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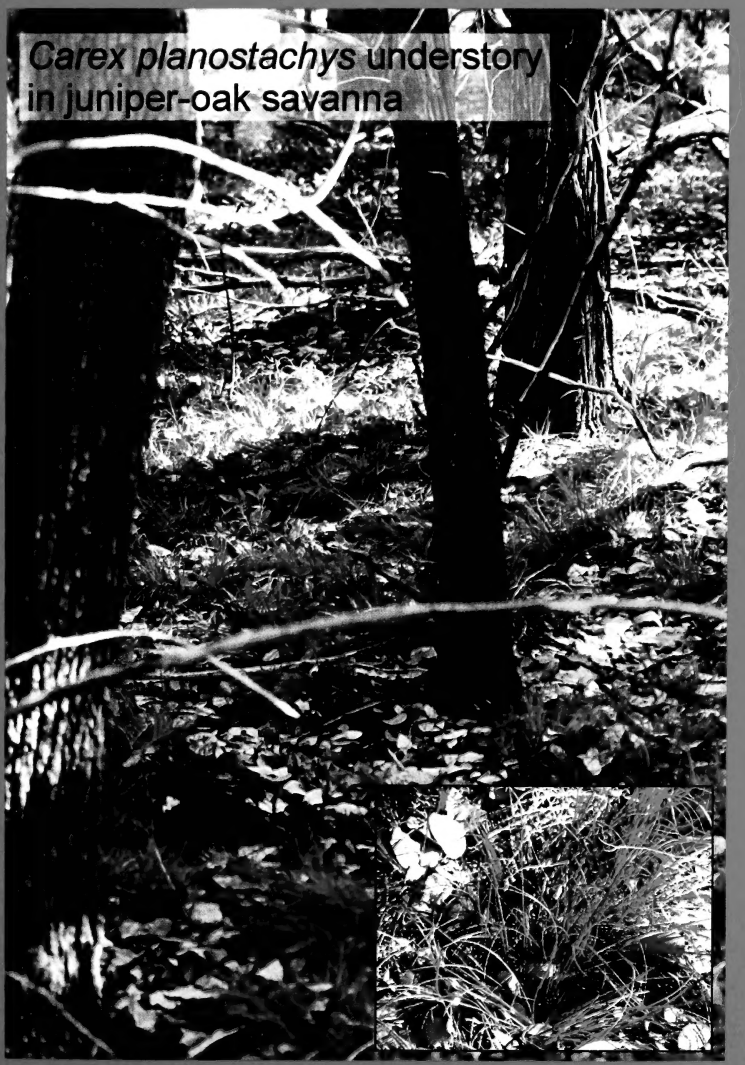

PHYTOLOGIA

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Robert P. Adams
Phytologia, Box 727, Gruver, TX 79040
Robert_Adams@baylor.edu

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Cover photo: *Carex planostachys* as an understory plant in juniper-oak savanna on the Edwards Plateau, Texas. Photo by E. R. Wayne.

GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS GRANDIS* (CUPRESSACEAE) II.

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

The volatile leaf oils of *J. grandis* in California were analyzed from throughout its range. A clinal trend was found northward from the High Sierras to Stampede Meadows and thence to Beckwourth. The Beckwourth oil, although most similar (0.745) to *J. grandis* from the High Sierras, had a 0.728 similarity to *J. occidentalis* (Yolla Bolly Mtns.). The oils in the Beckwourth population are intermediate between *J. grandis* and *J. occidentalis* indicating hybridization. A northward clinal pattern of higher similarities of *J. grandis* to *J. occidentalis* is suggestive of past introgression. The San Bernardino Mtns., *J. grandis* populations' oils were more similar to *J. occidentalis* (0.743), than to *J. grandis* at nearby 9 Mile canyon (0.540). Confounding the situation, DNA analyses, grouped the San Bernardino Mtns. juniper with *J. osteosperma*. *Phytologia 94(1): 3-21 (April 2, 2012).*

KEY WORDS: *Juniperus grandis (= J. occidentalis* var. *australis), J. californica, J. occidentalis, J. osteosperma,* Cupressaceae, terpenes, geographic variation.

Previously, Adams and Kauffmann (2010a) reported on geographic variation in the leaf essential oils of *J. grandis* R. P. Adams (= *J. occidentalis* var. *australis* (Vasek) A. & N. Holmgren). They found (Fig. 1) that the leaf oils of *J. grandis* contained two chemical races: High Sierra populations with oils dominated by δ-3-carene (17.9-30.0%) and low in sabinene, and the San Bernardino Mtns. population with oil low in δ-3-carene, but very high in sabinene (24.3%). Adams and Kauffmann (2010a) found the leaf oil of putative *J. grandis* of the Yolla Bolly Mtns. was actually more similar to the oil of *J. occidentalis*

than *J. grandis*. Subsequent DNA sequencing gave support that the Yolla Bolly Mtns. juniper is a divergent form of *J. occidentalis* (Adams and Kauffmann, 2010b; Adams 2011).

At the northern end of the range of *J. grandis*, populations appear to descend from the High Sierras (Fig. 2). Vasek (1966) felt that *J. grandis* (as *J. occ.* var. *australis*) intergraded into *J. occ.* var. *occidentalis* in the region north of the High Sierras. In my previous study (Adams and Kauffmann, 2010a) I did not sample from this region.

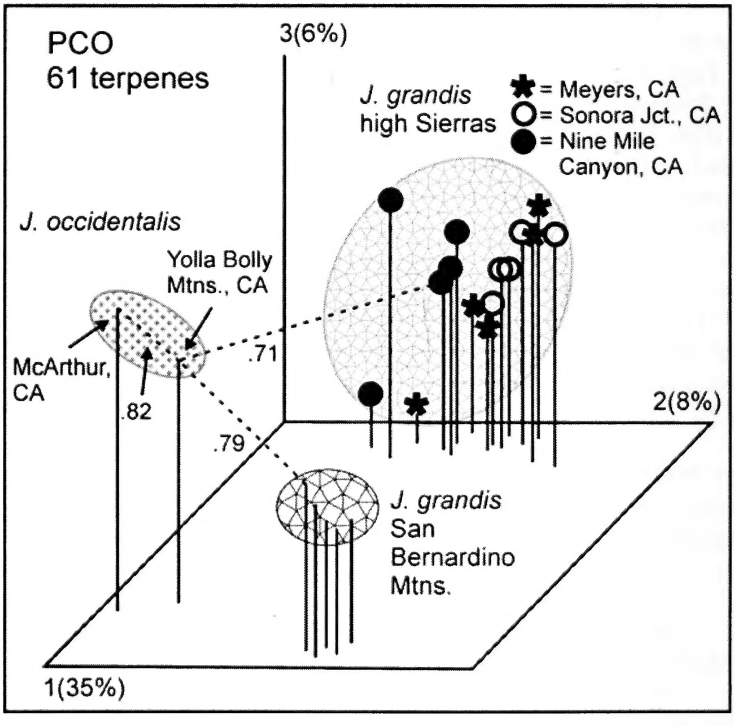

Figure 1. PCO based on 61 terpenes from *J. grandis* (20 individuals) and *J. occidentalis* populations (McArthur, Yolla Bolly Mtns., CA). The dotted lines are minimum links that connect the groups. The numbers by the dotted lines are the similarity (0.0 - 1.0 scale).

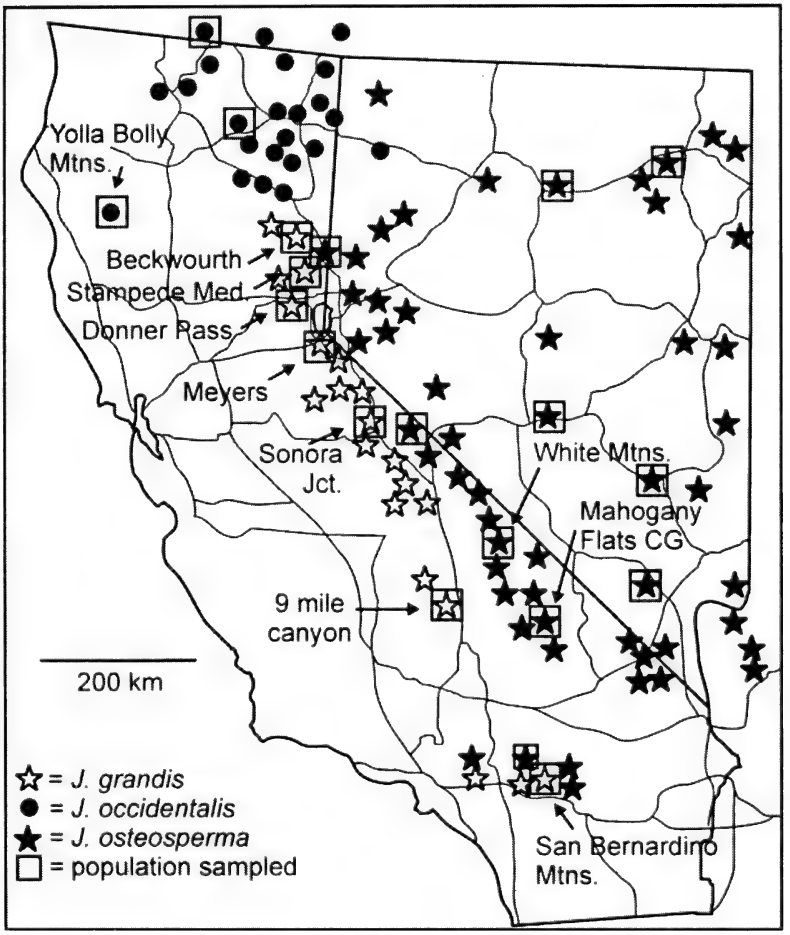

Figure 2. Distributions of *J. grandis*, *J. occidentalis* and *J. osteosperma*, modified from Vasek (1966) and Adams and Kaufmann (2010a). Note the northern-most populations of putative *J. grandis* sampled: Donner Pass, Stampede Meadows, and Beckwourth, CA.

In the present study, I report on analyses of populations of putative *J. grandis* from Donner Pass, Stampede Meadows, and Beckwourth, CA as a well as an additional population from Onyx

Summit, San Bernardino Mtns. where the 'purest' *J. grandis* is thought to grow (F. C. Vasek, personal communication).

MATERIALS AND METHODS

Plant material: *J. grandis, Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086N, 120° 01.244'W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289'N, 111° 35.598'W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003'N, 118° 02.078'W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34° 17.533'N, 116° 49.153'W, 2053 m, San Bernardino Co., CA; *J. grandis, Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086N, 120° 01.244'W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289'N, 111° 35.598'W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003'N, 118° 02.078'W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34° 17.533'N, 116° 49.153'W, 2053 m, San Bernardino Co., CA; *Adams 12319-12322,* Onyx Summit on CA 38, 34° 11.524'N; 116° 43.227' W.2600 m, San Bernardino Co., CA; *Adams 12328-12331, 12367,* Donner Pass Summit on old US50, 39° 18.999' N; 120° 19.581' W. 2180 m, Placer Co., CA; *Adams 12332-12336,* on Stampede Meadows Rd. (Co. rd 894Aa1t), 5 mi. n of I80. 39° 24.966' N; 120° 05.249' W, 1660 m, Nevada Co., CA; *Adams 12337-12341,* 4.7 mi. n of Beckwourth on Beckwourth-Genesee Rd., 39°52.433'N; 120° 24.345'W, 1770 m, Plumas Co., CA.

J. occidentalis, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392'N, 120° 41.207'W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44° 53.676'N, 120° 56.131'W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44° 02.390'N, 121° 20.054'W, 1132 m, Deschutes Co., OR; *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, Deschutes Co., OR; *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR66, 42° 08.044'N, 122° 34.130'W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA299, 10 km e of McArthur, CA, 41° 05.313'N, 121° 18.921'W, 1091 m,

Lassen Co., CA; *Adams 11995-11998 (Kauffmann A1-A3, B1),* Yolla Bolly-Middle Eel Wilderness, 40° 06' 34"N, 122° 57' 59W, 1815- 2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178'N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867'N, 120° 28.456' W, 1695 m, Lassen Co., CA.
J. osteosperma, Hancock Summit, mile 38 on US375, 37° 26.404'N, 115° 22.703'W, 1675 m, Lincoln Co. NV; *Adams 11125-11127*, McKinney Tanks Summit on US 6, 38° 07.005'N, 116° 54.103'W, 1933 m, Nye Co., NV. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type 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 (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf oil of *J. grandis* from Donner Pass was quite similar to that of nearby Meyers, CA, High Sierra type oil (Table 1), being high in α-pinene, δ-3-carene, β-phellandrene, and very low in β-pinene. The leaf oil from nearby Stampede Meadows was very similar to the High Sierra oil (cf. Stamp vs. Meyers, Table 1). However, the leaf oil from Beckwourth was quite different from the High Sierra oils (cf. Meyers, Stampede Meadows and Beckwourth, Table 1). The Beckwourth trees have a number of components similar to *J. grandis*: p-cymene, β-phellandrene, p-menth-1,5-dien-8-ol isomer, terpinen-4-ol, m-cymen-8-ol, citronellol, piperitone, unknown 1388, γ-cadinene, and elemicin. Other compounds are similar to *J. occidentalis*: tricyclene, α-thujene, α-pinene, camphene, sabinene, and cis-sabinene hydrate. Intermediate concentrations and complemented terpenes would be expected in hybrids. It is interesting that the lower amounts of α-pinene and α-fenchene is similar to the oil of *J. occidentalis* (Table 1), but the high concentration of β-phellandrene (20.1%) is more like High Sierra, *J. grandis*.

The oils of *J. grandis* are differentiated into three groups (Fig. 3): the High Sierras group (including the sub-group of Donner Pass), Beckwourth, CA, and the San Bernardino Mtns. One individual from 9 Mile Canyon had an unusual oil profile and is only loosely clustered with other High Sierra trees (Fig. 3).

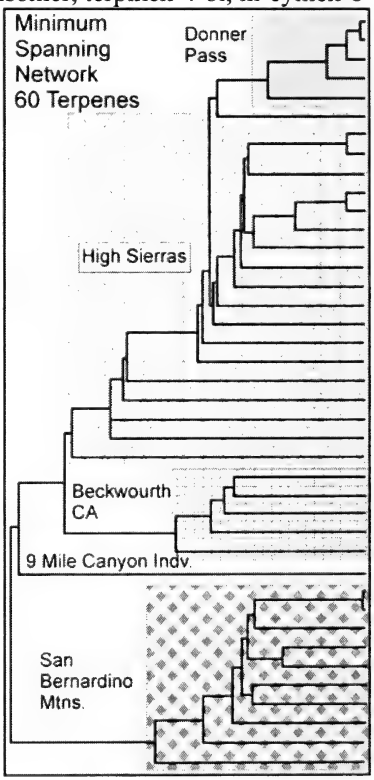

Figure 3. Minimum spanning network based on 60 terpenes.

Principal coordinates analysis (PCO) revealed additional perspectives (Fig. 4) among the groups. The Donner Pass oil is very similar to High Sierras oil (Fig. 4). As found in Table 1, the San Bernardino Mtns. oil is more similar to the Beckwourth oil than the oils from the High Sierras (Fig. 4).

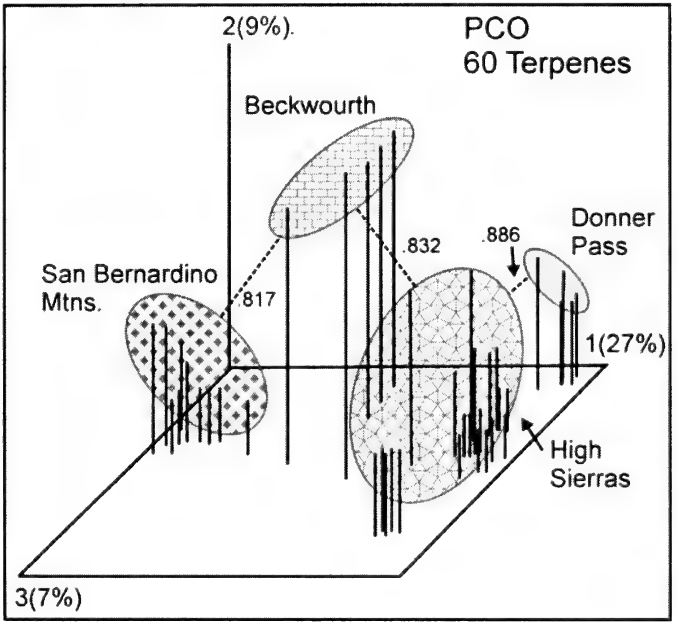

Figure 4. Principal Coordinates analysis (PCO) of 40 J. grandis oils based on 60 terpenes. The dashed lines are the linkage (similarity) between the four major groups.

A PCO using 63 terpenes with eight population average oils of *J. grandis,* plus two populations of *J. occidentalis* (McArthur and Yolla Bolly, CA) and two populations of *J. osteosperma* (McKinney Tanks and Hancock Summit, NV), shows (Fig. 5) the intermediate nature of the leaf oil from the Beckwourth population between Stampede Meadows and *J. occidentalis* (Yolla Bolly). The San Bernardino Mtns. population's oil is most similar to *J. occidentalis*, and more similar to the Beckwourth oil than to the High Sierras oil (Fig. 5, Table 1).

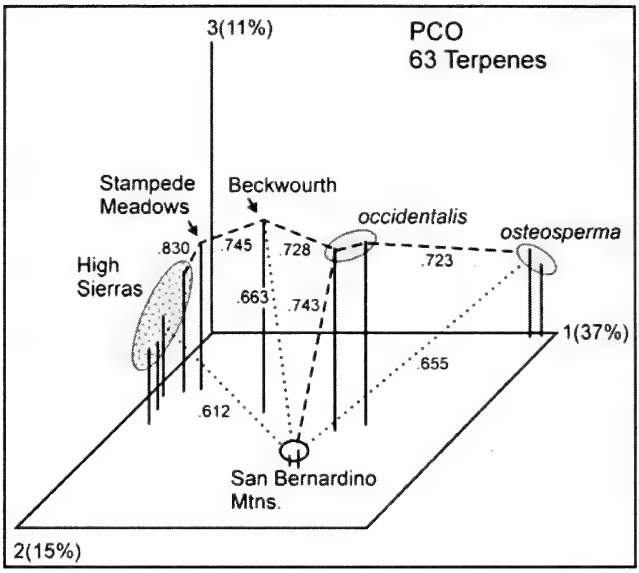

Figure 5. PCO based on 63 terpenes. The dashed lines are the minimum spanning network and the dotted lines show secondary links. The numbers near the lines are the similarities of the oils.

Contoured clustering (Fig. 6) shows the clinal nature of the variation in *J. grandis* from the High Sierras to the northernmost population (Beckwourth). The disjunct nature of the similarity between *J. occidentalis* (Yolla Bolly, McArthur) and the San Bernardino Mtns. (*J. grandis*) populations is apparent (Fig. 6).

Adams (1982), using morphological data from synthetic crosses in sunfish, and terpenoids in natural hybrids of *J. horizontalis* and *J. scopulorum*, compared a number of multivariate methods for the detection of hybridization. He found that Principal Coordinates (PCO) ordinated the parents on the first axis and the hybrids on the second axis, with backcrossed individuals between the parents and hybrids (Adams, 1982, Figs. 4, 9).

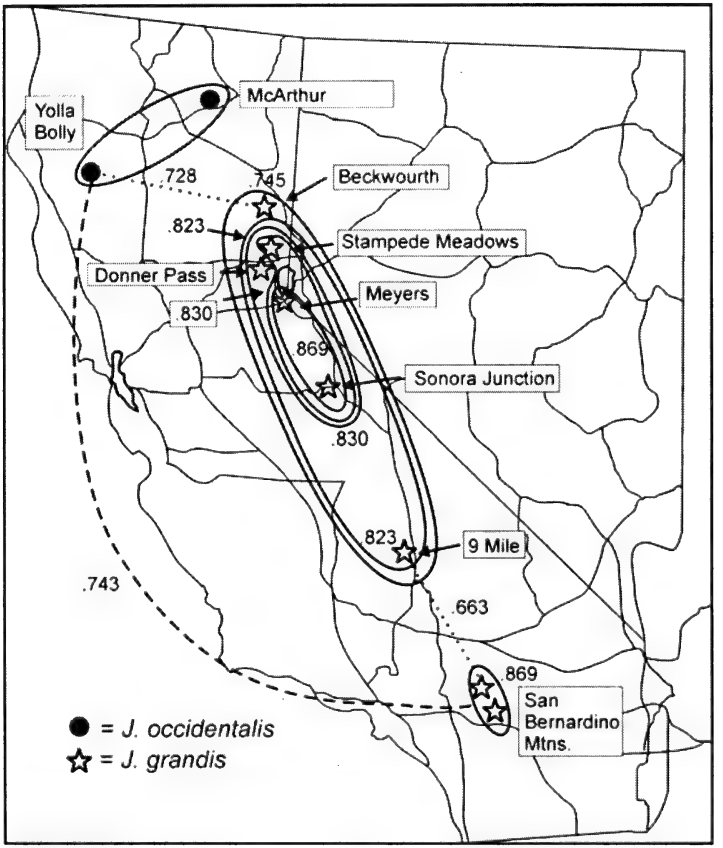

Figure 6. Contoured clustering based on 63 terpenoids. The dashed and dotted lines show unusual links. The numbers next the lines are the similarities.

PCO of *J. grandis* (Meyers, Sonora Junction) individuals from the Beckwourth and Stampede Meadows individuals, and ten populations of *J. occidentalis* accounted for 33% of the variance in PCO 1 (separating *J. grandis* and *J. occidentalis*, Fig. 7). PCO 2 (10% of variance) separates the Beckwourth individuals from *J. grandis* and *J. occidentalis*. As Adams (1982) found, the ordination forms a V

shape, with the putative hybrids intermediate, but not on a line between putative parents (Fig. 7). Examination of Table 1, shows a number of compounds that are present in the Beckwourth trees that are found in one of the putative parents. The oils of the divergent trees from the Stampede Meadows population appear to differ little from typical *J. grandis* in this ordination. It should be noted that the bark on trees in the Beckwourth population was shaggy and gray with cinnamon beneath, not the typical cinnamon bark color as found in the High Sierras.

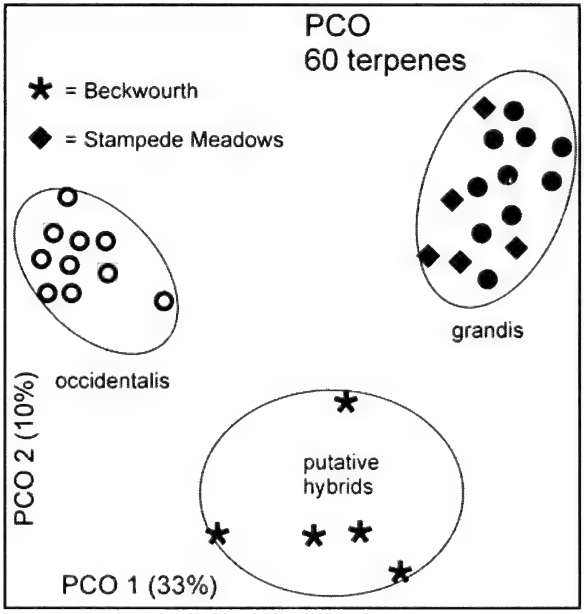

Figure 7. PCO based on 60 terpenes, ordinating *J. grandis, J. occidentalis* and putative hybrids from Beckwourth.

Another method to examine the clinal variation in *J. grandis* is by a linkage map among populations (Fig. 8). As one proceeds northward from Sonora Junction (Sj) to Meyers (My), to Donner Pass (Dp), Stampede Meadows (Sm) to Beckwourth (Bc) one finds progressively lower similarities (Fig. 8). However, the fact that similarities are lower as one proceeds from the central High Sierras

(My, Sj) to Sm and Bc does not necessarily imply introgression from *J. occidentalis*. Examination of the secondary links from *J. occidentalis* at Yolla Bolly (YB) show (Fig. 8) the highest similarity is to Beckwourth (Bc, .728), Donner pass (Dp, .701), Stampede Meadows (Sm, .650) and finally to the High Sierras (My, .630). This trend does appear to support introgression from *J. occidentalis* into *J. grandis*.

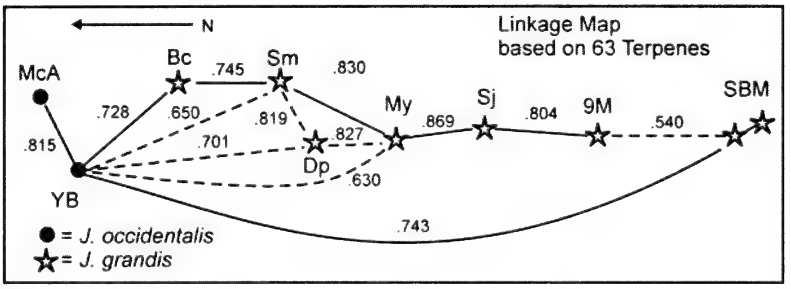

Figure 8. Linkage map based on 63 terpenes. The numbers next to the lines are the similarities. The dashed lines are secondary links.

The disjunct similarity between the *J. grandis* in the San Bernardino Mtns. and Yolla Bolly *(J. occidentalis*) is difficult to explain. Ancient hybridization and introgression could explain the pattern. Alternatively, if *J. occidentalis*-like individuals once extended much further southward along the Sierra Nevada foothills during the Pleistocene, then the population in the San Bernardino Mtns. could have been established and become isolated in more recent times. To confound the matter, DNA sequencing did not resolve putative *J. grandis* (San Bern. Mtns., Fig. 9) from *J. osteosperma* (Adams and Kauffmann, 2010b).

Thus, we are faced with conflicting data sets for the putative *J. grandis* from the San Bernardino Mtns. These trees' morphology (cinnamon-colored bark, leaves and females cones) is as found in the High Sierras, but the oils are very much more like *J. occidentalis,* and the cpDNA (so far) is not different from *J. osteosperma* (Fig. 9). Clearly the San Bernardino Mtns. *J. grandis* group presents an unusual situation that will require additional research to resolve.

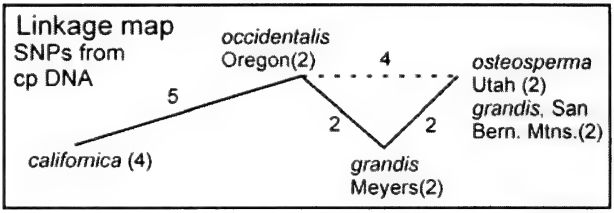

Figure 9. Linkage map based on SNPs from petN-psbM, trnD-trnT and trnG-trnG sequences (Adams and Kauffmann, 2010b). The numbers next the lines are the number of SNPs.

ACKNOWLEDGEMENTS

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Table 1. Leaf essential oil compositions for three populations of *J. grandis*, (Meyers, CA; Stampede Meadows, CA, Beckwourth, CA and Big Bear City, San Bernardino Mtns., CA) plus *J. occidentalis* (Yolla Bolly, Trinity Alps, CA, and McArthur, CA). Compounds in boldface appear to separate taxa and were used in numerical analyses. KI = Kovats Index (linear) on DB-5 column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. Those compounds that appear to distinguish taxa are in boldface.

KI	Compound	grandis Meyers	grandis Stamp	grandis Beckw.	grandis Big Bear	occid Trin Alp	occid Mc Art.
921	**tricyclene**	**-**	**-**	**0.1**	**0.3**	**t**	**1.1**
924	**α-thujene**	**-**	**-**	**0.4**	**2.3**	**1.8**	**1.0**
932	**α-pinene**	**14.0**	**6.8**	**2.5**	**7.1**	**5.1**	**5.0**
945	**α-fenchene**	**1.5**	**1.2**	**t**	**0.2**	**-**	**t**
946	**camphene**	**-**	**-**	**0.3**	**0.3**	**0.3**	**1.0**
953	thuja-2,4-diene	t	-	-	-	-	t
961	**verbenene**	**2.9**	**2.0**	**0.6**	**0.3**	**0.7**	**-**
969	**sabinene**	**-**	**t**	**10.7**	**24.3**	**20.4**	**12.0**
974	**β-pinene**	**1.3**	**0.8**	**0.6**	**0.5**	**0.7**	**0.4**
988	myrcene	3.1	3.4	3.5	1.7	3.0	1.3
1001	δ-2-carene	1.1	0.7	0.8	0.1	0.3	t
1002	α-phellandrene	1.6	1.6	4.0	0.4	1.2	0.8
1008	**δ-3-carene**	**27.3**	**17.0**	**1.6**	**2.8**	**4.4**	**1.0**
1014	α-terpinene	0.4	0.3	1.1	3.0	3.2	1.7
1020	**p-cymene**	**1.4**	**0.2**	**2.0**	**6.5**	**5.5**	**10.7**
1024	**limonene**	**1.2**	**t**	**t**	**1.6**	**0.7**	**0.9**

KI	Compound	grandis Meyers	grandis Stamp	grandis Beckw.	grandis Big Bear	occid Trin Alp	occid Mc Art.
1025	**β-phellandrene**	**10.6**	**16.1**	**20.1**	**1.5**	**6.7**	**3.5**
1044	(E)-β-ocimene	t	t	0.2	0.3	0.5	0.1
1054	**γ-terpinene**	**0.3**	**0.6**	**1.6**	**4.9**	**5.3**	**3.0**
1065	**cis-sabinene hydrate**	**-**	**-**	**0.5**	**1.9**	**1.2**	**0.9**
1086	terpinolene	3.7	2.3	1.7	1.9	2.4	1.3
1092	96, 109,43,152, C10-OH	0.9	1.2	-	-	-	-
1095	**trans-sabinene hydrate**	**-**	**-**	**0.4**	**1.8**	**t**	**0.7**
1095	linalool	t	0.7	0.5	-	1.5	0.5
1100	55,83,110,156, unknown	-	-	-	-	-	0.3
1102	isopentyl-isovalerate	-	-	-	-	-	-
1112	trans-thujone	-	-	-	0.2	-	t
1118	cis-p-menth-2-en-1-ol	0.8	1.7	2.7	0.7	1.0	0.7
1122	α-campholenal	t	-	-	-	-	-
1132	cis-limonene oxide (furanoid)	t	-	-	-	-	-
1136	trans-p-menth-2-en-1-ol	0.9	1.4	2.0	0.8	0.9	0.9
1141	**camphor**	**-**	**0.5**	**0.3**	**1.2**	**t**	**2.5**
1144	**neo-isopulegol**	**0.5**	**0.5**	**-**	**-**	**-**	**-**
1145	camphene hydrate	t	t	0.2	0.2	-	0.2
1154	**p-menth-1,5-dien-8-ol iso.**	**0.6**	**0.8**	**t**	**-**	**-**	**-**
1154	sabina ketone	-	-	t	0.9	0.3	0.4
1161	p-menth-1,5-dien-8-ol iso.	0.3	-	-	-	-	-
1165	borneol	-	-	-	0.1	t	2.2
1166	coahuilensol	t	0.7	1.2	-	2.4	0.6

KI	Compound	grandis Meyers	grandis Stamp	grandis Beckw.	grandis Big Bear	occid Trin Alp	occid Mc Art.
1174	**terpinen-4-ol**	**0.4**	**0.8**	**3.7**	**9.3**	**9.8**	**6.7**
1176	**m-cymen-9-ol**	**0.4**	**1.2**	**0.4**	**-**	**-**	**-**
1176	cryptone	-	-	t	-	-	-
1179	p-cymen-8-ol	0.4	1.1	0.2	1.0	0.9	0.5
1186	α-terpineol	1.2	0.3	-	0.3	0.5	0.4
1195	myrtenol	-	-	-	0.2	-	-
1195	cis-piperitol	0.4	0.6	0.6	0.2	0.1	0.2
1204	verbenone	-	-	-	-	-	-
1207	trans-piperitol	0.9	1.4	1.0	0.6	0.5	0.3
1215	trans-carveol	-	-	-	-	-	-
1219	coahuilensol, me-ether	0.4	1.7	1.5	-	2.7	1.1
1223	**citronellol**	**t**	**0.4**	**0.4**	**0.2**	**-**	**8.4**
1230	**trans-chrysanthenyl acetate**	**3.9**	**3.2**	**0.5**	**0.4**	**-**	**-**
1238	cumin aldehyde	-	-	-	0.3	0.7	0.2
1239	carvone	t	0.3	-	-	-	-
1249	piperitone	1.2	2.0	0.9	-	0.5	0.2
1253	trans-sabinene hydrate ac	-	-	-	0.6	-	-
1254	linalool acetate	-	-	-	-	0.1	0.1
1255	4Z-decenol	0.4	-	-	-	-	-
1257	methyl citronellate	0.2	0.2	-	0.1	-	-
1260	152,123,77,109, C10-OH	-	-	-	0.2	-	-
1274	neo-isopulegyl acetate	0.3	0.2	-	-	-	-
1283	α-terpinen-7-al	-	-	-	-	-	-

KI	Compound	grandis Meyers	grandis Stamp	grandis Beckw.	grandis Big Bear	occid Trin Alp	occid Mc Art.
1284	**bornyl acetate**	**0.4**	**0.8**	**5.2**	**2.2**	**t**	**9.5**
1285	**safrole**	**0.3**	**0.1**	**-**	**-**	**-**	**-**
1298	carvacrol	0.2	0.3	0.3	0.2	0.7	0.4
1298	3'-methoxy-acetophenone	-		-	0.2	-	-
1319	149,69,91,164, phenolic	0.8	-	-	-	-	-
1322	**methyl-geranate**	**-**	**1.4**	**16.3**	**1.8**	**0.8**	**1.0**
1325	p-mentha-1,4-dien-7-ol	-	-	-	0.7	0.1	t
1332	cis-piperitol acetate	0.4	-	-	-	-	-
1343	trans-piperitol acetate	0.3	-	-	-	-	-
1345	α-cubebene	-	-	-	t	t	t
1350	citronellyl acetate	-	-	-	-	-	-
1374	α-copaene	-	-	t	0.2	0.6	1.0
1387	β-bourbonene	0.5	-	-	0.3	t	0.2
1387	β-cubebene	-	-	-	-	-	-
1388	**79,43,91,180, unknown**	**0.3**	**0.3**	**0.1**	**-**	**0.1**	**-**
1389	**111,81,151,182, unknown**	**1.0**	**1.2**	**0.2**	**0.4**	**0.1**	**-**
1403	methyl eugenol	t	0.2	-	-	-	-
1417	(E)-caryophyllene	-	-	-	0.2	-	-
1429	cis-thujopsene	-	-	-	-	-	0.9
1430	β-copaene	-	-	-	t	-	-
1448	cis-muurola-3,5-diene	t	-	-	0.2	-	-
1451	trans-muurola-3,5-diene	-	-	-	-	0.1	0.1
1452	α-humulene	-	-	-	-	-	-

KI	Compound	grandis Meyers	grandis Stamp	grandis Beckw.	grandis Big Bear	occid Trin Alp	occid Mc Art.
1465	cis-muurola-4,5-diene	-	-	-	0.1	t	0.1
1468	pinchotene acetate	-	0.8	1.4	-	2.0	0.6
1471	**121,105,180,208,phenol**	**0.3**	**0.7**	**1.3**	**0.3**	**-**	**-**
1471	dauca-5,8-diene	-	-	-	0.2	-	-
1475	trans-cadina-1(6),4-diene	-	-	-	-	t	0.3
1478	**γ-muurolene**	**-**	**0.2**	**t**	**0.2**	**0.1**	**0.8**
1484	germacrene D	0.2	0.2	t	0.3	t	0.3
1491	43,207,161,222, C15-OH	-	-	-	0.3	-	-
1493	trans-muurola-4(14),5-diene	-	0.3	t	0.2	0.7	0.4
1493	epi-cubebol	-	0.4	0.2	0.5	0.4	0.4
1500	α-muurolene	0.3	0.3	t	-	0.6	1.1
1513	**γ-cadinene**	**1.3**	**0.6**	**0.3**	**1.2**	**1.8**	**3.7**
1518	epi-cubebol	0.4	1.2	0.4	1.5	t	0.4
1521	**trans-calamenene**	**-**	**0.6**	**t**	**2.3**	**-**	**-**
1522	δ-cadinene	1.1	0.7	0.8	-	2.2	4.1
1533	trans-cadina-1,4-diene	-	-	-	0.1	t	0.1
1537	α-cadinene	t	t	-	0.2	t	0.4
1544	α-calacorene	-	-	-	-	t	0.3
1548	elemol	-	0.1	0.7	0.9	-	-
1555	**elemicin**	**1.5**	**0.7**	**0.5**	**-**	**-**	**-**
1559	germacrene B	-	-	-	0.1	-	-
1561	**1-nor-bourbonanone**	**-**	**-**	**-**	**1.1**	**-**	**-**
1561	(E)-nerolidol	-	-	t	-	-	-

KI	Compound	grandis Meyers	grandis Stamp	grandis Beckw.	grandis Big Bear	occid Trin Alp	occid Mc Art.
1574	germacrene-D-4-ol	0.7	0.8	0.4	-	0.5	0.6
1582	caryophyllene oxide	t	t	-	0.3	-	-
1586	gleenol	-	-	-	-	t	0.3
1587	trans-muurol-5-en-4-α-ol	-	-	-	t	-	-
1607	β-oplopenone	0.4	0.4	0.1	0.8	0.4	0.4
1618	1,10-di-epi-cubenol	t	-	-	-	t	0.2
1627	1-epi-cubenol	t	0.7	0.3	0.5	1.3	1.6
1630	γ-eudesmol	-	-	t	t	-	-
1638	epi-α-cadinol	0.7	0.6	0.3	0.6	0.4	1.1
1638	epi-α-muurolol	0.7	0.6	0.4	0.6	0.6	1.2
1644	α-muurolol	t	0.2	t	0.1	t	0.7
1649	β-eudesmol	0.4	t	0.2	0.2	-	-
1652	α-eudesmol	-	-	0.2	0.6	-	-
1652	α-cadinol	1.6	1.3	0.9	0.7	0.8	1.8
1675	cadalene	-	-	-	0.1	t	0.3
1687	43,167,81,238, unknown	-	-	-	0.3	-	-
1688	shyobunol	0.2	t	-	-	-	-
1739	oplopanone	t	0.2	-	0.2	-	-
1987	**manoyl oxide**	**t**	**-**	**-**	**t**	**1.0**	**3.2**
2009	epi-13-manoyl oxide	-	-	-	-	t	t
2056	manool	t	0.4	-	-	-	-
2055	abietatriene	t	t	-	-	-	-
2298	4-epi-abietal	t	0.2	-	-	-	-

JUNIPERUS OCCIDENTALIS FORMA *CORBETII* R. P. ADAMS, A NEW SHRUBBY VARIANT: GEOGRAPHIC VARIATION IN LEAF ESSENTIAL OILS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

The volatile leaf oils of *J. occidentalis* were analyzed from throughout its range. The major differentiation found was the divergence of the Yolla Bolly population and the shrubby form that occurs about 30 km east of Bend, OR. The shrubby taxon is distinct in its habit and terpenes, having large amounts of p-cymene (20.0) and bornyl acetate (24.5%). A new form is named in honor of its discoverer: ***Juniperus occidentalis*** forma ***corbetii*** R. P. Adams, **forma nov.** *Phytologia 94(1): 22-34 (April 2, 2012).*

KEY WORDS: *Juniperus occidentalis, Juniperus occidentalis* forma *corbetii* R. P. Adams, **forma nov.,** *J. grandis,* Cupressaceae, terpenes, geographic variation.

Juniperus occidentalis, *J. grandis* (=*J. occidentalis* var. *australis* and *J. osteosperma* are three very closely related junipers in the western United States (Vasek; 1966, Adams, 2011). Although Adams and Kauffmann (2010a) and Adams (2012) reported on the compositions of the leaf oil of *J. occidentalis*, and hybridization with *J. grandis*, no extensive analysis of geographic variation in the leaf essential oils was reported. *Juniperus occidentalis* is a narrowly distributed species, growing largely east of the Cascade Mtns. and thence into nw California (Fig. 1). Recently, a shrubby form of *J. occidentalis* was discovered east of Bend, OR (Fig. 2). Careful field examination revealed that these shrubs are not just damaged (browsed, winter killed, etc.), but differ from the typical *J. occidentalis* that have a strong central axis. Thus, it seemed opportune to include this unusual population in this study of geographical variation.

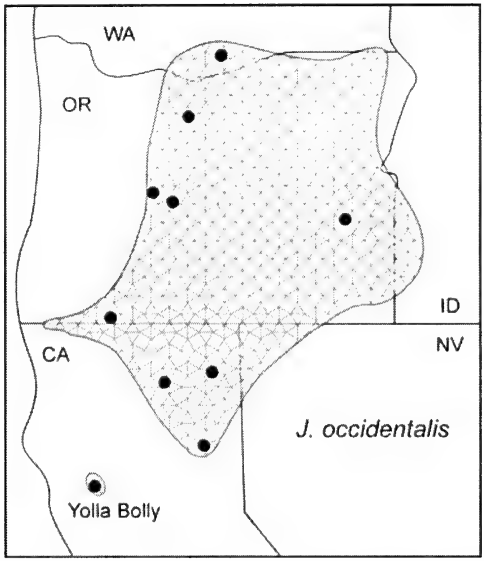

Figure 1. Distribution of *J. occidentalis* modified from Vasek (1966) and Adams (2011) showing sampled areas (dots). Note the southwestern-most population at Yolla Bolly (Trinity Alps, CA).

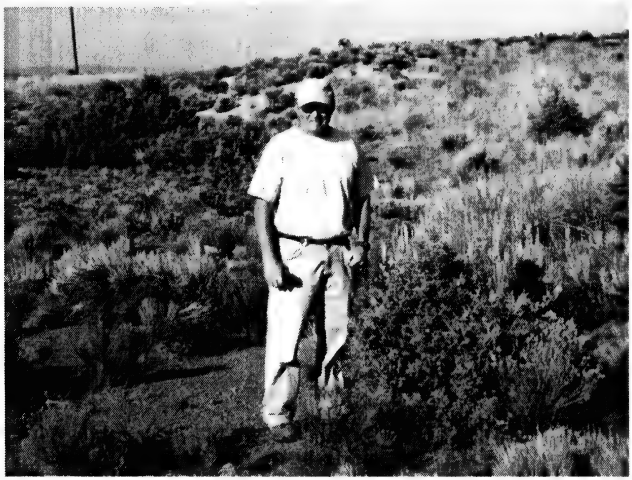

Figure 2. Mark Corbet with a shrubby form of *J. occidentalis,* 32 km east of Bend, OR (cf. *Adams 11949-11951).*

The purpose this paper is to report on geographic variation in the leaf essential oil of *J. occidentalis*. Analysis of hybridization with *J. osteosperma* in ne California and nw Nevada (Vasek, 1966, Terry, 2010; Terry et al. 2000) is beyond the scope of this paper.

MATERIALS AND METHODS

Plant material: *J. grandis, Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086N, 120° 01.244'W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289'N, 111° 35.598'W, 2585 m, Tuolumne Co.; CA.
J. occidentalis, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392'N, 120° 41.207'W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44° 53.676'N, 120° 56.131'W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44° 02.390'N, 121° 20.054'W, 1132 m, Deschutes Co., OR; *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, Deschutes Co., OR; *Adams 11952-11954,* 14 km e of Jct. OR66 & I5, on OR66, 42° 08.044'N, 122° 34.130'W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA299, 10 km e of McArthur, CA, 41° 05.313'N, 121° 18.921'W, 1091 m, Lassen Co., CA; *Adams 11995-11998 (Kauffmann A1-A3, B1),* Yolla Bolly-Middle Eel Wilderness, 40° 06' 34"N, 122° 57' 59W, 1815- 2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178'N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867'N, 120° 28.456' W, 1695 m, Lassen Co., CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type 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 (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled

to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The volatile leaf oil of *J. occidentalis* is dominated by sabinene, p-cymene, citronellol and bornyl acetate (Table 1). The leaf oil from the Yolla Bolly population is atypical in having more sabinene (20.4%), with a few compounds in common with *J. grandis* from Big Bear (verbenene, unknown 1389, Table 1). The shrubs east of Bend, OR have large amounts of p-cymene (20.0) and bornyl acetate (24.5%).

Principal coordinates analysis using 42 terpenoids resulted in eigenroots that accounted for 22, 15 and 15% of the variance. Ordination of the populations shows coordinate 1 separates the Yolla Bolly population from the other populations (Fig. 3). The shrubs east of Bend are clearly separated (Fig. 3).

Contoured clustering shows (FIg. 4) the sharp differentiation between the typical pyramidal trees at Bend and the shrubs east of Bend and the divergence of the Yolla Bolly (YB) population, joining last at a 0.670 similarity. Small amounts of differentiation is seen on the margins of the central region at Susanville, CA (Sv), Ashland, OR (As) and to a larger degree, the Klickitat, WA (Kw) population (Fig. 4). The oils of *J. occidentalis* appear to be very uniform throughout its range in eastern Oregon (Fig. 4).

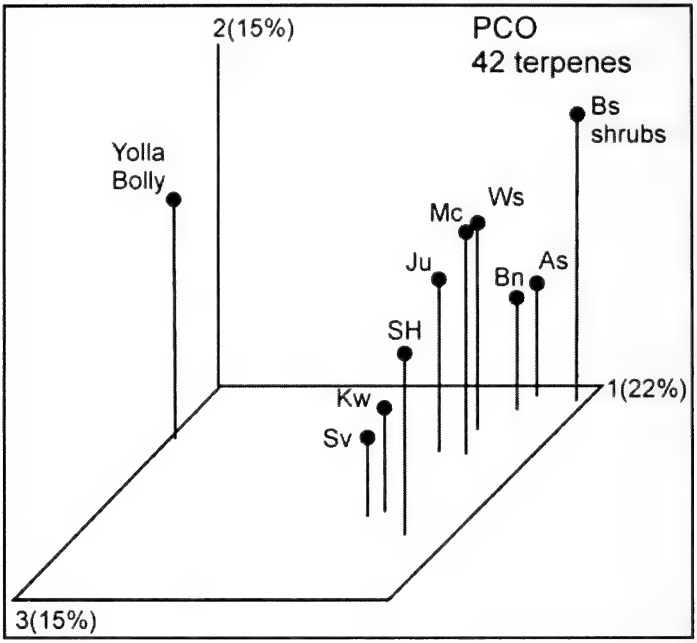

Figure 3. PCO based on 42 terpenes of 10 populations of *J. occidentalis*. Sv = Susanville, CA, Kw = Klickitat, WA, SH = Sage Hen Pass, CA, Ju = Juntura, OR, Mc = McArthur, CA, Ws = Wasco, OR, Bn = Bend, OR, As = Ashland, OR, Bs shrubs = shrubs, east of Bend, OR.

Because hybridization was found in the Beckwourth, CA area (Adams, 2012), it seemed important determine if any of the *J. occidentalis* populations show any evidence of increased similarity to *J. grandis*, suggestive of introgression. PCO ordination between *J. occidentalis* and *J. grandis* shows no intermediate *J. occidentalis* populations (Fig. 5), although the Yolla Bolly population shows some increased similarity to *J. grandis* (as reported by Adams, 2012).

Overall, the leaf essential oils of populations of *J. occidentalis* were found to be rather uniform except for the populations at the extremity of the range, and for the shrubby form east of Bend.

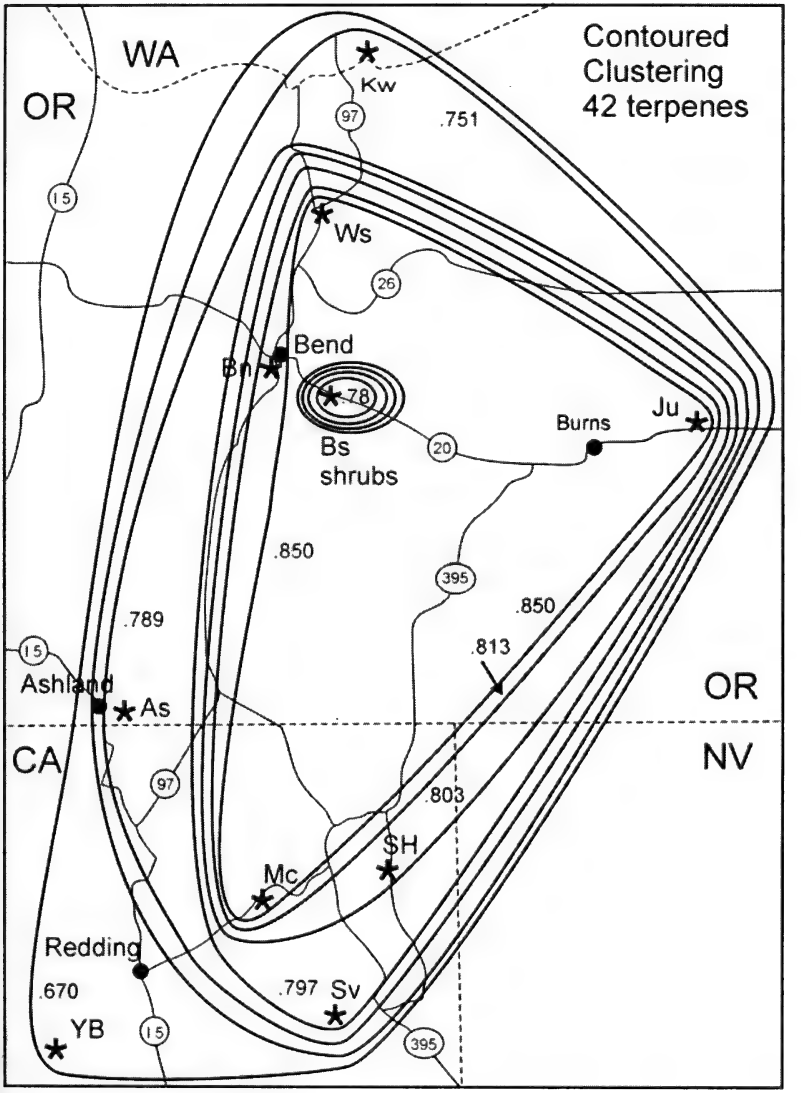

Figure 4. Contoured clustering based on 42 terpenes. See Fig. 3 for population identities.

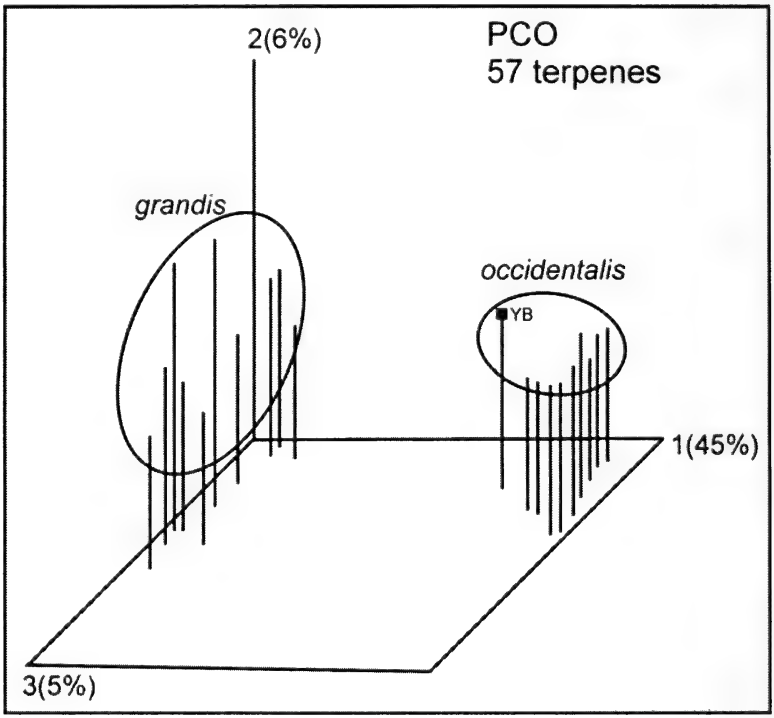

Figure 5. PCO based on 57 terpenes showing clear differentiation between *J. occidentalis* and *J. grandis*. YB is the Yolla Bolly population.

The shrubs of *J. occidentalis*, 32 km east of Bend, OR on hwy 20 appear to form a natural population that is reproducing itself. Due to the apparent differences in their habit from the normal *J. occidentalis* trees and their genetic differences in the expression of terpenoids, the shrubby junipers are worthy of recognition as a new forma:

Juniperus occidentalis forma **corbetii** R. P. Adams, **forma nov.** TYPE: United States, Oregon, Deschutes Co., 32 km E of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922'N, 120° 59.187'W, 1274 m, OR; 4 Aug 2009, *Robert P. Adams 11949*
(HOLOTYPE: BAYLU, PARATYPES: *Robert P. Adams 11950, 11951,* BAYLU).

Junipero occidentali similis sed differt habitu fruticoso et foliis confertim dispositis.

Similar to *Juniperus occidentalis* but differing habit, being a shrub with compact foliage.

The typical variety, with a strong central axis and pyramidal crown, grows on a nearby hillside, whereas f. *corbetii* grows along a dry wash on a mix of lava and sand. No female cones were found in this population.

ACKNOWLEDGEMENTS

Thanks to Mark Corbet for sharing in the discovery of this new shrub form with me and for his assistance in the field. Thanks to Guy Nesom for providing the Latin diagnosis. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Table 1. Leaf essential oil compositions for three populations of *J. occidentalis*, (Mc Arthur, CA, shrubs, e of Bend, OR, and Yolla Bolly, Y Bol) plus *J. grandis* from Big Bear, San Bernardino Mtns., CA. Compounds in boldface appear to separate taxa and were used in numerical analyses. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. For unknown compounds, four ions are listed, with the largest ion underlined.

		occid	occid.	occid	grandis
KI	Compound	Mc Art	shrub	Y Bol	Big Bear
921	**tricyclene**	**1.1**	**1.7**	**t**	**0.3**
924	α-thujene	1.0	0.9	1.8	2.3
932	**α-pinene**	**5.0**	**1.8**	**5.1**	**7.1**
945	α-fenchene	t	t	-	0.2
946	**camphene**	**1.0**	**1.2**	**0.3**	**0.3**
953	thuja-2,4-diene	t	-	-	-
961	**verbenene**	**-**	**-**	**0.7**	**0.3**
969	**sabinene**	**12.0**	**7.4**	**20.4**	**24.3**
974	β-pinene	0.4	0.2	0.7	0.5
988	myrcene	1.3	1.1	3.0	1.7
1001	**δ-2-carene**	**t**	**0.6**	**0.3**	**0.1**
1002	α-phellandrene	0.8	0.5	1.2	0.4
1008	**δ-3-carene**	**1.0**	**0.6**	**4.4**	**2.8**
1014	**α-terpinene**	**1.7**	**1.5**	**3.2**	**3.0**
1020	**p-cymene**	**10.7**	**20.0**	**5.5**	**6.5**
1024	limonene	0.9	0.7	0.7	1.6
1025	**β-phellandrene**	**3.5**	**2.0**	**6.7**	**1.5**
1044	(E)-β-ocimene	0.1	t	0.5	0.3
1054	γ-terpinene	3.0	2.5	5.3	4.9
1065	cis-sabinene hydrate	0.9	0.4	1.2	1.9
1086	terpinolene	1.3	1.4	2.4	1.9
1095	**trans-sabinene hydrate**	**0.7**	**t**	**t**	**1.8**
1095	**linalool**	**0.5**	**1.6**	**1.5**	**-**
1100	55,83,110,156, unknown	0.3	-	-	-
1112	trans-thujone	t	t	-	0.2
1118	cis-p-menth-2-en-1-ol	0.7	0.6	1.0	0.7
1136	trans-p-menth-2-en-1-ol	0.9	0.6	0.9	0.8
1141	camphor	2.5	1.3	t	1.2

		occid	occid.	occid	grandis
KI	Compound	Mc Art	shrub	Y Bol	Big Bear
1145	camphene hydrate	0.2	t	-	0.2
1154	sabina ketone	0.4	0.6	0.3	0.9
1165	**borneol**	**2.2**	**1.9**	**t**	**0.1**
1166	coahuilensol	0.6	0.7	2.4	-
1174	terpinen-4-ol	6.7	6.7	9.8	9.3
1179	p-cymen-8-ol	0.5	1.9	0.9	1.0
1186	α-terpineol	0.4	0.3	0.5	0.3
1195	**myrtenol**	**-**	**-**	**-**	**0.2**
1195	cis-piperitol	0.2	t	0.1	0.2
1207	trans-piperitol	0.3	t	0.5	0.6
1219	**coahuilensol, me-ether**	**1.1**	**0.6**	**2.7**	**-**
1223	**citronellol**	**8.4**	**-**	**-**	**0.2**
1230	trans-chrysanthenyl acetate	-	-	-	0.4
1238	cumin aldehyde	0.2	0.3	0.7	0.3
1249	piperitone	0.2	0.1	0.5	-
1253	trans-sabinene hydrate acetate	-	-	-	0.6
1254	linalool acetate	0.1	0.4	0.1	-
1257	methyl citronellate	-	-	-	0.1
1260	152,123,77,109, C10-OH	-	-	-	0.2
1284	**bornyl acetate**	**9.5**	**24.5**	**t**	**2.2**
1298	carvacrol	0.4	0.3	0.7	0.2
1322	methyl-geranate	1.0	0.5	0.8	1.8
1325	p-mentha-1,4-dien-7-ol	t	0.3	0.1	0.7
1345	α-cubebene	t	t	t	t
1374	**α-copaene**	**1.0**	**-**	**0.6**	**0.2**
1387	β-bourbonene	0.2	t	t	0.3
1388	79,43,91,180, unknown	-	-	0.1	-
1389	**111,81,151,182, unknown**	**-**	**-**	**0.1**	**0.4**
1417	(E)-caryophyllene	-	-	-	0.2
1429	cis-thujopsene	0.9	-	-	-
1430	β-copaene	-	-	-	t
1448	cis-muurola-3,5-diene	-	-	-	0.2
1451	trans-muurola-3,5-diene	0.1	t	0.1	-
1452	α-humulene	-	-	-	-
1465	cis-muurola-4,5-diene	0.1	t	t	0.1
1468	pinchotene acetate	0.6	0.6	2.0	-

KI	Compound	occid Mc Art	occid. shrub	occid Y Bol	grandis Big Bear
1471	121,105,180,208,phenol	-	-	-	0.3
1471	dauca-5,8-diene	-	-	-	0.2
1475	trans-cadina-1(6),4-diene	0.3	t	t	-
1478	γ-muurolene	0.8	0.4	0.1	0.2
1484	germacrene D	0.3	t	t	0.3
1491	43,207,161,222, C15-OH	-	-	-	0.3
1493	trans-muurola-4(14),5-diene	0.4	t	0.7	0.2
1493	epi-cubebol	0.4	t	0.4	0.5
1500	**α-muurolene**	**1.1**	**0.5**	**0.6**	**-**
1513	γ-cadinene	3.7	1.4	1.8	1.2
1518	epi-cubebol	0.4	0.4	t	1.5
1521	trans-calamenene	-	-	-	2.3
1522	**δ-cadinene**	**4.1**	**1.9**	**2.2**	**-**
1533	trans-cadina-1,4-diene	0.1	-	t	0.1
1537	α-cadinene	0.4	-	t	0.2
1544	α-calacorene	0.3	-	t	-
1548	elemol	-	0.4	-	0.9
1559	germacrene B	-		-	0.1
1561	1-nor-bourbonanone	-	-	-	1.1
1574	**germacrene-D-4-ol**	**0.6**	**t**	**0.5**	**-**
1582	caryophyllene oxide	-	-	-	0.3
1586	gleenol	0.3	t	t	-
1587	trans-muurol-5-en-4-α-ol	-	-	-	t
1607	β-oplopenone	0.4	t	0.4	0.8
1618	1,10-di-epi-cubenol	0.2	t	t	-
1627	1-epi-cubenol	1.6	0.7	1.3	0.5
1630	γ-eudesmol	-	t	-	t
1638	epi-α-cadinol	1.1	0.5	0.4	0.6
1638	epi-α-muurolol	1.2	0.5	0.6	0.6
1644	**α-muurolol**	**0.7**	**t**	**t**	**0.1**
1649	**β-eudesmol**	**-**	**0.9**	**-**	**0.2**
1652	α-eudesmol	-	-	-	0.6
1652	α-cadinol	1.8	1.0	0.8	0.7
1675	cadalene	0.3	t	t	0.1
1687	43,167,81,238, unknown	-	-	-	0.3
1739	oplopanone	-	-	-	0.2

		occid	occid.	occid	grandis
KI	Compound	Mc Art	shrub	Y Bol	Big Bear
1987	**manoyl oxide**	**3.2**	**3.0**	**1.0**	**t**
2009	epi-13-manoyl oxide	t	t	t	-

TAXONOMY OF THE *CROTON TEXENSIS* COMPLEX (EUPHORBIACEAE)

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

Croton texensis (Klotzch) Muell. Arg. is treated as having two allopatric, presumably intergrading, infraspecific categories: var. **texensis**, largely confined to the south-central U.S.A., and var. **utahensis** Cronq., confined to the southwestern U.S.A. and closely adjacent Mexico. A key to the taxa is provided, along with a map showing their distributions. *Phytologia 94(1): 35- 39 (April 2, 2012).*

KEY WORDS: Euphorbiaceae, *Croton, C. texensis*

CROTON TEXENSIS (Klotzsch) Muell. Arg., in DC. Prodr. 15(2): 692. 1866.
TYPE: "On the sand beaches of the Great Salt river, Arkansas," *Nuttall s.n.* (holotype G). The type of this name is probably from along the Salt Fork of the Arkansas River in north-central Oklahoma.

Hendecandra texensis Klotzsch, Wiegmann Archiv. Naturgeschichte 7: 252. 1841.
Type: TEXAS. *Drummond* (w/o additional data, Johnston 1959).

Croton muricatum Nutt., Trans. Amer. Phil. Soc. n.s. 5: 173. 1835 [non Vahl ex Geiseler, 1807].

Correll and Johnston (1970) provided a detailed description of *C. texensis* and this need not be enlarged upon here except to note that their descriptive account includes both of the varieties discussed herein, the two differing as follows:

1. Mature capsules to some extent warty, often markedly so, the warts usually tufted with stellate hairs; upper leaf surfaces usually densely stellate-pubescent; central U.S.A. from Nebraska and Wyoming to south-central Texas.....var. **texensis**
1. Mature capsules to some extent warty; upper leaf surfaces glabrous to moderately endowed with stellate hairs; Utah, Arizona, New Mexico, western Texas and adjacent Mexico.....var. **utahensis**

var. **texensis**

Var. *texensis* is an exceedingly common taxon, occurring predominantly in alluvial sands along streams or upon well-developed dunes. It is consistently reported as an erect annual herb to 1 m tall, becoming more robust as it approaches its more southern distribution. Correll and Johnston (1970) did note the capsules to be "usually slightly warty," but did not emphasize their distinctive presence, as done here. Var. *texensis* appears to intergrade with the more southwestern var. *utahensis* in northeastern New Mexico and the Panhandle region of Texas, hence their treatment as varieties.

Johnston (1959), in an excellent treatment of the complex, noted, "Many of the plants of the western portion of the range are of the form which has been called *C. virens* and *C. luteovirens*," but, Johnston thought these unworthy of recognition, at least at the specific level. He did, however, comment "*C. texensis* intergrades with *C. Parksii* (sic) and that the latter "might be reduced to synonymy under *C. texensis*, as has *C. virens*, or to varietal status." In my opinion, *C. parksii* is much better circumscribed by morphological characters than var. *utahensis*, but it does appear to grade into the var. *texensis*, but less perceptively so than does var. *utahensis*.

A single tetraploid chromosome count of n = 28 pairs has been reported for this taxon from the Panhandle of Texas (Randall Co., *Bacon & Hartman 1378*, TEX). Two diploid counts of n = 14 pairs have been reported for the var. *utahensis*, as noted below.

var. **utahensis** Cronq., Great Basin Naturalist 52: 76. 1992.

TYPE: **UTAH. Juab Co.:** sand dunes, ca 18 km airline N of Lynndyl, ca 1500 m, 28 Jul 1983, *Cronquist & Thorne 11839.* (holotype: US, isotype: UTC!).

Croton virens Muell. Arg., Linnaea 34: 142. 1865.
TYPE: El Paso Co.: "near El Paso," 1851-52, *Wright 1799* (B?) (Johnston 1959).

Croton luteovirens Woot. & Standl., Contr. U.S. Nat. Herb. 16: 145. 1913.
TYPE: New Mexico. Grant Co.: Rio Gila, 15 Aug 1902, *Wooton s.n.* (holotype US).

As noted by its creator (Cronquist 1992), this taxon differs from var. *texensis* only by having leaves with their upper surfaces glabrous, but he does note that some of these may have "at least a few stellate hairs (though these may eventually fall off)." He further states "The otherwise fairly widespread var. *texensis*, with the upper surfaces of the leaves evidently (and more or less persistently) stellate-hairy, is largely allopatric with var. *utahensis*, barely entering Utah in San Juan Co." Clearly Cronquist did not view *C. texensis* from throughout its range, confining his observations to Utah plants. At least he missed the warty capsules of var. *texensis*, as emphasized herein. A weak case might be made for the recognition of a var. *utahensis* with glabrous upper leaves, which grades into some newly created variety in the southwestern U.S.A. and Mexico, having pubescent leaves; however, leaf pubescence is so variable within the complex concerned, their seems little justification for such recognition (note that in the specimens cited below, Barkley collected, within the same population in Grant Co, N.M., both pubescent and markedly glabrous forms).

Occasional plants may be completely glabrous, or nearly so (e.g., *Barkley 14670B*, growing with typical form, *Barkley 14670*).

Two diploid counts of n = 14 pairs have been reported for this taxon (Eddy Co., N.M., *Hartman & Turner 3438*; and Samalayuca dunes, Chihuahua, Mex.; *Powell 2449;* vouchers at TEX).

REPRESENTATIVE SPECIMENS: **MEXICO: CHIHUAHUA.** Dune sands of Samalyaca (sic), 25 Oct 1966, *M.F. Roberty s.n.* (TEX); plus 15 or more collections from or near this site on file at LL-TEX.

SONORA. Mpio. de Guaymas: I km NE of Torim, Rio Yaqui valley, 10 m, 15 Dec 1988, *Felger 88-596* (TEX); S of Nogales "at km. 2354," 23 Jul 1959, *Gentry 17748* (LL); 6 mi S of Nogales, 7 Aug 1965 (TEX); Onavas, 10 Jul 1969, *Pennington 4* (TEX); Onavas, 550 ft, Aug 1968, *Pennington 335* (TEX); 1.5 km E of Tonichi, 180 m, 12 Feb 2991, *Van Devender 2001-54* (TEX); Colonia Morelos, 2600 ft, 24-27 Aug 1941, *White 4144* (LL); 10 mi E of Imuris, 7 May 1948, *Wiggins 11658* (TEX).

U.S.A.: ARIZONA. Apache Co.: Sanders, 5500 ft, 14 Aug 1973, *Moldenke 27730* (LL). **Gila Co.:** Pinal Mts., 6300 ft, 6 Aug 1968, *Gentry 2268* (TEX). **Maricopa Co.:** N of Winkelman, 5000 ft, 16 Aug 1973, *Keil 301* (TEX). **Navajo Co.:** "Mile-315 along US-40 east of Holbrook," 5400 ft, 25 Aug 1993, *Helmkamp 3-5* (TEX). **Pinal Co.:** Gila River at Kearny, 6 Jul 1968, *Crutchfield 3272* (TEX). **Santa Cruz Co.:** Nogales, 28 Jun 1067, *Moldenke 1858* (LL).

NEW MEXICO: Catron Co.: 4 mi W of Alma, 5295 ft, 5 Jul 1948, *Doolittle 11* (TEX). **Chaves Co.:** 30 mi E of Roswell, 2 Aug 1960, *Barkley & Davidson 866* (LL). **Dona Ana Co.:** sandy soil near El Paso, Tex, 20 Jul 1946, *Tharp et al. 36302* (TEX). **Eddy Co.:** 10 mi E of Loving, 20 Jul 1972, *Hartman 3438* (TEX). **Grant Co.:** 16 mi WNW Silver City, 24 Sep 1944, *Barkley 14644B* (TEX). **Guadalupe Co.:** Santa Rosa, 3 Sep 1929, *Whitehouse s.n.* (TEX). **Rio Arriba Co.:** Ojo Caliente, 23 Aug 1963, *Brown s.n.* (TEX). **Santa Fe Co.:** Sangre de Cristo Mts., 7200 ft, 14 Aug 1963, *Bennett 8267* (TEX).

As an aside, I am pleased to note that a plant from Chihuahua (Samalayuca dunes, cf. above citation), reported by Henrickson (2010) as possibly *C. bigbendensis* B.L. Turner, this disputed by Turner (2011), is in fact *C. t.* var. *utahensis*.

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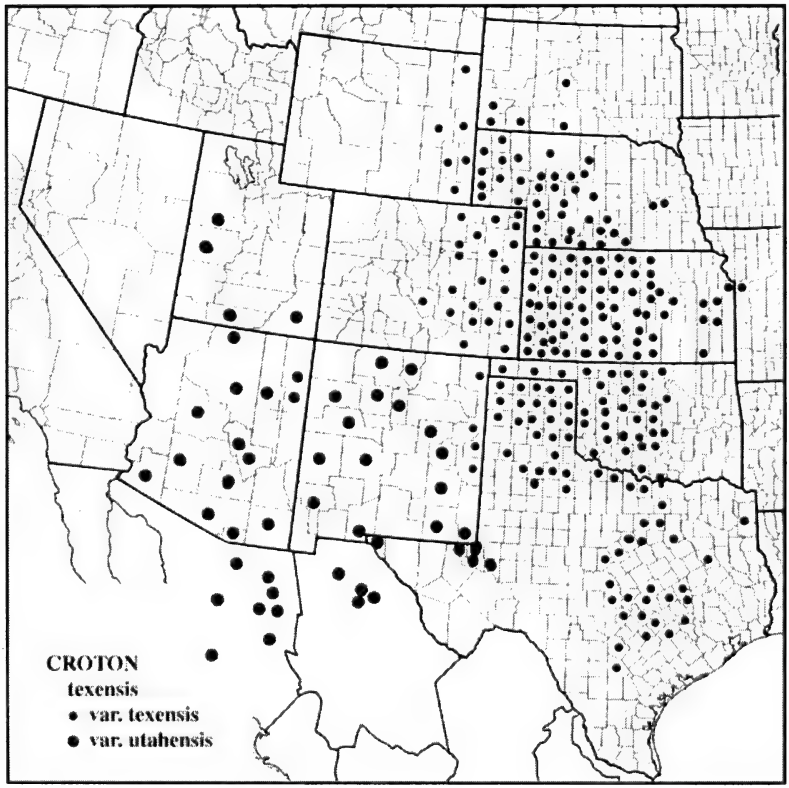

Fig. 1. Distribution of *Croton texensis*.

VARIATION IN LEAF ESSENTIAL OILS, DNA SEQUENCES AND MOPHOLOGY IN *JUNIPERUS DURANGENSIS*

Robert P. Adams
Biology Department, Baylor University, Waco, TX 76798, USA
Robert_Adams@baylor.edu

M. Socorro Gonzaléz Elizondo
CIIDIR Unidad Durango, Instituto Politecnico Nacional,
Sigma 119, Fracc. 20 de Noviembre II, Durango, Dgo., 34220 Mexico

and

Thomas A. Zanoni
New York Botanical Garden, 200th Street at Southern Blvd.
Bronx, NY 10458-5126

ABSTRACT

The leaf essential oil of *J. durangensis* is dominated by α-pinene (57.7%) and δ-3-carene (14.2%) with moderate amounts of verbenene, β-pinene, myrcene, limonene, β-phellandrene, terpinolene, linalool and elemol. The oil of the multi-seeded (5-9) Topia plants is similar to typical *J. durangensis*, except differing by containing several compounds not found in other *J. durangensis* oils: 1,8-cineole (2.8%, trace in other oils), cis-p-menth-2,8-dien-1-ol, germacrene B, patchouli alcohol, hexadecanol and sandaracopimarinal. The multi-seeded Topia junipers are recognized as a new variety, ***Juniperus durangensis*** var. ***topiensis*** R. P. Adams and S. Gonzalez, **var. nov**. *Phytologia 94(1): 40-52 (April 2, 2012).*

KEY WORDS: *Juniperus durangensis*, *J. durangensis var. topiensis,* terpenes, nrDNA, petN-psbM, trnD-trnT, trnL-trnF, trnS-trnG, SNPs, Cupressaceae, geographic variation.

Juniperus durangensis is in the serrate leaf margined junipers and appears to be most closely related to *J. martinezii* Peréz de la Rosa (Fig. 1). *Juniperus durangensis* Mart. is a tree or large shrub to 5 m that generally branches near the base and its seed cones contain 1-3(4) seeds (Adams, 2011).

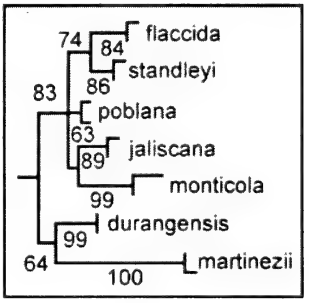

Figure 1. Clade from the serrate leaf margined junipers, from Adams and Schwarzbach (2011) showing the putative relationship of *J. durangensis* to *J. martinezii*.

It is often found on rhyolite, a nutrient poor rocky volcanic substrate, in Sierra Madre Occidental, a mountain range of western Mexico from Sonora and Chihuahua southward to Aguascalientes (Fig. 2). Adams (2009) reported DNA analysis of the multi-seeded (5-9 seeds), shrubs of *J. durangensis* growing near Topia, Durango. He considered those plants as conspecific with *J. durangensis*, differing by only 2 SNPs (in nrDNA and trnC-trnD). However, a subsequent visit to Topia, revealed that there are important differences between these plants and typical *J. durangensis*.

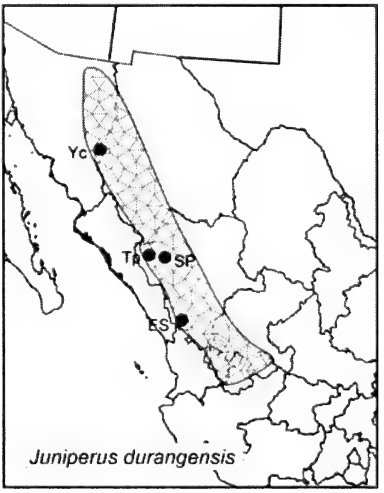

Figure 2. Distribution of *J. durangensis* with sampled areas.

Particularly noticeable is the large number of seeds per cone (Table 1) and multi-stemmed branching shrub habit in the Topia plants (Fig. 3). In addition, the plants were growing on lower elevations on the western flank of the Sierra Madre Occidental on tuffs (compacted volcanic ash or dust), not on rhyolite. Due to the morphological and edaphic differences seen at Topia, the previous study was expanded to additional populations of *J durangensis* and additional characters (leaf

essential oils, additional gene sequences and morphological data). These data are reported in this paper.

Table 1. Comparison of seeds/cone and habit in *J. durangensis*.

	seeds/cone	habit
Typical	1-3 (4)	small tree/shrub
Topia Tp, (Fig. 2)	(3) 5-9, 6.5 avg.	shrub
Santiago Papasquiraro(SP)	1.7 - 5 very variable	small tree/shrub

Figure 3. *J. durangensis* shrub habit growing at Topia.

MATERIALS AND METHODS

Specimens collected: *J. durangensis*, *Adams 8464, 8466, 8468,* 3 km sw of Yecora, 28° 22' N, 108° 57' W, 1570 m, Sonora, *Adams 6832-6834*, 52 km (road) w of El Salto, on Mex 40, 23° 41' N, 105° 44' W, 2700 m, Durango, MX; *Adams 11922, 11929, 11930*, 80 km (air), 137 km (road) east of Topia, 38 km (air), 116 km (road) w of Santiago Papasquiraro, 25° 03' 25.5" N, 105° 47' 45.2" W, 2670 m, Durango, *Adams 11923-25*, Topia, 25° 12.894' N, 106° 33.891' W, 1840 m, Durango, MX; *J. martinezii, Adams 5950, 5951, 8709,* 42 km n of Lagos de Moreno on Mex 80, thence east 10 km on road to La Quebrada Ranch, 21° 36' N, 101° 36' W, 2985 m, Jalisco, MX. Voucher specimens are deposited at BAYLU.

Analysis of oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type 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 (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

DNA Analysis - 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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM, trnDT, trnSG) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams and Schwarzbach (2011) for the ITS, petN-psbM, trn D-trnT and trnS-trnG primers utilized. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The composition of the leaf essential oil of *J. durangensis* from west of El Salto is very similar to the previous report (Adams et al. 1985) being dominated by α-pinene (57.7%) and δ-3-carene (14.2%) with moderate amounts of verbenene, β-pinene, myrcene, limonene, β-phellandrene, terpinolene, linalool and elemol. Several previously unknown compounds have now been identified. The oil of the Topia plants is also high in α-pinene (54.6%) and δ-3-carene (15.8%) with moderate amounts of β-pinene, myrcene, limonene, β-phellandrene, terpinolene and (E)-caryophyllene. In addition, its oil contains several compounds not found in other *J. durangensis* oils: 1,8-cineole (2.8%, trace in other oils), cis-p-menth-2,8-dien-1-ol, germacrene B, patchouli alcohol, hexadecanol and sandaracopimarinal (Table 2). The oils from the population east of Topia and Yecora are more similar to typical *J. durangensis* from El Salto, then to the Topia oil (Table 2), but they are much lower in δ-3-carene (7.1, 4.3%). An unusual aspect of the oils of *J. durangensis* is the number of components present in low concentrations that vary from present to absent across the populations. Some of these may due to enzyme non-specificity and/or free radical reactions. The differentiation of the Topia plants' oil is about the level one would expect in different varieties of *Juniperus*.

Sequencing nrDNA resulted in 1272 bp of aligned sequences which contained 9 single mutations and 10 multiple occurring mutations (informative). A minimum spanning network base on these 10 informative SNPs (Fig. 4) shows the separation of the closely related *J. martinezii,* but no grouping among the populations of *J. durangensis*.

Sequencing trnL-trnF (cpDNA) resulted in 695 bp of identical aligned sequences. Sequencing trnD-trnT (cpDNA) resulted in 656 bp of identical aligned sequences. Sequencing trnS-trnG (cpDNA) resulted in 818 bp of aligned sequences with only one SNP that separated *J. martinezii* from all *J. durangensis* samples.

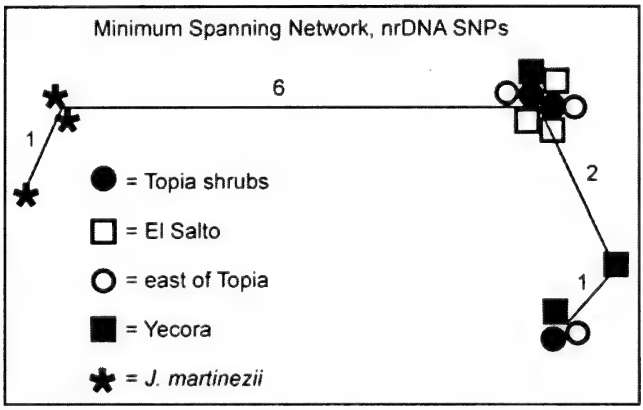

Figure 4. Minimum spanning network based on 10 SNPs from nrDNA. The numbers next to the links are the number of SNPs.

Sequencing petN-psbM yielded 847 bp of aligned sequences with 9 SNPs. Figure 5 shows that *J. martinezii* is separated by 6 SNPs from *J. durangensis.* The Topia plants are separated by 3 SNPs from other *J. durangensis* plants.

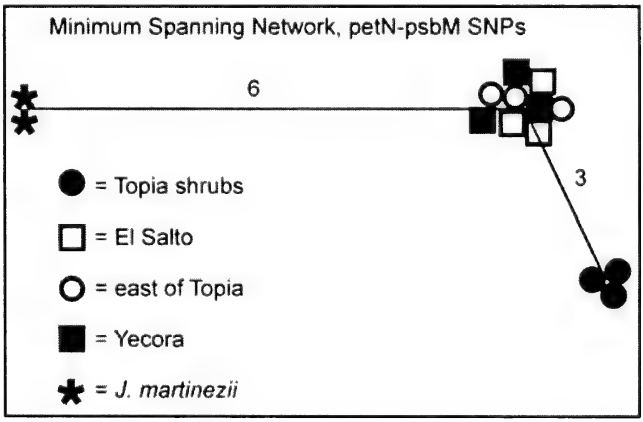

Figure 5. Minimum spanning network based on 9 SNPs found in petN-psbM (cpDNA).

Clearly the Topia junipers are closely related to typical *J. durangensis*. However, considering its much more shrubby habit, the difference in the number of seeds/ cone (considered one of the most important taxonomic characters in *Juniperus*), and its different habitat at Topia, it seems appropriate to recognize the junipers at Topia as a variety of *J. durangensis*:

Juniperus durangensis var. **topiensis** R. P. Adams & S. Gonzaléz, **var. nov**. TYPE: Mexico, Durango, Topia, 25° 12.894' N, 106° 33.891' W, 1840 m, *Adams 11923.*
(HOLOTYPE: BAYLU)., Fig. 6.

Junipero durangensi similis sed differt strobilis seminibus 5-9, et habitu fruticoso parvo multiramoso et ramulis laxiusculis.

Similar to *Juniperus durangensis*, but differing in having 5-9 seeds per cone and being a small, multi-branched shrub with less crowded branchlets.

Juniperus durangensis var. *topiensis* is currently known only from the type locality where, it is common on hillsides around Topia at about 1800-1900 m.

Other specimens studied: TOPOTYPES: *Adams 11924, 11925,* BAYLU, *S. Gonzaléz 7268a, 7268b*, BAYLU, CIIDIR, others to be distributed).

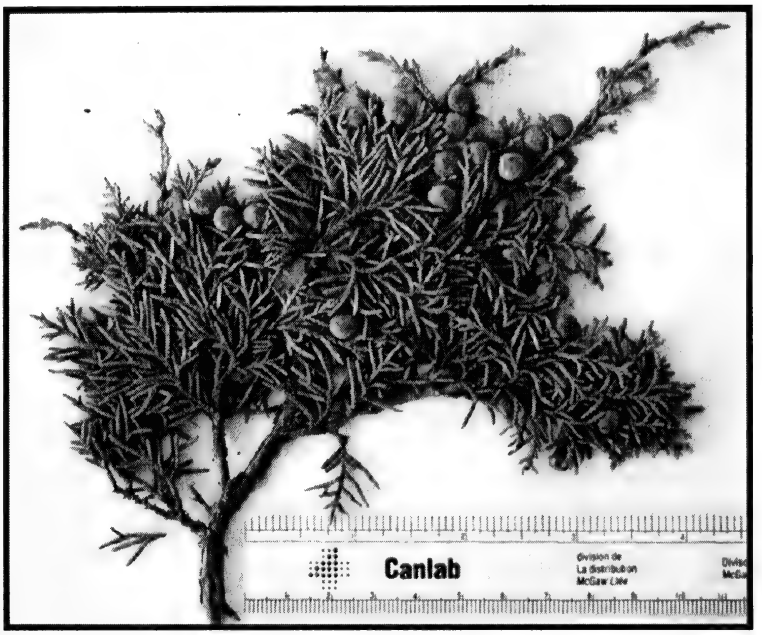

Figure 6. Holotype of *J. durangensis* var. *topiensis*.

ACKNOWLEDGEMENTS

Thanks to Guy Nesom for the Latin description and Andrea Schwarzbach, and Billie Turner for manuscript reviews. Thanks to Tonya Yanke for lab assistance and to Martha González-Elizondo for field work and helpful discussions on the new taxon. This research was supported in part with funds from Baylor University.

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Table 2. Leaf essential oil composition of populations of *J. durangensis*: west of El Salto (w El Salto), Topia shrubs, east of Topia, and Yecora. Compounds in boldface appear to separate taxa and were used in numerical analyses. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. For unknown compounds, four ions are listed, with the largest ion underlined.

KI	Compound	w El Salto	Topia shrubs	east of Topia	Yecora
921	tricyclene	0.2	0.1	0.1	0.1
924	α-thujene	t	t	-	t
932	α-pinene	57.7	54.6	54.3	58.8
945	α-fenchene	0.7	0.6	0.3	0.2
946	camphene	0.6	0.8	0.5	0.7
953	thuja-2,4-diene	t	0.1	0.1	t
961	**verbenene**	**1.6**	**0.3**	**1.7**	**4.0**
969	sabinene	t	t	t	t
974	β-pinene	1.7	2.7	2.2	2.9
988	myrcene	2.3	4.1	3.0	3.9
1002	α-phellandrene	t	t	t	0.1
1008	**δ-3-carene**	**14.2**	**15.8**	**7.1**	**4.3**
1014	α-terpinene	t	t	t	t
1020	p-cymene	0.2	t	0.3	0.3
1024	sylvestrene	t	t	t	t
1024	limonene	1.6	2.8	1.4	1.4
1025	β-phellandrene	1.7	2.8	1.3	1.4
1026	**1,8-cineole**	**t**	**2.8**	**t**	**t**
1044	(E)-β-ocimene	t	t	0.5	0.7
1054	γ-terpinene	0.2	0.1	0.2	0.2
1085	p-mentha-2,4(8)-diene	t	t	t	t
1086	terpinolene	1.8	2.1	0.8	1.1
1087	**p-cymenene**	**0.1**	**t**	**0.8**	**0.2**
1094	**unknown, 96, 109, 137, 152**	**0.6**	**t**	**1.4**	**0.2**
1098	**linalool**	**1.1**	**t**	**0.2**	**0.4**
1100	n-nonanal	-	0.1	0.1	0.1
1102	isopentyl-isovalerate	-	-	t	-
1113	endo-fenchol	0.1	t	t	-

KI	Compound	w El Salto	Topia shrubs	east of Topia	Yecora
1113	3-methyl-3-buten-methyl-butanoate	-	-	0.3	-
1122	α-campholenal	0.2	0.2	0.4	0.2
1135	trans-pinocarveol	0.3	0.2	0.5	0.2
1133	**cis-p-menth-2,8-dien-1-ol**	-	**t**	-	-
1137	cis-verbenol	-	-	t	-
1140	trans-verbenol	-	t	0.6	t
1141	camphor	0.3	0.1	-	0.4
1144	neo, iso-pulegol	0.7	-	-	-
1145	camphene hydrate	-	t	-	0.1
1148	citronellal	0.1	-	-	-
1154	karahanaenone	0.2	-	-	-
1155	iso, iso-pulegol	0.2	-	-	-
1158	trans-pinocamphone	-	t	t	t
1160	pinocarvone	t	-	t	-
1165	borneol	0.2	-	-	-
1166	δ-terpineol	0.1	-	-	-
1166	p-mentha-1,5-dien-8-ol	-	-	0.4	0.2
1172	cis-pinocamphone	t	t	t	0.1
1174	terpinen-4-ol	0.4	t	0.1	0.1
1178	naphthalene	-	0.1	t	t
1179	p-cymen-8-ol	0.1	t	0.2	t
1186	α-terpineol	0.2	0.2	0.3	0.1
1195	myrtenol	t	t	0.1	0.1
1195	methyl chavicol	-	-	0.3	0.1
1204	verbenone	0.2	0.1	0.3	0.1
1215	trans-carveol	t	t	t	-
1218	endo-fenchyl acetate	-	0.1	0.2	0.1
1223	citronellol	0.1	-	-	-
1232	thymol, methyl ether	t	-	0.8	t
1235	trans-chrysanthenyl acetate	-	-	-	1.2
1241	carvacrol, methyl ether	-	-	0.2	-
1254	linalyl acetate	-	t	-	t
1257	methyl citronellate	0.7	t	0.4	t
1274	pregeijerene B	-	-	-	t
1274	neo, iso-pulegol	-	-	0.2	-
1283	iso, iso-pulegol	-	-	t	t
1287	bornyl acetate	0.2	0.4	0.4	1.8

KI	Compound	w El Salto	Topia shrubs	east of Topia	Yecora
1298	trans-pinocarvyl acetate	-	-	t	-
1322	methyl geranate	0.1	0.2	-	-
1324	myrtenyl acetate	-	-	0.1	0.7
1326	unknown, 43, 92, 119, 152	-	-	-	0.7
1345	α-cubebene	-	-	0.1	0.2
1346	α-terpinyl acetate	-	-	-	t
1403	methyl eugenol	-	0.1	-	0.4
1407	longifolene	t	-	-	-
1417	(E)-caryophyllene	0.9	1.6	0.3	0.9
1448	cis-muurola-3,5-diene	t	-	-	-
1451	trans-muurola-3,5-diene	t	-	-	0.3
1452	α-humulene	0.7	t	-	0.4
1461	cis-cadina-1(6),4-diene	t	-	-	-
1465	cis-muurola-4(14),5-diene	t	-	-	-
1475	trans-cadina-1(6),4-diene	t	t	-	0.4
1478	γ-muurolene	-	t	t	-
1480	germacrene D	0.8	0.6	-	0.4
1493	trans-muurola-4(14),5-diene	0.1	t	-	0.7
1493	epi-cubebol	t	t	-	0.4
1500	α-muurolene	t	t	t	0.2
1513	β-curcumene	0.1	-	-	-
1513	γ-cadinene	0.4.	0.2	t	0.6
1513	endo-1-burbonanol	0.4	0.2	-	-
1513	cubebol	-	-	-	0.5
1514	trans-calamenene	t	-	-	0.5
1522	δ-cadinene	0.8	0.5	0.5	0.6
1528	zonarene	-	-	-	0.2
1544	α-calacorene	-	-	-	0.3
1548	elemol	1.0	0.3	4.3	0.1
1555	elemicin	-	-	t	-
1559	**germacrene B**	-	**0.3**	-	-
1561	(E)-nerolidol	-	-	-	0.1
1574	germacrene-D-4-ol	-	t	0.2	0.1
1582	caryophyllene oxide	0.1	0.6	0.2	1.1
1607	β-oplopenone	-	-	t	-
1608	humulene epoxide II	0.1	-	-	0.4
1627	1-epi-cubenol	0.2	0.7	-	1.0
1630	γ-eudesmol	-	t	1.6	-

KI	Compound	w El Salto	Topia shrubs	east of Topia	Yecora
1638	epi-α-cadinol	0.2	t	t	0.3
1638	epi-α-muurolol	0.3	t	t	0.4
1644	α-muurolol	t	t	t	t
1649	β-eudesmol	t	0.3	2.1	t
1652	α-eudesmol	t	0.2	1.3	-
1652	α-cadinol	0.6	0.3	1.2	0.5
1658	**patchouli alcohol**	**-**	**0.1**	**-**	**-**
1670	bulnesol	-	-	0.5	-
1688	shyobunol	t	0.6	0.2	0.1
1874	**hexadecanol**	**-**	**0.1**	**-**	**-**
1978	manoyl oxide	0.2	0.3	0.2	0.4
2026	geranyl linalool	0.2	-	-	-
2055	abietatriene	0.1	0.2	t	0.3
2184	**sandaracopimarinal**	**-**	**0.1**	**-**	**-**
2298	4-epi-abietal	0.2	-	0.2	0.2

GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *TAXODIUM* (CUPRESSACEAE)

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

Michael A. Arnold and Andrew R. King
Department of Horticultural Sciences, TAMU 2133
Texas A & M University, College Station, TX 77843-2133

and

Geoffrey C. Denny
G. C. Denny Horticulture, 12905 Co. Rd. 675, Parrish, FL 34219

ABSTRACT

Volatile leaf oils of *Taxodium distichum*, *T. d.* var. *imbricarium* and *T. mucronatum* were found to be high in α-pinene (27-83%), except from the Texas Hill country, where the oil was lower in α-pinene (5-17%) and very high in β-phellandrene (59-78%). Both *T. d.* var. *distichum* and var. *imbricarium* appear to have chemical races: high/low α-pinene and low/high limonene/β-phellandrene. Based on the leaf terpenoids, *T. mucronatum* in Mexico appears to have two variants: Durango and Oaxaca-Guatemala. The oils of *T. distichum* have two regional groups: South Central USA and Texas Hill Country - Rio Grande Valley. The oil of the putative *T. mucronatum* from Bolleros, MX was found to be more similar to *T. distichum* than to any *T. mucronatum* in the study. Putative *T. d.* var. *imbricarium* from Fowl River, AL was found to have oil like *T. d.* var. *distichum*, not like *T. d.* var. *imbricarium* from Tampa, FL. *Phytologia 94(1): 53-70 (April 2, 2012).*

KEY WORDS: *Taxodium distichum*, *T. d.* var. *imbricarium* (*T. ascendens*), *T. mucronatum*, terpenes, leaf essential oil composition, geographic variation.

Taxodium Rich. is a small genus with one to three species. Britton (1926), Dallimore & Jackson (1966) and Rehder (1940) all recognized three species: bald cypress, *T. distichum* (L.) Rich., pond cypress, *T. ascendens* Brongn. and Montezuma or Mexican bald cypress, *T. mucronatum* Ten. Watson (1985) treated *T. ascendens* as *T. d.* var. *imbricarium* (Nutt.) Croom. Both Farjon (2005) and Eckenwalder (2009) recognized *T. distichum*, *T. d.* var. *imbricarium* and *T. mucronatum*. Denny (2007) treated the genus as monotypic with one species, *T. distichum* and two varieties: var. *imbricarium* and var. *mexicanum* (Carr.) Gord. (= *T. mucronatum*). Denny (2007) and Denny and Arnold (2007) give a lucid discussion of the historical nomenclature of the genus.

There has been surprisingly little research on the leaf essential oils of *Taxodium*. A very early paper on the volatile oil from the seed cones of *T. distichum* (Odell, 1912) reported dextro pinene (85%), dextro limonene (5%), a "pseudo terpene alcohol (2%), carvone (3%) and a tricyclic sesquiterpene (3%) with a yield of 1-2%. Flamini et al. (2000) examined the essential oils from seed cones, leaves and branches from cultivated trees of *T. distichum* in North Tuscany, Italy. They found the oils from seed cones, leaves and branches were dominated by α-pinene (71.3, 79.9, 57.3%, respectively). The oils shared most components, but the seed cones were higher in limonene (18.7%). El Taunawy et al. (1999) reported that essential oil from seed cones of *T. distichum* grown in Giza, Egypt contained α-pinene (87.3%), camphene (1.0%), β-pinene (1.7%), myrcene (2.0%), limonene (1.3%) and thujopsene (3.7%) with 38 trace components. Ogunwande et al. (2007), in a study of cytotoxic effects of *T. distichum* seed cone and leaf oils from a tree cultivated in Ibaden, Nigeria, reported 60.5% α-pinene, 17.6% thujopsene, and 29 other compounds in seed cones, but only 0.9% α-pinene in leaves! In fact, α-pinene accounted for nearly all the monoterpene fraction.

No study of leaf oils of *Taxodium* from native materials in the United States, Mexico and Guatemala was found in the literature. The purpose of this study was to report on leaf essential oils of *Taxodium distichum*, *T. d.* var. *imbricarium* and *T. mucronatum* and examine geographic variation in these oils.

MATERIALS AND METHODS

Plant material: Seeds were collected by Denny (2007) in late summer and fall, 2003 and germinated and grown in containers in spring, 2004. Subsequently, seedlings were transplanted to the Texas A & M University Horticulture Farm, College Station, Texas (30°37'38.222" N, 96°22'18.505" W). The site soil was a Boonville Series, fine, smectitic, thermic chromic vertic albaqualf, with a mean soil pH of 6.4. From the 22 *Taxodium* collection sources, 9 were sampled for this study (Fig. 1) as follows:

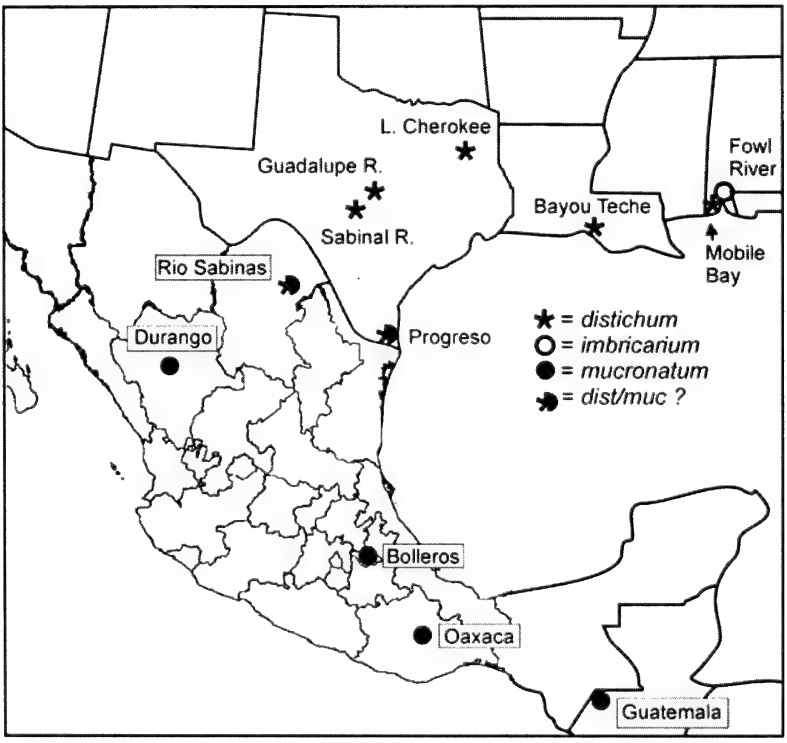

Figure 1. Locations of populations sampled for this study. Sites at Tampa, FL not shown, see text for details.

T. mucronatum: Rio Nazas, Durango, MX, MX2M, 25° 18' 36" N, 104° 38' 24" W, *Adams 12833-12837*; Rio Sabinas, Coah., MX, *Adams 13039-13043*, Bolleros, DF, MX, MX3M, 19° 30' 0" N, 98° 54' 36" W, *Adams 12838-12842;* Progreso, TX, MX5M, 26° 4' 12" N, 97° 54' 36" W, *Adams 12843-12847.*

T. distichum: Guadalupe River, TX, TX2D, 30° 4' 12" N, 99° 17' 24" W, *Adams 12848-12852*; Sabinal River, TX, TX5D, 29° 9' 36" N, 99° 28' 12" W, *Adams 12853-12857;* Lake Cherokee, TX, EP1D, 32° 20' 24" N, 94° 42' 0" W, *Adams 12858-12862;* Bayou Teche, LA, EP3D, 29° 5' 24" N, 91° 12' 6" W, *Adams 12863-12867*; Mobile Bay, AL, EP4D, 30° 36' 0" N, 87° 54' 36" W, *Adams 12868-12872.*

T. distichum var. *imbricarium*: Fowl River, AL, EP5I, 30° 27' 0" N, 99° 06' 36" W, *Adams 12873-12877.*

In addition, samples were collected from natural populations as follow:

T. mucronatum: KM 295 on Pan-American Highway, 42 km S of Guatemala-Mexico border, Guatemala, 15° 24' 05" N, 91° 41' 33" W, *Adams 6857*, Rio del Oro, 36 km S of Oaxaca on hwy 190, Oaxaca, MX, 16° 55' 52" N, 96° 25' 31" W, *Adams 6873*;

T. distichum: Hillsborough River, Hillsborough Co., FL, 28° 09' 05.15" N, 82° 13' 37.24" W, *Adams 12828*, Hillsborough River, Hillsborough Co., FL, 28° 01.164' N, 82° 27.881' W, *Adams 12829-12830,* Hillsborough River, Hillsborough Co., FL, 27° 59.796' N, 82° 28.010' W, *Adams 12831-12832*;

T. d. var. *imbricarium*: edge of swamp, Hillsborough Co., FL, 28° 11' 39.80" N, 82° 30' 54.09" W, *Adams 12823-12827.* Voucher specimens are deposited in the Herbarium, Texas A & M University (for plot materials) and Baylor University (for Adams field collected materials).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). Oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. Extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. Oils were analyzed on a

HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as percent total oil) were coded and compared among the taxa by the Gower metric Gower (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The volatile leaf oils of *Taxodium distichum*, *T. d.* var. *imbricarium* and *T. mucronatum* (Table 1) were found to be high in α-pinene (27-83%), except from the Texas Hill Country, where the oil was lower in α-pinene (5-17%) and very high in β-phellandrene (59-78%). The leaf terpenoids of *T. mucronatum* in Mexico appears to be of two geographic variants: Durango and Oaxaca-Guatemala, with the oil from Durango being very distinct (Table 1). Population samples from Bolleros, Durango, Progresso, Sabinal River, Guadalupe River and Fowl River had very little variation. Therefore, average component values were used for these populations in the numerical analyses. Both *T. d.* var. *distichum* and var. *imbricarium* (FL) appear to have chemical races based on high/low α-pinene and low/high limonene/β-phellandrene (LMNN/BPHL).

PCO analysis revealed (Fig. 2) that the oils of *T. distichum* appear to be composed of two regional groups: South Central USA and Texas Hill Country - RGV (Rio Grande Valley). Putative *T. mucronatum* from Bolleros, MX is more similar to *T. distichum* than to any *T. mucronatum* in the study. Rio Sabinas samples consisted of four low α-pinene samples that were very similar to the Hill Country/ RVG

samples and one high α-pinene tree that has affinities to the Durango and Bolleros trees (Fig. 2). Guatemala - Oaxaca plants form a group (Fig. 2). Durango trees have the most distinct oil (Table 2, Fig. 2).

The low α-pinene trees from the South Central USA cluster with Texas Hill Country - RGV trees (Fig. 2), but have a link to the high α-pinene South Central USA plants. It may be that these low α-pinene trees represent a genotype transplanted from the Hill Country, introgression or a chemical polymorphism. The Progresso, TX and Rio Sabinas (low α-pinene) trees cluster with the Texas Hill country trees (Sabinal R., TX; Guadalupe R.) and not with any *T. mucronatum* in the study (Fig. 2).

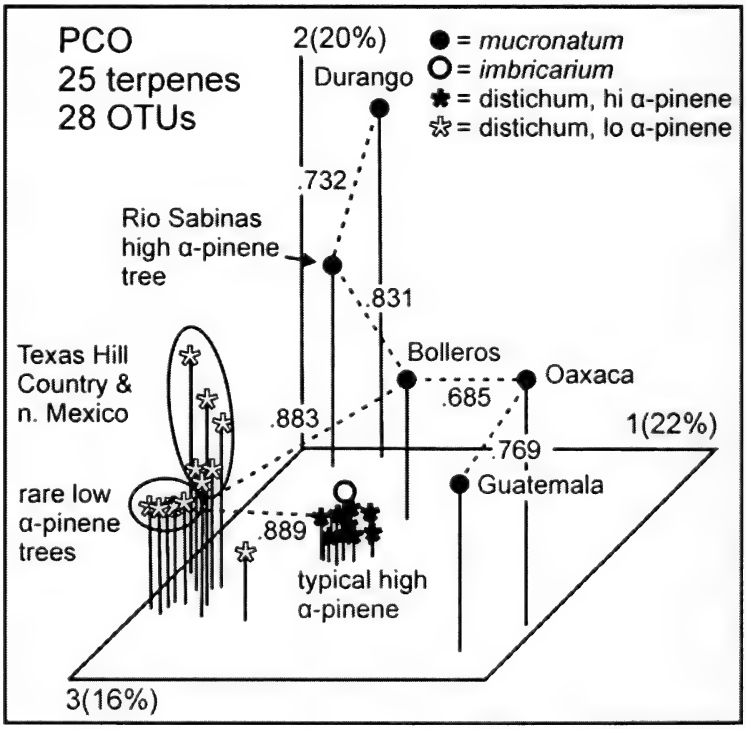

Figure 2. PCO based on 25 terpenes. Numbers next to dotted lines (minimum links) are similarities.

The imbricate leafed trees (cf. *T. distichum* var. *imbricarium*) from Fowl River, AL, have a leaf oil like that of *T. d.* var. *distichum* (Fig. 2).

To supplement the aforementioned samples, additional materials of *T. d.* var. *imbricarium* and *T. d.* var. *distichum* were collected from near Tampa, FL. Surprisingly, the oils of var. *imbricarium* contained both hi- and low α-pinene types (Table 2). A minimum spanning network without the intermediate oils, shows that the oils of var. *imbricarium* (FL) have a distinctive terpene pattern (Fig. 3, Table 1, see germacrene D, α-muurolene, δ-cadinene, α-cadinol). However, *imbricarium* (AL) is essentially the same as var. *distichum* from the South Central USA.

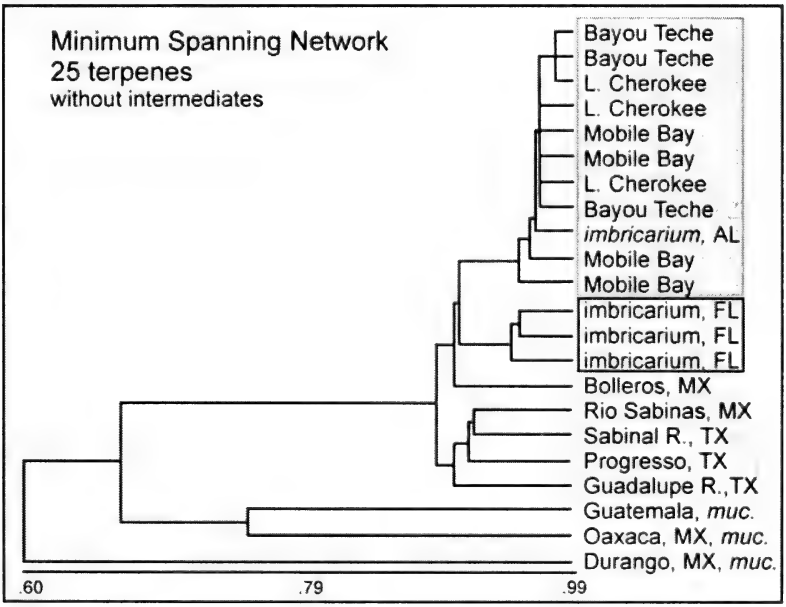

Figure 3. Minimum spanning network showing the placement of var. *imbricarium*, FL.

PCO of these 22 OTUs (Fig. 4) shows that the oils of the Florida var. *imbricarium* trees are most similar to var. *distichum* from

the South Central USA. The Texas Hill Country-n Mexico oils are quite distinct, as are the Durango and Guatemala-Oaxaca oils (Fig. 4).

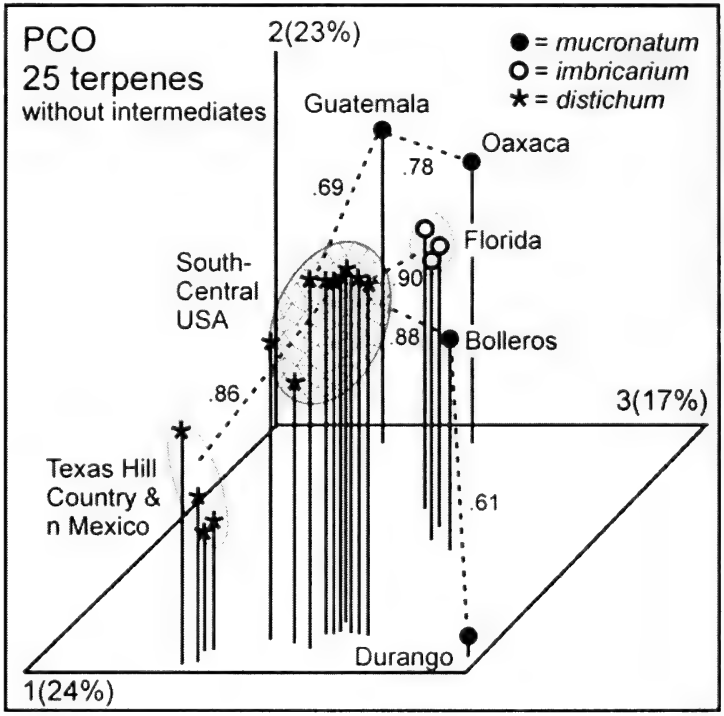

Figure 4. PCO of 22 OTUs based on 25 terpenes. Note the linkage of var. *imbricarium*, Florida with the South Central USA, var. *distichum* trees.

All of the trees analyzed in the Texas Hill Country-RGV-Rio Sabinas populations (20 trees) were very uniform and low in α-pinene, except one unusual individual at Rio Sabinas. However, all the Central South region populations contained mostly high α-pinene trees with one or two low α-pinene trees (Fig. 5). PCO of these individual *T. distichum* trees (Fig. 5) clearly separates low and high α-pinene trees (axis 1, 40%). Four low α-pinene individuals [Lake Cherokee (2), Bayou Teche (1), Mobile Bay (1)] cluster with typical Texas Hill Country - n Mexico trees (Fig. 5). No intermediate oil types are seen in

Fig. 5, suggesting that these individuals are not hybrids or introgressants, but more likely a chemical polymorphism. Additional sampling will be necessary to fully address this problem.

