Overview of *Kallstroemia* (Zygophyllaceae) in the USA and Mexico, and description of a new species: *Kallstroemia porteri*

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

ABSTRACT

As treated by Porter (1969), *Kallstroemia* was recognized as having 7 species in the USA and 8 species in Mexico, many of these occurring on both sides of the border. I have described an additional novelty, **K. porter** B.L. Turner, **sp. nov**., this occurring in central Mexico, bringing the number of species in the latter to 9. Distribution maps for all of the taxa occurring in the two regions are provided. Published on-line www.phytologia.org *Phytologia 98(2): 89-99 (Apr 4, 2016).* ISSN 030319430.

KEY WORDS: *Kallstroemia*, Mexico, USA, distribution maps

For its time, Porter (1969) provided an extraordinary taxonomic study of the genus *Kallstroemia*, recognizing 17 species, most of these native to Mexico and/or the USA. Distribution maps were provided for all of the taxa, based upon the collections of numerous institutions. I have updated all of the maps, largely from collections assembled since his time, these mostly on file at LL-TEX or reported in the literature of various Floras and/or from the USDA web sites.

KALLSTROEMIA PORTERI B.L. Turner, **sp. nov. Fig. 1**

Annual, stems prostrate, 10-20 cm long, moderately pubescent with upwardly appressed or upwardly-recurved hairs, ca 0.5-1.0 mm long, with occasions spreading hairs 1.0 -1.5 mm long; stipules linear, 2-4 mm long, ca 0.5 mm wide; leaves obovate, 3-4 cm long, 2-3 cm wide, sparsely pubescent above and below with mostly appressed hairs; leaflets 3 pairs, obovate-oblong, 10-15 mm long, 5-10 mm wide; flowering peduncles 5-10 mm long; flowers ca 6 mm across; sepals subulate, 2-3 mm long, ca 0.5 mm wide, in flower longer than style, in fruit clasping or loosely clinging to the mericarps and of ca the same length; petals orange, broadly obovate, 2-3 mm long, ca 1 mm wide; stamens as long as style; anthers ovate, ca 0.5 mm wide, yellow; ovary densely white-pubescent, ca 1 mm in diameter; style ca 1 mm long; stigma ovate or sub-capitate, ca 0.5 mm long, smooth; fruit ovoid, ca 6 mm wide, 4 mm high, strigillose-pubescent; beak 3 mm long, ca as long as body of fruit.

In the treatment of Porter (1969), **K. porteri** will key to or near **K. hirsutissima**, which it resembles, but the former has smaller flowers (ca 5 mm across vs 5-10 mm) and smaller orange petals (vs yellow), these broadly obovate in shape (vs linear-ovate). The petals of **K. hirsutissima** mostly vary in length from 3-6 mm long (rarely abortive), but so far as known, are never as small or broadly obovate and orange as in the novelty; according to Porter, **K. hirsutissima** is part of the Chihuahuan Desert flora (Maps 6, 12), "Found from sea level to about 1700 m, mainly at higher elevations." In contrast, according to label data, **K. porteri** occurs from 2000-2800 m in more forested habitats of central Mexico (Map 14).

It should be noted that Porter himself examined one of the sheets of this taxon (*McVaugh 16628,* cited below) but failed to render an identification, placing a "?" between the genus name and that of the species.

TYPE: **MEXICO. PUEBLA: Mpio. Caltepec,** "Cerro El Gavilan, al SE de Caltepec," ca 2320 m, 18° 12' N, 97° 30' W, reportedly locally abundant in igneous soils, 14 Jul 1986, *Pedro Tenorio L. 11799* [with A. Salinas T. & Dawn Frame] (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. AGUASCLIENTES:** "Highway to Ojuelos," 9 mi E of Aguacalientes, 2000 m, "Brush-covered hills among shrubs and small trees...Abundant, intermixed with K. rosei." 8-9 Aug 1958, *McVaugh 16628* (TEX). **GUANAJUATO: Mpio. San Luis de la Paz**, "Cerca de Pregon," 2100 m, 22 Aug 1988, *Rzedowski 47068* (TEX). **PUEBLA: Mpio. Caltepec,** Barranca del Agua Frio, 1.5 km W of San Luis Atolotitlan, 2100-2800 m, 5 Jul 1983, *Tenorio L. 4038* (TEX).

It's a pleasure to name this taxon for Duncan M. Porter, who contributed an exceptional study of the genus in 1969, for its time very through at every level. He is still deeply involved in things systematic as an Emeritus Professor of Biological Sciences at Virginia Tech University.

ACKNOWLEDGEMENTS

Jana Kos provided editorial input; dot maps are largely based upon the citations of Porter (1969) and specimens on file at LL-TEX (cf. Turner et al. 2003), except as noted in the above first paragraph.

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Turner, B.L. et al. 2003. *Kallstroemia*, in Sida, Bot. Misc. 24, Atlas of the Vascular Plants of Texas 1: 616-617.

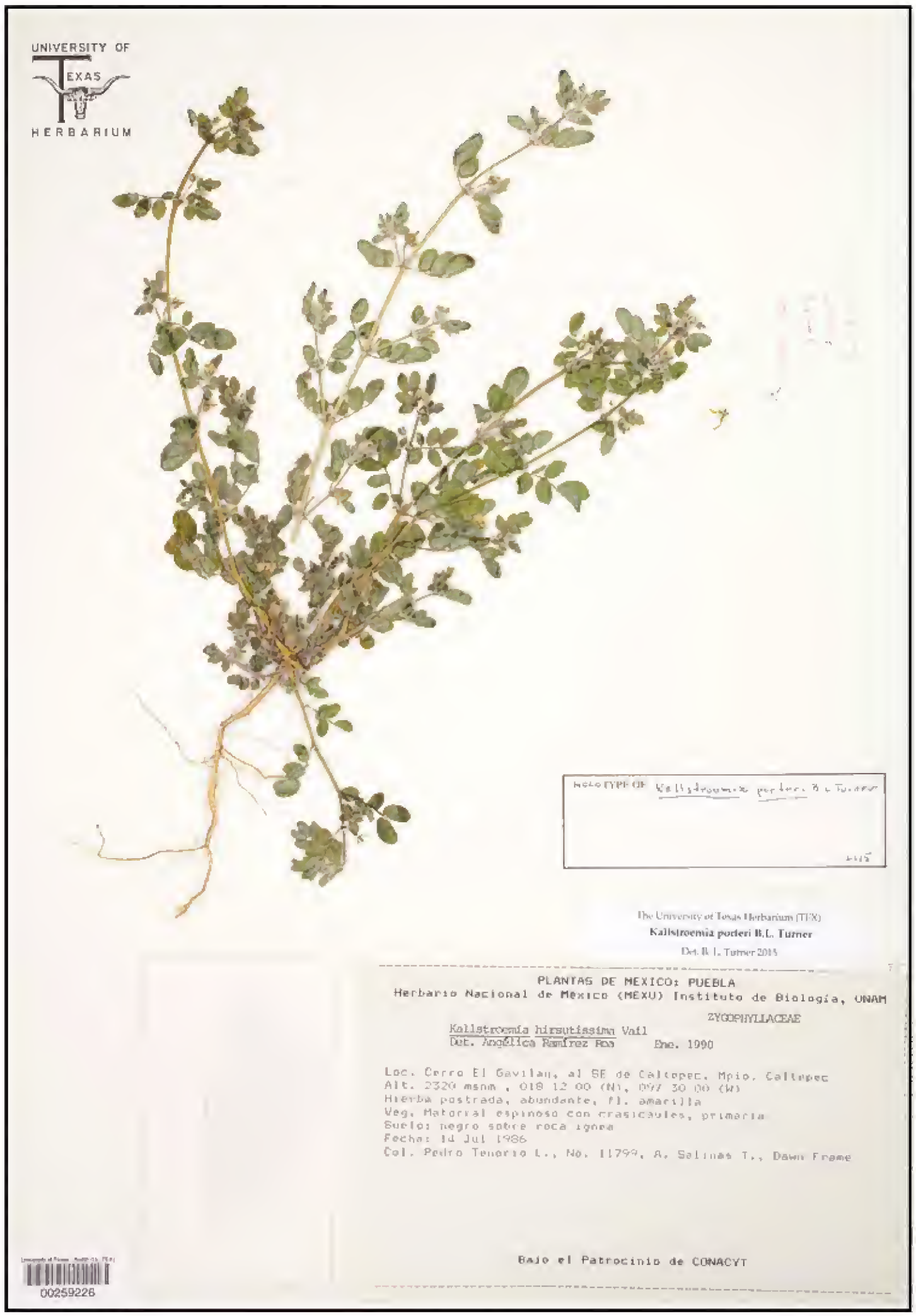
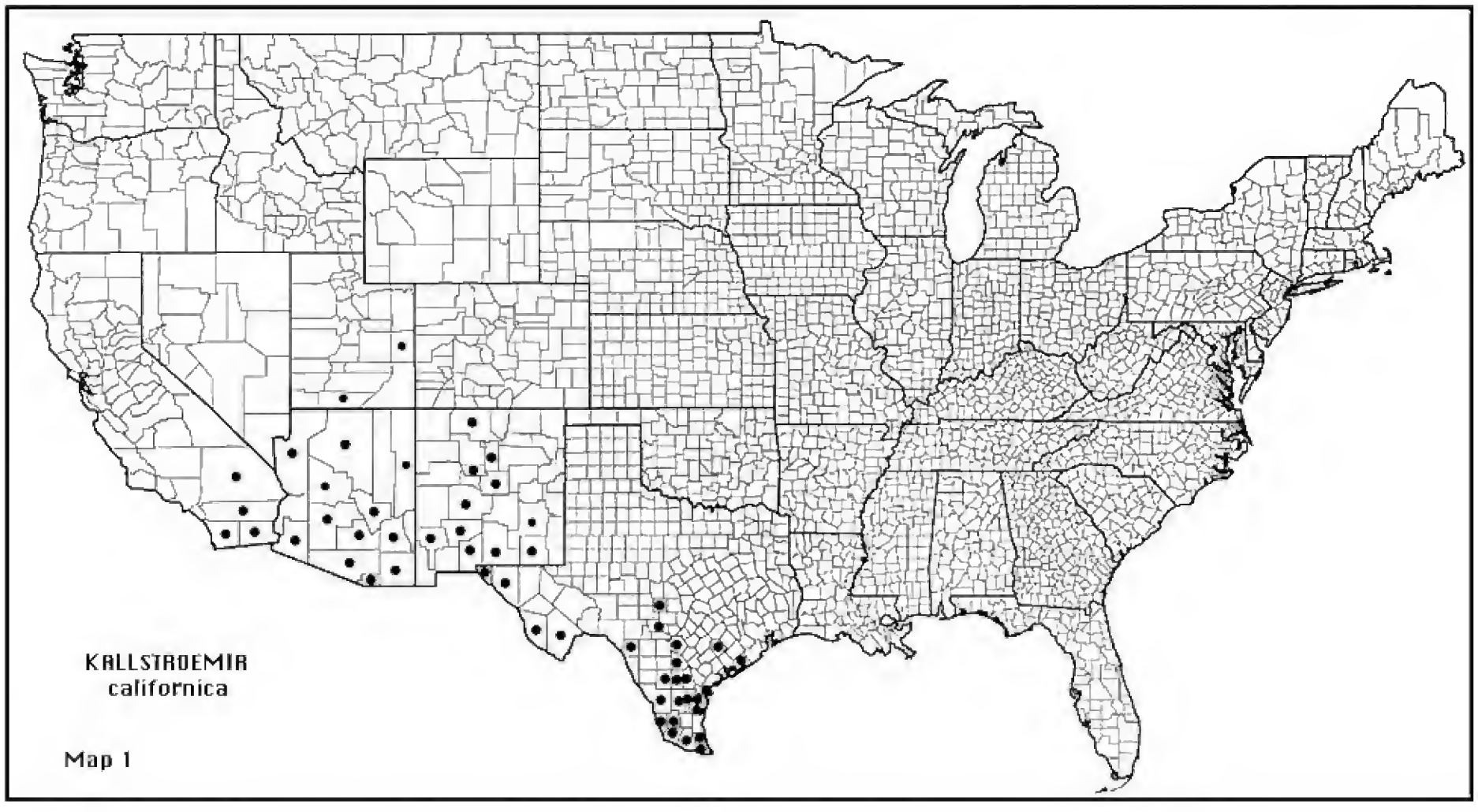
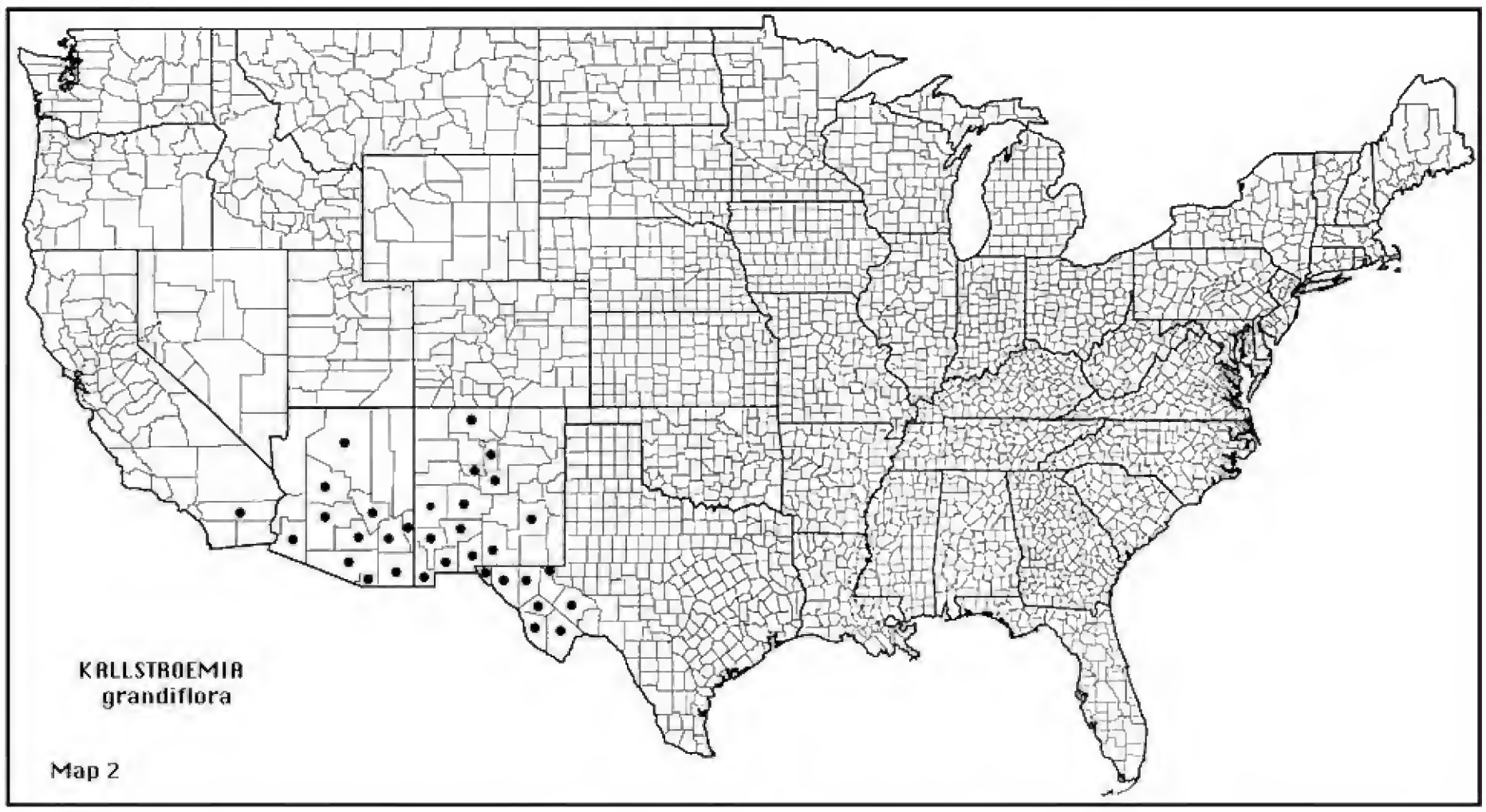


