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Systematics of smooth leaf margin *Juniperus* of the western hemisphere based on leaf essential oils and RAPD DNA fingerprinting

Robert P. Adams*

Plant Biotechnology Center, Baylor University, Box 669, Gruver, TX 79040, USA

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Abstract

The composition of the leaf essential oils of 13 taxa of the smooth leaf margin *Juniperus* in sect. *Sabina* from the western hemisphere are reported and compared. In addition, DNA fingerprinting revealed similar patterns among these species. Based on these data, a new species, very similar to *J. blancoi* and *J. scopulorum*, is recognized from northern Mexico: *Juniperus mucronata sp. nov.* R.P. Adams. Although the terpenes and morphology support the recognition of *J. barbadensis* var. *lucayana* (see Adams, R.P., 1995a, Revisionary study of Caribbean species of *Juniperus* (Cupressaceae). Phytologia, 78, 134–150), the RAPDs do not favor that alignment. Therefore, *J. lucayana* will be recognized as a distinct species at present. The recognition of *J. ekmanii* and *J. urbaniana* as varieties of *J. gracilor* is supported by the RAPDs data and these varieties will be maintained in the treatment of *Juniperus*.

Keywords: *Juniperus*; Cupressaceae; *Juniperus mucronata* R.P. Adams; Essential oils; Terpenes; RAPD; DNA fingerprinting; Systematics

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

* Corresponding author. Tel.: +1-806-733-5558; fax: +1-806-733-5605.
E-mail address: rpadams@juno.com (R.P. Adams)

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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*), with 10–12 species) and *Sabina* (the remaining, approximately 50 species).

Gaussen (1968) divided section Sabina into two groups: leaves minutely serrate versus entire (non-serrate) margins. Gaussen (1968) placed *J. convallium, J. mekongensis* (= *J. convallium*, Farjon, 1998), *J. phoenicea, J. saltuaria, J. seravschanica, J. pseudosabina, J. wallachiana, and J. zaidamensis* (= *J. przewalskii*, Farjon, 1998) in the entire leaf margined group with species from the western hemisphere. The author recently re-examined the aforementioned species, and found that very small and irregular teeth were found on leaves of *J. phoenicea*, and extremely small and very irregular serrations were found on *J. pseudosabina* (Adams, unpublished).

A study using RAPDs (Adams and Demeke, 1993), confirmed that section *Sabina* could be divided into junipers with serrate and those with entire (smooth) leaf margins plus *J. phoenicea* and *J. pseudosabina*. The truly serrate leaf margined junipers (excluding *J. phoenicea* and *J. pseudosabina*, which I would call 'pseudoserrate' as their DNA (Adams and Demeke, 1993) clearly points to their affinity with the smooth leaf margined junipers) are confined to the western hemisphere.

The smooth leaf margined *Juniperus* seem to be divided into those of the eastern and western hemispheres (Adams and Demeke, 1993). The smooth leaf margined (series entire) junipers of the western hemisphere are (Adams, 1993, 1995a; Zanoni and Adams, 1976): *J. barbadensis* L. endemic to St. Lucia, BWI; *J. barbadensis* var. *lucayana* (Britton) R.P. Adams, Bahama Islands, Cuba and Jamaica; *J. bermudiana* L. endemic to Bermuda Island, *J. blancoi* Mart., Mexico; *J. gracilior* Pilger, endemic to Hispanola, *J. gracilior* var. *ekmanii* (Florin) R.P. Adams, endemic to Hispanola, *J. gracilior* var. *urbaniana* (Pilger and Ekman) R.P. Adams, endemic to Haiti; *J. horizontalis* Moench, northern United States and southern Canada; *J. saxicola* Britt. P. Wilson, endemic to Cuba; *J. scopulorum* Sarg., western United States and Canada, northern Mexico, *J. virginiana* L., eastern United States and southeastern Canada, and *J. virginiana* var. *silicicola* (Small) E. Murray, southeastern United States, coastal fore-dunes.

In addition, we recently examined juniper plants from western Chihuahua/ eastern Sonora, Mexico (near Maicoba) that had mucronate tipped leaves, which will be referred to as 'mucronata' in this paper. These plants were very similar to *J. blancoi/ J. scopulorum* and are included in this study to ascertain their taxonomic status.

The volatile leaf oils of the Caribbean junipers (*J. barbadensis* and *J. barbadensis* var. *lucayana, J. bermudiana, J. gracilior, J. gracilior* var. *ekmanii, J. gracilior* var. *urbaniana, J. saxicola*) and *J. virginiana* and *J. virginiana* var. *silicicola* have been recently reported (Adams, 1995a). The oils of *J. blancoi, J. horizontalis* and *J. scopulorum* were reported some time ago (Adams et al., 1981), so a reexamination of these is timely.

In this paper, both the volatile leaf oils and DNA fingerprints will be examined for the smooth leaf margined *Juniperus* of section *sabina* of the western hemisphere to aid in understanding of the circumspection of the species.

