t	0.3
2080	Abietadiene	0.3	—	—	t	0.2	0.1	0.7	0.7	1.8	—	t	t	t	t	0.3
2103	Diterpene	0.6	—	—	—	—	—	—	—	—	—	—	—	5.1	—	—
2205	*cis*-Totarol, methyl ether	0.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2234	*trans*-Totarol, methyl ether	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2302	*trans*-Totarol	0.4			—	—	—	—	—	—	—	—	t	—	—	—

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.

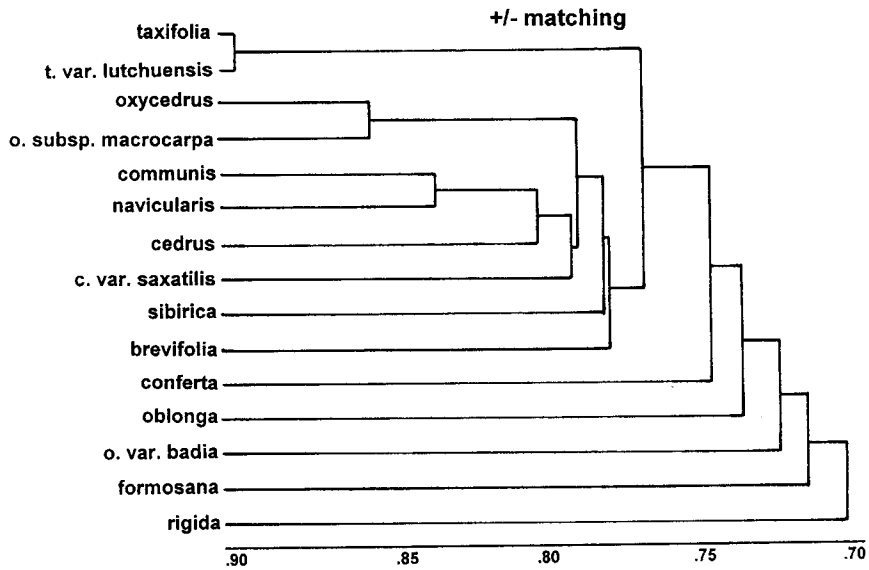

Fig. 1. Minimum spanning network for the taxa based on 105 terpenes for which similarity measures were computed as simple presence/ absence (±) matches. See text for discussion.

In contrast to the oils data, the DNA data shows much more groupings (Fig. 2). However, several taxa are quite distinct: *J. brevifolia*, *J. cedrus*, *J. navicularis*, *J. oxycedrus*, *J. o.* subsp. *macrocarpa* and *J. o.* var. *badia* (Figs. 2 and 3). Two of the east Atlantic island endemics, *J. brevifolia* and *J. cedrus* form a very loose group. *Juniperus navicularis* has been treated as *J. oxycedrus* subsp. *transtagana* (Table 2), but it is quite distinct in its oils, DNA, morphology and habitat. It grows in the Pliocene sands of southwestern Portugal as a small, fastigiate shrub and a understory plant in *Pinus* forests. It clearly deserves recognition at the specific level (Table 2).

Juniperus oxycedrus subsp. *macrocarpa* is quite distinct in having very large female cones, and large flat needles as well as being distinct in its DNA and oils. The taxon merits recognition at the specific level as *J. macrocarpa* (see Table 2). *Juniperus oxycedrus* var. *badia* is a large tree from central Spain that is very distinct in both its oils and DNA and warrants recognition at the specific level: *J. badia* (see Table 2).

The strong clustering of the east Asian species (*J. conferta*, *J. formosana*, *J. rigida*, *J. taxifolia* and *J. taxifolia* var. *lutchuensis*) and the clustering of the *J. communis-sibirica-oblonga* group (Figs. 2 and 3) present some difficulties in assessing the taxonomic ranks of these taxa. Fig. 3 shows that these complexes form clumps among the junipers of this section. In the case of the *communis* complex, there seems to be three groups: (1) *J. communis* var. *communis* and *J. c.* var. *saxatilis*; (2) *J. sibirica* (*J. communis* var. *saxatilis*, Table 2); and (3) *J. oblonga* (*J. communis* var. *oblonga*, see Table 2). If one recognizes *J. oblonga* at the specific level, then one should probably recognize *J. sibirica*, to be consistent (Table 2). *Juniperus communis* has several other infraspecific