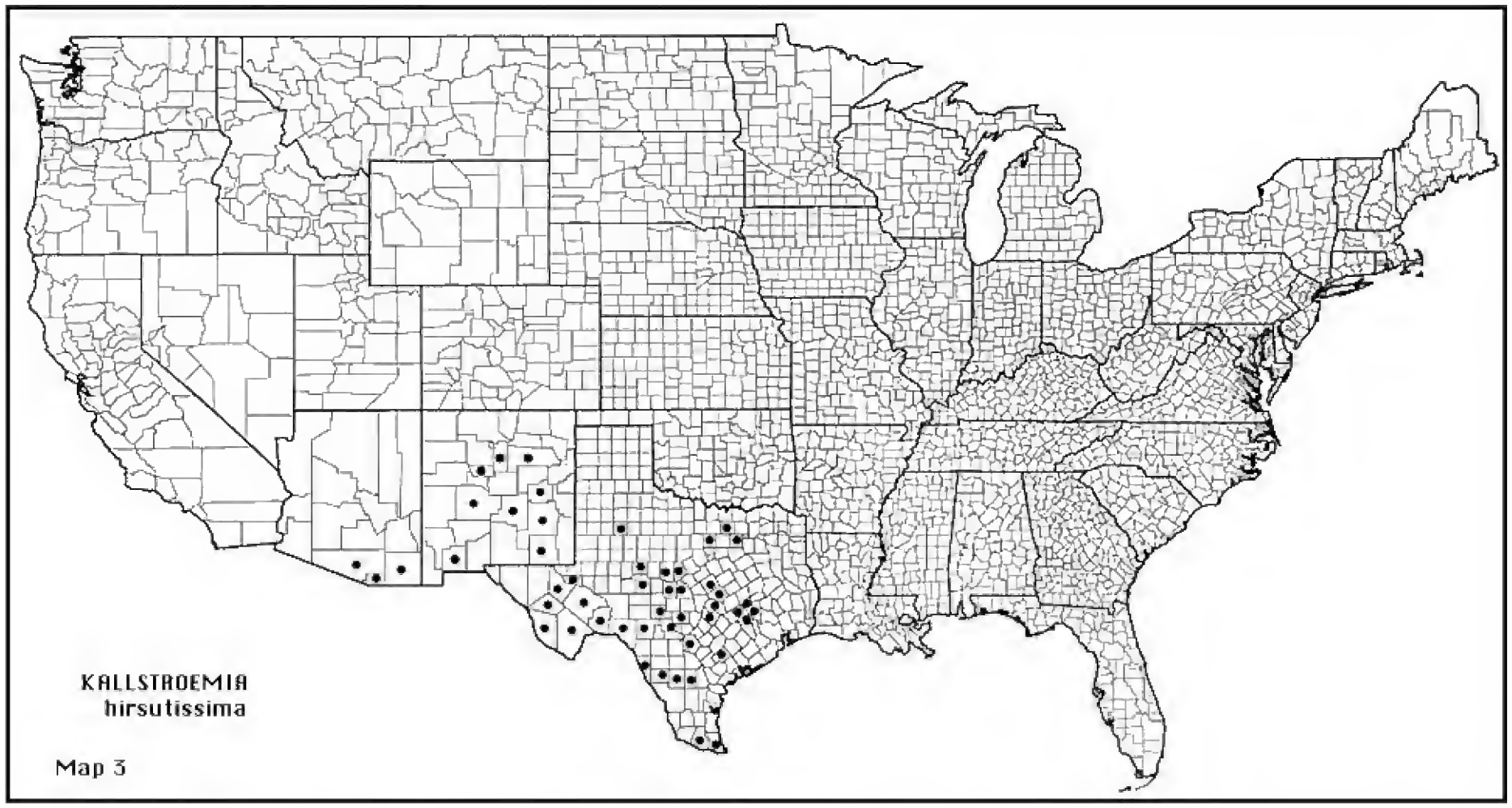
Fig1. Holotype, *Kallstroemia porteri.*



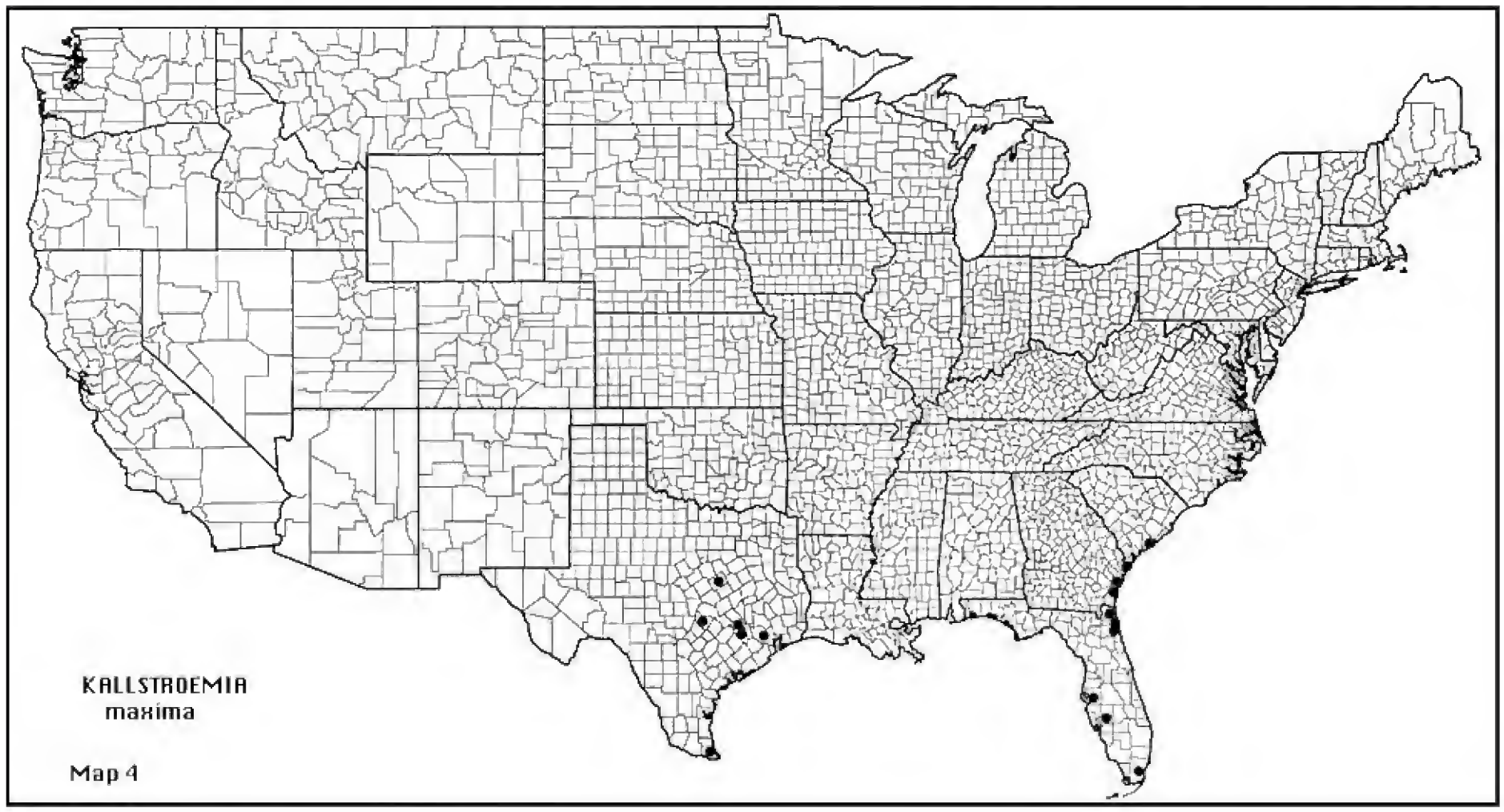
Map 1



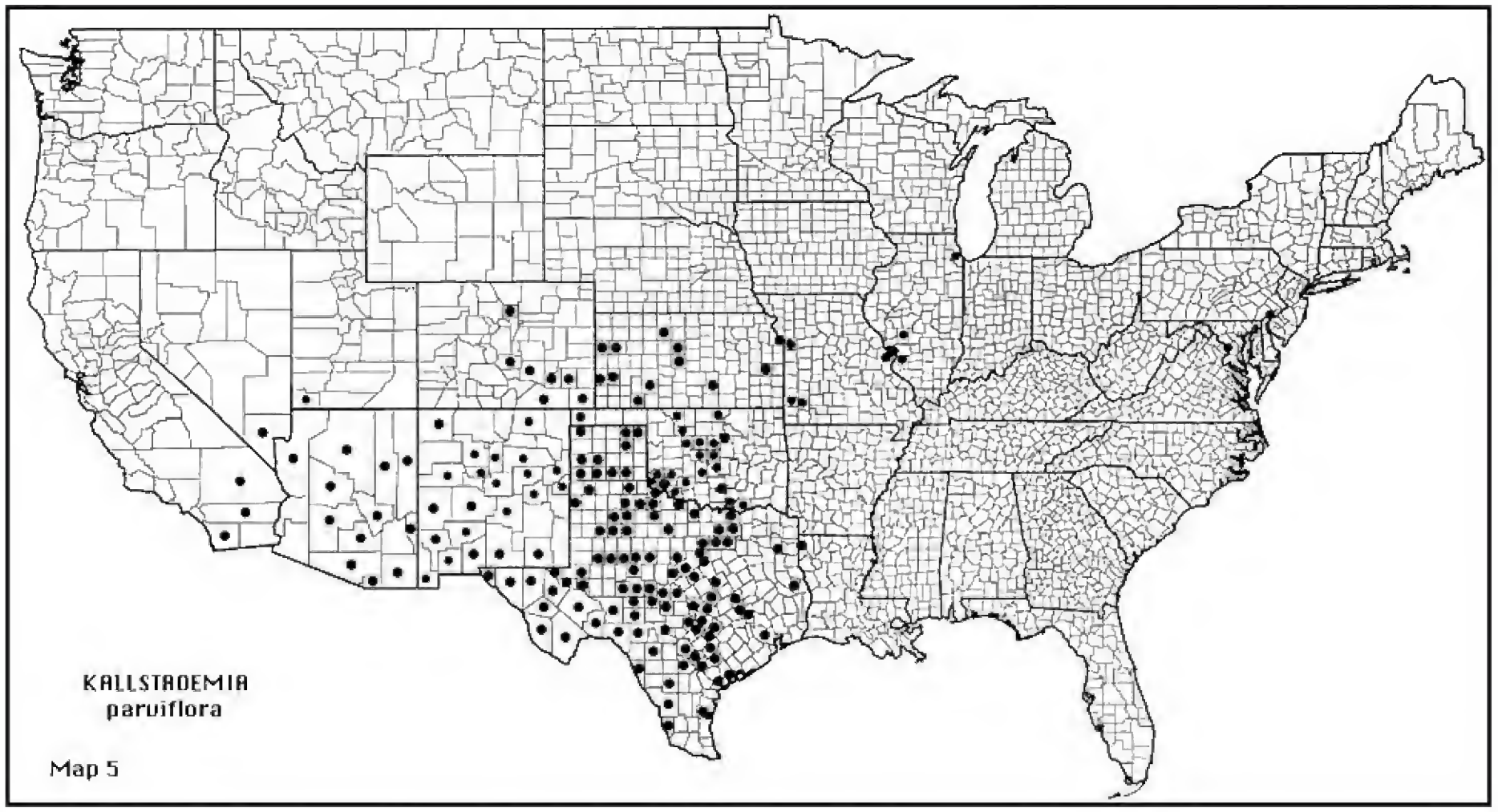
Map 2



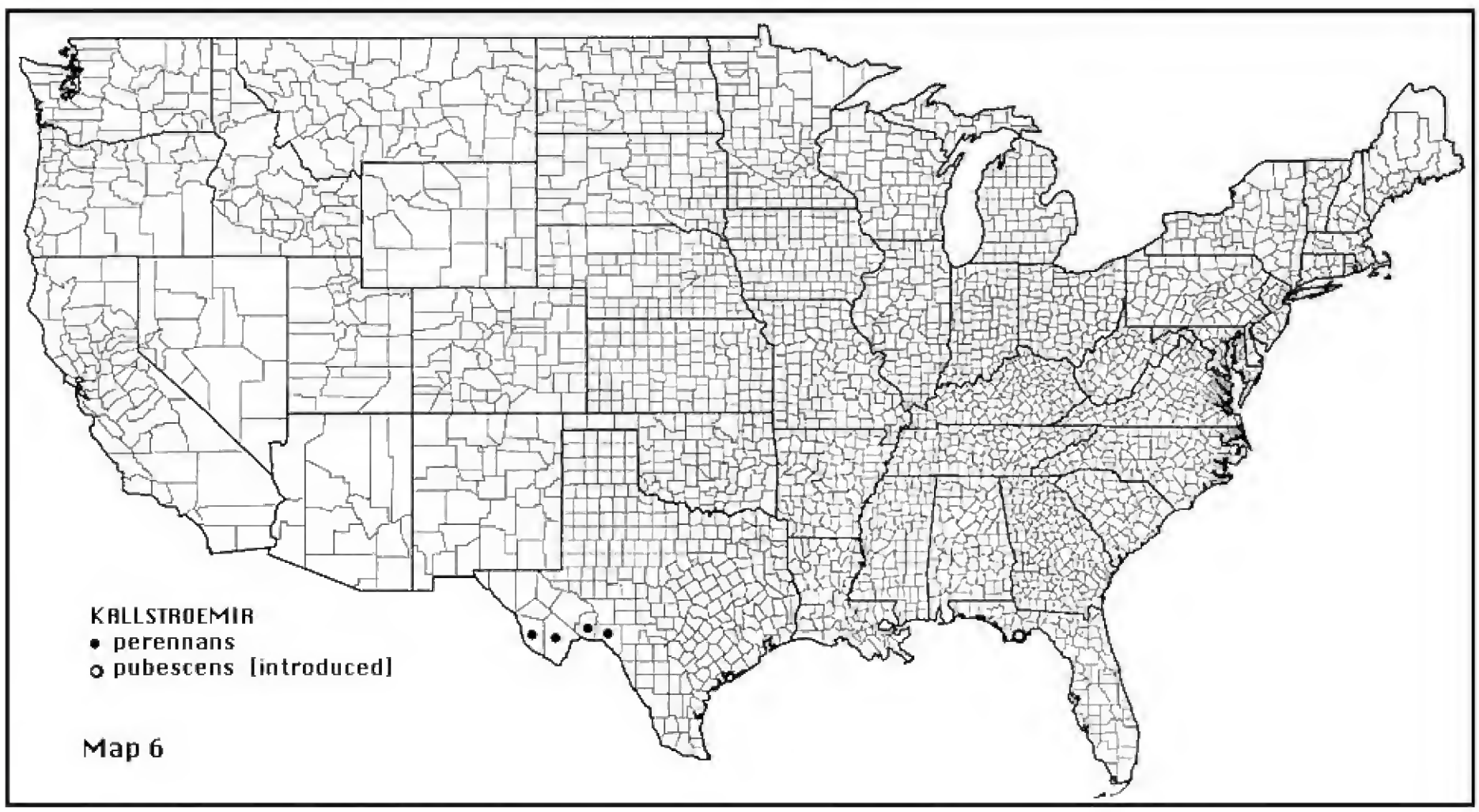
Map 3



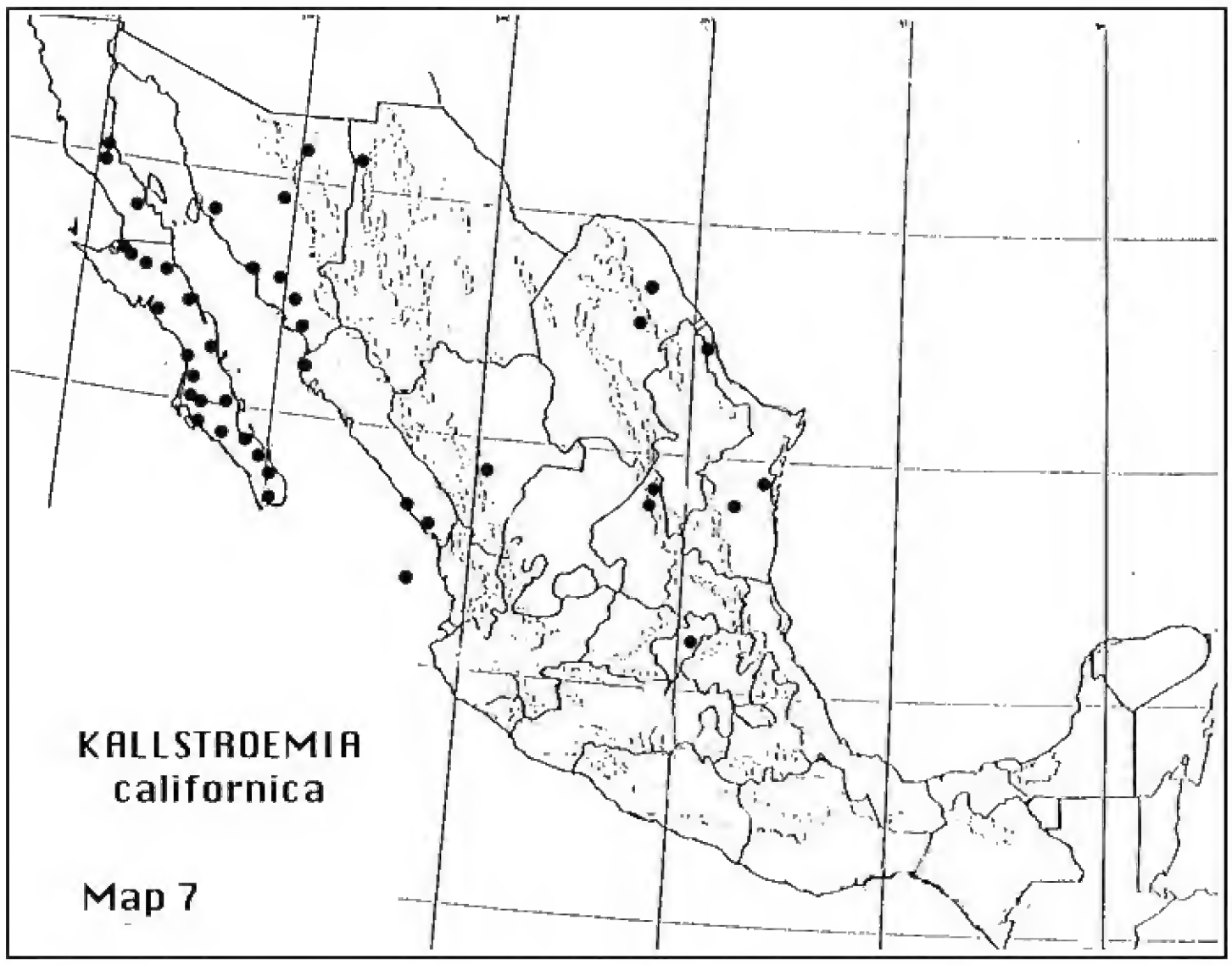
Map 4


KALLSTROEMIA
paruiflora

Map 5

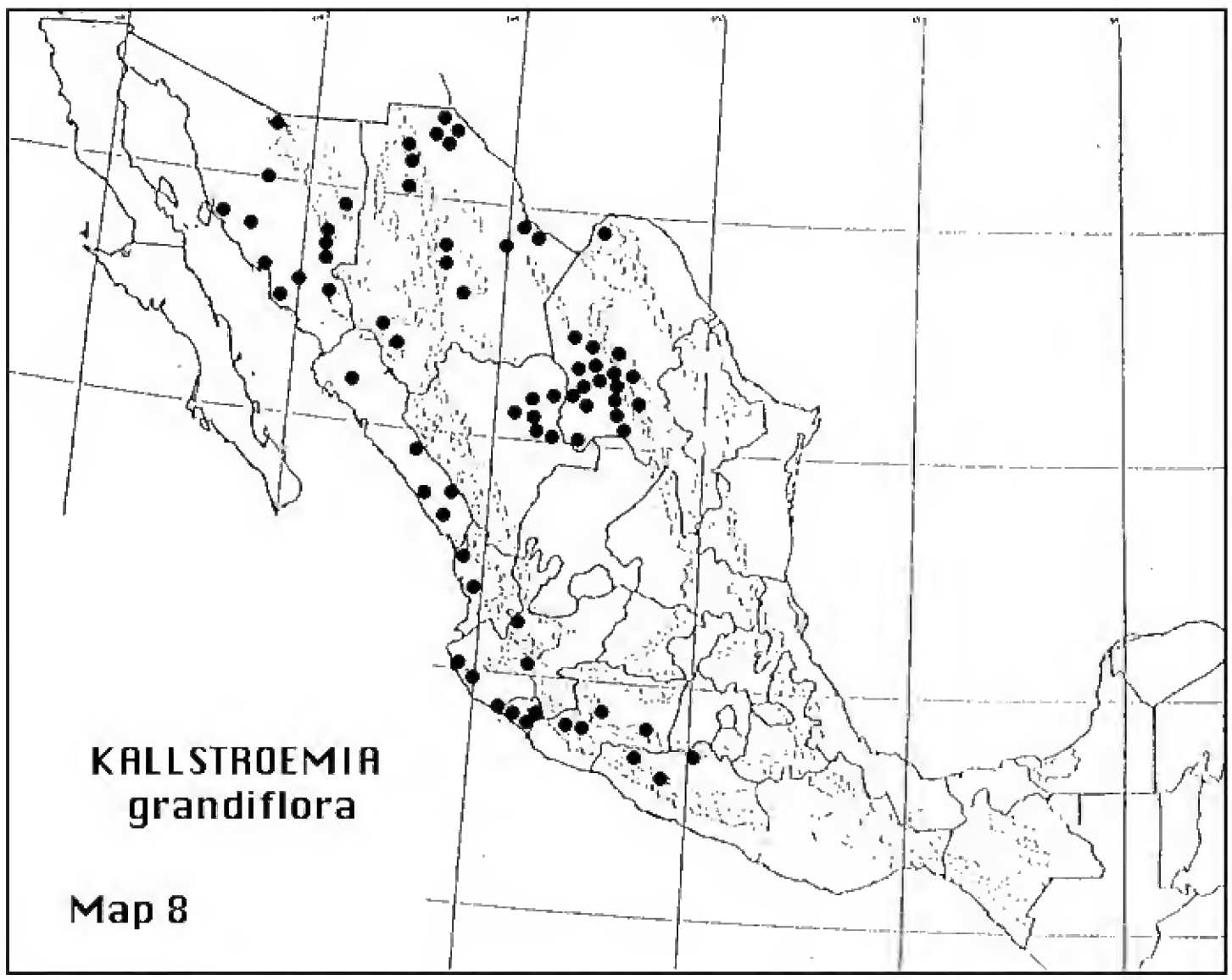

KALLSTROEMIA
• perennans
o pubescens [introduced]

Map 6



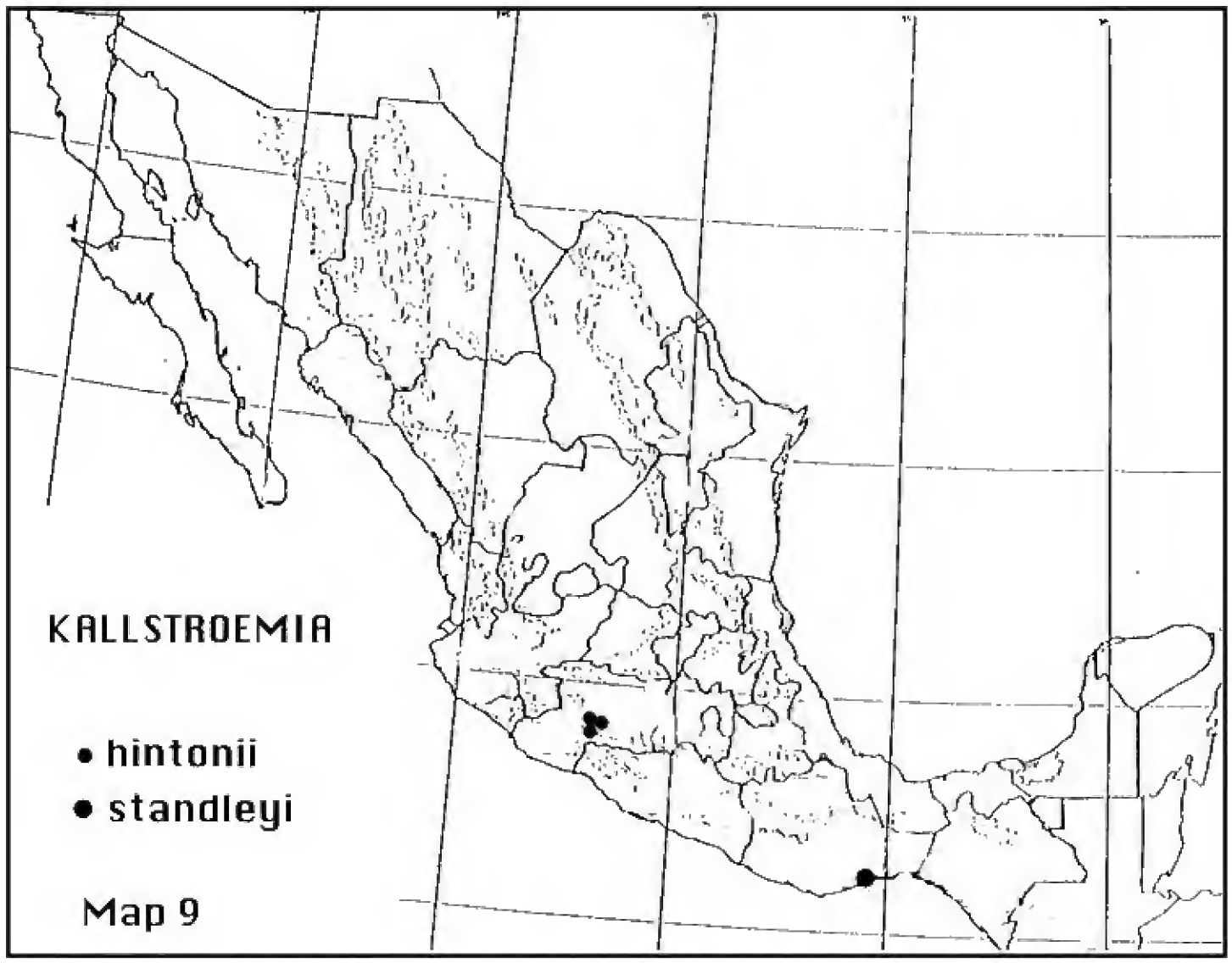
KALLSTROEMIA
californica

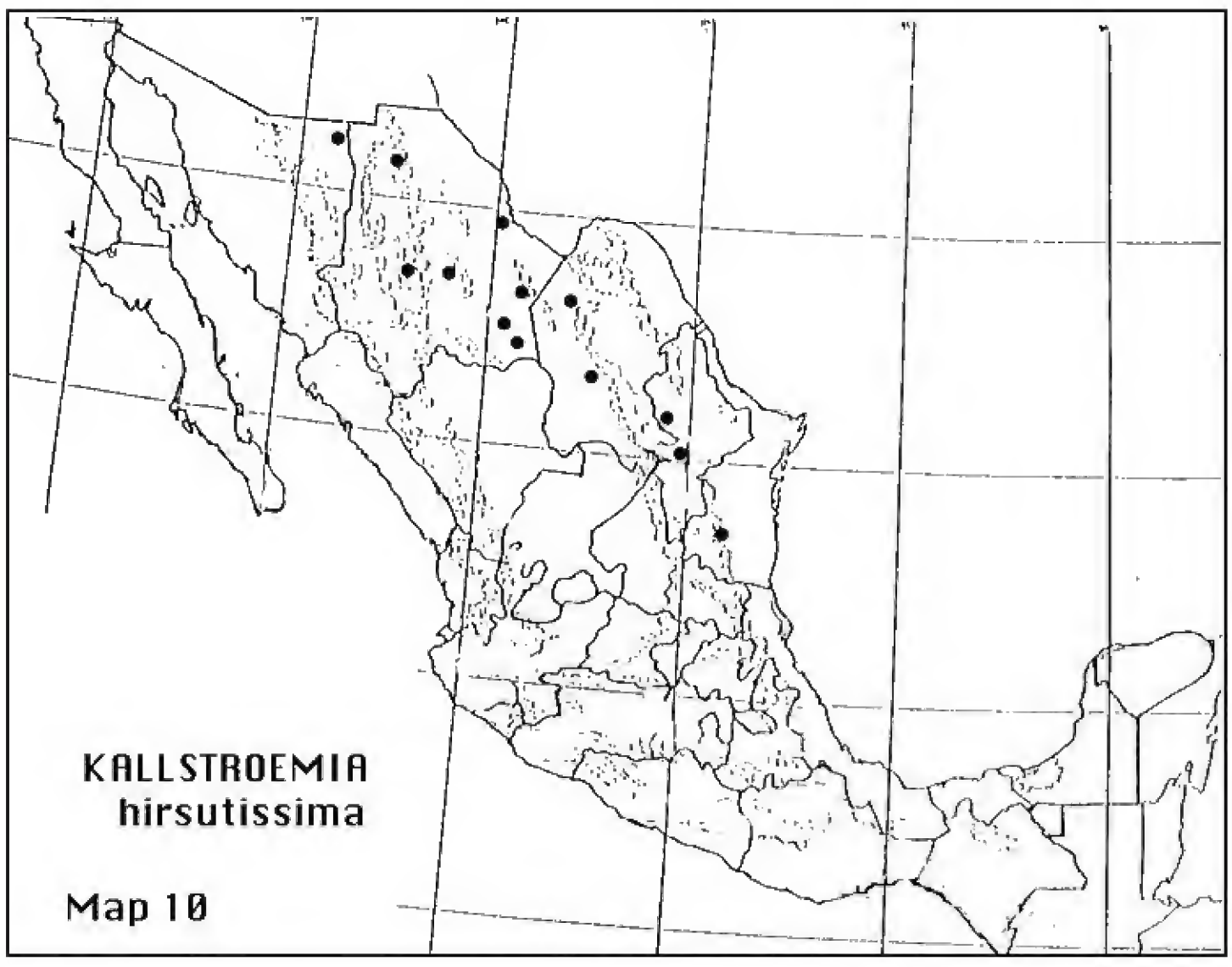
Map 7

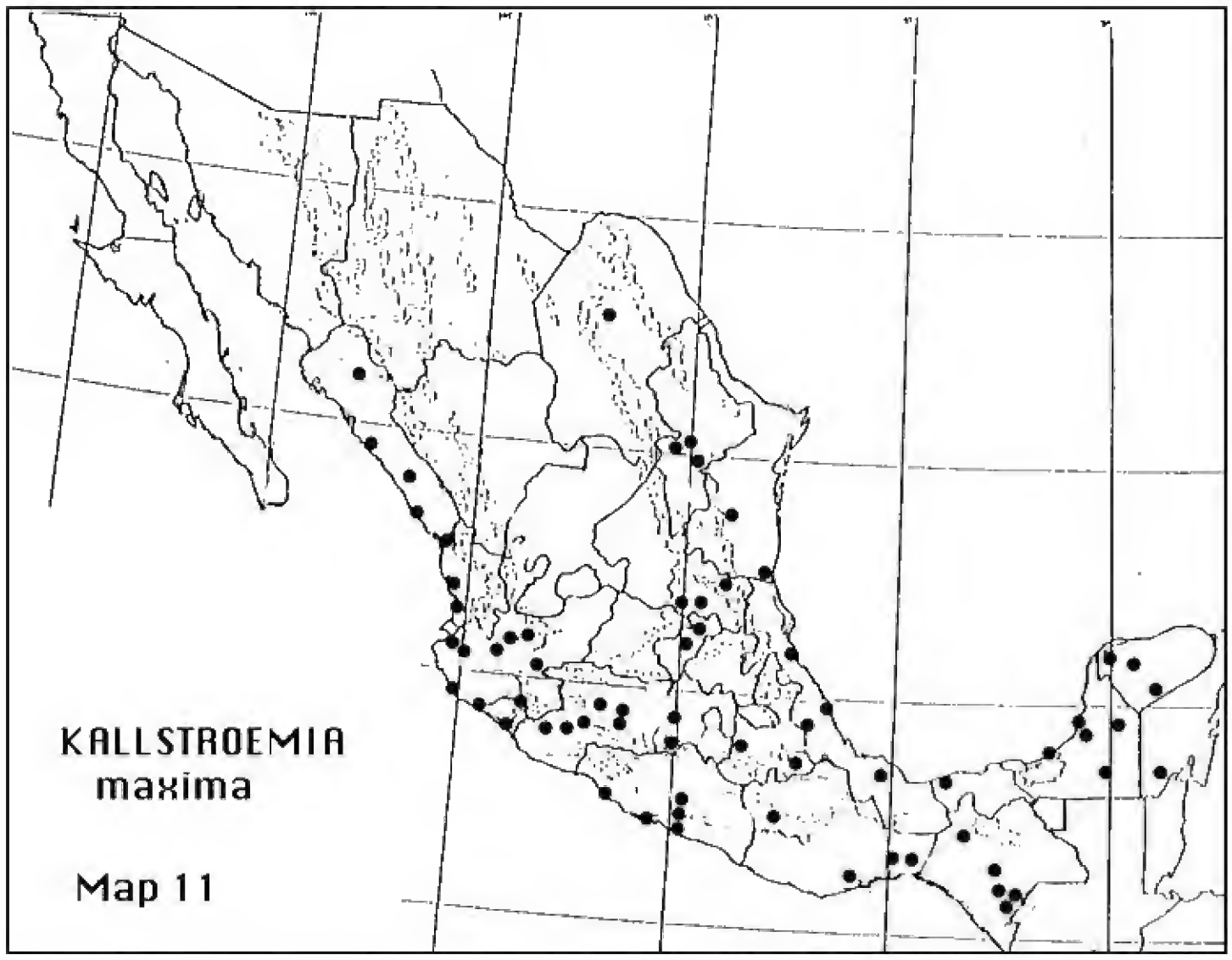


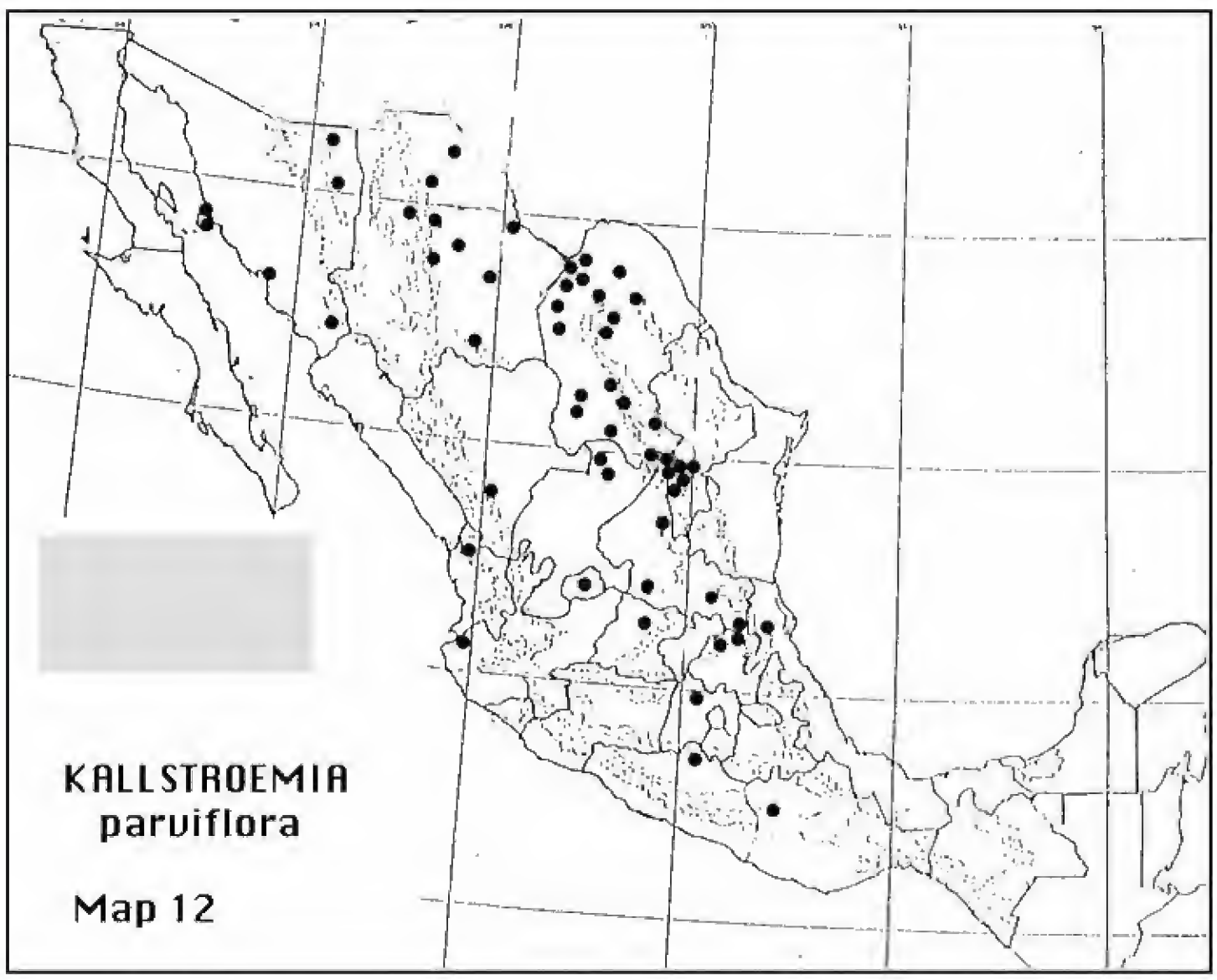
KALLSTROEMIA
grandiflora

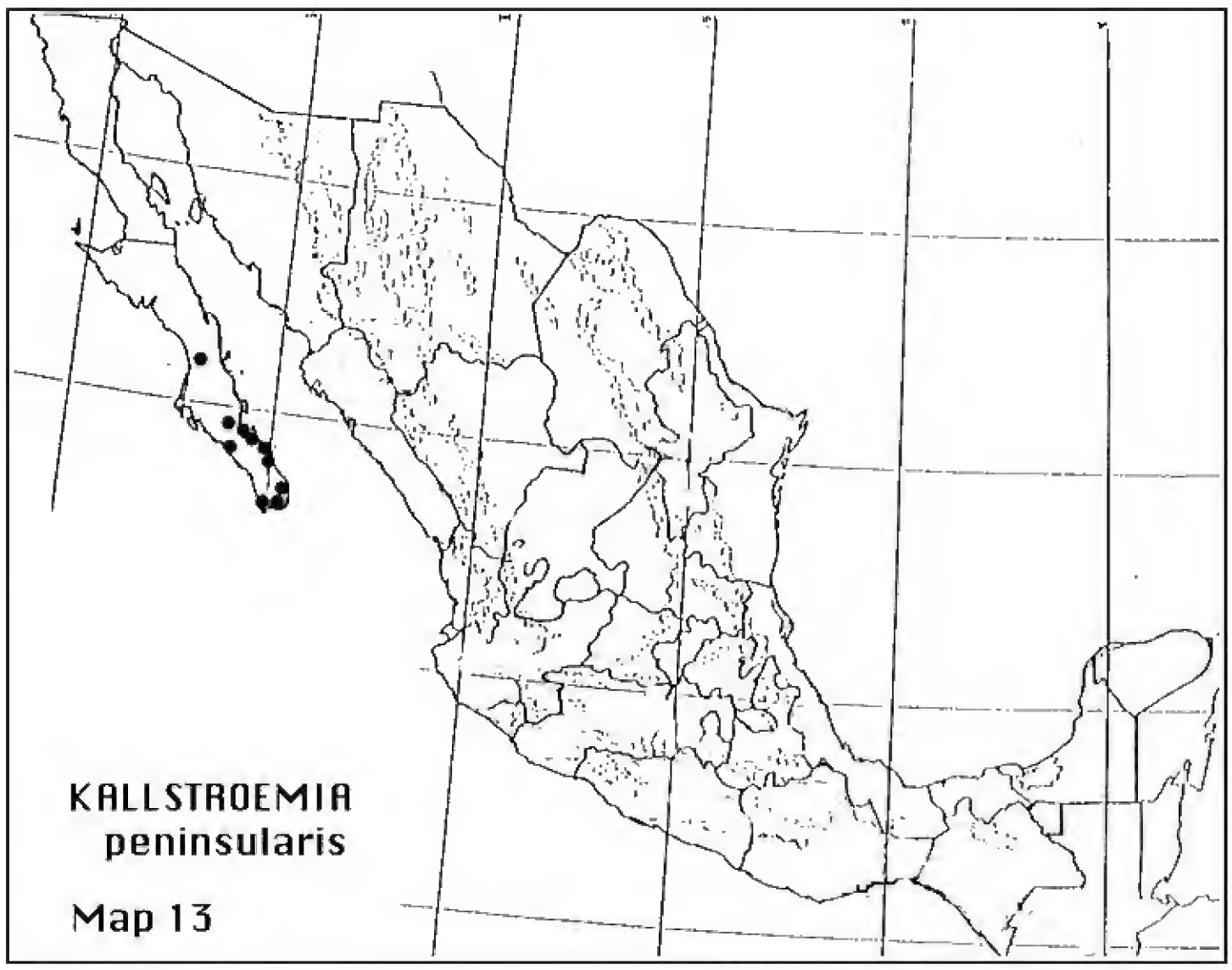
Map 8

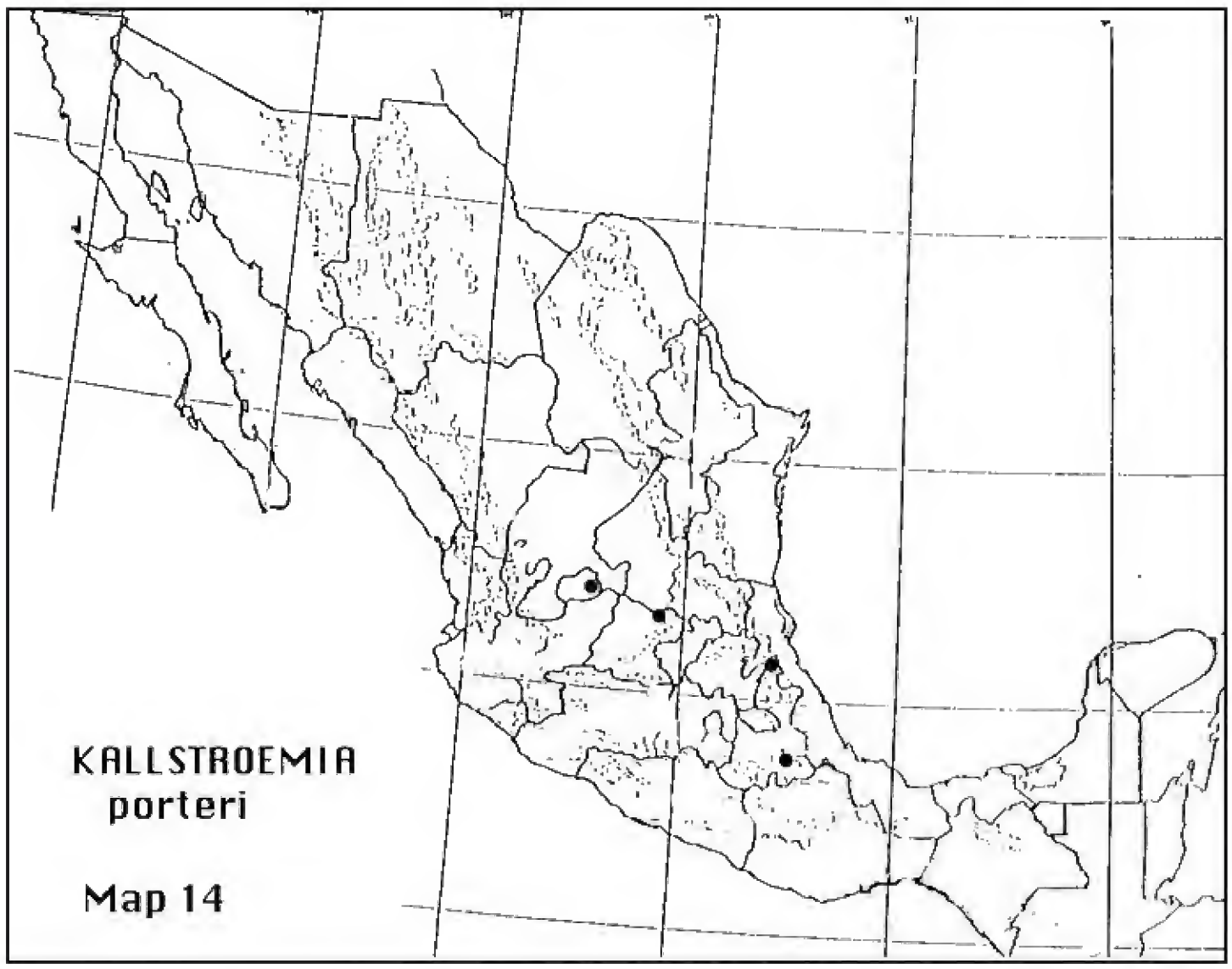

KALLSTROEMIA
• hintonii
• standleyi
Map 9

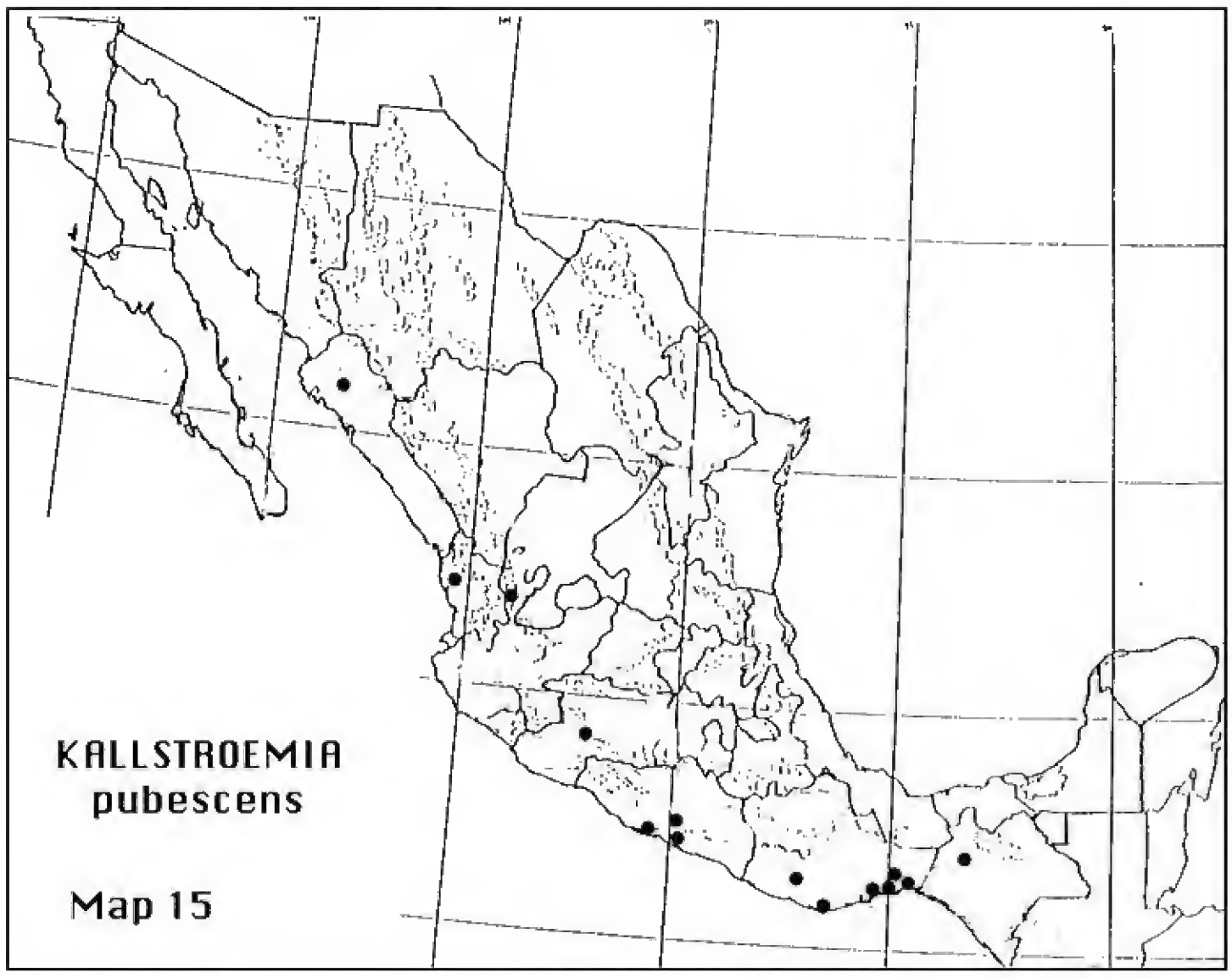

KALLSTROEMIA
hirsutissima
Map 10

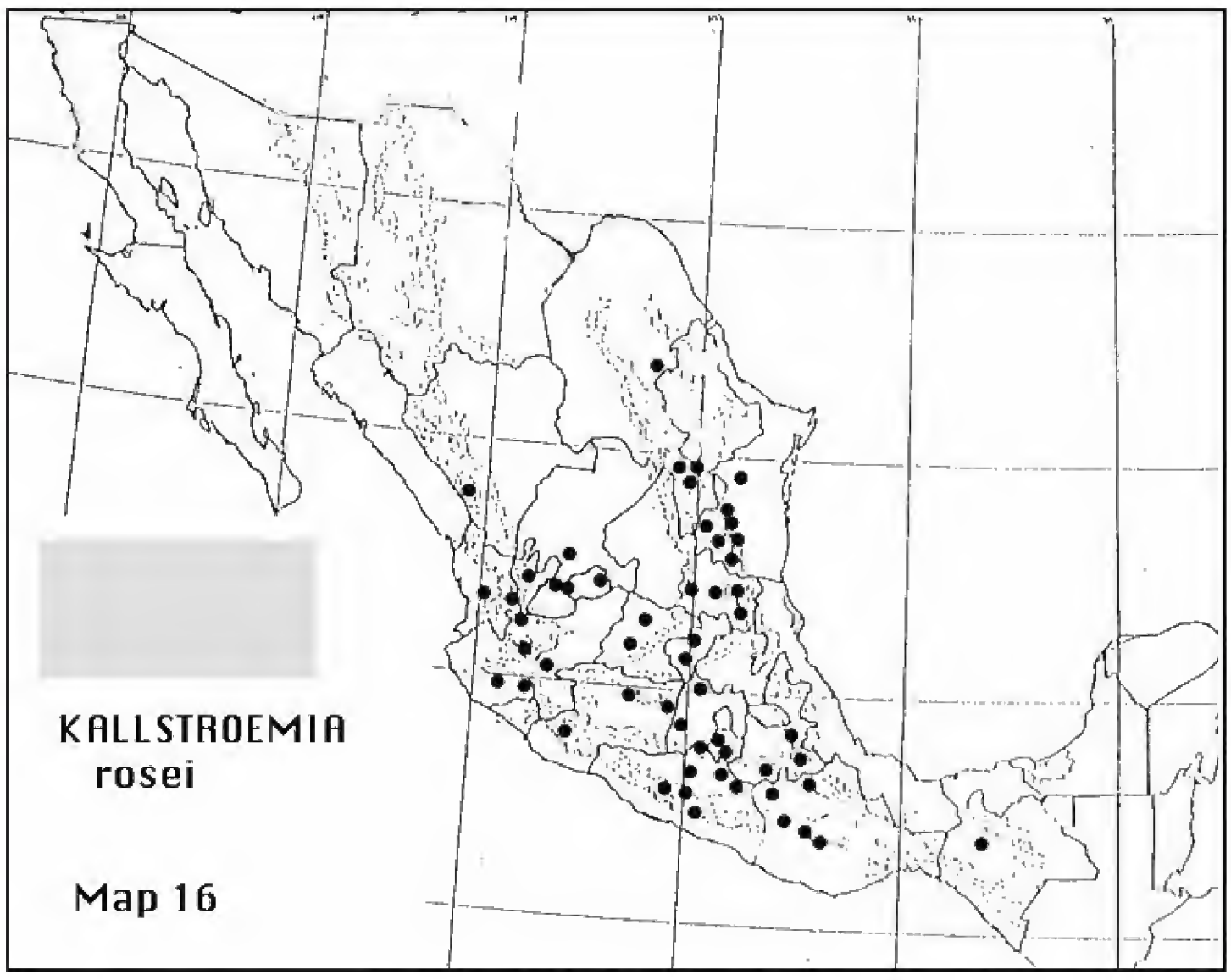

KALLSTROEMIA
maxima
Map 11


KALLSTROEMIA
parviflora
Map 12


KALLSTROEMIA
peninsularis
Map 13


KALLSTROEMIA
porteri
Map 14


KALLSTROEMIA
pubescens
Map 15


KALLSTROEMIA
rosei
Map 16

Chloroplast capture in *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev, from the Balkan peninsula: A new variety with a history of hybridization with *J. thurifera*

Robert P. Adams
Biology Department, Baylor University, Gruver Lab, Gruver, TX 79040, USA
Robert_Adams@baylor.edu

Andrea E. Schwarzbach
Department of Health and Biomedical Sciences, University of Texas Rio Grande Valley
Brownsville, TX, 78520, USA.

and

Alexander N. Tashev
University of Forestry, Dept. of Dendrology, 10, Kliment Ochridsky Blvd., 1756 Sofia, Bulgaria

ABSTRACT

An example of chloroplast capture has been identified in *Juniperus sabina* from Bulgaria and Greece in the Balkan peninsula. Nuclear DNA and overall morphology clearly indicate a close relationship to *Juniperus sabina*, whereas the cpDNA from these populations is very uniform and is nearly identical to that of *J. thurifera*, an unrelated species currently growing in France, Spain and Morocco. The new taxon is recognized as *Juniperus sabina* var. *balkanensis* R. P. Adams and A. Tashev. At present, this new variety is known only from locations in Bulgaria and Greece. Published on-line www.phytologia.org *Phytologia 98(2): 100-111 (Apr 4, 2016).* ISSN 030319430.

