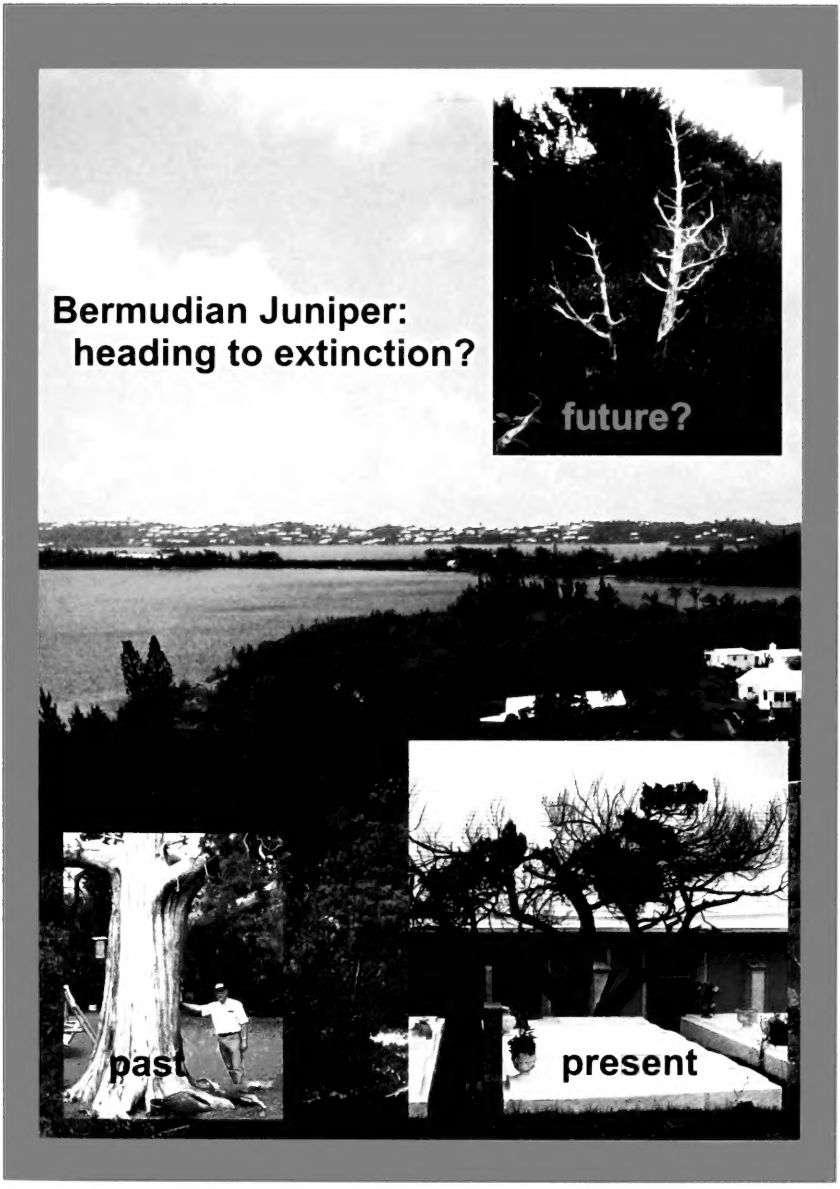
PHYTOLOGIA

An international journal to expedite plant systematic phytogeographical and ecological publication

www.phytologia.org

Vol. 90, No. 2, pp 121 - 252 August, 2008



PHYTOLOGIA

(ISSN 00319430)

Phytologia, a journal for rapid publication in plant systematics, phytogeography and vegetation ecology, is published three times a year.

Secretary-Treasurer - Subscriptions
Robert P. Adams
Phytologia, Box 727, Gruver, TX 79040
Robert_Adams@baylor.edu

Phytologia

Contents

Contents continued on next page.

Cover Photo: *Juniperus bermudiana*, endemic to Bermuda. Adams and Wingate examine hybridization with introduced junipers. p. 123. R. P. Adams, photos.

