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The serrate leaf margined *Juniperus* (Section Sabina) of the western hemisphere: systematics and evolution based on leaf essential oils and Random Amplified Polymorphic DNAs (RAPDs)

Robert P. Adams

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

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Abstract

The volatile leaf essential compositions of all 17 serrate leaf margin species of *Juniperus* (sect. *Sabina*) of the western hemisphere are reported and compared: *J. angosturana*, *J. ashei*, *J. californica*, *J. coahuilensis*, *J. comitana*, *J. deppeana*, *J. durangensis*, *J. flaccida*, *J. gamboana*, *J. jaliscana*, *J. monosperma*, *J. monticola*, *J. osteosperma*, *J. occidentalis*, *J. pinchotii*, *J. saltillensis*, and *J. standleyi*. A number of previously unidentified compounds of the leaf essential oils have now been identified. In addition, DNA data (RAPDs) of all these species were analyzed. Both the leaf essential oils and DNA show these species to be quite distinct with few apparent subgroups, such that the species groupings were not strong in either data set. These data support the hypothesis that this group of junipers originated in Mexico as part of the Madro-Tertiary flora by rapid radiation into new arid land habitats, leaving few extant intermediate taxa.

Keywords: *Juniperus*; Cupressaceae; Terpenes; DNA; RAPDs; Systematics; Essential oil

1. Introduction

The group of serrate leaf margined *Juniperus* species of the western hemisphere appears to be a natural division of *Juniperus*, section *Sabina* (Adams and Demeke, 1993). These junipers are characterized by having microscopic (40×) serrrations

* 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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Table 1
Classifications of the serrate leaf junipers taxa in North America

Martinez (1963)	Zanoni and Adams (1976)
Subsection monospermae	Monosperman group
J. californica Carr.	*J. ashei* Buch.
J. comitana Mart.	*J. comitana*
J. erythrocarpa Cory.(= *J. coahuilensis* (Mart.) Gaussen ex. R. Adams, in part)	*J. erythrocarpa* (= *J. coahuilensis* in part)
J. gamboana Mart.	*J. gamboana*
J. monosperma (Engelm.) Sarg.	*J. monsperma*
var. *gracilis* Mart.	var. gracilis (= *J. angosturana* RP Adams)
	J. pinchotii Sudw.
	J. saltillensis MT Hall
Subsection monticolae	Monticola group
J. durangensis Mart.	*J. durangensis*
J. monticola Mart. (plus forma *compacta* Mart., forma *orizabensis* Mart.)	*J. monticola*
J. standleyi Steyer.	*J. standleyi*
Subsection deppeanae	Deppeana group
J. deppeana Steudel.	*J. deppeana*
var. *pachyphaea* (Torrey) Mart.	var. *robusta*
var. *robusta* Mart.	var. *patoniana* (Mart.) Zanoni
var. *zacatecensis* Mart.	var. *zacatecensis*
J. patoniana Mart.	*J. jaliscana*
Subsection flaccidae	Flaccida group
J. flaccida Schlecht.(plus var. *problana* Mart.)	*J. flaccida* (plus var. problana)
Subsection jaliscanae	Not included in Zanoni and Adams (1976): *J. californica* Carr., *J. occidentalis* Hook. and *J. osteosperma* (Torr.)Little
J. jaliscana Mart.	

(teeth) on the scale leaves and these taxa are generally xerophytic, occurring in the great North American deserts and arid mountains adjacent to the deserts. These junipers range from northern Guatemala, into Mexico, thence northward into the southwestern United States, as far north as Oregon (*J. occidentalis*) and eastward to Arkansas (*J. ashei*). The group is thought to had been a part of the Madro-Tertiary flora dating from pre-Eocene (Axlerod, 1958). As the neotropical tertiary geoflora expanding into the drying conditions that created the southwestern deserts, Axlerod (1958) hypothesized that there was a rapid evolution of new species. North-central Mexico has the largest number of *Juniperus* species in the western hemisphere and this is one of three centers of origin for extant Juniperus species. The other two centers of diversity/ origins are the northern Mediterranean region and western China.

The first systematic treatment of these junipers was made by Martinez. His final treatment (Martinez, 1963) is shown in Table 1, along with the treatment by Zanoni and Adams (1976) based on morphology and essential oils. Martinez (1963) merely drew the groups in a circle, whereas Zanoni and Adams (1976) drew a hypothetical phylogenetic tree showing *J. flaccida* and *J. deppeana* diverging early but with the

other branches being questionable. They concluded that these junipers were loosely associated and a clear phylogenetic pathway was not now apparent.

As a group, these *Juniperus* species have been the most intensively analyzed of the junipers. Extensive studies involving thousands of samples using both terpenoids and morphology have been performed (Adams, 1975a, 1977, 1993, 1994; Adams et al., 1981, 1983, 1984a, b; Zanoni and Adams, 1975, 1976, 1979). These papers mostly utilized terpenoid data at the populational or infraspecifc level. The terpenoid data were found to be very useful at this taxonomic level and occasionally at the specific level. But, as a general rule, the terpenoids have not been very useful at the specific level in this section of *Juniperus* (Zanoni and Adams, 1976). With the advent of DNA-based technology, we have found that DNA fingerprinting (Random Amplified Polymorphic DNAs, RAPDs) has been very useful to both supplement and complement morphological and terpenoid data (Adams and Demeke, 1993; Adams et al., 1993; Adams, 1994, 1999, 2000a, b, c). This is the fifth paper in the series (Adams, 1999, 2000a, b, c) to serve as the basis for a modern monographic treatment of the genus *Juniperus*.