KEY WORDS: *Juniperus sabina var. balkanensis* var. nov., *J. sabina*, *J. thurifera,* relictual hybrid, nrDNA, petN-psbM, trnSG, trnDT, trnLF, leaf terpenoids, morphology, chloroplast capture.

For the past decade, the use of nrDNA and chloroplast (cp) markers has dominated phylogenetic research. However, 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 recent 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.

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.

The genus *Juniperus* consists of approximately 75 species (Adams, 2014), all of which grow in the northern hemisphere, although, *J. procera* Hochst. ex Endl. also grows southward along the rift mountains in East Africa into the southern hemisphere (Adams, 2014). The recent molecular phylogeny of the genus (Adams and Schwarzbach, 2013b) divides *Juniperus* into three sections: *Caryocedrus* (one species, *J. drupacea* Labill.); *Juniperus* (= *Oxycedrus*) with 14 species and *Sabina* (the remaining 60 species).

Section *Sabina* can be further divided into junipers with serrate and those with entire (smooth) leaf margins. The serrate-leaf margined junipers are confined to the western hemisphere, except for *J. phoenicea*, which may have a greater affinity to the smooth-leaf margined junipers (Adams and Schwarzbach, 2013b).

The *Juniperus* of section *Sabina*, of the eastern hemisphere, can be further divided into two groups based on the number of seeds per female cone (often called berries) and female cone shape. The single seed/cone (single-seeded) *Juniperus* of the eastern hemisphere have cones that are ovoid with a noticeable pointed tip, whereas the multi-seeded *Juniperus* are generally globose and often have an irregular surface. *Juniperus sabina* L. is a smooth leaf-margined, multi-seeded juniper of the eastern hemisphere. It is very widely distributed from Spain through Europe to Kazakhstan, western China, Mongolia and Siberia (Fig. 1). *Juniperus sabina* has a range that is discontinuous between Europe and central Asia; the species is generally a shrub less than 1 m tall and ranges up to 1-2 m wide. But in the Sierra Nevada of Spain, it forms a horizontal shrub.

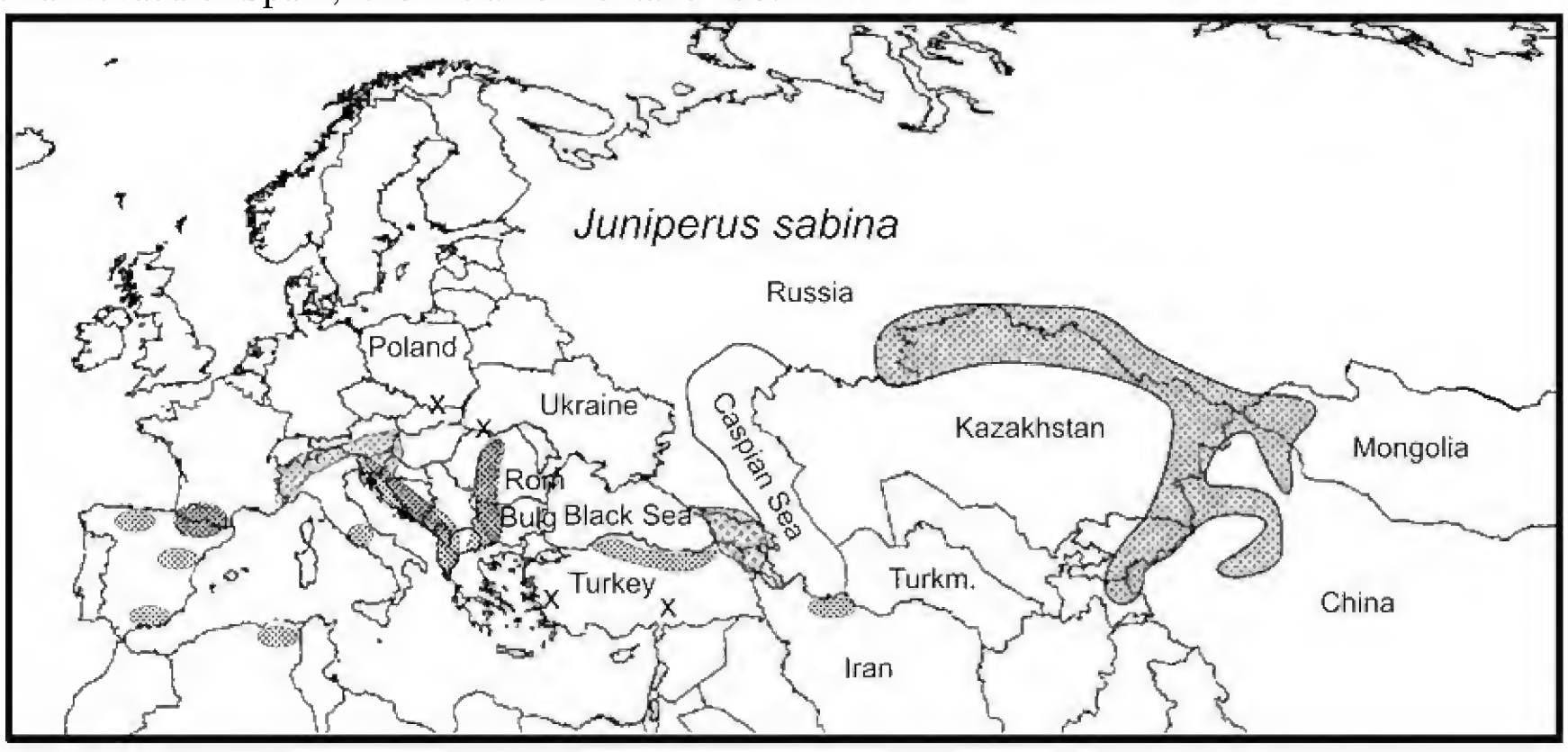


Fig. 1. Distribution of *J. sabina*. x = outlying populations of *J. sabina*.

DNA sequencing (Adams and Schwarzbach, 2013a), based on new collections of *J. sabina* var. *arenaria* from Lake Qinghai and a river bank in Gansu, as well as additional samples from Mongolia, has led to a different picture of the relationships in the *chinensis-erectopatens-davurica-sabina* complex (Fig. 2). Notice that *J. erectopatens* was 100% (posterior probabilities) supported as a distinct clade, as previously shown in both essential oils and RAPD data (Adams, 1999). There was no support for treating *J. erectopatens* as a synonym of *J. chinensis* (Farjon, 2005). *Juniperus erectopatens* is a cryptic species in its morphology, but it is quite distinct as an evolutionary unit in its terpenes, RAPD markers and DNA sequence data. *Juniperus chinensis* (and *J. procumbens*) were also well supported (100%) as being

distinct from *J. sabina* and *J. davurica* (Fig. 2), again as has been shown by their essential oils and RAPD data (Adams, 1999). Among the *J. sabina* , *J. davurica, J. d.* var. *arenaria* and *J. d.* var. *mongolensis* samples, there was some support for infraspecific taxa. The *J. sabina* plants from Mongolian sand dunes have recently been recognized as a new variety, *J. sabina* var. *mongolensis* R. P. Adams (2006).

It is noteworthy that Adams and Schwarzbach (2013a) included only one OTUs of *J. sabina* (Fig. 2) and that was from Europe (Switzerland). It grouped in a clade with *J. davurica* (Fig. 2).

Adams, Nguyen and Liu (2006) used Principal Coordinate analyses (PCO) of the leaf essential oils of these taxa to confirm (Fig. 3) that *J. chinensis* was distinct from the *J. sabina* - *davurica* complex (oval, Fig. 3). Adams, Nguyen and Liu (2006) also found that *J. davurica* var. *davurica* (DV, Fig. 3) had a terpenoid composition very similar to that of *J. d.* var. *arenaria* (AR, Qinghai sand dunes) and *J. d.* var. *mongolensis* plants growing on Mongolian sand dunes southwest of Ulan Batar (MS).

The Iberian *J. sabina* (SN, PY) plants' terpenoids were quite different (Fig. 3) from those of nearby Switzerland (SW) and central Asia (AM,TS, KZ).

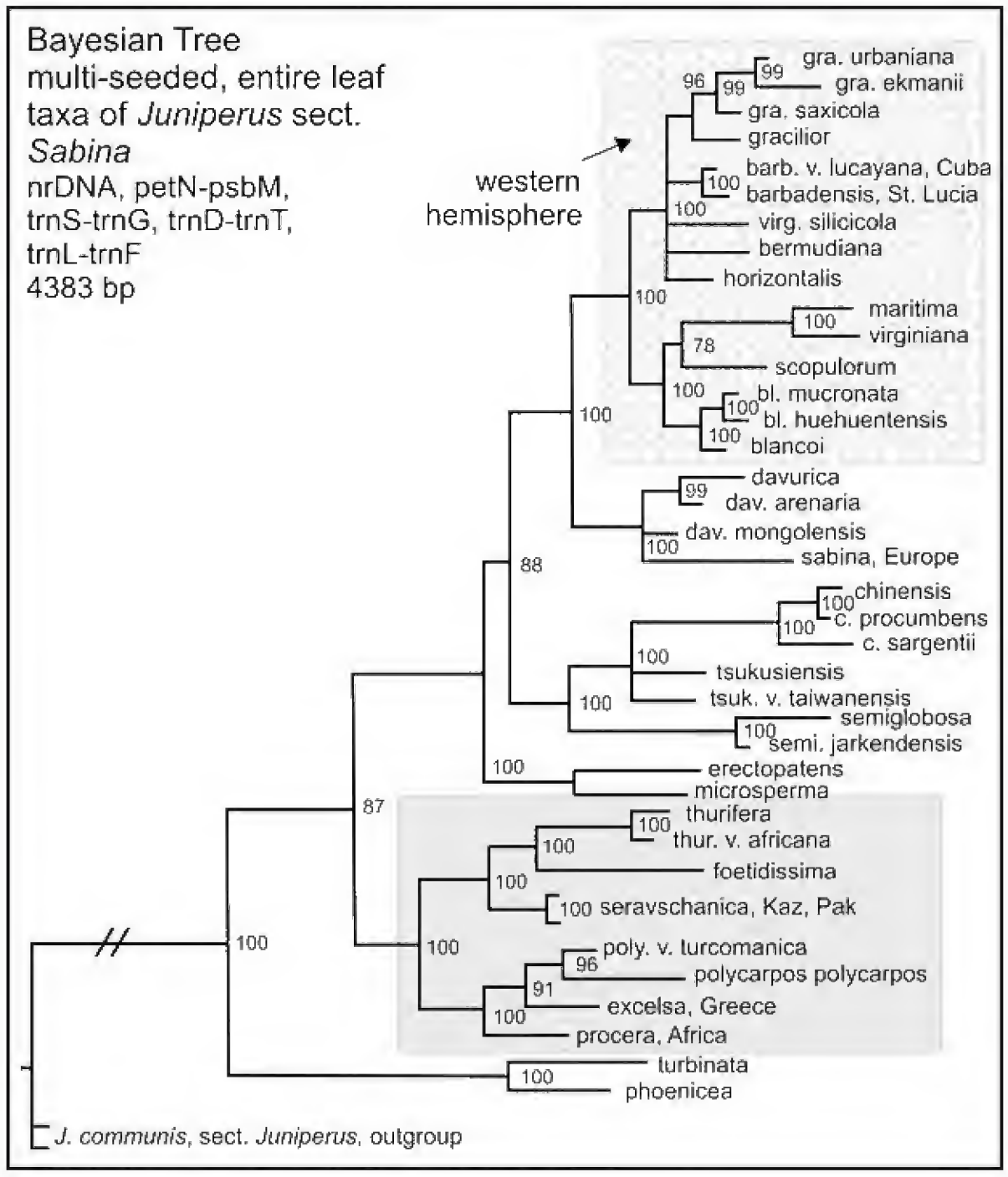


Fig. 2. Bayesian tree of multi-seeded, entire leaf taxa of *Juniperus* sect. *sabina* (from Adams 2014). Notice the clade of *davurica* and *sabina*. Numbers are posterior probabilities as percent.

In addition, Adams, Nguyen and Liu (2006) found the essential oils of *J. sabina* (sensu stricto) ranged (Fig. 3) from Kazakhstan (KZ), neighboring Xinjiang (TS) and the Altai Mtns., Mongolia (AM) to Switzerland (SW) to the Iberian Peninsula [Pyrenees (PY), Sierra Nevada, Spain (SN)].

Adams et al. (2007) examined RAPDs in the same populations studied for leaf volatile oils (Adams, Nguyen and Liu, 2006). They found that RAPDs data separated *J. sabina* (Europe, Tian Shan) from the more easterly, *J. s.* var. *davurica, J. s.* var. *arenaria and J. s.* var. *mongolensis*. There also appeared to be a trend in both the terpenoid and RAPDs from *J. sabina* in Spain, to the Pyrenees and Switzerland, thence to the Tian Shan, Xinjiang (Figs. 3, 4).

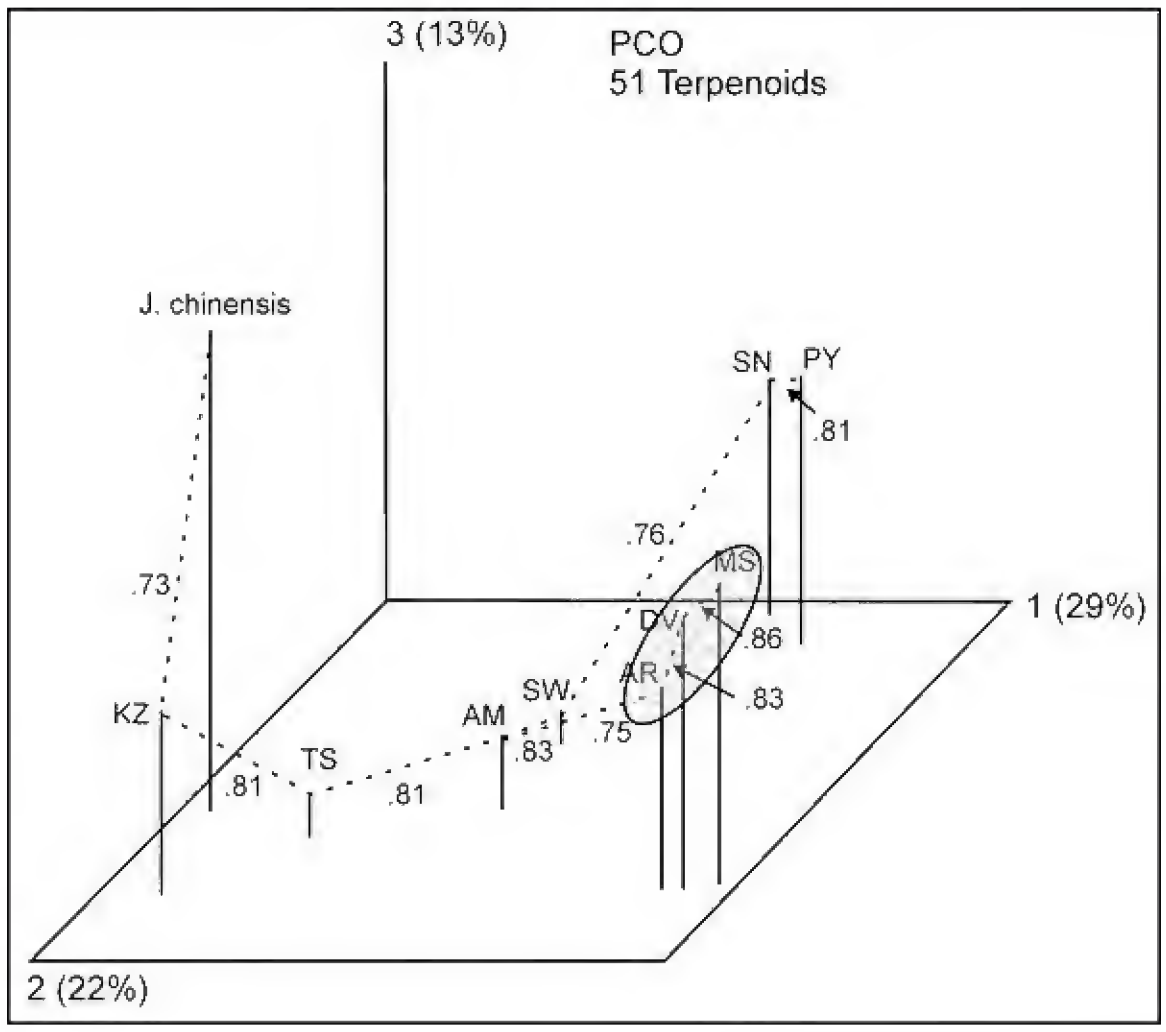


Fig. 3. Principal Coordinate Ordination (PCO) (from Adams, Nguyen and Liu, 2006) based on 51 terpenoids. Notice the distinct ordination of *J. chinensis* and the prostrate junipers of n China and Mongolia (AR = *J. d.* var. *arenaria*; DV = *J. davurica*; MS = *J. d.* var. *mongolensis*).
J. sabina:
KZ = Kazakhstan;
TS = Tian Shan Mtns., Xinjiang, China;
AM = Altai Mtns., w Mongolia;
SW = Switzerland;
PY = Pyrenees;
SN = Sierra Nevada, Spain

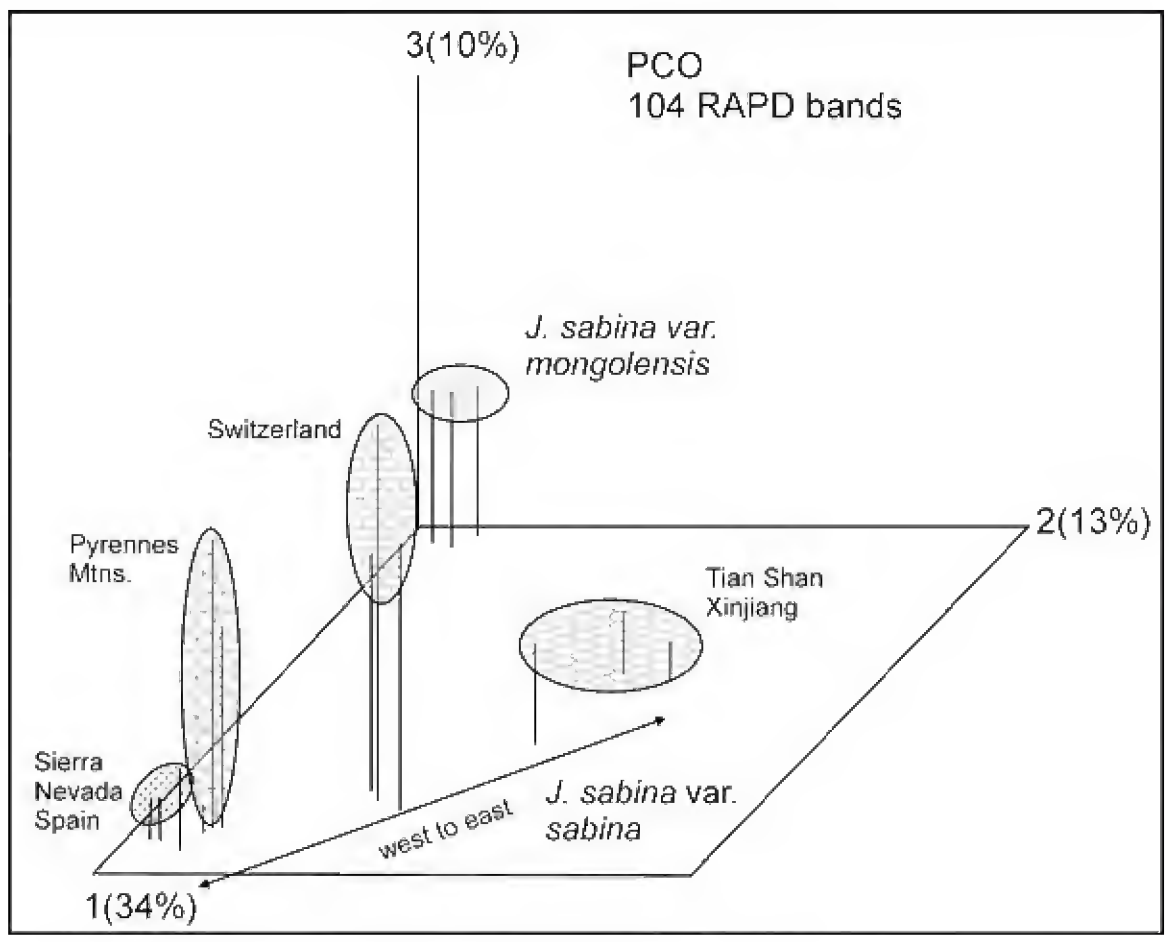


Fig. 4. PCO showing west to east clinal variation using RAPD bands, within *J. s.* var. *sabina* (from Adams et al. 2007).

Recently, in routine analyses of *J. sabina* from Bulgaria and northern Greece, we found plants that appeared to have non-*J. sabina* chloroplasts (based on petN-psbM, trnSG, trnDT and trnLF sequences). The purpose of the present paper is to report on these unusual plants and the taxonomy and evolution of this taxon.

MATERIAL AND METHODS

Specimens used in this study (species, popn. id., location, collection numbers): *J. chinensis*, CH, Lanzhou, Gansu, China, *Adams 6765-6767*; *J. sabina*: (SN), Sierra Nevada, Spain, *Adams 7197, 7199, 7200*; (PY), Pyrenees Mtns., Spain/ France border, *Adams 7573-7577*; (SW), Switzerland, *Adams 7611, 7612, 7614, 7615*; TS, Tian Shan Mtns., Xinjiang, China, *Adams 7836-7838*; Mongolia, Altai Mtns., *Adams 7585-7587*; Kazakhstan, Paniflor, *Adams 7811-7812*; Azerbaijan: *Adams 14316-14320*;
J. davurica (DV), 15 km se Ulan Bator, Mongolia, *Adams 7252, 7253, 7601*; *J. davurica* var. *arenaria* (AR) sand dunes, Lake Qinghai, Qinghai, China, *Adams 10347-10352*; river bank, Gansu, *J-Q. Liu and Adams 10354-10356*; *J. davurica* var. *mongolensis* (MS) sand dunes, 80 km sw Ulan Bator, Mongolia, *Adams 7254-7256*;
Collections of new taxon with non-*J. sabina* cpDNA: (acronyms used in Fig. 7)
B1-B5 Eastern Rhodopes, Bulgaria, *Adams 13725-13729 (A. Tashev 2012-1-5)*;
B6 Central Stara Plania, Sokolna reserve, Bulgaria, *Adams 14721 (A. Tashev 2015 Balkan 1*;
B7-B9, Ba, Bb Rila Mountain, Bulgaria, *Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2)*;
G1-G5 Mt. Tsena, Greece, *Adams 14727-14731* (*A. Tashev 2015 So. 1-5 Tsena*);

Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU) and Herbarium (University of Forestry, Sofia, Bulgaria).

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. R7 (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, Bartel and Price, 2009; Adams, 1975; Veldman, 1967).

RESULTS

Sequencing nrDNA for the *J. sabina* from Bulgaria and Greece resulted in 1270 bp of data. Bayesian analysis (including all the smooth leaf, globose cone *Juniperus* of section *Sabina*), shows (Fig. 5) that the *J. sabina* 'balkanensis' plants are in a diverse clade with most of the other *J. sabina* accessions. They are not distinct. In addition, *J. sabina* from Azerbaijan and Switzerland, plus one plant from the Altai Mtns., Mongolia form a distinct, disjunct clade. The other sample from Altai Mtns. is in a clade with *J. sabina* from the Pyrenees. Unfortunately, the use of nrDNA alone is just not sufficient to resolve this group of smooth junipers. This group includes *J. chinensis* and all the smooth leaf junipers from the

western hemisphere. Compare Fig. 5 with Fig. 2, that, based on combined nrDNA plus 4 cp regions, clearly resolved the smooth leaf junipers of the western hemisphere).

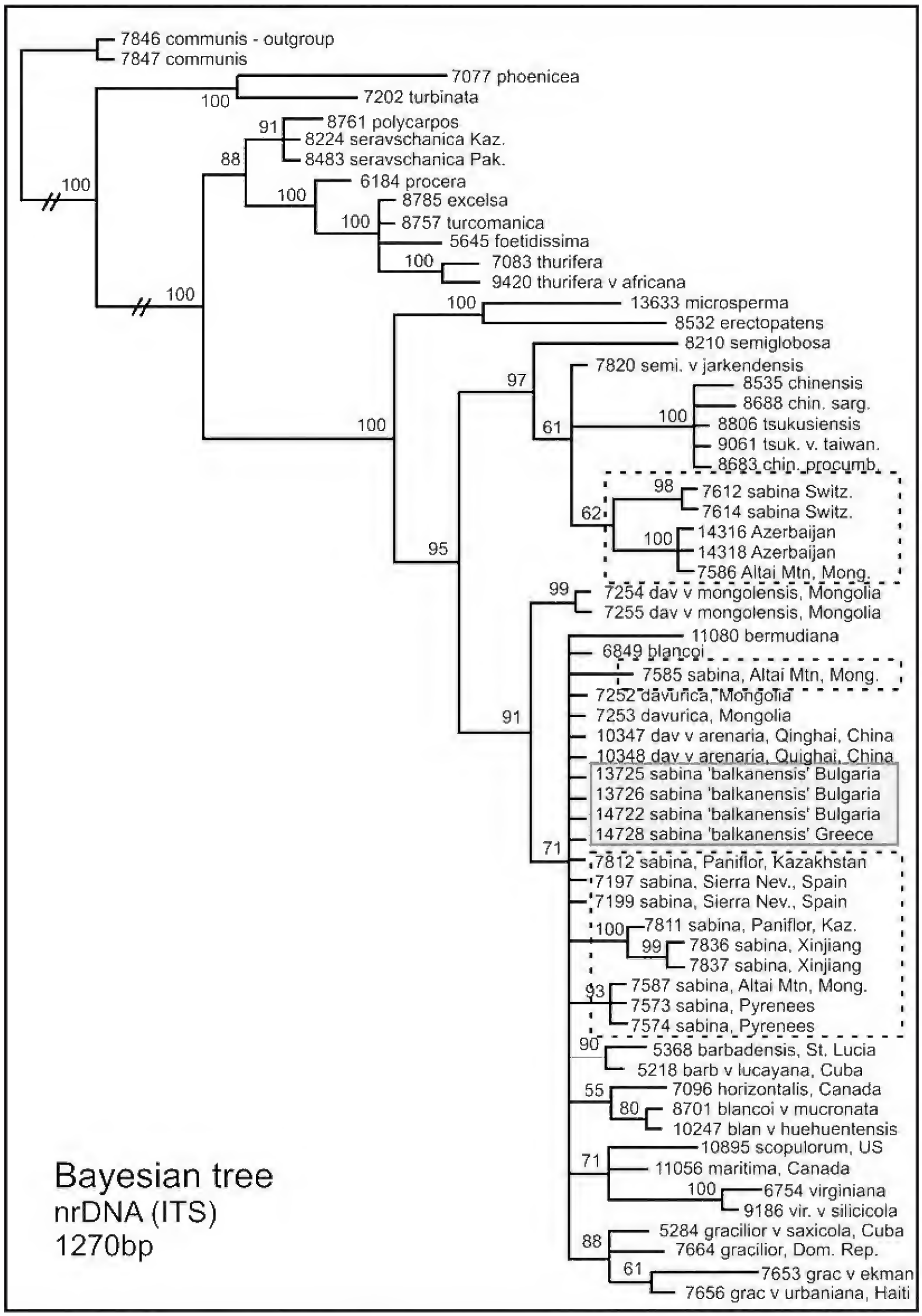


Figure 5. Bayesian analysis of the smooth leaf margined junipers of section Sabina using nrDNA (1270 bp). The numbers at the branch points are posterior probabilities (as percent). 'balkanensis' OTUs are in the shaded block. Other *J. sabina* OTUs are in the dashed line boxes.

Sequencing four cp regions (petN-psbM, trnSG, trnDT, and trnLF) resulted in 3114 bp of data. Bayesian analysis (including all the smooth leaf *Juniperus* of section *Sabina*), shows (Fig. 6) that the *J. sabina* 'balkanensis' plants are in a clade with *J. thurifera* (var. *thurifera* and var. *africana*). Clearly the chloroplast of 'balkanensis' is allied most closely with that of *J. thurifera*, not *J. sabina*. It seems likely that the 'balkanensis' chloroplast was captured from an ancestor of *J. thurifera* because *J. thurifera* (extant) is nested within 'balkanensis'. If 'balkanensis' captured its chloroplast from an extant *J. thurifera*, one would expect all of 'balkanensis' to be nested within *J. thurifera*. Further indicative of an ancient hybridization event is the measurable level of variation found in generally conserved chloroplast DNA among 'balcanensis' accessions.