2. Materials and methods

Specimens used in this study: *J. barbadensis*, Adams 5367–5370, St. Lucia, BWI; *J. barbadensis* var. *lucayana*, Adams 2875–2884, Jamaica and 5281–5282, Isle de Pinos, Cuba; *J. bermudiana*, Adams 2553, 2555–2567, Bermuda Island; *J. blancoi*, Adams, 6503–6504, 6849–6851, El Oro, Mexico; *J. gracilior*, Adams 2785–2794, 7664–7667, Constanza, Dominican Republic; *J. gracilior* var. *ekmanii*, Adams 3106, 3107, 7653, 7654, Pic La Selle, Haiti; *J. gracilior* var. *urbaniana*, Adams 7656–7658, Pic La Selle, Haiti; *J. horizontalis*, Adams 7096–7098, Saskatoon, Saskatchewan, Canada; *J. saxicola*, Adams 5284, 5285, Pico Turquino, Cuba; *J. scopulorum*, Adams 2010–2024, Durango, Colorado and 7063–7066, Soda Springs, Idaho, USA; *Juniperus* "mucronata", Adams 8701–8705, G.M. Ferguson 1448, 1456–1459; 1479, Maicoba, Mexico; *J. virginiana*, Adams 2405–2423, Washington, DC and 6753–6755, Waco, Texas, USA; *J. virginiana* var. *silicicola*, Adams 2716–2725, Murrells Inlet, South Carolina and 2775, 2776, Oak Hill, Florida, USA 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 (48 h, 100°C) for determination of oil yields. After GCMS analyses of each individual oil sample, 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 (= SE54), 0.26 mm × 30 m, 0.25 μm coating thickness, fused silica capillary column (see Adams, 1995b for operating details). Identifications were made by library searches of our volatile oil library, LIBR(TP) (Adams, 1995b), 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′): 131: GAA ACA GCG T; 134: AAC ACA CGA G; 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; 227: CTA GAG GTC C; 237: CGA CCA GAG C; 239: CTG AAG CGG A; 244: CAG CCA ACC G; 250: CGA CAG TCC C; 265: CAG CTG TTC A; 268: AGG CCG CTT A; 327: ATA CGG CGT C.

PCR was performed in a volume of 15 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, 0.3 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 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). In addition, for the terpenoid data, similarities were computed as simple presence–absence data. 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 1. Several trends are apparent from the terpenoid data. Notice that the oils of *J. gracilior* (GR), *J. gracilior* var. *ekmanii* (EK) and *J. gracilior* var. *urbaniana* (UR) are dominated by bornyl acetate (30 - 38%). A minimum spanning network based on the oils of the smooth leaf margined junipers is shown in Fig. 1. The smooth leaf margined junipers are divided into the mainland group (*J. blancoi, horizontalis*, 'mucronata', *scopulorum, virginiana* var. *virginiana* and var. *silicicola* plus *saxicola* from Cuba) and the junipers from the Caribbean Islands (plus *J. bermudiana* from Bermuda Island). Adams (1995a) previously recognized *J. ekmanii* and *J. urbaniana* as a varieties of *J. gracilior* on the basis of quantitative analyses of essential oils and morphology. These taxa form a very distinct group (Fig. 1) based on their qualitative oils compositions. In addition, *J. barbadensis* (St. Lucia) forms a group with *J. lucayana* (Isle de Pinos, Cuba). This led to the combination of *J. barbadensis* var. *lucayana* (Britt.) R.P. Adams (Adams, 1995a) based on the oils and morphology. The mucronate leaf tip juniper from Maicoba, Mexico is barely distinct from *J. scopulorum* in its oil (Fig. 1).

A minimum spanning network based on 132 RAPDs (Fig. 2) reveals several similar patterns as found in the oils (Fig. 1). *Juniperus gracilior, J. g.* var. *ekmanii* and *J. g.* var. *urbaniana* form a loose group (Fig. 2). A major difference in the terpene and RAPDs data is that *J. barbadensis* var. *lucayana* is linked with *J. bermudiana* (Fig. 2) in the RAPD data, whereas *J. b.* var. *lucayana* clustered with *J. barbadensis* using the terpene data (Fig. 1). *Juniperus saxicola* is linked with the Caribbean junipers in the RAPDs data. *Juniperus virginiana* and *J. virginiana* var. *silicicola* fail to cluster together in the RAPDs data (Fig. 2).

Juniperus horizontalis was the most distinct species in its RAPDs (Fig. 2). The mucronate tip leaf juniper from Maicoba was quite distinct in its RAPDs and linked loosely with *J. blancoi* (Fig. 2).

In order to better visualize the relationships among *J. blancoi, J. horizontalis, J. scopulorum* and the mucronate juniper, a data set using just these four taxa was

Table 1
Comparisons of the per cent total oil for leaf essential oils for *J. barbadensis* (BA), *J. barbadensis* var. *lucayana* (LU), *J. bermudiana* (BR), *J. blancoi* (BL), *J. gracilior* (GR), *J. gracilior* var. *ekmanii* (EK), *J. gracilior* var. *urbaniana* (UR), *J. horizontalis* (HZ), *J. saxicola* (SX), *J. scopulorum* (SC), mucronate tip Juniper (MU), *J. virginiana* (VG) and *J. virginiana* var. silicicola (SI).Components that tend to separate the species are highlighted in boldface