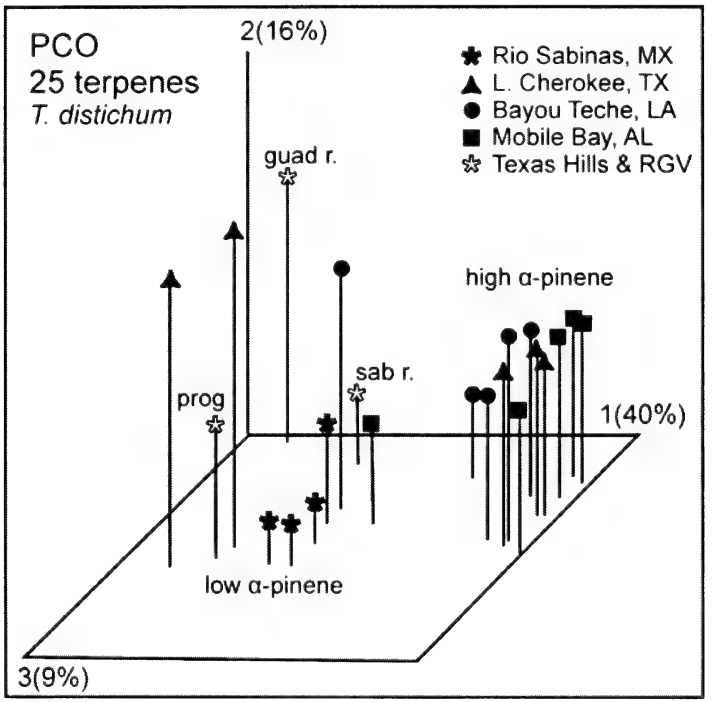

Fig. 5. PCO ordination of individuals of *T. distichum* based on 25 terpenes.

The *Taxodium* from near Tampa, Florida present an interesting problem. *Taxodium d.* var. *imbricarium* has adult foliage with imbricate leaves. Examination of trees on the edge of a swamp north of Tampa revealed that young trees first appear to produce alternate, lanceolate leaves, then produce adult, imbricate leaves. Many of the putative *T. d.* var. *distichum* trees, growing in the river rapids and at streamside on the Hillsborough River, had typical alternate, lanceolate leaves, but some trees had imbricate leaves. This heteroblastic change has also been observed during nursery production of *T. d.* var.

imbricarium seedlings. It appears that var. *distichum* and *T. mucronatum* are fixed in neoteny for the alternate, lanceolate leaves. This is similar to the situation in *Juniperus saxicola* Britt. and P. Wilson that is fixed for juvenile (whip or decurrent) leaves, as opposed to having the adult, scale-like leaves found on the adult foliage of the other *Juniperus* L. in section Sabina (Adams, 2011).

PCO analysis of var. *imbricarium* (Florida), var. *distichum* (Florida) and other var. *distichum* (AL and LA) shows (Fig. 6) the distinctness of var. *imbricarium* (FL) from var. *distichum*. In addition,

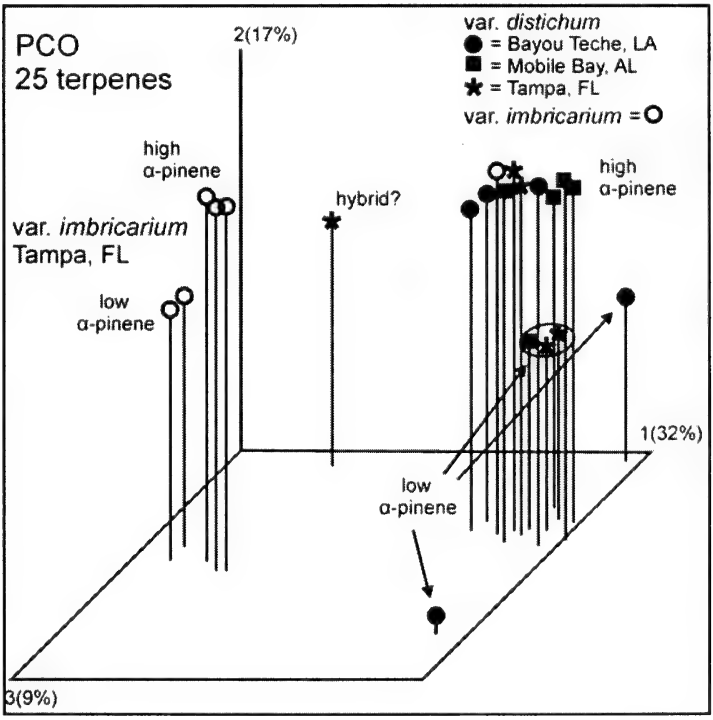

Figure 6. PCO of var. *imbricarium* (Florida) and var. *distichum* plants.

high/ low α-pinene var. *imbricarium* form a loose cluster, as do high/ low α-pinene var. *distichum* plants. Two trees from the Bayou TecheTeche population with low α-pinene are ordinated as outliers. These may be just trees with unusual oils. In addition, one of the var. *distichum*, Tampa, FL ordinates in a hybrid position midway between var. *distichum* and var. *imbricarium*.

Contouring the clustering order reveals (Fig. 7) the South Central USA, *T. distichum* group, the Texas Hill Country-northen Mexico group and the affinity of the Bolleros, MX population to *T. distichum*. These data suggest that *T. distichum* may extend into eastern Mexico.

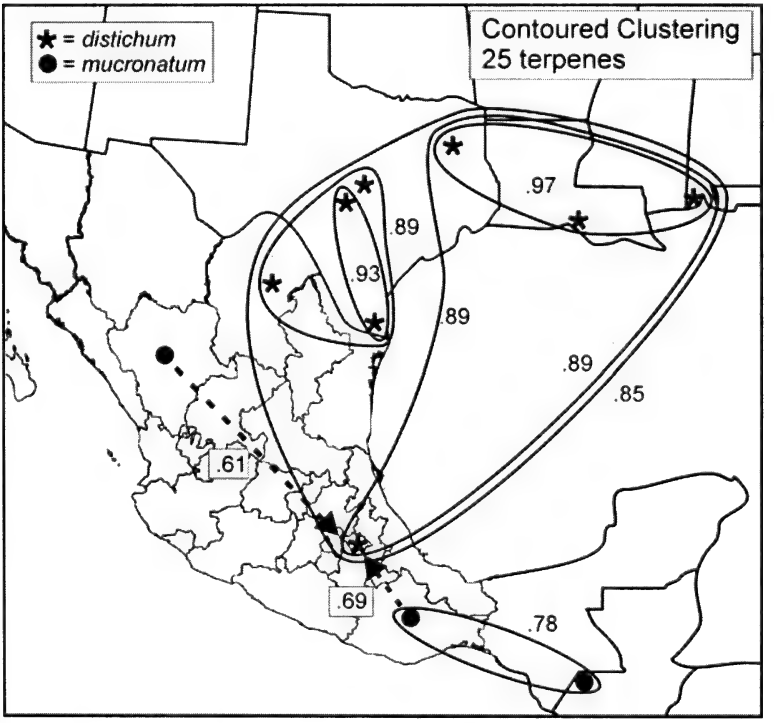

Figure 7. Contoured clustering based on 25 terpenes.

Of course, the Bolleros, MX population, with oils like var. *distichum* might have been derived from germplasm of var. *distichum* introduced from the USA (Fig. 7).

Based on analyses of the oils, it appears that *T. distichum* var. *distichum* from the South Central USA is distinct from var. *imbricarium* in Florida, but individuals with imbricate leaves from Fowl River, AL (cf. var. *imbricarium*) had oil patterns like var. *distichum*. *Taxodium d.* var. *distichum* appears to have 2 geographic chemical groups in the area studied: South Central USA and Texas Hill Country-northern Mexico (Fig. 7). Two types of oil patterns appear to be indigenous to Mexico: Durango and Oaxaca-Guatemala (Fig. 7). Additional research (in progress) using DNA sequencing will be needed to resolve these relationships.

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Table 1. Leaf essential oil composition of populations of *Taxodium.* muc Dur = *mucronatum*, Durango, MX; muc Oax = *mucronatum*, Oaxaca, MX; muc Bol = *mucronatum*, Bolleros, MX; dist Guad = *distichum*, Guadalupe River, TX, dist Mob = *distichum*, Mobile Bay, AL, imb FL = var. *imbricarium*, Tampa, FL. Compounds in boldface appear to separate taxa and were used in numerical analyses. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. For unknown compounds, four ions are listed, with the largest ion underlined.

KI	compound	muc Dur	muc Oax	muc Boll	dist Guad	dist Mob	imb FL
921	tricyclene	0.2	0.6	0.3	t	0.2	0.3
924	**α-thujene**	**1.1**	**-**	**-**	**0.4**	**t**	**-**
932	**α-pinene**	**27.3**	**42.9**	**76.6**	**5.3**	**72.8**	**79.3**
946	camphene	0.9	0.2	1.0	t	0.7	0.4
969	**sabinene**	**16.9**	**0.4**	**0.2**	**1.9**	**0.2**	**0.3**
974	**1-octen-3-ol**	**-**	**-**	**-**	**-**	**-**	**0.6**
974	β-pinene	0.9	1.2	3.0	0.3	2.1	1.4
988	myrcene	3.8	2.2	4.2	3.9	3.0	2.3
1002	α-phellandrene	0.2	t	t	t	t	t
1014	**α-terpinene**	**4.4**	**t**	**0.1**	**0.9**	**t**	**0.1**
1020	**p-cymene**	**0.5**	**1.2**	**1.9**	**0.1**	**t**	**t**
1024	**limonene**	**1.2**	**0.6**	**1.0**	**t**	**6.0**	**0.9**
1025	**β-phellandrene**	**1.2**	**0.6**	**0.9**	**61.5**	**5.0**	**0.6**
1044	(E)-β-ocimene	t	-	-	t	-	-
1054	**γ-terpinene**	**7.3**	**0.1**	**0.1**	**1.6**	**0.1**	**0.2**
1065	cis-sabinene hydrate	0.3	-	-	t	-	-
1086	**terpinolene**	**2.3**	**0.3**	**0.5**	**0.8**	**0.4**	**0.9**
1098	linalool	-	t	t	-	-	-
1098	**trans-sabinene hydrate**	**0.3**	**-**	**-**	**t**	**-**	**-**
1100	n-nonanal	-	-	-	t	-	-
1102	perrilene	t	-	-	-	-	-
1114	endo-fenchol	t	-	-	-	-	-
1118	**cis-p-menth-2-en-1-ol**	**0.9**	**-**	**-**	**0.2**	**-**	**-**
1122	α-campholenal	-	t	t	-	-	-
1135	trans-pinocarveol	-	t	t	-	-	t
1136	**trans-p-menth-2-en-1-ol**	**0.5**	**-**	**-**	**0.1**	**-**	**-**
1141	camphor	-	t	t	-	-	t
1145	camphene hydrate	-	t	t	-	-	t

KI	compound	muc Dur	muc Oax	muc Boll	dist Guad	dist Mob	imb FL
1165	borneol	t	0.2	0.1	-	-	-
1166	p-mentha-1,5-dien-8-ol	-	-	-	t	t	0.1
1174	**terpinen-4-ol**	**15.5**	**0.2**	**0.3**	**3.3**	**0.2**	**0.4**
1186	**α-terpineol**	**1.1**	**0.8**	**0.8**	**0.3**	**0.8**	**1.0**
1195	**cis-piperitol**	**0.2**	-	-	-	-	-
1207	**trans-piperitol**	**0.3**	-	-	**t**	-	-
1215	trans-carveol	-	-	-	t	-	-
1287	**bornyl acetate**	-	**1.7**	**1.2**	**0.1**	**0.6**	**0.4**
1298	trans-pinocarvyl acetate	0.1	0.2	0.2	t	0.1	t
1324	myrtenyl acetate	-	-	-	-	t	0.1
1339	trans-carvyl acetate	-	-	-	0.2	t	-
1345	α-cubebene	-	t	t	-	-	-
1346	α-terpinyl acetate	t	-	-	-	-	-
1373	α-ylangene	-	t	-	-	-	-
1374	α-copaene	-	t	-	-	-	-
1387	β-bourbonene	-	0.1	t	-	-	-
1390	β-elemene	-	0.2	t	-	-	-
1396	duvalene acetate	t	-	-	0.2	0.2	t
1410	α-cedrene	t	-	-	t	-	-
1419	β-cedrene	0.1	0.2	0.1	t	-	-
1417	**(E)-caryophyllene**	-	**1.6**	**0.2**	**0.9**	**0.9**	**1.0**
1429	**cis-thujopsene**	**3.3**	**6.9**	**3.2**	**1.7**	-	-
1452	α-humulene	-	0.3	t	0.1	0.1	0.2
1476	β-chamigrene	0.2	-	-	t	-	-
1478	γ-muurolene	-	1.2	0.2	-	-	0.1
1480	**germacrene D**	**0.2**	**0.8**	-	-	-	**4.0**
1495	γ-amorphene	-	0.3	-		-	-
1500	**α-muurolene**	-	**0.8**	**0.3**		-	**0.1**
1500	β-himachalene	0.3	-	-	0.1	-	-
1502	α-chamigrene	0.1	-	-	-	-	-
1504	**cuparene**	**0.2**	**0.4**	**0.2**	**0.2**	-	-
1505	β-bisabolene	-	t	-	-	-	-
1512	α-alaskene	t	-	-	-	-	-
1513	γ-cadinene	-	0.8	t	-	-	0.1
1522	**δ-cadinene**	**t**	**1.9**	**t**	-	-	**0.5**
1532	**γ-cuparene**	**0.2**	-	-	**t**	-	-
1533	**trans-cadina-1,4-diene**	-	**0.4**	**t**	**t**	-	-
1537	**α-cadinene**	-	**0.1**	-	-	-	-
1542	δ-cuparene	t	-	-	t	-	-

KI	compound	muc Dur	muc Oax	muc Boll	dist Guad	dist Mob	imb FL
1550	occidenol	-	0.2	-	-	-	-
1561	(E)-nerolidol	t	-	-	0.1	-	-
1562	β-calacorene	-	t	-	-	-	-
1574	germacrene D-4-ol	-	-	-	-	-	t
1582	**caryophyllene oxide**	**0.2**	**1.1**	**0.3**	**7.3**	**3.6**	**0.2**
1589	allo-cedrol	0.1	-	-	0.2	-	-
1599	**widdrol**	**2.0**	**0.6**	**0.7**	**1.2**	**-**	**-**
1600	**cedrol**	**0.8**	**1.2**	**1.5**	**1.3**	**-**	**-**
1607	β-oplopenone	0.1	-	-	-	-	-
1608	**humulene epoxide II**	**-**	**0.1**	**t**	**0.8**	**-**	**-**
1618	junenol	-	-	-	-	-	t
1638	epi-α-cadinol	-	0.5	-	-	-	0.2
1638	epi-α-muurolol	-	0.5	-	-	-	0.2
1639	**caryophylla-4(12),8(13) -dien-5-α-ol**	**-**	**-**	**-**	**0.6**	**0.3**	**0.3**
1644	α-muurolol	-	0.3	-	-	-	0.1
1649	β-eudesmol	-	0.2	-	-	-	-
1652	**α-cadinol**	**0.3**	**2.0**	**t**	**-**	**-**	**1.0**
1653	**3-thujopsenone**	**-**	**-**	**-**	**0.3**	**-**	**-**
1662	**43,79,187,220**	**-**	**-**	**-**	**0.6**	**0.2**	**0.3**
1674	**43,79,187,220**	**-**	**-**	**-**	**0.6**	**0.3**	**0.4**
1683	**epi-α-bisabolol**	**0.4**	**0.4**	**0.3**	**0.4**	**-**	**-**
1685	**germacra-4(15),5,10(14) -trien-1-al**	**0.1**	**0.6**	**-**	**-**	**-**	**0.6**
1905	isopimara-9(11),15-diene	0.1	-	-	0.2	0.1	0.2
1959	**hexadecanoic acid**	**-**	**1.7**	**0.2**	**-**	**-**	**-**
1978	manoyl oxide	t	-	0.1	0.3	-	t
2055	abietatriene	t	0.3	0.1	0.2	t	t
2056	manool	-	0.2	-	-	-	-
2087	abietadiene	t	-	-	-	-	-
2132	**linoleic acid**	**-**	**1.7**	**-**	**-**	**-**	**-**
2184	sandaracopimarinol	-	t	-	-	-	-
2231	diterpene, **41**,81,273,318	-	0.7	-	-	-	-
2268	diterpene, **41**,81,273,318, iso.	-	0.8	-	-	-	-
2329	**6,7-dehydro-ferruginol**	**-**	**1.1**	**t**	**t**	**t**	**t**
2331	**trans-ferruginol**	**-**	**7.5**	**0.1**	**t**	**0.2**	**0.3**
2350	diterpene acid,41,55,301,316	-	1.4	-	-	-	-
2365	communic acid	-	0.4	-	-	-	-

Table 2. Leaf essential oil composition plants with high/low amounts of α-pinene /low/high limonene-β-phellandrene (LMNN/BPHL). dist Prog = *distichum*, Progresso, TX, dist Guad = *distichum*, Guadalupe R., Mob hi LI = Mobile Bay, high LMNN/BPHL, Mob hi AP = Mobile Bay, high α-pinene, imb hi AP = *imbricarium* (FL), high α-pinene, imb hi LI = *imbricarium* (FL)/, high LMNN/BPHL.

KI	compound	dist Prog	dist Guad	Mob hi LI	Mob hi AP	imb hi AP	imb hi LI
921	tricyclene	t	t	0.1	0.2	0.3	0.1
924	**α-thujene**	**0.3**	**0.4**	t	**t**	-	**t**
932	**α-pinene**	**5.1**	**5.3**	**25.6**	**72.8**	**79.3**	**51.0**
946	camphene	t	t	0.4	0.7	0.4	0.3
969	**sabinene**	**1.4**	**1.9**	**0.4**	**0.2**	**0.3**	**0.2**
974	β-pinene	0.2	0.3	1.2	2.1	1.4	1.8
988	myrcene	3.4	3.9	2.8	3.0	2.3	3.8
1002	α-phellandrene	t	t	t	t	t	**t**
1014	**α-terpinene**	**0.2**	**0.9**	**0.1**	**t**	**0.1**	**0.1**
1020	p-cymene	t	0.1	t	t	t	**t**
1024	**limonene**	**t**	**t**	**29.0**	**6.0**	**0.9**	**11.0**
1025	**β-phellandrene**	**78.2**	**61.5**	**28.7**	**5.0**	**0.6**	**10.0**
1054	**γ-terpinene**	**1.2**	**1.6**	**0.3**	**0.1**	**0.2**	**0.2**
1086	**terpinolene**	**0.6**	**0.8**	**0.4**	**0.4**	**0.9**	**0.9**
1118	**cis-p-menth-2-en-1-ol**	**t**	**0.2**	-	-	-	-
1174	**terpinen-4-ol**	**2.6**	**3.3**	**0.5**	**0.2**	**0.4**	**0.3**
1186	α-terpineol	0.3	0.3	0.5	0.8	1.0	1.0
1287	bornyl acetate	0.3	0.1	0.8	0.6	0.4	0.4
1298	trans-pinocarvyl acetate	t	t	t	0.1	t	**t**
1396	duvalene acetate	0.1	0.2	0.3	0.2	t	**t**
1417	(E)-caryophyllene	0.3	0.9	0.6	0.9	1.0	0.2
1429	**cis-thujopsene**	**t**	**1.7**	-	-	-	-
1452	α-humulene	t	0.1	t	0.1	0.2	0.2
1478	**γ-muurolene**	-	-	-	-	**0.1**	**0.4**
1480	**germacrene D**	-	-	-	-	**4.0**	**4.5**
1500	**α-muurolene**	-	-	-	-	**0.1**	**0.2**
1504	cuparene	-	0.2	-	-	-	-
1513	**γ-cadinene**	-	-	-	-	**0.1**	**0.3**
1522	**δ-cadinene**	-	-	-	-	**0.5**	**0.9**
1574	germacrene D-4-ol	-	-	-	-	t	0.2
1582	**caryophyllene oxide**	**2.5**	**7.3**	**3.6**	**3.6**	**0.2**	**t**
1589	allo-cedrol	-	0.2	-	-	-	-

KI	compound	dist Prog	dist Guad	Mob hi LI	Mob hi AP	imb hi AP	imb hi LI
1599	**widdrol**	**t**	**1.2**	**-**	**-**	**-**	**-**
1600	**cedrol**	**t**	**1.3**	**-**	**-**	**-**	**-**
1608	**humulene epoxide II**	**0.3**	**0.8**	**-**	**-**	**-**	**-**
1638	**epi-α-cadinol**	**-**	**-**	**-**	**-**	**0.2**	**0.5**
1638	**epi-α-muurolol**	**-**	**-**	**-**	**-**	**0.2**	**0.4**
1639	**caryophylla-4(12),8(13) -dien-5-α-ol**	**t**	**0.6**	**0.3**	**0.3**	**0.3**	**-**
1644	α-muurolol	-	-	-	-	0.1	0.2
1652	**α-cadinol**	**-**	**-**	**-**	**-**	**1.0**	**2.3**
1653	**3-thujopsenone**	**t**	**0.3**	**-**	**-**	**-**	**-**
1662	**43,79,187,220**	**t**	**0.6**	**0.2**	**0.2**	**0.3**	**-**
1674	**43,79,187,220**	**t**	**0.6**	**0.3**	**0.3**	**0.4**	**-**
1683	**epi-α-bisabolol**	**-**	**0.4**	**-**	**-**	**-**	**-**
1685	**germacra-4(15),5,10(14) -trien-1-al**	**-**	**-**	**-**	**-**	**0.6**	**1.1**
1789	1-octadecene	t	t	0.2	t	-	-
1905	isopimara-9(11),15-diene	0.2	0.2	0.2	0.1	0.2	t
1978	**manoyl oxide**	**0.2**	**0.3**	**-**	**-**	**t**	**-**
2055	**abietatriene**	**0.1**	**0.2**	**t**	**t**	**t**	**-**
2105	**isoabienol**	**-**	**-**	**-**	**-**	**-**	**1.0**
2184	**sandaracopirmarinal**	**-**	**-**	**-**	**-**	**-**	**0.5**
2329	**6,7-dehydro-ferruginol**	t	**t**	**0.2**	**t**	**t**	t
2331	**trans-ferruginol**	t	**t**	**0.3**	**0.2**	**0.3**	**1.7**

DIFFERENCES IN GAS EXCHANGE RATES PROVIDE INSIGHT INTO THE DISTRIBUTION OF C_3 SEDGES AND C_4 GRASSES IN CENTRAL TEXAS SAVANNAS

E. R. Wayne
Department of Arts and Sciences, Texas A & M University, San Antonio, TX 78224

and

O. W. Van Auken
Department of Biology, The University of Texas at San Antonio, San Antonio, TX 78245 oscar.vanauken@utsa.edu

ABSTRACT

The Edwards Plateau of central Texas is mainly a savanna community with *Juniperus ashei-Quercus virginiana* woodlands and C_4 grasslands. C_4 grasses are common in the open grasslands but not below the woodland canopy while some C_3 sedges are found below the canopies and at the canopy edges. This study measured and compared gas exchange rates of these C_3 sedges and C_4 grasses to look for potential causes in distribution differences. In general, the response variables were 4 to 6.5 times higher for the grasses in high light while the sedges had higher values than the grasses in low light. The C_4 grass, *Bouteloua curtipendula* had the highest overall responses, while the C_3 sedge, *Carex planostachys* below the canopy had lowest values. Grasses had high A_{max} values (maximum photosynthetic rates), light saturation and respiration rates, suggesting they are sun species. The sedges had low values similar to shade species. Light levels appear to affect the distribution of both groups of plants but in different ways. Light attenuation seems to promote the sedges below the canopy and at the same time excluding grasses from the canopy habitat, while the reverse seems to be true in open grassland habitats. *Phytologia 94(1): 71-90 (April 2, 2012).*

KEY WORDS: C_3 sedges, C_4 grasses, savanna, gas exchange, photosynthesis, CO_2 uptake, woodlands, light response curves, *Juniperus-Quercus* woodlands, central Texas, sw North America

Complex gradients of abiotic and biotic factors or combinations occur throughout the World, including the central Texas Edwards Plateau, and these gradients determine the kind of communities and species that are present (Begon, Townsend, and Harper, 2006). This area of central Texas consists of savannas that are biphasic communities and have been difficult to study (House et al., 2003). Usually the grassland or gap phase is studied separately from the woodland phase (Van Auken, 2000; Van Auken and McKinley, 2008). In some central Texas woodland phase *Juniperus* communities, a drought tolerant sedge (*Carex planostachys* - cedar sedge, Cyperaceae) is found in the understory with high density, cover and dry mass (Wayne and Van Auken, 2008; 2010). Causes for differences in *C. planostachys* distribution, cover, and biomass seem to be related to the reduced light levels and enhanced soil moisture below the *Juniperus* canopy. This is unlike a majority of sedges that occur in cool and/or wet environments usually at higher latitudes and altitudes (Ball, 1990). Other sedges including *Carex tetrastachya* and *C. perdentata* are also reported in the Edwards Plateau region (Correll and Johnston, 1979). They seem to occur below the canopies of several deciduous tree species early in spring after bud break when canopy leaves are still emerging and light levels are less attenuated, but in deeper soils similar to some eastern deciduous forest understory species (Hull, 2002).

Physiological differences between plants native to open, full sun habitats compared to those found in shady, understory communities are fairly well known (Begon, Townsend, and Harper, 2006; Valladares and Niinemets, 2008). There are many papers concerning the C_4 grasses that occur in Texas full sun, grasslands and prairies and the grasslands of central North America (Gould, 1975; Correll and Johnston, 1979; Van Auken, Bush, and Diamond, 1994; Knapp and Medina, 1999; McCarron and Knapp, 2001). Most of these C_4 grasses seem to be sun species, but there are few papers about their gas exchange rates being suppressed at low light levels (McCarron and Knapp, 2001). Also, there is little information about their performance in the shade of woodland canopies specifically at low light levels (see Wayne and Van Auken 2009) and no published studies that we could find comparing the C_3 sedges and the C_4 grasses found in these communities.

Gas exchange rates reported for species found in sun or shade in these savanna ecosystems seem mixed. Rates for *Abutilon theophrasti* including A_{max} (maximum photosynthesis) and dark respiration (R_d) were high indicating a sun species (Van Auken and Bush, 2011). Similar measurements for *Malvavicus arborius*, *Verbesina virginica*, *Sophora secundifolora* and others were intermediate and suggest they could grow in full sun, shade or transitional light habitats (Furuya and Van Auken, 2009; Furuya and Van Auken, 2010; Gagliardi and Van Auken, 2010; Van Auken and Bush, 2011). Light compensation (L_{cp}), light saturation (L_{sat}), conduction (g_{leaf}), and transpiration (E) measurements all suggest that these species are either sun plants or intermediate sun species. One apparent high density but unusual understory species found in this area is *Carex planostachys* which has gas exchange rates that are similar to those reported for understory eastern deciduous forest species (Hull, 2002).

The purpose of this study was to measure and compare gas exchange rates of three C_3 sedges including *Carex planostachys*, *C. tetrastachya*, and *C. perdentata* that occur mostly below canopies in central Texas woodlands with four C_4 grasses including *Aristida purpurea, Bouteloua curtipendula*, *Bothriochloa laguroides*, and *B. ischaemum* that occur in the open gaps or grassland phase in this same area to see if gas exchange rates could help explain their distributions.

METHODS AND MATERIALS

Study site - The central Texas Edwards Plateau is a biphasic savanna community (Correll and Johnston, 1979; Van Auken, Ford, and Allen, 1981; Van Auken and McKinley, 2008; Furuya and Van Auken, 2009). The major vegetation type is *Juniperus-Quercus* savanna and is representative of savannas and woodlands found throughout this region. However, there are more woodlands with higher woody plant density, and smaller and fewer gaps in the east compared to savanna communities farther to the west (Van Auken and Smeins, 2008). This study was conducted on the southern edge of the Edwards Plateau near the University of Texas at San Antonio in Bexar County (29°34'53"N, 98°37'49"W). Soils at the study site vary, but in general are characterized as gentle to rolling, clayey-skeletal, smectitic, thermic

lithic calciustolls in the Tarrant association or moderately deep, dark-colored, nearly level alluvial soils in the Lewisville series (Taylor, Hailey, and Richmond, 1962; NRCS, 2006). Tarrant soils ranged from 0 to 25 cm in depth and the Lewisville soils were up to ca. 61 cm in depth. Regional climate is classified as sub-tropical – sub-humid with a mean annual temperature of 20 °C (Arbingast et al., 1976). Monthly mean temperature ranges from 9.6 °C in January to 29.4 °C in July (NOAA, 2004). Annual precipitation in the study area is 78.7 cm, bimodal, with peaks occurring in May and September with monthly means of 10.7 cm and 8.7 cm, respectively.

Vegetation on the shallow upland soil in the wooded habitat is dominated by *J. ashei* Buchh. (ashe juniper) and *Quercus virginiana* Mill. (live oak=*Q. fusiformis*) (Van Auken and Smeins, 2008) and representative species in the open grassland are *Aristida purpurea* Nutt. var. *longiseta* (Steud.) Vasey (Red three-awn), *Bouteloua curtipendula* (Michx.) Torr. (side-oats grama), *Bothriochloa laguroides* (de Candolle) Herter var. *torreyana* (von Steudel) Allred and Gould (silver bluestem), *Bothriochloa ischaemum* (L.) Keng var. *songarica* (Rupr.) Celerier and Harlan (KR or King Ranch bluestem), various other C_4 grasses and a variety of herbaceous annuals (Terletzky and Van Auken, 1996; Dibbs, Lipscomb, and O'Kennon, 1999; Barnes et al., 2000). *Carex planostachys* Kunze (cedar sedge) occurs mainly in wooded habitats in this area but occasionally in open grasslands (Wayne and Van Auken, 2008, 2009, 2010). In addition, in shaded deciduous woodland with deeper soils, two other sedges occur, *C. perdentata* S. D. Jones (sand sedge) and *C. tetrastachya* Scheele (britton sedge) (Ball, Reznicek, and Murray, 2003). The dominant woody species in these woodlands with deeper soil are *Q. virginiana, Ulmus crassifolia* Nutt. (cedar elm), *Celtis laevigata* Willd. (Texas sugarberry) and *Prosopis glandulosa Torr.* (honey mesquite).

Sampling procedures - Photosynthetic response curves were carried out on three randomly selected plants for each species, using mature, fully elongated leaves. Measurements were made when plant growth was most active, late March 2006 for the sedges and mid-July 2006 for the grasses. Previous observations verified that *C. perdentata* and *C. tetrastachya* were dormant by mid-April. Before making any measurements, all plants were watered to ensure full hydration (Peek et

al., 2002). Response curves for *C. planostachys* were made for plants below a *J. ashei* canopy and in the open grassland. Response curves for the other two sedges were made below a deciduous canopy prior to full leaf expansion of the canopy species. Response curves for all of the C_4 grasses were made in a gap between *J. ashei* canopy woodlands.

Gas-exchange measurements - Steady-state gas-exchange response curves as a function of light levels (photosynthetic active radiation, PAR, $\mu mol \cdot m^{-2} \cdot s^{-1}$) were completed to determine the maximum photosynthetic rate (A_{max}, $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), the light saturation point (L_{sp}, $\mu mol \cdot m^{-2} \cdot s^{-1}$), the quantum yield, the light compensation point (L_{cp}, $\mu mol \cdot m^{-2} \cdot s^{-1}$), the dark respiration rate (R_d, $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) and stomatal conductance (g_{leaf}, $mol\ H_2O \cdot m^{-2} \cdot s^{-1}$). Photosynthetic active radiation was measured using an integrated external quantum sensor, LiCor® LI-170 (Lincoln, NE). Plants were dark adapted for 30 minutes prior to making measurements for the response curves. Response curves were conducted within two hours prior to solar noon. Three to four intact, fully elongated, mature leaves were selected on a plant and placed on the surface of the cuvette of a LiCor® 6400 portable photosynthetic meter with the adaxial surface up, prior to closing the chamber. Both the cuvette and plant were covered with shade cloth to exclude external light. Leaf width was measured and total leaf area was calculated from the parallel portion of the leaves in the chamber. Gas-exchange measurements were standardized to a constant leaf area based on measurements for each sample.

Light levels were provided with an integrated red-blue LED light source attached to the cuvette. Chamber temperature and humidity were controlled at initial ambient levels. Cuvette CO_2 was maintained at 390 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$. The loaded cuvette was allowed to equilibrate prior to inducting the response curve. Response curves were initiated at high light levels to ensure stomatal opening and photoactivated Rubisco (Givnish, Montgomery, and Goldstein, 2004). After a steady state was achieved light levels were ramped down in 17 or 18 steps to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Sedges reached a steady state rapidly, < 1 minute, but grasses required up to 10 minutes for full induction. A stable coefficient of variation (< 0.3 %) was obtained at each level before data logging, ca. 2–3 min. Response curves were initiated at 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the sedges and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the grasses.

Natural log transformations were made on the averaged fitted data for CO_2 uptake for each species to better understand the species response as a function of light level. Comparisons were made between all of the sedges and all of the grasses.

Data Analysis - To compensate for within position differences between plant responses, A_{max} was averaged across the five highest asymptotic gas-exchange values (A_{net}) for each plant replication (Hull, 2002). Photosynthetic response data was fit with an empirical non-rectangular hyperbola function $A = A_{max} X [1 - (1 - R_d/A_{max})^{(1-PPF/Lcp)}]$ to model the nonlinear response of carbon uptake to change in light level (Bond et al., 1999). The quantum yield equation was derived by regressing the gas-exchange rate A_{net} from 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR upward until the regression coefficient of the slope decreased (Hull, 2002). L_{sp} for each replication was calculated from the regression of the initial linear portion of the response curve and was in agreement with the derived value of 90 % A_{max} (Bond et al., 1999). L_{cp} was calculated from the quantum yield equation by setting $A_{net} = 0$ $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$. R_d was calculated by averaging the A_{net} rate for each plant replication at PAR = 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The non-linear analysis described by Peek et al. (2002) was used to account for repeated measures. To test for differences within functional groups, grass or sedge, the Wald test for equality of variance was used on the estimated covariance parameters. A_{max} and g_{max} (raw data) were also evaluated for statistical differences with a repeated measures ANOVA and contrast statements to determine where differences occurred. L_{sp} and R_d were treated as stationary points and evaluated for statistical differences with ANOVA and linear regression (SAS, 2005). Comparisons between species A_{max}, L_{sp}, and R_d were conducted by testing for differences using ANOVA. Means separation tests were used when significant differences were detected.

RESULTS

C_3 sedge comparisons - Ambient light levels in March were high in habitats without a *Juniperus* canopy (1165 ± 484 $\mu mol \cdot m^{-2} \cdot s^{-1}$, mean ± SE) and reduced under a *Juniperus* canopy (318 ± 132 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Light levels below the leafless deciduous canopy were higher than light levels below a *Juniperus* canopy (Table 1). A_{max} values (Table 1, Fig. 1a) ranged from a low of 4.9 ± 0.3 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ for

C. planostachys below the *Juniperus* canopy to a high of 9.0 ± 0.8 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for *C. tetrastachya* below the canopy. The ANOVA

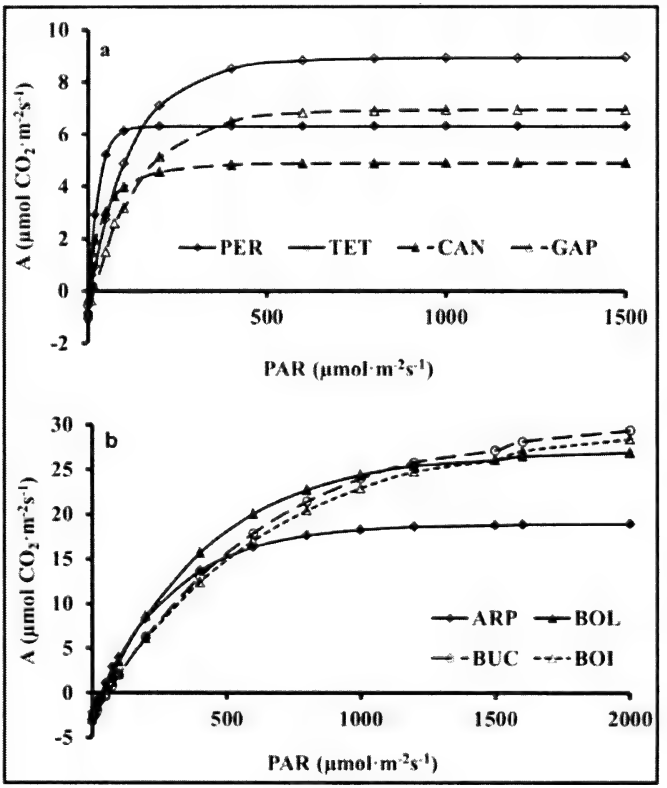

Figure 1. Mean light response curves for CO_2 uptake to incremental change in light levels for a) C_3 sedges *C. planostachys* below the *J. ashei* canopy (CAN) and intercanopy gap (GAP) and *C. perdentata* (PER) and *C. tetrastachya* (TET) below a leafless deciduous woodland canopy, and b) for the C_4 grasses in the grassland *A. purpurea* (ARL), *B. laguroides* (BOL), *B. ischaemum* (BOI) and *B. curtipendula* (BUC). Response curves were fitted to a non-rectangular hyperbola function. Measurements were made during active growth, March (sedges) and July (grasses) 2006, on fully expanded leaves. Standard error bars are not presented because they were smaller than the symbols used.

Table 1. Mean response parameters for C_3 sedges and C_4 grasses derived from light response curves for plants occurring in contrasting light environments. Means (±*SE*) are ambient photosynthetically active radiation (PAR), maximum photosynthetic rate (A_{max}), light compensation point (L_{cp},), dark respiration (R_d,), light saturation (L_{sp},), and maximum rate of stomatal conductance (g_{max}). A repeated measure ANOVA with p-1 contrasts was used to detect significant differences within columns and *P*-values are provided. Means with different letters within a column are significantly different.

Sedge Species	PAR	A_{max}	L_{cp}	R_d	L_{sp}	g_{max}
C. planostachys	1165	7.0	17	1.0	151	0.12
Gap *	±484	±0.9 **b**	±3	±0.2	±16	±0.01**a**
C. planostachys	318	4.9	4	0.4	151	0.07
Canopy *	±132	±0.3 **c**	±2	±0.0	±43	±0.01 **c**
C. tetrastachya	1322	9.0	7	0.6	187	0.09
	±199	±0.8 **a**	±2	±0.1	±24	±0.01 **b**
C. perdentata	800	5.8	3	0.9	114	0.08
	±152	±1.7 **b**	±1	±0.1	±42	±0.00**bc**
Col. *P*		****		ns	ns	****
Grass Species	PAR	A_{max}	L_{cp}	R_d	L_{sp}	g_{max}
A. purpurea	1576	19.2	33	2.3	428	0.23
	±14	±0.5 **c**	±4	±0.5	±31	±0.02 **b**
B. laguroides	1619	28.0	42	2.9	561	0.36
	±26	±1.1 **b**	±2	±0.3	±100	±0.70 **a**
B. ischaemum	1662	30.0	52	2.5	732	0.17
	±82	±1.4**ab**	±15	±0.3	±13	±0.01 **c**
B. curtipendula	1633	31.6	58	3.0	630	0.25
	±98	±0.5 **a**	±10	±0.1	±78	±0.01**b**
Col. *P*		****		ns	ns	**

* From Wayne and Van Auken 2009. ** = $P < 0.05$, **** = $P < 0.0001$, ns = not significant.

with repeated measures to test for differences in A_{max} was significant (F = 22.86, $P < 0.0001$), and with the exception of the *C. planostachys* in the gap and *C. perdentata* below the canopy, all paired comparisons were significantly different (Table 1). L_{cp} was lowest for *C. perdentata* at 3 ± 1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and highest for *C. planostachys* in the gap at 17 ± 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, but statistical differences were not detected (Table 1, Fig. 1a). The R_d was smallest for *C. planostachys* below the canopy at 0.4 ± 0.0 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ and highest for *C. planostachys* in the gaps at 1.0 ± 0.2 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ (Table 1, Fig. 1a) but not significantly different (F = 4.03, $P > 0.05$). The L_{sp} also did not vary significantly (ANOVA, F = 0.81, $P > 0.05$), but L_{sp} values ranged from 114 ± 42 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *C. perdentata* to 187 ± 24 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for *C. tetrastachya* (Table 1). The repeated measures *ANOVA* indicated that g_{max} varied significantly (F = 8.89, $P \leq 0.0001$), but values did not differ greatly (Table 1, Fig. 2a). At light extinction (0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF), conductance did not vary across any of the species of *Carex* examined (F = 0.81, $P > 0.05$, Fig 2b), with values ranging from 0.08 ± 0.00 $mol\ H_2O \cdot m^{-2} \cdot s^{-1}$ (*C. perdentata*) to 0.12 ± 01 $mol\ H_2O \cdot m^{-2} \cdot s^{-1}$ (*C. planostachys* in the gaps).

C_4 grass comparisons - Ambient light levels were high and consistent across all measurements (Table 1). The repeated measures *ANOVA* indicated significant differences in A_{max} between the C_4 grasses (F = 35.73, $P < 0.0001$, Table 1, Fig. 1b). A_{max} ranged from 19.2 ± 0.5 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ for *A. purpurea* to 31.6 ± 0.5 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ for *B. curtipendula*. The repeated measures *ANOVA* indicated no significant differences for the L_{cp}. However, it was lowest for *A. purpurea* at 33 ± 4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and highest for *B. curtipendula* at 58 ± 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The repeated measures *ANOVA* indicated no significant differences between R_d for the grasses, but was smallest for *A. purpurea* at 2.3 ± 0.5 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ and highest for *B. curtipendula* at 3.0 ± 0.1 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ (Table 1, Fig. 1b). The L_{sp} did not vary significantly between grasses (ANOVA, F = 3.78, $P > 0.05$), but ranged from 428 ± 31 to 732 ± 11 $\mu mol \cdot m^{-2} \cdot s^{-1}$. There were significant differences in stomatal conductance (ANOVA, F = 3.33, $P < 0.05$), lowest for *B.*

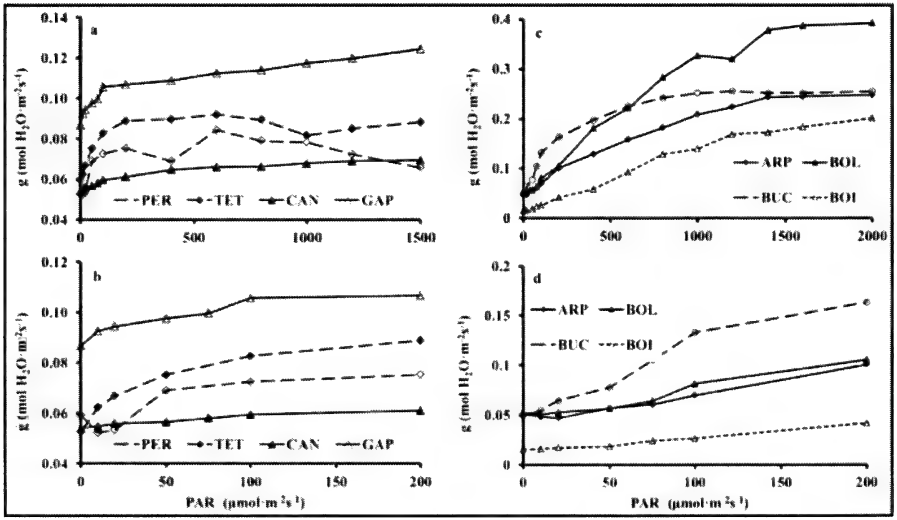

Figure 2. Mean stomatal conductance response curves (mol $H_2O \cdot m^{-2} \cdot s^{-1}$) to the incremental change in light intensity for C_3 sedges from a) 0-1500 PAR and b) a magnified view from 0-200 PAR and for C_4 grasses from c) 0-2000 PAR and d) a magnified view from 0-200 PAR. Abbreviations are as in Fig. 1. Measurements were made during active growth on fully expanded leaves in March 2006 for sedges and in July 2006 for grasses. Standard error bars are not presented because they were smaller than the symbols used.

ischaemum at 0.17 ± 0.01 mol $H_2O \cdot m^{-2} \cdot s^{-1}$ and highest for *B. laguroides* at 0.36 ± 0.07 mol $H_2O \cdot m^{-2} \cdot s^{-1}$ (Table 1, Fig. 2c). The paired comparisons indicated that there were significant differences between all species except *A. purpurea* and *B. curtipendula*. At light extinction (0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF) conductance did not vary across grass species (F = 0.95, $P > 0.05$, Fig. 2d), ranging from a low of 0.02 ± 0.00 mol $H_2O \cdot m^{-2} \cdot s^{-1}$ for *B .ischaemum* to ca.0.05 mol $H_2O \cdot m^{-2} \cdot s^{-1}$ for the other grasses.

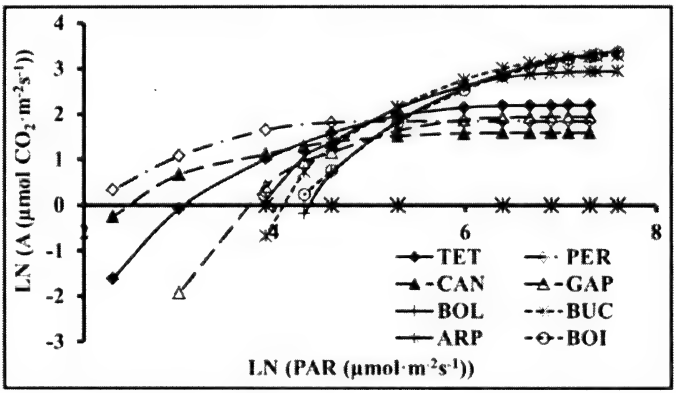

Figure 3. Natural log transformations of axes from the fitted response curves presented for the C_3 sedges and the C_4 grasses in Fig. 1. Asterisks on x-axis (from left to right) indicate light levels of 50, 100, 200, 500, 1000, 1500 and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Positions were curves intersect suggest adaptive crossover.

Comparison of species performance - At high light, above approximately 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (ln 6, Fig. 3) to 1500 – 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (ln 8, Fig. 3), the grasses had higher CO_2 uptake (between approximately 20 and 35 $\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$), while the rate for the sedges was much lower (approximately 4 – 9 $\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$). CO_2 uptake was approximately equal for the grasses and sedges between a PAR of 100 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 3, ln ≥ 5 but < 6). When light levels were reduced to 50 – 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, CO_2 uptake for the grasses was very low, at or below their CO_2 uptake rate at their light compensation point, but the sedges had positive CO_2 uptake rates between approximately 2 and 6 $\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$ (Fig. 3, ln ≤ 5). The

sedges continued to fix CO_2 until they reached their L_{cp} at between 20 and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

DISCUSSION

Theory suggests that plants occurring in habitat with a woodland or forest overstory are shade tolerant plants or shade plants and have low rates of CO_2 uptake, low light compensation and saturation, along with a low dark respiration rate; while shade intolerant plants or sun plants have considerably higher rates (Björkman et al., 1972; Givnish, 1988; Valladares and Niinemets, 2008). The photosynthetic response profiles for the C_3 *Carex* species we studied were considerably lower than the response profiles for the C_4 grass species examined when light levels exceeded ca. 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which is what is reported for sun and shade species from other areas.

The photosynthetic rates for the C_4 grasses examined were consistent with values reported for C_4 grass in water-limited northern prairie (Knapp, 1993; Turner, Kneisler, and Knapp, 1995; McCarron and Knapp, 2001; Fay et al., 2002). The photosynthetic rates for the C_3 sedges were similar to values reported for sedges in water stressed environments (Busch, 2001; Wayne and Van Auken, 2009). For both *C. perdentata* and *C. tetrastachya* the time of year seemed to be important for their growth and gas exchange responses, but was not examined in this study. Herbaceous annuals found in the understory of deciduous woodlands or forests are usually physiologically active at times when the solar track is low and there is an absence of canopy leaves, resulting in an environment suitable for the growth of shade tolerant herbaceous species (Baldocchi et al., 1984; Hull, 2002; Muth and Bazzaz, 2002). *Carex planostachys*, in central Texas woodlands, is physiologically active throughout the year below the evergreen canopy, but gas exchange is reduced during the hottest and driest time of year (Wayne and Van Auken, 2004, 2009). Although there are many sedge that occur in open habitats, these habitats are in higher latitudes or altitude and are cooler and wetter, reducing the negative impacts of water stress on photosynthesis (Busch, 2001). Thus, from a light tolerance perspective, the sedges studied were tolerant of low light, or shade species, while the grasses were shade intolerant or sun species.

Stomatal responses – Both light and soil water are required for carbon uptake and plant growth. Most plants have a stomatal response coupled to water availability or elevated rates of transpiration, which triggers a stomatal response to regulate aperture size (Chaves, Maroco, and Pereira, 2003; Osborne et al., 2003; Tuzet, Perrier, and Leuning, 2003). Like *C. perdentata*, the decline in conductance at elevated light levels for *C. tetrastachya* suggests there is a stomatal limitation regulating the photosynthetic response. In addition, the stomatal response observed at low light levels suggests that *C. tetrastachya* is coupled more strongly to light levels, but at higher light levels it uncouples. Some plants, such as *C. planostachys*, have conductance rates that appear fully coupled to the photosynthetic process or light availability rather than water induced stress (Wayne and Van Auken, 2009). This phenomenon may change with time of year, increasing daily temperatures and the severity of drought (Ogle and Reynolds, 2002). In the present study, *C. planostachys* did not appear to have any stomatal limitation regulating photosynthesis. The overall benefit of stomatal regulation maintaining water status comes at a cost of reduced carbon gain (Pearcy, 2000; Chaves, Maroco, and Pereira, 2003).

The C_4 grasses investigated had substantially higher rates of conductance at PAR > 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and stabilized approaching 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, except for *Bothriochloa ischaemum*. The conductance values were also consistent with values reported for C_4 grasses in northern prairies (Knapp, 1993). There was marginal evidence that *A. purpurea* conductance was coupled to soil moisture like *C. perdentata* (see Fig. 2b and d). Assuming stability when light was at higher levels, a benefit of a reduced or stable conductance, and corresponding transpiration, is a water conserving response identified for grasses as a means to limit plant water stress (Knapp, 1993; Chaves, Maroco, and Pereira, 2003; Lecain et al., 2003), this also appears to be true for *C. perdentata* and *C. tetrastachya*.

Species performance - Differences in response profiles between species are attributed to factors related to natural selection for a habitat type, usually high light or low light (Pearcy, 2000; Hull, 2002). It could be assumed there is an ecological or competitive advantage particular in the habitat that a species occupies, to have optimal photosynthesis and carbon-uptake in that habitat. In oak savanna in southern Wisconsin, C_4 grasses, having high light

requirements, are ideally suited for survival in open areas lacking a woody canopy. Sedges and C_3 grasses with reduced light requirements are more often found in shaded habitats and appear to be shade tolerant (Leach and Givnish, 1999). Sedges such as *C. planostachys, C. perdentata* and *C. tetrastachya*, with tolerance to low light, appear better suited to habitats that have a woody canopy to reduce light levels. Comparisons of response profiles among species in the present study (see Fig. 3), demonstrated interesting trends that suggest the C_3 sedges and C_4 grasses should occur in distinct ecological habitats, which seems to be true. Grasses perform better at high light and appear to be photosynthetically suited to open areas or gaps with high light levels. At lower light levels, ca. 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ or lower, the rate of grass CO_2 uptake decreased rapidly. Below 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the shade tolerant C_3 sedges have the higher CO_2 uptake rates and probably higher growth rates; thus they seem better suited to habitats where canopy structure attenuates incoming light. When light levels reached substantially low values, ca. 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, *C. perdentata* has the highest performance. Similar responses were observed in northern grasslands where canopy shade from taller grass plants modified the microclimate for plants nearer the soil, promoting cost savings in photosynthetic rates resulting from reduced stress (Turner and Knapp, 1996). *Carex perdentata* with a low compensation point may have rapid photosynthetic induction to maximize photosynthetic gain in light-flecks in the reduced light environment, however, this is not known.

Carex planostachys is tolerant of low light levels and severe aridity, and occurs on the shallow soils common to many central Texas *Juniperus* woodlands. The light response characteristics of *C. perdentata* and *C. tetrastachya* suggest they should also be able to fix carbon and grow below a *Juniperus* canopy as well; however, their tolerance to drought is probably low but currently unknown. For the C_4 grasses, soil depth, texture and organic content are factors that have an important role in explaining differences in their growth in semi-arid and arid habitats through their influence on plant water stress (Turner, Kneisler, and Knapp, 1995; Leach and Givnish, 1999; Weiher et al., 2004; Rosenthal, Ludwig, and Donovan, 2005). However, in the present study, light levels were critical.

The light compensation points, along with light saturation levels, and respiration rates are strong predictors of species distribution

in various habitats (Givnish, Montgomery, and Goldstein, 2004; Begon, Townsend, and Harper, 2006; Valladares and Niinemets, 2008), with many species being tolerant of either high or low light levels. Canopy overstory appeared to be an important influencing factor on *Carex* plant responses and thus distribution. Other factors including soil depth and the soil water holding capacity are probably also important in determining where the *Carex* species studied are found at least in central Texas. In arid or semiarid savanna systems, it is more likely that any sedges that are present would occur below a woody canopy that reduces light levels (Leach and Givnish, 1999) or at times of the year when temperatures are cooler and water stress is reduced (Knapp, 1993; Knapp et al., 1993). The dominance of C_4 grasses in the gaps is likely related to the higher light levels, temperatures, and increased aridity that occur in this habitat (Paruelo and Lauenroth, 1996). The C_4 grasses should also be capable of survival at the canopy edge where light levels are intermediate and equivalent to their saturation point (Leach and Givnish, 1999; Wayne and Van Auken, 2009). Most grasses though are inhibited in areas that have a woody canopy (Peltzer and Köchy, 2001) probably due to reduced light levels (Naumburg, De Wald, and Kolb, 2001).

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SEASONAL VARIATION IN THE LEAF ESSENTIAL OIL OF *TAXODIUM DISTICHUM* (CUPRESSACEAE)

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

The leaf essential oil of *Taxodium distichum* is dominated by α-pinene (63-69%) with moderate amounts of limonene, β-phellandrene, myrcene and β-pinene. Oil yield increased from April (3.45 mg/g DM) to May (6.64) then rapidly declined to a somewhat steady state in the summer and fall. The major component, α-pinene, exhibited no significant variation on a percent total oil basis, but did vary on a mg/g DM basis. Caryophyllene oxide and germacrene D reached a maximum in June and July, respectively, then declined in the fall (Sep, Oct). Several components declined as percent total oil from early leaf set (Apr) to leaf yellowing (Oct): β-pinene, myrcene, limonene, β-phellandrene, terpinolene and (E)-caryophyllene. Three terpene acetates and a sesquiterpene aldehyde increased to their maximum levels in Sep-Oct. Seasonal variation in the constituents was not statistically different between % total oil and mg/g DM basis. *Phytologia 94(1):91-102 (April 2, 2012).*

KEY WORDS: *Taxodium distichum,* leaf essential oils, seasonal variation.

The composition of leaf essential oils can be influenced by season, rainfall, sunlight and other growth factors (von Koenen, 2001). In peppermint, Burbott and Loomis (1967) found that monoterpenes were stored for energy sources and utilized as needed. Adams (1970) found the leaf oil of *Juniperus pinchotii* varied from summer to winter with more variation in the summer than winter. Powell and Adams (1973) found that significant seasonal differences in the oil components

of *Juniperus scopulorum* were correlated with growth, temperature, and yield of oil. Seasonal variation on a g/g dw basis was more variable than on a percent basis (Powell and Adams, 1973). Adams and Hagerman (1976) examined the volatile leaf oil of *J. scopulorum* in young and mature leaves from the same greenhouse grown trees. They found 19 of the 36 compounds examined showed significant differences between young and mature leaves. Shanjani et al. (2010) examined seasonal changes in the volatile oils from both leaves and seed cones of *J. excelsa* (later shown to be *J. polycarpos*, Adams and Shanjani, 2011) in Iran. They found considerable seasonal variation in the seed cone oils with much less variation in the leaf oils. In general, in conifers, it appears that sampling leaves for volatile leaf oils for chemosystematic studies is less variable if done in during the fall and winter seasons.

However, not all conifers have leaves that persist throughout the year. *Taxodium* is a conifer genus in which the leaves are deciduous (except in mild, sub-tropical and tropical sites). *Taxodium distichum* (L.) Rich., bald cypress, is a common tree that grows from Texas to Florida along rivers. It is very commonly cultivated. Several trees are cultivated on Lake Tanglewood, TX in the Texas Panhandle. The leaves on these trees appear in April and generally turn yellow in October. Thus, these cultivated trees present an excellent opportunity to study the seasonal accumulation and changes of terpenes in their leaf oils. The purpose of this study was to determine the changes in the leaf essential oil of *T. distichum* throughout the growing season in the Texas Panhandle.

There has been a surprisingly little amount of research on the leaf essential oils of *Taxodium*. Odell (1912) reported on the volatile oil from the seed cones of *T. distichum* and Flamini et al. (2000) examined the essential oils from seed cones, leaves and branches from cultivated trees of *T. distichum* in North Tuscany, Italy. El Taunawy et al. (1999) reported that the essential oil from seed cones of *T. distichum* grown in Giza, Egypt. Ogunwande et al. (2007) reported the oils of *T. distichum* seed cone and leaves from a tree cultivated in Ibaden, Nigeria. The composition of *T. distichum* and *T. mucronatum* trees native to the western hemisphere has recently been reported (Adams et al., 2012).

MATERIALS AND METHODS

Plant material - *T. distichum*, *Adams 12730, 12441*, 318 N. Shore Dr., Lake Tanglewood, Amarillo, Randall Co., TX. A voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g.) and air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate and Principal Component analyses were performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf essential oil of *T. distichum* is dominated by α-pinene (63-69%, Table 1) with moderate amounts of limonene, β-phellandrene, myrcene and β-pinene. Oil yield increased from April (3.45 mg/g DM) to May (6.64) then rapidly declined to a somewhat steady state in the summer and fall (Table 1, Fig. 1). Interestingly, the major component, α-pinene, exhibited no significant variation on a percent total oil basis

(F= 0.9, ns, Table 2), but did vary significantly on a mg/g DM basis (Table 3). Caryophyllene oxide showed a lag correlation with oil yield reaching a maximum (3.8%) in June, then declining (Table 1, Fig. 1). Germacrene D has a similar pattern reaching a maximum in August, then declining (Table 1, Fig. 1). Several components declined as percent total oil from early leaf set (Apr) to leaf yellowing (Oct): β-pinene, myrcene, limonene, β-phellandrene, terpinolene and (E)-caryophyllene (Table 1). These patterns are shown in Figure 2, with (E)-caryophyllene and terpinolene declining during summer growth. Myrcene was stable in April-May-June, then declined in July, and was again stable in Aug-Sep-Oct. A third pattern is shown in Fig. 3, with trans-pinocarvyl acetate rapidly increasing in the fall (Sep-Oct) along with the cis isomer (cis-pinocarvyl acetate) to a lesser degree (Fig. 3). Germacra-4(15),5,10(14)-trien-1-al produced a slightly different pattern (Fig. 3), increasing in the late summer, then with a significant decline in October.

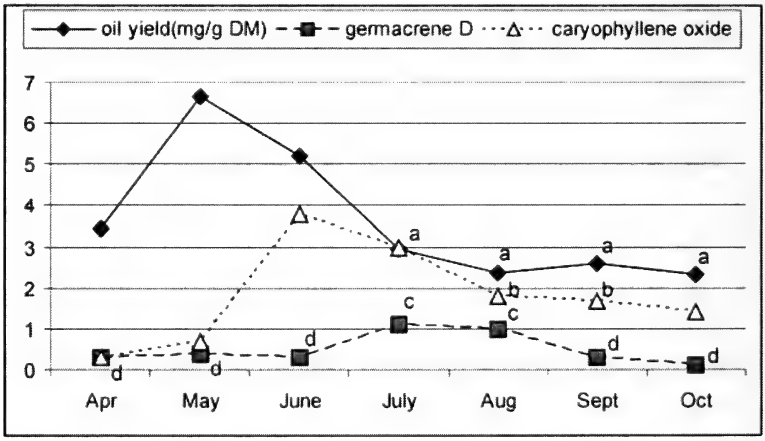

Figure 1. Seasonal variation in oil yield (mg/g DM), germacrene D and caryophyllene oxide (% total oil basis). Data points that share the same letter are not significantly different by the SNK test (P=0.05).

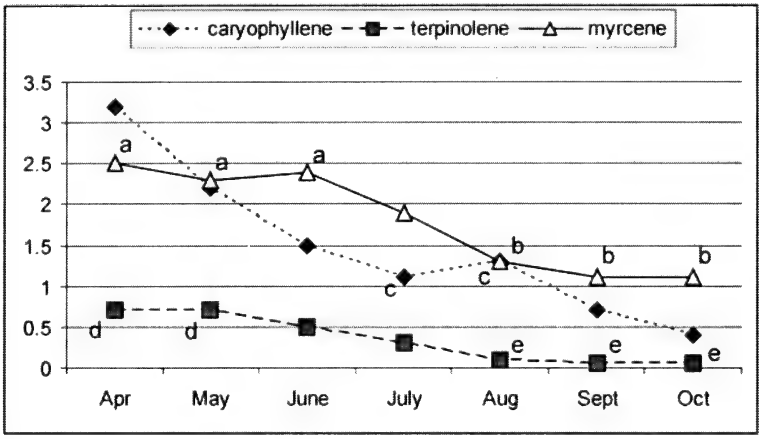

Figure 2. Seasonal variation in (E)-caryophyllene, terpinolene and myrcene (% total oil basis). Data points that share the same letter are not significantly different by the SNK test (P=0.05).

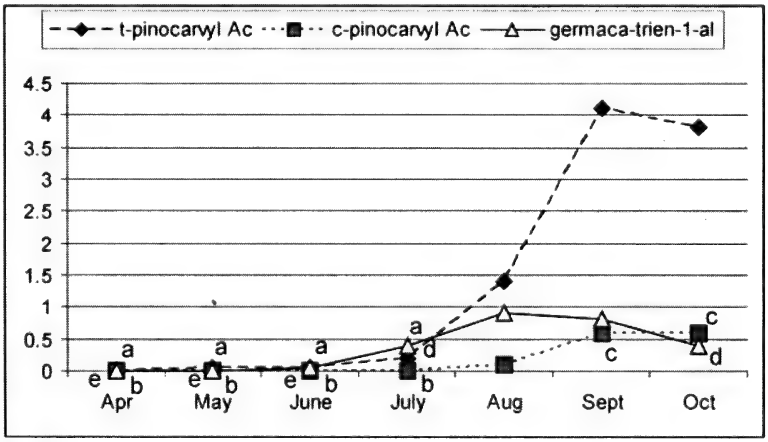

Figure 3. Seasonal variation in trans- and cis- pinocarvyl acetate and germacra-4(15),5,10(14)-trien-1-al (% total oil basis). Data points that share the same letter are not significantly different by the SNK test (P=0.05).

ANOVA, using oil yield (mg/g DM) and 15 major components (as % total oil), revealed that all exhibited highly significant seasonal differences except α-pinene (Table 2). The percent of α-pinene remained from 63.5 to 69.2% throughout the growing season, even in the yellowing October leaves. The average F value was 117.7. The pattern of trans- and cis- pinocarvyl acetate, trans-carvyl acetate and germacra-4(15),5,10(14)-trien-1-al are quite apparent in Table 2.

The data were recomputed on a mg/g DM basis and ANOVA performed. This resulted in an average F value of 116.6 that was not significantly different from the average F based on % total oil data (Table 2). Overall, the trends in the data expressed as mg/g DM are similar (Table 3) to the results found using % total oil (Table 2).

In order to examine the correlation patterns among terpenes and oil yield, Principal Component Analysis (PCA) was performed on the % total oil data. As expected, the terpene acetates (trans- and cis-pinocarvyl acetate, trans-carvyl acetate) were highly correlated as seen in Figure 4. Oil yield was correlated with β-pinene and limonene (Fig. 4). The components that decreased as oil yield decreased in the season (β-pinene, limonene, β-phellandrene, terpinolene, myrcene, caryophyllene) cluster (show a positive correlation) with oil yield (Fig. 4). In contrast, components that increased as oil yields declined are negatively correlated with oil yield and cluster at the far end of axis 1(Fig. 4). Interestingly, PCA using mg/g DM data gave essentially the same ordination as using percent data (results not shown).

Notice that 85% of the variance among components was removed in the first three axes in PCA. This seems to imply that there may be only a few dominant biosynthetic pathways that control the terpene pattern in this particular *Taxodium*.

To examine the overall similarities among samples, PCO was performed on the similarity matrix. Ordination reveals that the samples cluster by date of collection, except for the Sep-Oct samples that seem to form a continuum (Fig. 5). PCO divides the collections into three

groups: spring - early summer (Apr-Jun), summer (Jul-Aug), and fall (Sep - Oct). The fall group appears to be the most uniform (Fig. 5). Fall appears to be the best time to sample for chemosystematic studies, but an early freeze would likely prove to be a serious problem.

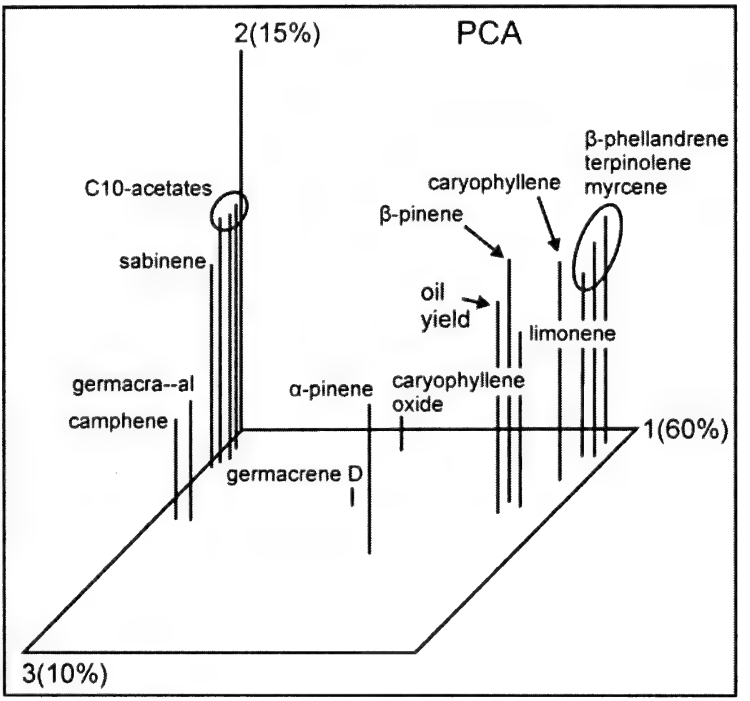

Figure 4. PCA of 21 samples using 16 characters.

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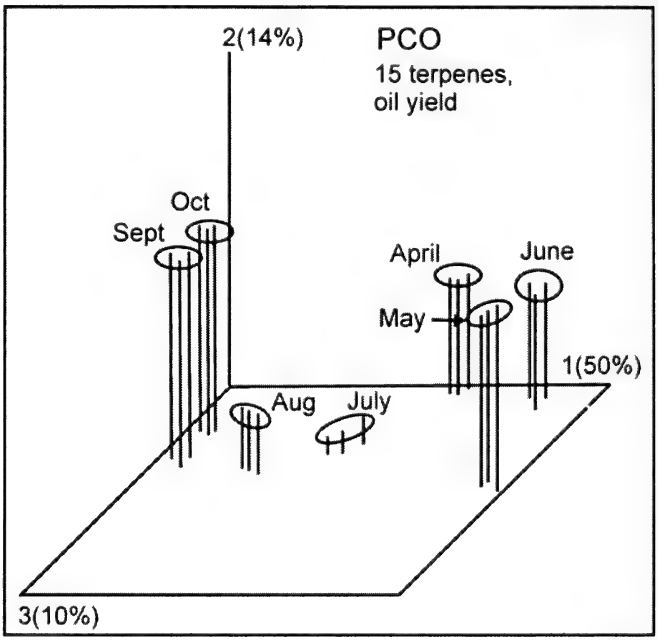

Figure 5. PCO showing samples cluster by dates of collection.

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Table 1. Composition of volatile leaf oils by collection dates for T. distichum. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t).

KI	compound	Apr	May	June	July	Aug	Sept	Oct
	oil yield (mg/ g DM)	3.45	6.64	5.20	2.94	2.37	2.60	2.32
921	tricyclene	0.2	0.3	0.2	0.4	0.3	0.2	0.3
924	α-thujene	t	t	t	t	t	t	t
932	α-pinene	66.4	69.2	63.5	67.1	65.7	64.2	69.2
946	camphene	0.6	0.8	0.9	1.1	1.1	1.2	1.2
969	sabinene	0.5	0.2	0.1	0.2	0.7	0.8	0.9
974	β-pinene	1.4	0.9	0.9	0.9	0.9	0.8	0.8
988	myrcene	2.5	2.3	2.4	1.9	1.3	1.1	1.1
1014	α-terpinene	0.1	t	0.5	t	t	t	t
1020	p-cymene	t	t	t	t	0.1	0.2	0.2
1024	limonene	11.0	10.0	10.0	9.5	8.9	8.2	8.4
1025	β-phellandrene	10.0	9.6	9.4	6.8	6.0	7.2	5.6
1054	γ-terpinene	0.2	0.2	t	t	0.1	0.1	t
1086	terpinolene	0.7	0.7	0.5	0.3	0.1	t	t
1122	α-campholenal	-	-	-	-	-	t	t
1135	trans-pinocarveol	-	-	-	-	-	0.1	0.1
1137	trams-verbenol	-	-	-	-	t	t	t
1160	pinocamphone	-	-		-	t	0.1	t
1166	p-mentha-1,5-dien-8-ol	-	-	-	-	t	t	t
1174	terpinen-4-ol	0.2	0.1	t	t	t	t	t
1186	α-terpineol	0.4	0.4	0.3	0.2	0.1	t	t
1200	dodecane	t	-	t	t	0.1	0.1	0.1
1287	bornyl acetate	0.3	0.3	0.4	0.4	0.6	0.6	0.8
1298	trans-pinocarvyl acetate	-	t	t	0.2	1.4	4.1	3.8
1311	cis-pinocarvyl acetate	-	-	-	-	0.1	0.6	0.6
1324	myrtenyl acetate	-	t	-	t	0.2	0.4	0.3
1339	trans-carvyl acetate	-	t	-	t	0.2	0.5	0.4
1346	α-terpinyl acetate	-	t	-	t	t	t	t
1365	cis-carvyl acetate	-	-	-	-	t	t	t
1396	duvalene acetate	-	-	t	t	0.1	0.1	t
1417	(E)-caryophyllene	3.2	2.2	1.5	1.1	1.3	0.7	0.4
1431	β-copaene	-	t	t	t	t	0.1	t
1452	α-humulene	0.3	0.3	t	0.1	0.1	t	t
1478	γ-muurolene	-	-	-	t	t	t	t
1480	germacrene D	0.3	0.4	0.3	1.1	1.0	0.3	0.1
1513	γ-cadinene	-	t	t	t	t	t	t
1522	δ-cadinene	t	0.1	t	t	0.1	0.1	t
1582	caryophyllene oxide	0.3	0.7	3.8	3.0	1.8	1.7	1.4
1608	humulene epoxide II	t	t	0.1	0.4	0.4	0.3	0.2

KI	compound	Apr	May	June	July	Aug	Sept	Oct
1639	caryophylla-4(12), 8(13)-dien-5-α-ol	-	-	t	0.1	0.2	0.1	t
1685	germacra-4(15),5,10 (14)-trien-1-al	-	-	t	0.4	0.9	0.8	0.4
1789	1-octadecene	-	-	t	t	t	t	t
1905	isopimara-9(11),15-diene	t	t	t	t	t	0.1	t
1992	ethyl hexadecanoate	-	-	-	-	0.1	t	-
2055	abietatriene	t	t	t	0.1	0.2	0.1	t
2331	trans-ferruginol	t	t	t	0.2	0.2	0.1	t

Table 2. ANOVA for major components of the volatile leaf oils of *T. distichum*. ns = non significant, * = P 0.05, ** = P 0.01.

compound	Apr	May	June	July	Aug	Sept	Oct	F sig.
oil yield(mg/g DM)	3.45	6.64	5.20	2.94	2.37	2.60	2.32	57. **
α-pinene	66.4	69.2	63.5	67.1	65.7	64.2	69.2	0.9 ns
camphene	0.6	0.8	0.9	1.1	1.1	1.2	1.2	6.6 **
sabinene	0.5	0.2	0.1	0.2	0.7	0.8	0.9	103. **
β-pinene	1.4	0.9	0.9	0.9	0.9	0.8	0.8	21. **
myrcene	2.5	2.3	2.4	1.9	1.3	1.1	1.1	37. **
limonene	11.0	10.0	10.0	9.5	8.9	8.2	8.4	4.1 **
β-phellandrene	10.0	9.6	9.4	6.8	6.0	7.2	5.6	20. **
terpinolene	0.7	0.7	0.5	0.3	0.1	t	t	131. **
trans-pinocarvyl acetate	-	t	t	0.2	1.4	4.1	3.8	458. **
cis-pinocarvyl acetate	-	-	-	-	0.1	0.6	0.6	260. **
trans-carvyl acetate	-	t	-	t	0.2	0.5	0.4	250. **
(E)-caryophyllene	3.2	2.2	1.5	1.1	1.3	0.7	0.4	120. **
germacrene D	0.3	0.4	0.3	1.1	1.0	0.3	0.1	125. **
caryophyllene oxide	0.3	0.7	3.8	3.0	1.8	1.7	1.4	108. **
germacra-4(15),5, 10(14)-trien-1-al	-	-	t	0.4	0.9	0.8	0.4	182. **
average F								117.7

Table 3. Composition by mg/ g DM for components greater than 0.5% concentration.

compound	Apr	May	June	July	Aug	Sept	Oct	F sig.
oil yield(mg/g DM)	3.45	6.64	5.20	2.94	2.37	2.60	2.32	58. **
α-pinene	2.29	4.59	3.30	1.97	1.56	1.67	1.60	67. **
camphene	0.02	0.05	0.05	0.03	0.03	0.03	0.03	34. **
sabinene	0.02	0.01	0.01	0.01	0.02	0.02	0.02	49. **
β-pinene	0.05	0.06	0.05	0.03	0.02	0.02	0.02	35. **
myrcene	0.09	0.15	0.12	0.06	0.03	0.03	0.02	124.**
limonene	0.38	0.66	0.52	0.27	0.21	0.21	0.20	68. **
β-phellandrene	0.35	0.64	0.50	0.20	0.14	0.19	0.13	98. **
terpinolene	0.02	0.05	0.03	0.01	t	t	t	172.**
trans-pinocarvyl acetate	-	t	t	0.01	0.03	0.11	0.09	220.**
cis-pinocarvyl acetate	-	-	-	-	t	0.02	0.02	241.**
trans-carvyl acetate	-	t	-	t	t	0.02	0.02	127.**
(E)-caryophyllene	0.11	0.15	0.08	0.03	0.03	0.02	0.02	175.**
germacrene D	0.01	0.03	0.02	0.03	0.02	0.01	0.01	89. **
caryophyllene oxide	0.01	0.05	0.20	0.09	0.04	0.04	0.03	150.**
germacra-4(15),5, 10(14)-trien-1-al	-	-	t	0.01	0.02	0.02	0.01	158.**
average F								116.6

SYNOPSIS OF *OBELIDIUM* (CHYTRIDIOMYCOTA)

Will H. Blackwell, Peter M. Letcher and Martha J. Powell
Department of Biological Sciences, University of Alabama,
Tuscaloosa, AL 35487, USA

ABSTRACT

Three species of *Obelidium*—*O. mucronatum* Nowakowski (1876), *O. hamatum* Sparrow (1937), and *O. megarhizum* Willoughby (1961)—have been described and are reviewed. Our collection of *O. megarhizum* from a North Carolina river is a new geographic record. A possible taxon of *Obelidium*, represented by specimens initially assigned with a question mark (Sparrow, 1937) to *O. mucronatum*, is discussed but not recognized because of insufficient evidence. Generic distinction of *Obelidium* has become less clear over time; consideration is thus also given to related chitinophilic genera. *Siphonaria petersenii* Karling (1945) merits special attention because of similarities to *Obelidium*. Additional molecular data will be required to conclusively resolve the systematics of *Obelidium* and related genera. *Phytologia 94(1): 103-117 (April 2, 2012).*

KEY WORDS: Chitin, chytrid, epibiotic, inoperculate, insect exuviae, interbiotic, mucro, resting spore, rhizoids, sporangium, zoospores.

Obelidium taxa are interbiotic or epibiotic, chitinophilic, eucarpic, monocentric, inoperculate Chytridiomycetes; the ellipsoid-ovate-spheroid, spine-bearing sporangium develops a subapical to lateral zoospore discharge pore (Sparrow, 1960; Karling, 1977). *Obelidium* and similar genera, saprophytic on kinds of insect exuviae, are obtained in culture with chitin bait (e.g., clarified shrimp exoskeleton), and may sometimes be cultured on chitin agar (Karling, 1967). *Obelidium* was established on *O. mucronatum* Nowakowski (1876)—characterized by a solid, apical sporangial spine (mucro) and a cup- or stalk-like sporangial base. The taxonomy of *Obelidium* became more uncertain as additional taxa (*O. hamatum* and *O. megarhizum*) were described, and another possible taxon discussed (first assigned, questionably, to *O. mucronatum*). We hope this revision of *Obelidium*

will encourage further ultrastructural and molecular work on the genus.

POSSIBLE GENERIC RELATIONSHIPS

Sparrow (1960, p. 429) noted similarities of the following four exuviae-inhabiting genera: *Obelidium*, *Siphonaria*, *Rhizoclosmatium*, and *Asterophlyctis*—listing allegedly unifying features: "method of development, general structural features, possession of a subsporangial apophysis, type of zoospore discharge, and habitat." These features, however, are indefinite or problematical. Regarding method of development, even within one species (*Obelidium mucronatum*) more than one mode of development was noted (Sparrow, 1938); Karling (1945) postulated two types of life cycles for *Siphonaria*. A further example of developmental variation is that the sporangium of *Asterophlyctis sarcoptoides* may form either from the zoospore cyst or from a swelling of the germ tube (cf. Antikajian, 1949; Karling, 1967)—see Karling (1977) versus Dogma (1974) concerning the systematic disposition of *Asterophlyctis*. "General structural features" refer to an overall resemblance of these genera on insect exuviae. However, some forms—e.g., *Asterophlyctis sarcoptoides*, with its angular, lobed, or even "stellate" sporangia—are distinctive. Sporangia of *Asterophlyctis irregularis* Karling (1967) possess tapering, peg-like projections. *Obelidium* taxa and *Siphonaria petersenii* have "spiny" sporangia, whereas other taxa of these four genera generally lack spines. *Obelidium mucronatum* and *Siphonaria petersenii* are, in fact, morphologically similar, as we discuss. A subsporangial apophysis is often present in these genera, but there are exceptions. *Obelidium mucronatum*, considered apophysate, occasionally lacks an apophysis; *O. hamatum* and *Siphonaria petersenii* apparently lack an apophysis; *Rhizoclosmatium* possesses an apophysis—often fusiform and transversely oriented (cf. Karling, 1977). In Sparrow's (1960, see p. 405) key to genera of Rhizidiaceae, *Obelidium* is distinguished from *Rhizoclosmatium* and *Asterophlyctis* by "Rhizoids arising from a....cuplike basal portion of the sporangium" in *Obelidium*, versus "Rhizoids arising from an apophysis" in *Rhizoclosmatium* and *Asterophlyctis*. This "distinction" is problematic; not only does *Obelidium mucronatum* often possess an apophysis, but the rhizoids, though usually initiating development first, may give the appearance of having arisen from the apophysis (cf. Sparrow, 1938, p. 4 and fig. 14).

Zoospore discharge in these genera is considered subapical or lateral; however, two species of *Siphonaria* do not discharge in this manner, and discharge in *Rhizoclosmatium* is often basal (Sparrow, 1960). Even habitat, presumed to involve chitin substrate, may not always be similar; *Rhizoclosmatium* (Sparrow, 1960; Karling, 1967) generally inhabits insect exuviae, but *R. marinum* occurs on the alga, *Codium*—Karling (1977), though, questioned generic placement of *R. marinum*.

The supposed production of resting spores (in the above four genera) by sexual means (cf. Karling, 1945)—allegedly initiated in these genera by rhizoidal anastomosis—is somewhat unusual; the majority of chytrid resting spores are asexually produced. Resting spore formation in *Asterophlyctis* (Antikajian, 1949; Karling, 1967) may in fact be asexual, and resting spores are unknown in taxa described as *Obelidium*—however, further attempts at discovering rhizoidal anastomosis, and potential heterothallism, are worthy of pursuit. The four putatively related genera are apparently inoperculate. Considered of major significance in delimiting larger groupings of chytrids (cf. Sparrow, 1960), the presence or absence of an operculum is now understood to have more relevance at the generic level. Operculate genera may occur in predominantly inoperculate groups, and vice versa (cf. Powell et al., 2011). The lack of an operculum, thus, does not necessarily predict relationship. In brief, analysis of details of traits of the four genera suggests that little meaningful morphology remains by which to reliably establish generic distinction, or confirm relationship. These four genera probably are related, though, and additional features suggest this. The developing sporangium in these genera typically possesses a large primary nucleus (cf. Karling, 1967), persistent until almost the point of zoospore cleavage. There is also reasonable consistency in the observation that zoospores are released from the sporangium together (and begin movement) in a temporary vesicle, before dispersing individually (cf. Karling, 1967). A similar structural type of zoospore apparently occurs in these genera (Letcher et al., 2005). These additional morphological traits are not, though, confined to these four genera. A general molecular similarity can be asserted for these genera (cf. James et al., 2006), *Asterophlyctis* being perhaps the most distantly related among them. Other genera, however, play into the molecular picture as well; isolates identified as *Rhizoclosmatium,*

Podochytrium and *Phlyctorhiza* positioned in the same clade as did *Siphonaria petersenii* and *Obelidium mucronatum* (James et al., 2006).

TAXONOMIC SYNOPSIS

OBELIDIUM Nowakowski. Cohn, Beitr. Biol. Pflanzen 2: 86, 1876.

Type of genus = Type of *Obelidium mucronatum* Nowak., 1876. Thallus eucarpic, monocentric, chitinophilic, typically developing in an interbiotic or epibiotic fashion, apophysate or apophysis lacking. Sporangium usually extramatrical, occasionally partially or wholly intramatrical, inoperculate, often mucronate (i.e., with an apical spine-like process), differentiating from the encysted zoospore, typically with a large primary nucleus persisting until the onset of zoospore formation; sporangial wall remaining thin or exhibiting uniform or basal thickening (in the last case, becoming cup- or stalk-like at the base). Rhizoids robust or more delicate, extensively or more infrequently branched, with or without major rhizoidal axes, intra- or extra-matrical, rarely sparsely developed. Zoospores posteriorly uniflagellate (characteristic of Chytridiomycota), spherical to ellipsoid, each with a single obvious lipid globule (uniguttulate), typically released as a group (often in a temporary vesicle) through a subapical to lateral discharge pore before dispersing individually, movement hopping or occasionally amoeboid. Resting spores not seen. Saprophytic on shed or fragmented exoskeletal material, particularly insect exuviae.

Species of *Obelidium*

(*Siphonaria petersenii* considered because of similarity in morphology)

A_1. Sporangium ovate, ellipsoid or spheroid, usually not or only the base embedded in host matrix, relatively thin-walled or wall becoming distinctly thickened and differentiated toward the base; spines (or similar pointed excrescences) one (apical) or a number (variously distributed), in any case borne on main body of sporangium, solid; zoospores mostly spheroidal; rhizoidal system usually well-developed, richly branched or with dominant trunks penetrating substrate..... B

B_1. Sporangium with one apical spine-like process (mucro) or, less commonly, two oppositely placed subapical processes; lateral sporangial spines lacking; sporangial base thin-walled or thickened and cup-, vase- or stalk-like; apophysis often present, sometimes obscure; sporangium and zoospores without special pigmentation..... C

C_1. Sporangial base thickened, cup-like, a pinched or stalk-like portion sometimes evident above the basal cup, the apical mucro prone to be elongate and spine-like; rhizoids usually similar in appearance, profusely branched, endobiotic or interbiotic.....*O. mucronatum* (1)

C_2. Sporangial base relatively thin-walled, not specially differentiated, the apical mucro usually pyramidal in form (though sometimes taper-tipped); rhizoids mostly endobiotic, with one or two main trunks extending into the substrate; secondary rhizoidal branches relatively sparse, often obscure in substrate.....*O. megarhizum* (2)

B_2. With a long apical and several lateral sporangial spines; sporangial base not thickened or cup-like; any stalk-like structure is beneath the sporangium, of rhizoidal origin, and not thickened; apophysis lacking; sporangium often with golden-orange pigmentation; zoospore lipid-globule often golden-red.....*S. petersenii*

A_2. Sporangium often obovate-oblong, main portion becoming uniformly thickened, lacking spines; two short lateral spines (barbs, with protoplasmic contents) borne oppositely on thin-walled, somewhat elongate, stalk-like portion of sporangium (this embedded in substrate); zoospores ellipsoidal; rhizoids delicate, sparse.....*O. hamatum* (3)

(1) ***Obelidium mucronatum*** Nowakowski. Cohn, Beitrag zur Biologie der Pflanzen 2: 86, 1876.

Type: Nowakowski's (1876) figs. 1-5, of Taf. V, accepted as the type.

Thallus typically interbiotic or epibiotic. Sporangium 20-56µm long, 7-20µm broad, broadly ovate or subspheroid to more narrowly elongate-ellipsoid, extramatrical or rarely intramatrical, without special color, with a single solid apical spine (mucro), less frequently with two (subapical, oppositely placed) spines; mucro simple, typically not more than one-third the sporangial height, refractive, often the first part of the

sporangium to differentiate; base of sporangial wall usually becoming thickened and cup-, vase- or funnel-like in appearance, 4-12μm broad; a stalk-like portion (immediately above the cup-like sporangial base) may also develop, this as much as 10μm long. Rhizoids intra- or extra-matrical, typically well-branched, often becoming extensively developed with finer branches spreading as much as 100μm; the primary rhizoidal axis occasionally enlarging proximally and bearing smaller, reduced branches; rhizoids rarely less branched, somewhat more delicate and sparsely developed. A small subsporangial apophysis usually present (but may be obscured by the cup-like sporangial base), developed from the inflated upper portion of the germ tube or the upper portion of the rhizoidal branches to which it is attached. Zoospores spherical to slightly ellipsoid, 2.5-3.5μm, with a flagellum ca. 20μm long and a colorless (but refractive) lipid globule, typically released *en masse* through a broad, subapical to lateral, circular exit pore before dispersing individually; a few zoospores may occasionally remain trapped inside the sporangium. Resting spores unknown. Thalli on exuviae of midges, other dipterans, and types of caddisflies. (**Fig. 1**)

Distribution: Reports (see Sparrow, 1960) from: Germany, Denmark, portions of Russia, and the United States (Michigan, Sparrow, 1938; Karling, Louisiana, 1948). Later reported: Karling (1967), New Zealand; Czeczuga et al. (2005), Poland (Supraśl River).

Discussion: *Obelidium mucronatum* is a variable species (Sparrow, 1938). Karling (1967) felt that its variation might encompass other species of the genus (with which we disagree, as discussed under these species). A central part of the identity of *O. mucronatum*, as established by Nowakowski (1876), has been the solid apical spine (mucro or "spike") terminating the zoosporangium (cf. Nowakowski, Taf.5, fig.1). Sparrow (1937) informally described, and illustrated (figs. a-i of his text Fig. 5), specimens from Massachusetts (on caddisfly integument, "pond near Hyannis"), referring to them as "*Obelidium* (?) *mucronatum*;" these specimens, exhibiting the cup-like sporangial base of *O. mucronatum*, differed by lack of an apical mucro and by a characteristically more delicate rhizoidal system. After examining typical *O. mucronatum* from Michigan, Sparrow (1938) indicated that the non-mucronate (Massachusetts) specimens should not be included in this species, and possibly constituted a new species of *Obelidium*—which he declined to describe without further study. Later, Sparrow (1960) was more equivocal on the disposition of the non-mucronate

material—zoospore discharge was still not observed—and questioned its retention in *Obelidium*. Since no additional information is available on this non-mucronate taxon, no resolution can be made as to its identity, other than to affirm that it could doubtfully be included in *O. mucronatum*, or even in the genus *Obelidium*.

(2) *Obelidium megarhizum* Willoughby, Trans. Brit. Mycol. Soc. 44: 588, text-fig. 1 & pl. 37, 1961 (with Latin description).
Type: Willoughby: Herb. I.M.I. 85111

Thallus epiboitic or restrictedly interbiotic. Sporangium extramatrical or intramatrical at the base, ovoid or ellipsoid (occasionally narrowly so) or spherioid (often assuming a broadly ovate or spheroidal shape just prior to zoospore discharge), 11-40μm long, 6-35μm broad, uniformly relatively thin-walled, with no portion of the wall especially differentiated except for the single solid apical mucro; this mucro, often refractive and of generally pyramidal form, is 2.5-8μm long and 1.5-6μm broad at the base. Apophysis sometimes evident, 5.5-8.5μm in diameter. Rhizoids relatively coarse, with one or two dominant trunks ca. 2-6.5μm broad, which may extend as much as 160μm into the substrate; lateral rhizoidal branching (sometimes obscure in the chitinous substrate) is seemingly relatively sparse. Zoospores generally spherical, 3.5-4.5μm, sometimes relatively numerous, discharged subapically (through a circular pore, 5.5-15μm broad, formed by liquefaction of a portion of the sporangial wall), becoming active within a group prior to individual dispersal. Resting spore not seen. Isolated on termite wings from culture of submerged lake mud, and on purified shrimp chitin from culture of twigs and mud from edge of a river. (**Figs. 2** and **5-11**)

Distribution: England, Malham Tarn, Yorkshire, L. G. Willoughby (reported 1961); United States, North Carolina, Rutherford County, edge of Broad River, below Bill's Mountain, 3.1 mi. northeast on Hwy 64 from Hwy 9 intersection, collected by W. Blackwell and M. Powell (WB#266G), April 9, 2005.

Discussion: Willoughby (1961) distinguished *Obelidium megarhizum* from *O. mucronatum* by the occurrence of one or two coarse, deeply penetrating, but sparingly branched, main rhizoidal trunks (as opposed to a spreading, more profusely branched rhizoidal system) and by the comparatively thin sporangial-wall base, not differentiated (not thickened and cup- or stalk-like) in comparison to

the rest of the sporangial wall. Karling (1967, 1977) indicated that these supposed *megarhizum*-features might fall within the variation of *O. mucronatum*. However, Karling's (1967) illustrations (e.g., his fig. 47) of putative rhizoidal identity between these species are not convincing; he did not deal with the other main distinguishing feature of *O. megarhizum*, i.e., the thin-walled sporangial base. Our observations of specimens of *O. megarhizum* from North Carolina support distinction of these species by the two traits suggested by Willoughby (1961). Additionally, the apical mucro in *O. megarhizum* does not typically attain the slender, elongate appearance often observed in *O. mucronatum* (and in *Siphonaria petersenii*). And, *O. megarhizum* is epibiotic (see also Willoughby's plate 37), or else more limitedly capable of interbiotic growth than *O. mucronatum*—consistent with Karling's (1967) notation, re: Willoughby's material, that rhizoidal axes were more strictly intramatrical in *O. megarhizum* than in *O. mucronatum*. The only interbiotic growth we observed in *O. megarhizum* was occasional extension of the subsporangial rhizoidal stalk beyond the chitinous substrate (**Figs. 8, 9**). Karling (1967) noted that Willoughby's (1961, fig. 1c) illustration of a "central vacuole" in a young thallus of *O. megarhizum* was probably the primary nucleus. A vacuole in a developing thallus is illustrated in our **Figs. 9** and **11**.

(3) *Obelidium hamatum* Sparrow, Proc. Amer. Phil. Soc. 78: 52, 1937 (with Latin description).

Type: Sparrow's (1937) figs. j-m of text Fig. 5 accepted as type. Thallus relatively small; main body of sporangium mostly epibiotic, 10-12μm long, 8-9μm broad, obvoid or somewhat oblong to ovoid, developing a uniformly somewhat thickened wall; lower (non-zoosporogenous) portion of sporangium thin-walled, tapering and stalk-like, 8-12μm long by ca. 4μm broad, extending into host substrate, bearing two short, oppositely placed, lateral, barb-like branches (at or just above mid-portion). Rhizoids rather poorly developed, delicate and sparsely branched (even unbranched), extending farther into substrate from the acute basal tip of the stalk-like portion of the sporangium. Apophysis lacking. Zoospores often ellipsoid, ca. 4μm by 2μm, with a single lipid globule evident, discharged laterally (often at the base of the ovate portion of the sporangium), sometimes creeping and amoeboid in behavior on surfaces encountered. Resting spore not observed. On dipteran (e.g., midge) exuviae. (**Fig. 3**)

Distribution: The type locality, collection by Sparrow ("8-IX-34"), was "Clark's Pond," New Hampshire (Sparrow, 1937). Sparrow (1960) reported additional specimens (mostly empty sporangia) in samples of exuviae from Michigan (no specific locality given).

Discussion: Sparrow (1937) formally described *O. hamatum*, and continued to recognize it as a distinct species the following year (Sparrow, 1938). Sparrow (1960) later expressed doubt as to its relationship (even inclusion in *Obelidium*), though having observed additional (if limited), similar specimen material. Karling (1967) implied that variations seen in *O. mucronatum* might cast doubt on recognition of *O. hamatum*; he did not, however, clearly document why *O. hamatum* should not be distinguished from *O. mucronatum*, and his illustrations of variation in *O. mucronatum* (reference being to his figs. 25 and 46) do not resolve the issue. Karling appeared to suppose that spines on the sporangial stalk of *O. hamatum* might be either aborted or incipient rhizoidal branches (based on forms seen in *O. mucronatum*), but such was never demonstrated. Karling (1977) became more uncertain as to whether *O. hamatum* should be a distinct species, though seeming to retain it in *Obelidium*. Neither Sparrow (1960) nor Karling (1977) suggested where *O. hamatum* might be placed, if not included in *Obelidium*. Thus, we are left with this situation: *O. hamatum* is obviously morphologically distinct—uniformly thickening, obovate sporangium; thin-walled stalk with paired "barbs;" feebly developed rhizoidal system; more ellipsoidal zoospores (cf. Sparrow, 1937)—compared with other species of *Obelidium*; yet, it resembles *Obelidium* more than species of related genera. Hence, we recognize *O. hamatum* as a species of *Obelidium* until contrary evidence is available.

Tentative placement:

Siphonaria petersenii Karling, Amer. J. Bot. 32: 580, 1945 (with Latin diagnosis).

Type: Karling's (1945, see reference above) figs. 1-26, p. 582, accepted as the type.

Thallus inter- epi- or intra-biotic. Sporangium 10-36µm long, 5-20µm broad, usually ovate or ellipsoid (rarely transversely elongated) or elongate-pyriform, typically extramatrical, often somewhat golden or orange in color, with a long (as much as 15µm) slender, unbranched, solid apical spine and 3-12 variously placed lateral spines (these usually somewhat shorter, simple or occasionally bifurcate); sporangial wall

not becoming thickened; sporangial base not specially differentiated. Rhizoids mostly intramatrical, directly connected to sporangial base, at first monoaxial but soon branching and tending to spread in a robust manner (often 80μm into the substrate), the ultimate branches fine. Apophysis lacking. Zoospores spheroidal, 3-3.5μm, a single, often golden-red lipid globule evident; zoospore discharge subapical, communal (in a vesicle) before individual dispersal. Resting spores result from a sexual process initially involving rhizoidal anastomosis, spherical to oval or somewhat angular, 6 to 15μm, reddish-brown, the thickened wall often bumpy or with low spines. Numerous thalli may develop on exuviae of dipterans such as mayflies. **(Fig. 4)**

Distribution: Reported from New York, Connecticut, and the type locality in Brazil (Karling, 1945, Flores Nabuco, Amazonas).

Discussion: Were it not for its lateral sporangial spines, *Siphonaria petersenii* (Karling, 1945) resembles *Obelidium mucronatum*. There is similarity in growth form, substrate preference, sporangial shape, and the spreading, well-branched rhizoidal system. An apophysis is not present in *S. petersenii*, whereas *O. mucronatum* is considered apophysate; however, the apophysis in *O. mucronatum* may be obscure, or even lacking (Sparrow, 1938). The subapical discharge of zoospores in *S. petersenii* is similar to *O. mucronatum* (not to the apical or basal discharge of other *Siphonaria* species, cf. Sparrow, 1960); both have a similar zoospore type (Letcher et al., 2005). In a study incorporating ultrastructural and molecular data (Vélez et al., 2011), including a relatively limited number of taxa, *O. mucronatum* and *S. petersenii* paired very closely. However, in James et al. (2006)—including more taxa, but focusing on molecular data—the relationship of these two species was less conclusive; each ultimately paired more closely with other taxa. Further molecular resolution will be required if these species are to be formally placed in the same genus. Resting spores have been observed (Karling, 1945) in *Siphonaria petersenii*, but not in taxa described as *Obelidium*; it is uncertain if this represents a real difference, or that resting spore stages (perhaps uncommon if involving heterothallism) have not yet been encountered in *Obelidium*. Were *Obelidium* species and *Siphonaria petersenii* placed in the same genus, a morphologically logical unit would result (for taxa with spiny sporangia). Although sporangia of *Asterophlyctis irregularis* bear tapered, peg-like projections, other morphological features (e.g., basal or subbasal zoospore discharge) do not suggest inclusion in *Obelidium*.

ACKNOWLEDGMENTS

We thank Dr. Sonali Roychoudhury, Patent Agent, NY, and Dr. W. Wallace Martin, Randolph-Macon College, VA, for review of this manuscript. Work supported in part by NSF Grant #DEB-0949305.

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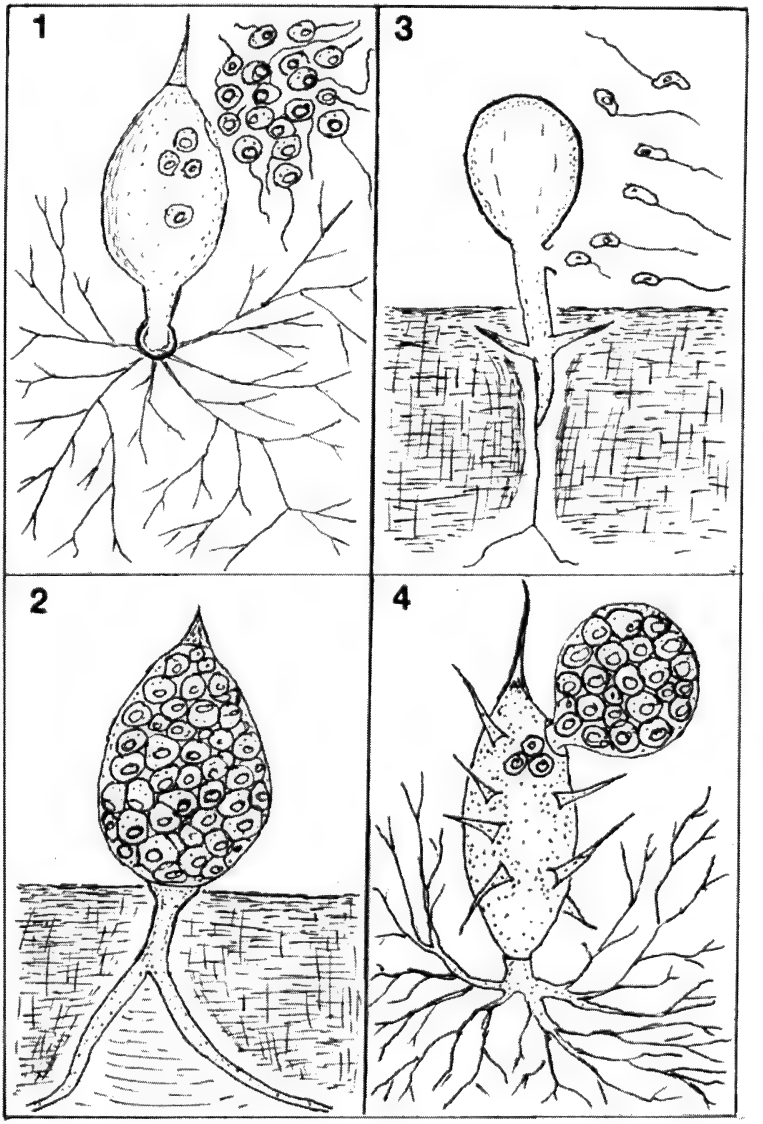
Figs. 1-4 (facing page).

Fig.1. Thallus of *Obelidium mucronatum*. Sporangium showing apical spine (mucro) and lower, stalk-like portion subtended by cup-like base; branching rhizoidal system seen extending out below this base. Spherical zoospores released, from subapical discharge pore, seen as they disperse individually (several remain trapped in sporangium).

Fig.2. *Obelidium megarhizum*. Sporangium assuming ovate form as its protoplast has undergone cleavage to form zoospores. Rhizoidal axis evident below sporangium; two prominent trunks penetrate substrate.

Fig.3. *Obelidium hamatum*. Obovate sporangium has released ellipsoid zoospores at junction with thinner-walled stalk (bearing two lateral "barbs") terminating in a sparse rhizoidal system.

Fig.4. *Siphonaria petersenii*. Ellipsoid sporangium (with spines), releasing zoospores in a vesicle. Rhizoidal system joining non-thickened sporangial base.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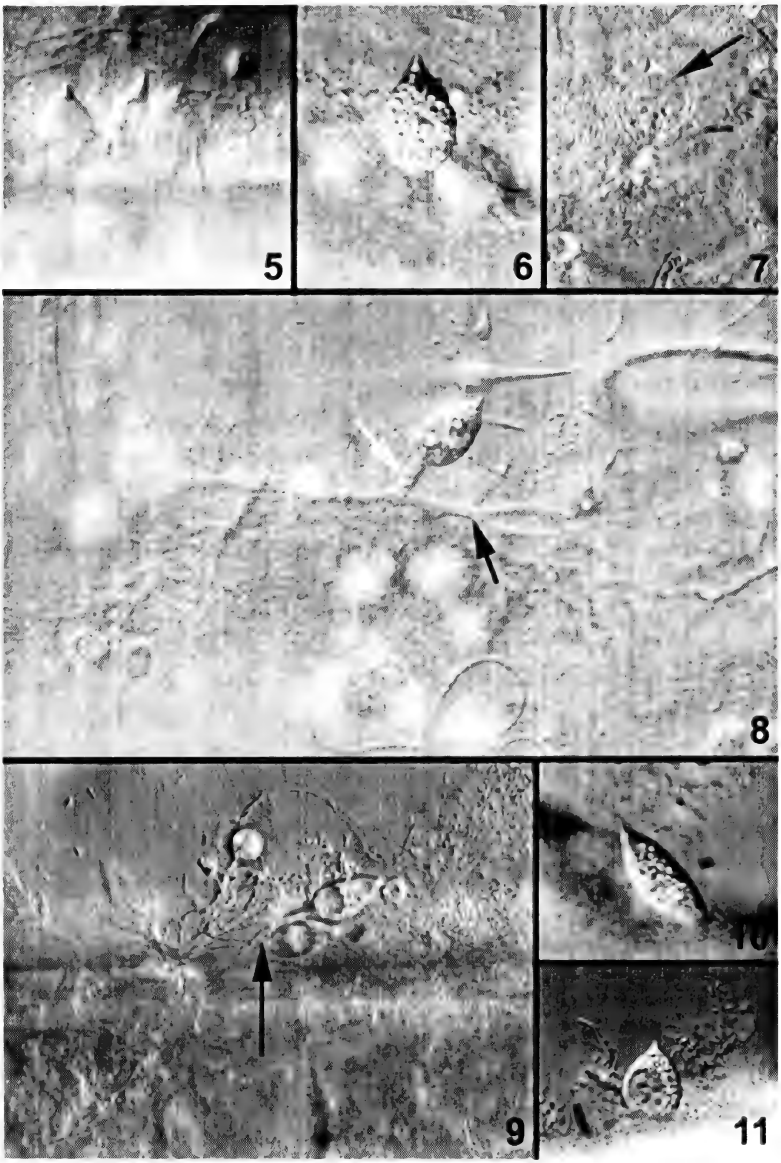
Figs.5-11 (facing page).
Obelidium megarhizum (photomicrographs, North Carolina specimens, on chitin)**:**
Fig. 5. Epibiotic sporangia.
Fig. 6. Rounded sporangium with cleaved zoospores.
Fig. 7. Sporangium after zoospore release; discharge pore (arrow) subapical to mucro.
Fig. 8. Limited interbiotic growth by extension of rhizoidal stalk (white arrow); a main rhizoidal trunk (dark arrow).
Fig. 9. Interbiotic cluster (rhizoidal stalk, arrow); vacuole in lower portion of largest sporangium.
Fig. 10. Ellipsoid sporangium.
Fig. 11. Developing, ovate sporangium; vacuole evident.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GEOGRAPHIC VARIATION IN THE LEAF ESSENTIAL OILS OF *JUNIPERUS OSTEOSPERMA* (CUPRESSACEAE) II.

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

The volatile leaf oils of *J. osteosperma* were analyzed from its western range. Four major geographical groups were found: Nevada, San Bernardino Mtns.- Mountain Pass, CA, Thistle, UT and Oak Creek Canyon, AZ. The AZ population is likely a Pleistocene relict that may account for its unusual oil. The terpene data did not indicate hybridization of *J. osteosperma* with *J. grandis* or *J. californica* in the San Bernardino Mtns. Populations from NW Nevada, reported to hybridize with *J. grandis* and *J. occidentalis,* were not included in the study but will be analyzed in a future report. *Phytologia 94(1): 118-132 (April 2, 2012).*

KEY WORDS: *J. osteosperma, J. grandis, J. occidentalis, J. californica,* Cupressaceae, terpenes, geographic variation.

Previously, Adams (1994) analyzed geographic variation in the leaf essential oils of *J. osteosperma* (Torr.) Little and reported differences among the five populations analyzed. More recently, Adams and Kauffmann (2010) analyzed 9 Nevada and California populations of *J. osteosperma* as part of a study on *J. grandis*. They reported some variation in the leaf oils of *J. osteosperma*, but did not delve deeply into geographic variation, as their focus was on *J. grandis* oils.

Terry et al. (2000) found cpDNA (trnL-trnF, trnS-trnG) haplotypes of *J. occidentalis* in Nevada populations of *J. osteosperma*, with lower frequencies occurring in Utah, Colorado, and Wyoming. Subsequently, Terry (2010) analyzed trnl-trnF and trnS-trnG (cpDNA) haplotypes and reported similar results (Fig. 1). Notice, all 15 trees of

J. occidentalis in Oregon have the same haplotype and that this haplotype is also present in northwest Nevada. Hybridization in this area was first reported by Vasek (1966) and confirmed by Terry et al. (2000) and Terry (2010). Subsequently, Terry (2010) also concluded that there was introgression from *J. occidentalis* into *J. osteosperma.*

The present study examines geographic variation in the leaf volatile oil components of *J. osteosperma.* Because terpenes are products of gene expression and interact directly with herbivores, insects and diseases in the environment, they have proved useful in the study of evolution. Our present understanding of nucleotide substitutions and indels in introns and inter-genic regions makes it difficult to discern their actual role, if any, in speciation. The area of

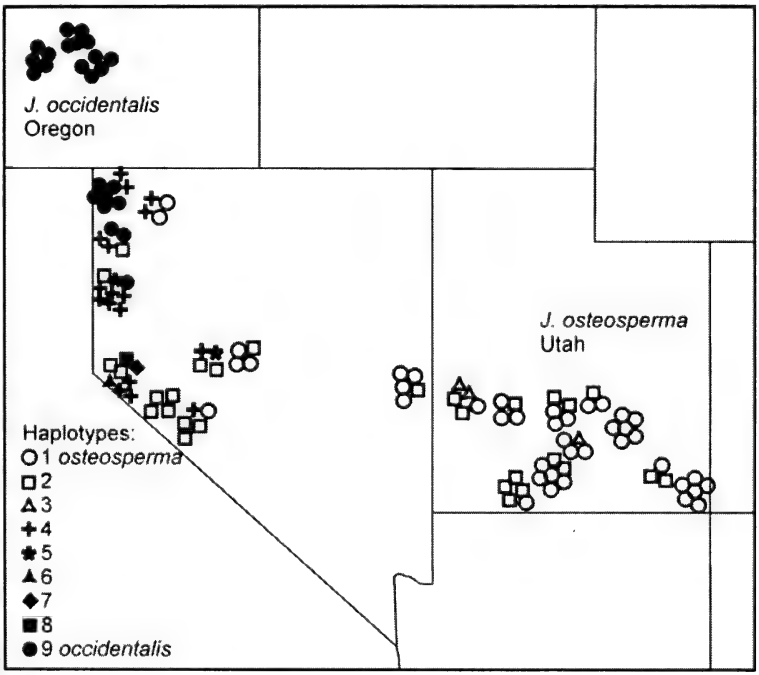

Figure 1. Distribution of haplotypes (trnL-trnF and trnS-trnG) in *J. occidentalis* and *J. osteosperma* (information from Terry, 2010).

putative hybridization in northwest Nevada is excluded from the present study and will be published in subsequent papers.