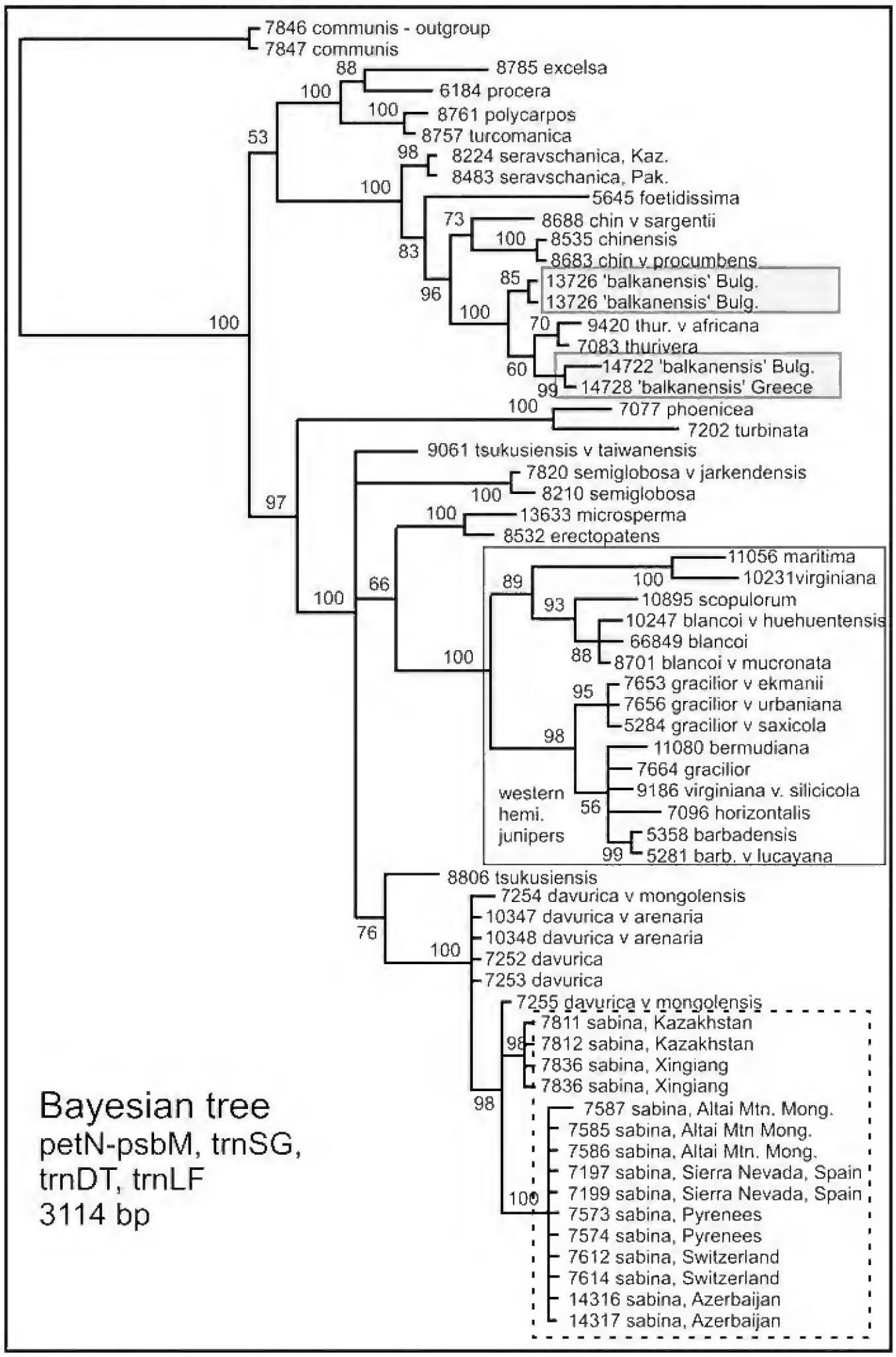


Figure 6. Bayesian analysis based on four cp regions.

It might be noted that *J. sabina* from Kazakhstan and Xinjiang form a clade (Fig. 6). The use of four cp regions resulted in a clade of the junipers from the western hemisphere (box, Fig. 6).

In order to more closely investigate the amount of divergence of the 'balkanensis' chloroplast from that of present day *J. thurifera*, a minimum spanning network was computed using both SNPs and indels, herein called mutations. This analysis found 52 mutations within the set: *J. sabina* (sensu stricto), *J. sabina* 'balkanensis' and *J. thurifera*. The minimum spanning network (Fig. 7) shows that all the 'balkanensis' plants differ by only 6-8 mutations from *J. thurifera* chloroplast. However, the nearest link connecting 'balkanensis' to *J. sabina* (*sensu stricto*) is 36 mutations!

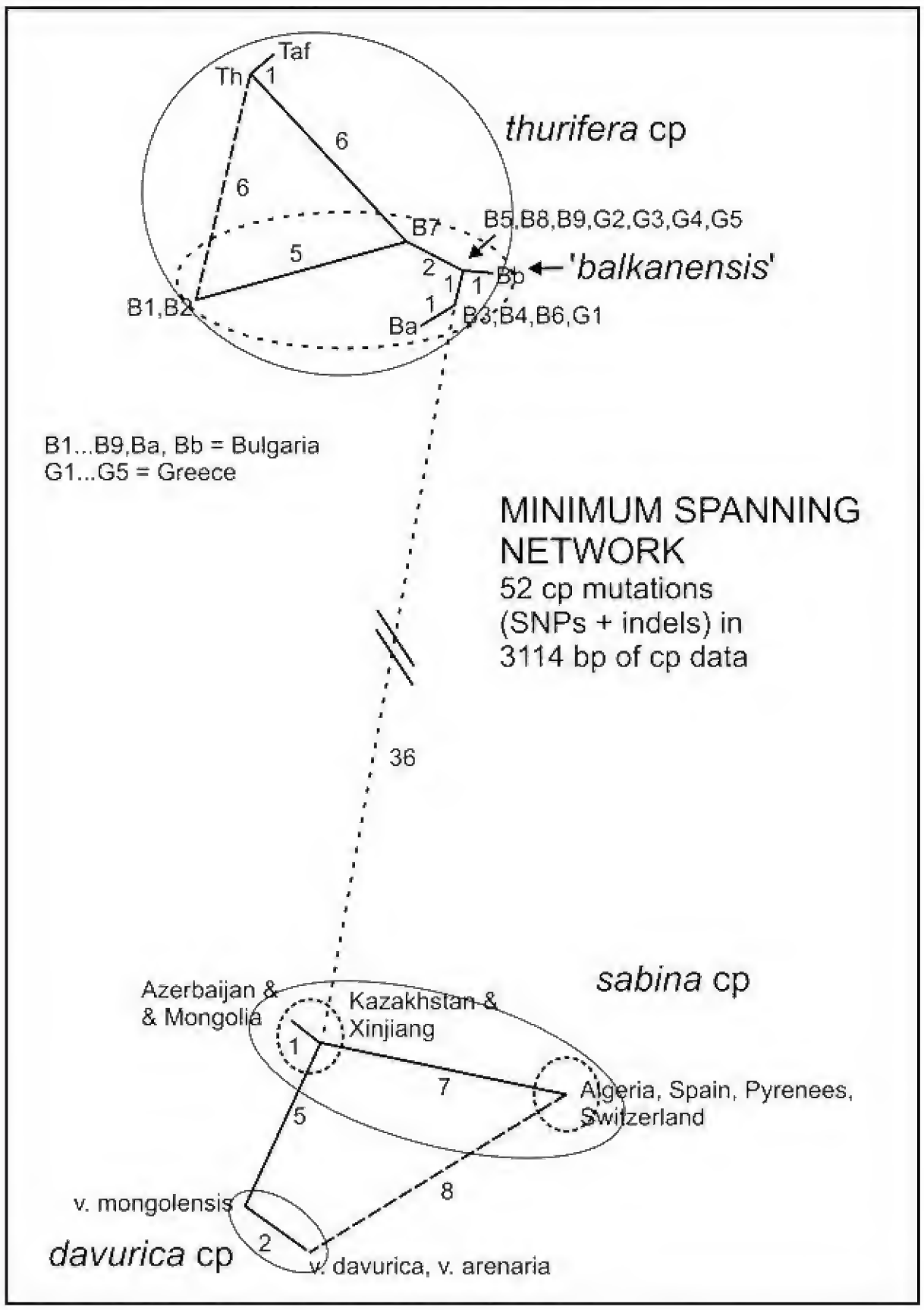


Figure 7. Minimum spanning network based on 52 mutations (SNPs + indels) in 4 cp markers (3114 bp). The numbers next to the lines are the number of mutations for that link. The dotted line connects the *thurifera* cp taxa to the *sabina* cp taxa by 36 mutations. The dashed line is the second nearest neighbor of *J. sabina* to *J. davurica* cp type. (8 mutations).

Notice (Fig. 7) that Azerbaijan/ Mongolia accessions group with Kazakhstan/ Xinjiang and this group differs by 7 mutations from the Europe/ Algeria group. This suggests that *J. sabina* in central Asia may be a different variety of *J. sabina*. That needs to be looked at in more details but this is beyond the scope of the present report.

The current data suggest that *J. sabina* 'balkanensis' captured the chloroplast of an ancestor of the *thurifera* lineage during an ancient hybridization event at a time when species distributions overlapped. Since it displays a general morphology similar of *J. sabina* this hybridization event was likely followed by successive backcrosses to *J. sabina* after the hybridization event, resulting largely in a nuclear genome as well as a morphology similar to *J. sabina* (*sensu stricto*). In fact, we do see that in the nrDNA analysis (Fig. 5), where 'balkanensis' is clearly interspersed in a clade with other *J. sabina*. So it is not surprising that a comparison of the morphology of 'balkanensis' and *J. sabina* has, to date, revealed only a few quantitative differences (Table 1). It may be that further morphological analysis will find additional differences but that seems unlikely, as no known genes for morphology reside in the chloroplast.

Table 1. Comparison of the morphology of *J. sabina* var. *balkanensis* and *J. sabina* (sensu stricto).

	sabina var. *balkanensis*	*sabina* var. *sabina*
foliage	fine, green	coarse, yellow-green
scale leaf tips	obtuse to acute	acute
scale leaf glands	not apparent	apparent
whip leaf glands	flat to sunken, most 3/4 length of leaf, elongate	level or above the surface mostly less than 3/4 length of leaf, oval to elongate
seed cones	mostly reniform (bi-lobed), some ovate;1,2 seeded	mostly ovate, some reniform mostly ovate; 1,2,3 seeded

There are many reasons to recognize a given taxon (species, subspecies, etc.). Often it is just a logical way to organize morphological groups into named taxa. In this case, although 'balkanensis' is cryptic in its morphology, it seems worthy of recognition so as to call attention to this unusual evolutionary entity as:

***Juniperus sabina* var. *balkanensis* R.P.Adams and A. N. Tashev, var. nov.** Fig. 8.
Type: Greece, Northern Central Greece. Region Central Macedonia. Mount Tsena near the village of Notia. 41°08' 29.4" N; 22° 14'42.2" E., 1630 m, *R. P. Adams 14730* (*Alexander Tashev Tsena 4*), 31 Aug. 2015 (HOLOTYPE: BAYLU, ISOTYPE University of Forestry, Dept. of Dendrology, Sofia, Bulgaria).

Prostrate shrubs similar to *J. sabina*, but differing in having a *J. thurifera*-like chloroplast, coarser foliage, leaf tips more acute, whip leaf glands flat to sunken, and more elongate.

Other specimens studied: TOPOTYPES: *Tashev Tsena 1,2,3,5 (Adams 14727, 14728. 14729. 12731)* at BAYLU and Herbarium, University of Forestry, Sofia, Bulgaria.

Additional specimens studied: B1-B5 Eastern Rhodopes, *Adams 13725-13729 (A. Tashev 2012-1-5*); Bulgaria. B6 Central Stara Plania, Sokolna reserve, *Adams 14721 (A. Tashev 2015 Balkan 1,* Bulgaria; B7-B9, Ba, Bb Rila Mountain, *Adams 14722-14726 (A. Tashev 2015 Rila 1.1-1.3, 2.1-2.2)* Bulgaria. G1-G5 Mt. Tsena, n Greece, *Adams 14727-14731* (*A. Tashev 2015 So. 1-5 Tsena*); Greece at Baylor University Herbarium (BAYLU) and Herbarium, University of Forestry, Sofia, Bulgaria.

At present, *J. sabina* var. *balkanensis* is known only from sloping rocky limestone, at 1240 - 1630m, in the mountains of Bulgaria and northern Greece (Fig. 9). It may occur northward into Romania,

westward into Macedonia and/ or eastward into northern Turkey. Additional research is in progress to more accurately determine its range (Fig. 10).

Figure 8. Holotype of ***Juniperus sabina* var. *balkanensis*** (*Adams 14730*)

Fig. 9. Habit and habitat of *J. s.* var. *balkanensis* in the eastern Rhodopes mountains, Bulgaria. *Juniperus communis*, columnar trees are in the background.

The distribution of *J. sabina* var. *balkanensis* and *J. sabina* is shown in Fig. 10. The distribution of *J. thurifera* is presented in the insert, lower left (Fig. 10). At present the distributions of *J. s.* var. *balkanensis* and *J. thurifera* do not appear to overlap, negating hybridization. However, there were large changes in plant distributions in the Pleistocene and earlier, that would have given opportunity for a *J. thurifera*-like ancestor to co-occur with *J. sabina*. This would have presented an opportunity for chloroplast capture by *J. sabina*.

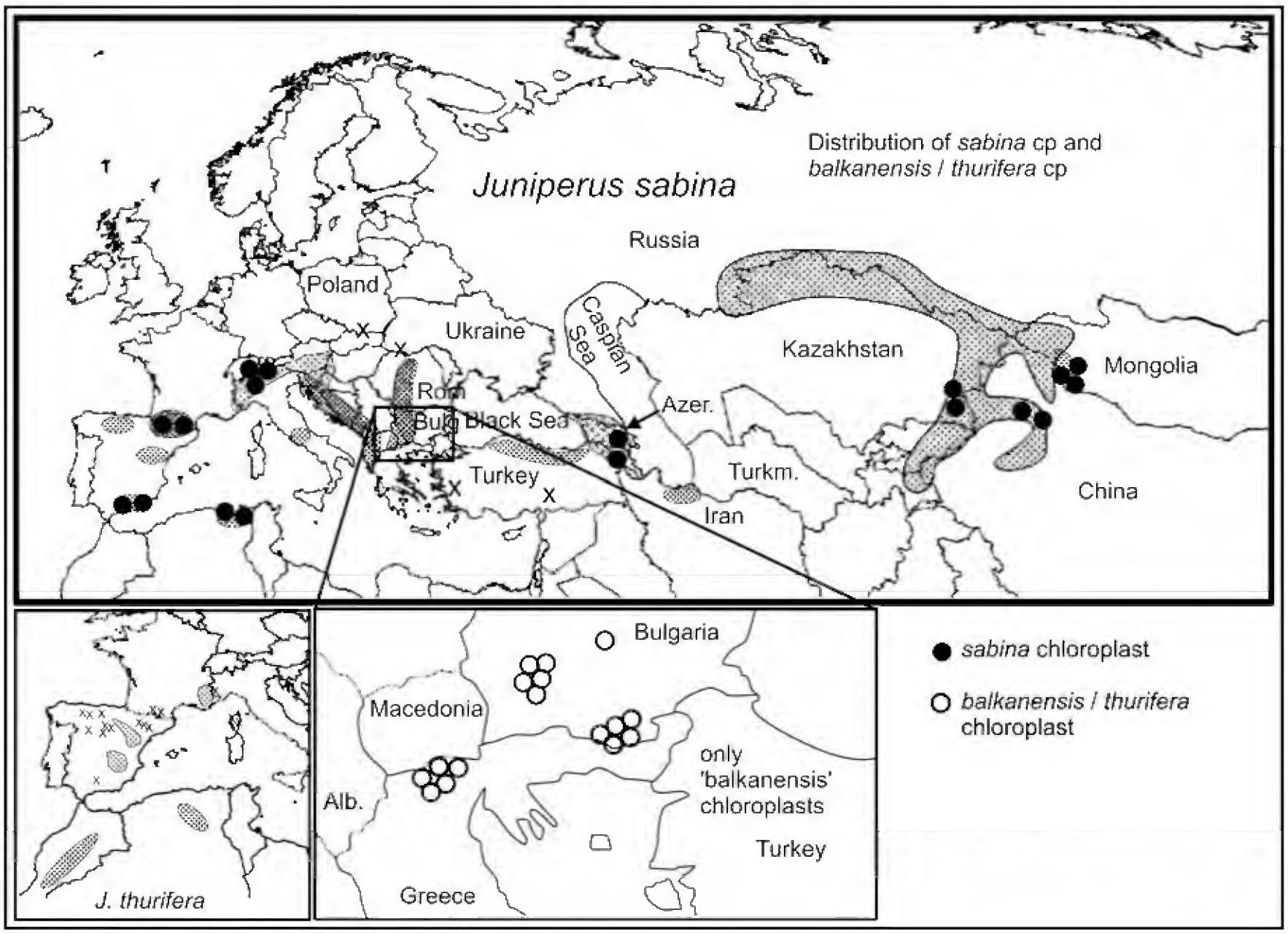


Figure 10. Distribution of *J. sabina* var. *balkanensis* and typical *J. sabina* chloroplast. The distributions of *J. thurifera* and var. *africana* (in north Africa) are shown in the insert on the lower left.

ACKNOWLEDGEMENTS

Thanks to G-L Chu, China; A. D. Dembitsky, Kazakhstan; A. Lara, Spain; S. Shatar, Mongolia; Y. Turuspekov, Kazakhstan; J-P. Zhong, and J. Liu, China for assistance on field trips. This research was supported with funds provided by Baylor University.

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Geographical variation in the leaf volatile oils of *Grindelia ciliata* and *G. adenodonta* (Asteraceae)

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

ABSTRACT

The composition of the leaf volatile oils of *Grindelia ciliata* was analyzed and found to be dominated by limonene (31.6 - 43.7%), bornyl acetate (19.2 - 31.6%), α-pinene (14.6 - 23.6%) and β-pinene (8.3 - 12.0%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B. Yields varied from 0.27 to 0.46% (DW). By comparison, the oil of *G. adenodonta* contained limonene (44.6%), bornyl acetate (13.2%), α-pinene (18.0%) and β-pinene (7.4%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B, with a larger yield of 1.39%. Patterns of geographic variation in yields and limonene are presented. Published on-line www.phytologia.org *Phytologia 98(2): 112- 117 (Apr 4, 2016).* ISSN 030319430.

KEY WORDS: *Grindelia ciliata, G. adenodonta,* Asteraceae, volatile leaf oil, terpenes, geographic variation, Spanish Gold, giant gumweed.

Grindelia (gumweed) is a large genus of about 75 species with an amphitropical distribution with half of the species occurring in North America and Mexico and the remaining species in South America (Moore, et al., 2012). Steyermark (1934) recognized 45 species, plus 40 varieties and 25 forms (110 taxa). Strother and Wetter (2006) estimated the genus to contain some 30 species; they recognized only 18 species in the Flora of North America. Bartoli and Tortosa (2012) recognized 41 species, 10 varieties and 2 forms in North America based on morphology. Moore et al. (2012) utilized nrDNA and the ETS region as well as psaI-accD cpDNA to analyze selected taxa of *Grindelia* from both North and South America. They found strong support for two sister clades in North and South America. The North American clade seemed to be divided into two groups by the continental divide.

Nesom, Suh and Simpson (1993) submerged the monotypic genus *Prionopsis* (*P. ciliata*) into *Grindelia* as *G. ciliata* (Nutt.l) Spreng. [syn: *G. papposa* (Nutt.) Nesom & Suh]. *Grindelia ciliata* reportedly grows as an annual or biennial. It is widely distributed in Texas, Oklahoma and Kansas, se Colorado, e New Mexico, s Nebraska, s and se Iowa (rare) with putative outlying records from Illinois, Missouri, Arkansas and Louisiana. Previously, we reported on variation in yields of 'bio-crude' (pentane extracts) of *G. ciliata* (Adams et al., 2015).
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Grindelia ciliata is a large plant (up to 2 m), that grows in disturbed sites in various soils and precipitations. It appears to have potential as a semi-arid land bio-crude crop plant. In contrast to most *Grindelia* species, in *G. ciliata*) the leaves and buds are not gummy or with exuded resin, yet, the bio-crude yields are comparable to sticky or gummy *Grindelia* species. *Grindelia adenodonta* (Steyerm.) Nesom is endemic to Texas and grows in the same area as *G. ciliata* near Newcastle - Graham, TX. However, in contrast to *G. ciliata*, it has sticky or gummy leaves. Moore et al. (2012) has shown that these species form a distinct clade as most closely related species. We decided therefore to investigate the volatile leaf oil of *G. adenodonta*. Searches of the literature found no reports on the leaf volatile oils or terpenoids of either *G. ciliata* or *G. adenodonta.*

The purpose of the present paper is to report, for the first time, the composition of the volatile leaf oils of *G. ciliata* and *G. adenodonta*. In addition, we report on geographic variation in the essential oils in natural populations of *G. ciliata.*

MATERIALS AND METHODS

Fresh leaves and specimens of *G. ciliata* were collected from the following populations:

Beav OK, 9 mi N of OK/TX border, on US83, at a ravine on terrace n of Beaver River, mostly single stemmed, locally common on sandy soil. 36° 35' 14" N, 100° 49' 42" W, 2893 ft, 19 Aug 2015, *Adams 14631*, BO1-10,

Cim KS, 3 mi N of KS/OK border on KS Hwy 23, n of Cimarron River on a terrace, locally common, mostly single stemmed, on sandy soil. 37° 01' 27" N, 100° 29' 39.5" W, 2378 ft, 19 Aug 2015, CK1-10C,

Dodge, on US 56, 3 mi w of jct US 56 and US 54, on sandy soil in ditch, Dodge City, KS, scattered but locally common, mostly single stemmed, 37° 43' 13" N, 100° 04' 11" W, 2510 ft, 19 Aug 2015, DCK1-10,

AMR TX, about 17 mi ne of Amarillo, TX, 35° 25' 34" N, 101° 38' 07" W, 3520 ft. on Tex 136, most plants branched. Common on west side of hwy from this location to near Fritch, TX (to last ravine) on Tex 136. in prairie grass, loam soil. 21 Aug 15, FHR1-10,

BOR TX, 1 mi s of Borger, TX on Tex 207 on road cut, sandy but caliche on top, mostly branched plants. 35° 38' 17" N, 101° 23' 50" W, 3203 ft, 22 Aug 2015. *Adams 14636,* BOR1-10,

NewCas, around oil tanks, on red loam, half of the plants were branched, on Bullock Road, near Newcastle TX, 33° 09' 34" N, 98° 41' 54" W, 1217 ft., 30 Aug 2015, *Adams 14642,* BR1-10,

CHD TX, on vacant lot in Childress, red sand, 100s of plants, many branched, on US 287, 34° 24' 47" N, 100° 10' 02" W, 1737 ft, 30 Aug 2015, *Adams 14644*, CHD1-10,

McG, on vacant lot, sandy-loam, ~10 plants, on US84, 7 m n of McGregor, 8 mi. s of Waco, TX, 31° 28' 48" N, 97° 17' 35" W, 540 ft., 27 Aug 2015, *Adams 14641,* MCG 1-6,

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

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

RESULTS

The composition of the leaf volatile oils of *Grindelia ciliata* was found (Table 1) to be dominated by limonene (31.6 - 43.7%), bornyl acetate (19.2 - 31.6%), α-pinene (14.6 - 23.6%) and β-pinene (8.3 - 12.0%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B. Yields varied from 0.27 to 0.46% (DW). By comparison, the oil of *G. adenodonta* (Table 1) contained limonene (44.6%), bornyl acetate (13.2%), α-pinene (18.0%) and β-pinene (7.4%) with moderate amounts of camphene, myrcene, (E)-β-ocimene and germacrene B, with a larger yield of 1.39%. The leaves of *G. ciliata* are not sticky (i.e., with a gummy exudate), whereas the leaves of *G. adenodonta* are covered with exudate and very sticky. It is interesting that these two most closely related species (Moore et al. 2012), also have leaf essential oils that are nearly identical. The oils mostly differ quantitatively except for the presence of some minor components (Table 1).

Because one would expect considerable biochemical changes between leaves and buds, both leaves and buds were collected from a single individual and analyzed separately. Interestingly, the buds volatile oil was lower in yield than leaves (0.32 vs. 0.52%, Table 2). There were also some changes in concentrations (Table 2) between buds and leaves: limonene 43.9, 33,.7%), bornyl acetate (17.8 - 21.4%), α-pinene (15.7, 20.6%) and β-pinene (7.3, 11.3%), borneol (0.4, 1.2%), germacrene D (3.1, 2.2%) and dicyclohexyl-propanedinitrile (0.9, 0.4%).

The yields of volatile oil varied (Fig. 1) from 0.22 (BO Beaver River, OK) and 0.30% (Mcg McGregor, TX) to the largest yields in the High Plains: 0.52% (BO Borger, TX), 0.46% (CK Cimarron River, KS) and 0.45% (AMR Amarillo, TX). Nothing about the habitats at BO and CK indicated that the oils yields would be so different. Both populations were in sandy-loam on south facing slopes with sage.

The % limonene shows (Fig. 2) a different pattern with the highest concentration in DCK (Dodge City, KS) and NC (Newcastle, TX). The remaining populations were fairly uniform in limonene, ranging from 31.6 to 34.3% (Fig. 2).

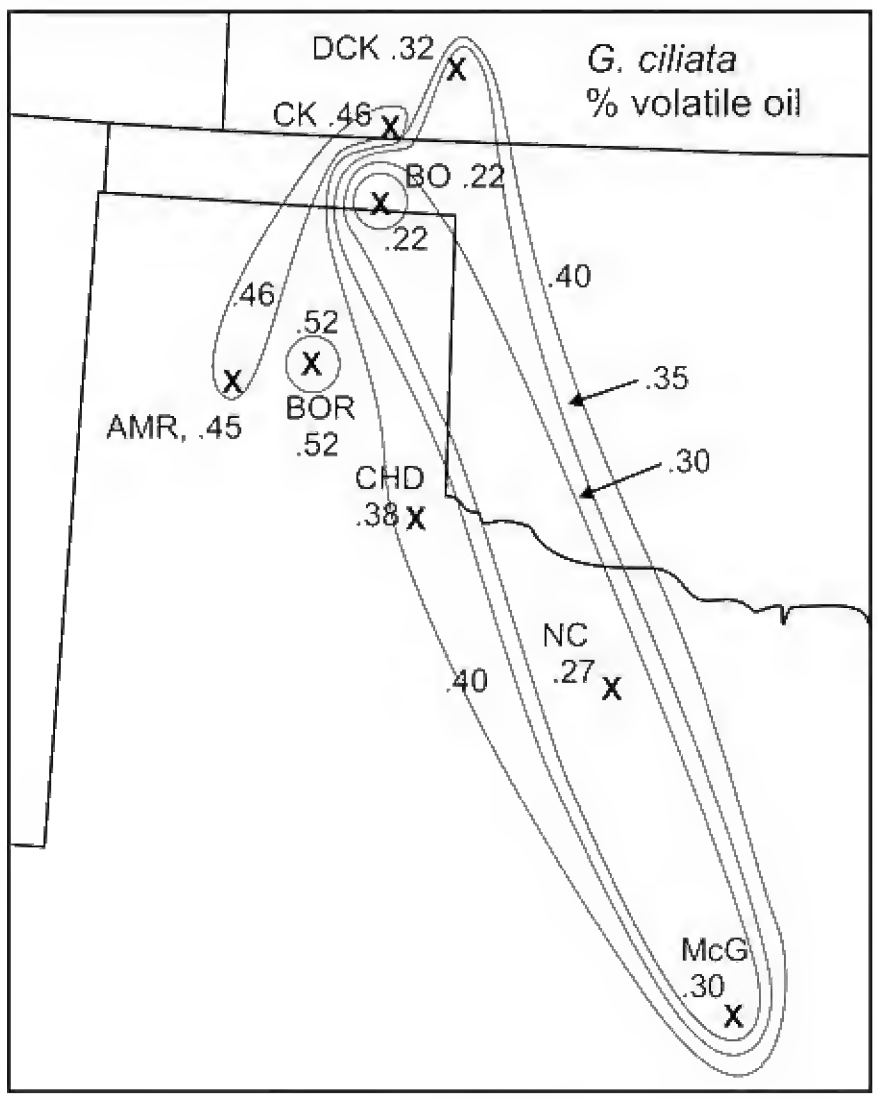

Figure 1. Contour map of yield (% volatile oil).

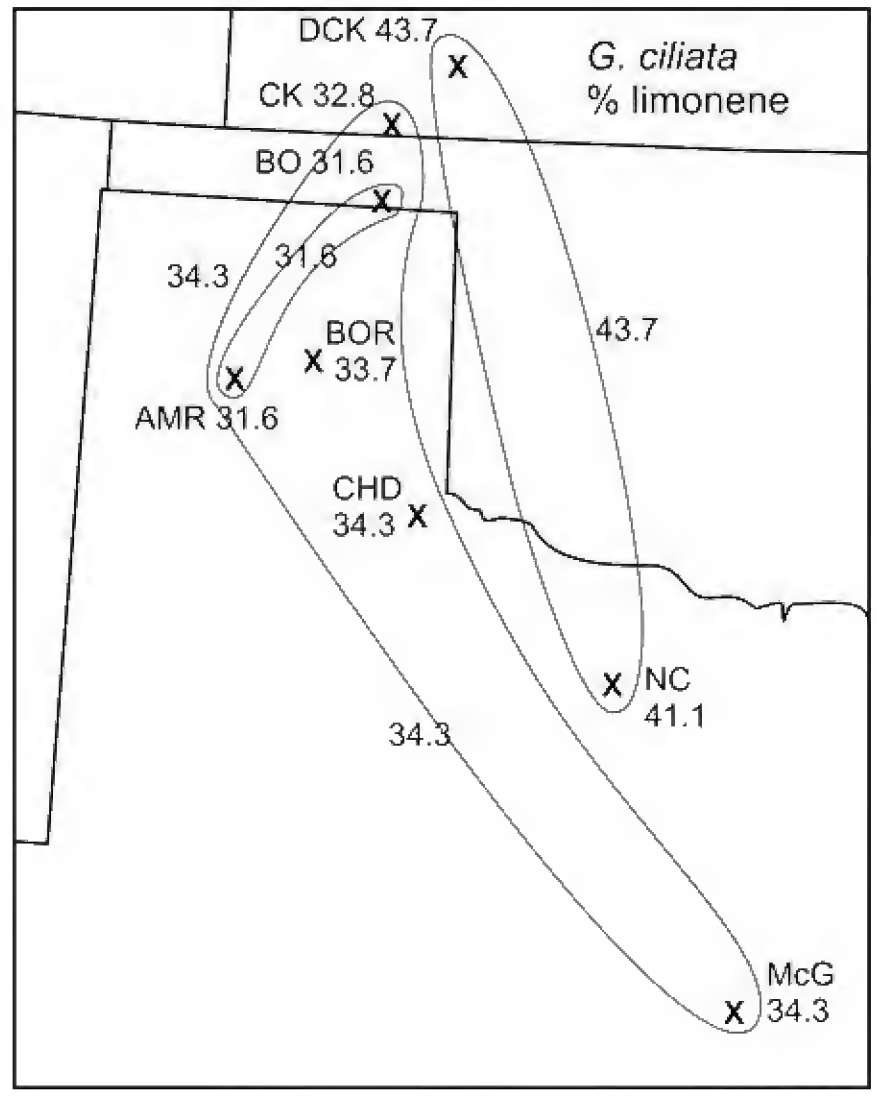

Figure 2. Contour map of % limonene.

ACKNOWLEDGEMENTS

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Table 1. Geographical variation in the volatile leaf oils of *Grindelia ciliata*. *Grindelia adenodonta*, Graham, TX, is included for comparison. McG TX = McGregor; TX; NewCas = Newcastle, TX; CHD Tx = Childress, TX; AMR = Amarillo, TX; Bor Tx = Borger, TX; Beav OK = Beaver River, OK; Cim KS = Cimarron River, KS; Dodge = Dodge City, KS. adeno = *G. adenodonta*, Graham, TX. Major components are in bold.

KI	compound	McG Tx 14641	NewCas 14642	CHD Tx 14644	AMR Tx 14635	Bor TX 14636	Beav Ok 14631	Cim Ks 14632	Dodge 14633	adeno 14643
	% yield	**0.30**	**0.27**	**0.38**	**0.45**	**0.52**	**0.22**	**0.46**	**0.34**	**1.39**
921	tricyclene	t	t	t	t	t	t	t	t	t
924	α-thujene	t	t	t	t	t	t	t	t	t
932	**α-pinene**	**17.9**	**14.6**	**19.9**	**20.9**	**20.6**	**15.3**	**23.6**	**15.4**	**18.0**
946	**camphene**	**2.5**	**2.2**	**2.9**	**3.0**	**2.9**	**2.2**	**3.2**	**2.0**	**2.7**
969	sabinene	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.3	0.3
974	**β-pinene**	**10.9**	**9.4**	**10.4**	**12.0**	**11.3**	**8.3**	**11.3**	**8.0**	**7.4**
988	myrcene	1.4	1.4	1.3	1.2	1.3	1.2	1.4	1.4	1.2
1000	n-decane	t	t	t	t	t	t	t	t	t
1002	α-phellandrene	0.1	t	0.1	t	t	t	t	t	t
1005	o-cresyl methyl ether	t	t	t	t	t	t	t	t	-
1024	**limonene**	**34.3**	**41.1**	**34.3**	**31.6**	**33.7**	**31.6**	**32.8**	**43.7**	**44.6**
1044	**(E)-β-ocimene**	**1.5**	**2.8**	**0.8**	**1.4**	**1.1**	**1.1**	**1.4**	**1.6**	**0.3**
1054	γ-terpinene	0.1	t	0.1	t	t	t	t	t	-
1065	cis-sabinene hydrate	0.2	0.2	0.1	0.2	0.2	t	0.1	t	-
1086	terpinolene	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.3	0.2
1100	undecane	0.2	0.2	0.1	0.1	0.2	0.2	t	t	-
1110	octen-3-yl acetate, 1-	t	t	0.1	0.2	t	0.2	t	0.1	-
1122	α-campholenal	-	-	-	-	-	-	-	-	0.2
1135	trans-pinocarveol	-	-	-	-	-	-	-	-	0.3
1140	trans-verbenol	-	-	-	-	-	-	-	-	0.6
1141	camphor	0.3	t	0.2	0.3	0.2	0.3	0.2	t	-
1160	pinocarvone	0.3	0.2	0.1	0.2	0.2	0.2	t	t	0.3
1165	borneol	1.9	2.0	1.1	1.1	1.2	1.8	1.1	0.5	-
1172	cis-pinocamphone	-	-	-	-	-	-	-	-	0.3
1174	terpinen-4-ol	0.2	0.2	0.1	0.2	0.2	0.3	0.4	0.1	0.1
1184	dill ether	t	t	t	t	0.1	t	t	0.1	-
1186	α-terpineol	0.2	0.3	0.2	0.2	0.2	0.2	t	0.2	0.1
1195	myrtenal	0.1	0.1	t	0.1	0.1	0.1	0.1	t	0.1
1195	myrtenol	0.1	0.1	t	0.1	0.1	0.1	0.1	t	0.1
1204	verbenone	-	-	-	-	-	-	-	-	0.2
1215	trans-carveol	-	-	-	-	-	-	-	-	0.1
1239	carvone	-	-	-	-	-	-	-	-	t
1284	**bornyl acetate**	**20.4**	**20.3**	**23.9**	**21.7**	**21.4**	**31.6**	**19.9**	**19.2**	**13.2**
1417	(E)-caryophyllene	t	t	t	t	t	t	t	0.1	-
1453	geranyl acetone	t	0.1	t	t	t	0.2	t	0.2	0.3
1478	γ-muurolene	t	t	t	t	t	t	t	t	-
1480	**germacrene D**	**2.3**	**1.2**	**1.0**	**1.5**	**2.2**	**1.7**	**1.3**	**2.3**	**0.6**
1489	β-selinene	t	t	t	t	t	t	t	t	-
1522	δ-cadinene	t	t	t	t	t	t	t	t	-
1574	germacrene-D-4-ol	t	t	t	t	t	t	t	t	-
1582	caryophyllene oxide	t	t	t	t	t	t	t	t	t
1638	epi-α-cadinol	t	t	t	t	t	0.1	t	t	-
1640	epi-α-muurolol	t	t	t	t	t	0.1	t	t	-
1645	cubenol	t	t	t	t	t	t	t	t	-
1649	**β -eudesmol**	**0.1**	**t**	**t**	**0.3**	**t**	**0.3**	**t**	**t**	**2.9**
1652	α -cadinol	0.1	t	t	t	t	t	t	t	-
1685	germacra-4(15),5, 10(14)-trien-1-al	0.3	0.4	0.4	0.3	0.3	0.4	0.3	0.4	0.2
1961	propanedinitrile, dicyclohexyl- (NIST)	0.2	0.2	t	0.3	0.4	0.2	0.2	0.9	-
2300	tricosane	t	t	t	t	t	t	t	t	-

Table 2 Comparison of volatile oil from buds and leaves from the same plant from Borger, TX. Components with considerable differences are in bold.