KI	Compound	GR	EK	UR	BR	LU	BA	SX	SI	VG	BL	MU	SC	HZ
854	**2E-Hexenal**	**0.1**	**t**	**t**	–	**0.5**	**0.1**	–	–	**t**	–	–	–	–
857	**3Z-Hexenol**	**0.7**	**t**	**t**	–	–	**0.2**	–	–	–	–	–	–	–
899	**Heptanal**	–	–	–	–	–	–	–	–	–	**0.5**	**1.0**	**0.2**	**0.3**
901	**Unknown, FW 125, 43, 55, 67**	**0.7**	**t**	–	–	–	–	–	–	–	–	–	–	–
912	**Unknown FW 125, 43, 55, 67**	**0.6**	**t**	–	–	–	–	–	–	–	–	–	–	–
926	Tricyclene	0.7	3.1	1.5	0.3	0.2	t	t	t	t	–	t	t	–
931	α-Thujene	0.3	0.7	0.8	0.1	0.6	0.5	0.2	0.1	0.2	1.5	2.6	1.1	1.0
939	**α-Pinene**	**1.2**	**6.4**	**1.6**	**14.3**	**36.7**	**4.7**	**4.6**	**0.9**	**1.3**	**1.9**	**3.9**	**4.5**	**3.9**
953	α-Fenchene	–	–	–	–	t	t	–	–	–	–	t	–	t
953	Camphene	0.9	2.4	0.6	0.5	0.4	0.1	t	0.1	0.1	–	t	t	t
957	Thuja-2,4(10)-diene	–	–	–	0.1	0.2	–	–	–	–	–	–	–	–
976	Sabinene	6.3	15.0	12.2	2.7	8.5	26.2	6.2	0.6	6.4	34.5	36.8	35.6	38.6
978	1-Octen-3-ol	t	t	t	1.6	0.1	0.9	–	0.9	–	t	–	–	t
980	β-Pinene	t	0.3	0.2	t	1.2	t	–	t	0.1	t	0.3	0.2	0.5
991	Myrcene	1.6	4.5	3.6	3.0	3.7	2.7	0.6	0.5	0.7	2.5	4.9	1.5	3.5
996	**Hexanoic acid, 4-methyl, methyl ester**[a]	–	–	–	–	–	–	–	–	**0.1**	**0.2**	**1.4**	–	–
1001	δ-2-Carene	0.1	0.3	0.6	–	0.1	–	–	t	0.1	0.7	0.4	–	0.1
1005	α-Phellandrene	0.1	0.1	t	t	–	0.1	t	–	–	0.1	–	t	t
1011	δ-3-Carene	–	–	–	t	–	–	–	t	0.1	–	t	0.1	0.5
1018	α-Terpinene	1.4	0.8	1.4	0.4	0.7	0.8	0.6	t	0.2	1.6	1.8	1.1	1.0
1026	p-Cymene	1.2	0.4	2.2	0.5	0.1	1.2	0.2	t	0.1	0.1	0.3	0.4	0.1
1031	Limonene	6.2	15.8	12.2	23.5	20.8	42.6	1.0	13.1	15.3	1.7	4.9	7.9	6.8
1031	β-Phellandrene	–	–	t	t	–	–	t	–	–	0.1	t	t	0.1
1032	1,8-Cineole	–	–	–	–	–	–	–	–	0.3	–	–	–	t
1034	2-Heptyl acetate	–	–	–	–	–	–	–	–	–	0.1	–	–	–
1050	(*E*) – β-Ocimene	–	0.1	–	–	0.2	0.4	0.1	–	t	0.1	0.7	0.1	0.2
1062	γ-Terpinene	2.7	1.8	2.8	0.7	1.1	1.4	1.2	0.1	0.3	2.6	2.9	1.9	1.6
1068	*cis*-Sabinene hydrate	1.0	0.7	1.0	0.1	0.4	1.2	0.5	–	0.3	1.5	2.6	1.9	1.1