The volatile leaf oils and the associated literature have been reported as follows: *J. angosturana* (= *J. monosperma* var. *gracilis* Mart.) (Adams et al., 1981); *J. ashei* (Adams et al., 1980a); *J. californica* (Adams et al., 1983); *J. coahuilensis* (= *J. erythrocarpa* Cory. in part) (Adams et al., 1981); *J. comitana* (Adams et al., 1985b); *J. flaccida* (Adams et al., 1984a); *J. deppeana* (Adams et al., 1984b); *J. durangensis* (Adams et al., 1985a); *J. gamboana* (Adams et al., 1985b); *J. jaliscana* (Adams et al., 1985a); *J. monosperma* (Adams et al., 1981; Adams et al., 1983); *J. monticola* (Adams et al., 1980b); *J. occidentalis* (Adams et al., 1983); *J. osteosperma* (Adams et al., 1983); *J. pinchotii* (Adams et al., 1981); *J. saltillensis* (Adams et al., 1980); *J. standleyi* (Adams et al., 1985b);

The purpose of this paper is to examine the systematics of the serrate leaf margined *Juniperus* in section *Sabina* of the western hemisphere. Two data sets will be examined: the complete set of the comprehensive leaf oil composition for all the serrate leaf junipers of the western hemisphere: *J. angosturana* R. P. Adams, *J. ashei Buch.*, *J. californica* Carr., *J. coahuilensis* (Mart.) Gaussen ex. R. P. Adams, *J. comitana* Mart., *J. deppeana* Steudel var. *deppeana*, *J. durangensis* Mart., *J. flaccida* Schlecht. var. *flaccida*, *J. gamboana* Mart., *J. jaliscana* Mart., *J. monosperma* (Engelm.) Sarg., *J. monticola* Mart. forma *monticola*, *J. osteosperma* (Torr.) Little, *J. occidentalis* Hook. var. *occidentalis*, *J. pinchotii* Sudw., *J. saltillensis* M. T. Hall, and *J. standleyi* Steyerm. and data obtained from Random Amplified Polymorphic DNAs (RAPDs) for these same taxa. Based on these data, the systematics of this group of *Juniperus* is discussed.

2. Materials and methods

Specimens used in this study (it should be noted that the specimens listed are merely some recent collections for DNA work and reconfirmation of terpenoid compositions, at least 20 individuals and often hundreds, have been used in studies in previous research, see references above): *J. angosturana*, Adams 6881–6885, San Luis Potosi, Mexico; *J. ashei*, Adams 6746, 6751–52, Texas, USA; *J. californica*, Adams, 2598–2600, 2601–06, 8695–97, California, USA and 2607–09, 8698–8700, Arizona, USA;

J. coahuilensis, Adams 6829–31, Durango, Mexico; *J. comitana*, Adams 6858–62, Chiapas, Mexico; *J. deppeana* var. *deppeana*, Adams 7632–34, New Mexico, USA; *J. durangensis*, Adams 6832–35, Durango, Mexico; *J. flaccida* var. *flaccida*, Adams 6892–96, Nuevo Leon, Mexico; *J. gamboana*, Adams 6863–67, Chiapas, Mexico; *J. jaliscana*, Adams 6846–48, Jalisco, Mexico; *J. monosperma*, Adams 7638–40, New Mexico, USA; *J. monticola* f. *monticola*, Adams 6874–78, Hidalgo, Mexico; *J. osteosperma*, Adams 6811–13, Utah, USA; *J. occidentalis* var. *occidentalis*, Adams 8592–94, Oregon, USA; *J. pinchotii*, Adams 7483–87, 8736–45, Texas, USA; *J. saltillensis*, Adams 6886–90, Nuevo Leon, Mexico; *J. standleyi*, Adams 6852-56, Huehuetango, Guatemala. 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, 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 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, (Adams, 1995), using the Finnigan library search routines based on fit and purity, coupled with retention time data of reference compounds. It should be emphasized that all of the entries in the library (nearly 1600 at present) are based on authenticated compounds that have been assembled by the author over the past 20 years. In cases of questionable identification, these references compounds can be re-analyzed (co-chromatographed) to establish identity. Any compounds identified merely by MS are so noted in Table 2 as tentatively identified.

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; 338 CTG TGG CGG T; 347 TTG CTT GGC G; 375 CCG GAC ACG A; 376 CAG GAC ATC 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

Table 2
Comparisons of the percent total oil for leaf essential oils for *J. pinchotii*(PN), *J. saltillensis*(SA), *J. californica*(CA), *J. osteosperma*(OS), *J. standleyi*(ST), *J. gamboana* (GM), *J. deppeana*(DP), *J. jaliscana*(JA), *J. durangensis*(DR), *J. coahuilensis*(CH), *J. monticola*(MT), *J. monosperma*(MN), *J. angosturana*(AN), *J. flaccida*(FL), *J. comitana*(CM), *J. ashei*(AS), *J. occidentalis*(OC).

