Mortality and growth of C*haptalia texana* (silver-puff) in full sun and canopy shade

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ABSTRACT

We completed a transplant experiment with *Chaptalia texana* Greene (silver-puff, Asteraceae) to determine its habitat preference. Sixty days after seedling emergence in a greenhouse, plants were transplanted into the field at Hardberger City Park in San Antonio, Texas. One half of the 40 plants were planted below a *Juniperus/Quercus* woodland canopy and half into an adjacent open grassland. Total mortality was 70% with 12 plants that survived 192 days to the end of the experiment. Nine plants below the canopy and three in the open grassland survived. Growth increased rapidly during the first 60-90 days of the experiment (spring). Full sun (open or grassland) plants were smaller than those below the canopy. Plant response variables decreased during the latter part of the experiment (summer and early fall). Survival and growth variables for canopy plants were greater than for open plants. We believe *C. texana* is found mostly below woodland canopies, but time to demonstrate this seemed to exceed one growing season. The amount and timing of rainfall and the presence of neighbors are also important. Published on-line www.phytologia.org *Phytologia 98(3): 156-163 (July 6, 2016).* ISSN 030319430.

KEY WORDS: canopy, open, sun species, shade species, sunflower, *Juniperus/Quercus* woodland, grassland, neighbors

Chaptalia texana Greene (silver-puff) is a member of the sunflower family (Asteraceae). The genus includes about 56 species, with two found in the southern United States, and the remainder in Mexico, Central America and much of South America (Correll and Johnston 1979; Enquist 1987; USDA 2009). In southwestern North America, *Chaptalia texana* occurs in parts of central and western Texas, southern New Mexico, northern, central and southern Mexico. In central Texas, it is reported in savanna communities, usually below the canopy of *Juniperus/Quercus* (juniper/oak) mottes or woodlands (Van Auken and Bush 2015). It is an herbaceous perennial and grows as a rosette in the woodland phase of some of these central Texas savanna communities, flowering and producing numerous seeds that will germinate within 16 days (Van Auken 2013).

Chaptalia texana seems to be a shade adapted species that can carry out photosynthesis below a woodland canopy (Van Auken and Bush 2015). Differences in photosynthetic rates of plants growing in full sun habitats compared to those found in shady habitats are fairly well known (Hull 2002; Begon et al. 2006; Valladares and Niinemets 2008). Gas exchange rates for shade leaves of *C. texana* placed in full sun were as high as full sun leaves of some sun species (Van Auken and Bush 2015). Thus, its maximum carbon uptake rate is higher than typical understory plants at high light levels (Hull 2002). Carbon uptake rates suggest that this species should occur in grasslands or open areas, but it was not found there.

Few ecological studies have been located that consider the habitat preference of various species of *Chaptalia* or why they seem to be found below canopies rather than grassland environments. The successional status, disturbance requirements, densities and resource requirements of the various species

of *Chaptalia* are unclear. One study of coastal plain longleaf and slash pine forests found *C. tomentosa* after winter fires with 50% cover reduction eight years later with grass regrowth (Lemon 1949). Reports of *Chaptalia* species in South American grasslands showed that frequent disturbances including fire, clipping or herbivory of adjacent species promoted *Chaptalia* density (Fidelis et al. 2010; Joiner et al. 2011). Accordingly, it seems to require disturbances but its ecological niche and factors affecting its distribution are not well understood (Begon et al. 2006). *Chaptalia texana* is mostly found below canopies suggesting that light level is the obvious but not necessarily the only factor controlling its habitat.

THEORY: Our theory is that *Chaptalia texana* is a canopy understory or shade species and will not grow in high light open grassland habitats.

METHODS

This project was completed in "Phil Hardberger Park" in San Antonio, Texas, USA (N-29°33'41.3", W-98°31'11.8"). Subsurface of the study area is Cretaceous limestone, and soils are usually shallow, rocky or gravelly, dark colored, calcareous with neutral or slightly basic pH. They are clayey-skeletal, smectitic, thermic, lithic, calciutolls usually Austin silty clays, Whitewright-Austin complex, or Eckrant cobbly clay (Taylor et al. 1962; NRCS 2006). The Park is approximately 20 km south of the Edwards Plateau region of central Texas just south of the Balcones Escarpment in north central Bexar County (Correll and Johnston 1979; Van Auken et al. 1981; Van Auken and McKinley 2008). Elevation is approximately 350 m above mean sea level (AMSL) (Taylor et al. 1962; NRCS 2006). January mean temperature is approximately 9.6°C and July mean is 29.4°C (NOAA 2004). Precipitation is bimodal totaling 78.7 cm/yr with peaks in May and September (10.7 cm and 8.7 cm), highly variable, with little summer rainfall and high evaporation (Thornthwaite 1931; NOAA 2004).

Chaptalia texana is a native, herbaceous, perennial that grows as a rosette of basal leaves with scapose, monocephalous stems (single flowering head or inflorescence) (Nesom 1995). It is similar to other species of *Chaptalia*. The flowering scapes can be 13-34 cm tall at flowering (anthesis) and become taller in fruit. Leaves are obovate to ovate or elliptical and relatively thick with dense, gray-white pubescence below and dark green and glabrescent above (Enquist 1987; Nesom 1995). This species is reported from thin, rocky, limestone soils and mostly from oak, pine-oak and juniper-oak woodlands (Enquist 1987; Nesom 1995; Harms 2011). It theoretically flowers year round, but mainly when temperatures and rainfall are moderate. Achenes (one seeded fruits) start to germinate soon after maturation with 100% germination at 25°C in low light 16 days after the start of incubation, with slight or no innate seed dormancy (Van Auken 2013).

Area vegetation of the study site was savanna or woodland with *Juniperus/Quercus* (juniper and oak) being dominant throughout this region (Van Auken et al. 1981; Van Auken and McKinley 2008). High density woody species were *Juuiperus ashei* (Ashe juniper) and *Quercus virginiana* (=*Q. fusiformis*, Live oak) followed by *Diospyros texana* (Texas persimmon) and *Sophora secundiflora* (Texas mountain laurel). *Ulmus crassifolia* (cedar elm) is sometimes found in these communities at lower density and on the deeper soils (Gagliardi and Van Auken 2010). There were also former grasslands of various sizes that are woodlands today with *Prosopis glandulosa* (mesquite), *Aloysia gratissima* (whitebrush) and *Diospyros texana* as major woody species (Van Auken and Leonard 2016). These areas seem to be on deeper soils and were not used in the current study. Within the *Juniperus/Quercus* woodlands there are sparsely vegetated intercanopy patches or gaps on shallow soil (openings in the woodlands) (Van Auken 2000). This is where the high light or open treatments were placed.

The highest density herbaceous species below the canopy were *Carex planostachys* (Cedar sedge) (Wayne and Van Auken 2008), *Verbesina virginica* (Gagliardi and Van Auken 2010) and occasionally *Chaptalia texana*. In the gaps, *Aristida longiseta* (Red three-awn), *Bouteloua curtipendula* (Side-oats

grama), *Bothriochloa* (=*Andropogon*) *laguroides* (Silver bluestem), *Bothriochloa ischaemum* (KR bluestem), various other C_4 grasses, and a variety of herbaceous annuals are common (Van Auken 2000).

Experimentally, there were two treatments, canopy or no canopy (+ or - canopy). The experiment included 20 replications for each treatment for a total of 40 pots or plants. Plants were started from seed and grown for 60 days in 10.1 x 10.1 cm peat pots (in a greenhouse) in native area soil from the study site (dried, sifted Whitewright-Austin complex) with 100 ml of a complete nutrient solution added initially (Van Auken, et al. 2005). Plants were randomized and planted in the field February 26, 2013. All plants were watered initially and then every other day with 500 ml of tap water for two weeks. After that, plants were given 500 ml tap water once/week to maintain the soil at or near field capacity. Rosette diameter, number of leaves, as well as the size of the largest leaf were measured monthly. Largest leaf and plant area were calculated. Live and dead plants were counted monthly. Upon harvesting, when growth had stopped (day 192), plants were clipped at the soil surface just below the rosette and dried at 75°C to a constant level and then dry mass was determined. Roots were not collected. Mid-day light levels on a clear spring day were measured at each plant position using a LI-COR® LI-190 SA integrating quantum sensor. Soil depth was determined using a piece of re-bar pounded into the ground, removed and measured (Wayne and Van Auken 2008). The study site was re-visited 56 d after the end of the experiment to look for missing plants.

Analysis of variance and *Student's t-test* was used for parametric results and X^2 tests were used for non-parametric results (Sall et al. 2001). This was used to test the effect of canopy position on response variables. Least square regressions were completed to examine how response variables changed in time. Data were compared to various functions and significance level for all tests was 0.05.

RESULTS

The experiment was planted on February 26, 2013 and harvested 192 days later on September 6, 2013. At the end of the experiment, overall mortality was 70% or 28/40 dead and survival was 30% with 12/40 total plants surviving. Mortality of *Chaptalia texana* increased through the experiment (Figure 1) and appeared to be greatest on day 192 with a total of 70% dead. Below the canopy in shade, mortality was 10/20 or 50% mortality. Total plant mortality was a significant linear function (R^2=0.80 and P<0.05), but the R^2 and P value were improved using a 2nd or 3rd order polynomial function (Figure 1). Time (days) explained 95% of the variation of mortality of *C. texana* using a 2nd or 3rd order polynomial function. On day 192, there was a significant difference between percent survival in the open versus canopy plants (X^2, $P < 0.05$). At this time, total survival was 30% and survival in the open was 15% while below the canopy it was 45%.

Several plant growth factors were measured during the experiment including number of leaves, length and width of the largest leaf, and length and width of the largest plant. Leaf area and plant area were calculated. These factors were regressed on time in days that they were measured or counted. Linear as well as logarithmic and polynomial (2nd, 3rd and 4th order) regressions were examined. None of the linear and logarithmic regressions were significant (P>0.05 in all cases).

The mean number of leaves for all living plants in time was a highly significant third order polynomial. The R^2 for all plants was 0.91. For plants grown in the open (no canopy) the R^2 was 0.94 and for plants growing below the canopy it was 0.88. Thus, the R^2 for the polynomial functions explained 88-94% of the variation in number of leaves of *C. texana* over the time in days of the experiment (Figure 2). Mean number of leaves on canopy plants was greatest on day 52 with 7.4 leaves per plant compared to 3.6 leaves per plant in the no canopy treatment (Figure 2). Mean number of leaves on open grown plants declined to approximately 0.1 leaf/per plant by day 192, because of leaf and plant mortalities. Mean number of leaves on the plants below the canopy decreased to about 1.5 leaves/plant.

The largest leaf area and mean plant area were significantly related to time and were 3rd or 4th order polynomial functions (Figure 3 shows plant area, leaf area followed the same trend but is not presented). These polynomial regressions explained 73-95% of the variation of that factor in time. Mean number of leaves, leaf area and plant area increased from the start of the experiment in March of 2013 through the early spring months (day 52-73) and then declined through late summer and early fall.

Considering final plant dry mass, canopy plants were larger (0.406 g/plant) than plants in the open (0.155 g/plant) (*Student's t-test* with *P* values < 0.05). There was more dry mass produced by the plants below the canopy, 7.71 g total dry mass compared to 2.48 g total in the open or 3.1 times more produced below the canopy. Cause of the difference was more plants surviving and growing below the canopy. Light level in the open areas was 1985±242 µmoles/m^2/s compared to canopy at 130±64 µmoles/m^2/s and significantly different (*Student's t-test, P* values < 0.05). Soil depth was the same below the canopy (19.4±4.5 cm) as it was in the open (20.0±6.1 cm) (*Student's t-test, P* > 0.05).

DISCUSSION

Chaptalia texana has been found below the canopy of *Juniperus ashei/Quercus virginiana* woodlands, but not in associated grasslands (Van Auken and Bush 2015). When *C. texana* was planted, in the open (no canopy) mortality was higher than for canopy plants (Figure 1). No *C. texana* plants were noted in any grassland during the current experiment or in a prior study (Van Auken and Bush 2015). Literature reports are ambiguous concerning its habitat (Enquist 1987; USDA 2009; Harms 2011). The importance of light level was noted, with no *C. texana* plants found in the high light open grassland habitat and fewer survived when they were planted in the grassland compared to plants planted below the canopy. Soil depth did not seem to be a factor, as open or grassland soil was as deep as soil below the canopy (20.0±6.1 cm versus 19.4 ± 4.5 cm), but light levels were significantly higher in the open grassland.

Neighbors, especially the common C_4 warm season grasses, appeared to be essential in preventing the establishment, growth and presence of *C. texana*, in C_4 grasslands but was not examined in the current experiment. Earlier work suggested the various species of *Chaptalia* are found below *Quercus* or *Pinus-Quercus* woodlands or savannas (Nesom 1984; 1995; Flora of North America 2003) and *Juniperus-Quercus* woodlands and savannas (Harms 2011; Van Auken and Bush 2015). In parts of the range of the genus *Chaptalia*, some species or individuals of all species of *Chaptalia* may establish and grow in low density grasslands outside of the woodland canopy. However, all of the *C. texana* plants that we found were below the *Juniperus-Quercus* woodland canopy (current study and Van Auken and Bush 2015). Thus, the presence of neighbors seems to be a more subtle but not a less important factor in influencing or determining the density and the distribution of *C. texana* in these communities. Neighbor effects seemed to be combined with one or more other factors, which were not readily apparent in the current or previous study. The various C_4 grasses in the open and the C_3 sedge, *Carex planostachys,* below the canopy may be more efficient in taking up water and possibly nutrients and thus reduce the possibility of *C. texana* easily establishing and persisting in habitats where these species are found (Wayne and Van Auken 2009). The inhibiting effects of the C_4 grasses seems to be paramount, but may be transitory and neighbor effects were not determined in the current research.

Growth of *C. texana* plants early in the current experiment (first five months) was greater for plants below the canopy, while plants in the open did not change very much compared to their initial size (Figures 3). However, by the end of the experiment plants in both treatments decreased in size. Plants below the canopy grew to be about seven times larger than at the start by two months into the experiment (Figure 3), but then decreased in size toward the end of the growing season. Open plants grew less in the first three months, then lost leaves and area until they had an average of < 1 leaf plant. After the experiment was terminated and after fall rains we revisited the study site and found some *C. texana*

plants. This was a surprise, because we did not expect to find any *C. texana* plants. Apparently some *C. texana* plants lose all of their leaves during drought and survive until rainfall reappeared. However, we could not relate these plants to our dead or lost study plants. Loss of leaves is known to happen for a number of herbaceous plants like the rain lilies (*Cooperia*), wild onions (*Allium*), the sego lily (*Calochortus*) and the C_4 grasses (Enquist 1987; Epple and Epple 1995). However, as far as we know, it has never been reported for *C. texana* or any other species of *Chaptalia.* Survival of *C. texana* in the rhizome stage was not expected.

A canopy habitat preference was demonstrated for *C. texana.* However, to determine neighbor effects will take more experimental time, a larger number of replications and probably a canopy that allows additional light to reach the understory species. Canopy light level in the current study was 130±64 μmoles/m^2/s which was < 10% of light levels found below a canopy where nearby populations of *C. texana* were previously noted and < 50% of measured light saturation for shade leaves of *C. texana* (Van Auken and Bush 2015).

Sorting out the causes that determine why a species is present in a given habitat is challenging (Begon et al. 2006; Smith and Smith 2012). While *C. texana* is usually found growing in shade, light levels and gas exchange characteristics are not the only factors controlling its habitat preference (Van Auken and Bush 2015). Further appraisal of the drought tolerance of *C. texana* is necessary to determine if they are restricted to the canopy because they cannot compete with associated species especially in the open or is it other factors. Other environmental conditions may limit *C. texana* to shaded understory habitats but they are not known at this time. A factor that may partially control the distribution of *C. texana* is covering or being buried by leaf litter. Covering *C. texana* by the leaves of various oaks especially *Quercus virginiana* seemed to occur but was not measured. Similar sun or shade distribution patterns have been reported for other species, but boundaries were caused by differential herbivory (Louda and Rodman 1996; Maron and Crone 2006; Leonard and Van Auken 2013).

Chaptalia texana growth may be limited by water availability or water use by more drought tolerant C_4 grasses that keep *C. texana* restricted to canopy habitats where these grasses cannot grow because of low light (Wayne and Van Auken 2009). Possibly higher soil water levels found below the canopy, which would be available to *C. texana,* are important. These conditions are dynamic and individuals respond to them all of the time, which makes it difficult to know which one or ones are controlling the species' responses and thus community composition. Because a species is present in a community today does not mean it was there yesterday or will be there tomorrow and determining the controlling factor or factors is difficult.

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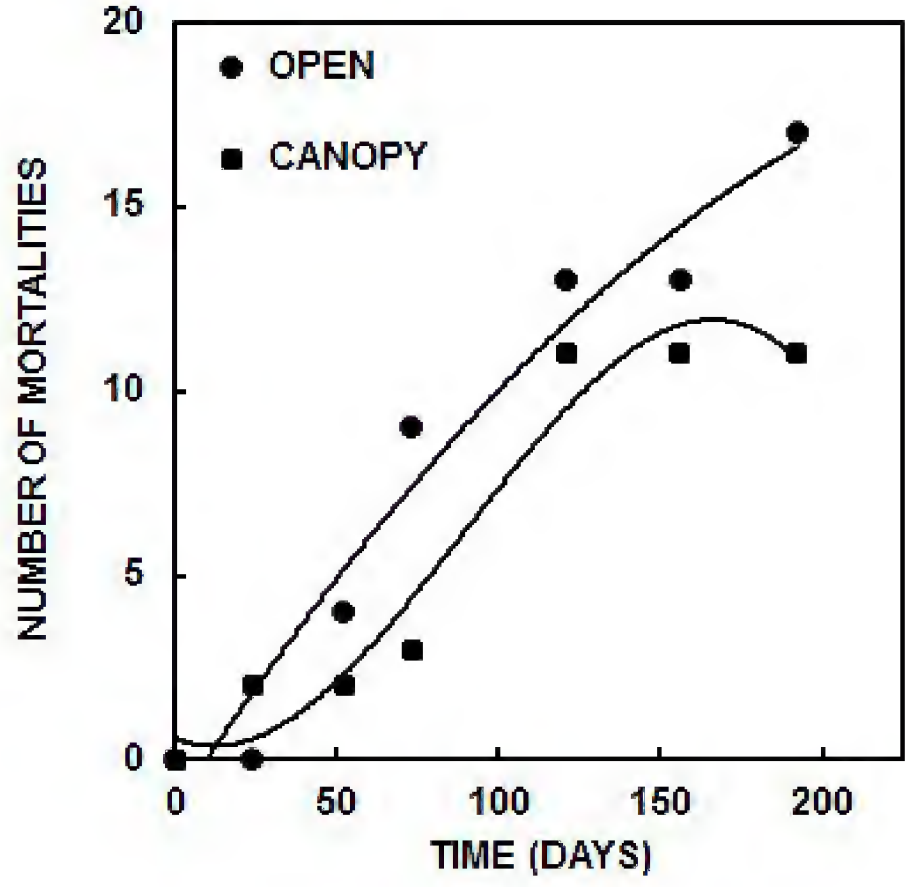

Figure 1. Mortality of Chaptalia texana plants at Hardberger City Park in San Antonio, Texas, USA is presented. Mortality of 40 plants (20 plants below a canopy and 20 plants in the open) was followed for 192 days in 2013. Total mortality at experiment end was 70% (12/40). Mortality in the open at the end of the experiment was 85% (17/20) and it was 55% (11/20) for plants below the canopy. Plotted lines are polynomial functions (no canopy or open, $y=-0.0002x^2+0.1345x-1.2786$, $R^2=0.95$, $P<0.001$; canopy, $y=-6E-06x^3+0.0017x^2-0.0379+0.5909$, $R^2= 0.95$, $P<0.0001$).

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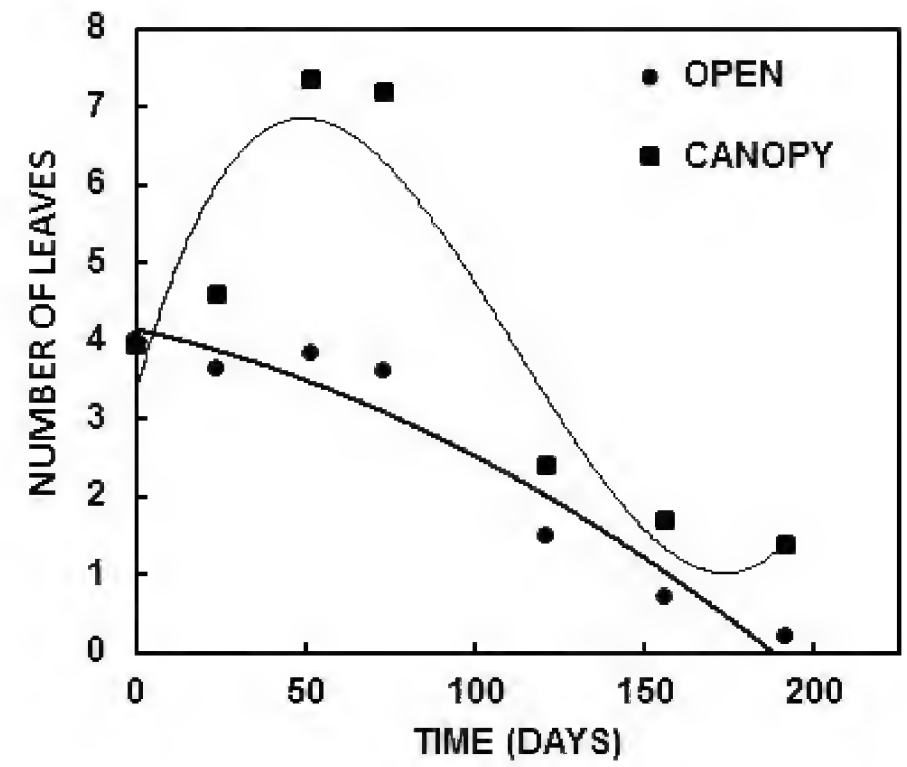

Figure 2. Presented is the mean number of leaves present on Chaptalia texana plants at Hardberger City Park in San Antonio, Texas, USA is. All leaves greater than 0.5 cm were counted approximately once per month over the course of the experiment. Plotted lines are polynomial functions (no canopy or open, $y=-7E-05x^2-0.0097x+4.1457$, $R^2=0.94$, $P<0.0001$; canopy $y=6E-06x^3-0.002x^2+0.156x+3.362$, $R^2=0.88$, $P<0.001$).

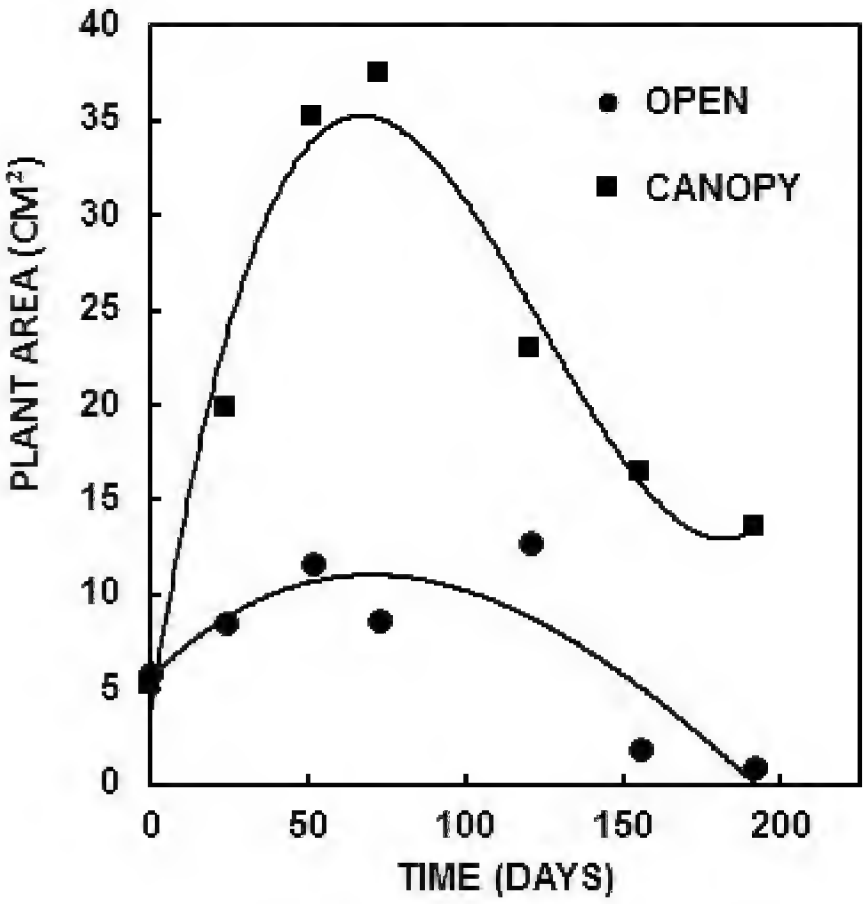

Figure 3. Presented is the mean area (cm2) of Chaptalia texana plants at Hardberger City Park in San Antonio, Texas, USA. Length of the two largest plant dimensions were measured and area was calculated. Plants were measured approximately once per month over the course of the experiment. Plotted lines are polynomial functions (no canopy or open, $y=2E-06x^3-0.0014x^2+0.1651x+5.5883$, $R^2=0.73$, $P<001$; canopy, $y= 3E-05x^3-0.011x^2+1.0756+3.6675$, $R^2=0.95$, $P<0.0001$).

A survey of percent-filled and empty seeds in multiple years observations of *Juniperus arizonica* and *J. osteosperma*

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ABSTRACT

The percent-filled seeds are presented for *J. arizonica* and *J. osteosperma* (Salt Lake City, Utah and Sedona, AZ) covering 3 and 4 years respectively. The % filled seeds for *J. arizonica* varied as: 34.4%(2010); 33.4% (2011); and 12.4% (2013). For *J. osteosperma*, Salt Lake City, Utah, the % filled varied as: 0.0% (2010), 0.4% (2011), 0.0% (2013), 0.0% (2014). In contrast, *J. osteosperma*, Sedona, AZ, the % filled varied as: 79.0% (2010), 7.2% (2011), 0.0% (2013). A summary of % filled seed is presented for 13 *Juniperus* species of North America. Published on-line **www.phytologia.org** *Phytologia 93(3): 164-169 (July 6, 2016).* ISSN 030319430

KEY WORDS: *Juniperus arizonica, J. ashei, J. californica, J. coahuilensis, J. communis* var. *depressa, J. deppeana, J. grandis, J. monosperma, J. occidentalis, J. osteosperma, J. pinchotii, J. scopulorum, J. virginiana,* filled and empty seeds, X-ray analysis.

Recently, we (Adams and Thornburg, 2011; Adams, Thornburg and Corbet, 2014) have reported a general survey of the incidence of filled vs. empty seeds for most of the *Juniperus* species of the western United States. This note is to report on additional observations on the incidence of filled seed in *Juniperus arizonica* and *J. osteosperma*.

MATERIAL AND METHODS

Plant specimens collected:

J. arizonica, *Adams 12505-12509*, 5 normal female trees, Cottonwood, AZ, 3 Nov 2010, *Adams 13178-13182*, 5 normal female trees, Cottonwood, AZ, 28 Nov 2011, *Adams 12510-12516*, 7 'male' trees with seed cones, Cottonwood, AZ, 3 Nov 2010, *Adams 14528-14532,* Cottonwood, AZ, 30 Jan 2014, *D. Thornburg, ns*,

J. osteosperma, Adams 12408-12412, Big Cottonwood Canyon, Salt Lake City, UT, 4 Sep 2010, *Adams 13188-13192,* Big Cottonwood Canyon, Salt Lake City, UT, 4 Dec 2011, *Adams 12323-12327*, *Adams 14190-14194,* Big Cottonwood Canyon, Salt Lake City, UT, 16 Mar 2014, *Adams 14539-14543,* Big Cottonwood Canyon, Salt Lake City, UT,*15 Mar 2015,*

Big Bear Basin, CA, 20 Jul 2010 (too early to be filled?), *Adams 12546-12550*, n of Sedona, AZ, 34.491521° N, 111.690468° W, Nov 2010, *Adams 13174-13177*, n of Sedona, AZ, 34.491521° N, 111.690468° W, Nov 2011,

Voucher specimens are deposited in the herbarium (BAYLU), Baylor University, Waco, TX.

X-ray analysis of the seeds was performed by the US Forest Service, National Seed Laboratory, Dry Branch, GA.

RESULTS AND DISCUSSION

The % filled seeds in *J. arizonica* near Cottonwood, AZ varied (Table 1) as: 34.4%(2010); 33.4% (2011); and 12.4% (2013). The % filled ranged (within a population) from 24 - 56% (2010), 20 - 49% ((2011) and 0 - 40% (2013). Clearly, the 2013 seed crop was poorly filled. The odd, *J. arizonica* male trees, each with a few seed cones, averaged 52.5% filled (Table 1).

Table 1. The % filled seeds from normal, female *J. arizonica* trees and 7 'male' trees bearing a few seed cones near Cottonwood, AZ.

J. arizonica, 10 normal, female trees, Cottonwood, AZ, David Thornburg property.

	# cones	seeds/cone	#seeds X-rayed	% filled	coll. **3 Nov 2010**
12505	50	1.22	50	28	
12506	50	1.00	50	30	
12507	50	1.11	50	34	
12508	50	1.14	50	56	
12509	50	1.00	50	24	avg = 34.4%
	# cones	seeds/cone	#seeds X-rayed	% filled	coll. **28 Nov 2011**
13178	50	1.00	50	21	
13179	50	1.00	50	39	
13180	50	1.00	50	49	
13181	50	1.00	50	20	
13182	50	1.00	50	38	avg = 33.4%
	# cones	seeds/cone	#seeds X-rayed	% filled	coll. **17 Jan 2014 (2013 seed)**
14528	50	1.00	50	0	
14529	50	1.00	50	40	
14530	50	1.00	50	0	
14531	50	1.00	50	0	
14532	50	1.00	50	22	avg = 12.4%

J. arizonica, 7 male trees, each with a few female cones, Cottonwood, AZ, David Thornburg property.

	# cones	seeds/cone	#seeds X-rayed	% filled	coll. **3 Nov 2010**
12510 tree 1	13	1.08	14	42.8*	
12511 tree 2	50	1.04	50	88.0*	
12512 tree 3	1	1.00	1	100.0	
12513 tree 4	1	1.00	1	0.0	
12514 tree 8	1	1.00	1	100.0	
12515 tree 10	37	0.92	34	29.4*	
12516 tree 17	30	1.00	30	50.0*	avg. = 52.6% (for * trees)

The Utah juniper, *J. osteosperma*, is the dominant tree in many parts of Utah and Nevada and extends into northern Arizona, southern California, and western New Mexico (Adams, 2014). Seeds were collected from a small population (50 - 100 trees) growing at the mouth of the Big Cottonwood Canyon, SLC, Utah. No filled seeds were found in 2010 and only 1 seed (in 50, = 2%) was found in 2011 (Table 2). New collections found 0.0% (2013) and 0.0% (2014). So, of the 1,252 seeds x-rayed over four years (2010, 2011, 2013, 1214) only 2 seeds were filled! This is an extreme situation. The site is not too unusual and the cause for the very low seed set is not known, although Fuentes and Schupp (1998) suggested that empty seeds reduce seed predation by birds in *J. osteosperma*. They reasoned that birds finding mostly empty seeds on a tree would abandon that tree, thus, the few filled seeds might escape bird seed predation.

Table 2. The % filled seeds for *J. osteosperma* in Utah.

J. osteosperma, Big Cottonwood Canyon, SLC, UT coll. **4 Sep 2010**

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled	
12408	90	0	1.00	90	0.0	
12409	102	1	1.01	103	0.0	
12410	101	0	1.00	101	0.0	
12411	102	0	1.00	102	0.0	
12412	100	6	1.06	106	0.0	avg = 0.0%

J. osteosperma, Big Cottonwood Canyon, SLC, UT coll. **4 Dec 2011** ex Andy Hornbaker

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled	
13188	50	0	1.00	50	2.0	
13189	50	0	1.00	50	0.0	
13190	50	0	1.00	50	0.0	
13191	50	0	1.00	50	0.0	
13192	50	0	1.00	50	0.0	avg = 0.4%

J. osteosperma, Big Cottonwood Canyon, SLC, UT coll. **16 Mar 2014 (2013 seed crop)**

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled	
14190	50	1	1.02	50	0.0	
14191	50	2	1.04	50	0.0	
14192	50	0	1.00	50	0.0	
14193	50	0	1.00	50	0.0	
14194	50	2	1.04	50	0.0	avg = 0.0%

J. osteosperma, Big Cottonwood Canyon, SLC, UT coll. **15 Mar 2015 (2014 seed crop)**

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled	
14539	50	0	1.00	50	0.0	
14540	50	0	1.00	50	0.0	
14541	50	0	1.00	50	0.0	
14542	50	0	1.00	50	0.0	
14543	50	0	1.00	50	0.0	avg = 0.0%

Seeds from a population north of Sedona, AZ were collected in 2010, 2011 and 2013. The % filled seeds varied (Table 3) as 79.0% (2010), 7.2% (2011), and 0.0% (2013). It is interesting that trees from the same population varied so much by years. By comparison, a population at Big Bear Basin, San Bernardino Mtns., CA had 51.4% (2010). Note (Table 3) than in the Big Bear population, four trees were very uniform ranging from 61.5 to 66.7% filled seeds, whereas one individual had no filled seeds. This reinforces the idea of collecting from several tree sources to obtain viable seeds for germination.

Fuentes and Schupp (1998) examined the incidence of filled seeds in a semi-arid species, *J. osteosperma* from Utah. Their 34 trees varied from 0.0 to 16.5% filled seeds (avg. = 5.61%). So it is interesting that the Sedona, AZ, for 2011 samples display a range of variation similar to their Utah samples, but 2010 and 2013 are quite different from the 2011 data.

Table 3. The % filled seeds for *J. osteosperma* at Grasshopper Point, Sedona Arizona.

J. osteosperma, Sedona, AZ, coll. **Nov 2010** ex David Thornburg

	# cones	seeds/cone	#seeds X-rayed	% filled	
12546	50	1.00	49	89.8	
12547	49	1.02	48	85.4	
12548	49	1.02	44	95.5	
12549	47	1.06	48	54.2	
12550	47	1.06	50	70.0	avg = 79.0%

J. osteosperma, Sedona, AZ, coll. **Nov 2011** ex David Thornburg

	#seeds X-rayed	% filled	
13173	50	4.0	
13174	50	2.0	
13175	50	7.0	
13176	50	16.0	
13177	50	6.8	avg = 7.2%

J. osteosperma, Sedona, AZ, coll. **30 Jan 2014 (2013 seed crop)** ex David Thornburg

	#seeds X-rayed	% filled	
14534	50	0.0	
14535	50	0.0	
14536	50	0.0	
14537	50	0.0	
14538	50	0.0	avg = 0.0%

J. osteosperma, Big Bear Basin, CA, coll. **20 Jul 2010**

	# cones	# w 2 seeds	seeds/cone	#seeds X-rayed	% filled	
12323	50	2	1.04	52	61.5	
12324	50	12	1.24	62	64.6	
12325	50	0	1.00	50	64	
12326	52	2	1.04	54	66.7	
12327	45	0	1.00	45	0.00	avg = 51.4%

The amount of variation in % filled seeds is remarkable, varying both by year and by location. The % filled seeds for 13 *Juniperus* species from the US and Canada are shown in Table 4. These values range from 0.0 (*J. osteosperma*, Utah) to 79.0% (*J. osteosperma*, Sedona, AZ). Of interest is the variation from 2010 to 2011, with *J. osteosperma* (Utah) having 0.0 in 2010 and 0.4% in 2011. Yet, *J. osteosperma* (Sedona, AZ) had 79.0% filled in 2010, but only 7.2% (2011) and 0.0% (2014).

Interestingly, *J. deppeana* had a similar pattern: 38.2% filled in 2010 and 0.0 % filled in 2011. But, *J. arizonica*, collected nearby, had the same % filled in 2010 (34.4%) and 2011 (33.4%) (Table 4).

Juniperus virginiana, an easy juniper to germinate from seeds, had only 1.2% (2010), which is surely not typical for that species. Observations on % filled seed based only one year's seed crop should be taken with considerable caution, as other years are likely to be quite different.

Table 4. Comparison of % filled seeds for 13 *Juniperus* species in North America.

Species, location	% filled (year)
J. arizonica, Cottonwood, AZ	34.4 (2010), 33.4 (2011), 12.4% (2013)
J. ashei, Westlake Hills, Austin, TX	27.2 (2010)
J. californica, Bodfish, CA	58.4 (2012)
J. californica, Victorville, CA	63.2 (2012)
J. californica, Bagdad, AZ	77.6 (2012)
J. coahuilensis, Alpine, TX	22.8 (2010)
J. communis var. *depressa*, Winnipeg, Can.	11.6 (2010)
J. deppeana, 14 miles SE Camp Verde, AZ	38.2 (2010), 0.0 (2011)
J. grandis, Onyx Summit, CA	13.7 (7/2010), 15.6 (11/2010)
J. monosperma, Lake Tanglewood, TX	36.8 (2010)
J. occidentalis, Bend, OR	5.6 (2010)
J. occidentalis, sw of Susanville, CA	5.9 (2010)
J. osteosperma, Big Cottonwood Canyon, UT	0.0 (2010), 0.4 (2011), 0.0 (2013), 0.0 (2014)
J. osteosperma, Sedona, AZ	79.0 (2010), 7.2 (2011), 0.0 (2013)
J. osteosperma, Big Bear Basin, CA	51.4 (2010)
J. pinchotii, Palo Duro Canyon	2.8 (2010)
J. pinchotii, from Warren (2001)	
Palo Duro Canyon	17.0
Justiceburg, TX	9.5
San Angelo, TX	18.1
Guadalupe (Salt Flat, TX)	9.9
J. scopulorum, Cimarron Canyon, NM	43.4 (2010)
J. virginiana, Lockhart, TX	1.2 (2010)

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Keys to the flora of Florida - 32, Zamia (Zamiaceae)

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ABSTRACT

Zamia (Zamiaceae) in Florida is represented by two species. The native *Zamia integrifolia* is treated as consisting of five varieties, with var. ***umbrosa*** differently aligned, var. ***floridana*** reranked, var. ***silvicola*** reaffirmed but re-ranked, and var. ***broomei*** described as new. *Zamia furfuracea* is accepted as a naturalized introduction. Justification is given for the new taxa and new ranks. A recent nomenclatural change is explained. An amplified key is provided to the Florida taxa recognized here. Published on-line www.phytologia.org *Phytologia 98(3): 170-178 (July 6, 2016).* ISSN 030319430.