HYBRIDIZATION BETWEEN *JUNIPERUS BERMUDIANA* AND *J. VIRGINIANA* IN BERMUDA

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

David Wingate
P. O. Box DD224, St. Davids, Bermuda

ABSTRACT

In 1942 two scale insects were accidentally introduced into Bermuda with devastating effects on *Juniperus bermudiana*, endemic to Bermuda. In an effort to repopulate junipers on Bermuda, two cultivated junipers from Florida were introduced by J. D. C. Darrell in 1940s (Darrell's cedar) and Reeve Smith in the 1950s (Smith's cedar). Analysis of SNPs of nrDNA and trnC-trnD cp DNA determined that Darrell's cedar is *Juniperus virginiana* var. *silicicola* and Smith's cedar is *Juniperus v.* var. *virginiana*. SNPs analysis reveal what appears to be F_1 hybrids between Darrell's cedar (*J. v.* var. *silicicola*) and *J. bermudiana*. In addition, two individuals were found that contained nucleotides from Darrell's cedar, Smith's cedar and *J. bermudiana*, suggestive of hybridization with backcrossing to the third taxon.

KEY WORDS: *Juniperus bermudiana,* Bermuda, nrDNA, trnC-trnD cp DNA, SNPs, Cupressaceae

---

The early British sailors often stopped in Bermuda to make repairs on their ships from Bermuda cedar (*J. bermudiana)* because the island was completely covered with Bermuda cedar (Groves, 1955).

Unfortunately, *Juniperus bermudiana*, endemic to Bermuda, has been subject to attack by two scale insects, *Lepidosaphes newsteadi* and *Carulaspis minima*, these apparently introduced from the U.S. mainland prior to 1942 (Bennett and Hughes, 1959; Groves, 1955). The two insects cause defoliation and death. Groves (1955) estimated that 90% of the trees were dead by 1955. In 1978 William E. Sterrer,

Bermuda Biological Station (pers. comm.), estimated that perhaps 99% of the original trees were exterminated.

Adding to the problem, J. D. C. Darrell obtained a juniper species from the Royal Palms Nursery in Florida in the 1940s and brought it back to Bermuda (Whitney, 1955). It flourished and has been widely planted as an ornamental, or as a replacement for dying Bermuda cedar. It has become known as "Darrell's cedar". The latter is characterized by finer needles and denser foliage than Bermuda cedar. Both *J. bermudiana* and Darrell's cedar shed their pollen in February and March. Darrell's cedar sets seed and appears to be fertile; it also appears to hybridize with Bermuda cedar, given time, and this will likely lead to the complete loss of the Bermuda cedar germplasm. If "Darrell's cedar" came from Florida, it is certainly very closely related to the Bermuda cedar (Adams, 2004) and it is likely interfertile with the Bermuda cedar.

In a second effort to revive cedars, Reeve Smith brought male juniper(s) into Bermuda in the 1950s (Whitney, 1955). Smith's cedar was thought to be 'Barbados cedars' (*J. barbadensis*). Because Smith's cedar(s) were all males, they had to be reproduced by cuttings.

The present study was designed to utilize (Single Nucleotide Polymorphisms, SNPs) of nr DNA and trnC-trnD, cp DNA to determine the affinities of Darrell's Smith's cedar and Smith's cedar and to assess hybridization with *J. bermudiana*.

MATERIALS AND METHODS

Specimens collected: Taxon, acronym, collector number, location: *Juniperus barbadensis* (BA), *Adams 5367-5371;* Petit Piton, St. Lucia, BWI; *Juniperus bermudiana* (BM), *Adams 11080-11082*, Bermuda; *J. gracilior* var. *ekmanii* (EK), *Adams 7653-7654*, 3-4 km ne Mare Rouge, Pic la Selle, Haiti; *J. g.* var. *gracilior* (GR), *Adams 7664-7667,* w of Constanza, Dominican Republic; *J. g.* var. *urbaniana* (UR) *Adams 7656-7658,* 4-5 km ne Mare Rouge, Pic la Selle, Haiti; *J. lucayana* (LU): *Adams 5259-5280*, Havana Botanical Garden (seed from Sierra de Nipe), Cuba; *Adams 5281-5282*, Havana Botanical