—*continued*

Table 1—*continued*

KI	Compound	GR	EK	UR	BR	LU	BA	SX	SI	VG	BL	MU	SC	HZ
1074	*trans*-Linalool oxide (furanoid)	0.3	–	–	–	–	–	–	–	0.1	–	–	–	–
1088	Terpinolene	0.7	0.6	0.9	0.8	1.3	0.8	0.5	0.3	0.4	1.1	1.6	1.1	0.8
1091	**2-Nonanone**	–	–	–	–	–	–	–	**t**	–	**2.6**	**1.8**	**0.4**	–
1091	6,7-Epoxymyrcene	0.2	t	t	–	–	–	–	–	–	–	–	–	–
1097	*trans*-Sabinene hydrate	0.7	0.3	0.4	t	0.3	1.0	0.5	–	–	1.3	1.7	1.3	0.9
1098	**Linalool**	**2.4**	**0.3**	–	**1.3**	–	–	–	**0.6**	**5.7**	**1.0**	**3.0**	**2.0**	**0.5**
1102	***n*-Nonanal**	–	–	–	–	**t**	–	–	**0.1**	**0.1**	**0.3**	**0.2**	–	**t**
1111	1-Octen-3-yl acetate	–	–	–	–	–	0.2	–	–	–	–	–	–	–
1111	*cis*-Rose oxide	0.1	t	t	–	–	–	–	–	–	–	–	–	–
1114	*trans*-Thujone (= β-thujone)	–	–	–	0.1	–	–	t	–	–	–	–	0.1	t
1116	**Terpene-OH,43,79,67,53,152**	**1.9**	**0.7**	**0.1**	–	–	–	–	–	–	–	–	–	–
1121	*cis*-*p*-Menth-2-en-1-ol	0.8	0.3	0.5	0.3	0.2	0.6	0.4	–	0.1	0.6	0.5	0.5	0.5
1125	**α-Campholenal**	–	–	–	**0.5**	**0.1**	–	**0.1**	–	–	–	–	–	–
1134	*cis*-Limonene oxide	–	–	–	–	–	0.1	–	–	–	–	–	–	–
1139	*trans*-Pinocarveol	–	–	–	0.8	–	–	–	–	–	–	–	–	–
1140	*trans*-*p*-Menth-2-en-1-ol	0.7	0.1	0.3	–	0.2	0.5	0.4	–	–	0.3	0.2	0.3	0.2
1143	Camphor	1.0	3.5	1.9	10.5	–	–	2.4	0.4	4.0	–	–	0.2	–
1144	***trans*-Verbenol**	–	–	–	–	**0.2**	**0.1**	–	–	–	–	–	–	–
1148	**Camphene hydrate**	**1.5**	**1.0**	**0.6**	–	–	–	–	**t**	**0.2**	–	–	–	–
1153	Citronellal	–	–	–	–	–	–	–	–	0.1	–	–	–	–
1156	Sabina ketone	0.4	t	t	–	–		–	–	–	–	–	–	–
1163	Pinocarvone	–	–	–	0.2	–	–	0.1	–	–	–	–	–	–
1165	Borneol	2.2	2.4	2.0	3.1	0.1	–	–	–	0.8	–	–	–	–
1167	δ-Terpineol	–	–	–	–	–	–	0.1	–	–	–	–	–	–
1170	2-(2-Propenyl)-phenol[a]	–	–	–	–	–	–	–	t	0.7	–	–	–	–
1171	Umbellulone	0.1	t	t	t	t	t	–	–	–	t	t	t	t
1177	Terpinen-4-ol	11.5	3.2	6.0	1.8	2.7	9.2	4.6	0.2	1.7	7.6	6.5	8.4	5.7
1179	Naphthalene	–	–	0.2	0.3	–	–	–	–	–	–	–	–	–
1183	*p*-Cymen-8-ol	–	–	–	0.2	–	0.2	0.1	–	–	–	–	–	–
1184	**Dill ether**	**0.4**	**0.1**	**t**	–	–	**t**	–	–	–	–	–	–	–
1189	α-Terpineol	0.7	0.2	1.2	0.2	0.3	0.3	0.3	t	0.1	0.3	–	0.3	0.2
1191	Myrtenal	–	–	–	0.2	–	0.1	t	–	–	–	–	–	–

1191	Myrtenol	t	–	–	0.2	t	–	–	–	–	t	–	–	–
1193	4Z-Decenal	–	–	–	–	–	–	–	–	–	–	t	–	t
1193	*cis*-Piperitol	0.4	–	–	–	0.1	0.1	–	–	–	t	–	t	t
1195	**Methyl chavicol(=estragole)**	–	–	–	–	–	–	–	**0.7**	**0.1**	–	–	–	–
1200	*trans*-Dihydrocarvone	t	t	t	–	–	–	–	–	–	–	–	–	–
1201	Terpene-OH,41,67,81,93,121	0.9	–	–	–	–	–	–		–	–	–	–	–
1204	Verbenone	–	–	–	–	t	–	0.3	–	–	–	–	–	–
1205	*trans*-Piperitol	0.4	–	–	–	t	0.2	–	–	t	0.1	–	0.1	0.1
1217	*trans*-Carveol	–	–	–	0.6	t	0.1	t	–	–	–	–	–	–
1220	endo-Fenchyl acetate	–	–	–	–	–	0.1	–	–	–	–	–	–	–
1228	Citronellol	0.9	t	t	–	0.1	0.2	0.4	0.2	3.1	t	t	0.6	0.4
1229	*cis*-Carveol	–	–	–	0.4	–	–	–	–	–	–	–	–	–
1252	**Piperitone**	**0.4**	**0.2**	**1.1**	–	–	–	–	**t**	**0.2**	**t**	**0.2**	–	–
1252	*trans*-Sabinene hydrate acetate	–	–	–	–	–	–	–	–	–	–	–	t	–
1257	**4Z-Decen-1-ol**	–	–	–	–	–	**0.1**	**0.3**	–	–	**t**	**t**	**0.2**	**t**
1258	*trans*-Myrtanol	–	–	–	–	–	–	–	–	0.2	–	–	–	–
1261	Methyl citronellate	–	–	–	–	0.2	–	–	–	0.1	t	–	t	–
1265	Terpene-OH,152,123,91,77,109	–	–	–	–	–	–	–	–	0.5	–	–	–	–
1267	Terpene-OH,43,91,79,119,152	0.7	t	t	–	–	–	–	–	–	–	–	–	–
1274	**Terpene-OH,79,91,105,147,133**	–	–	–	–	–	–	**0.9**	**3.9**	**5.3**	**3.0**	**2.2**	**7.6**	–
1285	**Bornyl acetate**	**31.3**	**30.8**	**38.4**	**7.8**	**1.7**	**0.6**	**0.3**	**t**	**t**	**0.3**	**0.4**	**1.0**	**0.2**
1285	*trans*-Linalool oxide acetate (pyanoid)	–	–	–	–	–	–	–	–	–	–	t	t	–
1285	**Safrole**	–	–	–	**1.2**	–	–	–	**27.2**	**18.6**	–	–	–	–
1291	2-Undecanone	–	–	–	–	–	–	–	–	–	t	–	–	–
1297	*trans*-Pinocarvyl acetate	–	–	–	0.2	t	0.1	–	–	–	–	–	–	–
1314	2E,4E-Decadienal	–	–	–	–	–	–	–	0.1	–	–	–	–	–
1323	Methyl geranate	–	–	–	–	0.2	–	–	–	t	–	–	–	–
1337	*trans*-Carvyl acetate	–	–	–	–	t	t	–	–	–	–	–	–	–
1354	Citronellyl acetate	–	–	–	–	-	–	–	–	0.1	–	–	–	–
1356	Eugenol	–	–	–	–	–	–	–	–	0.1	–	–	–	–
1380	Daucene	–	t	–	–	–	–	–	–	–	–	–	–	–
1389	β-Cubebene	–	–	t	–	–	–	–	–	–	–	–	–	–
1401	**Methyl eugenol**	**0.6**	**t**	**0.9**	**t**	–	–	–	**15.7**	**3.3**	–	–	–	–
1418	(*E*)-Caryophyllene	0.1	–	t	0.2	t	0.3	0.2	0.2	0.1	t	0.7	0.2	–
1429	***cis*-Thujopsene**	–	–	–	**2.7**	–	**0.3**	**0.2**	–	–	–	–	–	–