KEY WORDS: ***Zamia****, Zamiaceae, Florida flora.*

INTRODUCTION

Although the cycads (Cycadaceae, Stangeriaceae, Zamiaceae) are of ancient origin, with fossil traces deep into the Pennsylvanian and abundant presence throughout the Jurassic, the rise of other conifers followed by the now-omnipresent angiosperms has greatly reduced their significance among the world's flora. Even more, recent human actions, either by displacement through land-clearing or by selective removal, have caused most species to become rare or endangered. Yet their very rarity, together with their unique structure and botanical properties, has augmented their interest to both horticulture and to science and justifies the importance that we understand the characteristics of the different taxa, the names that they bear, and their distribution.

The genus *Zamia* (Zamiaceae) has recently been reported to consist of 71 species, with eight species native to the West Indies and Bahamas (Osborne et al., Mem. N.Y. Bot. Gard. 2012). These taxa appear to be of quite recent origin (Nagalingum et al., Science 334. 2011), at least relative to the primordial status of their progenitors, perhaps a consequence to the geological recency of the islands of the West Indies with separation and isolation of small soon-speciating populations, reinforced by the heavy seeds, ill adapted to cross-water migration. Unquestionably the arrival of a new vector -- aboriginal man -- facilitated the shuffling of these discrete populations, with many species, possibly of those most utilized as a food source, now found on more than a single island. The resultant variability permitted an early author (DeCandolle, 1868) to recognize 14 species from the region. It is from this melange that the cycads native to Florida must have come.

These Florida cycads have attracted a series of thoughtful investigations: early history and taxonomy of *Zamia* within the state (J. K. Small, J. N.Y. Bot. Gard. 22: 121-137. 1921; ibid. 27: 121-129. 1926; *Manual S.E. Flora.* 1933); Florida taxa treated as threatened (D. B. Ward, *Rare & Endangered Biota of Florida*, 5: 123-124. 1979); taxonomy of the West Indian entities (J. E. Eckenwalder, J. Arnold Arbor. 61: 701-722. 1980); a modern review of Florida *Zamia* (D. W. Stevenson, J. Arnold Arbor., suppl. ser. 1: 367-384. 1991); a popular overview of Florida *Zamia* (T. H. Broome, *The Coontie of Florida.* Online. 1998); a floristic treatment (R. P. Wunderlin & B. E. Hansen, *Flora of Florida.* 2000). But over the years misunderstandings of typifications and simple differences in taxonomic judgment have left a trail of often contradictory views.

Two species of *Zamia* -- one of them introduced -- are now found in Florida. Though these plants are instantly recognizable as to genus, the native population is variable, with several scientific names

applied at different times. Three names are currently in use for the single native species: *Zamia pumila* L. (1762), *Zamia integrifolia* L.f. in Ait. (1789), and *Zamia floridana* A. DC. (1868). The present task is to examine these and other names that have been used here, to determine their legitimacy, to apply them to appropriate natural populations within the state, to name and describe a taxon believed to be new, and to construct a key that will permit these populations to be identified.

History

Although plants that we would now recognize as members of the genus *Zamia* were in cultivation in Holland and perhaps elsewhere in Europe by the end of the 17th century, the first species to be given a scientific name under the practices that are now codified as the *International Code of Botanical Nomenclature* (I.C.B.N.) was *Zamia pumila*, described and named by Carl Linnaeus in 1762. Linnaeus in the mid-18th century, flush with the prestige and credibility won by his earlier publications and especially his landmark *Species Plantarum* ed 1 (1753), by then a professor at the University of Uppsala, Sweden, and far from the lush gardens of Holland where he had studied and worked as a young man, was still in pursuit of his goal of naming and describing all the plant species of the world. But of *Zamia* he had no specimen. His only resource must have been his memory and perhaps notes of the plants grown in Leiden and Amsterdam, and those books by other authors that described the plants he wished to include.

Linnaeus then followed the practice employed by him in his 1753 publication: when no specimen was available -- paraphrase. By adroitly selecting words and phrases from these "pre-Linnaean" authors, by assigning an appropriate single word as the epithet, and by citing earlier works as his source, he could create concise accounts that were quite usable in identifying and naming the intended plant.

This is the course Linnaeus pursued in addressing his perhaps half-forgotten cycad in *Species Plantarum* ed 2 (1762). He named the new genus "*Zamia*," gave it a brief description, and cited four earlier publications with appropriate descriptions and illustrations. For the species itself he gave no description other than the epithet *pumila* or "dwarf." [This epithet was no doubt chosen in contrast to a much larger cycad he had known when in Holland, named by him *Cycas frondibus pennatis* (1737) and later by his son as *Zamia furfuracea* L.f. in Ait. (1789).] He thus created the name and circumscription of *Zamia pumila* L. (1762).

Other authors recognized that the West Indies and Bahamas hosted a wide range of related forms; many were soon named (DeCandolle, 1868; Osborne et al., 2012). And the name *Zamia pumila* was variously applied, depending on individuals' interpretations. Stability of a sort was gained by Eckenwalder (1980: 702) who defined the species as "the common West Indian cycad that has traditionally been known (in part) by that name." The ambiguity of this formulation is the more striking in that Eckenwalder also chose to treat all West Indian cycads as a single species.

Eckenwalder (1980: 715) also recorded the many names applied to West Indian cycads, citing types or designating lectotypes as needed. He lectotypified *Zamia pumila* L. by one of the references cited by Linnaeus, that of Jan Commelijn (1697). The plate included as part of this reference is of a five-leaved plant with narrowly ovate, long-tapering leaflets, the apex acute and the margins bearing widely spaced small teeth.

Specimens corresponding to Commelijn's plate are rare. None were located (by a hurried on-line search) in North American herbaria. But the herbarium of Kew Gardens, London, has an ancient specimen identified as *Zamia pumila*. [Faint writing indicates it had come from the Leiden Botanical Garden, Netherlands, which in the early 18th century had participated in a vigorous program of plant acquisition from the West Indies.] This specimen consists of two sheets, bearing the upper and lower halves of a large leaf, and each detail of the leaflets -- the acute apex, sharp-toothed margins -- closely matching Commelijn's drawing.

Commelijn stated his plant to have come from "*Insulae Hispaniolae*" (Dominican Republic, Haiti). No plant of that form is now known from the West Indies. It is unclear whether the plant of the

illustration (and of Kew) had come originally from Hispaniola and is now extinct, or if its source was perhaps Central America where plants of similar form are known (cf. *Zamia fischeri* Miq.). But the Commelijn plate, designated as lectotype by Eckenwalder, confirmed by the biological reality of a known plant form, and wholly unlike any plant known from Florida, absolutely excludes the name *Zamia pumila* from application to any member of the state's native flora.

Nomenclature

In contrast to the unquestionably non-Florida origin of the type of *Zamia pumila*, both *Z. integrifolia* and *Z. floridana* are typified by Florida materials. *Zamia integrifolia*, the older name (1789), was based on a cultivated plant from "East Florida" (probably not a geographic designation, but the political district at that time of all peninsular Florida). *Zamia floridana* (1868) was based on a specimen collected at "Fort Brooke," near present-day Tampa on the west coast of peninsular Florida. The type specimens underlying *Zamia integrifolia* and *Z. floridana* are so similar that a recent author (Stevenson, Encephalartos 9: 3-7. 1987) could "not find a substantial difference between the two." Thus these two names must represent only a single species.

There has been dispute as to the correct name to be used for the Florida plant. The earlier name, *Zamia integrifolia* (1789), would seemingly prevail, under the rules for priority. Yet the present author has long held that *Zamia integrifolia* L.f. in Ait. (1789) was nomenclaturally superfluous when published. It was believed that Linnaeus filius (in Aiton) had erred (by modern rules) by citing in his synonymy an available pre-existing name, *Zamia pumila* L. (1762). The I.C.B.N. (Art. 52.1) states that if an old name cited in synonymy should have been used for the new taxon, the new name is superfluous and illegitimate. Further, the I.C.B.N. (Art. 6.4) mandates that a name illegitimate when published cannot (barring special actions) later become legitimate. A full argument in support of the name *Zamia floridana* was then presented (Ward, Phytologia 9: 95-104. 2009).

However the I.C.B.N. permits an override of its rules by appeal to a standing professional committee. Two taxonomists who favored the earlier name, D. W. Stevenson and J. L. Reveal (Taxon 60: 594-595. 2011), petitioned the Special Committee for Spermatophyta (as established by the International Association for Plant Taxonomy); they asked that the name *Zamia integrifolia* be conserved. Their justification was, in essence, that *Z. integrifolia* has been used more frequently than *Z. floridana* and its preservation will provide nomenclatural stability. The Committee ruled in favor of the petition, thereby negating the dicta of the I.C.B.N. and establishing that *Z. integrifolia* is the correct binomial for the familiar Florida Coontie.

Zamia integrifolia

Within *Zamia integrifolia* (s.l.), as observed in Florida, there is sufficient variability to have attracted taxonomic attention. Notably, J. K. Small (1933) divided the Florida plants into four species -- *Z. integrifolia* (s.s.), *Z. angustifolia*, *Z. silvicola*, and *Z. umbrosa*. These names and the discrete populations they presumably represent have not wholly withstood the test of further examination.

Zamia angustifolia Jacq. (1789) is a legitimate name, correctly applied to plants of the Bahamas and West Indies (Osborne et al., 2012), and used by Small (1933) for plants of the Everglade hammocks; he characterized these plants as having leaflets 3-6 mm. wide (a trait found somewhat widely in the peninsula). But Small stated he made this identification without access to ovulate cones. True *Zamia angustifolia* is characterized by dark gray to black strobili with short-acuminate sterile apices (Stevenson, Fairchild Trop. Gard. Bull. 42: 23-27. 1987). [*Z. integrifolia* has reddish-brown ovulate strobili with truncate or blunt apices.] And no plants identifiable as true *Z. angustifolia* appear to be known to South Florida botanists.

Observation of Florida *Zamia*, both in cultivation and in the wild, has convinced the present writer that the morphological differences to be observed in this genus are discrete and consistent within a given population and not simple developmental variability. Intermediates are encountered, but the great bulk of the total population can be assigned to just a few recognizably distinct morphotypes. These

imperfectly dissimilar groupings are best studied by being given varietal recognition. They are here presented here in order of descending confidence.

Var. **integrifolia**

The typical variety of *Zamia integrifolia* must rest on the type employed by Linnaeus filius in 1789. At that time Linnaeus' son was working in London with William Aiton, the "King's Gardener." Aiton was then compiling a treatment of the many plants in cultivation at Kew, and Linnaeus assisted by preparing selected groups, including the cycads.

The material used by Linnaeus -- almost certainly an entire living plant -- is now represented (Natural History Museum - BM) by a single leaf with 26 leaflets and a male strobilus. The leaflets are narrow, about 8 mm. wide, but uniformly slightly spatulate. This leaflet-form can be matched only occasionally within the widespread, presumably typical Florida *Zamia*, and apparently not within the other varieties recognized here. But, considering that the plant grew in greenhouse conditions in a northern latitude, there seems no merit in suggesting the widely distributed Florida variant is improperly typified.

The exact source of the Kew plant is not recorded, but can be deduced. In 1767 a plant of *Zamia* was given to Alexander Garden of Charleston, and hence to Aiton at Kew, by Andrew Turnbull, the founder of New Smyrna, Volusia County (Small, 1921). The port of St. Augustine would have been familiar to Dr. Turnbull; his plantation lay isolated some 110 km. to the south. Years of disturbance near St. Augustine has eliminated any semblance of undisturbed vegetation. But just west of New Smyrna, in the thin sandy woods near the abandoned fields where Dr. Turnbull's Minorcan serfs raised indigo, plants of *Zamia* still thrive. Collections from this source may serve as topotypes of *Z. integrifolia* var. *integrifolia* (cf. S.C. Provost s.n., Nov. 2015 - FLAS).

With the source of Aiton's greenhouse-grown *Zamia integrifolia* plausibly near New Smyrna, a better understanding may be given to its typical form. A midwinter survey (Dorothy Leeper Spruce Creek Reserve, by S.C.P.) found plants with parallel-margined leaflets 13-14 cm. long, ±13 mm. broad, and mature cones 6-8 cm. long.

Var. **umbrosa**

Zamia umbrosa Small (1921) is a legitimate name assigned to a Florida cycad. This name has generally been disregarded or assigned to synonymy under *Z. integrifolia* (Osborne et al., 2012). But it is here argued once again that the name represents a population worthy of taxonomic acknowledgment.

Appreciation by botanical collectors that there were two entities of *Zamia* in Florida began well before the second taxon was formally named. In the early 20th century, H. J. Webber (U.S. Dept. Agric. bull. 1: 81. 1901; Small's *Flora S.E. U.S.* 1903: 32) identified two species, one as *Zamia floridana* with a stated range of "southern peninsular Florida," the second as *Zamia pumila* from "middle peninsular Florida, particularly on the east coast." They were distinguished largely by leaflet width, as 3-7 mm. vs. 8-16 mm. Clearly Webber's taxa were the present *Z. integrifolia* and *Z. umbrosa*. Small himself noted (1921: 134) that there was a sheet in the Torrey herbarium (now NY) bearing leaves "representing two species of zamia;" the collectors were known but not their sources.

The present author has maintained for many years (Ward, 1979) that the variant of *Zamia* found primarily in the upper eastern Florida peninsula was distinguishable at some level from the more widespread, largely more western and southern taxon. In the late 1960s and early 1970s an effort was made to establish and maintain greenhouse plants of *Zamia* from throughout their Florida ranges. [This study has been briefly reported elsewhere (Ward, Novon 11: 360-365. 2001), justifying the formation of the combination *Z. floridana* var. *umbrosa*.] At peak, 29 plants were held, from five counties (Citrus, Dixie, Flagler, Glades, Marion), each presumed to represent a different population. At midwinter yearly intervals, measurements were taken of 5 leaves per plant, of one leaflet per leaf. Leaflet length and width were measured, and their l/w ratios were recorded.

The experimental design followed here permitted the partitioning of variance among populations, among plants per population, and among leaves per plant. [The present author had a deep appreciation of such statistical manipulation, stemming from his graduate studies involving *Sisyrinchium* (Ward, 1959).] As expected, no significance in leaflet width was demonstrated among leaves per plant, nor among plants per population. But significance was found *among* populations. A range test saw no difference among four populations (Citrus, Dixie, Glades, Marion), but found a significant difference (at 5%) separating the fifth population (Flagler). The Flagler population was within the geographic range ascribed to *Z. umbrosa*; the other populations fell within the distribution of *Z. integrifolia*.

A more sophisticated study of leaf measurements by S. J. Newell (Amer. J. Bot. 76: 1518-1523. 1989) addressed plants from five populations of *Zamia* in Florida (and three populations in Puerto Rico). Two of Newell's Florida populations were located in northeastern coastal counties (St. Johns, Flagler); three populations were in the southernmost peninsula (Dade County), two of which (Long Pine Key) were chosen to reflect shade and full-sun aspects of the habitat. Though some of Newell's comparisons showed significance, others did not. Notably, her two northern populations showed mean leaflet widths (10.8 mm., 12.5 mm.) greater than her three southernmost populations (7.1 mm., 5.9 mm., 8.6 mm.). Again, the St. Johns and Flagler plants were within the range of *Z. umbrosa*; the others corresponded to *Z. integrifolia*.

A character useful in determination of *Zamia umbrosa* is the presence on many leaflets of small protuberances on and near the leaflet apex. These have been termed "teeth" or "callous bumps." But unlike the structures called "teeth" among angiosperms, these small hardened projections from the leaf margin are not an inherent part of the leaf architecture. Rather, in *Zamia* they are protruding tips of the veins. From soon after the veins pass out of the rachis and into the blade of the leaflet, they no longer interconnect; they continue as simple, parallel strands until they terminate at the leaflet margin. This pattern of xylem/phloem bundles has been termed "multiple parallel (non-anastomosing) first-order venation." The "callous teeth" are the tips of these bundles, made apparent by the shrinkage or ablation of the intervening mesophyll tissue. This anatomical structure, aberrant to the forms of venation described elsewhere (Hickey, Amer. J. Bot. 60: 17-33. 1973), merits greater investigation than is appropriate here.

One must be wary of the significance of a possible developmental stage that is manifest in one presumed taxonomic entity but is absent or less evident in other closely related entities. Yet the presence of these curious "callous bumps," together with the demonstrated wider leaflets, and supported by the allopatric upper east coast distribution, justifies the judgment of earlier authors that Small's *Zamia umbrosa* merits taxonomic recognition.

Zamia umbrosa has elsewhere been treated at varietal rank (Ward, Novon 11: 363. 2001), as *Z. floridana* var. *umbrosa*. But the replacement of *Zamia floridana*, through conservation, by *Zamia integrifolia*, requires the procedure be repeated, with a corrected basis.

> ***Zamia integrifolia*** L.f. in Ait. var. ***umbrosa*** (J. K. Small) D. B. Ward, comb. nov. Basionym: *Zamia umbrosa* J. K. Small, J. New York Bot. Gard. 22: 136. 1921. TYPE: U.S.A. Florida: Volusia Co., hammock between Volusia and Ocean City, 4 May 1921, *Small 8679*: lectotype (designated by Eckenwalder, 1980), NY; isolectotypes, DUKE, FLAS, GH.

Var. **broomei**

A variant of *Zamia integrifolia* occasionally encountered in the wild is characterized by narrow leaflets, about half the width commonly seen in the more widespread, presumably typical form. Plants of this form are often locally frequent in the lower Suwannee River basin -- Dixie, Gilchrist, Levy, Alachua counties, in northwest peninsular Florida. Habitats observed are dry oak hammocks and sandy "high pine." The narrow leaflets produce sparse foliage, and only rarely are such plants seen in cultivation. The distinguishing characters given here do not permit separation from Small's interpretation of *Zamia angustifolia* as seen in the southernmost peninsula; further observations will be necessary before the

relationship of these two populations is understood. That this more northern variant may carry a defined taxonomic property, the following new variety is here described.

> ***Zamia integrifolia*** L.f. in Ait. var. ***broomei*** D. B. Ward, var. nov. TYPE: U.S.A. Florida: Infrequent in dry second-growth oak hammock, 9700 block, S.W. 44th Ave. (Haile Plantation), southwest Alachua County, 2 Jan 2015, *Ward 10791*: holotype, FLAS; isotype, NY. Differs from type of the species in leaflets narrow (5-7 mm. wide), leaves borne erect. Named in acknowledgment of services to the cycad community by Thomas H. Broome, owner of The Cycad Jungle, Lakeland, FL.

Var. **floridana**

Zamia floridana A. DC. (1868), as noted above, is a legitimate name, though no longer correct when applied at specific rank. But since priority applies only within rank, the epithet remains available at varietal rank.

Observations in western peninsular Florida have encountered plants that appear to differ in one quantitative character, and possibly in habitat, from plants here treated as typical var. *integrifolia*; larger strobili and a shell-mound substrate. These plants have long been known in Levy County, especially in the vicinity of Cedar Key, but no record seems to have been made of their relatively massive female strobili, which reach 18 cm. in length and 8 cm. in diameter. [A smaller cone 10 cm. long held 34 seeds beneath 62 partially nonfunctional megasporophylls.] In contrast, strobili seen elsewhere are smaller, those of the eastern coast no more than half these dimensions.

These large-cone populations have been found only on Native American (probably Calusa) shell middens. The significance of this substrate -- in constrast to the thin sands underlying typical *Zamia integrifolia* -- lies in the source of the type of *Z. floridana*. The collector, Gilbert Hulse, in the early 1830s was stationed at Fort Brooke, a Seminole war military encampment at the head of Tampa Bay on the west coast of peninsular Florida. And Fort Brooke was located on an extensive Indian midden, composed primarily of oyster and other marine shells! The edaphic conditions of Fort Brooke must have been very similar to the shell middens presently seen near Cedar Key. Indeed, one of the plants sent to John Torrey by Hulse was stated to have been found "upon the beds of oyster shells" (Small, 1921: 133).

It is difficult to resist the possibility that the Calusa, builders of other massive shell mounds along the Florida gulf coast, brought with them a strain of cycad from the West Indies that differed from variations grown elsewhere in Florida. [This commerce between Florida and the West Indies continued into historic times; in June 1774 William Bartram (Travels, 1791: 227), while on the lower Suwannee River, met a party of Calusa just returned from Cuba.]

Rather than coin a new epithet for the large-cone Levy County cycad, it seems preferable to retain one of known provenance even though of uncertain equality. The strobilus of Hulse's *Zamia* is unknown to us; DeCandolle's description noted it to be 2.5 cm. long, an immature or otherwise unmeaningful dimension. (The type itself cannot presently be accessed; Stevenson's 1987 observation omitted this datum.) Assignment of an epitype permits the Levy County strobilus dimensions to be associated with the Tampa Bay type.

> ***Zamia integrifolia*** L.f. in Ait. var. ***floridana*** (A. DC.) D. B. Ward, comb. et stat. nov. Basionym: *Zamia floridana* A. DC., Prodromus 16(2): 544. 1868. TYPE: U.S.A. Florida: Hillsborough Co., "Fort Brooke," *Hulse s.n.* Lectotype, G-DC; isotype, NY (noted by Eckenwalder, 1980). Epitype, Florida, Levy County, "Shell Mound," *Ward 10796*, FLAS (with female strobilus).

Var. **silvicola**

Zamia silvicola Small (1926) is also a legitimate name, though its application to a Florida population is uncertain. Small described the species without an exact source, and Eckenwalder (1980: 716) identified a collection from "Spanish Mound" (now Crystal River Archaeological State Park), Citrus

County as the holotype. But Small also gave photographs of his new species in hammocks of the Everglades, Dade County. He noted it to be "the most robust *Zamia* in Florida," and characterized it by relatively long (12-17 cm.), broad (10-15 mm.) leaflets. Quite uniformly, this name has been dismissed as a synonym of *Z. integrifolia*. However plants closely corresponding to Small's description are known, both in the wild and in cultivation. Their frequent use in horticulture is surely a response to their luxuriant, close-spaced, broad-leaflet foliage (quite the antonym of words describing var. *broomei*). The presumption is the cultivated specimens have been selected from the native Florida population. They are rather uncertainly distinguished from the more widespread, presumably typical variant by the longer, often broader leaflets (and often longer leaves). But the possibility that they represent merely robust, older specimens has not been wholly excluded. A varietal rank for *Z. silvicola* will encourage further attention and understanding.

But Small's epithet, "*silvicola*," may not have priority at varietal level. J. Schuster (in Engler, Pflanzenr. IV, 1: 151. 1932) formed the name *Zamia floridana* var. *purshiana*, a name Eckenwalder (1980: 716) placed in synonymy with *Z. silvicola*. Though later in date than Small's 1926 name, the I.C.B.N. does not permit priority to apply outside of rank; thus Schuster's epithet is prior when used as a variety. But without knowledge of his type and with uncertainty as to its application, it has seemed best to reject the Schuster name.

> ***Zamia integrifolia*** L.f. in Ait. var. ***silvicola*** (J. K. Small) D. B. Ward, comb. et stat. nov. Basionym: *Zamia silvicola* J. K. Small, J. New York Bot. Gard. 27: 128. 1926. TYPE: U.S.A. Florida: Citrus Co., "Spanish Mound" near Crystal River, 20 Nov 1921, *Small, Small & DeWinkeler 10060*: holotype (noted by Eckenwalder, 1980), NY.

Zamia furfuracea

Zamia furfuracea, the "Cardboard Palm," as this introduced species is commonly called, has become a popular dooryard and landscape ornamental in South Florida. Its red seeds are well understood to be poisonous and from time to time the local papers tell of a dog fatality, though seemingly not of humans. The seeds are abundantly produced and, even without an effective dispersal mechanism, often become lodged in nearby waste areas. Perhaps the first recognition of this cycad outside of cultivation was in 2000 (Wunderlin & Hansen, *Flora of Florida* 1: 302). Records of naturalization are now known from several south peninsula counties, within the range permitted this cold-sensitive plant. This Mexican species must now be accepted as a member of the Florida flora.

Distribution of Florida taxa

Plants native to Florida fall largely into recognizable geographic zones or areas. There are the northern species, subject perhaps to chilling requirements and thus limited in their southern extension into the state. The tropical species are restrained by thermal limits, often extending farther north along the coasts than within the peninsula. Endemics especially occur on the Apalachicola River bluffs, with others along the panhandle gulf coast. There are many exceptions, but commonly a species long-present in the state will have a distribution that at least approximates other native species.

Introduced species, that is, species arriving in the state after European contact, commonly will have erratic distributions. They may be abundant in one area, yet absent in other areas of similar edaphic factors. It is apparent that introduced species often have not yet reached all suitable sites.

Zamia integrifolia is unquestionably a native of Florida; it was certainly here centuries before European contact. Yet its distribution within the state suggests that of a recent introduction, with plants abundant in one area yet wholly absent in other seemingly suitable areas. And within the species the varieties are similarly inconsistent in their distribution. It is a simple, yet likely correct deduction that these weakly defined varieties are the consequence of multiple introductions from the West Indies, limited in the rate of their spread by their heavy seeds and restricted movements of their vectors, the Native American tribes.

The five varieties of *Zamia integrifolia* recognized here certainly differ in their distribution. Typical var. *integrifolia* is widespread in the peninsula, though often absent in areas where it might be expected. It is present on both coasts, more sparingly in the interior, and extending down the east coast to the vast stands once found on the Dade County rocklands. It seems to be this variety that is most heavily represented by the many thousands of commercial plants now planted on highway medians and landscaping. Var. *umbrosa* in nature is restricted to the upper eastern peninsula. [Small (1921: 137) noted plants in cultivation in Gainesville and Ocala. A Gainesville plant surely seen by him was still present into 2014.] Var. *broomei* appears confined to thin sandy woods of the lower Suwannee River basin. Var. *floridana* thus far has been seen only in Levy County, on the upper gulf coast, though to be expected further south on extant shell mounds. And var. *silvicola* has a questioned disjunct distribution between coastal Citrus County and the everglades of Dade County.

Zamia furfuracea, as a cold-sensitive plant of recent introduction, is to be expected in any south peninsula, non-hydric site wherever chance has yet placed its seeds.

Concluding remarks

Persons who study and grow Florida cycads fall into two camps. There are the taxonomists, few in number, experienced in determining workable classifications, seemingly inflexible in their judgment that all Florida *Zamia* constitute only a single taxon. And there are the horticulturists, intimately familiar with the plants both in the field and in cultivation, who maintain there are several *Zamia* in Florida that can be distinguished by anyone who takes the time to look. This present treatment attempts to place a foot in both camps, by giving systematic recognition to some of the insecurely defined populations believed in by growers and enthusiasts. (Even here, not all forms are addressed; there is said to be a "deciduous" plant, one whose leaves turn brown and die in the fall.) But perhaps the present paper pushes professional judgment as far as is defensible.

Amplified key

Taxonomic judgments based on gross morphology are appreciably less reliable in the cycads than is encountered with other vascular species. The length of a pinecone, size of an apple, width of a maple leaf is relatively independent of the size and age of the supporting plant. But the *Zamia* cone and the length of its leaf clearly are influenced by the age -- and presumably energy storage -- of the host. Thus simple linear measurements may mislead. Ratios (length/width ratios, as used in the following key) may minimize these distortions.

ZAMIA L. Coonties

1. Petioles with stout prickles; leaflets 25-50 mm. wide (l/w = ±3), thick and rigid, ovate, apically erose (with many protruding vein-tips); seeds red (orange). Trunk erect, stocky (pachycaulous), unbranched, to 1.5 m. Waste areas, disturbed scrubland. South peninsula (n. to Lake); rare and scattered.
CARDBOARD PALM. **Zamia furfuracea** L. f. in Ait.

1. Petioles without prickles; leaflets 5-16 mm. wide, firm but flexible, oblong to linear; seeds orange. Trunk subterranean or low emergent, sometimes dichotomous-forked, forming mounds. [*Zamia pumila*, misapplied]
FLORIDA COONTIE, FLORIDA ARROWROOT. **Zamia integrifolia** L. f. in Ait.
   a. Leaflets usually with slightly protruding vein-tips ("teeth," "callous bumps") on and near rounded apex; median leaflets 12-16 mm. wide (l/w = ±8); leaves to 1.5 m. long, dark green; older plants massively multistemmed, forming large clumps. Dry woodlands. Upper east peninsula (St. Johns, Putnam, e. Marion=Juniper, s. to Brevard); infrequent. Long in cultivation in northeast Florida, under the name "Palatka Giant." [*Zamia umbrosa* Small; *Zamia floridana* var. *umbrosa* (Small) D. B. Ward]
   EAST COAST COONTIE. var. **umbrosa** (Small) D. B. Ward

   a. Leaflets without protruding vein-tips on acute to obtuse apex; leaves to 1.0 m. long, medium green.
      b. Median leaflets linear, 5-8 mm. wide (l/w = ±18); female strobili 8-10 cm. long; foliage generally erect; plant rarely multistemmed. Dry oak hammocks, sandy high pine. Upper west peninsula (Alachua=Haile, San Felasco); infrequent. var. **broomei** D. B. Ward
      b. Median leaflets narrowly oblong to spatulate, 8-16 mm. wide; foliage generally spreading; plant usually multistemmed.
         c. Median leaflets 14-18 cm. long, 12-16 mm. wide (l/w = ±10); female strobili "9-14 cm. long" (Small, 1926). Moist hammocks, river bluffs. Upper west peninsula (Levy=Fanning Springs; Citrus=Crystal River), apparently disjunct to south peninsula (Dade); rare. *Zamia silvicola* Small var. **silvicola** (Small) D. B. Ward
         c. Median leaflets 8-12 cm. long, 8-14 mm. wide (l/w = ±12).
            d. Female strobili 8-18 cm. long; substrate shell mounds. Aboriginal shell middens. Upper west peninsula (Levy=Cedar Key); rare. [*Zamia floridana* A. DC.] var. **floridana** (A. DC.) D. B. Ward
            d. Female strobili 4-8 cm. long; substrate sandy soils. Pinelands, thin woodlands. Widespread: upper west peninsula (Taylor, Suwannee, w. Marion, south along west coast (Pinellas, Charlotte), upper east coast (Volusia), to south peninsula (Highlands, St Lucie, Dade); frequent. Formerly abundant in southeast peninsula, the basis for a large arrowroot starch industry. Now in common use as groundcover, foundation plantings, etc.
            FLORIDA COONTIE (typical). var. **integrifolia**

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.

I have been helped in the field by Kim M. Cordasco, Robert T. Ing, Steven C. Provost, Sylvia W. Schultz, and Gordon C. Ward, and with observations or suggestions by Tom H. Broome, Alan W. Meerow, Bart M. Schutzman, and Dennis W. Stevenson.

Juniperus arizonica (R. P. Adams) R. P. Adams, new to Texas.

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ABSTRACT

Juniperus arizonica, previously known only from Arizona and New Mexico, has now been verified, by DNA sequencing, to occur in trans-Pecos Texas in the Franklin Mtns., Hueco Mtns., Hueco Tanks State Park, Quitman Mtns., Eagle Mtns. and Sierra Vieja Mtns. primarily on igneous material. These trans-Pecos juniper populations have previously been identified as *J. coahuilensis*. Revised distribution maps are presented for *J. arizonica* and *J. coahuilensis*. Published on-line **www.phytologia.org** *Phytologia 98(3)179-185 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Juniperus arizonica, J. coahuilensis,* Cupressaceae, revised distribution maps, petN-psbM DNA.

Juniperus arizonica and *J. coahuilensis* are essentially cryptic species in Arizona, New Mexico, Texas and Mexico (Figs. 1, 2). The taxa appear to differ in the relative length of the whip leaf glands (shorter in *J. arizonica*). However, in routine examination specimens at UNM (University of New Mexico Herbarium) to verify SEINET distribution maps for the new Flora of New Mexico, I found the relative whip leaf gland length of *J. arizonica* was quite variable, overlapping that of *J. coahuilensis*. These taxa have very distinct differences in their DNA and they are in separate clades (Figure 3). The cp region petN-psbM is especially efficient in separating these taxa, as 5 SNPs occur in the 794 bp region. Additional examination of specimens at UA (Univ. Arizona), SJNM (San Juan College), UTEP (Univ. Texas at El Paso), and SRSC (Sul Ross State University) revealed that many of the *J. coahuilensis* specimens were not morphologically distinct from *J. arizonica*. To reconcile this problem, a survey was initiated to sequence petN-psbM from about 10 mg of specimen leaves to verify its identity. The purpose of this paper is to report on the revised distribution based on petN-psbM sequencing of herbarium material and new collections in trans-Pecos Texas.

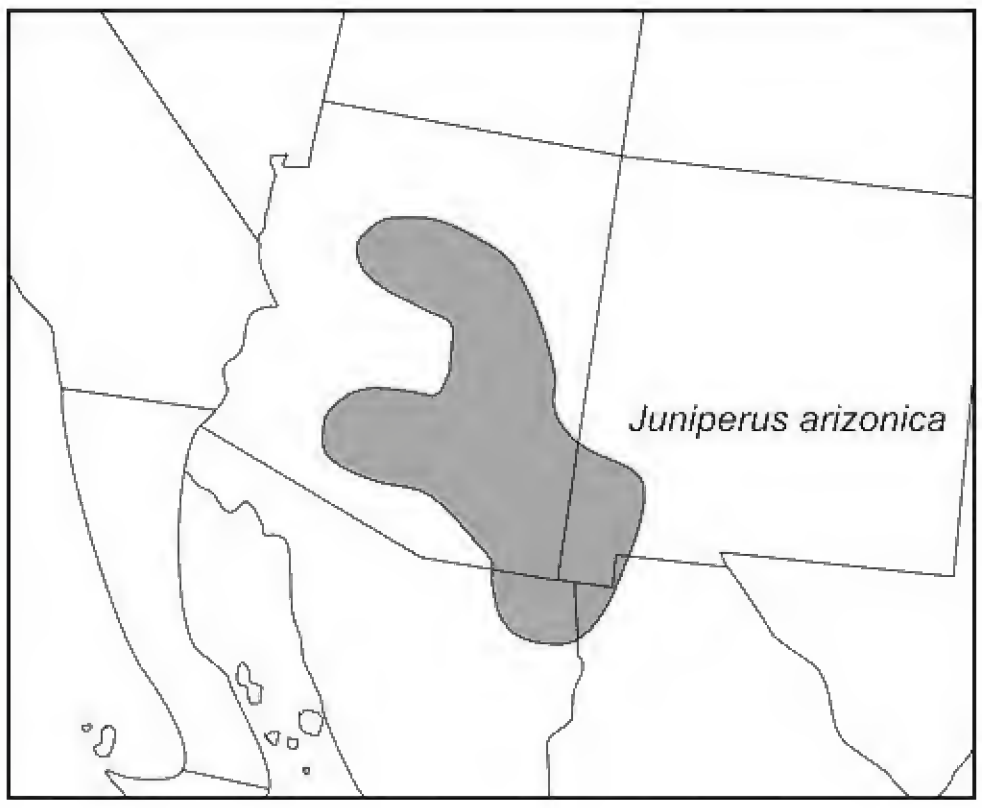


Figure 1. Distribution of *J arizonica* (Adams 2014).

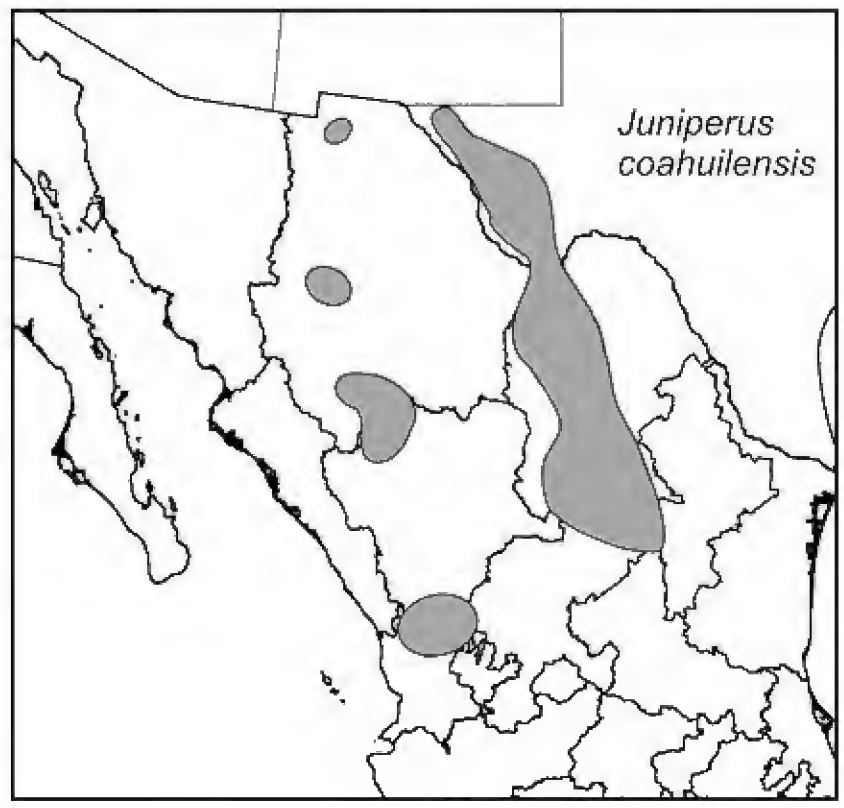


Figure 2. Distribution of *J coahuilensis* (Adams 2014).