MATERIALS AND METHODS

Plant material (Fig 2): ***J. osteosperma****, Adams 1689-1699, 1701-1705,* on US 6, Thistle, 40° 00' 6.9" N, 111° 29' 4.6" W, 1650 m, Utah Co., UT, *Adams 12067-12071*, 4 km n of Sedona, AZ, at Grasshopper Point,

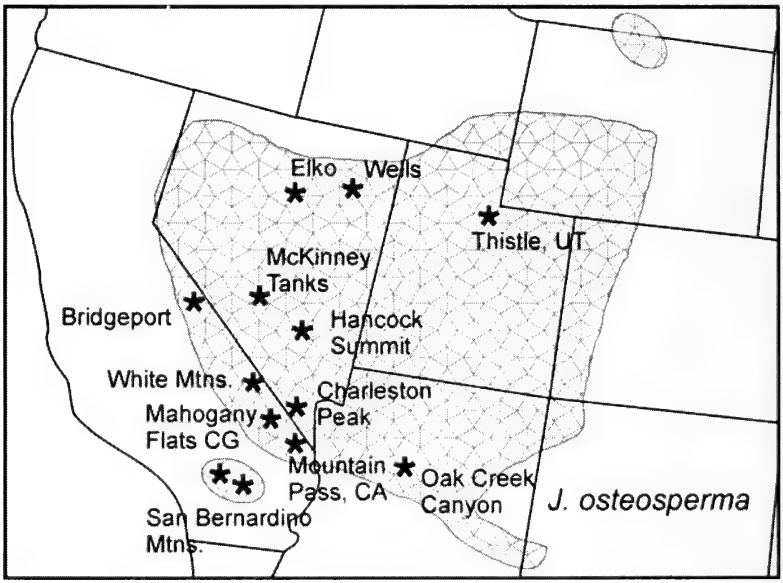

Figure 2. Distribution of *J. osteosperma* with populations sampled in this study.

on Alt US 89, 34.888° N, 111.733° W, 1380m, Coconino Co., AZ, *Adams 10272-10276,* on NV157, Charleston Mtns., 36° 16.246' N, 115° 32.604' W, 1795 m, Clark Co., NV; *Adams 11122-11124*, Hancock Summit, mile 38 on US 375, 37° 26.404' N, 115° 22.703' W, 1675 m, Lincoln Co. NV; *Adams 11125-11127*, McKinney Tanks Summit on US 6, 38° 07.005' N, 116° 54.103' W, 1933 m, Nye Co., NV; *Adams 11134-36*, 8 km s of Bridgeport, on US395, 38° 12.639' N, 119° 13.846' W, 2004 m, Mono Co., CA; *Adams 11141-11143,* 13 km w of Elko, on

I 80, 40° 45.598' N, 115° 55.942' W, 1535 m, Elko Co., NV; *Adams 11144-11146*, 8 km e of Wells, on I 80, 41° 06.533' N, 114° 51.441' W, 1876 m, Elko Co., NV; *Adams 11960-11962,* 56 km n of Reno, NV; on US 395, 39° 54.458' N, 120° 00.322' W, 1383 m, Lassen Co., CA; *Adams 11973-11977,* 10 km n of CA 168 on White Mtn. Rd., 37° 20.143' N, 118° 11.346' W, 2607 m, Inyo Co., CA; *Adams 11978-11982,* Mahogany Flats Campground, Panamint Mtns., 36° 13.783' N, 117° 04.102' W, 2477 m, Inyo Co., CA, *Adams 12323-12327*, Basin, San Bernardino Mtns., 34° 16.910' N, 116° 45.306' W, 1820 m, San Bernardino Co., CA, *Adams 12210-12214*, ca. 1 km e of CA 18, ca. 16 km s of jct CA 18 & CA 247, n slope San Bernardino Mtns., 34° 21.213' N, 116° 50.607' W, 1393 m, San Bernardino Co., CA, *Adams 12215-12219*, on I15, at Bailey Rd., 35° 27.938' N, 115° 31.709' W, 1431 m, San Bernardino Co., CA.

J. grandis, *Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003' N, 118° 02.078' W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34° 17.533' N, 116° 49.153' W, 2053 m, San Bernardino Co., CA; *Adams 11963-11967*, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; *Adams 11968-11972*, 16 km w of Sonora Jct., on CA Hwy. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA, *Adams 11984-11988*, Nine Mile Canyon Rd., 20 km w of Jct. with US 395, 35° 54.003' N, 118° 02.078' W, 2059 m, Tulare Co., CA; *Adams 11989-11993*, 5km n Big Bear City on CA 18, 34° 17.533' N, 116° 49.153' W, 2053 m, San Bernardino Co., CA; *Adams 12319-12322,* Onyx Summit on CA 38, 34° 11.524'N; 116° 43.227' W.2600 m, San Bernardino Co., CA; *Adams 12328-12331, 12367,* Donner Pass Summit on old US50, 39° 18.999' N; 120° 19.581' W. 2180 m, Placer Co., CA; *Adams 12332-12336,* on Stampede Meadows Rd. (Co. rd 894A alt), 5 mi. n of I80. 39° 24.966' N, 120° 05.249' W, 1660 m, Nevada Co., CA; *Adams 12337-12341,* 4.7 mi. n of Beckwourth on Beckwourth-Genesee Rd., 39°52.433' N, 120° 24.345' W, 1770 m, Plumas Co., CA.

J. occidentalis, *Adams 11940-11942,* 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392' N, 120° 41.207' W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km

ne of Madras, OR; 44° 53.676' N, 120° 56.131' W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44° 02.390' N, 121° 20.054' W, 1132 m, Deschutes Co., OR; *Adams 11949-11951,* 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922' N, 120° 59.187' W, 1274 m, Deschutes Co., OR; *Adams 11952-11954,* 14 km e of Jct. OR66 & I 5, on OR66, 42° 08.044' N, 122° 34.130' W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA 299, 10 km e of McArthur, CA, 41° 05.313' N, 121° 18.921' W, 1091 m, Lassen Co., CA; *Adams 11995-11998 (Kauffmann A1-A3, B1),* Yolla Bolly-Middle Eel Wilderness, 40° 06' 34" N, 122° 57' 59" W, 1815-2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178' N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867' N, 120° 28.456' W, 1695 m, Lassen Co., CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type 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 (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The oils of *J. osteosperma* are dominated by camphor (19.7 - 60.2%) and bornyl acetate (4.4 - 19.7%, Table 1), with moderate amounts of sabinene, α-pinene, borneol and terpinen-4-ol. For comparison, typical oils of *J. grandis* and *J. occidentalis* (Table 1) have little camphor (0, 2.5%) or borneol (0, 2.2%). The oil of *J. occidentalis* has large amounts of sabinene, p-cymene, citronellol and bornyl acetate (Table 1), whereas *J. grandis* oil is dominated by δ-3-carene, α-pinene and β-phellandrene (Table 1).

To examine geographic trends in the leaf essential oils, contours of the cluster levels were plotted (Figure 3). The overall trend is that *J. osteosperma* oils in the central portion of Nevada are very uniform (notice contour similarity levels of 0.84 - 88, Fig. 3). The major divergences are the Thistle, Utah population, the San Bernardino

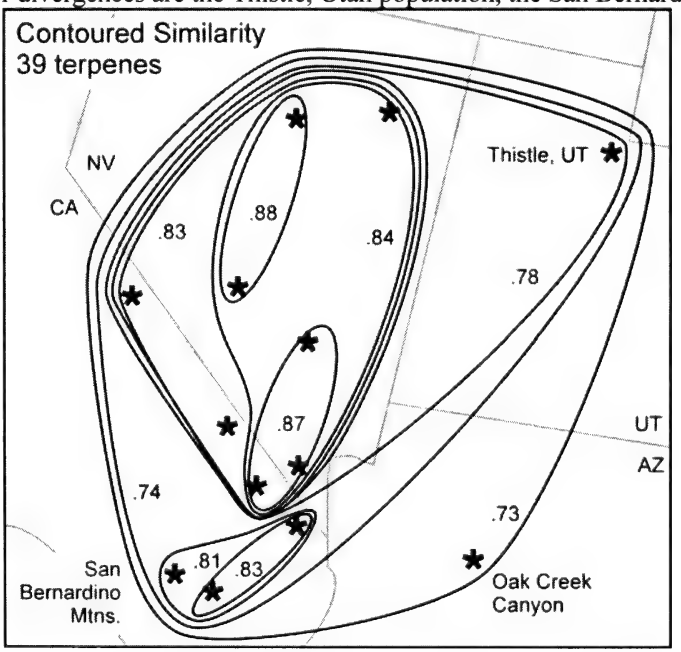

Figure 3. Contoured similarities of populations (see Fig. 2) of *J. osteosperma* based on 41 terpenes.

Mtns. - Mountain Pass, CA populations with the Oak Creek Canyon, AZ population being the most differentiated (Fig. 3). Comparison of the McKinney Tanks, Utah, San Bernardino Mtns., and Oak Creek Canyon AZ oils (Table 1) shows differences in sabinene, myrcene, camphor, terpinen-4-ol and bornyl acetate, but overall, these oils are very similar.

Principal Coordinate analysis of the terpene similarities matrix resulted in eigenroots that accounted for 24, 17, 10 and 9% of the variation among populations of *J. osteosperma*. Ordination reveals four groups: Nevada, San Bernardino Mtns. - Mountain Pass, CA, Utah and, the most differentiated population, Oak Creek Canyon, AZ. The Bridgeport population was not very different in the contoured similarities (Fig. 3), so this may be a feature of the ordination of 4 dimensions into 3 dimensions.

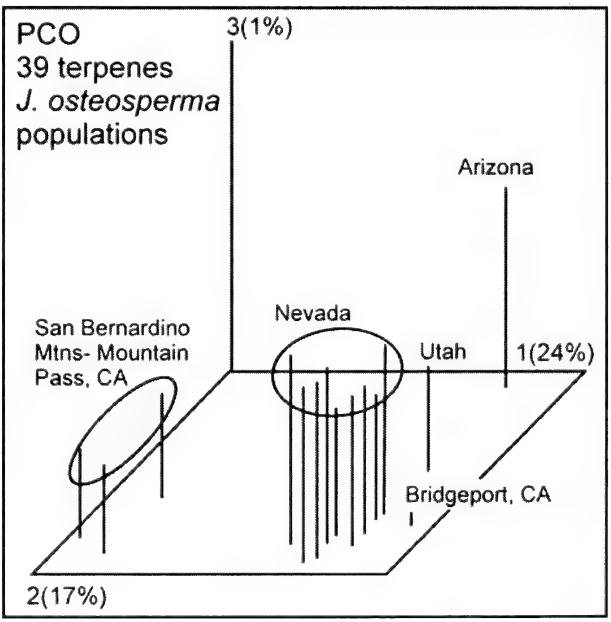

Figure 4. PCO of 13 *J. osteosperma* populations based on 39 terpenes.

Of immediate interest is whether the divergence of *J. osteosperma* in the San Bernardino Mtns.-Mountain Pass is due to introgression from *J. grandis* in the San Bernardino Mtns., where the two species are essentially sympatric in the Basin. Terpenoids have been useful for the detection of hybridization due to their complementary inheritance (Adams 1983, Irving and Adams, 1973). PCO was performed using *J. grandis* from the San Bernardino Mtns. The resulting ordination is shown in Fig. 5. There is no evidence that the San Bernardino Mtns. *J. osteosperma* populations are any more similar to *J. grandis* than the other *J. osteosperma* populations, far removed from the San Bernardino Mtns. (Fig. 5). Thus, the divergence of the San Bernardino Mtns. group does not appear to be due to hybridization with *J. grandis*.

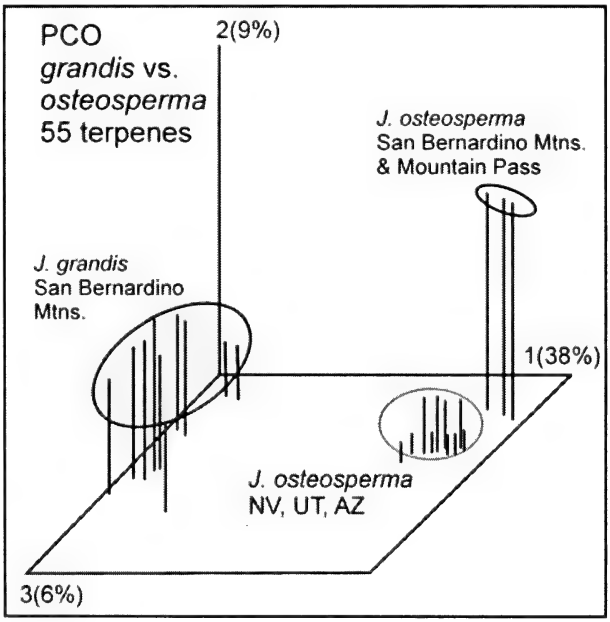

Figure 5. PCO analysis of *J. osteosperma* vs. *J. grandis* individuals from the San Bernardino Mtns.

Juniperus osteosperma grows on the north side of the San Bernardino Mtns. along CA 18 at 1393 m, in a very dry, desert

environment often occupied by *J. californica* (which grows at lower elevation nearby). In fact, trees at this population have been misidentified as *J. californica* in herbaria (pers. obs.). The population of *J. osteosperma* along I15 at Mountain Pass, CA is also near the *J. californica* populations, so it is of interest to compare *J. californica* with *J. osteosperma* so as to assess possible introgression into *J. osteosperma* in the San Bernardino Mtns. - Mountain Pass populations. PCO utilizing *J. californica* from Palmdale and Yucca Valley resulted in a clear separation of the San Bernardino Mtns. - Mountain Pass *J. osteosperma* populations from *J. californica*, with no evidence that the *J. osteosperma* populations are introgressants from *J. californica* (Fig. 6).

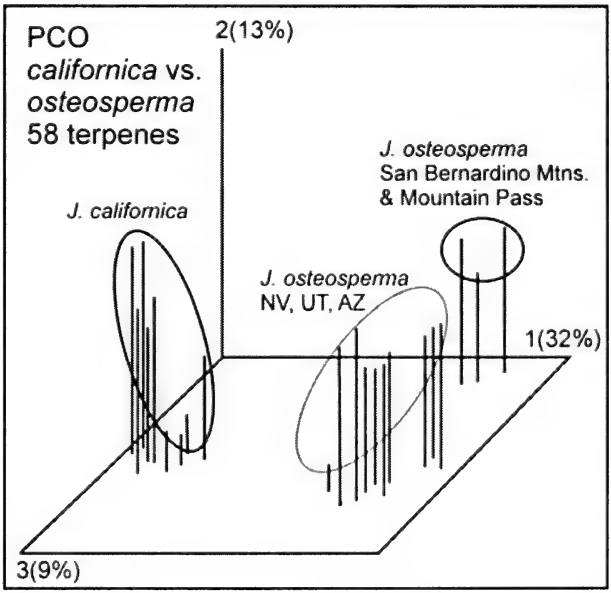

Figure 6. PCO of *J. californica* and *J. osteosperma* based on 58 terpenes.

In summary, geographic variation found in the volatile leaf oils of *J. osteosperma* consists of four major groups: Nevada, San Bernardino Mtns.- Mountain Pass, CA, Thistle, UT and Oak Creek Canyon, AZ. The AZ population may be a Pleistocene relict which

would account for its unusual oil. Life zones descended 300-1100m in the southwestern US during the Pleistocene (Adams 2011), so *J. osteosperma* was likely growing at much lower elevations in Arizona. No evidence of hybridization was found between *J. osteosperma* and *J. grandis* or with *J. californica* in the San Bernardino Mtns. Populations from NW Nevada reported to hybridize with *J. grandis* and *J. occidentalis* (Vasek, 1966; Terry et. al. 2000; Terry, 2010) were not included in the study, but these will be analyzed in a future report.

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Table 1. Leaf essential oil compositions for *J. osteosperma* (McK =McKinney Tanks, NV, UT = Thistle, UT, SB = San Bernardino Mtns., Basin, AZ = Oak Creek Canyon, AZ, *J. occidentalis* (Bend, OR) and *J. grandis* (Meyers, CA). Compounds in boldface appear to separate *J. osteosperma* populations.

KI	Compound	ost McK	ost UT	ost SBM	ost AZ	occ Bnd	gran Mey
921	**tricyclene**	**0.8**	**0.3**	**0.3**	**1.0**	**1.1**	**-**
924	α-thujene	0.5	0.3	0.5	0.2	1.0	-
932	**α-pinene**	**4.4**	**1.1**	**5.6**	**2.0**	**5.0**	**14.0**
945	α-fenchene	-	-	t	-	t	1.5
946	**camphene**	**1.1**	**0.5**	**0.4**	**1.0**	**1.0**	**-**
953	thuja-2,4-diene	t	t	0.2	0.1	t	t
961	verbenene	-	-	-	-	-	2.9
969	**sabinene**	**10.2**	**8.3**	**7.5**	**1.4**	**12.0**	**-**
974	β-pinene	0.2	0.1	0.2	0.1	0.4	1.3
988	**myrcene**	**1.7**	**1.0**	**1.1**	**0.6**	**1.3**	**3.1**
1001	δ-2-carene	-	-	t	-	t	1.1
1002	α-phellandrene	0.3	0.1	0.3	t	0.8	1.6
1008	δ-3-carene	-	t	t	t	1.0	27.3
1014	**α-terpinene**	**1.3**	**0.5**	**1.6**	**0.3**	**1.7**	**0.4**
1020	p-cymene	2.4	1.6	2.8	1.5	10.7	1.4
1024	limonene	2.1	1.6	2.1	2.4	0.9	1.2
1025	β-phellandrene	3.2	1.7	2.0	1.5	3.5	10.6
1044	(E)-β-ocimene	t	t	0.2	t	0.1	t
1054	**γ-terpinene**	**2.1**	**1.2**	**2.6**	**0.6**	**3.0**	**0.3**
1065	cis-sabinene hydrate	0.8	1.7	1.0	0.3	0.9	-
1078	camphenilone	t	t	t	t	-	-
1086	terpinolene	1.4	0.6	1.2	0.4	1.3	3.7
1090	6,7-epoxy-mycene	0.1	t	t	t	-	-
1092	96, 109,43,152	-	-	-	-	-	0.9
1095	linalool	t	t	t	t	0.5	t
1098	trans-sabinene hydrate	1.0	2.1	1.4	0.4	0.7	-
1100	55,83,110,156	-	-	-	-	0.3	-
1102	isopentyl-isovalerate	0.2	t	t	-	-	-

KI	Compound	ost McK	ost UT	ost SBM	ost AZ	occ Bnd	gran Mey
1112	3-me-3-buten-me-butanoate	0.4	t	0.2	t	-	-
1112	trans-thujone	-	-	-	t	t	-
1118	**cis-p-menth-2-en-1-ol**	**0.6**	**1.1**	**0.8**	**0.4**	**0.7**	**0.8**
1122	α-campholenal	0.3	0.2	0.6	0.4	-	t
1136	trans-p-menth-2-en-1-ol	-	-	-	-	0.9	0.9
1141	**camphor**	**23.7**	**19.6**	**25.6**	**60.2**	**2.5**	**-**
1144	neo-isopulegol	-	-	-	-	-	0.5
1145	**camphene hydrate**	**1.5**	**2.7**	**1.3**	**2.0**	**0.2**	**t**
1154	sabina ketone	0.8	1.4	1.1	0.5	0.4	-
1165	**borneol**	**6.0**	**4.3**	**7.2**	**3.0**	**2.2**	**-**
1166	coahuilensol	-	-	-	-	0.6	t
1174	**terpinen-4-ol**	**8.3**	**10.7**	**12.6**	**3.2**	**6.7**	**0.4**
1176	m-cymen-9-ol	-	-	-	-	-	0.4
1179	p-cymen-8-ol	0.5	1.4	1.0	0.5	0.5	0.4
1186	α-terpineol	0.4	0.7	0.5	0.4	0.4	1.2
1195	myrtenol	0.2	0.4	0.3	0.3	-	-
1195	cis-piperitol	0.3	0.4	t	t	0.2	0.4
1204	verbenone	0.2	0.3	0.6	0.8	-	-
1207	trans-piperitol	0.3	0.3	0.7	-	0.3	0.9
1215	trans-carveol	0.6	0.7	1.0	1.0	-	-
1219	**coahuilensol, me-ether**	**0.2**	**-**	**0.2**	**0.4**	**1.1**	**0.4**
1223	citronellol	t	t	0.7	0.4	8.4	t
1230	43,119,152,194	-	-	-	-	-	3.9
1238	cumin aldehyde	0.3	0.3	0.4	0.1	0.2	-
1239	carvone	0.6	0.8	0.6	0.8	-	t
1249	piperitone	t	-	t	-	0.2	1.2
1254	linalool acetate	-	-	-	-	0.1	-
1255	4Z-decenol	-	-	-	-	-	0.4
1257	me-citronellate	-	-	-	-	-	0.2
1274	neo-isopulegyl acetate	-	-	-	-	-	0.3
1283	α-terpinen-7-al	0.2	-	0.5	-	-	-
1284	**bornyl acetate**	**16.6**	**19.7**	**5.5**	**4.4**	**9.5**	**0.4**
1285	safrole	-	-	-	-	-	0.3

KI	Compound	ost McK	ost UT	ost SBM	ost AZ	occ Bnd	gran Mey
1298	carvacrol	t	0.2	t	t	0.4	0.2
1319	149,69,91,164	0.4	t	0.6	0.4	-	0.8
1322	me-geranate	-	-	-	-	1.0	-
1325	p-mentha-1,4-dien-7-ol	0.5	0.5	1.0	0.1	t	
1332	cis-piperitol acetate	-	-	-	-	-	0.4
1343	trans-piperitol acetate	-	-	-	-	-	0.3
1374	α-copaene	-	-	-	-	1.0	-
1387	β-bourbonene	-	-	-	-	0.2	0.5
1388	79,43,91,180	-	-	-	-	-	0.3
1389	111,81,151,182	-	-	-	-	-	1.0
1429	cis-thujopsene	0.7	-	-	-	0.9	-
1451	trans-muurola-3,5-diene	-	-	-	-	0.1	-
1465	cis-muurola-4,5-diene	-	-	-	-	0.1	-
1468	**pinchotene acetate**	**0.5**	**-**	**0.3**	**1.0**	**0.6**	**-**
1475	trans-cadina-1(6),4-diene	-	-	-	-	0.3	-
1478	γ-muurolene	-	-	-	-	0.8	-
1484	germacrene D	-	-	-	-	0.3	0.2
1493	trans-muurola-4(14),5-diene	-	-	-	-	0.4	-
1493	epi-cubebol	-	-	t	-	0.4	-
1500	α-muurolene	t	t	-	-	1.1	0.3
1513	γ-cadinene	t	t	t	-	3.7	1.3
1518	epi-cubebol	-	-	-	-	0.4	0.4
1522	**δ-cadinene**	**0.2**	**0.3**	**0.2**	**-**	**4.1**	**1.1**
1533	trans-cadina-1,4-diene	-	-	-	-	0.1	-
1537	α-cadinene	-	-	-	-	0.4	t
1544	α-calacorene	-	-	-	-	0.3	-
1548	elemol	0.9	0.6	2.5	1.6	-	-
1555	elemicin	-	-	-	-	-	1.5
1574	germacrene-D-4-ol	t	0.2	0.2	-	0.6	0.7

KI	Compound	ost McK	ost UT	ost SBM	ost AZ	occ Bnd	gran Mey
1582	caryophyllene oxide	t	0.1	0.1	t	-	t
1586	gleenol	-	-	-	-	0.3	-
1607	β-oplopenone	t	t	t	t	0.4	0.4
1608	humulene epoxide II	t	t	t	0.1	-	-
1618	1,10-di-epi-cubenol	-	-	-	-	0.2	t
1627	1-epi-cubenol	-	-	-	-	1.6	t
1630	γ-eudesmol	0.2	t	0.3	0.2	-	-
1638	epi-α-cadinol	t	0.2	0.1	-	1.1	0.7
1638	epi-α-muurolol	t	0.2	0.2	-	1.2	0.7
1644	α-muurolol	-	t	t	-	0.7	t
1649	β-eudesmol	0.2	t	0.4	0.2	-	0.4
1652	α-eudesmol	0.2	0.3	0.3	0.1	-	-
1652	α-cadinol	0.2	0.4	0.5	0.2	1.8	1.6
1670	bulnesol	t	t	0.2	0.1	-	-
1675	cadalene	-	-	-	-	0.3	-
1684	2Z,6Z-farnesal	-	-	0.2	-	-	-
1688	shyobunol	-	-	-	-	-	0.2
1739	oplopanone	t	t	t	t	-	t
1987	manoyl oxide	-	-	-	-	3.2	t
2009	epi-13-manoyl oxide	-	-	-	-	t	-
2056	manool	-	-	-	-	-	t
2055	abietatriene	-	-	-	-	-	t
2298	4-epi-abietal	-	-	-	-	-	t
2312	abieta-7,13-diene-3-one	0.1	t	0.2	0.4	-	-

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified cpds. less than 0.5% are not reported.

CHEMOSYTEMATICS OF DOUGLAS FIR *(PSEUDOTSUGA MENZIESII)*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

A comparison of the essential oil from fresh and air-dried leaves of Douglas fir (*Pseudotsuga menziesii*) revealed relatively minor changes in the oil composition (on a percent total oil basis), except for terpinen-4-ol, which declined from 12.2% to 7.8 and 8.6% when dried at 21°C and 42°C (Table 1). Citronellyl and geranyl acetates appear to increase with drying and storage for the first week, then are stable. It seems that careful air drying of Douglas fir leaves can result in the conservation of the terpenoid profile in the composition. This appears to be a solution to the problem of the transport of fresh materials across international borders. *Phytologia 94(1):133-138 (April 2, 2012).*

KEY WORDS: *Pseudotsuga menziesii,* Douglas fir, oils from dried leaves, chemosystematics.

Recently, Adams (2010, 2011) reported the effects of air-drying leaves on the essential oil composition of *Juniperus pinchotii* Sudw. and *J. virginiana* L. He found that gently air-drying (21-42°C) had generally small effects on the oil compositions in these *Juniperus* species, similar to the results reported for *J. thurifera* (Achak et al., 2008, 2009).

Our lab is currently involved in a study of Douglas fir (*Pseudotsuga menziesii*) from throughout its range. The transport of fresh materials from Mexico to our lab has presented considerable difficulties with government customs agents. However, herbarium vouchers are generally (in the author's experience) permitted without

too much difficulty. Part of the ease of importing herbarium specimens is because specimens are frozen to kill insects, then air dried. In order to facilitate this project, a small study was undertaken to evaluate the effects of leaf-drying on the essential oils from leaves of Douglas fir.

The purpose of this study was to determine if the changes in oil composition upon air-drying the leaves of Douglas fir would preclude their use in chemosystematics.

MATERIALS AND METHODS

Plant material - *Pseudotsuga menziesii*, *Adams 12918*, Olympic National Forest, Port Angeles, WA, 48° 02' 48.1" N, 123° 25' 04.08" W, 525 m. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Fresh (200 g.) and air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven-dried (48h, 100° C) for the determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

Table 1 shows the composition of the leaf oil of *Pseudotsuga menziesii,* and a comparison of components, over the 5 month storage period. The compounds are remarkably stable during the drying and

storage tests. The major components (α-pinene, sabinene, β-pinene, γ-terpinene, terpinolene) show small changes, except for terpinen-4-ol, which declined from 12.2% to 7.8 and 8.6% when dried at 21°C and 42°C, then increased after one and 2 months (Table 1). Citronellyl and geranyl acetates appear to increase with drying and storage for the first week, then they are stable.

Comparing the storage tests oils, with var. *menziesii* (var. *menz*, Table 1) and var. *glauca* (var. glauc, Table 1) shows that oils from both fresh and dried leaves of coastal Douglas fir (var. *menziesii*, Olympic Natl. Forest) are clearly in the published ranges of von Rudloff (1973).

Three kinds of variation seem apparent (Fig. 1): α-pinene, β-pinene and sabinene; γ-terpinene and terpinolene; and terpinen-4-ol. The decline of terpinen-4-ol in leaves air-dried (24 h, at 21°C and 42°C), and similar decline in the 5 month sample (Table 1), is not understood and deserves additional study.

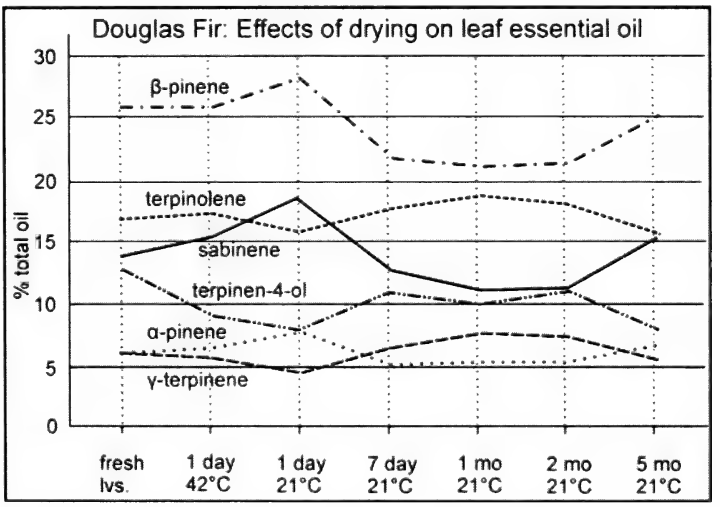

Figure 1. Variation in the major terpenoids of Douglas fir with leaf drying.

In conclusion, it appears that careful air-drying of Douglas fir leaves can result in the conservation of the terpenoid profile in the composition. This appears to be a solution to the problem of transport of fresh materials across international borders.

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Table 1. Comparison of leaf oil compositions for *Pseudotsuga menziesii* var. *menziesii* (Olympic National Forest, WA): fresh leaves, air dried 42°C then distilled, air dried at 21°C, then stored at 21°C for 1 day, 1 month, 2 months and 5 months. var. *menz.* = coastal type (var. *menziesii*), von Rudloff (1973), var. *glauc* = Rocky Mtn. type (var. *glauca*), von Rudloff (1973).

KI	compound	fresh lvs.	21°C 24h	42°C 24h	21°C 1 mo	21°C 2 mo	21°C 5 mo	var. *menz.*	var. *glauc*
884	santene	-	-	-	-	-	-	-	3-5
921	tricyclene	t	t	t	t	t	t		2.4-4
924	α-thujene	0.4	0.6	0.7	0.6	0.6	0.8	-	
932	α-pinene	6.2	7.6	6.5	4.8	4.8	6.4	7-15	15-20
946	camphene	0.4	0.4	0.4	0.3	0.3	0.4	0-0.2	20-30
969	sabinene	14.0	18.6	15.4	11.2	11.2	15.5	2-15	0.1-0.5
974	β-pinene	25.8	28.3	25.9	21.2	21.3	25.2	25-30	5-10
988	myrcene	1.3	1.5	1.5	1.2	1.2	1.3		
1002	α-phellandrene	0.2	0.2	0.3	0.5	0.4	0.3		
1008	δ-3-carene	0.5	0.7	0.8	0.5	0.4	0.3		
1012	1,4-cineole	t	t	t	t	t	t		
1014	α-terpinene	3.4	2.7	3.3	4.3	4.2	3.5	2-5	0-0.3
1020	p-cymene	0.1	0.1	0.2	0.3	0.2	0.4		
1024	limonene	0.8	0.8	1.0	1.0	1.0	0.9	0.5-1.5	5-10
1025	β-phellandrene	1.7	1.8	2.0	2.0	2.0	1.9		
1054	γ-terpinene	5.8	4.5	5.6	7.4	7.2	5.6	3-8	0.1-1
1065	cis-sabinene hydrate	0.4	0.3	0.4	0.4	0.4	0.4		
1086	terpinolene	16.8	15.8	17.3	18.5	18.0	15.6	5-20	0.5-3
1098	trans-sabinene hydrate	0.3	0.2	0.4	0.2	0.2	0.3		
1098	linalool	0.2	0.1	0.1	0.3	0.3	0.2		
1118	endo-fenchol	t	t	t	t	t	t		
1118	cis-p-menth-2-en-1-ol	0.7	0.5	0.6	0.6	0.7	0.6		
1130	1-terpineol	t	0.1	0.1	0.1	0.2	0.1		
1136	trans-p-menth-2-en-1-ol	0.5	0.4	0.4	0.6	0.7	0.5		
1145	camphene hydrate	t	t	t	t	t	0.1		
1148	citronellal	0.6	0.5	0.3	0.7	0.8	0.5		
1165	borneol	t	t	t	t	t	t		
1174	terpinen-4-ol	12.2	7.8	8.6	10.2	11.4	8.2	5-15	0.5-3
1186	α-terpineol	1.9	0.9	1.0	1.1	1.3	0.3	1-3	0.2-1
1195	cis-piperitol	t	t	t	0.2	0.1	t		
1207	trans-piperitol	0.2	0.1	0.2	0.2	0.2	0.2		
1223	citronellol	0.5	0.4	0.3	0.8	0.5	0.8	1-5	0.1-1
1287	bornyl acetate	0.1	0.1	0.1	0.2	0.2	0.1	0-0.3	20-30
1350	citronellyl acetate	1.9	1.9	2.9	2.5	2.6	2.6	2-4	0.1-2
1379	geranyl acetate	1.9	1.7	2.3	2.4	2.7	2.1	1-3	0.1-1

KI	compound	fresh lvs.	21°C 24h	42°C 24h	21°C 1 mo	21°C 2 mo	21°C 5 mo	var. *menz.*	var. *glauc*
1452	α-humulene	0.1	0.1	0.1	0.3	0.3	0.2		
1483	α-amorphene	t	t	t	t	t	t		
1483	germacrene D	t	t	t	t	t	t		
1638	epi-α-cadinol	t	t	t	t	t	t		
1638	epi-α-muurolol	t	t	t	t	t	t		
1652	α-cadinol	0.1	0.1	t	0.1	0.1	0.2		
2300	tricosane (C23)	0.1	0.1	0.1	t	t	0.1		

KEYS TO THE FLORA OF FLORIDA - 30, *LIATRIS* (COMPOSITAE)

Daniel B. Ward
Department of Botany, University of Florida
Gainesville, Florida 32611, U.S.A.

ABSTRACT

Liatris (Compositae) is represented in Florida by 14 species, four of which are treated as of two varieties, with *L. spicata* var. ***savannensis*** and *L. gracilis* var. ***gholsonii*** recognized as new combinations. Four species and two varieties are endemic, and two of the endemic species are rated as endangered. One species is excluded. An amplified key is given to the Florida taxa. *Phytologia 94(1): 139-146 (April 2, 2012).*

KEY WORDS: *Liatris*, Compositae, Florida flora.

For two-thirds of a century the painstakingly detailed monograph of *Liatris* (Compositae) by Lulu O. Gaiser (Rhodora 48: 165-183, 216-263, 273-326, 331-382, 393-412. 1946) has been the accepted standard. Gaiser reported 13 species for Florida, all well-defined and with adequately discrete ranges. Though the names may have been changed, the taxa she recognized were not far dissimilar from those of J. K. Small (1933).

But time, further observations, and especially a willingness to recognize at specific rank obscure taxa that might once have been disregarded or ranked as varieties, have put Gaiser's work in need of subtle revisions. Guy L. Nesom (Fl. N. Amer. 23: 512-535. 2006) has produced a synopsis of *Liatris* across the continent, and Richard P. Wunderlin (Guide Vasc. Plants Fla. 1998, et seq.) has briefly summarized the Florida species. Still, there is room for further interpretation, to bring the treatment of *Liatris* into line with other genera of the present series.

Here, 14 species are recognized as native to Florida. The peculiar *Liatris ohlingerae*, endemic to the mid-peninsula scrub and separated by Small as *Ammopursus ohlingerae* (Bull. Torrey Bot. Club 51: 392-393. 1924), is retained in *Liatris*, in agreement with Nesom and Wunderlin, and with R. M. King & H. Robinson (Taxon 19: 6-11. 1970) who reported setae on the achene to be of a distinctive type in common with other *Liatris*. Its morphology and life history have been closely examined by Olga Lakela (Sida 1: 240-247. 1964) -- who retained the Sand-torch in *Ammopursus* -- and Alan Herndon (Fla. Fish and Wildl. Conserv. Comm., Tallahassee. 1999).

Other adjustments involve more recently recognized taxa, one worthy of specific rank, others less well defined. *Liatris provincialis*, an endemic of the Florida panhandle well documented by R. K. Godfrey (Amer. Midl. Nat. 66: 466-470. 1961), seems adequately separated from the more widespread *L. chapmanii*. Its range coincides quite exactly with that of other endemics of the mid-panhandle coastal lowlands (*Cuphea aspera*, *Harperocallis flava*, *Hypericum chapmanii*, *Pinguicula ionantha*, etc.). *Liatris elegans* var. *kralii* of the western panhandle, Alabama, and Georgia, was prudently reported by Mark H. Mayfield (Sida 20: 597-603. 2001) as a variety. The similar *Liatris pauciflora* and *L. secunda* have elsewhere been combined at varietal level (D. B. Ward, Novon 14: 365-371. 2004).

Two other recent novelties are believed also to merit only infraspecific rank. *Liatris gholsonii*, of the Apalachicola River bluffs in the central panhandle, differs from *L. gracilis* in several quantitative but intergrading characters, making identification uncertain if knowledge of the source is unknown. Though the Apalachicola bluffs are the home of several impressive endemics (*Torreya taxifolia*, *Taxus floridana*, *Croomia pauciflora*, etc.), these are of relic status, ancient species far removed in both geography and morphology from their congeners, while the new *Liatris*, with its scant separation from typical *L. gracilis*, must be presumed to be of relatively recent origin. In age it is perhaps to be compared with the many, modestly differentiated endemics of the geologically recent, glacially immersed coastal lowlands of the central Florida panhandle.

Liatris savannensis of the southwestern peninsula may readily pass for the rather frequent and quite widespread *L. spicata*. The differentiating characters, though often apparent, intergrade to such an extent that judgment as to taxon is uncomfortably subjective.

Liatris gracilis Pursh var. ***gholsonii*** (L. C. Anderson) D. B. Ward, comb. et stat. nov. Basionym: *Liatris gholsonii* L. C. Anderson, Sida 20: 98. 2002. TYPE: U.S.A. Florida: Liberty Co., shaded upper slopes of No Name Ravine on the Nature Conservancy's Apalachicola Bluffs and Ravines Preserve, 13 Sept 2001, *L.C. Anderson 19932* (holotype, BRIT; isotypes, FSU, MO, NY).

Liatris spicata (Linnaeus) Willdenow var. ***savannensis*** (Kral & Nesom) D. B. Ward, comb. et stat. nov. Basionym: *Liatris savannensis* R. Kral & G. L. Nesom, Sida 20: 1574. 2003. TYPE: U.S.A. Florida: Charlotte Co., wet pine/cabbage palm flats, ca. 3 mi. S of Punta Gorda, 7 Oct 1979, *R. Kral 64559* (holotype, US; isotypes, FSU, NY, VDB, VSC).

The name of one species has been poorly understood and is often incorrectly reported as *Liatris graminifolia*. Thomas Walter (1788; 197) reported a Carolina plant as *Anonymos graminifolia*. His name is of course illegitimate (Walter named 28 of his genera "*Anonymos*") and he left no type, but his epithet is often assumed to have been legitimitized by Willdenow (1803), as "*L. graminifolia* (Walt.) Willd." But Walter's *Anonymos graminifolia* was surely *Vernonia angustifolia*, not a *Liatris* (Ward, 2007: 409). And Willdenow's *L. graminifolia* was original, not a transfer, and is now considered a synonym of *L. pilosa* (Ait.) Willd., a northern species (G. L. Nesom & J. M. Stucky, Sida 21: 815-826. 2004). The Florida (and Georgia) plant, otherwise without a name, thus becomes *Liatris elegantula* (Greene) K. Schum.

LIATRIS Gaertn. ex Schreb. Blazing-stars[1]

1. Inner phyllaries with tips expanded and petal-like (purple, mauve, or sometimes white), far more prominent than the florets; pappus bristles plumose; rootstock globose. Perennial herb, to 1 m. Dry pinelands. Summer-fall. [*Lacinaria elegans* (Walt.) Kuntze]
Liatris elegans (Walt.) Michx.

   a. Heads sessile or nearly so; tips of phyllaries recurved. Panhandle and north peninsula (s. to Pasco Co.); frequent (infrequent to rare in peninsula). var. **elegans**

   a. Heads on short peduncles; tips of phyllaries ascending. Panhandle (Okaloosa, Santa Rosa, Washington cos.); rare. var. **kralii** M. H. Mayfield

1. Inner phyllaries firm and non-petaloid, less prominent than the florets.

   2. Pappus bristles plumose, the lateral cilia many times longer than diameter of the shaft; heads large, to 2.5 cm. high, few (often only 3-4 per stem); phyllaries acute, squarrose, fringed with white hairs; florets purple; rootstock globose. Perennial herb, to 0.8 m. Grassy pinelands. Panhandle (e. to Gadsden Co.); infrequent. Summer. [*Lacinaria squarrosa* (L.) Hill]
Liatris squarrosa (L.) Michx.

   2. Pappus bristles barbellate, the lateral cilia less than 3 times longer than diameter of the shaft.

      3. Rootstock (true roots) elongated and jointed; heads large (2.5 cm. high), borne erect on 2-5 cm. peduncles; phyllaries loosely appressed; florets pale purple. Perennial herb, to 0.6 m. White sand scrub. Central peninsula (Highlands, Polk cos.); rare. Summer-fall. Endemic. ENDANGERED (Federal, State listings). [*Ammopursus Ohlingeri* (Blake) Small]
SAND-TORCH. **Liatris ohlingerae** (Blake) B. L. Robinson

      3. Rootstock globose (in *L. garberi*, elongate and branched, but heads 1.0 cm. high); heads sessile or on short ascending or spreading peduncles.

         4. Heads as broad as long, 15-40 flowered; phyllaries loosely erect or partly recurved, rounded at the tips.

5. Phyllaries entirely green or with very narrow membranous margins; heads 15-25 flowered. Perennial herb, to 1.5 m. Open sandy banks. West and central panhandle (Okaloosa, Gadsden, Wakulla cos.); rare. Summer-fall. [*Lacinaria Tracyi* Alex. in Small] **Liatris earlei** (Greene) K. Schum.

5. Phyllaries with broad scarious usually pigmented margins; heads 20-40 flowered. Perennial herb, to 1.5 m. Open mixed woods. North Florida (s. to Alachua Co.); rare. Summer-fall. [*Lacinaria scariosa*, misapplied] **Liatris aspera** Michx. var. **intermedia** (Lunell) Gaiser

4. Heads longer than broad, 3-18 flowered; phyllaries erect, rounded to acute.

6. Lower leaves long, abruptly changing into much shorter setaceous, closely appressed cauline leaves.

7. Leaves filiform, or a few basal ones markedly wider (to 3 mm.), usually sparsely ciliate near base. Perennial herb, to 1.8 m. Dry pinelands, clearings, flatwoods. Panhandle, south to mid-peninsula (Highlands), disjunct to south peninsula (pine islands of Dade Co.); frequent (rare in s. peninsula). Fall. [*Lacinaria tenuifolia* (Nutt.) Kuntze] **Liatris tenuifolia** Nutt.

7. Leaves linear, 2-8 mm. wide, glabrous. Perennial herb. Moist to dry pinewoods. Perennial herb, to 1.8 m. Peninsula (Dade, n. to Columbia, Baker cos.), westward on coastal dunes (to Bay, Franklin cos.); frequent. Fall. A southern vicariad of *L. tenuifolia*, now overlapping and intergrading. [*Liatris tenuifolia* var. *quadriflora* Chapm.; *Lacinaria tenuifolia*, misapplied] **Liatris laevigata** Nutt.

6. Lower leaves gradually reduced upward, or if abruptly reduced, the cauline leaves foliaceous and spreading.

8. Phyllaries broad, rounded at apex, often with scarious margins.

9. Heads pedunculate (to 2 cm.) and widely spreading, or rarely sub-sessile; stem lightly hoary-pubescent; leaves glabrous or ciliate toward the base. Perennial herb, to 1.0 m. Fall. **Liatris gracilis** Pursh

a. Lower cauline leaves lanceolate, mostly <12 mm. wide; lower floral bracts <2 mm. wide; phyllaries usually obtuse. Dry longleaf-pine forests, flatwoods, occasionally persisting on roadsides. Throughout; frequent to common (rare in Keys). [*Lacinaria gracilis* (Pursh) Kuntze; *Lacinaria laxa* Small] var. **gracilis**

a. Lower cauline leaves elliptic, mostly >12 mm. wide; lower floral bracts >2 mm. wide; phyllaries usually acuminate. Dry deciduous woodlands on upper slopes of bayhead ravines and crest of bluffs. Central panhandle (Liberty Co.); rare. Endemic. [*Liatris gholsonii* L. C. Anderson] var. **gholsonii** (L. C. Anderson) D. B. Ward

9. Heads sessile, or sometimes short-pedunculate; stem and leaves essentially glabrous.

10. Heads sessile or on short peduncles; inflorescence a loose spike, the interval between heads often equal their length; lower leaves narrowly lanceolate. Perennial herb, to 1.0 m. Rocky hammocks. Central panhandle, south along west coast (to Manatee Co.); infrequent. Fall. [*Lacinaria graminifolia* (Willd.) Kuntze; *Liatris graminifolia*, misapplied] **Liatris elegantula** (Greene) K. Schum.

10. Heads uniformly sessile; inflorescence a dense spike, the interval between the heads much shorter than their length; lower leaves linear, scarcely tapering for most of length. Perennial herb, to 1.8 m. Wet to dry pinelands and savannas. Summer-fall. **Liatris spicata** (L.) Willd.

a. Stems eglandular; leaves inconspicuously or not punctate; margins eciliate or with short hairs (<0.8 mm.); corolla tubes glabrous within. Nearly throughout (excl. Keys); frequent. [*Lacinaria spicata* (L.) Kuntze] var. **spicata**

a. Stems minutely glandular; leaves prominently punctate; margins with long hairs (>1.0 mm.); corolla tubes pilose within. Southwest peninsula (Hillsborough to Lee Co.); infrequent. Endemic. [*Liatris savannensis* Nesom & Kral]
var. **savannensis** (Nesom & Kral) D. B. Ward

8. Phyllaries narrow, acute, without scarious margins.

11. Heads stout, with 6-7 florets; phyllaries hirsute along midrib; rootstock (true roots) forked, elongate. Perennial herb, to 0.6 m. Low pinelands and wet prairies. South peninsula (excl. Keys), north to mid-peninsula (Hillsborough, Orange, Brevard cos.); frequent. Summer-fall. Endemic. [*Lacinaria chlorolepis* Small; *Lacinaria Garberi* (Gray) Kuntze]
Liatris garberi Gray

11. Heads slender, with 3-6 florets; phyllaries glabrous or with minute marginal cilia; rootstock (lower stem) globose.

12. Heads sessile or sub-sessile, forming an erect symmetrical spicate inflorescence.

13. Heads rigidly ascending, crowded and overlapping; involucres 18-20 mm. high; leaves with short stiff pubescence. Perennial herb, to 1.2 m. Well drained sand of scrub and longleaf pinelands. Panhandle (e. to Leon Co.), disjunct to peninsula (Putnam to Dade cos.); frequent. Summer-fall. Endemic. [*Lacinaria Chapmanii* (Torr. & Gray) Kuntze]
Liatris chapmanii Torr. & Gray

13. Heads strongly divergent from the rachis, often loosely spaced; involucres 12-15 mm. high; leaves glabrous. Perennial herb, to 0.8 m. Coastal dunes and islands, longleaf-pine ridges. Central panhandle (Franklin, Wakulla cos.); rare (locally abundant). Summer-fall. Endemic. ENDANGERED (State listing). **Liatris provincialis** Godfrey

12. Heads pedunculate, often shortly so, all rotated to upper side of the curving rachis, forming a loose arching racemose inflorescence. Perennial herb, to 0.8 m. Dry longleaf-pine sandhills. Frequent. Summer-fall. **Liatris pauciflora** Pursh

    a. Stem and rachis glabrous. Eastern panhandle (Taylor, Madison cos.), south to central peninsula (Hillsborough, Polk cos.). [*Lacinaria pauciflora* (Pursh) Kuntze] var. **pauciflora**

    a. Stem and rachis densely short-pubescent. Western panhandle (e. to Washington Co.). [*Lacinaria secunda* (Ell.) Small] var. **secunda** (Ell.) D. B. Ward

Excluded names:

Liatris squarrulosa Michx.
Northern, to mid Georgia. Reported for Florida (Cronquist, 1980), for Wakulla Co. (Clewell, 1985), and for Gadsden and Okaloosa cos. (Wunderlin, 1998). R. K. Godfrey (pers. comm., Feb 1987) suggested that all are *L. aspera*. More probably, plants so named are best assigned to *L. earlei*.

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[1] This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

This key to *Liatris* was begun in October 1966 and has undergone numerous revisions as further wisdom has been obtained over the years. I am grateful to Robert K. Godfrey and Loran C. Anderson, to John Beckner, to Arthur Cronquist, to Robert L. Wilbur, and especially to Erdman West, for their willingness to assist me in deciphering the taxonomy, distribution, and nomenclature of the Florida Blazing-stars. Sadly, I must note that more than half of these good people are now gone. I must dally no longer lest those remaining not know of my gratitude.

NOTICE

NEW TAXA DESCRIPTIONS: LATIN OR ENGLISH, IT IS YOUR CHOICE

At the Nomenclatural Section of the XVIII International Botanical Congress, Melbourne, Australia, 18 - 22 July 2011, the congress voted to allow English to be used as an alternative language for producing valid descriptions and diagnoses of new taxa of organisms covered by the *International Code of Botanical Nomenclature* (to be renamed International *Code of Nomenclature for algae, fungi and plants*). Commencing on Jan. 1, 2012, either English or Latin can be used to provide a description or diagnosis for new taxa.

In accordance with these changes Phytologia will adhere to the new standards and accept either English or Latin for the description or diagnosis for new taxa beginning with this issue:
Phytologia 94(1): 147 (April 2, 2012).

Robert P. Adams, Editor
Phytologia
Robert_Adams@baylor.edu

Correction

Figure 1 (p. 317) of Adams, R. P. and P. S. Shanjani. 2011. Identification of the Elburz Mountains, Iran juniper as *Juniperus polycarpos* var. *polycarpos*. Phytologia 93(3): 316-321, inadvertently had the keys' icons reversed for var. *seravschanica* and var. *turcomanica*.

Below is the corrected figure:

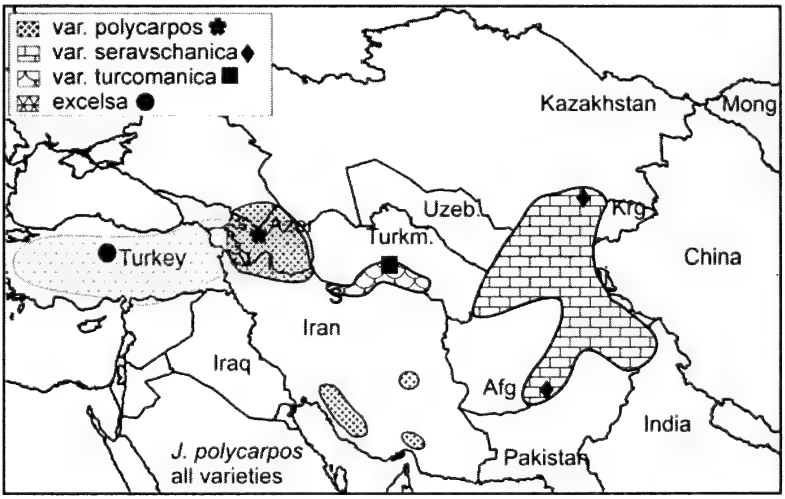

Figure 1. Distributions of *J. excelsa* (Greece not shown), *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica* (adapted from Adams, 2011). Symbols indicate the populations sampled for each taxon. S is the site of the Iranian juniper samples in the Elburz Mtns., Iran.

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Cover photo: *Taxodium distichum*, Guadalupe River, near Comfort, TX photo by Bill Rosendahl.

KEYS TO THE FLORA OF FLORIDA - 31, *ARISAEMA* (ARACEAE)

Daniel B. Ward
Department of Botany, University of Florida
Gainesville, Florida 32611, U.S.A.

ABSTRACT

Arisaema (Araceae) is represented in Florida by 3 species, two of which are treated as of two varieties. The nomenclature of *Arisaema triphyllum* and its variants is discussed. *Arisaema triphyllum* var. *acuminatum* is justified as worthy of recognition. *Arisaema dracontium* var. ***macrospathum*** is recognized as a new combination. *Arisaema quinatum* is noted as distinct and rare. An amplified key is given to the Florida taxa. *Phytologia 94(2): 151-158 (August 1, 2012).*

KEY WORDS: *Arisaema*, Araceae, Florida flora.

Arisaema (Araceae) is a rather large genus (150 species - Mabberley, 1997), numerous in Africa and Asia and only nominally represented in North America. The eastern U. S. species have been addressed twice, by Donald G. Huttleston (M.S. and Ph.D. theses, Cornell Univ.) and by Miklos Treiber (Ph.D. thesis, Univ. North Carolina), but neither study has been published. The three species native to Florida present problems both in taxonomy and nomenclature.

ARISAEMA TRIPHYLLUM. Twice Huttleston (Bull. Torrey Bot. Club 76: 407-413. 1949; ibid. 108: 479-481. 1981) touched on his earlier, more comprehensive thesis, by publishing brief justification of his new combinations, *Arisaema triphyllum* ssp. *pusillum* (Peck) Huttleston, ssp. *stewardsonii* (Britt.) Huttleston, and ssp. *quinatum* (Nutt.) Huttleston. [It is noted that as a student under Dr. R. T. Clausen, Huttleston did not have the option of employing the rank of variety.] These three taxa as well as typical *A. triphyllum* are generally

recognized in eastern U. S. floras, at times at ranks other than subspecies.

To anchor further discussion, it is necessary to establish what is meant by "typical" *Arisaema triphyllum*. In the Northeast, three moderately well-defined, long-recognized variants of the plant known as the Jack-in-the-pulpit or Indian Turnip have been distinguished. In the early 20th century these were known as: *A. triphyllum* (L.) Schott, the most common and most widespread variant, with a wide spathe flange, of mesic woodlands; *A. pusillum* (Peck) Nash, also of wide distribution, with a narrow flange, a smaller plant of wetter soils; and *A. stewardsonii* Britton, northern, with fluted spathes, of acidic soils. [Distinguishing characters are summarized by Huttleston (1949).] *Arisaema triphyllum* was assumed to be the variant known to Linnaeus (1753: 965). [*Arisaema quinatum*, a similar but separate taxon, was also known, as was the quite different Green-dragon, *Arisaema dracontium*.] But this stable nomenclature was upended by M. L. Fernald (Rhodora 42: 247-254. 1940) who argued that Linnaeus' type was the variant known as *A. pusillum*. If this were the case, the three taxa would become *A. atrorubens*, *A. triphyllum* (incl. *A. pusillum*), and *A. stewardsonii*. Fernald continued with this interpretation in his influential Manual (1950), and has been followed by some (e.g., Steyermark, 1963 (Missouri); Scoggin, 1982 (Canada).

But Fernald's argument hinges on the identification of Linnaeus' type. Of the single herbarium specimen in the Linnaean herbarium and several references (with their associated specimens) that formed the original material of *Arum triphyllum* Linnaeus (Sp. Pl. 965), Fernald called Linnaeus' specimen (1079.2, LINN) the type. He believed it to be "exactly similar" to a John Clayton specimen from Virginia in the herbarium of Gronovius (BM), and perhaps assumed it to be from the same source. [Gronovius had two Clayton specimens. -- #66 and #539 -- and gave each of them its own phrase-name. Fernald apparently was referring to #66, but the photo he was using may have been of #539. The discrepancy is not consequential.] Fernald failed to note the specimen in the Linnaean herbarium was marked "HU," Linnaeus' abbreviation for "Horto Upsaliensi," the Uppsala botanical

garden; it was not a Clayton sheet from Virginia. But by his designation of the Linnaean specimen as the type, Fernald locked the meaning of *Arum triphyllum* (hence, *Arisaema triphyllum*) to the Uppsala garden plant. Thus the epithet "*triphyllum*" (at specific, subspecific, or varietal rank) must be applied to the form represented by the specimen in the herbarium of Linnaeus, not the specimens of Gronovius.

Fernald believed Linnaeus' specimen represented the variant then known as *Arisaema pusillum*. He then felt justified in applying "*triphyllum*" to that plant, with "*pusillum*" reduced to synonymy, and calling the former *A. triphyllum* by a later name, *A. atrorubens* (Ait.) Blume.

The Linnaean specimen of *Arum triphyllum* (1079.2, LINN) consists of a single three-parted leaf and two opened spathes, showing spadices. [It is shown in part by Fernald, 1940, and in full by Jarvis, 2007: 319.] Huttleston (1949), however, was unable to agree with Fernald's identification; he cited several characters that he had found useful, but none were conclusive. He then proceeded to use the epithet in the traditional way, as described above.

As part of the project to typify all Linnaean names, James L. Reveal, Charles E. Jarvis, and Fred R. Barrie (Taxon 39: 355-357. 1990) published a fully detailed proposal to conserve the name *Arum triphyllum* with a different type. Implicit in their argument -- though not quite stated -- is the belief that the Linnaean specimen is indeed *not* the "typical" form of *A. triphyllum*, and that another type must be chosen so as to avoid accepting Fernald's nomenclature. Their proposal was considered by the Committee for Spermatophyta; questions were raised as to the taxonomy of the species and the accuracy of the identifications of the potential types, and the proposal was not recommended (Brummitt, Taxon 42: 875. 1993).

Failure of the Reveal et al. proposal to re-typify Linnaeus' *Arum triphyllum* has left botanists with an inherently unstable nomenclature for the variations of the Jack-in-the-pulpit. Yes, it is

agreed that Linnaeus' specimen is the type of *A. triphyllum*. But no, there is no agreement as to which form of the species is represented by that specimen. Prudence, not nomenclatural edict, suggests that the assignment of names as used by Huttleston (though not necessarily their rank) be followed, as is done here. Other recent notable floristic treatments have done likewise (cf. S. A. Thompson, Fl. N. Amer. 22: 139-141. 2000).

With the variant represented by the name *Arisaema triphyllum* understood, the question arises as to which variants are present in Florida. Here, recent practice has been to report *A. triphyllum* only as a single undivided entity (cf. Clewell, 1985; Wunderlin, 1998; Wunderlin & Hansen, 2003), usually with *A. pusillum* and/or *A. acuminatum* in synonymy.

J. K. Small (1903, 1933) thought otherwise. He recognized *Arisaema acuminatum* Small as a Florida endemic, typified by a plant from mid-peninsula (Clearwater, Pinellas Co.). He distinguished it from *A. triphyllum* -- also a Florida plant -- on the basis of a wholly-green, proportionately narrow spathe hood ("over twice as long as broad") with long-acuminate tip (vs. purple or brown-striped, shorter, broader hood with acute to short-acuminate tip). He excluded *A. pusillum* from the Florida flora, giving it a range only south to northern Georgia. [*Arisaema stewardsonii*, still more northern in range, does not reach the Manual coverage.]

Huttleston (1981) found *Arisaema triphyllum* ssp. *triphyllum* to be tetraploid and described it as having leaflets glaucous beneath with pronounced spathe-tube flanges; its range extended into north Florida. He described typical ssp. *pusillum* as diploid with leaflets green beneath and modest spathe-tube flanges, and included within it most plants from the Florida peninsula. But, after repeated trips to Florida (and ca. 250 chromosome counts - Huttleston, pers. comm., Mar. 1981), he could not fully dismiss Small's Florida variant. He found a diploid population occurring throughout peninsular Florida and extending north along the Georgia coast that combined the

characteristics of the two taxa. He concluded these plants to be of hybrid origin.

The plants Huttleston considered of hybrid origin coincide with the plants Small treated as *Arisaema acuminatum*. The range they occupy is the same as many other Florida-Georgia species. They are uniform in morphology throughout the peninsula (though less so in the panhandle where typical *A. triphyllum* also occurs). They form stable, sexually reproducing populations. Hybridity seems improbable; uniform diploid counts are scarcely to expected between diploid and tetraploid taxa. It may be that the Florida population has been derived from typical *A. pusillum* (or vice versa). But its stable format and real but modest differences have made it worthy of retention at varietal rank.

A differentiating character noted by Huttleston but difficult to quantify is relative robustness of the two variants. *Arisaema pusillum* north of Florida is usually a small plant, often under 20 cm. tall, with a delicate, slender stem. *Arisaema acuminatum* in the Florida peninsula is commonly somewhat stout, the stem firm, not notably flexible, sometimes above 50 cm. J. K. Small (J. N.Y. Bot. Gard. 28: 39. 1927) reported *A. acuminatum* in the Turnbull Hammock, eastern Volusia County, as: "the plants...varied in height from waist high to as high as one's head." Small was not a large man; even so, this observation indicates a height above 1.5 m. However, neither later observations nor preserved materials are available for confirmation.

ARISAEMA DRACONTIUM. The distinctive Green-dragon, *Arisaema dracontium* (L.) Schott, scarcely varies throughout most of its broad range in eastern and central North America. But where it extends into Florida and through Texas into Mexico, differences in morphology are to be found. In some Florida populations, as in apparently all northern populations, the spathe is tightly furled around the lower portion of the spadix, leaving free only the long-extended sterile tip. In other Florida populations the distal portion of the spathe is more or less expanded and leaflike, often to a width of 20 mm. or more.

A similar varient with leaflike spathes is quite widespread in Mexico, and has been named *Arisaema macrospathum* Bentham. Typical *Arisaema dracontium* is also present. The two have at times been held separate (cf. Conzatti, Fl. Tax. Mex. 1946); but the distinction has been limited to number of leaf segments (6 to 7 in *A. macrospathum*, 9 to 14 in *A. dracontium*). The wider spathe is very evident in isotypes of Hartweg's 1837 collections in Michoacan, as identified by Bentham (1840). [Each specimen seen has 7 leaf segments. But one must doubt the utility of this character in view of the Florida observation that number of segments increases with age and size of the plant and change in gender (from male to female), and that Bentham noted the plant to be "masculo," suggesting with time the number of segments might well increase.] Huttleston (thesis, 1953) doubted the two taxa were sufficiently different to be recognized as species, but did not publish the new combination (which would have been *A. dracontium* ssp. *macrospathum* (Benth.) Huttleston). That new combination at varietal level is provided here.

Arisaema dracontium (L.) Schott var. ***macrospathum*** (Bentham) D. G. Huttleston ex D. B. Ward, comb. et stat. nov. Basionym: *Arisaema macrospathum* Bentham, Pl. Hartw. 52. 1840. TYPE: Mexico, Michoacan, Morelia,"in sylvis umbrosis," [1837], *K. T. Hartweg 394* (holotype, BM (not seen); isotypes, E, G, L).

ARISAEMA QUINATUM. The least well known, most range-restricted species of *Arisaema* in the eastern states is *A. quinatum* (L.) Schott. In Florida it has been found only in the central panhandle; it is disjunct to Georgia and Louisiana. Though reduced to subspecific status by Huttleston (1981) and sometimes casually reduced to synonymy under *A. triphyllum*, its erect, blunt spathe and five-parted leaves are distinctive.

ARISAEMA Mart. Jack-in-the-pulpit, Green-dragon[1]

1. Leaves usually solitary, with 8-12 leaflets; terminal portion of spadix filiform, long-extended beyond spathe, flexuous. Perennial herb. Rich woodlands. Summer.
GREEN-DRAGON. **Arisaema dracontium** (L.) Schott

a. Spathe tightly inrolled around spadix, to 1.5 cm. broad when unfurled. Panhandle and north Florida (s. to Hernando Co.); infrequent. [*Muricauda dracontium* (L.) Small]
var. **dracontium**

a. Spathe flared distally, forming a blade, to 2.5 cm. broad. North and central peninsula (Alachua Co., to Pasco, Orange cos.); frequent. [*Arisaema macrospathum* Benth.]
var. **macrospathum** (Benth.) D. B. Ward

1. Leaves 2 (in mature plants), each with 3 or 5 leaflets; terminal portion of spadix stout, blunt, enclosed within or extending scarcely beyond mouth of spathe.

2. Leaflets usually 5; blade of spathe obtuse to abruptly acute, ascending to erect. Perennial herb. Mesic woodlands. Central panhandle (Walton, Liberty, Leon, Jefferson cos.); rare. Spring-summer. [*Arisaema triphyllum* ssp. *quinatum* (Nutt.) Huttleston]
PRESTER JOHN. **Arisaema quinatum** (L.) Schott

2. Leaflets usually 3; blade of spathe long-acute to acuminate, downcurved over mouth of spathe. Perennial herb. Spring-summer.
JACK-IN-THE-PULPIT. **Arisaema triphyllum** L.

a. Leaflets glaucous beneath; spathes green or purple, the tip long-acute; plant to 0.4 m. tall. Mesic woodlands. Panhandle and north Florida (to Alachua Co.); infrequent.var. **triphyllum**

a. Leaflets green beneath; spathes uniformly green, the tip acuminate; plant to 1.2 m. tall. Low woodlands. North Florida, south to lower peninsula (Collier Co.); frequent. [*Arisaema acuminatum* Small; *Arisaema pusillum*, misapplied]
var. **acuminatum** (Small) Engl.

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[1] This paper is a continuation of a series begun in 1977. The "amplified key" format employed here is designed to present in compact form the basic morphological framework of a conventional dichotomous key, as well as data on habitat, range, and frequency. Amplified keys are being prepared for all genera of the Florida vascular flora; the present series is restricted to genera where a new combination is required or a special situation merits extended discussion.

Donald G. "Dutch" Huttleston (1928-2000) and I were graduate school contemporaries at Cornell University in the early 1950s, sharing the special guidance of our thesis advisor, Robert T. Clausen. I remember with fondness Dutch's quick wit and bottomless poetry repertoire. I only wish -- as must we all -- that his later career had given him incentive to publish the solid research of his two theses (*Arisaema*, 1948; Araceae, 1953). But by inserting his name in the authorship of the new combination published here, I can commemorate his scholarship and his incompleted study.

I am grateful to John Beckner for his observations of *Arisaema* throughout Florida, to Robert L. Wilbur for his knowledge of the Carolina Jacks, and to Kent Perkins for literature acquisition.

TAXODIUM (CUPRESSACEAE): ONE, TWO OR THREE SPECIES? EVIDENCE FROM DNA SEQUENCES AND TERPENOIDS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

Michael A. Arnold and Andrew R. King
Department of Horticultural Sciences, TAMU 2133
Texas A & M University, College Station, TX 77843-2133

Geoffrey C. Denny
G. C. Denny Horticulture, 12905 Co. Rd. 675, Parrish, FL 34219

and

David Creech
SFA Mast Arboretum, Box 13000, Stephen F. Austin University
Nacogdoches, TX 75962-3000

ABSTRACT

DNA sequences (3547 bp) of nrDNA and cp regions (petN-psbM, trnS-trnG, ycf-psbA) were utilized to examine variation in *Taxodium distichum*, *T. d.* var. *imbricarium* and *T. mucronatum* using *Glyptostrobus pensilis* as an out-group. In contrast to considerable geographical variation found in the terpenoids (Adams et al. 2012), no informative mutations were found in these DNA sequences. The 16 SNPs found in nrDNA did not support three species of *Taxodium*. No variation was found in the cpDNA regions. DNA sequencing and terpenoids support the recognition of *Taxodium* as a monotypic genus with three varieties, var. *distichum* (L.) Rich. var. *imbricarium* (Nutt.) Croom and var. *mexicanum* (Carr.) Gord. *Phytologia 94(2): 159-168 (August 1, 2012).*

KEY WORDS: *Taxodium distichum*, *T. d.* var. *imbricarium* (*T. ascendens*), *T. d.* var. *mexicanum* (*T. mucronatum)*, nrDNA, cpDNA.

Taxodium Rich. is a small genus with one to three species. Britton (1926), Dallimore and Jackson (1966) and Rehder (1940) recognized three species: bald cypress, *T. distichum* (L.) Rich, pond cypress, *T. ascendens* Brongn. and Montezuma or Mexican bald cypress, *T. mucronatum* Ten. Watson (1985) treated *T. ascendens* as *T. d.* var. *imbricarium* (Nutt.) Croom. Based on morphology, Farjon (2005) and Eckenwalder (2009) recognized *T. distichum*, *T. d.* var. *imbricarium* and *T. mucronatum*. However, Denny (2007) treated the genus as monotypic with one species, *T. distichum* and three varieties: var. *imbricarium* and var. *mexicanum* (Carr.) Gord. (= *T. mucronatum*). Denny (2007), and Denny and Arnold (2007), give a lucid discussion of the historical nomenclature of the genus.

Recently, we reported on the leaf essential oils of *Taxodium distichum*, *T. d.* var. *imbricarium* (Adams et al. 2012). The geographical trends in the oils are shown in Fig. 1. *T. mucronatum* in

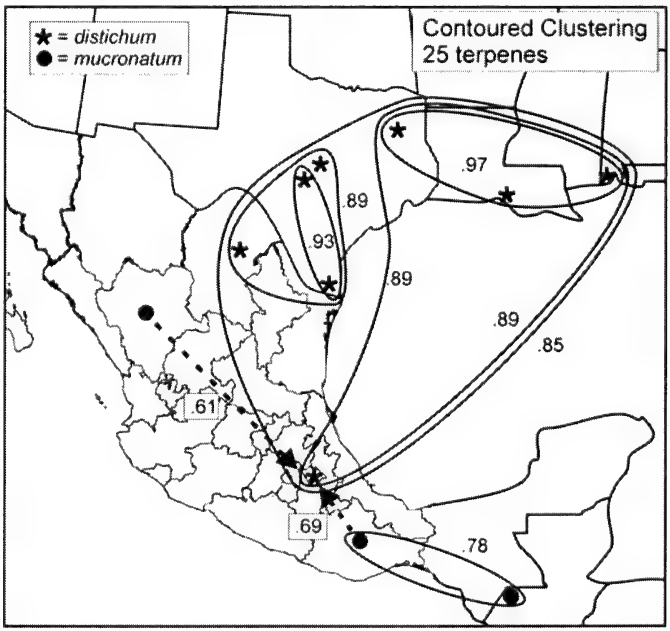

Figure 1. Contoured terpenoid similarities (from Adams et al. 2012). The number next to a contour line is the clustering similarity.

Mexico appears to be of two oil types: Durango and Oaxaca-Guatemala. The oils of *T. distichum* are in two regional groups: South Central USA and Texas Hill Country - Rio Grande Valley. The oil of the putative *T. mucronatum* from Bolleros, MX is more similar to *T. distichum* than to any *T. mucronatum* in that study (Adams et al. 2012). Putative *T. d.* var. *imbricarium* from Fowl River, AL was found to have oil like *T. d.* var. *distichum* not like *T. d.* var. *imbricarium* from Tampa, FL. Both *T. d.* var. *distichum* and var. *imbricarium* were found to have chemical races: high/low α-pinene and low/high limonene/β-phellandrene.

The purpose of this paper to is to report on variation in DNA sequences (3547 bp) of nrDNA and three cp regions (petN-psbM, trnS-trnG, ycf-psbA) and relate this DNA data to the taxonomy of *Taxodium*.

MATERIALS AND METHODS

Plant material: Seeds were collected by Denny (2007) in late summer and fall, 2003 and germinated and grown in containers in spring, 2004. Subsequently, seedlings were transplanted to the Texas A & M University Horticulture Farm, College Station, Texas (30°37'38.222" N, 96°22'18.505" W).

T. mucronatum: Rio Nazas, Durango, MX, MX2M, 25° 18' 36" N, 104° 38' 24" W, *Adams 12833-12837*; Rio Sabinas, Coah., MX, *Adams 13039-13043*, Bolleros, DF, MX, MX3M, 19° 30' 0" N, 98° 54' 36" W, *Adams 12838-12842;* Progreso, TX, MX5M, 26° 4' 12" N, 97° 54' 36" W, *Adams 12843-12847.*

T. distichum: Guadalupe River, TX, TX2D, 30° 4' 12" N, 99° 17' 24" W, *Adams 12848-12852*; Sabinal River, TX, TX5D, 29° 9' 36" N, 99° 28' 12" W, *Adams 12853-12857;* Lake Cherokee, TX, EP1D, 32° 20' 24" N, 94° 42' 0" W, *Adams 12858-12862;* Bayou Teche, LA, EP3D, 29° 5' 24" N, 91° 12' 6" W, *Adams 12863-12867*; Mobile Bay, AL, EP4D, 30° 36' 0" N, 87° 54' 36" W, *Adams 12868-12872.*

T. distichum var. *imbricarium*: Fowl River, AL, EP5I, 30° 27' 0" N, 99° 06' 36" W, *Adams 12873-12877.*

In addition, samples were collected from:

T. distichum: Hillsborough River, Hillsborough Co., FL, 28° 09' 05.15" N, 82° 13' 37.24" W, *Adams 12828*, Hillsborough River,

Hillsborough Co., FL, 28° 01.164' N, 82° 27.881' W, *Adams 12829-12830,* Hillsborough River, Hillsborough Co., FL, 27° 59.796' N, 82° 28.010' W, *Adams 12831-12832*;

T. d. var. *imbricarium*: edge of swamp, Hillsborough Co., FL, 28° 11' 39.80" N, 82° 30' 54.09" W, *Adams 12823-12827.*

Seeds were removed from a herbarium specimen (TEX) of *M Veliz 17213*, 31 Aug 2006, Chimaltenango, Guatemala, Lab Acc. *12591* for DNA extraction.

Specimens of *Glyptostrobus pensilis* K. Koch were obtained from the Stephen F. Austin State University, Mast Arboretum for use as an out-group to *Taxodium* (Kusumi et al. 2000). Voucher specimens are deposited in the Herbarium, Texas A & M University (for plot materials) and Baylor University (for Adams field collected materials).

DNA Analysis - 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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM, trnS-trnG) or K (nrDNA, ycf-psbA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams and Schwarzbach (2011) for the ITS, petN-psbM, and trnS-trnG primers utilized. Primers used for ycf exon 3 - spacer - psbA: ycf1843F GCT CCA AGC AAT TAT ATC GAA GCA CA; psbA2516R ATG ATC TTT ACT TCT GGT TCC GGT GA. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco, CA) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams, 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the

maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

Sequencing nrDNA yielded 65 SNPs (4 single events) with 61 potentially informative SNPs. 55 of these SNPs differentiated *Glyptostrobus pensilis* from *Taxodium* (Fig. 2). For the remaining 15

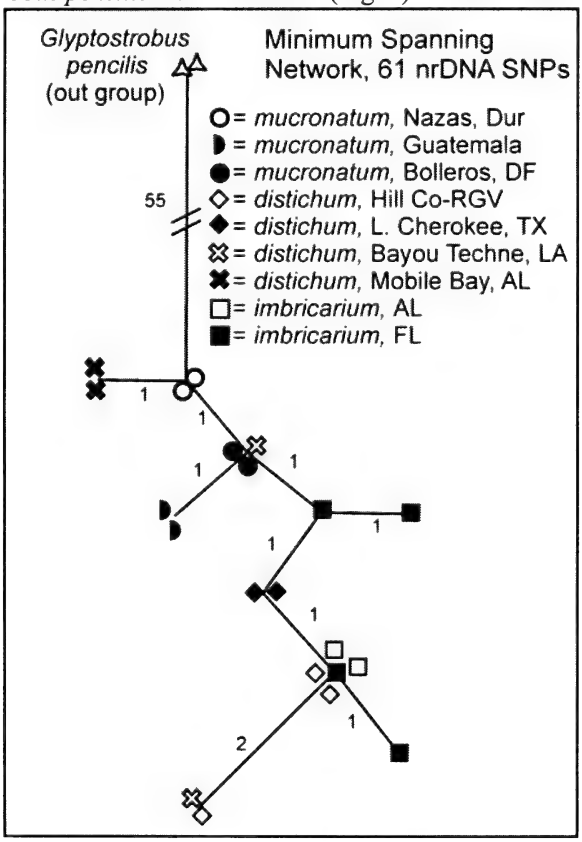

Figure 2. Minimum spanning network based on 61 SNPs from nrDNA. The numbers next to the links are the number of SNPs.

SNPs, the pattern appears to be mostly random (Fig. 2). Although the *T. mucronatum* from Durango, Bolleros and Guatemala differ by only 1 SNP, they also differ by only one SNP from *T. distichum*, Mobile Bay, AL and *T. d.* var. *imbricarium*, FL. *Taxodium d.* var. *imbricarium*, FL is interspersed with *T. distichum*, Hill Country- RGV and Lake Cherokee, TX (Fig. 2). There is no apparent grouping of the three taxa.

Sequencing of petN-psbM (955 bp) gave 14 informative SNPs (9 substitutions and 5 indels), all of which occurred between *Glyptostrobus* and *Taxodium*. There was no variation among the *Taxodium* accessions, except for a 63 bp insertion in var. *imbricarium* (FL, *Adams 12825*)

The trnS-trnG intron (821bp) had 12 SNPs (9 substitutions and 3 indels). All 12 SNPs were between *Glyptostrobus* and *Taxodium*. No variation was found among the *Taxodium* accessions.

Sequencing of ycf exon 3 - spacer - psbA (591bp) yielded 9 SNPs (8 substitutions and 1 indel). All 9 SNPs were between *Glyptostrobus* and *Taxodium*. No variation was found among the *Taxodium* accessions, except in the long stretch of poly mono-nucleotide Ts (imb = imbricarium, dist = distichum, muc = mucronatum): 19T - imb FL 12826; 18T - dist Guad R 12848; 17T - dist 12864 AL, imb FL 12824, 12825, muc 12591 Guat., dist Sab R 12853, muc Bolleros 12838, 12839, muc Nazas 12833, 12834; 16T - dist Progresso 12843; 15T - dist AL 12868, dist LA 12862, dist L. Cherokee 12858, 12861, imb AL 12873, 12874, imb FL 12823; and 14T - dist AL 12872. There seems no regional or taxonomic pattern in the length of the poly T section. It should be noted that poly mono-nucleotides of greater than 8-10 nucleotides are difficult to sequence due to slippage of the *Taq* enzyme. But in each instance, the poly T region was sequenced in both directions and the number of Ts verified in both sequences. This may just be a hyper-variable region with no taxonomic significance.

Tsumura et al. (1999) used cleaved amplified polymorphic sequences (CAPS) to examine var. *distichum* and var. *imbricarium* in Florida (one population, Fargo, GA, was on the Georgia / Florida

border). They were able to classify each population of the two varieties except the Fargo, GA population that clustered by itself. They concluded that CAPS did support the recognition of varieties (var. *distichum* and var. *imbricarium*), but not distinct species.

Kusumi et al. (2010) examined populational variation along the Mississippi River in *T. distichum,* plus one population of *T. d.* var. *imbricarium* near New Orleans. They did not comment on the taxonomic implications of their data.

Lickey and Walker (2002) used allozymes to examine var. *distichum* and var. *imbricarium* and showed var. *imbricarium* to be somewhat distinct from var. *distichum* (clustering with var. *distichum* from Stone Mt., MS). A population of *T. mucronatum* (Sonora, MX) clustered well within *T. distichum.* Interestingly, they found that var. *distichum* from the Guadalupe River, TX had a unique allozyme pattern in their data set. They concluded that gene flow is occurring between the two varieties and that the taxa are likely varieties not species.

In our previous study on leaf terpenoids, we found (Fig. 3) considerable differentiation in the Guatemala - Oaxaca, and Durango populations from the central group of *T. distichum* in the USA. The var. *imbricarium* from Florida was only slightly different from var. *distichum* in the south-central USA (Fig. 3, 0.90) as was the Hill country - RGV populations (Fig. 3, 0.86). The high similarity of the Bolleros (DF, MX) to south-central USA (Fig. 3, 0.88) may be due to the movement of germplasm by people as the trees are widely cultivated in Mexico. However, it is clear that there has been evolutionary differentiation in *Taxodium* of southern Mexico - Guatemala and Durango in the leaf terpenoids.

In view of the lack of variation in the DNA sequences (4 gene regions, 3547 bp) found in this study, there is no support for the recognition of *T. ascendens* (*T. d.* var. *imbricarium*) and *T. mucronatum* as distinct species. In short, the present data supports the recognition of *Taxodium* as a monotypic genus with three varieties: var. *distichum* (L.) Rich.; var. *imbricarium* (Nutt.) Croom; and var. *mexicanum* (Carr.) Gord. as advocated by Denny and Arnold (2007).

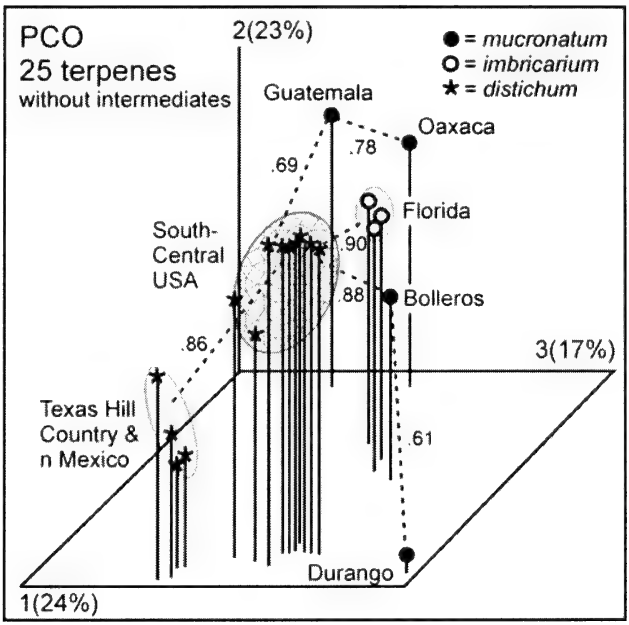

Figure 3. PCO based on 25 terpenes without intermediate terpene patterns. Adapted from Adams et al. (2012).

Key to the varieties of *Taxodium distichum* (from Denny and Arnold, 2007):

1a. Determinate short shoots mostly ascending in a vertical plane; awl-like leaves narrowly lanceolate, 2.5 - 10 mm long, appressed, and imbricate in 5 to 8 ranks on shoots.....var. *imbricarium*

1b. Determinate short shoots mostly spreading in a horizontal plane; flattened leaves narrowly linear, 5 - 15 mm long, divergent, and appearing two-ranked on shoots.....2

2a. Leaves deciduous; branches (catkins) containing male cones, short and crowded, often divided into compact secondary branches..... .....var. *distichum*

2b. Leaves semi-evergreen; branches (catkins) containing male cones, long and slender, open, made up of single cones or clusters of several cones.....var. *mexicanum*

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A NEW SPECIES OF *TRIDAX* (ASTERACEAE, MILLERIEAE) FROM OAXACA, MEXICO

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

A new taxon, **Tridax paneroi** B.L. Turner, **sp. nov**. is described from western Oaxaca, Mexico. It is closely related to the rarely collected species, *T. purpurea* and *T. oaxacana,* but readily distinguished from both by several characters. A photograph of the type is presented, along with a map showing distributions of the taxa concerned. *Phytologia 94(2): 169-173 (August 1, 2012).*

KEY WORDS: Asteraceae, Heliantheae, *Tridax*, *T. purpurea, T. oaxacana*, Mexico, Oaxaca

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Identification of Mexican Asteraceae, and preparation of a treatment of The Comps of Mexico (cf Turner 2010), has occasioned the present paper.

TRIDAX PANEROI B.L. Turner, **sp. nov. Fig. 1**

Resembling *Tridax oaxacana* B. L. Turner but with larger, more lanceolate leaves, 4-6 times as long as wide; (vs more nearly ovate and 2-3 times as long as wide); petioles 1-3 cm long (vs petioles 0-2 mm long), and disc florets greenish-yellow (vs purple-black).

Tap-rooted perennial herbs,1.0-1.5 m high. Mid-stems 4-5 mm thick, simple, mostly unbranched, pubescent with eglandular hairs ca 1 mm long, these occasionally interspersed with a few glandular hairs. Leaves (at or near mid-stem) 6-10 cm long, 1.5-3.0 cm wide; blades linear-lanceolate, having a single prominent mid-rib, sparsely pubescent above and below, especially along the margins, the latter remotely

serrate; petioles 1-3 cm long, grading into the blades. Capitulescence a terminal, markedly open, long, lax panicle of 3-4 heads, the ultimate peduncles 6-15 cm long, glandular-pubescent to nearly glabrous. Heads campanulate, ca 2 cm high, 1.5 cm wide. Involucral bracts, numerous, glabrous, 2-6 seriate, markedly imbricate, their apices obtuse or rounded. Receptacle convex, ca 3 mm across; pales linear-lanceolate, ca 10 mm long, their apices acute. Ray florets 3, pistillate, fertile; ligules purple ("magenta" or "morales-rosa"), ca 1 cm long, 0.5-0.8 cm wide; tubes ca 8 mm long. Disk florets 30-40 per head; tubes ca 2 mm long, appressed-pubescent; throats 6-7 mm long, pubescent below, glabrous above, the 5 lobes ca 1.5 mm long. Anthers pale-purplish, the apical appendages glabrous, eglandular. Achenes, of ray and disc florets similar; body ca 4 mm long, densely upwardly appressed-pubescent; pappus of ca 20 pubescent, subulate, scales ca 1.2 mm long. Chromosome number, n = 10 pairs, this from the type itself (Strother and Panero, 2001).

The species is named for my fellow colleague and exceptional scholar of the Asteraceae, Prof. Jose Panero, University of Texas, Austin.

TYPE: **MEXICO. OAXACA: Distrito Tlaxiaco. Mpio. San Pedro Molines,** "Km 64 carretera Tlaxiaco-Putla," 17 13 34 N, 97 43 04.5 W, 1950 m, "Creciendo en bosque de pino-encino." 16 Oct 1994, *Jose L. Panero 5049* (Holotype: TEX; isotypes MEXU, MSC).

ADDITIONAL SPECIMEN EXAMINED: **MEXICO. OAXACA:** reportedly, same location as holotype, but 2200 m, 14 Oct 1991, *Panero 2474* (TEX).

In my original description of *Tridax oaxacana* (Turner, 1988), I compared it to *T. purpurea* Blake, the latter a poorly collected species previously accounted for by only a few collections from Guatemala (Nash and Williams, 1976; Powell 1965); Strother (1999) called attention to a recent record from Chiapas, Mexico, this the basis for the collection shown in Fig. 3. *Tridax paneroi* has the stiffly erect habit of *T. oaxacana*, but the greenish-yellow heads of *T. purpurea*. The following key, along with a map showing distributions (Fig. 2), should help identify the taxa concerned:

1. Disk corollas purple-black; larger leaves ovate, 4-7 cm long, the petioles 0-2 mm long.....**T. oaxacana**
1. Disk florets greenish-yellow; larger leaves lanceolate, 7-10 cm long, the petioles 3-10 mm long...(**2**)

2. Plants sprawling or subdecumbent, 0.4-0.7 m high; eastern Chiapas and Guatemala.....**T. purpurea**
2. Plants stiffly erect, 1.0-1.5 m high; western Oaxaca.....**T. paneroi**

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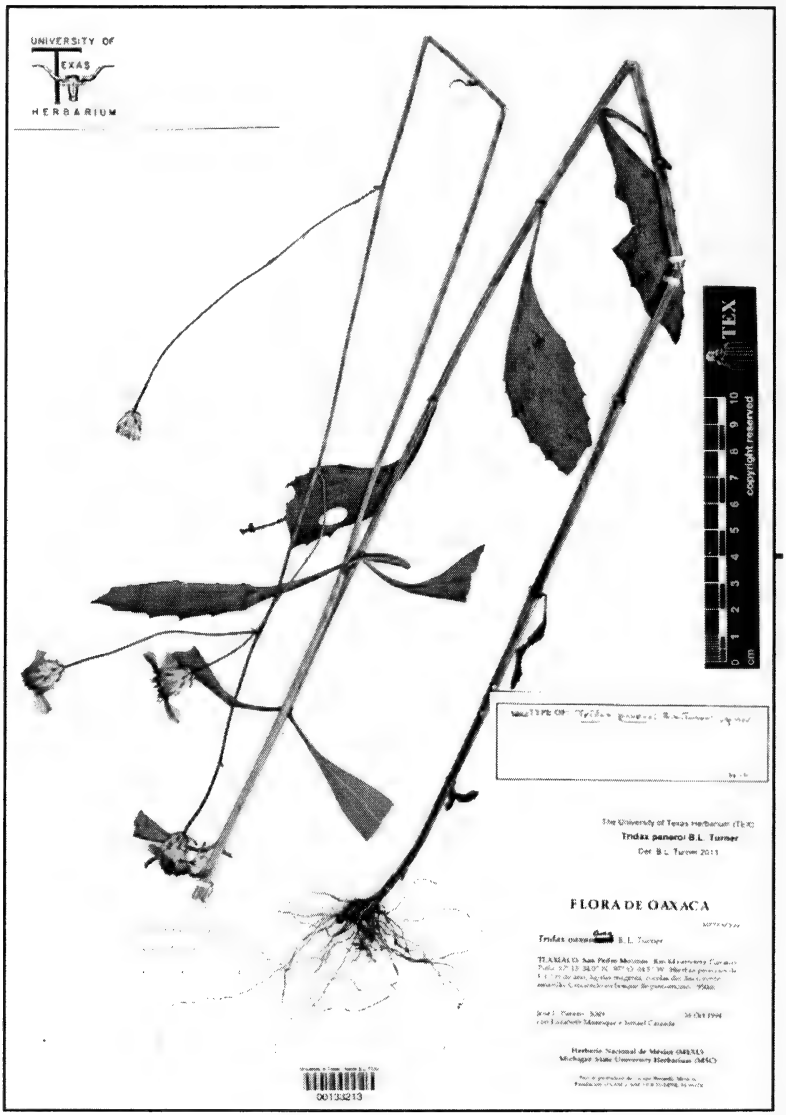

Fig. 1. Photograph of *Tridax paneroi* (Holotype: TEX).

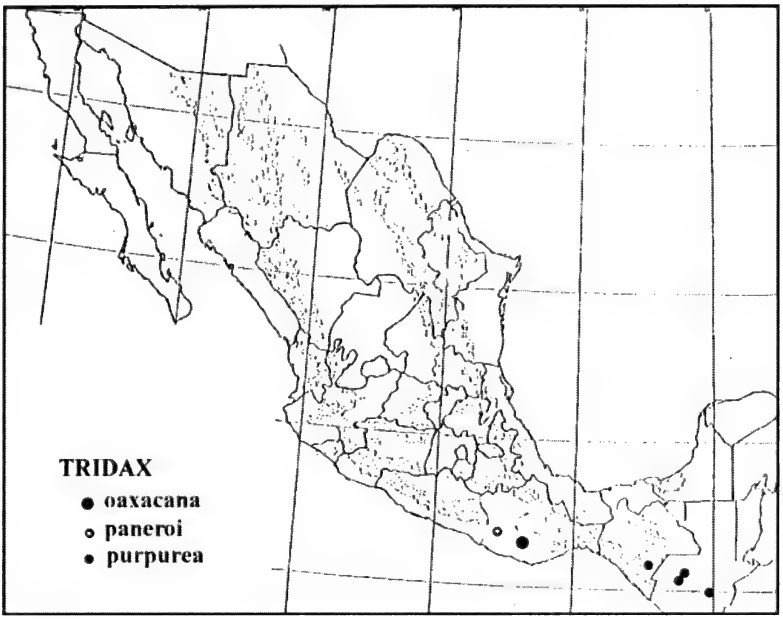

Fig. 2. Distribution of *Tridax paneroi* and cohorts.

ANALYSIS OF PUTATIVE HYBRIDS OF *HESPEROCYPARIS GLABRA* X *H. PYGMAEA* BY LEAF ESSENTIAL OILS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA, email Robert_Adams@baylor.edu

Frank Callahan
P. O. Box 5531, Central Point, OR 97502

and

Richard Kost
P. O. Box 475, Astoria, OR 97103-0475

ABSTRACT

The leaf volatile oils of three putative, spontaneous hybrids between *Hesperocyparis glabra* and *H. pygmaea* were analyzed and compared to typical oils of the parents as well as twelve F_2s derived from one putative hybrid. The oils of the putative hybrids contained components from both *H. glabra* and *H. pygmaea* (cf. karahanaenone, umbellulone). Terpenoids present in both parents were present in intermediate or transgressive amounts, confirming the concerned plants to be natural hybrids between *H. glabra* and *H. pygmaea*. The oils from twelve F_2 individuals displayed about equal numbers of intermediate and transgressive components. The use of terpenoids for the analysis of hybridization is discussed. *Phytologia 94(2): 174-192 (August 1, 2012)*

KEY WORDS: *Hesperocyparis* (=*Cupressus*), *H. glabra*, *H. pygmaea*, hybrids, essential oil, terpenes, inheritance, genetics.

There are few studies on the genetics of terpenes in conifers. Hanover (1966) analyzed the genetics of monoterpenes from the oleoresin in clones, F_1 hybrids and S_1 progeny of *Pinus monticola*. He found the inheritance of each terpene (except camphene) to be additive

with some heterotic or epistatic effects. Re-analysis of the Hanover (1966) data for parents and F_1 progeny (Fig. 1) shows that α-pinene is intermediate in 6/17 and transgressive in 11/17 F_1 individuals. β-pinene had 7/17 intermediate and 10/17 transgressive (Fig. 1). δ-3-carene appears to be mostly intermediate (14/17) with only 3/17 being transgressive (Fig. 1), as was the case for limonene (11/17 intermediate, 6/17 transgressive).

Hanover (1971) expanded his study on *P. monticola* and concluded that :

1. Monoterpenes were under strong, predictable genetic control involving one to several loci.
2. One compound, β-pinene, consistently occurred in larger concentrations in the progeny, which may be due to age effects.
3. A strong positive correlation was found between concentrations of δ-3-carene and terpinolene and negative correlations between α-pinene/ β-pinene and myrcene/ δ-3-carene. Otherwise, the compounds appeared to be independently inherited.
4. Negative correlations were found between monoterpene concentrations and progeny height growth rate.

To determine the number of genes controlling terpenes, Irving and Adams (1973) crossed *Hedeoma drummondii* x *H. reverchonii* and analyzed the parents, F_1, and F_2 progeny. They reported the terpenes were controlled by a minimum of 1 to 7 genes.

Squillace (1971) examined inheritance of monoterpenes in oleoresin of *Pinus elliotii* and concluded that β-pinene and myrcene were controlled by two alleles at a single locus, with high amounts being dominant over low. It is interesting to note that this same pattern is evidenced in Fig. 1. Notice, that of the 30 transgressive individuals, 27 are in larger concentrations than the parents (Fig. 1).

Both quantitative and dominance has been reported in the inheritance of terpenes of Douglas fir (von Rudloff, 1984; von Rudloff and Rehfeldt, 1980) and Scots Pine (Pohjola, et al., 1989).

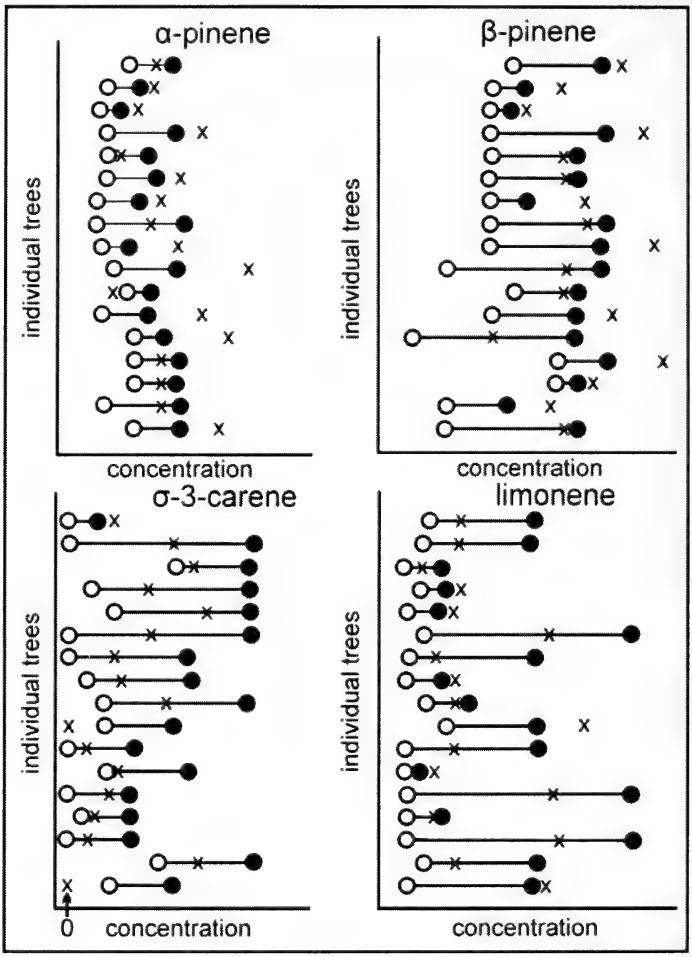

Figure 1. Graphs for four terpenes for 17 F_1 trees. Open and closed circles are the concentrations for parents 1 and 2 in that cross. x is the concentration in the F_1 individual tree. (data from Hanover, 1966).

In the Cupressaceae, there have been very few studies on the inheritance of terpenes. One significant study in *Cupressus* (now *Hesperocyparis*) is that of Lawrence et al. (1975). They examined the

leaf oils of *H. sargentii*, *H. macnabiana* and their putative natural hybrids. In a single population, they analyzed oils from 36 trees and concluded that 12 were *H. sargentii*, 13 were *H. macnabiana* and 10 were intermediate in their oils. PCO analysis of their data (Lawrence et al., 1975, Table 2) found the first and second eigenroots accounted for 27 and 17% of the variance among their samples. These low amounts of variance are likely due to the fact that only two samples of *H. sargentii* and one of *H. macnabiana* were present in the data set. Ordination (Fig. 2) reveals the putative hybrids are quite dispersed between the parental species. From this ordination, it would appear that the putative hybrids (x) likely contained some F_{2s} and backcrossed individuals. However, if transgressive inheritance is involved, that could also explain the wide variation in the putative hybrids.

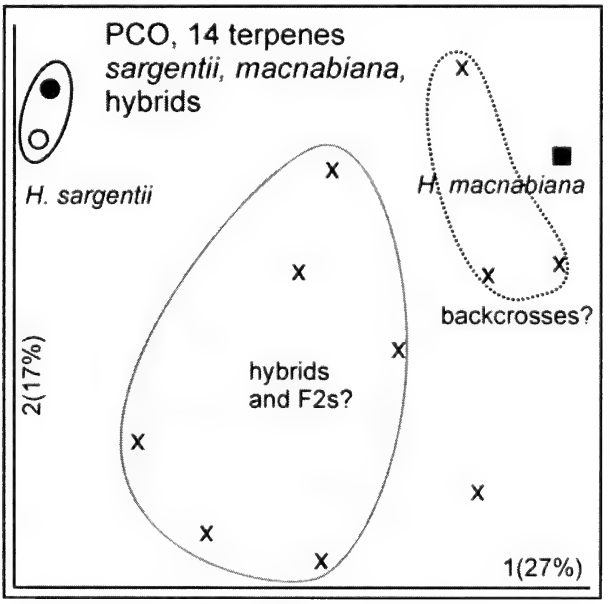

Figure 2. PCO of *H. sargentii, H. macnabiana* and putative hybrids (x). Data from Lawrence et al. (1975, Table 2).

Data from Lawrence et al. (1975, Table 2) was plotted to show the relationship of individual components to the parental species (Fig. 3). Many of the terpenes (7/13) are clearly transgressive. Only 4 of the

13 terpenes are mostly intermediate (camphene, sabinene, myrcene and β-phellandrene). The proportion of transgressive terpenes (7/13) is similar to that found in *P. monticola* (11/17, Fig. 1 above, Hanover,

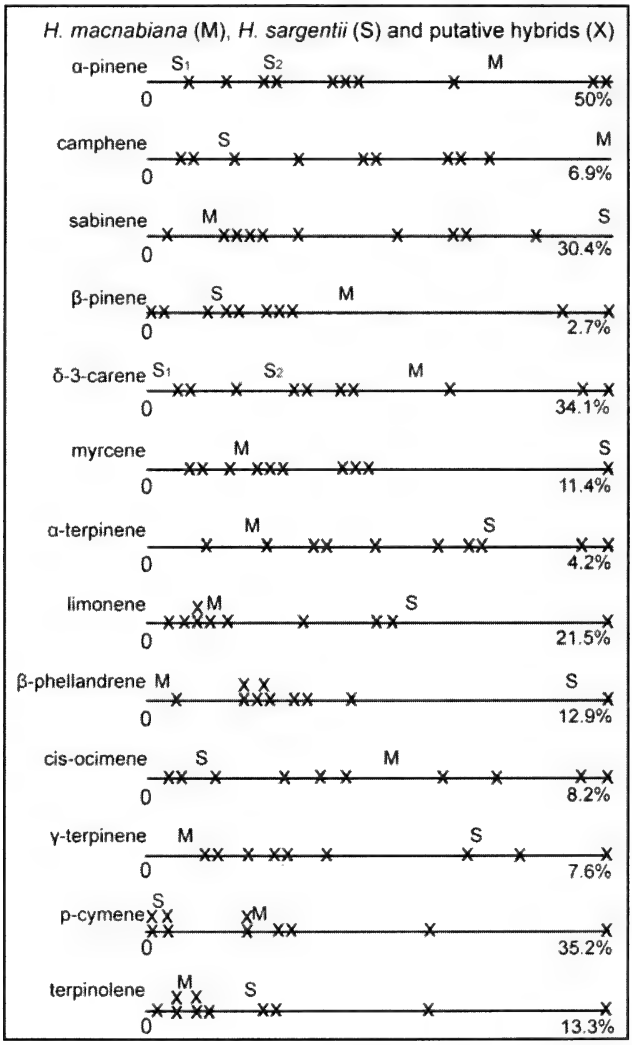

Figure 3. Plot of *sargentii* x *macnabiana* hybrids (data from Lawrence at al. 1975, Table 2). S_1 and S_2 are high/low α-pinene/δ-3-carene popns.

1966). It appears that mixing biosynthetic genes can lead to over-expression of some terpenes.

These 7 transgressive terpenes (α-pinene, β-pinene, δ-3-carene, α-terpinene, limonene, cis-ocimene, p-cymene, terpinolene) plus tricyclene (also transgressive) were removed from the data set and a new PCO performed. This resulted (Fig. 4) in more defined intermediate individuals (presumed hybrids and F_{2s}) and better clustering of the putative backcrosses with *H. macnabiana*. This exercise clearly demonstrates the difficulty of proving hybridization.

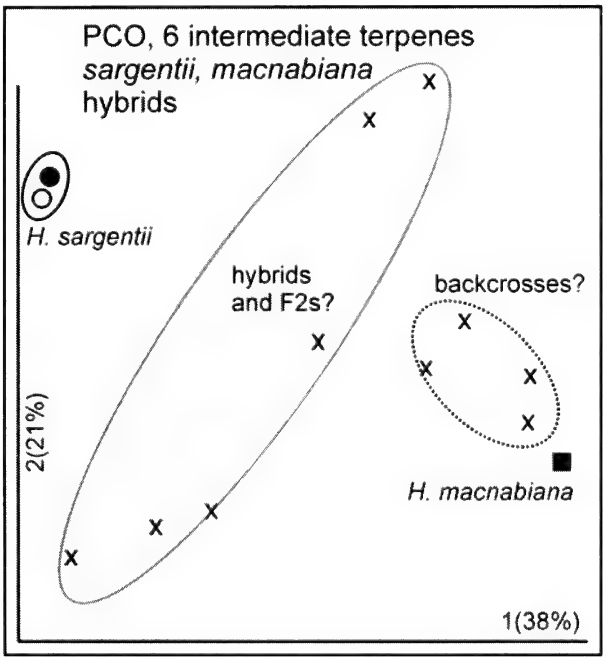

Figure 4. PCO based on 6 mostly intermediate terpenes. Compare with figure 2 using both transgressive and intermediate terpenes.

In 1940, the University of Washington Arboretum accessioned seeds of *H. glabra* and *H. pygmaea* and planted two very glaucous *H. glabra* trees next to a dark green *H. pygmaea* tree near the golf

course in the late 1950s. Richard and Merlin Kost collected seed cones from the *H. pygmaea* (mother tree) in early 1980s. Germination of seeds from these cones produced a few, very unusual, seedlings that were robust with somewhat glaucous foliage (unlike the mother tree, *H. pygmaea*, that was dark green). Two of these seedlings (now large trees) are growing in the Kost Arboretum near Astoria, OR and a third seedling (now a large tree) is growing at the Callahan Arboretum near Central Point, OR. In 2010, leaf samples were taken from these three trees (ca. 42 yrs. old). The original two *H. glabra* trees died in 1974 and the *H. pygmaea* mother tree died in 1997 (pers. comm., Randall Hitchin, Washington Bot. Gard.; unfortunately, these putative parents were not available to study. It appears possible the pollen parents of the seedlings from the *H. pygmaea* tree were from the adjacent *H. glabra* trees. The Callahan tree is very robust and has produced fertile seed for several years. Seedlings and young trees (putative F_2) are very robust and have been cultivated for use as horticultural plants. The nearest cypress is *H. stephensonii* that grows about 3000' from the Callahan putative F_1 tree.

The purpose of this paper is to report on the volatile leaf oils of the putative hybrids, F_2, and parental oils from *H. glabra* and *H. pygmaea*.

MATERIALS AND METHODS

Specimens collected: ***H. glabra***, *Adams 11690-11695*, Dry Beaver Creek, Sedona, 34° 46.131' N, 111° 45.779' W, 1197 m, Yavapai Co., AZ, ***H. pygmaea***, *Adams 11480, 11483* (ex *Bartel 1601a, d*), Albian Ridge, Mendocino Co., CA, *Adams 11484, 11485* (ex *Bartel 1602a, b*), Little River Airport, Mendocino Co., CA, *Adams 11492, 11493* (ex Bartel 1603c, d), Caspar Little Lake, Mendocino Co., CA. putative ***H. glabra x pygmaea*** **hybrids**: *Adams 12444, 12451*, Richard Kost Arboretum, Astoria, OR, *Adams 12452*, Frank Callahan Arboretum, Central Point, OR. **F_2 plants from *Adams 12452***, *Adams 12453-12454, 12458-12475*, Frank Callahan Arboretum, Central Point, OR. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type 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 (100°C, 48 h) for determination of oil yields.

Chemical Analysis - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as percentage of total oil and as mg per g dry foliage weight) compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric (Gower, 1971; Adams, 1975) were computed among all individuals using character weighting of F-1 (F from ANOVA) for PCO . PCA was performed to examine the patterns of association among individual terpenes in the hybrids (formulation of Gower, 1966 and Veldman, 1967).