Garden (seed from Isle de Pinos), Cuba; *J. saxicola* (SX) *Adams 5284-5285,* w slope of Pico Turquino, Prov. Granma/Santiago de Cuba boundary, Cuba; *J. virginiana* var. *virginiana* (VG) *Adams 6753-6755*; on I35, Hewitt, TX; *J. v.* var. *silicicola* (SI) *Adams 9186-9188,* Ft. Desoto Park, Mullet Key, Florida. Darrell's Cedar (DC): *Adams 11111-11114*, Bermuda; Smith's Cedar (SC): *Adams 11088-11090*, Bermuda; Putative hybrids: *Adams 11093, 11094, 11101, 11102, 11106, 11107*, Bermuda. Herbarium vouchers for all of the aforementioned collections are deposited at 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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer [final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM $MgCl_2$, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4% (by vol.) DMSO].

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

trnC-trnD: CDFor: CCA GTT CAA ATC TGG GTG TC
CDRev: GGG ATT GTA GTT CAA TTG GT

CDFor, CDRev primers from Demesure et al. (1995).

CD10F: AAA GAG AGG GAT TCG TAT GGA
CD3R: AAC GAA GCG AAA ATC AAT CA

CD10F and CD3R primers from Andrea Schwarzbach (pers. comm.).

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a

band of approx. 1120 bp. The internal trnC-trnD primers, CD10F-CD3R produced a band of approx. 800 bp. In each case the band was excised and purified using a Qiagen QIAquick gel extraction kit.

The gel-purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Analyses of 1119 bp of nrDNA (ITS) sequences revealed 24 SNPs among the taxa, including a 3 bp deletion in both samples of *J. g.* var. *ekmanii* and a 1 bp insertion in all six samples of *J. v.* var. *virginiana* and *J. v.* var. *silicicola*. PCO of the SNPs resulted in 5 eigenroots that were larger than the average diagonal value. These 5 eigenroots accounted for 42.49, 20.48, 12.98, 8.54 and 5.80% of the variation among the OTUs or a total of 90.21%. From this factor analysis there appear to be 4 majors groups and 2 minor groups. Ordination (Fig. 1) shows four major groups: (*J. virginiana, J. v.* var. *silicicola* and Darrell's cedar), all the Caribbean junipers, *J. bermudiana*, and Smith cedar. The identical vertical bars imply that

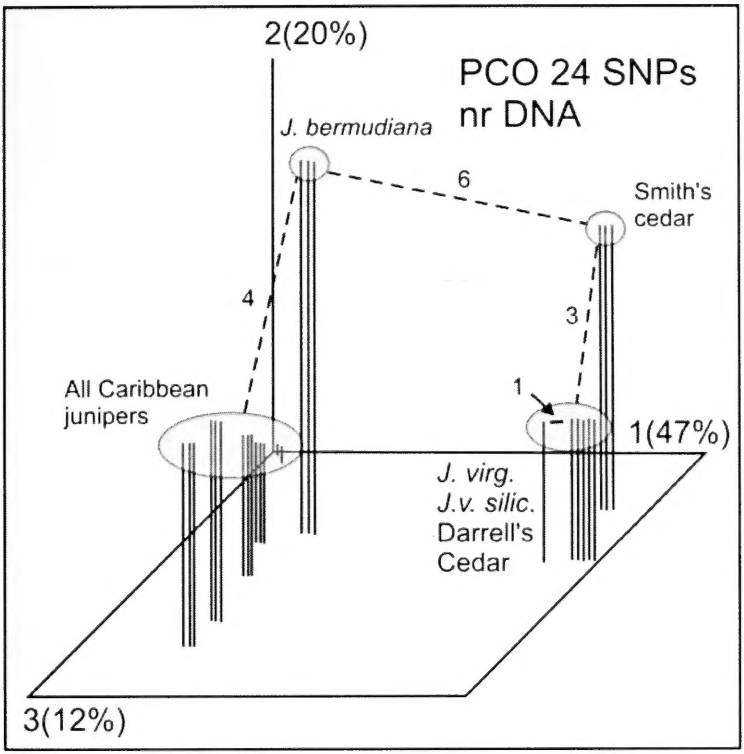


Figure 1. PCO of Caribbean taxa and *J. bermudiana*, Darrell's Cedar and Smith's Cedar. Number next to dashed line is the number of bp differences between groups.