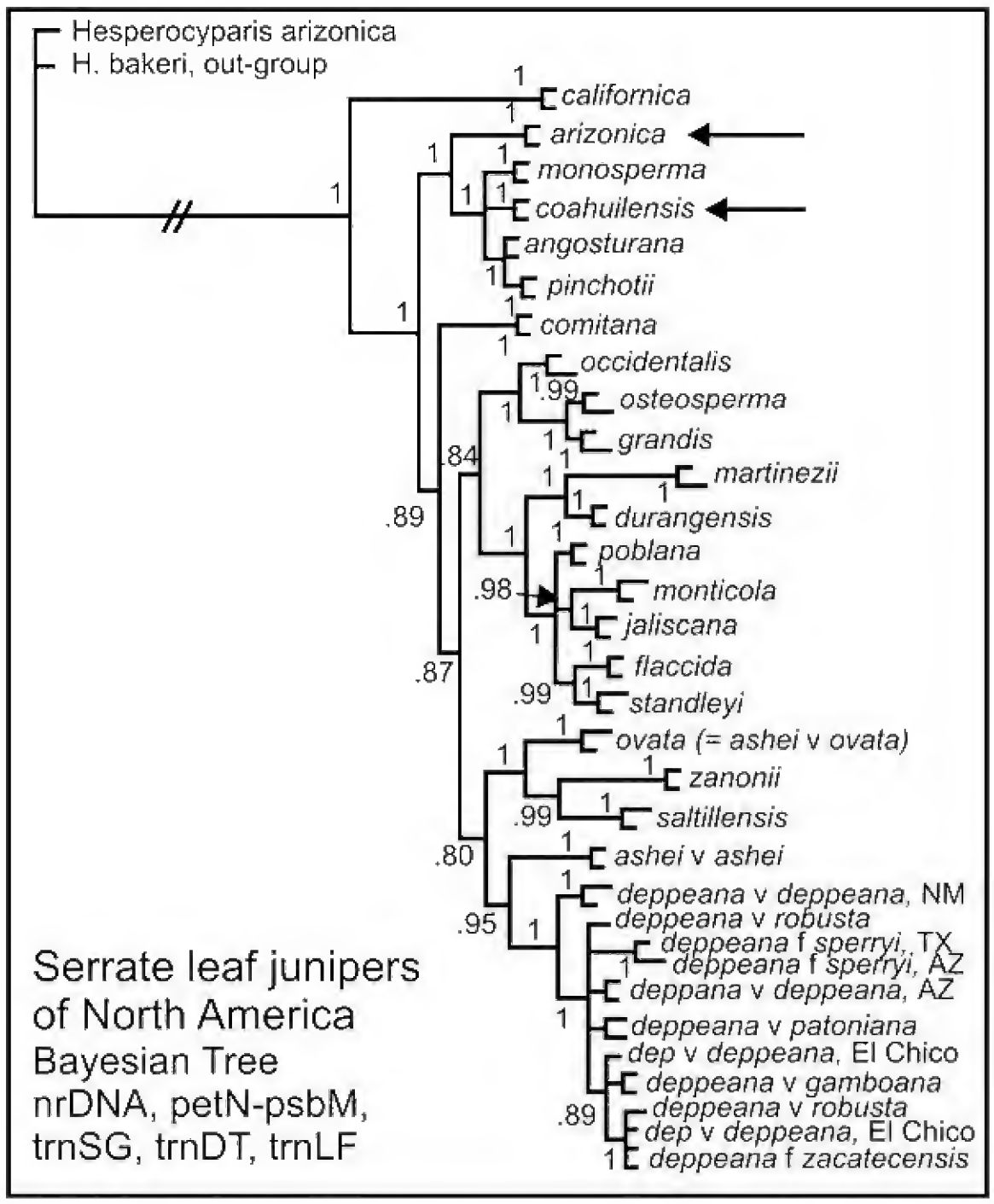


Figure 3. Bayesian tree for the serrate *Juniperus* of the North America. Note the position of *J. arizonica* and *J. coahuilensis* in well supported, separate clades. (from Adams 2014)

MATERIALS AND METHODS

Plant material:

UNM:

J. arizonica by petN DNA, Dona Ana Co., NM, Potrillo Mtns, 31.865257° N, 106.9397° W, 4500', UNM 101883, *Worthington 19806*, 13 Jul 1991,Lab Acc. *Robert P. Adams 14696,*

J. arizonica by petN DNA, Hidalgo Co., NM, Animas Mtns, 31.61176° N, 108.7791° W, 5750', Seinet Cat # 57778, *Wagner 1283*, 22 Jul 1975, Lab Acc. *Robert P. Adams 14697*,

J. arizonica by petN DNA, Luna Co., NM, Tres Hermanos Mtns, 31.9010° N, 107.7794° W, 4250', Seinet Cat # 85666, *J L Carter 1246*, 14 Aug 1993, Lab Acc. *Robert P. Adams 14698,*

J. arizonica by petN DNA, Luna Co., NM, Florida Mtns, 32.1266° N, 107.6413° W, 5800', UNM 85854, *J L Carter 1247,* 14 Aug 1993, Lab Acc. *Robert P. Adams 14699,*

J. monosperma by petN and trnLF, Luna Co., NM, Cooke's Range Mtns, 32.5386° N,107.7055° W, 5848' (Goggle Earth) Seinet Cat # 85987, JP Hubbard sn, 25 Apr 1978, Lab Acc. *Robert P. Adams 14700,*

J. arizonica by petN DNA, Hidalgo Co., NM. Animas Mtns, 31.5938° N, 108.7684° W, 6000', Seinet Cat # 57776, *Wagner 1005*, 17 Jun 1975, Lab Acc. *Robert P. Adams 14701,*

J. monosperma by petN and trnLF, Dona Ana Co., NM, blue cones, about 100 yds e of St. Nicholas Camp, San Andreas Mtns., ca 32.580 N, 106.5283 W, 5-6000', UNM 55607, lvs for DNA taken 10/29/2015 Jim Von Loh 27, 25 Mar 1975, Lab Acc. *Robert P. Adams 14702,*

J. monosperma by petN and trnLF, Otero Co., NM, Alamo Mtns., ca. 32° 01' 46" N, 105° 38' 20" W, 6500' (Google Earth), J S Findley ns, 16 Arp 1962, Lab Acc. *Robert P. Adams 14703,*

J. arizonica by petN DNA, no cones, Luna Co., NM, Florida Mtns., ca. 32.1266° N, 107.6413° W, 5800', UNM 31814, *J S Findley ns,* 31 Jan 60, Lab Acc. *Robert P. Adams 14704,*

J. arizonica by petN DNA, no cones, Hidalgo Co., NM, Animas Peak, Animas Mtns., 31.5813° N, 108.7843° W, 8452' (Google Earth), Seinet Cat # 25131, *WC Martin 4678*, 29 Oct 1960, Lab Acc. *Robert P. Adams 14705*,

J. arizonica by petN DNA, no cones, Hidalgo Co., NM, Floor of Skeleton Canyon, Peloncillo Mtns., ca. 31.590° N,109.028° W, 4900' (Google Earth), Seinet cat # 22333, *E F Castetter 11257*, 20 Aug 1956, Lab Acc. *Robert P. Adams 14706,*

J. arizonica by petN DNA, no cones, Hidalgo Co., NM, e side of McGee Peak, ca. 4 mi. s of Steins, Peloncillo Mtns., 32.1666° N,108.992° W, 5150 Seinet cat # 112666, *RC Sivinski 6275*, 21 Mar 2007, Lab Acc. *Robert P. Adams 14707,*

J. arizonica by ptN DNA, Hidalgo Co., NM, 0.8 mi. e of gate to Guadalupe Ranch, Guadalupe Canyon, 31.3613° N,109.0438° W, 4400', Seinet cat # 85707, *JL Carter 274*, 14 Aug 1991, Lab Acc. *Robert P. Adams 14708,*

SJNM:

J. arizonica by petN DNA, Hidalgo Co., NM, Big Hatchet Mtns.,with Quercus, Parthenium, Ocotillo, Mesquite, Agave 31.6249° N, 108.36425° W, 5350', *Ken Heil 9254*, 28 May 2010, Lab Acc. *Robert P. Adams 14716,*

J. arizonica by petN DNA, Grant Co., NM, ca 1.5 mi. s of NM hwy 9, near 'Old Hachiti' townsite. Chihuahuan desert scrub - creosote, Lycium koberlina and Dalea formosa. 31.9139° N, 108.41472° W, 4745', *Ken Heil 32357,* 29 Apr 2010, Lab Acc. *Robert P. Adams 14717,*

UA:

J. arizonica by petN, UA363788, Sonora, Sáric Municipio, Rancho La Tinaja, Arroyo El Silencio, 31.36° N, 111.4° W, 1035 m, *T. R. Van Devender, 2002-913*, 10/6/2002, Lab Acc. *Robert P. Adams 14777*,

J. arizonica by petN, UA372017, Sonora, Nogales Municipio, Canada El Aguaje de Zorrillo, near Rancho Esmeralda, 31.205° N, 111.1° W, 1133 m, *A. L. Reina G., 2004-952*, 8/18/2004, Lab Acc. *Robert P. Adams 14778*,

J. arizonica by petN, UA373061, Sonora, Agua Prieta Municipio, NE of Sierra Anibacachi, Rancho La Calera, ca. 10 km (by air) SW of Agua Prieta, 31.233° N, 109.6° W, 1287 m, *T.R. Van Devender, 2004-843*, 8/15/2004, Lab Acc. *Robert P. Adams 14779*,

J. arizonica by petN, UA396885, Sonora, Agua Prieta, Municipio, Rancho El Diablo, Arroyo Cajón Bonito, Cuenca Los , Ojos, 31.291° N, 109° W, 1252 m, *A. L. Reina-G., 2010-473*, 5/16/2010 Lab Acc. *Robert P. Adams 14780*,

J. arizonica by petN, UA400214, Sonora, Agua Prieta Municipio, Rancho El Pinito, Arroyo Cajón Bonito, 56.5 km(by air) ESE, 31.191° N,108.9° W,1432 m, *T. R. Van Devender, 2009-1366*, 9/23/2009, Lab Acc. *Robert P. Adams 14781*,

J. arizonica by petN, UA405935, Sonora, Bavispe Municipio, 9.0 km (by air) S of Colonia Morelos, 59.5 km (by air) NE of.., 30.746° N, 109.2° W, 1009 m, *A. L. Reina-G, 2010-295*, 3/21/2010, Lab Acc. *Robert P. Adams 14782*,

J. arizonica by petN, UA406347, Sonora, Imuris Municipio, Arroyo el Catrín, Rancho El Salto, 31.3 km (by air) ESE of í..., 30.684° N, 110.6° W, 1256m, *T. R. Van Devender, 2010-1086*, 10/4/2010, Lab Acc. *Robert P. Adams 14783*,

J. arizonica by petN, UA406806, Sonora, Arizpe Municipio, 3.8 km (by air) ENE of Arizpe along Río Sonora, 30.349° N, 110.1° W, 833m, *A. L. Reina G., 2011-18 2/8/2011*, Lab Acc. *Robert P. Adams 14784*,

J. arizonica by petN, UA407978, Sonora, Imuris Municipio, Remedios, Arroyo Los Remedios, 30.762° N, 110.7° W, 1044m, *T. R. Van Devender, 2005-635*, 4/8/2005, Lab Acc. *Robert P. Adams 14785*,

J. arizonica by petN, UA408981, Sonora, Arizpe Municipio, Sierra San Antonio, Arroyo Tirinagua, 30.375° N, 110.4° W, 1200m, *George M. Ferguson, 2371,* 5/5/2000, Lab Acc. *Robert P. Adams 14786*,

J. arizonica by petN, UA409144, Sonora, Arizpe Municipio, Sierra San Antonio, Arroyo Tirinagua, 30.374° N, 110.4° W, 1230m, *George M. Ferguson, 3117*, 5/2/2011, Lab Acc. *Robert P. Adams 14787*;

J. arizonica by petN, UA410254, Sonora, Magdalena, Magdalena Palm Canyon. 30.47° N, 110.8° W, 1150m, *Benjamin T. Wilder, 10-582*, 8/30/2010, Lab Acc. *Robert P. Adams 14788*,

J. arizonica by petN UA410257, Sonora Magdalena, 6.5 mi E of Magdalena, 30.564° N, 110.9° W, 830 m, *Benjamin T. Wilder, 10-495*, 8/27/2010, Lab Acc. *Robert P. Adams 14789*,

J. arizonica by petN, Mexico, Chihuahua, Municipio Janos, Sierra San Luis, along road in N tributary of Arroyo Las Chimeneas (Cajon Bonito drainage), foothills on W side of range at 1.0 km N Rancho San Antonio, Sonora, 31.2303° N, 108.864° W, 1690 m, *George M. Ferguson, 990,* 27 May 1996, Lab Acc. *Robert P. Adams 14790*,

J. arizonica by petN, Mexico, Sonora, Municipio Agua Prieta, Sierra Guadalupe (=Peloncillo Mts.), along Mex hwy 2 at milepost 104, airline 2.5 km N and 3.5 km E confluence Arroyo El Diablo-Cajon Bonito. 1460 m, 31° 18' 45" N, 109° 00' 30" W, *George M. Ferguson 1730*, 27 June 1999, Lab Acc. *Robert P. Adams 14791*,

J. coahuilensis by petN, Mexico, Durango, Municipio Villa Ocampo, ca. 13 km SE of Canutillo, along Mex hwy 45, at milepost 326.5, 26° 20' 23.7" N, 105° 13' 58.7" W, 1800 m , *George M. Ferguson 2177*, 2 July 1999, Lab Acc. *Robert P. Adams 14792*,

J. arizonica by petN, female, Mexico, Chihuahua, Municipio Ascencion, along Mex hwy 2 at milepost 127.5, at 5 km NE jct to Microondas Palomas, and 16 km W jct of hwy to Palomas. on limestone 31° 23' 39.3" N, 107° 44' 48.9" W, 1340 m, *George M. Ferguson 2107a*, 17 June 1999, Lab Acc. *Robert P. Adams 14793*,

J. arizonica by petN, male Mexico, Chihuahua, Municipio Ascencion, along Mex hwy 2 at milepost 127.5, at 5 km NE jct to Microondas Palomas, and 16 km W jct of hwy to Palomas. on limestone 31° 23' 39.3" N, 107° 44' 48.9" W, 1340 m, *George M. Ferguson 2107b*, 17 June 1999, Lab Acc. *Robert P. Adams 14794*,

J. arizonica by petN, New Mexico, Hidalgo Co., Big Hatchet Mountains, Thompson Canyon, on limestone, 31° 37' 02.5" N, 108° 22' 51.5" W, 6200', *George M. Ferguson 2544*, 22 Sept 2001, Lab Acc. *Robert P. Adams 14795*,

J. arizonica by petN, Mexico, Sonora, Municipio Huachinera, 4 km (by road) E Huachinera, 0.5 km W of Rancho San Ignacio de Cobora, 30° 12' 20"N,108° 55' 00" W, 1150 m, *George M. Ferguson 2852*, 1 August 2006, Lab Acc. *Robert P. Adams 14796*,

J. arizonica by petN, Texas, Hudspeth Co., Eagle Mtns., 0.5 mi NNE of Oxford Spring, 6 mi (by air) SSE Eagle Peak, 4400', 30.824 N, 105.0351 W, *George M. Ferguson 3570,*25 May 2014, Lab Acc. *Robert P. Adams 14877*,

SRSC:

J. pinchotii by petN and trnLF, Brewster Co., TX, Dead Horse Rg., non-glaucous, 29° 24' N, 103° 00' W, 5250', *J Fenstemacher1240*, 2005, Lab Acc. *Robert P. Adams 14836*,

J. pinchotii by petN and trnLF, Brewster Co., TX, 10 mi s Alpine, rare non-glaucous, *Powell 5185*, 1985, Lab Acc. *Robert P. Adams 14837,*

J. coahuilensis by petN, Brewster Co., TX, Basin, Big Bend National Park, ca. 29° 18' 23" N, 103° 18' 06" W, 5250' (Google Earth), *SC Bartel 575*, 2001, Lab Acc. *Robert P. Adams 14838,*

J. pinchotii by petN and trnLF, Presidio Co., TX, Friedrich Mesquite Ranch, Cinco de Mayo Pasture, 5800', *Warnock 590*, Oct 1990, Lab Acc. *Robert P. Adams 14839,*

J. arizonica by petN, large trees,1m diam!, Sierra Vieja, Indian Peak Canyon, 30° 33' 25" N, 104° 40' 15" W, 5185', *Powell 6838*, 2010, Lab Acc. *Robert P. Adams 14840,*

DNA, all degraded! Jeff Davis Co., TX, Haystack Mtn, 1959, coah, TJ Allen 247, May 1959! Lab Acc. *Robert P. Adams 14841,*

J. arizonica by petN, Hudspeth Co., TX, Eagle Mtns, *BG Hughes 392*, 1991, Lab Acc. *Robert P. Adams 14842*,

UTEP

DNA all degraded! El Paso Co., TX, Indian Spring, Franklin Mtns, 31° 54' 19" N, 106° 28' 09" W, 4900', *Worthington 2266*, 1978, Lab Acc. *Robert P. Adams 14843,*

J. arizonica by petN, El Paso Co., TX, Franklin Mtns, no fruit, 31° 52' 40" N, 106° 28' 56" W, 5600', *Worthington 1838*, 1978, Lab Acc. *Robert P. Adams 14844,*

J. arizonica by petN, Hudspeth Co., TX, Hueco Mtn, (not Hueco Tanks), 31° 49' 12" N, 106° 07' 11" W, 4300', *Worthington 1844,* 1978, Lab Acc. *Robert P. Adams 14845,*
J. arizonica by petN, Hudspeth Co., TX, Eagle Mtns, Wind Canyon, 30° 54' N, 105° 04' W, 6000', *N Hutchings #5*, 1972, Lab Acc. *Robert P. Adams 14846,*
J. coahuilensis, by petN, Indio Mtns, Hudspeth Co, TX, 30° 47' 05" N, 104° 58' 20" W, 4350', *CS Licks 933*, 1988, Lab Acc. *Robert P. Adams 14847,*
J. arizonica by petN, Franklin Mtns. El Paso Co., TX, 31° 55' 15" N, 106° 30' 13" W, 5700', *Worthington 2112*, 1978, Lab Acc. *Robert P. Adams 14848,*

New Collections by RP Adams:

J. arizonica by petN, Hudspeth Co., TX, common on degraded granite, north face of Quitman Mtns., with desert-scrub. On south side of I10, ~6.3 mi. w of Sierra Blanca, TX, 31°12' 25" N; 105° 27' 51" W, 4629', *Robert P. Adams 14798-14806,* 12 March 2016,
J. coahuilensis, by petN, Brewster Co, TX, abundant in grassland, 11.2 s of Alpine, TX on Tex 118. 30° 14' 08" N; 103° 34' 00" W, 5222', *Robert P. Adams 14807-14811,* 15 March 2016,
J. coahuilensis, by petN, Brewster Co, TX, 11.0 mi w of Alpine on US 90, abundant in grassland, in Paisano Mtns., 30° 17' 42" N; 103° 48' 02" W, 4967', *Robert P. Adams 14812-14816,* 15 March 2016,
J. coahuilensis, Jeff Davis Co., TX, common locally, in grassland. 4.2 mi se of Ft. Davis, on Tex 118, e 1.0 mi into Chi. Desert Res. Inst., 39° 09' 27.54" N; 86° 18' 23.31" W, 5050', *Robert P. Adams 14817-14821*, 16 March 2016,
J. coahuilensis, by petN, Presidio Co., TX, common in grassland, 19.4 mi. s of Marfa, on US 67, 30° 04' 07" N; 104° 10' 19" W, 5137', *Robert P. Adams 14822-14826*, 16 March 2016,
J. arizonica by petN El Paso Co., TX, uncommon, 50- 100 trees seen, on granite, Hueco Tanks St. Park, 31° 54' 49.7" N; 106° 02' 6.8" W, 4560', *Robert P. Adams 14827-14835*, 18 March 2016.
J. monosperma, Dona Ana Co. NM, common, blue berries, ca. 1 mile nw of Lower Ash Spring, San Andres Mountains, 32° 38.131' N, 106° 32.785' W, 5622', *Kelly Allred sn*, 18 Nov 2015 Lab Acc. *Robert P. Adams 14718.*

Voucher specimens for new collections are deposited in the Herbarium, Baylor University (BAYLU).

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.

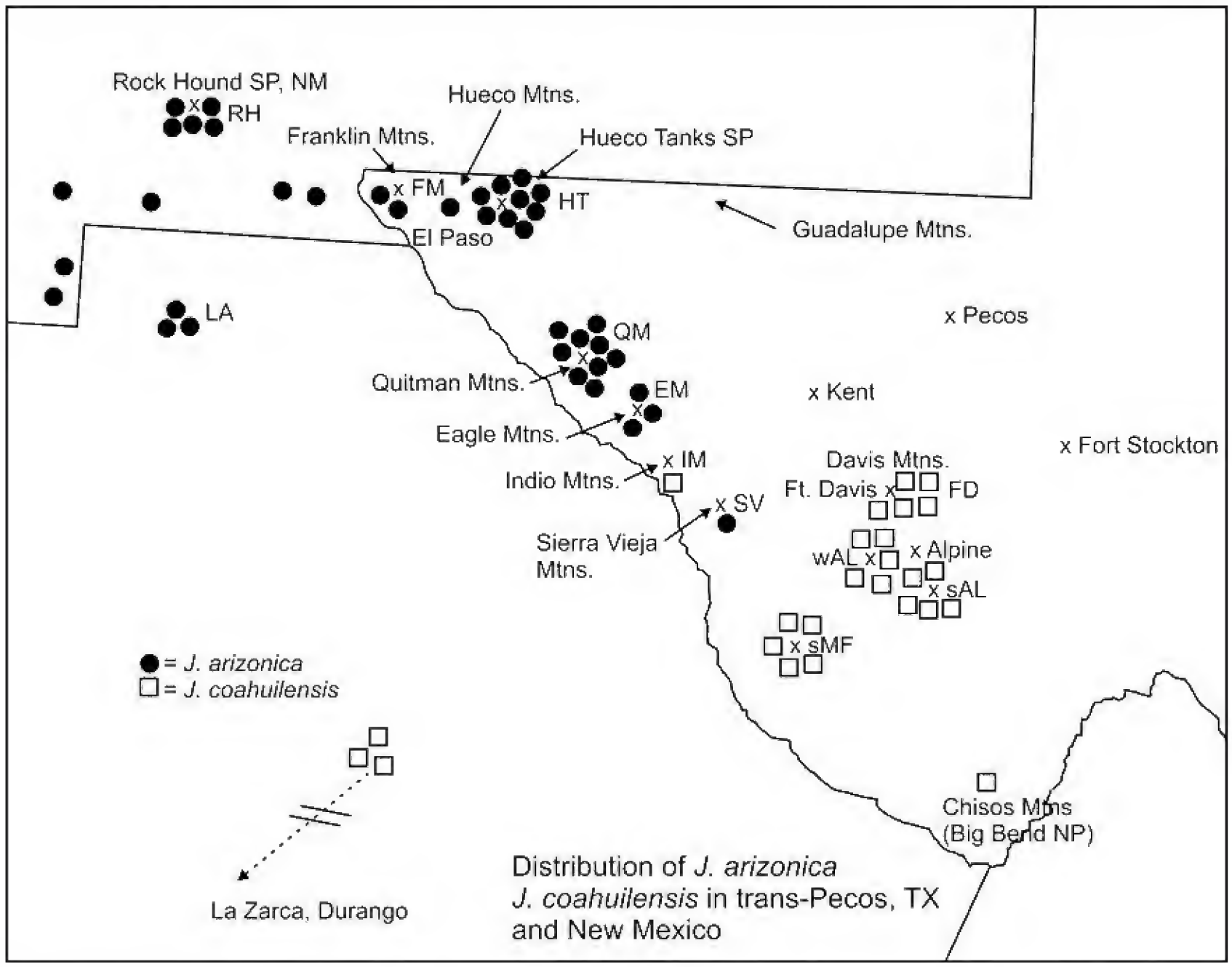
Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN-psbM), D (maldehy) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM $MgCl_2$ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the petN-psbM primers utilized.

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.).

RESULTS AND DISCUSSION

Sequencing petN-psbM yielded 794 bp with 5 SNPs separating *J. arizonica* and *J. coahuilensis*. Based on these data, samples were classified accordingly (see MATERIALS AND METODS). Figure 4 shows the distribution of *J. arizonica* and *J. coahuilensis* in trans-Pecos Texas and New Mexico based on petN-psbM data. The junipers at all the Texas and new Mexico locations (except Rock Hound SP) of *J. arizonica*, have previously been classified as *J. coahuilensis*.

In Texas, *J. arizonica* was found in the Franklin Mtns., Hueco Mtns., Hueco Tanks State Park, Quitman Mtns., Eagle Mtns. and Sierra Vieja Mtns. primarily on igneous material. Worthington (pers. comm.) said that granitic outcrops (such as Huecho Tanks) are common in southwestern New Mexico where he has found junipers. It is interesting that on the George Ferguson specimens from Sonora (Materials and Methods, above), he notes the substrates as limestone. *Juniperus coahuilensis* in trans-Pecos seems to grow mostly in grasslands over limestone and volcanic soils. As far as known, the two species are not sympatric, however, the area from Sierra Vieja to Indio Mtns. to Quitman Mtns. may



contain sympatric populations.

Figure 4. Distribution of *J. arizonica* and *J. coahuilensis* based on petN-psbM data. All the Texas and new Mexico locations of *J. arizonica*, (except Rock Hound SP) have previously been called *J. coahuilensis*.

Revised species distribution maps for *J. arizonica* and *J. coahuilensis* are shown in Figures 5 and 6. The Xs show the new populations discovered (and discussed in this paper) in trans-Pecos Texas. It seems likely that hybridization and, possibly, introgression is occurring, but that is beyond the scope of this paper.

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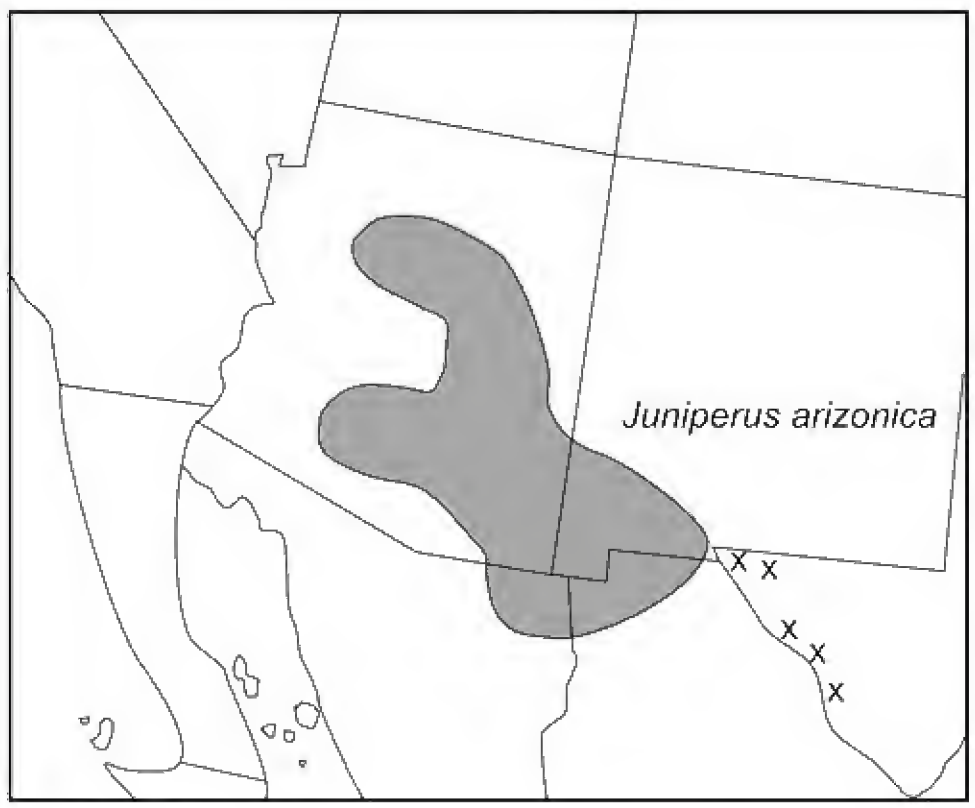

Figure 5. Distribution of *J. arizonica*.

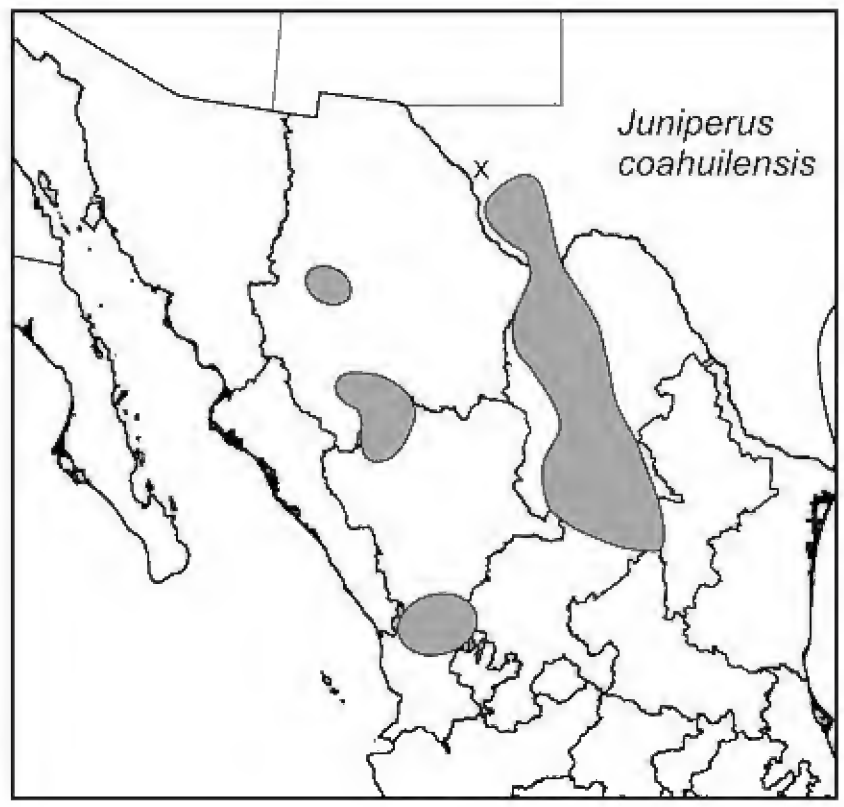

Figure 6. Distribution of *J. coahuilensis*.

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Measurements and taxonomy in *Arceuthobium* (Viscaceae).

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ABSTRACT

The population complexities within *Arceuthobium campylopodum* sensu Gill (1935) have led to two recent analyses reaching different conclusions even though mainly or at least in part based on standardized internodal measurements. These measurements cannot be utilized to formulate taxonomic conclusions because stem internodes continue to elongate from year to year. It is recommended to refrain from using infraspecific categories until the relevant variation patterns, both in hosts and parasite populations, are better known. *Phytologia 98(3): 186-189 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Arceuthobium campylopodum*, Viscaceae, internodal measurements.

The complex of populations representing *Arceuthobium campylopodum* Engelm. in the sense of Gill (1935) has taxonomically challenged innumerable workers, resulting in a very extensive collection of synonyms and other nomenclatural combinations. It is a controversy of long standing. That it is not close to a resolution is shown by the fact that two major and contrasting approaches have been published within a couple of years of each other, each representing much meticulous work, one basically molecularly oriented but also building on earlier morphometric information (Nickrent 2012), the other with extensive morphometric data that cover 5 pages of tables and two geographic maps (Mathiasen & Kenaley 2015). The former study recognizes 13 subspecies within (but not limited to) the western United States. The latter one concerns only the three groups that are mostly limited to *Pinus ponderosa* Doug. ex Loud., *Larix occidentalis* Nutt., and *Tsuga heterophylla* (Raf.) Sarg., and are the most northerly representatives of the complex. The coastal plants of the Pacific Northwest, furthermore, have previously been further divided into *A. tsugense* (Rosend.) G. N. Jones subspecies *mertensianae* Hawksw. & Nickr. (Hawkswoth et al. 1992) and the taxonomically dubious *A. tsugense* subspecies *contortae* Wass & Math. (Wass & Mathiasen 2003).

I wish to start my comments with a disclaimer. It is not my purpose to claim that any one taxonomic solution to this baffling complex of plants is better than any other, even though I also will state my personal preference. Rather, my main purpose is to comment on the reliability of the mensural data that have in the past been gathered and are said to support both the positions in the two major recent papers.

I also hasten to say that I am not an expert in the intracacies of either molecular taxonomy or morphometric analysis, but I strongly suspect that the significant issues lie elsewhere. At this moment, I shall accept at face value the authentic nature of the techniques employed and the data gathered, and focus on some serious flaws in the claimed *significance* of mensural data.

First of all, a semantic issue that may appear to be a quibble. The words "morphometric" and "morphological" should not be used interchangeably, as Mathiasen and Kenaley do throughout. The former term refers to measurements, but the latter has a much broader meaning, including some things like branching patterns, flower position, leaf shape and position, and (in *Arceuthobium*) emergence patterns from hosts. The curious fan-shaped (flabellate) branching pattern of *A. campylopodum*, for example, is a gross *morphological* feature that is identical throughout the complex, and is also seen in *A.*

vaginatum (Willd.) Presl. This morphological feature is diametrically opposed to that of *A. americanum* Nutt., but comparisons with the smaller, highly reduced species are essentially impossible. Technically, I would maintain, in contrast to what Mathiasen and Kenaley write, that there are no strictly morphological differences in the elements of the *A. campylopodum* complex beyond possible mensural ones.

Measurements

The idea that standardized measurements of internodes can be used to distinguish *Arceuthobium* taxa was first introduced in Hawksworth & Wiens' work on Mexican species (1965) and later refined more generally (1972, 1996). In the latter monograph, while admitting that shoot internodes may elongate for several years, these authors nevertheless defended the taxonomic utility of such measurements, narrowing the latter to the third internode of a shoot without providing a rationale for that particular choice. They stated that "The overall mature internode dimensions among various species differ so significantly that internodal elongation does not negate the usefulness of the character in these cases."

Since 1996, internodal length measurements have been employed for this purpose in Western North America (see Mathiasen & Kenaley 2015), and they form a major structural part of the taxonomic conclusions in the latter study. As mentioned above, Hawksworth & Wiens' early data are also said to support the very different conclusions reached by Nickrent (2012; see his Fig. 1). In all cases, the assumption clearly is that the third internode of a plant can reliably be utilized as a standard. This laudable goal of standardization is based on a patently false foundation. In fact, the idea was demonstrated to be untenable nearly 50 years ago in a major paper that has not, curiously, been cited in any of the above studies (Kuijt 1969) – a paper, ironically, that deals specifically with the very question at issue.

In that study, careful measurements were made of the lengths of internodes especially of a related species, *A. americanum*, a species showing a highly unusual sexual dimorphism in its inflorescences. The curious male inflorescnce unit is a one-flowered structure of a single internode, its two distal scale leaves in the following season subtending two more such units; this is repeated from year to year, although the initial situation is somewhat different. Thus, a continual forking results. The important point here is that the single inflorescence internode in each case elongates somewhat every successive year: it was demonstrated that a single such internode may increase its lenth by a factor of *ten or more* over a period of 5 years. (The female inflorescences undergo a separate, more modest internodal elongation in most species, separating the maturing fruits, but eventually are dropped). Since male inflorescences in *A. campylopodum* are very different in structure, no such age determination is possible in that species. However, it is quite clear that the phenomenon of yearly internodal elongation is also a fact in *A. campylopodum* and other large species. For one thing, the basal internodes of large, older plants are always much longer than those in their first flowering season, even if part of the same colony. "Seasonal extension of all internodes can be accepted as a fact in all large species of *Arceuthobium*" (Kuijt 1969).

It can be seen immediately that this fact completely deprives third-internode measurements of their significance. Such internodes – all internodes – are not of the same length from year to year, or even within a single growing season. Significantly, in none of the above papers are the seasons or dates of measurements mentioned; in the Hawksworth & Wiens data reproduced by Nickrent (2012), neither sample sizes nor variances were mentioned. In fact, Mathiasen & Kenaley specifically write that in each collection the "dominant plant (largest plant)" was used for measurements, surely admitting bias in the light of the known internodal elongation that takes place.

A number of additional measurements were used by Mathiasen & Kenaley (2015). Several of these can be criticized on the same basis as internodal length (plant height, basal diameter, and others). Others are expressed even in 0.1 mm units, the accuracy of which is dubious. Nickrent (2012) already pointed out that staminate flower width appears to show very little variation.

But let us assume, for a moment, that the measurements in Mathiasen & Kenaley's paper are accurate, and even that the differences between their three groups are as consistent as stated there. The question at this point would be: do such differences necessitate the groups' recognition at either the specific or subspecific level? The answer surely is negative: this is where taxonomic judgment enters. There are innumerable instances in the taxonomic biological literature of complexes that defy an immovable hierarchic solution. The classical instance is that of the ring species (Rassenkreis) of gulls, *Larus spp.*, but several other such instances are known from the animal world. The first botanical instance of such a "ring species" was recently described by Cacho & Baum (2012). I am not implying that the situation in *A. campylopodum* is comparable; I am merely pointing out that population complexities exist that cannot be fully accommodated by a standard Linnean hierarchy. The many known botanical examples of hybrid swarms, sometimes even involving three species, might be cited as comparable situations.