KI[a]	Compound	PN	SA	CA	OS	ST	GM	DP	JA	DR	CH	MT	MN	AN	FL	CM	AS	OC
926	Tricyclene	0.4	0.6	0.2	0.5	0.4	0.3	3.1	0.1	0.2	t	0.8	0.1	0.1	0.2	0.1	2.9	2.5
931	α-Thujene	0.5	0.2	0.2	0.2	1.4	t	0.5	t	t	1.3	—	t	t	0.1	—	—	1.4
939	α-Pinene	2.0	2.7	40.0	3.0	4.1	31.0	13.2	43.2	47.5	2.3	9.9	49.2	23.1	52.4	65.7	0.6	9.7
953	α-Fenchene	t	—	t	—	t	t	0.7	t	0.8	—	—	t	0.6	—	—	—	0.1
953	Camphene	0.4	0.6	0.3	0.5	0.5	0.6	1.5	0.5	0.5	t	1.0	0.5	0.2	0.7	1.0	2.3	1.1
957	Thuja-2,4(10)-diene	—	—	t	0.1	—	t	0.1	t	0.1	—	—	0.1	t	0.1	0.1	—	t
967	Verbenene	—	1.6	—	—	0.3	1.8	0.2	0.1	1.8	—	1.2	0.4	1.7	1.4	—	—	—
976	Sabinene	22.0	7.1	7.6	5.3	31.9	0.1	8.1	t	t	20.5	t	0.2	t	0.2	0.1	—	23.3
980	β-Pinene	0.1	1.1	0.9	0.1	0.2	3.9	0.9	2.6	2.0	0.2	0.7	1.5	1.1	5.9	6.4	0.1	0.4
985	6-Methyl-5-hepten-2-one	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	t	—
991	Myrcene	2.0	2.0	1.4	0.8	3.4	4.6	2.9	4.6	2.8	2.4	2.4	2.1	2.8	5.6	6.8	0.8	1.7
1000	Hexanoic acid, 4-methyl, Methyl ester[b]	—	t	—	—	—	—	—	—	—	—	0.2	—	—	0.2	—	—	—
1001	δ-2-Carene	—	—	0.2	t	0.4	t	0.2	—	—	—	0.1	t	—	—	—	t	0.1
1005	α-Phellandrene	t	t	t	0.1	0.1	0.3	1.3	t	—	0.2	0.1	0.6	0.1	0.1	0.1	0.5	0.8
1011	δ-3-Carene	0.5	0.4	0.1	0.1	1.3	1.6	7.6	t	14.9	—	—	1.1	11.5	t	0.1	t	1.1
1018	α-Terpinene	1.3	0.4	0.6	0.3	1.9	t	0.7	0.1	t	3.1	0.1	0.1	0.1	t	—	t	2.2
1026	*p*-Cymene	0.1	0.1	0.3	1.5	0.1	t	1.8	0.2	0.2	0.2	0.2	0.7	0.2	0.1	t	2.6	13.5
1027	Sylvestrene	—	—	—	—	—	—	—	—	0.1	—	—	—	0.1	—	—	—	—
1031	Limonene	3.6	3.0	2.9	3.6	3.0	0.2	0.3	24.0	1.6	2.2	10.0	1.5	0.5	3.5	1.4	4.6	0.1
1031	β-Phellandrene	0.1	0.3	0.4	t	0.8	4.9	10.7	0.1	1.7	0.5	0.4	6.2	0.6	3.6	1.9	—	5.0
1032	1,8-Cineole	0.1	0.3	—	—	—	t	t	—	0.1	0.5	—	—	0.4	0.1	—	—	—
1050	(E)-β-Ocimene	—	0.1	t	0.1	0.2	0.8	0.3	0.1	—	0.2	0.1	t	0.2	1.7	0.1	—	0.1
1057	*n*-Pentyl isobutyrate	—	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1062	γ-Terpinene	2.2	0.7	1.1	0.6	3.1	t	1.1	0.4	0.3	4.8	0.4	0.7	0.5	0.2	0.1	0.3	3.4
1068	*cis*-Sabinene hydrate	1.2	0.4	0.4	0.8	1.4	—	0.7	—	—	1.8	—	t	—	—	—	—	0.9
1072	Unknown,41,55,101,159 FW 158	—	0.2	—	—	—	—	—	—	—	—	1.1	—	—	—	—	—	—
1074	*trans*-Linalool oxide (furanoid)	—	—	—	0.2	—	—	—	—	—	t	t	—	t	0.1	—	t	—
1083	Camphenilone	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t	—
1086	*p*-Mentha-2,4(8)-diene	—	—	—	—	—	—	—	—	0.1	—	—	—	0.1	—	—	—	—
1088	Fenchone	—	—	—	—	—	—	—	—	—	—	—	0.6	—	—	—	t	—
1088	Terpinolene	0.8	0.6	0.5	0.4	1.3	0.6	1.6	0.9	2.1	1.6	0.5	0.6	2.3	0.5	0.7	0.3	1.1
1095	α-Pinene oxide	—	3.2	—	—	—	0.9	—	—	0.9	—	0.5	0.6	0.6	1.5	—	—	—