KI	compound	Bor Tx 14627B	Bor TX 14636 L
	% yield	**0.32**	**0.52**
921	tricyclene	t	t
924	α-thujene	t	t
932	**α-pinene**	**15.7**	**20.6**
946	camphene	2.0	2.9
969	sabinene	0.2	0.4
974	**β-pinene**	**7.3**	**11.3**
988	myrcene	1.4	1.3
1000	n-decane	t	t
1002	α-phellandrene	0.2	t
1005	o-cresyl methyl ether	t	t
1024	**limonene**	**43.9**	**33.7**
1044	(E)-β-ocimene	1.4	1.1
1054	γ-terpinene	t	t
1065	cis-sabinene hydrate	t	0.2
1086	terpinolene	0.2	0.2
1100	undecane	t	0.2
1110	octen-3-yl acetate, 1-	t	t
1141	camphor	t	0.2
1160	pinocarvone	t	0.2
1165	**borneol**	**0.4**	**1.2**
1174	terpinen-4-ol	t	0.2
1184	dill ether	0.1	0.1
1186	α-terpineol	t	0.2
1195	myrtenal	t	0.1
1195	myrtenol	t	0.1
1284	**bornyl acetate**	**17.8**	**21.4**
1417	(E)-caryophyllene	0.1	t
1453	geranyl acetone	0.2	t
1478	γ-muurolene	t	t
1480	**germacrene D**	**3.1**	**2.2**
1489	β-selinene	t	t
1522	δ-cadinene	t	t
1574	germacrene-D-4-ol	t	t
1582	caryophyllene oxide	t	t
1638	epi-α-cadinol	0.1	t
1640	epi-α-muurolol	0.1	t
1645	cubenol	t	t
1649	β -eudesmol	t	t
1652	α -cadinol	t	t
1685	germacra-4(15),5, 10(14)-trien-1-al	0.2	0.3
1961	**propanedinitrile, dicyclohexyl- (NIST)**	**0.9**	**0.4**
2300	**tricosane**	**0.4**	**t**

A nomenclatural note on *Struthanthus acuminatus* (Loranthaceae)

Job Kuijt
649 Lost Lake Road, Victoria, BC V9B 6E3, Canada
jobkuijt@uvic.ca

ABSTRACT

A new combination is presented: ***Struthanthus acuminatus*** (Ruiz & Pavon) Kuijt and the synonymy reviewed. Published on-line www.phytologia.org *Phytologia 98(2): 118 (Apr 4, 2016).* ISSN 030319430.
KEY WORDS: *Loranthus (Struthanthus) acuminatus, Struthanthus divaricatus,* nomenclature.

One of the earliest recorded species of South American Loranthaceae was a plant characterized by strikingly acuminate leaves, appropriately called *Loranthus acuminatus* Ruiz & Pavon (1802). It was subsequently listed under *Loranthus* section *Struthanthus* by Blume (1830), and has regularly appeared in the botanical literature as *Struthanthus acuminatus* (Ruiz & Pavon) Blume as, for example, in Abbiatti's (1946) treatment of the mistletoes of Argentina. However, Blume did not actually make this combination, but simply listed the species epithet under *Loranthus* section *Struthanthus* (Several other, parallel taxa are stated to be sections as well). The fact that he continued to think of this (and many other) species as *Loranthus* is also clear from his inclusion of "Lor." preceeding the list of epithets including "*acuminatus*". To clarify Blume's taxon, the following *comb. nov.* is presented along with full synonymy:

STRUTHANTHUS ACUMINATUS (Ruiz & Pavon) Kuijt, *comb. nov.*

Basionym: *Loranthus acuminatus* Ruiz & Pavon, Fl. Peruv. Chil. 3: 49. 1802.
Synonyms: *Notanthera acuminatus* (Ruiz & Pavon) G. Don, Gen. Syst. 3: 429. 1843.
Loranthus cubeboides Rusby, Bull. Torrey Bot. Club 27: 135. 1900.
Struthanthus divaricatus Rusby, Descr. S. Amer. Pl. 12--13. 1920, *syn. nov.*

I am herewith adding *S. divaricatus* as a new synonym after inspecting images of its holotype, *Buchtien 3158* (NY) and two isotypes (US). The charcteristic leaf morphology and elongate, open racemes of pedunculate triads correspond closely to the holotype of *Loranthus acuminatus* as preserved in Madrid (See Abbiatti 1946, Pl. XIII). The Buchtien gathering was collected in a locality called Contana, at 2450 m, which I have not been able to locate. I would suggest the locality may be in or near Prov. Tarija in southern Bolivia, for the known Argentinian localities of the species listed in Abbiatti are from Prov. Salta, adjacent to Prov. Tarija.

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I am obliged to Amy Weiss (NY) for help in locating the Buchtien specimens.

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Ruiz, H., & J. A. Pavon. 1802. Fl. Peruviana, et Chilensis 3: 39.

Comparison of volatile oils of *Juniperus coahuilensis* in fresh seed cones vs. cones in fresh gray fox scat

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

Shirley Powell and A. M. Powell
Herbarium, Sul Ross State University, Box C-64, Alpine, TX 79832 USA ampowell@sulross.edu

ABSTRACT

The composition of volatile oil from *Juniperus coahuilensis* berries (seed cones) in fox scat is very similar to that of berries taken directly from trees. The most notable difference is that the percent yield is significantly higher from scat than intact fresh berries. This may be partially explained by the fact that the scat berry has been partially masticated and thus amenable to steam during distillation, whereas the fresh seed cones have their skin intact, impeding the loss of terpenes in distillation. A second factor is that sugars, starch and protein may have been partially removed during digestion. Thus, the yield from the 'depleted' berry would naturally contain a higher percent volatile oil relative to remaining cellulosic pulp. The gray fox does not extract most of the terpenes from juniper berries. The nearly intact berries, containing most of the terpenes, are excreted in the scat. This undoubtedly results in the loss of some or considerable amounts of nutrients, but the food source is so plentiful, that the loss of nutrients, due to incomplete digestion, may not be significant to the health of the gray fox. Published on-line www.phytologia.org *Phytologia 98(2): 119-127 (Apr 4, 2016).* ISSN 030319430.
KEY WORDS: *Juniperus coahuilensis,* volatile oils, gray fox scat, terpenes, composition.

Based on identification of plant material in gray fox (*Urocyon cinereoargenteus*) scat, White et al. (1999) found that gray fox obtained over half (51.3%) of its diet from *Juniperus osteosperma* berries (seed cones) in eastern Utah. They also reported that gray fox is adept at climbing trees and may have used juniper trees for resting, food source, or as escape cover. Analysis of the berry hull (pulp surrounding the seed) in different seasons, over two years, showed the hull was about 63 - 68% of the whole seed cone. The hulls (pulp) contained 3.8 - 5.2% protein, 15.9 - 28.3% starch and sugars, 25.8 - 26.8% crude fat, and 27.4 - 32.1% ADF (Acid Detergent Fiber). The juniper seeds were not digested by gray fox.

The summer of 2013 presented a remarkable year for the production of a bumper crop of seed cones (berries) of *Juniperus coahuilensis* near Alpine, TX (Fig. 1). Notice the limbs are loaded with berries and leaning. Many branches were so loaded with berries that they drooped to the ground.

Careful observation revealed that in Nov. and Dec. 2013, gray foxes were feeding almost exclusively on the seed cones of *J. coahuilensis* and these seed cones accounted for ca. 90% of the volume in the scat (Fig. 2).

Fig. 1. *J. coahuilensis* with branches loaded with fruits.

Gray foxes were often seen feeding on freshly fallen berries on the ground (Fig. 3), or up in a juniper eating the berries directly from the limbs (Fig. 4).

Fig. 2. Fresh gray fox scat with nearly intact juniper berries (seed cones).

Fig. 3. Gray fox eating *J. coahuilensis* berries on the ground.

In addition to gray fox, mule deer (*Odocoileus hemionus*) ate berries from the ground (Fig. 5) or even resorted to considerable effort to eat berries directly from the trees (Fig. 6). Western blue birds also fed on *J. coahuilensis* berries, both on the ground (Fig. 7) and in trees.

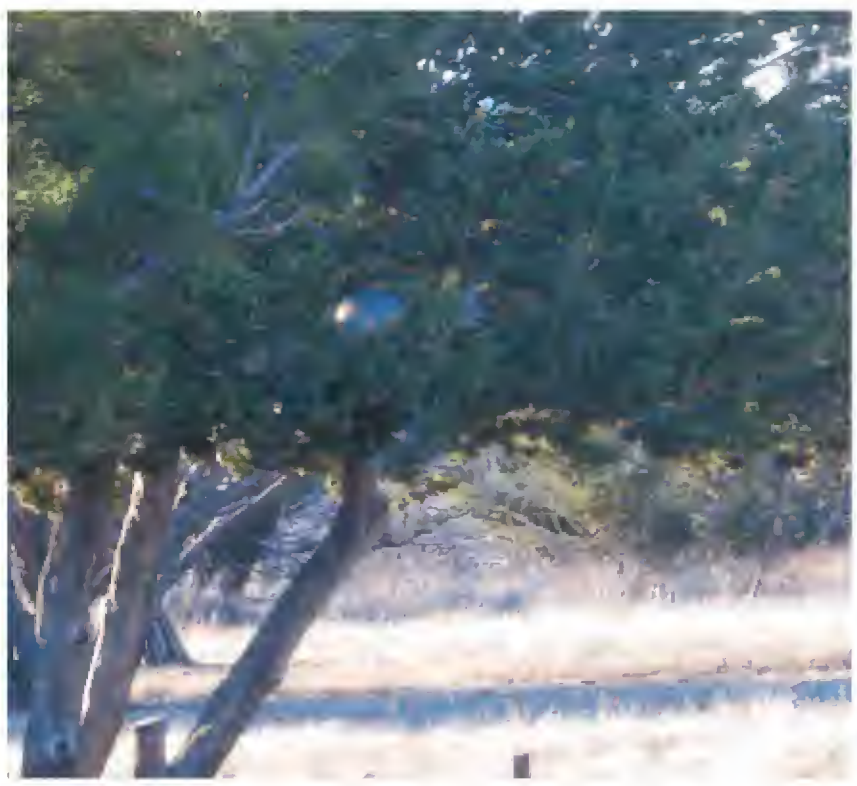

Fig. 4. Gray fox in juniper tree eating berries.

Fig. 5. Mule deer eating berries from the ground.

Cunningham, Kirkendall and Ballard (2006) reported *Juniperus monosperma* berries accounted for 0.0 - 22.5% of the gray fox's diet and from 0.0 to 18.25% of the diet of coyotes in central Arizona. Thacker et al. (2011), by analyzing the terpenes in fecal pellets of greater sage-grouse in Utah, correctly identified the sagebrush species that the sage-grouse was feeding on using crude terpene profiles.

As far as known, there are no reports on the composition of essential oil of *J. coahuilensis* berries present in fresh gray fox scat. The composition of the volatile leaf oil of *J. coahuilensis* has been reported (Adams, 2000, 2014), but there are no reports on the composition of the volatile oil of the berries (seed cones).

Fig. 6. Mule deer expending great effort to reach berries in the *J. coahuilensis* trees.

Fig. 7. Western blue birds eating *J. coahuilensis* berries on the ground.

The changes in terpenes during passage through the gray fox digestive tract are not known. The purpose of the present paper is to compare the volatile oil compositions of fresh *J. coahuilensis* seed cones vs. fresh fox scat in which ca. 90% of the scat consisted of *J. coahuilensis* seed cones.

MATERIALS AND METHODS

Fresh, mature seed cones were collected from *J. coahuilensis* trees in Dec., 2013 at the home of Mike and Shirley Powell, approx. 8 mi se of Alpine, on Tex 118, thence e 2 mi on Mile High Rd., 30° 16.147' N, 103° 33.522' W, 5324 ft (1623 m). *Adams 14649-14653* ripe seed cones of *J. coahuilensis*; *Adams 14654-14658*, fresh fox scat was collected at the small location and frozen. Leaves for volatile oil were collected from *J. coahuilensis*, 85 km north of La Zarca, Durango, Mexico, *Adams 6829-6831.*

Fresh frozen *J. coahuilensis* seed cones (13-33 g) were co-steam distilled with 2 mg of undecane and 2 mg methyl decanoate (internal standards) for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). In the same manner, individual frozen fox scat (3.88 - 8.74 g) were distilled. The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted scat and leaves were oven dried (48h, 100° C) for the determination of oil yields.

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

RESULTS AND DISCUSSION

The compositions of the berries in the fox scat are very similar to berries taken directly from trees (Table 1). The most notable difference is that the percent yield is significantly higher from scat than intact fresh berries (Table 2). This may be partially explained by the fact that the berry skin and layer of wax was somewhat disrupted by mastication in the scat (Fig. 8), whereas, fresh seed cones remained intact during distillation. Thus, the pulp was more easily subjected to steam in the scat berries. A second factor is that sugars, starch and protein may have been partially removed during digestion. Thus, the yield from the 'depleted' berry would naturally contain a higher percent volatile oil relative to remaining cellulosic pulp. A third factor might be the enzymatic removal of glucosides attached to terpene-glucosides, leaving free terpenes that easily volatilize during distillation. A fourth factor is that the scat berries and tree berries likely did not come from the same tree. Notice the composition of the berries from tree 14649 is more like that of the scat berries for α-pinene, terpinolene and terpinen-4-ol. It is possible that the scats collected did not come from any of the trees from which berries were collected.

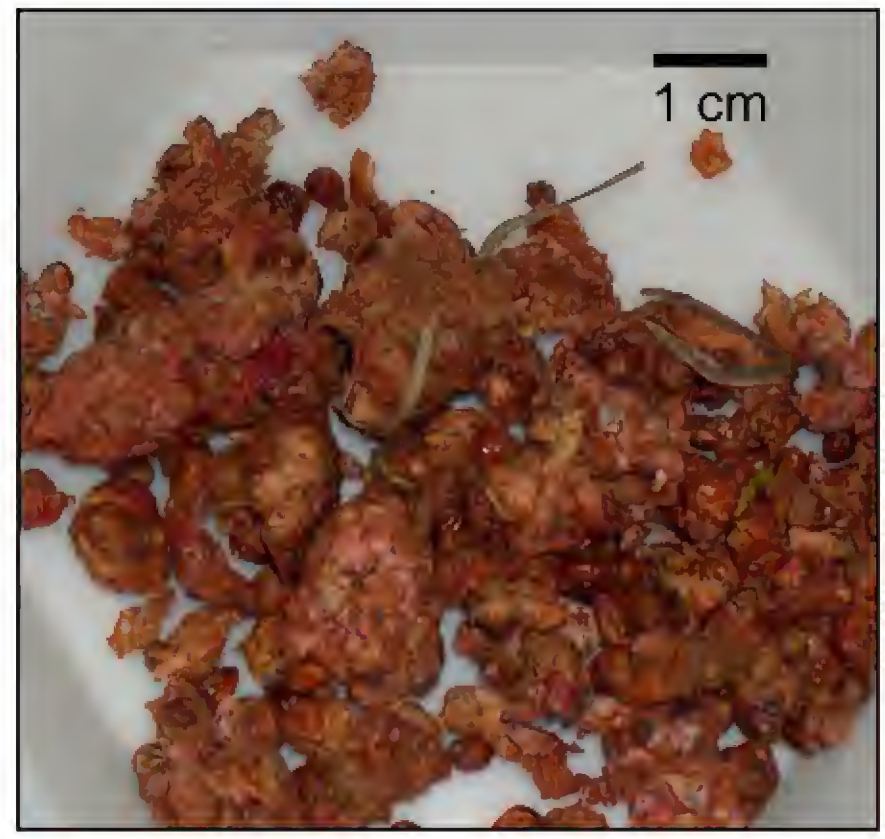

Fig. 8. Fox scat with *J. coahuilensis* berries.

From the current limited study, one can not say which factor was most important (or if there may be additional factors, not considered).

Several terpenes are in larger concentration (Table 2) in the scat: α-pinene, sabinene, and the three diterpenes - abietadiene, 4-epi-abeital, and abieta-7,13-dien-3-one. Two terpenes are larger in the cones: terpinolene and terpinen-4-ol (Table 2).

The volatile berry oils differ in many components, both quantitatively and qualitatively, from the volatile leaf oil of *J. coahuilensis* from La Zarca, Mexico (Table 3). This likely reflects both ontogenetic variation between berries and leaves as well as geographic variation in the leaf oils of *J. coahuilensis* (Figs. 4, 5, Adams, 2000). Although, Adams (2000) found the leaf oil from Alpine, TX to be very similar to that from Ciudad Chihuahua (CM), north of La Zarca.

Although there appear to be no reports on the fate of terpenes in gray fox, there are a few such papers on small mammals. McLean et al. (1993) fed *Eucalyptus radiata* leaves to ringtail possums and found terpene derived metabolites increased in the urine. They concluded possums detoxify dietary terpenes by polyoxygenating the terpenes so the highly polar metabolites will be water soluble and excreted in urine.

Boyle et al. (2000) fed p-cymene to Koala and found no p-cymene or metabolites in the feces, but oxidized metabolites of p-cymene were excreted in urine; a very similar mechanism as found in possum (McLean et al., 1993).

Other methods of detoxifying *Juniperus* terpenes in woodrats (*Neotoma*) are discussed by Adams et al. (2014, 2016).

In conclusion, it appears, in this preliminary study, that gray fox does not extract most of the terpenes from juniper berries. The nearly intact berries, containing most of the terpenes, are excreted in the scat. This undoubtedly results in the loss of some or considerable amounts of nutrients, but the food source is so plentiful, that the loss of nutrients, due to incomplete digestion, may not be significant to the health of the gray fox.

ACKNOWLEDGEMENTS

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Table 1 Comparison of the volatile oil compositions of fresh seed cones vs. fox scat of *J. coahuilensis*. Components with considerable variation between scat and seed cones (berries) are in bold.

KI	Compound	scat 14654	scat 14655	scat 14656	scat 14657	scat 14658	cones 14649	cones 14650	cones 14651	cones 14653
	percent yield (% ODW)	**1.38**	**1.95**	**1.54**	**1.52**	**1.32**	**0.49**	**0.42**	**0.26**	**0.30**
921	tricyclene	t	t	t	t	t	t	t	t	t
924	α-thujene	2.0	1.8	2.1	2.3	1.8	1.5	1.6	2.1	1.1
932	**α-pinene**	**14.4**	**13.0**	**17.7**	**8.9**	**10.2**	**16.7**	**6.0**	**3.0**	**3.2**
946	camphene	0.1	0.2	0.2	0.2	0.2	0.3	0.3	t	t
961	verbenene	-	-	-	-	-	0.8	-	-	0.2
969	**sabinene**	**46.9**	**44.0**	**51.9**	**55.4**	**50.0**	**21.9**	**19.9**	**20.1**	**22.1**
974	β-pinene	0.9	0.9	0.9	1.0	0.7	0.9	0.5	0.2	0.3
988	myrcene	0.8	0.9	1.0	1.8	0.8	0.5	0.3	0.5	0.8
1002	α-phellandrene	t	t	t	t	t	t	0.2	0.1	0.2
1014	α-terpinene	0.8	1.0	0.8	1.1	1.1	1.5	2.9	3.9	2.8
1020	p-cymene	0.5	0.5	0.7	0.6	0.6	0.6	1.2	1.1	1.0
1024	limonene	2.1	1.7	2.2	2.3	2.0	2.6	1.7	2.4	1.5
1025	β-phellandrene	1.4	1.0	1.5	1.6	1.3	1.8	1.1	1.5	1.0
1044	(E)-β-ocimene	t	t	t	t	t	t	t	t	t
1054	γ-terpinene	1.4	1.9	1.4	2.1	1.9	2.8	5.0	6.7	5.3
1065	cis-sabinene hydrate	1.7	1.7	1.1	1.5	1.5	1.6	2.0	1.8	2.0
1086	**terpinolene**	**0.4**	**0.5**	**0.4**	**0.7**	**0.5**	**1.0**	**1.4**	**1.8**	**1.6**
1098	trans-sabinene hydrate	3.5	3.5	1.5	2.4	2.4	2.8	3.1	3.9	2.8
1099	α-pinene oxide	0.4	0.4	0.2	0.1	t	0.3	0.2	t	t
1112	trans-thujone	0.1	0.1	0.1	t	t	0.2	0.3	0.3	0.3
1118	cis-p-menth-2-en-1-ol	0.3	0.4	0.3	0.4	0.5	0.7	1.3	1.4	1.6
1122	α-camphenal	0.6	1.0	0.5	0.3	0.6	2.0	2.5	1.3	1.3
1123	terpene,67,81,,156,168	0.8	1.3	0.7	0.6	0.8	1.4	1.4	1.3	1.4
1135	trans-pinocarveol	0.7	0.7	0.4	0.4	0.8	2.6	1.7	1.3	1.8
1137	trans-sabinol	0.6	1.2	0.8	0.5	0.9	1.1	2.1	1.2	1.7
1137	trans-verbenol	2.0	2.1	1.5	0.8	1.5	5.3	3.1	1.1	1.8
1154	sabina ketone	1.5	1.4	1.2	1.0	1.4	1.9	4.0	3.7	3.9
1160	pinocarvone	0.2	0.3	0.2	t	0.2	0.6	0.6	0.2	0.4
1166	p-mentha-1,5-dien-8-ol	0.1	0.2	0.2	t	0.2	0.7	0.9	0.3	0.6
1169	terpene,92,81,134,152	0.7	1.0	0.6	0.6	1.0	0.9	1.8	1.6	1.7
1174	**terpinen-4-ol**	**3.1**	**4.4**	**2.3**	**4.2**	**4.4**	**6.8**	**13.9**	**18.1**	**20.2**
1181	thuj-3-en-10-al	0.4	0.5	0.4	0.3	0.6	0.5	1.1	1.1	1.2
1186	α-terpineol	0.3	0.3	0.2	0.2	0.3	0.5	0.6	0.7	0.8
1195	myrtanal	1.2	1.5	1.1	1.1	1.5	2.3	3.7	3.1	4.0
1196	myrtanol	0.4	0.5	0.4	t	0.4	1.0	0.7	0.3	0.1
1204	verbenone	0.6	0.7	0.4	0.3	0.5	1.7	1.2	0.9	1.1
1215	trans-carveol	0.4	0.4	0.3	0.2	0.4	1.2	1.0	0.6	0.7
1223	citronellol	t	1.0	0.2	t	0.4	1.2	0.3	0.5	0.3
1235	cis-chrysanthenyl acetate	0.2	t	0.1	t	0.3	t	-	-	-
1238	cumin aldehyde	0.2	0.2	0.2	t	0.3	0.3	0.6	0.4	0.4
1239	carvone	0.2	0.2	0.2	0.1	0.3	0.4	0.5	0.5	0.4
1269	perilla aldehyde	0.1	0.2	0.2	t	0.2	0.3	0.6	0.4	0.4
1284	bornyl acetate	0.3	0.3	0.3	0.1	0.2	0.5	1.0	0.7	0.7
1289	p-cymen-7-ol	0.3	0.4	0.3	0.2	0.8	0.6	0.4	0.2	0.6
1325	p-mentha-1,4,dien-7-ol	0.5	0.4	0.3	0.1	0.4	0.9	1.5	1.6	1.4
1400	tetradecane	t	t	t	t	t	t	t	t	t
1417	(E)-caryophyllene	t	t	t	0.2	t	t	t	t	t
1480	germacrene D	t	t	t	0.9	t	t	t	t	t
1513	γ-cadinene	t	t	t	0.2	t	t	t	t	t
1522	δ-cadinene	t	t	t	0.1	t	t	t	t	t
1548	elemol	0.4	0.5	0.2	0.5	0.4	0.4	0.2	0.2	0.1
1574	germacrene D-4-ol	t	t	t	t	t	t	t	t	t

KI	Compound	scat 14654	scat 14655	scat 14656	scat 14657	scat 14658	cones 14649	cones 14650	cones 14651	cones 14653
1582	caryophyllene oxide	0.3	0.3	0.2	0.2	0.3	0.4	0.2	0.3	t
1608	humulene epoxide	0.3	0.2	0.2	0.2	0.2	0.5	0.2	0.2	t
1627	1-epi-cubenol	t	0.2	t	t	t	t	t	t	t
1649	β-eudesmol	t	0.2	t	t	t	t	t	t	t
1652	α-eudesmol	t	0.1	t	t	t	t	t	t	t
1685	germacra-4(15),5,10(14)-trien-1-al	t	t	t	t	t	-	t	t	t
1959	hexadecanoic acid	t	0.2	t	t	t	0.2	0.2	0.7	0.2
2022	cis-abieta-8,12-diene	t	t	t	t	t	-	-	t	-
2055	abietatriene	t	t	t	t	t	-	-	t	-
2087	**abietadiene**	**0.3**	**0.2**	**t**	**0.2**	**0.4**	**-**	**-**	**t**	**-**
2298	**4-epi-abietal**	**0.2**	**0.3**	**t**	**0.3**	**0.2**	**-**	**-**	**t**	**-**
2312	**abieta-7,13-dien-3-one**	**0.4**	**0.4**	**t**	**0.4**	**0.7**	**-**	**-**	**0.2**	**-**
2343	4-epi-abietol	t	t	t	t	t	-	-	t	-
2401	abietol	t	0.2	t	t	0.2	-	-	t	-

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

Table 2. Statistical analysis of selected components of oils in scat vs. seed cones.

KI	Compound	scat avg	cones avg	P value	significance
	percent yield (% ODW)	1.54	0.39	2 x 10-5	**
932	α-pinene	12.84	7.22	0.68	ns
969	sabinene	49.64	21.00	1 x 10-5	**
1086	terpinolene	0.50	1.45	3x10-4	**
1174	terpinen-4-ol	3.58	14.75	2x10-3	**
2087	abietadiene	0.07	0.01	8x10-3	**
2298	4-epi-abietal	0.20	0.02	8x10-3	**
2312	abieta-7,13-dien-3-one	0.39	0.03	1.5x10-2	*

Table 3. Comparison of the volatile oil compositions of fresh seed cones and leaf oil from La Zarca, MX, *Adams 6829* (Adams, 2000). Components with considerable difference between seed cone oils and leaf oil are in bold.

KI	Compound	cones 14649	cones 14650	cones 14651	cones 14653	leaf oil 6829
	percent yield (% ODW)	**0.49**	**0.42**	**0.26**	**0.30**	**1.23**
921	tricyclene	t	t	t	t	t
924	α-thujene	1.5	1.6	2.1	1.1	1.6
932	**α-pinene**	**16.7**	**6.0**	**3.0**	**3.2**	**2.6**
946	camphene	0.3	0.3	t	t	t
961	**verbenene**	**0.8**	**-**	**-**	**0.2**	**-**
969	**sabinene**	**21.9**	**19.9**	**20.1**	**22.1**	**35.5**
974	β-pinene	0.9	0.5	0.2	0.3	0.5
988	myrcene	0.5	0.3	0.5	0.8	2.6
1002	α-phellandrene	t	0.2	0.1	0.2	0.3
1014	α-terpinene	1.5	2.9	3.9	2.8	3.6
1020	p-cymene	0.6	1.2	1.1	1.0	0.3
1024	limonene	2.6	1.7	2.4	1.5	1.8
1025	β-phellandrene	1.8	1.1	1.5	1.0	1.7
1026	1,8-cineole	-	-	-	-	0.5
1044	(E)-β-ocimene	t	t	t	t	0.2
1054	γ-terpinene	2.8	5.0	6.7	5.3	5.6
1065	cis-sabinene hydrate	1.6	2.0	1.8	2.0	1.5
1067	cis-linalool oxide (furano-)	-	-	-	-	t
1086	**terpinolene**	**1.0**	**1.4**	**1.8**	**1.6**	**1.9**
1098	trans-sabinene hydrate	2.8	3.1	3.9	2.8	2.2
1099	α-pinene oxide	0.3	0.2	t	t	-
1112	trans-thujone	0.2	0.3	0.3	0.3	t
1118	cis-p-menth-2-en-1-ol	0.7	1.3	1.4	1.6	0.9
1122	**α-camphenal**	**2.0**	**2.5**	**1.3**	**1.3**	**-**
1123	**terpene,67,81,109,156,168**	**1.4**	**1.4**	**1.3**	**1.4**	**-**
1135	**trans-pinocarveol**	**2.6**	**1.7**	**1.3**	**1.8**	**-**
1136	**trans-p-menth-2-en-1-ol**	**-**	**-**	**-**	**-**	**0.6**
1137	**trans-sabinol**	**1.1**	**2.1**	**1.2**	**1.7**	**-**
1137	**trans-verbenol**	**5.3**	**3.1**	**1.1**	**1.8**	**-**
1142	**camphor**	**-**	**-**	**-**	**-**	**0.4**
1144	**neo-isopulegol**	**-**	**-**	**-**	**-**	**0.4**
1145	**camphene hydrate**	**-**	**-**	**-**	**-**	**t**
1148	**citronellal**	**-**	**-**	**-**	**-**	**4.1**
1154	**sabina ketone**	**1.9**	**4.0**	**3.7**	**3.9**	**-**
1155	iso-isopulegol	-	-	-	-	0.1
1160	**pinocarvone**	**0.6**	**0.6**	**0.2**	**0.4**	**-**
1165	borneol	-	-	-	-	t
1166	p-mentha-1,5-dien-8-ol	0.7	0.9	0.3	0.6	-
1166	coahuilensol	-	-	-	-	t
1169	**terpene,92,81,134,152**	**0.9**	**1.8**	**1.6**	**1.7**	**-**
1174	terpinen-4-ol	6.8	13.9	18.1	20.2	12.4
1181	**thuj-3-en-10-al**	**0.5**	**1.1**	**1.1**	**1.2**	**-**
1186	α-terpineol	0.5	0.6	0.7	0.8	0.5
1195	cis-piperitol	-	-	-	-	0.2
1195	**myrtanal**	**2.3**	**3.7**	**3.1**	**4.0**	**-**
1196	**myrtanol**	**1.0**	**0.7**	**0.3**	**0.1**	**t**
1204	**verbenone**	**1.7**	**1.2**	**0.9**	**1.1**	**-**
1207	**trans-piperitol**	**-**	**-**	**-**	**-**	**0.3**
1215	**trans-carveol**	**1.2**	**1.0**	**0.6**	**0.7**	**-**
1223	**citronellol**	**1.2**	**0.3**	**0.5**	**0.3**	**4.9**
1235	cis-chrysanthenyl acetate	t	-	-	-	-

KI	Compound	cones 14649	cones 14650	cones 14651	cones 14653	leaf oil 6829
1238	**cumin aldehyde**	**0.3**	**0.6**	**0.4**	**0.4**	**-**
1239	**carvone**	**0.4**	**0.5**	**0.5**	**0.4**	**-**
1269	**perilla aldehyde**	**0.3**	**0.6**	**0.4**	**0.4**	**-**
1274	**pregeijerene B**	**-**	**-**	**-**	**-**	**0.4**
1284	**bornyl acetate**	**0.5**	**1.0**	**0.7**	**0.7**	**t**
1289	**p-cymen-7-ol**	**0.6**	**0.4**	**0.2**	**0.6**	**-**
1325	**p-mentha-1,4,dien-7-ol**	**0.9**	**1.5**	**1.6**	**1.4**	**-**
1387	β-cubebene	-	-	-	-	t
1389	β-elemene	-	-	-	-	0.1
1400	tetradecane	t	t	t	t	-
1417	(E)-caryophyllene	t	t	t	t	t
1448	cis-muurola-3,5-diene	-	-	-	-	0.2
1452	α-humulene	-	-	-	-	t
1468	pinchotene acetate	-	-	-	-	t
1475	trans-cadina-1(6),4-diene	-	-	-	-	0.2
1480	germacrene D	t	t	t	t	-
1489	β-selinene	-	-	-	-	t
1493	trans-muurola-4(14),5-diene	-	-	-	-	0.2
1496	valencene	-	-	-	-	0.2
1500	α-muurolene	-	-	-	-	t
1513	γ-cadinene	t	t	t	t	0.2
1522	δ-cadinene	t	t	t	t	0.1
1545	selina-3,7(11)-diene	-	-	-	-	t
1548	elemol	0.4	0.2	0.2	0.1	5.8
1574	germacrene D-4-ol	t	t	t	t	t
1582	caryophyllene oxide	0.4	0.2	0.3	t	-
1608	humulene epoxide	0.5	0.2	0.2	t	-
1627	1-epi-cubenol	t	t	t	t	0.5
1630	γ-eudesmol	-	-	-	-	1.0
1649	β-eudesmol	t	t	t	t	1.2
1652	α-eudesmol	t	t	t	t	1.3
1670	bulnesol	-	-	-	-	0.5
1685	germacra-4(15),5,10(14)-trien-1-al	-	t	t	t	-
1746	**8-α-11-elemodiol**	**-**	**-**	**-**	**-**	**0.3**
1792	**8-α-acetoxyelemol**	**-**	**-**	**-**	**-**	**0.7**
1959	hexadecanoic acid	0.2	0.2	0.7	0.2	-
1987	manoyl oxide	-	-	-	-	t
2022	cis-abieta-8,12-diene	-	-	t	-	-
2055	abietatriene	-	-	t	-	t
2087	**abietadiene**	**-**	**-**	**t**	**-**	**t**
2298	**4-epi-abietal**	**-**	**-**	**t**	**-**	**t**
2312	**abieta-7,13-dien-3-one**	**-**	**-**	**0.2**	**-**	**t**
2313	abietal	-	-	-	-	t
2331	trans-ferruginol	-	-	-	-	t
2343	4-epi-abietol	-	-	t	-	-
2401	abietol	-	-	t	-	-

Reconsideration of the inclusiveness of genus *Plasmophagus* (Chytridiomycota, *posteris traditus*) based on morphology

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

ABSTRACT

Plasmophagus De Wildeman (1895), a presumably primitive fungal genus, has been considered to contain one or three species. Judging it to contain more than the original species (*P. oedogoniorum* De Wildeman, 1895), however, is a relatively recent taxonomic assessment. Dick (2001) recognized three species—placing genus *Plasmophagus*, though of uncertain position, in association with (but not necessarily included in) what one would interpret as the Oomycota (cf. "Peronosporomycotina" Dick). Our analysis of original (and other) literature and illustrations led to the realization that, as presently recognized (cf. Dick, 2001; *Index Fungorum*, as of this writing), *Plasmophagus* is a heterogeneous assemblage. Each of three supposed species apparently belongs to a different genus; two of these, including the original species, are in (probably closely related) genera, that may be considered (based on classical characters) to belong to Phylum Chytridiomycota, Kingdom Fungi; the third alleged species is best placed in a genus in Phylum Oomycota, Kingdom Straminipila (cf. "Chromista," some authors). *Plasmophagus* is consistent as a genus only in its initial, monotypic sense, and aligns (traditional classification) with Chytridiomycetes rather than Oomycetes. Molecular investigations could clarify the systematic placement of *Plasmophagus*, i.e., if it should possibly be in the Opisthosporidia/Cryptomycota (in which a potentially related genus, *Rozella*, has now been reclassified). Published on-line www.phytologia.org *Phytologia 98(2): 128-136 (Apr 4, 2016).* ISSN 030319430.