Remarkably, the missing element in all cited papers is the possibility of infrataxon variation in host susceptibility and its possble bearings on the patterns observed in the field. Could the host species not influence morphometric data? Admittedly, the demonstration of such variation would be exceedingly difficult, but most students with field experience have seen suggestive evidence. It would by no means be extraordinary for such an effect to exist. The most convincing published example is seen in Fig. 10 of Kuijt (1955), where one spruce tree is very heavily infected and broomed while its close neighbor, with branches interlocking, is completely free of the parasite, *A. americanum*. We cannot deny the possibility that some populations of a conifer might be more, or less, susceptible than others to *A. campylopodum*, giving deceptive impressions in the field. The absence of data on this issue casts a shadow on the above taxonomic conclusions in *Arceuthobium*.

In Mathiasen & Kenaley paper (2015), it is stated that the authors purposely "did not include samples of plants collected from hosts other than principal hosts for each dwarf mistletoe because there is some evidence that plants are smaller on less susceptible hosts (Mathiasen & Daugherty 2009b)." I would argue that this is a procedural error, and shall illustrate my contention with an example. The plants that locally parasitize *Larix occidentalis* in the southern interior of British Columbia and those that commonly grow on *Tsuga heterophylla* in coastal regions constitute two of the three major groups in Mathiasen & Daugherty's contribution. It would be difficult, in the forested regions of the Province, to find two areas more ecologically unlike. Each group not uncommonly parasitizes *Pinus contorta* Dougl. ex Loud., on which it also can perpetuate itself (in at least some coastal locations, surely for many hundreds of years). The pivotal question is: can we distinguish plants in these two different occurrences on *P. contorta*? If the mistletoes concerned, when growing on the same host species, would show significant differences, a taxonomic decision might be more convincing. Several parallel questions could be raised in more southern areas, with other "principal hosts". That sort of comparison, in my opinion, would be more meaningful than comparing the plants on two different principal hosts; it would at least bypass, in the case mentioned, the potential host influences of *L. occidentalis* and *T. heterophylla*, even though significant environmental influences, and host variation within *P. contorta*, remain possible.

Color variation

There is little doubt that coastal plants often tend to be greener than plants of the other two groups treated in Mathiasen & Daughtery (2015), even though some coastal plants are also distinctly yellow-green (Wass & Mathiasen 2003). Here also, the environment, including exposure to the sun, might exert some influence.

CONCLUSIONS

1. Morphometric measurements of internodes in *Arceuthobium campylopodum* cannot support taxonomic conclusions, because the internodes continue to elongate in successive growing seasons.

2. The population complexities of *A. campylopodum* are such that no one infraspecific hierarchy may be acceptable. It is advisable to avoid infraspecific categrories until the relevant variation patterns of both parasites and hosts are better known, as such categories tend to reflect a deceptive sense of accuracy.

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Geographic variation in the leaf essential oils of *Hesperocyparis* in Arizona, New Mexico, Texas and Mexico

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ABSTRACT

The leaf essential oils were analyzed from *Hesperocyparis* (=*Cupressus*) *arizonica* from Arizona, New Mexico and Texas populations and compared to putative *H. arizonica, H. benthamii* and *H. lindleyi* from Mexico as well as to *H. lusitanica* from the type locality. Clustering revealed six groups: *H. arizonica* (AZ, NM, TX); *H. lindleyi*; an un-named Coahuila group; *H. a.* f. *minor*/f. *glomerata, H. benthamii* and *H. lusitanica.* The leaf oils of the *H. arizonica - lindleyi* group are dominated by umbellulone (9.6 - 32.3%), limonene (2.5 - 19.0%), β-phellandrene (4.0 - 18.5%), sabinene (1.1 - 10.7%) with moderate amounts of terpinen-4-ol (4.1 - 9.4%), isoabienol (t - 4.9%) and phyllocladanol (0 - 4.6%). Nezukol was found to be very variable, ranging from 0.5 to 15.2%. The leaf oils of *H. arizonica* from Arizona, Cooke's Range, NM and Big Bend, TX form a distinct cluster and were surprisingly uniform, in spite of the large distances between the Arizona - NM populations and Big Bend, TX. Published on-line www.phytologia.org *Phytologia 98(3): 190-202 (July 6, 2016).* ISSN 030319430.
KEY WORDS: *Hesperocyparis* (=*Cupressus*) *arizonica, H. lindleyi, H. benthamii, H. lusitanica,* terpenoids, geographic variation, taxonomy.

In the latest nomenclature of the cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere (exclusive of *Xanthocyparis vietnamensis* and *Callitropsis nootkatensis*). Bartel made the new combinations of *Hesperocyparis arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel in addition to the other cypresses the Western Hemisphere .

Analyses using RAPDs fingerprinting (Bartel et al., 2003) showed *H. glabra* to be distinct from *H. arizonica.* Contouring the RAPDs clustering of the populations revealed the geographic disjunction between *H. arizonica* and *H. glabra.* It appears that *H. glabra* is restricted to the Interior Biogeographic Provinces (BP) (Arizonan, which is largely below the Mogollon Rim), while *H. arizonica* is found within the "Sky Islands" of the Madrean BP (Bartel, 1993). The Madrean BP, which occurs throughout much of north-central Mexico, only enters the US in southeastern Arizona and extreme southwestern New Mexico. Wolf (1948), Schoenike et al. (1975), Little (2005), Rehfeldt (1997) and others have all concluded that *H. arizonica* does not range north of Greenlee County nor west of Pima County. Bartel (1993) mapped the distributions of *H. glabra* and *H. arizonica* (in Arizona).

Adams et al. (2010) reported on variation in the leaf oils of *H. arizonica* and *H. glabra* (in Arizona). They found the leaf terpenoids clearly separated these taxa but the study was restricted to populations in Arizona. A preliminary comparison of the leaf oils of *H. arizonica* (actually a cultivated *H. glabra* tree in Waco, TX), *H. benthamii*, a putative *H. lindleyi* from Creel, Chih. and *H. lusitanica* has been previously reported (Adams et al. 1997).

This paper presents the leaf oil compositions and analyses of geographical variation of *H. arizonica* from Arizona, New Mexico and Texas populations as compared to putative *H. arizonica/H. lindleyi* from Mexico. In addition, an updated report on the volatile leaf oil compositions of *H. benthamii, H. lindleyi* and *H. lusitanica* are presented.

MATERIALS AND METHODS

Collection site information for samples utilized in this study.
***Hesperocyparis arizonica*, United States:**
BC *Adams 11665-11669*. upper Bear Canyon, 11.8 mi n of Houghton Rd along Catalina Hwy, 32° 21.801' N, 110° 42.765' W, 1695 m, Santa Catalina Mtns., Pima Co., AZ;
CF *Adams 11670-11674*, n side of US191 in dry creek bed, 13 mi. n of Clifton, 33° 08.429' N, 109° 22.537' W, 1636 m, Greenlee Co., AZ;
DG *Adams 11675-11679*, Stronghold Canyon East, 8.5 mi w of US 191, along Ironwood Rd., 31° 55.540' N, 109° 58.007' W, 1501 m, Dragoon Mtns., Cochise Co., AZ;
CR *Ferguson 4028 - 4033* (= Lab acc. *Adams 14767-14772*), north slope of Cooke's Range, n of Cooke's Peak, 32° 34' 32.4" N, 107° 43' 41.2" W, 7345 ft., Luna Co., NM; (Note: Co. corrected from Grant to Luna, digitally, 16 July 2016 by RPA, ed.
BB *Joe Sirotnak ns* 1-5 (= Lab acc. *Adams 14585-14589*), Boot Spring, Chisos Mtns., Big Bend Natl. Park, 29° 14' 30.264" N, 103° 17' 49.4874" W, 6800 ft;
***H. arizonica / H. lindleyi / H. benthamii*, Mexico:**
BN *H. benthamii, Adams (with Tom Zanoni) 6879* (bulk collection, 5 trees), 8 km NW of Pachuca, Hidalgo, foliage planate, common with *Abies religiosa*, in El Chico Natl. Park, approx. 10 km from jct. with Mex. 105, 2920 m, ca 20° 09' 06" N, 98° 41' 45" W, ex Google Earth;
CM *Adams 6821-6823*, Creel, Chihuahua, 27° 44' n, 107° 38' w, 2250 m;
C1-C5 *Gonzalez et al. 8345a-e* (= Lab acc. *Adams 14598-14602*), Sierra La Concordia, Cañón de Agua Verde; predio San Marcos del Encino, al S de La Casita, Coahuila, 25° 10' 04" N, 101° 26' 11" W, 2202 m;
G1-G5 *Gonzalez et al. 8350-8354* (= Lab acc. *Adams 14603-14607*), *Cupressus* (*Hesperocyparis*) *arizonica* f. *glomerata* from type locality, Río Jaral, cerca del puente Santa Bárbara sobre la carretera a San Miguel de Cruces, al W de Estación Coyotes, Durango, 24° 00' 44" N, 105° 26' 33" W, 2168 m;
Y1-Y2 *Gonzalez et al. 8191-8192* (= Lab acc. *Adams 14609-14610*), Maicoba, al NE, por la carretera 16 (Hermosillo-Chihuahua), al SW del límite Sonora-Chihuahua, 28° 25' 26" N, 108° 34' 18" W, 1560 m, Yecora, Sonora;
C6 *Gonzalez et al. 8343* (= Lab acc. *Adams 14611*), Sierra la Concordia, s of General Cepeda and n of La Casita, 25° 13' 08" N, 101° 26' 10" W, 2022 m, Coahuila.
L1-L5 *Zamudio 17098, 17099a,b,c,d.* (= Lab acc. *Adams 14885-14889*) cultivated from type locality of *Hesperocyparis lindleyi*, between Angangueo and Tlalpujahua, Michoacan, ca. 19° 39' 45" N, 100° 15' 39" W, 3100 m (Google Earth). Cultivated at Patzcuaro, Michoacan, 19°32' N 101°36' W, 2160 m
M1-M6 *Gonzalez et al. 8390a,b,c,d,e,f.* (= Lab acc. *Adams 14890-14895*) *Cupressus* (*Hesperocyparis*) *arizonica* f. *minor* from type locality, Cruz de Piedra, Durango, 23° 49' 49" N, 105° 15' 22" W, ca. 2270 m.
***H. lusitanica*, Portugal:**
LU *H. lusitanica* (Miller) Bartel, *Adams 7071, 7072, 7073, 7071* collected from one of the original trees at Busaco (= Bussaco), Portugal at a monastery, sign on tree *7071* read 'Planted 1644', *7071* tree ca 30-40 m tall, 1m DBH, 349 yr old (in 2 Feb 1993), 540 m; *7072* and *7073* from younger trees (8m tall x 12 cm

DBH (progeny) planted? or naturally? established from the original trees within the grove of the 6 old, original trees. This grove of cypress is thought to have been established from seed from Mexico.
All specimens are deposited in the BAYLU and CIIDIR herbaria.

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 5-15 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 5 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

From Table 1, one can see that the volatile leaf oils are dominated by umbellulone (9.6 - 32.3%), limonene (2.5 - 19.0%), β-phellandrene (4.0 - 18.5%) and sabinene (1.1 - 10.7%) with moderate amounts of terpinen-4-ol (4.1 - 9.4%), isoabienol (t - 4.9%), and phyllocladanol (0 - 4.6%). Nezukol was found to be very variable ranging from 0.5 to 15.2% (Table 1).

A minimum spanning network, based on 49 terpenoids, revealed six groups: *H. arizonica* (AZ, NM, TX); *H. lindleyi*; an un-named Coahuila group; *H. a.* f. *minor*/f. *glomerata*; *H. benthamii*, and *H. lusitanica*. In addition, *H. arizonica* from Arizona, Cooke's Range, NM, and Big Bend, TX form a distinct cluster (Fig. 1). Interestingly, one plant from Coahuila (C2, 14599) also joins this cluster. The other five plants from Coahuila (C1, C3, C4, C5, C6) form a very distinct cluster (Fig. 1). The Yecora plants are loosely associated with the Coahuila plants.

Hesperocyparis benthamii, El Chico NP, Hgo. and *H. lusitanica*, Bussaco, Port. loosely cluster indicating theirs oils are quite differentiated from *H. arizonica*, *H. lindleyi* and the other cypresses in this study. The distinct oils of *H. benthamii* and *H. lusitanica* are apparent (Fig. 1, Table 3). It is interesting that to date, the population from which *H. lusitanica* seeds were collected and established at a monastery in Bussaco, Portugal in 1644, has yet to be found in Mexico. Terry, Bartel and Adams (2012), using sequences from seven cp, nrDNA and NEEDLY, found *H. lusitanica* in the *arizonica* clade, but not clearly associated with any other species. It might be noted that *H. lindleyi* was not in the study, nor were any other Mexican cypresses. So it is of interest that the terpenoid data do not place *H. lusitanica* close to any oils in the present study, yet by DNA data, it is clearly nested deep in a clade with the Western Hemisphere cypresses (Terry, Bartel and Adams, 2012), not with the Eastern Hemisphere cypresses (*Cupressus, sensu stricto*). Clearly, Terry, Bartel and Adams (2012), with the utilization of considerable DNA sequencing, showed that many of the cypress species in the *arizonica* clade are scarcely distinct and these taxa might well be treated as conspecific. However, it might be noted that verified H. lindleyi was not included in that study. This seems especially true in the Mexican cypresses that seem to intergrade in their oils.

The question of the validity of *Cupressus lusitanica* Miller, is interesting, in that the naming of cultivated plants as distinct species is controversial, especially if that taxon can not be veritied as growing in nature, without cultivation. Marion Ownbey (1950), in a study of difficult, naturalized hybrids of *Tragopogon*, argued that to recognize a taxon as specifically distinct, it must be a natural group, characterized by: 1. A combination of distinctive morphological features (and/or chemical/ DNA features, *my addition here*); 2. The taxa are reproducing under natural conditions; and 3. There is not free gene exchange between the taxa concerned. One may argue about point 3, as many species do produce hybrids which are fertile and can produce even hybrid swarms by back crosses, yet the species remain clear and distinct in most places where they coexist. Ownbey's second premise: taxa are reproducing under natural conditions, is scarcely the case for *C.(H.) lusitanica* at Bussaco, where they are prized and nurtured since 1644. Thus, if *C. (H.) lusitanica* is not found in Mexico, a case might be made that the name is invalid. An interesting idea, beyond the scope of this study.

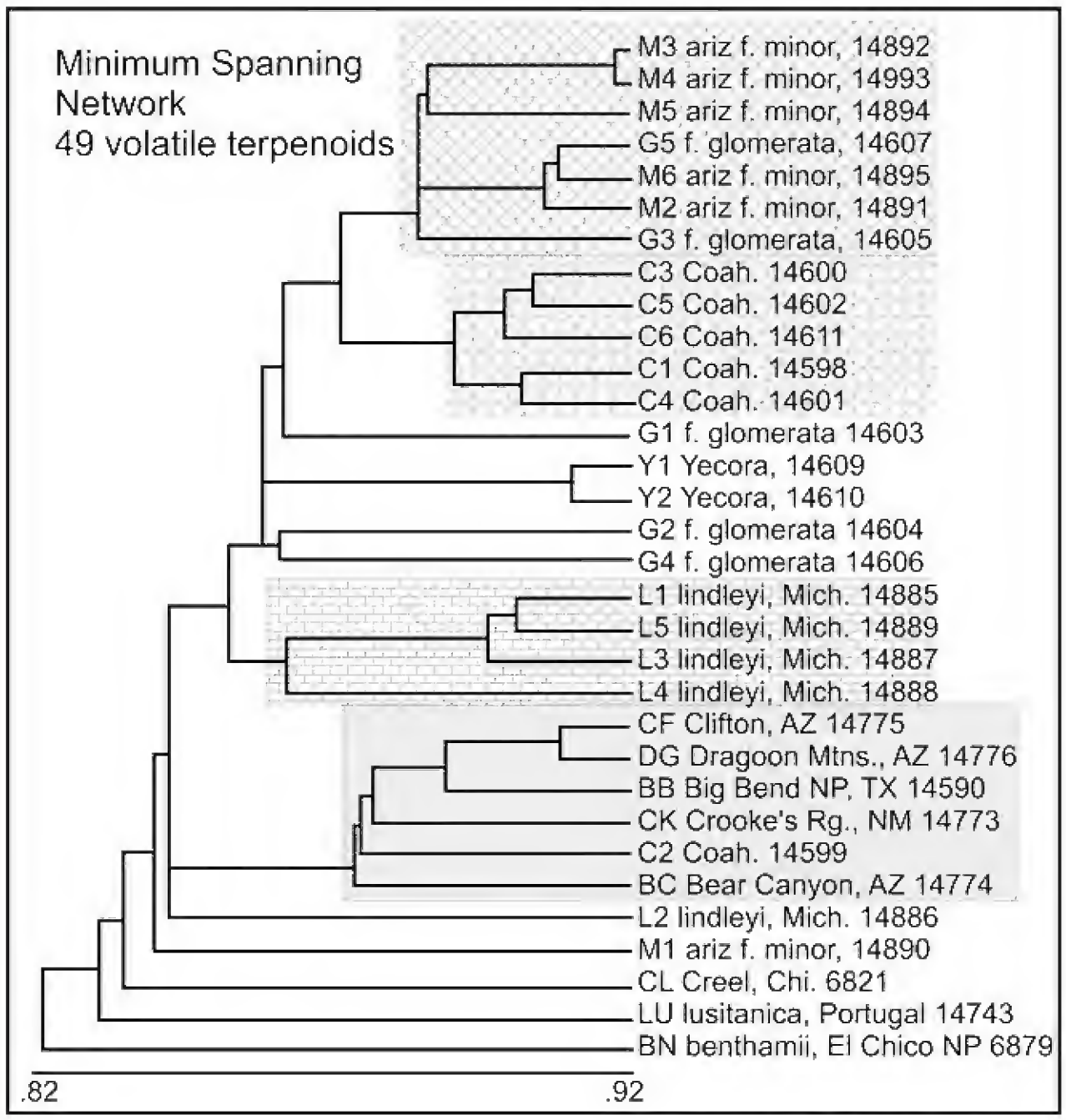

Figure 1. Minimum spanning network based on 49 volatile oil components.

Construction of 2-dimensional minimum spanning network, with distances = [(Sr(max) - Sr(i)] x 100, shows a more details in the magnitude of the links to nearest neighbors (Fig. 2). The six groupings are still present, but the diversity within the groups is now more apparent. The variation in the oils of the *H. arizonica* f. *minor/ glomerata* group is large (Fig. 2) with G1, G2 and G4 having somewhat different oils from the core group (M3, M4, M2, M6, G6). The Coahuila group have five uniform members (C1,C3,C4,C5,C6), but C2, from the same population near Saltillo, has oil that is more like that of *H. arizonica* (Fig. 2). The *lindleyi* group has three very similar members (L1,L3,L5) and two divergent oils in L2 and L4.

Nearly all the groups from Mexico contained chemical polymorphisms. This is shown in table 2 for *H. lindleyi* (L1, L2, L4) and *H. arizonica* f. *minor* (M1, M2, M3, M5). Limonene (and β-phellandrene,

not shown) varied from 3.7% to 14.9% (a 4-fold range) as did camphor (0.2 - 11.6%, 58 fold), abietadiene (4.9 - 18.1%, 3.7 fold), and especially nezukol, that varied from 0.0 (absent) to 12.7%. The chemical polymorphisms found in these groups (and others in this study) make it very difficult to utilize terpenoids for systematic purposes. This is unfortunate, as terpenoids can be quite useful in conifers for the analysis of population differentiation and in systematics (see Adams, 2014 for review of use in *Juniperus*).

Again, one sees the divergence of *H. benthamii* and *H. lusitanica* (Fig. 2), whose oils are quite different from any oils in the present study.

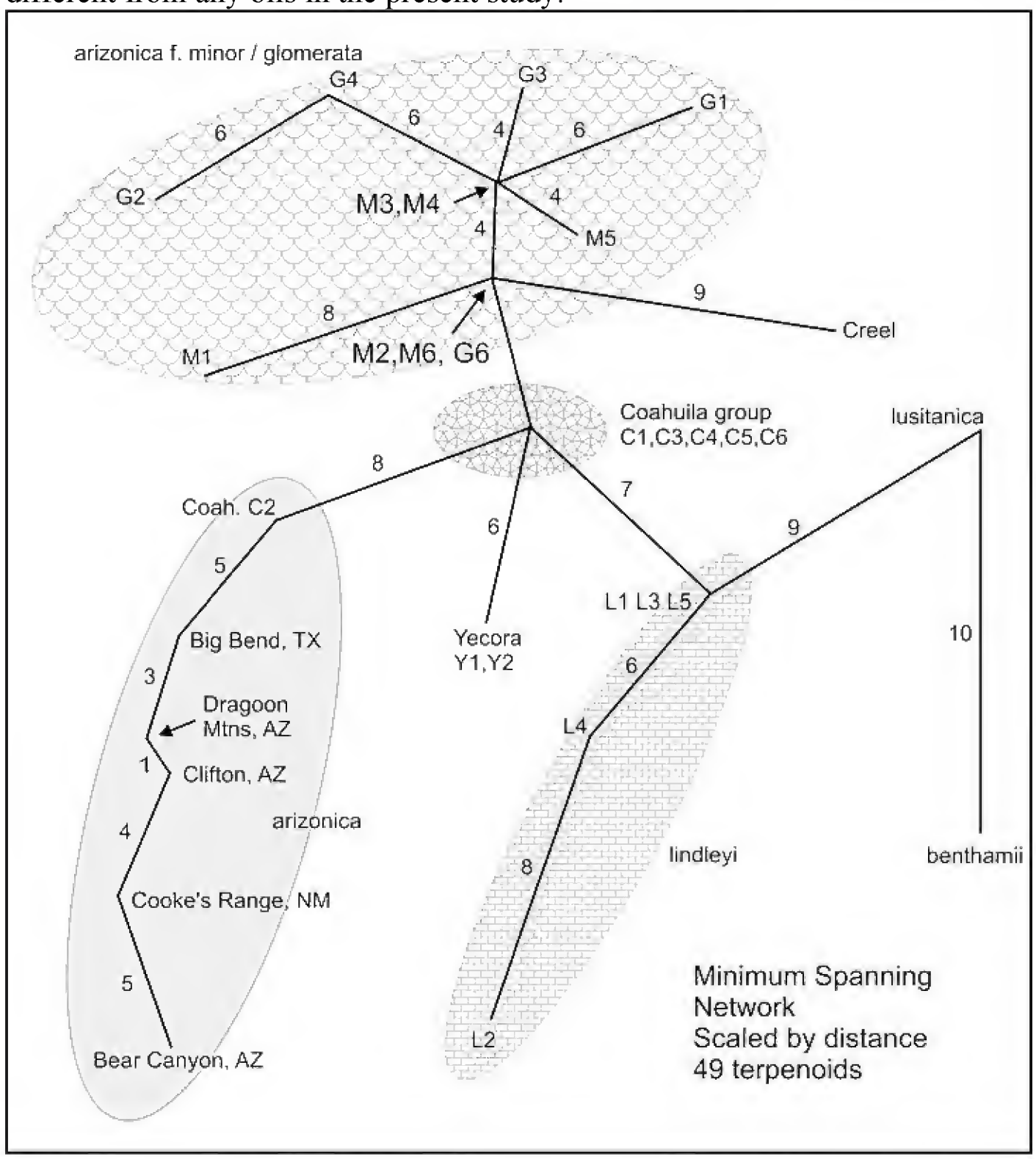

Figure 2. Minimum spanning network with OTUs by distance for *H. arizonica*, *H. lindleyi*, *H. benthamii* and *H. lusitanica* based on 49 leaf terpenoids. The number on a link is distance = [(Sr(max) - Sr(i)] x 100.

To visualize the geographic trends, the minimum spanning network was superimposed on a geographic map (Figure 3). The *H. arizonica* group is clearly defined (dashed ellipse, Fig. 3). It is surprising that the Big Bend population was not very different from the Arizona - New Mexico oils., in contrast to the RAPDs data that showed a clear differentiation by the Big Bend plants from Arizona populations (Bartel et al., 2003). Perhaps the similarities in oil compositions are maintained by selection pressure.

The unusual Coahuila plant (C2) is 0.87 similar to Big Bend, but only 0.84 similar to plants in the same Coahuila population (Fig. 3). The other, typical, Coahuila plant oils are most similar to those of f. *minor/ glomerata* (0.87). The Yecora oil is most similar to the Coahuila oil (0.86, Fig. 3). The Creel oil is

not very similar to any of the oils, and joins in the network with a low similarity of 0.83 to the f. *minor/ glomerata* group (Fig. 3).

The *H. lindleyi* group's oil is not very similar to other oils, and joins at 0.85 similarity to the Coahuila group (Fig. 3). Notice that *H. lusitanica* (cult, Portugal, exact geographic origin in Mexico is not known) nearest link is to *H. lindleyi* (L4) and that *H. benthamii* links to *H. lusitanica.*

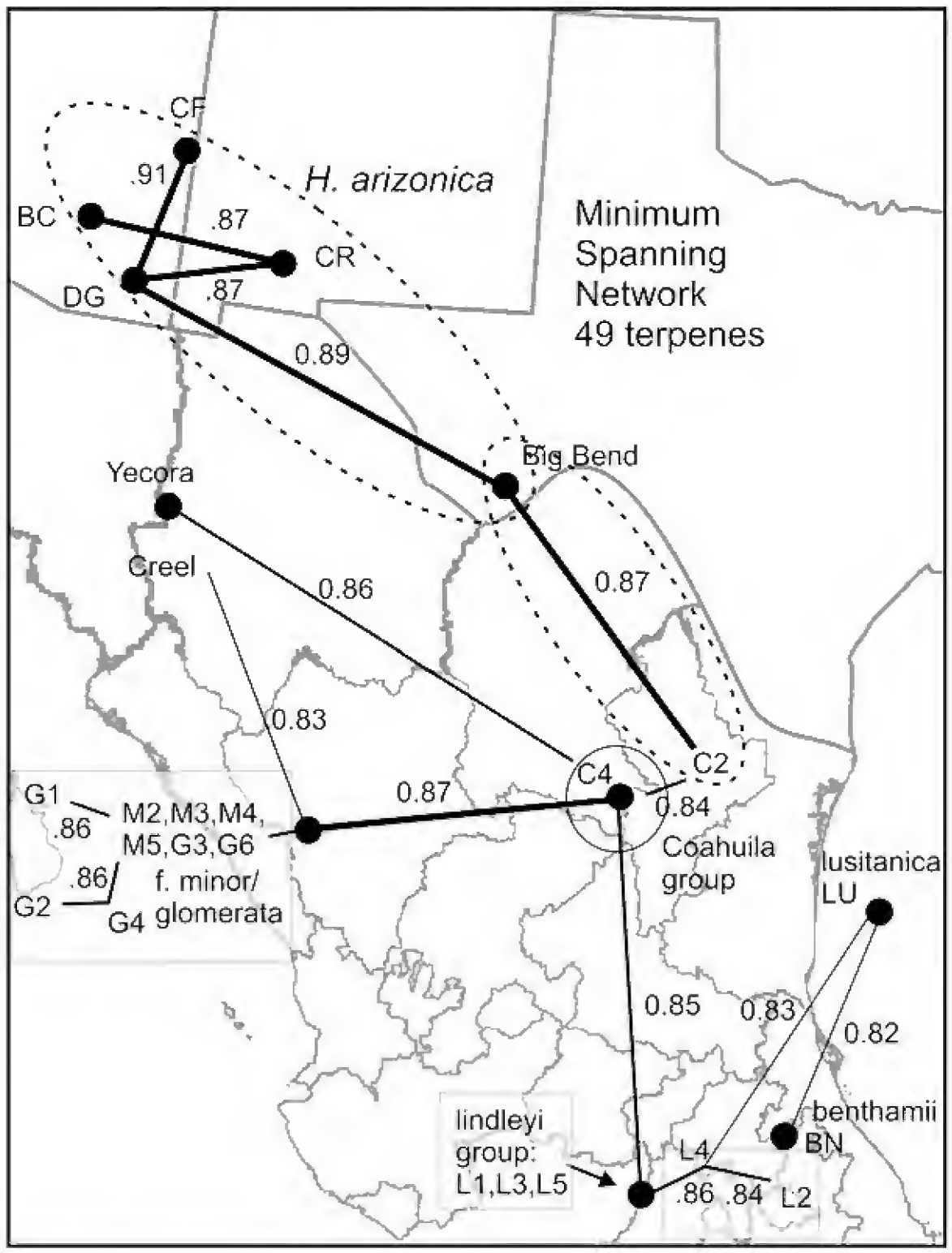

Figure 3. Minimum spanning network with oil similarities next to links.

The similarities among the oils in plants separated by great distances are likely due to a more continuous distribution during the Pleistocene when life zones descended hundreds of meters and discontinuous populations were joined.

The compositions of the leaf volatile oils of *H. arizonica, H. benthamii, H. lindleyi* and *H. lusitanica* are reported in Table 3 so as to correct the previous, erroneous report (Adams et al. 1997).

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Table 1. Leaf essential oil compositions for *H. arizonica* and affiliates. Ariz = composite Dragoon, Bear Canyon and Clifton, AZ populations; CR = Cooke's Rg., NM, BBNP = Big Bend Natl. Pk, Boot Spring; Creel = Creel, Chihuahua; Coah C1 = 14598, C1, Coahuila (typical), Coah C2 = 14599, Coahuila (like BBNP oil); minor = *arizonica* f. *minor*, lindleyi = *H. lindleyi*. Compounds in bold show large differences between the taxa.

KI	compound	CR	Ariz	BBNP	Coah C2	Coah C1	minor	lindleyi	Creel
921	tricyclene	t	0.1	t	t	0.1	t	t	0.6
924	α-thujene	1.0	0.9	0.9	1.0	0.9	1.0	0.6	1.2
932	α-pinene	6.5	3.9	2.9	6.1	3.0	3.4	5.0	4.7
946	camphene	0.1	t	t	t	0.2	t	0.2	0.8
969	**sabinene**	**6.2**	**4.3**	**10.7**	**9.3**	**9.2**	**4.8**	**6.0**	**1.1**
974	β-pinene	0.2	0.2	0.1	0.3	0.1	0.1	0.3	0.1
988	myrcene	1.9	1.7	2.4	2.8	2.0	2.1	2.4	2.8
1002	α-phellandrene	0.1	0.1	0.1	0.3	0.2	0.2	0.1	0.3
1008	**δ-3-carene**	**0.6**	**0.3**	**0.2**	**0.7**	**0.1**	**0.3**	**5.1**	**0.1**
1014	α-terpinene	1.4	1.4	1.8	2.4	1.7	1.9	1.3	1.8
1020	p-cymene	1.7	1.1	0.9	0.5	0.6	1.0	0.6	0.8
1024	**limonene**	**3.4**	**4.2**	**3.7**	**4.4**	**2.5**	**11.0**	**5.7**	**19.0**
1025	**β-phellandrene**	**5.2**	**4.2**	**5.6**	**6.4**	**4.0**	**11.0**	**2.9**	**18.5**
1054	γ-terpinene	2.0	1.8	2.5	3.0	2.2	2.1	1.7	2.6
1065	cis-sabinene hydrate	0.8	0.6	0.9	0.7	1.4	0.8	0.8	t
1086	terpinolene	1.7	1.7	2.0	2.8	2.2	2.1	2.5	2.6
1098	trans-sabinene hydrate	0.7	0.6	0.6	0.5	0.5	0.5	0.3	0.2
1099	linalool	0.3	0.3	0.3	0.2	0.2	1.9	2.2	0.2
1118	cis-p-menth-2-en-1-ol	0.8	0.7	0.9	0.8	0.6	0.7	0.5	0.4
1136	trans-p-menth-2-en-1-ol	0.5	0.5	0.6	0.6	0.3	0.5	0.4	0.3
1141	**camphor**	**0.2**	**0.6**	**0.3**	**0.3**	**2.6**	**0.2**	**2.9**	**1.5**
1145	camphene hydrate	0.6	0.3	1.0	0.2	0.3	0.3	0.3	0.3
1167	**umbellulone**	**18.4**	**19.0**	**20.0**	**19.3**	**32.3**	**27.9**	**17.6**	**9.6**
1174	**terpinen-4-ol**	**7.8**	**5.9**	**9.4**	**7.3**	**5.0**	**5.6**	**5.1**	**4.4**
1179	p-cymen-8-ol	1.0	1.3	0.9	0.3	0.2	0.6	0.3	0.2
1186	α-terpineol	0.7	0.7	0.8	0.7	1.0	1.5	1.2	0.7
1195	cis-piperitol	0.2	0.2	0.2	0.2	0.1	0.2	0.1	t
1205	trans-piperitol	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.1
1223	citronellol	t	0.1	0.2	t	t	-	-	-
1249	piperitone	0.2	0.2	t	-	-	0.2	0.2	-
1254	linalool acetate	-	0.1	-	-	-	0.1	0.2	-
1287	bornyl acetate	0.1	0.1	t	t	0.3	t	0.3	0.9
1289	thymol	0.1	0.2	-	-	-	0.2	t	-
1299	terpinen-4-yl acetate	0.9	0.9	0.8	0.5	0.2	0.4	0.3	0.4
1346	α-terpinyl acetate	3.0	2.6	2.0	1.6	1.1	1.5	1.6	2.0
1374	α-copaene	-	-	-	-	-	0.1	-	0.5
1417	(E)-caryophyllene	0.1	-	-	0.2	-	0.3	0.1	0.1
1448	**cis-muurola-3,5-diene**	**0.1**	**0.2**	**t**	**0.6**	**-**	**-**	**1.0**	**-**
1452	α-humulene		-		-		0.2	0.1	-
1465	**cis-muurola-4(14),5-diene**	**0.2**	**0.5**	**0.2**	**1.4**	**-**	**-**	**2.5**	**-**
1469	β-acoradiene	-	-	-	-	-	0.2	-	-
1478	γ-muurolene	t	t	t	t	0.2	0.1	-	-
1500	α-muurolene	t	0.1	t	0.4		0.2	-	0.4
1513	γ-cadinene	0.2	0.2	t	t	0.4	0.5	t	0.6
1518	endo-1-bourbonanol	-	t	-	-	1.1	-	-	-
1521	trans-calamenene	0.1	t	0.3	0.1	0.6	t	t	0.8

KI	compound	CR	Ariz	BBNP	Coah C2	Coah C1	minor	lindleyi	Creel
1522	δ-cadinene	0.2	0.8	0.3	0.2	0.6	0.6	0.4	0.9
1537	α-cadinene	-	t	-	-	-	-	-	-
1548	elemol	t	0.2	0.3	0.2	t	0.4	-	0.3
1550	cis-muurol-5-en-4-β-ol	-	-	-	-	-	-	0.3	-
1559	cis-muurol-5-en-4-α-ol	-	-	-	-	-	-	0.5	-
1574	germacrene D-4-ol	0.5	0.9	0.7	-	1.1	0.3	-	0.1
1582	caryophyllene oxide	-	-	-	-	-	0.1	t	-
1600	cedrol	-	-	-	-	-	0.3	-	-
1607	β-oplopenone	0.1	0.2	t	-	t	-	-	0.1
1618	1,10-di-epi-cubenol	t	0.1	-	0.3	-	-	-	0.1
1627	1-epi-cubenol	t	t	0.3	-	0.3	-	-	1.4
1632	**α-acorenol**	**-**	**-**	**-**	**-**	**-**	**1.5**	**-**	**-**
1636	β-acorenol	-	-	-	-	-	0.2	-	-
1638	epi-α-cadinol	0.3	0.6	0.4	0.1	0.5	0.3	0.1	2.2
1638	epi-α-muurolol	0.3	0.6	0.5	0.1	0.5	0.2	0.1	2.1
1644	α-muurolol	0.1	-	0.2	t	0.2	0.1	t	0.8
1652	α-cadinol	0.8	1.6	1.5	0.2	1.0	0.7	0.5	6.2
1688	cis-14-nor-murrol-5-en-4-one	-	-	-	-	-	-	0.2	-
1740	(E)-isoamyl cinnamate	0.1	0.1	-	-	-	-	-	t
1748	(Z)-isoamyl cinnamate	0.2	0.2	t	t	-	-	-	t
1793	(pentenyl cinnamate isomer)	t	t	t	t	0.1	0.2	t	0.5
1887	oplopanonyl acetate	0.9	1.0	0.5	t	t	0.2	-	0.6
1905	isopimara-9(11),15-diene	0.6	0.4	0.2	0.2	0.2	-	t	-
1907	pimara-8(9),15(16)-diene	0.2	0.2	t	-	t	-	-	-
1933	isohibaene	1.1	0.9	0.6	0.7	0.4	-	-	-
1941	sandaracopimara-8(14),15-diene	0.4	0.3	t	t	t	-	-	-
1958	**iso-pimara-8(14),15-diene**	**2.7**	**1.0**	**1.5**	**1.6**	**0.8**	-	t	**-**
1966	**isophyllocladene**	**4.0**	**3.7**	**2.3**	**2.3**	**1.2**	-	t	**t**
1978	manoyl oxide	1.8	2.0	1.5	1.8	0.7	0.5	0.7	t
1987	13-epi-manoyl oxide	0.3	0.5	0.4	0.6	0.2	0.1	-	-
2014	palustradiene	-	-	-	-	-	-	0.4	-
2022	cis-abieta-8,12-diene	-	-	-	-	-	-	0.3	-
2034	kaur-16-ene	0.5	0.4	0.4	0.4	0.2	-	-	-
2055	**abietatriene**	**0.1**	**0.2**	**0.3**	**0.4**	**0.4**	**0.1**	**0.9**	**t**
2087	**abietadiene**	**t**	**0.4**	**2.1**	**t**	**0.1**	**0.1**	**9.2**	**t**
2090	diterpene, 55,41,272,290	0.4	0.4	t	0.2	0.2	-	-	-
2105	**isoabienol**	**0.3**	**0.6**	**3.0**	**4.9**	**2.4**	**0.4**	**1.3**	**t**
2132	**nezukol**	**11.6**	**15.2**	**4.1**	**4.1**	**7.6**	**3.0**	**4.4**	**0.5**
2153	abieta-8(14),13(15)-diene	-	-	-	-	-	-	0.5	-
2209	**phyllocladanol**	**1.5**	**1.3**	**t**	-	-	-	-	-
2184	sandaracopimarinol	-	-	-	-	-	-	0.2	-
2282	sempervirol	0.2	0.3	0.5	1.2	0.6	0.4	0.4	1.1
2314	trans-totarol	0.1	0.2	0.4	0.9	0.5	0.8	0.8	0.6
2331	trans-ferruginol	t	0.1	0.2	0.5	0.2	0.1	0.2	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.