Table—*continued*

Table 2—*continued*

KI[a]	Compound	PN	SA	CA	OS	ST	GM	DP	JA	DR	CH	MT	MN	AN	FL	CM	AS	OC
1097	*trans*-Sabinene hydrate	1.0	0.1	0.5	1.0	1.0	—	0.4	—	—	2.6	—	—	—	—	—	—	0.7
1098	Linalool	—	0.1	t	0.4	2.0	5.8	1.7	0.2	1.9	t	1.1	0.1	0.3	4.6	1.4	1.4	0.3
1099	Perillene	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—
1102	*n*-Nonanal	—	—	—	—	t	—	—	—	—	—	—	0.1	0.1	—	—	—	—
1103	Isopentyl isovalerate	—	—	—	0.1	—	—	—	—	—	—	t	t	—	—	—	0.2	—
1111	*cis*-Rose oxide	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1112	Endo-fenchol	—	—	—	—	—	—	—	t	t	—	—	t	—	—	t	—	—
1114	*trans*-Thujone(= β-thujone)	t	—	t	0.2	t	—	—	—	—	t	—	—	—	—	—	—	t
1116	3-Methyl butanoate, 3-methyl-3-Butenyl-	—	—	—	—	—	—	—	—	—	—	t	0.6	—	0.2	—	0.1	—
1121	*cis-p*-Menth-2-en-1-ol	0.4	0.2	0.2	0.7	0.7	0.1	1.0	t	t	1.0	0.1	0.4	0.1	0.2	t	0.3	0.6
1124	Chrysanthone	—	—	—	—	—	—	0.1	—	—	—	—	t	—	—	—	—	—
1125	α-Campholenal	t	t	t	0.3	—	t	0.6	0.1	0.3	—	—	0.3	t	0.3	0.2	0.1	—
1134	*cis*-Limonene oxide	—	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—
1134	*cis-p*-Mentha-2,8-dien-1-ol	—	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—
1139	*trans*-Pinocarveol	—	—	t	t	—	—	t	0.1	0.2	—	—	0.8	—	0.3	0.2	—	—
1140	*trans-p*-Menth-2-en-1-ol	0.2	—	0.2	—	0.5	t	0.9	—	—	0.6	t	—	—	—	—	0.1	0.5
1140	*trans*-Sabinol	—	—	—	—	—	—	—	—	—	—	—	t	t	—	—	—	—
1141	*cis*-Verbenol	—	—	—	—	—	—	—	—	t	—	—	—	—	0.1	—	—	—
1143	Camphor	31.4	42.1	16.2	33.4	0.3	1.1	19.0	0.1	0.2	0.4	0.9	1.9	0.9	0.9	0.1	64.9	1.7
1144	*trans*-Verbenol	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	0.3	—	—
1146	Isopulegol	—	—	—	—	—	—	—	—	1.5	0.5	0.5	—	—	0.1	—	—	—
1148	Camphene hydrate	0.8	1.2	0.3	1.7	0.1	0.4	0.8	0.1	—	—	1.0	0.2	—	0.3	0.2	1.7	0.2
1153	Citronellal	0.7	0.9	1.0	—	t	0.1	—	—	0.1	4.8	t	—	—	0.1	—	—	—
1156	Sabina ketone	—	—	—	0.8	t	—	0.2	—	—	—	—	—	—	—	—	—	0.3
1156	iso-isopulegol	—	—	0.1	—	—	0.1	—	—	0.5	0.2	—	t	—	0.1	—	—	—
1156	Isoborneol	0.1	0.1	—	—	—	—	—	—	—	—	t	—	—	—	—	0.1	—
1159	*p*-Mentha-1,5-dien-8-ol	—	—	—	—	—	—	0.5	—	0.2	—	—	—	0.2	—	—	—	—
1160	*trans*-Pinocamphone	—	—	0.1	t	—	—	—	—	—	—	—	0.2	—	—	—	—	—
1162	Pinocarvone	—	—	t	t	—	—	t	—	t	—	—	0.2	—	t	—	—	—
1165	Borneol	1.6	0.8	0.2	5.8	0.3	0.2	0.1	0.2	0.1	—	1.8	0.4	t	0.8	t	2.0	1.3
1167	δ-Terpineol	—	—	—	—	—	0.1	0.5	0.1	0.4	—	—	—	t	t	t	—	—
1170	Terpene alcohol	t	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	t
1173	*cis*-Pinocamphone	—	0.1	0.1	—	—	t	t	—	—	—	—	0.1	t	0.2	t	—	t
1177	Terpinen-4-ol	7.5	1.9	3.4	7.4	9.7	0.4	2.7	0.5	0.4	13.9	0.5	0.5	0.3	0.4	0.2	0.2	4.6