—*continued*

Table 1—*continued*

KI	Compound	GR	EK	UR	BR	LU	BA	SX	SI	VG	BL	MU	SC	HZ
1450	*trans*-Muurola-3,5-diene	–	–	0.3	–	–	–	–	–	–	–	–	–	–
1461	*cis*-Muurola-4(14),5-diene	–	–	–	–	–	–	–	–	–	–	–	–	0.2
1473	*trans*-Cadina-1(6),4-diene	t	t	0.2	–	–	–	–	–	–	–	–	–	–
1477	γ-Muurolene	–	–	–	–	–	–	–	–	–	–	t	–	0.2
1480	Germacrene D	–	t	0.3	0.1	1.6	0.1	3.9	0.4	–	0.1	0.2	0.2	0.1
1491	***trans*-Murrola-4(14),5-diene**	**0.1**	**0.1**	**0.8**	–	–	–	–	–	–	–	**0.2**	–	**0.1**
1493	4-epi-Cubebol	t	–	0.1	–	–	–	–	–	–	–	–	t	0.3
1493	*cis*-Cadina-1,4-diene	–	t	–	–	–	–	–	–	–	–	–	–	–
1495	(*E*)-Methyl isoeugenol	–	–	–	–	–	–	–	0.2	–	–	–	–	–
1499	α-Muurolene	t	t	t	–	0.2	t	t	t	0.1	t	0.3	t	0.4
1513	γ-Cadinene	t	t	0.6	–	0.2	–	0.1	t	0.3	–	0.5	0.2	1.1
1513	Cubebol	t	–	–	–	–	–	–	0.2	–	–	–	–	–
1524	δ-Cadinene	t	0.3	0.6	t	0.8	t	0.6	0.3	0.5	–	1.0	0.3	2.1
1538	α-Cadinene	–	–	–	–	–	–	–	–	–	–	–	–	0.2
1549	**Elemol**	–	–	**0.7**	–	**t**	–	**8.2**	**11.5**	**7.2**	**1.9**	**1.7**	**3.1**	**5.0**
1554	**Elemicin**	**0.7**	**t**	**t**	–	–	–	–	**2.7**	**1.9**	–	–	–	–
1564	(*E*)-Nerolidol	–	–	–	–	–	–	0.5	–	–	–	–	–	–
1570	Z-3-Hexenyl benzoate	–	–	–	t	–	–	–	–	–	–	–	–	–
1574	Germacrene D-4-ol	–	0.1	t	–	0.6	t	0.5	0.4	1.1	t	3.6	1.0	5.5
1581	**Caryophyllene oxide**	**t**	–	–	**t**	**0.3**	**0.2**	**t**	**0.2**	**0.1**	–	–	–	–
1596	Cedrol	0.1	t	t	0.2	–	0.4	–	–	–	–	–	–	–
1606	Humulene epoxide II	t	–	–	–	–	–	–	–	–	–	–	–	–
1606	β-Oplopenone	t	t	t	0.2	0.3	0.1	0.7	0.2	0.2	–	0.7	0.2	3.2
1610	Sesquiterpene,41,91,105,222?	–	–	–	–	0.5	–	–	–	–	–	–	–	–
1627	1-epi-Cubenol	0.2	–	0.6	–	–	–	–	–	–	–	–	–	0.2
1630	α-Acorenol	–	–	–	–	–	–	–	0.5	0.2	–	–	–	–
1630	γ-Eudesmol	–	–	t	–	–	–	3.0	1.4	0.9	0.3	t	0.3	0.2
1640	epi-α-Cadinol	t	t	t	–	0.4	t	0.7	0.4	0.4	–	1.1	0.2	1.2
1640	epi-α-Muurolol	t	t	t	–	0.5	t	1.0	0.4	0.5	–	t	0.2	1.6