RESULTS AND DISCUSSION

The compositions of the oils of *H. glabra, H. pygmaea*, and putative F_1s are given in Table 1. Twenty three compounds are present in one species but absent in the other species. However, 12 of these compounds (Table 1) are in low concentrations (0.1%) and determining that a compound is absent is questionable, as GCMS sensitivity is so great, even a few molecules can be detected under ideal conditions. There are 11 compounds that are substantially different (conc. in *H. glabra, H. pygmaea*): karahanaenone (0, 14.6%), umbellulone (8.8%, 0), α-cedrene (0.5, 0), β-cedrene (0.3%, 0), cis-muurola-3,5-diene (4.3%, 0), cis-murrola-4(14),5-diene (11.8%, 0), cedrol (1.2%, 0), α-acorenol (3.2%, 0), β-acorenol (0.6%, 0), α-cadinol (0.9%, 0), iso-

pimara-8(14), 15-diene (0, 0.3%), abietadiene (0.9%, 0) and phyllocladanol (0.5%, 0). Interestingly, only two of these (karahanaenone, iso-pimara-8(14), 15-diene) are present in *H. glabra*, with 9 present in *H. pygmaea*. Two sets of components present in *H. pygmaea* are isomers, and thus may be largely controlled by the same gene (with modifiers): (α-cedrene, β-cedrene), (α-acorenol, β-acorenol), so the differences may overestimated. Karahanaenone and umbellulone are unusual components in species of *Hesperocyparis* and seem good taxonomic markers. For karahanaenone, the putative F_1s are intermediate (12.1, 1.6, 6.8%). The putative F_1s are also intermediate in their umbellulone concentrations (3.6, 7.2, trace). This supports the theory that C1, K1 and K2 are indeed hybrids between *H. glabra* and *H. pygmaea*. Adams (1982) used leaf terpenoids from *Juniperus* to compare Wells' hybrid distance diagrams, PCA, PCO, and canonical variate analysis. He found that PCO, using character weights of F-1 (F from ANOVA between the putative parents), was the most effective method tested. PCO using 23 terpenoids, shows the putative F_1s cluster near *H. pygmaea*, with K2 being very similar to *H. pygmaea* (Fig. 5). Putative F_1s C1 and K1 are more intermediate in their oils. It is very

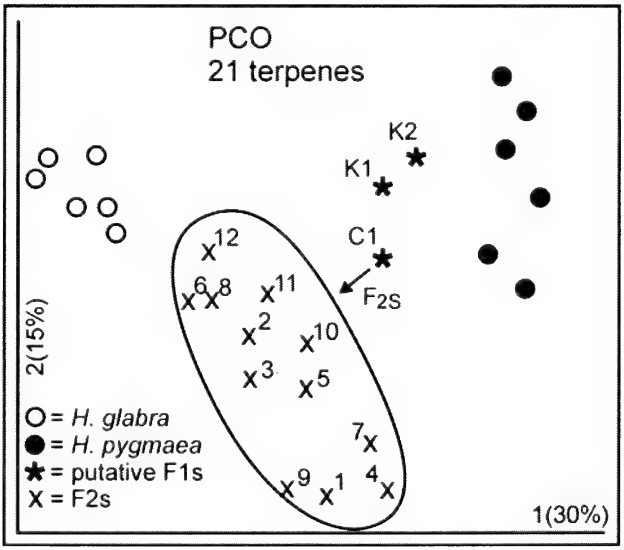

Figure 5. PCO of *H. glabra*, *H. pygmaea*, putative F_1s and F_2s (derived from C1).

important to note that the putative parents of C1, K1, and K2 were not available for study, as they died several years ago and it is unlikely that the oils of the surrogate samples representing *H. glabra* and *H. pygmaea* are exactly like the real parents. The oils of F_2s derived from individual C1 (Fig. 5) have considerable variation. Four F_2s (2, 8, 11, 12) have oils similar to *H. glabra* and four (3, 5, 6, 10) are quite intermediate. Four F_2s (1, 4, 7, 9) are very different. The variation in the F_2s (Fig. 5) is similar to that seen in *H. sargentii* - *H. macnabiana* (Figs. 2, 3).

Eleven compounds were intermediate in the F_1s and F_2s (Table 1) between the parents: α-thujene, α-pinene, sabinene, α-terpinene, limonene, β-phellandrene, camphor, terpinen-4-ol, α-terpineol, α-terpinyl acetate, and nezukol. These compounds were deemed to be quantatively inherited and are graphed in Fig. 6. Sabinene, limonene, β-phellandrene and camphor were transgressive in the F_2s (Fig. 6). Often one or two individual F_2s contained large, transgressive amounts as in the case of camphor (F_2 #4: 31.8%, F_2 #1: 23%), and limonene (F_2 #5: 20.8%, F_2 #2: 15.5%.

Ten compounds that were present/absent in one putative parent were graphed in Figure 7. For five compounds, most of the F_2s have zero or traces amounts (karahanaenone, α-cedrene, cis-muurola-3,5-diene, cis-muurola-4(14), 15-diene, iso-pimara-8(14), 15-diene). Four compounds have one extremely transgressive individual: camphene hydrate (F_2 #4: 1.5%), umbellulone (F_2 #8: 20.5%), α-acorenol (F_2 #6: 8.1%) and β-acorenol (F_2 #6: 1.6%).

When a compound occurs in a greater concentration (transgressive) than either of the parents, it seems logical to score that individual as being similar to the appropriate parent with high amounts of this component. One way to accomplish this it to truncate the transgressive amount to the level found in one parent. PCO analysis with the 8 transgressive compounds (sabinene, limonene, β-phellandrene, camphor, camphene hydrate, umbellulone, α-acorenol, β-acorenol) truncated at the highest level found in either parent resulted in the removal of more variance in the second eigenroot (17 vs. 15%). Ordination (Fig. 8) shows the F_1s are better resolved from *H. pygmaea* and the F_2s are clustered about the same.

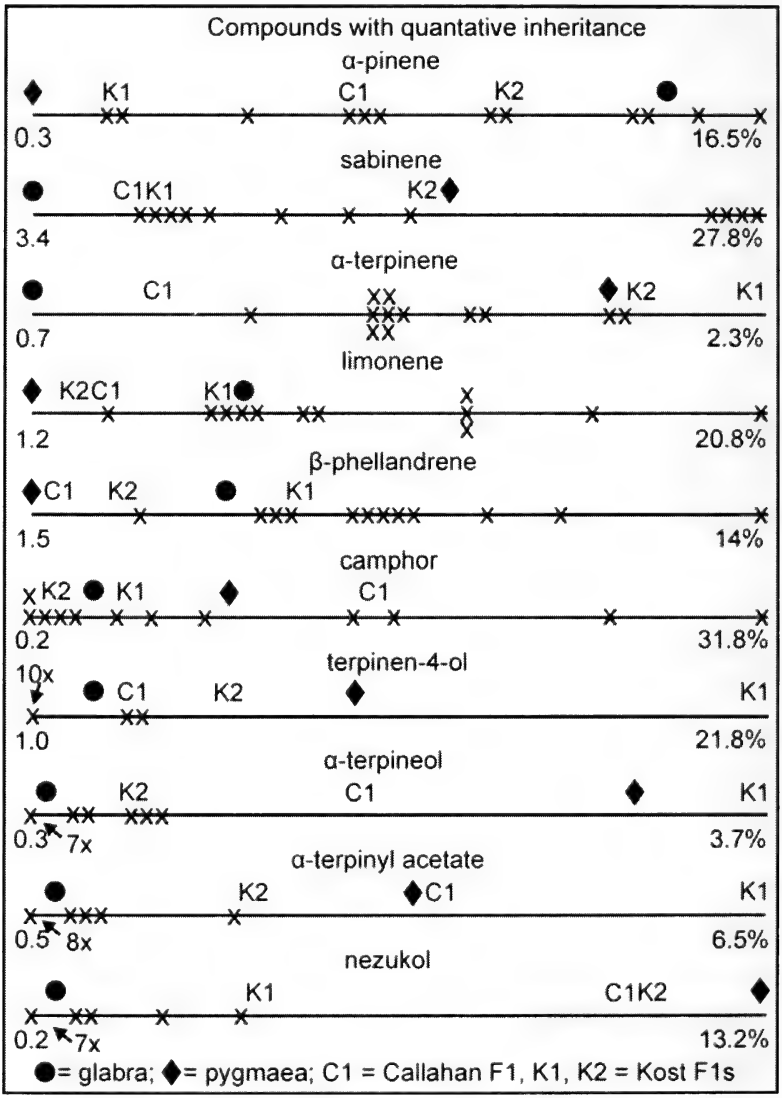

Figure 6. Graph of *H. glabra* and *H. pygmaea*, putative F_1s and F_2s (derived from C1) for terpenes with quantitative inheritance.

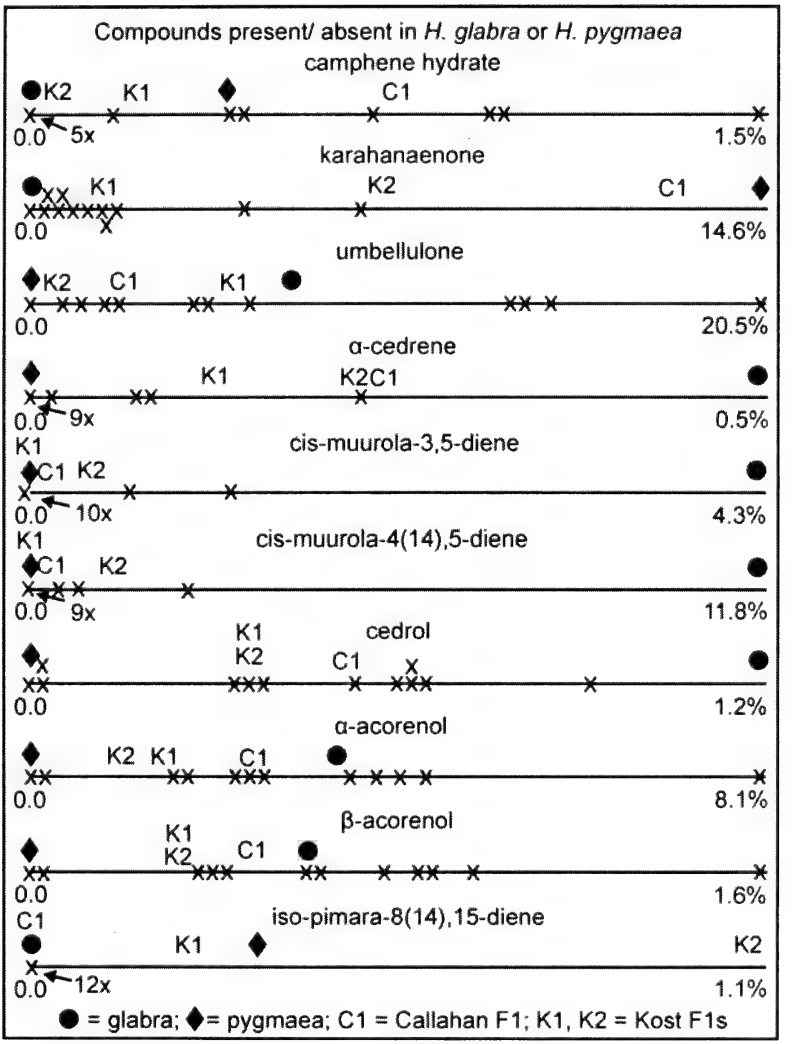

Figure 7. Graph of *H. glabra* and *H. pygmaea*, putative F_1s and F_2s (derived from C1) for terpenes that were present/ absent in one parent.

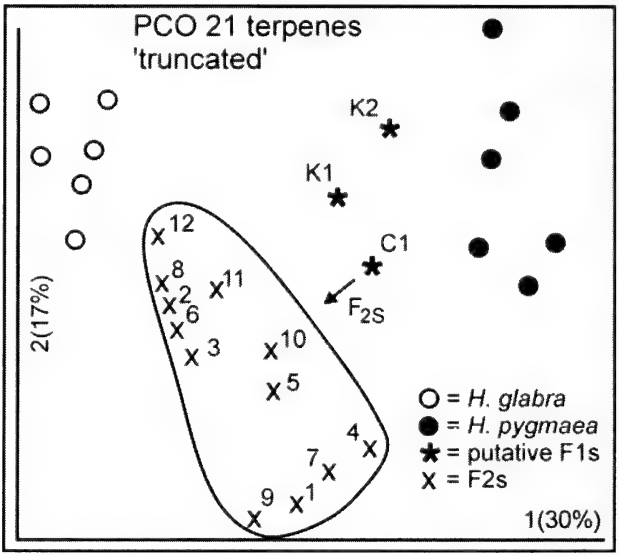

Figure 8. PCO using 21 terpenes with 8 transgressive compounds truncated at the highest level found in either parent.

The nature of inheritance in the present case is similar to that of *H. sargentii* x *H. macnabiana* (Lawrence et al., 1975) and *Pinus monticola* (Hanover, 1966), in that about equal numbers of terpenes are intermediate between parents (versus being transgressive to one of the parents). It is not clear if truncating transgressive characters is appropriate. Data from parents, and their artificial F_1s and F_2s are needed to confirm this methodology. From the present analysis of *H. glabra* x *H. pygmaea*, it may be that *H. sargentii* x *H. macnabiana* plants labeled 'backcrosses' (Fig. 4) are in fact first generation hybrids.

Although it is unfortunate that the parents of the putative hybrids, C1, K1 and K2 died and were not available for analysis, it does appear that these individuals are of hybrid origin. The analysis of F_2s derived from individual C1 demonstrates the large amount of variation one might expect among F_2 individuals, as well as the transgressive nature of many terpenoids.

In a review of transgressive variation (in both plants and animals), Rieseberg et al. (1999) examined 171 studies and found that at least one transgressive trait was reported in 155 (91%) of the studies. Of the 1229 traits studied, 44% were transgressive in the hybrids. Transgressive variation occurred most frequently in intraspecific, inbred, domesticated plants and least frequently in interspecific crosses between outbred, wild animal species.

Clearly, terpene data from parents, and their artificial F_1s (and F_2s) are needed to further elucidate the magnitude of transgressive variation and to evaluate numerical methods for the analysis of putative natural populations.

ACKNOWLEDGEMENTS

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Table 1. Comparisons of the leaf essential oil compositions of putative *H. glabra* x *H. pygmaea* hybrids with the oils of *H. glabra* (glab), and *H. pygmaea* (pyg). Cal F1 = *Adams 12452*, putative *H. glabra* x *pygmaea*, F. Callahan Arb., Ks1 = *Adams 12444*, tree 1, putative *H. glabra* x *pygmaea*, R. Kost Arb., Ks2 = *Adams 12451*, tree 2, putative *H. glabra* x *pygmaea*, R. Kost Arb. Compounds that are present in only one of the parental taxa and in the putative hybrid are in boldface. Compounds that are intermediate in concentration are in italics.

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
846	(E)-2-hexenal	0.3	0.2	0.4	2.7	1.1
908	isobutyl-isobutyrate	-	0.2	0.1	t	-
921	tricyclene	0.1	0.1	t	t	0.1
924	*α-thujene*	*0.6*	*0.2*	*0.2*	*0.7*	*0.6*
932	*α-pinene*	*12.6*	*6.5*	*1.5*	*9.0*	*0.3*
945	α-fenchene	0.1	t	-	0.2	0.1
946	camphene	0.2	0.2	t	0.1	0.3
953	thuja-2,4-diene	t	-	-	-	-
969	*sabinene*	*3.4*	*5.9*	*6.7*	*15.2*	*15.2*
974	β-pinene	0.4	0.3	0.1	1.1	0.3
988	myrcene	1.7	1.8	1.7	2.5	2.6
994	2-octanol	t	-	t	-	-
1002	α-phellandrene	0.2	0.1	0.3	0.2	0.6
1008	δ-3-carene	1.8	0.2	t	5.8	1.3
1014	*α-terpinene*	*0.7*	*0.9*	*2.3*	*2.0*	*1.9*
1020	p-cymene	0.7	0.2	0.3	0.3	0.5
1024	*limonene*	*4.0*	*2.4*	*3.5*	*2.0*	*1.2*
1025	*β-phellandrene*	*4.0*	*1.6*	*5.2*	*2.0*	*1.5*
1026	1,8-cineole	-	-	t	-	0.1
1054	γ-terpinene	1.1	1.6	4.4	3.3	3.1
1065	cis-sabinene hydrate	0.2	0.5	0.9	0.3	0.5
1086	terpinolene	1.0	0.1	2.5	1.8	1.3
1098	trans-sabinene hydrate	0.1	0.6	0.7	0.2	0.3
1099	linalool	0.2	0.6	0.7	0.6	0.7
1100	**n-nonanal**	**-**	**0.2**	**-**	**t**	**0.1**
1112	**trans-thujone**	**-**	**0.1**	**-**	**t**	**0.1**
1118	cis-p-menth-2-en-1-ol	0.2	0.4	1.4	0.3	0.5
1122	**α-campholenal**	**0.1**	**t**	**t**	**t**	**-**
1136	trans-p-menth-2-en-1-ol	0.2	t	1.1	0.3	0.3

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
1141	*camphor*	*1.3*	*13.3*	*1.8*	*0.7*	*8.7*
1145	**camphene hydrate**	**-**	**0.7**	**0.2**	**t**	**0.4**
1148	citronellal	t	t	1.4	0.4	t
1154	**karahanaenone**	**-**	**12.1**	**1.6**	**6.8**	**14.6**
1165	borneol	-	t	-	t	t
1167	**umbellulone**	**8.8**	**3.8**	**7.2**	**t**	**-**
1174	*terpinen-4-ol*	*2.7*	*5.4*	*21.8*	*7.8*	*9.5*
1179	p-cymen-8-ol	0.5	0.2	-	-	t
1186	*α-terpineol*	*0.5*	*2.0*	*3.7*	*1.1*	*3.2*
1195	cis-piperitol	-	0.1	0.3	t	t
1195	myrtenal	0.1	-	-	-	-
1205	trans-piperitol	0.3	0.2	0.6	0.1	0.2
1206	verbenone	0.2	-	t	-	-
1223	citronellol	2.4	2.3	2.2	0.3	2.2
1232	thymol, methyl ether	t	-	-	-	-
1239	**carvone**	**t**	**0.1**	-	-	**-**
1241	**carvacrol, methyl ether**	**-**	**0.1**	-	**0.1**	**t**
1249	piperitone	t	0.1	0.2	t	0.1
1287	**bornyl acetate**	**-**	**t**	**0.6**	**0.4**	**t**
1293	2-ethyl-isomenthone	0.2	-	-	-	-
1295	**3-thujanol acetate**	**-**	**0.2**	**1.6**	**-**	**0.1**
1293	2-undecanone	-	-	-	-	t
1299	terpinen-4-yl acetate	0.5	0.1	0.8	0.2	0.1
1339	trans-carvyl acetate	t	-	-	-	-
1346	*α-terpinyl acetate*	*0.6*	*4.3*	*6.5*	*2.6*	*4.2*
1350	citronellyl acetate	-	-	0.2	t	t
1379	geranyl acetate	-	-	0.7	-	-
1403	methyl eugenol	-	-	0.3	-	t
1410	**α-cedrene**	**0.5**	**0.2**	**0.1**	**0.2**	**-**
1419	**β-cedrene**	**0.3**	**0.1**	**t**	**0.1**	**-**
1448	**cis-muurola-3,5-diene**	**4.3**	**t**	**-**	**0.2**	**-**
1452	α-humulene	t	-	-	-	-
1465	**cis-muurola-4(14),5-diene**	**11.8**	**0.1**	**-**	**0.6**	**-**
1465	α-acoradiene	-	0.1	0.1	-	-
1474	10-epi-β-acoradiene	-	t	-	-	-
1479	ar-curcumene	-	t	-	-	-
1480	germacrene D	-	-	t	-	t
1482	citronellol isobutyrate	0.2	-	-	-	-
1501	epi-zonarene	3.9	-	-	-	-

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
1504	cuparene	-	t	-	-	-
1505	β-bisabolene	-	-	-	-	t
1506	(Z)-α-bisabolene	-	0.1	-	-	-
1512	α-alaskene	0.2	0.1	-	-	-
1521	trans-calamenene	2.7	t	-	-	-
1522	δ-cadinene	t	-	-	-	-
1533	10-epi-cubebol	0.4	-	-	-	-
1536	italicene ether	0.9	t	-	-	-
1544	α-calacorene	0.2	-	-	-	-
1550	cis-muurola-5-en-4-β-ol	1.6	t	-	-	-
1559	cis-muurola-5-en-4-α-ol	1.9	t	-	-	-
1559	germacrene B	-	-	-	-	t
1561	(E)-nerolidol	-	0.1	-	-	t
1564	β-calacorene	0.1	-	-	-	-
1582	caryophyllene oxide	t	-	-	-	-
1600	**cedrol**	**1.2**	**0.5**	**0.3**	**0.3**	**-**
1608	humulene epoxide II	0.1	-	-	-	-
1618	epi-cedrol	-	t	t	-	-
1618	1,10-di-epi-cubenol	0.4	-	-	-	-
1627	3-oxobutyl-isomenthone	0.6	-	-	-	-
1632	**α-acorenol**	**3.2**	**2.6**	**1.8**	**1.5**	**-**
1636	**β-acorenol**	**0.6**	**0.5**	**0.3**	**0.3**	**-**
1638	epi-α-cadinol	0.3	-	-	-	-
1638	epi-α-muurolol	0.3	-	-	-	-
1645	cubenol	t	-	-	-	-
1652	**α -cadinol**	**0.9**	**t**	t	-	**-**
1675	cadalene	0.5	-	-	-	-
1683	epi-α-bisabolol	-	0.1	0.2	-	t
1685	α-bisabolol	-	0.5	-	-	-
1688	cis-14-nor-muurol-5-en-4-one	0.1	-	-	-	-
1722	2Z,6E-franesol	-	-	1.0	-	-
1724	(Z)-nuciferol	-	0.4	-	-	t
1754	β-(Z)-curcumen-12-ol	-	0.4	-	-	t
1887	oplopanonyl acetate	0.1	-	-	-	-
1896	rimuene	-	-	-	0.1	t
1900	pimara-9(11),15-diene	-	t	-	-	1.1
1907	pimara-8(9),15(16)-diene	-	0.1	0.1	0.5	-
1933	isohibaene	t	0.5	0.1	0.5	-
1905	**isopimara-9(11),15-diene**	**-**	**0.4**	**t**	**0.4**	**0.2**

RT	compound	glab	Cal F1	Ks1 F1	Ks2 F1	pyg
1948	pimaradiene	-	0.1	-	t	t
1958	**iso-pimara-8(14),15-diene**	**-**	**-**	**0.2**	**1.1**	**0.3**
1960	iso-sandaracopimara-8(14),15	t	-	-	-	t
1966	isophyllocladene	0.4	3.2	0.6	2.9	0.4
1978	manoyl oxide	0.8	2.7	-	3.1	0.5
1987	iso-pimara-7,15-diene	-	-	0.3	-	-
2009	13-epi-manoyl oxide	t	0.6	-	0.4	0.1
2016	**phyllocladene**	**-**	**0.4**	**-**	**0.3**	**0.1**
2055	abietatriene	0.2	0.2	t	0.2	0.1
2087	**abietadiene**	**0.9**	**0.1**	**t**	**t**	**-**
2091	**iso-nezukiol**	**-**	**0.3**	**-**	**1.2**	**0.2**
2105	isoabienol	0.1	-	-	-	t
2132	*nezukol*	*0.6*	*11.7*	*4.4*	*12.1*	*13.2*
2184	**sandaracopimarinal**	**-**	**0.2**	**0.1**	**0.2**	**0.1**
2209	**phyllocladanol**	**0.5**	**t**	-	-	**-**
2282	sempervirol	0.1	0.5	0.7	0.8	0.3
2314	trans-totarol	0.1	0.1	0.3	0.3	t
2331	trans-ferruginol	t	t	0.1	0.3	t

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

SYSTEMATIC STUDY OF THE *KOANOPHYLLON PALMERI* COMPLEX (ASTERACEAE: EUPATORIEAE)

Billie L Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

The *Koanophyllon palmeri* complex is treated as consisting of three taxa: *K. palmeri* (A. Gray) King & H. Rob. var. *palmeri*; *K. palmeri* **var. tonsa** (B.L. Rob.) B.L. Turner, **comb. nov**.; and **Koanophyllon pochutlana** B.L. Turner, **sp. nov.** A phototype of the latter is provided, along with distribution maps, and a key to the taxa concerned. *Phytologia 94(2): 193-198 (August 1, 2012).*

KEY WORDS: *Eupatorium, Koanophyllon*, *K. palmeri*, Asteraceae, Eupatorieae, Mexico

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Routine identification of Mexican comps has occasioned the present paper.

In my seminal treatment of the tribe Eupatorieae for Mexico, I treated *Koanophyllon* within the broad fabric of *Eupatorium* (Turner 1997). I have subsequently come to accept the fragmentation of that genus, as espoused by King and Robinson (1987).

Key to the Koanophyllon palmeri complex:

1. Heads with 10-11 florets; inner bracts broadly ovate, ca twice as long as wide; Oax.....**K. pochutlana**
1. Heads with 4-6(7) florets; inner bracts narrowly ovate, ca 3 times as long as wide; Son to Gue.....**K. palmeri** (**2**)

2. Involucral bracts mostly 1-2 mm long; pappus bristles ca 1.5 mm long .....**var. tonsa**
2. Involucral bracts mostly 2.6-4.0 mm long; pappus bristles 2.5-3.5 mm long .....**var. palmeri**

var. PALMERI (A. Gray) King & H. Rob., Phytologia 22: 150. 1971. *Eupatorium palmeri* A. Gray, Proc. Amer. Acad. Arts 21: 383. 1886.

TYPE: **MEXICO. CHIHUAHUA: Mpio. Batopilas,** mountains above Batopilas, Aug-Nov 1885, *Palmer 144, 260* (Lectotype: *Palmer 144*, GH!)

Son, Chi, Sin, Dur, Jal, Col and closely adjacent USA, barrancas, in mostly pine-oak forests, 10-1700 m; Sep-Nov. **Fig. 2**

Slender arching shrub or shrublets 1-3 m high. **Stems** striate, puberulent. **Leaves** thin, opposite, 5-12 cm long, ca 2.5 cm wide; petioles 5-20 mm long; blades ovate-lanceolate, 3-nervate from or near the base, puberulent and glandular-punctate beneath, the margins crenulate to serrulate, the apices attenuate. **Capitulescence,** a terminal, congested corymbose-panicle, the ultimate peduncles mostly 0-1 mm long. **Heads,** 5-6 mm high; corollas, cream, whitish, yellowish or reddish-brown, ca 3.5 mm long. **Receptacles** ca 0.3 mm across, glabrous. **Involucres** mostly 3-4 mm high; inner bracts 3-4 mm long, ca 0.6 mm wide, mostly 3-nervate. **Florets,** 4-7 per head. **Achenes** 2.0-2.5 mm long, hispidulous; pappus of 40-50 bristles, 2.5-3.5 mm long.

McVaugh (1984) placed this taxon in synonymy under his broad concept of *Eupatorium solidaginifolium* A. Gray [= *Koanophyllon solidaginifolium* (A. Gray) King & H. Rob.], a decidedly different species of more eastern distribution. He also neglected to account for the var. *tonsa.*

var. TONSA (B.L. Rob.) B.L. Turner, **comb. nov.**
Based upon *Eupatorium palmeri* var. *tonsum* B.L. Rob., Proc. Amer. Acad. Arts 42: 43. 1906.

TYPE: **MEXICO. GUERRERO:** "El Ocote," 300 m, 10 Nov 1998, *E. Langlasse 616* (Lectotype GH!, selected here; photolectotype, TEX!)

Pacific slopes, s Sin, Nay, Jal, Col, Mic, Gue, tropical deciduous forests, 10-800 m; Oct-Dec. **Fig. 2**

Suffruticose herbs or shrublets 1-3 m high. **Stems** sparsely pubescent with upwardly appressed, white, hairs. **Leaves** 5-7 cm long, 2-5 cm wide, opposite throughout; petioles 1-2 cm long, pubescent like the stems; blades narrowly to broadly ovate, pubescent beneath, mainly along the ribs, the surfaces markedly glandular-punctate; margins weakly crenulate to entire. **Capitulescence** a pyramidal, corymbose, panicle ca 10 cm long, 4-6 cm wide, the ultimate peduncles 1-2 mm long. **Involucres** composed of 5-7 lanceolate bracts, mostly 3-nervate, 1.5-2.0 mm long, ca 0.4 mm wide in 2-3 series, their apices sharply acute. **Receptacles** ca 0.2 mm across, glabrous. **Florets** 4-5 to a head; corollas glabrous, white, ca 1.5 mm long, the 5 lobes markedly beset with amber globules. **Style branches** clavate apically. **Anthers** included, ca 1 mm long, the appendages minute, ca as long as wide. **Achenes** hispid, 4-5 sided, ca 2 mm long; pappus of 20-30 persistent bristles ca 1.5 mm long.

This is a weakly differentiated taxon, largely distinguished from the typical variety by its smaller heads, smaller inner involucral bracts and shorter pappus. Most collections of var. *tonsa* have been gathered from relatively low elevations along the Pacific slopes; when occurring in close geographical proximity, the var. *palmeri* usually is found in more interior locations at higher sites. Intergradation appears to occur between the two taxa in regions of near contact.

KOANOPHYLLON POCHUTLANA B.L. Turner, **sp. nov. Fig. 1**

Resembling *Koanophyllon palmeri* (A. Gray) King & Rob. but the florets more numerous (10-11 per head vs 4-7), involucral bracts shorter (ca 2 mm long vs 3-4 mm), smaller achenes (ca 1.5 mm long vs 2.5-3.5 mm) and the pappus shorter (ca 1.5 mm long vs 2.5-3.5 mm).

TYPE: **MEXICO. OAXACA: Mpio. San Miguel del Puerto,** "1.63 km (LR) 154 [degrees] de rancho Dioon. Arroyo Arena," ca 790 m, 15 58 33.7 N, 96 6.53 W, 18 Nov 2003, *Alfredo Saynes V. 4078* [con A. Nava et al.] (holotype: TEX).

Known only by the type collection. **Fig. 2**

Scandent shrubs to 3 m high. **Stems** densely to sparsely pubescent with short, mostly up-curved, hairs. **Leaves** opposite, 6-9 cm long, 3-4 cm wide; petioles 1.0-1.5 cm long; blades ovate, 3-nervate from or near the base or nearly so. **Capitulescence** a terminal cymose panicle ca 11 cm high, 8 cm wide, the ultimate peduncles ca 1 mm long. **Heads** ca 5 mm high, the inner bracts ovate, ca 2 mm long, 0.8 mm wide, the apices mostly broadly acute and mostly 1-nervate. **Florets** 10-11 per head; corollas white, ca 5 mm long, glabrous, the 5 lobes atomiferous-glandular. **Achenes** black, ca 1.5 mm long, moderately pubescent with short, appressed, hairs; pappus of 30-40 tawny-white bristles ca 1.5 mm long.

Named for the Distrito Pochutla, where first collected.

The novelty appears to stand somewhere between *Koanophyllon palmeri* var. *tonsa* and *K. solidaginifolia*, having the more numerous florets and apically broad inner bracts of the latter, but the smaller heads and achenes of the former.

ACKNOWLEDGEMENTS

Distribution maps are based upon specimens on file at LL-TEX, those cited by McVaugh (1987), and USDA records available on web sites.

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Fig. 1. *Koanophyllon pochutlana* (holotype).

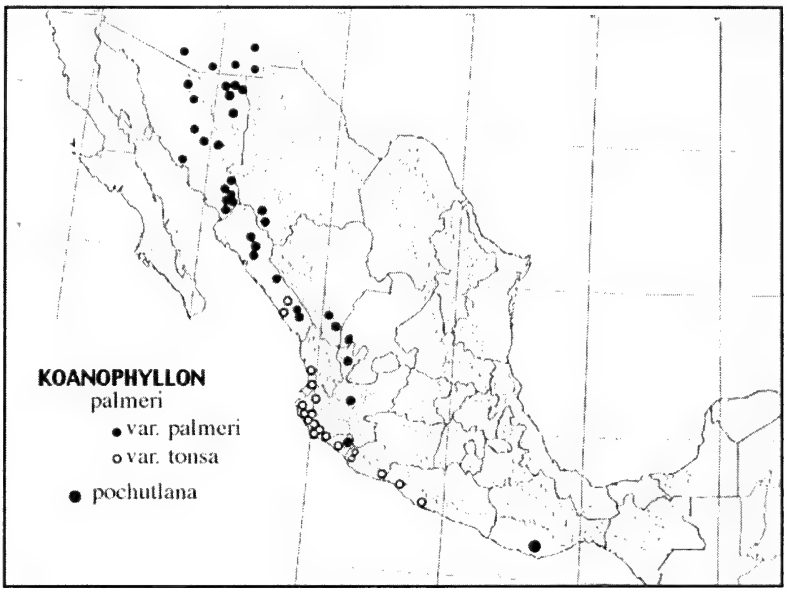

Fig. 2. Distribution of the *Koanophyllon palmeri* complex

GEOGRAPHIC VARIATION IN LEAF ESSENTIAL OILS OF DOUGLAS FIR (*PSEUDOTSUGA MENZIESII*)

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA Robert_Adams@baylor.edu

J. Jesús Vargas-Hernández
Programa Forestal - Campus Montecillo, Colegio de Postgraduados km 65.5 Carretara México - Texcoco, México C.P. 56230 México

M. Socorro González Elizondo
CIIDIR Unidad Durango, Instituto Politecnico Nacional, Sigma 119, Fracc. 20 de Noviembre II, Durango, Dgo., 34220 Mexico

Gay Hunter
Museum Curator, Olympic National Park
600 East Park Ave., Port Angeles, WA 98362

Thomas A. Fairhall
1136 Water St., Suite 101, Port Townsend, WA 98368

David Thornburg
220 W. Winchester Lane, Cottonwood AZ

and

Frank Callahan
Callahan Seeds, P. O. Box 5531, Central Point, OR 97502

ABSTRACT

The volatile leaf oils of Douglas fir (*Pseudotsuga menziesii*) were analyzed from throughout its range. The major differentiation found was the divergence of the inland populations (var. *glauca*) from the coastal and Sierra Nevada populations (var. *menziesii*). The oils of var. *menziesii* differed from var. *glauca* in their major components: camphene (0.4-17, 25-30%), β-pinene (25-38, 2.5-12%), sabinene (4-12, trace-0.5%), α-terpinene (2.1-3.3, 0.2-0.0%), γ-terpinene (3.4-5.5, 0.1-0.3%),

terpinolene (9.5-14.6, 1.2-1.6%), cis-p-menth-2-en-1-ol (0.7-0.8, 0.0-trace), terpinen-4-ol (10-12.1, trace-0.5%), and bornyl acetate (0.2-1.3, 14.6-44.7%). The oil of the Sierra Nevada population (Sierra Nevada race of Snajberk and Zavarin, 1976) was quite similar to typical coastal Doug Fir from WA and OR and not as distinct as found in the oleoresin oils of Snajberk and Zavarin. Overall the oils of the inland variety (var. *glauca*) varied from Yellowstone southward with the AZ - NM oils forming a group along with the southern Mexico oils except for the population at Cerro Potosi that had a different oil. The Cerro Potosi oil differed from other inland (var. *glauca*) oils in having larger amounts of α-pinene, β-pinene, δ-3-carene and smaller amounts of camphene, limonene and bornyl acetate. The leaf oil of var. *oaxacana* did not differ from var. *glauca* from nearby populations in southern Mexico. The leaf essential oils of *P. macrocarpa* are also reported. *Phytologia 94(2):199-218 (August 1, 2012).*

KEY WORDS: *Pseudotsuga menziesii,* var. *menziesii,* var. *glauca,* var. *oaxacana,* leaf essential oils, geographic variation, Douglas Fir.

Douglas Fir [*Pseudotsuga menziesii* (Mirb.) Franco] is a wide ranging, common forest tree in North America (Fig. 1). The nomenclatural history of the name is a morass (see http://www.plantsystematics.org/reveal/pbio/LnC/dougfir.html), but seems to be settled by James Reveal. In a recent treatment, Eckenwalder (p. 572, 2009) recognizes two varieties: var. *menziesii* and var. *glauca* (Mayr) Franco [cited as (Beissn.) Franco in Eckenwalder, 2009]. Eckenwalder (2009) did not recognize var. *oaxacana* Debreczy & Racz, described from Oaxaca (Debreczy and Racz, 1995).

The leaf essential oils of *P. menziesii* have been exhaustively studied by von Rudloff (von Rudloff, 1972, 1973, 1984, von Rudloff and Rehfeldt, 1980) who carefully documented the large differences in oils between coastal (var. *menziesii*) and inland (var. *glauca*) in many compounds including camphene (0.3-8, 20-30%), β-pinene (15-30, 5-10%), sabinene (2-12, 0.1-0.5%), α-terpinene 1-3, 0-0.3%), γ-terpinene (2-8, 0.1-1%), terpinolene (5-15, 0.5-3%), terpinen-4-ol (5-15, 0.5-3%), and bornyl acetate (0.5-5, 20-30%). However, von Rudloff's studies

were limited to the Pacific Northwest and northern US-Canada (south to Wyoming).

A second team from USDA, Forest Products, Richmond, CA (Snajberk and Zavarin), made extensive studies of the terpenoids from the oleoresin of Douglas Fir (Snajberk, Lee and Zavarin, 1974, Snajberk and Zavarin, 1976, Zavarin and Snajberk, 1973, 1975). In their most comprehensive study (Snajberk and Zavarin, 1976), they found four chemical races: coastal, northern inland, southern inland and Sierra Nevada. These are shown in Fig. 1, along with populations used in the present study. Notice the populations sampled in the present study are from each race: coastal (2), northern inland (1), Sierra Nevada (1), southern inland (2), plus Mexico (6) as well as *P. macrocarpa*. Douglas Fir in Mexico is principally found in small restricted populations, except for a larger area in Chihuahua in northern Mexico (Fig. 1). In spite of the very exhaustive studies of leaf and wood oils in the United States and Canada, nothing has been reported about variation in Douglas Fir oils in Mexico.

However, there has been work at the molecular level on Douglas Fir in Mexico. Li and Adams (1989) reported that allozymes divided the Douglas Fir into northern coastal (var. *menziesii*) and inland (var. *glauca*) with two subgroups (northern and southern inland). They did not find evidence of a subgroup of Sierra Nevada Douglas Fir as Snajberk and Zavarin (1976) found, based on the oleoresin oils. In addition, Li and Adams (1989) found a distinct pattern in the allozymes from population 103 at General Cepeda, Coah., MX and speculated that it might be *P. flahaultii* Flous (also recognized by Martinez, 1963). However, a nearby collection (104, La Encantada, near Zaragoza, NL) clustered closely with *P. menziesii* from New Mexico. So that case seems unresolved.

Gugger et al. (2010) examined mtDNA and cpDNA sequences and found support for coastal (var. *menziesii*) and inland (var. *glauca*) in the United States and Canada. No evidence was found for a Sierra Nevada taxon, but mtDNA suggested the inland (var. *glauca*) might be divided into northern and southern groups. In a subsequent study, Gugger et al. (2011) examined Douglas Fir from Mexico. They found considerable divergence in cpDNA from Cerro Potosi, NL and Jamé,

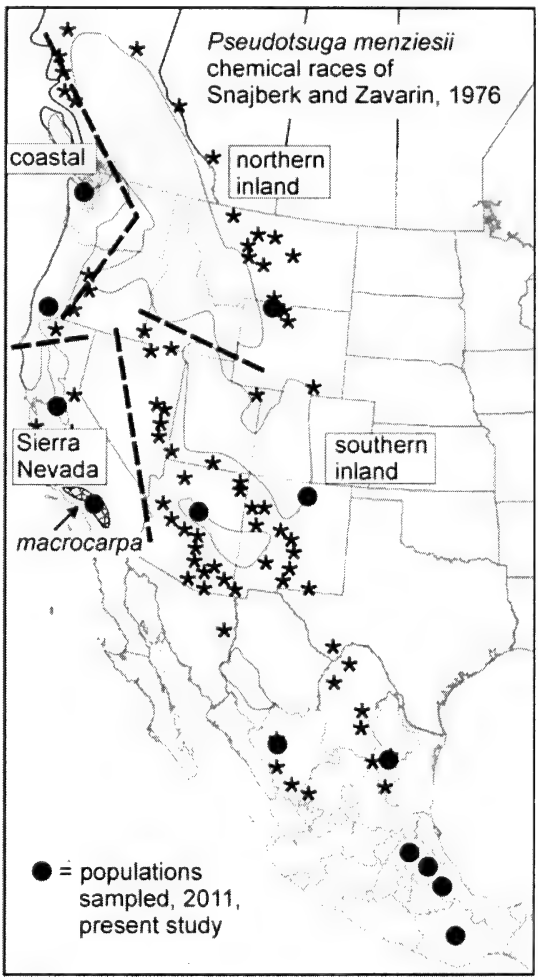

Figure 1. Distribution of *P. menziesii* with chemical races of Snajberk and Zavarin, 1976 and populations sampled in the present study.

Coah. from other Mexico populations. CpSSRs supported two clades in Mexico (Gugger, Fig. 4c), but that pattern was not supported in mtDNA (Gugger, Fig. 4a) or cpDNA (Gugger, Fig. 4b) data; in summary, they concluded that "Mexican populations were genetically

distinct from USA and Canadian populations, but more closely related to the Rocky Mountain variety than the coastal variety". As Gugger et al. (2010) did not show data from Mexico, and Gugger et al. (2011) showed only data from that country, it is difficult to ascertain the relationship of Mexican populations to those of the USA.

Phenotypic analyses (Reyes Hernández et al. 2006) revealed that *Pseudotsuga* populations of northern Mexico are morphologically similar to *P. menziesii* var. *glauca* from southwestern USA, but the populations from central Mexico differed. They also found a population of NE Mexico (San Francisco) morphologically separated from the rest, even from those of the same geographical region, suggesting an effect of isolation. This population is just 15 km NW from the one from El Potosi analyzed here.

The purpose this paper is to report on geographic variation in the leaf essential oils of *Pseudotsuga menziesii* from the USA and Mexico.

MATERIALS AND METHODS

Plant material: *P. menziesii* var. *menziesii* (coastal/ Sierra Nevada): *Adams 12918-12922*, Olympic National Forest, Port Angeles, WA, 48° 02' 48.1" N, 123° 25' 04.08" W, 525 m; *Adams 12745-12757*, on serpentine, Oregon Mtn., OR, 41° 59' 59.1" N, 123° 47' 10.2" W, 895 m; *Adams 12779-12783*, 6 km e of Buck Meadows, CA, 21 km w of Yosemite NP on US 120, 37° 49.579' N, 119° 58.421' W, 1150 m. var. *glauca*: *Adams 12556-12560*, 13 km w of Cimarron, NM on US 64, 36.54684° N, 105.03321° W, 2125 m; *Adams 12744-12748* (ex *D. Thornburg, 1-5*), 9 km ne of Pine, AZ on Hwy 87, 34° 27.422' N, 111° 24.115' W, 2250 m; *Adams 12818-12822*, 20 km e of Yellowstone NP, on US 14 at the Palisades, 44.45448° N, 109.78182° W, 1910 m; *Adams 13056-13060*, (ex *M. Socorro González Elizondo 7777a-e*), Cerro Potosi, NL, 24° 53' 9" N, 100° 13'14" W, 3141 m.; *Adams 13061-13066*, (ex *Martha González Elizondo 4408-4409, 4413-4416*) Los Altares, Dur., 25°2'56" N, 105°59'48" W, 2310 m; *Adams 13082-13087* (ex *Vargas-Hernandez J1-J6*), El Chico Natl. Park, Mineral del Chico, Hgo., 20° 10' 16" N, 98° 43' 55" W, 2,765m, Nov. 4, 2011; *Adams 13088-13094* (ex *Vargas-Hernandez C1-C7*), Cuatexmola,

Ixtacamaxtitlan, Puebla, 19° 30' 22" N, 97° 50' 21" W, 2,980m, Oct. 30, 2011; *Adams 13095-13100* (ex *Vargas-Hernandez* T1-T6), Ejido Paso National, Tlachichuca, Puebla, 19° 17' 47" N, 97° 19' 45" W, 3,000m, Sep. 30, 2011; var. *oaxacana*: *Adams 13101-13103, 13105-13106* (ex *Vargas-Hernandez I1-I6*, Paraje Peña Prieta, Oaxaca, 17° 09' 38" N, 96° 38' 07" W, 2,700m, Oct. 21, 2011. *P. macrocarpa*: *Adams 12776-12778*, USFS Eddy Arboretum, Placerville, CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU, CIIDIR and CHAPA).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type 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 (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams Essential Oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf essential oils of *P. menziesii* var. *menziesii* (coastal) were found (Table 1) to be nearly identical to the reports of von

Rudloff (1972, 1973). This is remarkable considering the changes in gas chromatography from packed to fused capillary columns. The oils of var. *menziesii* differed (Table 1) from var. *glauca* in their major components: camphene (0.4-17, 25-30%), β-pinene (25-38, 2.5-12%), sabinene (4-12, trace-0.5%), α-terpinene (2.1-3.3, 0.2-0.0%), γ-terpinene (3.4-5.5, 0.1-0.3%), terpinolene (9.5-14.6, 1.2-1.6%), cis-p-menth-2-en-1-ol (0.7-0.8, 0.0-trace), terpinen-4-ol (10-12.1, trace-0.5%), and bornyl acetate (0.2-1.3, 14.6-44.7%). The oil from the Yosemite NP, Sierra Nevada (Yose in Table 1) population (cf. Sierra Nevada race of Snajberk and Zavarin, 1976) was quite similar to typical coastal Doug Fir from WA (ONF, Table 1) and not as distinct as found in the oleoresin oil of Snajberk and Zavarin. However, comparing coastal (ONF) and Sierra Nevada (Yose, Table 1) oils reveals the coastal oil is higher in sabinene (12.9, 4.0%), δ-3-carene (1.8, 0.5%), γ-terpinene (5.4, 3.4%), terpinolene (14.6, 9.5%) and geranyl acetate (2.1, 0.9%), but lower in β-pinene (25.5, 38.0%), bornyl acetate (0.2, 1.3%) and citronellyl acetate (1.2, 2.8%). The inland group (var. *glauca*, Yell, AZ, NM, A Du, El C, Oax, Table 1) shows some differences between the northern inland (Yell) and southern inland oils (excluding Cerro Potosi) for camphene (17.0 vs. 24-30%), α-pinene (2.5 vs 3.2-9.1%) and bornyl acetate (44.7 vs. 16-32%).

The Cerro Potosi oil (C Po, Table 1) differed from other inland (var. *glauca*) oils in having larger amounts of α-pinene, β-pinene, δ-3-carene and smaller amounts of camphene, limonene and bornyl acetate. The leaf oil of var. *oaxacana* (Oax, Table 1) did not differ from var. *glauca* oils in nearby populations in southern Mexico.

Principal Coordinate (PCO) analysis of 24 terpenoids (boldface, Table 1) excluded one (Z-β-ocimene) whose maximum value was too small (0.4%). PCO of the resulting 23 terpenoids gave eigenroots that accounted for 48.7, 15.4, 7.9, 7.5 and 5.0% of the variation before the eigenroots asymptoted. The overall trends among the oils are seen in Fig. 2 where the major Principal Coordinate (PCO) accounted 49% of the variance among the populations. The first axis separated coastal (var. *menziesii*) and inland (var. *glauca*) populations. Notice that *Pseudotsuga macrocarpa* is well resolved (Fig. 2).

To examine variation among the *P. menziesii* populations, *P. macrocarpa* was removed form the data and a new PCO performed. PCO analysis of 24 terpenoids (boldface, Table 1) excluded Z-β-ocimene (max. value 0.4%) and humulene epoxide II (max. value 0.0). PCO of the resulting 22 terpenoids gave eigenroots that accounted for 57.6, 9.4, 8.4, 5.9 and 4.2% of the variation before the eigenroots asymptoted.

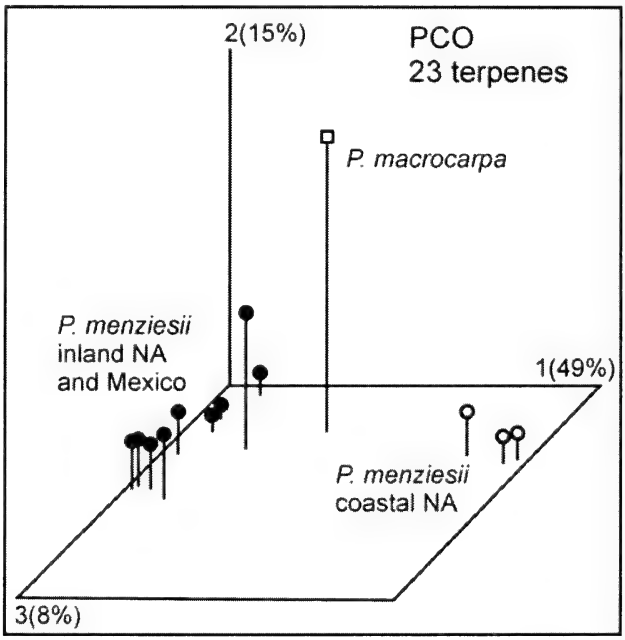

Figure 2. PCO of *P. menziesii* populations and *P. macrocarpa* based on 23 terpenes.

This resulted in an even greater amount of variance removed by PCO axis 1 (58%, Fig. 3). One can now see some differentiation among the inland populations. Note particularly the much lower similarity of Cerro Potosi oil to other inland populations (0.774), than seen between other inland populations (0.892, 0.888). There appears to be a slight north - south cline from Yellowstone - AZ, NM - Mexico populations (see tricyclene, α-pinene, camphene, β-pinene and bornyl acetate, Table 1).

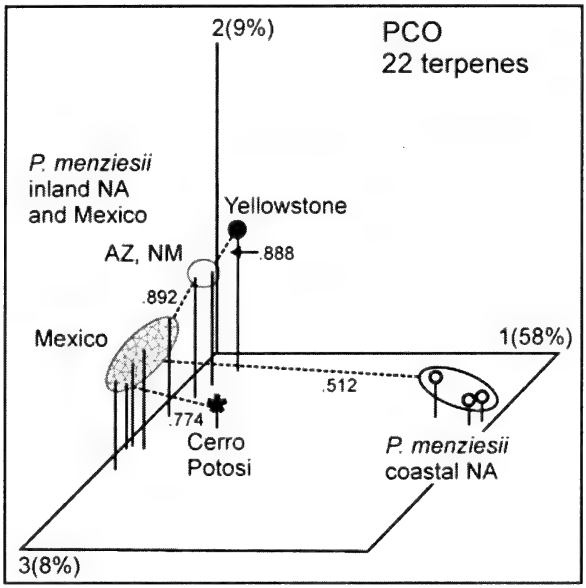

Figure 3. PCO of 12 populations of *P. menziesii* using 22 terpenes. Dashed lines are minimum spanning links. Numbers next to the lines are similarities.

Another way to visualize the clustering and similarities is by use of a phenogram. Figure 4 shows the clustering of populations based on 22 terpenes. The coastal - inland split is the major trend.

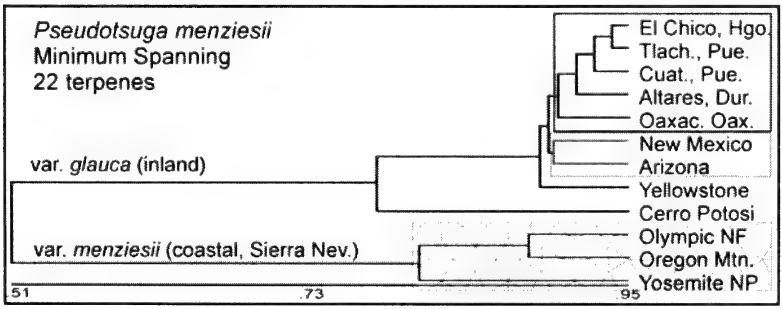

Figure 4. Minimum spanning diagram based on 22 terpenes for populations of Douglas Fir.

One can also see that the oil from the Cerro Potosi population is the most unusual oil in the inland (var. *glauca*) group (Fig. 4). In addition, the divergence of the Yosemite NP (Sierra Nevada) population is clearly seen. The Mexico populations (excluding Cerro Potosi) are very uniform in their oils, including var. *oaxacana* (Oaxac. in Fig. 4). There is no support for the recognition of var. *oaxacana*, but it may be divergent in molecular characters.

To better understand the variation, geographic clustering was preformed. The resulting diagram (Fig. 5) clearly shows the uniformity of the oils in the inland group and the divergence of the oil of Cerro Potosi from other Mexico populations. Again, the oil of var. *oaxacana* from Oaxaca is shown to be very similar to nearby populations of var. *glauca*.

These oils data are similar to the cpDNA sequencing data of Gugger et al. (2011), showing Cerro Potosi population to be different from other Mexico populations. A recent study (Wei et al. 2011) found a similar pattern in mtDNA (coastal and inland groups), but they found the cpDNA of inland Mexico populations to differ from inland USA populations. Additional molecular studies (in progress) are needed to clarify the taxonomic relationship of Cerro Potosi and inland USA populations.

ACKNOWLEDGEMENTS

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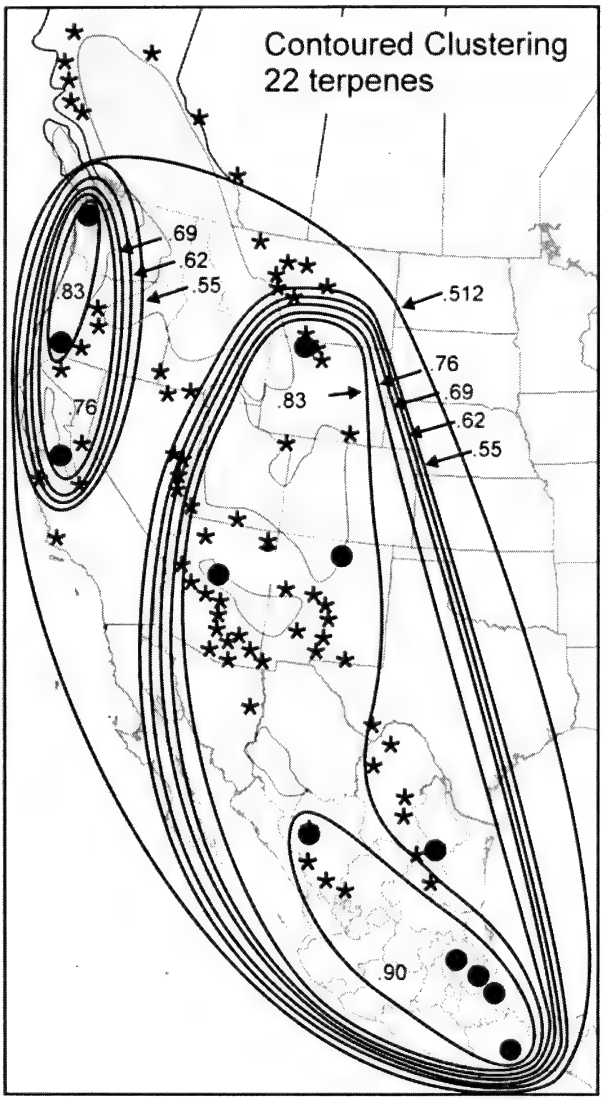

Figure 5. Contoured clustering (minimum spanning) based on equal interval clustering (0.07 similarity step intervals). Based on Fig. 4. data.

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Table 1. Comparison of leaf oil compositions for *Pseudotsuga menziesii* oils. var. *menziesii*: ONF = Olympic National Forest, Yose = Yosemite NP; var. *menziesii* var. *glauca*: Yell = Yellowstone NP, AZ = Mogolllon Rim, AZ, NM = Cimarron, NM, C Po = Cerro Potosi, NL, A Du = Altares, Dur.; var. *oaxacana*: Oax = Paraje Pena Prieta, Oax., El C= El Chico Nat. Pk., Hgo., Oax., Mac = *P. macrocarpa*. Compounds in bold face appear to separate the taxa and were used in numerical analyses.

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
884	**santene**	**t**	**-**	**1.8**	**2.1**	**1.7**	**1.2**	**3.1**	**4.1**	**4.8**	**-**
900	n-nonane	-	-	-	-	-	t	-	-	-	-
921	**tricyclene**	**t**	**0.1**	**1.0**	**2.2**	**2.5**	**1.3**	**3.1**	**2.8**	**3.0**	**0.1**
924	α-thujene	0.4	0.6	-	-	-	0.1	-	-	-	-
932	**α-pinene**	**6.4**	**7.9**	**7.0**	**9.7**	**4.5**	**18.0**	**13.5**	**10.6**	**11.6**	**14.0**
946	**camphene**	**0.4**	**1.3**	**17.0**	**24.6**	**26.4**	**13.8**	**30.3**	**30.0**	**28.2**	**1.0**
969	**sabinene**	**12.9**	**4.0**	**0.1**	**t**	**t**	**0.5**	**0.3**	**0.1**	**0.4**	**t**
974	**β-pinene**	**25.5**	**38.0**	**2.5**	**8.1**	**3.2**	**12.3**	**8.1**	**9.1**	**7.8**	**16.2**
988	myrcene	1.6	1.8	1.9	1.5	2.0	7.8	3.0	7.0	1.6	9.2
998	n-octanal	-	-	t	-	-	-	-	-	-	-
1002	α-phellandrene	0.2	0.2	0.1	0.1	t	0.1	0.1	0.1	t	t
1008	**δ-3-carene**	**1.8**	**0.5**	**0.6**	**0.7**	**0.8**	**3.9**	**1.9**	**0.1**	**0.3**	**0.2**
1014	**α-terpinene**	**3.3**	**2.1**	**-**	**t**	**t**	**0.2**	**0.1**	**t**	**t**	**t**
1020	p-cymene	0.7	0.9	0.1	t	t	0.2	0.1	t	0.1	t
1024	**limonene**	**1.6**	**2.0**	**6.6**	**5.0**	**6.0**	**2.8**	**6.0**	**6.0**	**5.4**	**20.6**

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
1025	**β-phellandrene**	**2.3**	**2.4**	**5.1**	**3.4**	**4.3**	**5.4**	**5.5**	**4.9**	**3.9**	**14.0**
1032	**(Z)-β-ocimene**	**t**	**-**	**-**	**-**	**-**	**0.3**	**t**	**t**	**t**	**2.9**
1044	(E)-β-ocimene	0.1	0.2	-	t	0.5	1.3	2.3	0.9	0.1	0.2
1054	**γ-terpinene**	**5.4**	**3.4**	**0.1**	**0.1**	**0.2**	**0.3**	**0.2**	**0.1**	**0.1**	**0.1**
1065	cis-sabinene hydrate	0.3	0.4	-	-	-	-	-	-	-	-
1086	**terpinolene**	**14.6**	**9.5**	**1.2**	**1.0**	**1.2**	**1.6**	**1.5**	**1.2**	**1.3**	**0.6**
1089	6-camphenone, isomer	-	-	0.3	0.1	t	t	t	0.2	t	-
1095	trans-sabinene hydrate	0.2	t	-	-	-	-	-	-	-	-
1095	linalool	0.2	1.7	1.3	1.5	3.2	t	0.7	0.4	-	0.4
1100	undecane	-	t	-	-	-	1.2	-	-	-	-
1100	n-nonanal	-	-	-	-	-	t	-	-	-	-
1118	**cis-p-menth-2-en-1-ol**	**0.8**	**0.7**	**-**	**t**	**t**	**t**	**-**	**-**	**-**	**-**
1118	endo-fenchol	t	0.1	t	0.1	t	t	t	t	-	t
1122	α-campholenal	0.5	-	0.1	-	-	0.1	-	-	t	0.1
1123	methyl octanoate	-	0.1	-	t	-	-	-	-	-	-
1131	myroxide	-	0.2	-	-	-	-	-	-	-	-
1135	trans-pinocarveol	-	-	-	-	-	t	-	-	t	-
1136	trans-p-menth-2-en-1-ol	-	0.4	-	t	t	-	-	-	-	-

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
1141	camphor	t	t	0.2	0.5	0.2	t	0.4	0.5	0.3	t
1145	**camphene hydrate**	**t**	**0.1**	**2.0**	**1.7**	**1.2**	**0.3**	**1.2**	**0.6**	**0.1**	**0.1**
1148	**citronellal**	**0.5**	**0.4**	**0.3**	**0.3**	**0.2**	**0.1**	-	-	-	-
1155	isoborneol	t	-	t	-	-	-	-	0.2	-	-
1165	**borneol**	**-**	**-**	**1.3**	**2.2**	**1.5**	**0.4**	**1.3**	**0.8**	**0.4**	**-**
1169	ethyl benzoate	-	-	-	-	-	t	-	-	-	-
1174	**terpinen-4-ol**	**12.1**	**10.1**	**0.5**	**0.2**	**0.2**	**0.4**	**0.3**	**0.2**	**t**	**0.3**
1179	p-cymen-8-ol	t	0.1	t	-	-	t	t	t	t	t
1186	**α-terpineol**	**2.4**	**2.3**	**0.3**	**0.2**	**0.8**	**0.4**	**0.5**	**0.3**	**0.2**	**2.9**
1190	methyl salicylate	-	0.4	0.1	t	t	-	-	-	-	-
1195	methyl chavicol	-	-	-	-	-	t	-	-	-	-
1196	cis-piperitol	t	-	-	-	-	-	-	-	-	-
1201	n-decanal	-	-	-	-	-	t	-	-	-	t
1204	verbenone	-	-	-	-	-	t	-	-	-	-
1207	trans-piperitol	t	-	-	-	-	-	-	-	-	-
1218	endo-fenchyl acetate	-	-	0.2	-	t	0.3	-	-	-	-
1223	citronellol	0.2	0.9	0.3	1.5	0.2	0.3	t	0.5	t	-
1232	thymol, methyl ether	-	-	-	-	-	-	-	-	-	0.2

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
1249	piperitone	t	t	-	t	0.2	0.1	-	-	t	-
1274	neo-isopulegol acetate	-	t	-	-	-	-	-	-	-	-
1287	**bornyl acetate**	**0.2**	**1.3**	**44.7**	**32.4**	**28.9**	**14.6**	**16.0**	**20.7**	**25.2**	**0.1**
1298	trans-pinocarvyl acetate	-	t	-	-	-	-	-	-	-	-
1300	tridecane	-	-	-	-	-	0.1	-	-	-	-
1324	myrtenyl acetate	-	t	-	-	-	0.1	-	-	-	t
1342	43,93,121,194	-	-	0.3	0.1	0.1	-	-	-	-	-
1345	α-cubebene	-	-	-	-	-	0.3	-	-	-	-
1350	α-longipinene	-	-	-	-	-	-	-	-	-	0.2
1350	**citronellyl acetate**	**1.2**	**2.8**	**0.2**	**0.1**	**0.4**	**0.2**	**t**	**t**	**t**	**t**
1359	neryl acetate	-	-	-	-	-	-	-	-	-	t
1374	α-copaene	-	-	-	-	t	0.4	-	-	t	-
1379	**geranyl acetate**	**2.1**	**0.9**	**0.2**	**-**	**0.3**	**0.1**	**t**	**0.1**	**t**	**6.1**
1387	β-bourbonene	-	-	-	-	-	t	-	-	-	-
1387	β-cubebene	-	-	-	-	-	0.1	-	-	-	-
1389	longifolene	-	0.2	-	t	t	0.2	-	-	t	0.6
1417	**(E)-caryophyllene**	**-**	**-**	**-**	**-**	**-**	**0.5**	**-**	**-**	**-**	**0.2**

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
1430	β-copaene	-	-	-	-	-	t	-	-	-	-
1432	trans-α-bergamotene	-	-	-	-	-	-	-	-	t	-
1451	trans-muurola-3,5-diene	-	-	-	-	-	0.2	-	-	-	-
1452	α-humulene	t	-	-	-	-	0.1	-	-	-	0.8
1465	ethyl cinnamate	-	-	-	-	-	t	-	-	-	-
1471	massoia lactone	-	-	-	-	-	t	-	-	-	-
1475	trans-cadina-1(6).4-diene	-	-	-	-	-	0.4	-	-	-	-
1478	γ-muurolene	-	-	-	-	t	0.1	t	t	t	-
1480	germacrene D	t	0.2	-	t	0.3	0.4	0.1	0.3	0.7	0.7
1493	trans-muurola-4,5-diene	-	-	-	-	-	0.4	-	-	-	-
1493	epi-cubebol	-	-	-	-	-	0.4	-	-	-	-
1500	α-muurolene	-	-	-	-	t	0.5	0.1	-	0.2	-
1505	β-bisabolene	t	-	-	-	-	-	-	-	-	-
1513	γ-cadinene	-	-	-	-	-	-	t	-	0.2	-
1514	cubebol	-	-	-	-	-	1.0	-	-	-	-
1522	δ-cadinene	t	t	t	-	t	1.5	0.2	0.3	0.5	0.3
1533	trans-cadina-1,4-diene	-	-	-	-	-	0.2	-	-	-	-

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
1541	(E)-α-bisabolene	-	-	-	-	-	-	-	t	0.1	0.2
1561	(E)-nerolidol	-	-	-	-	-	0.1	-	-	-	-
1574	germacrene D- 4-ol	-	-	-	-	-	-	-	-	-	-
1582	caryophyllene oxide	-	-	-	-	-	0.1	-	-	-	-
1608	β-atlantone	-	-	-	-	-	-	-	-	-	0.1
1608	**humulene epoxide II**	-	-	-	-	-	-	-	-	-	**0.6**
1616	43,81,161,222	-	0.2	-	-	-	-	-	-	-	-
1627	1-epi-cubenol	-	-	-	-	-	0.6	-	-	-	-
1632	α-acorenol	-	t	-	t	t	-	-	-	-	0.8
1638	epi-α-cadinol	-	t	t	t	t	0.2	0.1	0.1	0.1	0.2
1638	epi-α-muurolol	-	t	t	t	t	0.2	0.1	0.1	0.1	0.2
1644	α-muurolol	-	-	-	-	t	t	t	t	t	-
1646	119,107,91,202	-	-	-	-	-	-	-	-	-	0.8
1652	α-cadinol	0.1	t	0.3	0.2	0.4	0.2	0.2	0.2	0.3	0.7
1711	pentadecanal*	-	-	-	-	-	0.2	t	t	t	-
1759	benzyl benzoate	-	t	-	t	t	-	-	-	-	-
1814	hexadecanal	-	-	-	-	-	-	t	-	t	-
1864	benzyl salicylate	-	t	-	-	-	-	-	-	-	-
1887	octadecadiene*	-	-	-	-	-	t	-	-	-	-

KI	cpd	ONF	Yose	Yell	AZ	NM	C Po	A Du	El C	Oax	Mac
1889	heptadecatrienal*	-	-	-	-	-	0.1	-	t	-	-
1937	cembrene	-	-	-	t	t	0.1	-	-	-	0.2
1943	iso-cembrene	-	-	-	-	t	-	-	-	-	t
1987	manoyl oxide	-	-	-	-	t	-	-	-	-	-
1992	ethyl hexadecanoate	-	-	-	-	-	0.3	-	t	-	-
2014	palustradiene	-	-	-	t	t	t	-	-	-	-
2048	thunbergol	-	-	-	t	t	0.1	-	-	-	0.5
2055	abietatriene	-	-	-	-	-	t	-	-	-	-
2056	manool	-	-	t	t	t	1.0	-	-	t	t
2087	abietadiene	-	-	-	-	t	t	-	-	-	-
2149	abienol	-	-	-	-	-	t	-	-	-	-
2165	9Z,12Z,15Z-octadeca-trienoic acid, ethyl ester*	-	-	-	-	-	0.2	-	-	-	-
2300	tricosane(C23)	0.1	t	-	t	t	-	-	-	-	0.3
2313	abietal	-	-	-	-	t	-	-	-	-	-

KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported. For unknown compounds, four ions are listed, with the largest ion underlined. *tentatively identified.

TAXONOMY OF *JUNIPERUS* IN IRAN: INSIGHT FROM DNA SEQUENCING

Robert P. Adams
Biology Department, Baylor University, Box 97388,Waco, TX 76798, USA, Robert_Adams@baylor.edu

and

Fatemeh Hojjati
Dept. of Plant Sciences, University of Tehran, Box 14155-6455, Tehran, Iran

ABSTRACT

Sequence data from four gene regions (nrDNA, petN-psbM, trnD-trnT, trnS-trnG, 3,705 bp) revealed that junipers from the southern mountain ranges of Iran are very diverse. The combined NJ tree (3,705 bp) showed strong support for the distinct nature of *J. excelsa*, *J. polycarpos*, *J. p.* var. *turcomanica*, and *J. p.* var. *seravschanica*. The samples from NW Iran are *J. polycarpos* and samples from NE Iran are clearly *J. p.* var. *turcomanica*, as are the samples from Fasa in SW Iran. The samples from nearby south central Iran (Khabr) are part of a clade with *J. p.* var. *seravschanica.* However, the divergence of the Khabr and Kuhbanan junipers from *J. p.* var. *seravschanica* (9 bp or more) is so great that a new taxon may be present in southern Iran. *Phytologia 94(2): 219-227 (August 1, 2012).*

KEY WORDS: *Juniperus polycarpos* var. *polycarpos, J. p.* var. *seravschanica, J. p. var. turcomanica, J. excelsa,* Cupressaceae, Iran, nrDNA, petN-psbM, trnD-trnT, trnS-trnG.

The distributions of *J. excelsa* M.-Bieb. and *J. polycarpos* K. Koch in Iran and the surrounding region are not well understood. Adams (2011) noted the occurrence of *J. excelsa* in Turkey and thence eastward into Armenia with the *J. p.* var. *polycarpos* mapped into the southern Iran mountains (Fig. 1). However the taxa are very similar in

morphology and specimens in southern Iran do not seem typical of *J. p.* var. *polycarpos* (Adams, 2011).

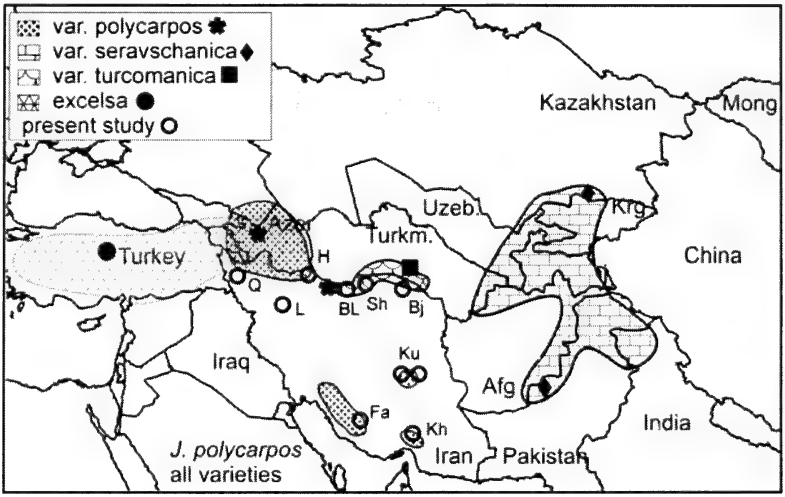

Figure 1. Distributions of *J. excelsa* (Greece not shown), *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica*. (adapted from Adams, 2011). Symbols indicate the populations sampled for each taxon.

Recently, Hojjati et al. (2009) reported on variation in isozymes of *Juniperus* from throughout Iran (Fig. 2). They concluded that their samples from northwestern Iran were *Juniperus polycarpos* (group C, Fig. 2); *J. p.* var. *turcomanica* (B. Fedtsch.) R. P. Adams (group D, Fig. 2) in northeastern Iran, and an undescribed, cryptic species from southern Iran (group A, Fig. 2). In addition, they concluded *J. excelsa* (*sensu stricto*) was not present in Iran. However, they did not include typical *J. excelsa* (cf. Turkey, Fig. 1) nor *J. p.* var. *seravschanica*, Fig. 1). In an early study of the taxonomic utilization of isozymes in *Juniperus*, Kelley and Adams (1978) found that isozymes were not useful in detecting known geographic variation in *J. ashei* Buch. nor for use in the taxonomy of *Juniperus*. They concluded that the homology of co-migrating bands is a potentially serious problem for the taxonomic use of isozymes.

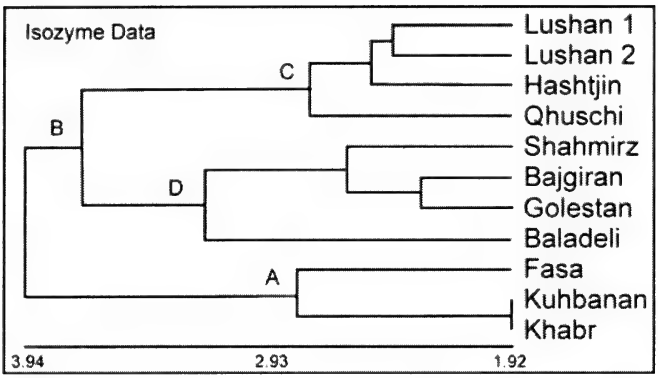

Figure 2. Phenogram of 11 populations of Juniperus from Iran based on isozymes using Euclidean distances. Adapted from data in Hojjati et al. (2009).

Recently, Adams and Shanjani (2011) have shown that DNA sequencing is very useful to elucidate these difficult central Asia taxa.

The purpose of this study was to utilize DNA sequence data from nrDNA, petN-psbM, trnD-trnT, trnS-trnG regions to analyze *Juniperus* from throughout Iran.

MATERIALS AND METHODS

In order to address variation in Iran junipers, samples were selected from specific populations (Fig. 2) that were examined by Hojjati et al. (2010). DNA was extracted from plant materials from the following Hojjati populations (Popn. # and symbols are compatible with Hojjati et al., 2009):

Popn 1 L1, Lushan: *Adams 12789-12791*, 36° 40' 27"N, 49° 38' 49.5" E, Oct, 2006, Popn 2:L2, Lushan *Adams* 12792-94 (3), 36° 40' 50" N, 49° 42' 24" E, Oct. 2006, Popn 3: H, Hashtjin *Adams 12795-12797*, 37° 26' 59" N, 48° 24' 13" E, Oct., 2006, Popn 4: Q, Qushchi *Adams 12798*, 38° 01' 20.3" N, 44° 57' 45.5" E, Oct., 2006, Popn 5: Sh, Shahmirzad *Adams 12799-12801*, 35° 50' 55" N, 53° 26' 24.2" E, Nov, 2006, Popn 6: Bj, Bajgiran *Adams 12802-12804*, 37° 25' 9.8" N, 58° 32' 0.2" E,

Nov, 2006, Popn 7: G, Golestan *Adams 12805-12807*, 37° 19' 46.3" N, 56° 02' 34.2" E, Nov, 2006, Popn 8: BL, Baladae *Adams 12808*, 36° 14' 34.4" N, 51° 50' 20.4" E, Oct, 2006, Popn 9: F, Fasa *Adams 12809-12811*, 29° 09' 57.8" N, 53° 40' 7.8" E, Dec, 2006, Popn 10: Ku, Kuhbanan Adams 12812-12814, 31° 28' 21.5" N, 55° 52' 58.9" E, Jan, 2007, Popn 11: Kh, Khabr *Adams 12815-12817*, 28° 51' 8.4" N, 56° 22' 51.7" E, Jan, 2007.

Authentic, typical taxonomically identifiable reference taxa, were included from *J. excelsa,* n of Eskisehir, Turkey, *Adams 9433-9435*, *J. polycarpos* var. *polycarpos*, Lake Sevan, Armenia, *Adams 8761-8763*, *J. p.* var. *seravschanica*, Quetta, Pakistan, *Adams 8483-8485*, Dzhabagly, Kazakhstan, *Adams 8224-8226*; Elburz Mtns., Iran, *Shanjani s. n.*, [=*Adams 12603, 12604*]. Voucher specimens are deposited at Baylor University (BAYLU).

DNA Analysis - 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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA). PCR amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM, trnDT, trnSG) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams et al. (2011) for the ITS, petN-psbM, trn D-trnT and trnS-trnG primers utilized. The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. (South San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments and NJ trees were made using MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/). Minimum spanning networks were constructed from SNPs data using PCODNA software (Adams et al., 2009). Associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower

metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The NJ tree based on 3,705 bp shows (Fig. 3) strong support (100%) for the distinct nature of *J. excelsa*, *J. polycarpos*, *J. p.* var. *turcomanica*, and *J. p.* var. *seravschanica*. The samples from NW Iran (L, H, Q) are nested with *J. polycarpos* as found in the isozyme data (Fig. 2). Samples from NE Iran (Sh, BS, BL) are clearly *J. p.* var. *turcomanica* (Fig. 3) as are the samples from Fasa in SW Iran. The

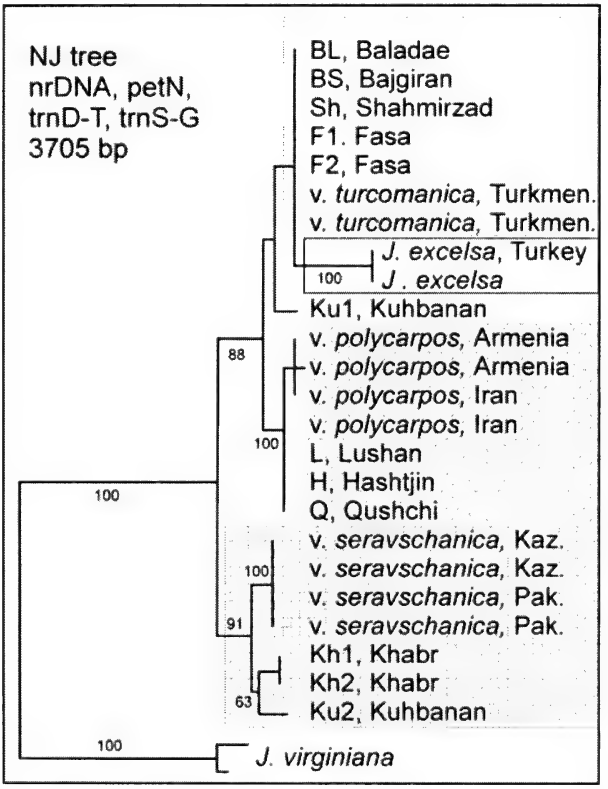

Figure 3. NJ tree based on four gene regions. See text for discussion.

samples from nearby south central Iran (Khabr) are part of a clade with *J. p.* var. *seravschanica* from Pakistan and Kazakhstan. There is support (63%) for a clade composed of Khabr (Kr1, Kr1) and Kuhbanan (Ku2). The Kuhbanan region seems to contain two taxa, as represented by Ku1 and Ku2 (Fig. 3).

To examine the amount of differentiation in DNA, both substitutions and indels were coded and a minimum spanning network was constructed (Fig. 4). The vast majority (65/103) of SNPs separate the out-group (*J. virginiana*). No variation was found in v. *seravschanica* from Pakistan and Kazakhstan (Fig. 4). The Khabr plants are separated by 9 SNPs from var. *seravschanica* and by 6 SNPs from the KU2 individual. The *seravschanica* complex is the most distinct (23 SNPs, Fig. 4), followed by *J. excelsa* (Turkey, 15 SNPs, Fig. 4). The NW Iran samples (L, H. Q) differ by zero or one SNP from var. *polycarpos*, Armenia (Fig. 4). The samples from NE Iran (BL, BS, Sh) are closely linked to var. *turcomanica*, Turkmenistan, along with plants from Fasa in SW Iran (F1, F2, Fig. 4). However, an individual (Ku1) from Kuhbanan differs by only 5 SNPs from Fasa.

To visualize the geographical trends, the minimum spanning network was overlaid on a geographic map (Fig. 5). This visual image highlights the uniformity of var. *polycarpos* from Armenia into NW Iran and var. *turcomanica* in NE Iran and thence into the Fasa population in SW Iran (Fig. 5). Note the area in N Iran, where possibly sympatric var. *polycarpos* (Sj) and var. *turcomanica* (BL) differ by 9 bp. The Fasa population is large (see distribution map, Fig. 1). It is not known at this time if this large region is composed of only var. *turcomanica*.

It is interesting the Khabr plants and one Kuhbanan plant differ by only 9 SNPs from var. *seravschanica* in nearby Pakistan. It appears that two taxa are present in the Kuhbanan region. Our two samples (Ku1, Ku2) differ by 24 SNPs (Fig. 5), comparable to the difference between Fasa - Khabr (30 SNPs, Fig. 5). Additional studies are needed to more fully resolve these taxonomic and distributional questions (in progress by authors).

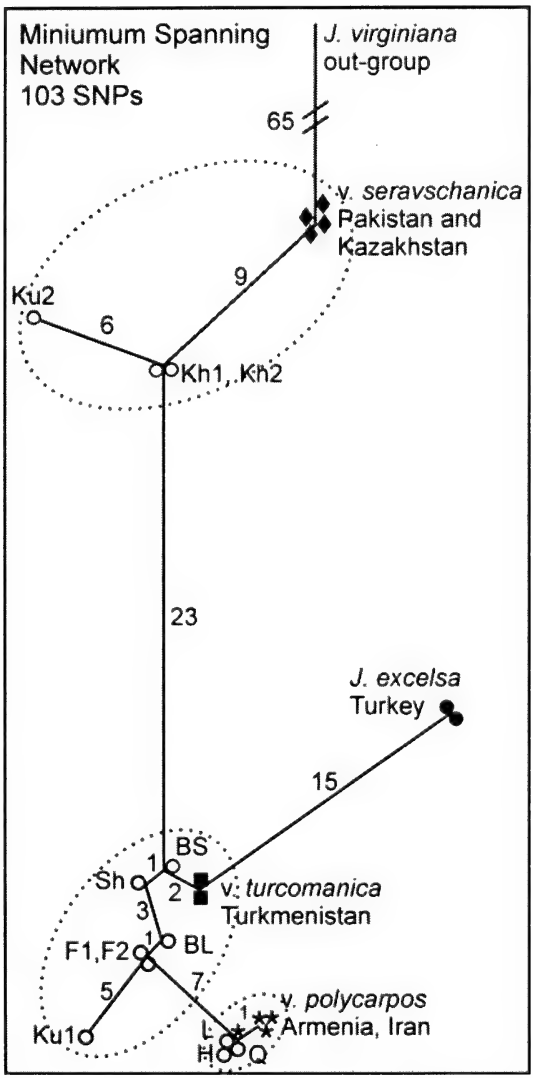

Figure 4. Minimum spanning network based on 103 SNPs. The numbers next to the links are the number of SNPs.

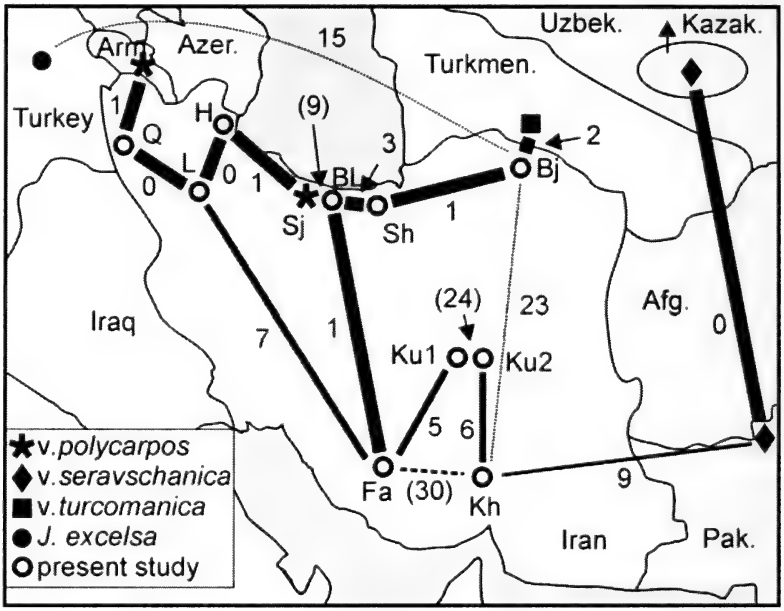

Figure 5. Minimum linkage map based on 37 SNPs. Line widths are proportional to similarity. Numbers next to links are the number of SNPs differing adjacent nodes. Notice the southern Iran populations of Khabr (Kh) and Kuhbanan (Ku2) are loosely linked var. *seravschanica* (diamonds) whereas the other S Iran population (Fasa, Fa) is most closely linked to var. *turcomanica* (Turkmenistan)differs by on 1 SNP from BL and.

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THREE NEW SPECIES OF *AGERATINA* (ASTERACEAE, EUPATORIEAE) FROM MEXICO BELONGING TO THE *A. CAPILLIPES* COMPLEX

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

Three new taxa are recognized within the *Ageratina capillipes* King & H. Rob. complex (sensu Turner 1997): **Ageratina iltisii** B.L. Turner, **sp. nov.,** from Sierra Manantlan, Jalisco; **Ageratina microcephala, sp. nov.,** from Oaxaca, and **Ageratina reserva, sp. nov.,** from Chiapas. Photographs of the types are provided, along with a map showing their distributions. *Phytologia 94(2):228- 236 (August 1, 2012).*

KEYWORDS: Asteraceae, Eupatorieae, *Ageratina*, *A. capillipes*, Mexico, Oaxaca

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Routine identification of Mexican comps has occasioned the present paper.

In my seminal treatment of *Ageratina* for Mexico, I treated *A. capillipes* in a broad morphological sense, largely because of the rarity of collections available to me at the time. I refer to these taxa here as the *A. capillipes* complex, all of the taxa being herbaceous perennials having foliage with relatively long petioles, possessing relatively small heads and largely restricted to the Pacific slopes of Mexico and Guatemala. Recent collections from Oaxaca have prodded me to take a more critical look at the complex.

The following simplified key should help identify the taxa concerned:

1. Leaf blades 3-nervate from the very base.....**(3)**
1. Leaf blades 3-5 nervate from above the base.....**(2)**

2. Leaf blades 5-7 cm wide; Oaxaca.....**A. microcephala**
2. Leaf blades 2-4 cm wide; Chiapas and Guatemala.....**A. capillipes**

3. Petioles1/2 as long as the blades, or more; El Triumfo, Chiapas.....
.....**A. reserva**
3. Petioles much shorter than indicated in the above; Jalisco...**A. iltisii**

AGERATINA CAPILLIPES King & H. Rob., Phytologia 69: 64. 1990.

TYPE: **GUATEMALA. Chimaltenango:** road to Iximche Ruins, Tecpan, 19-23 Jan 1966, *Molina et al. 16080* (Holotype US; photoholotype TEX).

In addition to the type itself, the authors of the above cited the following specimen from Mexico:
CHIAPAS. 14.1 mi NW of Motozintla, 11/2/79, *Croat 17287* (MO,US).

I have examined an additional collection from this same region: **CHIAPAS, Mpio. Motozintla,** "4.6 mi SW of cemetery in Motozintla, on dirt road to El Porvenir." 4/1/92, *Soule 3099* (TEX).

As noted below, I mapped (Turner 1997) an additional collection from Chiapas as *A. capillipes*; this is treated below as an undescribed taxon, *A. reserva.*

AGERATINA ILTISII B.L. Turner, **sp. nov. Fig. 1**
TYPE: **MEXICO. JALISCO:** "Forested hills ca. 1.5 km N of **TERRERO** on rd. to La Laguna, ca. 27 km NW of Colima. Top of **CERRO GRANDE**, a massive limestone plateau, 18-15 km NW of Colima," 19 27 30 N, 103 57 12 W, 2300 m, 18 Dec 1988, *H. Iltis, Cuevas G. & Gusman H. 30142* (Holotype; TEX; isotype TEX).

Perennial, seemingly tap-rooted herbs to 60 cm high, the roots or underground shoots, densely white-pubescent with spreading hairs. **Mid-stems** ca 2 mm thick, moderately pubescent with mostly upturned hairs. **Leaves** (mid-stem) opposite, 6-10 cm long, 3-4 cm wide; petioles 3.0-3.5 cm long; blades broadly ovate, glabrous below and above, or nearly so, 3-nervate from the very base, their margins irregularly serrate. **Capitulescence** terminal, cymose-paniculate, 3-10 cm high, 4-6 cm across, the ultimate peduncles mostly 8-10 mm long, pubescent like the stems. **Heads** ca 6 mm high, 4-6 mm wide; involucral bracts linear-lanceolate, mostly 3-4 mm long, ca 0.5 mm wide, glabrous or nearly so, their apices acute. Receptacles plane, ca 1 mm across. Florets ca 20 per head; corollas white, ca 3.5 mm long; tubes ca 1.5 mm long; throats ca 1.5 mm long, the 5 lobes pubescent. **Achenes** black, ca 1.5 mm long, moderately pubescent; pappus of ca 20 fragile bristles ca 3 mm long.

The species is named for Hugh Iltis, exceptional systematist and long-time, much admired, friend of the present author, whose collections from Mexico, especially Jalisco, are well known.

In McVaugh's (1984) treatment of *Eupatorium* (including *Ageratina*) for Novo-Galeciana, this novelty will key to or near *E. aschenborniana* (= *Ageratina pichinchensis*, sensu Turner 1997), a shrubby taxon with larger, more numerous-flowered heads, although he calls attention to the doubtful identification of specimens from the vicinity of Nevado de Colima, such plants having larger heads than typical for the species. In this connection, while Jalisco is given as the state of collection, it should be noted that on the label of the type itself, there is the notation "(Colima? Border disputed)."

In my treatment of *Ageratina* for the Comps of Mexico (Turner 1997), I included specimens of the present novelty under my broad fabric of *A. capillipes*, as noted in the above introduction.

AGERATINA MICROCEPHALA B.L. Turner, **sp. nov. Fig. 1**

TYPE: **MEXICO. OAXACA: Distrito Pochutla, Mpio. San Miguel del Puerto,** "El Aguacate, sitio de Muestreo," ca 1186 m, "Bosque templado de tilia mexicana, sobre cerro." 16 01 06.7 N, 95 07 06.6 W,

03/02/2011, *Arturo Sanchez Martinez 3086* [with J. Lucas, J. Pascual, Alex] (Holotype: TEX).

Perennial herbs, to 60 cm high. **Mid-stems,** ca 3 mm across, moderately puberulent. **Leaves**, opposite, 10-13 cm long, 5-7 cm wide; petioles ca 3 cm long, pubescent like the stems; blades broadly ovate, tapering upon the petioles, glabrous or nearly so; margins irregularly serrate. **Capitulescence** a terminal, cymose, congested panicle, 5-10 cm high, 7-12 cm across, the ultimate peduncles puberulent like the stems, having mostly upswept hairs, among these a smidgen of minute glandular hairs, and endowed with linear-lanceolate bracts 1-3 mm long. **Heads** ca 5 mm high and as wide; involcres campanulate, weakly imbricate, if at all, ca 4 mm high; bracts linear-lanceolate, 2-4 mm long, mostly 2-nervate, ca 0.5 mm wide, glabrous, or nearly so, their apices acute to obtuse. **Florets** ca 30 to a head; corollas white, ca 2.5 mm long; tubes ca 1 mm long; throat ca 1.5 mm long, the lobes 5, deltoid. **Achenes** (immature) ca 2 mm long, sparsely pubescent; pappus of ca 20, readily deciduous, ciliate, pink or white, bristles ca 3 mm long.

The species is named for its small heads, among the smallest of the genus *Ageratina* known to this author; it stands in marked contrast with the recently described *A. megacephala* B.L. Turner from Distrito Putla, Oaxaca (Turner 2010).

Ageratina microcephala might be confused with the taller, more stiffly erect, *A. peracuminata* King & H. Rob., the type collected in Oaxaca ca 50 miles north of Puerto Escondido. The two have similar capitulescences with relatively small heads, but the latter taxon is readily distinguished by its more robust, suffruticose, habit, somewhat larger heads, larger subdeltoid leaves, having more elongate petioles, the ultimate peduncles somewhat longer and not possessing minute glandular hairs along its puberulent axis.

ADDITIONAL SPECIMENs EXAMINED: **MEXICO. OAXACA: Distrito Pochutla, Mpio. San Miguel del Puerto,** "Cerro El Lobo," ca 1814 m, 06/12/02, *Pascual 675* (TEX). **Distrito Santiago Juxtlajuaca, Mpio Santiago Juxtlajuaca,** "5 km del poblado El

Manzanal, carretera a Infiernillo," ca 1920 m, 15/12/95, *Calzada 20627* (TEX).

AGERATINA RESERVA B.L. Turner, **sp. nov. Fig 3**

Suffruticose perennial herb or "shrub" to 1 m high. **Mid-stems** ca 2 mm thick, pubescent with upturned, multiseptate, trichomes. **Leaves** opposite, 7-11 cm long, 3.5-4.0 cm wide; petioles 4-6 cm long, pubescent like the stems; blades broadly ovate to subdeltoid, 3-nervate from the very base, glabrous above and below, the margins irregularly serrate. **Capitulescences** cymose-paniculate, 2-4 cm high, 3-5 cm across, arranged both terminal and axillary, the ultimate peduncle mostly 3-5 mm long, pubescent like the stems. **Heads** narrowly campanulate, ca 5 mm high, and as wide. **Receptacle** plane, ca 0.5 mm across. **Florets** ca 20 to a head; corollas white, ca 2.5 mm long; tubes glabrous, ca 1.5 mm long; throat ca 1 mm long, the 5 lobes minutely and sparingly pubescent, if at all; achenes black, ca 1.5 mm long, sparingly ciliate, mostly along the ridges.

TYPE: **MEXICO. CHIAPAS: Mpio Jaltenango/Mapastepec,** "RESERVA EL TRIUMFO, POLIGONO 1." Canada Honda, 1400 m, 15 39 N, 92 48 W, "Nov-Dec 1989," *M. Heath & A. long 377* (Holotype: TEX).

The species name derives from the Reserva El Triumfo, from whence the type.

In my treatment of *Ageratina* subgenus Ageratina for the Comps of Mexico (Turner 1998), material of this novelty, because of its possession of both terminal and axillary capitulescences, will key to *A. lasia*, a taxon restricted to Michoacan. If the capitulescence character is ignored, the taxon will key to or near *A. capillipes*, as noted in the above. If keyed within the subgenus Neogreenella (because of its minute hairs on corolla lobes) it will key to, or near the widespread, *A. rubricaulis*. *Ageratina reserva* is readily distinguished from the latter by its leaves, having 3 nerves arising from the very base, and exceptionally long petioles, the latter mostly as long as the blades.

The distribution maps (Map 1) are based upon specimens on file at LL-TEX.

ACKNOWLEDGEMENTS

Thanks to my academic colleague, Jana Kos, for editing the paper and helpful suggestions.

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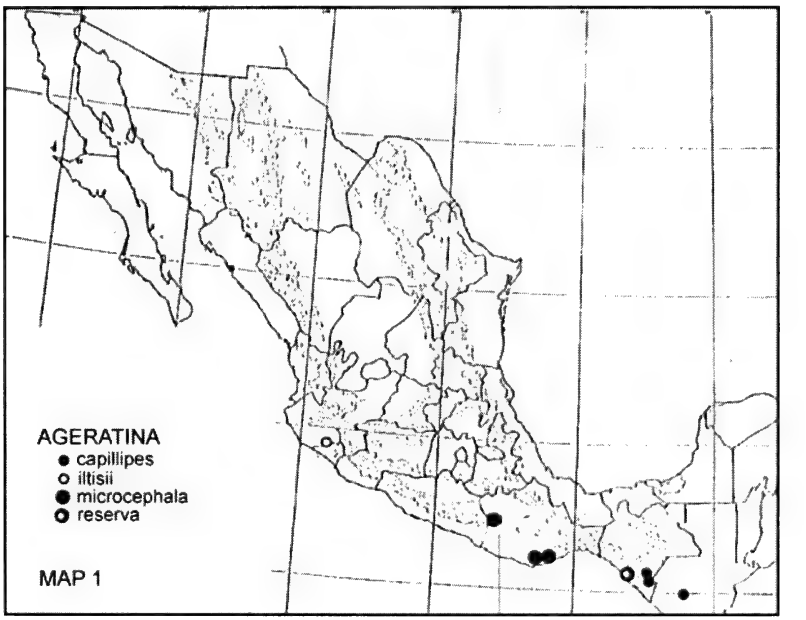

Map 1. Distributions of *A. capillipes, A. iltisii, A. microcephala* and *A. reserva.*

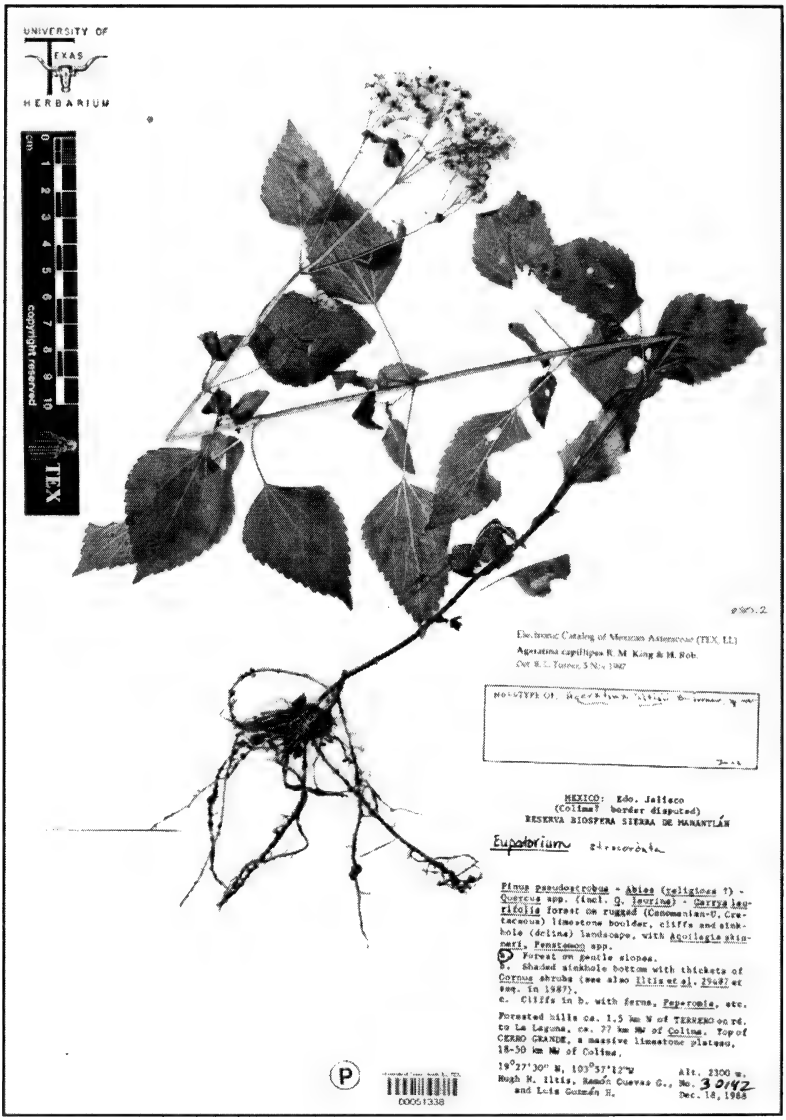

Fig. 1. *Ageratina iltisii* (Holotype).

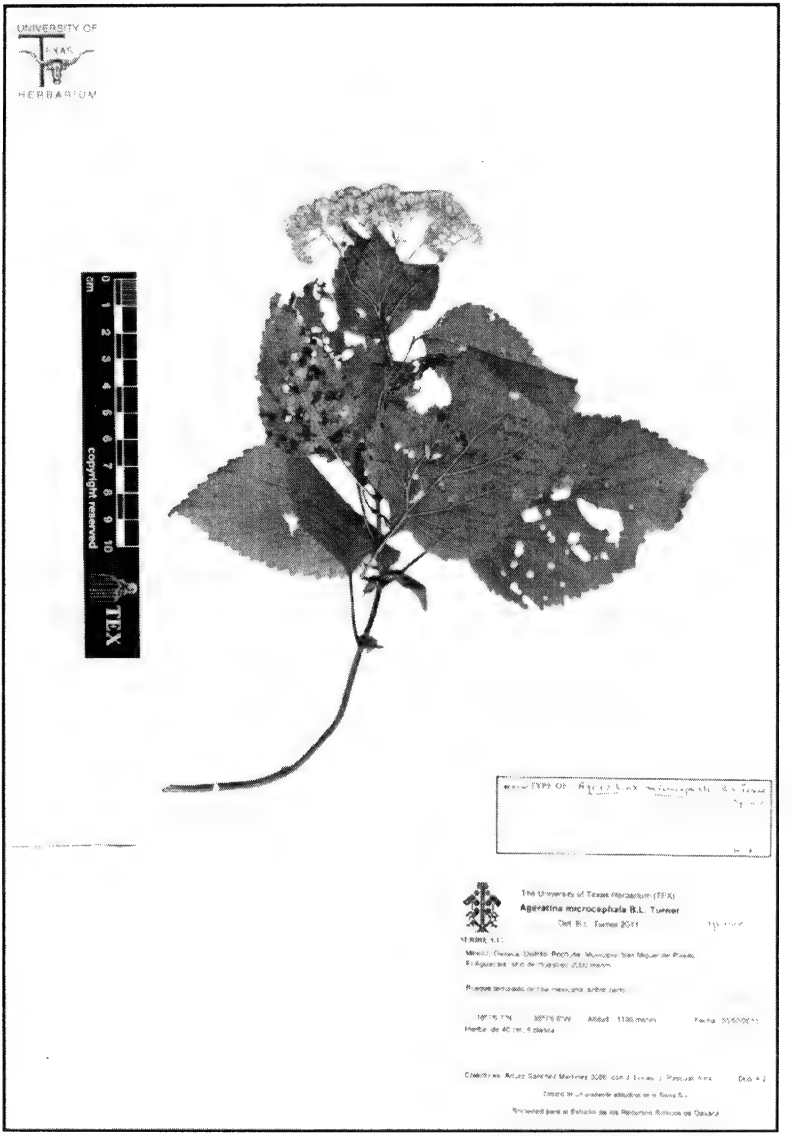

Fig. 2. *Ageratina microcephala* (Holotype).

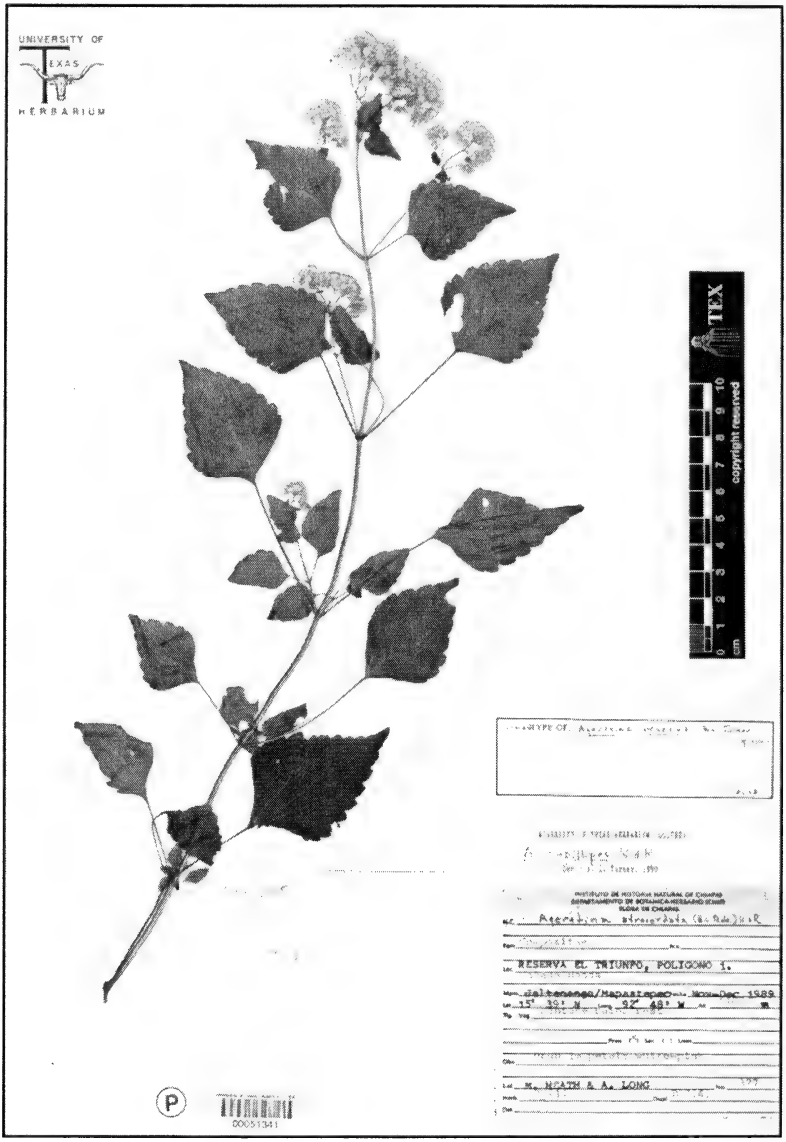

Fig. 3. *Ageratina reserva* (Holotype).

MEXICAN SPECIES OF *HELIOMERIS* (ASTERACEAE: HELIANTHEAE)

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

The genus *Heliomeris* is treated as having three species in Mexico: *H. hispida, H. multiflora* (including *H. longifolia*) and *H. obscura*. A key to the species and an abbreviated synonymy is provided, along with maps showing their distribution. *Phytologia 94(2): 237-244 (August 1, 2012).*

KEY WORDS: Asteraceae, Heliantheae, *Heliomeris, Viguiera*, Mexico

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The following, forthcoming, treatment of *Heliomeris* for Mexico follows the format established in my treatment of the Comps of Mexico (Turner 1979), the most recent issue appearing in Turner (2009).

HELIOMERIS Nutt.

Annual or, tap-rooted, erect perennial herbs to 1 m high. Leaves alternate or opposite, linear to ovate, variously pubescent. Heads, in open paniculate cymes, or solitary. Involucres, hemispheric to campanulate. Receptacles convex to conical, paleate throughout. Ray florets, pistillate, neuter; ligules, yellow. Disc florets mostly numerous, perfect, fertile; corollas yellow, the tube short and abuptly flaring into a broad cylindrical throat. Anthers, black, or reddish-brown. Achenes, relatively small, glabrous, epappose. Base chromosome number, x = 8.

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Schilling, E.F. and Panero, J.L. 2011. A revisd classification of subtribe Helianthinae (Asteraceae: Heliatheae) 11. Derived lineages. Bot. J. Linn. Soc. 167: 311-331.

Yates, W.F. and C.B. Heiser. 1979. Synopsis of *Heliomeris* (Compositae). Indiana Acad. Sci. 88: 364-372.

Heliomeris was treated within the large genus *Viguiera* by Blake (1918), but subsequent workers have treated the group as distinct, this largely supported by DNA studies (Schilling and Jansen 1989; Panero 2007; Schilling and Panero 2011). Schilling (2006) recognized *Heliomeris* as having five species, all restricted to the western U.S.A. and Mexico. The genus is readily separated from *Viguiera* by its epappose, glabrous achenes.

Key to species

1. Leaves and stems coarsely hispid-pubescent throughout, the longer hairs 1.5-2.0 mm long; a relatively rare species of wet places or standing water; Son, Chi.....**V. hispida**
1. Leaves and stems not as above, at least some, or most of the hairs softer and shorter, 0.5-1.5 mm long .....**(2)**

2. Leaf blades broadly ovate to deltoid, 1-2 times as long as wide, the margins serrate; se Pue and adjacent Oax .....**V. obscura**
2. Leaf blades lanceolate to linear-lanceolate, 3-8 times as long as wide, the margins entire or nearly so; widespread .....**V. multiflora**

HELIOMERIS HISPIDA (A. Gray) Cockerell, Torreya 18: 183. 1918. **Map 1**

Gymnolomia hispida (A. Gray) Rob. & Greenm.
Gymnolomia hispida var. *ciliata* Rob. & Greenm.
Heliomeris hispida var. *ciliata* (Rob. & Greenm.) Cockerell
Heliomeris multiflora var. *hispida* A. Gray
Viguiera ciliata var. *hispida* (A. Gray) Blake

Known in Mexico by only a few collections from n Son, also closely adjacent U.S.A., low swales with standing water and along stream sides, oak woodlands, 1200-1600 m; Sep-Oct.

Annual herbs to 60 cm high, resembling **H. multiflora** but very rough-hispid with rigid white hairs to 2.5 mm long; chromosome number, n = 8 pairs (*Keil 13588*, TEX).

In southeastern-most Arizona, along the San Pedro River (ARIZ), the species appears to grade into, or form hybrids with, *H. multiflora.*

HELIOMERIS MULTIFLORA Nutt., J. Acad. Nat. Sci. Philad., ser. 2, 1: 171. 1848. **Map 2**

This is an exceedingly widespread variable species occurring in the Rocky Mountains from the Canadian border southwards to central Mexico. In Mexico I recognize 2 varieties, as follows:

1. Heads 4.0-5.5 cm across the expanded rays; outer involucral bracts reflexed, 8-15 mm long; subalpine areas, Mount Mohinora, Chi..... .....var. **macrocephala**
1. Heads 1.5-4.0 cm across the expanded rays; outer involucral bracts erect, mostly 3-8 mm long; pine-oak forests, so far as known, not subalpine; widespread .....var. **multiflora**

var. **macrocephala** Heiser, Indiana Acad. Sci. 88: 368. 1979.
Viguiera multiflora var. *macrocephala* (Heiser) B.L. Turner

Known only from subalpine forests of Chi (spruce-fir forests of Sierra Mohinora) and outlier populations in the U.S.A. (Cochise Co., Ariz.), reportedly between 3000-3300 m; Aug-Oct.