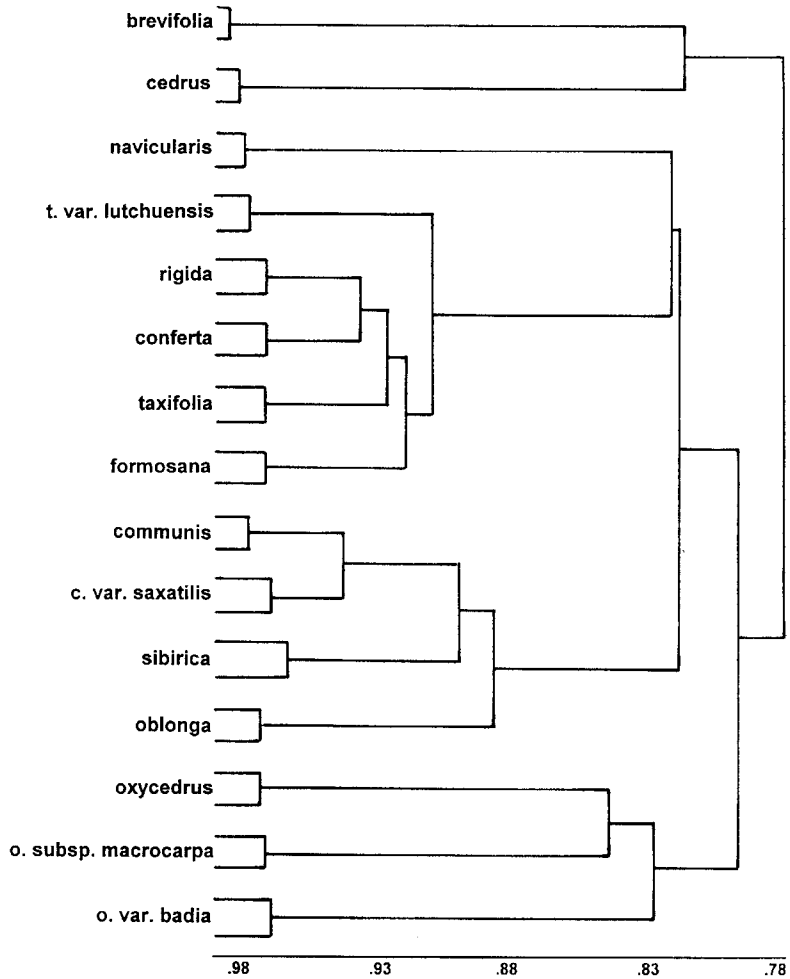

Fig. 2. Minimum spanning network for *Juniperus* sect. *Juniperus* based on 193 RAPD DNA bands. Several major groupings are apparent. See text for discussion.

taxa not examined in this study: var. *depressa* Pursh. (Adams, 1993), subsp. *hemispherica* (J. and C. Presl.) Nyman (Franco, 1964); var. *hondoensis* Satake (Ohwi, 1965); var. *nipponica* (Maxim.) Wils.(Ohwi, 1965) and var. *megistocarpa* Fernald and H. St. John (Adams, 1993). A detailed study of infraspecific variation within *J. communis* is presently in progress but is beyond the scope of this report.

If one recognizes *J. sibirica* at the specific level, then it seems (see Fig. 2) that *J. taxifolia* var. *lutchuensis* should, like wise, be recognized (*J. lutchuensis*, see Table 2). *Juniperus lutchuensis* is known only from Oshima Island, and the Ryukyu Islands of Japan (Kitamura and Murata, 1979).

Table 2
Comparison of Farjon's (1998) recent treatment and the current results based on DNA data and essential oils. Syn = Farjon placed the taxon in synonymy

Farjon (1998)	DNA and essential oils
J. brevifolia (Seub.)Antoine	*J. brevifolia* (Seub.)Antoine
J. cedrus Webb & Berthol.	*J. cedrus* Webb & Berthol.
J. communis L.	*J. communis* L.
J. communis var. *saxatilis* Pall	*J. communis* var. *saxatilis* Pall.
J. communis var. *oblonga* (M.-Bieb.) Loud.	*J. oblonga* M.-Bieb.
Syn: *J. communis* var. *saxatilis* Pall.	*J. sibirica* Brugsd.
J. formosana Hayata	*J. formosana* Hayata
J. oxycedrus L.	*J. oxycedrus* L.
J. oxycedrus subsp. *badia* (H. Gay) Debeaux	*J. badia* H. Gay
J. oxycedrus subsp. *macrocarpa* Sibth. & Sm.)Neilr.	*J. macrocarpa* Sibth and Sm.
J. oxycedrus subsp. *transtagana* Franco	*J. navicularis* Gand.
J. rigida Siebold & Zucc.	*J. rigida* Siebold & Zucc.
J. rigida subsp. *conferta* (Parl.) Kitam.	*J. rigida* var. *conferta* (Parl.) Patschke
J. taxifolia Hook & Arn.	*J. taxifolia* Hook & Arn.
Syn: *J. taxifolia* Hook and Arn.	*J. lutchuensis* Koidz.