1642	Cubenol	t	–	t	–	–	–	–	–	–	–	–	–	–
1645	α-Muurolol (= torreyol)	–	–	t	–	0.3	–	t	–	–	–	t	–	0.4
1649	β-Eudesmol	t	t	0.2	–	–	–	3.6	2.5	1.2	0.4	0.2	0.3	0.6
1652	α-Eudesmol	t	t	0.2	–	–	–	3.3	3.9	1.4	0.5	t	0. 3	0.5
1653	α-Cadinol	t	t	t	0.2	2.3	0.1	3.3	t	1.5	–	1.7	0.6	4.5
1666	Bulnesol	–	–	t	–	–	–	1.6	1.5	1.6	0.4	0.3	0.6	0.9
1685	**Eudesma-4(15),7-dien-1-β-ol**	–	–	–	**0.5**	**1.7**	**0.3**	**3.2**	–	–	–	–	–	–
1733	Oplopenone	–	–	–	–	–	t	–	–	–	–	–	–	–
1789	**8-α-Acetoxyelemol**	–	–	–	–	–	–	**3.3**	**2.7**	**4.9**	**6.8**	**2.3**	**8.7**	–
1881	**Oplopanonyl acetate**	–	–	–	–	–	–	–	–	–	–	–	–	**0.6**
1930	Rosa-5,15-diene	–	–	–	–	–	–	–	–	–	1.1	–	–	–
1961	Sandaracopimara-8(14),15-diene	–	–	–	t	–	t	t	–	–	–	–	–	–
1989	Manoyl oxide	t	–	–	t	–	–	–	t	–	–	–	–	–
2054	Abietatriene	t	t	t	0.5	0.2	t	t	t	–	t	t	–	–
2055	**Manool**	–	–	–	–	–	–	**3.7**	–	**t**	**16.2**	**1.4**	–	–
2080	Abietadiene	t	–	–	1.6	t	t	2.1	–	–	–	t	–	–
2147	**Abieta-8(14),13(15)-diene**[a]	–	–	–	–	–	–	**0.4**	–	–	–	–	–	–
2180	**Diterpene,41,91,79,271,257,286**	–	–	–	–	–	–	**0.5**	–	–	–	–	–	-
2220	**Diterpene,243,41,271,286, 91**	–	–	–	–	–	–	**1.0**	–	–	–	–	–	–
2227	**Diterpene,91,41,133,187,286**	–	–	–	–	–	–	**1.6**	–	–	–	–	–	–
2278	*cis*-Totarol	0.3	–	t	2.2	2.5	0.2	1.2	0.4	t	0.8	t	–	–
2288	**4-epi-Abietal**	–	–	–	–	–	**0.1**	**8.9**	**0.1**	**0.2**	**0.6**	**0.6**	–	**1.5**
2302	*trans*-Totarol	0.6	–	t	2.1	1.5	0.2	1.7	0.3	0.1	0.4	t	–	–
2325	*trans*-Ferruginol	t	–	–	t	0.2	–	–	–	–	t	–	–	–
2361	**Diterpene,135,286,91,148,187**	–	–	–	–	–	–	**0.6**	–	–	–	–	–	–
2477	**Diterpene, 43,255,270,187,131**	–	–	–	–	–	–	**2.7**	–	–	–	–	–	–

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.

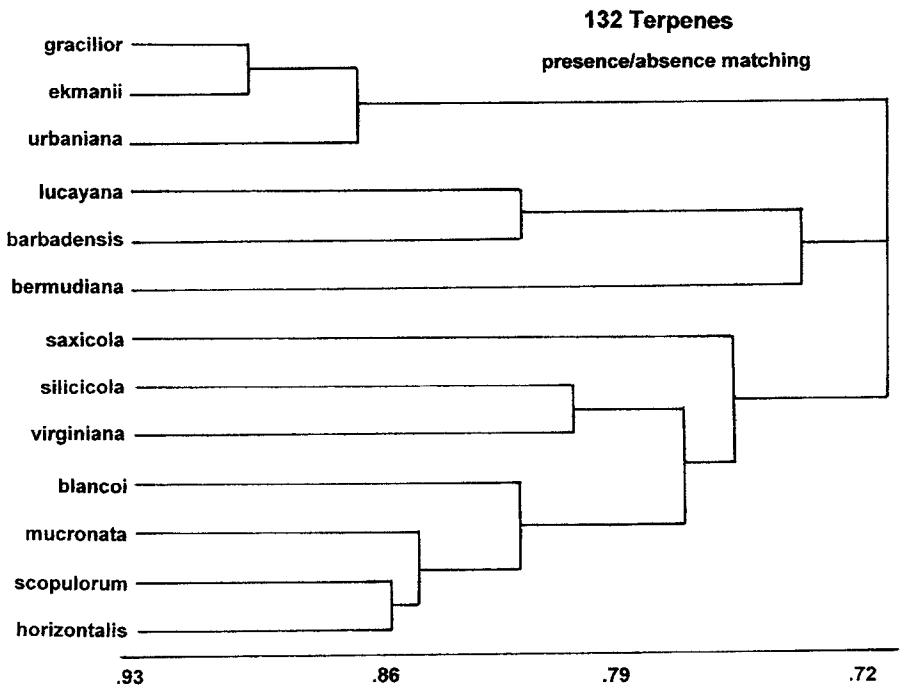

Fig. 1. Minimum spanning network based on 132 terpenoids, with similarities computed as presence/absence data. See text for discussion.

constructed and principal coordinate analysis (PCO) was performed. Fig. 3 shows the ordination of these taxa based on the terpenes data. *Juniperus horizontalis* appears quite distinct. The mucronate juniper (mucronata in Fig. 3), appears to be intermediate between *J. scopulorum* and *J. blancoi* in its terpenes in showing a triangle of relationships (0.80 similarity between *J. blancoi* and *J. scopulorum*, not shown). Notice that the similarity of the mucronate juniper to *J. scopulorum* (0.86) is the same as the similarity of *J. horizontalis* (a universally recognized species) to *J. scopulorum* (0.86).