(Fig. 1) there was no variation among these samples. One of the *J. v.* var. *silicicola* samples had a 1 bp difference from other samples of *J. v.* var. *silicicola* and *J. virginiana*. Smith's cedar appears to be a form of *J. virginiana* var. *virginiana* or *J. v.* var. *silicicola* based on ITS SNPs.

Analysis of trnC-trnD, cpDNA revealed 6 SNPs and PCO of these data yielded two eigenroots of 81 and 16%. Figure 2 shows that all the individuals in the analysis had a 553 bp sequence, in contrast to *J. virginiana* and Smith's cedar that both had 798 bp of sequence data.

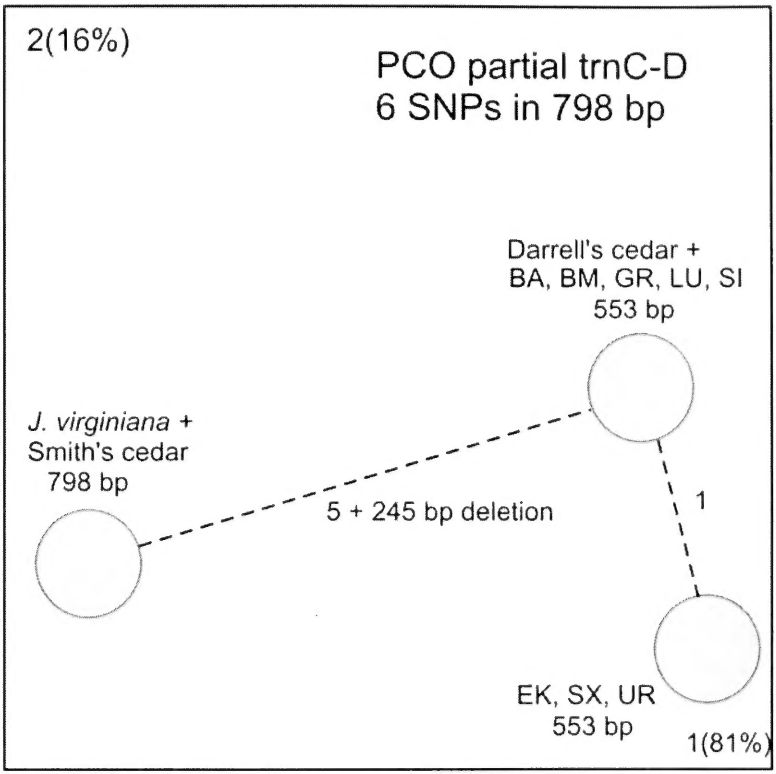


Figure 2. PCO of trnC-trnD SNPs. Numbers next to the dashed lines of the number of SNPs.

Clearly, Smith's cedar is allied with *J. virginiana* and Darrell's cedar is allied with the Caribbean junipers (including *J. v.* var. *silicicola*).

Figure 3 shows a summary of the ITS and trnC-trnD data. It is clear that Smith's cedar is a form of *J. virginiana* and Darrell's cedar is a form of *J. v.* var. *silicicola*.