1180	*m*-Cymen-8-ol	—	—	—	t	—	—	0.6	—	0.2	—	—	—	t	—	—	—	—
1183	*p*-Cymen-8-ol	—	t	t	1.2	—	—	1.1	t	0.2	—	t	t	t	t	t	0.3	0.5
1186	*m*-Terpineol[b]	t	t	—	—	—	—	0.2	—	0.2	—	—	—	0.7	—	—	—	—
1189	α-Terpineol	0.3	0.2	0.2	0.4	0.4	0.3	1.9	0.5	0.4	0.6	0.2	0.2	0.4	0.4	0.5	0.1	0.2
1191	Myrtenol	—	—	—	0.2	—	—	—	—	—	—	—	t	—	—	t	—	—
1193	4Z-Decenal	—	0.1	—	—	—	—	—	—	—	—	—	—	0.3	0.1	—	—	—
1193	Myrtenal	t	—	t	0.2	—	—	t	—	t	—	—	—	—	—	t	t	—
1193	*cis*-Piperitol	t	—	t	—	0.1	—	0.2	—	—	0.2	—	—	—	—	—	—	—
1195	Methyl chavicol	—	—	—	—	—	0.2	—	—	—	—	—	—	t	t	0.2	—	—
1200	*trans*-Dihyrdocarvone	—	0.3	t	—	—	—	—	—	—	—	—	—	—	0.1	—	—	—
1204	Verbenone	—	0.2	0.1	1.4	—	t	0.7	t	0.2	—	—	0.4	0.6	0.2	0.1	0.1	—
1205	*trans*-Piperitol	—	0.1	—	—	0.3	—	—	—	—	0.3	—	—	—	—	—	—	—
1217	*trans*-Carveol	—	—	t	1.3	—	—	0.2	—	0.2	—	—	t	—	0.1	t	0.5	—
1220	Endo-fenchyl acetate	—	—	—	—	—	t	—	0.1	—	—	0.1	—	—	—	0.2	t	—
1222	Aromatic, 105, 133, FW 148	—	—	—	—	—	—	—	—	—	—	—	—	0.9	—	—	—	—
1228	Citronellol	3.0	1.5	9.0	—	0.2	0.3	—	—	0.4	6.4	t	—	—	0.2	—	—	—
1229	*cis*-Carveol	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—	—	0.1	—
1235	*trans*-Chrysanthenyl acetate	—	0.5	—	—	—	0.5	0.2	—	—	—	1.1	—	1.3	0.1	—	—	—
1235	Thymol, methyl ether	—	—	—	—	0.2	—	—	0.1	t	—	—	—	—	—	—	—	—
1239	Cumin aldehyde	—	—	—	0.2	—	—	0.2	—	—	—	—	—	—	—	—	—	t
1242	Carvone	—	—	t	0.2	—	—	0.2	—	—	—	—	t	—	t	—	0.8	—
1251	Alcohol, 119,109, 91, FW 152	—	—	—	—	—	—	—	—	0.4	—	—	—	—	0.3	—	—	—
1252	Carvenone	—	0.5	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—
1252	Piperitone	—	—	0.2	0.1	—	t	0.7	—	—	—	0.1	0.3	0.1	—	—	t	t
1257	Linalyl acetate	–	—	—	—	—	—	0.4	—	—	—	0.1	—	—	—	—	—	—
1258	4Z-decen-1-ol	—	t	—	—	—	—	—	—	0.2	—	—	—	—	0.2	—	—	—
1261	Methyl citronellate	—	—	t	—	—	—	—	—	1.0	—	0.1	—	—	—	—	—	—
1264	Alcohol, FW 152, 123, 79	—	—	—	—	—	—	0.4	—	—	—	—	0.8	—	—	—	—	—
1272	*p*-Menth-1-en-7-al	—	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—
1274	Unknown, 79, 91, 147, FW 162	—	0.4	0.3	—	—	—	—	—	—	0.3	0.7	2.8	—	—	—	—	—
1281	Isopulegyl acetate	—	—	—	—	—	0.7	—	—	—	—	0.8	—	—	—	—	—	—
1285	Bornyl acetate	7.1	1.0	0.9	15.0	13.0	11.4	1.9	1.7	0.3	t	39.1	1.3	1.3	0.7	3.2	9.2	8.1
1286	Safrole	—	—	—	—	—	—	—	—	—	—	—	—	0.1	—	—	—	—
1287	*p*-Cymen-7-ol	—	—	—	0.6	—	—	—	—	—	—	—	—	—	—	—	—	—
1290	Thymol	t	0.1	—	—	—	—	—	—	—	—	t	—	t	—	—	—	—
1290	*trans*-Sabinyl acetate	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—
1297	*trans*-Pinocarvyl acetate	—	—	t	—	—	—	—	—	—	—	—	t	—	—	—	—	—
1298	Carvacrol	—	—	—	—	—	t	—	—	—	—	0.1	—	0.1	—	—	—	—
1302	Alcohol, 135, 77, FW 150	—	—	—	—	1.0	—	—	—	—	—	—	—	—	—	—	—	—
1314	2E, 4E-Decadienal	—	—	t	—	—	—	—	—	—	0.3	—	0.2	—	t	—	—	—