The RAPDs data depicts a very similar relationship among these taxa (Fig. 4). As with the terpenes, the RAPDs show (Fig. 4) *J. horizontalis* is most dissimilar from the group (0.77 to *J. blancoi* and 0.75 to *J. scopulorum*). Notice that *J. blancoi*, the mucronate juniper and *J. scopulorum* form a triangle (0.81 similarity between *J. blancoi* and *J. scopulorum*, not shown in Fig. 4) and the mucronate juniper is only slightly more similar to *J. blancoi* (0.82) than to *J. scopulorum* (0.81).

Based on the morphology, terpenes and RAPDs, it seems appropriate to recognize the mucronate tipped juniper as: *Juniperus mucronata* R.P. Adams, *sp. nov.*, TYPE: Mexico, Maicoba, 1180 m. R.P. Adams 8704 (holotype at SRCG)

Arbores axe centrali forti (usque 15 m *alti), corona pyramidali; foliis apicibus mucronatis; ligno centrali (duramine) purpureo vivido; ramulis foliatis pendulis;*

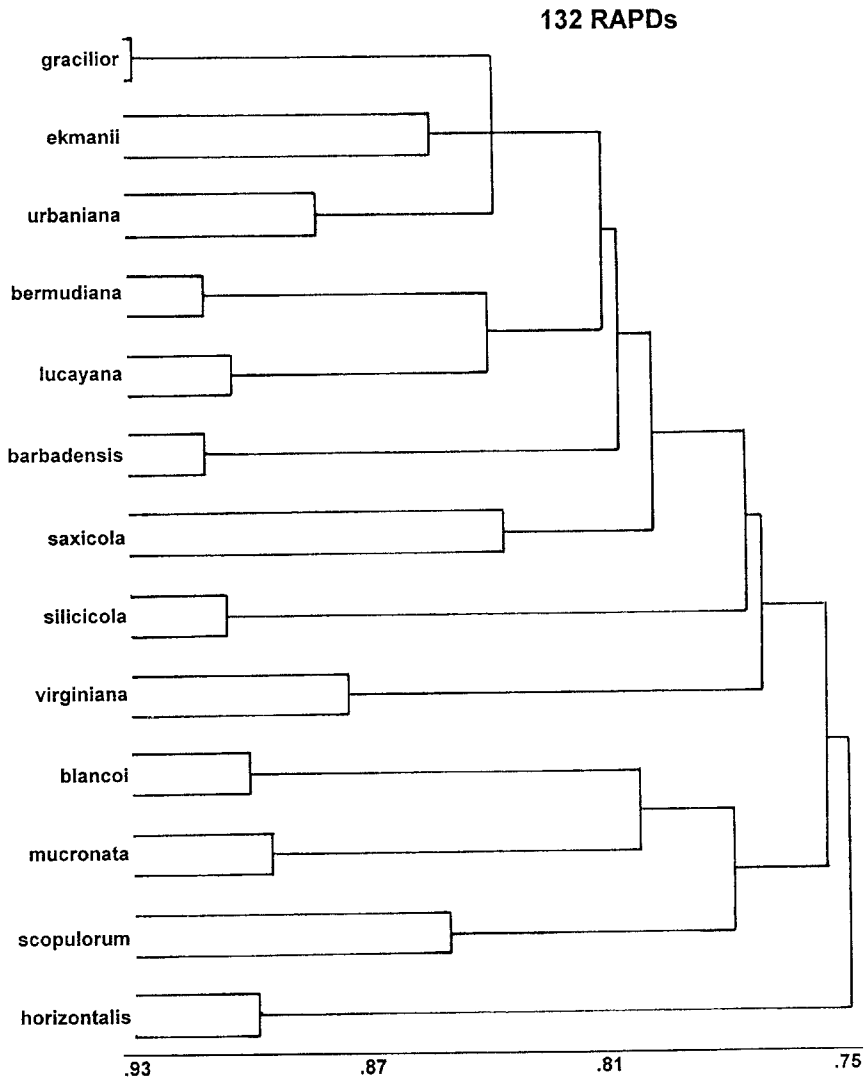

Fig. 2. Minimum spanning network based on 132 RAPD bands. Each OTU is represented by two individuals. See text for discussion.

cortice trunci brunnea in ligulam decorticanti; strobilis femineis pulpa succulenta molli globosis vel reniformibus (ubi 2-seminalis) in maturitate anthracinis dealbatis 4–6 mm *longis* 5–8 mm *latis, maturitatem attingentem tempore anni; pedunculis rectis; seminibus* 1–2 *(saepe* 1 *per abortionem) profunde sulcatis brunneis globosis* 3–4 mm *longis* 2–3 mm *latis, cicatrice hili per* $\frac{1}{3}$–$\frac{1}{2}$ *longitudinem seminis.*

Trees with a strong central axis (to 15 m), crown pyramidal; both scale and whip leaves mucronate tipped, heartwood bright purple; foliage pendulous; trunk bark