Both Darrell's and Smith's cedars are resistant to the introduced scale leaf insects. This is readily understandable as they

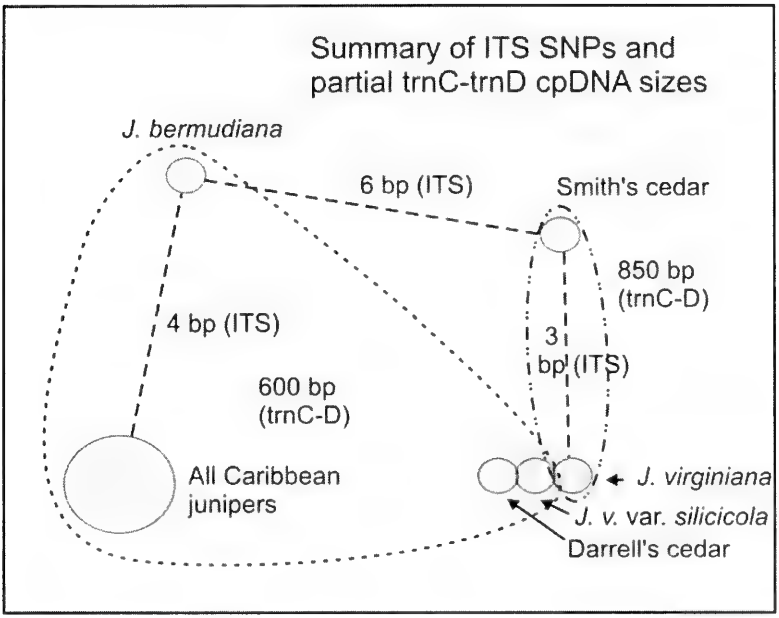


Figure 3. Summary of ITS and trnC-trnD data.

were introduced from the mainland and, as elements of *J. virginiana*, have co-evolved resistance to these scale insects. However, this presents a problem in Bermuda, as Darrell's and Smith's cedars are often planted instead of *J. bermudiana* because they are resistant to scale insects and thus, grow well on Bermuda.

The second problem is that hybridization between Darrell's, Smith's and Bermuda cedars seems likely. If hybrids are fertile, introgression of *J. virginiana* genes into *J. bermudiana* could occur.

HYBRIDIZATION

Darrell's cedar has finer foliage, leaves in threes, and is more bushy (less of a central axis) than *J. bermudiana*. Several trees were sampled that appeared to be morphologically intermediate between Darrell's cedar and *J. bermudiana*. Table 1 shows that plants 11101,

11102 and 11107 (Tivoli North) appear to be hybrids between *J. bermudiana* and Darrell's cedar. Plants 11093 and 11094 that were growing about 100 m from a row of male, Smith's cedars, each have the cpDNA (trnC-trnD, 850 bp) of Smith's cedars, but they also have nuclear DNA markers from *J. bermudiana* (79 C; 332 deletion; 589 A; 699 T; 944 A and 1059 T) and from Darrell's cedar (121 G; 441 T). Darrell's cedar has been planted in the vicinity, so it appears that the plants may be hybrids backcrossed to Darrell's cedar.

Plant 11106 (Aileen Morrison's house) is nearly pure *J. bermudiana*, but it appears that it may have some DNA markers of Darrell's cedar (note the small amounts of several nucleotides typical of Darrell's cedar, Table 1). Because nrDNA is composed of thousands of copies, it might take many generations of concerted evolution to remove these polymorphisms.

Table 1. Comparisons of SNPs from ITS and trnC-trnD fragment lengths for putative hybrids with *J. bermudiana*, Darrell's cedar (DC), Smith's cedar (SC), *J. virginiana*, and *J. v.* var. *silicicola*.