KEY WORDS: Chytrid, *Coleochaete*, *Draparnaldia*, host-cell, *Oedogonium*, Olpidiaceae, *Olpidiopsis*, *Olpidium*, Opisthosporidia, parasite, *Rozella*, Rozellopsidales, sporangium, *Tribonema*, zoospores.

In a comparatively recent taxonomic/nomenclatural treatment, Dick (2001, pp. 380-381) included genus *Plasmophagus* (*insertae sedis*, not assigned to a family; *sphalm.* Pseudosphaeritaceae, Beakes et al., 2014, p. 50) in his newly described Order Rozellopsidales (Order also *incertae sedis*). Though at first glance seemingly an "oomycetous grouping," Dick (2001) did not include Order Rozellopsidales within Class Peronosporomycetes (this Class roughly equivalent to a restrictive concept of the traditional Class, Oomycetes; see explanatory paragraph in Beakes et al., 2014, p. 52). More confusingly, Dick (2001, p. 274) apparently included his new Order (ostensibly containing *Plasmophagus*) within, but at the same time "provisionally excluded" it from, the broader grouping, Subphylum Peronosporomycotina (in "Phylum" Heterokonta, cf. p. 275). Subphylum Peronosporomycotina (described by Dick, 2001, p. 288) may be considered largely equivalent to Phylum Oomycota (cf. Beakes et al., 2014, p. 52). We note in passing that some workers (e.g., Petersen and Rosendahl, 2000) have employed "Peronosporomycetes" as a Class within Phylum Oomycota. Regardless, even in a wider survey of pertinent 21st century literature, no conclusive placement of genus *Plasmophagus* (*totus*) is evident.

For more than a century, *Plasmophagus* was monotypic, containing only the original species, *P. oedogoniorum* De Wildeman (1895). Without explanation, Dick (2001, p. 380-381) recognized three species in the genus: "*Plasmophagus oedogoniorum* É. de Wildeman," "*Plasmophagus deformans* (I. L. Serbinov) M. W. Dick," and "*Plasmophagus coleochaetis* (F. K. Sparrow, R. A. Paterson & R. M. Johns) M. W. Dick;" the last two alleged species of *Plasmophagus*, transferred from other genera, represent new combinations effected by Dick (2001). As regards authorship of *P. deformans*, we note that the spelling "Serbinov" (given by Dick) should be "Serbinow." Dick (2001) indicated that "*Rozella* sp. (Schulz, 1922)" represented an "unidentified species referable to *Plasmophagus*." By listing the Schulz reference

under "*Plasmophagus coloechaetis*," Dick (2001) may have been implying that a taxon in Schulz (1922) might be referable here (i.e., to *P. coleochaetis*). Our examination of the Schulz (1922) paper, however, revealed no organism definitely referable to *Plasmophagus*—and certainly not to "*Plasmophagus*" (*Rozella*) *coleochaetis*, since Schulz' 1922 article dealt with desmids (*Coleochaete*, not a desmid, was not found in this article). We also examined Schulz' 1923 paper—dealing primarily with parasites of desmids (and a member of the Zygnemataceae)—and likewise found no definitive match for *Plasmophagus*.

Based on traditional morphology, it appears that two of the species included by Dick (2001) under *Plasmophagus*—*P. oedogoniorum* (the "type") and *P. coleochaetis*—are members of the Chytridiomycetes (Chytridiomycota), cf. De Wildeman (1895), Fitzpatrick (1930), Sparrow (1960) and Karling (1977); accordingly, these two taxa would be considered to belong to Kingdom Fungi. However, the third species, *P. deformans*, has been deemed (statedly or implicitly) to have a placement within the Oomycetes (Oomycota), cf. Sparrow, 1943, 1960 and Karling, 1981; if so, this (third) taxon would presently be encompassed within Kingdom Straminipila (cf. Dick, 2001; or Kingdom Chromista, cf. Cavalier-Smith, 2001), not Kingdom Fungi (i.e., as now more restrictively recognized, cf. Blackwell and Powell, 1995; Cavalier-Smith, 2001). Attempting to properly sort out these points (and address associated nomenclatural questions) constituted the basis for undertaking the present investigation.

CLASSICAL TAXONOMIC HISTORY AND NOMENCLATURE OF *PLASMOPHAGUS*
(Figs. 1-9: Illustrations of Organisms and Structures Discussed)

The genus *Plasmophagus* was established in 1895 by De Wildeman on the basis of a single species, *P. oedogoniorum* (**Figs. 1-3**), found in *Oedogonium* sp. This parasite was also putatively found (**Fig. 4**) in *Tribonema bombycina* (Sparrow, 1933). *Plasmophagus* was placed in the chytridiaceous family, Olpidiaceae (cf. Fitzpatrick, 1930; Sparrow, 1960)—the simple, single-celled thallus in this family lacks the rhizoidal system ("vegetative structures") characteristic of a majority of Chytridiomycete families (cf. Sparrow, 1960, p. 120-122; Blackwell et al. 2006, p. 94, 97) and is holocarpic (entirely converting to a sporangium in asexual reproduction). At first amoeboid, the thallus of *Plamophagus* eventually more or less fills the algal host-cell (distinguishing it from genus *Olpidium*), allegedly becoming walled in the process; however, the "sporangial wall" (or containing membrane) of the parasite does not fuse (or completely fuse) with the host cell-wall—in supposed distinction to a similar genus, *Rozella*, also traditionally placed in the Olpidiaceae, in which such (complete) fusion is said to occur (Sparrow, 1960; Karling, 1977). Some hypertrophy of the host-cell is a consequence of infection by *Plasmophagus* (as in the case of infection by some species of *Rozella*). The small, posteriorly uniflagellate zoospores (longer than broad; with a single refractive globule) are released, laterally (though sometimes toward one end of the cell), through a modest discharge-pore; the discharge papilla is small, or obscure, barely penetrating the host-wall to the outside. Resting spores were not observed.

Five years after publication of Sparrow's second edition of *Aquatic Phycomycetes* (1960), Sparrow, Paterson and Johns (1965) published "Additions to the Phycomycete Flora of the Douglas Lake Region," Michigan. Included in Sparrow et al. (1965) was a description of a new species of *Rozella*, *R. coleochaetis* (**Fig. 5**), by Sparrow (*solum*). *Rozella*, though with more species, has been considered generally similar to *Plasmophagus* (cf. Sparrow, 1960; Karling, 1977). In both genera, the sporangium may essentially occupy the space within the host cell. In his key to genera of the traditional Chytridiomycete family, Olpidiaceae, Sparrow (1960) distinguished these genera by the fact that, in *Rozella*, the sporangial "wall" of the parasite (*Rozella*) and the host cell-wall supposedly become fused, with no space existing between them. In *Plasmophagus*, although the sporangium often comes to more or less fill the host-cell, fusion (or complete fusion) of the "walls" (parasite and host) is not attained. Regardless of the specifics of development (i.e., exactly "how it happens"), the morphological distinction (of *Rozella* and *Plasmophagus*) is that, in *Plasmophagus*, limited "space" between parasite and host may be visible in places (**Figs. 1, 3**). Concerning the species of *Rozella* (*R. coleochaetis*) found in "free-

filament portions" of the cushion-like green-alga *Coleochaete*, Sparrow (*in* Sparrow et al., 1965) concluded that complete fusion of host and parasite partitions must somehow take place, because, at maturity, their "walls" were "indistinguishable"—in other words, it wasn't possible (in a developed thallus) to tell where the boundary of the parasite ended, and the host began. Indeed, no separated "wall-areas" are evident in illustrations of *Rozella coleochaetis* Sparrow (Sparrow et al., 1965, figs. A-E), and one observes in these drawings only a unitary containing-structure (see also our **Fig. 5**). We find no basis, regarding the nature of "wall-fusion" or other traditional characters, for transfer of *Rozella coleochaetis* to genus *Plasmophagus*—viz. *P. coleochaetis* (Sparrow) Dick—as (questionably) effected by Dick (2001).

The posteriorly uniflagellate zoospores of *Rozella coleochaetis* suggest a chytridiomycetous (Fungal) classification for this taxon—not a placement within the Peronosporomycotina [Oomycota, Straminipila], which would seem at first glance to have been indicated by Dick (2001, p. 380-381) in his "circumscription" of *Plasmophagus* (this including *R. coleochaetis*, as *Plasmophagus coleochaetis*). As mentioned, though, Dick (see his pp. 274, 371) left the "relationship" of Order Rozellopsidales (to which genus *Plasmophagus* was assigned by him) "*insertae sedis;*" not only was the Order *not* included in the Peronosporomycetes, it was "provisionally excluded" from the broader grouping, Peronosporomycotina. Dick (2001) did not indicate where Order Rozellopsidales should be placed; in a footnote (p. 254) Dick noted that this Order contained both uniflagellate and biflagellate taxa [encompassing, thus, possibly unrelated organisms]. Beakes et al. (2014, p. 52) noted there had been additional molecular insights since Dick's (2001) compilation, raising questions about certain higher level taxa recognized by Dick, and suggesting the need for additional resolution of less-studied Orders [such as the Rozellopsidales]. In further uncertainty, *Plasmophagus* (including *Rozella coleochaetis*, according to Dick) was indicated as *insertae sedis* within the Order Rozellopsidales. In any event, as for possible actual placement of *Rozella coleochaetis*, not only are its zoospores posteriorly uniflagellate (consistent with chytrid zoospores), but the somewhat elongate, generally straight, form of these small spores compares well with zoospore-form of other species of *Rozella*—e.g., *R. allomycis* and *R. polyphagi*—*Rozella* being historically classified in the Olpidiaceae/Chytridiomycetes (cf. Sparrow, 1960). The initial taxonomic assignment of *R. coleochaetis* to *Rozella* (and to Chytridiomycota, *a maioribus traditus*) by Sparrow (Sparrow et al., 1965), seems to be supported by "thallus-fusion with the host-cell" (paragraph above) *and* zoospore morphology. Again, we find no substantive basis for Dick's (2001) transfer of *R. coleochaetis* to *Plasmophagus*.

Even if one were to accept Dick's (2001) unexplained transfer of *Rozella coleochaetis* to *Plasmophagus*, matters of nomenclatural concern remain. Dick credited publication of *Rozella coleochaetis* to "F. K. Sparrow, R. A. Paterson & R. M. Johns" (authors of the 1965 paper; cf. Sparrow et al., 1965, above). However, Sparrow *alone* is author of this species—*in* Sparrow, Paterson & Johns (1965)—and the specific epithet ("*coleochaetis*") should be attributed only to Sparrow. *Index Fungorum* (IF), an online resource for fungal names, indeed recognized authorship as just "Sparrow," but listed the spelling of the epithet (under *Plasmophagus*) as *P.* "*coleochaetes*" (Sparrow) M. W. Dick (2001), rather than *P.* "*coleochaetis*." One might suspect perhaps that this spelling ("*coleochaetes*") of the epithet in IF would be based on the way Sparrow (*in* Sparrow et al., 1965) was thought to have originally spelled the name (*sic*, "*Rozella coleochaetes*," IF). However, Sparrow (in 1965) in fact spelled the original epithet "*coleochaetis*," not "*coleochaetes*." Dick (2001) simply utilized Sparrow's original spelling ("*coleochaetis*") in his (otherwise questionable) transfer to *Plasmophagus*.

As mentioned, Dick (2001) included three species in *Plasmophagus*, two species in addition to the chytridiaceous, "type" species (*P. oedogoniorum* De Wildeman, 1895). Of the additional two species included (transferred in) by Dick, *P. coleochaetis* (as discussed) seems best assigned to *Rozella* (Chytridiomycetes *sensu lato*); but, the other species, *Plasmophagus deformans* (Serbinow) Dick, is of questionable relationship. *Plasmophagus deformans* was based on a taxon, with biflagellate zoospores, described by Serbinow (1907, p. 153-154) under the name "*Pseudolpidium* (?) *deformans*" [later considered to belong to genus *Olpidiopsis*]. This organism is parasitic in lateral branches of the alga,

Draparnaldia (**Fig. 6**), in which it causes cell-hypertrophy. Though *P. deformans* (**Figs. 7-9**) was viewed as chytridiaceous by Serbinow, the critical distinction between posteriorly-uniflagellate forms (probable Chytridiomycetes) and biflagellate (often laterally biflagellate) forms—these "biflagellates" turning out *not* to be chytrids—was not necessarily clear in 1907, and developed over a period of years (cf. Scherffel, 1926; Sparrow, 1943). Sparrow (1943, 1960) placed genus *Pseudolpidium* in the Olpidiopsidaceae of the Lagenidiales—these being Oomycetes, *not* Chytridiomycetes. Sparrow (1943, p. 609) questioned the status of some species of *Pseudolpidium*, yet recognized the genus *pro parte* (pp. 636-638); he listed *P. deformans* as an "imperfectly known" species under this genus, and did not include it in the key to species. Later, Sparrow (1960, p. 955), still (questioningly) recognizing *Pseudolpidium*—referring to the genus as "a dumping ground" for several incompletely known species—included *P. deformans* in the genus (this time, including it in his species key). Karling (1981) transferred *Pseudolpidium deformans* to *Olpidiopsis* (also a genus of Oomycota)—viz., *Olpidiopsis deformans* (Serbinow) Karling—*not* to *Olpidium*. Sparrow (1960) considered the two flagella of zoospores of *Olpidiopsis deformans* (i.e., *Pseudopidium deformans*, cf. Sparrow, 1960) to be laterally inserted; Karling (1981) thought them possibly laterally inserted, but added a question mark (and did not illustrate them as lateral; his plate 5, fig. 184). Regarding illustrations of "*P. deformans*," Serbinow's (1907) figure 28 (of Plate III/IV) shows the biflagellate nature of the zoospores, and that the insertion of the two, possibly unequal flagella is perhaps apical or sub-apical (**Fig. 9**). In any case, the biflagellate character of the zoospores indicates that they are *not* chytridiaceous (i.e., not fungal) zoospores, and would support a placement of "*Olpidiopsis deformans*" (i.e., *Plasmophagus deformans*, cf. Dick, 2001; i.e., *Pseudolpidium deformans*, cf. Serbinow, 1907; Sparrow, 1960) within the Oomycota (and hence within Kingdom Straminipila).

Thus, although Dick (2001)—in what is apparently the most recent taxonomic treatment of the genus—included genus *Plasmophagus* in an Order (Rozellopsidales) of uncertain position (and *Plasmophagus* was not assigned by Dick to a family within this Order), he nonetheless loosely associated this Order (and genus) with Peronosporomycotina (i.e., Oomycota). However, whereas Dick included three species under *Plasmophagus*, only one of these (*P. deformans*) is referable to a genus of Oomycota. The other two species included by Dick (*P. oedogoniorum* and *P. coleochaetis*)—though in different (perhaps closely related) genera—are better referred, based on traditional morphology and classification, to the Chytridiomycota. Since the "type species," *P. oedogoniorum*, is determinable as a Chytridiomycete (*praeteritum tempus*), *Plasmophagus* should not be considered an Oomycete genus—and Dick's "provisional exclusion" of the Order containing this genus from the Peronosporomycotina, though he provided no reason for this, would (in so far as his tentative statement went) be upheld.

SUMMARY OF TRADITIONAL SYSTEMATIC INFORMATION

Genus *Plasmophagus* as recognized by Dick (2001), though "encompassing" only three species (*P. oedogoniorum*, *P. coleochaetis*, and *P. deformans*), is a disparate assemblage. Each species, in fact, probably represents a different genus—one of these (*P. deformans*) being unrelated to the other two.

Plasmophagus (De Wildeman, 1895) initially contained a single species (*P. oedogoniorum*) and seemingly fitted well in the simple-structured, presumably primitive Chytridiomycete family, Olpidiaceae (cf., Fitzpatrick, 1930; Sparrow, 1960; and Karling, 1977, provisionally). *Plasmophagus* (*sensu originalis*) has been distinguished (Sparrow, 1960) from *Olpidium* (i.e., *Olpidium* as traditionally understood) by a sporangium which virtually fills the host-cell, and from *Rozella* by the fact that the "sporangial wall" does not (uniformly) "fuse" with the host cell-wall.

Sparrow's (*in* Sparrow et al., 1965, pp. 117-118) description of *Rozella coleochaetis* should have had little effect on the taxonomy of genus *Plasmophagus*. Sparrow pointed out that, whereas *Rozella coleochaetis* resembled *Plasmophagus oedogoniorum*, *R. coleochaetis* differed "in having complete fusion of host and parasite wall." Though *Rozella* and *Plasmophagus* were similar, they were recognized

as distinct genera (cf. Sparrow, 1960) based on alleged degree of "wall-fusion." In any case, Dick (2001) provided no rationale for his transfer of *Rozella coleochaetis* Sparrow to *Plasmophagus*. Based on Sparrow's (1965) original description and illustrations, *R. coleochaetis* should remain in *Rozella* (as it has been classically recognized, by morphological characteristics). *Rozella*, traditionally recognized as a Chytridiomycete genus, may actually place in a related grouping (or "clade"), as will be discussed.

The third alleged species of *Plasmophagus*, *P. deformans* (Serbinow) Dick (2001), has been the most difficult to determine. This species is apparently not a Chytridiomycete, and not even in Kingdom Fungi. It should, rather, be included with those organisms recognized as Oomycetes [Oomycota], cf. Sparrow (1960) and Karling (1981), these encompassed within Kingdom Straminipila (Dick, 2001; or Kingdom "Chromista," if one prefers a broader kingdom concept, cf. Cavalier-Smith, 2001; Blackwell, 2009). The further placement of "*P. deformans*" seems best at present in genus *Olpidiopsis* (Oomycetes), see Karling (1981), *not Olpidium*. We cannot agree with Dick's (2001) transfer of *Olpidiopsis deformans* to *Plasmophagus* (since *Plasmophagus* was founded as a chytridiaceous genus).

In summary of traditional information, *Plasmophagus*, as recognized by Dick (2001) and enumerated in *Index Fungorum* (presently), is polymorphic, containing: (1) one chytrid-like, original species, in Kingdom Fungi; (2) a second, initially "chytrid" species which belongs to a similar, possibly related, fungal genus, *Rozella*; and (3), a third, "oomycete" species which apparently belongs to a fundamentally different genus, *Olpidiopsis* (not *Olpidium*), this in a different kingdom (Straminipila, not Fungi). If *Plasmophagus* is to be definably recognized, it should (based on traditional taxonomic characters, and in the absence of molecular data) be returned to its initial, monotypic status (containing only *P. oedogoniorum*). Though classically considered to be a chytrid, it remains to be seen if *P. oedogoniorum* will ultimately place within the Chytridiomycota, or in a related group of organisms (if certain molecular information can be obtained).

CURRENT STATUS OF CLASSIFICATION

Although *Plasmophagus* and *Rozella* were earlier classified as members of the Olpidiaceae (Sparrow, 1960), in the Chytridiales, Barr (1980) transferred *Rozella* to a newly erected order, Spizellomycetales. However, molecular phylogenetic analysis has revealed that *Rozella* as well as *Olpidium* place in lineages outside of the Chytridiomycetes (James et al. 2006). *Olpidium* (at least certain species) is in a lineage that diverges among zygomycetous fungi. *Rozella* is now classified (based in particular on molecular study of *R. allomycis*) in a clade, sister to the traditionally recognized Kingdom Fungi, which Karpov et al. (2014) circumscribed as Superphylum Opisthosporidia—*Rozella* is classified in Phylum Cryptomycota within this superphylum (Opisthosporidia). Determining the molecular comparison of *R. coleochaetis* to, for example, *R. allomycis*, will prove essential for final confirmation of the placement of *R. coleochaetis* in genus *Rozella*.

Additional information has also come from transmission electron microscopy, especially as regards modified understandings of the host-parasite interfaces of two species of *Rozella* (Held, 1981; Powell, 1984). Rather than forming its own sporangial wall during the vegetative stage, as earlier interpreted (e.g., Sparrow, 1938, 1960; Karling, 1977), *R. allomycis* (Held, 1981) and *R. euglenae* (Powell, 1984) were found to develop as endoplasmodial parasites which make direct contact with host cytoplasm. It appears that this endoplasmodial stage of *Rozella* phagocytizes the host protoplasm (Powell, 1984) and ultimately enlarges until it totally fills the host compartment—and that this parasite does not develop its own wall but uses the host wall (as a containing vessel) as it cleaves its zoospores (Held, 1981); these posteriorly uniflagellate zoospores are subsequently typically discharged, to the exterior of the host, by a pore generated in the host cell-wall.

If *Plasmophagus* is related to *Rozella* (as implied; cf. Sparrow, 1960), *Plasmophagus* may also ultimately place phylogenetically outside of the Chytridiomycetes, and among the Opisthosporidia. Supporting this possibility, *Nucleophaga amoebae*—traditionally classified in the Olpidiaceae, and an organism considered possibly related to both *Rozella* and *Plasmophagus*—has recently been revealed as a member of the Opisthosporidia clade (Corsaro et al., 2014). However, the phylogenetic affinity of *Plasmophagus* can only be definitively determined by collecting it and sequencing its genes, an intriguing challenge. For the time being, *Plasmophagus* remains recognized as a member of the Chytridiomycetes.

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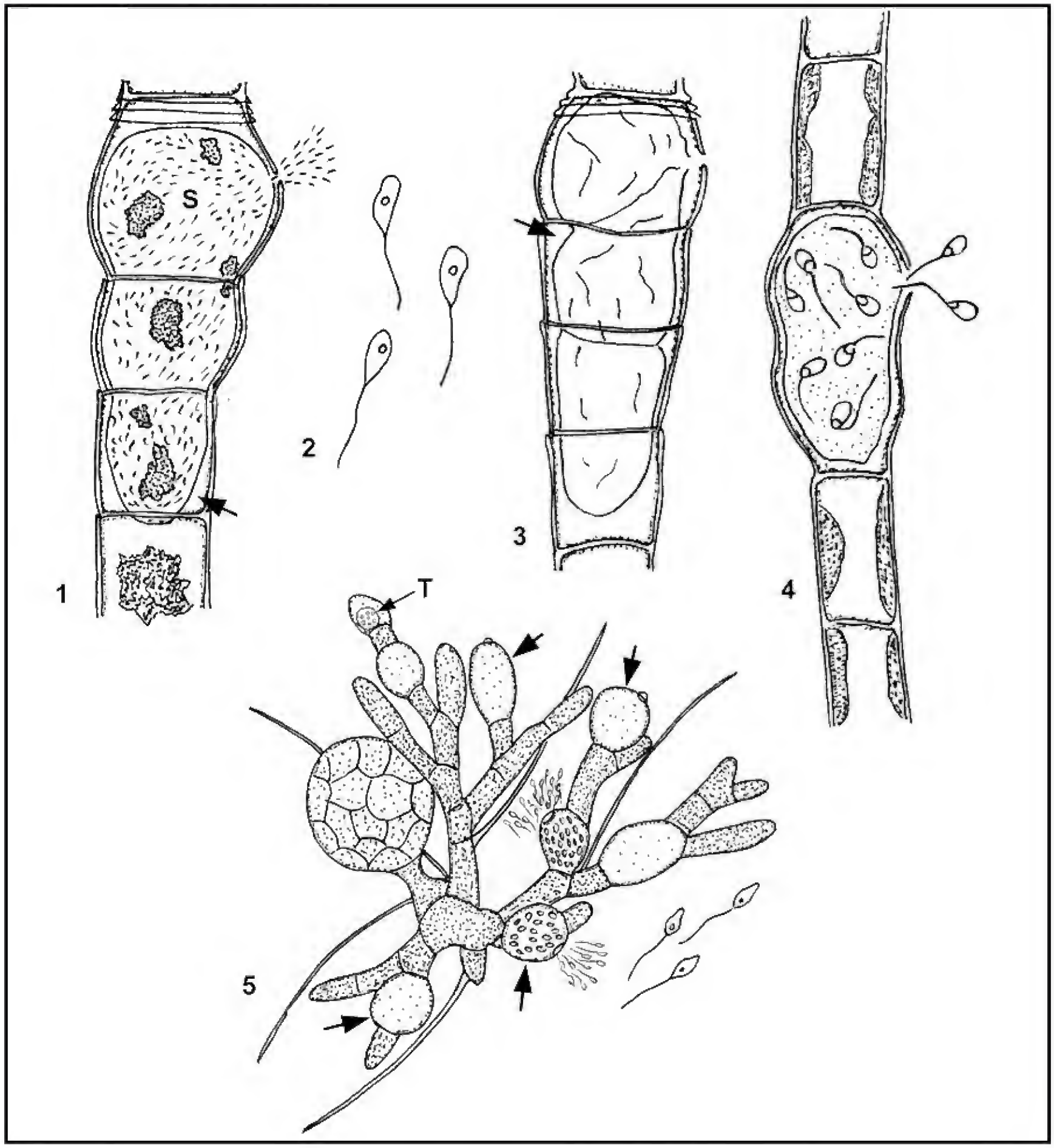

Figs. 1-4: *Plasmophagus oedogoniorum.* Fig. 1: Sporangia of *P. oedogoniorum* parasitic in successive *Oedogonium* cells; sporangium (S) mostly fills algal cell, but "free space" evident in places (arrow); note small zoospores, released from lateral pore in uppermost cell. Fig. 2: Elongate form of the posteriorly uniflagellate zoospores. Fig. 3: Older, empty sporangia; "space" between parasite and *Oedogonium*-host more evident at this stage (arrow). Fig. 4: Putative *P. oedogoniorum* in alga, *Tribonema*; note zoospore release from lateral pore. **Fig. 5**: Sporangia of *Rozella coleochaetis* (arrows), each completely filling a cell of the algal host (*Coleochaete)*, no "free space" evident*;* note small, elongate zoospores (generally similar to those of *Plasmophagus*) released from two sporangia (mid-, lower-right); *R. coleochaetis* young thallus (T) -- Figs. 1-3 after De Wildeman, 1895; Fig. 4 modified from Sparrow 1933; Fig. 5 modified from Sparrow et al., 1965.

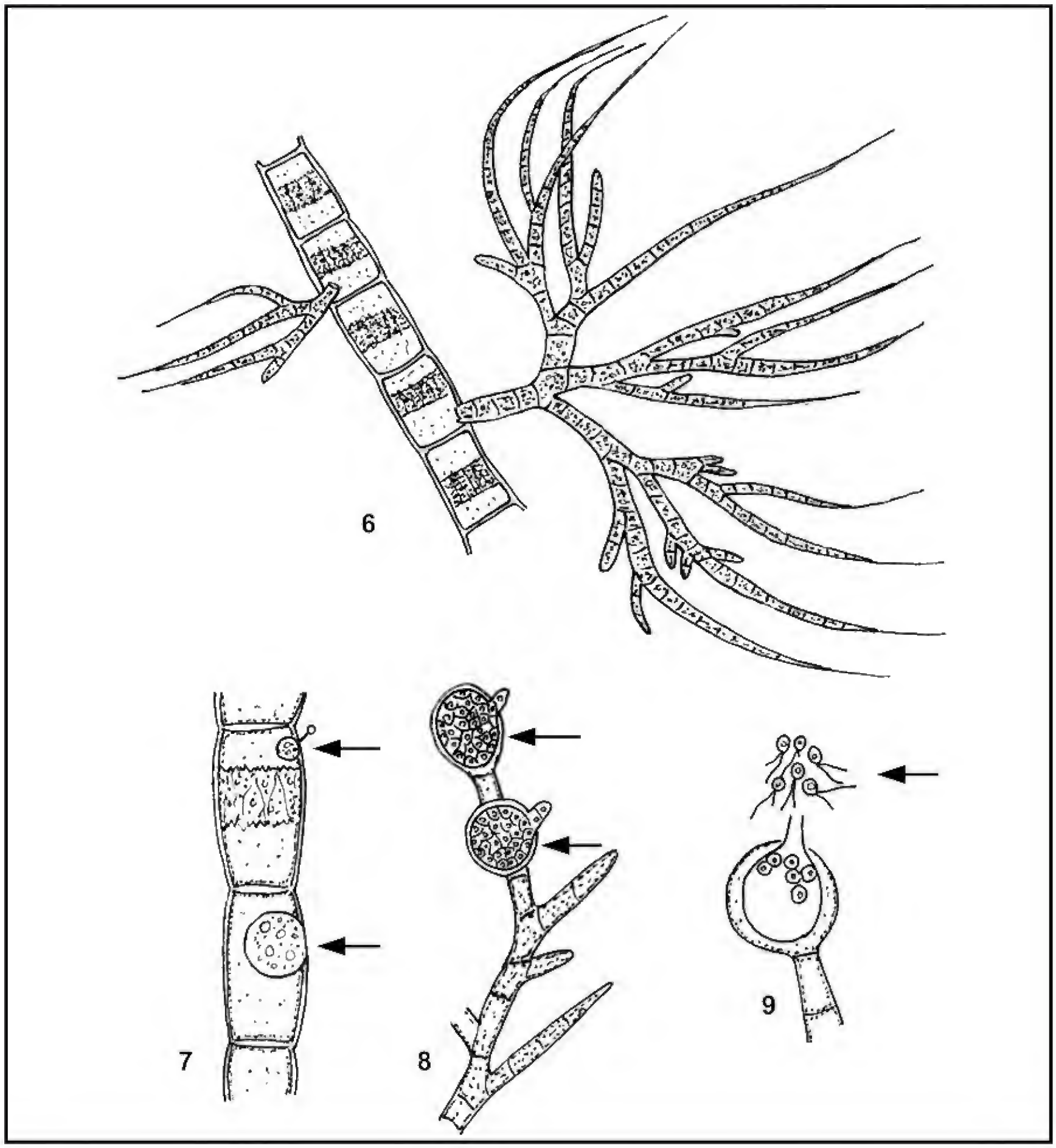

Fig. 6: *Draparnaldia*, the algal host for *Pseudolpidium deformans* ("*Plasmophagus*" *deformans*, Dick, 2001); note the main, algal axis and smaller diameter, tip-pointed, lateral branches (cells of these lateral branches are potential sites of infection by *P. deformans*). **Figs. 7-9**: *Pseudolpidium deformans* (= *Olpidiopsis deformans*, Karling, 1981). Fig. 7: Infection of two cells of *Draparnaldia* lateral filament (arrows); upper algal-cell shows slender zoospore-germ-tube still present; cell below shows young, endobiotic sporangium. Fig. 8: Developed sporangia (arrows) of *P. deformans*, zoospores not yet released. Fig. 9: Release of zoospores from mature sporangium, the zoospores developing motility; zoospores (arrow) are biflagellate; note that the two, possibly unequal flagella of each zoospore are apparently apically or sub-apically inserted. -- Figs. 7-9 based on Serbinow, 1907.