Table 2. Variation in selected terpenoids in *H. lindleyi* (L1, L2, L4) and *H. arizonica* f. *minor* (M1, M2, M3, M5).

KI	compound	L1	L2	L4	M1	M2	M3	M5
1024	limonene				3.7	9.8	14.9	11.4
1099	linalool	1.1	1.7	0.4				
1141	camphor	0.2	11.6	2.0				
1346	α-terpinyl acetate	2.2	0.0	2.7	1.8	1.9	0.5	2.4
1600	cedrol				1.1	0.0	0.0	0.4
1632	α-acorenol				4.5	0.0	2.1	2.1
1978	manoyl oxide				0.4	0.03	0.8	2.4
2087	abietadiene	9.2	4.9	18.1				
2105	isoabienol	0.2	1.6	2.3				
2132	nezukol	4.1	2.7	6.3	0.0	0.0	7.9	12.7

Table 3. Leaf essential oil compositions for *H. arizonica*, *H. benthamii*, *H. lindleyi,* and *H. lusitanica*. ariz = composite oil from Dragoon, Bear Canyon and Clifton, AZ populations; benth = *H. benthamii*, El Chico NP; lusit = *H. lusitanica*, Bussaco, Portugal (ex Mexico); lind = *H. lindleyi*, Angangueo, Michoacan. Major components are in bold. This replaces and corrects the Adams et al. (1977) report of these oils.

KI	compound	lindleyi	arizonica	lusitanica	benthamii
921	tricyclene	t	0.1	t	0.2
924	α-thujene	0.6	0.9	0.3	0.2
932	**α-pinene**	**5.0**	**3.9**	**7.8**	**1.2**
945	α-fenchene	t	-	t	t
946	camphene	0.2	t	t	0.3
969	sabinene	6.0	4.3	6.7	4.3
974	β-pinene	0.3	0.2	0.5	0.5
988	myrcene	2.4	1.7	2.0	2.2
1002	α-phellandrene	0.1	0.1	t	0.1
1008	δ-3-carene	5.1	0.3	3.5	1.2
1014	α-terpinene	1.3	1.4	0.6	1.4
1020	p-cymene	0.6	1.1	0.3	0.6
1024	**limonene**	**5.7**	**4.2**	**1.2**	**2.0**
1025	**β-phellandrene**	**2.9**	**4.2**	**1.2**	**2.0**
1026	1,8-cineole	-	t	0.9	-
1014	(E)-β-ocimene	t	-	0.1	-
1054	γ-terpinene	1.7	1.8	1.1	2.2
1065	cis-sabinene hydrate	0.8	0.6	0.4	t
1086	terpinolene	2.5	1.7	1.1	1.8
1087	2-nonanone	-	t	0.2	-
1098	trans-sabinene hydrate	0.3	0.6	0.5	-
1098	2-nonanol	-	-	0.4	-
1099	linalool	2.2	0.3	-	t
1112	trans-thujone	t	t	t	t
1118	cis-p-menth-2-en-1-ol	0.5	0.7	0.3	0.5
1123	(4-propyl heptane)	-	-	0.4	-
1136	trans-p-menth-2-en-1-ol	0.4	0.5	0.3	0.2
1140	trans-verbenol	0	-	0.2	-
1141	camphor	2.9	0.6	-	t
1145	camphene hydrate	0.3	0.3	0.3	t
1167	**umbellulone**	**17.6**	**19.0**	**2.0**	**5.3**
1174	**terpinen-4-ol**	**5.1**	**5.9**	**3.7**	**3.1**
1178	naphthalene	t	-	0.3	-
1179	p-cymen-8-ol	0.3	1.3	t	-
1186	α-terpineol	1.2	0.7	0.6	0.5
1195	cis-piperitol	0.1	0.2	-	-
1198	shisofuran	-	0.2	-	-
1205	trans-piperitol	0.3	0.3	t	t
1206	verbenone	-	-	t	-
1215	trans-carveol	-	t	-	-
1223	citronellol	-	0.1	t	-
1232	thymol, methyl ether	-	0.1	-	-
1239	carvone	t	t	-	-
1241	carvacrol, methyl ether	-	t	-	-
1249	piperitone	0.2	0.2	-	-
1254	linalool acetate	0.2	0.1	-	-
1287	bornyl acetate	0.3	0.1	t	t
1289	thymol	t	0.2	t	-

KI	compound	lindleyi	ariz	lusit	benth
1299	terpinen-4-yl acetate	0.3	0.9	0.2	-
1315	(2E,4E)-decadienal	-	t	-	-
1319	(2E,4E)-decadienol	-	t	-	-
1346	α-terpinyl acetate	1.6	2.6	0.7	0.1
1396	duvalene acetate	-	-	0.2	-
1407	longifolene	-	-	0.2	-
1417	(E)-caryophyllene	0.1	-	0.5	0.5
1448	cis-muurola-3,5-diene	1.0	0.2	0.6	0.3
1452	α-humulene	0.1	-	0.7	0.3
1465	cis-muurola-4(14),5-diene	2.5	0.5	1.7	1.6
1478	γ-muurolene	-	t	t	-
1479	ar-curcumene	-	-	0.1	-
1500	epi-zonarene	-	t	0.4	1.1
1500	α-muurolene	-	0.1	0.4	-
1513	γ-cadinene	t	0.2	-	t
1514	β-curcumene	-	-	0.1	-
1518	endo-1-bourbonanol	-	t	-	-
1521	trans-calamenene	t	t	0.3	t
1522	δ-cadinene	0.4	0.8	0.2	0.8
1533	10-epi-cubebol	t	-	0.2	t
1537	α-cadinene	-	t	-	-
1548	elemol	-	0.2	-	-
1550	cis-muurola-5-en-4-β-ol	0.3	0.1	0.6	-
1559	cis-muurola-5-en-4-α-ol	0.5	0.2	0.8	t
1565	dodecanoic acid	-	-	-	0.3
1574	germacrene D-4-ol	-	0.9	-	-
1582	caryophyllene oxide	t	-	0.7	0.2
1600	cedrol	-	-	0.6	-
1607	β-oplopenone	-	0.2	-	-
1608	humulene epoxide II	-	t	0.3	0.2
1618	1,10-di-epi-cubenol	-	0.1	0.1	t
1627	1-epi-cubenol	-	t	-	0.2
1632	α-acorenol	-	-	2.1	-
1636	β-acorenol	-	-	0.4	-
1638	epi-α-cadinol	0.1	0.6	0.2	0.2
1638	epi-α-muurolol	0.1	0.6	0.2	0.2
1644	α-muurolol	t	-	t	t
1652	α-cadinol	0.5	1.6	0.9	0.5
1685	germacra-4(15),5,10(14)-trien-1-a	-	-	0.3	-
1688	cis-14-nor-muurol-5-en-4-one	0.2	0.1	-	-
1724	(E)-nuciferol	-	-	0.1	-
1740	(E)-isoamyl cinnamate	-	0.1	0.2	-
1748	(Z)-isoamyl cinnamate	-	0.2	-	-
1793	(pentenyl cinnamate)	t	-	-	-
1887	oplopanonyl acetate	-	1.0	-	-
1905	isopimara-9(11),15-diene	t	0.4	-	-
1907	pimara-8(9),15(16)-diene	-	0.2	-	-
1933	isohibaene	-	0.9	-	-
1941	sandaracopimara-8(14),15-diene	-	0.3	-	-
1958	iso-pimara-8(14),15-diene	t	1.0	0.6	0.7
1959	hexadecanoic acid	-	-	-	0.6
1966	isophyllocladene	t	3.7	-	-

KI	compound	lindleyi	ariz	lusit	benth
1978	manoyl oxide	0.7	2.0	1.5	-
1987	13-epi-manoyl oxide	-	0.5	-	-
2014	palustradiene(abieta-8,13-diene)	0.4	-	1.0	0.5
2022	cis-abieta-1,12-diene	0.3	-	0.6	0.2
2034	kaur-16-ene	-	0.4	-	-
2055	abietatriene	0.9	0.2	2.8	1.8
2087	**abietadiene**	**9.2**	**0.4**	**26.0**	**15.9**
2090	diterpene. 55,41,272,290	-	0.4	-	-
2105	isoabienol	1.3	0.6	1.5	0.2
2132	**nezukol**	**4.4**	**15.2**	**2.4**	**1.2**
2153	abieta-8(14),13(15)-diene	0.5	-	0.9	0.4
2184	sandaracopimarinal	0.2	-	0.6	0.4
2203	diterpene,43,232,275,290	-	-	-	1.0
22 16	diterpene,41,183,141,253,286	-	-	-	0.6
2209	phyllocladanol	-	1.3	-	-
2269	sandaracopimarinol	0.2	-	0.2	0.3
2282	sempervirol	0.4	0.3	2.1	3.9
2300	diterpene,41,107,245,288	-	-	1.3	1.8
2314	**trans-totarol**	**0.8**	**0.2**	**3.8**	**10.1**
2331	trans-ferruginol	0.2	0.1	1.1	2.4
2401	abietol	-	-	t	0.2
2439	diterpene,41,69,301,316	-	-	0.5	1.1

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.

A new endemic subspecies of *Stictocardia* (Convolvulaceae) from the Marquesas Islands, French Polynesia

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ABSTRACT

Study of herbarium specimens for Convolvulaceae from French Polynesia brought to light several specimens with a distinctive morphological appearance. These are here described as *Stictocardia tiliifolia* subsp. *marquesensis*, subsp. nov., from the Marquesas Islands. Morphological evidence is presented in support of this conclusion. Published on-line www.phytologia.org *Phytologia 98(3): 203-206 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Stictocardia tiliifolia* subsp. *marquesensis*, subsp. nov., biodiversity, Oceania

In the course of preparing an account of the Convolvulaceae for the *Flore de la Polynésie française* (Florence 1997 & 2004), the available herbarium collections from BISH and P were studied between 2005 and 2013. Selected additional specimens from K, PTBG, and US were examined online, via loan, or in person. Then in 2014, when a fairly complete draft manuscript was in hand, the Convolvulaceae specimens from PAP were examined. While most of the specimens could be immediately recognized and confidently assigned to familiar Pacific taxa, a few specimens collected by J.-C. Thibault and J. Florence from the Marquesas proved to be problematic. Over time the duplicates in various herbaria for these problematic collections had been named by G. Staples as *Ipomoea violacea*, *Stictocardia tiliifolia*, or as *Ipomoea* Indet., but when material from PAP was compared with the corresponding duplicates in BISH it became clear that there was a distinctive facies about them, and they were set aside for closer scrutiny. Photographs by J.-F. Butaud of living plants on the islands of Eiao and Tahuata (Marquesas), and his recent collections from there, included in the PAP loan, added further evidence that the Marquesan plants were different from typical *Stictocardia tiliifolia* from elsewhere in the Pacific. In total, four collections are available and a new subspecies is described here, based on the available material.

Stictocardia tiliifolia is distributed in the tropics world-wide but is presumed to have an Old World origin with later, human-mediated dispersal to the Neotropics (Austin & Eich 2001) where the species is now naturalized. Throughout its global range, *S. tiliifolia* has a remarkably uniform appearance with very little morphological variation. Against this context, the Marquesan specimens at hand are consistently different and have a distinctive facies that can be recognized immediately.

Careful comparison of the four Marquesan collections with typical material for *Stictocardia tiliifolia* from French Polynesia (Austral Islands, Society Islands) and Hawai'i showed that in nearly all characters they match the typical phenotype for the species but they differ consistently in the denser indumentum on the vegetative plant body and in their pure white corollas. Because these Marquesan populations are geographically disjunct and isolated from the range for typical *S. tiliifolia* and their morphological differences are small but consistent, the rank of subspecies is appropriate for them.

Stictocardia tiliifolia (Desr.) Hallier f. subsp. ***marquesensis*** Staples & Butaud, *subspecies novum*

Differing from the typical subspecies *tiliifolia* by the persistent whitish villous indumentum on all vegetative parts of the plant (stems, innovations, leaves, petioles, peduncles, pedicels) and by the pure white corolla with a broader throat and spreading limb.
Figure 1A, -C, -D.
Type: Marquesas Islands. Eiao, Tetiaenui plateau, dans les parages, 350 m elev., 25 July 1987, *J.-C.Thibault 1066* (holotype PAP [bar code PAP001844; FPF sticker No. 034938]; isotype P, bar code P04399830).

Distribution. Found only in the Marquesas Archipelago, and so far known from Eiao and Tahuata; possibly also Hiva Oa and Nuku Hiva (see below).

Additional specimens examined:
French Polynesia: Marquesas Islands. Eiao, Tohuanui, secteur Est, ravin, 475 m elev., 8 July 1988, *Florence & Teikiteetini 9375* (BISH, P, PAP); Eiao, Plateau Vaituha, 200 m au Nord de l'arrivée sur le plateau en venant de Vaituha, 385 m elev., 18 June 2010, *Butaud & Jacq 2684* (PAP); Tahuata, Kiinui, amont de la route traversière, début de piste pour Amatea, 422 m elev., 30 June 2010, *Butaud & Girardi 2688* (BISH, PAP).

One additional sterile specimen that may belong here follows:
French Polynesia: Marquesas Islands. Hiva Oa, Makemake Valley, in native forest, ca 300 m elev., 22 Feb. 1929, *Mumford & Adamson 16* (BISH).

In addition to these vouchered collections, sight records documented by digital photographs have been made by J.-F. Butaud from Nuku Hiva [Vaituku valley, 22 March 2008, 270 & 345 m elevation, (leaves, old fruits)]; Hiva Oa [Haamanaua, small valley West of Eiaone, 23 Feb 2010, 235 m elevation, dry forest (seedling)]; and Tahuata [Hanatefau valley, 2 Aug 2013, around 200 m elevation, along the road in an anthropogenic forest (leaves, flowers)]. To be sure, only the last report, with flowers, can confidently be identified as subsp. *marquesensis*, the other two are provisionally included.

Sight records by Butaud that are undocumented by voucher specimen or digital photos include sterile plants on Tahuata: Hanatuuna valley, Tehotomei'a gulch, 31 July 2013, 422 m elev., in *Hibiscus tiliaceus* wet forest (leaves); Haaoipu valley, 2 Aug. 2013, 134 m elevation, along a trail inside a coconut forest (leaves). These are provisionally placed with subsp. *marquesensis*.

Ecology. Growing at elevations from 135 to 475 m on volcanic, more or less rocky, ferralitic to brown humic soils, under all exposures. This plant can be found in disturbed, anthropogenic areas such as overgrazed and eroded fields with isolated shrubs like *Annona squamosa* L., *Cordia lutea* Lam. and *Leucaena leucocephala* (Lam.) de Wit (Eiao); extensive cattle pasture with introduced species like *Cocos nucifera* L., *Cynodon dactylon* (L.) Pers., *Desmodium incanum* (G.Mey.) DC., *Digitaria didactyla* Willd., *Cyperus brevifolius* (Rottb.) Hassk., *Cyperus mindorensis* (Steud.) Huygh, *Mangifera indica* L., *Mimosa pudica* L., *Ocimum gratissimum* L., *Oxalis corniculata* L., and *Stachytarpheta cayennensis* (Rich.) Vahl (Tahuata); on a trail in the coconut forest (Tahuata); or along a road crossing old fallow forest of *Hibiscus tiliaceus* L., *Inocarpus fagifer* (Parkinson) Fosberg, and *Mangifera indica*, between two villages (Tahuata). But this subspecies is also growing in native dry forest dominated by the trees *Hibiscus tiliaceus, Sapindus saponaria* L., *Thespesia populnea* (L.) Sol. ex Corrêa, and *Xylosma suaveolens* G.Forst. (Hiva Oa); or more usually in mesic to wet, dense *Hibiscus tiliaceus* forest with *Macropiper latifolium* (L.f.) Miq. in the understorey and ferns like *Angiopteris evecta* (G.Forst.) Hoffm., *Arachniodes aristata* (G.Forst.) Tindale, *Asplenium australasicum* Hook., *Diplazium harpeodes* T.Moore, and *Tectaria*

jardinii (Mett. ex Kuhn) E.D.Br. plus herbs like *Oplismenus hirtellus* (L.) P.Beauv. and *Stephania japonica* (Thunb.) Miers as well as other vines (Tahuata, Nuku Hiva).

Phenology. Flowering in June, July; fruiting in June.
The flowers of subsp. *marquesensis* have been observed open after 5 PM, and as early as 7 AM, which suggests night-flowering rather than diurnal flowering as in subsp. *tiliifolia*. The pure white corolla, broader throat with stamens presented higher in the throat of the flower (subsp. *tiliifolia* has stamens borne lower down in the corolla tube) suggests a moth pollination syndrome, rather than bee pollination that is typical for subsp. *tiliifolia*.

Vernacular names:

The Marquesan names reported for this plant are *puhipuhi* on Hiva Oa [ex label *Mumford & Adamson 16*]; *puhipuhi* on Tahuata and *'aupuhi* on Ua Pou (Sachet, 1975 citing unpublished documents of Frère (brother) S. Delmas from the Mumford and Adamson collections). These names were not confirmed with contemporary Marquesan resource persons, who seem to have put nearly all the Convolvulaceae together under the general name "*pohue*."

Conservation Status:

Vulnerable under criterion B (VU B2 ab(ii,iii,iv,v)) and criterion D (VU population < 1000). This subspecies is endemic to the Marquesas Islands where it is known from four islands and seven locations. Its extent of occurrence (EOO) reaches 6000 km² for an area of occupancy (AOO) of 28 km² using cells 2 km wide. The size of its population is not known exactly but can be estimated at less than 1000 mature individuals (it was collected only once by the ornithologist J.-C. Thibault, and once by the botanist J. Florence who made several trips in the Marquesas; it was never collected by NTBG botanists who made intensive collections all over the Marquesan archipelago; however it could be more abundant than supposed as it looks very similar to other, more common, Convolvulaceae when not flowering). This vine, which is sometimes expansive in disturbed areas is also present in the common native *Hibiscus* forest; it appears very rare in both vegetation types and could be threatened directly by grazing mammals and indirectly by destruction of its habitats (overgrazing, fire, invasive plant species), which are considered highly fragmented.

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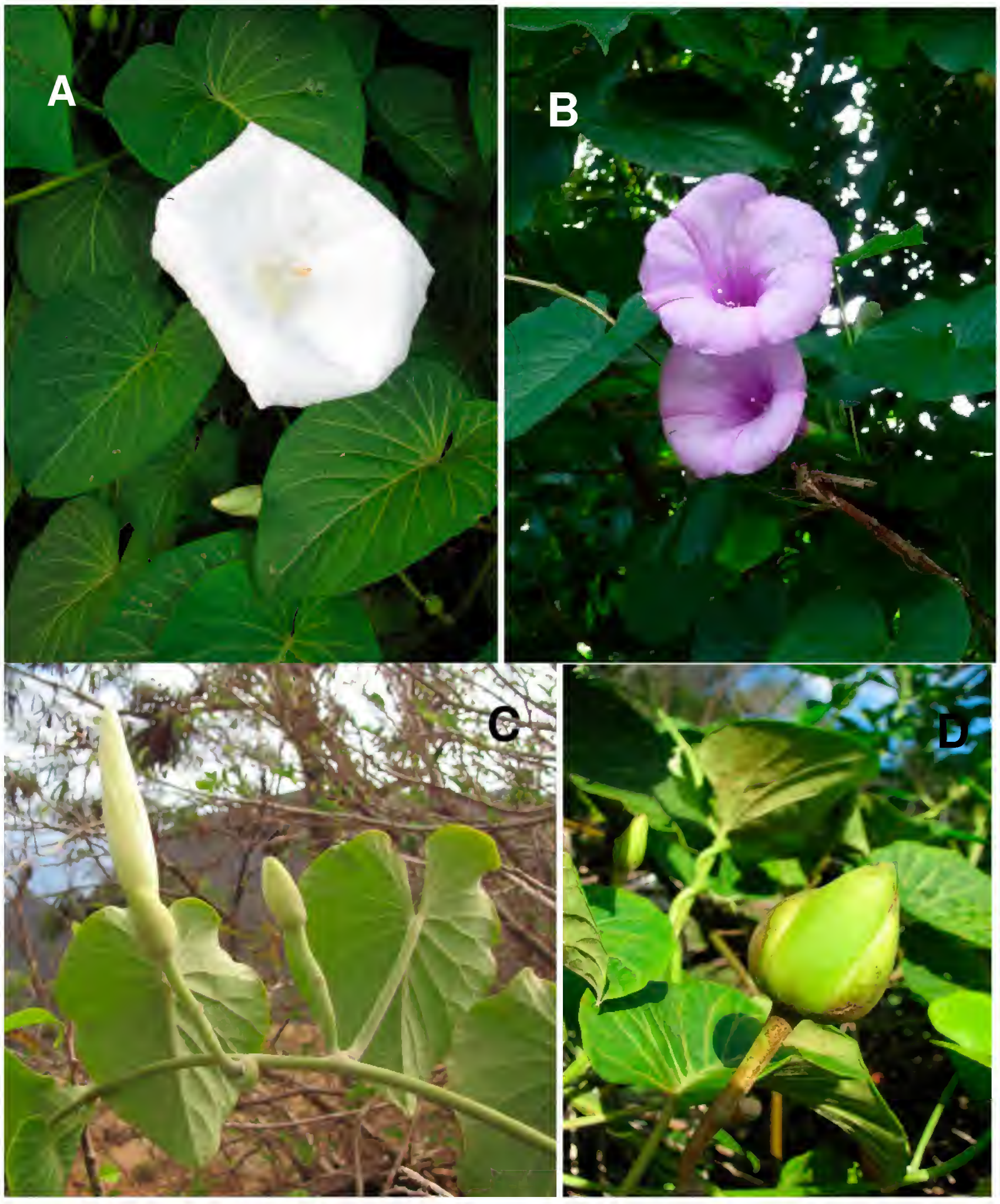

Figure 1. Comparative photographs of living plants of both subspecies of *Stictocardia tiliifolia in situ* with locality and voucher specimens that document the photos.

A. Corolla of *S. tiliifolia* subsp. *marquesensis* in frontal view (Tahuata, *Butaud & Girardi 2688,* photo by J.-F. Butaud);

B. Corolla of *S. tiliifolia* subsp. *tiliifolia* in frontal view (Hawai'i, Oahu, *Staples 1564,* photo G. Staples);

C. Foliage of *S. tiliifolia* subsp. *marquesensis* showing villous indumentum and flower buds (Eiao, *Butaud & Jacq 2684,* photo by J.-F. Butaud);

D. Fruit of *S. tiliifolia* subsp. *marquesensis* enclosed by accrescent leathery calyx (Tahuata, *Butaud & Girardi 2688,* photo by J.-F. Butaud).

Comparison of intensely sweet volatile leaf oils of *Lippia dulcis* (Verbenaceae) with low and high camphor from Brazil and Mexico

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ABSTRACT

Lippia dulcis is a sweet-tasting, woody herb but the sweetness is often mixed with a camphorous taste. *Lippia dulcis* oil from Brazil was found to be low in camphor (trace), whereas the oil from Mexico had considerable camphor (33.9%). The *Lippia* oil from Brazil was high in 6-methyl-5-hepten-2-one (10.5%), α-copaene (8.6%), (E)-caryophyllene (10.6%), bicyclogermacrene (6.6%), δ-cadinene (7.2%), epi-α-bisabolol (6.5%) and hernandulcin (8.8%). In addition, it contained β-cedrene and α-calacorene, compounds found in cedarwood oils. The *Lippia* oil from Mexico was high in camphene (12.7%), limonene (4.6%), camphor (33.9%), α-copaene (4.0%), (E)-caryophyllene (6.0%) and hernandulcin (5.9%). In addition, it contained alkanes and acids (docosane, tricosane, tetracosane, pentacosane, linoleic acid and octadecanoic acid) that were not found in the *Lippia* oil from Brazil. The Brazil germplasm with low-camphor and high hernandulcin is worthy of conservation, as it could be an important alternative source of sweeteners.
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KEY WORDS: *Lippia dulcis,* leaf terpenoids, hernandulcin, Brazil, Mexico, steam distillation, supercritical CO_2, degradation.

Lippia dulcis Trevir. (Verbenaceae) is a sweet-tasting, woody herb sold as hierbia dulce, hierbia buena, yerba dulce, Orozuz and yet other names in Mexico and central America (Compadre, Robbins and Kinghorn, 1986). The sweet taste is due to the presence of hernandulcin, a bisabolane-type sesquiterpene. It has been rated as 1000 times sweeter than sucrose (Compadre, Robbins and Kinghorn, 1986). Unfortunately, most of the oils also contain copious amounts of camphor, so the sweetness is mixed with a camphorous taste.

The nomenclature has been subject to controversy and has been reviewed by Adams et al. (2014). But it might be mentioned that O'Leary and Mulgura (2012), in their revision of the genus *Phyla*, explicitly excluded *Phyla dulcis* and *P. stochaedifolia* from the genus, stating "these are considered here to be better placed under *Lippia*, given that both species lack malpighiaceous hairs, which are characteristic of the genus *Phyla* and are woody shrubs rather than the herbaceous habit noted for all *Phyla* species considered herein." In the present report, it is treated as *Lippia dulcis*.

Reports on the composition of the leaf terpenoids of *L. dulcis* have been variable. Nayal et al. (2009) and Gornmann et al. (2008) reported 32.6% camphor and 10% hernandulcin from *L. dulcis* grown from seeds from M. P. Gupta, Panama. However, the same laboratory (Nayal et al. 2009) reported 0.02% camphor and 14.5% hernandulcin from plants grown from Panama seeds (ex M. P. Gupta seed lot). It may be that the report by Gommann et al. (2008), from that same laboratory, erroneously reported the composition of Mexico *L. dulcis* for their 'Panama' plants.

Kaneda et al. (1992) found no camphor but 0.154% hernandulcin in market plants from Valle de Anton, Cocle, Puerto Rico, sold for the treatment of respiratory ailments. This plant (identified as *L. dulcis*) was listed in the Flora of Panama as *Phyla scaberrima* (A. L. Juss.) Moldenke. Kaneda et al. (1992) also identified a new sweet sesquiterpene, (+)-4β-hydroxyhernandulcin as well as (-) epi-hernandulcin from their Panama plants. Mori and Kato (1986a,b) synthesized all four isomers of hernandulcin and noted that only the 6S, 1'S isomer (i.e., (+) hernandulcin) was sweet.

The presence of a large amount of camphor in the Mexican plants is of considerable interest since there are conflicting reports of none (or trace amounts of camphor in plants) from Panama, Puerto Rico and Columbia (Table 1). Because camphor is very heat-stable, it seems unlikely that the trace or absence of camphor in the Panama, Puerto Rico, Columbia and Brazil samples is due to decomposition; more likely, it is due to the lack of camphor in these plants. Although all the studies cite "plants identified by taxonomist," it is very possible that some of the samples may have been mis-identified or there may be chemical races or chemotypes present in *L. dulcis,* as suggested by Souto-Bachiller et al. (1997). Souto-Bachiller et al. (1997) concluded that 'tzonpelic xihuitl' ascribed to Francisco Hernandez by Aztec physicians more than 400 years ago (Anderson, 1977) is, in fact, 'yerba dulce' of Puerto Rico. Research on geographic variation in the leaf oils of *L. dulcis* is needed to clarify the problem.

Souto-Bachiller et al. (1996) collected seeds in 1990 from plants in Orocovis, Puerto Rico. They obtained high hernandulcin yields (18-26 mg/g), with no camphor from germinated shoots (6-8 weeks old). After repeated sub-culturing for five years, there were little effects on the oil composition, implying that the oils are genetically stable.

Table 1. Reports on the amounts of camphor and hernandulcin in *Lippia dulcis*.

publication	plant source	camphor %	hernandulcin %	extraction
Compadre et al. 1986	Mexico (local markets)[1]	53.2	0.004[#]	steam distilled, 2h
Nayal et al. 2009	Mexico (Helenion Nursery, Germany)[2]	32.6	10.1	distilled, 4h
Gornmann et al. 2008	Panama (seeds, M. P. Gupta, Panama)[3]	32.6	10.0	steam distilled, 4h
Nayal et al. 2009	Panama (seeds, M. P. Gupta, Panama)[2]	0.02	14.5	distilled, 4h
Kaneda et al. 1992	Puerto Rico (market,Valle de Anton)[1]	none	0.154	petroleum ether
Souto-Bachiller et al. 1997	Puerto Rico (plants, ex Orocovis)[1]	<0.01	22.0	pentane & CH_2Cl_2
Moreno-Murillo etal. 2010	Colombia (plants, ex Tenza Valley)[4]	none	1.1*	hydro-distillation, 3h
Oliveira, et al. 2010.	Brazil(local plants?)[1]	none	19.2	supercritical CO_2
Adams et al. (2014)	Mexico (seeds, Chiltern Seeds, UK)[5]	21.2	9.2	pentane overnight
Adams et al. (2014)	Mexico (seeds, Chiltern Seeds, UK)[5]	33.9	4.5	steam distilled, 4h

[1]dried and milled; [2]air dried, 30°C and cut; [3]dried and cut; [4]fresh or dried?; [5]fresh leaves.
*(ca. 4-5%, hernandulcin was mostly decomposed during GC analysis)
[#]severely decomposed during GC analysis.

Recently, Attia, Kim and Ro (2012) reported on molecular cloning of (+)-epi-β-bisabolol synthase as a precursor to the biosynthesis of hernandulcin.

Compardre et al. (1986) discovered that hernandulcin decomposes upon heating to 140°C. They tried to compensate for this problem by running their GC injector at 70°C, but this is too low to quantitatively transfer a board mixture of volatile components to the GC column. Souto-Bachiller et al. (1997) found a solution to the problme: they ran a narrow bore injector liner (0.75 mm bore) so that the dead volume is small and the sample quickly transferred from their injector (220°C) to the cool (60°C) column. Even using this method, they appeared to have decomposition of hernandulcin, as indicated by the presence of 6-methyl-5-hepten-2-one and 3-methyl-2-cyclohexen-1-one (putative decomposition

products of hernandulcin). Souto-Bachiller et al. (1997) extracted with pentane and dichloromethane (sequentially with combined extracts) because they thought that distillation would lead to decomposition. It may be that they were considering water-distillation where the plants are placed in water and boiled to co-produce steam and volatile oil. Water-distillation (or hydro-distillation) is well known to produce artifacts due action of acids from the leaching of organic leaf acids into the water (Adams, 1991). A safer steam distillation can be performed in all glass units, with the plant materials suspended above boiling water, so that the oil is not exposed to leached-out organic acids. As the maximum temperature reached is 100°C, and contact is only with glass, this type of steam distillation eliminates decomposition for all but the most labile components found in nature. See Adams (1991) for a diagram of this type of steam distillation apparatus.

Adams et al. (2014) examined the effects of inlet injector temperature on the degradation of hernandulcin using a series of analyses, by increasing the injector temperature (Fig. 1). Hernandulcin content was lowest at 100°C, then increased to 160°C, then declined from 200°C to 220°C (Fig. 1). It seems likely that the high variance at 100°C and lower amount of the less volatile sesquiterpene, hernandulcin, is due to incomplete volatilization in the injector and selective loss of hernandulcin in the split line. The decline at 220°C is due to decomposition of hernandulcin (Fig. 1). The rather constant nature of 6-methyl-5-hepten-2-one (Fig. 1), followed by the sudden increase at 220°C, seems to indicate that most 6-methyl-5-hepten-2-one is a natural product in the oil and only a small portion was derived from the decomposition of hernandulcin (220°C, Fig. 1). Alternatively, there could have been some decomposition of hernandulcin during harvest, storage and/ or extraction.

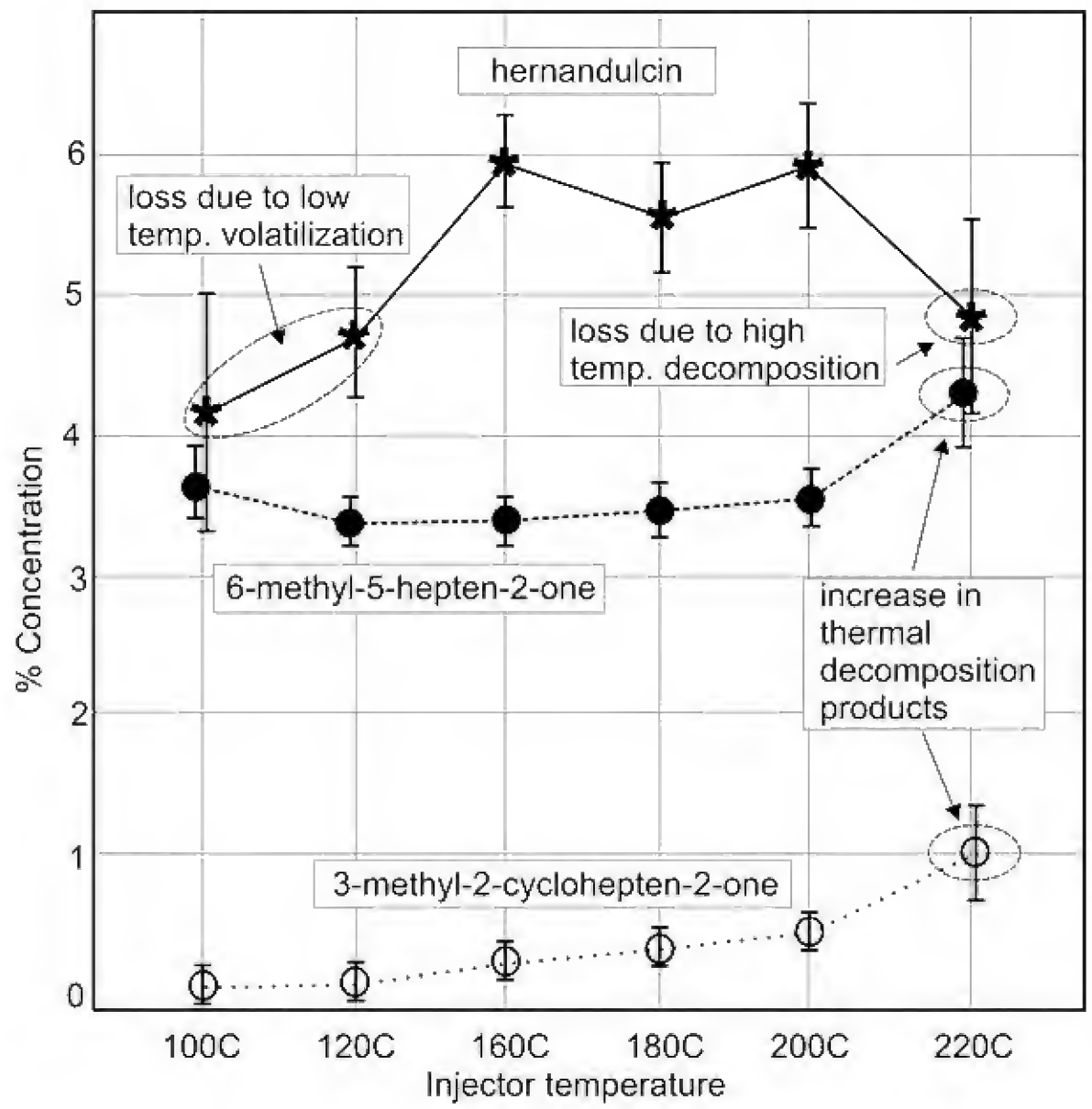

Figure 1. Plots of hernandulcin versus putative decomposition products: 6-methyl-5-hepten-2-one and 3-methyl-2-cyclohepten-2-one with changes in the injector temperature for GC analyses. (from Adams et al., 2014).

The concentration of 3-methyl-2-cyclohepten-2-one was very stable from 100°C to 200°C, then increased at 220°C (Fig. 1). This suggests that the increase at 220°C is due to the decomposition of hernandulcin. Small amounts of 3-methyl-2-cyclohepten-2-one may be naturally present in the oil.

Oliveira et al. (2010) reported 19.2% hernandulcin but no camphor in plants grown in Brazil extracted by supercritical CO_2. These plants appear unusual in having little or no camphor (Table 1). Low camphor *Lippia dulcis* plants have been reported from Panama (0.02%, Nayal et al., 2009); Puerto Rico (none, Kaneda et al, 1992, or <0.01%, Souto-Bachiller et al. 1997); Columbia (none, Moreno-Murillo et al., 2010) and Brazil (none, Oliveira et al. 2010).