	ITS position									
taxon/sample	79	121	332	441	589	699	944	1059	trnC-D(bp)	Classification
J. bermudiana	C	C	-	C	A	T	A	T	600	*J. bermudiana*
Darrell's Cedar	T	G	G	T	C	C	G	A	600	*J. v.* var. *silicicola*
Smith's Cedar	T	C	G	C	C	C	G	A	850	*J. v.* var. *virginiana*
J. virginiana	T	G	G	G	C	C	G	A	850	*J. virginiana*
J. v. silicicola	T	G	G	G/C	C	C/T	G	A	600	*J. v.* var. *silicicola*
11093	C/T	C/G	G/-	C/T	A/C	C/T	A/G	A/T	850	J. *berm.* x DC x SC?
11094	C/T	C/G	G/-	C/T	A/C	C/T	A/G	A/T	850	J. *berm.* x DC x SC?
11101	C/T	C/G	G/-	C/T	A/C	C/T	A/G	A/T	600	J. *berm.* x Darrell's
11102	C/T	C/G	G/-	C/T	A/C	C/T	A/G	A/T	600	J. *berm.* x Darrell's
11107	C/T	C/G	G/-	C/T	A/C	C/T	A/G	A/T	600	J. *berm.* x Darrell's
11106	C	C	-	C(T)*	A	T(C)	A(T)	T(A)	600	*J. berm* backcrossed to DC?

*base in () was a small peak, less than 20% the height of the parent peak.

Old *J. bermudiana* tree at Devonshire Church cemetery, Bermuda, *Adams 11080*

Darrell's cedar (*J. v.* var. *silicicola*) planted along Cedar Ave., Hamilton, Bermuda. cf *Adams 11112-11114.*

Smith's cedar, *J. virginiana.* at Ms. Nea Smith's house. cf *Adams 11088-11092.*

Putative hybrid of *J. bermudiana* x Darrell's cedar *(J. v.* var. *silicicola*), cf. *Adams 11101.*

Trees at Aileen Morrison's house.
Left: *J. bermudiana*, putative backcross with Darrell's cedar. *Adams 11106*

Right: young *J. bermudiana* tree. This tree appears to be showing disease symptoms. Note loss of foliage at the top.

Planted trees along Cedar Ave., Hamilton, Bermuda.

Foreground: diseased *J. bermudiana* tree. Note the loss of foliage on the upper branches.

Background: healthy Darrell's cedar (*J. v.* var. *silicicola*)

As old diseased *J. bermudiana* trees died on Cedar Ave., Hamilton, Bermuda, they have often been replaced with Darrell's cedar. Darrell's cedar grows very well as it is resistant scale insects that it co-evolved with on the United States mainland.

ACKNOWLEDGEMENTS

Thanks to Tonya Yanke for lab assistance. This research funded in part by Baylor University. Thanks to B. L. Turner and Andrea Schwarzbach for manuscript reviews.

LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 2004. Junipers of the World: The genus *Juniperus*. Trafford Publ., Vancouver, B. C.

Bennett, F.D. and I.W. Hughes. 1959. Biological control of insect pests in Bermuda. Bulletin of Entomology Research 50: 423-436.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Groves, G.R. 1955. The Bermuda cedar. World Crops 7:1-5.

Veldman D. J., 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

Whitney, C. M. 1955. The Bermuda Garden. p. 34., The Garden Club of Bermuda, Bermuda.

Point of View

JUNIPERUS BERMUDIANA: A SPECIES IN CRISIS, SHOULD IT BE RESCUED FROM INTRODUCED JUNIPERS?

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

In this issue we (Adams and Wingate, pp. 123-133) have presented data that show that *J. bermudiana* is being subjected to hybridization and likely introgression from Darrell's cedar (*J. virginiana* var. *silicicola*) and Smith's cedar (*J. v.* var. *virginiana*). Because Dr. Wingate and I have differing philosophies on the conservation of species, we decided to omit conclusions from the previous paper (Adams and Wingate, pp. 123-133, this issue). Instead, we are presenting two 'Point of View' to summarize our conclusions.

In 1942 two scale insects were accidentally introduced into Bermuda with devastating effects on *Juniperus bermudiana*, endemic to Bermuda. In an effort to repopulate junipers on Bermuda, two cultivated junipers were introduced by J. D. C. Darrell in the 1940s (Darrell's cedar) and Reeve Smith in the 1950s (Smith's cedar) from Florida. Analysis of SNPs of nrDNA and trnC-trnD cp DNA revealed that Darrell's cedar is *Juniperus virginiana* var. *silicicola* and Smith's cedar is *Juniperus v.* var. *virginiana.*

Although this research is preliminary in scope, several facts seem apparent to the author:

1. Darrell's cedar is *J. v.* var. *silicicola.* Smith's cedar is *J. virginiana* var. *virginiana.* Both introduced cedars are resistant to the introduced scale insects and are, therefore, competitive with the endemic *J. bermudiana.* Darrell's cedar and Smith's cedars are widely cultivated and desirable due to their robust growth.