Table—*continued*

Table 2—*continued*

KI[a]	Compound	PN	SA	CA	OS	ST	GM	DP	JA	DR	CH	MT	MN	AN	FL	CM	AS	OC
1321	Aromatic, 149, FW 164	—	—	t	0.5	0.5	—	—	—	—	—	—	t	—	—	—	—	—
1322	Methyl geranate	—	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—	—	0.5
1350	α-Terpinyl acetate	—	—	—	—	—	—	3.7	—	—	—	0.1	—	—	—	—	—	—
1351	α-Cubebene	0.1	—	—	—	—	—	—	—	t	—	—	—	0.3	0.2	—	—	t
1354	Citronellyl acetate	—	—	0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1376	α-Copaene	t	—	—	—	0.2	t	—	—	t	—	—	—	—	—	—	—	0.5
1378	Ethyl 4E-decenoate	—	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—	—	—
1384	β-Bourbonene	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	t
1390	β-Cubebene	0.1	—	—	—	0.1	t	—	—	t	t	—	—	0.4	—	—	—	t
1401	Methyl eugenol	—	—	0.4	—	—	0.2	—	—	—	—	—	—	—	0.1	0.2	—	—
1402	Longifolene	—	—	—	—	0.3	—	t	—	t	—	—	—	—	—	—	—	—
1409	α-Cedrene	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1418	(E)-Caryophyllene	t	—	t	—	0.1	0.4	—	0.2	1.4	t	t	t	t	0.3	0.2	—	t
1429	*cis*-Thujopsene	—	—	0.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1444	Citronellyl propanoate	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—
1444	*cis*-Murrola-3,5-diene	—	—	—	—	—	0.3	—	0.4	t	—	—	—	—	—	—	—	—
1450	*trans*-Muurola-3,5-diene	0.2	t	—	—	—	t	t	—	t	0.2	—	—	0.9	—	—	—	t
1454	α-Humulene	t	—	t	—	—	t	—	0.3	1.0	—	—	t	—	—	—	—	—
1461	*cis*-Muurola-4(14),5-diene	—	—	—	—	—	0.9	—	1.1	0.3	—	—	—	—	—	—	—	—
1471	Unknown, 43, 133, 105, 149	—	—	—	0.5	—	—	—	—	—	—	—	—	—	—	—	—	—
1473	*trans*-Cadina-1(6),4-diene	0.1	—	—	—	—	0.1	t	—	0.2	0.1	—	—	0.8	—	—	—	—
1477	γ-Muurolene	—	—	—	—	—	0.1	—	0.1	t	—	—	—	—	—	—	—	0.3
1480	Germacrene D	t	—	—	—	0.4	0.4	—	1.5	1.3	—	—	—	—	0.1	0.7	—	—
1485	β-Selinene	—	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—	—
1491	*trans*-Muuola-4(14),5-diene	0.5	t	—	—	t	0.2	t	—	0.3	0.4	—	—	2.2	—	—	—	0.1
1491	Valencene	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.1	—	—
1493	*cis*-Cadina-1,4-diene	t	—	—	—	—	—	t	—	—	—	—	—	—	—	—	—	0.1
1493	4-Epi-cubebol	—	—	—	—	t	0.1	—	—	0.2	0.2	—	—	0.9	—	—	—	—
1499	Epi-zonarene	—	—	—	—	—	t	—	0.2	—	—	—	—	—	—	—	—	—
1499	α-Muurolene	t	—	—	—	—	0.5	—	0.2	0.2	—	—	t	t	t	—	—	0.4
1512	β-Curcumene	—	—	—	—	t	—	—	—	0.2	—	—	—	—	—	—	—	—
1513	γ-Cadinene	0.4	—	—	t	—	1.7	t	0.9	1.1	—	—	—	—	—	—	—	1.7
1513	Cubebol	—	t	—	—	0.5	—	—	—	—	0.4	—	—	2.0	—	—	—	—
1515	Endo-1-bourbonanol	—	—	—	—	—	—	—	—	0.1	—	—	—	—	—	—	—	t
1521	*trans*-Calamenene	—	—	—	—	—	t	—	t	—	—	—	—	—	—	—	—	—
1524	δ-Cadinene	0.1	—	—	t	0.3	2.5	t	1.1	1.1	0.2	—	—	1.4	t	0.2	—	1.4