Souto-Bachiller et al. (1997) described their collection location of *L. dulcis* as "in Sector Negro, Orocovis, we found abundant samples of this species at the country estate of Mrs. Maria Ortolaza, Road No. 143, Km 30.7. A specimen is deposited at the herbarium, Biology Dept., UPRM." After correspondence with James Ackerman, Dir. of UPRRP Natural History Collections, Univ. of Puerto Rico, in 2014, a graduate student, Fabiola Areces, visited the estate of Mrs. Ortolaza in Nov., 2014 and found that Mrs. Ortolaza had died and that her estate was now in disrepair. A search for plants of *Lippia dulcis* at the estate revealed that the garden plot had been abandoned and was grown over with wild vegetation. No *Lippia dulcis* plants could be found. Thus, the garden plants of *Lippia dulcia,* may remain the only known source of this rare low-camphor genotype.

We recently analyzed leaves from the plants that Oliveira et al. (2010) used for supercritical CO_2 extraction. The purpose of this study was to compare the steam distilled oils of the Brazilian *Lippia dulcis* with the volatile leaf oil of Mexican materials.

MATERIALS AND METHODS

Plant material: Lippia dulcis seeds were obtained from Chiltern Seeds, UK, via M. Attia, University of Calgary, Canada and grown in the greenhouse. Vegetatively propagated plants were grown under partial shade in pots or in the field of the experimental farm at Prairie View A&M University. Fresh leaves were collected from young plants. In addition, we were able to obtain leaves of *Lippia dulcis* from plants grown in a garden in Florianopolis, Brazil.

Essential oils analysis - 30.1 g FW (4.79 g DW) of fresh, greenhouse, mature leaves with 2 mg of methyl decanoate added (as an internal standard) steam distilled for 4 h using a modified circulatory Clevenger-type apparatus (Adams 1991). Extracts were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20°C until analyzed. Extracted leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt./(oil wt. + oven dried extracted foliage wt.)]. The extracted oils were analyzed on a HP5971 MSD mass spectrometer: 0.2 ul of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60° - 246°C at 3°C/min. (62 mins.), carrier gas He, flow 34.96 cm/sec or 1.02 ml/min, injector 160°C, detector 240°C, 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, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

RESULTS AND DISCUSSION

The volatile oils yields were very different, with Brazil *Lippa* yielding 0.37% and Mexico, 2.13% (Table 2) The oils were quite different with most monoterpenes being a trace or absent in the Brazilian *Lippia* oil (Table 2). The *Lippia dulcis* oil from Brazil was the low in camphor (trace), whereas the oil from Mexico had considerable camphor (33.9%).

The *Lippia* oil from Brazil was high in 6-methyl-5-hepten-2-one (10.5%), α-copaene (8.6%), (E)-caryophyllene (10.6%), bicyclogermacrene (6.6%), δ-cadinene (7.2%), epi-α-bisabolol (6.5%) and

hernandulcin (8.8%). In addition, it contained β-cedrene and α-calacorene, compounds found in cedar wood oils. It is note-worthy that not only is camphor very low, but all the monoterpenes are also low or missing in the Brazil oil. It appears the entire monoterpene pathway has been blocked in the Brazil plants.

The *Lippia* oil from Mexico was high in camphene (12.7%), limonene (4.6%), camphor (33.9%), α-copaene (4.0%), (E)-caryophyllene (6.0%) and hernandulcin (5.9%). In addition, it contained alkanes and acids (docosane, tricosane, tetracosane, pentacosane, linoleic acid and octadecanoic acid,) not found in the *Lippia* oil from Brazil.

The exact origin of the plants from Brazil is not known. They were obtained from a nursery garden dealer, so they are likely items of trade commerce. Because *Lippia dulcis* is thought to be non-native to Brazil, it may be this germplasm came from low-camphor plants in Panama, Columbia or even from Puerto Rico. In any case, this germplasm with low-camphor and high hernandulcin is worthy of conservation as it could be an important alternative source of sweeteners.

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Table 2. Comparison oil compositions of Brazil *Lippia dulcis* (cultivated) vs. Mexico *Lippia* or Orozuz steam distilled (4h). t < 0.1%, NI = not integrated. GC injector run at 160°C.

KI	Compound	Brazil	Mexico
	percent oil yield (DM basis)	0.37%	2.13%
846	(E)-2-hexenal	0.2	t
850	(Z)-3-hexenol	t	t
921	**tricyclene**	**-**	**0.1**
932	**α-pinene**	**-**	**2.1**
946	**camphene**	**-**	**12.7**
974	1-octen-3-ol	0.2	t
974	**β-pinene**	**-**	**0.6**
981	6-methyl-5-hepten-2-one	10.5	3.4
988	myrcene	0.1	1.4
1024	**limonene**	**t**	**4.6**
1036	benzene acetaldehyde	t	-
1046	3-me-2-cyclohexene-1-one	0.5	0.4
1086	**terpinolene**	**-**	**1.7**
1095	linalool	1.3	0.4
1100	2-methyl-butyl-2-methyl-cyclohexen-1-one	0.2	-
1103	2-methyl-butyl isovalerate	t	-
1126	4-acetyl-1-methyl-1-cyclohexene	t	-
1141	**camphor**	**t**	**33.9**
1148	citronellal	t	-
1165	**borneol**	**-**	**0.5**
1174	terpinen-4-ol	t	
1179	p-cymen-8-ol	-	0.1
1186	**α-terpineol**	**t**	**0.1**
1199	3-methyl-3-buten-1-ol, tiglate	t	-
1223	**citronellol**	**t**	**-**
1235	**neral**	**0.1**	**-**
1249	**geraniol**	**t**	**-**
1264	**geranial**	**0.2**	**-**
1284	bornyl acetate	t	-
1309	p-vinyl-guaiacol	t	-
1345	α-cubebene	0.3	0.1
1374	α-copaene	8.6	4.0
1387	β-bourbonene	1.0	0.5
1387	β-cubebene	0.1	0.1
1409	α-gurjunene	0.2	0.1
1417	(E)-caryophyllene	10.5	6.0
1419	**β-cedrene**	**0.2**	**-**
1432	α-trans-bergamotene	0.9	0.3
1440	(Z)-β-farnesene	0.5	0.2
1452	α-humulene	0.5	0.3
1454	(E)-β-farnesene	5.3	2.3
1464	9-epi-(E)-caryophyllene	0.7	0.3
1469	7-epi-1,2-dehydro-sesquicineole	1.3	0.3
1478	γ-muurolene	0.8	0.3
1480	germacrene D	3.6	1.1
1500	**bicyclogermacrene**	**6.6**	**-**
1500	α-muurolene	1.1	0.6
1505	β-bisabolene	2.6	1.0
1514	epi-cubebol	0.3	0.1
1522	δ-cadinene	7.2	3.8
1544	**α-calacorene**	**0.8**	**-**
1561	(E)-nerolidol	1.1	0.3
1577	**spathulenol**	**1.9**	**-**

KI	Compound	Brazil	Mexico
1582	caryophyllene oxide	1.4	0.6
1622	sesquiterp.,85,94,136,218	1.6	0.7
1630	muurola-4,10(14)-dien-1-β-ol	0.4	0.2
1639	caryophylla-4(12),8(13)-dien-5-β-ol	-	0.1
1644	α-muurolol	-	t
1662	**hernandulcin isomer 1,43,109,218,236**	**2.1**	**1.7**
1667	**isomer of 1662; hernandulcin isomer**	**1.2**	**1.0**
1668	β-atlantone	0.7	-
1683	α-bisabolol	-	0.1
1685	epi-α-bisabolol	6.5	2.6
1753	**sesquiterp.,41,82, 109, 232**	**0.7**	**-**
1766	**sesquiterp.,41, 109, 150, 228**	**0.6**	**-**
1794	**sesquiterp.,41, 175, 218, 248**	**0.6**	**-**
1851	**(+) hernandulcin**	**8.8**	**5.9**
1865	(-) epi-hernandulcin	1.4	0.7
1959	hexadecanoic acid	0.3	1.2
2113	hydrocarbon,71,123,55,296	0.2	0.8
2132	**linoleic acid**	**-**	**2.5**
2158	**octadecanoic acid**	**-**	**1.0**
2200	**docosane**	**-**	**t**
2300	**tricosane**	**-**	**t**
2400	**tetracosane**	**-**	**0.9**
2500	**pentacosane**	**-**	**0.1**

KI = linear Kovats Index on DB-5, 30m column.

The effects of gibberellic acid (GA3), Ethrel, seed soaking and pre-treatment storage temperatures on seed germination of *Helianthus annuus* and *H. petiolaris*

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ABSTRACT

A moderate concentration of GA3 (500 ppm) with one wk stratification at 4°C was very effective in increasing the germination rate of recalcitrant native sunflower seeds (80% vs. 30% control). Stratification (1 wk at 4°C) increased germination, regardless of the seed treatment. Ethrel (25 ppm) treatment was effective, but not as much as GA3 (500 ppm). Soaking sunflower seed in water for 12 or 16 hr resulted in no seed germination. Published on-line www.phytologia.org *Phytologia 98(3): 213-218 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Helianthus annuus*, *H. petiolaris*, seed germination, dormancy, gibberellic acid (GA3), Ethrel, Florel, Ethephon.

Seed dormancy in wild sunflowers can be a problem for studies of hybridization and in crop breeding. In a recent seed shipment from GRIN, USDA, 15 accessions of *Helianthus annuus* had germination rates ranging from 13% (PI673305, Utah) to 91% (PI413066, Obregon, Mexico). Two *H. petiolaris* accessions (PI451978, Ellsworth, KS, PI413175, Spencer, NE) both had 14% germination rates. Seiler (1993) reported germination rates of 84% (*H. agrophyllus*), 76% (*H. debilis* ssp. *silvestris*), 51% (*H. petiolaris*) and 44% (*H. annuus*). He noted that dry storage at 20, 0, or -20°C did not affect the germination rate. In addition, Seiler, also found that the length of storage (0, 120, 360 days) did not significantly affect the germination rate.

Seed stratification (5°C) was found (Bratcher, Dole and Cole, 1993) to increase the germination of *H. maximiliani* from 35% (no stratification) to 87% (2 weeks, 5°C), but longer stratification (up to 10 weeks) made little difference in germination.

Soaking in Ethrel (that produces ethylene), water , 25% acetone soaking, or potassium nitrate (0.2%) all increased germination in *H. annuus*, with soaking in plain water (24-36h, 20°C) giving the largest increase in germination (Maiti et al. 2006a). Interestingly, tests based on 12 genotypes, gave mixed results, with most genotypes improving germination by 10 to 43%. However, the germination of two of the genotypes actually declined with water soaking from 27% to 20% and 33% to 17%. Maiti et al. (2006b) extended their study on water soaking (priming) by the examination of germination after 5, 10, 15, 20, 25, 30, 35 and 40 hrs of soaking (at RT?). They found, with genotype VSF-15046 (*H. annuus*), the maximum germination occurred for seeds soaked 15 and 20 hrs, then declined with increaded soaking time. However, 15 hr water soaking using seed of VSFH-1008 from 13 locations gave greater germination in 10/13 seed lots, no difference for one location and a decline in germination for seeds from two locations.

Ethephon (Ethrel) has been used to break dormancy in sunflower. Kumari and Singh (2000) sprayed seed heads (21 Days After Anthesis, DAA) and obtained an increase in germination from 35.5% to 69.1% at 250 ppm spray. Gibberellic acid (GA3) has been used to enhance germination (Deno, 1993). Pallavi et al. (2010) tested gibberellic acid (GA3), Ethrel (Ethephon),potassium nitrate, water soaking, dry heating, microwave and smoking treatments on the germination of sunflower seed (hybrid KBSH-44).

They reported that GA3 (100 ppm); Ethrel (25 ppm); water soaking (24h); dry heating (80°C, 10 min); and smoking (3h) were all very effective (230 to 240% increase in germination) for KBSH-44 seed.

This is a brief, but sufficient review of methods to enhance sunflower seed germination for the reader to grasp that there are many methods, and some work better than others, but seldom does one method work on all genotypes of a species. Thus, the search for a universal method to apply to recalcitrant seed collections appears to be near, but not quite attained.

The purpose of the present paper is to report on sunflower seed germination tests using various concentrations of GA3 and Ethrel as well as water priming (soaking in water) and temperature stratification.

MATERIALS AND METHODS

All seeds were obtained from GRIN (Germplasm Resources Information Network), USDA.
H. annuus: PI413039, Gettysburg, SD; PI413035, Kearney, NE.
H. petiolaris, PI451978-NC7, Ellsworth, KS.
All seeds were surface sterilized by:
1. Washing with soap/tap water;
2. Dipping in 70% ethanol, 30 sec;
3. Sterilizing by soaking in 20% Chlorox (8.25% sodium hypochlorite) for 30 min.;
4. Thoroughly rinsing in sterilized ddwater (Protocol from Singhung Park, Kansas State University).

Experiment 1. Effects of soaking in 500 and 1000 ppm GA3 at RT(21°C), and soaking in DI (deionized) water, 12 h vs. 16 hr. GA3, gibberellic acid, PlantHarmones.net, 90%. dissolved in 1.0 g in 5 ml ethanol, add to 1 995 ml DI water to produce 1000 ppm stock. Dilute 1/2 with DI water for 500 ppm stock.
Experiment 2. Effects of stratification in 500 and 1000 ppm GA3 for 1 week at 4°C vs. 21°C.
Experiment 3. Effects of stratifying in 29, 100, and 200 ppm Ethrel, 1 week at 4°C. Florel is used to prevent nuisance fruit, remove mistletoe, induce flowering, reduce plant height, increase branching and increase seed germination. Florel is sold as a mixture of 3.9% Ethel [(2-chloroethyl) phosphoric acid] and 96.15 'inert' ingredients. Ethrel ex Florel, Monterrey Florel Brand Growth Regular, HydroGalaxy.com. Florel.

RESULTS

GRIN reported that the germination of *H. petiolaris* (PI451978), ex Ellsworth, KS, was low (14%) using their sunflower germination test protocol: seed soaked in hydrogen peroxide (3%), 5 min., rinsed in water, then soaked in 25 ppm Ethrel, 12hr at RT, then chilled 7-14 days (4° C), then planted onto wetted filter paper (Laura Marek, Lisa Pfiffner, GRIN, pers. comm.). No doubt, germination in *H. petiolaris* would have been lower if Ethrel and stratification were not used (Maiti et al. 2006a; Kumari and Singh, 2000).

Table 1 shows that germination of *H. petiolaris*, on DI (deionized water) wetted filter paper at RT, gave a very low germination rate (10% vs. 14% for GRIN treatment). Saturating the filter paper with 1000 ppm GA3 gave a large increase in germination rate (35%) and an even larger rate (70%) using 500 ppm GA3. Soaking in DI, 12 or 16 hr did not produce any germination (Table 1).

Varying gibberellic acid (GA3) concentration (Deno, 1993) and stratification temperatures using *H. annuus*, PI413039, ex Gettysburg, SD, produced considerable differences (Table 2, Fig. 1). The DI control had 30% germination vs. 50% (1000 ppm GA3, 1 wk, 21°C), 70% (1000 ppm GA3, 1 wk, 4°C), 65% (500 ppm GA3, 1 wk, 21°C) and 80% (500 ppm GA3, 1 wk, 21°C). Notice (Fig. 1) that germination

for both 1000 and 500 ppm GA3 increased when stratification was cold (4°C). Likewise, both 21°C and 4°C tests increased with lower GA3 (500 ppm, Fig. 2). At least in these preliminary tests, the optimum conditions are 500 ppm GA3 with 1 wk at 4°C. Additional, replicated tests are needed (in progress) to determine if a lower concentration of GA3 might be even more effective.

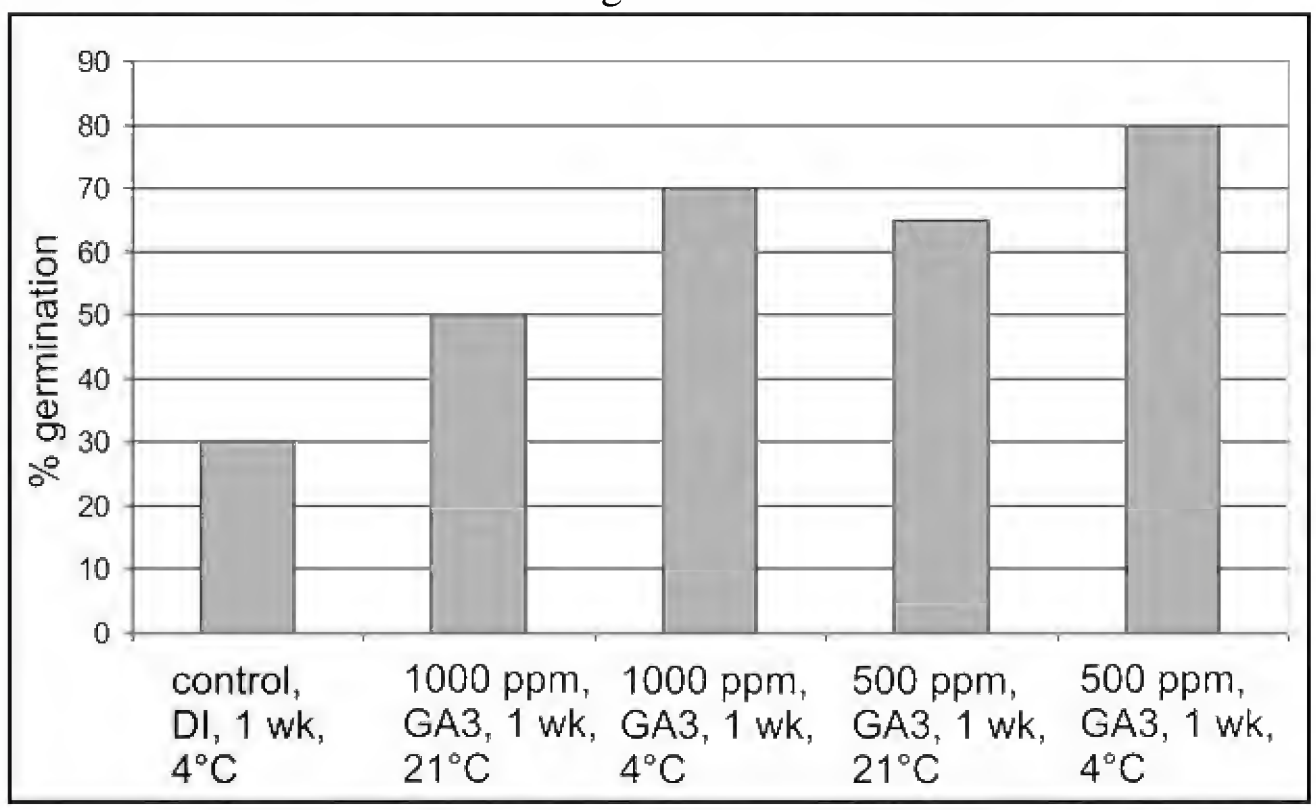

Figure 1. The effects of GA3 concentration and stratification temperature on germination, *H. annuus*, PI413039, ex Gettysburg, SD.

Testing the effects of Ethrel concentrations found the highest germination of *H. annuus*, PI413035, ex Kearney, NE, to be 25 ppm Ethrel (Table 3, Figure 2). This is the concentration used by GRIN and their germination of this lot of PI413035 was 51% (vs. 55% in our test).

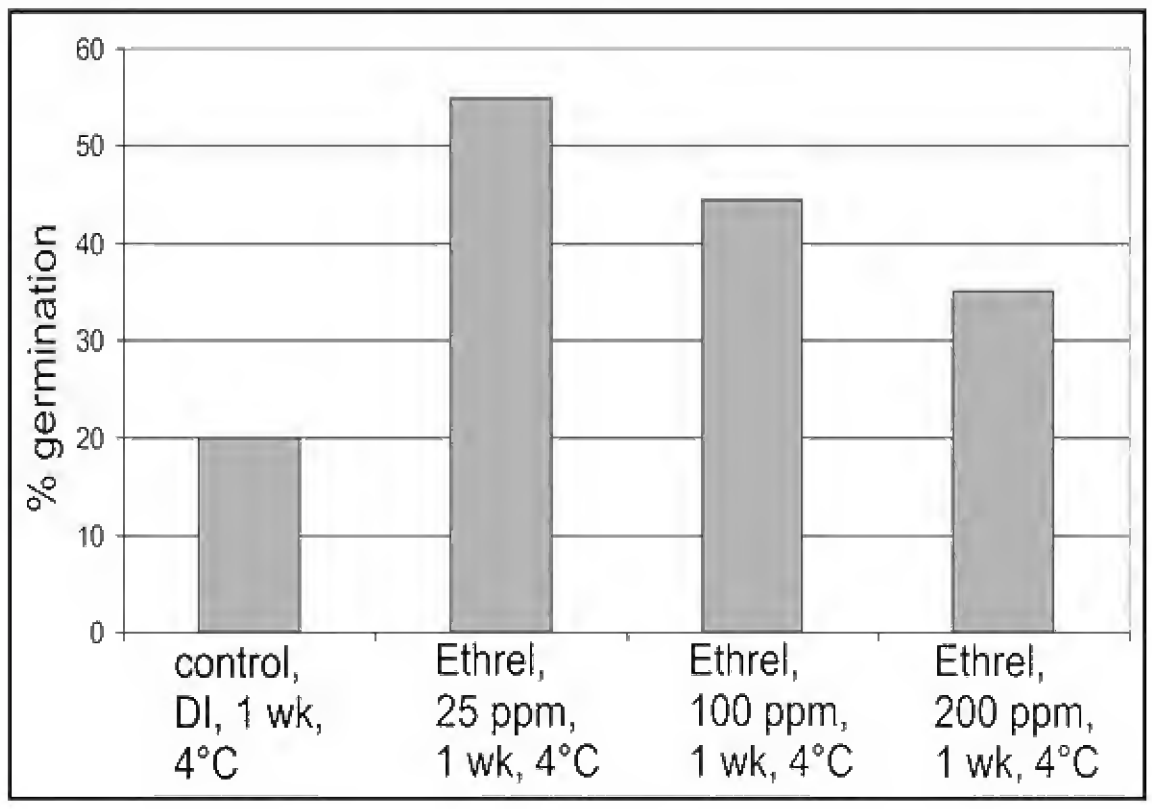

Figure 2. The effects of Ethrel concentration and stratification temperature on germination, *H. annuus*, PI413035, ex Kearney, NE.

In summary, this preliminary report found that a moderate concentration of GA3 (500 ppm), with 1 wk at 4°C, was very effective in increasing the germination (80% vs. 30% control) of recalcitrant native sunflower seeds. Stratification (1 wk at 4°C) increased germination, regardless of the seed treatment. Ethrel (25 ppm) treatment was effective, but not as much as GA3 (500 ppm). Soaking sunflower seeds in water for 12 or 16 hr resulted in no germination.

ACKNOWLEDGEMENTS

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Table 1. Tests of the effects of gibberellic acid (GA3) concentration and soaking on germination of *H. petiolaris*, PI451978-NC7, Ellsworth, KS. Tests: 21 days, RT, ambient room light, 20-21° C.

Treatment	germination	reference
GRIN (germination basis): soak in hydrogen peroxide (3%), 5 min., rinse in water, soak in 25 ppm Ethrel, 12hr at RT, chill 7-14 days (**4° C**?), plant on filter paper (GRIN, USDA)	**25 ppm Ethrel, 1 wk, 4° C** GRIN, Control #1: 14% (% viable = 93%)	GRIN, USDA, Laura Marek and Lisa Pfiffner, (pers. comm.)
1. dry seed, control, filter paper saturated with DI water	**DI water, RT** Lab, Control #2: 2/20 (10%)	Seiler (1993)
2. filter paper saturated with 1000 ppm GA3, RT	**1000 ppm GA3, RT** 7/20 (35%)	Deno, 1993
3. filter paper saturated with 500 ppm GA3, RT	**500 ppm GA3, RT** **14/20 (70%)**	Deno, 1993, 1/2 X GA3
4. seed soaked in DI water, 12 hr., then placed on DI saturated filter paper, RT	**soaked DI, 12 hr, RT** 0/18 (0%)	Maiti, et al. (2006a)
5. seed soaked in DI water, 16 hr., then placed on DI saturated filter paper, RT	**soaked DI, 16 hr, RT** 0/17 (0%)	Maiti, et al. (2006a)

Table 2. Tests of the effects of gibberellic acid (GA3) concentration on germination of *H. annuus*, PI413039, Gettysburg, SD. Tests: 21 days, RT, ambient room light, 20-21° C.

Treatment	germination	reference
GRIN (germination basis): soak in hydrogen peroxide (3%), 5 min., rinse in water, soak in 25 ppm Ethrel, 12hr at RT, chill 7-14 days (**4° C**?), plant on filter paper (GRIN, USDA)	**25 ppm Ethrel, 1 wk, 4° C** GRIN, Control #1: 49% (% viable = 97%)	GRIN, USDA, Laura Marek and Lisa Pfiffner, (pers. comm.)
1. seed stored on filter paper saturated with DI water in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**DI water, 1 wk., 4° C** Lab, Control #2: 6/20 (30%)	Seiler (1993)
2. seed stored on filter paper saturated with1000 ppm GA3, in a plastic bag, 1 wk., **21°C**, then planted on filter paper, sat. with DI water, RT	**1000 ppm GA3, 1 wk., 21° C** 10/20 (50%)	Kumari and Singh (2000) Maiti et al. (2006b)
3. seed stored on filter paper saturated with 1000 ppm GA3, in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**1000 ppm GA3, 1 wk., 4° C** **14/20 (70%)**	Deno, 1993
4. seed stored on filter paper saturated with 500 ppm GA3, in a plastic bag, 1 wk., **21°C**, then planted on filter paper, sat. with DI water, RT	**500 ppm GA3, 1 wk., 21° C** 13/20 (65%)	Deno, 1993
5. seed stored on filter paper saturated with 500 ppm GA3, in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**500 ppm GA3, 1 wk., 4° C** **16/20 (80%)**	Deno, 1993

Table 3. Tests of the effects of Ethrel concentration on germination of *H. annuus*, PI413035, Kearney, NE. Tests: 21 days, RT, ambient room light, 20-21° C.

Treatment	germination	reference
GRIN (germination basis): soak in hydrogen peroxide (3%), 5 min., rinse in water, soak in 25 ppm Ethrel, 12hr at RT, chill 7-14 days (**4° C**?), plant on filter paper (GRIN, USDA)	**25 ppm Ethrel, 1 wk, 4° C** GRIN, Control #1: 51% (% viable = 96%)	GRIN, USDA, Laura Marek and Lisa Pfiffner, (pers. comm.)
1. control, seed stored on filter paper saturated with DI water in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**DI water, 1 wk., 4° C** Lab, Control #2: 4/20 (20%)	Seiler (1993)
2. seed stored on filter paper saturated with 29 ppm Ethrel (to prod. ethylene), in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**29 ppm Ethrel, 1 wk., 4° C** **11/20 (55%)**	Kumari and Singh (2000)
3. seed stored on filter paper saturated with 100 ppm Ethrel (to prod. ethylene), in a plastic bag, 1 wk., **4° C**, then planted on filter paper, sat. with DI water, RT	**100 ppm Ethrel, 1 wk., 4° C** 8/18 (44.4%)	Kumari and Singh (2000)
4. seed stored on filter paper saturated with 200 ppm Ethrel (to prod. ethylene), in a plastic bag, 1 wk., **4°C**, then planted on filter paper, sat. with DI water, RT	**200 ppm Ethrel, 1 wk., 4° C** 7/20 (35%)	Kumari and Singh (2000) Maiti et al. (2006b)

Two new cases of chloroplast capture in incongruent topologies in the *Juniperus excelsa* complex: *J. excelsa* var. *turcomanica* comb. nov. and *J. excelsa* var. *seravschanica* comb. nov.

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ABSTRACT

Juniperus excelsa, *J. polycarpos, J. turcomanica*, *J. seravschanica* and *J. procera* were analyzed for incongruent topologies between their nrDNA and cp DNA (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF). Incongruent topologies suggest that there are two cases of chloroplast capture in the *J. excelsa* complex: *J. p.* var. *turcomanica* appears to have recently captured its chloroplast from *J. polycarpos* or an ancestor; and *J. seravschanica* seems to posess an anciently captured chloroplast from an ancestor of *J. foetidissima*/ *J. thurifera*. Thus, *J. p.* var. *turcomanica* and *J. seravschanica* seem to defy an uncluttered taxonomic classification. Two new varieties are recognized: *J. excelsa* var. *turcomanica* (B. Fedtsch.) R. P. Adams, **comb. nov.** and *J. excelsa* var. *seravschanica* (Kom.) R. P. Adams, **comb. nov.** This constitutes *J. excelsa* M.-Bieb. with four varieties: var. *excelsa*, var. *polycarpos* (K. Koch) Silba, var. *turcomanica* (B. Fedtsch.) R. P. Adams and var. *seravschanica* (Kom.) R. P. Adams. Due to incongruent topologies, *J. excelsa* is presently a polyphyletic species. Published on-line **www.phytologia.org** *Phytologia 98(3):219-231 (July 6, 2016).* ISSN 030319430.

KEY WORDS: *Juniperus excelsa, J. polycarpos* var. *polycarpos, J. polycarpos* var. *turcomanica, J. seravschanica, J. procera,* chloroplast capture, incongruent topologies, DNA sequencing, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF.

Recently, Adams, Schwarzbach and Tashev (2016) reported a case of putative chloroplast capture by plants of *J. sabina* in Bulgaria and northern Greece. The Balkan plants had nrDNA exactly the same as other *J. sabina* plants in other regions, but their cp DNA differed by only 6 MEs(SNPs + indels) from that of *J. thurifera*, but 36 MEs from typical *J. sabina* cp. To call attention to these unusual individuals, with a geographic range, the authors recognized the plants as *J. sabina* var. *balkanensis* R. P. Adams and A. Tashev. By naming a new variety, the authors hope this will initiate additional research on this unusual situation.

The idea of chloroplast capture is not new, even two decades ago, Rieseberg and Soltis (1991) warned of chloroplast capture (both recent or ancient via hybridization) that provides incongruent topologies in phylogenetic trees between nuclear and cp data. They found evidence of chloroplast capture in 37 cases and, of those, 28 were thought to be conclusive (Table 1, Rieseberg and Soltis, 1991). With the explosion of the use of nrDNA and cp markers, there are hundreds of examples of chloroplast capture today. A few examples of putative chloroplast capture include *Heuchera* (Soltis and Kuzoff, 1995), *Brassica napus - B. rapa* (Haider et al. 2009), and *Osmorhiza* (Yi et al. 2015).

There are fewer examples of chloroplast capture in conifers. In *Pinus* and other conifers, Hipkins et al. (1994) concluded that "past hybridization and associated 'chloroplast capture' can confuse the phylogenies of conifers." Bouille et al. (2011) found significant topological differences in phylogenetic trees based on cpDNA (vs. mtDNA sequences) in *Picea* that suggested organelle capture.

In *Juniperus*, Terry et al. (2000) suggested that chloroplast capture was involved in the distribution of cp haplotypes in *J. osteosperma* in western North America. More recently, Adams (2015a, b) found widespread hybridization and introgression between *J. maritima* and *J. scopulorum* in the Pacific northwest, with introgression from *J. maritima* into *J. scopulorum* eastward into Montana. The

disparity between cpDNA and nuclear markers (nrDNA and maldehy) suggested that cp capture had occurred. Incongruent topologies between nrDNA and cpDNA has been found for *J. horizontalis* and *J. virginiana* var. *silicicola*, that have the common cpDNA found in the Caribbean junipers, but have nrDNA like *J. blancoi* and *J. virginiana*, respectively (Adams 2014, Adams, Schwarzbach and Morris 2008).

Although chloroplast capture, on its face, seems unlikely, Tsitrone et al. (2003) proposed a model of chloroplast capture that provides some basis for the concept.

Farjon (1992), in a seminal paper on the taxonomy of multiseed junipers of Southwest Asia and east Africa examined hundreds of specimens of *Juniperus excelsa*, *J. polycarpos, J. turcomanica*, *J. seravschanica* and *J. procera.* Farjon (1992, 2005, 2010) treated *J. polycarpos, J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpos*. Thus, he recognized only *J. excelsa* and *J. excelsa* subsp. *polycarpos*. His taxonomic treatment appears to be in common usage in the mid-East at this time. Farjon (1992) noted that branch morphology of *J. excelsa* and *J. e.* subsp. *polycarpos* are, in part, clinal and the taxa overlap in areas. Farjon (1992) keyed these taxa as:

Ultimate branchlets slender to very fine, 0.7-1 mm diam., often disposed in dorsiventral sprays; scale leaves very small, on ultimate branchlets 0.6-1.1 mm long, appressed; female cones 6-11 mm diam.....*J. excelsa* subsp. *excelsa*.

Ultimate branchlets coarser, thicker, 1-1.3 mm diam., more irregularly disposed; scale leaves larger, on ultimate branchlets 1.2-1.6 mm long, appressed or sometimes free at apex; female cones 6-14 mm diam.....*J. excelsa* subsp. *polycarpos* (including *J. turcomanica* and *J. seravschanica*).

However, Adams et al. (2008), Adams and Schwarzbach (2012), Adams et al. (2014, 2016a), Adams and Hojjati (2012, 2014) and Adams (2013, 2014), utilizing DNA sequence data, recognized *J. excelsa,* in addition to *J. polycarpos, J. p.* var. *turcomanica* and *J. seravschanica.* They found *J. seravschanica* to be in a separate clade with *J. foetidissima* and *J. thurifera* (Fig. 1), whereas, *J. excelsa*, *J. polycarpos,* and *J. p.* var. *turcomanica* were in a clade with *J. procera* (Fig. 1). Based largely on these DNA differences, they concluded *J. seravschanica* was not con-specific with *J. excelsa* subsp. *polycarpos*, but is a distinct species. They also found that *J. e.* subsp. *turcomanica* was more allied with *J. polycarpos* (i.e., as *J. p.* var. *turcomanica*).

Recently, I have been examining specimens of *J. excelsa* and *J. polycarpos* from Asia Minor. The DNA classification of these taxa (Table 1) appears to bear little correlation with the branchlet sizes, so critical for their identification. This was especially noticeable in specimens from eastern Turkey and Azerbaijan. These specimens often had

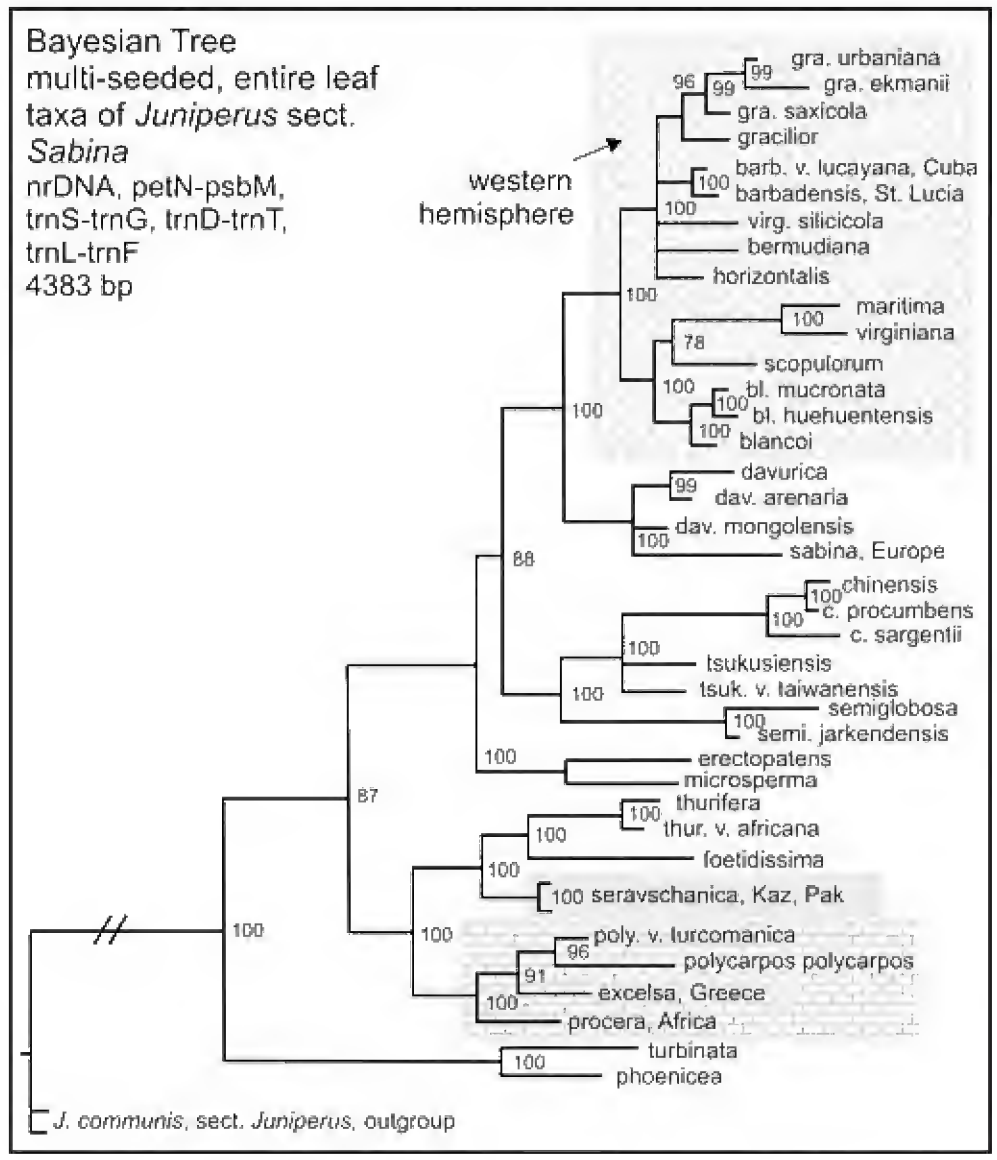

Figure 1. Bayesian analysis based on nrDNA and 4 cp genes. Numbers at the branch points are posterior probabilities. Modified from Adams et al. (2013).

very fine foliage and keyed to *J. excelsa*, yet in many cases, their DNA placed them in *J. polycarpos* (Table 1).