2. Hybridization between *J. bermudiana* and Darrell's and Smith's cedars is occurring. There is also evidence of introgression. Darrell's cedar is hybridizing with *J. bermudiana* and results in scale-resistant plants that grow well in Bermuda. It appears to be just a matter of time

until there will be no genetically pure individuals of *J. bermudiana* on Bermuda.
3. Many residents of Bermuda apparently do not recognize the differences between their endemic juniper and the introduced juniper species. Therefore, it would seem unlikely that public interest will develop in removing the introduced junipers and conserving the native junipers.

4. Although *J. bermudiana* has gone through a tremendous genetic bottleneck, trees were found that were in good health and appear to have resistance to the scale insects. The effects of the scale insects are not completely devastating as many very old, large trees remain cultivated in cemeteries and in private yards. Another significant factor has been the introduction of several unrelated, weedy tree species that are very aggressive and compete for space and nutrients.

Can *J. bermudiana* germplasm be conserved in Bermuda? Yes. Will the germplasm be conserved? That is a difficult projection to make. It will take a massive effort to remove Darrell's and Smith's cedars from Bermuda. Some Darrell's cedar trees are now 50-60 years old and prized by residents (particularly along Cedar St. in Hamilton, where dead *J. bermudiana* trees have been replaced with scale resistant Darrell's cedars). Because hybridization is occurring, suspected hybrids and introgressive plants would also need to be removed. Removing all such cedars will likely be opposed by residents.

Can *J. bermudiana* germplasm be conserved in other locations? In the mid-19th century, seeds of *J. bermudiana* from Bermuda (via Kew Gardens) were taken to St. Helena and Ascension Islands in the south Atlantic ocean, between Africa and Brazil. They were planted to produce timber trees and *J. bermudiana* has thrived on these islands (Phillip Ashmole and Andrew Darlow, Invasive Species Officer, St. Helena Island, and Susanna Musick, Head, Conservation Dept., Ascension Island, pers. comm.). However, *J. virginiana* has also been introduced on St. Helena (Phillip Ashmole, pers. comm.) so there is the potential for hybridization. These islands, due to their extreme geographic isolation, may be a repository for *J. bermudiana* germplasm.

Botanic gardens hold promise for *ex situ* conservation of *J. bermudiana*, but because nearly all botanic gardens grow *J. virginiana*, collecting seed from *J. bermudiana* might result in hybrid plants. Note that both *J. bermudiana* and *J. virginiana* are dioecious species. Thus, pollination of a female *J. bermudiana* from a male *J. virginiana* is quite likely (particularly if no male *J. bermudiana* is nearby). The safest method of propagation would be by taking cuttings from *J. bermudiana*.

Should we care if a species goes extinct? Extinction is the norm; survival is the exception for species throughout geologic time. Yet, there seems to be a common philosophy among humans that we should not hasten the extinction of species. There is also a philosophy that we are part of the environment and the evolutionary mix, so let "nature take its course". However, that philosophy can degenerate into a "live today; nature will take care of tomorrow".

There are, however, practical reasons to conserve species. First, biodiversity in our ecosystem generally leads to stability and a stable environment is mostly to our benefit. Second, species possess a unique genome with germplasm that has co-evolved with nature. They contain a storehouse of phytochemicals that have biological activity such as anti-cancer, anti-malaria, anti-microbial, etc. that act as lead structure compounds for drug development. Third, species contain many allelic forms of genes that can provide important germplasm resources for breeding and genetic engineering. For example, the genetic resources of the wild wheats from central Asia are constantly being utilized in modern wheat breeding to develop agronomic characteristics such as anti-lodging, seed shatter resistance, insect and disease resistance, to name but a few. Without the conservation of the wild species, breeders would very limited. Fourth, species are critical for phylogenetic and evolutionary research. One can only imagine the impact of having access to some of the extinct species' genomes in our present day studies of evolution.