Much-resembling var. **multiflora** but the leaves broader (15-40 mm wide), ovate to lanceolate-elliptic, having softer appressed hairs, the heads much larger, and the outer bracts much longer and mostly reflexed. Additionally, the disc florets are more numerous and their corollas larger.

In Mexico, a number of collections (4 or more) of this taxon have been made, all from the subalpine areas of Sierra Mohinora (3000-3300 m). Heiser, in his original description, noted that "*Correll and Gentry 23230 (*TEX) from three miles south of La Rocha is somewhat transitional between var. *macrocephala* and var. *multiflora.*" The latter collection, from ca 7000 ft, seems more typically *H. multiflora* than var. *macrocephala*; indeed, the latter might be treated at the specific level, being much more distinct than the various varieties proposed for *H. multiflora* in his treatment of *Heliomeris* for the North American Flora (Schilling 2006). The latter worker did not account for the, presumably relic, population in Cochise Co., Arizona, U.S.A. (*Psedotsuga* woodlands in the Huachuca Mts.) but I can find little to separate such plants from the two remote areas concerned.

var. **multiflora**
Gymnolomia annua Rob. & Greenm.
Gymnolomia brevifolia Greene ex Woot. & Standl.
Gymnolomia longifolia Rob. & Greenm.
Gymnolomia multiflora (Nutt.) Benth.
Gymnolomia multiflora var. *annua* M.E. Jones
Heliomeris brevifolia (Woot. & Standl.) Cockerell
Heliomeris longifolia (Rob. & Greenm.) Cockerell
Heliomeris longifolia var. *annua* (M.E. Jones) W.F. Yates
Heliomeris multiflora Nutt.
Heliomeris multiflora var. *brevifolia* (Woot. & Standl.) Yates

Viguiera annua (M.E. Jones) Blake
Viguiera longifolia (Rob. & Greenm.) Blake
Viguiera ovalis Blake
Viguiera shrevei Steyermark

Son, Chi, Coa, Nue, Dur, Zac, San, Gua, Jal, Mic, Cps and adjacent U.S.A., grass lands and pine-oak forests, 1000-2400 m; Jun-Nov.

Erect perennial herbs to 1 m high; leaves linear-lanceolate, 5-10 cm long, 0.5-1.0 cm wide; petioles 1-5 mm long; blades with 1 or 3 major nerves, pubescent with short, soft or hispid, mostly appressed hairs, the margins entire or nearly so; heads mostly 10-50 in a loose terminal, leafy, capitulescence; involucres 2-3 seriate, the bracts narrowly lanceolate, herbaceous throughout, subequal, appressed or the outer series rarely reflexed; receptacle globose or nearly so, paleate; ray florets 8-15, the ligules 10-15 mm long, yellow; disk florets numerous, yellow; achenes 2-3 mm long, glabrous, epappose; chromosome numbers, n = 8 or 16 pairs.

A widespread highly variable species, as might be ascertained from the synonymy given.

Heliomeris longifolia is essentially the same as *H. multiflora*, said to differ from the latter by its mostly annual habit, somewhat smaller heads and longer, somewhat narrower leaves (Schilling 2006). Out of deference to previous workers, I have long accepted the two taxa as distinct species, but believe their recognition is largely arbitrary. If combined, as done here, the earlier name is *H. multiflora*, this typified by specimens collected in the Rocky Mountains of the U.S.A. *Heliomeris longifolia* is typified by material collected in western Texas (30 miles east of El Paso) by Wright. In the latter area, specimens occur (SRSC, TEX) that may be seemingly annual or perennials, the heads of various sizes and the leaves of various widths. Strother (1999), in his treatment of Chiapas plants, in which *H. longifolia* was recognized, echoed the same reservations regarding such recognition: "The type of *Heliomeris longifolia* may prove to be conspecific with that of *H. multiflora*."

Recognition of *H. longifolia* var. *annua*, sensu Yates and Heiser (1979) seems also arbitrary, the type from Defiance, New Mexico said to differ from typical *H. longifolia*, by its branching habit, somewhat smaller leaves and smaller heads (Schilling 2006). I have been unable to segregate the taxa concerned with reasonable conviction.

HELIOMERIS OBSCURA (Blake) Ckll., Torreya 18: 183. 1918.
Map 1

Known only from se Pue and adjacent Ver, xerophytic shrublands, 2000-2200 m; Sep-Oct.

Annual tap-rooted herbs 30-50 cm high; much-resembling **V. multiflora,** but markedly different because of its broadly ovate or deltoid leaves, these sometimes weakly 3-lobed; the margins decidedly serrate; chromosome number, n = 13 or 14 pairs.

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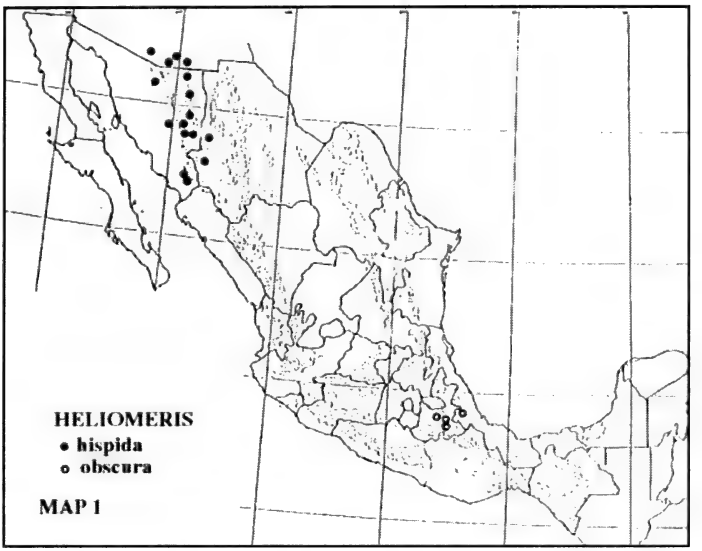
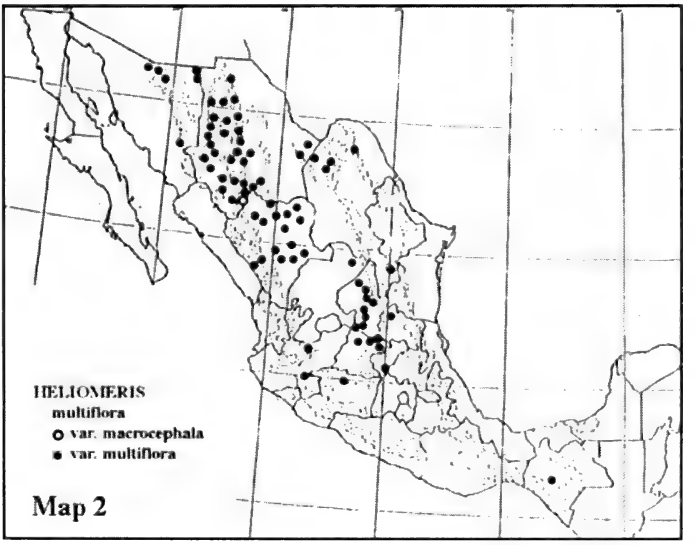
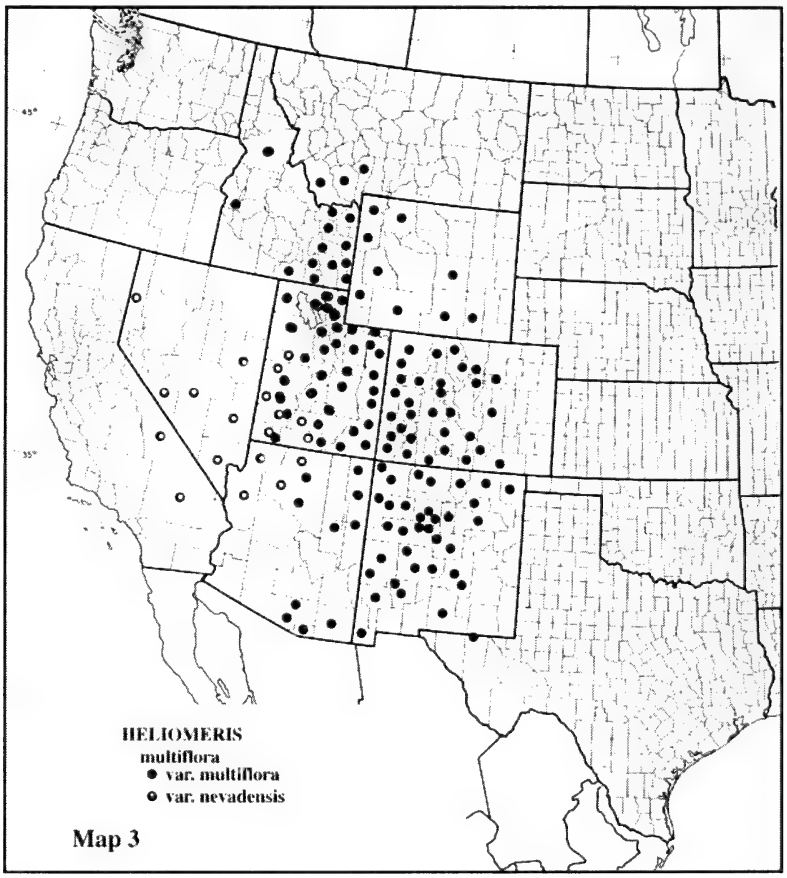
Turner, B.L. 2009. Subfamily Mutisioideae, in Comps of Mexico, Phytologia Memoirs 14: 1-130.


HELIOMERIS
• hispida
o obscura
MAP 1


HELIOMERIS
multiflora
o var. macrocephala
• var. multiflora
Map 2


HELIOMERIS
multiflora
var. multiflora
var. nevadensis
Map 3

STATUS OF *MORUS MURRAYANA* (MORACEAE)

Dayle E. Saar, Nathan C. Bundy, and Leslie J. Potts
Department of Biological Sciences, Murray State University,
Murray, KY 42071 USA
dsaar@murraystate.edu *or* dayle.saar@gmail.com

and

Michael O. Saar
Dept. of Energy, Argonne National Lab, Argonne, IL 60439 USA

ABSTRACT

A reexamination of *Morus murrayana* with more individuals from a wider geographic range, coupled with an additional molecular marker, has led to the conclusion that *M. murrayana* should be revised as ***M. rubra var. murrayana***. Leaf vein patterns are shown to be a much more accurate character for species delineation between *M. rubra* and *M. alba* than the commonly used comparisons of leaf pubescence, as verified by DNA-identified individuals. *Phytologia 94(2): 245-252 (August 1, 2012).*

KEY WORDS: Moraceae, mulberry, *Morus*, vars. *murrayana*, *rubra*, *alba*, Kentucky, internal transcribed spacer, ITS, *trn*L-F

Morus murrayana D.E. Saar & S.J. Galla (Murray State's Mulberry) was named (Galla et al., 2009) based on unique morphological characters and sequences from nuclear DNA (internal transcribed spacer region (ITS) of nuclear ribosomal DNA (nrDNA)). Distinctions between native *M. rubra* L. (Red Mulberry) and the invasive, non-native *M. alba* L. (White Mulberry) continue to be blurred due to the almost exclusive use of pubescence as the diagnostic character in plant keys (e.g., Jones, 2005; Mohlenbrock, 2002; Wunderlin, 1997; Swink & Wilhelm, 1994; Gleason & Cronquist, 1991; Elias, 1987; Radford et al., 1968; Steyermark, 1963; Britton & Brown, 1913). *M. alba* is a highly variable species, even within its native range in Asia (Chen Renfang, Southwest University, China, pers. com. to DES). This variability includes leaf pubescence, with the result

that many pubescent individuals of *M. alba* have been incorrectly identified as *M. rubra*, as is evident on many herbarium specimens. This includes individuals of *M. alba* where the top surface of the leaves are somewhat scabrous. The taxonomy of *M. murrayana* was further complicated by its large leaves and long fruits, neither of which is described in the literature. Further, *M. murrayana* does not have straight secondary veins that end in a marginal tooth, as is illustrated in the literature for *M. rubra* (e.g., Wunderlin, 1997; Britton & Brown, 1913).

Further studies utilizing more individuals from a much larger distribution area, with the additional molecular data from a chloroplast marker (*trn*L-F intergenic spacer), have led us to the conclusion that *M. murrayana* is a variety of *M. rubra*. We also found that the leaf vein pattern is a much more reliable diagnostic character than pubescence, when compared to DNA-identified individuals.

Morus rubra* var. *murrayana (D.E. Saar & S.J. Galla) D.E. Saar, **comb. & stat. nov.**

Arboles ad 20 m alto; folia alternatum, unifolius-quinquelobus, lamina ad 38 cm longus, serrulatus; fructus ad 4 cm longus, nigellus purpureus.

TYPE: **USA. KENTUCKY: Calloway County.** Frequent in open mesic woodlands dominated by *Quercus* spp. and *Carya* spp. along both sides of Watersport Rd. between gate to Racer Point and boat landing on Kentucky Lake, near Hancock Biological Station, Murray State University, ca. 25 km NW of Murray, KY (36° 43.87' N; 088° 07.35' W), 13 May 2006, *Dayle E. Saar 3606* (Holotype: MUR; isotypes: F, MO, NY, TENN, US).

Basionym: *Morus murrayana* D.E. Saar & S.J. Galla. Phytologia 91: 105-116. 2009. Holotype: as listed above; isotypes: as listed above.

MATERIALS AND METHODS

Trees of *M. rubra* and *M. alba* were observed and sampled from KY, TN, AL, NC, VA, OH, PA, NY, MA, MI, IL, WI, IA, MN,

and ON, Canada, during the summers of 2009, 2010, and 2011. Plant material was stored in silica gel and herbarium vouchers are at MUR.

DNA was extracted using Quagen DNeasy kits. Potential chloroplast markers were screened from these chloroplast regions: *ndh*C-*trn*V (Timme et al., 2007), *ndh*G-*ndh*I (Panero & Crozier 2003), *rbc*L-*acc*D (Panero & Crozier, 2003), *rpl*16 (Crawford & Mort, 2005), *trn*L-*rpl*32 (Timme et al., 2007), *trn*L-F (Taberlet et al., 1991), and *trn*Y-*trn*E (Timme et al., 2007). DNA was sequenced in the DNA Core Facility at Northern Illinois University, DeKalb, Illinois, on a Beckman-Coulter capillary sequencer. All sequences were aligned with Clustal X software (Thompson et al., 2003). Resulting sequences, along with the nuclear markers (ITS) utilized in Galla et al. (2009), were compared to the voucher specimens collected during field work.

RESULTS

Most primer pairs screened in this study either do not amplify despite several modifications to the PCR parameters, do not demonstrate informative interspecific differences, or produce multiple bands on electrophoresis gels. A six-base insertion occurs in sequences of *M. rubra* in the *trn*L-F intergenic spacer, which was used to differentiate it from *M. alba*, along with additional ITS sequences generated from this study.

When DNA-identified individuals were plotted on a map of the US, it appears that the actual range for *M. rubra* is smaller than published range maps (e.g., Wunderlin, 1997; Elias, 1987) (unpublished climate/species correlations forthcoming). This is probably due to the incorporation of individuals of *M. alba* that were misidentified as *M. rubra* when developing the current maps.

DISCUSSION

Comparisons between DNA-identified individuals and their respective vouchers indicate that *M. alba* is a highly variable species. While most leaves are glabrous and often lustrous on the upper side, this species can also exhibit leaves that are slightly scabrous. Field observations also identified this condition, particularly on vigorous

growth produced after the initial early season leaf-out. When this scabrous feature was encountered (MOS & DES, pers. ob.), leaves produced earlier in the season were often glabrous and moderate to highly lustrous on the same tree.

As mentioned previously, current keys focus on pubescence as the diagnostic character for distinguishing *M. rubra* from *M. alba*. Plant key descriptions are summarized as follows:

- *M. alba* leaves are glabrous above and often lustrous, glabrous below or pubescence restricted to scattered hairs in vein axils or scattered along larger veins.
- *M. rubra* leaves are scabrous above and undersides are pubescent throughout.

Given the above descriptions, individuals of *M. alba* with scabrous and/or pubescent leaves would be identified as *M. rubra*. This is particularly true in areas where *M. rubra* does not actually occur but is thought to be present, based on inaccurate range maps. This was the case with the presumed *M. rubra* sequenced in the study of *M. murrayana* by Galla et al. (2009). The individuals of *M. rubra* sequenced in the 2009 study for comparison to *M. murrayana* were from northeastern IL, where we now believe "true" *M. rubra* var. *rubra* does not exist, although they were from an area well within the published range. The identity of these trees as *M. rubra* was also confirmed locally by leading professional botanists, further underscoring the need for reliable field characters.

Based on our comparisons between DNA sequences and their respective herbarium vouchers, we have concluded that leaf vein patterns are more accurate for species delineation than pubescence. Laterals from the midvein, or secondary veins, for *M. rubra* curve towards the tip of the leaf as they approach the leaf margin and connect to the next lateral. The Leaf Architecture Working Group (LAWG) (1999) describes this pattern as the brochidodromous subtype of pinnate venation. The secondary veins of *M. alba* are fairly straight and terminate in a marginal tooth. This is pattern is classified as the craspedodromous subtype of pinnate venation (LAWG, 1999). The large tertiary veins off the lowest lateral vein can occupy up to a third of the blade surface and curve to the next tertiary vein on both *M. rubra*

and *M. alba*, and therefore are not useful for species delineation. Both varieties of *M. rubra* have the curved brochidodromous vein pattern. *M. rubra* var. *murrayana* is differentiated from *M. rubra* var. *rubra* by its longer fruits and tendency for much larger leaves.

M. rubra var. *murrayana* occurs in open mesic to wet-mesic woodlands in western KY, NW TN, and S IL. It rarely occurs in deep shade or in areas with moderate to high disturbance. Additional field work is needed to better define its geographic range.

Key to Native, Invasive, and Hybrid Species in North America:

1. Leaves (without petioles) 2-5 cm in length, strongly bicolored (dull dark green above, pale green below); shrubs or small straggly trees to 7 m; trees of the American SW and N Mexico. . *M. microphylla*
1. Leaves 3.8-14 cm long or longer, not strongly bicolored; trees. . . 2

    2. Secondary leaf veins, sometimes branched, ending in a marginal tooth, never curving to connect with the next secondary vein distal to the leaf base; leaf surface glabrous and often lustrous to slightly scabrous above, glabrous to variously pubescent below, the latter more often on later growth; mature fruit short cylindrical to slightly ovoid, white through pink to blackish purple. . . . . *M. alba*
    2. Secondary veins curve before reaching margins and connect to next secondary vein distal to the leaf base, only tiniest veinlets end in a tooth, or with both straight and curved vein patterns present, usually on different leaves; mature fruit short to long cylindrical, pink through blackish purple. 3

3. Both vein patterns present, or with curved vein pattern (only) and at least a slight luster on upper leaf sides; mature fruit to 3 cm long but usually shorter, pink to blackish purple. . . . . hybrid of *M. rubra* and *M. alba**
3. Leaves completely without luster, curved vein pattern present on all leaves; mature fruit blackish purple. 4

4. Leaves to 16 cm long but often <10 cm, acute to acuminate at tip, mature fruit to 3 cm long but usually shorter. . . . . *M. rubra* var. *rubra*

4. Leaves to 38 cm long, outer three leaves on branchlets almost always ≥ 16 cm, fruit to 4 cm long and 1.5 cm wide but often thinner, with some size variation on a single individual. . . . . *M. rubra* var. *murrayana*

* Hybrid individuals matching this description may be *Morus rubra* x *M. alba*, *M. alba* x *M. rubra*, or a hybrid of *M. rubra* and *M. alba* followed by back crosses with one or both species, or with other hybrids.

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MULTIVARIATE DETECTION OF HYBRIDIZATION USING CONIFER TERPENES I: ANALYSIS OF TERPENE INHERITANCE PATTERNS IN *CRYPTOMERIA JAPONICA* F_1 HYBRIDS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

and

Yoshihiko Tsumura

Dept. of Forest Genetics, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, 305-8687, Japan.

ABSTRACT

The leaf volatile oils of two cultivars of *Cryptomeria japonica*, cv. Haava and cv. Kumotooshi were analyzed, along with their 22 hybrids. The compositions of leaf oils of cv. Haava and cv. Kumotooshi and several hybrids are reported. The oil of Haava contains appreciable amounts of cis-thujopsene, widdrol and cedrol (not found in Kumotooshi oil) that appear to be inherited as a group in the hybrids in a Mendelian fashion, with a second (dominant/ recessive) gene involved. PCO (Principal Coordinates analysis) using character weights of Fs (Fs from ANOVA between the parents) was found to be the most effective method to separate the parents and their hybrids. PCA (Principal Components Analysis) and PCO using equally weighted characters were found to be ineffective in detecting hybrids. The hybrids clustered in two groups: those with and those without the cis-thujopsene/ widdrol/ cedrol suite. Several hybrids' oils were very similar to the Haava parents' oil. *Phytologia 94(2):253-275 (August 1, 2012).*

KEY WORDS: *Cryptomeria japonica,* cv. Haava, cv. Kumotooshi, hybrids, essential oil, terpenes, inheritance, genetics.

There are few studies on methods for the detection of hybridization using conifer terpenes from known crosses. Adams (1982) used leaf terpenoids to compare Wells' hybrid distance diagrams, PCA, PCO, and canonical variate analysis, but he had to use putative natural hybrids in *Juniperus*. He found that PCO, using character weighting of F-1 (F ratios from ANOVA between the putative parents), was the most effective method tested.

Hanover (1966) analyzed the genetics of monoterpenes from the oleoresin in clones, F_1 hybrids and S_1 progeny of *Pinus monticola*. He found the inheritance of each terpene (except camphene) to be additive, with some heterotic or epistatic effects. Re-analysis of the Hanover (1966) data for parents and F_1 progeny (Fig. 1) shows that α-pinene is intermediate in 6/17 and transgressive in 11/17 F_1 individuals. β-pinene had 7/17 intermediate and 10/17 transgressive (Fig. 1) values. δ-3-carene appears to be mostly intermediate (14/17) with only 3/17 being transgressive (Fig. 1), as was the case for limonene (11/17 intermediate, 6/17 transgressive).

Hanover (1971) expanded his study on *P. monticola* and concluded that :

1. Monoterpenes were under strong, predictable genetic control involving one to several loci.
2. One compound, β-pinene, consistently occurred in larger concentrations in the progeny, which may be due to age effects.
3. A strong positive correlation was found between concentrations of δ-3-carene and terpinolene and negative correlations between α-pinene/ β-pinene and myrcene/ δ-3-carene. Otherwise, the compounds appeared to be independently inherited.
4. Negative correlations were found between monoterpene concentrations and progeny height growth rate.

To determine the number of genes controlling terpenes, Irving and Adams (1973) crossed *Hedeoma drummondii* x *H. reverchonii* and analyzed the parents, F_1, and F_2 progeny. They reported the terpenes were controlled by a minimum of 1 to 7 genes. Tucker and Kitto (2011) and Tucker (2012) have recently published an informative review of the genetics of *Mentha* and discusses transgressive variation.

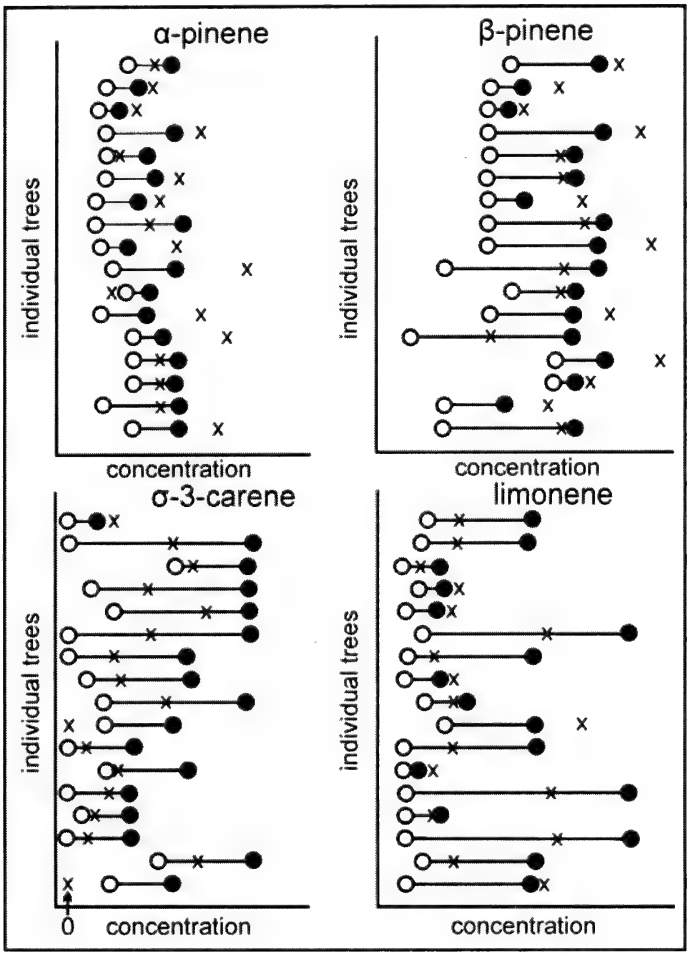

Figure 1. Graphs for four terpenes for 17 F_1 trees. Open and closed circles are the concentrations for parents 1 and 2 in the cross. x is the concentration in the F_1 individual tree (data from Hanover, 1966).

Squillace (1971) examined inheritance of monoterpenes in oleoresin of *Pinus elliotii* and conclude that β-pinene and myrcene were controlled by two alleles at a single locus, with high amounts being

dominant over low. Interestingly, this same pattern is evident in Fig. 1. Notice, that of the 30 transgressive individuals, 27 are in larger concentrations than the parents (Fig. 1).

Both quantitative variation and simple dominance has been reported in the inheritance of terpenes of Douglas fir (von Rudloff, 1984; von Rudloff and Rehfeldt, 1980) and Scots Pine (Pohjola, et al., 1989).

In the Cupressaceae, there have been very few studies on the inheritance of terpenes. One significant study in *Cupressus* (now *Hesperocyparis*) is that of Lawrence et al. (1975). They examined the leaf oils of *H. sargentii*, *H. macnabiana* and their putative natural hybrids. They analyzed oils from 36 trees in a single population and concluded that 12 were *H. sargentii*, 13 were *H. macnabiana* and 10 were intermediate in their oils. PCO analysis, using their terpene data with equal weights (Lawrence et al., 1975, Table 2), shows (Fig. 2) that

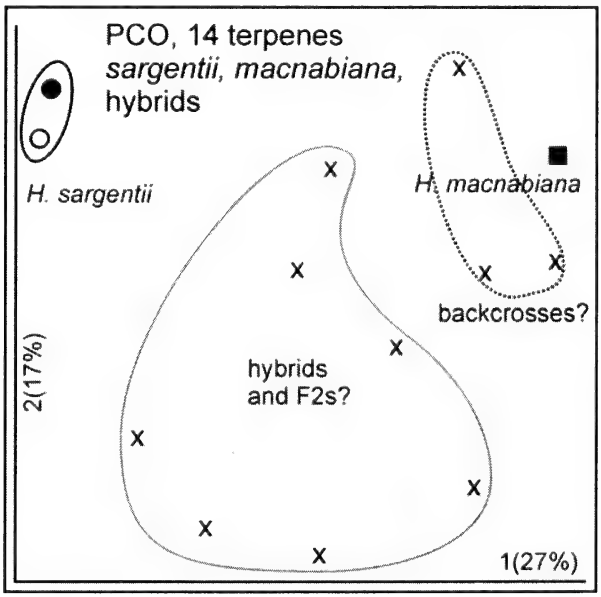

Figure 2. PCO of *H. sargentii, H. macnabiana* and putative hybrids (x). Data from Lawrence et al. (1975, Table 2).

the first and second eigenroots accounted for 27 and 17% of the variance among their samples. These low amounts of variance are likely due to the fact that only two samples of *H. sargentii* and one of *H. macnabiana* were present in the data set. Ordination (Fig. 2) reveals that the putative hybrids are quite dispersed between the parental species. From this ordination, it would appear that the putative hybrids (x) likely contain some F_{2s} and backcrossed individuals. However, if transgressive inheritance is involved, that could also explain the wide variation in the putative hybrids.

Seven of thirteen (7/13) terpenes (from Lawrence et al. 1975, Table 2) are transgressive. Only 4 of the 13 terpenes are mostly intermediate (camphene, sabinene, myrcene and β-phellandrene). The proportion of transgressive terpenes (7/13) is similar to that found in *P. monticola* (11/17, Fig. 1 above; Hanover, 1966). It appears that mixing biosynthetic genes can lead to over and under-expression of some terpenes.

Cryptomeria japonica D. Don (Sugi) is a monotypic genus (Farjon, 2005; Tsumura, 2011), endemic to Japan. Farjon (2005) argues that *C. fortunei* Hooibr. is conspecific, and a study (Kusumi et al. 2000) based on DNA sequencing, found no support for the recognition of *C. fortunei* separate from *C. japonica*. *Cryptomeria japonica* appears to have been introduced into China many years ago (Farjon, 2005) and is now widely cultivated in Japan, Taiwan, Korea, China and the Azores Islands (Tsumura, 2011). It is a very important commercial forest tree in Japan and the object of many detailed studies (see review, Tsumura, 2011) at the Forestry and Forest Products Research Institute and other institutes in Japan. The composition of the wood oil has been reported in the careful work of Nagahama and colleagues (Nagahama, 1964; Nagahama and Tazaki, 1993; Nagahama et al. 1996, 1998). Nagahama and Tazaki (1993) reported on the oil from wood of 14 sources and found an interesting polymorphism in appreciable amounts of cis-thujopsene/ cedrenes/ cedrol/ eudesmols/ elemol in 8 samples, and either absence or trace amounts in 6 samples.

The leaf oil of *Cryptomeria japonica* has been less examined. Shieh et al. (1981) reported taxon to have considerable amounts of elemol, cedrol, α-eudesmol, β-eudesmol, β-eudesmol that were

confirmed combined GCMS, IR and NMR, but the other components were only identified by GCMS. The development of new libraries utilizing both GCMS and retention time data (Adams 2007) make identification much more certain than in 1981. Nagahama et al. (1993) analyzed sesquiterpenes in leaf oils from 5 cultivars and found all contained appreciable amount of elemol, germacrene D-4-ol and hedycaryol with smaller amounts the eudesmols and α-cadinol. The leaf oil of two cultivars (Garin and Tosaaka) contained cedrol. Nagahama et al. (2001) compared the sesquiterpenes and diterpenes from leaves of 6 elite clones of *Cryptomeria japonica* and found that all were very high in *ent*-kaurene (kaur-16-ene), with moderate amounts of germacrene D-4-ol. Two clones had cedrol and α-thujopsenol. Most of the clones had elemol, eudesmols, and considerable hedycaryol.

More recently, Cheng et al. (2005) reported that the leaf oil of *Cryptomeria japonica* was dominated by *ent*-kaur-16-ene (40.6%), valencene (19.9%), eudesma-3,7(11)-diene (8.4%) and α-eudesmol (5.9%). However, the oil components were identified by the use of Wiley/NBS database of mass spectra, which, in the experience of the senior author, is not reliable for the unequivocal identification of terpenoids.

The purposes of the present paper are to report on a complete analysis of the volatile leaf essential oil of two cultivars of *Cryptomeria japonica* and their F_1 hybrids, and to compare various multivariate methods in the recognition of hybrids using terpenoid data.

MATERIALS AND METHODS

Leaves (2 branchlets, 15-20 cm long) were collected from *Cryptomeria japonica* cv. Haava (*Adams 13142*) and cv. Kumotooshi (*Adams 13143*) and 22 F_1 hybrids (#48, Adams 13144; #70, Adams 13145; #83, Adams 13146; #56, Adams 13147; #77, Adams 13123; #23, Adams 13149; #37, Adams 13150; #65, Adams 13151; #62, Adams 13152; #81, Adams 13153; #4, Adams 13224; #10, *Adams 13225*; #27, *Adams 13226*; #36, *Adams 13227*; #60, *Adams 13228*; #73, *Adams 13229*; #74, *Adams 13275*; #76, *Adams 13231*; #78, *Adams 13232*; #78, *Adams 13233*; #80, *Adams 13234*; #89, *Adams 13235,* growing at the Forestry and Forest Products Research Institute,

Tsukuba, Ibaraki, Japan. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Air dried (30°C, 24h) leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus and trapped in a layer of diethyl ether (Adams, 1991). Nagahama et al. (1993) noted that steam distillation (actually hydrodistillation, where the leaves are placed into the boiling flask and may be subjected to reactions with the plant acids) resulted in the loss of elemol, germacrene D-4-ol and hedycaryol compared to hexane extraction. It should be noted that the modified Clevenger-type apparatus, having a diethyl ether solvent trap (see Fig. 4, Adams, 1991) utilizes a chamber to suspend the plant material, so only steam (not plant acids) is in contact with the materials. However, Nagahama et al. (1993) further demonstrated that germacrene D-4-ol and hedycaryol declined by 16% and 40%, respectively, when subjected to neutral steam. Because each sample in this study was distilled by neutral steam, the amount of degradation of germacrene D-4-ol and hedycaryol is proportional in all samples. The oil samples were concentrated with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using the HP Chemstation software with a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column run under the same conditions as the GCMS analysis (above).

Terpenoids (as percentage of total oil) were compared between the parents (3 replicate analyses) by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric similarities (Gower, 1971; Adams, 1975) were computed among all individuals using character weighting of F-1

(F from ANOVA), and equal weights (wts = 1.0). Principle Component Analysis (PCA) and Principal Coordinate Ordination (PCO) were performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The compositions of the leaf essential oils of *C. japonica* cv. Haava and cv. Kumotooshi are given in Table 1. The oil of cv. Haava is dominated karu-16-ene (47.7%) with moderate amounts of sabinene and cis-thujopsene as well as α-cuprenene, widdrol and cedrol. The oil of cv. Kumotooshi is also dominated by karu-16-ene (28.4%), but contains moderate amounts of α-pinene, sabinene, limonene, β-phellandrene, bornyl acetate, elemol, and α-, β- and γ-eudesmols. The oils are highly significantly different in 21 components (Table 1) and significantly different in 5 components.

The oils in the hybrids were found to comprise two groups: 16 that showed complementation (cf. H x K 23, Table 1) and 6 that did not have the cis-thujopsene/ widdrol/ cedrol suite from parent cv. Haava (cf. H x K 74, Table 1).

Of the 17 major compounds, 7 were intermediate in concentration in the hybrids and 10 were transgressive. The distributions of values of components having intermediate inheritance (bornyl acetate, elemol, α-, β- and γ-eudesmols, bulnesol and kaur-16-ene) are shown in Figure 3. The concentration of bornyl acetate is skewed towards cv. Haava (Fig. 3). However, the other 6 compounds are generally scattered between parents, suggestive of multi-genic inheritance. Kaur-16-ene is generally intermediate, but three samples were slightly beyond the parents (Fig. 3).

The distributions of 8 of the 10 major compounds with transgressive inheritance are shown in Figure 4. Camphene and α-pinene are mildly transgressive in that most samples fall on or between the parents. Sabinene is the most extreme transgressive component, with parents' values of 8.5 and 11.6%, but hybrids ranged from 7.4 to 17.9%. cis-thujopsene, widdrol and cedrol all show similar patterns

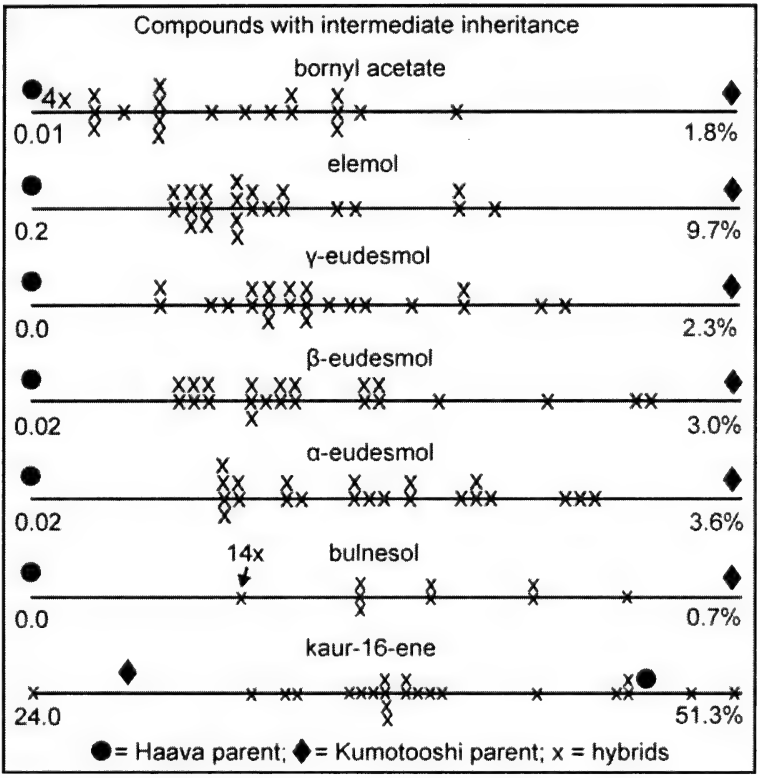

Figure 3. Distribution of compounds with intermediate inheritance.

with 6 of the same individuals having 0.0 amounts and 16 having amounts intermediate to greater than the Haava parent (Fig. 4). The ratio of 6:16 is very near 1:3 for a single locus, dominant gene (cis-thujopsene, widdrol, cedrol synthesized) vs. recessive gene (compounds absent). Due to the structural differences, it is very unlikely that cis-thujopsene, widdrol, and cedrol are produced by a single gene. It is more likely that the pathway is switched on by a gene leading to the synthesis of cis-thujopsene, widdrol and cedrol (along with other compounds associated with cedrol in the Cupressaceae: α- and β-cedrene, widdra-2,4(14)-diene, α-, γ-, and δ-cuprenene,

thujopsan-2-α-ol, and allo-cedrol). Notice that all of these compounds are present in Haava and absent in Kumotooshi parents (Table 1). In a related genus, *Juniperus*, these compounds are nearly always found only in the heartwood and not in the leaves (Adams, 2011), whether such compounds are found in the wood of Haava is not known, but Nagahama et al. (2001) reported α- and β-cedrene, thujopsene (cis?), cuparene and cedrol in the wood oil of cv. Tosaaka, whereas these

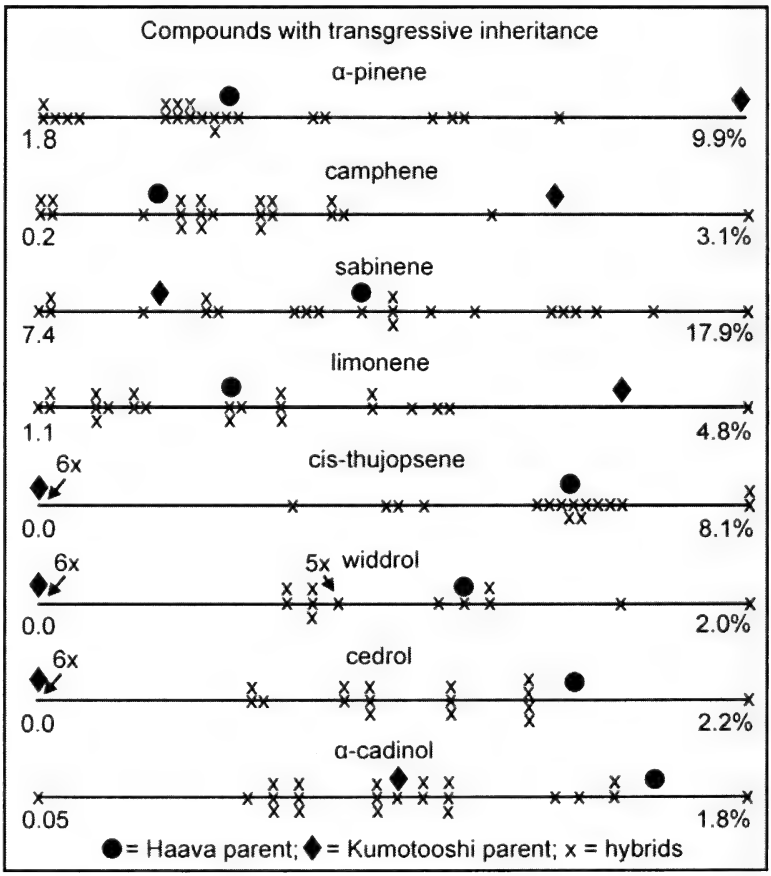

Figure 4. Distributions of compounds with transgressive inheritance.

compounds were absent in 5 other cultivars examined (they did not analyze the wood oils of cv. Haava or cv. Kumotooshi). Nagahama and Tazaki (1993) found cedrol, etc. to be present in the wood oil of cv obisugi, but absent in 6 other Sugi accessions.

To examine correlations among the components, PCA was performed on 29 terpenoids (> 0.4% conc.) utilizing components from three replicates of each parent and 22 hybrids. The first component (PC) accounted for 38% of the variance among the samples and the second PC removed 18%. A plot of the components on the first 2 PCs (Fig. 5) shows several inheritance patterns. Notice the tight clusters

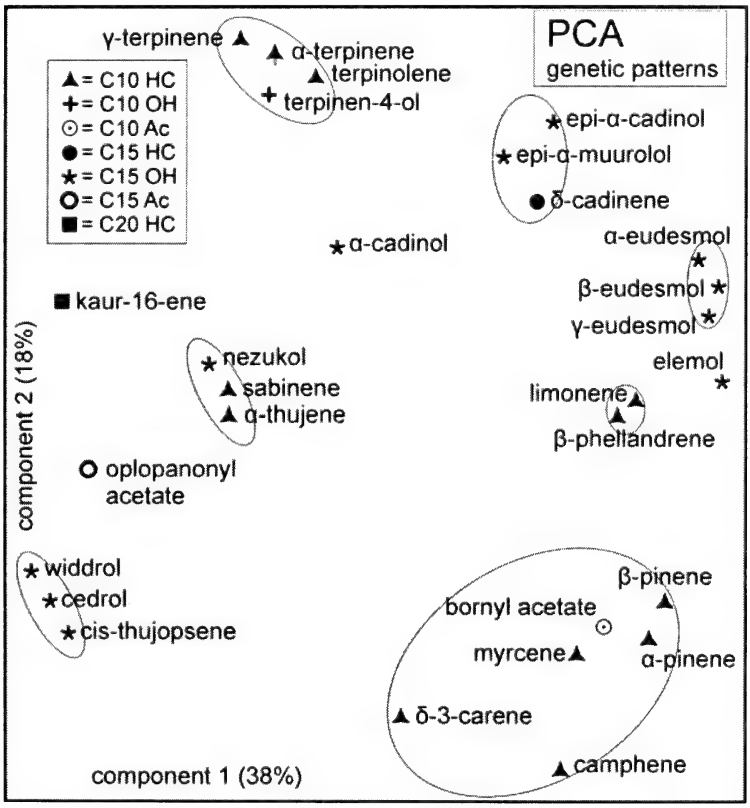

Figure 5. PCA inheritance patterns of 29 terpenoids.

clusters of cis-thujopsene/ cedrol/ widdrol; nezukol/ sabinene/α-thujene; α- and γ-terpinene/ terpinolene/ terpinen-4-ol; epi-α-cadinol/ epi-α-muurolol/ δ-cadinene; α-, β- and γ-eudesmols; and limonene/ β-phellandrene. The monoterpenes (α- and β-pinene, myrcene, δ-3-carene and camphene) form a loose group with bornyl acetate.

Histograms of cis-thujopsene, sabinene and kaur-16-ene are shown in Figures 6, 7, and 8. The histogram of cis-thujopsene (Fig. 6) shows a 3:1 inheritance. However, a 3:1 Mendelian inheritance would presume that each parent was heterozygous. Yet, the absence of cis-thujopsene/ widdrol/ cedrol) in parent Kumotooshi (Fig. 4) suggests it is homozygous recessive. There appears to be a modifier gene involved.

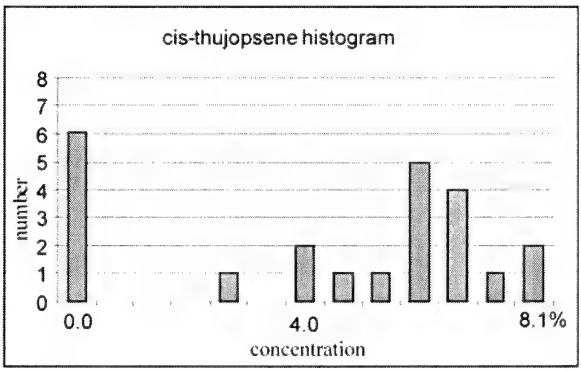

Figure 6. Histogram of cis-thujopsene (widdrol and cedrol had similar histograms).

The histogram of sabinene shows a second mode of variation in that the distribution is nearly continuous (Fig. 7). This suggests multi-genic control.

The histogram for kaur-16-ene displays a third pattern of variation that suggests two or more genes controlling the expression of kaur-16-ene.

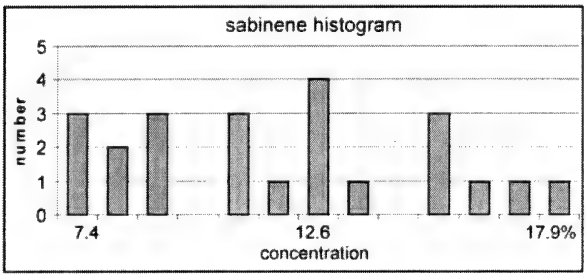

Figure 7. Histogram for sabinene.

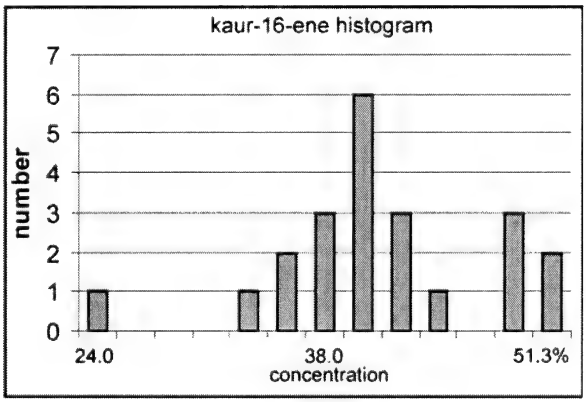

Figure 8. Histogram for kaur-16-ene.

The detection of hybrids in nature using terpenoids can present a challenge. Ordination of the parents and their artificial hybrids using PCA (Fig. 9) gives an incomplete separation. This was also found by Adams (1982) in putative natural hybridization of *Juniperus* species. Notice (Fig. 9) that the hybrids (cf. 80, 89) are depicted very near parent H (Haava). However, the group of 6 individuals, that do not contain the dominant compounds (cis-thujopsene, cedrol, widdrol), are intermediate between parents H and K (Fig. 9). In general, Adams (1982) found that correlation was not as useful as similarities in classifying hybrids.

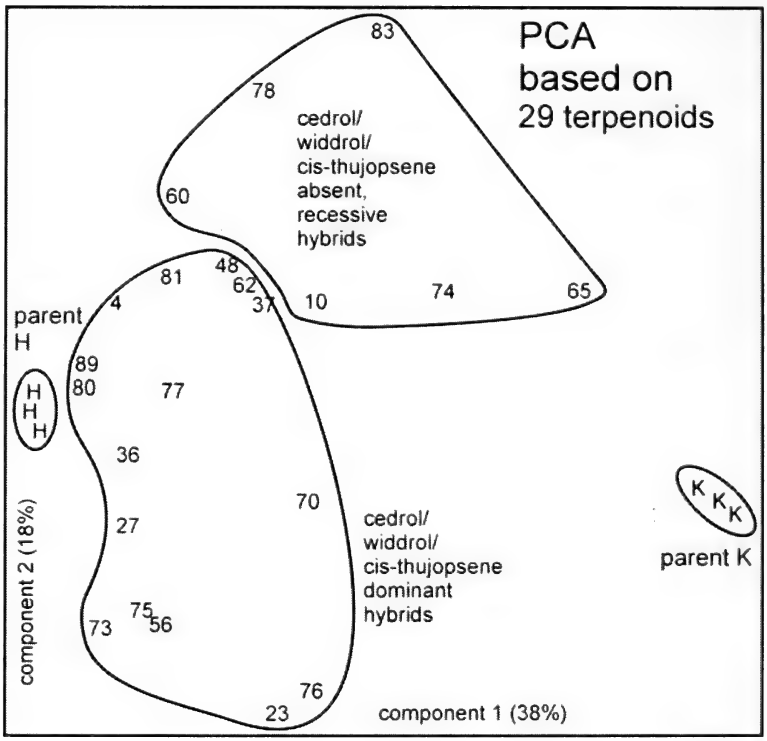

Figure 9. PCA of parents and hybrids using 29 terpenoids.

PCO using equally weighted terpenoids (Fig. 10) in the calculation of similarity measures gave some improvement over PCA (cf. Figs. 9, 10). The cis-thujopsene/ widdrol/ cedrol-absent hybrids are more intermediate between the parents. This PCO accounted for 27% and 13% of the variance among samples. However, plants 80, 4, 89, and 36 are very near the H (Haava) parent and their separation little improved from the PCA analysis (cf. Figs. 9, 10).

Adams (1982) investigated the use of statistically derived character weighting and found that weighting the similarity by F ratios (F from ANOVA between the parents) improved the detection of

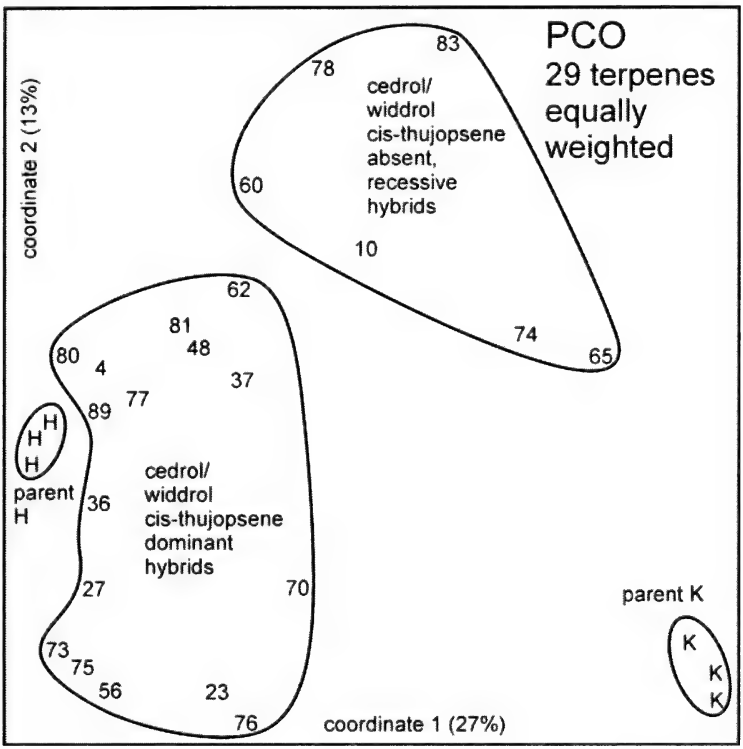

Figure 10. PCO based on 29 terpenoids, equally weighted.

hybridization. PCO using F ratio weighted characters (Fig. 11) tightened the cluster of the cis-thujopsene/ widdrol/ cedrol-absent group and placed them in a very intermediate position between the parents. F weighed PCO accounted for 43% and 11% of the variance among samples. Thus, the use of F ratio weighting increased the separation between parents H and K from 27% to 43% making the ordination of the hybrids much more distinct. The group cis-thujopsene/ widdrol/ cedrol group is still quite near parent H (Fig. 11).

It may be that compounds that are clearly inherited as dominant/ recessive should be remove from the data. To test this idea,

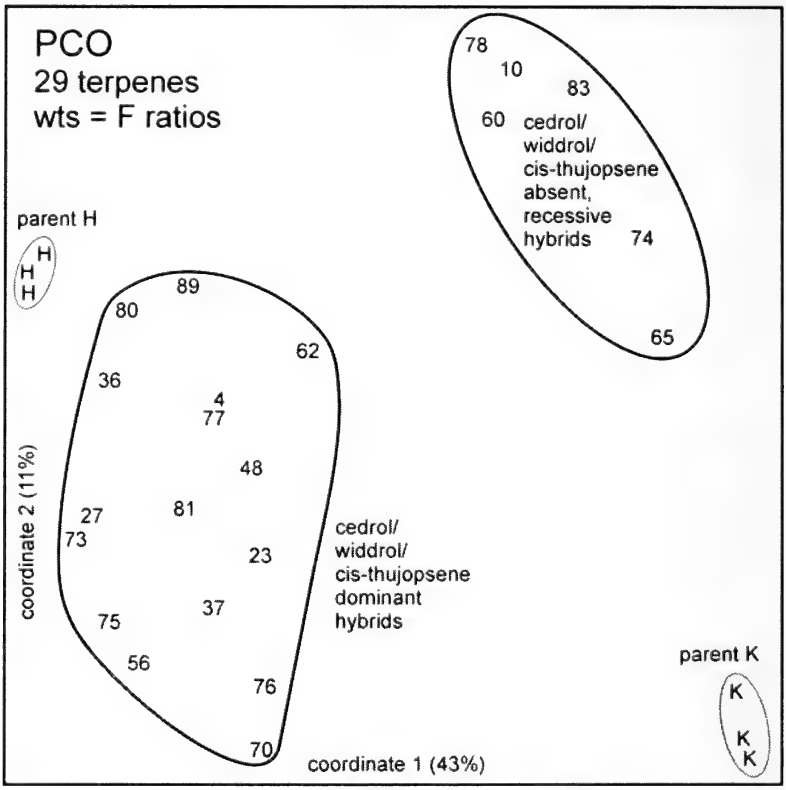

Figure 11. PCO based on 29 terpenes using F ratios for character weights.

cis-thujopsene, widdrol and cedrol were removed from the data set and a new PCO was run. Most of the hybrids that contained cis-thujopsene, widdrol and cedrol were moved into the intermediate group (Fig. 12). However, 6 hybrids (23, 27, 56, 73, 75, 76) remained near parent H (Fig. 12). It may be that some maternal inheritance is involved in these individuals. In general, the detection of hybrids is much better with the three dominant/ recessive cis-thujopsene/ widdrol/ cedrol components removed from the analyses. Although this was easily done with artificial hybrids, it may be more difficult to determine dominant/

recessive components in hybridization (including backcrossing) in natural populations.

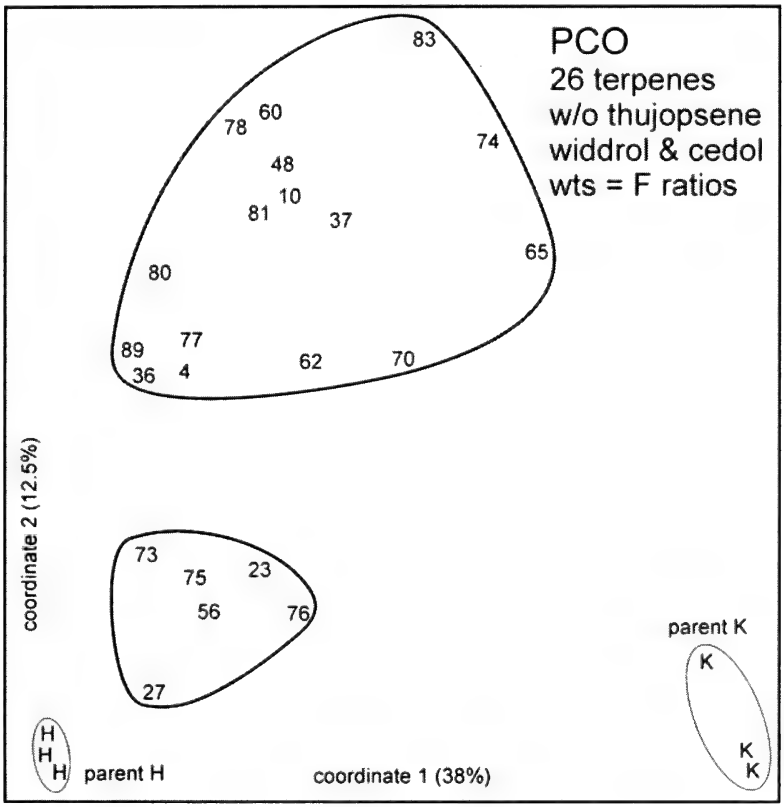

Figure 12. PCO with weighting by Fs, without cis-thujopsene, widdrol and cedrol.

In summary, the detection of hybrids by chemical means from artificial crosses of two cultivars of *C. japonica* was not easy, due to transgressive variation and Mendelian heritance of several compounds found only in one parent (cv. Haava). The use of F weighted similarities greatly improved the detection of hybrids and the removal

of dominant/ recessive components further added in the identification of hybrids. Additional artificial crosses of conifers will need to be examined to develop robust methods for the analysis of putative, natural hybridization situations.

ACKNOWLEDGEMENTS

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Table 1. Comparison of leaf essential oils of *Cryptomeria japonica*, cv. Haava, Kumotooshi, and selected hybrids: H x K 23, hybrid with compounds from each parent present, H x K 74, hybrid without cis-thujopsene/widdrol/cedrol suite of components (from Haava). Components that clearly differ between Haava and Kumotooshi are in bold face. F significance (F sig.): * = P 0.05, ** = P 0.01, ns = not significant, nt = not tested.

KI	compound	Haava	Kumo	F sig.	HxK 23	HxK 74
921	tricyclene	t	0.3	nt	0.4	0.1
924	α-thujene	0.8	0.6	10.8*	1.1	0.5
932	**α-pinene**	**3.9**	**9.9**	**93.1****	**8.2**	**4.8**
946	**camphene**	**0.4**	**2.4**	**236****	**3.1**	**0.5**
969	**sabinene**	**11.6**	**8.5**	**18.0***	**17.9**	**8.3**
974	**β-pinene**	**0.3**	**0.8**	**102****	**0.5**	**0.3**
988	**myrcene**	**2.2**	**3.6**	**34.6****	**4.1**	**1.8**
1002	α-phellandrene	t	t	nt	-	t
1008	**δ-3-carene**	**0.3**	**0.9**	**120****	**1.4**	**0.1**
1014	α-terpinene	1.2	1.1	4.0 ns	1.1	0.9
1020	p-cymene	0.3	0.1	nt	0.3	0.1
1024	**limonene**	**2.1**	**4.2**	**66.2****	**1.5**	**3.0**
1025	**β-phellandrene**	**1.6**	**2.7**	**29.4****	**1.2**	**2.0**
1044	(E)-β-ocimene	t	t	nt	-	t
1054	γ-terpinene	2.1	1.7	7.0 ns	2.0	1.5
1065	cis-sabinene hydrate	0.4	0.3	nt	0.5	0.2
1086	terpinolene	0.8	0.9	2.1 ns	0.7	0.6
1098	trans-sabinene hydrate	0.4	0.4	nt	0.4	0.2
1110	1-coten-3-yl acetate	t	t	nt	-	-
1112	trans-thujone	t	t	nt	t	t
1118	cis-p-menth-2-en-1-ol	0.2	0.1	nt	0.2	0.1
1136	trans-p-menth-2-en-1-ol	0.1	t	nt	0.2	0.1
1141	camphor	-	t	nt	t	-
1145	camphene hydrate	-	t	nt	t	-
1165	borneol	-	-	nt	0.1	-
1174	terpinen-4-ol	2.9	2.3	8.3 *	3.2	2.0
1186	α-terpineol	0.1	0.1	nt	0.1	0.1
1254	linalyl acetate	t	t	nt	0.1	0.2
1287	**bornyl acetate**	**t**	**1.8**	**292****	**0.1**	**0.3**
1289	trans-sabinyl acetate	t	t	nt	-	t
1346	α-terpinyl acetate	t	0.2	nt	0.2	t
1389	β-elemene	-	0.1	nt	-	-

KI	compound	Haava	Kumo	F sig.	HxK 23	HxK 74
1407	longifolene	-	0.1	nt	-	-
1373	α-ylangene	t	-	nt	-	-
1396	α-chamipinene	t	-	nt	-	-
1410	α-cedrene	t	-	nt	t	-
1413	β-funebrene	t	-	nt	t	-
1419	β-cedrene	t	-	nt	0.2	-
1429	**cis-thujopsene**	**5.9**	**-**	**340****	**5.7**	**-**
1454	(E)-β-farnesene	t	-	nt	-	-
1478	γ-muurolene	0.3	0.2	nt	0.3	-
1481	widdra-2,4(14)-diene	0.3	-	nt	0.3	-
1480	germacrene D	t	0.1	nt	0.7	-
1485	β-selinene	-	t	nt	-	-
1493	trans-muurola-4,5-diene	-	0.2	nt	-	-
1500	**α-muurolene**	**-**	**0.4**	**298****	**0.3**	**0.1**
1505	**α-cuprenene**	**0.8**	**-**	**304****	**0.3**	**-**
1513	γ-cadinene	0.4	0.4	nt	0.4	0.5
1522	δ-cadinene	1.4	1.5	0.92ns	0.9	1.6
1532	γ-cuprenene	0.3	-	nt	0.4	t
1537	α-cadinene	t	0.1	nt	t	0.1
1542	δ-cuprenene	t	-	nt	t	-
1548	**elemol/hedycaryol**	**0.2**	**9.7**	**271****	**2.4**	**6.8**
1574	germacrene-D-4-ol	0.8	0.9	nt	1.2	1.3
1586	thujopsan-2-α-ol	0.1	-	nt	-	-
1589	allo-cedrol	t	-	nt	-	-
1599	**widdrol**	**1.2**	**-**	**432****	**0.6**	**-**
1600	**cedrol**	**1.6**	**-**	**675****	**0.7**	**-**
1607	β-oplopenone	0.1	-	nt	t	-
1630	**γ-eudesmol**	**-**	**2.3**	**339****	**0.7**	**1.8**
1638	epi-α-cadinol	0.6	0.7	16.2*	0.3	0.8
1638	epi-α-muurolol	0.5	0.7	3.5ns	0.3	0.8
1644	α-muurolol	t	t	nt	t	t
1649	**β-eudesmol**	**t**	**3.0**	**296****	**0.9**	**2.6**
1652	**α-eudesmol**	**t**	**3.6**	**268****	**1.0**	**3.0**
1652	**α-cadinol**	**1.6**	**0.9**	**42.4****	**0.5**	**1.1**
1670	**bulnesol**	**-**	**0.7**	**308****	**0.2**	**0.5**
1685	α-bisabolol	0.1	t	nt	0.1	-
1887	**oplopanonyl acetate**	**0.4**	**-**	**295****	t	-
1932	beyerene	-	t	nt	-	-
1932	C_{20}, 41,55,257,272	-	0.5	nt	-	0.1
1958	isopimara-8(14),15-diene	0.2	0.2	nt	0.1	0.3

KI	compound	Haava	Kumo	F sig.	HxK 23	HxK 74
1999	kaur-15-ene	0.2	0.3	nt	0.3	0.3
2009	epi-13-manoyl oxide	-	t	nt	t	-
2034	**kaur-16-ene**	**47.7**	**28.4**	**38.8****	**24.0**	**47.7**
2055	abietatriene	0.4	0.3	nt	0.3	-
2132	nezukol	0.8	0.6	12.0*	0.1	0.7
2331	trans-ferruginol	0.2	t	nt	0.2	0.2

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

SENEGALIA ARISTEGUIETANA (L. CÁRDENAS) SEIGLER & EBINGER (FABACEAE: MIMOSOIDEAE): AN UNCOMMON SPECIES OF CENTRAL AMERICA AND NORTHERN SOUTH AMERICA

David S. Seigler*
Department of Plant Biology, University of Illinois, Urbana, IL 61801
*Correspondent: seigler@life.illinois.edu

and
John E. Ebinger
Emeritus Professor of Botany, Eastern Illinois University
Charleston, IL 61920

ABSTRACT

A new combination is made in *Senegalia*: *Senegalia aristeguietana* (L. Cárdenas) Seigler & Ebinger, based on *Acacia aristeguietana* L. Cárdenas, Ernstia 2(1-2):31. 1992. We have collections from southern Mexico (Chiapas), Honduras, Panama, Colombia and Venezuela. A detailed description of the taxon is given. *Phytologia 94(2): 276-279 (August 1, 2012).*

KEY WORDS: *Senegalia aristeguietana comb. nov.,* Fabaceae.

During our studies of *Acacia* s. l. in the New World, we found a few specimens that we originally thought represented a new species in the genus *Senegalia.* Recently we discovered that this entity had already been described by Cárdenas (1992) as a member of the genus *Acacia* from a single state in Venezuela. The name proposed by Cárdenas (1992), *Acacia aristeguietana* fits, in all distinguishing characteristics, the specimens that we had originally considered to represent a new species. Unfortunately, when we examined the Kew isotype of this species, we erroneously synonymized it with *Senegalia polyphylla* (DC.) Britton & Rose (Seigler et al. 2006). The new combination is made below along with a detailed species description, specimens examined, and discussion of the probable relationship of this taxon to closely related species.

Senegalia aristeguietana (L. Cárdenas) Seigler & Ebinger, **comb. nov.**
Basionym: *Acacia aristeguietana* L. Cárdenas, Ernstia 2(1-2):31.1992. – TYPE: VENEZUELA. TÁCHIRA: Las Dantas, vía entre Peracal y Rubio, alt. 1000 m, 25 Aug 1991, *L. Cárdenas & O. Tapias* 3864 (holotype: MY; isotype: K! (000530832), MEXU (PVT633146).

Climbing **shrub** or **liana** to 35 m long; twigs light to dark purple-brown, not flexuous, terete to slightly ridged, glabrous to puberulent; short shoots absent; prickles light purple-brown, flattened, straight to recurved, woody, 1-4 × 1-5 mm at the base, glabrous to lightly puberulent, persistent, widely scattered along the twig, petiole, and rachis. **Leaves** alternate, 120-300 mm long; stipules light brown, linear, symmetrical, flattened, straight, herbaceous, 1-3 × 0.1-0.4 mm near the base, puberulent, early deciduous; petioles shallowly adaxially grooved, 25-55 mm long, lightly to densely puberulent; petiolar gland solitary, located near the base of the petiole, sessile and fused throughout, oblong, 2-7 mm long, apex mostly bulbous, wrinkled, glabrous; rachis adaxially grooved, 70-250 mm long, lightly to densely puberulent, an orbicular gland 0.6-1.2 mm across between the upper 1 to 6 pinna pairs, apex usually flat, glabrous; pinnae 15 to 30 pairs per leaf, 40-110 mm long, 4-13 mm between pinna pairs; paraphyllidia 0.4-0.8 mm long, commonly absent; petiolules 1.0-2.5 mm long; leaflets 50 to 90 pairs per pinna, opposite, 0.6-1.2 mm between leaflets, linear, 3.6-6.5 × 0.6-1.3 mm, glabrous, lateral veins not obvious, 1 vein from the base, base oblique and truncate on one side, margins lightly ciliate, apex obtuse to acute, midvein subcentral. **Inflorescence** a densely 20- to 35-flowered globose head 8-13 mm across, in terminal racemose or paniculate clusters, the main axis to 1.5 m long; peduncles 6-25 × 0.4-0.6 mm thick, puberulent; receptacle subglobose; involucre commonly absent, rarely a single small bract located near the top of the peduncle, early deciduous; floral bracts spatulate, 0.3-0.7 mm long, puberulent, early deciduous. **Flowers** sessile, white; calyx 5-lobed, 1.3-2.2 mm long, puberulent; corolla 5-lobed, 2.2-3.5 mm long, mostly puberulent, lobes one-quarter the length of the corolla; stamen filaments 4.5-6.5 mm long, distinct; anther glands present; ovary pubescent, on a stipe to 1.8 mm long. **Fruits** light brown to reddish brown, straight, flattened, not constricted between the seeds, oblong, 100-230 × 27-35, coriaceous, transversely striated, puberulent, eglandular, dehiscent

along both sutures; stipe 10-15 mm long; apex obtuse to slightly beaked. **Seeds** unknown. Flowers Aug-Oct. Chromosome number: Not determined. Distribution: Wet tropical forests and disturbed second growth forest from sea level to 1100 m in southern Mexico (Chiapas), south through Panama to Colombia and Venezuela.

Specimens Examined: CENTRAL AMERICA: HONDURAS: Atlántida: 10 km E of Tela, orillas Río Piedras Gordas, alt. 5 m, 15 Aug 1981, *J.L.Segovia 53* (MO); 10 km E of Tela, orillas Río Piedras Gordas, alt. 5 m, 15 Aug 1981, *J.V.Soto 47* (MO); **Colon:** Ciudad de Trujillo. Barrio Cristales, 17-20 Jul 1973, *C.Nelson & J.R.Martínez 1255* (MO). **MEXICO: Chiapas:** 4 km W of Crucero Corozal, Palenque-Boca Lacantum road, alt. 180 m, 19 Sep 1984, *E.Martínez S. 7599* (ASU, MO, WIS); 4 km SE of Palenque, 20 Sep 1979, *O.Téllez & E.Martínez 848* (CM). **PANAMA: Darién:** Vicinity of El Real, alt. 15 m, 7 Oct 1938, *P.H.Allen 961* (MO); 1-3 km S of El Real, near sea level, 7 Jan 1975, *A.Gentry 13470* (MO). **SOUTH AMERICA: COLOMBIA: Antioquia:** Trail from Tirana Creek to Providencia, alt. 400-700 m, 25 Oct 1972, *D.D.Soejarto 3495* (F). **Bolivar:** 16 km NE of San Jacinto, Cerro Maco, alt. 650 m, 8 Aug 1985, *J.L.Zarucchi & H. Cuadros 4052* (ILL); **Sucre:** Coloso, alt. 200 m, 12 Mar 1992, *L.H.Soto & H.Giraldo 4* (MO). State not listed: 1760-1808, *J.C.Mutis 3626* (US). **VENEZUELA: Aragua:** Parque Nacional de Rancho Grande, June 1940, *E. Delgado 282a* (US). **Distrito Capital:** Parque Nacional de El Pinar, Caracas, Jul 1940, *E.Delgado 293* (F); Lower Cotiza, near Caracas, alt. 900-1000 m, 20 Aug 1918, *H.Pittier 8041* (F, MO, MT, US); Near Petare, 11 Sep 1921, *H.Pittier 9789* (MT, NY, US). **Carabobo:** Vicinity of Las Trincheras, road between Caracas-La Guaira, 15 Jun 1922, *H.Pittier 10379* (NY, US). **Lara:** Entre Terepaima y Cabudare, alt. 500-900 m, 5-10 Aug 1970, *J.A.Steyermark & F.Delascio, G.C.K. & E.Dunsterville 103668* (CM, NY). **Miranda:** Caracas-Maracay, between kms 37-38, 16 Sep 1972, *L.Cárdenas de Guevara 1376* (F); near Petare, 11 Sep 1921, *H.Pittier 9789* (US); Near Arenazas, road Caracas-Tuy, 26 Sep 1926, *H. Pittier 12219* (A, CM, F, MO, NY, PH, US). **Táchira:** Capacho Nuevo, 17 Aug 1975, *L.Cárdenas 2191* (F); La Puenta, Municipio Palmira, 29 Oct 1976, *L.Cárdenas de Guevara, A.Costero & A.González 2485* (F).

On the label of one specimen (*Allen 961* from Panama) this taxon is described as a giant thorny liana in the top of trees to 35 m

with the white flowers in panicles to 1.5 m long. This little known, but widely distributed, species that occurs from northern South America (Colombia and Venezuela) north to southern Mexico (Chiapas) was not described until 1992. The species does not appear to be common anywhere except possibly in northern Venezuela (Cárdenas 1992). The oblong petiolar gland is located near the base of the petiole and the apex is bulbous and wrinkled a characteristic typical of most species apparently related to *Senegalia amazonica* (Benth.) Seigler & Ebinger. The combined characteristics of the globose inflorescences, the numerous pinna pairs (17-30), and the widely scattered prickles separate this species from other members of this group of species.

ACKNOWLEDGMENTS

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Seigler, D. S., J. E. Ebinger, and J. T. Miller. 2006. The genus *Senegalia* (Fabaceae: Mimosoideae) from the New World. Phytologia 88(1): 38-93.

TAXONOMY OF *JUNIPERUS,* SECTION *JUNIPERUS*: SEQUENCE ANALYSIS OF nrDNA AND FIVE cpDNA REGIONS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA email Robert_Adams@baylor.edu

and

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

ABSTRACT

A robust analysis of *Juniperus,* sect. *Juniperus* is presented based on nrDNA and five cpDNA regions. The section is clearly divided into two groups composed of blue and red seed cone species and is composed of four major clades: *J. communis* and allies; *J. cedrus - oxycedrus* allies; *J. brevifolia - deltoides - navicularis;* and a loose clade of *J. formosana*, *f.* var. *mairei*, and *J. communis* var. *jackii*. *Juniperus c.* var. *jackii* was found to be the most divergent taxon in the blue seed cone group and is recognized at the specific level: ***Juniperus jackii* (Rehder) R. P. Adams, comb. nov.** *Juniperus formosana* and var. *mairei* were found to be very distinct and the DNA data supports the recognition of *J. mairei* Lemee & H. Lev. The DNA data also support the recognition of *J. lutchuensis* Koidz. and *J. communis* var. *hemisphaerica* (J. & C. Presl) Parl. The putative *J. communis* var. *saxatilis* from Kamchatka peninsula, Russia was found to be unique in its DNA sequence and was recognized as: ***Juniperus communis* var. *kamchatkensis* R. P. Adams, var. nov.** *Phytologia 94(2): 280-297 (August 1, 2012).*

KEY WORDS: Phylogeny, *Juniperus*, section *Juniperus*, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, ycf3 intron 2, trnK-matK, *J. jackii, J. c.* var. *kamchatkensis*.

The genus *Juniperus* consists of approximately 68 species and 37 varieties (Adams, 2011), all of which grow in the northern hemisphere, although *J. procera* Hochst. ex Endl. extends southward into the southern hemisphere along the rift mountains in east Africa (Adams and Demeke, 1993). The genus is divided into three sections: *Caryocedrus* (one species, *J. drupacea* Labill.); *Juniperus* (= *Oxycedrus*, 11 species) and *Sabina* (56 species). Section *Juniperus* is circumboreal (Fig. 1), whereas sect. *Caryocedrus* is restricted to the eastern Mediterranean region.

Mao et al. (2010) presented an abbreviated phylogeny of *Juniperus* as part of a study focused on intercontinental dispersal. The study did not include all species of *Juniperus*, as a complete phylogeny was not the goal of their study. The purpose of the current paper is to

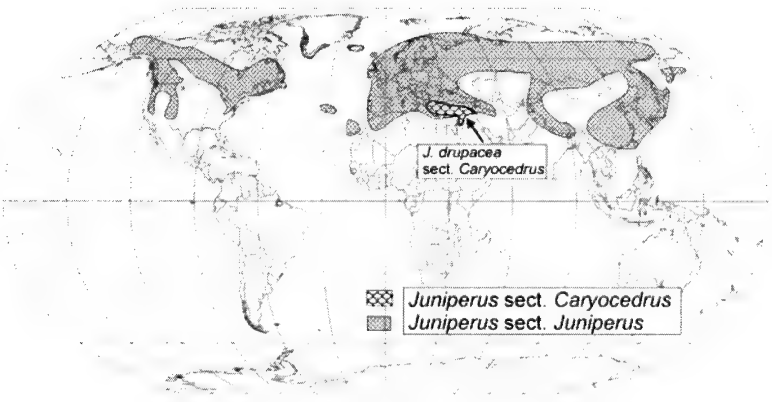

Figure 1. Distributions of *Juniperus* sect. *Caryocedrus* and sect. *Juniperus* (adapted from Adams, 2011).

present a robust analysis based on the most informative nuclear (nrDNA) and cpDNA regions (petN-psbM, trnS-trnG, trnD-trnT, ycf3 intron 2, trnK-matK) of section *Juniperus*, with particular attention to include all known species in this section plus, *J. drupacea* (section *Caryocedrus*) as an outgroup.

MATERIALS AND METHODS

Specimens used in this study: *J. brevifolia* (Seub.) Ant., *Adams 8152,* Serra da Tronqueira, San Miguel Island, Azores Islands; *J. cedrus* Webb & Berthol., *Adams 11510*, La Palma, Canary Islands, Spain; *J. communis* L. var. *communis*, *Adams 7846*, Stockholm, Sweden; *J. c.* var. *charlottensis* R. P. Adams, *Adams 10304*, Queen Charlotte Island, BC, Canada; *J. c.* var. *depressa* Pursh, *Adams 7802*, Victor, CO, USA; *J. c.* var. *hemisphaerica* (J. & C. Presl) Parl. in Candolle, *Adams 9045,* Mt. Etna, Sicily, Italy (type loc.); *J. c.* var. *hemisphaerica*, *Adams 7194*, Sierra Nevada, Spain; *J. c.* var. *jackii* Rehder, *Adams 10287*, sw Oregon, USA; *J. c.* var. *megistocarpa*, Fernald & H. St. John, *Adams 8575*, Magdalen, Isl., Quebec, Canada; *J. c.* var. *nipponica* (Maxim.) E. H. Wilson, *Adams 8579*, Japan; *J. c.* var. *oblonga* hort. ex Loudon, *Adams 8764,* Armenia; *J. c.* var. *saxatilis* Pall., *Adams 7589*, Altai Mtns., Mongolia; *J. c.* var. *saxatilis*, *Adams 11206,* Norway; *J. c.* var. *saxatilis*, *Adams 9182*, Kamchatka, Russia; *J. c.* var. *saxatilis*, *Adams 10188,* Sakhalin Island, Russia; *J. c.* var. *saxatilis*, *Adams 10890*, Redfish Lake, ID, USA; *J. c.* var. *saxatilis, Adams 8686*, Japan; *J. deltoides, Adams 9431*, Turkey; *J. formosana* Hayata var. *formosana*, *Adams 9071*, Taiwan; *J. f.* var. *mairei* (Lemee & Lev.) R. P. Adams & C-F. Hsieh, *Adams 6772,* Gansu, China; *J. macrocarpa* Sibth. & Sm., *Adams 7205*, 15 km w Tarifa, Paloma sand dunes, Spain; *J. maderensis* (Menezes) R. P. Adams, *Adams 11497*, Madeira Island, Portugal; *J. navicularis* Gand., *Adams 8240*, Lisbon, Portugal; *J. oxycedrus* L. var. *oxycedrus*, *Adams 9039*, 4 km e Forcalquier, France; *J. o.* var.. *badia* H. Gay, *Adams 7795*, Jaen, Spain; *J. rigida* Mig. in Sieb., *Adams 8544*, Gifu Prefecture, Japan (provided by Jin Murata); *J. r.* var. *conferta* (Parl.) Patschka, *Adams 8585*, Tottori Sand Dunes, Japan (provided by Jin Murata); *J. taxifolia* Hook. & Arn. var. *taxifolia*, *Adams 8448*, Bonin Islands, Japan (provided by Jin Murata); *J. t.* var. *lutchuensis* (Koidz.) Satake, *Adams 8541*, Japan. Section *Caryocedrus*: *J. drupacea* Labill., *Adams 8795, 8796*, Achladokampos Pass, 18 km e Tripolis, Greece. Voucher specimens are deposited in the herbarium, BAYLU, Baylor University.

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 use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

PCR amplification Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010). The 5'trnK-matK-3'trnK region was amplified with primers based on Johnson and Soltis (1994) and modified for use in Cupressaceae (matK1437F: TTGGAAGTTTCGTTCGCAAT; matK1291R: GTAGGGCACTCGTATATCTG; trnK3914F(2565): TGGGTTGC TAACTCAATGG; trnKRcup: AGCTCGTCGGATGGAGTGG. PCR reactions were conducted in 50 µl reactions using 40 ng of genomic DNA, containing 0.5 U Phusion polymerase (NEB, Ipswich, MA) with 1× Phusion HF Buffer (containing 1.5 mM $MgCl_2$), 0.2 mM dNTPs, 0.2 µM each primer. Since the Phusion polymerase has been designed for reduced cycling times, we used increased denaturation and annealing temperatures and shortened thermal cycling times (per the manufacturer's recommendations) as follows: an initial denaturation at 98°C for 30s; 35 cycles of 98°C for 10 s, 60°C for 30 s, and 72°C for 165 s; hold at 72°C for 5 min; and an indefinite hold at 4°C.

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. 5.4 (Drummond et al. 2011), the MAFFT alignment program and the PAUP* program, version 4.0b10 (Swofford 2003) for neighbor joining, parsimony, and maximum likelihood tree searches. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and

Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975). The Splitstree v.4 program was used to calculate a splits network tree (Huson and Bryant 2006).

RESULTS AND DISCUSSION

Eight gene regions were surveyed to determine applicability (Table 1). Based on these data, one nuclear region (nrDNA) and five cpDNA regions (petN-psbM, trnS-trnG, trnD-trnF, trnK-matK, ycf3-intron 2) were used in the analyses.

Table 1. Comparison of gene regions and variation in section *Juniperus* (and *J. drupacea*, section *Caryocedrus*). ycf2-IGS-psbA and trnL-trnF were not included in the final analysis. subs = nucleotide substitutions, % inf = % potentially informative = total / length (bp) as percent.

gene region	length(bp)	subs	indels	total	% inf
nrDNA	1278	80	16	96	7.51
petN-psbM	850	21	22	43	5.06
trnS-trnG	830	16	23	39	4.70
trnD-trnF	676	16	10	26	3.84
trnK-MatK	2354	27	3	30	1.27
trnL-trnF	679	8	9	17	2.50
ycf3 intron 2	877	6	6	12	1.37
ycf3 - IGS - psbA	558	2	4	6	1.07

The aligned concatenated data set was composed of 6862 bp from nrDNA, and five cpDNA regions (petN-psbM, trnS-trnG, trnD-trnF, trnK-matK, ycf3-intron 2) sequences. Bayesian analysis shows section *Juniperus* to be clearly divided (Fig. 2, 100% support) into the blue and red seed cone groups (as previously found, Mao et al. 2010). However, in the red seed cone group, there are two sub-clades of *oxycedrus-cedrus* allies and *deltoides-brevifolia-navicularis* (Fig. 2, 100% support). The *J. communis* taxa from North America, form two

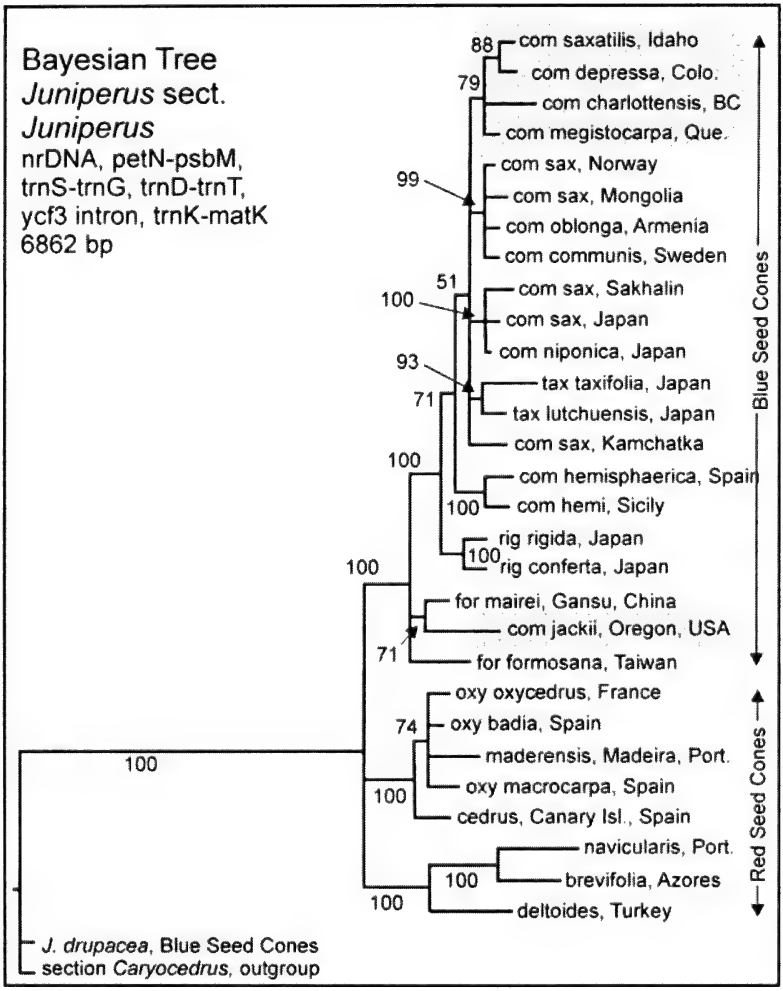

Figure 2. Bayesian tree for all proposed *Juniperus* sect. *Juniperus* taxa. Numbers at the branch points are posterior probabilities (as percent).

clades: var. *jackii*, Oregon and var. *saxatilis*, Idaho; var. *depressa* Colorado, var. *charlottensis*, BC, Canada and var. *megistocarpa*, Quebec, Canada (Fig. 2). *Juniperus taxifolia* and *J. t.* var. *lutchuensis* (Japan) are nested within the main *J. communis* clade. *Juniperus c.* var. *hemisphaerica* (treated as *J. c.* var. *saxatilis* by Adams 2011), has 100% support as a distinct clade (Fig. 2). The two varieties of *J. formosana* (v. *formosana*, Taiwan, v. *mairei*, Gansu, China) are in separate clades (Fig. 2.).

An alternative portrayal of the phylogeny is by a split tree diagram (Fig. 3); in this, the division between the blue and red seed cone junipers is very clear. Four major groups are evident in the split tree as shown by circles (Fig. 3). Two groups are of particular interest: the very closely related *J. communis* and allies form a group and the diverse collection of *J. formosana, J. f.* var. *mairei* and *J. communis* var. *jackii* (Fig. 3) form another. The divergence of the *oxycedrus-cedrus* and *brevifolia-deltoides-navicularis* clade is very pronounced.

Taxonomic Considerations

Aside from elucidating phyletic relationships, DNA sequence data is useful for clarification of taxonomic decisions. For example, the taxonomy of sect. *Juniperus* has been controversial for decades. Many of the taxa are highly variable (particularly in *J. communis* and allies), and taxa are defined on the basis of a single, often quantitative, morphological character (such as width of stomatal band).

In order to examine the divergence among taxa in sect. *Juniperus*, the entire data set of mutations were utilized. This included 221 nucleotide substitutions and 67 indels. All indels were treated as present/ absent regardless of their length. This set of 288 mutational events (ME) were utilized to construct minimum spanning networks (MSN). MSN are particularly useful to ascertain the magnitude of genetic mutations that have accumulated between the lowest levels of classification such as varieties, subspecies and forms. These data are important because such categories are the most controversial and are often based on only one or two morphological characters. The MSN of the red seed cone taxa (Fig. 4) shows most of the recognized species separated by 10- 30 MEs (mutations).

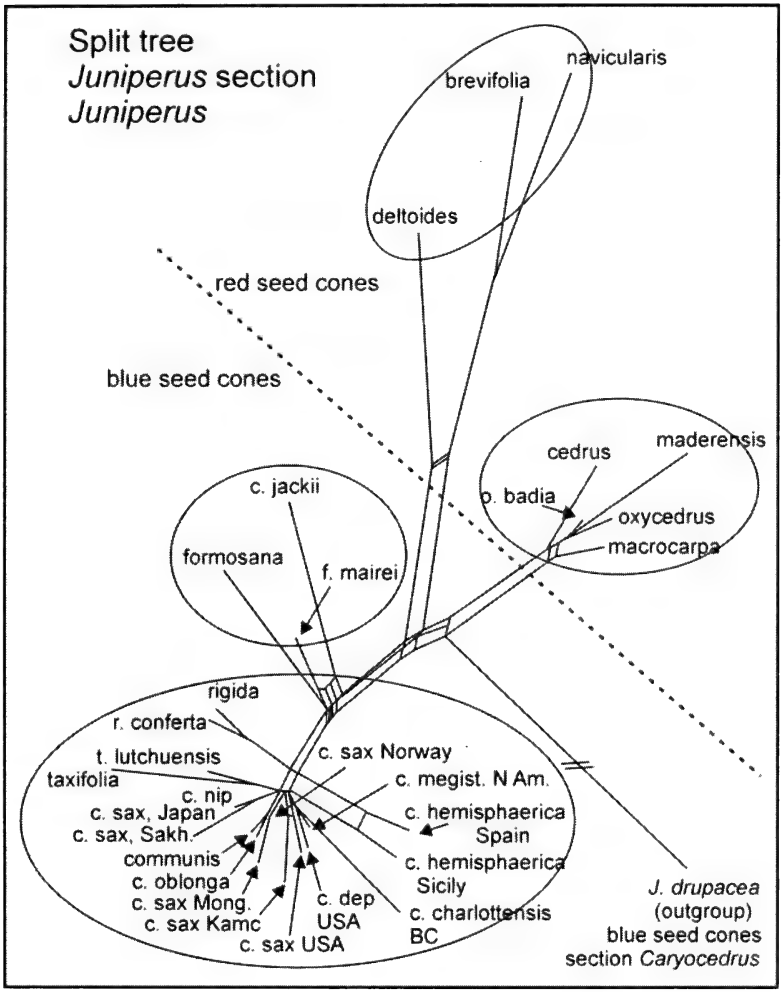

Figure 3. Splits tree analysis of *Juniperus* sect. *Juniperus* evolution.

It is interesting that the nearest link to a non-red seed cone taxon is that of *J. formosana* var. *mairei,* whose mature seed cones are reddish-blue under a bright blue glaucous coating. It should be noted that the Bayesian tree did not include data from indels, so that may, in part, account for this oddity. *Juniperus deltoides*, *J. brevifolia* and *J. navicularis* are especially distinct in the Bayesian tree (Fig. 2), and they are separated by 40 MEs (Fig. 4), and from each other by 20 and 32 MEs. The *cedrus* - *oxycedrus* group is much less diverse (Fig. 4). It is well accepted that *J. oxycedrus* and *J. cedrus* are distinct species (Adams, 2011, Eckenwalder, 2009, Farjon, 2005, 2012) and these differ by 10 MEs (Fig. 4). Note particularly that *J. maderensis* (not accepted

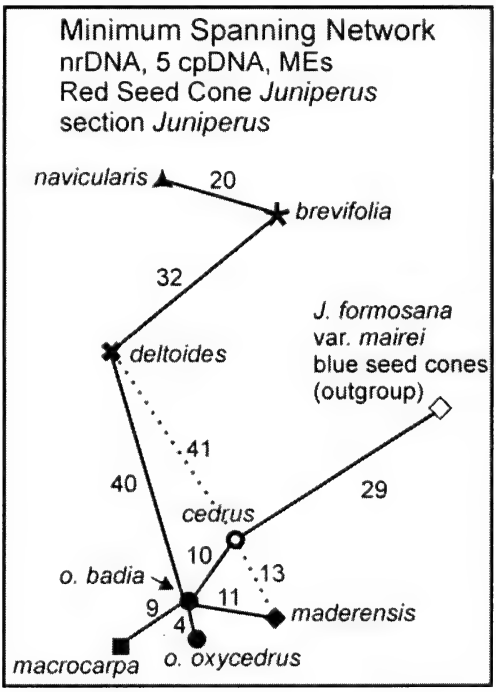

Figure 4. MSN (Minimum spanning network) for the red seed cone junipers, based on 221 nucleotide substitutions and 67 indels. Numbers next to the links are the number of Mutational Events (MEs). *J. formosana* var. *mairei* is the nearest blue seed cone species. Dotted lines show the second nearest links.

by Farjon, 2005, 2012, see Table 2) differs by 11 MEs, giving support for the continued recognition of this species, endemic and threatened on Madeira Island. It is important to note that *J. maderensis* is about equally removed from *J. cedrus* (13 MEs) and *J. oxycedrus* var. *badia* (11 MEs), further supporting their recognition as separate species. The reader is referred to Adams, et al. (2010) for a more detailed analysis of *J. cedrus* and *J. maderensis* from Canary and Madeira Islands.

Juniperus macrocarpa is often treated as *J. oxycedrus* var. or subsp. *macrocarpa* (Table 2), because it differs by its very large seed cones with three raised cone scales and its unusual sea-coast habitat. It is surprising that it is not universally accepted as a species based solely on its morphology. The present study found *J. macrocarpa* to be separated by 9 MEs from *J. oxycedrus* var. *badia* (Fig. 4). Considering morphological and ecological differences as well as the 9 MEs, the continued recognition of *J. macrocarpa* is supported (Table 2).

Juniperus oxycedrus var. *badia* is recognized by its smaller seed cones and shorter leaves than found in *J. oxycedrus* var. *oxycedrus* (Adams 2011). However, in practice, these characters tend to overlap and var. *badia* is scarcely distinct. This study confirms the very close relationship of these being separated by 4 MEs (Fig. 4) out of 6862 bp. Recognition of var. *badia* is only weakly supported by the sequence data (Table 2).

The blue seed cone junipers in sect. *Juniperus* consist of a diverse assemblage. The *J. communis* and allies group are especially difficult and subject to endless nomenclatural changes due to the fact that the habit and leaf characters of varieties (in North America) or subspecies (in Europe) are very variable. In many populations of *J. c.* var. *communis* (typically a small, upright tree) and *J. saxatilis* (a shrub), one finds forms ranging from trees with a strong central axis to decumbent or trailing shrubs. Even in 'pure' var. *communis* populations, the tree habit is often not completely expressed. It seems likely that this 'key' character is controlled by only a few genes or by gene-environmental interactions.

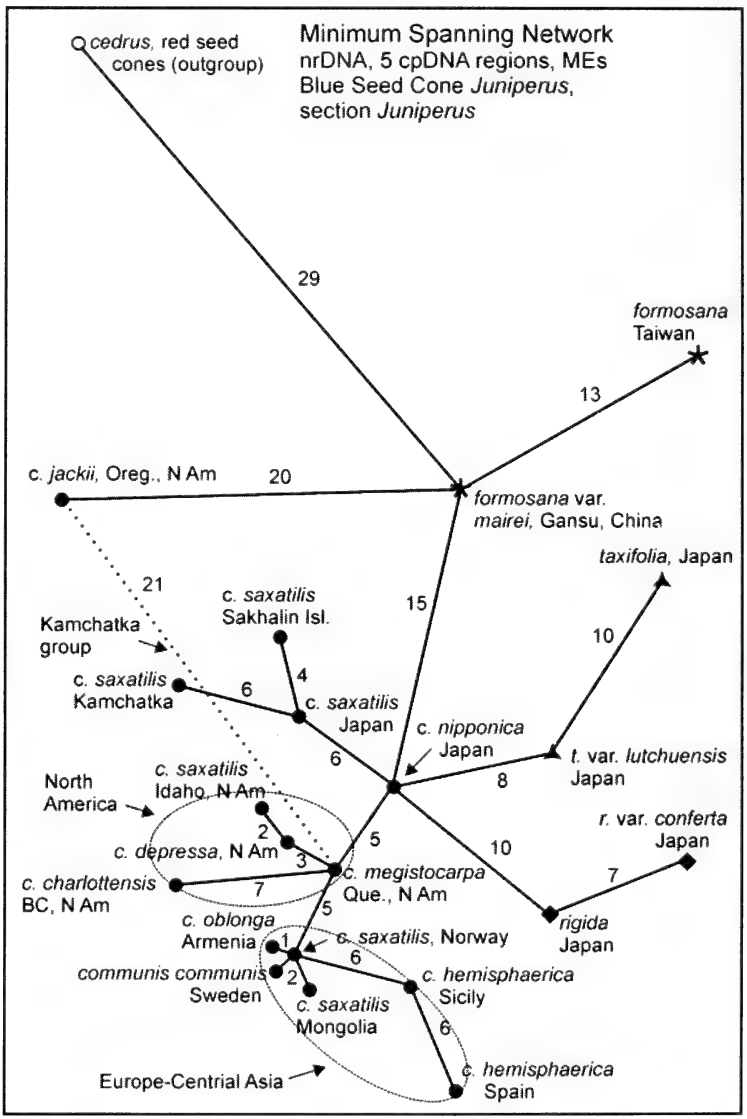

Figure 5. MSN of the blue seed cone junipers (see notes Fig. 4). *J. cedrus* is the nearest of the red seed cone species.

Table 2. Taxonomy of taxa in sect. *Juniperus* by Adams (2011) and Farjon (2010). See also Farjon (2005) for detailed comments. New taxa and taxa newly supported by this study.

Adams(2011)	Farjon (2005, 2010)	Supported, this study
J. brevifolia	*J. brevifolia*	*J. brevifolia*
J. cedrus	*J. cedrus*	*J. cedrus*
J. communis	*J. communis*	*J. communis*
v. *charlottensis*	v. *saxatilis*	v. *charlottensis*
v. *depressa*	v. *depressa*	v. *depressa*
v. *hemisphaerica*	v. *communis*	v. *hemisphaerica*
v. *megistocarpa*	v. *megistocarpa*	v. *megistocarpa*
v. *nipponica*	v. *nipponica*	v. *nipponica*
v. *oblonga*	v. *communis*	**v. *communis***
v. *saxatilis* (Europe,	v. *saxatilis*	**v. *communis*** (Europe, central Asia)
v. *saxatilis* (Japan, Sakhalin Isl.)	v. *saxatilis*	**v. *nipponica*? or v. *kamchatkensis***
v. *saxatilis*(Kamchatka)	v. *saxatilis*	**v. *kamchatkensis***
v. *saxatilis* (N Am.)	v. *saxatilis*	***v. depressa***
v. *jackii*	v. *saxatilis*	***J. jackii***
J. deltoides	*J. oxycedrus*	*J. deltoides*
J. formosana	*J. formosana*	*J. formosana*
v. *mairei*	*J. formosana*	***J. mairei***
J. macrocarpa	*J. oxycedrus. subsp. macrocarpa*	*J. macrocarpa*
J. navicularis	*J. oxycedrus. subsp. transtagana*	*J. navicularis*
J. oxycedrus	*J. oxycedrus*	*J. oxycedrus*
v. *badia*	*subsp. badia*	***J. oxycedrus***
J. rigida	*J. rigida*	*J. rigida*
v. *conferta*	subsp. *conferta*	v. *conferta*
J. taxifolia	*J. taxifolia*	*J. taxifolia*
v. *lutchuensis*	*J. taxifolia*	*J. lutchuensis* or *J. t.* v. *lutchuensis*

The MSN for the blue seed cone sect. *Juniperus* shows considerable differentiation (20 MEs) for *J. communis* var. *jackii*, endemic to serpentine and ultra-mafic rocks in Oregon and northern California (Fig. 5). Its nearest link is with *J. formosana* var. *mairei*, Gansu, China (with a link of 20 MEs differences), but note (Fig. 5) that it is 21 MEs from *J. c.* var. *megistocarpa* N. America, and 22 MEs removed (data not shown) from *J. c.* var. *communis*, Sweden. It is apparently not closely related to any extant juniper. The sequence data strongly support the recognition of var. *jackii* at the specific level (Table 2), ecology and morphology support the recognition of the taxon as:

***Juniperus jackii* (Rehder) R. P. Adams, comb. nov.**

Basionym: *Juniperus communis* var. *jackii* Rehder Mitt. Deutsch. Dendrol. Ges. 1907 (16): 70 (1907). Type: Siskiyou Mtns., on the road from Waldo, Oregon to Crescent City, CA, 3000 ft., 25 Aug., 1904, *J. G. Jack (A. Rehder) s. n.*, (lectotype: A!, designated by Farjon (2005). Named in honor of J. G. Jack.

The next largest link shown in figure 4 is between *J. formosana* var. *mairei* and *J. communis* var. *nipponica* (15 MEs) followed by the gap between *J. f.* var. *mairei* and *J. f.* var. *formosana* (13 MEs). These data indicate that *J. f.* var. *mairei* is not conspecific with *J. formosana* and supports the recognition of this taxon as distinct species:

***Juniperus mairei* Lemee & H. Leveille**, Monde Pl. 2(16): 20 (1914). Maire's juniper, Type: Yunnan, Jong-tohouan, *J. mairei* Lemee & H. Lev., *E. E. Maire s. n.*, (holotype A! barcode *#38339*, isotype E?)

J. chekiangensis Nakai

J. formosana f. *tenella* Handel-Mazzetti

J. formosana var. *mairei* (Lemee & Lev.) R. P. Adams & C-F. Hsieh

Juniperus rigida and *J. r.* var. *conferta* differ by 10 MEs from *J. communis* var. *nipponica*, Japan (Fig. 5). The sequencing data supports the recognition of *J. rigida* (Table 2). *Juniperus r.* var. *conferta* has also been treated as a *J. conferta* Parl. The taxa differ in several

morphological and ecological characters (Adams, 2011) as well as by 7 MEs in this data set. However, it seems prudent to recognize *J. r.* var. *conferta* at this time (Table 2).

Juniperus taxifolia and *J. t.* var. *lutchuensis* present a more difficult taxonomic problem. They were found to form a clade within the *J. communis* clade (Fig. 2) and MSM analysis reveals *J. t.* var. *lutchuensis* is a little closer to *J. c.* var. *nipponica* (8 MEs, Fig. 5) than to *J. taxifolia* (10 MEs, Fig. 5). The divergence of *J. taxifolia* from *J. t.* var. *lutchuensis* (10 MEs) is similar to that found in other closely related species pairs (*macrocarpa* - *oxycedrus*, 9; *maderensis* - *oxycedrus* - 11; *cedrus* - *oxycedrus* 10, *rigida* - *communis* 10). The taxa appear to occupy similar habitats and are very similar in their morphology. At present, it seems prudent to maintain *J. t.* var. *lutchuensis* (Table 2).

The *J. communis nipponica* - *saxatilis* complex from Japan (and nearby Sakhalin Island and the Kamchatka peninsula) is closely allied with North America (5 MEs, Fig. 5), while differing by 4-6 MEs between taxa. The putative *J. c.* var. *saxatilis*, Japan is 6 MEs from *J. c.* var. *nipponica*, compared to 8 MEs (data not shown) to *J. c.* var. *saxatilis*, Norway and 8 MEs (data not shown) to *J. c.* var. *depressa*, N. America. The putative var. *saxatilis* from Japan, Kamchatka and Sakhalin does not appear to be part of the traditionally recognized *J. c.* var. *saxatilis* from Europe and central Asia. It appears that the key character defining var. *saxatilis*, stomatal band twice as wide as each green leaf margin (Adams, 2011), may have evolved independently several times. There is, at present, no easy solution to this taxonomy. Comparison of their leaf morphology (Table 3) shows differences in the stomatal band width (relative to the green leaf margins), leaf cross sections and overall leaf shape. The leaves of var. *saxatilis* from mainland Japan are quite similar to var. *saxatilis* from Europe, including having keeled leaves the latter was thought to be a useful character to separate var. *saxatilis* from var. *nipponica* (Adams, 2011). Morphology and DNA sequence data separate the taxon on Kamchatka from *J. c.* var. *saxatilis*, Japan and *J. c.* var. *nipponica* Europe, including having keeled leaves the latter was thought to be a useful

Table 3. Comparison of the leaf morphology of *J. c.* var. *saxatilis*, Japan and Kamchatka, and *J. c.* var. *nipponica*, Japan.

	var. *saxatilis*		var. *nipponica*
	Japan	Kamchatka	Japan
Stomatal band width vs. green leaf margin	2x	1-1.5x	0.5-0.25x
Leaf cross-section	flat to slightly concave with keel	concave with keel	very concave, sunken stomatal band, with keel
Leaf shape	straight to curved	straight to slightly curved	curved, boat shaped

character to separate var. *saxatilis* from var. *nipponica* (Adams, 2011). Morphology and DNA sequence data separate the taxon on Kamchatka from *J. c.* var. *saxatilis*, Japan and *J. c.* var. *nipponica* (Table 3). These differences warrant the recognition of the taxon on Kamchatka as a new variety:

***Juniperus communis* var. *kamchatkensis* R. P. Adams, var. nov.**
Type: Russia, near Esso, Kamchatka peninsula, 56° N, 159° E, 550-700 m., *J. W. Leverenz 5* (= *Adams 9164*) (HOLOTYPE: BAYLU).

Shrubs, similar to *J. communis* var. *saxatilis*, but differing in having straight to slightly curved leaves, with cross section concave with keel and stomatal band 1 - 1.5 x width of green leaf margins. Seed cones purple-blue when mature.

Other specimens studied: TOPOTYPES: *J. W. Leverenz 6* (*Adams 9181*), *J. W. Leverenz 7* (*Adams 9182*), *J. W. Leverenz 8* (*Adams 9183*).

Juniperus communis var. *kamchatkensis* is known only from the type locality and vicinity in Kamchatka; usually beneath *Betula platyphylla*, *Populus tremula* and *Salix* sp. in ravines with large rocks.

In spite of the fact that DNA sequence data (Fig. 5) shows var. *saxatilis*, Japan, not to be closely related to var. *saxatilis* of Europe and central Asia (Fig. 5), no reliable morphological character was found to separate the Europe - central Asia plants from those on Japan and Sakhalin Island.

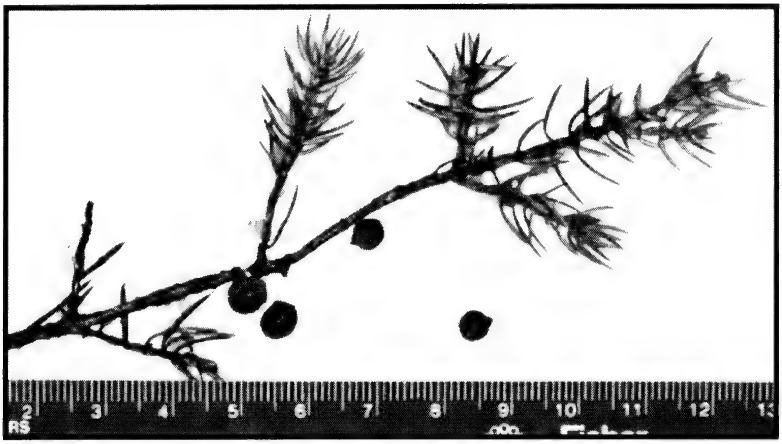

Figure 6. Holotype of *Juniperus communis* var. *kamchatkensis*.

The North American *J. communis* taxa (vars. *charlottensis*, *depressa, megistocarpa* and *saxatilis,* Fig. 5) are shown to differ by at least 5 MEs from Japan and Europe taxa. The var. *charlottensis* is the most distinct (7 MEs), whereas vars. *depressa, megistocarpa* and *saxatilis* differ by only 2 to 3 MEs; var. *megistocarpa* is very distinct in having very large seed cones (9-13 mm and larger than leaf length, Adams, 2011) and grows on coastal sand dunes. Although the DNA differences are not large (3 MEs) the continued recognition of this variety is warranted. In North America, var. *depressa* and var. *saxatilis* differ principally by the width of stomatal bands (compared to the green leaf margin). The var. *saxatilis*, Idaho, USA, differs by only 2 MEs from var. *depressa*, USA, but by 6 MEs to var. *nipponica*, Japan and var. *saxatilis*, Norway (data not shown). Clearly it is part of var. *depressa* and not var. *saxatilis* (*sensu stricto*).

Juniperus c. var. *communis*, trees in Sweden, var. *saxatilis*, Norway and Mongolia, and var. *oblonga*, Armenia form a very tight group differing by 1 to 2 MEs (Fig. 5). Clearly there is no support for the recognition of var. *oblonga* (Fig. 5, Table 2). The separation of var. *communis* from var. *saxatilis* (in Europe and central Asia) is largely based on the tree vs. shrub habits. However, as previously mentioned,

many populations of *J. c.* var. *communis* have individuals that are semi-trees and/or both large and small shrubs. It appears that, in spite of strong DNA evidence, the shrubs in Europe and central Asia will continue to be called var. *saxatilis*.

Of special interest is the var. *hemisphaerica* from the type locality (Mt. Etna, Sicily) and Sierra Nevada, Spain. These plants differ by 6 MEs from var. *saxatilis* (Fig. 5) and between each other. The DNA data support the recognition of var. *hemisphaerica* (Table 2) in Sicily and perhaps a new variety in Sierra Nevada, Spain.

One is always looking for more data to resolve difficult problems. However, it may be that these unresolved taxonomic problems in *Juniperus* section *Juniperus* are actually evolutionary branches in the midst of complex speciation processes.

ACKNOWLEDGEMENTS

Thanks to Jin Murata, Japan for providing specimens from Japan. Thanks to Tonya Yanke and Julie Morris for lab assistance, and Baylor for funds. This research was supported in part by funds from NSF projects DEB-0316685 to RPA and AES as well as DEB-0629402 to AES.