Juniperus formosana occurs in both central-eastern China and Taiwan. Its specific status has not been controversial and its oil is very distinct (Table 1, Fig. 1). So it seems prudent to continue to recognize *J. formosana* at the specific level. This leaves *J. taxifolia* with a DNA similarity index of about 0.91 as being in the transitional level between a variety and species. The taxon is endemic to the Bonin Islands, quite far south of the larger islands of central Japan. The oils of *J. taxifolia* and *J. t.* var. *lutchuensis* are very similar (Table 1, Fig. 1), yet the DNAs are quite different. In fact, the DNA of *J. taxifolia* is most similar to *J. conferta* (another shrub). This data set is based on 193 RAPD DNA bands. The presence or absence of just a few bands could make a difference in the classification. Although, the continued recognition of *J. taxifolia* (Table 2) as the specific level is supported by these data, additional information on this taxon may change its taxonomic status in the future.

This study supports the recognition of *J. conferta* as a variety of *J. rigida* (*J. rigida* var. *conferta*, Table 2). Variety rather than subspecies is used as the ranking category to be consistent throughout the genus *Juniperus*.

In previous studies comparing leaf essential oils and DNA fingerprints (Adams, 1999a,b), there has been good agreement between these kinds of data. In the present case, these junipers are generally very similar in their oils. No large trends are readily apparent in the oils. Numerous trace components are present (because the oils are generally dominated by one or a very few simple monoterpenes such as the pinenes, sabinene, limonene, etc.). Perhaps these trace components are products of free radical reactions or side products arising from the non-specificity of enzymes and have generated 'noise' in the data that obscures the major trends. We know that there are

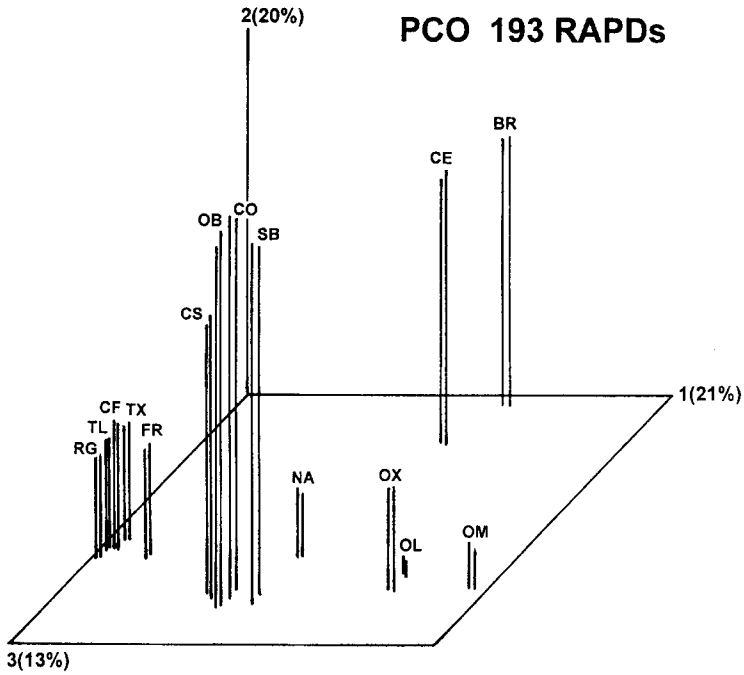

Fig. 3. Principal coordinate analysis (PCO) based on 193 RAPD DNA bands. Two major species complexes are seen: *J. rigida* complex (far left) and *J. communis* complex, front center. Note that the *J. brevifolia* (BR) and *J. cedrus* (CE) are rather distant from all the other taxa. See Table 1 for codes.

limits in comparing (for example) the presence of α-pinene across the gymnosperms as a taxonomic character. However, the analysis of quantitative variation of terpenoids within a species, if environmental factors are accounted for, can be quite useful in assaying infraspecific trends (Adams, 1991b).

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