Taxonomy and distribution of *Euphorbia chaetocalyx*, *E. crepidata* and *E. fruticulosa* (Euphorbiaceae)

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

ABSTRACT

The taxonomy and distribution of three species of the genus *Euphorbia* (*E. chaetocalyx*, *E. crepidata* and *E. fruticulosa*) from north-central Mexico and closely adjacent USA are elucidated.

KEY WORDS: Euphorbiaceae, *Chamaesyce, Euphorbia*, Chihuahua, Coahuila, Texas. Published on-line www.phytologia.org *Phytologia 98(2):137-141 (Apr 4, 2016).* ISSN 030319430.

Attempts to identify anomalous Mexican gypsophiles has occasioned the present paper, the following species being especially worrisome; these have been treated as belonging to either *Chamaesyce* or *Euphorbia* by various workers, but I follow the nomenclature suggested by the DNA studies of Yang et al. (2012).

Euphorbia chaetocalyx (Woot. & Standl.) Tidestr., Proc. Biol. Soc. Washington 48: 40. 1935.

Johnston (1975) recognized two varieties within this taxon, as follows:

1. Glandular appendages usually with 3 linear-lanceolate lobes; north central Mexico and closely adjacent Texas .....var. **triligulata**
1. Glandular appendages otherwise; south central USA.....var. **chaetocalyx**

var. **chaetocalyx** Woot. & Standl., Contr. U. S. Natl. Herb. 16:144. 1913. **Map 1**
Chamaesyce chaetocalyx (Woot. & Standl.) Tidest.

This is the common variety of the species, widely distributed over the southwestern USA.

var. **triligulata** (Wheeler) M.C. Johnst., Wrightia 5: 139. 1975. **Map 2**
Chamaesyce chaetocalyx var. *triligulata* (Wheeler) Mayfield
Chamaesyce triligulata (Wheeler) B.L. Turner
Euphorbia fendleri var. *triligulata* Wheeler

In my elevation of this taxon to specific status (Turner et al. 2003) I had only examined a few exceptional sheets from Brewster Co. Texas, such as pictured by Poole et al. (2007) and those from closely adjacent Mexico. Subsequent study of additional material from more western trans-Pecos, Texas has shown the taxon to intergrade with typical populations of *E. chaetocalyx*, especially along the Rio Grande, hence my acceptance of its varietal status herein. I should note that my colleague, James Henrickson would agree with my present taxonomy, for he prepared an excellent unpublished account of the taxon in 2003 entitled "Regarding the validity of *Euphorbia* (*Chamaesyce*) *triligulata* (M. C. Johnson) B. L. Turner," in which he refuted its specific status; he pasted this epistle on a herbarium sheet, depositing it in the herbarium proper, my not having seen this until the spring of 2016. I consider his views to be "right on."

Euphorbia crepitata Wheeler, Contr. Gray Herb. 127: 60. 1939. **Map 3**
Chamaesyce crepitata (Wheeler) Mayfield

This species is readily distinguished by its glutinous lower stems, as well noted by Johnston (1977); he recognized two infraspecific taxa from among the complex:

1. Leaves mostly 3-9 mm long, 0.5-5.0 mm wide; involucral glands usually markedly appendaged; plants mostly some distance sw of Cuatro Cienagas, Coa.....var. **longa**
1. Leaves mostly smaller; glands weakly appendaged, if at all; plants mostly in and about the vicinity of Cuatro Cienagas.....var. **crepitata**

var. **crepitata**
Chamaesyce crepitata (Wheeler) Mayfield
TYPE: MEXICO. " 4 mi W of Cuatro Cienagas," 24 Aug 1938, *I.M. Johnston 7160* (GH).

var. **longa** M.C. Johnston, Wrightia 5: 139. 1975.
Chamaesyce crepitata var. *longa* (M.C. Johnst.) Mayfield

TYPE: MEXICO. COAHUILA: **Mpio. San Pedro**, 50 km NE of San Pedro de las Colonias, near Puerto de Ventanillas (26 00 N, 102 44 W), 1240 m, gypsum soils, 17 Aug 1973, *Hendrickson* (sic) *12502* (TEX).

The two taxa, while largely allopatric, intergrade throughout their regions of near contact, as indicated in Map 3.

Euphorbia fruticulosa Engelm. ex Boiss., Prodr. [DC] 15: 38. 1862. **Map 4**

Johnston (1975) recognized two varieties within this complex:

1. Stems, foliage and fruits glabrous.....var. **fruticulosa**
1. Stems, foliage and fruits markedly pilose,.....var. **hirtella**

var. **fruticulosa**
Chamaesyce fruticulosa (Engelm. ex Boiss.) Millsp.

This is the commonly collected variety, represented by numerous sheets at TEX, the type reportedly collected by *J. Gregg (506)* in 1848-49 in the vicinity of Saltillo, Mexico.

var. **hirtella** M.C. Johnst., Wrightia 5: 141. 1975.
Chamaesyce fruticulosa var. *hirtella* (M.C. Johnst.) Mayfield

Distribution of the two taxa is shown in map 4. There is no intergradation between these at all, in spite of the numerous sheets of var. **fruticulosa** available.

This taxon is represented by two sheets at TEX (holotype and isotype, the latter not mentioned in Johnston's original description). The only other collections known to me are those of Palmer, collected in 1890 from near the type locality of var. **hirtella**, this also called to the fore by M. H. Huft by annotation of the holotype at TEX, noting that he had examined two Palmer collections at US, such also mentioned by Johnston in his discussion of the type locality (this being in the "Sierra de Solis," (ca 25 40 N, 103 10 W). Indeed, it would seem that var. **hirtella** is a populational thing, after persisting over such a long period of time and having been collected at least twice, it might be deserving of specific status; **or** else it is a most remarkable pilose **forma** of the typical variety. As noted above, I could find no intermediates of

any kind among the many sheets of typical **fruticulosa** at TEX. DNA and additional field study of the taxon is much needed!

ACKNOWLEDGEMENTS

My editorial assistant, Jana Kos provided helpful input. Distribution maps are based upon specimens at LL-TEX and in the case of Map 1, from additional USDA distributional data on the WWW.

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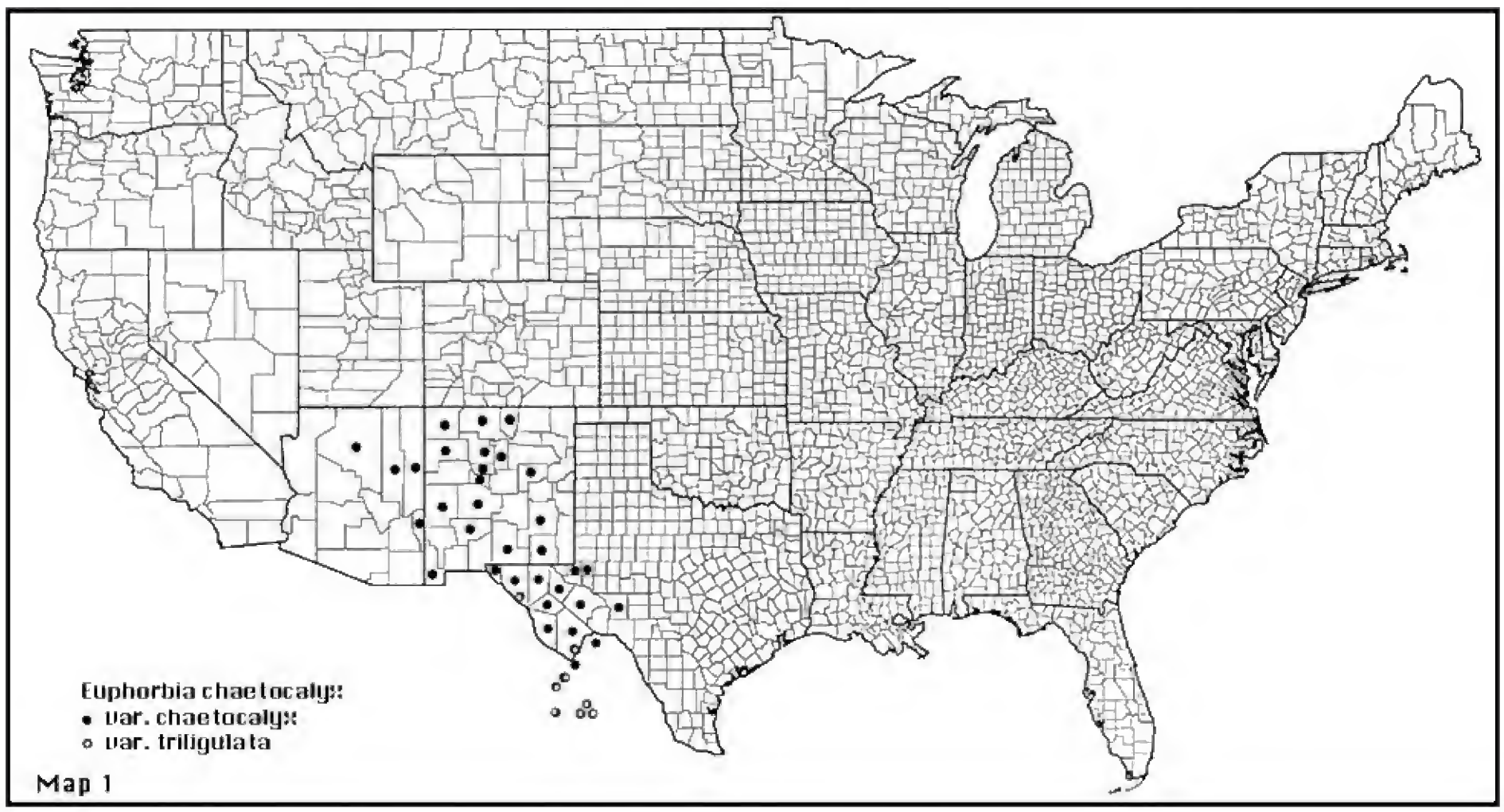
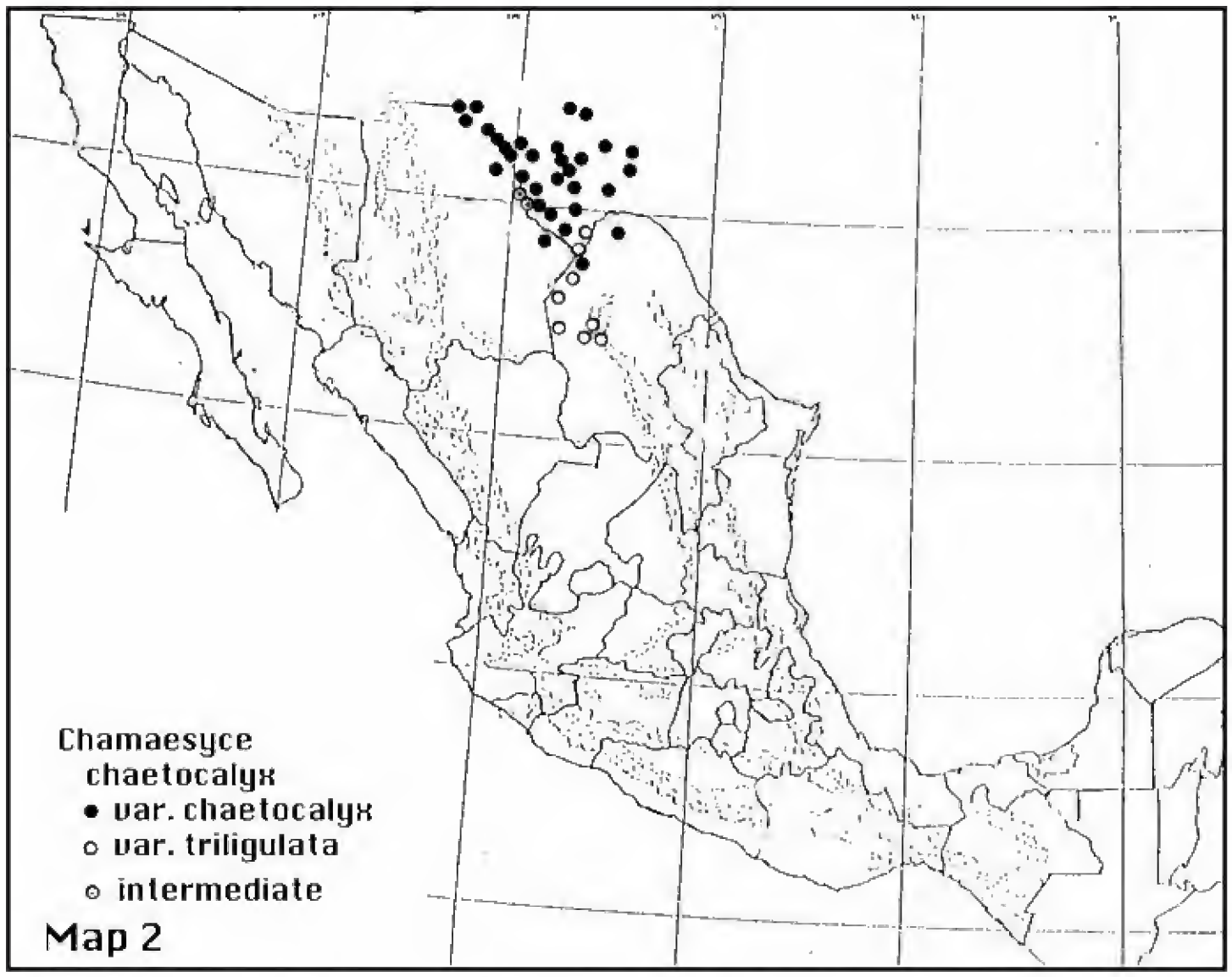
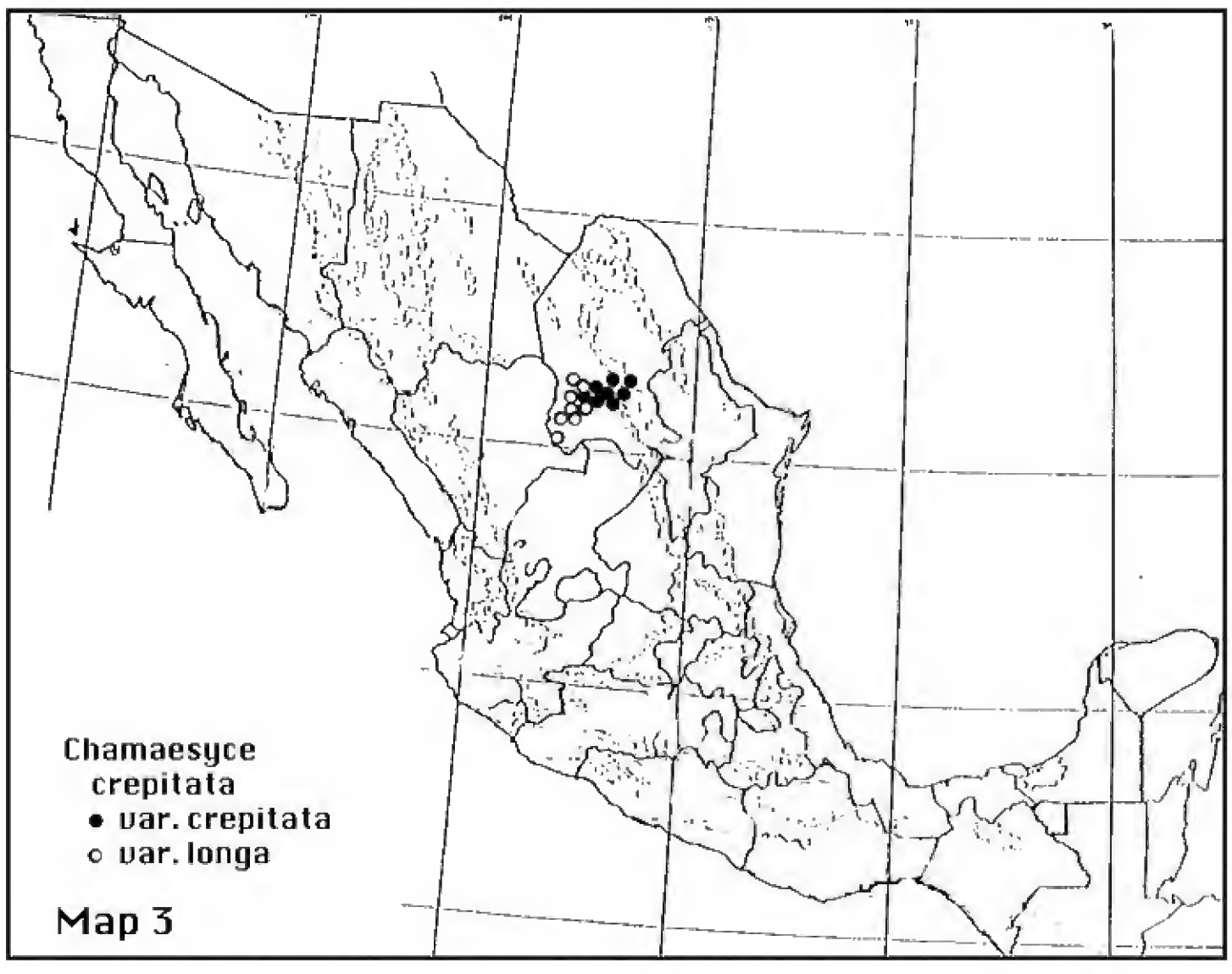


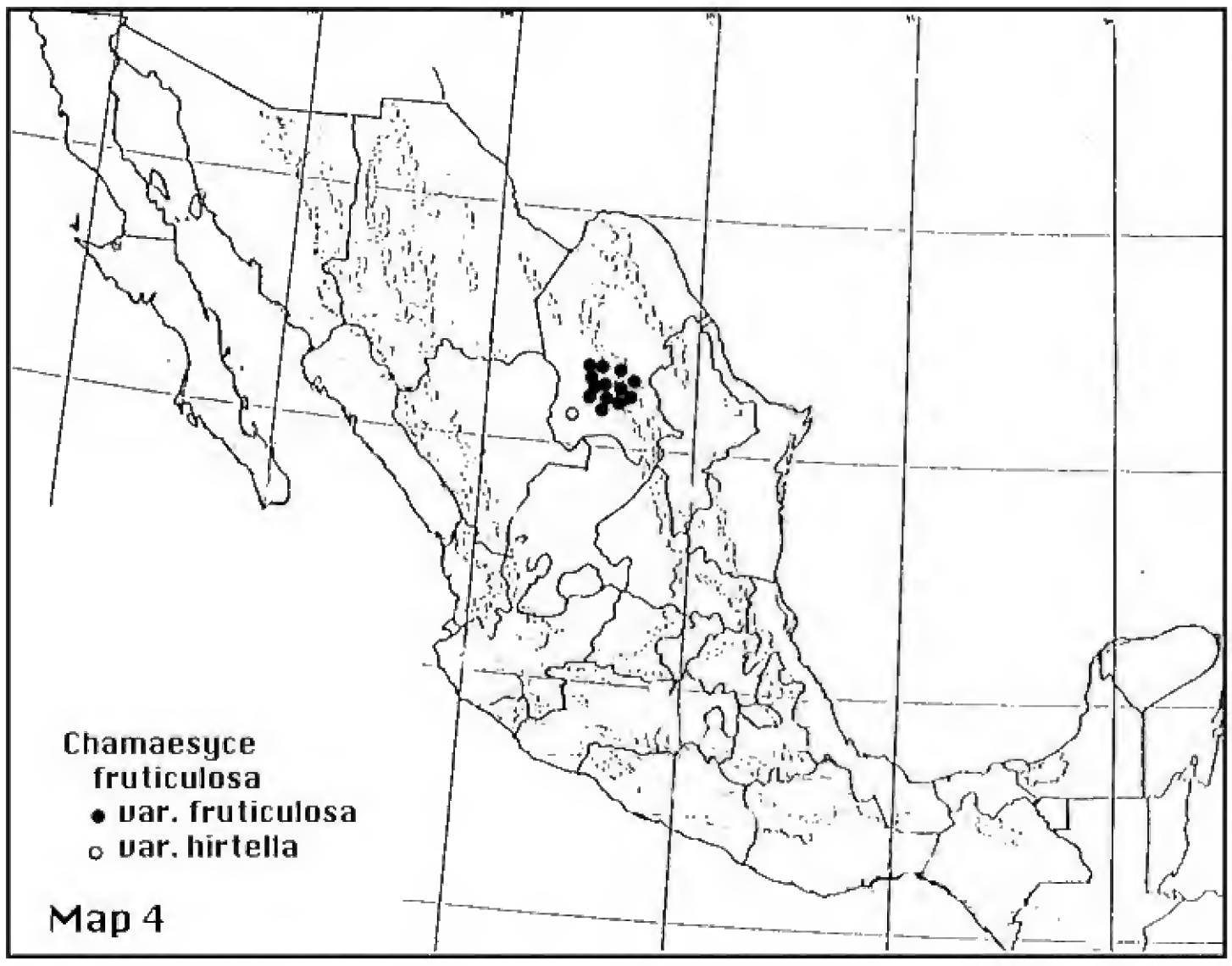
Map 1


Chamaesyce
chaetocalyx
• var. chaetocalyx
○ var. triligulata
⊙ intermediate

Map 2


Chamaesyce
crepitata
• var. crepitata
○ var. longa

Map 3


Chamaesyce
fruticulosa
• var. fruticulosa
o var. hirtella
Map 4

A third endemic *Dendrophthora* (Viscaceae) from Cerro Jefe, Panama

Job Kuijt
649 Lost Lake Road, Victoria, BC V9B 6E3, Canada
jobkuijt@uvic.ca

and

Jerry and Linda Harrison
PTY 9617, 2250 NW 114th Ave., Unit 1P, Miami, FL 33192, USA

ABSTRACT

A rare new species of *Dendrophthora*, **D. primaria** J. Kuijt (Viscaceae), **sp. nov**., is described and illustrated. It is believed to be endemic to the Cerro Jefe area, as are two previously described species of the genus and several other mistletoes in Loranthaceae. Published on-line www.phytologia.org *Phytologia 98(2): 142-145 (Apr 4, 2016).* ISSN 030319430.

KEY WORDS: *Dendrophthora primaria*, Viscaceae, Panama, Cerro Jefe, endemic.

In a previous contribution (Kuijt et al. 2015), we described and illustrated two new species of *Dendrophthora* (Viscaceae) that are believed to be endemic to the Cerro Jefe area of central Panama. At that time, we had already located a third undescribed species but, since no male material had been found, we postponed its publication until we could study male material, its placement in either *Dendrophthora* or *Phoradendron* being contingent on the anthers being unilocular or bilocular, respectively (Kuijt 2003).

Unfortunately, repeated searches in the area have not been able to locate male material, and we wish to register this rare species as a *Dendrophthora* even though the possibility of eventually transferring it to *Phoradendron* must remain open. Our experience suggests that the present assignment is the more likely generic position.

DENDROPHTHORA PRIMARIA J. Kuijt, **sp. nov. Figs. 1 & 2.**

Diagnosis Robust, grayish green plants. Leaf blades symmetrical, ovate to lanceolate, pendulous and often somewhat naviculate in shape, apex acute or acuminate, venation pinnate, inconspicuous. Dioecious. Female inflorescence with mostly 4 fertile internodes, flowers/fruits 2--4 per fertile bract, peduncle with 4--7 sterile basal internodes. Fruit ellipsoid, orange, 5 x 3 mm.

Description **Robust, glabrous, yellowish- to grayish-green plants,** often dichotomous by terminal abortion, occasionally with a terminal inflorescence, each innovation bearing 3 or 4 pairs of blunt cataphylls, the basal ones ca. 3 mm above the base, the uppermost ones near, or slightly below, the middle of the innovation and sometimes caducous. Distally, the innovation bears a single pair of foliage leaves between which the shoot apex commonly aborts, new innovations developing in the axis of foliage leaves. **Internodes terete**, red to yellowish brown, the nodes somewhat swollen. **Leaf blade** to 11 cm long and 5 cm wide, ovate to lanceolate, pendulous, coriaceous, yellowish or greyish green when fresh, drying cinnamon brown, tapering to an acute or acuminate apex, at maturity often somewhat naviculate in shape; margin leathery, red or reddish brown when fresh; petiole 10--15 mm, red, tapering into the obtuse to acute leaf base; venation pinnate but inconspicuous. Dioecious, inflorescences mostly at older foliar nodes, in small clusters. **Female inflorescence** (Fig. 2) to 3 cm long, with 4--7 sterile internodes together 1 cm long in fruit; fertile internodes mostly 4, each bearing 2--4 flowers in triseriate pattern; stigma and

inside of petals bright red, external surface reddish. **Fruit** ellipsoid, 5 x 3 mm, twice as long as the thickness of the rachis, orange with dark tip. **Male inflorescence** not known.

TYPE: **PANAMA. PANAMÁ:** Cerro Jefe, ca. 23 km from turn off to Los Altos de Cerro Azul from "Fucer," reached from town of "24 Diciembre" off Inter-American Hwy; proceed to "first cut" off road ca. 50 m beyond entrance gate to conservation area along Paseo Cerro Jefe, 900 m, 09°13'17.01" N, 79°23'30.05"W, on *Lonchocarpus atropurpureus*, 28 Jun 2014, *J. & L. Harrison 638* (Holotype UCH: Isotypes MO, PMA, US).

The dichotomous branching pattern seen in *Dendrophthora primaria* is also found in the two previously described Cerro Jefe species (Kuijt et al. 2015), and it is therefore useful to delineate contrasts between these three species. *Dendrophthora fortis* has broadly ovate, flat leaves with rounded apices, standing rigidly sideways, and showing numerous, very prominent, palmate veins; inflorescences bear 9--12 (female) or ca. 18 (male) flowers per fertile bract; and the globular, light pink fruits are about 3 mm in diameter (Kuijt et al. 2015). In contrast, *D. primaria* has ovate to lanceolate, often somewhat naviculate leaves that are mostly pendulous, with acute to acuminate apices and inconspicuous, pinnate venation; it bears female inflorescences with no more than 4 flowers per fertile bract, the ellipsoid, orange fruits being 5 x 3 mm in size. *Dendrophthora perlicarpa* is distinct from *D. primaria* in having its leaves transversely placed and showing 3 or 5 somewhat inconspicuous, palmate veins; its female inflorescences have 6 or 7 flowers per fertile bract; fruits are spherical, a clear pearly white, and 5--6 mm in diameter. All three species show at least some dichotomous branching, *D. perlicarpa* with occasional percurrent portions; innovations bear several prominent pairs of cataphylls to about halfway to the next foliage leaves.

The new species is clearly a rare entity and is here placed in *Dendrophthora* even though the crucial male flowers have not yet been located; we are confident that, when male plants are available, the anther will be found to be unilocular, a difference constituting the major distinction from the related *Phoradendron*. Our reasons for this assignment are partly its evident general similarity to the other two endemic Cerro Jefe species, especially in the matter of multiple cataphylls and dichotomous branching habit. The elevation at which the plant was found also supports this assignment, as *Dendrophthora* is primarily a high altitude genus in continental settings, while *Phoradendron* is almost exclusively low altitude in preference. The local exception to the latter pattern is the ubiquitous *P. piperoides* (Kunth) Trel. which is easily separated from the three local *Dendrophthora* species by its biseriate flowers, monoecy, and exclusively percurrent branching, each pair of foliage leaves there alternating with a single pair of very low intercalary cataphylls.

The discovery of *Dendrophthora primaria* reinforces the high degree of mistletoe endemism of the Cerro Jefe area. As mentioned above, two other species of the genus have recently been described that are endemic or essentially so (Kuijt et al. 2015). Additionally, *Psittacanthus pusillus* Kuijt and *Peristethium primarium* (Kuijt) Kuijt (both Loranthaceae) are known to be restricted to the area (Kuijt 2009, 2011). Another, previously unreported species of *Psittacanthus* for Panama, *P. costaricensis* Kuijt, is abundant locally on Cerro Jefe but otherwise known from only a couple collections from adjacent Costa Rica (Kuijt 2009). A specimen of the latter, *Harrison & Harrison 652*, has been deposited at UCH.

Etymology. The epithet "*primaria*" is a transcription of "Jefe."

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Kuijt, J. 2011. Reinstatement and expansion of the genus *Peristethium* (Loranthaceae). Ann. Missouri Bot. Gard. 98: 542-577.

Kuijt, J., J. Harrison and L. Harrison. 2015. Endemism in two new species of *Dendrophthora* (Viscaceae) from Cerro Jefe, Panama. Phytologia 97: 139-144.

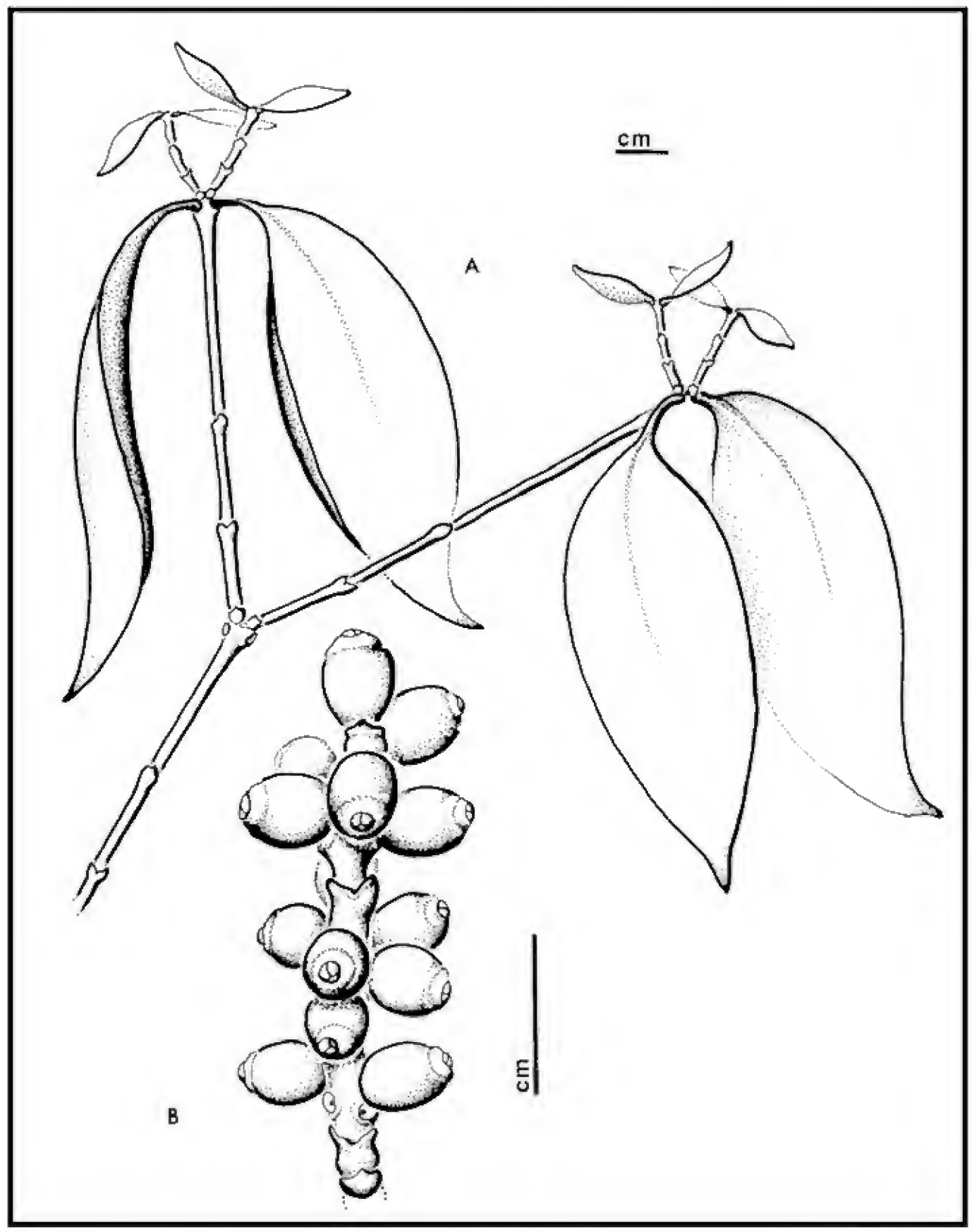

Fig. 1. *Dendrophthora primaria. Harrison & Harrison 638* (UCH). A. Habit. B. Infructescence.

Fig. 2. *Dendrophthora primaria*, female inflorescences. *Harrison & Harrison 638* (UCH).

Evidence of relictual introgression or incomplete lineage sorting in nrDNA of *Juniperus excelsa* and *J. polycarpos* in Asia Minor

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

Metin Armagan
Yüzüncü Yıl Üniversitesi, Eğitim Fakültesi B Blok Kampüs, Van, Turkey

Adam Boratynski*
Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035, Kornik, Poland

Bouchra Douaihy
Department of Life and Earth Sciences, Faculty of Sciences, Lebanese University, Tripoli, Lebanon

Magda Dou Dagher-Kharrat
Laboratoire Caractérisation Genomique des Plantes, Université Saint-Joseph, Campus Sciences et Technologies, Mar Roukos, Mkalles, BP 1514, Riad el Solh, Beirut 1107 2050, Lebanon

Vahid Farzaliyev
Central Botanical Garden, Azerbaijan National Academy of Science,
Badamdar shosse 40, Baku, Azerbaijan, AZ 1073.