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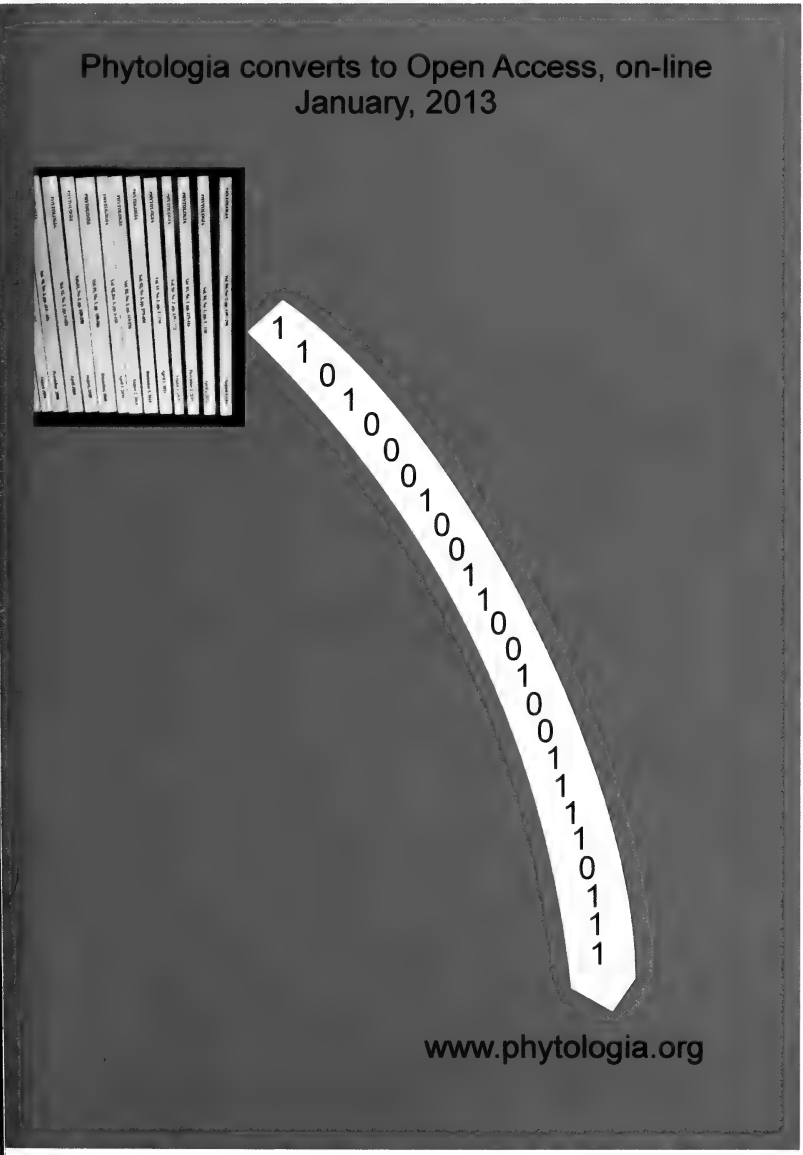

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Dr. Robert P. Adams, editor

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Cover photo: Conversion of Phytologia to digital, Open Access

ARTEMISIA WOODII (ASTERACEAE), A NEW RANK FOR A NARROW ENDEMIC OF THE YUKON TERRITORY, CANADA

Chance W. Riggins
Department of Plant Biology
University of Illinois at Urbana-Champaign
Urbana, IL 61801, U.S.A.
email criggins@life.illinois.edu

ABSTRACT

Results from molecular phylogenetic investigations of Old and New World *Artemisia* species, and Beringian species in particular, have led to the reevaluation of certain taxa with broad disjunctions. One such taxon, *Artemisia rupestris* spp. *woodii* Neilson, is endemic to the southwestern Yukon Territory, Canada and represents the only North American occurrence of the Eurasian species *A. rupestris*. Based on a reexamination of specimens, molecular evidence, and geographic considerations, subspecies *woodii* is judged to be distinct from its Eurasian congeners and is elevated to specific rank as: **Artemisia woodii** (Neilson) C. Riggins, **stat. nov**. *Phytologia 94(3): 301-304 (December 1, 2012).*

KEY WORDS: *Artemisia*, Asteraceae, Anthemideae, Yukon Territory, Canada

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The Eurasian species *A. rupestris* L. is represented in North America by a single taxon, *A. rupestris* ssp. *woodii* Neilson, which is only found in two highly localized areas of southwestern Yukon Territory, Canada (Hoefs et al., 1983). As part of ongoing investigations of New and Old World *Artemisia* species, the status of this taxon was reevaluated in light of molecular nrITS and plastid DNA evidence (Riggins and Seigler, in press) and a detailed assessment of morphological characters. During the course of these studies it became apparent that this subspecies is distinct from the Eurasian *A. rupestris* and associated North American taxa within *Artemisia* (sensu Shultz, 2006) and so is here elevated to specific status as follows:

Artemisia woodii (Neilson) C. Riggins, **stat. nov.**

BASIONYM: *Artemisia rupestris* ssp. w*oodii* Neilson, Canadian Field-Naturalist 82: 114-119. 1968. Type: CANADA. YUKON: Sheep Mountain, Kluane Range, vicinity of Kluane Lake, 20 August, 1967, *Neilson 1242* (holotype US, digital image!; isotypes CAN, DS, GH, S, UC).

Neilson (1968) originally described *A. rupestris* ssp. *woodii* from a collection made in the Kluane Lake area of the Yukon Territory, stating that "This plant has no morphologically similar relative in North America." He distinguished ssp. *woodii* from the typical Eurasian *A. rupestris* primarily by having broader, rounder phyllaries with scarious, iridescent margins and a reduced number of bracts subtending the capitula. Neilson also noted that the leaf lobes of ssp. *woodii* tended to be more blunt or obtuse in comparison to the acute tips common in the Eurasian material. While acknowledging a superficial resemblance to *A. frigida* Willd. in habit, he further commented that ssp. *woodii* differed from the former by consistently having coarse hairs on the receptacle (sometimes absent in populations of *A. frigida*) and dark green, sticky-glandular leaves.

Almost simultaneous with Neilson's publication, Welsh (1968) described *A. frigida* var. *williamsae* Welsh from a collection on the lakeshore at Kluane Lake that featured near glabrous receptacles and larger capitula than typical *A. frigida.* Welsh (1974) later considered *A. rupestris* ssp. *woodii* as a synonym for *A. frigida* var. *williamsae*, but Cody (2000) correctly pointed out that the two taxa are not one and the same and that "a form [of *A. frigida*] with glabrous receptacles found in the Kluane Lake area (var. *williamsae* Welsh) should not be confused with the densely glandular-dotted *A. rupestris* ssp. *woodii*, which is endemic to alpine areas in southwestern Yukon Territory." Comparisons of the holotype (BRY; *Williams 1369*) and an isotype (OSU) of var. *williamsae* support Cody's observations. In addition to the presence of viscous glands, *A. woodii* is further distinguished from *A. frigida* by its bright to dark green appearance, its narrow racemiform or spiciform synflorescences (usually paniculiform in *A. frigida*), and slightly larger involucres (5-6 x 6-8 mm) subtended by short linear green bracts. Although both taxa do share a general area

of sympatry in the Yukon Territory, there are no apparent signs of intergradation in the material I have examined.

Artemisia woodii is a low mat-forming perennial found on dry well-drained slopes with a southern exposure ca. 800-1900 m in the mountains around Kluane Lake and in one other locality in southwestern Yukon Territory (Hoefs et al., 1983; Cody, 2000). These populations are approximately 5200 km from the nearest easternmost Eurasian populations of *A. rupestris* from the Vilyuy River tributary of the Lena River (see range maps in Hultén, 1968 and Jäger, 1987). *Artemisia rupestris* has its main range centered in the steppe and alpine zones of central Siberia (Altai) with extensions from Kazakstan to Mongolia and northern Afghanistan, but disjunct populations are also found in the Baltic Region of Northern Europe (where the type was first described), central Germany, and in the Ural Mountains. While the ploidy of *A. woodii* is unknown at present, the diploid (2n=18) condition seems to predominate in Eurasian populations of *A. rupestris*, although there is one count from the Russian Altai that appears to be hypotetraploid (2n=34) (Ehrendorfer, 1964; Goldblatt and Johnson, 2010). It is in the Altai and Lake Baikal areas that plants of *A. rupestris* tend to display the greatest variation in morphology and ecological preference, but there are consistent differences in habit, leaf morphology, and floral bract number in comparison to specimens of *A. woodii*.

In summary, the aforementioned distinctions coupled with molecular evidence that places the Eurasian *A. rupestris* and the North American *A. woodii* in separate clades (Riggins and Seigler, in press) warrant their recognition as distinct species.

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I thank Dr. Bruce Bennett, of the Yukon Conservation Data Centre in Whitehorse, Yukon, for reviewing the manuscript and generously sharing his field notes and images of this species. Dr. Lee Crane, Illinois Natural History Survey, is also acknowledged for kindly reviewing the manuscript. Thanks also to the curators of ALA, ALTA, CAN, ILLS, and UBC for the loan of specimens for study.

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TAXONOMY OF *EUCNIDE BARTONIOIDES* (LOASACEAE) COMPLEX IN TEXAS

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

`The taxonomic status of *Eucnide bartonioides* in Texas is evaluated. It is concluded that two populational systems occur in the state, a small flowered complex largely confined to the Edwards Plateau, and a large flowered complex occurring to the west of the former. A formal name, **E. b.** var. **edwardsiana** B.L. Turner, is provided for the small flowered populations. A photograph of the holotype is provided, along with maps showing distribution of the complex. *Phytologia 94(3): 305-309 (December 1, 2012).*

KEY WORDS: Loasaceae, *Eucnide, E. bartonioides*, Edwards Plateau, Texas

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Eucnide bartonioides Zucc. is a widespread, variable species occurring from south-central Texas to San Luis Potosi in northeastern Mexico (Thompson and Ernst 1967, Fig. 2, 3). Most specimens possess relatively large yellow flowers (petals 2.5-4.0 cm long), but as Thompson and Ernst note, "The size of the corolla (and the leaves) is quite variable. The small flowered forms can be confusingly similar to depauperate plants of *Eucnide lobata* even though these two species normally are quite distinct."

The present paper has to do with the biological status of small flowered plants from the Edwards Plateau of central Texas, which were called to the fore by Turner et al. (2003), but are treated herein as a distinct entity, as follows:

EUCNIDE BARTONIOIDES VAR. EDWARDSIANA B.L. Turner, **var. nov.** Fig. 1

Cliff-dwelling perennial herbs to 30 cm high and as wide. **Leaves** mostly 8-16 cm long; petioles 4-8 cm long, pubescent with acerose trichomes ca 1 mm long; blades asymmetrically cordate at base, 5-6 cm long, 4-8 cm wide, having 5-9 crenulate lobes 0.5- 2.0 cm long, sparingly pubescent above and below. **Flowers** axillary; peduncles mostly 2-5 cm long, rarely extending to 12 cm long with age, pubescent like the stems. **Sepals** 5, lanceolate, ca 8 mm long. **Petals** yellow, lanceolate, 10-20 mm long, 5-8 mm wide. **Stamens** 20-40, 19-20 mm long; anthers ca 1 mm long. **Capsule** 5-6 mm long, 5-6 mm wide, densely pubescent with acicular hairs ca. 1 mm long. **Seeds** numerous, ca 0.75 mm long, having 4-5 parallel ridges. **Chromosome number**, undetermined.

TYPE: **U.S.A. TEXAS: COMAL CO.,** "Ca 5 airline mi N of New Braunfels; off River Rd., 3.3 mi N of jct Loop 337; at 1st crossing of Guadalupe River, then (on E side of R) 0.2 mi S along campground rd." On west-facing, limestone bluffs (ca 50 ft high) in a band comprising the middle 1/3rd of bluff in friable limestone. 9 May 1999, *Matt W. Turner 93* (Holotype: TEX; isotypes [7] to be distributed).

REPRESENTATIVE SPECIMENS: **U.S.A. TEXAS: COMAL CO.,** Comanche Springs, New Braunfels, Jun 1850, *Lindheimer 814* (TEX). **EDWARDS CO.,** 12 mi NW of Barksdale, "Blue Hole" on Cedar Creek, 25 Jul 1946, *Correll13450A* (LL). **HAYS CO.:** ca 3.8 mi SE of Jct Rte 150 and F.M.3237 at Hays City, 700-750 ft, 2 Apr 2008, *Carr 26642* (TEX). **LLANO CO.:** "ca 2 mi N of Tow on sheer schistosic bluff along Colorado River." 30 Jul 1970, *Crutchfield 3639* (TEX). **TRAVIS CO.:** "limestone cliffs above Cow Creek on Lake Travis," 22 May 1956, *Tharp s.n.* (TEX).

In the Edwards Plateau region of Texas (Fig. 2), which houses most of the populations of var. *edwardsiana,* the specimens bear uniformly small flowers. In Edwards Co. var. *edwardsiana* appears to intergrade with var. *bartonioides* (*Turner 99-351*, TEX), such phenomena extending into Val Verde Co. (*Reed 643*, TEX); because of this I have opted to treat the taxa as allopatric varieties.

It should be noted that I sent a collection (*Crutchfield 3639*) of var. e*dwardsiana* to Prof. Henry Thompson, guru of the genus at the time of my first interest in the complex, asking his opinion re the small-flowered collections from the Edwards Plateau. He responded (letter attached to the Crutchfield collection at TEX) that he and his co-worker, Wally Ernst were aware of the small-flowered populations of the Austin area, but opted not to name these. He went on to say, however,

> With hindsight and the new collection that you have sent I feel that the small flowered plants, at least the ones geographically isolated in the Austin Area (Comal, Travis and now Llano counties) should be recognized taxonomically. At the very least the Austin Area plants are a small flowered, probably highly autogamous race that have become established beyond the limits of large flowered *bartonioides*.

Numerous collections since the above communication have prompted the present contribution.

The novelty is named for the Edwards Plateau, and not for the county (Edwards) in which it appears to grade into var. *bartonioides.*

ACKNOWLEDGEMENTS

I am grateful to my long-time field companion, Jana Kos, for reviewing the paper and providing helpful comments. Distribution maps are based upon specimens on file at TEX, and records provided by Thompson and Ernst (1967).

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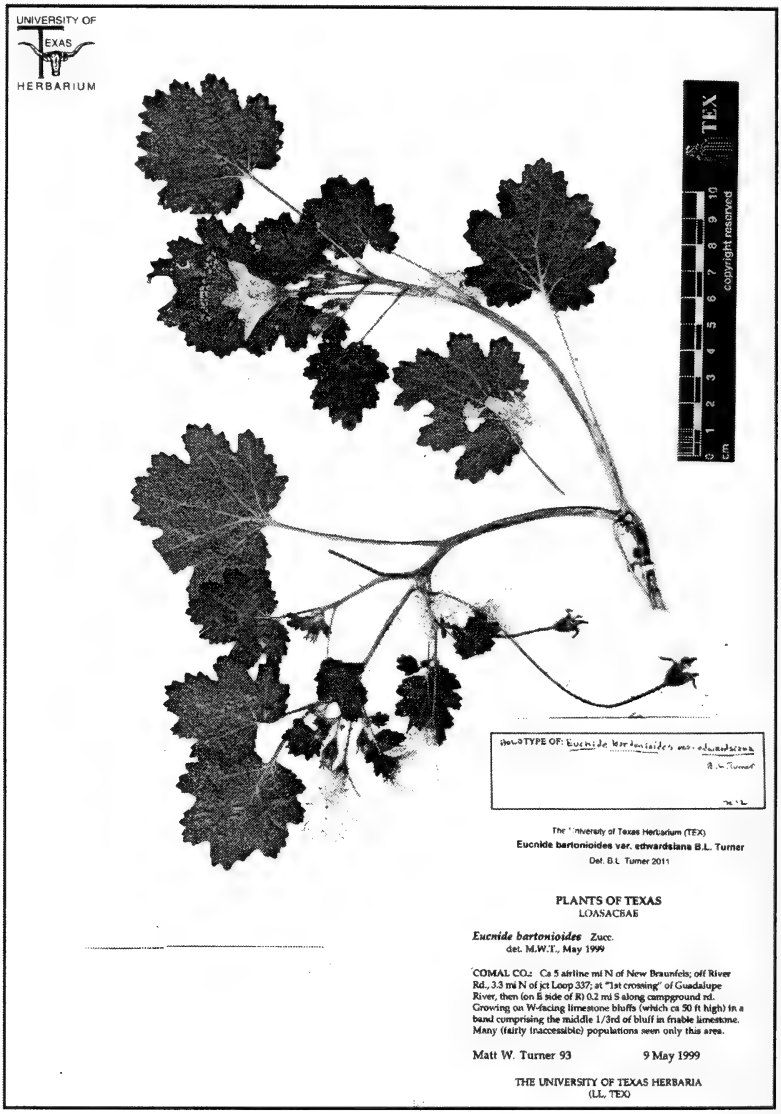

Fig. 1. *Eucnide bartonioides* var. *edwardsiana* (Holotype: TEX).

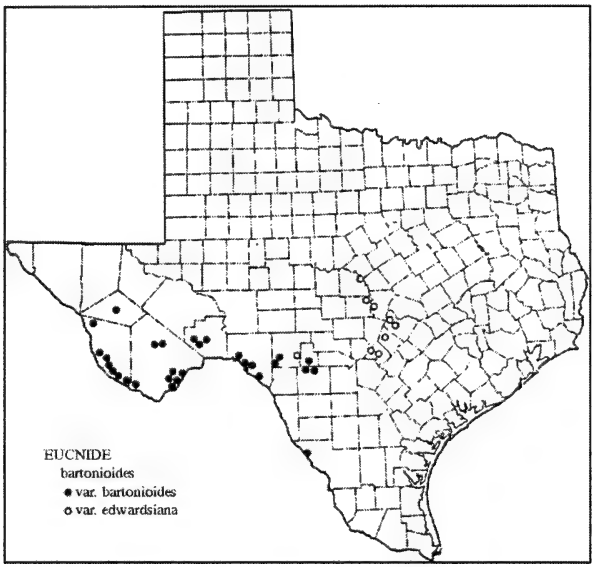

Fig. 2. Distribution of *Eucnide bartonioides* in Texas.

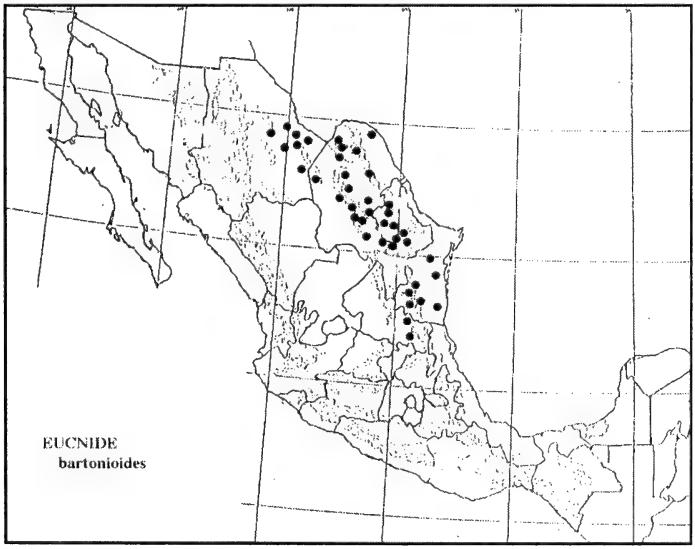

Fig. 3. Distribution of *Eucnide bartonioides* in Mexico.

GEOGRAPHIC VARIATION IN LEAF OILS OF *JUNIPERUS DELTOIDES* FROM BULGARIA, GREECE, ITALY AND TURKEY

Robert P. Adams
Baylor University, Biology Department, One Bear Place, #97388,
Waco, TX 76798, USA, email Robert_Adams@baylor.edu

and

Alexander N. Tashev
University of Forestry, Dept. of Dendrology
10, Kliment Ochridsky Blvd., 1756 Sofia, Bulgaria

ABSTRACT

The volatile leaf oil of *J. deltoides,* Bulgaria, was compared to *J. deltoides* oils from Greece, Italy and Turkey as well as the oil of *J. oxycedrus,* France. Leaf terpenoids showed a similar pattern somewhat intermediate between Greece and Turkey *J. deltoides*. The oil was unusual in having less α-pinene (12.6%) and more manoyl oxide (16%) than in any other *J. deltoides* sources examined. The plants had protuberances on the seed cones and leaves with deltate bases, typical of *J. deltoides*. *Phytologia 94(3): 310 - 318 (December 1, 2012).*

KEY WORDS: *Juniperus deltoides,* Bulgaria, Greece, Italy, Turkey, *J. oxycedrus,* terpenes, taxonomy.

Recent studies (Adams, 2004; Adams, et al., 2005) utilizing nrDNA sequencing, RAPDs, leaf terpenoids and morphology, clearly demonstrate that *J. oxycedrus* (*sensu stricto*) is restricted to the western Mediterranean; whereas, another, morphologically similar species, *J. deltoides* R. P. Adams occupies the eastern Mediterranean region. Adams (2011) recognized both *J. deltoides* and *J. oxycedrus* in his monograph of *Juniperus*. However, examination of junipers on the margins of the range of *J. deltoides* on the boundary between the taxa is useful to understand the distribution (Fig. 1).

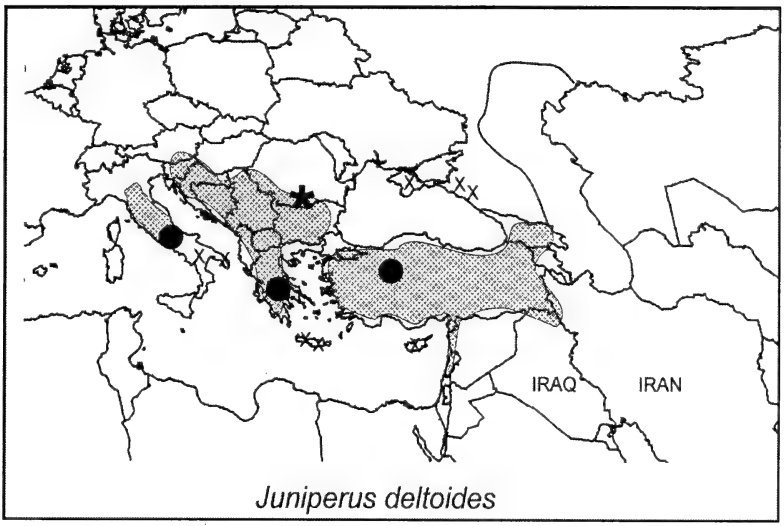

Figure 1. Distribution of *J. deltoides* with the Bulgaria population indicated by a star; Italy, Greece and Turkey populations are indicated by closed circles.

Recent collections of *J. deltoides* from Bulgaria (star, Fig. 1) afforded the opportunity to compare the leaf essential oils with other populations previously sampled (Adams et al. 2005; Adams, R. P. and T. Matraci, 2011; Adams, R. P., S. Terzioğlu and T. Mataraci, 2010; closed circles, Fig. 1). The purposes of this paper are to report on the leaf oil of *J. deltoides* from Bulgaria and compare it with other populations of *J. deltoides*.

MATERIALS AND METHODS

Plant material: *J. deltoides*: *Adams 9445-47,* 2 km w Raiano, 42° 05.768' N, 13° 47.757' E, 525 m, Italy, *Adams 9436-38*, 14 km e Arachova, 38° 26.720' N, 22° 41.678' E, 420 m, Greece, *Adams 9430-9432*, 30 km n Eskisehir, 39° 57.300' N, 30° 41.027' E, 1064 m, Turkey, *Adams and Tashev 13126-13130*, Devin region, 47° 44' 54" N, 24° 23' 02" E, 857 m, Bulgaria; *J. oxycedrus*, *Adams 9039-9041*, 4 km e

Forcalquier, 44° 04.06' N, 5° 59.19' E, 490 m, France. Voucher specimens are deposited at Baylor University (BAYLU).

Isolation of Oils - Air dried leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus with an ether trap (Adams, 1991). The oil samples were concentrated (ether reduced to 90% conc.) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1 sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

Specimens of *J. deltoides* from Bulgaria had the typical protuberances on the seed cones (Fig. 2, left) compared to smooth seed cones of *J. oxycedrus* (Fig. 3). In addition, their leaves were scarcely tapered to deltate at the point of attachment (Fig. 2, right) compared to the leaves of *J. oxycedrus,* that has leaves noticeably constricted (tapered) at the point of attachment (Fig. 3).

The leaf oil from Bulgaria contained all the characteristic components of *J. deltoides* (cis-p-mentha-2,8-dien-1-ol, carvone, (2E)-decenal, α-copaene, α-muurolene, α-copaen-11-ol, α- and β-calacorene, cis-calamenen-10-ol and cadalene, Table 1) and lacked components typical of *J. oxycedrus* (1-octen-3-ol, α-terpinyl acetate, 2-tridecanone, germacrene B, salvial-4(14)-en-1-one, hexadecane, sesquiterpene

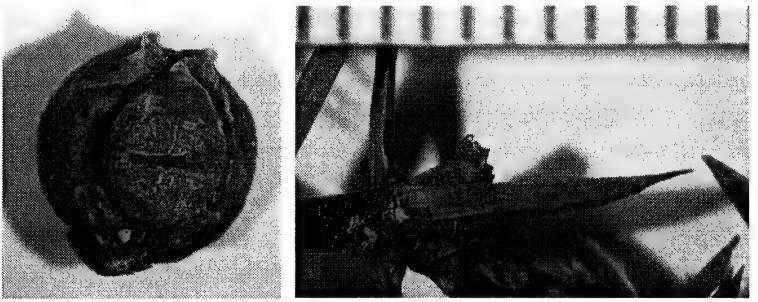

Figure 2. *J. deltoides*, Bulgaria. Left: seed cone showing protuberances. Right: leaf base showing the delta shape of the leaf at the point of attachment.

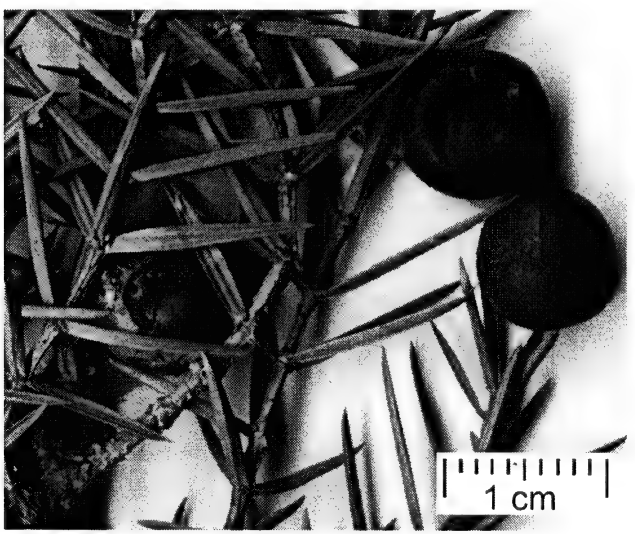

Figure 3. *J. oxycedrus*. Note the lack of protuberances on the seed cones and the leaves that are constricted (tapered) at the point of attachment (from Adams 2011).

alcohol 1619, C15-dienol acetate 1674, nootkatone, nonadecane, epi-13-manoyl oxide, sandaracopimarinal, 1-docosene, docosane, phytol acetate and tricosane, Table 1). However, the Bulgarian oil was unusual in having less α-pinene (12.6%) and more manoyl oxide (16%) than found in the other populations of *J. deltoides* examined (Table 1).

ACKNOWLEDGEMENTS

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Table 1. Comparisons of the per cent total oil for leaf oils components of *J. deltoides*, Bulgaria, Turkey, Greece, and Italy compared to *J. oxycedrus*, France. Components that tend to separate the taxa and populations are highlighted in boldface. KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
927	tricyclene	0.1	0.1	t	t	0.1
930	α-thujene	t	-	t	t	t
939	**α-pinene**	**32.7**	**12.6**	**22.7**	**15.3**	**53.2**
953	α-fenchene	0.3	-	0.1	0.5	0.1
954	camphene	0.6	0.3	0.2	0.2	0.6
960	thuja-2,4(10)-diene	0.4	-	-	-	t
975	sabinene	0.2	0.1	0.1	0.2	0.5
979	**1-octen-3-ol**	-	-	-	-	**0.1**
979	β-pinene	3.0	0.3	2.4	1.8	2.1
991	myrcene	3.8	0.3	2.3	1.7	2.8
1002	δ-2-carene	0.9	-	t	-	t
1003	α-phellandrene	1.8	0.2	0.3	0.5	t
1011	**δ-3-carene**	**3.7**	**t**	**3.3**	**10.0**	**5.1**
1017	α -terpinene	0.1	-	-	-	t
1025	p-cymene	2.3	0.5	0.3	0.6	0.3
1029	**limonene**	**6.0**	**6.0**	**23.7**	**22.8**	**3.5**
1030	β-phellandrene	11.5	5.5	4.8	2.5	0.8
1050	(E)-β-ocimene	-	-	0.2	0.2	t
1060	γ-terpinene	0.2	-	0.1	0.1	0.1
1089	terpinolene	2.0	0.5	1.1	1.6	0.7
1099	linalool	0.7	-	0.3	t	t
1101	n-nonanal	0.5	0.2	0.2	0.2	t
1122	cis-p-menth-2-en-1-ol	0.3	-	-	t	-
1123	trans-p-mentha-2,8-dien-1-ol	t	0.3	0.2	-	-
1126	α -campholenal	1.3	1.3	0.3	0.2	0.8
1137	trans-pinocarveol	1.3	1.3	0.3	-	0.4
1138	**cis-p-mentha-2,8-dien-1-ol**	**0.1**	**0.1**	**0.3**	**0.2**	-
1141	cis-verbenol	0.4	0.4	0.1	-	t
1145	trans-verbenol	1.8	3.2	0.5	0.2	0.6
1163	trans-pinocamphone	0.1	-	-	0.2	-
1165	pinocarvone	0.6	0.5	-	-	t

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
1170	p-mentha-1,5-dien-8-ol	1.1	0.4	0.2	0.3	0.5
1175	cis-pinocamphone	0.1	0.1	-	-	-
1177	terpinen-4-ol	0.6	0.1	0.4	0.3	0.3
1181	naphthalene	0.3	-	0.2	-	0.1
1183	p-cymen-8-ol	1.0	0.3	0.2	0.2	t
1187	trans-p-mentha-1(7),8-dien-2-ol	-	-	0.2	-	-
1189	α-terpineol	1.2	0.4	0.3	0.1	0.6
1196	**myrtenal**	**0.6**	**0.4**	**0.3**	**-**	**t**
1205	verbenone	0.7	0.7	0.3	0.2	0.3
1217	trans-carveol	0.5	1.1	1.1	0.7	0.1
1229	cis-carveol	t	0.5	0.4	0.3	-
1242	cumin aldehyde	0.1	-	-	-	-
1243	**carvone**	**0.3**	**0.8**	**0.8**	**0.7**	**-**
1253	piperitone	t	-	-	-	-
1257	linalyl acetate	t	-	-	-	0.3
1264	**(2E)-decenal**	**0.2**	**0.1**	**0.2**	**0.2**	**-**
1289	bornyl acetate	0.9	0.6	0.4	0.3	0.7
1298	trans-pinocarvyl acetate	0.1	0.1	-	-	-
1298	carvacrol	t	0.1	-	-	-
1299	(2E,4Z)-decadienal	0.4	-	t	t	-
1317	(2E,4E)-decadienal	0.8	-	t	t	0.1
1342	trans-carvyl acetate	t	t	-	t	-
1349	**α-terpinyl acetate**	**-**	**-**	**-**	**-**	**0.2**
1377	**α-copaene**	**0.2**	**0.3**	**0.3**	**0.3**	**-**
1381	geranyl acetate	t	-	t	-	-
1388	β-bourbenene	0.2	0.1	0.2	0.1	0.3
1408	longifolene	0.6	-	-	-	-
1419	(E)-caryophyllene	1.2	0.8	0.2	1.1	0.4
1431	cis-thujopsene	0.1	-	t	-	-
1455	α-humulene	0.8	0.5	0.2	0.8	0.3
1480	γ-muurolene	t	-	-	t	0.1
1485	germacrene D	0.5	0.2	0.7	1.4	2.3
1486	ar-curcumene	-	1.0	0.7	0.3	-
1496	**2-tridecanone**	**-**	**-**	**-**	**-**	**0.3**
1500	**α-muurolene**	**0.4**	**1.0**	**02**	t	-
1514	γ-cadinene	0.4	1.2	0.9	0.8	0.7
1523	δ-cadinene	0.4	0.7	1.1	1.1	0.4
1541	**α-copaen-11-ol**	**0.1**	**0.5**	**1.1**	**0.6**	**-**

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
1546	**α-calacorene**	**0.5**	**0.6**	**0.7**	**0.6**	-
1561	**germacrene B**	-	-	-	-	**0.1**
1566	**β-calacorene**	**0.3**	**0.5**	t	**0.4**	-
1563	(E)-nerolidol	-	-	1.5	-	-
1567	dodecanoic acid	-	0.8	1.4	-	0.4
1583	caryophyllene oxide	3.2	6.0	0.6	5.9	0.4
1595	**salvial-4(14)-en-1-one**	-	-	-	-	**0.4**
1600	**hexadecane**	-	-	-	-	**0.3**
1601	cedrol	0.1	t	0.3	t	t
1608	humulene epoxide II	1.1	2.8	0.3	2.8	0.3
1619	**sesquiterpene alcohol, M226**	-	-	-	-	**0.3**
1627	1-epi-cubenol	0.1	-	-	-	-
1630	muurola-4,10(14)-dien-1-β-ol	-	-	0.3	-	-
1640	epi-α-cadinol	t	0.2	0.3	0.3	0.3
1641	epi-α-muurolol	t	0.2	0.3	0.3	0.3
1651	β-eudesmol	-	-	-	-	t
1654	α-cadinol	-	-	0.3	0.4	1.6
1661	**cis-calamenen-10-ol**	**t**	**0.4**	**0.2**	**0.5**	-
1670	caryophylene<14-OH-(Z)->	-	0.6	-	-	-
1674	**C15-dienol acetate, M+224**	-	-	-	-	**1.6**
1677	**cadalene**	**0.1**	**0.5**	**0.5**	**0.4**	-
1686	germacra-4(15),5,10(14)-trien-1-al	-	-	0.3	-	1.6
1700	heptadecane	-	-	t	-	0.3
1702	10-nor-calamenen-10-ene	-	0.1	0.1	t	-
1717	(2E, 6E)-farnesol	-	1.0	t	-	0.3
1722	(2Z, 6E)-farnesol	-	-	1.0	-	0.4
1740	(2E,6Z)-farnesal	-	-	0.3	-	-
1758	tetradecanoic acid	-	-	0.5	0.3	-
1800	octadecane	-	t	0.1	-	t
1807	**nootkatone**	-	-	-	-	**0.1**
1900	**nonadecane**	-	-	-	-	**0.1**
1959	hexadecanoic acid	-	1.1	-	-	-
1966	sandaracopimara-8(14),15-diene	-	-	0.7	-	0.1
1998	**manoyl oxide**	**1.3**	**16.0**	**6.8**	**5.8**	**6.2**
2014	palustradiene (=abieta-8,13-diene)	-	1.1	-	-	-
2017	**epi-13-manoyl oxide**	-	-	-	-	**0.1**

KI	Compound	delt Turk	delt Bulg	delt Grec	delt Italy	oxy Fra
2022	cis-abieta-8,12-diene	-	0.3	-	-	0.1
2026	geranyl linalool	-	-	-	-	-
2057	abietatriene	0.1	5.5	2.0	2.3	1.2
2088	abietadiene	-	7.6	1.4	1.9	1.3
2154	abieta-8(14),13(15)-diene	-	-	0.7	-	0.2
2185	**sandaracopimarinal**	-	-	-	-	**0.2**
2190	**1-docosene**	-	-	-	-	**0.1**
2200	**docosane**	-	-	-	-	**0.1**
2218	**phytol acetate**	-	-	-	-	**0.1**
2220	dehydro-totarol*	-	2.3	-	-	-
2300	**tricosane**	-	-	-	-	**0.2**
2313	trans-totarol	-	0.6	-	-	-
2313	abietal	-	0.5	0.5	0.4	-
2331	trans-ferruginol	-	0.4	-	-	-

RECENSION OF THE MEXICAN SPECIES OF *ZALUZANIA* (ASTERACEAE: HELIANTHEAE)

Billie L. Turner
Plant Resources Center
The Univ. of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

Preparation of a treatment of the tribe Heliantheae for the Comps of Mexico has prompted the following paper, this occasioned by the description of a single novelty in the genus, **Zaluzania durangensis** B.L. Turner, **sp. nov.** Additionally, I have chosen not to recognize *Z. augusta* var. *rzedowskii,* treating this as synonymous with the typical var. *augusta.* So treated, Mexico contains 11 species of *Zaluzania*, most of these occurring in montane habitats of north-central Mexico. A figure of the novelty is provided, along with a key to species and maps showing distributions, all of this in the format of my Comps of Mexico (cf. Turner 2009, 2010). *Phytologia 94(3): 319-333 (December 1, 2012).*

KEY WORDS: Asteraceae, Heliantheae, *Zaluzania,* Mexico

ZALUZANIA Pers.

Suffruticose, perennial herbs, shrublets or shrubs to 4 m high. Stems simple and arising from fleshy tuberous roots, or much-branched from woody tap-roots. Leaves mostly alternate, simple or pinnately parted. Heads discoid or radiate, few to numerous in terminal corymbose panicles. Involucres campanulate, 2-3 seriate, the bracts subequal. Receptacle conical, paleate, the pales persistent and clasping the achenes. Ray florets, when present, mostly pistillate, rarely neuter, fertile, the ligules white or yellow. Disk florets numerous, the corollas white or yellow, usually "capping" the achenes, often conspicuously so. Achenes clavate or narrowly obovate, black, glabrous or somewhat

pubescent, mostly epappose, or those of the ray with 2-6 short inconspicuous scales. Base chromosome number, x = 18.

Type species, *Zaluzania triloba* (Ort.) Pers.

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A genus of 11 species, with the exclusion of the South American elements referred to *Kingianthus* by Robinson (1978a,b). The species are mostly shrubs or suffruticose herbs largely confined to rather dry habitats. Sharp (1935) and McVaugh (1984) retained the monotypic genus *Hybridella* within *Zaluzania*, but I follow Olsen (1979) and Panero (2007) in its treatment as a good genus. Olsen also transferred *Z. grayana* (previously accepted by Sharp) into *Viguiera*. Robinson (1981) treated *Zaluzania, Hybridella* and *Chromolepis* as the only members of his subtribe Zaluzaniinae; Panero (2007), however, included only *Hybridella* and *Zaluzania* in the latter subtribe.

KEY TO SPECIES

1. Leaves simple, entire to dentate or with shallow lobes.....(**3**)
1. Leaves deeply dissected, either tripartite or pinnately parted.....(**2**)

2. Stems and foliage uniformly pubescent with appressed soft short hairs .....**Z. parthenioides**
2. Stems and foliage pubescent with an uneven mixture of short glandular trichomes and coarse hispid hairs, the latter 1-2 mm long .....**Z. triloba**

3(**1**). Heads discoid; w Chi, n Dur .....**Z. discoidea**
3. Heads radiate; Coa to Tam, southwards .....(**4**)

4. Disk florets white; ray florets white, or absent.....**Z. pringlei**
4. Disk and ray florets yellow.....(**5**)

5. Leaves not bicolored, about equally green or greenish on both surfaces, coarsely hispid or hispidulous beneath, not at all white-tomentulose.....(**8**)
5. Leaves to some extent bicolored, moderately to densely white-tomentulose beneath, greener above.....(**6**)

6. Ray florets with ligules mostly 4-10 mm long; involucres mostly 5-7 mm wide.....(**10**)
6. Ray florets with ligules mostly 10-15 mm long; involucres (pressed) mostly 9-15 mm wide.....(**7**)

7. Leaves lanceolate, mostly 4-6 times as long as wide....**Z. mollissima**
7. Leaves broadly ovate, mostly 2-3 times as long as wide..... .....**Z. megacephala**

8(**5**). Leaves thick, the blades abruptly deltoid or cordate, scarcely, if at all, tapering upon the petioles; e Pue and adjacent Ver ....**Z. subcordata**
8. Leaves relatively thin, the blades ovate, clearly tapering upon the petioles; widespread.....(**9**)

9. Leaves white-tomentose beneath; petioles not clearly winged;

wide spread.....**Z. augusta**
9. Leaves otherwise; petioles winged for 1-2 cm; Chi, Dur.....
.....**Z. durangensis**

10(**6**). Petioles unwinged, not clasping the stem; Dur, e Jal.....
.....**Z. delgadoana**
10. Petioles winged, clasping at the base; Mor, Pue, Gue, Ver and Oax .....**Z. montagnifolia**

ZALUZANIA AUGUSTA (Lag.) Sch.-Bip., Flora 44: 562. 1861.
Map 1
Zaluzania augusta var. *rzedowskii* McVaugh

Dur, Zac, Agu, San, e Jal, Gua, Que, Hid, Mic, Mex, Mor and Ver, Central Plateau, dry hillsides, 1500-2500 m; Sep-Nov.

Shrubs or shrublets 1-3 m high; stems much-branched, puberulent, glabrate with age; leaves 1.5-7.0 cm long, 0.5-4.0 cm wide; petioles 2-10 mm long; blades ovate to ovate-lanceolate, the surface greenish above, white-tomentose below, the margins entire to crenulate, serrate, or rarely lobed; heads radiate, numerous in cymose panicles, the ultimate peduncles mostly 5-15 mm long; involucres 3-4 mm long, the bracts subequal; ray florets 8-11, the ligules yellow, 5-7 mm long; disk florets 50-70, the corollas yellow; achenes 1.5-2.0 mm long, those of the ray with usually 4 scales (rarely absent); chromosome number, n = 18 pairs.

This is a widespread variable species under which McVaugh (1984) and Olsen (1979) recognized two varieties, the typical var. *augusta* and var. *rzedowskii*. The characters used in there recognition (largely pappus scales and leaf color) are extremely variable and seem not to circumscribe meaningful biological entities, there being much intergradation of the traits concerned.

Olsen (1979) keys and describes *Z. augusta* as having entire leaf margins, but numerous collections from throughout the range have serrate or sub-serrate margins.

ZALUZANIA DELGADOANA B.L. Turner, Phytologia 58: 228. 1985. **Map 2**

Known only from s Dur and n Jal; arid shrublands, 1800-2100 m; Sep-Nov.

Shrubs 1-3 m high; stems much-branched, densely hirsutulous; leaves 3-4 cm long, 1.5-3.0 cm wide; petioles 6-10 mm long; blades deltoid-triangular to nearly cordate, abruptly truncate, not tapering upon the petioles, densely short-hirsutulous on both surfaces, the lower surfaces markedly venose and atomiferous-glandular, the margins crenulo-dentate; heads radiate, in rounded terminal cymules; involucres 3.5-4.5 mm high, 2-3 seriate, the bracts subequal; ray florets 5-8, pistillate, fertile, the ligules yellow, 4-5 mm long; disk florets numerous, the corollas yellow, capping the achenes; achenes of ray and disk florets similar, black, glabrous, epappose.

This is a very distinct species, perhaps closest to *Z. montagnifolia*. The single Jal collection differs in having somewhat larger heads and more densely pubescent leaves.

Olsen (1979), in his treatment of *Z. megacephala* var. *coahuilensis,* notes, "One collection [NY, US], *Pennell 18521*, from Durango, appears to be far out of range for this taxon." At the time of this statement, Olsen was unaware of my up-coming description of *Z. delgadoana* Turner (1985), which I take the specimens concerned to be, largely based upon description and location (Map 2).

ZALUZANIA DISCOIDEA A. Gray, Proc. Amer. Acad. Arts 21: 388. 1886. **Map 2**

w Chi and n Dur in pine-oak woodlands, 1900-2200 m; Oct-Sep.

Perennial herbs to 2 m high; stems thick, simple, striate, densely soft-puberulent; leaves 8-15 cm long, 4-10 cm wide; petioles 1-2 cm long; blades broadly ovate to deltoid or subcordate, bicolored, the lower surface densely white-tomentose, the margins dentate to shallowly lobulate; heads discoid, numerous in terminal corymbose

panicles, the ultimate peduncles mostly 5-15 mm long; involucres 5-6 mm high, the bracts white-puberulent, subequal; disk florets numerous, the corollas yellow, capping the achenes; body of achenes ca 2 mm long, glabrous; chromosome number, n = 18 pairs.

This species is readily recognized by its discoid heads and large, bicolored, leaves.

ZALUZANIA DURANGENSIS B.L. Turner, **sp. nov. Fig. 1, Map 2**

Zaluzaniae discoideae A. Gray similis sed differt foliis ovatis (vs cordatis) non bicoloribus (vs valde bicoloribus) et capitulis radiatus (vs discoideis).

Perennial herbs, 0.8-1.7 m high. **Stems** mostly unbranched, stiffly erect, purplish, ca 5 mm thick. **Leaves** ovate, alternate throughout, 6-10 cm long, 3-5 cm wide; petioles 1-10 mm long, tapered upon by the blades for 1-2 cm; blades ovate, sparsely pubescent to glabrate above and below, the lower surfaces glandular-punctate with black dots, the margins weakly serrulate. **Capitulescence,** a terminal corymbose panicle of ca 15 heads, the ultimate peduncles 1-3 cm long, puberulent with appressed hairs. **Heads** 1.5-2.0 cm across the extended rays. **Involucral bracts** 2-3 seriate, 4-5 mm long, pubescent like the peduncles. **Ray florets** ca 8, neuter; ligules yellow, ca 8 mm long, 3-4 mm wide, ca 8-nervate; tube ca 1 mm long. **Receptacle** conical, ca 2.5 mm across, the chaff lanceolate. **Disk florets** numerous; corollas 2-3 mm long, yellow, pubescent. **Anthers** markedly black. **Achenes** (immature) epappose, pubescent.

TYPE: **MEXICO. DURANGO**: "Santiago Papasquiaro. 159 km al W de Santiago Papasquiaro sobre la carretera a Canelas." Bosque de pino-encino, 2600 m, 28 Aug 1991, *Jose L. Panero 2248C* [with S. Gonzalez & S. Acevedo]. (Holotype: TEX).

ADDITIONAL SPECIMEN EXAMINED [immature]: **MEXICO. CHIHUAHUA.** "Llano Grande; 5-6,000 ft." 23 Jul 1965, *Pennington 104* (TEX).

The most striking character of this species is the foliage, possessing nearly glabrous, dark green leaves, the blades tapering upon the petioles, often completely so; the holotype was initially identified by its collector as *Z. megacephala,* which it superficially resembles.

The above cited Pennington collection from "Llano Grande," located in southern Chihuahua (Pennington 1963), gives the Indian name and use as, "oreja del venado; a pasturage plant."

ZALUZANIA MEGACEPHALA Sch.-Bip., Flora 44: 563. 1861.
Map 3

Zaluzania cinerascens Sch.-Bip.
Zaluzania coulteri Hemsl.
Zaluzania megacephala var. *coahuilensis* Olsen

Coa, Nue, Tam, Que, Hid, e Jal and Mex, pine-oak forests, 1900-2900 m; Jul-Oct.

Perennial erect herbs to 1.5 m high, the stems simple and arising from short rhizomes which bear 1 or more fleshy tubers; leaves broadly ovate to lanceolate, mostly 3.5-8.0 cm long, 1.5-5.0 cm wide, the blades abruptly or gradually tapering upon the short petioles, moderately to densely and softly pubescent, especially below, sometimes forming a soft white tomentum beneath, the margins serrulate; heads in terminal subfasciculate, corymbose, rounded panicles, the ultimate peduncles mostly 0.5-2.0 cm long; involucres 2-3 seriate, the bracts 4-8 mm long, somewhat graduate to subequal; ray florets 8-11, the ligules yellow, 5-14 mm long; disk florets numerous, the corollas yellow, capping the achenes; achenes black, glabrous or nearly so, 1.0-2.6 mm long, epappose; chromosome number, n = 18 pairs.

This is a widespread variable species. Olsen (1979) recognized two varieties, largely based upon leaf shape and vestiture, but these appear to be but individual or populational forms, their being much variation in the characters concerned. Populations of *Z. megacephala* from nw Dur, reported as var. *coahuilensis* by Olsen (1979), appear to be *Z. durangensis*

ZALUZANIA MOLLISSIMA A. Gray, Proc. Amer. Acad. Arts 15: 35. 1880. **Map 3**

Coa, Zac, and San, calcareous soils of Chihuahuan Desert, 1500-2400 m; Jul-Sep.

Much-branched, rounded shrublets or shrubs, 1-3 m high; very similar to **Z. augusta** but the heads larger with longer rays, as noted in the key to species; the primary leaves are somewhat thicker and larger with usually two rounded lobes at the base; chromosome number, n = 27 pairs.

As noted above, this taxon is close to *Z. augusta* but differs in leaf shape (primary leaves), head size and chromosome number (hexaploid in *Z. mollissima,* tetraploid in *Z. augusta* - assuming a base number of x = 9). Olsen (1979) notes that *Z. mollissima* occurs within a few miles of *Z. augusta,* but this is not shown on his map (Fig. 2) showing their distributions.

ZALUZANIA MONTAGNIFOLIA (Sch.-Bip.) Sch.-Bip., Flora 44: 563. 1861. **Map 4**

Zaluzania asperrima Sch.-Bip.

Pue, Ver, Gue and Oax, mostly subtropical deciduous forests,1500-2200 m; Sep-Dec.

Shrubs to 4 m high; stems much-branched, striate, hirsutulous, with age glabrate; leaves alternate, 5-9 cm long, 1.5-4.8 cm wide; petioles 1-2 cm long, strongly winged, dilated at the base; blades ovate to deltoid, pubescent above and below, the lower surface densely glandular-punctate with amber glands, the margins variously dentate; heads radiate, numerous in stiffly divaricate corymbose panicles; involucres 2-3 seriate, 3-5 mm high, the bracts subequal; ray florets 5-11, the ligules yellow, mostly 5-8 mm long; disk florets 50-80, the corollas yellow; achenes 1.5-2.0 mm long, glabrous, epappose; chromosome number, n = 18 pairs.

See additional comments under *Z. pringlei.*

ZALUZANIA PARTHENIOIDES (DC.) Rzedowski, Brittonia 20: 167. 1968. **Map 4**

Aiolotheca parthenioides DC.
Zaluzania robinsonii W.M. Sharp

Coa, Nue, Tam, Agu, Zac, San, Gua, Que and Hid, mostly Central Plateau of the Chihuahuan Desert regions, 1500-2100 m; Jul-Dec.

Perennial, ashen-colored, suffruticose herbs to 1 m high; much resembling *Z. triloba* but amply distinct, both in its mostly trilobate, less dissected, leaves and smaller heads and receptacles, but it is most readily distinguished from the latter by its uniformly soft, white-puberulent, vestiture, not at all like the coarse, glandular-hispid, vestiture of *Z. triloba*; chromosome number, n = 18 pairs.

Olsen (1979) treated this taxon as synonymous with *Z. triloba*. While noting the very different vestitures that seem to mark the two taxa, he concluded that the "characters used to separate these taxa intergrade completely." He further contended that plants "growing in the sun usually have the characters attributable to *Z. triloba*, while plants found growing in the shade have the characters attributable to *Z. parthenioides*." I do not find intergrades between these at all and conclude that he was dealing with very well marked sympatric species that do not appear to intercross, even when growing together. Similar conclusions have also been reached by Rzedowski, and yet others.

ZALUZANIA PRINGLEI Greenm., Proc. Amer. Acad. Arts 39: 101. 1903. **Map 5**

Mor, w Pue and Gue, subtropical deciduous forests, 1000-1500 m; Oct-Dec.

Shrubs 2-3 m high, the lower stems up to 5 cm in diameter; leaves mostly 4-8 cm long, 2-4 cm wide; petioles 5-15 mm long, often winged; blades ovate to deltoid, thick, 3-nervate to seemingly pinnately nervate, coarsely pubescent above with broad-based hairs, similarly pubescent beneath or merely white-puberulent without broad-based,

coarse hairs, the margins crenulodentate; heads discoid or radiate, 10-60 in terminal corymbose panicles, the ultimate peduncles mostly 5-15 mm long; involucres 4-5 mm high, the bracts sparsely puberulent, subequal; ray florets, when present, 1-5, the ligules white, 2-3 mm long; disk florets numerous, the corollas white, capping the achenes, sparsely to densely puberulent; achenes glabrous, mostly 1.5-2.2 mm long, epappose.

Zaluzania pringlei is closely related to *Z. montagnifolia*, but readily recognized by its white disc and ray florets; populations of both taxa occur near Chilpancingo, Gue, but appear not to hybridize or intergrade.

ZALUZANIA SUBCORDATA W. Sharp, Ann. Missouri Bot. Gard. 22: 111. 1935. **Map 5**

Pue and closely adjacent Ver and Oax, in dry oak-juniper woodlands, 1900-2200 m; Sep-Nov.

Shrubs 1-3 m high; stems white-tomentulose at first, but reddish-glabrate with age; leaves bicolored, thick, the blades deltoid, densely white-tomentose beneath, abruptly tapering upon the petiole, the margins dentate to nearly entire; much resembling *Z. augusta* but distinguished by its broader, deltoid to cordate blades and broad rounded capitulescence which extends somewhat above the leaves, the ultimate peduncles mostly 10-25 mm long; chromosome number, n = 36 pairs.

This is a rather localized, well-marked, taxon reportedly infrequent on hillsides about Esperanza, Pue.

ZALUZANIA TRILOBA (Ort.) Pers., Syn. Plant. 2: 473. 1807. **Map 6**

Acmella trilobata Spreng.
Anthemis sinuata La Llave & Lex.
Anthemis trilobata Ort.

Nue, Zac, Agu, San, Gua, Que, Hid, Mex, Tla, Pue, Ver and Oax, in dry calcareous or gravelly soils, 1800-2400 m; Aug-Nov.

Erect, suffruticose, perennial herbs to 1 m high; stems striate, brittle, coarsely pubescent with spreading multicellular whip-like trichomes 1-3 mm long, interspersed among numerous, short, glandular-trichomes; mid-stem leaves 6-12 cm long, 3-5 cm wide; petioles 1-4 cm long; blades tripartite or pinnately parted, pubescent on both surfaces like the stem; heads radiate, 3-50 in corymbose panicles, the ultimate peduncles mostly 1-8 cm long; involucres 2-3 seriate, the bracts 2-4 mm long, subequal; receptacles conical, ca 3.5 mm high, ca 2 mm wide, paleate, the pales persistent, 3-lobed at the apex; ray florets 8, the ligules yellow, 5-10 mm long; disk florets numerous the corollas yellow, 2.5-3.0 mm long, "capping" the apex of the achene; achenes black, glabrous, ca 2 mm long, epappose; chromosome number, n = 18 pairs.

Olsen (1979) combined *Z. parthenioides* with this species, but I think incorrectly, a conclusion shared with Rzedowski (1968). The two species are quite different in vestiture and yet other characters, as noted under *Z. parthenioides.*

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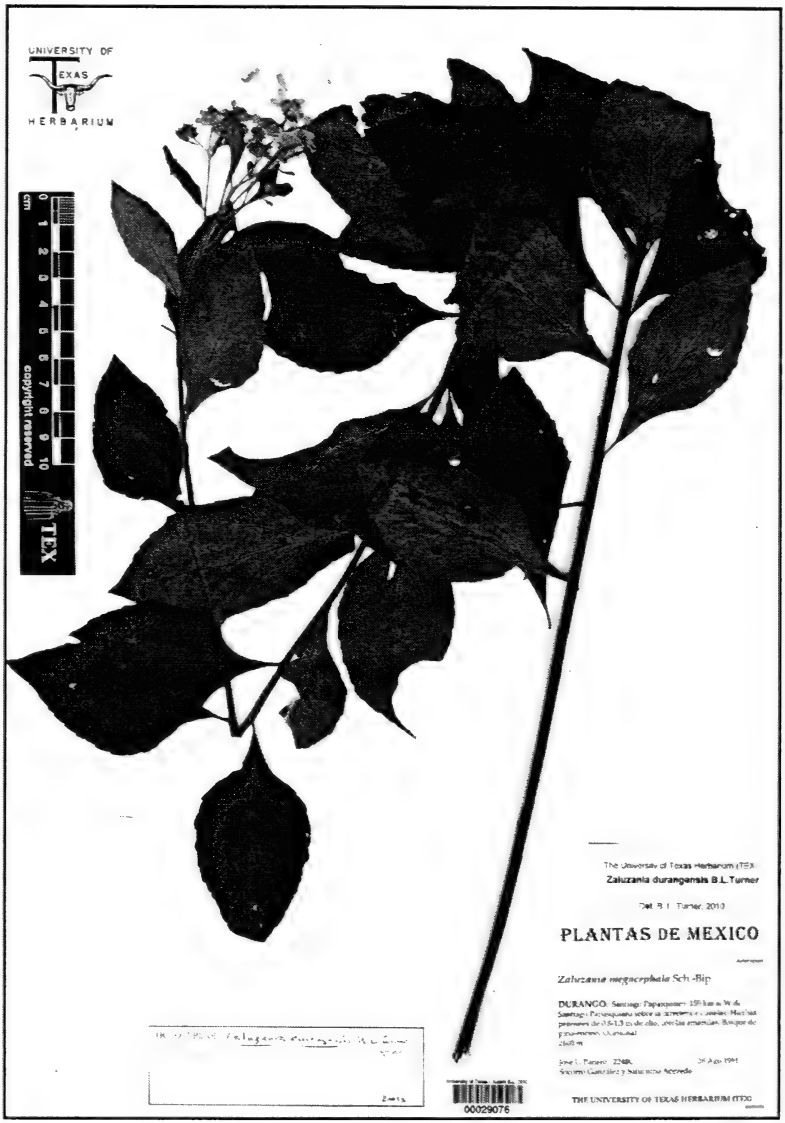
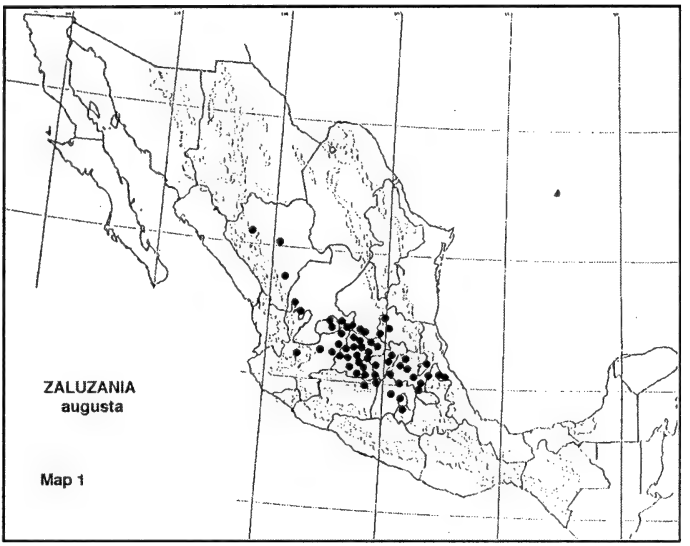
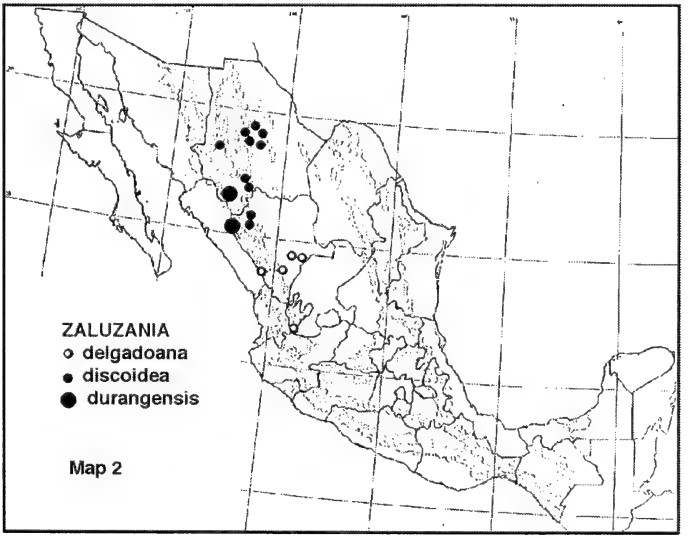


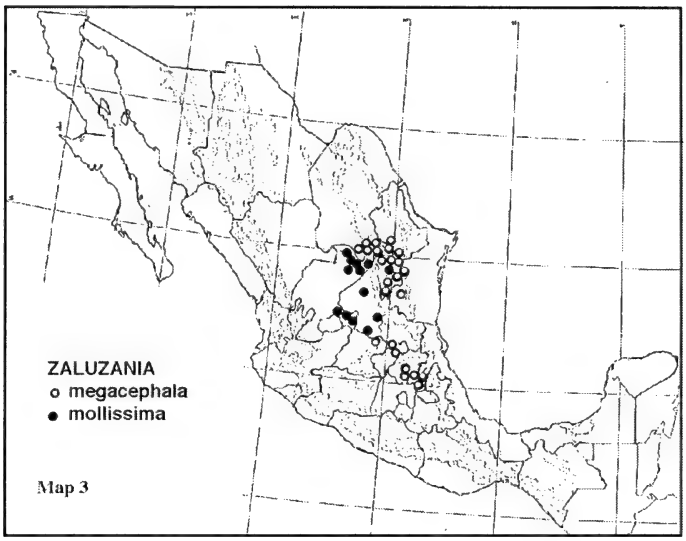
Fig. 1. *Zaluzania durangensis*, holotype.



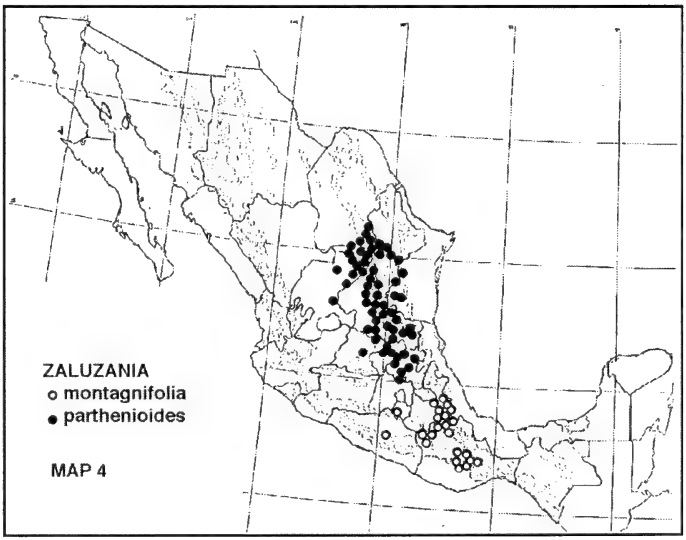
Map 1. *Z. augusta*



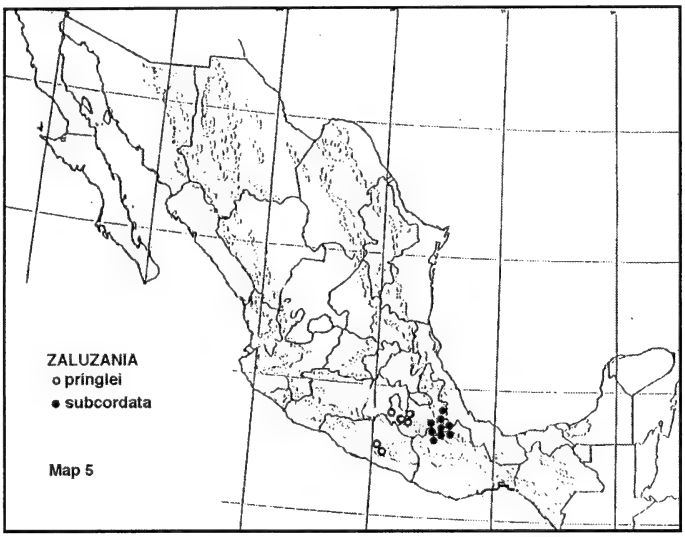
Map 2. *Z. delgadoana*, et. al.



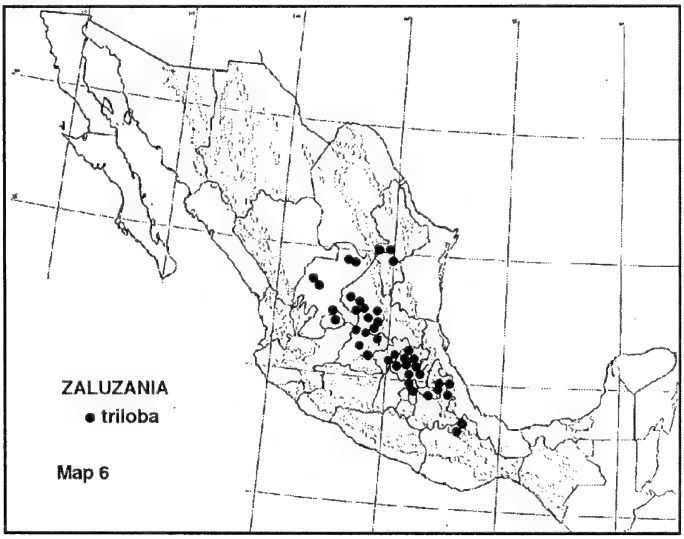
Map 3. *Z. megacephala* and *Z. mollissima*



Map 4. *Z. montagnifolia* and *Z. parthenoides*



Map 5. *Z. pringlei* and *Z. subcordata*



Map 6. *Z. triloba*

TERPENOID FINGERPRINTING TO DETERMINE AN ESCAPED *JUNIPERUS RIGIDA* VAR. *CONFERTA* IDENTITY

Robert P. Adams
Biology Department, Baylor University, Box 97388
Waco, TX, 76798, USA
Robert_Adams@baylor.edu

Richard E. Riefner, Jr.
Research Associate, Rancho Santa Ana Botanic Garden
1500 North College Avenue
Claremont, CA 91711-3157, USA.

ABSTRACT

An adventitious juniper was discovered on a sea cliff at Newport Beach, CA. Morphologically, the juniper appeared to be *J. rigida* var. *conferta*. In an attempt to determine if it arose from a locally planted cultivar, the volatile leaf oils of *J. rigida* var. *conferta*, cv. Blue Pacific, and cv. Emerald Sea were compared with the oil of the escaped juniper, the later was very similar to the oil of cv. Blue Pacific (commonly cultivated in s California) and appears to have arisen from cv. Blue Pacific grown for ground cover in residential communities on the bluffs above the cliff. In addition, the composition of the leaf oil of *J. rigida*, Japan is presented for comparison. *Phytologia 94(3) 334 - 342 (December 1, 2012).*

KEY WORDS: *Juniperus rigida var. conferta, J. rigida,* Cupressaceae, leaf essential oils, terpenes, escaped plants.

Recently, a prostrate juniper was discovered (RR) growing on a cliff below houses at Newport Beach, CA (Fig. 1). Morphologically, the juniper appeared to be *J. rigida* var. *conferta* (Parl.) Patschka or the 'shore juniper' from Japan. Shore juniper is widely cultivated in the area as a ground cover. A check with local

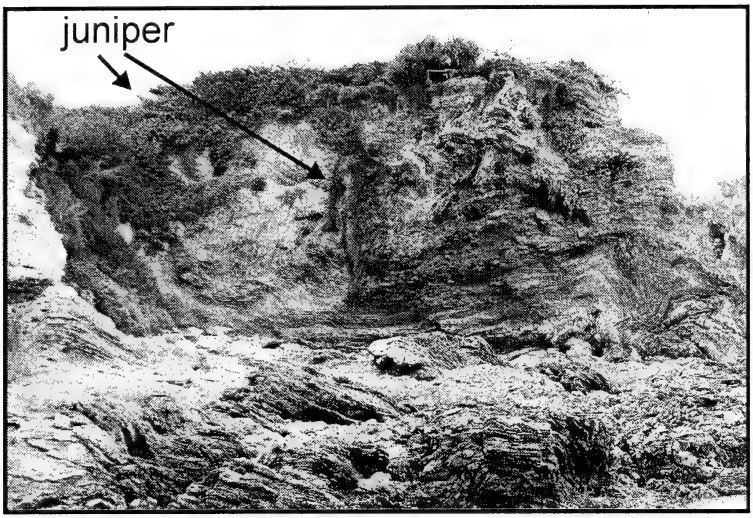


Figure 1. Juniper on seaside cliff in Newport Beach, CA.

nurseries revealed that cv. Blue Pacific is commonly sold in the area. Blue Pacific is a cultivar released by Monrovia Nursery. However, another cultivar (Emerald Sea) was introduced to the US from Japan in 1967 from Honshu by J. L. Creech (Meyer, 1979). Comparing the putative escaped cultivar with specimens of *J. r.* var. *conferta* specimens from Japan, cv. Blue Pacific and cv. Emerald Sea (ex Berkeley Bot. Garden) indicated that the plant from Newport Beach was most like cv. Blue Pacific obtained from ABC Nursery, Gardena, CA. However, as the plant from ABC Nursery was young and in a pot, some morphological plasticity would be expected, so unequivocal identification to cultivar level was not possible.

Junipers produce terpenoids in their leaves and wood that are often very specific, even to the level of cultivars (Fretz, 1976). An analysis of leaf oil of *J. rigida* var. *conferta* has been published (Adams, 1998, 2000), but that analysis was based on a cultivated plant from Kew Gardens. Doi and Shibuya (1972a, 1972b) reported on the sesquiterpenes and diterpenes from wood of *J. conferta* (*J. r.* var. *conferta*), but not on the leaf oil composition. In this paper we present analyses of the leaf oils of *J. r.* var. *conferta* from the Tottori

sand dunes in Japan, cv. Blue Pacific and cv. Emerald Sea, and compare these oils with those of the escaped juniper at Newport Beach, CA. In addition, we present analysis of the leaf oil of *J. rigida*, Honshu Prefecture, Japan. The leaf oil of *J. rigida* has been reported by Adams, Chu and Zhang (1995), but their sample from Japan was from cultivated material at the Arnold Arboretum. They reported considerable variation, especially in α-pinene and bornyl acetate. The composition of the leaf oil of *J. rigida* was previously reported by Yatagai, Sato and Takahashi (1985) and Yatagai and Takahashi (1988).

MATERIALS AND METHODS

Plant material - *Juniperus rigida,* Honshu Prefecture, Japan, *Adams 8544-8546* (ex Jin Murata), *J. r. conferta*: Tottori Sand Dunes, Tottori Prefecture, Japan, *Adams 8585-8589* (ex Jin Murata), cv. Blue Pacific, ABC Nursery, Gardena, CA, *Adams 13199*, cv, Emerald Sea, ex Berkeley Botanical Garden, *Adams 13242*, unknown cv., escaped cultivation, Newport Beach between Shorecliff Road and Cameo Shores Road, 33° 35' 6.465" N, 117° 51' 50.729" W, CA, *Adams 13198*, *Riefner 11-81*. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Fresh leaves (200 g.) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl 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.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 1991 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron

coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The oil from a natural population of *J. r.* var. *conferta* on the Tottori sand dunes, Japan was found to be very high in α-pinene (40.6%), with moderate amounts of β-pinene, myrcene, δ-3-carene, bornyl acetate and isoabienol (Table 1). This is in contrast to Yatagai, Sato and Takahashi (1985) who reported 13.5% α-pinene and 59.0% bornyl acetate and Adams, Chu and Zhang (1995), who found 15.4% α-pinene and 40.5% bornyl acetate in their plant from Japan. The present analysis from the Tottori sand dunes, Japan is very much like the analyses (Adams, Chu and Zhang, 1995) of *J. r.* var. *conferta* from ne China and Korea in having high amounts of α-pinene and low amounts of bornyl acetate. It appears that there may be chemical races of *J. rigida* var. *conferta* in Japan.

The escaped juniper from Newport Beach, CA has oil that is similar to that of cv. Blue Pacific (Table 1). Note the similarity in concentrations for α-pinene, camphene, sabinene, β-pinene, myrcene, limonene, β-phellandrene, terpinolene, trans-verbenol, endo-fenchyl acetate, (E)-caryophyllene, α-humulene, caryophyllene oxide, hexadecanoic acid, and isoabienol (Table 1). The oil of cv. Emerald Sea is dominated by isoabienol (63.9%) and is clearly, quite different from cv. Blue Pacific, or the escaped juniper (Table 1).

The oil of *J. rigida*, Honshu Prefecture, Japan, is similar in many respects to *J. r.* var. *conferta* (Table 1). However, a comparison between its DNA and var. *conferta* supports the recognition of var. *conferta* (Adams and Schwarzbach, 2012, Adams 2011).

In summary, both morphology and terpenoids confirm that the escaped cultivar is cv. Blue Pacific and likely came from cultivated plants at houses on the bluff-top in Newport Beach. Both websites at Missouri Botanical Garden and U. Arkansas list *J. conferta* (*J. r.* var. *conferta*) as having seed cones (fruit or berries). The escaped cultivar may have arisen by seeds from cultivated plants

nearby or by branchlets (cut or broken) that fell down the cliff and rooted. *Juniperus rigida* var. *conferta* has not been reported previously for CA (Adams and Bartel, 2012). Whether this juniper will become a serious invader is not known.

ACKNOWLEDGEMENTS

Thanks Holly Forbes, Berkeley Botanical Garden for providing a sample of cv. Emerald Sea.

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Table I. Comparison of the leaf oils of *J. rigida var. conferta*, Tottori sand dunes, Japan (CF), cv. Blue Pacific (BP), the Newport Beach, CA juniper (NpB), cv. Emerald Sea (EmS), and *J. rigida*, Honshu, Japan (RG). Those compounds that appear to distinguish the cultivars are in boldface.

KI	Compound	CF	BP	NpB	EmS	RG
921	tricyclene	0.3	t	t	t	0.1
924	α-thujene	t	t	t	-	-
932	**α-pinene**	**40.6**	**58.6**	**43.0**	**9.8**	**62.3**
945	α-fenchene	0.4	t	t	-	0.2
946	**camphene**	**0.7**	**0.5**	**0.5**	**0.1**	**0.5**
969	**sabinene**	**0.3**	**0.2**	**0.1**	**t**	**0.4**
974	**β-pinene**	**4.1**	**6.9**	**4.6**	**1.4**	**5.8**
988	**myrcene**	**6.9**	**8.4**	**7.1**	**1.6**	**6.1**
1001	δ-2-carene	-	-	-	-	0.1
1002	α-phellandrene	t	-	-	-	0.2
1008	δ-3-carene	9.1	t	t	-	4.2
1014	α-terpinene	-	-	-	-	t
1020	p-cymene	t	t	t	-	t
1025	sylvestrene	t	-	-	-	t
1024	**limonene**	**1.6**	**2.8**	**1.8**	**0.6**	**6.0**
1025	**β-phellandrene**	**2.5**	**1.3**	**1.0**	**0.2**	**5.5**
1049	pentyl isobutanoate	-	-	0.1	t	0.1
1054	γ-terpinene	t	t	t	t	0.1
1065	cis-sabinene hydrate	t	-	-	-	t
1085	p-mentha-2,4(8)-diene	t	-	-	-	-
1086	**terpinolene**	**1.8**	**0.2**	**0.3**	**t**	**0.9**
1095	linalool	0.2	t	-	t	t
1101	isopentyl isovalerate	t	t	-	-	t
1101	cis-thujone	t	-	-	-	-
1101	ipsenol	t	-	-	-	-
1111	endo-fenchol	-	t	0.4	t	-
1112	3-me-3-butenyl-me-butanoate	-	-	t	-	t
1122	α-campholenal	t	-	0.3	-	0.1
1132	cis-limonene oxide	t	-	-	-	-
1135	trans-pinocarveol	-	t	0.3	t	-
1137	**trans-verbenol**	**-**	**0.2**	**0.4**	**t**	**-**
1141	camphor	0.6	-	-	-	-

KI	Compound	CF	BP	NpB	EmS	RG
1145	camphene hydrate	0.2	t	0.3	t	-
1165	borneol	0.2	t	0.5	t	-
1174	terpinen-4-ol	0.2	t	t	t	0.2
1178	naphthalene	1.1	t	-	-	0.3
1186	α-terpineol	0.3	t	0.4	t	0.2
1218	**endo-fenchyl acetate**	**t**	**0.6**	**0.6**	**0.2**	**-**
1248	citronellol	0.3	-	0.4	-	0.3
1257	methyl citronellate	-	-	-	0.8	-
1287	**bornyl acetate**	**7.5**	**1.1**	**1.4**	**0.7**	**0.6**
1293	2-undecanone	-	t	0.4	-	-
1298	carvacrol	-	-	t	-	-
1315	(E,E)-2,4-decadienal	-	-	t	0.2	-
1324	myrtenyl acetate	0.2	-	-	-	-
1346	α-terpinyl acetate	t	t	0.1	0.3	0.2
1350	citronellyl acetate	t	t	t	0.3	-
1359	neryl acetate	t	t	t	t	-
1379	geranyl acetate	t	t	t	0.2	-
1417	**(E)-caryophyllene**	**t**	**0.3**	**1.3**	**-**	**0.2**
1452	**α-humulene**	**t**	**0.3**	**0.9**	**-**	**0.2**
1480	germacrene D	t	-	t	-	0.2
1505	β-bisabolene	-	0.3	0.5	0.2	-
1513	cubebol	0.2	-	-	-	0.2
1522	δ-cadinene	t	-	-	-	0.1
1548	elemol	t	0.2	t	t	-
1559	germacrene B	0.3	0.4	0.5	0.5	t
1561	(E)-nerolidol	2.1	-	-	2.3	0.2
1565	dodecanoic acid	-	t	0.6	-	-
1574	germacrene D-4-ol	-	-	-	-	t
1577	spathulenol	-	-	-	-	0.1
1582	**caryophyllene oxide**	**-**	**0.5**	**0.9**	**-**	**-**
1608	humulene epoxide II	-	0.3	0.7	0.2	t
1649	β-eudesmol	t	-	0.4	-	-
1652	α-eudesmol	-	-	0.3	-	-
1652	α-cadinol	t	-	-	-	0.1
1688	epi-α-bisabolol	1.4	2.0	1.7	2.7	0.8
1713	(2E,6Z)-farnesal		0.4	0.5	0.9	0.2
1722	(2Z,6E)-farnesol	2.6	0.7	t	0.7	0.4
1740	(2E,6E)-farnesal	t	0.5	0.7	1.3	0.2
1756	ambroxide	-	-	0.2	-	-
1933	cyclohexadecanolide	-	t	0.3	0.4	-

KI	Compound	CF	BP	NpB	EmS	RG
1959	**hexadecanoic acid**	**-**	**0.2**	**1.1**	**-**	**-**
1987	manool oxide	0.2	t	0.3	1.8	t
2014	palustradiene	-	-	0.3	-	-
2022	cis-abieta-8,12-diene	-	-	0.3	-	-
2055	abietatriene	1.6	1.5	0.9	1.4	0.3
2087	abietadiene	t	0.3	1.7	t	0.1
2105	**isoabienol**	**6.7**	**2.0**	**14.5**	**63.9**	**-**
2184	sandaracopimarinal	t	0.2	0.3	0.2	-
2313	abietal	-	0.3	0.7	0.4	-
2314	trans-totarol	0.3	-	-	-	t
2331	trans-ferruginol	2.0	5.6	2.8	4.0	0.3

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

TWO NEW SPECIES OF *VERBESINA* (ASTERACEAE: HELIANTHEAE) FROM OAXACA, MEXICO

Billie L. Turner
Plant Resources Center
The Univ. of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

Verbesina textitlana B.L. Turner, **sp. nov**. and **Verbesina etlana** B.L. Turner, **sp. nov.** are described from Oaxaca, Mexico. The former belongs to the Sect. Verbesinaria DC., sensu Robinson and Greenman (1899), where it nestles next to *V. oaxacana* and cohorts; the latter relates to *V. scabrida* Rzed. and cohorts. Photographs of the types are provided, along with maps showing distribution of the taxa concerned. *Phytologia 94(3): 343-349 (December 1, 2012).*

KEY WORDS: Asteraceae, Heliantheae, *Verbesina*, *V. scabrida*, *V. oaxacana*, Mexico, Oaxaca

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Routine identification of Mexican Asteraceae has revealed the following novelties:

VERBESINA TEXTITLANA B. L. Turner, **sp. nov. Fig. 1**

Resembling *Verbesina oaxacana* DC. but having more numerous, somewhat smaller heads, the leaves smaller, darker green, more sparsely pubescent beneath, and the pales with sharply recurved apices.

Perennial herbs or shrubs, 1-2 m high. **Stems** purplish, pubescent with spreading or up-swept hairs. **Leaves** alternate, dark green, pinnately veined, mostly 7-10 cm long, 2.5-5.0 cm wide; petioles 2-15 mm long; blades narrowly to broadly oval, widest at or near the middle, grading into the petioles, sparsely pubescent above and below, mainly along the ribs, their margins serrulate. **Capitulescence** a terminal, corymbose panicle of 20 or more heads, the ultimate peduncles mostly

1-4 cm long. **Heads** hemispheric, 10-15 mm across, 6-8 mm high. **Involucres** 3-4 mm high, the bracts ovate, arranged in 3-4 sub-equal series. **Receptacles** (in fruit) hemispheric, ca 2 mm across, 1 mm high; paleate, the pales linear-lanceolate, 3-4 mm long, the apices recurved. **Ray florets** ca 21, pistillate, fertile; lamina yellow, linear, 6-10 mm long. **Disk florets** numerous, yellow, the corollas ca 3 mm long; tubes pubescent, ca 1 mm long, the throat glabrous or nearly so. **Anthers** brown. **Achenes** (the body) ca 2 mm long, 1 mm wide, the pappus scales ca 1 mm long.

TYPE: **MEXICO. OAXACA: Mpio. Santiago Textitlan,** "Bosque de pino-encino, orilla de arroyo, suelo rojo." ca 1739 m, (16 41 24.4 N, 97 15 29.4 W), 8/10/2006, *Maria Ester Jacob Salinas 723* (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. OAXACA: Mpio. Santiago Textitlan,** "Paraje Zacaton, Bosque de pino-encino, suelo negro," en Canada, ca 2500 m, 7/12/2006, *Salinas 1136* (TEX); "Cueva de Cuche, Bosque de pino-encino. suelo negro, ca 2356 m, 26/08/2006, *Marcos 285* (TEX); "Paraje la Hierba Santa, Vegetacion secundaria de bosque de pino-encino. suelo negro, orillo de arroyo," ca 1893 m, 21/10/2006, *Olazo 755* (TEX); "Lachixao paraje Pena de Chivo, Bosque de pino-encino. Suelo café." ca 2317 m, 7/9/2006, *Vasquez 369* (TEX).

Marcos 285, cited in the above, appears to be a form of this taxon having winged stems. All other collections of *V. textitlana* had stems that lacked wings. However, similar wing-phenomena have been found in yet other taxa of *Verbesina*, hence my reluctance to place undue emphasis on the character concerned.

Verbesina textitlana is clearly related to *V. oaxacana* but is readily distinguished by its coarser habit, larger heads, and the characters brought to the fore in the above diagnosis. Specimens obtained from Mpio. Santiago Textitlan are all very similar; however, those from Mpio. San Miguel de Puerto appear to approach *V. oaxacana*, but have the more numerous heads and technical characters of *V. textitlana*. Distribution of the two taxa in Mexico is shown in Map 1.

The species name derives from the Municipio in which the type was collected.

VERBESINA ETLANA B.L Turner, **sp. nov. Fig. 2**

Resembling *Verbesina scabrida* Rzed. but a perennial herb 30-100 cm high (vs shrub or subshrub 1.5-2.5 m high), with smaller leaves, the capitulescence a loosely knit, terminal, cymose-panicle composed of 20-30 heads (vs numerous heads arranged in stiffly divaricately branched cymose-panicles), and the disc floret having densely pubescent tubes (vs glabrous or nearly so).

Perennial herbs, reportedly 30-100 cm high. **Leaves** opposite, 8-13 cm long, 2-4 cm wide; petioles 0-2 mm long; blades oblanceolate, reticulate venose beneath, sparsely pubescent above and below, especially along the veins. the margins entire or weakly serrate. **Capitulescence** a terminal cymose-paniculate panicle of 20-30 heads, 5-7 cm high, and as wide, the ultimate peduncles, appressed-pubescent, 5-15 mm long. **Heads** 5-6 mm high, and as wide. **Involucres** narrowly to broadly campanulate, 4-5 mm high, and as wide; outer involucral bracts, mostly linear-ovate, 3-4 mm long, ciliate with rounded apices. **Disk florets** ca 20; corollas ca 5 mm long; tubes markedly pubescent; throats sparsely ciliate. **Anthers** brown. **Achenes** (immature) narrowly winged, glabrous; pappus scales 2-3 mm long.

TYPE: **MEXICO. OAXACA: Distrito: Etla; Mpio. San Felipe Tejalapa.** La Pena. "Bosque de pino-encino. Sobre cerro." 17.3 37.3 N, 96 55 17.1 W, ca 2179 m, 06/10/2011, *Mario Cruz (MAC) 388* (holotype, TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. OAXACA: Distrito Etla. Mpio. San Felipe Tejalpa.** Tierra de los Sanchez el Capulin, ca 2575 m, 08/09/2011, *Morales 284* (TEX). **Distrito Pochutla, Mpio. San Miguel del Puerto.** El Encinal, "Bosque de Encino." 04/10/2003, *Pascual 858* (TEX).

Morales 284 described the plant as an herb 30 cm high; the other two collectors reckon the plant to be an herb 1 m high. All specimens (numerous) of *V. scabrida* (including *V. costata* Fay, a later

name), seen by the present author were said to be shrubs 1.5-2.5 m high. The collection from Distrito Pochutla has heads superficially resembling *V. scabrida*, but otherwise appears to have the parameters of *V. etlana*.

The species is named for the Distrito Etla, whence the type.

ACKNOWLEDGEMENTS

I am grateful to Jana Kos for reviewing the paper and helpful suggestions. Jose Villasenor Rios (pers. corr.) called to my attention the synonymy of *V. costata*. Distribution maps (Figs. 3 and 4) are based upon specimens at LL-TEX.

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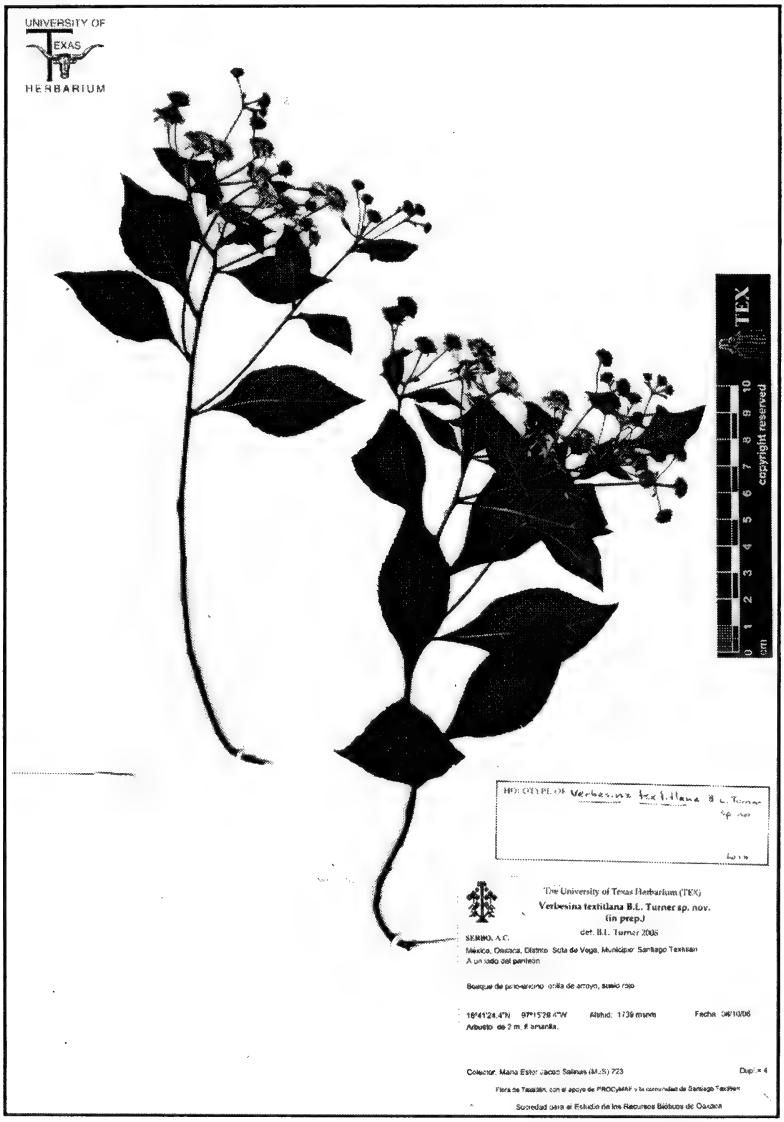

Fig. 1. *Verbesina textitlana* (holotype).

Fig. 2. *Verbesina etlana* (holotype).

Fig. 3. Distribution of *Verbesina textitlana* and *V. oaxacana.*

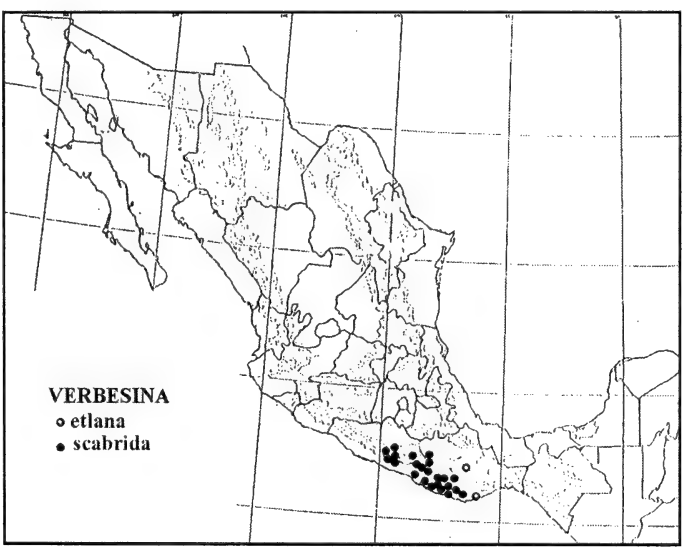

Fig. 4. Distribution of *Verbesina etlana* and *V. scabrida.*

TAXONOMY OF THE MULTI-SEEDED, ENTIRE LEAF TAXA OF *JUNIPERUS*, SECTION *SABINA*: SEQUENCE ANALYSIS OF nrDNA AND FOUR cpDNA REGIONS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA email Robert_Adams@baylor.edu

and

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

ABSTRACT

An analysis of the multi-seeded, entire leaf margined taxa of *Juniperus* sect. *Sabina* is presented based on nrDNA and four cpDNA regions. These DNA data revealed several previously unknown affinities with *J. sabina* and *J. chinensis*, better resolution of the *J. excelsa* - *polycarpos* complex and the Caribbean junipers, including the recognition of ***Juniperus gracilior* var. *saxicola*, comb. nov.** and ***Juniperus semiglobosa* var. *jarkendensis*, comb. nov.** *Juniperus sabina* var. *davurica* (Mongolia) was found to be clearly separated from *J. sabina* (Europe), supporting the recognition of *J. davurica* Pall., ***J. d.* var. *arenaria*, comb. nov**. and ***J. d.* var. *mongolensis*, comb. nov.** The taxonomy of the entire group is reviewed and necessary changes made in several taxa. *Phytologia 94(3): 350-366 (December 1, 2012).*

KEY WORDS: Taxonomy, *Juniperus*, section *Sabina*, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, *Juniperus gracilior* var. *saxicola* com. nov., *J. semiglobosa* var. *jarkendensis* comb. nov., *J. davurica* var. *arenaria*, comb. nov. and *J. davurica* var. *mongolensis*, comb. nov.

Recently, Mao et al. (2010) presented an abbreviated phylogeny of *Juniperus* as part of a study focused on intercontinental dispersal. As such, that report was incomplete as it did not include all taxa in the three sections, nor did it include taxonomically difficult infra-specific taxa.

To remedy these shortcomings, we have presented analyses of the serrate leaf taxa of *Juniperus,* sect. *Sabina* (Adams and Schwarzbach, 2011) and all taxa of *Juniperus* sect. *Juniperus* (Adams and Schwarzbach, 2012). The purpose of the current study is to continue this work by analyzing all the multi-seeded, entire leaf margined taxa of section *Sabina* using the most informative nuclear (nrDNA- ITS) and cpDNA regions (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF). The pseudo-denticulate species, *J. phoenicea* (Adams, 2011) is included, as its placement is uncertain (Mao et al. 2010).

MATERIALS AND METHODS

Specimens used in this study: *J. blancoi,* El Oro, Mex., *Adams 6849, J. b.* var. *mucronata,* Maicoba, Mex., *Adams 8702, J. b.* var. *huehuentensis,* w. Durango, Mex. *Adams 10247, J. barbadensis,* Petit Peton, St. Lucia, BWI, *Adams 5368, J. b.* var. *lucayana,* Isle de Pinos, Cuba, *Adams 5281, J. bermudiana,* Bermuda, *Adams 11083, J. chinensis,* Shizuoka Prefecture, Japan, *Adams 8535, J. c. var. sargentii,* Hokkaido, Japan (ex N. Yoshida) *Adams 8688, J. communis* L. *var. communis*, *Adams 7846, 7847*, Stockholm, Sweden (outgroup), *J. erectopatens,* s of Songpan, Sichuan, China, *Adams 8532, J. excelsa,* w of Lemos, *Greece, Adams 8785, 9433, J. foetidissima,* Mt. Parnassus, Greece, *Adams 5645, J. gracilior,* Dominican Republic, *Adams 7664, J. g.* var. *ekmanii,* Haiti, *Adams 7653, J. g.* var. *urbaniana,* Haiti, *Adams 7656, J. horizontalis,* Montana, USA, *Adams 7096, J. jarkendensis,* Kunlun Mtns., Xinjiang, China, *Adams 7820, J. maritima,* Brentwood Bay, VI, BC, Canada, *Adams 11056, J. phoenicea,* El Penon, Spain, *Adams 7077, J. p.* var. *turbinata,* w Setubal, Portugal, *Adams 7077,* Tarifa sand dunes, Spain, *Adams 7302, J. polycarpos,* Lake Sevan, Armenia, *Adams 8761, J. p.* var. *seravschanica,* s of Dzhabagly, Kazakhstan, *Adams 8224, J. p.* var. *seravschanica,* Quetta, Pakistan (ex A. Hafeez Buzdar) *Adams 8483, J. p.* var. *turcomanica,* Kopet Mtns., Turkmenistan, *Adams 8757, J. procera,* 6184, w of Addis Ababa,

Ethiopia, *Adams 6184, J. procumbens, 8683,* Hokkaido, Japan (ex N. Yoshida) *Adams 8683, J. sabina,* Baltschieder, Switzerland, *Adams 7611, J. s.* var. *arenaria,* Lake Qinghai sand dunes, Qinghai, China, *Adams 10347, J. s.* var. *davurica,* 15 km se Ulan Batar, Mongolia, *Adams 7353, J. s.* var. *mongolensis,* on sand dunes, 80 km sw Ulan Bator, Mongolia, *Adams 7354, J. scopulorum,* Kamas, UT, USA, *Adams 10895, J. semiglobosa,* Kyrgyz range, 60 km sw Bishket, Kyrgystan, *Adams 8210, J. saxicola,* Pico Turquino, Cuba, *Adams 5284, J. thurifera,* 2 km e Ruidera, Spain, *Adams 7083, J. t.* var. *africana,* 60 km e Marrakech, Morocco, *Adams 9420, J. tsukusiensis,* Yakushima Island, Japan (ex Jin Murata), *Adams 8806, J. tsukusiensis* var. *taiwanensis,* Mt Chingshui, Taiwan, *Adams9061, J. virginiana,* Waco, TX USA, *Adams 6754,* 32 km e Knoxville, TN, USA, *Adams 10234, J. v. var. silicicola,* Ft. DeSoto Park, Mullet Key, FL, USA, *Adams 9186-88.* Voucher specimens are deposited in the herbarium, BAYLU, Baylor University.

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 by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

PCR amplification, sequencing and data analyses - see Adams and Schwarzbach (2012).

RESULTS AND DISCUSSION

The multi-seeded, smooth leaf margined junipers are very diverse (Fig. 1) having several distinct groups: the eastern hemisphere e*xcelsa-polycarpos, chinensis, sabina* and the western hemisphere *virginiana* and Caribbean clades. *Juniperus erectopatens* exhibits an isolated position (Fig. 1); *J. phoenicea* was included, although it is loosely associated with this group (Fig. 1). Despite the large amount of data, the extremely closely related taxa of the Caribbean are not resolved, awaiting more detailed analyses (Fig. 1). The classical taxon, *J. polycarpos* var. *seravschanica* (Kazakhstan, Pakistan) is placed in a different clade from that of *J. polycarpos - excelsa* (Fig. 1).

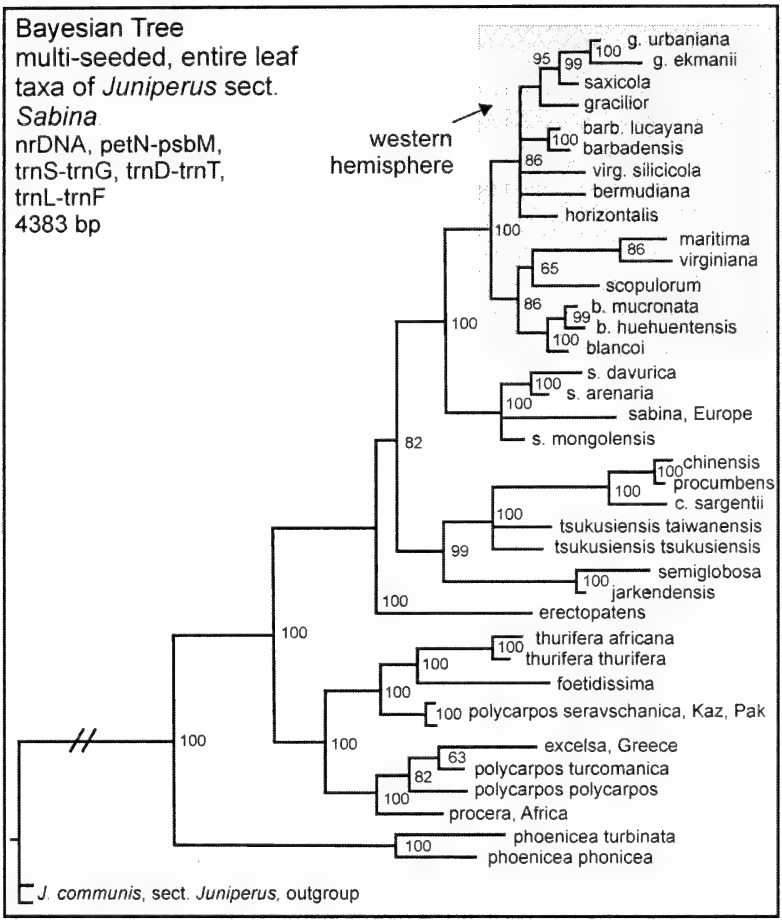

Figure 1. Bayesian tree for multi-seeded, entire leaf taxa, sect. *Sabina*. Numbers at the branch points are posterior probabilities (as percent).

Taxonomic considerations

To examine taxonomic ranks at the terminus of the tree, a minimum spanning network was constructed to include both SNPs and indels (termed Mutational Events, MEs). *Juniperus phoenicea* and *J. p.*

var. *turbinata* are separated by 66 MEs from their nearest neighbor (*chinensis* group, Fig. 2). The 23 MEs between var. *phoenicea* and var. *turbinata* is comparable to differences between recognized species Fig. 2). These data suggest that var. *turbinata* might be recognized at the specific level (***J. turbinata*** Guss.) as proposed by LeBreton and Paz (2001). Additional research into the nature of this difference is under investigation (RPA).

The *Juniperus tsukusiensis* group (var. *tsukusiensis, var. taiwanensis*) was treated as *J. chinensis* var. *tsukusiensis* by Adams and Farjon (Table 1), but later treated as *J. tsukusiensis* (Adams et al., 2011). It is separated from *J. jarkendensis* by 24 MEs, from the *J. davurica* group by 24 MEs and from *J. chinensis* by 32 MEs (Fig. 2). The decision by Adams et al. (2011) to recognize *J. tsukusiensis* and *J. t.* var. *taiwanensis* as separate taxa is strongly supported by our molecular data as well as morphological differences (Table 1).

The *Juniperus jarkendensis* / *J. semiglobosa* taxa are separated by 24 MEs (Fig. 2), but *J. jarkendensis* and *J. semiglobosa* differ by only 7 out of the total of 354 MEs. Furthermore, the taxa are very similar in morphology. The present data support recognition of *J. jarkendensis* as a variety of *J. semiglobosa*:

***Juniperus semiglobosa* var. *jarkendensis* (Komarov)** R. P. Adams, **comb. nov. Basionym**: *Juniperus jarkendensis* Kom., Not. Syst. Herb. Petrop. 4: 181 (1923). **Type**: China, Xinjiang, Kunlun Shan, Yarkant River, mtns. near Shache (Yarkant), *V. I. Robarovski 409* (holotype LE!). *J. sabina* var. *jarkendensis* (Kom.) J. Silba, Phytologia 68: 33 (1990). *Sabina vulgaris* var. *jarkendensis* (Kom.) C. Y. Yang in Fl. Reipub. Pop. Sin. 7: 360 (1978).

Juniperus sabina (Europe and central Asia) is separated by 24 MEs from the *davurica* group (*J. s.* var. *davurica*, var. *arenaria*, var. *mongolensis*) (Fig. 2).

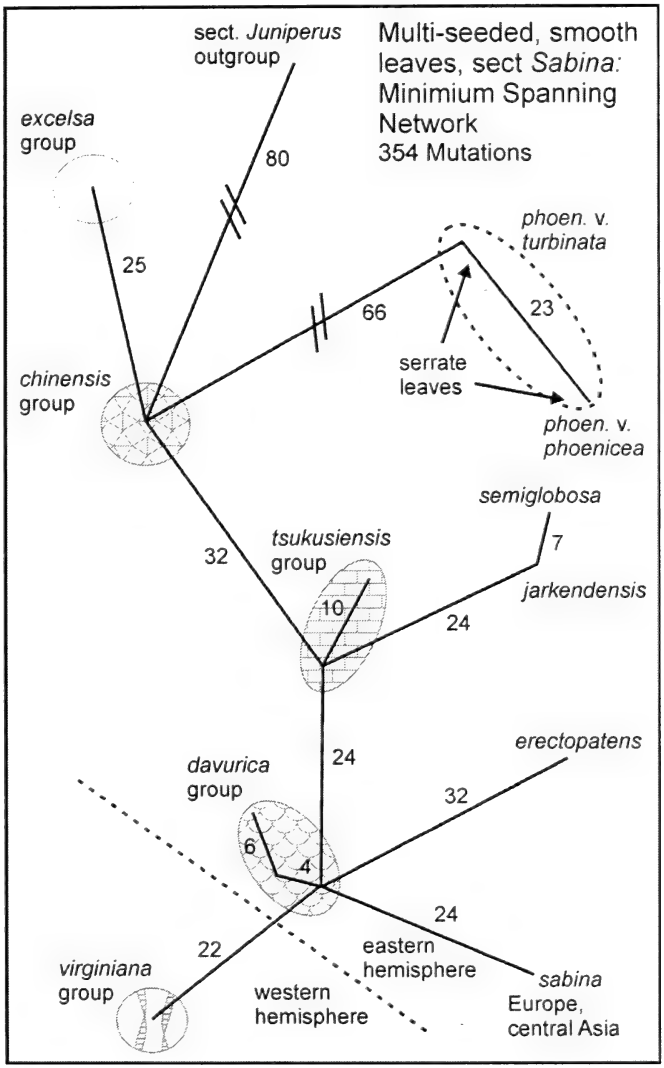

Figure 2. Minimum spanning network of the multi-seeded, smooth leaf junipers of sect. *Sabina*. Numbers next to links are the number of Mutational Events (MEs = #SNPs + # indels).

Finally, one should note that the nearest link to the *J. virginiana* group, endemic to North America, is the *J. davurica* group from Mongolia and Qinghai by only 22 MEs differences (Fig. 2), suggestive of migration from eastern Asia to North America (see also Mao et al., 2010).

The *Juniperus excelsa - chinensis* group is examined in detail in Figure 3. These groups differ by 25 MEs (Fig. 2). The *J. excelsa - chinensis* group is very diverse (Fig. 3) but *J. chinensis* and *J. procumbens* differ by only 1 ME (of 354). Although both Adams and Farjon (Table 1) recognize *J. procumbens*, the current sequence data do not support the separation of this taxon at the species level. *Juniperus procumbens* differs from *J. chinensis* by a prostrate habit with only decurrent (juvenile) leaves. Although the taxon is distinct due to these characteristics, the presence of only juvenile leaves (neoteny) and prostate habit may be controlled by only a few genes. It seems prudent to treat the taxon as ***J. c.* var. *procumbens* Siebold ex Endl.** (Table 1).

The DNA of *J. chinensis* var. *sargentii* differs by 35 MEs from *J. chinensis* var. *procumbens* and 17 MEs from *J. polycarpos* var. *seravschanica* (Fig. 3). Previously, Adams et al. (2011) found var. *sargentii* to differ from *J. chinensis* by 10 MEs, using a smaller data set, although single mutations within a taxon were excluded from their analysis. It appears that analysis using all MEs (this study) likely accentuates the differences among taxa, as single point mutations are included and some of these are likely not representative of a given taxon. These single mutations could not be removed from the present data set, as replicates were not analyzed for each taxon. At present, it seems prudent to leave this taxon as *J. c.* var. *sargentii.*

Juniperus foetidissima is quite distinct (Fig. 3) and well accepted as a distinct species (Table 1). The status of *J. thurifera* and var. *africana* is controversial (Table 1). The taxa are clearly very closely related, differing by only 4 MEs (Fig. 3). Adams (2011) recognized var. *africana* because it differed in its oil and ecology and to call attention to its conservation in the Atlas Mtns. The present data offer little support for the recognition of var. *africana.*

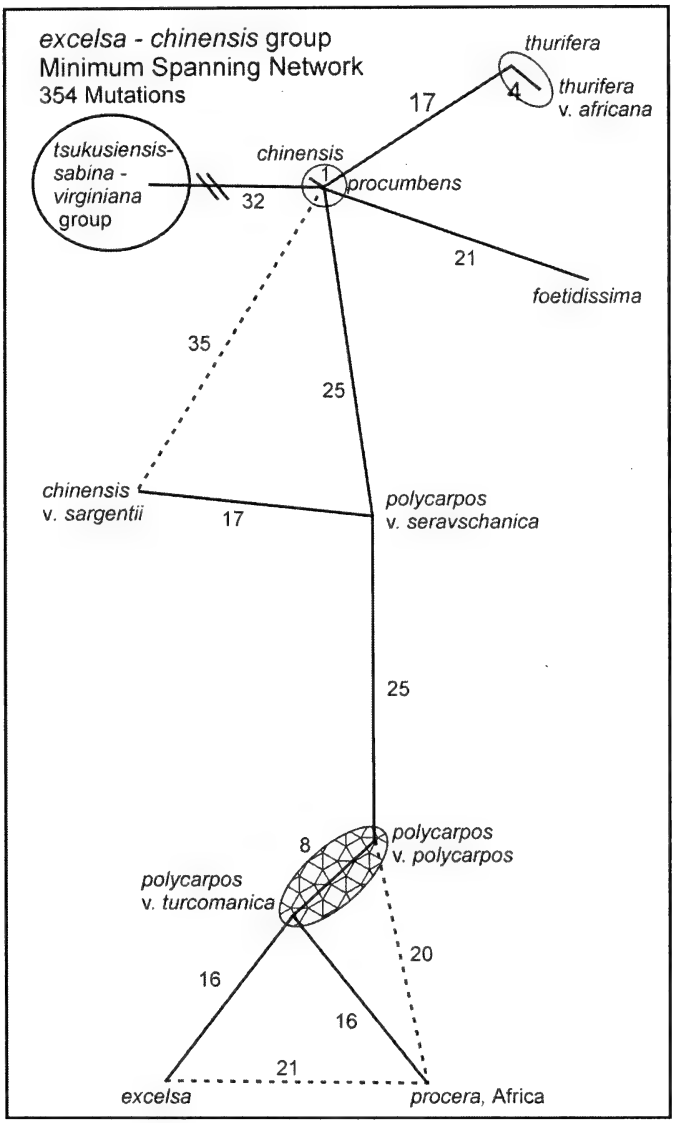

Figure 3. Minimum spanning network of the *excelsa* - *chinensis* group. Numbers next to links are the number of Mutational Events (MEs). Dashed lines are second nearest links.