1526	Zonarene	t	—	—	—	—	t	—	—	t	0.1	—	—	0.6	—	—	—	—
1529	Citronellyl *n*-butyrate	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1532	*Trans*-Cadina-1(2),4-diene	t	—	—	—	—	—	—	—	t	t	—	—	0.2	—	—	—	t
1538	α-Cadinene	—	—	—	—	—	0.1	—	—	t	—	—	—	—	—	—	—	t
1542	α-Calacorene	—	—	—	—	—	—	—	0.1	—	—	—	—	—	—	—	—	t
1542	Sesquiterpene, 43, 95, 207	—	—	—	—	—	0.5	—	—	—	—	—	—	—	0.2	—	—	—
1549	Elemol	2.6	1.5	2.1	0.6	3.5	t	t	—	t	7.6	5.4	3.7	8.5	0.1	0.8	—	—
1554	Elemicin	—	—	1.2	—	—	0.1	—	—	—	—	—	—	10.2	t	—	—	—
1556	Germacrene B	0.1	—	—	—	0.1	—	—	—	0.2	—	—	t	—	—	0.2	—	—
1564	(E)-Nerolidol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t	—	—
1574	Germacrene D-4-ol	—	—	—	t	—	0.5	t	0.2	1.2	—	—	—	—	—	—	—	0.3
1581	Caryophyllene oxide	—	—	—	t	—	0.2	t	0.1	0.1	—	—	—	—	0.3	0.2	—	—
1585	Gleenol	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t
1596	Cedrol	—	—	t	—	—	—	—	—	—	—	t	—	—	—	t	t	—
1606	β-Oplopenone	—	—	—	—	—	0.5	—	t	—	—	—	—	—	—	—	—	0.2
1606	Humulene epoxide II	—	—	—	—	—	—	t	t	0.1	—	—	—	0.1	—	—	—	—
1610	Unknown, 43, 119, 195	—	0.2	—	0.1	—	—	—	—	—	—	0.9	—	—	—	—	—	—
1627	1-Epi-cubenol	0.4	0.1	—	t	0.1	0.4	t	0.1	0.4	0.6	—	—	3.1	—	t	—	0.7
1630	Eremoligenol	—	—	—	—	—	—	—	—	—	—	0.1	0.5	—	—	—	—	—
1630	α-Acorenol	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1630	γ-Eudesmol	0.4	0.3	0.4	t	0.6	—	t	—	t	1.7	0.7	2.4	1.6	—	0.2	—	—
1640	Epi-α-cadinol	—	—	—	—	—	3.1	t	4.3	0.6	0.5	—	—	0.5	—	0.3	t	0.6
1640	Epi-α-Muurolol	—	—	—	—	—	3.1	—	—	0.6	0.4	—	—	0.5	—	0.2	—	0.7
1642	Cubenol	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—
1645	α-Muurolol (= torreyol)	—	—	—	—	—	1.0	—	0.7	t	—	—	—	0.3	—	t	—	0.2
1649	β-Eudesmol	0.6	0.4	0.8	t	0.8	—	t	—	t	2.8	1.1	6.5	1.7	—	0.5	—	—
1652	α-Eudesmol	0.7	0.6	0.7	t	1.7	—	t	—	—	7.1	1.9	4.8	3.0	—	0.5	—	—
1653	α-Cadinol	—	—	—	t	—	6.1	—	3.4	1.5	—	—	—	—	—	1.0	t	0.9
1666	Bulnesol	0.6	0.2	0.1	t	0.9	—	—	—	—	1.6	1.1	0.5	0.9	—	0.2	—	—
1674	Cadalene	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	t
1685	Eudesma-4(15), 7-dien-1-β-ol	—	—	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—
1733	Oplopenone	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—	—
1741	8-α-11-Elemodiol	—	0.2	—	—	—	—	—	—	—	0.3	—	—	—	—	—	—	
1789	8-α-Acetoxyelemol	—	0.5	0.1	—	—	—	—	—	—	1.3	2.0	1.7	t	—	—	—	—
1930	Rosa-5,15-diene	—	—	—	—	—	—	—	—	—	—	—	—	t	—	—	—	—
1960	Sandaracopimara-8(14),15-diene	—	—	—	—	—	—	—	—	—	—	—	—	—	0.2	—	t	—
1989	Manoyl oxide	0.7	3.6	0.2	—	1.4	0.4	0.8	0.4	0.6	t	1.6	t	0.3	6.4	0.2	2.6	2.7
1989	Isokaurene	—	—	—	—	—	—	—	—	—	—	1.5	—	—	—	—	—	—
2054	Abietatriene	t	0.4	t	—	t	0.2	—	0.7	0.3	t	1.6	t	0.2	0.4	t	t	—

Table—*continued*

Table 2—*continued*

KI[a]	Compound	PN	SA	CA	OS	ST	GM	DP	JA	DR	CH	MT	MN	AN	FL	CM	AS	OC
2054	Manool	—	—	—	—	—	0.2	—	—	—	—	—	—	—	—	—	—	—
2080	Abietadiene	0.3	—	—	—	t	—	—	t	t	t	0.8	—	—	—	—	0.1	—
2102	Diterpene, 41, 191, 257	—	0.5	—	—	—	—	—	—	—	—	—	—	0.9	—	t	—	—
2243	*Trans*-Totarol, methyl ether	—	—	—	—	t	—	—	—	—	—	—	—	—	—	—	—	—
2252	Methyl sandaracopimarate[b]	—	0.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2278	*cis*-Totarol	—	0.4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2288	4-epi-Abietal	0.3	—	—	—	0.2	—	—	t	0.4	t	—	—	t	—	t	—	—
2302	Abieta-7,13-dien-3-one	0.1	—	—	—	—	—	—	—	t	—	—	—	—	—	t	—	—
2302	*Trans*-Totarol	—	0.2	—	—	1.1	—	—	1.7	—	—	1.3	—	0.3	—	t	t	—
2325	*Trans*-Ferruginol	—	0.1	—	—	t	t	—	0.3	—	t	t	—	0.3	—	t	—	—

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

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 ($\pm$) 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 colorless to yellow in color. Table 2 gives the tabulated results. Several components previously unidentified have now been identified.

Some discussion is needed to facilitate recent nomenclature changes. *Juniperus erythrocarpa*, the taxon in trans-Pecos, Texas and Mexico is now treated as *J. coahuilensis*; *J. monosperma* var. *gracilis* Mart., from north-central Mexico, is now *J. angosturana* R. P. Adams; and *J. monsperma*, previously reported from Mexico, is now thought to be confined to the United States.

The oils of this section are quite diverse. The presence of very large amounts of camphor (64.9%, *J. ashei*) is common in this section (Table 2). In general, these junipers do not accumulate large amounts of diterpenes as seen in the junipers of the eastern hemisphere (Adams, 1999, 2000b). Except for a chemical polymorphism in *J. californica* (Adams et al., 1983) involving camphor, the pinenes, and terpinen-4-ol, no chemical races have been found in the junipers of this section. The presence of cedrol, a major constituent of the juniper wood oils (Adams, 1991), is found only as trace component in the leaves of a few taxa (Table 2). This is in contrast to many junipers of the eastern hemisphere where cedrol is a major component (up to 30%) of the leaf oils (Adams, 1999).