Salih Gucel
Environmental Research Institute, Near East University, North Nicosia, Cyprus

Tuğrul Mataraci
Eskidji Müz. AŞ., Sanayi Cad. Vadi Sokak No:2, Yenibosna-Bahçelievler, İstanbul, Turkey

Alexander N. Tashev
University of Forestry, Dept. of Dendrology, 10, Kliment Ochridsky Blvd., 1756 Sofia, Bulgaria

and

Andrea E. Schwarzbach
Department of Health and Biomedical Sciences, University of Texas - Rio Grande Valley, Brownsville, TX 78520, USA.

ABSTRACT

DNA analysis of *Juniperus excelsa* from throughout its range revealed that *J. polycarpos*, instead of *J. excelsa* occupies central and eastern Turkey. Based on nrDNA (ITS) data, it appears that relictual hybridization has occurred in southeastern Turkey between *J. polycarpos* and *J. turcomanica*. Surprisingly, evidence of incomplete lineage sorting or relictual hybridization between *J. polycarpos* and *J. seravschanica* was found in central Turkey and northwest Iran. Published on-line **www.phytologia.org** *Phytologia 98(2): 146-155 (April 4, 2016*).* ISSN 030319430 *digitally corrected-Adam Boratynski, and symbols added to Fig. 3, May, 10, 2016.

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

Recently, Adams et al. (2016) examined *J. excelsa* and putative *J. polycarpos* from the eastern Mediterreanea, eastward into Azerbaijan. A Bayesian consensus tree shows *Juniperus seravschanica, J. polycarpos, J. p.* var. *turcomanica, J. procera* and *J. excelsa* in well-supported clades. *J. excelsa* samples, newly collected from Bulgaria, Cyprus, and sw Turkey, are in a clade with other *J. excelsa* (Fig. 1). There is some minor variation among the *J. excelsa* samples, mostly notably in the Afqa, Lebanon population as previously reported (Douaihy et al., 2011, 2013).

All of the *J. polycarpos* samples from Azerbaijan are closely related with *J. polycarpos*, Armenia along with the El Njass, Lebanon (*Adams 14161*) sample (Fig. 1). Three other El Njass samples (*Adams 14158, 14158, 14160*) appear to be intermediate between *J. polycarpos and J. p.* var. *turcomanica* (Fig. 1).

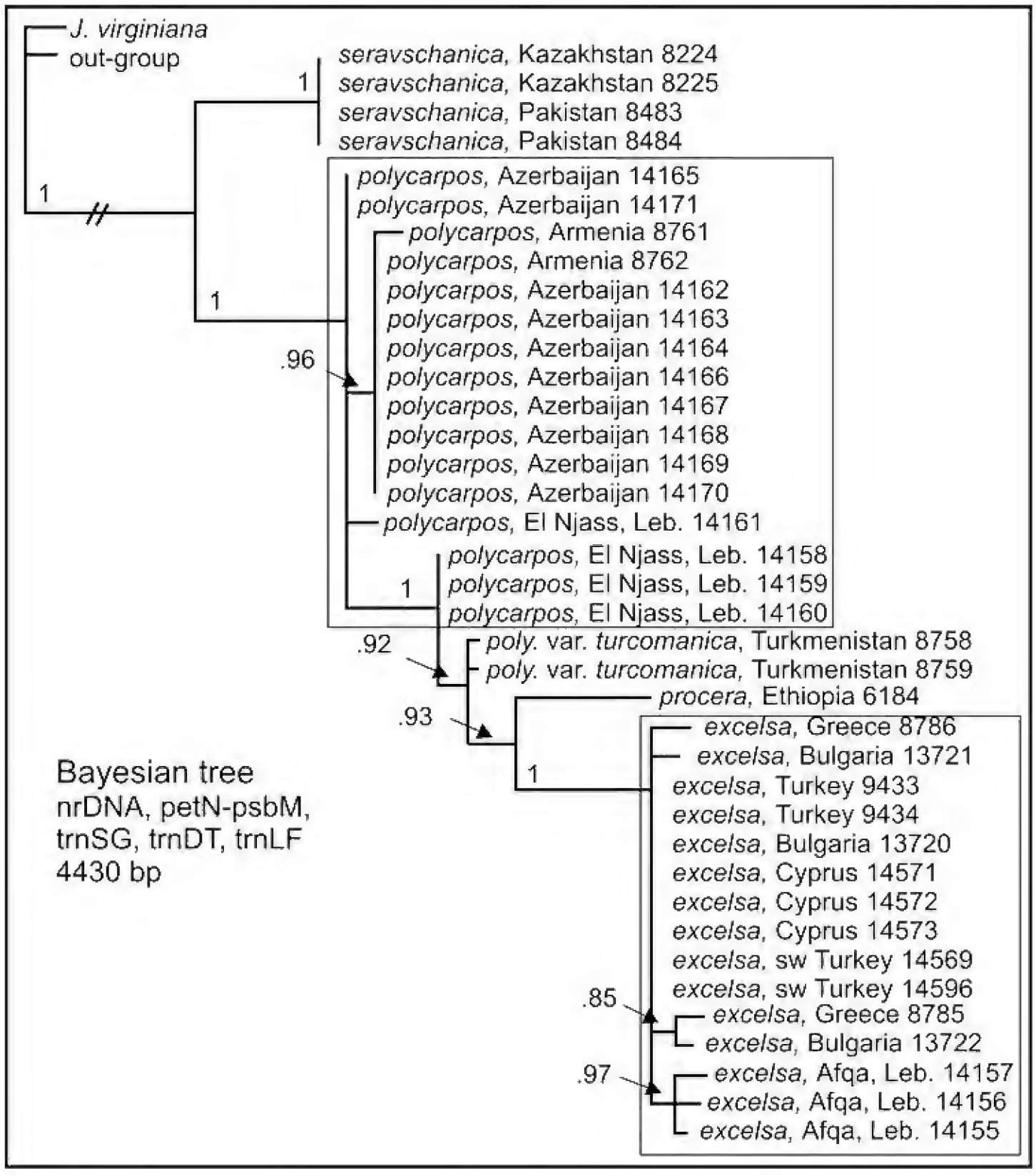

Figure 1. Bayesian analysis based on nrDNA, petN-psbM, trnSG, trnDT and trnLF. Numbers at the branch points are posterior probabilities.

Overlaying a minimum spanning network onto a distribution map gives one a perspective of the geographic trends (Fig. 2). The newly sampled *J. excelsa* populations from Bulgaria, Cyprus and sw

Turkey were identical or nearly identical to *J. excelsa* of Eskisehir, Turkey (Fig. 2). Both the Cyprus and southwestern Turkey populations of *J. excelsa* showed no differences (Fig. 2). The Bulgaria *J. excelsa* differed by none or one difference from Eskisehir, Turkey (Fig. 2).

As previously reported (Adams et al., 2014), the Afqa, Lebanon *J. excelsa* population differs by 2 MEs from Eskisehir, Turkey, which in turn, differs by only 1 ME from the Lemos, Greece population (Fig. 2). The other Lebanon populations that group with Afqa are probably *J. excelsa*.

However, the Wadi El Njass, Lebanon (2287 m) population, although near Afqa, grouped with *J. polycarpos* and differs by 1 to 3 MEs from *J. p.* var. *turcomanica*, Turkmenistan and by 1 to 2 MEs from *J. polycarpos,* Armenia (Fig. 2). The *J. excelsa*, Afqa population is only about 100 - 150 km from other *J. excelsa* populations (Fig. 2), but the Wadi El Njass, *J. polycarpos* population is 700 to 1000 km from the nearest *J. polycarpos* population, still, it differs by only 1 to 3 MEs.

Figure 2. Minimum spanning network mapped onto the distributions of *J. excelsa and J. polycarpos*. Numbers next to lines are the number of MEs (Mutational Events = base substitutions plus indels).

Adams et al. (2016) concluded that *J. excelsa*, as sampled in their study, was a fairly uniform species, except for the unusual situation in Lebanon, where *J. excelsa* and *J. polycarpos* (and likely *J. p.* var. *turcomanica*) grow near each other and may be hybridizing. However, the genetic composition of the eastern-most populations of *J. excelsa* in Turkey was unknown and deserved additional study.

Farjon (2005, 2010) treated *J. polycarpos, J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpos*. However, Adams et al. (2008), Adams and Schwarzbach (2012) and Adams (2013, 2014), utilizing DNA sequence data, recognized *J. excelsa,* in addition to *J. polycarpos, J. p.* var.

turcomanica and *J. seravschanica.* Adams and Hojjati (2012) and Adams, Hojjati and Schwarzbach (2014), using sequences from 4 gene regions, did not find *J. excelsa* in Iran, but did confirm *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica* in Iran. Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had none or only one SNP difference compared with *J. polycarpos* var. *polycarpos* from Armenia and was concluded to be *J. polycarpos* (Adams and Hojjati, 2012). Adams et al. (2014) found that putative *J. excelsa* in Azerbaijan was, in fact, *J. polycarpos* or in one case, a putative hybrid (*polycarpos* x *turcomanica*).

The distribution of *J. excelsa* in eastern Turkey has proved difficult to determine by modern methods of DNA sequencing, due to the lack of samples from these regions. Recently, materials were obtained of *J. excelsa* from central and eastern Turkey. This afforded the opportunity to further examine geographic variation of *J. excelsa* and *J. polycarpos*.

MATERIALS AND METHODS

Plant material - *J. excelsa:*
Bulgaria, Central Rhodopes, above the town of Kritchim, Reserve "Izgorialoto Gune", 42° 01' 22.0" N; 24° 28' 03.1" E, 356 m, *Alex Tashev, 2012-1-JE -5-JE*, 1 Sep 2012, Lab Acc. *Adams 13720-13724*,; **Crimea:** Karadigski Zapovidnyk, between Kurortne and Koktebel, 44.914° N, 35.215° E. 220m, *A. Boratynski, Y. Didukh, K. Romashenko, A. Romo, A. Susanna, 2001, KOR 49898*, Karadag near Kolhoznoe, 44° 28' 06" N, 33° 49' 54" E, ca 530m; *A. Boratynski, G. Iszkulo, A. Lewandowski, 2006, KOR 45630*; **Cyprus**: 34° 57' 45.82" N, 33° 59' 55.33" E, 1461m, *Salih Gucel ns*, 3 July 2015, Lab Acc. *Adams 14570-14574*; **Greece**: Lemos, ca 40° 49' N, 21° 03' E, 1100m, *Adams 5983-5985, 5987*; **Lebanon**: Afqa, 34° 04' 58.12"N, 35° 53' 08.52" E, 1306 m, *Bouchra Douaihy 1-3*, 4 Nov 2013, Lab Acc. *Adams 14155-14157*; **Turkey**: Antalya-Manavgat, Köprülü Canyon National Park, 37° 20' N; 31° 16' E, 550 m, *Tuğrul Mataraci 2015-18*, 24 May 2015, Lab Acc *Adams 14569*; Isparta-Eğirdir, junction of Kasımlar-Sütçüler road, 37° 28' N; 30° 59' E, 1180 m, *Tugrul Mataraci, 2015-7*, 24 May 2015, Lab Acc. *Adams 14596*; ~40 km north of Eskişehir, with Oaks, 39° 58.307'N; 30° 41.045' E, Turkey, 820m, *Adams 9433-9435;* Sirnak, se Turkey on Turkey/Iraq border, 37° 34' 08" N, 43° 09' 45" E, 1754m, *Metin Armagan ns*, Lab. Acc. *Adams 14709-14712*, Sirnak Turkey, on Turkey/Iraq border se Anatolia, Prov., near Beytussebap, GE ca 37° 37' 18"N, 42 52' 28" E, 1420m, *Metin Armagan ns*, Lab. Acc. *Adams14715*; 3 km from Unlupinar village towards Gumushane, 40° 14' 25.14" N; 39° 27' 19.17" E. ne Turkey, 1763m, *Ali Kandemir 10846*, Lab Acc. *Adams 14713*; around Lake Ardicli, near Ergan Mountains, near Erzincan. 39° 37' 47.45" N; 39° 29' 54.3" E. ne Turkey. 1797m, *Ali Kandemir 10850*, Lab Acc. *Adams 14714*; Material from specimens at the herbarium, Yüzüncü Yıl University, Van, Turkey: *Adams 14750-* 1986, 38° 28' 41.7" N, 43° 31' 20.6" E, 2800m; *Adams 14751-* 1989, 38° 08' 37.5" N, 42° 17' 15.2" E, 1550m; *Adams 14752*, 1995, 38° 36' 20.2" N, 38° 47' 35.0" E, 1700m; *Adams 14753-* 1996, 38° 40' 6.6" N, 38° 44' 58.6" E, 1600m; *Adams 14754-* 2001, 38° 20' 30.7" N, 43° 37' 44.9" E, 2000m; *Adams 14755-* 2006, 39°13'8.08"N 43° 23' 4.11"E, 1965m; *Adams 14756-* 2014, 38°04'33.6"N 43° 25' 32.0"E, 2010m; *Adams 14757-* 2014, 39° 07' 47" N, 38° 47' 0.5" E, 1275m; *Adams 14758-* 2014, 39° 07' 50.6" N, 39° 07' 27.2" E, 1720m; *Adams 14759-* 2014, 39° 19' 42.4" N, 39° 25' 41.3" E, 1860m; *Adams 14760-* 2014, 39° 21' 17.6" N, 39° 28' 28.8" E, 1890m;
***J. polycarpos*:**
Armenia: Lake Sevan, 1900m, *Adams 8761-8763*; **Azerbaijan**, 40° 44' 41.05" N; 47° 35' 19.14" E, 177-231m, *Vahid Farzaliyev 1-10*, Dec 2013, Lab Acc. *Adams 14162-14171*; **Lebanon:**, Wadi El Njass, 34° 20' 47.79"N, 36° 05' 45.54"E, 2287m, *Bouchra Douaihy 4-7*, 14 Nov 2013, Lab Acc. *Adams 14158-14161*;
***J. polycarpos* var. *turcomanica*: Turkmenistan**: Kopet Mtns., 38° 25.12'N, 56° 58.80'E, 1535 m, 22 May 1999, *Adams 8758-8790*;
***J. procera*: Ethiopia**: on the road to Guder, ca. 40 km w of Addis Abba, ca. 9° 02'N, 38° 24' W, 2400 m, *Adams 6184-6188*;

***J. seravschanica*: Pakistan**: near Quetta, Baluchistan, *A. Hafeez Buzdar ns*, 6 Apr 1998, Lab Acc. *Adams 8483-8485*; **Kazakhstan**: west end of Talasskiy Ala-Tau Range, ca. 2 km S. of Dzhabagly, 42^0 24.53'N, 70^0 28.50'E, 1770m, 12 Sept 1997, *Adams 8224-8226*.
Voucher specimens 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, 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 classification of samples on the basis of ITS and petN (cp data) is given in Table 1. First it should be noted that nrDNA does not distinguish *J. excelsa* (exc) from *J. p.* var. *turcomanica* (tur) (compare 1st table entry, Greece A G C C C T A exc vs. Turkmenistan (last entry) A G C C C T A tur.). Secondly, exc (or tur) is very distinct from pol in its nrDNA (exc: A G C C C T A vs. pol: C G T T T T C T). Thirdly, exc.(or tur) is very distinct from ser (exc: A G C C C T A vs. ser: C G C T T T C T). And, finally, nrDNA for pol has only one nucleotide different from ser (pol: C G **T** T T T C T vs ser: C G **C** T T T C T). Several plants had nrDNA from one taxon, but cp DNA from another taxon: El Njass (3 E,P); Metin e Turkey, *14757*, (S,P); Azerbaijan *14171* (S,P); Elburz Mtn., Iran *12504* (S,P); Lushan, Iran *12798* (S,P); Hastjin, Iran *12795* (S,P); and Qushchi, Iran, *12798* (S,P). Other plants appeared to be hybrids by nrDNA: El Njass, *14161*, PxE; Metin e Turkey *14753*, PxS; Metin e Turkey *14754*, PxS; Metin e Turkey *14758*, PxS; ne Turkey 14714, PxS; se Turkey/ Iraq border *14715*, PxT(or E); se Turkey/ Iraq border *14709*, PxT(or E); se Turkey/ Iraq border *14710*, PxT(or E); and Azerbaijan, *14165*, PxT(or E).

To visualize this variation, plants were mapped with their nrDNA and cp (petN) DNA coded (Fig. 3). It is sometimes difficult to determine whether a variation is due to incomplete lineage sorting or hybridization (see discussion in Naciri and Linder, 2015). In the present study, 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 (Fig. 4). 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.

Table 1. Classification of samples, on ITS and cp (petN) sequence data. exe = *excelsa*, pol = *polycarpos*, tur = *turcomanica*, ser = *seravschanica*. PxE = hybrid pol x exc; PxS = hybrid pol x ser; PxT(E) = pol x tur (or exc, as ITS for exe = tur).

source	acc #	230	232	238	354	427	732	8952	ITS	cp
n Greece, 1010m	8785	A	G	C	C	C	T	A	exc	exc
n Greece, 1010m	8786	A	G	C	C	C	T	A	exc	exc
n Greece, 903m	14742	A	G	C	C	C	T	A	exc	exc
Bulgaria, 365m	13720	A	G	C	C	C	T	A	exc	exc
Bulgaria, 365m	13721	A	G	C	C	C	T	A	exc	exc
Bulgaria, 365m	13722	A	G	C	C	C	T	A	exc	exc
Cyprus, 1461m	14570	A	G	C	C	C	T	A	exc	exc
Cyprus, 1461m	14571	A	G	C	C	C	T	A	exc	exc
Cyprus, 1461m	14572	A	G	C	C	C	T	A	exc	exc
Crimea, 220m	14906	A	G	C	C	C	T	A	exc	exc
Crimea, 530m	14907	A	G	C	C	C	T	A	exc	exc
sw Turkey, 550m	14569	A	G	C	C	C	T	A	exc	exc
sw Turkey, 1461m	14596	A	G	C	C	C	T	A	exc	exc
nw Turkey, Eskisehir, 820m	9433	A	G	C	C	C	T	A	exc	exc
nw Turkey, Eskisehir, 820m	9434	A	G	C	C	C	T	A	exc	exc
Afqa Leb M1, 1306m	14155	A	G	C	C	C	T	A	exc	exc
Afqa Leb M2, 1306m	14156	A	G	C	C	C	T	A	exc	exc
Afqa Leb M3, 1306m	14157	A	G	C	C	C	T	A	exc	exc
El Njass Leb M4, 2287m	14158	A	G	C	C	C	T	A	exc	poly
El Njass Leb M5, 2287m	14159	A	G	C	C	C	T	A	exc	poly
El Njass Leb M5, 2287m	14160	A	G	C	C	C	T	A	exc	poly
El Njass Leb M7, 2287m	14161	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxE**	poly
Metin e Turkey, 2800m	14750	na	na	na	na	T	C	T	poly	poly
Metin e Turkey, 1550m	14751	C	G	T	T	T	C	T	poly	poly
Metin e Turkey, 1700m	14752	C	G	T	T	T	C	T	poly	poly
Metin e Turkey, 1600m	**14753**	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
Metin e Turkey, 2000m	**14754**	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
Metin e Turkey, 1965m	14755	C	A	T	T	T	C	T	poly	poly
Metin e Turkey, 2010m	14756	C	G	T	T	T	C	T	poly	poly
Metin e Turkey, 1275m	14757	C	G	T	C	T	C	T	**serav**	poly
Metin e Turkey, 1720m	**14758**	**C**	**G**	**T**	**Y-C/T**	**T**	**C**	**T**	**PxS**	**poly**
Metin e Turkey, 1860m	14759	C	G	T	T	T	C	T	poly	poly
Metin e Turkey, 1890m	14760	C	G	T	T	T	C	T	poly	poly
ne Turkey , 1,753m	14713	C	G	T	T	T	C	T	poly	poly
ne Turkey, 1,783m	14714	C	**R-A/G**	T	**Y-C/T**	T	C	T	**PxS**[a]	poly
se Turkey/ n Iraq, 1,420m	14715	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT(E)**	poly
se Turkey/ n Iraq, 1,743m	14709	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT(E)**	poly
se Turkey/ n Iraq, 1,743m	14710	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT(E)**	poly
se Turkey/ n Iraq, 1,743m	14711	C	G	T	T	T	C	T	poly	poly
se Turkey/ n Iraq, 1,743m	14712	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14162	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14163	C	**R-A/G**	T	T	T	C	T	poly[a]	poly
Azerbaijan, 200m	14164	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14165	**Y-C/T**	G	**Y-C/T**	C	**Y-C/T**	**Y-C/T**	**W-A/T**	**PxT(E)**	poly
Azerbaijan, 200m	14166	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14167	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14168	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14169	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14170	C	G	T	T	T	C	T	poly	poly
Azerbaijan, 200m	14171	C	G	T	**C**	T	C	T	**serav**	poly
Armenia, 1900m	8761	C	G	T	T	T	C	T	poly	poly
Armenia, 1900m	8762	C	**A**	T	T	T	C	T	poly	poly
Elburz Mtn., Iran, 2033m	12603	C	G	T	T	T	C	T	poly	poly
Elburz Mtn., Iran, 2033m	12604	C	G	T	**C**	T	C	T	**serav**	poly
Lushan, Iran, 1120m	12789	C	G	T	**C**	T	C	T	**serav**	poly
Hastjin, Iran,1610m	12795	C	G	T	**C**	T	C	T	**serav**	poly
Qushchi, Iran, 1760m	12798	C	G	T	**C**	T	C	T	**serav**	poly
Pakistan, seravschanica	8483	C	G	T	**C**	T	C	T	**serav**	serav
Pakistan, seravschanica	8484	C	G	T	**C**	T	C	T	**serav**	serav
Kazakhstan, seravschanica	8224	C	G	T	**C**	T	C	T	**serav**	serav
Kazakhstan, seravschanica	8225	C	G	T	**C**	T	C	T	**serav**	serav
Turkmenistan, turcomanica	8757	A	G	C	C	C	T	A	turc=exc	turco
Turkmenistan, turcomanica	8758	A	G	C	C	C	T	A	turc=exc	turco

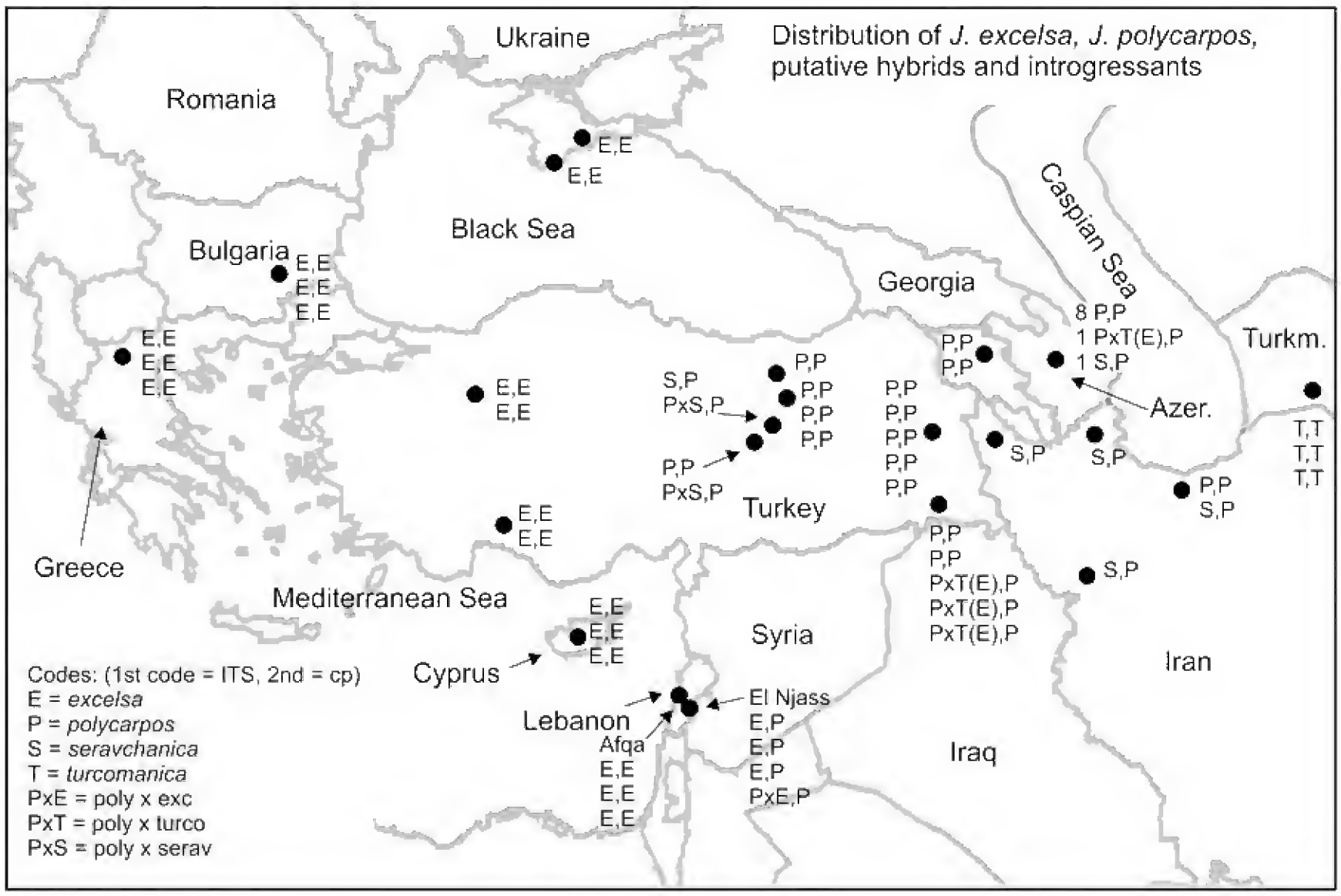

Figure 3. Distribution of *J. excelsa, J. polycarpos*, putative hybrids and introgressants based on ITS and cp sequences. The pair of capital letters (eg., E,E) gives the sample classification based on ITS (1st letter) and cp (2nd letter).

The situation in Lebanon seems to favor hybridization between *J. excelsa* and *J. polycarpos*, even though no pure *J. polycarpos* was found (Adams et al., 2014). Note the four plants in the El Njass population all have *J. polycarpos* cp, and one appears to be a ExP hybrid by nrDNA (Fig. 3). It seems likely that pure (P,P) *J. polycarpos* grows in the region, although these plants may have become extinct, in which case, this may be relictual hybridization.

Liao (1999), in a seminal paper on concerted evolution, defined it as "The molecular process that leads to homogenization of DNA sequences belonging to a given repetitive family". Liao reasoned that because rRNA functions only when assembled into large complexes, homogeneity of rRNAs is critical if all the steps of ribosome assembly and translation are to proceed normally. Liao (1999) says "One can therefore envision that a possible biological function of concerted evolution is to maintain homogeneous gene copies in a family so that homogeneous transcripts can be produced". However, it seems possible that there could be "silent" base substitutions that do not impact the shape or function of a rRNA. If so, these "silent" changes might persist indefinitely in a derived taxon.

Naciri and Linder (2015) estimated that typical tree species with N_e of 1 million individuals and a generation time of 10 yrs would require 50 M yr after speciation to reach full monophyly. Syring et al. (2007) concluded that the presence of shared nrDNA haplotypes among *Pinus* species was due to incomplete lineage sorting. They estimated that reciprocal monophyly will be more likely than paraphyly in 1.7 to 2.4 M yr, but complete genome-wide coalescence in species could take up to 76 M yr.

However, Bouillé and Bousquet (2005) examined trans-specific allelic polymorphism in three low-copy nuclear genes in different *Picea* species and they estimated that allelic coalescence time between randomly selected alleles in *Picea* was 10 to 18 million years ago. The effective population size can greatly effect coalescence times (Naciri and Linder, 2015), such that species with smaller effective population sizes would coalescence faster than species with larger effective population sizes.

Mao et al. (2010) published an ancestral reconstruction of *Juniperus* based on all three (3) known *Juniperus* fossils. They showed *J. excelsa* and *J. polycarpos* joined in a clade at approximately 7 M yr. If that result is correct, then the amount of time available for complete nrDNA coalescence in the *J. excelsa* - *J. polycarpos* clade seems insufficient, compared with the 10 to 18 M yr required in *Picea* species (Bouillé and Bousquet, 2005). The ca. 7 M yr in *J. excelsa/ polycarpos* is far less than the 76 M yr that Syring et al. (2007) suggested was needed for coalescence of nrDNA in *Pinus*. It is difficult to know how accurate the dates are in Mao et al. (2010) due to the very small number (3) of *Juniperus* fossils used. But, it does appear that there has been insufficient time for complete coalescence of nrDNA in the present day *J. excelsa/ J. polycarpos*. Incomplete lineage sorting would explain the presence of *J. seravschanica* (S) nrDNA in otherwise, typical *J. polycarpos* in central-eastern Turkey, Azerbaijan and northwest Iran (Fig. 3).

The currently understood distributions of *J. excelsa, J. polycarpos, J. seravschanica* and *J. p.* var. *turcomanica* are depicted in Figure 4. The dashed line in central Turkey indicates the boundary between *J. excelsa* and *J. polycarpos* is unknown at present. The population of *Juniperus* on the north coast of the Black Sea may be *J. excelsa* or *J. polycarpos*. Recent events have made it impossible to collect in that area.

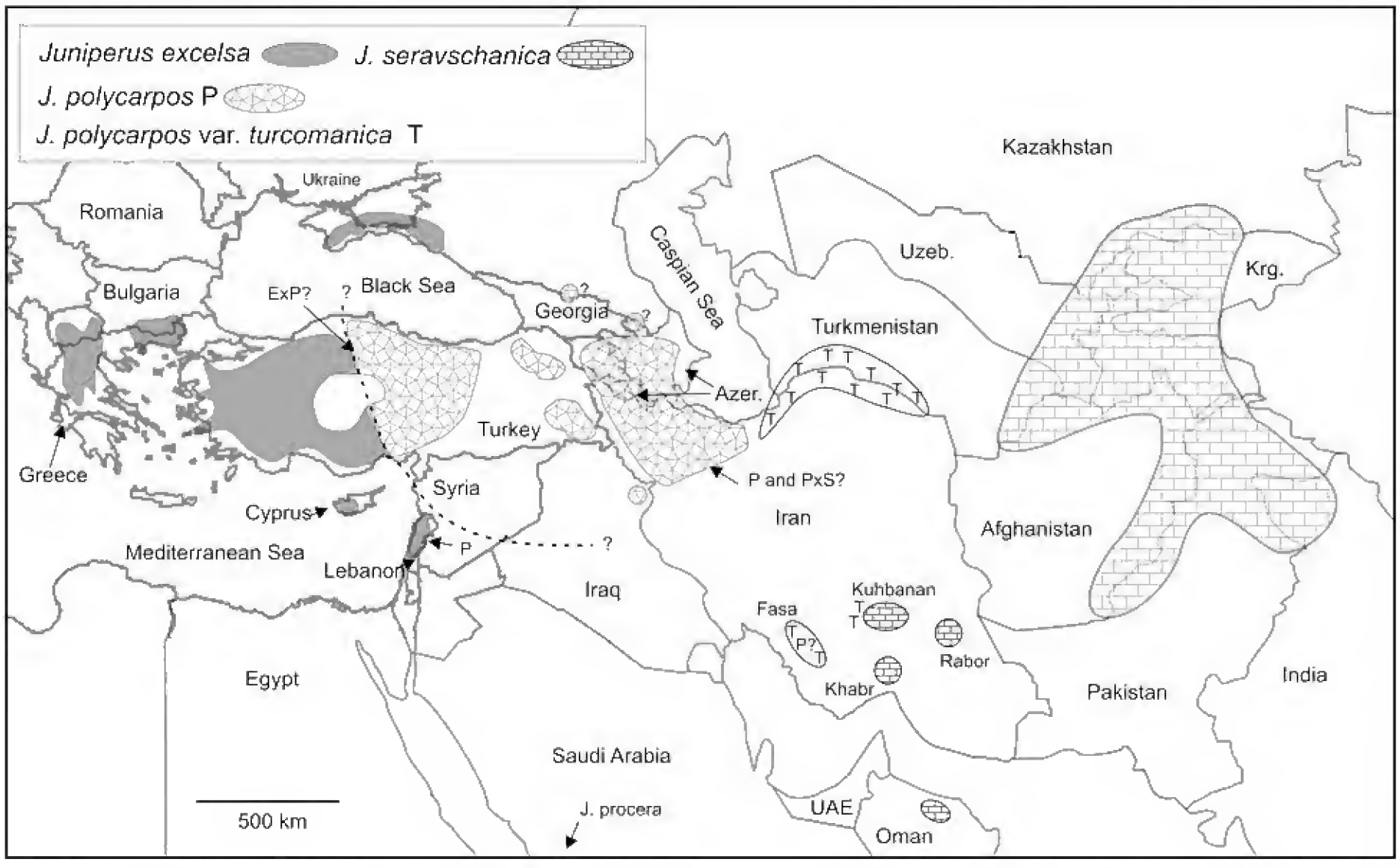

Figure 4. Distributions of *J. excelsa, J. polycarpos, J. p.* var. *turcomanica and J. seravschanica* as understood at present. The dashed line indicates the uncertain limits of *J. excelsa* and *J. polycarpos* in central Turkey. See text for discussion.

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