Juniperus polycarpos - *J. excelsa* are morphologically very similar and this has led to considerable disagreement about the recognition of *J. polycarpos* (Table 1). The problem is illustrated in the present data (Fig. 3) in which *J. polycarpos* var. *turcomanica* is separated by 16 MEs from both *J. excelsa* and *J. procera*. Historically, *J. procera* has been recognized as a species, in spite of the paucity of morphological differences separating it from *J. excelsa*. Adams et al. (1993) found that DNA fingerprints (RAPDs) and terpenoids clearly separated *J. excelsa* and *J. thurifera*. If one includes *J. polycarpos* under *J. excelsa*, then it seems to be consistent one should include *J. procera* under *J. excelsa* (Fig. 3). Further confounding the situation is the fact that *J. p.* var. *turcomanica* has foliage and nrDNA that are similar to *J. excelsa*, but its cpDNA (tnrC-trnD) is like of *J. polycarpos*. (Adams, 2011, Fig. 5.4.1). It may be that *J. p.* var. *turcomanica* is of hybrid origin between *J. excelsa* and *J. polycarpos* . At present, both *J. polycarpos* var. *polycarpos* and *J. p.* var. *turcomanica* are recognized (Table 1).

Plants from two populations of *J. polycarpos* var. *seravschanica* (Kazakhstan, Pakistan) differed by no MEs (data not shown), but the taxon differs by 25 MEs from *J. p.* var. *polycarpos* (Fig. 3). These data support the recognition of *J. seravschanica* Kom. (Table 1).

The *J. sabina* - *virginiana* group (Fig. 4) is diverse, having only a few species. Of particular interest is the large difference (24 MEs, Fig. 4) between *J. sabina* (Europe) and the *J. davurica* group in Mongolia and Qinghai, China. Adams et al. (2006) reported considerable variation in the leaf terpenoids from Europe to Mongolia. RAPDs data indicated that *J. sabina* from Europe and the Tian Shan Mtns., China differed from *J. sabina* in Mongolia and Qinghai, China (Adams et al. 2007). The current DNA data indicate that the taxonomic separation in *J. sabina* is an even greater extent with 24 MEs between *J. sabina* (Europe) and the *sabina* varieties in Mongolia and Qinghai, China (Fig. 4). In fact, the data offer no support that *J. sabina* (*sensu stricto*) occurs in Mongolia and Qinghai, China, but rather that *J. davurica* Pall. should be recognized in this region (Table 1). It is apparent that *J. sabina* var. *arenaria* and *J. s.* var. *mongolensis* are not

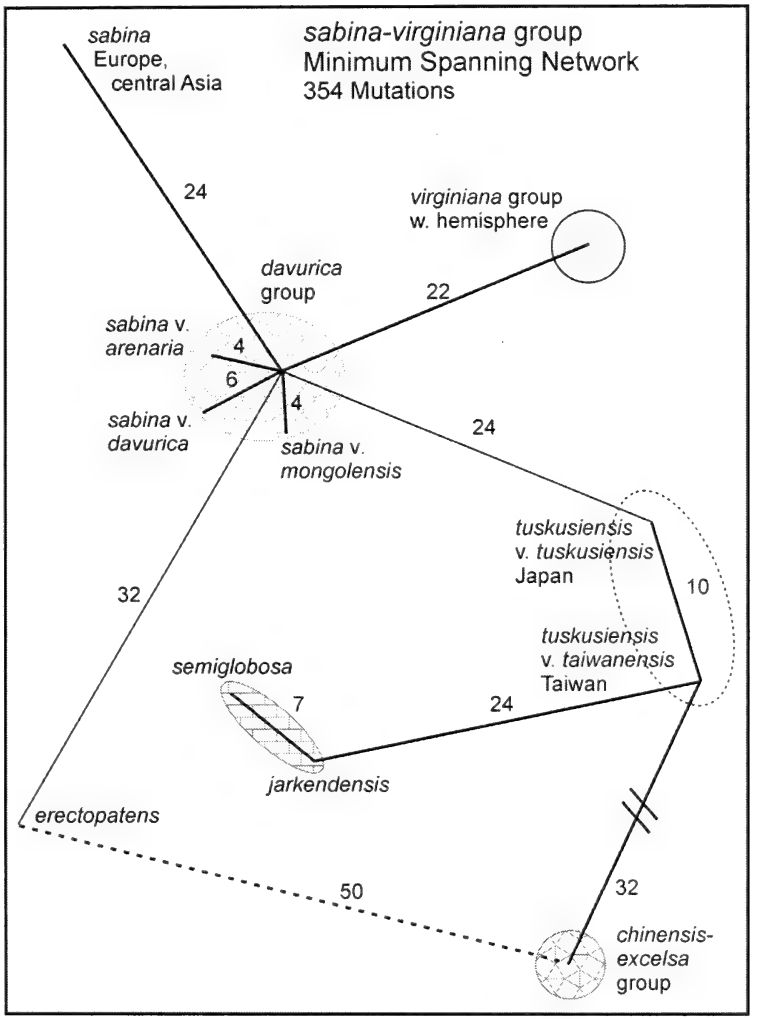

Figure 4. Minimum spanning network of the *sabina* - *virginiana* groups. Numbers next to links are the number of Mutational Events (MEs). Dashed line is second nearest link.

part of *J. sabina* (*sensu stricto*) but rather these are varieties of *J. davurica* (Fig. 4). Two nomenclatural adjustments are needed:

***Juniperus davurica* var. *arenaria* (E. H. Wilson)** R. P. Adams, **comb. nov. Basionym**: *Juniperus chinensis* var. *arenaria* E. H. Wilson, J. Arnold Arbor. 9: 20. 1928. **Type**: China, Qinghai, Qinghai Lake, *J. F. Rock 13346* (holotype A!; isotypes E, K). *J. arenaria* (E. H. Wilson) Florin, Acta Horti Berg. 14 (8): 353. 1948. *J. sabina* var. *arenaria* (E. H. Wilson) Farjon, Checklist of Conifers, ed. 2, 73 (2001).

Juniperus davurica* var. *mongolensis (R. P. Adams) R. P. Adams, **comb. nov. Basionym**: *Juniperus sabina* var. *mongolensis* R. P. Adams, Phytologia 88(2): 182. 2006. **Type**: Mongolia, 80 km sw of Ulan Batar, 1230 m, on sand dunes, 16 Jun 1994, *Adams 7255* (holotype BAYLU); topotypes *Adams 7254, 7256* (BAYLU).

The *J. tsukusiensis* group (var. *tsukusiensis var. taiwanensis*) is treated as *J. chinensis* var. *tsukusiensis* by Adams and Farjon (Table 1), but later treated as *J. tsukusiensis* (Adams et al., 2011), and is separated by 32 MEs from the *J. chinensis* group. The present data strongly support the recognition of *J. tsukusiensis* Masam. and *J. t.* var. *taiwanensis* (R. P. Adams and C-F. Hsieh) R. P. Adams (Table 1).

Juniperus erectopatens is an unusual taxon. The senior author discovered the taxon at the margins (wasteland) of a cultivated field south of Songpan, Sichuan, China. It is a small tree, resembling *J. chinensis* (as recognized by Farjon, Table 1). However, its oils and RAPDs clearly separate it from *J. chinensis* and any other taxa (Adams, 1999). The sequence data separate *J. erectopatens* by 32 MEs from the nearest taxon (*J. davurica*, Fig. 4) and 50 MEs from the *J. chinensis* group. These data support the recognition of *J. erectopatens* R. P. Adams (Table 1). More field work is desperately needed to understand better this taxon. It may be a product of hybridization, such that these 'apparent mutations' are actually DNA from another species.

The *J. virginiana* group is the least diverse and probably most recently diversified of the major groups (Fig. 5). Overall, the group is divided into the *J. blancoi* group, the Caribbean group (*gracilior-barbadensis*) and outliers, *J. virginiana, J. horizontalis, J. maritima* and *J. scopulorum* (Fig. 5). The *J. blancoi* group is located in western and central Mexico and consists of 3 closely related varieties, differing by only 3-4 MEs (Fig. 5). Both Adams and Farjon (Table 1) accept these taxa.

Juniperus horizontalis is a cool-season species of northern United States and Canada (Adams 2011), thought to have been derived from *J. scopulorum* or *J. virginiana*, so it is surprising to find it linked to the Caribbean and *blancoi* groups (Fig. 5).

The Caribbean group is closely related and difficult to separate by morphology. The Cuban *Juniperus saxicola* is a tree that has only juvenile leaves (neoteny), but is otherwise similar to *J. gracilior*. It differs from the shrubby *J. gracilior* var. *urbaniana* by only 3 MEs (Fig. 5). The DNA data suggest that *J. saxicola* and *J. g.* var. *ekmanii* are conspecific members of the *J. gracilior* group. However, tree habit and juvenile leaves seem sufficient to recognize *J. saxicola* at the varietal level:

Juniperus gracilior* var. *saxicola (Britton & P. Wilson) R. P. Adams, **comb. nov. Basionym**: *Juniperus saxicola* Britton & P. Wilson, Bull. Torrey Bot. Club 50: 35. 1923. **Type**: Cuba, Granma Prov., Sierra Maestra, Oriente, *J. S. S. (Frere) Leon 10798* (holotype NY!). *J. barbadensis* L. subsp. *saxicola* (Brittion & P. Wilson) Borhidi, Acta Bot. Acad. Sci. Hungarica 37: 90. 1992. *J. barbadensis* L. var. *saxicola* (Brittion & P. Wilson) Silba, J. Int. Conifer Preserv. Soc. 7(1): 25. 2000.

Juniperus barbadensis (sensu stricto) is known only from Petit Piton, St. Lucia, BWI (Adams, 2011), whereas *J. b.* var. *lucayana* is widespread growing on Hispanola, Jamaica, Cuba and the Bahama Islands. The taxa are nearly indistinguishable, differing only by glands conspicuous on old whip leaves (Adams 2012). The DNA data show these taxa differ by only 1 ME (of 354), providing no support to the continued recognition of *J. b.* var. *lucayana* (Table 1).

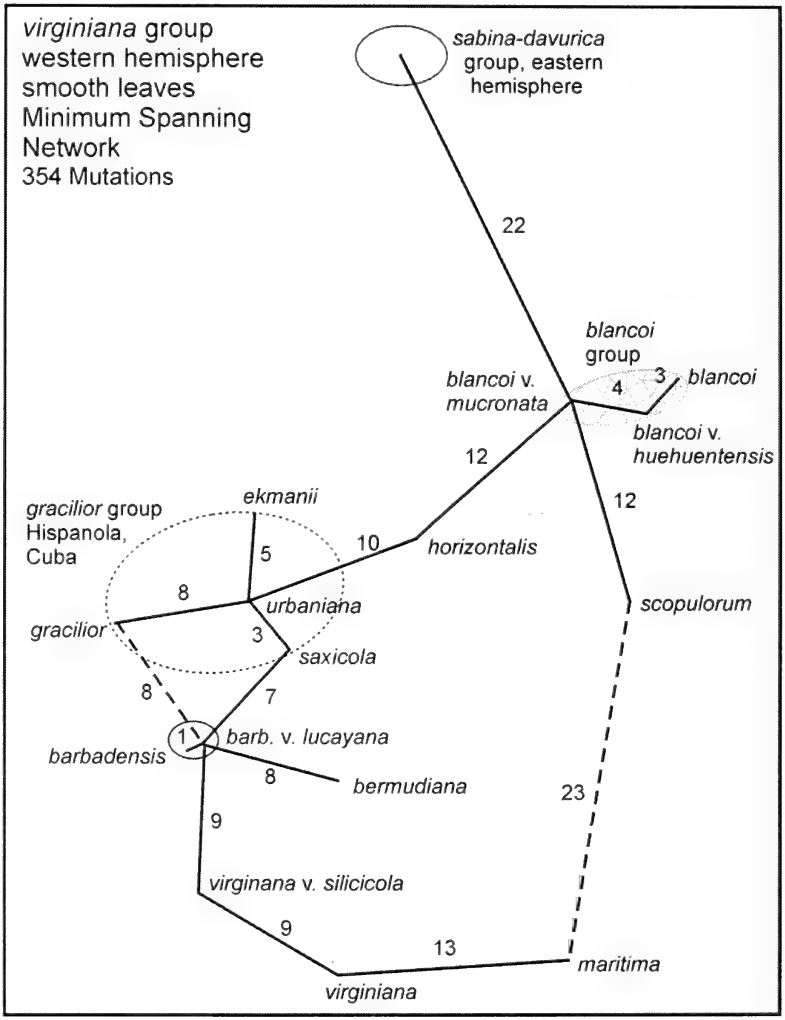

Figure 5. Minimum spanning network of the *virginiana* group. Numbers next to links are the number of MEs. Dashed lines are second nearest link.

Juniperus bermudiana is obviously closely related to *J. barbadensis* (Fig. 5), but growing in an isolated habitat has evolved shorter scales leaves appearing as a string of beads, not to mention differences in its leaf essential oils (Adams 2000; Adams and Wingate 2008; Adams 2008). Although its DNA differences are only 8 MEs, it seems prudent to retain it at the specific level (Table 1).

Juniperus virginiana var. *silicicola* is a curious taxon with affinities to both the Caribbean junipers and the mainland, *J. virginiana.* Adams et al. (2008) found that its nuclear nrDNA was identical to *J. virginiana,* but its cpDNA (trnC-trnD) was identical to the Caribbean species including, a 245 bp indel. That data was suggestive that *J. v.* var. *silicicola* may be of hybrid origin between *J. virginiana* and Caribbean *J. barbadensis* (or an ancestor). The present DNA data show the taxon to occupy an intermediate position between *J. virginiana* and *J. barbadensis* (Fig. 5).

Juniperus scopulorum is separated by 12 MEs from *J. blancoi* var. *mucronata* and 23 MEs from *J. maritima* (Fig. 5). The close relationship of *J. scopulorum* and *J. blancoi* is shown in their morphology, and by likely hybridization in the Colonia Pacheco area of northern Mexico in the Pleistocene with nearby populations of *J. b.* var. *mucronata* (Adams 2011b).

Juniperus maritima is separated by 13 MEs from *J. virginiana*, and 23 MEs from *J. scopulorum.* Several characters support the close relationship of *J. maritima* and *J. virginiana*: slender foliage, strong central axis, fruit maturing in one year, and growth in mesic habitats. If *J. maritima* were to be treated as a variety, it would have to be a variety of *J. virginiana*, not *J. scopulorum.* However, DNA data give strong support to the continued recognition of *J. maritima* at the specific level (Table 1).

Table 1. Comparison of Adams and Farjon taxonomic treatment of taxa in this study. Taxa with support for a modified taxonomic status are in bold.

Adams (2011)	Farjon (2005, 2010)	Supported, this study
J. barbadensis	*J. barbadensis*	*J. barbadensis*
J. b. var. *lucayana*	*J. b.* var. *lucayana*	***J. barbadensis***
J. bermudiana	*J. bermudiana*	*J. bermudiana*
J. blancoi	*J. blancoi*	*J. blancoi*
J. b. v. huehuentensis	*J. blancoi*	*J. b. var. huehuentensis*
J. b. var. *mucronata*	*J. b.* var. *mucronata*	*J. b.* var. *mucronata*
J. chinensis	*J. chinensis*	*J. chinensis*
J. c. var. *sargentii*	*J. c.* var. *sargentii*	*J. c.* var. *sargentii*
J. c. var. *tsukusiensis*	*J. c. var. tsukusiensis*	***J. tsukusiensis***
J. c. var. *taiwanensis*	*J. c.* var. *tsukusiensis*	***J. t.*** var. ***taiwanensis***
J. erectopatens	*J. chinensis*	*J. erectopatens*
J. excelsa	*J. excelsa* (in part)	*J. excelsa*
J. foetidissima	*J. foetidissima*	*J. foetidissima*
J. gracilior	*J. gracilior*	*J. gracilior*
J. g. var. *ekmanii*	*J. g.* var. *ekmanii*	*J. g.* var. *ekmanii*
J. g. var. *urbaniana*	*J. g.* var. *urbaniana*	*J. g.* var. *urbaniana*
J. horizontalis	*J. horizontalis*	*J. horizontalis*
J. jarkendensis	*J. semiglobosa*	***J. semiglobosa*** var. ***jarkendensis***
J. maritima	*J. scopulorum*	*J. maritima*
J. phoenicea	*J. phoenicea*	*J. phoenicea*
J. p. v. *turbinata*	*J. p.* subsp. *turbinata*	***J. turbinata***
J. procera	*J. procera*	*J. procera*
J. procumbens	*J. procumbens*	***J. chinensis* var. *procumbens***
J. polycarpos	*J. excelsa* subsp. *polycarpos*	*J. polycarpos*
J. p. v. *seravschanica*	*J. e.* subsp. *polycarpos*	***J. seravschanica***
J. p. v. *turcomanica*	*J. e.* subsp. *polycarpos*	*J. p.* var. *turcomanica*
J. sabina	*J. sabina*	*J. sabina*
J. s. var. *arenaria*	*J. s.* var. *arenaria*	***J. davurica* var. *arenaria***
J. s. var. *davurica*	*J. s.* var. *davurica*	***J. davurica* var. *davurica***

J. s. var. *mongolensis*	*J. s.* var. *arenaria*	***J. davurica*** var. ***mongolensis***
J. scopulorum	*J. scopulorum*	*J. scopulorum*
J. semiglobosa	*J. semiglobosa*	*J. semiglobosa*
J. saxicola	*J. saxicola*	***J. gracilior*** var. ***saxicola***
J. thurifera	*J. thurifera*	*J. thurifera*
J. t. var. *africana*	*J. thurifera*	***J. thurifera***
J. virginiana	*J. virginiana*	*J. virginiana*
J. v. var. *silicicola*	*J. v.* var. *silicicola*	*J. v.* var. *silicicola*

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TAXONOMIC STUDY OF THE *LASIANTHAEA CEANOTHIFOLIA* (ASTERACEAE: HELIANTHEAE) COMPLEX

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

Lasianthaea ceanothifolia is recognized as having three, largely allopatric, intergrading varieties in Mexico: var. **ceanothifolia**, var. **gracilis** and var. **gradata**. The long-standing var. *verbenifolia* is treated as a synonym of the typical variety. A key to the complex is provided, along with a map showing their distribution. *Phytologia 94(3): 367-371 (December 1, 2012).*

KEY WORDS: Asteraceae, *Lasianthaea, L. ceanothifolia*, Mexico

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Preparation of a treatment of the Mexican species of *Lasianthaea* has occasioned the present paper.

Becker (1979) provided an excellent treatment of the Mexican species of Mexico, this expanded upon by Turner (1988). The present contribution is to be included in my forth coming treatment of *Lasianthaea* for The COMPS OF MEXICO (cf. Turner 2009, etc.).

LASIANTHAEA CEANOTHIFOLIA (Willd.) K. Becker, Mem. N. Y. Bot. Gard. 31: 38. 1979.

This is a highly variable species within which Becker (1979) recognized four, more or less, intergrading varieties, as did McVaugh (1984); I would recognize but three intergrading varietal taxa, as follows:

1. Awns of disk achenes mostly 1.5-2.0(3.0) mm long; leaves softly pubescent beneath; Son, Chi, Sin and Dur.....var. **gradata**
1. Awns of disk achenes mostly 2.5-5.5 mm long; leaves variously pubescent beneath; Central Plateau s Sin and Nay southwards ...(**2**)

2. Middle and outer involucral bracts conspicuously many-nerved below, mostly glabrous except for the ciliate margins; s Sin, Dur, Nay, Jal and Col; mostly Pacific slopes .....var. **gracilis**
2. Middle and outer involucral bracts not conspicuously many-nerved below, their surfaces mostly pubescent; widespread from s Sin and Zac, southwards .....var. **ceanothifolia**

var. **ceanothifolia**

Lasianthaea ceanothifolia var. *verbenifolia* (DC.) K. Becker
Zexmenia ceanothifolia var. *conferta* A. Gray
Zexmenia cordifolia Blake
Zexmenia microcephala Hemsl.
Zexmenia verbenifolia (DC.) Blake

s Sin, Nay, s Zac, Agu, Gua, Jal, Mic, Mex, Mor, Ver, Gue, Oax and Cps, mostly tropical deciduous forests, 1000-2000 m; Sep-Dec.

Suffruticose shrublets or shrubs 0.5-6.0 m high; leaves opposite, 4-15 cm long, 1.5-5.0 cm wide; petioles 0-12 mm long; blades variously ovate, 3-nervate from or near the base, the undersurfaces relatively rough, the margins serrate; heads radiate, in terminal subumbellate clusters of 8-30, the peduncles mostly 1.2-3.0 cm long; involucres mostly 5-8 mm high, 3-8 mm wide, the bracts 3-5 seriate, graduate; ray florets 8-11, the ligules yellow, 4-8 mm long; disk florets mostly 10-30, the corollas yellow; disk achenes 3.5-5.0 mm long, the pappus of 2 awns 4-6 mm long, clearly exserted from the involucre at maturity; chromosome number, n = 10 pairs.

This is a widespread highly variable taxon, typified by collections from near Cuernavaca, Morelos, best recognized by its numerous, relatively small, narrow heads from which the achenal awns protrude at maturity giving the head a bristly appearance. It appears to intergrade with its more northern, lower elevational, allopatric, cohort,

var. **gracilis,** where their boundaries approach each other. Additionally, it apparently hybridizes upon occasion with yet other, very distinct, taxa such as **L. crocea**, as well noted by Becker (1979).

McVaugh (1984) accepted Becker's 4-varietal concept of **L. ceanothifolia**, distinguishing var. *verbenifolia,* the type, according to McVaugh (1984), possibly collected "near San Blas," Nayarit, Mexico. It differs from var. **ceanothifolia** largely by petiolar length [(1-5(12) mm long in the former, 5-20 mm long in the latter]. In my examination of numerous sheets of the complex from throughout Mexico, I could not see that such a correlation exists. McVaugh also reported that plants of var. *verbenifolia* grow in close proximity to var. **gracilis** in southern Nayarit, and that at that site, the latter taxon flowers "somewhat earlier" than the former. In my opinion, the area concerned is where var. **ceanothifolia** grades into var. **gracilis** (sensu the present author), and not much inference can be made as to the flowering periods of such populations.

I have not examined material of **L. ceanothifolia** from the state of Chiapas, but accept the identifications of Strother (1999), who cites a number of collections from the more eastern portions of the state, all assembled by Matuda.

var. **gracilis** (W.W. Jones) K. Becker, Mem. N. Y. Bot. Gard. 31: 44. 1979.

Zexmenia gracilis W.W. Jones
Zexmenia rotundata Blake

s Sin, Dur, Nay, Col, and Jal, in mostly tropical deciduous forests, 100-1000 m; Aug-Oct.

This is a relatively distinct taxon, as noted by Becker (1979). It is typified by collections from Nayarit, and is identified best by its striate, nearly glabrous, outer involucral bracts and mostly, sparsely pubescent, linear lanceolate leaf blades, these usually 3-4 times as long as wide; chromosome number, n = 10 pairs.

This variety, and perhaps others, occasionally forms hybrids with **L. helianthoides** (Becker, 1979) and presumably with yet other species.

var. **gradata** (Blake) K. Becker, Mem. N. Y. Bot. Gard. 31: 43. 1979.
Zexmenia gradata Blake

Son, Chi, Sin and Dur, tropical deciduous and pine-oak forests, 200-1300 m; Sep-Dec.

This variety is typified by collections assembled in Sinaloa. It resembles var. **ceanothifolia,** but the heads are smaller, on mostly shorter peduncles, bearing fewer (10-20) disc florets, the leaves more softly pubescent beneath, and the achenes having shorter awns, as noted in the above key to taxa.

Both Becker (1979) and McVaugh (1984) thought this variety to be the most distinct of the several infraspecific taxa proposed here. The taxon resembles var. **ceanothifolia** but is usually readily distinguished by its sub-pinninerved leaves which are somewhat softly pilose beneath, and by its disk achenes with awns 2-4 mm long, which do not extend much beyond the involucres at maturity, if at all.

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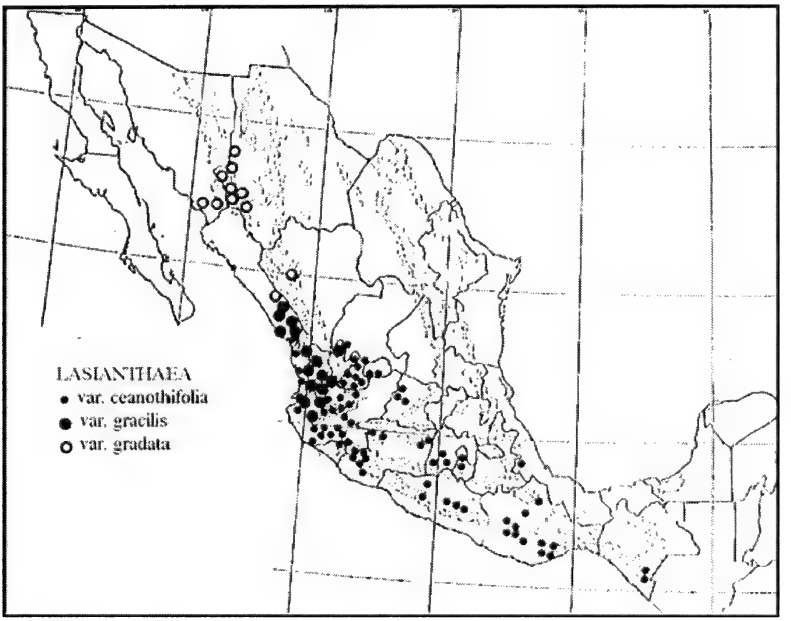

Fig. 1. Distribution of infraspecific taxa of *Lasianthaea* in Mexico.

CHEMOSYTEMATICS OF *JUNIPERUS*: EFFECTS OF LEAF DRYING ON ESSENTIAL OIL COMPOSITION III

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

The essential oils of leaves of *J. virginiana* were collected and analyzed as fresh vs. air dried and stored at ambient conditions (21° C) for up to 25 months before extraction. Changes occurred between months 8 and 25, implying loss due to volatilization and oxygenation. However, for taxonomic analysis involving species closely related to *J. virginiana*, the variations in the oils due to storage were minor. It appears that the oils from dried specimens can be used for studies among species with large differences in the essential oil compositions. Nevertheless, the present study does raise questions about the unexpected changes in leaf oils from specimens stored between 8 and 16 months. *Phytologia 93(1) 372-383 (December 1, 2012).*

KEY WORDS: *Juniperus,* oils from dried leaves, storage tests, chemosystematics.

In a previous study (Adams, 2010), leaves of *Juniperus pinchotii* Sudw. and *J. virginiana* L. were air dried (as herbarium specimens) and the oils analyzed from fresh vs. stored (ambient lab conditions, 21° C) specimens (stored for up to 8 months before extraction). The leaf oils of both species proved to be remarkably stable. For *J. virginiana*, ANOVA of 58 components revealed only 9 significant and 4 highly significant differences among the 7 sample sets. PCO of the samples showed some clustering by length of storage, but with considerable intermixing of samples.

However, in a more recent study on leaves stored for 16 months (Adams, 2011), ANOVA of 58 components revealed 4 significant and 19 highly significant differences among the 8 sample sets, with the major changes occurring between 8 and 16 months storage. PCO of the samples showed the 16 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raised concerns about mixing analyses of oils from fresh, recently dried and 16 mo. stored leaves of *Juniperus* for chemosystematic studies

Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22° C, 16 days) leaves of *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. and found only small differences.

The purpose of the present study is to report on changes in the composition of the steam distilled leaf oil of *J. virginiana* from specimens stored for 25 months.

MATERIALS AND METHODS

Plant material - *J. virginiana, Adams11768,* cultivated, nw corner of Gruver City Park, Hansford Co. TX, initial bulk collection: 23 Apr 2009. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (100 g.) and air dried (10-15 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library

search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principal Components Analysis (PCA) as formulated by Veldman (1967) was performed to examine correlations between components.

RESULTS AND DISCUSSION

Table 1 shows the composition of the leaf oils of *J. virginiana*, and a comparison of components over the 25 month storage period. In contrast to the previous study of 16 mo. (Adams, 2011), the percent oil yield did decline (significantly) in the 25 mo. sample (Table 1). It is unclear why there was no decline during the first 16 mo. of storage. Shanjani et al. (2010) reported that α-pinene (the major and most volatile component) declined from 23.9 to 14.2% when the foliage of *J. excelsa* was air dried. Achak et al. (2008) found oil yields to be greater from fresh than air dried leaves from 2 populations of *J. thurifera* var. *africana*, but with a lower yield in another population. Later, Achak et al. (2009) reported lower oil yields in dried leaves of *J. thurifera* var. *africana* and *J. oxycedrus*, but a much higher yield from dried leaves of *J. phoenicea*.

The compounds (as percent total oil) are remarkably stable during the drying and storage tests for the first 8 months but there are major changes between 8 and 25 months storage tests. In the tests up to 8 months storage, only 9 compounds significantly differed, and only 4 compounds differed highly significantly (Adams, 2010). However, distillation of leaves stored for 25 months revealed 1 significant and 30 highly significant differences (Table 1). Several compounds had large declines in concentration from 8 to 25 month: sabinene (17.6, 10.24),

limonene (14.6, 10.7), β-phellandrene (9.7, 7.1) and germacrene D-4-ol (3.8, 3.6). In contrast, several compounds increased: safrole (9.9, 10.7), methyl eugenol (2.2, 2.6), elemol (5.8, 10.6) and 8-α-acetoxyelemol (10.7, 11.8). Figure 1 (upper) shows the major compounds that declined. Notice that sabinene, limonene, and β-phellandrene show

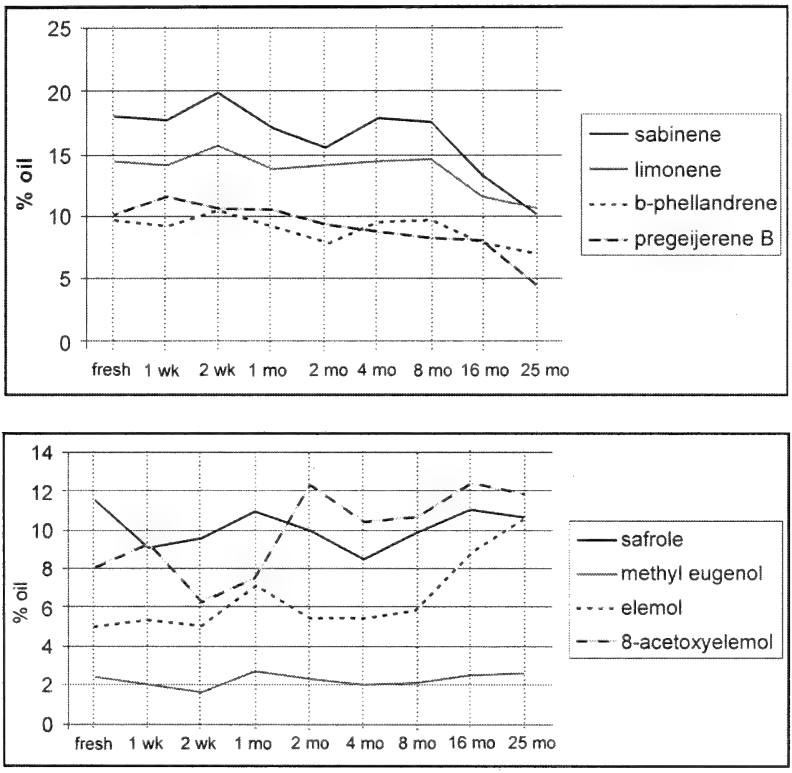

Figure 1. (upper) Changes in concentration (% total oil) for four major components that declined during leaf storage. (lower) Changes in concentration (% total oil) for four major components that increased during leaf storage.

similar patterns. Pregeijerene B shows a gradual decline from 1 month to 25 months.

The patterns for four of the major components that increased during the study are shown in figure 1 (lower). Safrole and methyl eugenol (both from the phenyl propanoid pathway) show similar patterns along with elemol. However, 8-α-acetoxyelemol (dashed line, Fig. 1, lower) increased from fresh to week 1, then declined, then increased to 2 month, then declined, then increased in month 16, and finally decreased in the final, 25 month, sample.

The leaf essential oils in *Juniperus* are stored in leaf glands. In *J. virginiana*, the leaf glands are generally not ruptured and often sunken beneath the waxy cuticle. With the loss of the more volatile monoterpenes and concurrent increase in the sesquiterpenes and diterpenes (Table 1), volatilization seems to be a factor in the changes in composition. The compounds showing the greatest increases (as percent total oil, Fig. 1, lower) are all oxygenated compounds. It seems possible that free radical oxygenation may occurring leading to an increase of these oxygenated compounds.

To estimate the impact of the utilization of oils from fresh versus dried and stored leaves, principal coordinates analysis (PCO) was performed. The PCO (Fig. 2) shows the major trend is the separation of the 16 mo. and 25 mo. samples on axis 1 (33% of the variance among samples). Overall, the samples stored from 1 wk. to 8 mos. seem to form a fairly uniform group.

To determine the utilization of oils from dried *J. virginiana* specimens in a taxonomic study, *J. virginiana* oils were compared with oils of *J. scopulorum* (Durango, CO), *J. blancoi* (Durango, MX), *J. b.* var. *huehuentensis* (Durango, MX) and *J. b.* var. *mucronata* (Maicoba, MX). The resulting PCO ordination (Fig. 3) shows that most of the variation (43%, axis 1) due to the separation of *J. virginiana* from the very closely related *J.* scopulorum and *J. blancoi*. It appears that for taxonomic use, the changes seen in months 16 and 25 are minor as compared to differences in the oils of closely related species.

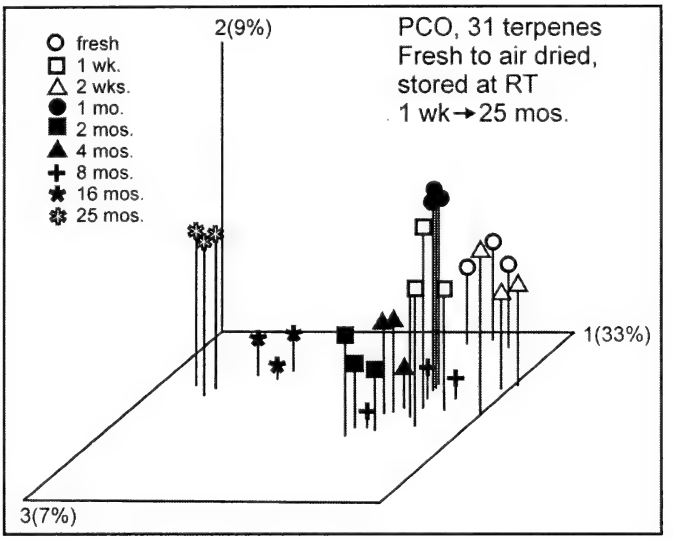

Figure 2. PCO of 9 sample sets ranging from fresh to storage for 25 months at ambient herbarium conditions (air conditioned, 21°C).

CONCLUSIONS

In this study, ANOVA revealed 1 significant and 30 highly significant differences among the 9 sample sets, with the major changes occurring between 8 and 25 months storage. PCO of the samples showed the 16 and 25 mo. samples to be clearly clustered. In contrast to the previous 8 mo. study (Adams, 2010), unexpected changes in the oils raise concerns about mixing analyses of oils from fresh, recently dried and 16 or 25 mo. stored leaves of *Juniperus* for populational chemosystematic studies. However, for taxonomic analysis involving species closely related to *J. virginiana*, the variation in the oils due to storage appeared to be minor. It appears that the use of oils from dried specimens can be used for studies among species with large differences in the essential oil compositions. Nevertheless, the present study does raise questions about the unexpected changes between 8 and 16 months of herbarium storage. It may be difficult to predict the stability of leaf essential oils in specimens over long periods of storage.

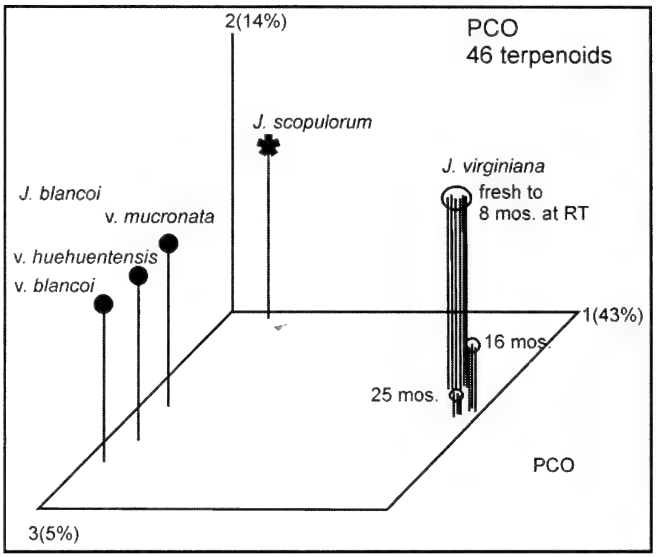

Figure 3. PCO of 9 sample sets of *J. virginiana* plus the oils of *J. scopulorum, J. blancoi, J. b.* var. *huehuentensis* and *J. b.* var. *mucronata*. Note the close clustering of all the *J. virginiana* samples.

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Table 1. Comparison of the composition of leaf oils from fresh leaves of *J. virginiana* vs. leaves dried and stored at 21° C. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	18mo	25mo	F sig
	percent yield	0.55	0.52	0.48	0.51	0.48	0.56	0.53	0.55	0.33	**
924	α-thujene	0.4	0.4	0.5	0.5	0.4	0.4	0.5	0.4	0.3	**
932	α-pinene	0.7	0.7	0.9	0.7	0.5	0.6	0.8	0.5	0.4	**
945	α-fenchene	t	t	t	t	t	t	t	t	t	nt
969	sabinene	18.0	17.7	19.8	17.1	15.5	17.9	17.6	13.3	10.24	**
974	β-pinene	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	ns
988	myrcene	1.2	0.9	1.1	0.8	0.7	0.7	0.5	0.2	0.2	**
990	74,87,43,115	0.5	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.3	**
1008	3-carene	0.6	0.6	0.6	0.5	0.5	0.7	0.9	0.4	0.4	**
1014	α-terpinene	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.4	ns
1024	limonene	14.4	14.2	15.6	13.8	14.0	14.4	14.6	11.7	10.7	**
1025	β-phellandrene	9.6	9.3	10.4	9.2	7.9	9.5	9.7	8.0	7.1	**
1054	γ-terpinene	0.6	0.5	0.5	0.6	0.5	0.6	0.5	0.6	0.7	*
1065	cis-sabinene hydrate	0.5	0.5	0.5	0.5	0.6	0.6	0.5	0.6	0.8	**
1086	terpinolene	0.8	0.7	0.8	0.7	0.7	0.8	0.7	0.5	0.4	**
1096	trans-sabinene hydrate	0.3	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.6	nt
1097	linalool	0.4	0.3	0.6	0.5	0.5	0.7	0.5	1.0	0.6	nt
1100	n-nonanal	t	t	0.2	t	0.2	t	t	t	t	nt
1118	cis-p-menth-2-en-1-ol	t	t	t	t	t	0.2	t	t	t	nt

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	25mo	F sig
1136	trans- p-menth-2-en-1-ol	t	t	t	t	t	t	t	t	t	nt
1148	citronellal	0.2	t	t	t	t	t	t	t	0.2	nt
1174	terpinen-4-ol	1.3	0.8	0.8	0.9	1.1	1.2	0.9	1.5	1.5	**
1186	α-terpineol	t	t	t	t	t	t	t	t	0.3	nt
1195	methyl chavicol	0.1	0.2	t	0.2	0.2	0.2	t	t	t	nt
1223	citronellol	0.2	t	t	t	0.2	0.2	t	t	0.2	nt
1261	152,123,81,77, aromatic	0.4	0.4	0.3	0.4	0.3	0.4	0.3	0.3	0.4	nt
1274	pregeijerene B	10.2	11.7	10.7	10.6	9.4	8.7	8.3	8.2	4.6	**
1285	safrole	11.6	9.1	9.6	10.9	10.0	8.5	9.9	11.1	10.7	**
1322	methyl geranate	0.1	t	t	t	0.1	0.1	t	t	t	nt
1350	citronellyl acetate	t	t	t	t	t	t	t	t	t	nt
1379	geranyl acetate	t	t	t	t	t	t	t	t	t	nt
1403	methyl eugenol	2.4	2.0	1.6	2.7	2.3	2.0	2.2	2.5	2.6	**
1417	(E)-caryophyllene	t	t	t	t	t	t	t	t	t	nt
1447	43,105,149,178, aromatic	0.3	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.6	nt
1465	cis-muurola-4(14),5-diene	t	t	t	t	t	0.2	t	0.2	0.6	nt
1491	epi-cubebol	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	nt
1500	α-muurolene	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.3	0.4	nt
1513	γ-cadinene	0.3	0.4	0.5	0.6	0.5	0.5	0.4	0.5	0.8	**
1522	δ-cadinene	0.8	0.7	0.8	1.0	0.8	0.9	0.9	1.0	1.6	**

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	25mo	F sig
1539	α-copaen-11-ol	t	0.3	t	t	t	t	t	t	t	nt
1548	elemol	5.1	5.3	5.1	7.2	5.4	5.5	5.8	8.8	10.6	**
1555	elemicin	0.8	0.8	0.5	0.8	0.9	0.7	1.1	0.7	0.5	**
1565	(3Z)-hexenyl benzoate	0.2	t	0.2	0.2	0.3	0.2	t	t	t	nt
1574	germacrene-D-4-ol	2.8	3.4	3.4	2.6	3.5	3.0	3.8	3.4	3.6	**
1630	γ-eudesmol	0.3	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.3	nt
1638	epi-α-cadinol	0.6	0.6	0.5	0.6	0.6	0.6	0.6	0.9	0.8	**
1638	epi-α-muurolol	0.6	0.6	0.5	0.7	0.6	0.6	0.7	0.8	0.8	**
1649	β-eudesmol	0.4	0.5	0.4	0.5	0.2	0.6	0.6	0.7	1.0	**
1652	α-eudesmol	0.6	0.7	0.6	0.6	0.7	0.7	0.8	0.9	1.3	**
1652	α-cadinol	1.0	1.0	0.8	1.0	1.0	1.1	1.2	1.4	1.0	**
1670	bulnesol	0.5	0.4	0.4	0.3	0.5	0.5	0.6	0.5	0.2	**
1688	shyobunol	t	t	t	t	0.2	0.2	t	t	t	nt
1746	8-α-11-elemodiol	t	t	0.2	t	0.3	0.4	0.3	t	t	nt
1761	iso to 8-α-acetoxyelemol	0.2	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.3	nt
1792	8-α-acetoxy-elemol	8.1	9.3	6.3	7.5	12.3	10.5	10.7	12.4	11.8	**
2054	41,81,137,270,	0.2	0.2	t	0.3	0.3	0.3	0.3	0.4	0.5	**
2087	abietadiene	t	t	t	t	t	t	t	t	t	**
2298	4-epi-abietal	0.4	0.3	0.3	0.2	0.4	0.4	0.3	0.5	0.6	**

KI	compound	fresh	1 wk	2 wk	1 mo	2 mo	4 mo	8 mo	16 mo	25mo	F sig
2312	abieta-7,13-dien-3-one	t	t	t	t	t	t	t	0.1	0.6	**

KI = Kovats Index (linear) on DB-5 column (see Adams, 2007). Unidentified compounds have the major ions listed. The first ion (underlined) is the base (100%) ion. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

A NEW SPECIES OF *CARDAMINE* (BRASSICACEAE) FROM NUEVO LEON, MEXICO

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

A novel taxon, **Cardamine cebollana** B.L. Turner, **sp. nov.**, is described from Sierra Cebolla, Mpio. Montemorelos, Nuevo Leon, Mexico. A photograph of the holotype is provided, along with a map showing its distribution, vis-a-vis, other taxa in the immediate area concerned. *Phytologia 94(3): 384-387 (December 1, 2012).*

KEY WORDS: Brassicaceae, *Cardamine*, Mexico, Nuevo Leon, Sierra Cebolla

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Routine identification of plant taxa from north-central Mexico has occasioned the present paper.

CARDAMINE CEBOLLANA B.L. Turner, **sp. nov. Fig. 1.**

Perennial, glabrous, herbs to 15 cm high arising from perennial taproots, 3-4 mm thick and the stems divaricately branched at its apex. **Basal leaves** 3-4 cm long, 1-2 cm wide, pinnately parted, so far as known, not persisting. **Stem leaves** (lower) similar to the basal, but larger (4-5 cm long), the 4-8 lateral segments oblanceolate, entire, or asymetrically basally lobed, about the same size as the terminal segments; petioles 2-3 cm long, the leaves gradually reduced upwards. **Inflorescence** a divaricately branched assemblage of ca 20 stems, the whole ca 8 cm high, and as wide, the branches not at all fractiflex. **Pedicels** (fruiting) 5-8 mm long. **Sepals** purple or purplish, ca 2 mm long. **Petals,** white, oblancelate, 2-4 mm long, 0.5-1.0 mm wide. **Anthers,** yellow, 4, ca 0.3 mm long. **Siliques** 1.5-2.0 cm long, ca 1

mm wide, erect, glabrous. **Seeds** ovoid, ca 1 mm long, 0.8 mm wide, glabrous, minutely rugose, 18-20 per fruiting body.
TYPE: **MEXICO. NUEVO LEON: Mpio. Montemorelos,** "6 Km SE of La Trinidad, in La Sierra Cebolla, just below the summit of one [sic] the picachos, on limestone, in fir-oak-Cupressus woodland." 2900 m, 25 11 N, 100 07 W, 5 Aug 1988, *T. F. Patterson 6150*(Holotype: TEX).

In the treatment of *Cardamine* for Mexico and Central America by Rollins (1993), largely because of its perennial habit and reddish sepals, *C. cebollana* will key to or near *C. eremita* Standl. & Steyerm., a localized, alpine, endemic of Guatemala.

Amongst Mexican taxa of *Cardamine*, the novelty appears to have no close relatives, although it resembles, vegetatively, *C. macrocarpa* Brandegee, but lacks the fractiflex racemes and large siliques of that species.

The novelty is named for the Sierra La Cebolla, to which it seems confined; the latter locale and immediate environs is home to a number of localized endemics, including *Pinarpappus pattersonii* B.L. Turner and *Senecio pattersonii* B.L. Turner (Turner 1988; Turner 1996) and, more importantly, the very localized, *Picea martinezii* T.F. Patterson (Patterson 1988).

ACKNOWLEDGEMENTS

I am grateful to my field companion, Jana Kos, for proof-reading the paper, and to my Academic son, Tom Patterson, for helpful comments.

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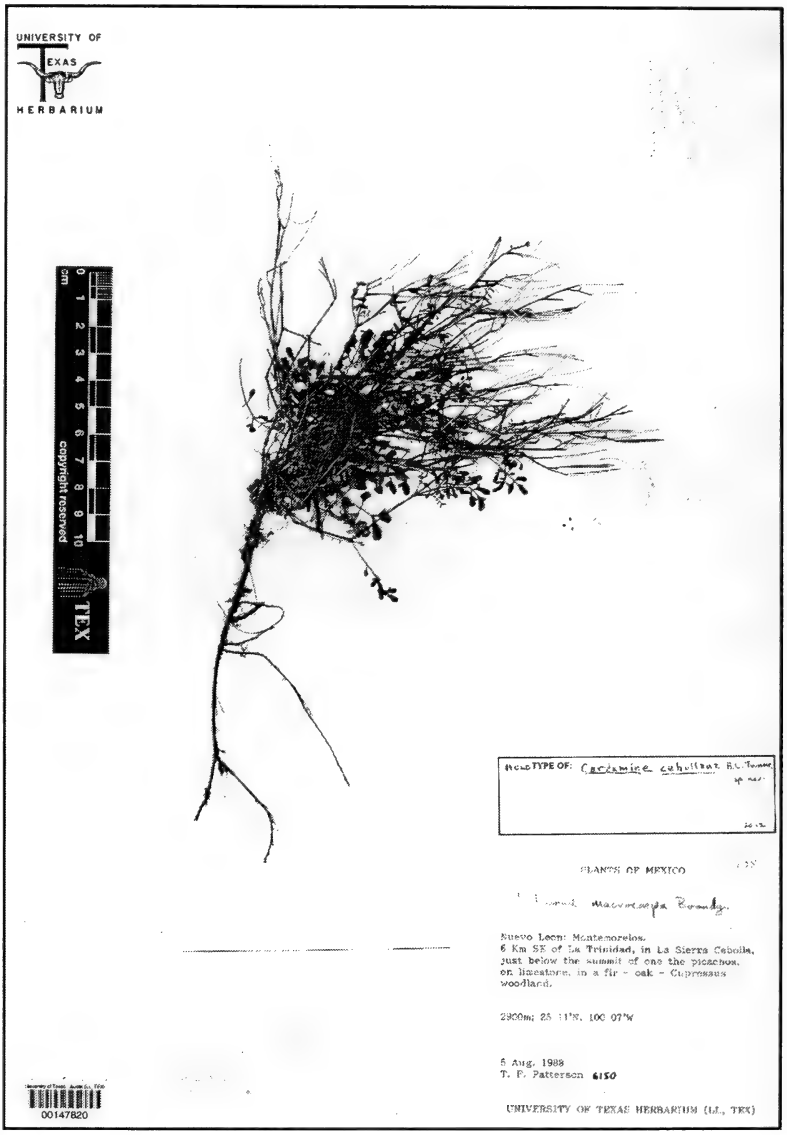


Fig. 1. Photograph of *Cardamine cebollana* (Holotype: TEX).

Fig. 2. *Cardamine cebollano* (details from type).

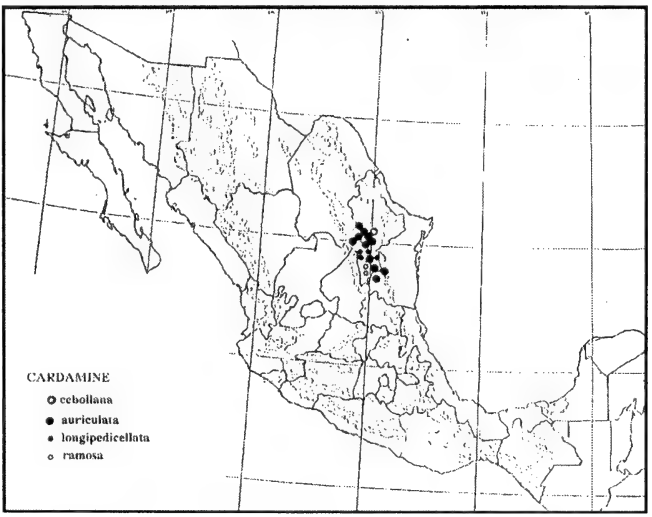


Fig. 3. Distribution of *Cardamine* spp. in vicinity of Sierra Cebolla.

TAXONOMY OF THE TURBINATE SHAPED SEED CONE TAXA OF *JUNIPERUS*, SECTION *SABINA*: SEQUENCE ANALYSIS OF nrDNA AND FOUR cpDNA REGIONS

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798, USA email Robert_Adams@baylor.edu

and

Andrea E. Schwarzbach
Department of Biomedicine, University of Texas at Brownsville, Brownsville, TX 78520, USA.

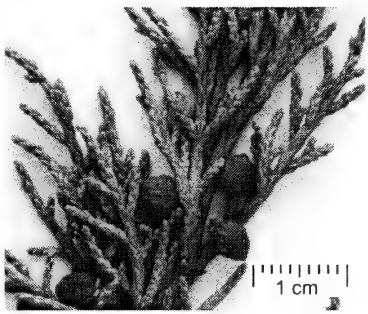
ABSTRACT

An analysis of the turbinate shaped seed cone taxa of *Juniperus* sect. *Sabina* is presented, based on nrDNA and four cpDNA regions. These DNA data revealed several previously unknown affinities. Plants collected as *J. przewalskii* f. *pendula* were found to be *J. convallium* (***J. convallium* forma *pendula*, comb. nov.**). Several infraspecific taxa were found to be so distinct to warrant recognition at the specific level: *J. fargesii* (=*J. squamata* var. *fargesii*); ***J. uncinata*, stat. & comb. nov.** (= *J. recurva* var. *uncinata*); *J. carinata* (= *J. pingii* var. *carinata*); ***J. rushforthiana*, stat. nov.** (= *J. indica* var. *rushforthiana*). *J. squamata* f. *wilsonii* is best treated as ***J. squamata* var. *wilsonii*, comb. nov**. *Phytologia 94(3): 388-403 (December 1, 2012).*

KEY WORDS: Taxonomy, *Juniperus*, section *Sabina*, turbinate seed cones, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, *J. convallium* f. *pendula,* comb. nov., *J. uncinata*, stat. & comb. nov., *J. squamata* var. *wilsonii*, com. nov., *J. rushforthiana,* stat. nov.

The turbinate shaped seed cone junipers of section *Sabina* comprise a distinct clade (Mao et al., 2010), having seed cones with elongated, pointed tips (Fig. 1). The cone elongation is more

apparent in immature cones. Although most of the taxa have only scale-like leaves in the adult foliage (Fig. 1), several taxa have only decurrent type leaves in the adult foliage (Fig. 2). Decurrent leaves (juvenile) have a sheath that clasps the stem and a free blade that may be appressed (*J. squamata* var. *squamata*, Fig. 2), free at about 30 - 45° (*J. sq.* f. *wilsonii*), free at about 60° - 90° (*J. sq.* var. *fargesii*), elongated and slightly free (10° - 20°) in *J. morrisonicola*, to very appressed as in *J. recurva* and *J. coxii* (Fig. 2). Because the seed cones are similar, decurrent leaf shape has been used to lump *J. squamata*, *J. sq.* f. *wilsonii* and *J. sq.* var. *fargesii* and *J. morrisonicola* (*J. sq.* var. *morrisonicola* (Hayata) H-L. Li & H. Keng).



J. convallium

Figure 1. *J. convallium* leaves and turbinate shaped seed cones.

Juniperus squamata has, historically, included several taxa with decurrent leaves that don't fit into other turbinate junipers. Part of the confusion seems to have arisen by the nature of the type specimen from a sheet that has three specimens from at least two and likely, three taxa (Fig. 3); Farjon (2005, p. 382) designated this sheet as the lectotype for *J. squamata*, but he failed to indicate which one of the specimens. As the sheet contains three plants, the lectotype is clarified here as: *W. S. Webb W 6043C* (lectotype K-W, right-most specimen on sheet *W 6043C*). The original description of Buch.-Ham. [Lambert, Descr. Pinus 2: 17 (1824)] reads "leaves 3, appressed, imbricate, ovate-oblong, acute, acuminate; withered persistent; inflexed when young with apex somewhat obtuse, fruits ovate and umbilicate at the top, branches and branchlets crowded, terete, stem prostrate." Clearly, the right-most specimen best fits the description of *J. squamata.*

A comparison of leaves from the type specimens of *J. squamata*, f. *wilsonii* and var. *fargesii* (Fig. 4) reveals the nature of the appressed leaf blades in *J. squamata* and f. *wilsonii*, and the free, very

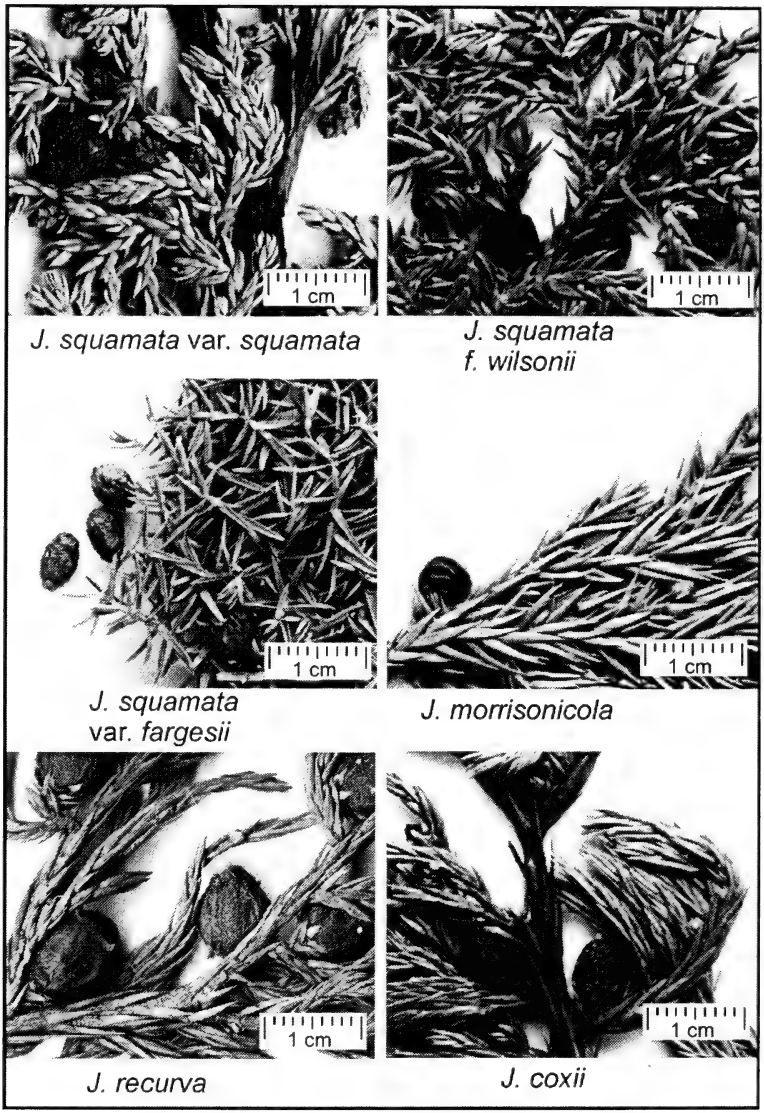


Figure 2. Comparison of the decurrent leaves of six turbinate seed cone taxa.

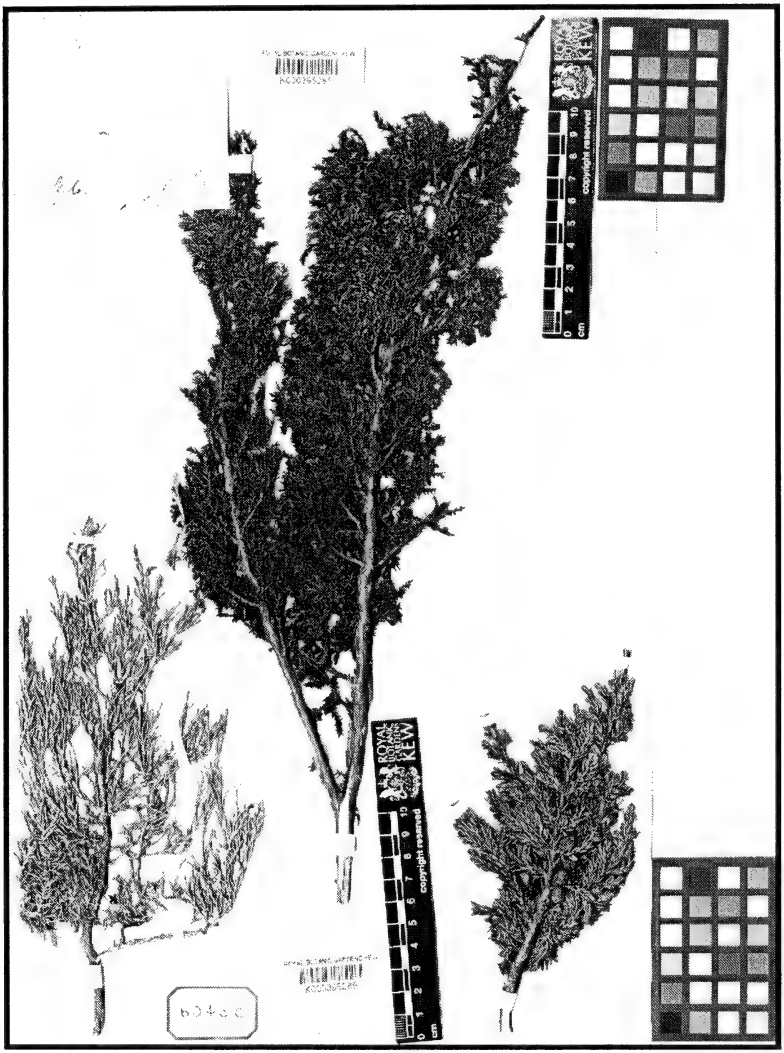


Figure 3. Photo of type of *J. squamata* Buch.-Ham., *W. S. Webb, W 6043C* at K (K000395285). Left-most: cf. *J. indica*, center: cf. *J. squamata* var. *fargesii*, right-most: *J. squamata*.

divergent leaf blades in *J. squamata* var. *fargesii*. The seed cones of *J. squamata* appear larger than those of *J. s.* f. *wilsonii* (Fig. 4).

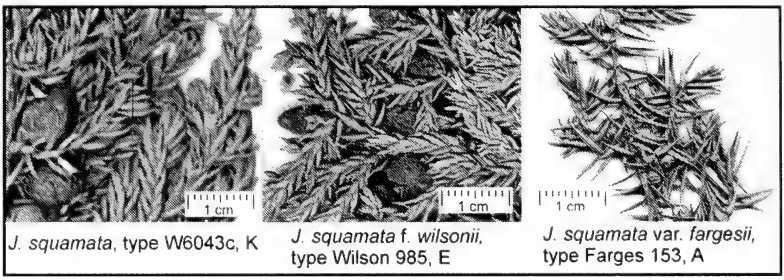


Figure 4. Comparison of leaves from the type specimens of *J. squamata*, f. *wilsonii* and var. *fargesii*.

Although Mao et al. (2010) analyzed several of the turbinate junipers (*J. convallium, J. coxii, J. indica, J. komarovii, J. pingii, J. przewalskii, J. pseudosabina, J. saltuaria, J. squamata, J. tibetica*), they did not include *J. indica* var. *caespitosa, J. i.* var. *rushforthiana, J. microsperma, J. morrisonicola, J. pingii* var. *carinata, J. przewalskii* f. *pendula, J. recurva* var. *recurva, J. r.* var. *uncinata, J. squamata* var. *fargesii and J. s.* f. *wilsonii*.

The purpose of the current study is to present analysis of all the turbinate seed cone taxa of section *Sabina* using the most informative nuclear (nrDNA - ITS) and cpDNA regions (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF).

Table 1. Comparison of Adams and Farjon taxonomic treatments of taxa in this study. Taxa with DNA sequencing support for a modified taxonomic status are in bold.

Adams(2011)	Farjon (2005, 2010)	Supported, this study
J. convallium	*J. convallium*	*J. convallium*
J. coxii	*J. recurva v. coxii*	*J. coxii*
J. indica	*J. indica*	*J. indica*
J. i. var. caespitosa	*J. i. var. caespitosa*	*J. recurva* var. *caespitosa*?
J. i var. rushforthiana	*J. indica*	***J. rushforthiana***
J. komarovii	*J. komarovii*	*J. convallium* var. *komarovii*?
J. microsperma	*J. convallium* var. *microsperma*	*J. microsperma*
J. morrisonicola	*J. squamata*	*J. morrisonicola*
J. pingii	*J. pingii*	*J. pingii*
J. p. var. carinata	*J. p. var. wilsonii*	***J. carinata***
J. przewalskii	*J. przewalskii*	*J. przewalskii*
J. p. f. pendula	*J. przewalskii*	***J. convallium f. pendula***
J. pseudosabina	*J. pseudosabina*	*J. pseudosabina*
J. recurva	*J. recurva*	*J. recurva*
J. r. var. *uncinata*	*J. recurva?*	***J. uncinata***
J. saltuaria	*J. saltuaria*	*J. convallium var. saltuaria*?
J. squamata	*J. squamata*	*J. squamata*
J. s. var. fargesii	*J. squamata*	***J. fargesii***
J. s. f. *wilsonii*	*J. pingii* f. *wilsonii*	***J. s.*** var. ***wilsonii***
J. tibetica	*J. tibetica*	*J. tibetica*

MATERIALS AND METHODS

Specimens used in this study: *J. convallium, Adams 6781-83,* 17 km e Tewo, Gansu, China, *J. communis* L. var. *communis*, *Adams 7846, 7847*, Stockholm, Sweden (outgroup), *J. coxii, Adams 8137-38,* clone from Type tree, Abbotsmarsh Arboretum, UK, ex Burma, Chimili valley, *J. indica, Adams 8775-77,* Dumpa, Jomson, Nepal, *J. indica* var. *caespitosa, Adams 7625-27,* near Yangjin Gompa, Nepal, *J. indica* var. *rushforthiana, Adams 8140-41,* Abbotsmarsh Arboretum, UK, ex Lingshi, Bhutan, *J. komarovii, Adams 8518-20,* near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. microsperma, Adams 8522-24,* near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. morrisonicola, Adams 8681-2,* Younger Bot. Gard., Scotland, ex Taiwan, *J. pingii, Adams 8506-7,* near White Horse Mtn., Deqin County, Yunnan, China, *J. pingii var. carinata, Adams 8497-99,* near White Horse Mtn., Deqin County, Yunnan, China, *J. pseudosabina, Adams 7808-10,* 30 km n Jarkent (Paniflor), Kazakhstan, *J. przewalskii, Adams 6775-77,* 25 km w Jone, Gansu, China, *J. przewalskii* f. *pendula, Adams 6779,* Langmusi, Gansu, China, *J. recurva, Adams 7215, 7217, 7219,* Cholan Pati lodge, Nepal, *J. recurva* var. *uncinata, Adams 7212-14,* Lauri Binayak, Nepal, *J. saltuaria, Adams 6789-91,* on Duoer River, 23 km e Forestry Station, Gansu, China, *J. squamata, Adams 6795-96,* Xian Bot. Garden, Shaanxi, China, *J. s. var. fargesii, Adams 8491-93,* near White Horse Mtn., Zhongdian County, Yunnan, China, *J. s.* f. *wilsonii, Adams 5521,* Accession 1010-64A, cultivated from seeds from *E. H. Wilson 985* (Holotype) collection, Arnold Arboretum, USA, ex. China, *J. tibetica, Adams 8512-16,* on Maerkan River, near Zhe Gu Mtn., Maerkan County, Sichuan, China. Voucher specimens are deposited in the herbarium, BAYLU, Baylor University.

DNA extraction, PCR amplification, sequencing and data analyses - see Adams and Schwarzbach (2012a).

RESULTS AND DISCUSSION

The turbinate seed cone junipers are relatively uniform with a few distinct groups (Fig. 5). As previously reported (Adams 2011), *J. recurva* and *J. indica* taxa are in a clade, but well resolved from *J.*

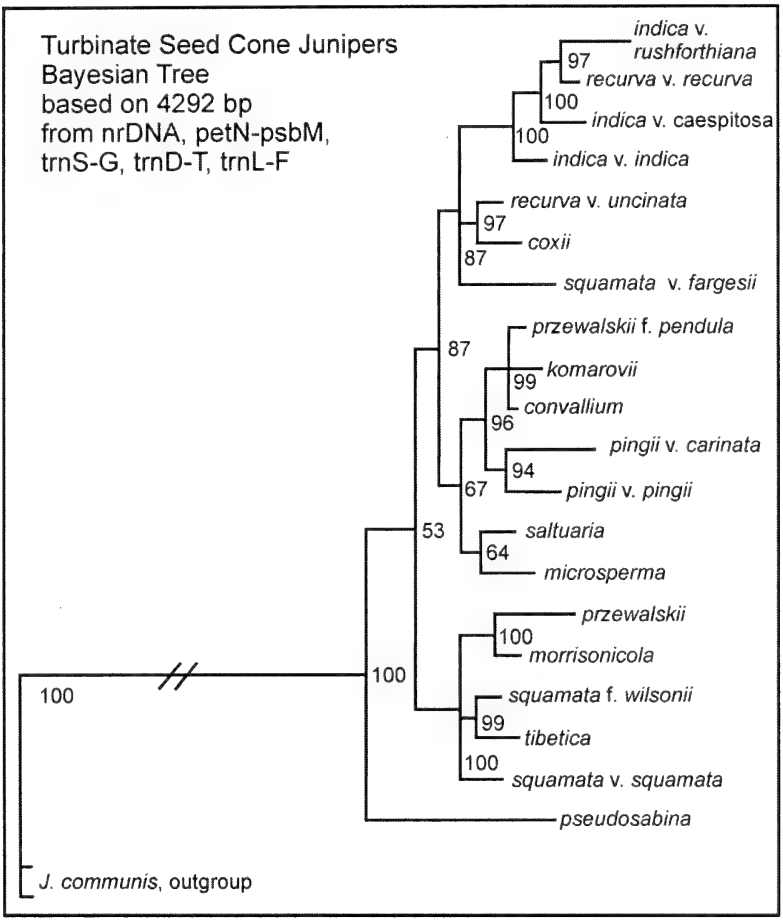


Figure 5. Bayesian tree for the turbinate seed cone taxa, sect. *Sabina*. Numbers at the branch points are posterior probabilities (as percent).

indica var. *rushforthiana* (Fig. 5). *Juniperus recurva* is a small tree with lax foliage and tightly appressed decurrent leaves (Fig. 2), and *J. indica* has generally erect foliage with typical scale-like leaves of (cf. Fig. 1), otherwise, the taxa are similar in morphology (Adams 2011). *Juniperus recurva* var. *uncinata* is in a clade with *J. coxii* and *J. squamata var. fargesii.*

Juniperus convallium, J. komarovii and the putative *J. przewalskii* f. *pendula* are in an unresolved clade (Fig. 5). *J. pingii* and *J. p.* var. *carinata* are in a clade, but are well differentiated. *J. microsperma* and *J. saltuaria* are associated with the *J. convallium - komarovii* clade (Fig. 5). Finally, it is noteworthy that *J. przewalskii, J morrisonicola, J. squamata* f. *wilsonii, J. s.* var. *squamata* and *J. tibetica* form a distinct clade (Fig. 5). *J. pseudosabina* is well supported as an outlier to the other turbinate junipers (Fig. 5).

Taxonomic Considerations

In addition to illuminating phylogeny, DNA sequence data are useful in validating and elucidating taxonomy. Coding all nucleotide substitutions and indels (as single mutations) resulted in 225 mutational events (MEs). These 225 MEs were used to construct a minimum spanning network (Fig. 6). Two patterns are immediately visible: the central nature of *J. convallium* among the taxa, and relative uniformly large differences between taxa (even taxa in the same species, such as *J. indica, J. i.* var. *caespitosa, J. i.* var. *rushforthiana*, Fig. 6).

The most obvious taxonomic anomaly is the difference of only 2 MEs (out of 225) between *J. convallium* and putative *J. przewalskii f. pendula* (Fig. 6). Clearly, this pendulous form is a part of *J. convallium* and should be recognized as:

Juniperus convallium* forma *pendula (Cheng & L-K. Fu) R. P. Adams, **comb. nov. Basionym:** *Sabina przewalskii* (Kom.) W-C., Cheng & L-K. Fu f. *pendula* Cheng & L-K. Fu, Acta Phytotax. Sin. 13(4): 86. 1975. Type: PE. *Juniperus przewalskii* f. *pendula* R. P. Adams and G-L. Chu, J. Essential Oil Res. 6:17. 1994.

The *J. convallium - komarovii - saltuaria* complex is separated by only 6 - 8 MEs (Fig. 6). Adams and Schwarzbach (2012a, 2012b) found varieties in *Juniperus* sections *Juniperus* and *Sabina* to differ by 6 - 8 MEs, whereas generally accepted species differed by 10 - 12 to 20 MEs (using the same set of gene regions). Both Adams (2011) and Farjon (2005, 2010) recognize these three as species in their monographic treatments (Table 1); it seems wise to continue such

recognition , although there is only marginal support for such treatment in the DNA data set.

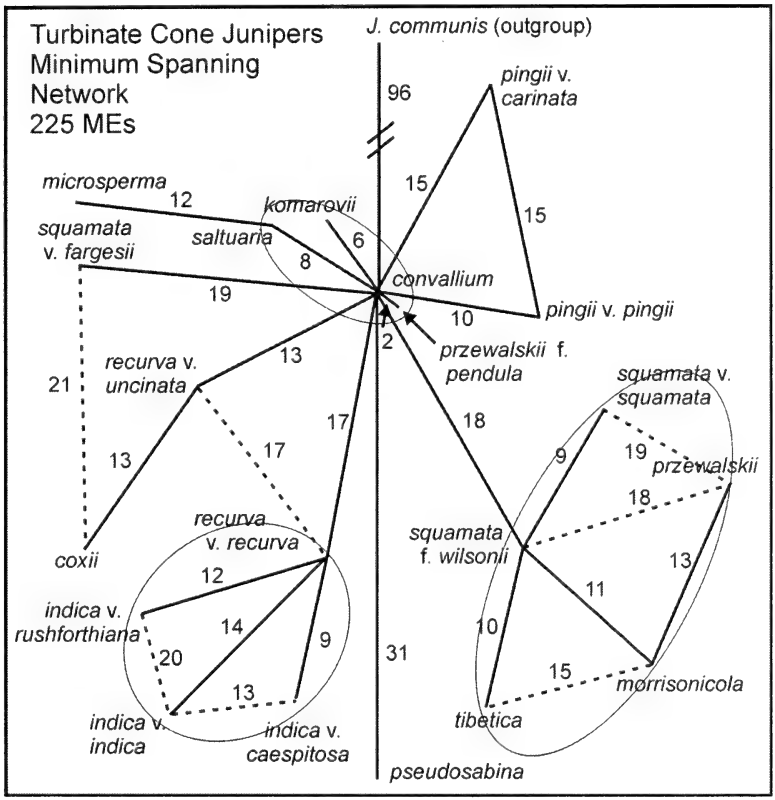


Figure 6. Minimum spanning network based on 225 MEs from nrDNA, and cpDNA (petN-psbM, trnS-G, trnD-T, trnL-F). Numbers next to the lines are the number of MEs separating taxa. Dashed lines are the second nearest link.

Juniperus pingii is a tall tree, with long, sharp-pointed decurrent leaves and *J. pingii* var. *carinata* is a small shrub with short, scale-like leaves (Fig. 7). *Juniperus pingii* differs by only 10 MEs

from *J. convallium* (Fig. 6), whereas *J. p.* var. *carinata* differs by 15 MEs from both *J. pingii* and *J. convallium* (Fig. 6). The taxa are easy to identify by their leaves and habit. The morphology and DNA data support the recognition of *J. carinata* R. P. Adams, [Biochem. Syst. Ecol. 28: 541 (2000)].

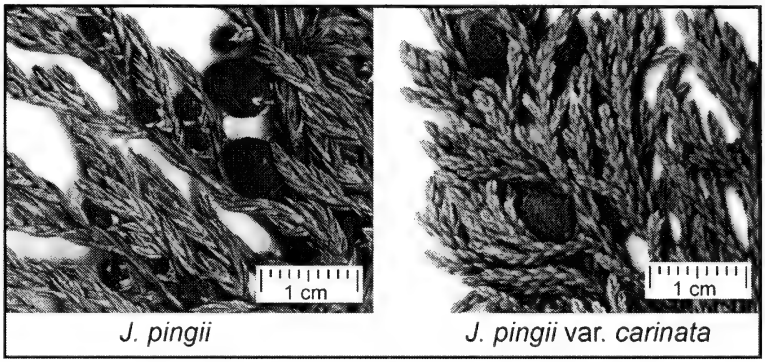


Figure 7. Leaves and seed cones of *J. pingii* and *J. p.* var. *carinata.*

Juniperus squamata var. *fargesii* has very unusual leaves with the blades divergent at 60 - 90° from the stem (Fig. 4) these differing from *J. squamata*. The placement of *J. s.* var. *fargesii*, in both the Bayesian tree and the network, is distantly separated from *J. squamata* (Figs. 5, 6). It is 19 MEs from *J. convallium* and 21 MEs from *J. coxii* (Fig. 6). Both morphology and DNA support the recognition of *Juniperus fargesii* (Rehder & E. H. Wilson) Kom.

Juniperus recurva var. *uncinata* is a shrub with hooked branchlets (Adams et al. 2009) from Nepal and appears, morphologically, to be closely related to *J. recurva* (Fig. 8). *Juniperus recurva* and *J. r.* var. *uncinata* hybridize in the area of Cholan Pati, Nepal (Adams et al. 2009). However in the present analysis, the taxa are in different clades (Fig. 5). *Juniperus r.* var. *uncinata* differs by 13 MEs from both *J. convallium* and *J. coxii*, and by 17 MEs from *J. recurva* (Fig. 6). Theses differences give strong support for the recognition of:

Juniperus uncinata (R. P. Adams) R. P. Adams, **stat. & comb. nov.**, **Basionym**: *Juniperus recurva* var. *uncinata* R. P. Adams, Phytologia 91(3): 365 (2009). Type: BAYLU.

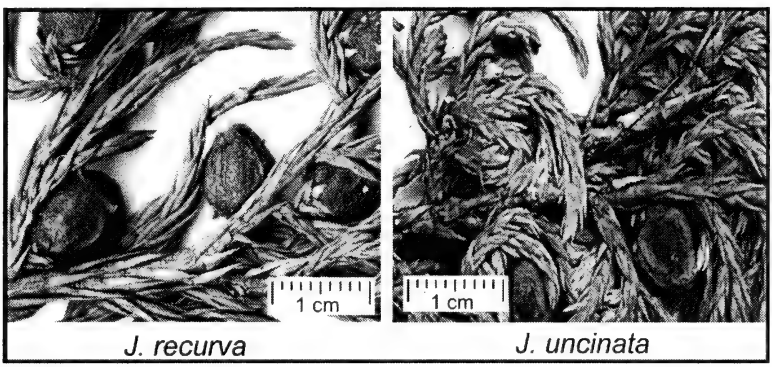


Figure 8. Comparison of the leaves and seed cones of *J. recurva* and *J. uncinata*. Note the hooked branchlets of *J. uncinata*.

Juniperus squamata f. *wilsonii* was collected by E. H. Wilson on his 1907-1909 China expedition. Seeds from this collection were germinated by Arnold Arboretum and three plants are currently in cultivation at Arnold Arboretum; DNA from one of these was utilized in the present study. The blade tips are more spreading in cultivation than in the type specimen (Fig. 9). *Juniperus s.* f. *wilsonii* is loosely associated with *J. squamata* (Fig. 6) by 9 MEs vs. 10 MEs to *J. tibetica* and 11 MEs to *J. morrisonicola*). The category *forma* is used to recognize a minor variant in a population that differs by only one or a few genes. In short, DNA data indicate that *J. s.* f. *wilsonii* is well differentiated from *J. squamata* (Fig. 6) and supports its recognition as a variety:

Juniperus squamata* var. *wilsonii (Rehder) R. P. Adams, **comb. nov.** **Basionym**: *Juniperus squamata* f. *wilsonii* Rehder, J. Arn. Arb. 1: 191 (1920). Type: A.

Sabina pingii (W. C. Cheng ex Ferre) W. C. Cheng & W. T. Wang var. *wilsonii* (Rehder) W.C. Cheng & L.K. Fu, Fl. Reipubl. Pop. Sin. 7: 356 (1978)

S. squamata (Buch.-Ham. ex D. Don) Antoine var. *wilsonii* (Rehder) W. C. Cheng & L.K. Fu, [Chin. title; see Fl. Sichuan 2:177 (1983)] 1: 320 (1972)
S. wilsonii (Rehder) W.C. Cheng & L.K. Fu, Forest Sci. Techn. 4: 455 (1981)
J. wallichiana Hook. f. & Thomson ex E. Brandis var. *loderi* Hornibr., Gard. Chron., ser. 3, 85: 50 (1929)
J. squamata Buch.-Ham. ex D. Don var. *loderi* (Hornibr.) Hornibr. in Chittenden, Rep. Cult. Conif.: 74 (1932)

Clearly, *J. s.* var. *wilsonii* is not part of *J. pingii* (Figs. 3, 4, Table 1) and the present DNA data give no support for the recognition of *J. pingii* f. *wilsonii* of Silba (1984).

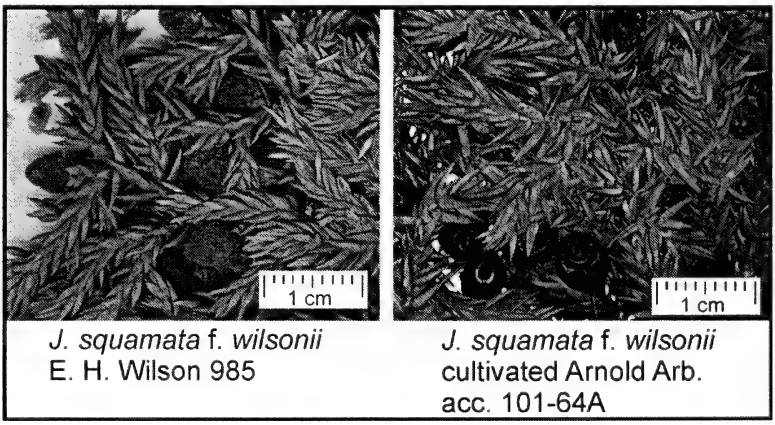


Figure 9. Comparison of *J. s.* f. *wilsonii*, specimen and cultivated.

It should be noted that the recognition of *J. s.* var. *wilsonii*, although well supported by the present DNA data, does not resolve the taxonomy of the *J. squamata - tibetica - morrisonicola - przewalskii* complex (Fig. 6). This group differs by 18 MEs from *J. convallium* (Fig. 6) with internal links of 9 to 13 MEs. At present, it seems prudent to continue to recognize these four species (Table 1).

The *J. indica - recurva* group differs by 17 MEs from *J. convallium* and from 9 to 14 MEs within the group (Fig. 6). *Juniperus*

indica var. *rushforthiana* differs by 12 MEs from *J. recurva* and 20 MEs from *J. indica* (Fig. 6) giving support for recognition as a species:

Juniperus rushforthiana (R. P. Adams) R. P. Adams, **stat. nov.**
Basionym: *Juniperus indica var. rushforthiana* R. P. Adams, Phytologia 90(2): 244 (2008). Type: BAYLU.

Juniperus indica var. *caespitosa* differs by 13 MEs from *J. indica* and 9 MEs from *J. recurva*. Additional sampling and sequencing is in progress to better understand this group.

A graphic summary of the current turbinate juniper taxonomy is shown in Figure 8.

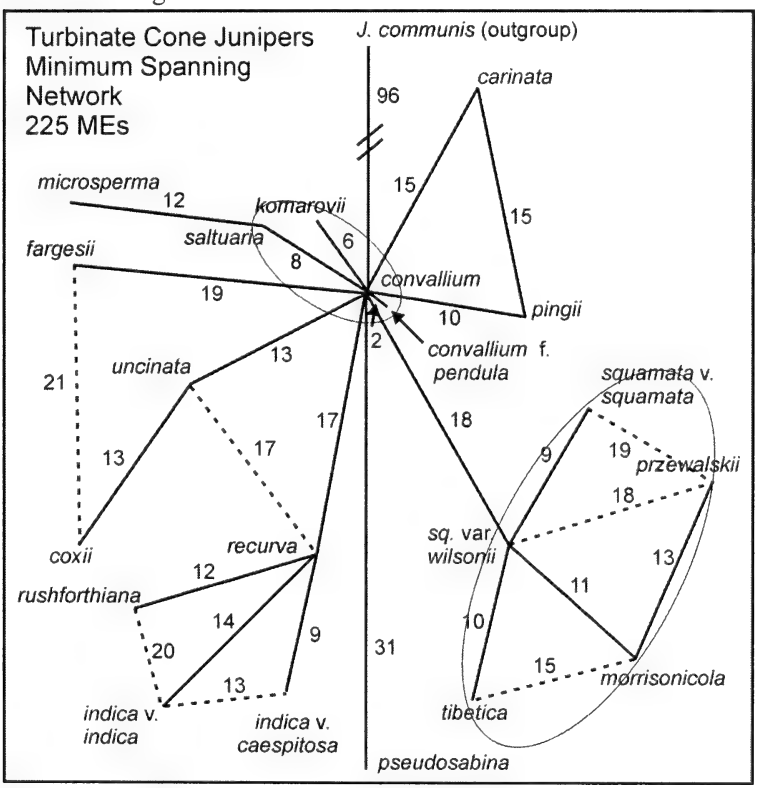


Figure 10. Minimum spanning network of the turbinate junipers as recognized in this study.

The turbinate junipers are found chiefly in western China and the eastern Himalayan Mtns. in small isolated populations that appear to be relictual. Clearing of land for agriculture, grazing by goats, use of foliage for incense (Nepal), and fuel wood utilization have decimated many of these species' populations. Whether these extremely small populations (often only a few individuals) are representative of the original taxa is not known, but genetic drift is very likely responsible for the large variation in morphology encountered. Additional studies involving extensive field work are needed in the turbinate junipers.

ACKNOWLEDGEMENTS

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PHYLOGENETIC PLACEMENT OF A RECENTLY DESCRIBED TAXON OF THE GENUS *PLEODENDRON* (CANELLACEAE)

Elizabeth A. Zimmer
Department of Botany, MRC 166, National Museum of Natural History, Smithsonian Institution, Box 7012, Washington, DC 20013-7012, USA, zimmerl@si.edu

Youngbae Suh
Natural Products Research Institute, Seoul National University, Seoul, Korea

Kenneth G. Karol
The Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies, New York Botanical Garden, Bronx, NY 10458-5126

ABSTRACT

In order to determine placement of the recently described plant species, *Pleodendron costaricense* (Canellaceae), five DNA regions were sequenced. For the new species, those included two from the nuclear rRNA coding region, ITS and 18S, and three from the chloroplast genome, the genes for *rbc*L and *atp*B and the spacer trnLF region. For the 18 taxa of Canellaceae and sister group Winteraceae, ITS and *trn*LF sequences were published (Karol et al. 2000), while the other three regions were sequenced for this study. The aligned sequences were combined and analyzed with parsimony, likelihood and Bayesian programs. The single tree produced in these analyses provided 100% support for placement of *P. costaricense* in a monophyletic group with *Pleodendron macranthum* and *Cinnamodendron ekmanii*. This result suggests that nomenclatural changes for those three species should be considered. *Phytologia 94(3): 404-412 (December 1, 2012).*

KEYWORDS: Canellaceae, *Pleodendron*, nuclear ribosomal DNA (nrDNA), chloroplast DNA (cpDNA), phylogenetic analyses

The family Canellaceae, along with the family Winteraceae, make up the order Canellales (Stevens 2010). Canellaceae is a relatively small family (~16-20 species) of woody plants. Like the Winteraceae, the Canellaceae have a disjunct distribution, with species in the New World (South America and the Caribbean) and Africa, including Madagascar (Kubitzki 1993). Although several papers have concentrated on molecular phylogenetic relationships of the Winteraceae (Suh et al. 1993; Karol et al. 2000; Doust and Drinnan 2004; Marquinez et al. 2009) using nuclear ribosomal and chloroplast DNA spacers, those investigations used one to six species of Canellaceae, each from a different genus, and primarily as outgroups. Recently, a new species of Canellaceae was discovered in Costa Rica and described as *Pleodendron costaricense* (Hammell and Zemora 2005). We wished to place this new species in the molecular phylogeny produced in our previous paper that highlighted Winteraceae. To accomplish this, we sequenced *P. costaricense* for the ribosomal DNA (nrDNA) spacer ITS and the chloroplast DNA (cpDNA) region *trn*LF, as in Karol et al. (2000). We also increased the number of phylogenetically informative characters for all 19 taxa, adding sequences for their chloroplast genes *rbc*L and *atp*B and for their 18S nuclear ribosomal region. The single tree produced in our analyses differs from that found in a recent article of Salazar and Nixon (2008).

MATERIALS AND METHODS

Species sampled and sources of plant material: With the exception of the new accession of *P. costaricense*, vouchered as *Zamora et al. 2986* (Hammell and Zamora 2005), all samples and voucher numbers were those listed in Table 1 of Karol et al. (2000).

DNA amplifications and sequencing: DNA isolation and subsequent amplification and sequencing for the nrDNA ITS and *trn*LF regions were conducted for the *Pleodendron costaricense* sample as described in Karol et al. (2000). The 18S rDNA, *rbc*L and *atp*B regions were amplified from the 18 DNA samples obtained for the Karol et al. (2000) study along with the new *Pleodendron costaricense* sample. Primers and amplification conditions for all 19 taxa used were those from Karol et al. (2000) and Soltis et al. (2000). Sequencing was

performed according to standard procedures on an ABI automated sequencing instrument. Alignments were produced with the program Se-Al vsn 2.0a11 Rambaut (2000). ITS and *trn*LF alignments, which include a significant number of insertion/deletion characters, were added with the alignment of Karol et al. (2000) as a guide. The 18S region, because of its slow rate of change, and the *atp*B and *rbc*L sequences that encode proteins, these sequences were straightforward and aligned by eye.

Phylogenetic analyses: Analyses were performed for each single gene, for the two nuclear sequences combined, for the three chloroplast sequences combined and for the complete concatenated data set. Parsimony analyses were performed in PAUP* (Swofford, 2002) using the Branch-and-Bound algorithm and default parameters. Winteraceae sequences were used as the outgroup for the Canellaceae. Parsimony bootstrapping was done for 1000 replicates, again with branch-and-bound settings. Maximum likelihood analyses on the concatenated and individual data sets were performed with PAUP*, on a 32 processor cluster, using parameters settings derived from three iterations of ModelTest (Posada and Crandall, 1998). GARLI (Zwickl, 2006) gave a similar tree. Bayesian analyses were performed on the data sets with MrBayes (Huelsenbeck and Ronquist, 2003), also on the computer cluster. ILD and SH tests (Farris et al.,1994; Kishino and Hasegawa, 1989) were performed to determine whether the data from all five DNA regions could be combined.

RESULTS AND DISCUSSION

As noted above, alignment of the new sequences were easily accomplished by eye. In the concatenated data set (available upon request), positions 1-1411 represent *rbc*L; 1412-2855 *atp*B; 2856-4568 18S; 4569-5555 *trn*LF; and 5556-6334 ITS. For the new 19 taxa data sets (i.e., for *rbc*L, *atp*B and 18S) only minor indels were identified. The first, in *rbc*L, is a three-codon difference just before the UUA stop codon; the Winteraceae have a GAU (Asp) GTC (Val) UUG (Leu) sequence, whereas the Canellaceae is missing those three codons. The second, an additional AAU (Asn) codon in position 34 of *atp*B, is also present in *Cinnamodendron ekmannii* and the two *Pleodendron* species, but absent in all other taxa. Of the seven single base differences in the

18S rDNA sequence, six are autapomorphies in Winteraceae, and the seventh is informative in Winteraceae (C) and Canellaceae (T) overall, but autapomorphic in *Takhtajania* (-) and in *Pleodendron costarciense* (A). No new indels, relative to those in Karol et al. (2000), were identified for *trn*LF with the inclusion of the new *P. costaricense* sequence. For ITS, the region with the most indels in the published 18 taxon data set, only two autapomorphies, both single base insertions in *P. costaricense* relative to the other species, were observed.

The five individual DNA regions, the combined three chloroplast DNA regions, and the combined two rDNA regions were first analyzed separately to check for incongruence. The results of these ILD and SH tests indicated that combining the data sets did not violate the null hypothesis. With unweighted maximum parsimony, using the Branch-and-Bound option in PAUP*, a single most parsimonious tree was obtained for the concatenated data set, as well as for ITS separately (Fig.1). For *trn*LF alone, two trees with the same topology as in Karol et al. (2000), were obtained. Individual 18S, *rbc*L and *atp*B DNA trees were more unresolved, with the 18S region exhibiting the most polytomies. Combining the nuclear rDNA regions also gave a single tree identical to the ITS tree. Combining the chloroplast DNA regions resulted in 36 most parsimonious trees whose consensus generally agreed with the nrDNA trees, but was also less well-resolved. Bootstrap support values based on 1000 replicates for the combined sequence tree are given (Fig.1). Likelihood and Bayesian analyses gave the same topology as did maximum parsimony. The bootstrap values for likelihood, and the posterior probabilities for the latter, are also given on the tree (Fig. 1). In the single tree produced, and with all three algorithms, *Pleodendron costaricense* was in a clade with *Pleodendron macranthum* and *Cinnamodendron ekmannii*, with 100% support values. However, that clade had *Pleodendron* as a paraphyletic lineage, with *P. macranthum* and *C. ekmanii* actually forming a clade with 100% support values, and *P. costaricense* basal to that clade. Relationships of the four other Canellaceae were those seen in the study of Karol et al. (2000), albeit with much stronger support for the monophyly and the exact branching relationships of *Warburgia salutaris*, *Cinnamosma madagascariensis* and *Capsicodendron denisii*. *Canella winterana* remained outside that clade, but with no support as to its ultimate affinity.

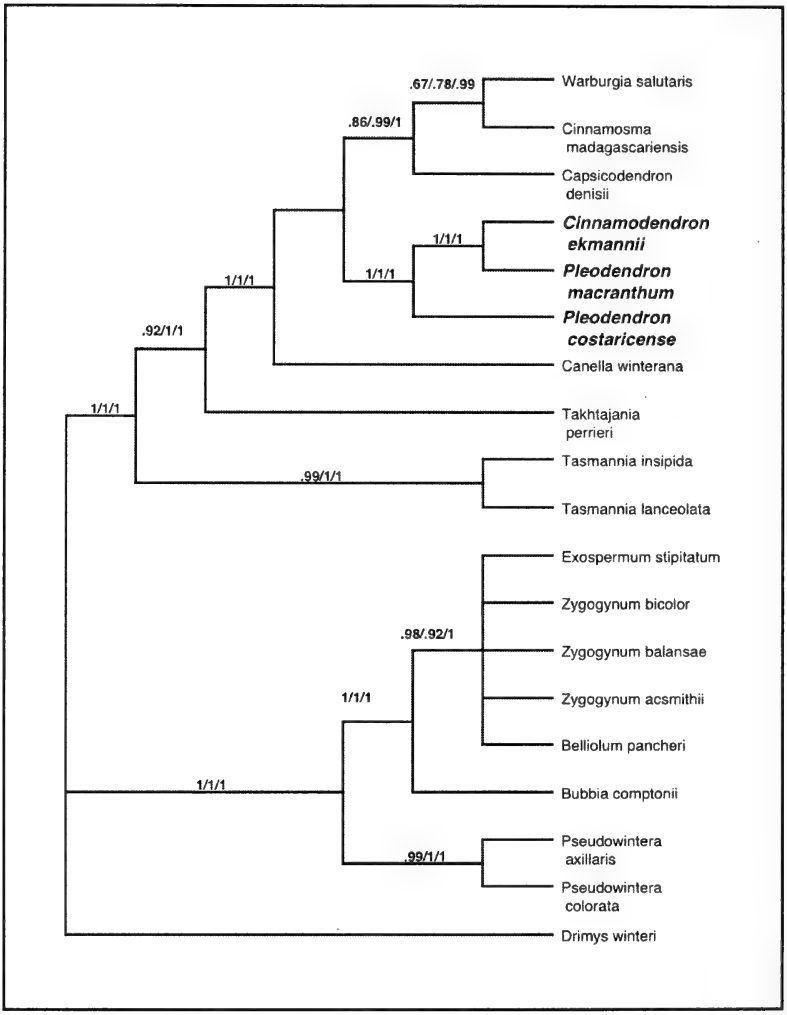

Figure 1. Most parsimonious single tree for Canellales based on combined data from five gene regions. Numbers above the nodes, from left to right, are for: maximum parsimony bootstrap support; maximum likelihood bootstrap support; Bayesian posterior probabilities.

The Winteraceae relationships, including *Takhtajania* as the basal lineage, and the polytomy seen for the closely related *Zygogynum/Exospermum/Belliolum* clade were basically those found in our previous study (Karol et al. 2000).

With the addition of data from three additional coding sequences, we continued to generate a phylogeny for the Canellales that agreed with our previous one in Karol et al. (2000). The placement of the new taxon described as *Pleodendron costaricense* was unequivocally shown to be sister to *Cinnamodendron ekmannii* and *Pleodendron macranthum* (Fig. 1). Given our results with the three taxa forming a completely supported monophyletic clade, these taxa should be combined into a single genus, presumably as *Cinnamodendron*, which has taxonomic priority (Kubitzki 1993).

Our study is in conflict with that of Salazar and Nixon (2008) for 49 morphological characters, and molecular data sets for five markers, three of which, ITS, *trn*LF and *rbc*L, are in common with ours. They also used the chloroplast *mat*K gene and the spacer *trn*D-*trn*T, whereas we included 18S rDNA and *atp*B. Their study included *Pleodendron costaricense* as well as several additional Antillean and South American species denoted as members of the genus *Cinnamodendron*. In addition to using Winteraceae as an outgroup, they also included four more distantly related "magnoliid" genera, *Illicium*, *Annona*, *Myristicum* and *Piper*. Unlike our results, Salazar and Nixon's phylogeny placed the two *Pleodendron* species as well-supported sister species which were in a monophyletic clade with the Antillean *Cinnamodendron*. Their consensus tree (of 42 most parsimonious ones) also placed *Capsicodendron* with the South American *Cinnamodendron*, unlike our single tree with *Capsicodendron* strictly aligned with *Warburgia* and *Cinnamosma*.

The discrepancy between the two studies is somewhat difficult to resolve. The differences for two markers are likely to cause this, as the topology of our tree is observed for ITS, *trn*LF and *rbc*L alone, with ITS providing the majority of base substitution and indel characters. At least some of the differences observed for our findings, versus those for the Salazar and Nixon, paper may be due to alignment issues for ITS and the chloroplast spacers. Unfortunately, their paper does not include

a Materials and Methods section describing their alignment and analyses procedures. Additionally, for the four outgroup magnoliids, we have not found it possible to align their ITS sequences with those of the Canellales (Suh et al. 1993). When their additional ITS, *trn*LF and *rbc*L sequences available in GenBank were included in our aligned data set for maximum parsimony and bootstrap analyses, we generated the same phylogeny as that in Fig.1 with respect to the *Pleodendron costaricense-Pleodendron macranthum-Cinnamodendron ekmanii* clade and the *Capsicodendron dinesii-Warburgia salutaris-Cinnamosma madagascariensis* one (data not shown). We did find that the Antillean *Cinnamodendron* species were allied with the currently named *Pleodendron* species, separate from the South American ones (which were allied with the *Capsicodendron dinesii-Warburgia salutaris-Cinnamosma madagascarensis* clade), as did Salazar and Nixon, so it is likely that their recommendation for naming a new genus for the South American group is appropriate.

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OBSERVATIONAL NOTE

WESTERN GRAY SQUIRRELS FORAGING CONES OF MCNAB CYPRESS

Frank Callahan
P.O. Box 5531, Central Point, OR 97502 USA
callahanseeds@gmail.com

ABSTRACT

This is the first known field report of the behavior of western gray squirrels cutting, transporting and consuming only the outer soft tissue of McNab cypress cones. Squirrels abandoned the partially eaten cones, which later desiccated and released the seeds for germination and potential tree establishment. Hopefully, this report will encourage additional field observations and reports on western gray squirrels and other cypress cone foragers. *Phytologia 94(3): 413-416 (December 1, 2012).*

KEY WORDS: *Hesperocyparis* (*Cupressus*) *macnabiana,* McNab cypress, western gray squirrel, seed dispersal.

McNab cypress (*Hesperocyparis macnabiana*) grows in northern California in the foothills bordering the Sacramento Valley. A disjunct population is also found on the south-facing slopes of Sprignett Butte in Jackson County, Oregon. The latter representing the northern limit of its distribution. This cypress is often found on nutrient imbalanced soils derived from ultramafic or basaltic parent materials derived from lava flows and not enriched by ash layers. Regarding dispersal mechanisms of seeds or cones of this species, my search of the literature has revealed no specific information.

I first noticed Western gray squirrels (*Sciurus griseus*) in northern California harvesting McNab cypress cones. After cutting cones from the trees, I also observed the gray squirrels carrying the cones to stumps or rocks before starting to feed. Gray squirrels (a prey

species) commonly transport cones to higher outposts where they can watch for potential predators.

Using field glasses, I spent the next hour watching their activity. During this time I observed squirrels take a cache of cones to a high rock and subsequently chew off the fleshy outside portion of each cone. Finally, they left the remains in the hot sun where they had been feeding.

Intrigued by the squirrels' behavior, I decided to investigate the effects on the cones. I discovered that many of the discarded cones opened as they dried out and were releasing many seeds. Initially, I was puzzled that the squirrels were not consuming the seeds. However, upon closer examination I discovered the seeds had sharp-bladed edges (Fig. 1). Once the squirrel chews off some of the waxy protective coating, the cone desiccates very rapidly - within hours as ground or rock temperatures can easily reach or surpass 140° F (60° C). I also noticed piles of loose seeds and rejected desiccated cones at many of the stations and young seedlings downhill from most of the sites. It appears the squirrels may not eat the seeds (either not in this instance or incompletely); this mirrors the activities of the Douglas squirrels use of *Sequoiadendron giganteum* cones as they also only eat the fleshy outer part of the cones and reject the fibrous inner cone elements (scales) that contain the seeds (Harvey et al 1980).

This leads me to hypothesize that the seeds might cut or irritate the squirrel's gums and are mostly avoided. If this hypothesis is correct, gray squirrel feeding behavior might provide an aid in cypress seed dispersal. My personal observations of McNab cypress recruitment, as evidenced by younger age classes, have been in the vicinity of gray squirrel feeding stations. Many of the young trees I have observed were located upslope from the main cypress stands; some as far as 60 m. Therefore, as a vector for dispersal, gray squirrels have likely moved seeds in the opposite direction of gravitational dispersal. To date, I have observed this behavior at two sites in Shasta County in northern California: Lack Creek and Round Mountain. At both sites, cypress trees ranged in age from seedlings and saplings to mature trees.

Figure 1. Composite of closed and opened cones and seeds of McNab Cypress. Note the sharp edges on the seeds.

Given that Arizona cypress (*H. arizonica*) cones (whose seeds also have sharp edges) make up more of the diet of Chiricahua fox squirrels (*Sciurus nayaritensis chiricahuae*) in southeastern Arizona than any other species (Koprowski and Corse 2001), the seeming avoidance of McNab seeds by Gray squirrels may be due to factors besides cutting their gums. Further study of gray squirrel feeding behavior, and its potential relationship to McNab cypress seed dispersal seems warranted.

As a side note, the largest known McNab cypress is located in the Lack Creek area. It is currently 21 m tall with a 21 m crown spread and an over 1.2 m basal diameter.

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PREDICTING AND QUANTIFYING POLLEN PRODUCTION IN *JUNIPERUS ASHEI* FORESTS

Landon D. Bunderson
Department of Biology, The University of Tulsa, Tulsa, OK 74104, landon-bunderson@utulsa.edu

Peter Van de Water
California State University, Fresno, 2576 E. San Ramon Ave., Mail Stop ST-24, Fresno, CA 93740

Harrington Wells and **Estelle Levetin**
Department of Biology, The University of Tulsa, Tulsa, OK 74104

ABSTRACT

Juniperus ashei pollen has been reported as a major airborne allergen in regions of Texas and Oklahoma. Pollen production across these populations was examined in order to support a pollen forecast system. Four locations in Texas and 2 locations in Oklahoma were chosen as study sites. Trees in each location were measured, cone production was evaluated based on a rating system, and percent tree cover was determined. Cone production was estimated by counting cones from $1/8^{th}$ sections of 10 representative trees. Additionally, vials of cones collected at each location were used to determine the number of pollen grains per cone. The 10 representative trees were used to test three models describing the relationship of pollen production to tree size: height, surface area, and volume. Using the pollen count data, tree measurements, the rating system, and the three models, three estimates of total pollen grains per hectare were produced. The highest producing area using the most conservative model predicted a total pollen production of 3.3×10^{13} pollen grains per hectare. Although there is great variability between the locations making it difficult to determine which factors are most important, differences in the amount of pollen production in each location could be partially predicted by percent juniper cover. *Phytologia 94(3): 417-438 (December 1, 2012).*

KEY WORDS: *Juniperus ashei*, pollen, allergy, pollination ecology

Airborne allergens are a major contributor to allergic disease. As such, airborne pollen concentrations have been monitored for decades in many parts of the world (Gregory, 1973; Lacey & Venette, 1995). Much effort has gone into predicting the season start, daily concentration, and peak date of various allergenic pollen types (Garcia-Mozo et al., 2002; Adams-Groom et al., 2002; Schappi et al., 1998; Norris-Hill, 1995; Raynor and Hayes, 1970; Garcia-Mozo et al., 2009). Although many studies have focused on local release and deposition, daily concentrations can be affected by both local and long-distance pollen producers (Skjøth et al., 2007; Van de Water and Levetin, 2001). Wind trajectories have been used to track the long-distance transport of pollen and mold spores (Gregory, 1973; Aylor et al., 1982; Van de Water and Levetin, 2001; Skjøth et al., 2007; Skjøth et al., 2008). These deterministic models predict trajectory, but are not predictive of how much pollen is transported. In order to produce a more precise pollen forecast, the disciplines of aerobiology, meteorology, plant phenology, and plant ecology must be combined (Levetin and Van de Water, 2003). More complicated prediction systems which use estimates of source contribution have been created to track dust, pollen, smoke, and other bioaerosols (Sofiev et al., 2006; Nickovic et al., 2001; Jain et al., 2007). Although little information exists on pollen source contribution, there are some noteworthy studies.

Anemophilous plants often produce large amounts of pollen and there have been attempts to quantify production per anther in several angiosperms (Subba Reddi and Reddi, 1986) and per cone in gymnosperms (Hidalgo et al., 1999). One study estimated source contribution of Poaceae pollen production in NW Morocco. This study identified various representative zones and estimated pollen production in each area. Number of pollen grains per anther, anthers per flower, flowers per spikelet, and number of spikelets per inflorescence were estimated. Also, the number of inflorescences per square meter was estimated using a quadrat system (Aboulaich et al., 2009). In a similar approach, potential pollen production of three *Cupressus* species was approximated by estimating cones per tree and pollen grains per cone (Hidalgo et al., 1999). Another approach to predicting pollen source is the use of plant distribution maps. Often, smaller surveys are used to determine prevalence of species of interest and then combined with less detailed distribution maps to create an extrapolated relative pollen

contribution map (Pauling et al., 2012; Sofiev et al., 2006). Once relative pollen concentrations have been determined with this method, modelers often use trial and error to determine input values. There has been no attempt to estimate pollen production within the genus *Juniperus*.

Juniperus ashei is among the most important aeroallergens in the Cupressaceae (Weber and Nelson, 1985). The airborne pollen *J. ashei* produces is well documented to affect inhabitants of cities and towns adjacent to juniper woodlands and, because juniper pollen can be transported over long distances, it also is well known to affect people in cities far from the pollen source. Instances of long distance transport of *J. ashei* pollen from the Edwards Plateau, Texas into Tulsa, OK and transport from the Arbuckle Mountains in south central Oklahoma into Tulsa, OK have been well documented (Levetin and Buck, 1986; Rogers and Levetin, 1998; Levetin, 1998). In fact, even though the nearest upwind source of *J. ashei* pollen is over 200 km away, it has been detected in Tulsa, Oklahoma for over 30 years (Levetin and Buck, 1986; Van de Water et al., 2003). *J. ashei* releases pollen from December to February (Pettyjohn and Levetin, 1997; Levetin and Buck, 1986). Since 1998, Levetin and Van de Water have used HY-SPLIT trajectory modeling to predict long-distance dispersal of *J. ashei* pollen (http://pollen.utulsa.edu/pollen.html).

Juniperus ashei inhabits large areas across Oklahoma and Texas. It is distributed throughout central Texas, New Mexico, northern Mexico, the Arbuckle Mountains of south central Oklahoma, and the Ozark Mountains of northern Arkansas and southwestern Missouri (Adams, 2008) *J. ashei* is dioecious, grows to 15 m in height, and inhabits limestone glades and bluffs at elevations of 150-600 m. Dry, eroded, nutrient-poor sites favor *J. ashei* and it often becomes the predominant species in such sites (Diamond et al., 1995). Other sites, including grasslands, have been subject to encroachment by *J. ashei* and as a result, lands are often actively managed to prevent such encroachment (Noel and Fowler, 2007). *J. ashei* encroachment is apparently due in large part to fire suppression (Noel and Fowler, 2007; Allred et al., 2012). One study from a location with shallow, limestone soils in Uvalde County, TX found that 90% of the 1,000 trees per ha were *J. ashei* (Hicks and Dugas, 1998).