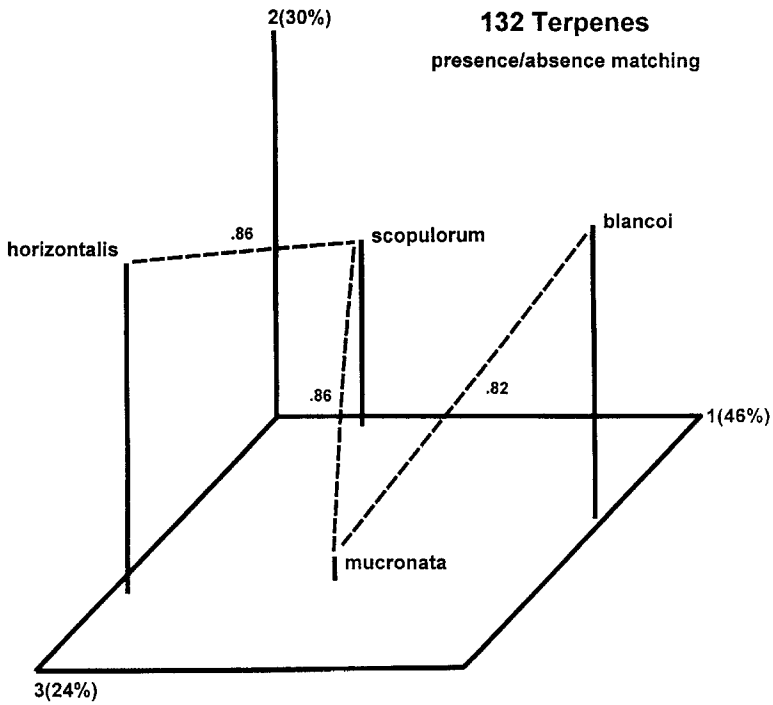

Fig. 3. Principal co-ordinate ordination based on 132 terpenoids, with similarities computed as presence/absence data. The dashed lines show the minimum spanning network and the number along the dashed lines are the similarities between OTUs. See text for discussion.

brown, exfoliating in strips; female cones with soft, flesh pulp, globose to reniform (when 2 seeded), dark bluish black, with light white bloom, 5–8 mm wide × 4 – 6 mm long, mature in one year; peduncle straight; seeds 1–2 (often one aborts, leaving only 1), brown, globose, deeply grooved, 3–4 mm long, 2–3 mm wide, hilium scar from $\frac{1}{3}$ to $\frac{1}{2}$ length of seed.

Additional specimens: Adams 8701–8703, 8705; G. M. Ferguson 1448, 1456–1459, 1479, Maicoba/Yecora, Sonora/Chihuahua border, Mexico.

Juniperus mucronata differs from *J. scopulorum* in that its fruit mature in one year, the leaf tips are mucronate (versus acute in *J. scopulorum*), and the heartwood is bright purple versus brown with a bluish cast in *J. scopulorum*. *Juniperus mucronata* differs from *J. blancoi* in that the leaf tips are mucronate (versus acute or rarely mucronate on whip leaves in *J. blancoi*), and the heartwood is bright purple versus brown with a purplish cast in *J. blancoi*. At present, the species is known only from the banks of the Maicoba River (30 – 60 m above the river) and other streams near the Sonora-Chihuahua border, where it occurs on basalt with *Cupressus lindleyii, J. deppeana and Quercus species*.

The taxonomic status of *J. lucayana* remains inexact. Although the terpenes and morphology support the recognition of *J. barbadensis* var. *lucayana* (see Adams,

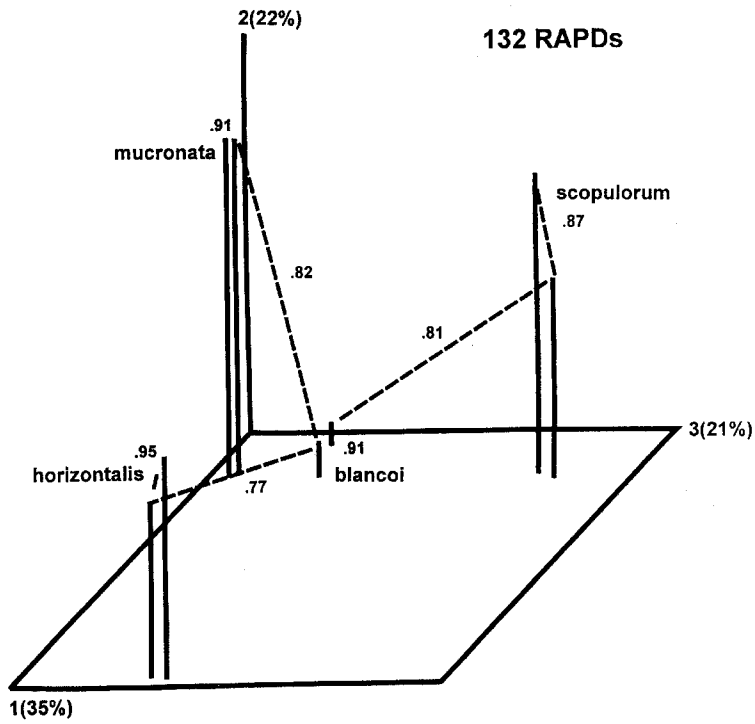

Fig. 4. Principal co-ordinate ordination based on 132 RAPDs. The dashed lines show the minimum spanning network and the number along the dashed lines are the similarities between OTUs. See text for discussion.

1995a), the RAPDs do not favor that alignment. It seems prudent to maintain *J. lucayana* as a distinct species at present.

The recognition of *J. ekmanii* and *J. urbaniana* as varieties of *J. gracilor* (Adams, 1995a) is supported by the RAPDs data and these varieties will be maintained in the treatment of Juniperus.

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