In Adams (2014), these taxa are keyed as follows:

24a.Ultimate branchlets 0.6 - 1 mm diam.; scale-leaves 0.5 - 1 mm; 2 - 5 seeds per cone, seed cones reddish-brown to purple-black; trees with pendulous foliage; endemic to e. Africa, Arabian Peninsula..... .....***J. procera***

24b.Ultimate branchlets 0.7 - 1 mm, scale-leaves 0.6 - 1.6 mm long, 3 - 6 (-8) seeds per cone, reddish-brown to purple black, trees and shrubs, foliage erect to pendulous

25a.Ultimate branchlets 0.7 - 1 mm diam.; scale-leaves very small, 0.6 - 1.1 mm long, appressed; seed cones 6 - 11 mm diam, globose. .....***J. excelsa***

25b.Ultimate branchlets 1 - 1.3 mm diam.; scale-leaves coarse, 1.2 - 1.6 mm long, appressed or apex free; seed cones 8 - 14 mm diam, globose

25.1a. Foliage slender, 0.7- 0.8 mm in cross section on ultimate branchlets, seed cones 7-9(-10) mm, scale leaves tightly appressed, giving a smooth branchlet, (1-)2-3(-4) seeds/cone...***J. polycarpos*** var**. *turcomanica***

25.1b. Foliage stout, 0.9-1 mm in cross section on ultimate branchlets, seed cones 9-11 mm or more, scale leaves with a beak or keel so branchlet appears as a string of beads, 3-6 seeds/cone

25.2a. Seed cones 9-11mm, at least some scale leaves with very narrow, elongated, brown glands, not ruptured. .....***J. polycarpos*** var**. *polycarpos***

25.2b. Seed cones 8-10 mm, scale leaves with clear, ellipsoid glands, often ruptured, with a clear exudate.....***J. seravschanica***

It is obvious from the above key, that these taxa are nearly impossible to distinguish by their morphology. So the question remains, Should these taxa be recognized at the specific level?

To visualize genetic variation in this region, plants were mapped with their nrDNA and cp (petN) DNA coded (Fig. 2). It is sometimes difficult to determine whether variation is due to incomplete lineage sorting or hybridization (see discussion in Naciri and Linder, 2015). The odd occurrence of *J. seravschanica* nrDNA in central-eastern Turkey plants seems more likely incomplete lineage sorting than hybridization, because no pure *J. seravschanica* grows sympatric with *J. polycarpos* in the area. Long distance cross-pollination is possible but unlikely, as the nearest known *J. seravschanica* is quite distant. In northwestern Iran, one P,P and three S,P plants were found. This may be due to either hybridization or incomplete lineage sorting. Additional samples are needed to better understand that region.

The purpose of this paper is to examine incongruent topologies in the *J. excelsa* complex and review the taxonomic status for these taxa.

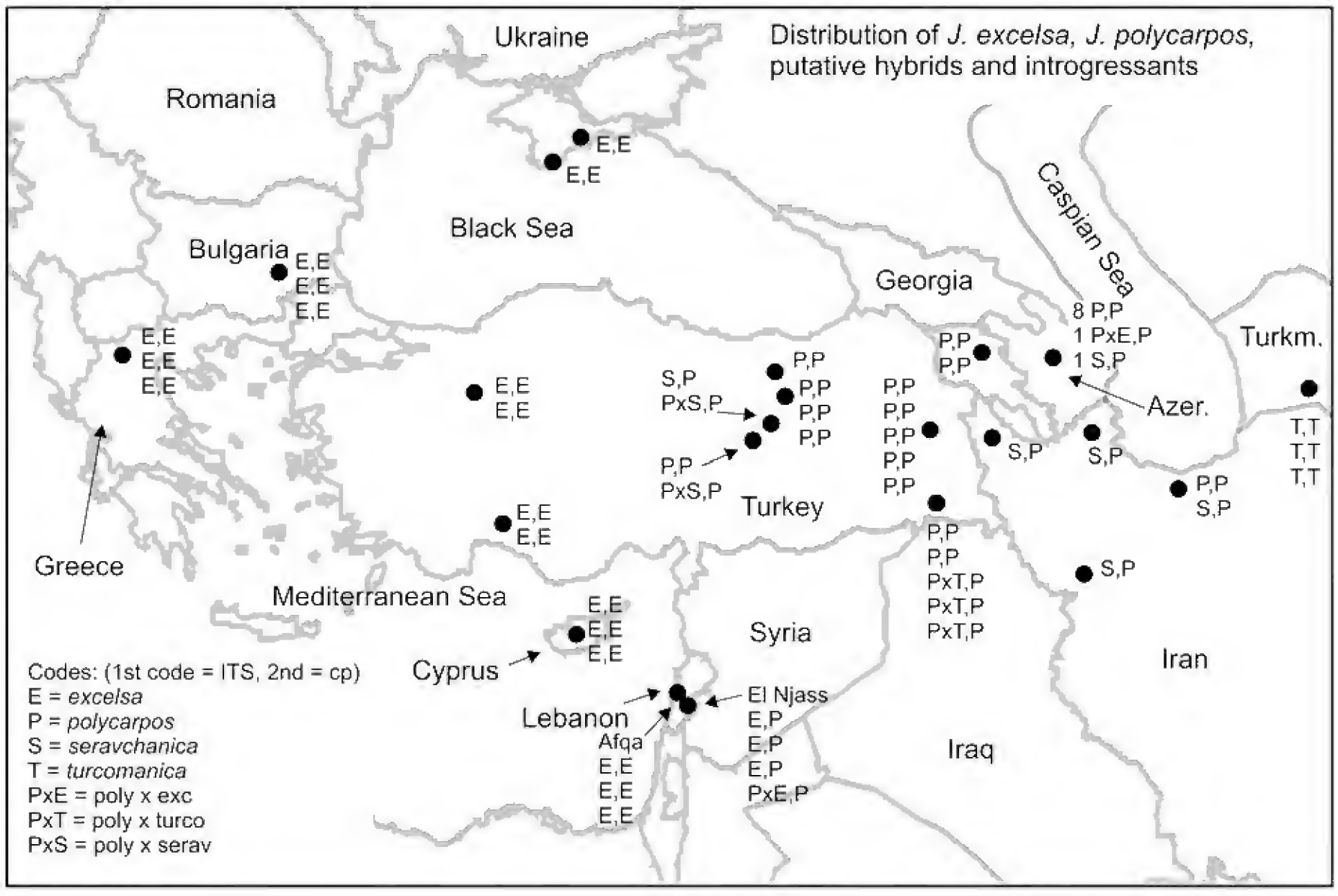

Figure 2. Distribution of *J. excelsa, J. polycarpos*, putative hybrids and introgressants based on ITS and cp sequences. The pair of capital letters (eg., E,E) gives the sample classification based on ITS (1st letter) and cp (2nd letter). Note: revised 30 May 2016, as ambiguity between ITS of *excelsa* and *turcomanica* has been resolved by the discovery of an indel (insertion) at position 526: *excelsa* = AACTCGCCCCT; *turcomanica* = AACTCG**G**CCCT. Adapted from Adams et al. (2016b).

MATERIALS AND METHODS

Plant material - See Adams et al. (2016b).

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. 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 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. R8 (Biomatters. Available from http://www.geneious.com/), the MAFFT alignment program. 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; Veldman, 1967).

RESULTS AND DISCUSSION

The overall DNA pattern among the taxa in the *J. excelsa-J. polycarpos-J. procera* and *J. seravschanica-J. foetidissima-J. thurifera* clades (Fig. 1), reveals that *excelsa* - *procera* are differ by 21 Mutation Events (SNPs + indels). In *Juniperus*, these five gene sequences have been used for the monograph and all species (Adams, 2014). It has been found that about 6-8 MEs differentiate varieties and species are delineated by about 9-15 MEs. Thus, Adams (2014 and refs. within) recognized *J. excelsa*, *J. procera*, *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica*.

In view of the fact that these five taxa are nearly impossible to identify from morphology alone, it seems timely to re-examine this set of DNA sequences, because the nuclear DNA data (nrDNA) does not tell the same story as the cp genome DNA (petN-psbM, trnSG, trnDT, trnLF). Figure 4 shows the Minimum Spanning Network (MSN) based on nrDNA. Notice that *J. polycarpos* and *J. seravschanica* nrDNAs differ by only 1 SNP. Also note that *J. seravschanica* differs by only 7 MEs from *J. foetidissima.* and by 8 MEs from *J. thurifera* (Fig. 4), such that *J. seravschanica* as similar to *J. foetidissima* as to *J. excelsa* and *J. p.* var. *turcomanica* (Fig. 4) in its nrDNA.

The MSN using four cp genes (3113 bp), shows several incongruent topologies: *J. polycarpos* and *J. seravschanica* are quite differentiated by 23 MEs (Fig. 5). In fact, the cp genes of *J. seravschanica* differ by 10 and 12 MEs from *J. foetidissima* and *J. thurifera*, respectively.

The nrDNAs of *J. polycarpos* and *J. p.* var. *turcomanica* differ by 8 MEs (6 SNPs + 2 indels), which is larger between many Juniper species. Yet, they have no differences in these four cp genes (3113 bp) (Fig. 5). This suggests chloroplast capture by *J. p.* var. *turcomanica* from *J. polycarpos*. resulting in var. *turcomanica* cpDNA being identical to that of *J. polycarpos* (Fig. 5). The fact that var. *turcomanica* cpDNA is identical to *J. polycarpos*, supports the concept that this cp capture is of recent origin.

The nrDNAs of *J. polycarpos* and *J. seravschanica* differ by only 1 SNP, yet their cpDNA differ by 23 MEs Fig. 4). *Juniperus seravschanica* differs by only 10 MEs from *J. foetidissima* and 12 MEs from *J. thurifera* (Fig. 5), but by 7 and 8 MEs in its nrDNA from *J. foetidissima* and *J. thurifera*, respectively. This suggests ancestral chloroplast capture by *J. seravschanica* from an ancestor of *J. foetidissima/ J. thurifera.*

It is useful to consider the overall genome organization. The major 'storehouse' of genes is in the nucleus. Sterck et al. (2007) reported over 26,500 genes in *Arabidopsis*, 41,000 in rice (*Oryza sativa*), 45,000 in popular (*Populus*), and 40,000 in *Medicago* and *Lotus*. For Norway spruce, *Picea abies*), Nystedt, et al. (2013) reported 28,354 well-supported genes.

The mitochondrion is another 'storehouse' of genes and a just-published study (Jackman, et al., 2016) on the *Picea glauca* mt genome, reported its size as 5.94 Mb (Mbases), containing only 106 protein-coding, 8 rRNA, and 29 tRNA genes (total 143 genes). Jackman, et al. (2016) also reported the size of the *P. glauca* cp genome as 123,266 bp, with 74 protein-coding, 4 rRNA, and 36 tRNA genes (114 genes).

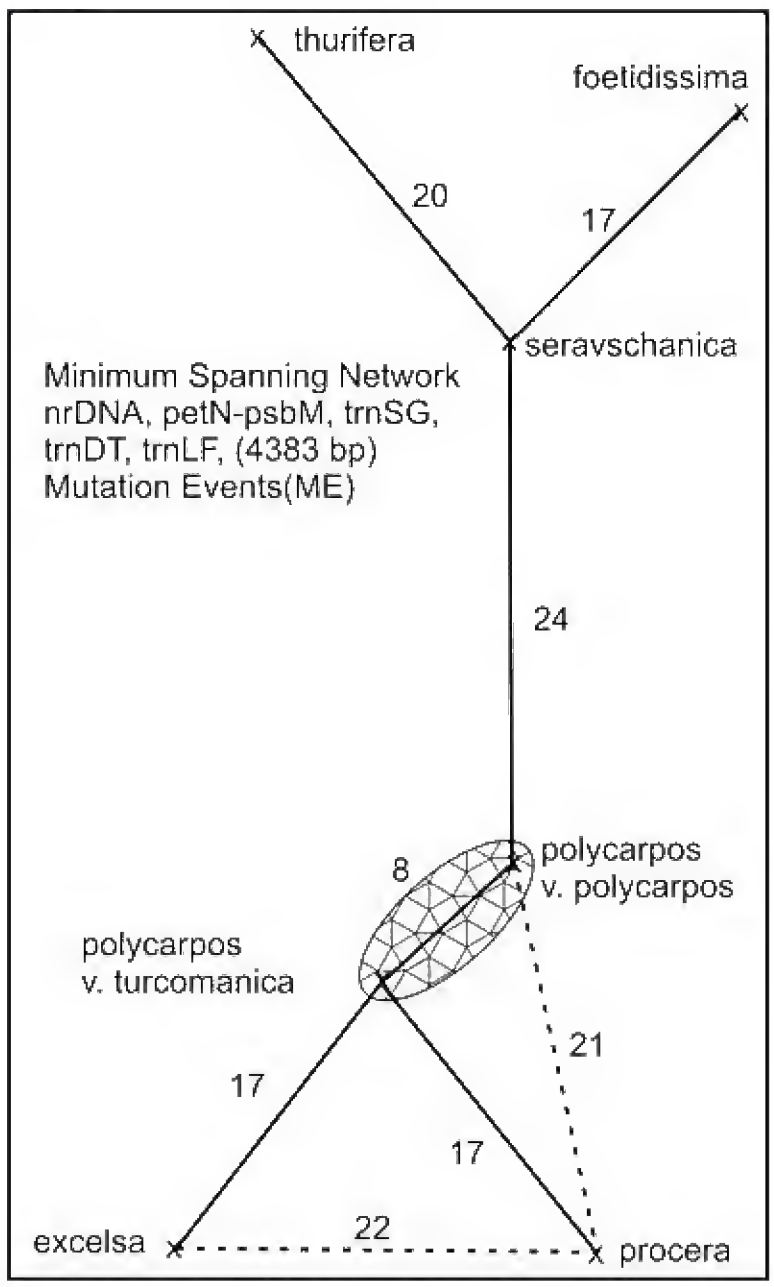

Figure 3. Minimum spanning network of the *excelsa* group. Numbers next to links are the number of Mutation Events (MEs). Dashed lines are second nearest links. Modified from Adams and Schwarzbach (2012).

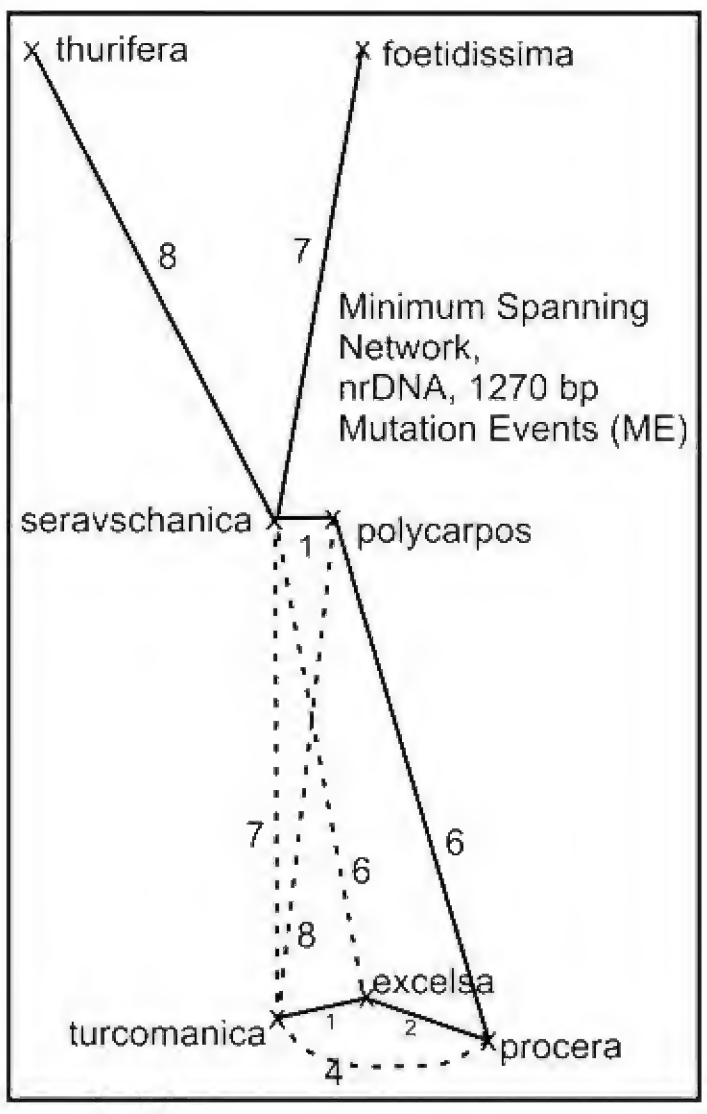

Fig. 4. MSN based on nrDNA. Dashed lines are second nearest links. Notice that *polycarpos* and *seravschanica* differ by one SNP in their nrDNA sequences.

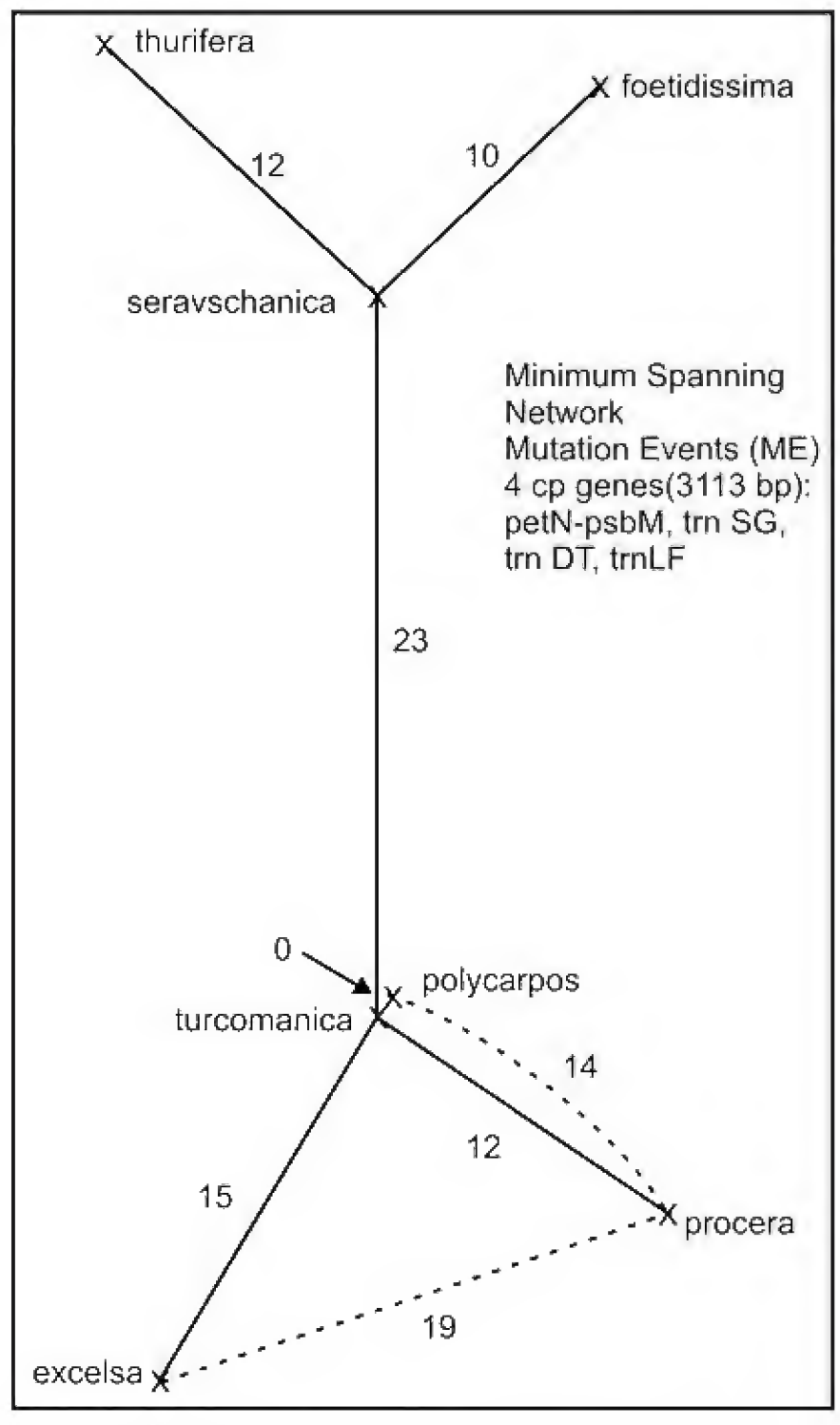

Fig. 5. MSN based on 4 cp gene regions. Note these 4 cp gene regions are identical for *J. polycarpos* and *J. p.* var. *turcomanica*. (i,e, 0 differences in 3113 bp).

A recent study (Guo et al. 2014) of cp genomes of four *Juniperus* species reported the sizes as: *J. bermudiana*, 127,659 bp, *J. monosperma*, 127,744 bp, *J. scopulorum*, 127,774 bp and *J. virginiana*, 127,770 bp. Each of the species had 82 protein-coding, 4 rRNA, and 33 tRNA genes (total 119 genes).

It is instructive to examine the percentage of the genomes that have been studied in the *J. excelsa - polycarpos* complex:

Genome	genes utilized	# genes used	#genes/ genome	% genes used
nuclear	nrDNA (1270bp)	1	ca. 28,000	0.0036%
mt genome	none	0	ca. 143 genes	0.0
cp genome	petN-psbM, trnSG, trnDT, trnLF(3113bp)	4	ca. 119 genes	3.36%

It is quite apparent that the sampling of the nuclear genes (0.0036%) is vastly under-represented, compared to the sampling of the cp genes (3.36%). Does the nrDNA sequence data well-represent variation among taxa in their 28,000 nuclear genes? It is thought that nrDNA is subject to concerted evolution (Liao, 1999) such that point mutations are harmonized to conserve the structure of ribosomes. It seems unlikely that protein-coding nuclear genes are subjected to concerted evolution. So, it is possible that nrDNA (RNA structural genes) might not show the same phylogenetic patterns as protein-coding nuclear genes. Due to the lack of single copy genes (SCNG) in conifers, few SCNG are currently utilized in evolutionary studies in conifers.

Adams (2009) examined variation among smooth-leaf margined *Juniperus* in Mexico using nrDNA, two SCNG (4CL, ABI3), and petN-psbM. Figure 6 show MSNs based on nrDNA (left), and two SCNGs (4CL and ABI3, right). Figure 7 shows MSN based on cp petN-psbM sequences. There is considerable agreement between the MSNs using nrDNA and petN-psbM. Both show *J. virginiana* as the most distinct taxon and both show *J. scopulorum*, distinct, but closely related to *J. blancoi* varieties. 4CL generated only 5 SNPs, so it may be unfair to compare with the other sequences that generated 9 to 17 SNPs. Nevertheless, 4CL does show *J. virginiana* distinct (Fig. 6, right), but fails to resolve *J. scopulorum* and *J. blancoi*. ABI3 gave a MSN that is different from any of the other MSNs (Figs. 6, 7). So, this limited comparison of nuclear and cp data gives some support that nrDNA may represent genomic DNA, but not unequivocal support.

Graphic comparison (Fig. 8) shows *excelsa* (E) distinct in terpenes and RAPDs but only somewhat distinct in morphology. nrDNA presents another pattern with two groups: (*excelsa-turcomanica*), (*polycarpos-seravschanica*).

petN-psbM depicts three groups: (*excelsa*), (*polycarpos=turcomanica*), (*seravschanica*) (Kazakhstan = Pakistan). Finally, the lower right (Fig. 8) presents the grouping based on all DNA data.

Incongruent topologies suggest that there are two cases of chloroplast capture in the *J. excelsa* complex: *J. p.* var. *turcomanica* has recently captured its chloroplast from *J. polycarpos* or an extinct ancestor; and *J. seravschanica* has an anciently captured chloroplast from an ancestor of *J. foetidissima*/ *J. thurifera*. Thus, *J. p.* var. *turcomanica* and *J. seravschanica* seem to defy an uncluttered taxonomic classification. Not only does incongruent topologies disrupt a linear, taxonomic classification, but the situation is further confused by wide-spread hybridization, incomplete linage sorting and reticulate evolution in the *J. excelsa* complex. The morphology is reticulated in a manner that defines a multi-dimensional set of relationships among different character sets.

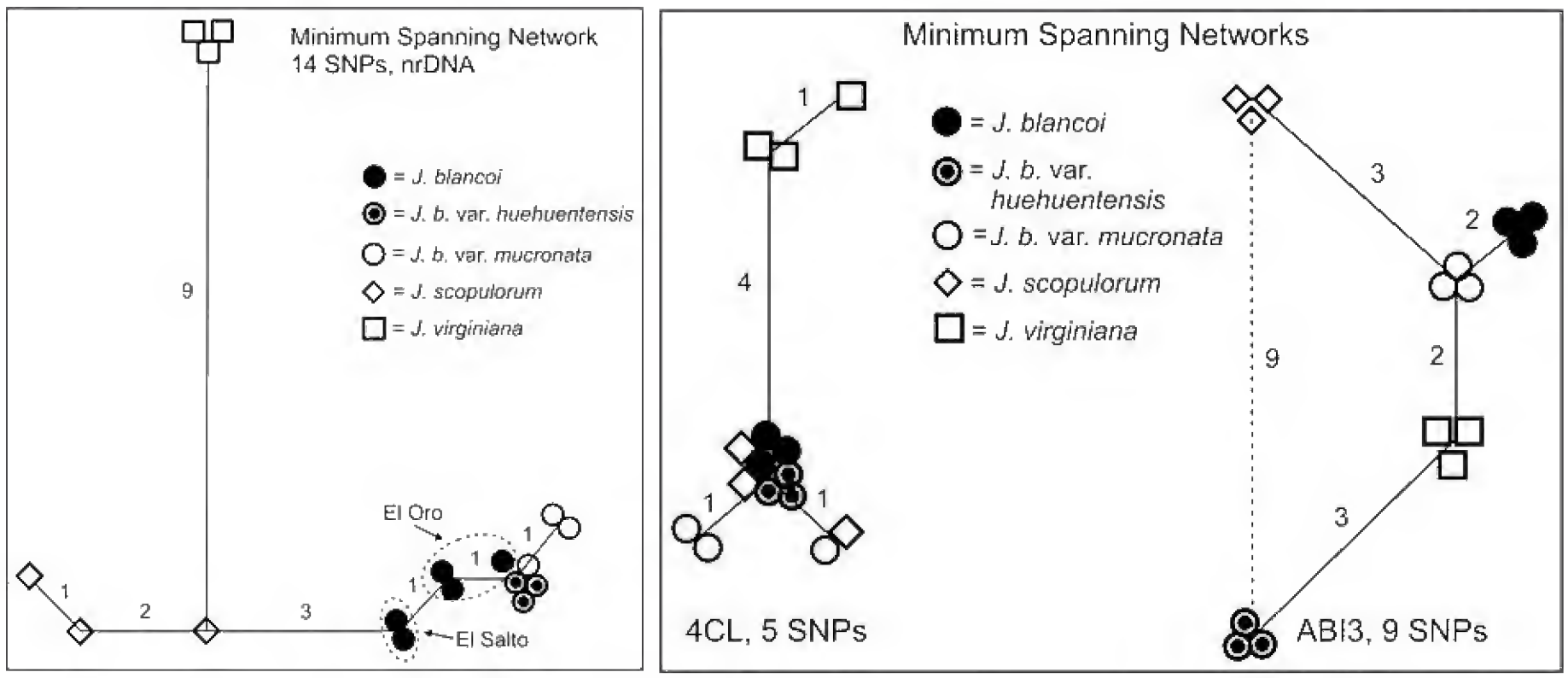

Fig. 6. Comparison of MSN based on nrDNA (left) and two SCNGs (4CL, ABI3, right). Modified from Adams (2009).

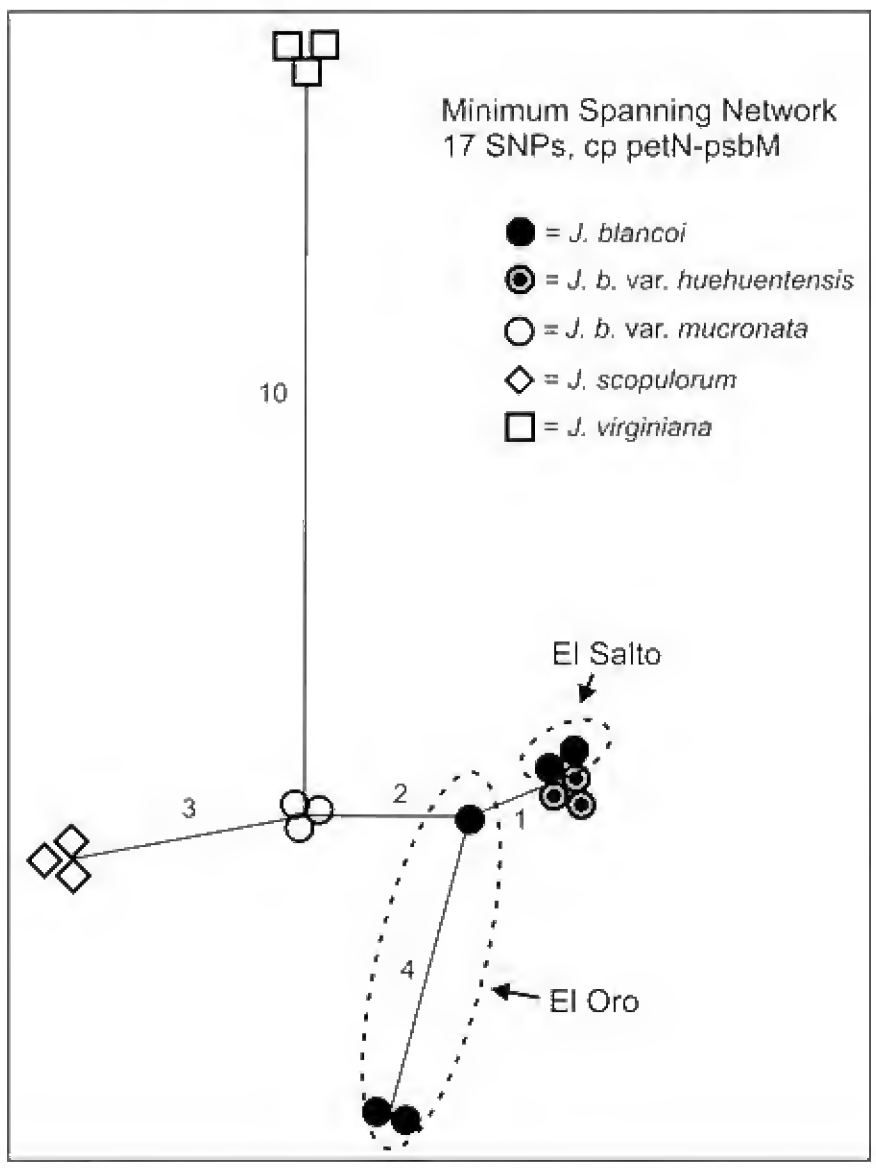

Figure 7. MSN based on petN-psbM. Modified from Adams (2009).

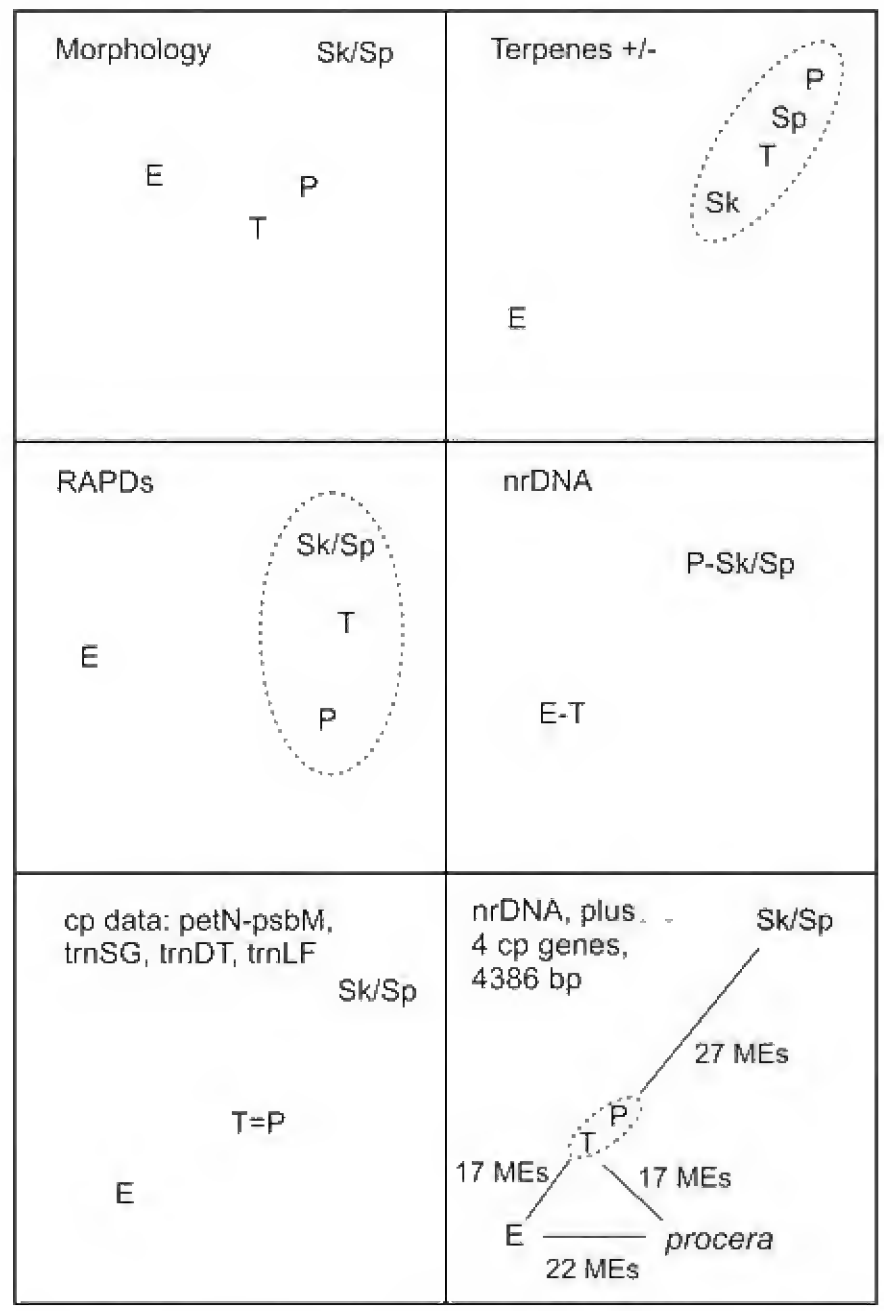

Figure 8. Graphic grouping of *excelsa* (E), *polycarpos* (P), *turcomanica* (T), and *seravschanica* (Sk, Kazak., Sp, Pakistan). Modified from Adams (2014).

Because these taxa are reproducing themselves in nature, occupy distinct geographical regions, and contain unique combinations of genetic material, it is important to recognize two new varieties in order to call attention to chloroplast capture and the unique evolutionary nature of these taxa. These taxa are not merely isolated hybrids (Table 1).

Juniperus excelsa M.-Bieb. var. ***turcomanica*** (B. Fedtsch.) R. P. Adams, **comb. nov.**

BASIONYM: *Juniperus turcomanica* B. Fedtsch. in Fedtschenko et al. Fl. Turkmenii 1:14. 1932. TYPE: Lost or destroyed (Imkhaniskaya, 1990). (LECTOTYPE: *D. P. Gedevanov & D. A. Dranitsyn 148, 3 v 1912*, Turkmenia, Kopet Dag. Dschalilu (chosen by Imkhaniskaya, 1990, LE!)

J. turcomanica B. Fedtsch. in Fedtschenko & al., Fl. Turkmenii 1:14 (1932)
Sabina turcomanica B. Fedtsch). Nevski, Trudy Bot. Inst. Akad. Nauk S.S.S.R. ser. 1, Fl. Sist. Vyss Rast. 4:218 (1937)
J. excelsa M.-Bieb. subsp. *turcomanica* (B. Fedtsch.) Imkhan., Bot. Zurn. 75 (3):408 (1990)

Distribution: Elburz and Kopet Mtns., of Iran and Turkmenistan.

Juniperus excelsa M.-Bieb. var. ***seravschanica*** (Kom.) R. P. Adams, **comb. nov.**

BASIONYM: *Juniperus seravschanica* Kom. Bot. Zurn. (Moscow & Leningrad) 17: 481. 1932. TYPE: Tadjikistan. Zaravshan Range: Zaravshan Valley, Darch, V. L. Komarov s.n. (LECTOTYPE: (chosen by Imkhaniskaya, 1990, LE!)

J. excelsa M.-Bieb. subsp. *seravschanica* (Kom.) Kitam., (Fl. Pl. W. Pakist. Afghan. 7. 1964)
J. polycarpos var. *seravschanica* (Komarov) Kitamura, Fl. Pl. W. Pakist. Afghan Add. & Corr. Fl. Afghan.: 68 (1966).
J. polycarpos K. Koch var. *seravschanica* (Kom.) Kitam., Add. & Corr. Fl. Afghan.: 68 (1966)
J. excelsa M.-Bieb. subsp. *seravschanica* (Kom.) Imkhan., Bot. Zurn. 75 (3): 407 (1990)
Sabina seravschanica (Kom.) Nevski, Trudy Bot. Inst. Akad. Nauk S.S.S.R., ser. 1, Fl. Sist. Vyss. Rast. 4:245 (1937)

Distribution: Central Asia to Iran and Oman.