The current study was undertaken to estimate *J. ashei* pollen production per tree as well as across the landscape in order to provide information for a pollen forecasting model. Hidalgo et al. (1999) used surface area of trees as a measure of pollen production. On the other hand, fecundity (at least in animals) is quite often related to volume of the individual (Wenner et al., 1991). In this study, both of these models of male fecundity are tested, as well as a simple linear relation between tree height and pollen production, using *J. ashei.* In addition, tree stand characteristics are tested as predictors for high rates of pollen production.

MATERIALS AND METHODS

Study Sites

The information from this study will be combined with a pollen air sampling study to support the previously mentioned pollen forecast model. The air sampling locations were positioned to cover the geographic range of *J. ashei.* Survey locations for this study were established near pollen sampling stations and were intended to be representative of the air sampling locations. Additional considerations were based on accessibility and recommendations of local representatives of the United States Departments of Agriculture and Interior as well as colleagues from local universities. Two locations in the Arbuckle Mountains of Oklahoma and four locations in the Edwards Plateau region of Texas were chosen as sampling sites. The sites were at Camp Classen (Classen) and Crossbar Ranch (Crossbar) in the Arbuckle Mountains near Davis, Oklahoma, San Marcos (San Marcos), TX, Llano River State Park near Junction, TX (Junction), the Texas Agrilife Research and Extension Center near Sonora, TX (Sonora), and the Balcones Canyonlands National Wildlife Refuge near Cedar Park, TX (Balcones). Most of the data were collected in December 2009 and January 2010. Some of the cone data for representative tree cone counts and pollen grains per cone counts were collected in December of 2010 and January 2011 which included an additional site for cone collection at the Cedar Ridge Preserve near Dallas, TX.

Representative quadrats measuring 100×100 m were chosen at each location. Within each quadrat six 10×10 m sub-quadrats were

randomly selected. Quantitative measures of size were recorded for all trees within a sub-quadrat. Tree height, trunk diameter and canopy diameter were measured. The majority of trees exhibited radial symmetry, so the canopy diameter was estimated by taking measurements in 2 directions perpendicular to one another (Hicks and Dugas, 1998). Since juniper trees are difficult to age (Panshin and Dezeeuw, 1964; Van Aukin, 1993), diameters were used as a measure of size class rather than age class. Many of the trees measured were too small to achieve valuable measurements at the standard breast height. Instead, trunk diameter was measured at a height of approximately 15 cm. Also, many *J. ashei* trees have multiple trunks. Because of this, diameters of multiple trunk trees were converted to basal area and summed (Rodgers et al., 1996).

Finally, the gender of each tree was noted and for male trees a subjective measure of pollen cone production was given: high cone producing (HCP), low cone producing (LCP) or no cone producing (0CP). The 0CP category consisted of trees that had very few male cones per tree which allowed for gender determination but did not contribute much to pollen load. This subjective measure was made because of the striking difference in number of cones on trees of the same height. The subjective measure was tested to determine whether the population was indeed made of two classes of male trees (HCP, LCP) which could improve prediction of total pollen production from the juniper population. Since parts of many trees were dead or dying, estimates of percent live vegetation per tree were made by visual inspection.

Percent canopy coverage of the quadrat area was determined using a line-intercept method (Floyd and Anderson, 1987). Three 100 m transects radiating outward from a central point were selected randomly as measures for canopy cover.

Quantifying Pollen Cone Production

Five trees were chosen from each of the HCP and LCP categories from the combined tree pool of all of the locations. Preliminary observations revealed that cone production varied more from top to bottom than it did left to right. This phenomenon has been observed in female cones of *Picea glauca* (Turkington et al., 1998).

Due to this type of cone distribution, representative trees were divided longitudinally into eighths. A $1/8^{th}$ section was harvested and the number of cones counted for that portion of the tree. All $1/8^{th}$ sections were collected from trees with no dead vegetation. Due to this requirement, it was difficult to find representative trees from each location.

Some large trees were so laden with cones that it was necessary to do a sub-count on the representative trees. In this case, approximately 1/3 of the cones in the 1/8th section were counted. The remaining cones were estimated by obtaining the weight of the green vegetation associated with 500 cones. This was repeated at least 10 times. The remainder of the cones and green vegetation were removed from the branches and weighed and the number of cones was extrapolated.

Pollen Grains Per Cone

Ten mature but unopened cones per tree (Hidalgo et al., 1999) from at least 5 *J. ashei* trees in each location were placed in sterile tubes. Vials were returned to the lab and refrigerated until processed. Each ten-cone sample was thoroughly crushed in a vial and suspended in 10 ml of a 50:50 glycerol and water solution. Approximately 10 µl of the solution was placed on each side of the hemocytometer (Pettyjohn and Levetin, 1997). For each 10-cone tube, this process was repeated six times. The number of pollen grains was estimated using standard hemocytometer dilution conversions. Not all cone vials were processed due to fungal growth in 28 of 40 vials.

Statistical Analysis

Three models predicting cone production per tree (cone production being proportional to height, surface area, or volume) were tested through linear regression on log transformed data (i.e. $\log(y) = \log(b) + a \log(x)$). A slope of 3 suggests a volume relationship, a slope of 2 suggests a surface area relationship, and a slope of 1 suggests a one-dimensional relationship (i.e., height). An ANOVA was used to test for significance of the regression for each slope (SAS JMP 2010).

Combining the cone count data and each model of cone production with the quadrat sampling data, total cone production was

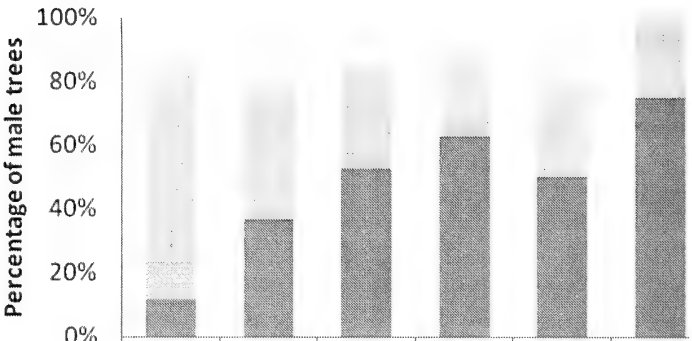
estimated. Adjustments were made to the cone production model by multiplying by the percent live vegetation per tree.

A chi-square test was used to determine whether the distribution of the HCP, LCP, and 0CP trees varied in their ratios across locations. Tree stand characteristics were compared using one-way ANOVAs and mean comparisons were made using a Tukey Kramer HSD test. Male to female ratio was analyzed using chi-squared distribution tests (test for 50:50, and test for different frequencies among locations). A regression analysis was performed to determine whether individual stand characteristics were good predictors of pollen production. The number of trees in each cone production rating (HCP, LCP, 0CP) for every sub-quadrat (36) was plotted against the mean or number value for each stand characteristic (36) for the regression analysis. All statistical analyses were performed using SAS JMP 2010.

RESULTS AND DISCUSSION

Stand Characteristics

Analysis of stand characteristics was based on trees that were ≥2 m tall. This threshold was determined by finding the shortest cone producing tree (2 m). The estimated number of HCP trees per hectare was greatest at Crossbar and Junction with 166.7 trees per hectare (Figure 1). The Balcones had the least HCP trees with 33 trees/ha but the highest number of LCP trees with 200 trees/ha (Figure 1). A chi square test to test the ratio of HCP, LCP, and 0CP trees between locations was not significant ($p>0.05$). Location and individual HCP, LCP, and 0CP groups were compared using a one-way ANOVA. Although the number of HCP trees per hectare varied widely between locations (Figure 1), differences were not significant. The number of LCP trees was significantly different ($F_{5,30} = 3,1$, $p<0.05$) and means comparisons reveal that the number of LCP at Balcones was significantly greater than at Sonora (Figure 1). No differences were found between locations for the number of 0CP trees.



	Balcones	Classen	Crossbar	Junction	San Marcos	Sonora
Male 0CP	50	67	50	33	50	0
Male LCP	200	133	100	67	83	17
Male HCP	33	117	167	167	133	50

Figure 1. Number and percentage of male HCP, LCP, and 0CP tree groups per hectare in each location. Bars represent percentage of trees in each group. Values in table are the number of trees per hectare in each group.

Juniperus ashei tree density was highest in San Marcos with 1500 mature trees per hectare and lowest in Sonora with 150 mature trees per hectare (Table 1). The remaining locations were between 683 and 1100 trees per hectare (Table 1). The one-way ANOVA indicated the differences were significant ($F_{5,30} = 3.8$, $p<0.01$) for tree density and means comparison show that the only significant difference was the density between Balcones and Sonora (Table 1). There was a positive correlation between tree density and 0CP, LCP, and HCP, but the relationship was not significant (Table 2).

In all 6 locations, the genus *Juniperus* was the dominant arboreal vegetation. *Juniperus ashei* and *J. pinchotii* were both present in Sonora with *J. pinchotii* making up a slightly higher percentage of the canopy cover. *J. ashei* was 2.7% of the cover and *J. pinchotii* was 3.3%. Allred et al. measured tree cover in Sonora and found that percent cover ranged from below 10% to around 30% (2012).

Differences between percent juniper canopy cover and percent total canopy cover were less than 6% for all locations except San Marcos (Table 1). San Marcos was the only location where a large percentage of the tree canopy cover consisted of species other than juniper. Of the total 67.3% cover, 35.2% was juniper while the remainder consisted mostly of *Quercus* species (Table 1). Total canopy cover was significantly different across locations ($F_{5,12} = 5.44$, $P < 0.01$). Means comparisons show that total cover was significantly higher in San Marcos and Balcones than in Sonora (Table 1). Crossbar and Junction were 46.8% and 44.1%. Classen and Sonora were 32.7% and 6%, respectively (Table 1). For juniper canopy cover, locations were significantly different ($F_{5,12} = 5.0$, $p<0.05$). Means comparisons reveals that juniper canopy cover at Balcones was significantly higher than Sonora (Table 1).

Table 1. *Juniperus ashei* stand characteristics by location: mean (standard deviation) and letter grouping based on Tukey-Kramer means comparisons*.

Location	Tree Height (m)	Canopy Diameter (m)	*J. ashei* Cover(%)	Total Cover (%)	Live Vegetation (%)	Mature Density†	Trunk Basal Area (cm)
Balcones	5.8(1.7)A	3.6(1.6)A	65.3(14.8)A	69.1(9.8)A	26.3(22.3)C	900(119)AB	309(473)A
Classen	3.0(0.7)B	2.4(0.8)B	29.0(21.5)AB	32.7(26.1)AB	65.1(21.8)A	733(69)AB	133(87)B
Crossbar	4.0(1.1)B	3.7(1.5)A	41.0(18.9)AB	46.8(12.1)AB	40.2(19.8)BC	1100(112)AB	283(274)A
Junction	3.5(0.9)B	2.5(1.2)B	40.0(20.1)AB	44.1(18.9)AB	73.3(11.5)A	683(52)AB	111(106)B
San Marcos	4.1(1.7)B	2.3(1.6)B	35.2(1.6)A	67.3(22.8)A	42.2(15.6)BC	1500(137)AB	85(154)B
Sonora	3.5(0.8)B	3.6(1.4)AB	2.7(4.0)B	6.0(4.5)B	58.3(15.4)AB	150(18)B	187(178)AB

*In each column values follow by the same letter are not significantly different.
†Mature tree density per hectare including male, female, and gender unidentifiable trees. Mature tree based on the shortest cone producing tree (>2 m).

Percent juniper cover was significantly correlated with number of LCP trees ($r = 0.90$, $p < 0.05$) (Table 2). Juniper canopy cover was the only stand characteristic that was significantly correlated with the number of LCP trees.

Mean tree height in Classen was 3 m, which was much shorter than the mean 5.8 m tree height in Balcones (Table 1). Mean tree height for all locations except Balcones was between 3 and 4.1 m (Table 1). A

one-way ANOVA showed that mean tree height was significantly different ($F_{5,298} = 24.8$, $p<0.0001$). Means comparisons show that trees in the Balcones were significantly taller than all other locations (Table 1). Classen trees were significantly shorter than those in the Balcones, Crossbar, and San Marcos. Canopy height was positively correlated with number of LCP trees, but the relationship was not significant. The number of 0CP and the number of HCP trees was not strongly correlated with tree height (Table 2).

Table 2. Correlation coefficients of stand characteristics and HCP, LCP, 0CP. Number of male trees in a given category (HCP, LCP, 0CP) of each sub-quadrat were plotted with a given stand characteristic of each sub-quadrat.

Male Cone Production Rating	Tree Density*	Tree Height*	Canopy diameter	Total %Cover	Juniper %Cover	%Live Vegetation	Trunk Basal Area
LCP	0.39	0.70	0.15	0.68	0.90*	-0.59	0.14
HCP	0.44	-0.49	-0.60	0.14	0.05	0.40	0.05
0CP	0.68	0.16	-0.42	0.64	0.64	-0.25	0.15

*$p<0.05$

Trees at Classen, Junction, and San Marcos had the smallest mean canopy diameters (2.4, 2.5, and 2.3 m respectively) and those at Crossbar had the largest mean canopy diameter (3.7 m). Mean canopy diameter of trees at Balcones and Sonora was 3.6 m (Table 1). The one-way ANOVA for canopy diameter across locations showed significant differences ($F_{5,298} = 7.7$, $p<0.0001$). Means comparisons showed tree diameters at Balcones and Crossbar were greater than Classen, Junction, and San Marcos. Canopy diameter was negatively correlated with 0CP and HCP trees with r values of -0.42 and -0.60 respectively but the relationship was not significant for either (Table 2).

Tree trunk basal area was highest in Balcones and Crossbar with 309 cm^2 and 283 cm^2, respectively. San Marcos had the smallest basal area at 85 cm^2. Although there were several large trees in San

Marcos, a large number of trees in the area had small trunk diameters and were near the minimum height of 2 m which is the reason for the low mean basal area. Basal areas at Junction, Classen, and Sonora were 111, 133, and 187 cm^2 respectively (Table 1). A one-way ANOVA showed that basal area differences were significant ($F_{5, 287} = 7.8$, $p<0.0001$). Means comparison showed trees at Balcones and Crossbar had a larger mean trunk size than Classen, Junction, and San Marcos. Sonora was not significantly lower or higher than any other location (Table 1). Trunk basal area was not correlated with 0CP, LCP, or HCP (Table 2).

Mean percent live tree vegetation varied from 26% in Balcones to 73% in Junction. San Marcos and Crossbar were similar with 42% and 40% respectively (Table 1). Live vegetation at Classen was 65% and at Sonora it was 58% (Table 1). A one-way ANOVA found that differences in percent live vegetation were significant ($F_{5, 241} = 38.7$, $p<0.0001$) and means comparison revealed that Junction and Classen percent live vegetation ratings were significantly higher than at San Marcos, Crossbar, and Balcones; and Sonora was significantly higher than Balcones (Table 1). Negative non-significant correlations exist between percent live vegetation and LCP and 0CP. While HCP trees were positively correlated with live vegetation, the correlation was not significant (Table 2).

Male trees were more abundant than female in Balcones, Classen, Junction, and San Macros. Across the six locations, percent male (m/(m+f)) varied from 45% in Crossbar to 71% in Balcones. In Sonora, there was an equal amount of male trees to female trees. Classen, Junction, and San Marcos had male percentages of 58, 62, and 63 respectively. A chi-squared analysis showed that the locations were not significantly different from each other ($p > 0.05$) nor were they significantly different from a 50:50 ratio ($p > 0.05$).

Tree stand characteristic results from this study are generally comparable with other studies. For example, mean tree heights from this study were in the same range as other studies. Two locations in Guadalupe River State Park, TX (30 km north of San Antonio) had mean tree heights of 6.2 m and 7.9 m. Another location in the same study in Bosque County, TX (west of Waco) had a mean tree height of

7.2 m (Mclemore et al., 2004). Hicks and Dugas found that trees in a location in Uvalde County, TX averaged 2.7 m in height (1998). Mean tree height in this study ranged from 3.0 m to 5.8 m (Table 1). Hicks and Dugas also found that there were approximately 1,000 trees per ha and 90% of those trees were *J. ashei* (1998). In other words, the juniper density was 900 trees/ha. Balcones tree density was also 900 trees/ha and the highest density area in this study was San Marcos which reached 1500 *J. ashei* trees/ha (Table 1).

Pollen Grains Per Cone

The average number of pollen grains per cone was significantly different across the population per the one-way ANOVA ($F_{11, 60} = 10.3$, $p<0.0001$). Means comparison shows that Sonora was higher than San Marcos and Junction (Table 3).

Table 3. *Juniperus ashei* pollen grains per cone by location.

Location	Pollen grains/cone
San Marcos	$3.74 \times 10^5 \pm 7.04 \times 10^4$
Junction	$3.63 \times 10^5 \pm 6.32 \times 10^4$
Sonora	$4.72 \times 10^5 \pm 4.23 \times 10^4$
Dallas	$4.02 \times 10^5 \pm 5.91 \times 10^4$

The mean across all sites was 402,000 pollen grains per cone and the standard deviation across all sites was 74,794. Hidalgo et al., found that the mean pollen per cone of *Cupressus sempervirens* was 365,722 and the standard deviation was 40,058 (1999). The mean pollen grain per cone value will be used in the pollen production estimate.

Pollen Cone Production Model

Observation at each location confirmed preliminary findings that cone production varied more from top to bottom than left to right. Male cones were counted from ten trees. The largest HCP tree countedwas 4.8 m tall with a mean canopy diameter of 5.0 m and estimated to have produced 1.38 million cones. By comparison, the tallest LCP tree was 5.2 m tall with a mean canopy diameter of 5.7 m and estimated to have produced 140,000 cones (Figure 2). Hidalgo et

al. estimated male cone production in three *Cupressus* species and found that mean cone production ranged from 176,233 cones per tree in the lowest cone producing species and 2,974,651 cones per tree in the highest producing species (1999). When cone count was plotted against tree height for the HCP and LCP cone producers, the slopes fell onto separate and distinct lines. Further, using the test statistic, Student's t, the slopes were found to be significantly different ($p<0.001$) thus justifying separate regression analyses for the two groups (Figure 2).

The HCP regression was significant ($F_{1,3} = 44.1$, $P<0.01$) as was the LCP regression ($F_{1,3} = 15.6$, $P<0.05$).

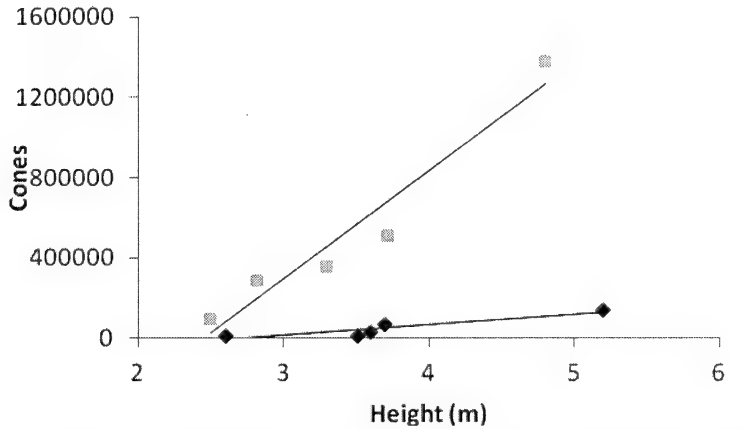


Figure 2. Height of representative trees and cone production for low cone producing (LCP) trees and high cone producing (HCP) trees. Estimates of cones were based on counts of 1/8 tree × 8. HCP = gray squares, LCP = dark diamonds.

The three models (cone production proportional to height, surface area, and volume) were tested through linear regression using log transformed data: log(cones) = a log(height) + log(b) (Table 4). Slope for the log transformed LCP data was approximately 3.39 (95% CI, -0.77, 7.54) and for HCP it was 3.73 (95% CI, 1.93, 5.53). Externally studentized residuals were calculated to test for outliers and none were identified. A slope of 3 suggests a volume relationship, a slope of 2 suggests a surface area relationship, and a slope of 1 suggests

a one-dimensional relationship (i.e., height). The 95% confidence interval for the log transformed HCP slope includes 2 and 3 while the LCP interval includes 1, 2, and 3. The estimate of total cone production, therefore, used the 3 models and location stand characteristics.

Potential pollen production was estimated for each location using the three models (Table 4). Using the values and equations from Table 4, and tree heights (x) from the six locations, the totals were multiplied by percent live vegetation estimates and the mean number of pollen grains per cone (402,000) and extrapolated and values are expressed in pollen grains per hectare. Total estimated pollen production varied widely (Figure 3). The volume model predicts San Marcos as the highest pollen producing location while the surface area and height models predict Junction as the highest (Figure 3). Tree heights and number of HCP trees per location were the driving factors. The HCP trees often produce an order of magnitude greater number of cones (pollen). There were 167 HCP trees/ha in Junction and Crossbar

Table 4. Height, surface area, volume, and full-log models of pollen cone production including equation and constants (a,b).

Model	Equation	LCP		HCP	
		a	b	a	B
Height	$Y = ax + b$	51,819	-135,132	538,770*	-1,320,926*
Surface Area	$Y = bx^2$	2	2750.86	2	33011.64
Volume	$Y = bx^3$	3	757.38	3	9890.16
Full-log	$Y = bx^a$	3.39	459.20	3.73	4102.04

*Full-log test of height, surface area, and volume models led to rejection of simple height relationship in HCP trees.

and only 133 HCP trees/ha in San Marcos (Figure 1). The HCP trees in San Marcos were taller (data not shown) on average than the Junction trees and Junction HCP trees were taller than Crossbar HCP trees which is the reason the volume model predicted more pollen grains in San Marcos. Rankings of the total production for Crossbar, Classen, Sonora, and Balcones were the same for all three models (Figure 3).

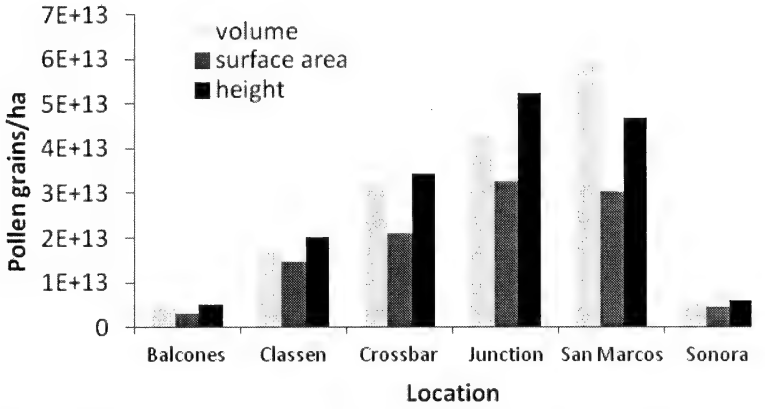


Figure 3. Estimated pollen production per hectare using volume, surface area, and height models.

A representative HCP and LCP tree was not collected in every location due to the requirement that the representative trees have a live vegetation rating of approximately 100%. Another shortcoming is that the tallest HCP tree with cone counts was 4.8 m which means that HCP trees taller than 4.8 m were predicted to follow the same cone to height relationship established by our slope without an actual count to support it (Figure 2). Many of the HCP trees in the San Marcos location were taller than 5 m and it is possible that the relationship between cone production and tree size was different for taller trees. It is interesting that Sonora and Balcones were very similar in their estimated production with vastly different stand characteristics (Table 1, Figure 3). The trees in Sonora were much shorter and smaller than the Balcones trees, but the number of HCP trees per ha was higher (Figure 1, Table 1). Although these data provide a pollen production range, the slopes of the HCP and LCP trees created by the 10 trees counted do not have multiple trees counted for the same height in each cone production class. This means that the model does not test the variability in the relationship between trees of the same height. Rather, it provides a range of possible pollen production based on a single count for each height. More representative tree cone counts would be necessary to determine which model is the most accurate. It is also possible that the

model varies by location or that groups of trees fit one model and other groups fit another.

This study compared plots at six woodland locations. It is important to point out that forest descriptions by location as well as pollen cone production estimates by location reflect immediate localities and not greater geographic areas. For example, Classen and Crossbar are approximately 5 kilometers apart yet have very different stand characteristics (Table 1). This is due in part to an age mosaic created by wild and prescribed fires as well as other human removal practices. Some stand characteristics are likely a function of precipitation, stand age, climate zone and soil types. For instance, the average precipitation from the west end of the Edwards Plateau to the east end of the Plateau ranges from 600 mm/yr to 900 mm/yr respectively which likely contributes to the tree density difference between locations (Owens et al., 2006).

Light was one major factor affecting pollen cone production and could also contribute to the number of pollen grains per cone. Incident light is increased when forest edges are created which promotes plant growth (Murcia, 1995). The effect of light availability was tested on seed production of balsam fir (*Abies balsamea*) and white spruce (*Picea glauca*) in western Quebec. While mean annual seed production is typically proportional to basal area, light conditions affected production. Sub-canopy trees were found to produce half as many seeds as canopy trees. Height at which seed cones are produced differed between sub-canopy and canopy white spruce trees with heights of 14 m and 3 m respectively (Greene et al., 2002). In the locations sampled for this study, more male cones were typically produced where there was full sun as on the upper portion of the trees or trees in the open and this is documented in other junipers (Raatikainen and Tanksa, 1993). The edge effect was especially apparent along roadways, but this study was not designed to test the edge effect. Density and number of HCP trees was not negatively correlated as one would expect, and this is probably in part due to edge effects along clearings. In other words, a high tree density area could be an area where trees were evenly spread or in clumps. It is interesting to note that in the least dense area (Sonora) 75% of all male trees were HCP trees (Figure 1).

The only significant relationship between stand characteristic and HCP, LCP, or 0CP trees was LCP and juniper percent cover (Table 2). If more sunlight results in more cones, then low cone producing (LCP) trees would be expected in areas with less light (i.e. tall trees, dense forest, high forest cover). Balcones produced the highest number of LCP trees and had the highest percent juniper cover (Table 1, Figure 1). Other trees associated with juniper forests in Oklahoma and Texas were various species of *Quercus* some of which may have less dense canopies than junipers. This could result in more light available in a stand with high percent canopy cover that is made up of *Quercus* as opposed to a high percent cover juniper monostand. For example, percent juniper cover in San Marcos was relatively low (35%) but overall cover was high (67%) and number of HCP trees was relatively high which may be due to better access to light (Table 1, Figure 1). Compare this to the Balcones location where there were very few HCP trees and juniper made up 65% out of the 69% total cover (Table 1).

Clearly, the canopy characteristics are many and varied across the *J. ashei* distribution and these six locations may not represent all possibilities. It should be noted that the large amount of variability within each location can have a significant effect on the various models presented. More data is necessary in order to determine the best model and to ensure that the range in each variable is representative. Another avenue of testing the models may be comparing aerobiological data with predicted pollen production. Although analysis of aerobiological data is ongoing, preliminary results indicated that the mean pollen concentrations were not well correlated with estimated pollen load from the models in this study (Levetin et al., 2011).

CONCLUSION

This study showed that *Juniperus ashei* trees have the potential of producing enormous amounts of pollen with up to 1.3 million pollen cones per tree and approximately 402,000 pollen grains per cone. While the large amount of pollen in juniper woodlands is difficult to quantify with a high degree of accuracy, loads per hectare can be estimated through statistical models based on tree and landscape characteristics. Typically, very dense areas are not producing as many

pollen cones. The data from this study also indicate that only a fraction of the trees on the landscape are producing most of the pollen. More field work is needed to distinguish between the pollen production models and to quantify the effect of forest edge on pollen production.

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SENEGALIA BERLANDIERI, S. GREGGII AND S. WRIGHTII HYBRIDS (FABACEAE: MIMOSOIDEAE) IN TEXAS AND ADJACENT MEXICO

David S. Seigler
Department of Plant Biology, University of Illinois, Urbana, IL 61801
seigler@life.uiuc.edu

John E. Ebinger
Emeritus Professor of Botany, Eastern Illinois University, Charleston, IL 61920

and

Catherine Glass
Pacific Northwest College of Art, Portland, OR 97209

ABSTRACT

Principal component (PCA) and principal coordinate analyses (PCoA) suggest that *Senegalia x emoryana* is of hybrid origin, the probable parents being *S. berlandieri* and *S. greggii.* Many individuals and populations involving the parental species and F_1 hybrids, as well as backcrosses to either of the two parents, have been observed by the authors and others. This hybrid occasionally dominates disturbed habitats, becoming more common than the parental species. A hybrid morphologically similar to *S. x emoryana* involving *S. berlandieri* and *S. wrightii* is also discussed. The hybrid between *S. berlandieri* and *S. wrightii* (herein described as *Senegalia x turneri* Seigler & Ebinger), in contrast to *Senegalia x emoryana*, does not appear to be common; we have found relatively few individuals, mostly in association with the parents. *Phytologia 94(3):439-455 (December 1, 2012).*

KEY WORDS: Senegalia, hybrids, *Senegalia x emoryana, Senegalia x turneri, S. berlandieri, S. greggii, S. wrightii*

The genus *Senegalia* is a segregate of plants from *Acacia* s.l. with woody prickles scattered on the stem and commonly the leaf petiole and rachis, and flowers with a ring of glands at the base of the long-stalked ovary. The genus consists of 100 species in New World tropical and subtropical areas ranging from the southwestern United States south to Argentina and in the West Indies (Seigler et al. 2006). Nearly 100 additional species are found in the Old World tropics and subtropics of Asia, Africa, and Australia.

Only rarely have hybrids between New World species of *Senegalia* been reported (Britton and Rose 1928, Turner 1959, Correll and Johnston 1970, Johnson 1974). In our experience, all observed hybrids in this genus involve *S. berlandieri* (Benth.) Britt. & Rose as one of the parents and either *S. reniformis* (Benth.) Britt. & Rose, *S. crassifolia* (A. Gray) Britt, & Rose, *S. greggii* (A. Gray) Britt. & Rose or *S. wrightii* (Benth.) Britt. & Rose as the other parental species. These hybrids are restricted to the southwestern United States and northern Mexico (Maslin and Stirton 1997, Seigler et al. 2006) and are usually associated with disturbance, normally being found in pastures, edges of roads, and other disturbed sites. The present study was undertaken to examine the morphological differences of hybrids and hybrid populations involving *S. berlandieri* and the apparently related species *S. greggii* and *S. wrightii*. These three species are common components of thorn-scrub communities in large parts of the southwestern United States and adjacent Mexico.

MATERIALS AND METHODS

Three separate analyses were conducted, one involving *Senegalia berlandieri, S. greggii* and their suspected hybrids, the other involving *S. berlandieri, S. wrightii* and their suspected hybrids, and a third including all three species and their suspected hybrids. These analyses were based on herbarium specimens of the putative parents and hybrids from Texas and adjacent Mexico (Appendix I). Many of the specimens used were collected by the authors, but other materials were also included.

The study involving the *Senegalia berlandieri* x *S. greggii* population was undertaken using specimens collected by the authors at

the Chaparral Wildlife Management Area near Artesia Wells, Dimmitt and LaSalle Counties, Texas, in the northern half of the South Texas Plains ecological region (Correll and Johnston 1970). This management area is deer-proof fenced, about 6,150 ha in size, and utilizes a high intensity, low frequency rotational grazing system with stocking rates of one animal unit per 12 ha (Ruthven 2001). The study involving the *Senegalia berlandieri* x *S. wrightii* population was undertaken using specimens collected by the authors at the Harris Ranch, near Cline, 20 miles W of Uvalde, Uvalde County, Texas at the northern edge of the South Texas Plains ecological region. Managed by the Texas A & M University, Agricultural Research and Extension Center, Uvalde, the ranch is not deer-proof fenced, about 6,764 ha in size, and utilizes a cattle stocking rate of one animal unit to 35 ha (Cooper et al. 2008).

Initially, the specimens were separated into taxonomic groups based on overall morphological similarity and scored for 13 characters (Appendix II). These data served as the source of characters for principal component (PCA) and principal coordinate analyses (PCoA). Three or more measurements were made for each continuous character of each specimen. These values were then plotted to confirm that gaps in the data exist.

A few species of *Senegalia* have short shoots at many of the nodes on which clusters of leaves occur. Short shoot leaves, are usually smaller, have fewer pinna pairs, and smaller leaflets than the solitary or primary leaves found on the nodes of new growth. Primary leaves are larger, but rare or not present on many herbarium specimens. Of the species and hybrids studied in this paper, both *S. greggii* and *S. wrightii* have short shoots on which these smaller leaves are common. All measurements of *S. greggii* and *S. wrightii* used in these analyses were taken from short shoot leaves.

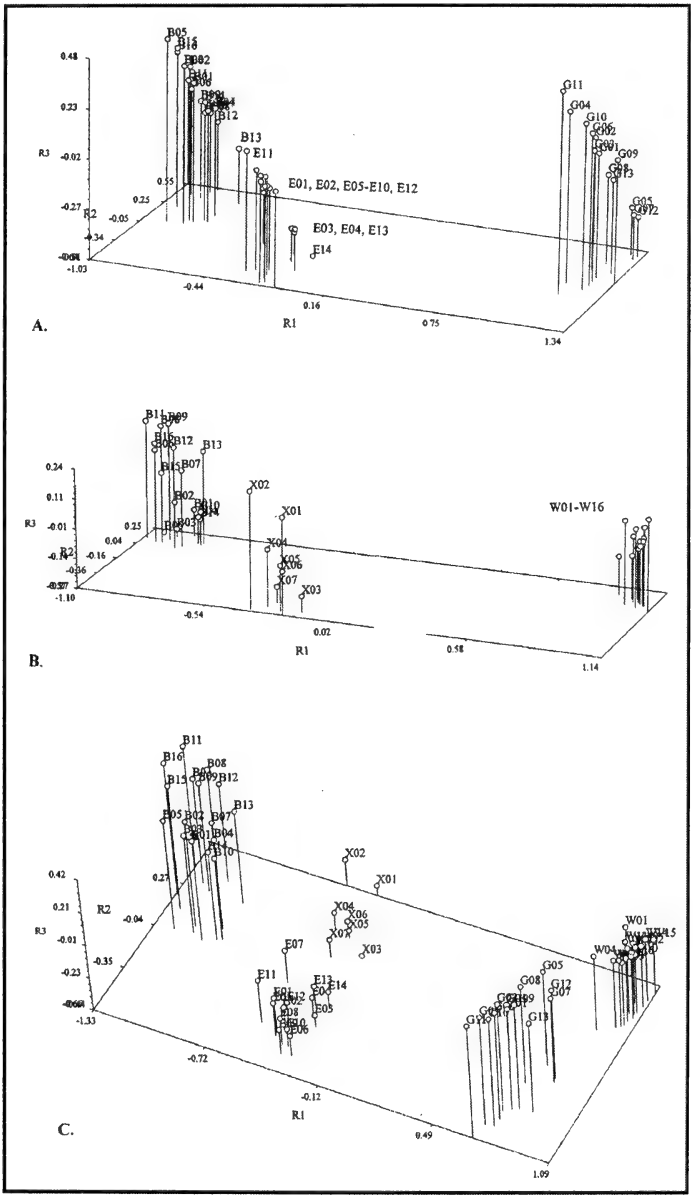
A PCA to identify groupings of the specimens examined was carried out. For this analysis, the data were first standardized and a correlation matrix, eigenvalues, and eigenvectors were calculated using NTSYS-pc verson 2.1 (Rohlf 2000). Eigenvectors were scaled by the square root of λ. The axes were rotated and the resulting loading values graphically represented as both two- and three-dimensional plots.

To carry out PCoA analyses, Gower's resemblance coefficients were calculated (Legendre and Legendre 1983; Podani 1999; Dickinson 2000). The nature of each character was designated by binary, multistate, or quantitative descriptors and all characters were weighted equally (Dickinson 2000). The data matrix was transformed by the DCENTER algorithm using distances squared and eigenvectors and eigenvalues calculated with NTSYS-pc verson 2.1 (Rohlf 2000). Eigenvectors were scaled by the square root of λ. The resulting loading values were graphically represented as both two- and three-dimensional plots.

Figure 1 (facing page). A. Three-dimensional plot for the principal component analysis using the 13 characters (Appendix II) of 16 specimens of *Senegalia berlandieri* (B01-B16), 13 specimens of *S. greggii* (G01-G13), and 14 specimens of probable hybrids (*S. x emoryana*) (E01-E14) from Chaparral Wildlife Management Area, Dimmitt and LaSalle Counties, Texas.

B. Three-dimensional plot for the principal component analysis using the 13 characters (Appendix II) of 16 specimens of *Senegalia berlandieri* (B01-B16), 16 specimens of S. wrightii (W01-W16), and 7 specimens of probable hybrids (*S. berlandieri* x *S. wrightii* = *S.* x *turneri*) from Harris Ranch, Uvalde County, Texas (X01-X07).

C. Three-dimensional plot for the principal component analysis using 13 characters (Appendix II) of 16 specimens of *Senegalia berlandieri* (B01-B16), 13 specimens of *S. greggii* (G01-G13), 16 specimens of *S. wrightii* (W01-W16), 14 specimens of *S. x emoryana* (E01-E14), and 7 specimens of *S. berlandieri* x *S. wrightii* (*S. x turneri*) (X01-X07) collected from throughout the range of *S. berlandieri* (Appendix I).


A.
R1
R2
R3
E01, E02, E05-E10, E12
E03, E04, E13
E14
B13
E11
G11
G04
B.
X01
X02
X03
X04
X05
X06
X07
W01-W16
C.
W01
G05
G08
G12
G07
G13

RESULTS

Senegalia berlandieri and S. greggii: The analysis involved 16 specimens of S*enegalia berlandieri*, 13 specimens of *S. greggii* and 14 probable hybrids collected at Chaparral Wildlife Management Area. The PCA based on 13 characters (Appendix II), and a PCoA based on Gower's similarity coefficients proved to be similar (Figure 1A). In the PCA, the first three principal components accounted for 94% of the total variance. Leaflet pairs/pinna (Lep), pinna length (Pil), and petiole gland length (Gll) (characters 10, 7, and 3) were most important for determining the component score of the first axis; leaflet length (Lel), leaflet shape (Les), and gland shape (Gls) (characters 13, 11, and 4) were most important for determining the second axis. The specimens used in this analysis represented distinct groupings in both PCA and PCoA. The clusters for each of the parental species were well separated from each other and the cluster corresponding to hybrids was spatially located between the putative parental species (Figure 1A).

***Senegalia berlandieri and S. wrightii*:** The analysis involved 16 specimens of S*enegalia berlandieri*, 16 specimens of *S. wrightii* and 7 probable hybrids (*S. berlandieri x S. wrightii*) collected at Harris Ranch. The PCA based on all 13 characters (Appendix II) and a PCoA based on Gower's similarity coefficients for species scored proved to be similar (Figure 1B). In the PCA, the first three principal components accounted for 96% of the total variance. Leaflet distance (Led), pinna pair number (Pip), and rachis length (Ral) (characters 9, 6, and 5) were most important for determining the component score of the first axis; leaflet apex shape (Lea), leaflet shape (Les), and petiole gland shape (Gls) (characters 8, 11, and 4) were most important for determining the second axis. The specimens used in this analysis represented distinct groupings in both PCA and PCoA. The clusters for each of the parental species were well separated from each other and the cluster corresponding to hybrids was spatially located between the putative parental species (Figure 1B).

Senegalia berlandieri, S. greggii, and S. wrightii: This analysis used herbarium specimens from throughout the range of these three species in south central and southern Texas and adjacent northern Mexico. The analysis involved 16 specimens of S*enegalia berlandieri*,

13 specimens of *S. greggii*, 16 specimens of *S. wrightii*, 14 specimens of *S. berlandieri x S. greggii* (*S. x emoryana*), and seven specimens of *S. berlandieri* x *S. wrightii* (Appendix I). No specimens of suspected backcrosses to either parent were included in the analysis. The PCA based on 13 characters (Appendix II), and a PCoA based on Gower's similarity coefficients for species scored proved to be similar (Figure 1C). In the PCA, the first three principal components accounted for 96% of the total variance. Short shoots (Shs), petiole gland shape (Gls), leaflet apex shape (Lea), and leaflet shape (Les) (characters 1, 4, 8, and 11) were most important for determining the component score of the first axis; leaflet length (Lel), leaflet distance (Led), and leaflet width (Lew) (characters 13, 9, and 12) were most important for determining the second axis. The specimens used in this analysis represented distinct groupings in both PCA and PCoA. The clusters for each of the parental species were well separated from each other and the clusters corresponding to hybrids were spatially located between the respective putative parental species (Figure 1C).

DISCUSSION

Senegalia berlandieri and S. greggii: Of these two taxa, *Senegalia greggii* has the most extensive distribution, known from southern California east through extreme southern Nevada and Utah, most of Arizona and New Mexico, through southern Texas, and south into Mexico in the states of Baja California Sur, Sonora, Chihuahua, Durango, Coahuila, Nuevo León and Tamaulipas. *Senegalia berlandieri*, in contrast, has a more restricted distribution in the United States, occurring in south central and southern Texas, and farther south than *S. greggii* in the states of Chihuahua, Durango, Zacatecas, Coahuila, Nuevo León, San Luis Potosí, Hidalgo, Guanajuato, Querétaro, and Tamaulipas, Mexico. The hybrid, *S. x emoryana,* is restricted to areas in which the parental species have an overlapping distribution, mostly in south central and southern Texas, and the states of Chihuahua, Coahuila, Durango, and San Luis Potosí, Mexico.

Senegalia x emoryana can easily be separated from both *S. berlandieri* and *S. greggii* using many of the characteristics listed in Appendix II. The most obvious and commonly used characteristics include: short shoot at most nodes of *S. greggii*, but are absent on *S. x*

emoryana and *S. berlandieri*; most leaves with 1-3 pinna pairs in *S. greggii*, 4-8 in *S. x emoryana*, and 9-15 on *S. berlandieri*; and many leaflets obovate to oblanceolate in *S. greggii,* and most leaflets linear to oblong in *S. berlandieri* and *S. x emoryana.* In floral material the globose inflorescence of *S. berlandieri* separates this species from *S. x emoyana* which has an elongated inflorescence that is less than twice as long as wide, and *S. greggii* which has an elongated inflorescence more than twice as long as wide. Backcrossed individuals are more difficult to identify, but these were only rarely encountered. The most common backcrossed specimens observed were between *S. berlandieri x S. x emoryana.* Separation was usually easy because *S. berlandieri* average 25 to 55 leaflets/pinna, whereas *S. x emoryana* averages 15 to 20 leaflets/pinna.

***Senegalia berlandieri and S. wrightii*:** Of these two taxa, *Senegalia wrightii* has a more extensive distribution in the United States than *S. berlandieri*, being known from southern Nevada and Arizona, and east through most of southern Texas (Little 1979). In Mexico, we have found specimens of *S. wrightii* from Baja California Sur, east through Chihuahua, Coahuila, Nuevo León, and Tamaulipas. It may occur further south into central Mexico but we have been unable to locate specimens. We have few specimens of the hybrid between these two taxa (*Senegalia berlandieri* and *S. wrightii*). In order to distinguish this hybrid from other taxa in the group it is important to select mature vegetative material; in particular, flowering material with immature leaflets often falls below 5.5 mm in length. In the original analysis, all of the proposed hybrid specimens were from the population at Harris Ranch near Cline, Texas. Presently, we have located additional specimens of this proposed hybrid, all from the South Texas Plains ecological region in southern Texas (Correll and Johnston 1970). Based on these specimens the proposed new hybrid is described. This hybrid is named after Dr. Billie L. Turner (Director Emeritus, University of Texas Herbarium) who has studied the *S. berlandieri, S. greggii, S. x emoryana* species complex and has annotated many specimens of this species complex at TEX (Turner 1959).

Senegalia x turneri Seigler, Ebinger, & Glass *nothomorph nov.*
Figure 2

TYPE: UNITED STATES. TEXAS: Uvalde Co.: Harris Ranch near Cline, 20 miles W of Uvalde on Rt. 90, 29°N 14' 38"; 100 °W 06' 02", 18 Aug 2003, D. S. Seigler & J. E. Ebinger 15815 (Holotype: ILL). Putative hybrid between *Senegalia berlandieri* and *Senegalia wrightii.*

Shrub or small **tree** to 5 m tall. Bark light to dark brown, shallowly furrowed. Twigs dark grayish brown, straight, usually puberulent. Short shoots mostly absent. Prickles brown below, apex dark brown, flattened, usually slightly recurved, woody, 1-5 x 1-5 mm at the base, usually glabrous, persistent, scattered along the twig, sometimes rare to absent. **Leaves** alternate, 25-70 mm long. Stipules light to dark brown, narrowly triangular to linear, symmetrical, flattened, straight, herbaceous, 0.5-2.1 x 0.3-1.1 mm near the base, puberulent, tardily deciduous. Petiole adaxially grooved, 5-20 mm long, puberulent; petiolar gland solitary, located on the upper half of the petiole, sessile in the expanded petiole groove with the margins raised, orbicular to elliptic, 0.8-2.1 long, apex depressed, glabrous. Rachis adaxially grooved, 15-45 mm long, puberulent, an oval to orbicular gland 0.4-1.2 mm long between the upper pinna pair, apex depressed, glabrous. Pinnae 2 to 7 pairs per leaf, 20-45 mm long, 4-14 mm between pinna pairs; paraphyllidia 0.2-0.5 mm long; petiolule 1.1-3.1 mm long. Leaflets 13 to 21 pairs per pinna, opposite, 0.6-2.1 mm between leaflets, oblong, 5.1-9.3 x 0.7-2.6 mm, glabrous to lightly appressed puberulent on both surfaces, lateral veins obvious, 1 to 4 veins from the base, base oblique and obtuse, margins ciliate, apex acute, midvein submarginal. **Inflorescence** a densely 35- to 85-flowered subglobose head, slightly longer than wide, 8-13 mm wide, usually solitary in the leaf axil. Peduncles 7-25 x 0.5-0.8 mm wide, puberulent; receptacle elongated, slightly enlarged. Involucre a single small bract on the upper half of the peduncle, early deciduous. Floral bracts linear to spatulate, 0.9-1.7 mm long, puberulent, early deciduous. **Flowers** sessile, white; calyx 5-lobed, 1-2 mm long, puberulent; corolla 5-lobed, 2-3 mm long, puberulent, lobes one-quarter the length of the corolla; stamen filaments 5-7 mm long, distinct; anther glands absent; ovary lightly pubescent, stipe to 0.6 mm long. **Legumes** light to dark

brown, straight to slightly curved, flattened, usually constricted between some seeds, oblong, 40-160 x 20-35 mm, coriaceous, lightly transversely striated, puberulent, eglandular, dehiscent along both sutures; stipe 5-20 mm long; apex obtuse, short beaked to lacking a beak. **Seeds** uniseriate, no pulp, brown, orbicular, flattened, 8-12 x 5-8 mm, smooth; pleurogram U-shaped, 1-3 mm across.

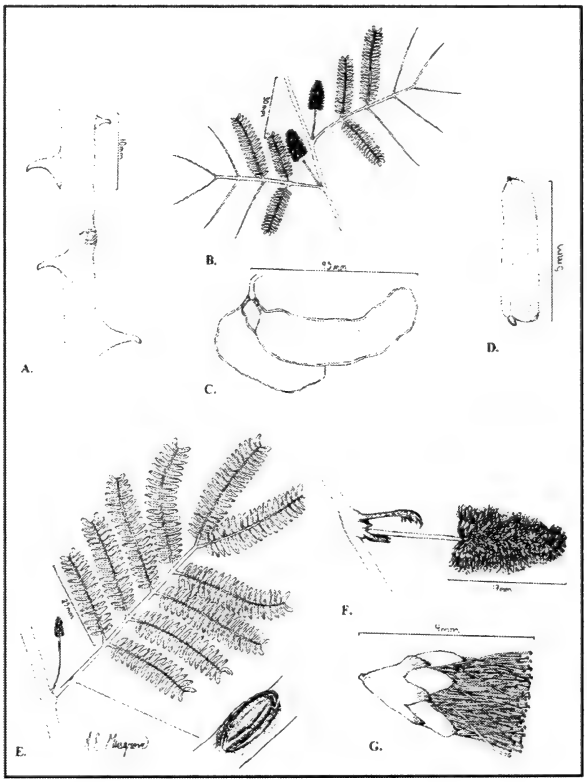


Figure 2 *Senegalia x turneri* Seigler, Ebinger & Glass. A: Twig with prickles (*E. J. Palmer 12330*). B. Habit sketch with inflorescences (*Seigler & Ebinger 15815*). C. Fruits (*Seigler & Ebinger 15815*). D. Leaflet (adaxial surface) (*Seigler & Ebinger 15799*). E. Leaf with petiolar gland (*Seigler & Ebinger 15799, 15808*). F. Inflorescence (*Seigler & Ebinger 15799, 15815*). G. Flower (*Seigler & Ebinger 15799, 15815*).

Flowers: April-June.

Distribution: Limestone outcrops in gravely, calcareous, and disturbed soils between sea level and 1700 m in southern Texas.

Specimens examined: UNITED STATES: Texas: Hidalgo Co.: Armando Vela property, 85 ft., 11 Nov 2003, *W.R.Carr & A.Vela 22700* (ILL, TEX). **Kinney Co.:** Kickapoo State Park, 6 Aug 1988, *T.Keeney 8620* (BRIT); 9 miles NE of Bracketville, 9 Jun 1955, *B.L.Turner 3879* (TEX). **Live Oak Co.:** 7 miles S of George West, 6 Apr 1953, *M.C.Johnston s.n.* (TEX); **Maverick Co.:** Eagle Pass, *V.Havard 1375* (MO). **Uvalde Co.:** Roadside, 2 miles S of Garner Park, 29 Apr 1973, *T.Keeney 833* (SMU); Vacant lot, Knippa, 13 Sep 1988, *T.Keeney 8613* (BRIT); Along Frio River, W of Knippa at the Dude Ranch off Cactus Flats Road, 12 Aug 1994, *T.Keeney 10410* (BRIT); Uvalde, 20 Jun 1917, *E. J. Palmer 12318* (MINN); Montell, 23 Jun 1917, *E. J. Palmer 12330* (MO). Winston Ranch, 4 miles S of Sabinal, Rt. 187, 10 May 2003, *M.Reed, H.Loring, E.Winston M, H.Wilson, T.Wilson & R.Corbett 2633* (BRIT); Harris Ranch near Cline on Rt. 90, 24 Jun 2002, *D.S.Seigler & J.E.Ebinger 15217* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15797* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15798* (ILL, MU); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15799* (ILL, NY, TEX); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15808* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15809* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15815* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15819* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15821* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15822* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15829* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15833* (ILL); Harris Ranch near Cline on Rt. 90, 18 Aug 2003, *D.S.Seigler & J.E.Ebinger 15838* (ILL). **Val Verde Co.:** Devil's River, 5 miles above Ft. Hudson, 3 May 1949, *W.V.Brown s.n.* (TEX); Rocky banks of Devils River, 26 Mar 1917, *E.J.Palmer 11379* (MINN); 4.2 miles N of US 90, N of Del Rio, on US 277-377,

1200 ft., 27 Mar 1986, *T.R.Van Devender & R.K.Van Devender 86-80* (ASU).

Key to the species and hybrids examined:

1. Short shoots present at many nodes, these with clusters of leaves; inflorescence a spike more than twice as long as wide.
    2. Most leaflets less than 5.5 mm long (2.8-5.5 by 0.9-3.2 mm); flower stalks 0- 0.6 mm long.....*Senegalia greggii*
    2. Most leaflets more than 5.5 mm long (5.5-9.2 by 2.2-4.5 mm); flower stalks mostly more than 0.7 mm long...*Senegalia wrightii*
1. Short shoots mostly absent; inflorescence globose or a short spike less than twice as long as wide.
    3. Most leaves with 9-15 pinna pairs; most pinnae with 30-55 pairs of leaflets; inflorescence globose.....*Senegalia berlandieri*
    3. Most leaves with fewer than 9 pinna pairs; most pinnae with 2-25 pairs of leaflets; inflorescence a short spike less than twice as long as wide.
        4. Most fully expanded mature leaflets more than 5.5 mm long (5.1-9.3 mm by 0.7-2.6 mm).....*Senegalia* x *turneri*
        4. Most fully expanded mature leaflets less than 5.4 mm long (3-6 by 0.7-1.7 mm).....*Senegalia x emoryana*

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Appendix I. Specimens examined and scored for the 13 characters used in this study.

Senegalia berlandieri: MEXICO: Coahuila: S of Monclova on Rt. 57, 30 May 1983, *D.S.Seigler, J.Kramer & E Carreira 12034* (ILL); S of Monclova on Rt. 57, 30 May 1983, *D.S.Seigler, J.Kramer & E Carreira 12040* (ILL). **San Luis Potosí:** 14 miles W of Río Verde, Rt. 70, 3 Jun 1991, *D.S.Seigler, J.E.Ebinger, H. D. Clarke & K.Readel 13705* (ILL). **UNITED STATES: Texas: Dimmit Co.:** Chaparral Wildlife Management Area, 19 May 2005, *D.S.Seigler, J.Miller & B.R.Maslin 15937* (ILL). **Duval Co.:** 6 miles S of Freer on Rt. 16, 20 May 1983, *D.S.Seigler, J.Kramer & E.Carreira 11937* (ILL). **Jim Wells Co.:** 9 miles N of Alice on US Rt. 281, 6 Jun 1991, *D.S.Seigler, J.E.Ebinger, H.Clarke & K.Readel 13765c* (ILL). **Kinney Co.:** 10 miles E of Brackettville on Ranch Road 334, 20 May 1976, *D.S.Seigler, S.Saupe, & H.Welt 9942* (ILL). **La Salle Co.:** Chaparral Wildlife Management Area, 25 May 2003, *D.S.Seigler & J.E.Ebinger 15676* (ILL). **McMullen Co.:** S edge of Tilden, Rt. 16, 10 Jul 1998, *D.S.Seigler & J.E.Ebinger 14337* (ILL); 3 miles E and 1 mile N of Tilden, 25 May 2001, *D.S.Seigler & J.E.Ebinger 15027* (ILL). **Medina Co.:** 2 miles W of D'Hanis, 4 Mar 1954, *O.E.Sperry 2975* (ILL). **Starr Co.:** access road to Falcon Dam, 18 Feb 2004, *D.S.Seigler, J.E.Ebinger & L.R.Phillippe 15896* (ILL). **Uvalde Co.:** Harris Ranch near Cline, 24 Jun 2002, *D.S.Seigler & J.E.Ebinger 15250* (ILL). **Val Verde Co.:** 16 miles W of Comstock, Rt. 90, 10 Jul 1998, *D.S.Seigler & J.E.Ebinger 14350* (ILL); Langtry, 21 May 1976, *D.S.Seigler, S.Saupe & H.Welt 9952* (EIU, ILL). **Webb Co.:** 5 miles NE of Laredo on US 59, 15 Sep 1979, *D.S.Seigler & D.A.Young 11369* (ILL).

Senegalia greggii: MEXICO: Nuevo León: Dirt road W of Canon Ojo De Agua near Bustamante, 17 May 1997, *C.Glass & G.Glass 359* (ILL). Near Grutas de García, NW of Monterrey, 800 m, 14 May 1991, *D.S.Seigler, J.Ebinger, H.Clarke & K.Readel 13366* (ILL). **UNITED STATES: Texas: Atascosa Co.:** Road to San Miguel Power Plant, 10 Jul 1998, *D.S.Seigler & J.E.Ebinger 14324* (ILL). **Brewster Co.:** Roadside, 15 miles S of Marathon, 18 May 1990, *J.E.Ebinger 24679* (EIU); Rio GrandeVillage, 11 Jul 1998, *D.S.Seigler & J.E.Ebinger 14394* (ILL). **Crockett Co.:** 2 miles S of Interstate 10, W of Ozona, 12 Jul 1998, *D.S.Seigler & J.E.Ebinger 14409* (ILL). **Dimmitt Co.:** Chaparral Wildlife Management Area, 19 May 2005, *D.S.Seigler,*

J.Miller & B.R.Maslin 15938 (ILL). **Jim Wells Co.:** 9 miles N of Alice on US 281, 6 Jun 1991, *D.S.Seigler, J.Ebinger, H.Clarke & K.Readel 13765v* (ILL). **La Salle Co.:** Chaparral Wildlife Management Area, 23 May 2003, *D.S.Seigler & J.E.Ebinger 15667* (ILL). **McMullen Co.:** S of Tilden on Rt.16, 11 May 1991, *D.S.Seigler, J.Ebinger. H.Clarke & K.Readel 13264* (ILL); 3 km. N of Tilden on Rt.16, 20 May 1983, *D.S.Seigler, J.Kramer & E.Carreira 11935* (ILL). **Pecos Co.:** 7 miles E of Bakersfield, 22 May 1977, *D.S.Seigler & S.G.Saupe 10449* (ILL). **Terrell Co.:** 20 miles E of Sanderson, Rt. 90, 10 Jul 1998, *D.S.Seigler & J.E.Ebinger 14360* (ILL).

Senegalia wrightii: MEXICO: Coahuila: Rt. 57, between Allende and Nueva Rosita, 480 m, 5 Jun 1997, *C.Glass & G.Glass 439* (ILL). **Nuevo León:** Near Grutas de García, 800 m, 14 May 1991, *D.S.Seigler, J.Ebinger, H.Clarke & K.Reade13359* (ILL); 10 miles N of Montemorelos on hwy. 85, 6 Jul 1983, *D.S.Seigler, J.Kramer & E.Carreira 12124* (ILL). **Tamaulipas:** 5 miles E of Lucio Blanco, near Linares, 305 m, 20 May 1997, *C.Glass & G.Glass 368* (ILL); Rt. 85, S of Linares, 640 m, 20 May 1997, *C.Glass & G.Glass 369* (ILL). **UNITED STATES: Texas: Callahan Co.:** 10 miles S of Baird, Rt. 283, 13 Jul 1998, *D.S.Seigler & J.E.Ebinger 14426* (ILL). **La Salle Co.:** Farm Road 624, E of Cotulla, 19 May 2005, *D.S.Seigler, J.T.Miller & B.R.Maslin 15939* (ILL). **Maverick Co.:** 10 miles E of Eagle Pass, 27 Jun 2002, *D.S.Seigler & J.E.Ebinger 15269* (ILL). **Schleicher Co.:** 7 miles SW of Fort McKavett, Rt. 864, 13 Jul 1998, *D.S.Seigler & J.E.Ebinger 14411* (ILL). **Shackelford Co.:** 16 miles S of Albany and 2 miles N of county line, Rt. 283, 13 Jul 1998, *D.S.Seigler & J.E.Ebinger 14425* (ILL). **Starr Co.:** 6 miles NW of Roma-Los Saens, Rt. 83, 20 May 1983, *D.S.Seigler, J.Kramer & E.Carreira 11953* (ILL). **Uvalde Co.:** Harris Ranch, near Cline, 20 miles W of Uvalde, 24 Jun 2002, *D.S.Seigler & J.E.Ebinger 15219* (ILL); 1 mile W of Blanco River, rest area on Rt. 90, E of Uvalde, 27 Jun 2002, *D.S.Seigler & J.E.Ebinger 15271* (ILL); 15 miles NW of Uvalde, off hwy 55, 27 May 2002, *D.S.Seigler & J.E.Ebinger 15688* (ILL). **Zapata Co.:** Arroyo Dolores where it is crossed by US 83, 25 miles N of Zapata, 18 Feb 2004, *D.S.Seigler, J.E.Ebinger & L.R.Phillippe 15883* (ILL). **Zavala Co.:** 6 miles N of La Pryor, US 83, 27 May 2003, *D.S.Seigler & J.E.Ebinger 15686* (ILL).

Senegalia x emoryana (Senegalia berlandieri x S. greggii): MEXICO: Chihuahua: 21 km. NW of Escalón, 1650 m, 7 Jul 1972, *F.Chiang, T.L.Wendt & M.C.Johnston 8308* (LL). **Coahuila:** 5 km. E of San José del Refugio on road to Santa Teresa, 1675 m, 5 Jul 1972, *F.Chiang, T.L.Wendt & M.C.Johnston 8266* (MEXU); Virgen de Guadalupe shrine, 3100 ft., 7 Aug 1973, *J.Henrickson 12036* (LL); Cañón de Fora, 1100 m, 7 May 1973, *M.C.Johnston, T.L.Wendt & F.Chiang C. 10908* (NY). **Durango:** 42 miles E of La Zarca, 21 Apr 1960, *J.Crutchfield & M.C.Johnston 5284* (MEX). **UNITED STATES: Texas: Brewster Co.:** SW end of Bullis Canyon of Rio Grande, 500-600 m, 8 Apr 1973, *M.C.Johnston, T.L.Wendt & F.Chiang C. 10592* (LL); Anderson's ranch, Marathon, 26 Jun 1929, *H.B.Parks, Jr. 4031* (F). **Dimmitt Co.:** Chaparral Wildlife Management Area, 19 May 2005, *D.S.Seigler, J.Miller & B.R.Maslin 15933* (ILL). **Jim Wells Co.:** 9 miles N of Alice, Rt. 281, 6 Jun 1991, *D.S.Seigler, J.E.Ebinger, H.Clarke & K.Readel 13769* (ILL). **McMullen Co.:** N of Tilden, hwy. 16, 20 Jul 2004, *D.S.Seigler & B.Maslin 12667* (ILL); 33 miles N of Freer, 22 May 1976, *D.S.Seigler, S.Saupe & H.Welt 10040* (ILL). **Presidio Co.:** 1 mile W of San Antonio Canyon, 3800 ft., 13 Jun 1977, *M.Butterwick & E.Lott 3849* (TEX). **Val Verde Co.:** 8 miles E of Langtry, Rt. 90, 7 Jul 1958, *D.S.Correll & I.M.Johnston 19412* (LL); 10 miles NW of Langtry, 1 May 1955, *B.L.Turner 3767* (TEX).

Senegalia x turneri (Sengalia berlandieri x S. wrightii):UNITED STATES: Texas: Hidalgo Co.: Armando Vela property, 85 ft., 11 Nov 2003, *W.R.Carr & A.Vela 22700* (ILL, TEX). **Kinney Co.:** 9 miles NE of Bracketville, 9 Jun 1955, *B.L.Turner 3879* (TEX). **Live Oak Co.:** 7 miles S of George West, 6 Apr 1953, *M.C.Johnston s.n.* (TEX). **Maverick Co.:** Eagle Pass, *V.Havard 1375* (MO). **Uvalde Co.:** Uvalde, 20 Jun 1917, *E. J. Palmer 12318* (MINN); Montell, 23 Jun 1917, *E. J. Palmer 12330* (MO). **Val Verde Co.:** Devil's River, 5 miles north Ft. Hudson, 3 May 1949, *W.V.Brown s.n.* (TEX).

Appendix II. Characters scored for the principal component (PCA) and principal coordinate analyses (PCoA) of the *Senegalia berlandieri, S. greggii,* and *S. wrightii* species complex.

1. Short shoots (Shs) 1 = absent or nearly so, 2 = common at stem nodes.
2. Petiole length in mm (Pel).
3. Petiole gland length in mm (Gll).
4. Petiole gland shape (Gls) 1 = elliptic, 2 = round or nearly so.
5. Rachis length in mm (Ral).
6. Pinna pair number (Pip).
7. Pinna length in mm (Pil).
8. Leaflet apex shape (Lea) 1 = acute, 2 = obtuse.
9. Leaflet distance in mm (Led).
10. Leaflets pairs/pinna (Lep).
11. Leaflet shape (Les) 1 = nearly all linear to oblong, 2 = many obovate to oblanceolate.
12. Leaflet width in mm (Lew).
13. Leaflet length in mm (Lel).

AGERATINA MARKPORTERI (ASTERACEAE: EUPATORIEAE), A NEW SPECIES FROM OAXACA, MEXICO

Billie L. Turner
Plant Resources Center
The University of Texas
Austin, TX 78712
billie@uts.cc.utexas.edu

ABSTRACT

A new taxon, **Ageratina markporteri** B.L. Turner, **sp. nov.** is described from northwestern Oaxaca, this previously identified as *A. yaharana* B.L. Turner, a species of south-central Guerrero. The novelty reportedly grows (at the type locality) in gypsum soils, and is readily recognized by its broadly ovate leaves, the undersurfaces minutely reticulate-veined and ornamented with numerous golden globules. A photograph of the type is provided, along with a map showing distributions of the two taxa concerned. *Phytologia 94(3): 456-458 (December 1, 2012).*

KEY WORDS: Asteraceae, Eupatorieae, Mexico, Oaxaca, *Ageratina, A. yaharana*

Preoccupation with the identification of the comps of Mexico has occasioned the present paper.

AGERATINA MARKPORTERI B.L. Turner, **sp. nov. Fig. 1**

Resembling *A. yaharana* but the leaves broadly ovate (vs ovate-deltoid to subcordate), blades longer than wide (vs as wide as long or more), having 5 principal nerves (vs 3).

Suffruticose herbs, or shrublets, mostly 1.0-1.5 m high. **Stems** (upper), minutely appressed pubescent. **Leaves** (upper), 3-7 cm long, 2-5 cm wide; petioles 1.5-3.0 cm long; blades broadly ovate, sparingly pubescent above, markedly minutely reticulate veined beneath and

endowed with numerous golden globules, 3-nervate from the base or somewhat above, the margins entire or nearly so. **Capitulescence** a broad rounded cymose panicle of 10-40 heads, the ultimate peduncles 1-5 mm long. **Heads**, 7-8 mm high, 4-5 mm wide; receptacle plane, ca 1 mm across, glabrous; involucral bracts 2-3 seriate, the innermost 3-4 mm long, ca 1 mm wide. **Florets** 10-20 per head; corollas white to pinkish-white, 4-5 mm long, the lobes glabrous, ca 0.75 mm long. **Achenes** ca 2 mm long, sparingly pubescent; pappus of ca 30 persistent bristles 4 mm long.

TYPE: **MEXICO. OAXACA: Distr. Nochixtlan,** "along Mexico Hwy 190 libre, 16 km N of the junction of 190 cuota and 190 libre at Nochixtlan;" 17 32.266 N, 97 21.449 W, ca 2230 m, " Large gypsum outcrop." 13 Dec 2005, *J. Mark Porter 14548* [with V. Steinmann] (Holotype TEX; isotype RSA).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. OAXACA: Distr. Huajuapan, Mpio. Santo Domingo Tonola,** Paraje "Amates Amarillos," ca 1634 m, 15/10/08, *Hernandez* (ATH) *525* (TEX); Paraje "Barranca del Limoncillo," ca 1588 m, 30/10/08, *Hernandez* (ATH) *682* (TEX). Paraje "Canon del Boqueron," ca 1347 m, 10/10/08, *Perez* (LAH) *511* (TEX); Paraje "Palo de Flor," 1698 m, *Perez* (LAH) *774* (TEX).

In my treatment of the Eupatorieae for Mexico, the novelty will key to or near *A. rubricaulis* (HBK) King & Rob., much as the subsequently named name *Ageratina yaharana* B.L. Turner (Turner 2008), to which I had originally assigned the Nochixtlan collections cited above. Recent acquisitions from elsewhere (the type!) led me to reconsider such identification, hence the present paper.

The original publication of *A. yaharana* is reproduced below; unfortunately the type locality of that taxon was omitted in my descriptive account, but accounted for on the photograph of the Type itself (Fig. 7), hence I reckon the name appropriately published, although a few nomenclatural pundits thought otherwise (pers. comm.).

The species is named for its earliest collector (known to me), Dr. Mark Porter, well known plant systematist, long associated with Rancho Santa Ana Botanical Gardens, Claremont, California.

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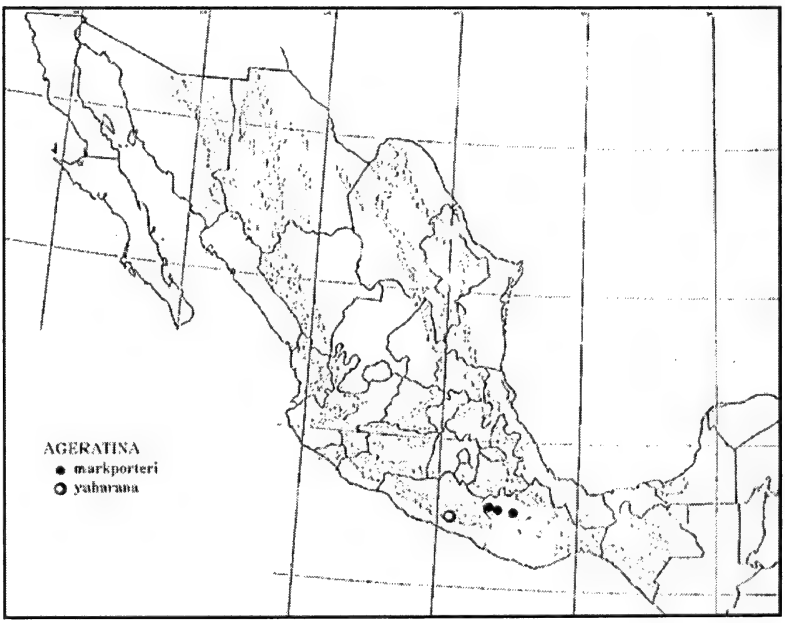

Figure 1. Distributions of *Ageratina markporteri* and *A. yaharana.*

NEW COMBINATIONS IN THE FLORIDA FLORA III

Daniel B. Ward
Department of Botany, University of Florida
Gainesville, FL 32611, U.S.A.

ABSTRACT

New combinations are made for the following species and varieties within the flora of Florida: *Aster shortii* var. *camptosorus*, *Borrichia arborescens* var. *glabrata*, *Carex amphibola* var. *godfreyi*, *Carex flaccosperma* var. *pigra*, *Carex granularis* var. *gholsonii*, *Carex oligocarpa* var. *calcifugens*, *Carex oligocarpa* var. *paeninsulae*, *Carex oligocarpa* var. *thornei*, *Chrysopsis floridana* var. *highlandsensis*, *Chrysopsis linearifolia* var. *dressii*, *Conradina grandiflora* var. *etonia*, *Croton linearis* var. *fergusonii*, *Hymenocallis latifolia* var. *puntagordensis*, *Ludwigia grandiflora* var. *hexapetala*, *Oldenlandia uniflora* var. *fasciculata*, *Panicum spretum* var. *leucothrix*, *Panicum spretum* var. *longiligulatum*, *Peperomia obtusifolia* var. *floridana*, *Phlox carolina* var. *angusta*, *Phlox nivalis* var. *hentzii*, *Psilocarya eximia*, *Rayjacksonia phyllocephala* var. *megacephala*, *Schwalbea americana* var. *australis*, *Scutellaria altamaha* var. *australis*, *Spiranthes lacera* var. *eatonii*, *Vernonia gigantea* var. *ovalifolia*. Lectotypes have been designated for: *Aster camptosorus* (= *Aster shortii* var. *camptosorus*), *Croton fergusonii* (= *Croton linearis* var. *fergusonii*), *Eriocarpum megacephalum* (= *Rayjacksonia phyllocephala* var. *megacephala*), *Hedyotis fasciculata* (= *Oldenlandia uniflora* var. *fasciculata*). *Phytologia 94(3): 459-485 (December 1, 2012).*

KEY WORDS: Amaryllidaceae, *Aster*, *Borrichia*, *Carex*, *Chrysopsis*, Compositae, *Conradina*, *Croton*, Cyperaceae, Euphorbiaceae, *Hymenocallis*, Labiatae, *Ludwigia*, *Oldenlandia*, Onagraceae, Orchidaceae, *Panicum*, *Peperomia*, *Phlox*, Piperaceae, Polemoniaceae, *Psilocarya*, *Rayjacksonia*, Rubiaceae, *Schwalbea*, Scrophulariaceae, *Scutellaria*, *Spiranthes*, *Vernonia*.

Efforts to understand and document the rich Florida flora continue to encounter names of species or varieties that seem misplaced

as to rank, or have been overlooked when genera are divided. A series of publications (Brown 2008; Kral 1999; Ward 2001; Wunderlin & Hansen 2001; Ward 2004; Ward & Hall 2004; Ward 2006a; Ward 2006b; Ward & Housel 2007; Ward 2008; Ward 2009a; Ward 2009b; Ward 2011; Weakley et al. 2011) has attempted to adjust the epithets of these names to the taxonomic level that their degree of morphological difference would seem to deserve. Here, a further 26 new combinations are formed, in pursuit of that goal.

These changes in rank from species (or subspecies) to variety, or the reverse, or transfers from one generic home to another, are surely of minor importance in the larger world. But they speak directly to that age-old quandary, "what is a species," where the words carry an ever-evolving intellectual gloss. Advanced systematics texts present sophisticated discussions of as many as 7 defined species concepts (e.g., Judd et al. 1999), some far more subtle than the once revered "biological species concept." Still, the workaday definition requires that each newly examined taxon must have characteristics of form and distinctness that are similar in magnitude to other, more familiar taxa, or that its rank and placement be adjusted until it reflects minimal anomalies.

Within these uneven ranks are discrepancies of what have here been called "orphan taxa," entities that were recognized historically in Florida but for one reason or another have not been transferred into the appropriate genus or species. More often entities bear a rank higher or lower than comparison with the characteristics of related taxa would justify. This group contains a subset of recently recognized taxa, by authors who are intimately familiar with the group (usually a genus or section), and who define as species small morphological variances that an earlier author would have interpreted as acceptable infraspecific variation.

A common feature of this last subset is that the author has focused narrowly, often exclusively, on the genus of his interest. With this concentration, it is perhaps difficult for him to appreciate where his definition of "species" differs from that of the scholars who preceded

him. His work product then becomes a taxon -- a "species" -- with conventional nomenclature, morphological terminology, and seeming legitimacy, but of small, subtle, marginally useful groupings. Some workers, perhaps unwilling frontally to acknowledge the distortion these interpretations place on their science, resort to calling them cryptic species or microspecies.

This proliferation of diminutive taxa has increasingly beset all ranks, from families to genera and to species. The lure of monophylly as an essential element of classification has encouraged and justified this fragmentation (though at times the reverse is employed, where familiar, readily recognized families or genera disappear by merger into larger taxa). But with this fragmentation has come reduced ability by others to exploit the once-exclusive purpose of plant classification, that of identification and information retrieval. If the distinguishing characters become increasingly slight, accurate identification is retarded. And if identification -- correct or otherwise -- yields an unfamiliar name, access to relevant information elsewhere is hindered.

The authors of certain of these microspecies compound the analysis of their taxa by publishing their names and descriptions in the author's own online journals. Some adequately, though narrowly, meet the standards of "effective publication" (McNeill et al. 2006), by distribution of a few hard-paper copies to appropriate libraries; others seem to be accessible only in an electronic medium. Personal journals such as these are often overlooked (or scorned!) by the larger botanical public, and tend to be ephemeral, sooner or later overstaying their author's patience and stamina. A much-needed proposal for constrainment and standardization of electronic publication is presently under examination (Watson 2010).

It is realistic to believe that the era of truly new discoveries in Florida of species-level native plants is drawing to a close. The day may have passed when a student new to America and speaking scarcely intelligible English could within weeks encounter the modest but sharply distinct endemic known as *Stylisma abdita* Myint (1966); or a specimen found in an herbarium could be recognized as a genus new to

the state with a new, endemic species, *Ziziphus celata* Judd & Hall (1984), later confirmed by discovery of living plants (DeLaney et al. 1989); or a recent graduate, fresh from his discovery of a forest tree clearly native to the state but previously unknown to Florida and to the eastern United States, *Ulmus crassifolia* Nuttall (McDaniel 1967), could publish a well-founded new genus, based on a new panhandle endemic, *Harperocallis flava* McDaniel (1968). Those exuberant days are in the past and it is unwise to simulate them by assigning less distinct taxa to the same taxonomic rank.

A plea could be made -- certain to be ignored -- that future students of floras of reasonably well-studied regions such as Florida, restrain themselves in the publication of new microspecies. When the urge becomes irresistible to show their latest findings to the botanical public, may they temper their pride by selecting an infraspecific rank, rather than burden the science with what is potentially an immeasurably large, crippling, abundance of new species names.

The proposed diminution here of some recently described Florida taxa is not to be interpreted as condemnation of all. *Forestiera godfreyi* L. C. Anderson (1985) is both distinct and disjunct from its western congeners. *Carex kraliana* Naczi & Bryson (Naczi et al. 2002) seems sufficiently different to justify specific status. *Chrysopsis delaneyi* Wunderlin & Semple (DeLaney et al. 2003) has a substantial morphological basis. *Crotalaria avonensis* DeLaney & Wunderlin (1989) has been known as a distinct form at least since 1962 (Ward 2010). These, with *Eriocaulon nigrobracteatum* Bridges & Orzell (1993), *Rhynchospora megaplumosa* Bridges & Orzell (2000), *Juncus paludosus* Bridges & Orzell (2008), and others, though by-and-large of lesser morphological prominence than earlier-described taxa of their genus, are adequately distinguished.

As before, the rank of variety is here preferred where an infraspecific category is desired. Appreciation is given to other authors (e.g., Holmgren 1994) who have placed on record their own support of the varietal rank over that of subspecies.

The following are Florida taxa that in each case appear to represent discrete groupings but have been recognized and published at a rank their differences do not justify. In most, the morphological basis for distinction between the taxa is not given, but is reserved for another forum.

AMARYLLIDACEAE

Hymenocallis latifolia (Mill.) Roem. var. **puntagordensis** (H. P. Traub) D. B. Ward, comb. et stat. nov. Basionym: *Hymenocallis puntagordensis* H. P. Traub, Plant Life 18: 71. 1962. TYPE: United States, Florida, Charlotte Co., Punta Gorda (orig. source), 29 Aug 1961 (cult.), *Traub 878a*, *878b*, *878c* (holotype: MO).

Recent presentations of *Hymenocallis* (Smith & Flory 2002; Smith & Garland 2003) enumerated 12 species within Florida (and 3 elsewhere). Of these, 4 are in addition to those known to Small (1933), himself an acute field observer and assertive taxonomic splitter. At first glance, *Hymenocallis* appears to show the signs of an over-fragmented genus. Its numerous species, many bearing unfamiliar names, are so subtly distinguished that even experienced field botanists (e.g., Godfrey & Wooten 1979) have despaired of forming a meaningful treatment. Herbarium materials are often unidentifiable, even to the author of the species (H. P. Traub, pers. comm., Jan 1965). One searches for groupings among the named entities that will approximate what is thought of as "species" within other genera.

But further consideration of these unfamiliar taxa shows in large part a conformation to the characteristics found of species in other genera. The entities are separated by several morphological features, the ranges of many coincide with the known ranges of other unrelated Florida species, and for some there is the implication of genetic isolation as indicated by differing chromosome counts (Flory 1978). The easy pathway -- to accept as proven the names and data of these unfamiliar species -- is probably justified for most.

Even so, one name stands out, as probably unworthy of specific rank. *Hymenocallis puntagordensis*, though in print for over forty years (Traub 1962), is a wholly unfamiliar name to southeastern botanists. It came to attention only in the 1990s through the successful efforts of Gerald Smith and Mark Garland (1996) to relocate the plant, and its brief mention in a guide to the state's flora (Wunderlin 1998). Other than its type (prepared from cultivated plants) and a few collections by Smith and Garland, it seems entirely unrepresented in Florida and large national herbaria. It appears to occur only on disturbed sites, along roadsides and railroad right-of-ways, near the city of Punta Gorda, in southwestern peninsular Florida.

These plants bear evergreen leaves, a trait of tropical species found among Florida species only in *Hymenocallis latifolia*. Indeed, more robust specimens bear a striking resemblance to that species; but Smith and Garland believe that closer examination reveals several differences: the margin of the staminal cup has prominent projecting points, the pollen is yellow, the ovaries are pyriform, and the leaves are narrowly strap-shaped (in contrast to only small marginal irregularities of the staminal cup, orange pollen, ovoid ovary, and broader leaves in *H. latifolia*). The habitat is strikingly different; *H. latifolia* is a coastal species, found on dunes and edges of saline swamps and swales.

Smith and Garland are equivocal as to the nativity status of *Hymenocallis puntagordensis*. They have suggested (1996), in light of its restriction to disturbed sites, that it may not be native to Florida. Even so, because of certain similarities with other Florida species (*H. henryi*, of the Florida panhandle; *H. palmeri*, a widespread native of the Everglades), they concluded it is native. They later (2003) back away from this status by wondering if "it may be a taxon naturalized from the Neotropics that has undergone natural selection..." Wunderlin (1998) recorded it as a native and -- logically, considering that its only known location is in Florida -- as an endemic.

This habitat and distribution suggest this taxon is significantly different from other Florida species-level taxa of the genus. Unlike certain other areas of the state where endemics abound (Ward 1979;

Christman & Judd 1990), low-lying western Charlotte County is nearly lacking in plant endemism. Though of course, everything has to grow somewhere, one is uncomfortable in ascribing a unique distribution to a native plant. It is far more to be expected that a species of long duration in the area will conform to the vagaries of climate, soil types, competition, sea-level change, and other factors that constrain the distribution of other native species.

In contrast, an introduced species is free of historic influences and is subject only to the circumstances that bring it into the area. With *Hymenocallis puntagordensis* found only in disturbed habitats, as is typical of recently introduced species, and with its distinctive evergreen foliage showing relation only to the widespread, variable, little studied *H. latifolia*, it seems best to interpret *H. puntagordensis* as a non-native, smaller variant of that pan-Caribbean coastal species.

COMPOSITAE

Aster shortii Lindl. in Hook. var. **camptosorus** (Small) D. B. Ward, comb. et stat. nov. Basionym: *Aster camptosorus* Small, Bull. Torrey Bot. Club 24: 339. 1897. TYPE: United States, Alabama, Lee Co., "Wright's Mill, five miles south of Auburn," 17 Oct 1896, *Baker 76* (lectotype, designated here: NY).

Aster shortii is a northern species, rare in Virginia, the Carolinas, and northern Georgia. Var. *camptosorus* in Florida is quite disjunct, very rare, all collections seemingly having come from a small area in western Gadsden County. The variety as expressed in Florida differs in that leaves are narrower and more glossy above. Burgess (1903) and Alexander (1933) recognized the taxon at specific rank. Jones, in 1986, annotated specimens (FLAS, NY) with the above combination, but did not publish it. More recent authors (cf. Brouillet et al. 2006; Nesom 1994) did not address variation within the species.

Borrichia arborescens (L.) DC. var. **glabrata** (Small) D. B. Ward, comb. et stat. nov. Basionym: *Borrichia glabrata* Small, Man. S.E. U.S. 1340. 1903. Type: United States, Florida, Monroe Co., "southern Florida and the Keys," 1892?, *Curtiss 1412* (holotype: NY).

In Florida, typical *Borrichia arborescens* extends north along both coasts and onto the panhandle; its leaves are silvery-pubescent. On the Keys, Small's *B. glabrata* is of limited distribution, usually sympatric with the typical form, but readily distinguished by its green, glabrous leaves. Semple (1978a) noted the two forms, but did not give them taxonomic recognition.

Chrysopsis floridana Small var. **highlandsensis** (DeLaney & Wunderlin) D. B. Ward, comb. et stat. nov. Basionym: *Chrysopsis highlandsensis* K. R. DeLaney & R. P. Wunderlin, Bot. Expl. 2: 2. 2002. TYPE: United States, Florida, Polk Co., Avon Park, 12 Nov 2001, *DeLaney 5113* (holotype: USF; isotype: USF).

Chrysopsis linearifolia Semple var. **dressii** (Semple) D. B. Ward, stat. nov. Basionym: *Chrysopsis linearifolia* Semple ssp. *dressii* Semple, Brittonia 30: 492-495. 1978. TYPE: United States, Florida, Brevard Co., Merritt Island, 2 Oct 1976, *Semple, Wunderlin, Poppleton & Norman 2530* (holotype: MO; isotypes: US, USF, WAT).

Two recent epithets in the genus *Chrysopsis* require adjustment. DeLaney & Wunderlin (2002) report what they believe to be a new species, *Chrysopsis highlandsensis*. They recognize it to be related to the peninsular endemic known as *C. floridana* Small, and re-identify most collections bearing that name from the south-central peninsula (primarily Highlands Co.) as their new species. Though they relegate prior collections from counties immediately to the west (Hillsborough, Manatee) to *C. floridana* s.s., they also report stations for this older species in close proximity to their novelty. The authors

do not indicate they saw the type of *C. floridana* or were aware of its source; Small's type came from Bradenton, Manatee Co., within the range they assign to typical *C. floridana.*

DeLaney & Wunderlin (2002) speak at length (20 pages) of the differences they observed between the two taxa: "...the two species differ markedly in overall appearance and capitulescence shape...rosette habit...mid-stem leaf shape...rosette leaf shape...pubescence type, and...other subtile [!] characteristics..." Their 12 photographs (some full-page) show differences, and there is no difficulty in accepting the two named populations as carrying different genotypes. Yet specimens (in FLAS) they did not see are often ambiguous and easily misassigned if their place of collection is hidden. The rank of species is not justified by these observed differences.

Chrysopsis linearifolia ssp. *dressii* Semple (1978b) is a distinct peninsular endemic taxon, geographically disjunct from typical *C. linearifolia* of the Florida panhandle. Yet a wider selection of Florida specimens (FLAS), not reviewed by its author, shows frequent ambiguity if the origin is hidden. Varietal rank retains the taxon, yet in a less obtrusive context.

Rayjacksonia phyllocephala (DC.) Hartman & Lane var. **megacephala** (Nash) D. B. Ward, comb. et stat. nov.
Basionym: *Eriocarpum megacephalum* Nash, Bull. Torrey Bot. Club 23: 107. 1896. TYPE: United States, Florida, Manatee Co., Sneed's Island, "near the mouth of the Manatee River," 21-23 Aug 1895, *Nash 2432* (lectotype, designated here: US; isolectotypes: F, MICH, MO, NY, P, PH).
= *Machaeranthera phyllocephala* var. *megacephala* (Nash) Shinners; *Haplopappus phyllocephalus* var. *megacephalus* (Nash) Waterfall; *Sideranthus megacephalus* (Nash) Small

When Lane & Hartman (1996) divided Cassini's *Haplopappus* by recognizing the new genus *Rayjacksonia*, they correctly transferred *Haplopappus phyllocephala* DC. But they slighted its larger-headed Florida native, *H. megacephalus* (Nash) Hitchc. by leaving it

synonymous with *R. phyllocephala*. Appropriate recognition was given by Shinners (1950) with his *Machaeranthera phyllocephala* var. *megacephala*; again by Waterfall (1960) with *Haplopappus phyllocephalus* var. *megacephalus*. With *Rayjacksonia* accepted at generic rank, Nash's epithet again needs transfer.

Vernonia gigantea (Walt.) Trel. ex Branner & Coville var. **ovalifolia** (Torr. & Gray) D. B. Ward, comb. et stat. nov. Basionym: *Vernonia ovalifolia* Torr. & Gray, Fl. N. Amer. 2: 59. 1841. TYPE: United States, Florida, Franklin Co.?, "Middle Florida," 1837?, *Chapman s.n.* (holotype: NY). = *Vernonia gigantea* ssp. *ovalifolia* (Torr. & Gray) Urbatsch

Urbatsch (1972) and Jones & Faust (1978) recognized both *Vernonia gigantea* ssp. *gigantea* and ssp. *ovalifolia*. The first is restricted in Florida to the central panhandle, the second is widespread in both panhandle and peninsula. A. W. Chapman, resident of Apalachicola and within range of both variants, first (1860) recognized both *V. ovalifolia* and *V. gigantea* (his *V. noveboracensis*), but later (1897) distinguished only *V. gigantea*. Wunderlin (1998) placed both variants under an undivided *V. gigantea*. The differences as documented by Urbatsch (1972: 236) are real, but modest.

CYPERACEAE

Carex amphibola Steud. var. **godfreyi** (Naczi) D. B. Ward, comb. et stat. nov. Basionym: *Carex godfreyi* Naczi, Contr. Univ. Michigan Herb. 19: 200. 1993. TYPE: United States, Florida, Lake Co., Astor Park, 22 Apr 1991, *Naczi 2781* (holotype: MICH; isotypes: FLAS, FSU, NCU, NY, US, VDB).

Carex flaccosperma Dewey var. **pigra** (Naczi) D. B. Ward, comb. et stat. nov. Basionym: *Carex pigra* Naczi, Novon 7: 67. 1997. TYPE: United States, Mississippi, Lowndes Co., Mahew, 15

May 1989, *Naczi 2174A* (holotype: MICH; isotypes: KNK, NCU, NY, US).

Carex granularis Muhl. ex Schkuhr in Willdenow var. **gholsonii** (Naczi & Cochrane) D. B. Ward, comb. et stat. nov. Basionym: *Carex gholsonii* Naczi & Cochrane, Novon 12: 524. 2002. TYPE: United States, Florida, Citrus Co., Crystal River, 24 Apr 1991, *Naczi 2787* (holotype: DOV; isotypes: FLAS, MICH, MO, NY, WIS).

Carex oligocarpa Schkuhr in Willd. var. **calcifugens** (Naczi) D. B. Ward, comb. et stat. nov. Basionym: *Carex calcifugens* Naczi, Novon 12: 512. 2002. TYPE: United States, Georgia, Screven Co., Blue Springs, "Blue Springs Landing on Savannah River," 2 May 1991, *Naczi 2840* (holotype: DOV; isotypes: FLAS, FSU, GA, GH, MICH, MO, NCU, NY, PH, TENN, UNA, US, USCH, VDB, VPI, VSC, WIN).

Carex oligocarpa Schkuhr in Willd. var. **paeninsulae** (Naczi, Bridges & Orzell) D. B. Ward, comb. et stat. nov. Basionym: *Carex paeninsulae* Naczi, Bridges & Orzell, Novon 12: 514. 2002. TYPE: United States, Florida, Clay Co., Green Cove Springs, "Magnolia Springs," 20 Apr 1991, *Naczi 2770* (holotype: DOV; isotypes: FLAS, FSU, GA, GH, MICH, MO, NY, VDB, WIN).

Carex oligocarpa Schkuhr in Willd. var. **thornei** (Naczi) D. B. Ward, comb. et stat. nov. Basionym: *Carex thornei* Naczi, Novon 12: 516. 2002. TYPE: United States, Alabama, Russell Co., Holy Trinity, "along S. side of Bluff Creek," 3 May 1996, *Naczi 5214* (holotype: DOV; isotypes: MICH, MO, NY, US, WIN).

Beginning in the early 1990s, Robert Naczi and colleagues published an impressive number of new southeastern species of *Carex*. For so many new species of that genus to be uncovered in the span of so few years, one would think that the sedges, admittedly without the

importance of many other genera, are so lacking in charm as to have been only superficially surveyed by previous workers.

This is surely not the case. Whatever their motivation, entire generations of cyperologists have labored in the field and herbarium and prepared scrumptious volumes of cleanly described, beautifully illustrated sedges. But how did these earlier workers overlook so many species? How could Naczi and his colleagues have sufficient skill and/or good fortune to be able to find seven new species of *Carex* for presentation in a single paper (2002, with Bryson and Cochrane)?

The answer, one fears, is that there has been a shift in the standards of what constitutes a species. It is possible that many of the newly described species of *Carex* will be found to represent geographically or environmentally separated and genetically isolated populations. But even so, and with acknowledgment that Naczi has provided detailed keys to separate his entities from their congeners, the differences are subtle. Until other persons have had opportunity to independently appraise these new entities, it seems best to to look with some caution at their significance. The ranking of "variety" preserves the present information, yet avoids over-emphasizing a taxon whose importance is not yet known.

Psilocarya eximia (Nees in Seem.) D. B. Ward, comb. nov. Basionym: *Spermodon eximius* Nees in Seem., Bot. Voy. Herald, 222. 1854. TYPE: Panama, "in palis prope urb.," 1846-1847, *Seemann 140* (holotype: BM; isotype: K).
= *Rhynchospora eximia* (Nees in Seem.) Boeck. [Seemann may have collected the specimen in Panama just before he joined the Herald expedition in Jan 1847. Nees, author of the name, was never in Panama.]

Psilocarya Torr. may be argued to be generically separable from *Rhynchospora* Vahl. [*Psilocarya* are annuals, with several to many flowers (and achenes) per spikelet and no perianth bristles; *Rhynchospora* s.s. are mostly perennials, with 1-2 flowers per spikelet

and perianth bristles often present at base of achene.] If retained at generic rank, three species of *Psilocarya* occcur in Florida. *Psilocarya nitens* (Vahl) Wood and *Psilocarya scirpoides* Torr. are widespread and frequent. A third species is rare, with few Florida collections; it was formerly (and incorrectly) known as *Psilocarya schiedeana* (Kunth) Liebm. (i.e., Small 1933; Godfrey & Wooten 1979). If treated as a *Rhynchospora* it becomes *R. eximia* (Nees) Boeck. But without transfer of Nees' epithet, it has no correct name in *Psilocarya.*

This species had earlier been treated (as a *Psilocarya*) by Liebmann, but rather than forming a new name he made a new combination, *P. schiedeana* (Kunth) Liebm. (1851), based on *Rhynchospora schiedeana* Kunth (1837), a very different plant (thus misapplied to the Florida species).

EUPHORBIACEAE

Croton linearis Jacq. var. **fergusonii** (Ferguson in Small) D. B. Ward, comb. et stat. nov. Basionym: *Croton Fergusonii* Ferguson in Small, Flora Southeastern United States 695. 1903. TYPE: United States, Florida, Palm Beach Co., Palm Beach, "sand ridges near the ocean," 2 May 1895, *Curtiss 5360* (lectotype, designated here: NY; isolectotype: MO?).

In his monograph of the genus *Croton*, Ferguson (1901) described two variants of *C. linearis*, the first typical of the species (as confirmed by comparison with its type), the second (non-typical) representative of a wider-leaved form. Ferguson did not name these other than as "Form A" and "Form B," though he noted them to be "probably...specifically distinct." Two year later he prepared the treatment of *Croton* for Small's "Flora" (1903); there his "Form A" was named *C. Fergusonii*, with "Small" as the author.

These two entities, as described in Ferguson (1901) and Ferguson in Small (1903), well fit the variants found along the southeastern Florida coast. Intermediates seem few. Yet Ferguson's

plant is clearly derivative of *C. linearis*, and is best treated as a variety of that species, as is done here.

The authorship of *Croton Fergusonii* is muddled. Small (1903: 693) credited the treatment of *Croton* to Ferguson; his data, though reworded, is largely from Ferguson's earlier (1901) monograph, and two other species were recorded as named by him. But Small's authorship was unambiguously assigned to *C. Fergusonii*, and Small (1933) later continued this accreditation. Unquestionably Ferguson would not have submitted his treatment under the name and authorship "*Croton Fergusonii* Ferguson;" he would have proposed some other epithet. Likely, Small, in appreciation of Ferguson's scholarly efforts and as a professional courtesy, simply substituted Ferguson's name for whatever epithet had been suggested for "Form B." If this be true, Small was merely the editor, and the true authorship was that of Ferguson. "Ferguson in Small" is sufficient acknowledgment for both.

GRAMINEAE

Panicum spretum Schult. var. **leucothrix** (Nash) D. B. Ward, comb. et stat. nov. Basionym: *Panicum leucothrix* Nash, Bull. Torrey Bot. Club 24: 41. 1897. TYPE: United States, Florida, Lake Co, "low pine land at Eustis," July 1894, *Nash 1338* (holotype: NY; isotypes: NCU, NY, TAES, US).
= *Panicum acuminatum* Sw. var. *leucothrix* (Nash) Lelong; *Dichanthelium leucothrix* (Nash) Freckmann; *Dichanthelium acuminatum* ssp. *leucothrix* (Nash) Freckmann & Lelong

Panicum spretum Schult. var. **longiligulatum** (Nash) D. B. Ward, comb. et stat. nov. Basionym: *Panicum longiligulatum* Nash, Bull. Torrey Bot. Club 26: 574. 1899. TYPE: United States, Florida, Franklin Co, Apalachicola, 1892, *Vasey s.n.* (holotype: NY). = *Panicum acuminatum* Sw. var. *longiligulatum* (Nash) Lelong; *Dichantheliu acuminatum* ssp. *longiligulatum* (Nash) Freckmann & Lelong

The genus *Panicum* in recent decades has inspired a number of independent reappraisals, each with its own philosophies and taxonomic conclusions. Lelong (1965, 1984), Freckmann (1967, 1981), Gould & Clark (1978), Hansen & Wunderlin (1988), and Freckmann & Lelong (2003) have all attempted to improve on the classic study by Hitchcock & Chase (1910). Only because of the variances of name and rank found among these worthies does it seem permissible to offer still another interpretation of certain taxa.

In the later years of the 19th century, George V. Nash applied his keen eye to the small differences to be found among the many southeastern *Panicum*. Two of his discoveries, *P. longiligulatum*, and *P. leucothrix*, though usually no longer given specific status, have survived recognition at lower ranks. These names, with others, form a small group of taxa held together by scarcely more than ligules of conspicuous hairs and mid-sized spikelets; the earliest name is *P. acuminatum* Sw. Ten of these variants are summarized by Freckmann & Lelong (2003), all treated as subspecies.

But examination of the southeastern members of this group suggests they may be separated into two adequately distinct species -- *P. acuminatum* and *P. spretum*. Both *P. leucothrix* and *P. longiligulatum* fall within the second species. Typical *P. spretum* is northern and seems absent from Florida. The Florida variants of *P. spretum* appear not to intergrade, but their differences are slight. Both have previously been treated as varieties of *P. acuminatum* (Lelong 1984), thus the change made here is only an accomodation to recognition of *P. spretum*.

These species, among many others, have in recent decades been treated as members of *Dichanthelium*, a genus apart from *Panicum* s.s. (Gould & Clark 1978; Hansen & Wunderlin 1988; Freckmann & Lelong 2003). There is merit in recognition of *Dichanthelium* as a distinct biological group. But its differences from *Panicum* are slight, of lesser magnitude than those separating other segregates such as *Setaria*, *Paspalidium* and *Brachiaria*. It is sufficient that *Dichanthelium* be recognized at subgeneric rank.

LABIATAE

Conradina grandiflora Small var. **etonia** (Kral & McCartney) D. B. Ward, comb. et stat. nov. Basionym: *Conradina etonia* Kral & McCartney, Sida 14: 393. 1991. TYPE: United States, Florida, Putnam Co., vic. Florahome, 20 Sept 1990, *McCartney s.n.* (holotype: SMU; isotype: VCB).

The treatment of *Conradina etonia* by McCartney and Kral (1991) is vastly detailed, suffering only by the absence of a similar treatment of typical *Conradina grandiflora* with which it may be compared. In compensation, the authors provide a lengthy commentary to establish the most apparent character differences between the two taxa.

But the cited contrasts are, in effect, damaging to the argument that the differences are of specific rank. Details of indumentum, of leaf venation, of size and pilosity, and of stamen pubescence, while wholly persuasive of the taxon's genetic separateness, do not rise to the level of difference to be found among related species. The sole population, in northwestern Putnam County, is a 90 km. outlier from the northernmost *Conradina grandiflora* in eastern Volusia County, a species whose scattered stands extend to southern Florida. This pattern of disjunction occurs with other taxa; the authors cite *Sabal etonia*, an endemic palm found throughout the Florida scrub and whose type locality is the nearby Etonia Creek for whom their new *Conradina* species is named. This isolation, coupled with the small size of the *C. etonia* population, gives ample opportunity for random selection to produce small deviations. That it is known from a single population, within a platted but yet undeveloped subdivision, raises fear that it is likely to vanish from the flora before more can be learned.

Scutellaria altamaha Small var. **australis** (Epling) D. B. Ward, comb. et stat. nov. Basionym: *Scutellaria altamaha* Small ssp. *australis* Epling, Univ. California Publ. Bot. 20: 89. 1942.

TYPE: United States, Alabama, Houston Co., "pine woods 10 miles south of Dothan," 10 Aug 1927, *Wiegand & Manning 2782* (holotype: GH; isotype: BH).

Specimens cited and mapped by Epling (1942) show a discontinuous distribution between his *Scutellaria altamaha* ssp. *altamaha* [North Carolina into central Georgia] and his *S. altamaha* ssp. *australis* [southern Alabama, panhandle Florida (disregarding a mapped but uncited out-of-range record of ssp. *australis* from vic. Tampa Bay)]. Later workers have either omitted *S. altamaha* or incorrectly merged it with the larger-flowered *S. incana* Biehl., a disjunct northern species. But the distinction between Epling's two subspecies of *S. altamaha* is unclear; he noted "plants of the two areas are not appreciably different."

ONAGRACEAE

Ludwigia grandiflora (Michx.) Greuter & Burdet var. **hexapetala** (Hook. & Arn.) D. B. Ward, comb. et stat. nov. Basionym: *Jussiaea hexapetala* Hook. & Arn. in Hook., Bot. Misc. 3: 312. 1833. TYPE: Uruguay, "in marshes," 1832, *Tweedie s.n.* (holotype: K; isotype: E). = *Ludwigia hexapetala* (Hook. & Arn.) Zardini, Gu & Raven; *Ludwigia grandiflora* ssp. *hexapetala* (Hook. & Arn.) Nesom & Kartesz

Authors differ as to the taxonomic rank of an introduced *Ludwigia* now appearing in Florida wetlands. All agree that *L. grandiflora* is present in the state. Zardini et al. (1991) maintain that a related, somewhat rarer, larger-flowered entity, *L. hexapetala*, is also in the state and is best held as a separate species. Nesom & Kartesz (2000) recognize this second entity, but as *L. grandiflora* ssp. *hexapetala*. And Wunderlin & Hansen (2003) combine the two without distinction. The differences as described by Nesom & Kartesz and Zardini et al. are real. But in consideration of the "quantitative and broadly overlapping" morphological distinctions between the two

(Nesom & Kartesz), they are here treated as var. *grandiflora* and var. *hexapetala*.

Neither variety of *Ludwigia grandiflora* is native to the southeastern United States; both taxa are from South America, but their histories suggest different dates of introduction. Var. *grandiflora*, the smaller-flowered form, has been in the Southeast since Michaux (1803) and Chapman (1860). Michaux's collection was from the seaport of Savannah, Georgia (Zardini et al. 1991), an obvious point of entry. [Michaux's journal (Sargent 1889) recorded his presence in Savannah on April 30, 1787, the only time he visited that city.] The larger-flowered var. *hexapetala* seems to lack early collections. The wide-ranging William Bartram in the 1770s and Ferdinand Rugel in the 1840s did not encounter the species. Both varieties are erratic in distribution, a common pattern with introductions. Both appear to be at least partly sympatric in southern Brazil and elsewhere in South America. The two taxa distinguishable in Florida may represent only "founder effect" selections from a less well differentiated parent population.

ORCHIDACEAE

Spiranthes lacera Raf. var. **eatonii** (P. M. Brown) D. B. Ward, comb. et stat. nov. Basionym: *Spiranthes eatonii* O. Ames ex P. M. Brown, North Amer. Nat. Orchid Jour. 5: 9. 1999. TYPE: United States, Florida, Dade Co., Orange Glade, 21 Feb 1905, *Ames 6905* (holotype: GH).

This orchid was discovered in South Florida in 1905 by A. A. Eaton (Brown 1999), and his specimens were annotated as *Spiranthes eatonii* by O. Ames. But Ames never published the name, and it is appropriate that Brown should do so. The plants appear to represent populations showing small morphological discontinuities with their related congeners.

PIPERACEAE

Peperomia obtusifolia (L.) A. Dietr. in L. var. **floridana** (Small) D. B. Ward, comb. et stat. nov. Basionym: *Peperomia floridana* Small, Torreya 26: 109. 1926. TYPE: United States, Florida, Dade Co., "Ross Hammock near Silver Palm School," 12 Nov 1906, *Small & Carter 2478* (holotype: NY).
= *Rhynchophorum floridanum* (Small) Small

Boufford (1982) and others have merged Small's *Peperomia floridana* within the widespread tropical *P. obtusifolia*. But Florida botanists (J. Beckner, A. Herndon, R. Woodbury) have long been of the opinion that the two are separable. Popenoe (1979) reported *P. obtusifolia* "is usually restricted to decaying bark of logs and stumps and is seldom found far above the ground," while *P. floridana* is epiphytic, and "prefers the sound bark of living wood and often occurs...in the upper branches of trees." *Peperomia obtusifolia* is rare, but is found in the Fakahatchee Strand of Collier Co. and on the east coast north to Brevard Co.; *P. floridana* is very rare, persisting only marginally in hammocks of Dade Co.

Restoration of *Peperomia floridana* to the ranks of recognized Florida plants, if only at varietal rank, follows closely upon the similarly justified separation of *P. cumulicola* as worthy of varietal distinction from typical *P. humilis* (Ward 2001).

POLEMONIACEAE

Phlox carolina L. var. **angusta** (Wherry) D. B. Ward, comb. et stat. nov. Basionym: *Phlox carolina* L. ssp. *angusta* Wherry, Baileya 4: 98. 1956 (nomen novum, a *Phlox glaberrima* var. *suffruticosa* subvar. *angustissima* Brand, Pflanzenr. IV. 250: 65. 1907). TYPE: United States, Mississippi ('Missouri'), Biloxi, [date?], *Tracy 5077* (holotype: G).

Phlox nivalis Lodd. ex Sweet var. **hentzii** (Nutt.) D. B. Ward, comb. et stat. nov. Basionym: *Phlox hentzii* Nutt., J. Acad. Nat. Sci. Phila. 7: 110. 1834. TYPE: United States, North Carolina, Durham Co., Chapel Hill, "Southern Pine-barrens," 1833, *Hentz s.n.* (holotype: GH). = *Phlox nivalis* ssp. *hentzii* (Nutt.) Wherry

For decades Edgar T. Wherry reigned as the authority among American students of the Polemoniaceae. Unlike earlier European authors, Wherry had opportunity to see in the field nearly all species he treated. He summarized his deep knowledge of *Phlox* in 1955 with his informative but flawed "The Genus Phlox." [Though he meticulously described each entity and cited the place of origin of each name, he aberrantly chose to use forbidden trinomials, a flaunting of accepted practice that brought quick condemnation (DeWolf 1956) and acquiescence (Wherry 1956).]

Wherry's personal knowledge of variations within each species must command respect. Though some authors (e.g., Wunderlin & Hansen 2003) have disregarded or submerged Wherry's many subspecies, some seem to retain enough morphological reality to merit recognition as varieties. Two are recognized here.

RUBIACEAE

Oldenlandia uniflora L. var. **fasciculata** (Bertol.) D. B. Ward, comb. et stat. nov. Basionym: *Hedyotis fasciculata* Bertol., Mem. Reale Accad. Sci. Ist. Bologna 2: 306. 1850. TYPE: (lectotype, designated here: Bertoloni, Tab. 17, fig. 2. 1850). [Bertoloni cited no type, nor source for his new species. However, his full-page plate is "original material," suitable for lectotypification. A specimen may also exist (BOLO?).] = *Hedyotis uniflora* var. *fasciculata* (Bertol.) W. H. Lewis, nom. nud. (1962).

The genus *Oldenlandia* as a segregate from *Houstonia* and *Hedyotis* is now generally accepted (Terrell 1996), with 4 species recognized for Florida (Terrell & Robinson 2006). *Oldenlandia uniflora*, a pantropic weed, is quite variable in its African homeland. Variation in Florida seems to be bimodal, differing in pubescence, leaf shape, and capsule size (Small 1933), and suggestive of founder-effect chance selection from foreign sources. Though intermediates are common, sufficient to cause rejection of infraspecific taxa by most authors, recognition of two varieties assists further study of Florida variation.

SCROPHULARIACEAE

Schwalbea americana L. var. **australis** (Pennell) D. B. Ward, comb. et stat. nov. Basionym: *Schwalbea australis* Pennell, Proc. Acad. Nat. Sci. Phil. 71: 289. 1920 ("1919"). TYPE: United States, Florida, Volusia Co., "damp pine barrens near Seville," 10 May 1900, *Curtiss 6742* (holotype: NY).

Although Pennell (1920, 1935) recognized both *Schwalbea americana* and *S. australis* as species, his differences as keyed are small. Authors (Godfrey 1981; Wunderlin 1998; Federal Register, 29 Sept 1992; etc.) who acknowledge only a single undivided species, have a point. But plants identifiable as *S. australis* are distinctly southern and appear to be non-overlapping in range.

Schwalbea americana (inclusive of any infraspecific variation) is a Federally-listed endangered plant. Var. *australis* is not only rare in Florida, but is greatly diminished from earlier years. Although herbarium records are from scattered locations nearly throughout the state (south to Highlands Co.), the plant apparently only persists in the central panhandle (R. Halsenbeck, pers. comm., Oct 1992).

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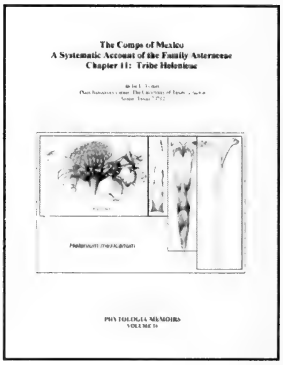

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