The overall similarities of the oils are shown by a minimum spanning network of these junipers, based on presence/absence matching (Fig. 1). One is immediately impressed that the taxa are all distinct. This is not surprising because the varieties of *J. deppeana*, *J. flaccida*, and *J. coahuilensis* and the forms of *J. monticola* were not included in this study. However, several species are, morphologically, quite similar

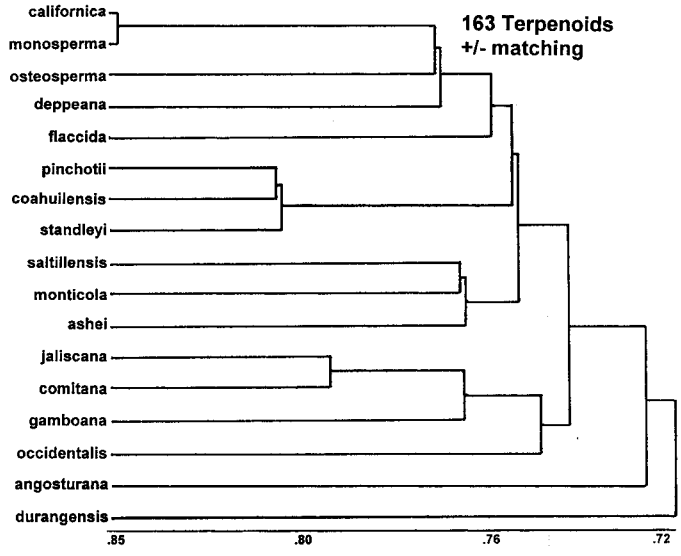

Fig. 1. Minimum spanning network based on 163 terpenoids, with similarities computed as presence/absence data.

(*angosturana-coahuilensis-monosperma-pinchotii*, *ashei-saltillensis*, *comitana-gamboana*, *californica-osteosperma*). This is not particularly reflected in their terpenoids based on presence/absence matching. *Juniperus pinchotii* and *J. coahuilensis* do form a loose cluster (with *J standleyi*) (Fig. 1) and *J. comitana* and *J. jaliscana* form another loose cluster. *Juniperus ashei* and *J. saltillensis* (plus *J. monticola*) form another loose cluster (Fig. 1) This pattern of very distinct species is similar to that found by Zanoni and Adams (1976).

The DNA (RAPDs) data revealed a similar pattern (Fig. 2) in that the taxa are each very distinct with little clustering. *Juniperus durangensis* and *J. jaliscana* are the most similar species (Fig. 2), but only slightly removed from *J. coahuilensis*, *J. deppeana*, *J. gamboana*, and *J. standleyi*. As with the terpenoids, the species in this section of *Juniperus* are distinct. There is some clustering of *J. pinchotii*, and *J. saltillensis*, followed with *J. californica* and *J.* osteosperma (Fig. 2). *Juniperus flaccida*, *J. comitana*, *J. ashei*, and *J. occidentalis* are very loosely associated with the other junipers (Fig. 2).

The evolution of these xerophytic junipers of the high deserts and arid mountains seems to present a somewhat reticulate pattern of evolution. One finds quadrangular bark (only found in this section of *Juniperus*), expressed in *J. angosturana*, *J. deppeana*, and *J. gamboana* (Zanoni and Adams, 1976). In the middle of the one seeded junipers cluster (Figs. 1 and 2), one finds the multi-seeded species, *J. deppeana*. If, as Axlerod (1958) suggests, there was a rapid speciation into the newly formed deserts, it is

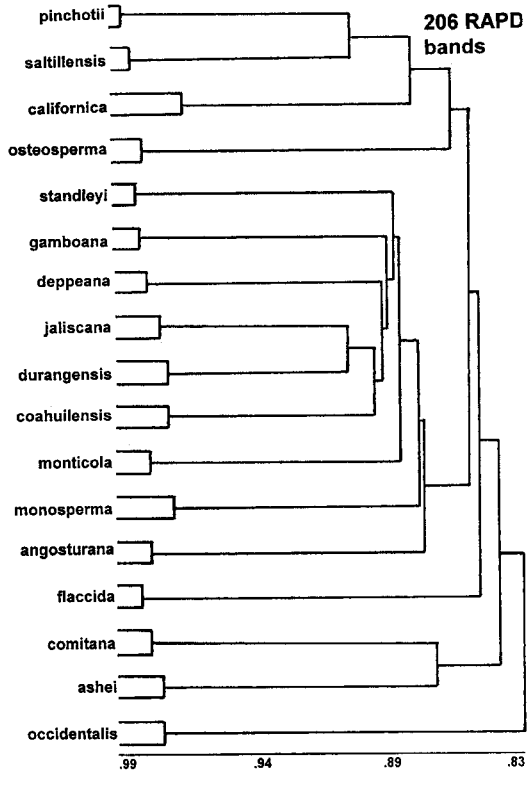

Fig. 2. Minimum spanning network based on 206 RAPD bands. Each OTU is represented by two individuals.

possible that the ancestral *Juniperus* species became extinct, leaving a number of adaptive lines of radiation. In addition, hybridization is likely to have been involved, further obscuring the patterns of adaptive radiation. Sequence data may help elucidate the problem, but for now, the essential oils and RAPDs both argue for a very loose assemblage of species that diverged into distinct taxa leaving no clear path of intermediate extant taxa.

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