It should be noted that this creates a polyphyletic species, *J. excelsa*, with varieties in two distinct clades (Fig. 1) when both nrDNA and cpDNA are utilized. So, one should view this work as interim and as a practical matter. It calls attention to this situation, that at present time, appears to escape traditional taxonomic classification.

This treatment gives a variable, polyphyletic species, *J. excelsa*, with three varieties (or subspecies if one prefers, but I prefer to use variety as that is used throughout the genus *Juniperus* and most other Cupressaceae). For those who can not accept polyphyletic species, they are free to use *J. seravschanica*, instead of *J. e.* var. *seravschanica,* and then both it and *J. excelsa* would be monophyletic.

Juniperus excelsa M.-Bieb. var. *excelsa* or subsp. *excelsa*
var. *polycarpos* (K. Koch) Silba (Phytologia Mem. 7: 34. 1984)
(or subsp. *polycarpos* (K. Koch) Tahkt. (Fl. Yerev. 53. 1972.))
var. *turcomanica* (B. Fedtsch.) R. P. Adams, **comb. nov.** this issue.
var. *seravschanica* (Kom.) R. P. Adams, **comb. nov.** this issue.
(or subsp. *seravschanica* (Kom.) Kitam., Fl. Pl. W. Pakist. Afghan. 7. 1964)

It is likely as NexGen sequencing develops, single copy nuclear genes will become widely identified and applied so the concept of *J. excelsa* will also change. As for *J. procera*, it appears to have such a strong geographic integrity, that it seems best to continue its recognition.

The currently understood distributions of *J. excelsa,* var. *polycarpos,* var. *seravschanica* and var. *turcomanica* are depicted in Figure 9. The dashed line in central Turkey indicates the boundary between *J. excelsa* and var. *polycarpos*.

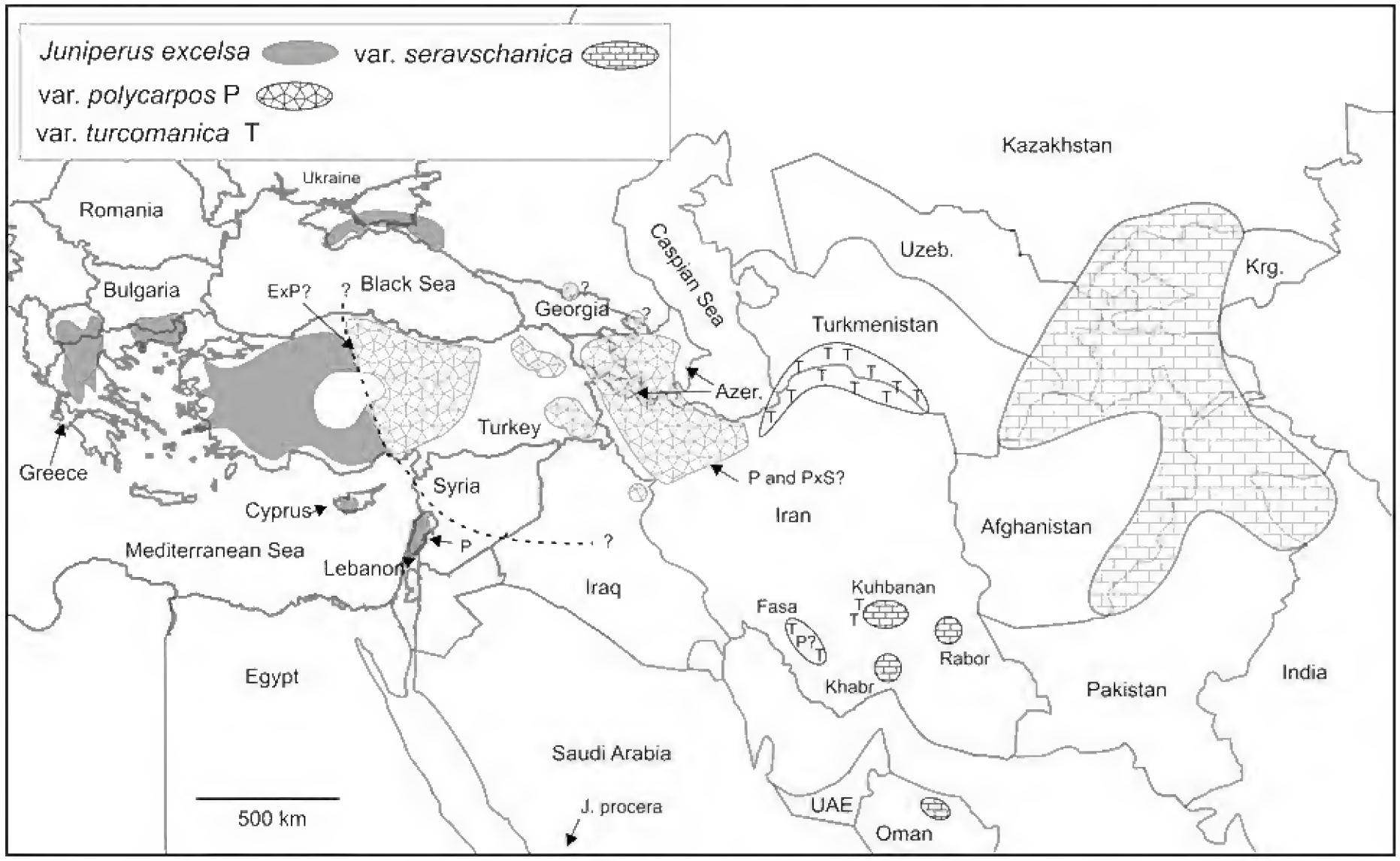

Figure 9. Distributions of *J. excelsa,* var. *polycarpos,* var. *turcomanica and* var. *seravschanica* as understood at present. The dashed line indicates the uncertain limits of *J. excelsa* and var. *polycarpos* in central Turkey. See text for discussion. From Adams et al. (2016b).

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Table 1. Classification based on ITS and cp (petN). exe = *excelsa*, pol = *polycarpos*, tur = *turcomanica*, ser = *seravschanica*. PxE = hybrid pol x exc; PxS = hybrid pol x ser; PxT = pol x tur, PxE = pol x exc. NB: indel at 527 separates exc from tur!

acc #	230	232	238	354	427	732	8952	ITS	cp
8785	A	G	C	C	C	T	A	exc	exc
8786	A	G	C	C	C	T	A	exc	exc
14742	A	G	C	C	C	T	A	exc	exc
13720	A	G	C	C	C	T	A	exc	exc
13721	A	G	C	C	C	T	A	exc	exc
13722	A	G	C	C	C	T	A	exc	exc
14570	A	G	C	C	C	T	A	exc	exc
14571	A	G	C	C	C	T	A	exc	exc
14572	A	G	C	C	C	T	A	exc	exc
14906	A	G	C	C	C	T	A	exc	exc
14907	A	G	C	C	C	T	A	exc	exc
14569	A	G	C	C	C	T	A	exc	exc
14596	A	G	C	C	C	T	A	exc	exc
9433	A	G	C	C	C	T	A	exc	exc
9434	A	G	C	C	C	T	A	exc	exc
14155	A	G	C	C	C	T	A	exc	exc
14156	A	G	C	C	C	T	A	exc	exc
14157	A	G	C	C	C	T	A	exc	exc
14158	A	G	C	C	C	T	A	exc	poly
14159	A	G	C	C	C	T	A	exc	poly
14160	A	G	C	C	C	T	A	exc	poly
14161	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxE**	poly
14750	na	na	na	na	T	C	T	poly	poly
14751	C	G	T	T	T	C	T	poly	poly
14752	C	G	T	T	T	C	T	poly	poly
14753	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
14754	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
14755	C	A	T	T	T	C	T	poly	poly
14756	C	G	T	T	T	C	T	poly	poly
14757	C	G	T	C	T	C	T	**serav**	poly
14758	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
14759	C	G	T	T	T	C	T	poly	poly
14760	C	G	T	T	T	C	T	poly	poly
14713	C	G	T	T	T	C	T	poly	poly
14714	C	**R-A/G**	T	**Y-C/T**	T	C	T	**PxS**[a]	poly
14715	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT**	poly
14709	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT**	poly
14710	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT**	poly
14711	C	G	T	T	T	C	T	poly	poly
14712	C	G	T	T	T	C	T	poly	poly
14162	C	G	T	T	T	C	T	poly	poly
14163	C	**R-A/G**	T	T	T	C	T	poly[a]	poly
14164	C	G	T	T	T	C	T	poly	poly
14165	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxE**	poly
14166	C	G	T	T	T	C	T	poly	poly
14167	C	G	T	T	T	C	T	poly	poly
14168	C	G	T	T	T	C	T	poly	poly
14169	C	G	T	T	T	C	T	poly	poly
14170	C	G	T	T	T	C	T	poly	poly
14171	C	G	T	**C**	T	C	T	**serav**	poly
8761	C	G	T	T	T	C	T	poly	poly
8762	C	**A**	T	T	T	C	T	poly	poly
12603	C	G	T	T	T	C	T	poly	poly
12604	C	G	T	**C**	T	C	T	**serav**	poly
12789	C	G	T	**C**	T	C	T	**serav**	poly
12795	C	G	T	**C**	T	C	T	**serav**	poly
12798	C	G	T	**C**	T	C	T	**serav**	poly
8483	C	G	T	**C**	T	C	T	**serav**	serav
8484	C	G	T	**C**	T	C	T	**serav**	serav
8224	C	G	T	**C**	T	C	T	**serav**	serav
8225	C	G	T	**C**	T	C	T	**serav**	serav
8757	A	G	C	C	C	T	A	turco	turco
8758	A	G	C	C	C	T	A	turco	turco

Diameter growth of *Acer grandidentatum* (Bigtooth maple) in isolated central Texas populations

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ABSTRACT

Acer grandidentatum Nutt. (Bigtooth maple, Aceraceae) populations are mostly found in the mountains of western North America. There are a few isolated populations in western and central Texas. We measured annual diameter growth of plants that were found in isolated populations in deep canyons of the Albert and Bessie Kronkosky State Natural Area in central Texas. Plants sampled ranged in size from 17.57 mm to 232.41 mm in basal diameter. We used dendrochronological techniques to estimate diameter growth which was 2.50 mm basal diameter/y and comparable to some other deciduous and evergreen woody plants. Growth in diameter was a linear function with stem age increasing as basal diameter increased (y= 0.40x, R^2=0.85, $P < 0.0001$). The largest tree found was estimated to be 113 years old. *Acer grandidentatum* growth suggests it is not an early successional sun species but a mature community understory shade species. The age-diameter function will be used to estimate the age of *A. grandidentatum* plants in a future ecological study to determine recruitment of *A. grandidentatum* into isolated populations in central Texas including the Albert and Bessie Kronkosky State Natural Area. Published on-line www.phytologia.org *Phytologia 98(3): 232-240 (July 6, 2016).* ISSN 030319430.

KEY WORDS: basal diameter, mean annual growth rate, conservation, ABK, Albert and Bessie Kronkosky State Natural Area, dendrochronology

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Acer grandidentatum Nutt. (Bigtooth maple, Aceraceae) populations occur in a wide range of montane habitats, especially protected canyons across western North America (Little 1972; USDA 2016). They are found at various elevations, soil depths, soil types, moisture regimes, and with a number of different plant species (see Gehlbach and Gardner 1983; Nelson Dickinson and Van Auken 2016). In western Texas, there are scattered, isolated populations of *A. grandidentatum* in the Chisos, Davis and Guadalupe mountains (Little 1972; USDA 2016). In central Texas, isolated *A. grandidentatum* populations are mostly found in steep, sheltered canyons of the Edwards Plateau physiographic region (Nelson Dickinson and Van Auken 2016).

The most well-known populations of *A. grandidentatum* in central Texas are in Lost Maples State Natural Area (McCorkle 2007; Heidemann 2011). However, in 2011 Texas Parks and Wildlife acquired the approximately 1520 ha (=3757 ac) Albert and Bessie Kronkosky property, known as the 3K ranch, now called the Albert and Bessie Kronkosky State Natural Area (Figure 1). There are a number of *A. grandidentatum* populations in the various canyons present in this area (Figure 2A). The understory of the *A. grandidentatum* communities was fairly open (Figure 2B). It is possible that the number of *A.*

grandidentatum trees at the Albert and Bessie Kronkosky State Natural Area exceeds the number found at Lost Maples State Natural Area, although the specific density characteristics of either population are unknown.

There are few studies of outlier populations of *A. grandidentatum* (Gehlbach and Gardner 1983; Nelson Dickinson and Van Auken 2016). Gas exchange rates of *A. grandidentatum* suggest it is a shade species (Nelson Dickinson 2011; USDA 2016). There are no studies that we have identified concerning growth rates of mature *A. grandidentatum* plants. There are a few studies of *A. saccharum* germination and seedling growth, but few concerning growth rates of mature trees (Duchesne et al. 2002; Watmough 2002; Duchesne et al. 2003). Most of the studies of *A. saccharum* were carried out in the northeastern U.S. or southeastern Canada. The rates of recruitment of juvenile *A. grandidentatum* into these isolated mature populations in central Texas are unknown. *Acer grandidentatum* trees flower and produce seed, and seed germination and seedling establishment is occurring (personal observations), which should lead to successful population regeneration. However, long term endurance of juvenile maples has not been observed at the Lost Maples State Natural Area (Nelson Dickinson and Van Auken 2016) or the Albert and Bessie Kronkosky State Natural Area and recruitment of juveniles into the adult population is hearsay at best. Furthermore, the timing and date of the last recruitment are unknown.

PURPOSE

The purpose of this study was to determine the diameter and age of *A. grandidentatum* plants in the Albert and Bessie Kronkosky State Natural Area and to estimate the mean annual growth rate of *A. grandidentatum* in these central Texas populations.

METHODS

The study site was in the Albert and Bessie Kronkosky State Natural Area in and around the "Tin Cup Canyon" (Figure 1), which is in the Edwards Plateau Physiographic region of Texas (approximately 29°44'25"N, 98° 50' 18"W). The study area was a woodland near an intermittent stream at the bottom of "Tin Cup Canyon" and on adjacent slopes of mostly north facing side canyons. Domestic grazing was the main industry of the general area, but in 1998 a 2.4 m high fence (deer fence) was constructed and grazing was halted in the Natural Area (Carpenter and Brandimarte 2014). For sampling, we identified standing dead and fallen *A. grandidentatum* plants in this area. The elevation is 484-614 m above mean sea level. Canyon bottoms have relatively deep calcareous silty clay soil, a Mollisol overlying limestone bedrock (SCS 1977). The soil series in the area are Eckrant-Rock outcrop associations, steep, consisting of clayey-skeletal, smectitic, thermic lithic haplustolls (SCS 1977). Mean annual temperature in the study area is approximately 18.3°C, ranging from near 0.7°C to 34.1°C, and mean annual precipitation is highly variable but approximately 72.4 cm/year with very little in July and August with May and September being wettest (World Climate 2011). Unknown densities of white-tailed deer and wild hogs are present in the Natural Area while densities of approximately one deer/5 ha are reported in adjacent regions (Armstrong and Young 2000; Fulbright and Ortega-S. 2005).

Tree sampling was conducted from March through May 2016. In the canyons where *A. grandidentatum* populations were found, we haphazardly selected samples. Samples were standing dead and fallen trees and had a variety of different diameters. Tree sections were collected from near the base. Samples were numbered and taken to the laboratory for processing. In the laboratory, collected stems were cut into thinner slices and air dried. After drying, slices were sanded thoroughly using coarse (p-grade 100), medium (p-grade 150) and then fine (p-grade 220) sandpaper until the annual rings could be clearly observed (Figure 3A). Earlywood, latewood, vascular rays and pith could be easily seen in many of the stems. Some samples had heart-rot (Figure 3B) and some had insect holes or borings and could not be used (Figure 3C). Many of the larger down stems that we cut could not be aged because of extensive

rot and insect damage (Figure 3C, some were worse and are not shown). Basal diameter of smaller stems was measured to the nearest 0.01 mm with a caliper (Mitutoyo-digimatic) in two perpendicular directions across the stem and averaged, while large stems were measured with a tape measure in two perpendicular directions and averaged. Visual age of samples was determined by counting the annual rings with a 20X magnifying glass and a 40X binocular microscope (Fritts 1976; Shen et al. 2016). The distribution of tree age versus diameter was examined and then regressed. The best fit regression equation with the 95 % confidence intervals are presented. The growth rate for the *A. grandidentatum* trees was calculated as the inverse of the slope of the regression line (1/0.40 in the current example).

RESULTS

A map of the location of the Albert and Bessie Kronkosky State Natural Area, the location in the state of Texas and the location in Kendall and Bandera Counties along state highway 46 is shown (Figure 1A&B). The study area included steep sided canyons shown in part of a topographic map with the approximate location of the research area indicated with a red oval, with the dark green line indicating the northern Natural Area boundary (Figure 1C). An aerial photograph (Figure 1D) shows the approximate study area with the same northern boundary (fence) and some of the *A. grandidentatum* trees in their fall colors, with the red oval showing the study area. A picture from ground level looking northwest over "Tin Cup Canyon" (Figure 2A) where many *A. grandidentatum* trees were found is presented, while the second photograph (Figure 2B) is a picture below the *A. grandidentatum* canopy depicting many of the mature tree stems and the very open nature of the community.

Many cross sections of the *A. grandidentatum* trees were quite clear (Figure 3A) and usually showed the pith, vascular rays, earlywood, latewood and the annual rings, and when the bark was intact the cambium, inner and outer bark could be identified (Figure 3B). Various stems had some rot, including heart rot, and some stems encountered could not be used and were discarded because of rot, numerous insect borings in the stem (probably beetles) and missing bark (Figure 3C). An X-Y plot of stem diameter in mm and age in years is also presented (Figure 4). Shown in the figure are 26 age/diameter points of various sized *A. grandidentatum* trees. The regression is a significant linear function with R^2=0.85 and $p<0.0001$. The equation for the line is y= 0.40x when the origin is forced through zero. The figure also shows the upper and lower 95 % confidence interval for the data. The growth rate for the *A. grandidentatum* trees was calculated as 2.50 mm/y.

DISCUSSION

All of the *Acer grandidentatum* trees aged and measured in the present study were in or around the "Tin Cup Canyon" of the Albert and Bessie Kronkosky State Natural Area (Figure 1). A moisture gradient may have been present and a possible cause of some of the variation in diameter, but was not examined. Seedlings of *A. grandidentatum* were present within the communities examined, but very few saplings or juvenile trees were observed, which suggested that some biotic or abiotic factor or factors prevented seedlings from being recruited into the canopy (personal observation). The same seems to be true for *A. saccarium* in central Missouri (Belden and Pallardy 2009). Similar studies reported lower mortality and greater growth in the understory for *A. saccarium* and other species if herbivory was prevented (Côté et al. 2004; Russell and Fowler 2004; Belden and Pallardy 2009; Leonard and Van Auken 2013; Nelson Dickinson and Van Auken 2016). Examining herbivory was not part of the present study; however, herbivory has been reported as a cause of recruitment failure for other deciduous species in the Edwards Plateau Physiographic Region (Van Auken 1988; Fuhlendorf et al. 1997; Russell et al. 2001; Russell and Fowler 2004).

We wanted to demonstrate the relationship between age and size (diameter) of *A. grandidentatum* in the Albert and Bessie Kronkosky State Natural Area (Figure 4). We hope to use this information to

show the recruitment history of this species in the Natural Area. This will require knowing the age-size frequency distribution of the various populations of *A. grandidentatum*, which are unknown at this time. Understanding recruitment of juveniles into the adult population in existing woodland and forest communities has proven difficult to understand. However, a large number of papers have been written about the topic (see Baker et al. 2005). Factors that control recruitment have also been difficult to understand as have the factors that control the growth of individual plants below the canopy of an adult population. Light levels are certainly important, but soil resources, neighbors and herbivores are important as well (see McKinley and Van Auken 2005; Van Auken 2009).

The age and diameter of *A. grandidentatum* could be a sigmoid relationship, but much of that curve could be linear depending on the ages of the plants sampled. In a previous study of *A. saccharum* (Duchesne et al. 2003), trees that were between 111 and 135 years old, considerable variation in age and size was found. In addition, many of the trees in their study couldn't be aged because of heart-rot or other unidentified factors causing variability in growth. In the present study we noted considerable heart-rot and the smallest individuals measured were not zero age, but 4-5 years old and approximately 17 mm in diameter, suggesting our methods may have missed the first several years of growth.

We believe that the possible decreased density of *A. grandidentatum* and other deciduous species in favor of increased density of *Juniperus ashii* reported at Lost Maples State Natural Area (Nelson Dickinson and Van Auken 2016) may be reflective of wider trends reported across North American woodlands. These studies have shown that the presence of predators is critical for maintaining mature plant populations (Beschta and Ripple 2009; Abrams and Johnson 2012; Ripple et al. 2014). *Acer grandidentatum* growth is about 33 % of the growth of *Pinus ponderosa* a gymnosperm an early successional sun species (Table 1). Its growth is greater than *Quercus gambelii* and *Larix olgensis* a deciduous species and a gymnosperm species respectively (Ryniker et al. 2006; Shen et al. 2016). *Acer grandidentatum* seems to be an understory species and a late successional species (Nelson Dickinson and Van Auken 2016).

Table 1. Growth rates of two conifers (gymnosperms) and two deciduous angiosperms.

Species	Age(y)	Growth Rate(mm/y)	Date Source
Pinus ponderosa	15-130	7.654	Personal observations
Larix olgensis	145-397	1.595	Shen et al. (2016)
Quercus gambelii	109-137	1.237	Ryniker et al. (2006)
Acer grandidentatum	4-113	2.500	This paper

We will use the age-diameter curve reported in the present study to demonstrate that recruitment failure for *A. grandidentatum*, which appears to be occurring, seems to be happening very early in the life cycle of *A. grandidentatum* in its Central Texas populations. This same phenomena is occurring for other woody angiosperms in this area and the cause is probably herbivory and the same for all of these central Texas woody species (Van Auken 1988; Fuhlendorf et al. 1997; Russell et al. 2001; Russell and Fowler 2004; Nelson Dickinson and Van Auken 2016).

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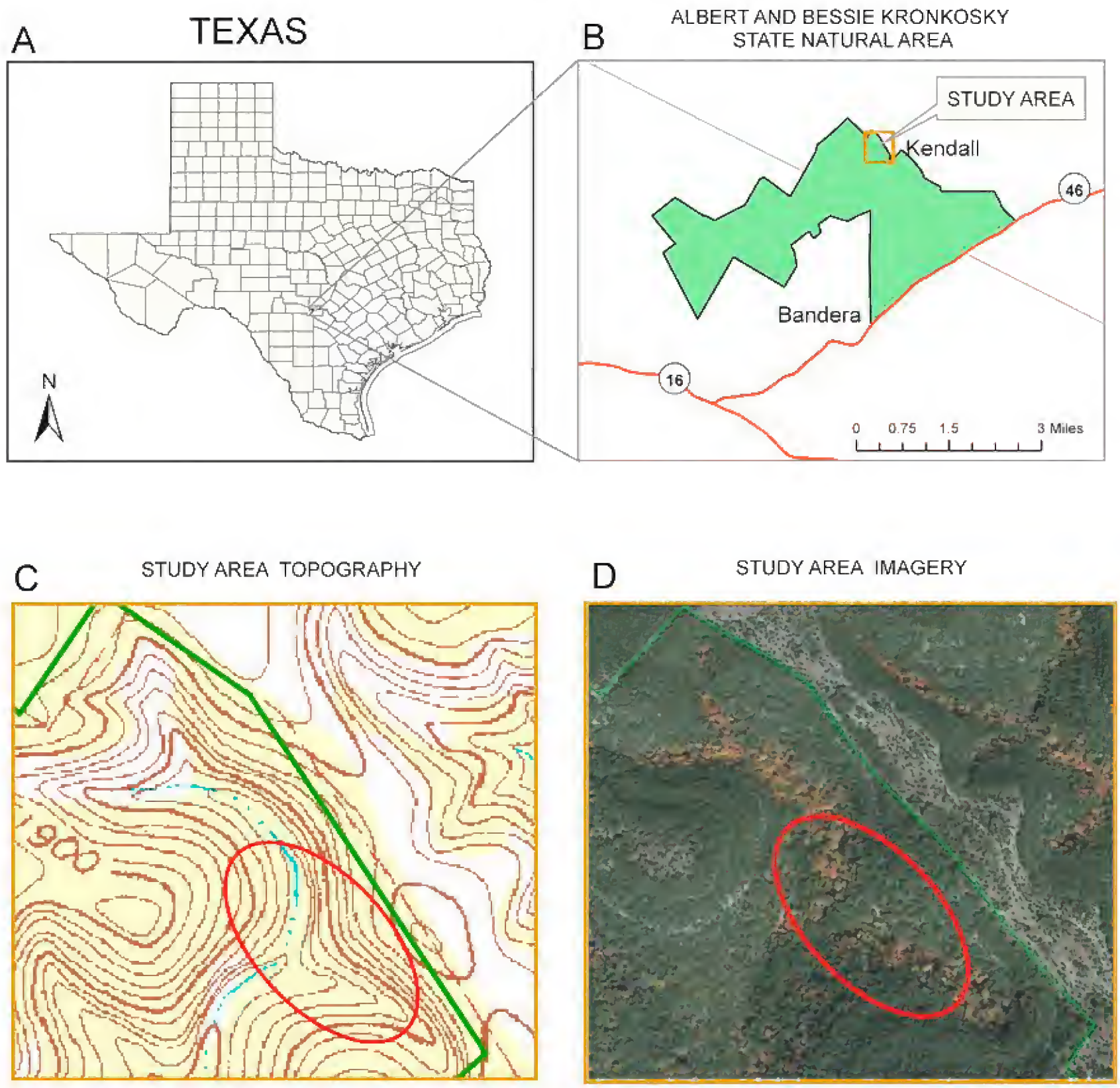

Figure 1. Map of research area in the Albert and Bessie Kronkosky State Natureal Area with (A) showing the location in the state of Texas. (B) Shows the location in Kendall and Bandera Counties along state highway 46. (C) Is part of a topographic map showing the approximate location of the study area red oval in the steep sided "Tin Cup Canyon" with the green or dark line depicting the northern boundary. (D) Is part of an aerial photograph showing the same northern boundary line (fence) with some of the bigtooth maples showing their fall colors and the red oval showing the approximate study area.

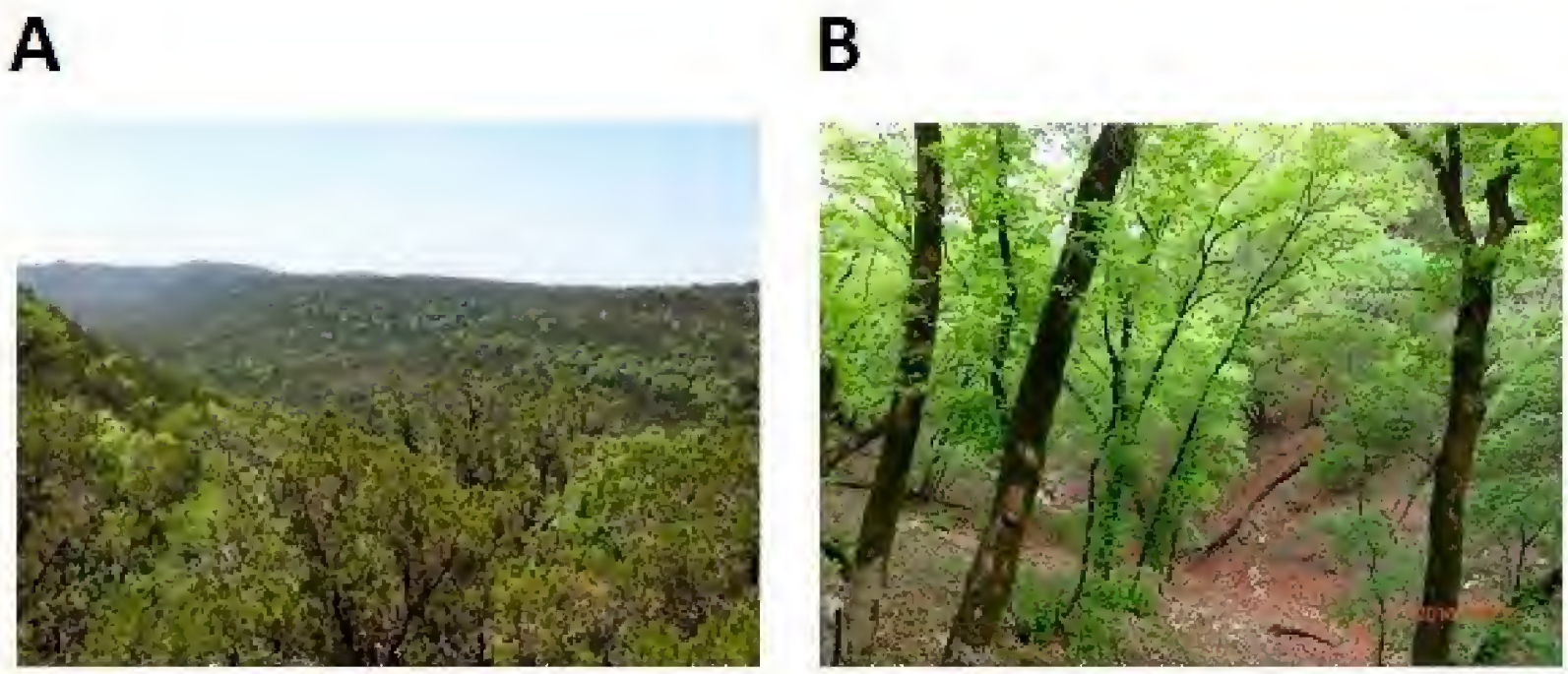

Figure 2. The first picture (A) shows a picture from ground level looking northwest over a canyon where many *A. grandidentatum* trees were found. The second photograph (B) is a picture below the *A. grandidentatum* canopy showing many of the mature tree stems and the very open nature of the community.

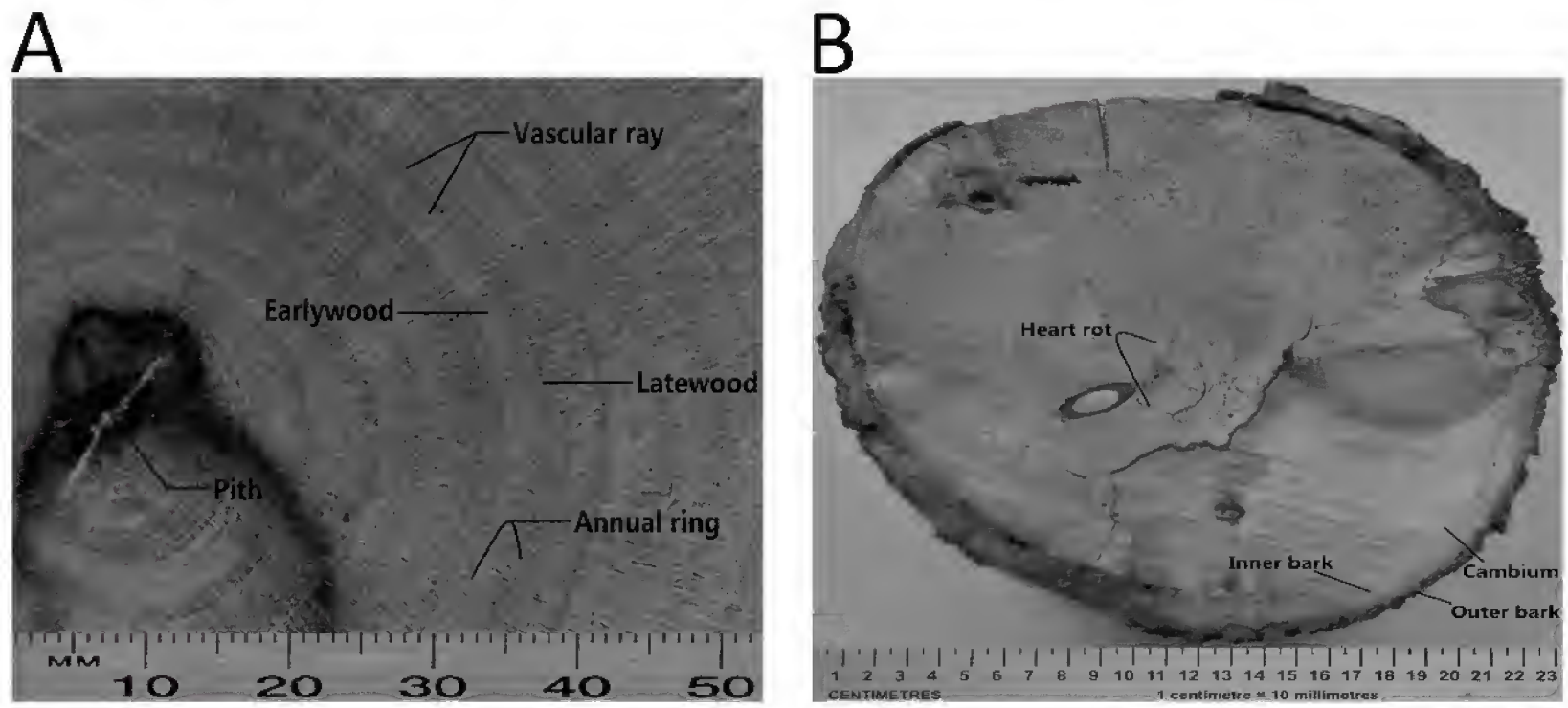

Figure 3. (A) This picture shows an intact stem cross-section with the pith, vascular rays, earlywood, latewood and a number of annual rings. (B) This picture shows a stem with heart rot, cambium, inner and outer bark. (C) This picture shows a completely dead stem with a number of insect holes in the stem (probably beetles) and the bark is missing. Note the scale (bottom) in all of the figures.

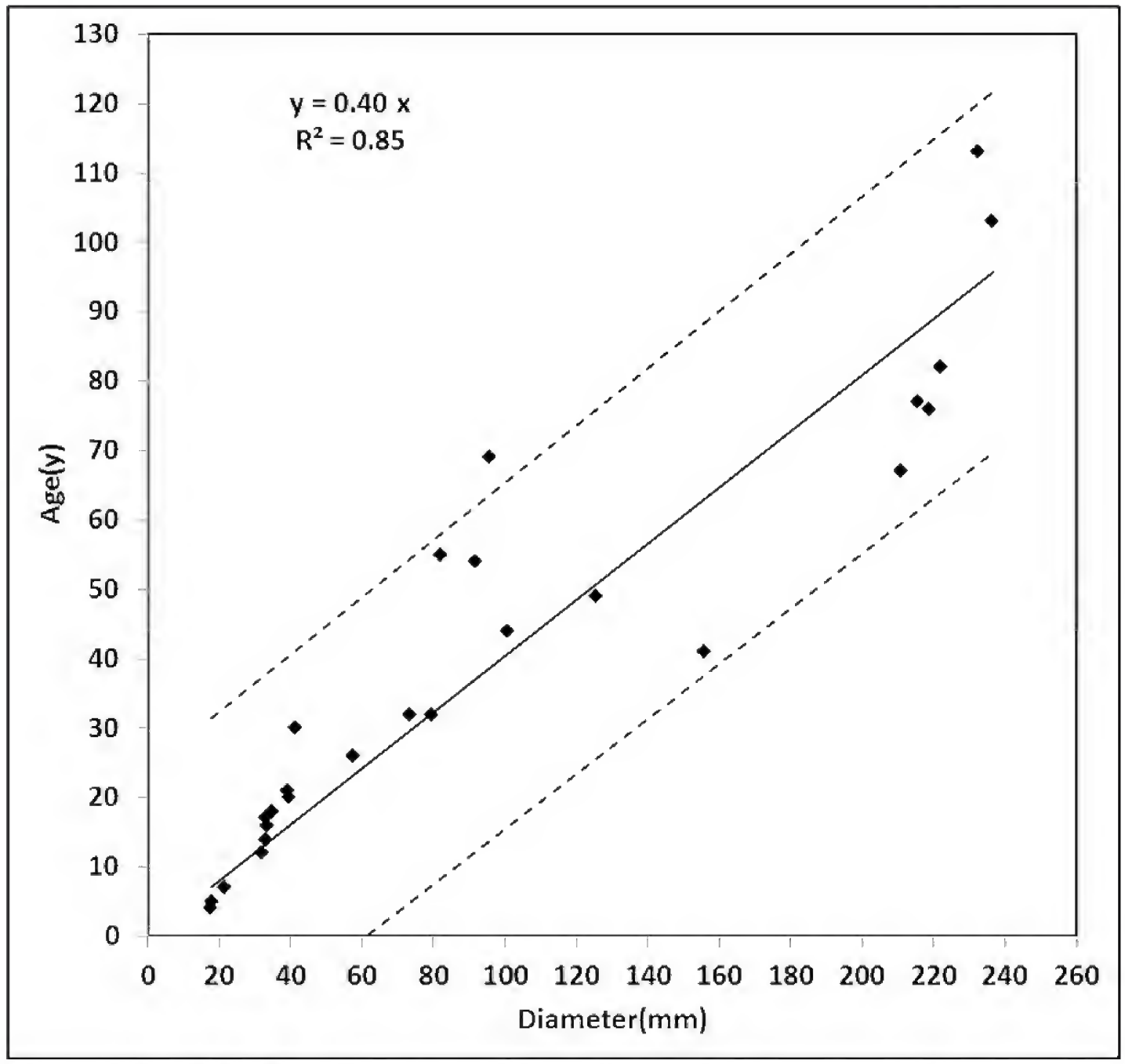

Figure 4. This figure represents a linear regression of age on diameter of the 26 samples of *Acer grandidentatum* trees measured. The two dashed lines stand for the upper and lower bounds of the 95% confidence interval for the regression. The mean value of the confidence interval for each bound is 24.22 units above or below the regression line.