In summary, the preservation of a seemingly 'insignificant' species can have very practical significance. However, whether the cost of preservation *in situ* or *ex situ* is acceptable depends on many social norms that are outside my expertise.

Point of View

CONSERVATION OF THE BERMUDA JUNIPER

David B. Wingate
wingate@northrock.bm

Shortly before or during the scale epidemic, two distinguishable varieties of *J. virginiana*: var. *virginiana* and var. *silicicola* were introduced from nurseries in Florida and locally propagated and planted from nursery stock. While they do not self-seed as readily on Bermuda as the Bermuda cedar, they are inherently scale resistant, and, thus, their uncommon and localized presence in the landscape became noticeable once the majority of the Bermuda cedars had died.

The selective impact of the scale epidemic, leaving about 5% of the original J. Bermudians forest unaffected, strongly suggests that a genetic trait for scale resistance had persisted sparsely in the population despite hundreds of thousands of years of evolution in a scale free environment. If this was the case natural selection will have quickly restored a scale resistant strain to dominance according to Stephen J. Gould's theory of punctuated equilibrium evolution. Concomitantly, hybridization with the recently introduced scale resistant junipers could be having the same effect. Whichever the case, *J. bermudiana* has remained numerically dominant over the introduced trees throughout this transition, so that all of the unique adaptive features of the Bermuda juniper should survive in the genetic code of the hybrids.

In view of Bermuda's very small size (20KM long and only 57 square Km in area) and the fact that junipers are wind pollinated and bird dispersed, and considering that the introduced *J. virginiana* varieties have now been on Bermuda for over 60 years and hybridize with *J. bermudiana*, it is undoubtedly far too late to preserve the pure *J. bermudiana* germplasm in the wild on Bermuda by attempting to cull out all of the *J. virginiana* and the hybrids. In any case, the public would be adamantly opposed to such a drastic measure. In my opinion the only sure way to preserve pure *J. bermudiana* stock for scientific research and biodiversity conservation purposes will be to propagate it by cuttings from surviving trees which predate both the scale epidemic and the introduction of *J. virginiana*.

SALVIA ACERIFOLIA (LAMIACEAE), A NEW SPECIES FROM MICHOACAN, MEXICO

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

ABSTRACT

Routine identification of Mexican plants has revealed the following novelty:

SALVIA ACERIFOLIA B.L. Turner, **sp. nov. Fig. 1**

Salvia subhastatae Epling similes sed differt laminas subter glabris (vs minute glanduloso-pubescentibus) marginibus grosse dentatis (vs minute crenulatis), calycibus minoribus, et corollis lavandulaceis (vs luteis).

Perennial herbs to 1 m (?) high. **Stems** weakly 4-sided, if at all, with a vestiture of multiseptate crinkly trichomes 2-3 mm high. **Leaves** at mid-stem mostly 10-15 cm long; petioles 5-10 cm long; blades 6-8 cm long, 5-6 cm wide, glabrous beneath, sparsely pubescent above, deltoid-cordate, their margins irregularly broadly dentate. **Spikes** 10-20 cm long, interrupted, the axis pubescent like the stems. **Floral bracts** (uppermost) glabrous, broadly ovate, 6-9 mm long, 4-8 mm wide, readily deciduous. **Flowers** arranged 2 to a node, their pedicels mostly 5-7 mm long. **Calyx** (flowering) 8-10 mm long, pubescent like the stems, 2-lipped, the upper lip 5-veined, lower lip somewhat smaller, bifid. **Corolla** purple, 22-25 mm long; throats 9-12 mm long, not papillose within; lower lip ca 12 mm long, 8 mm wide, clearly 5-lobed; upper lip ca 4 mm long, sparsely pubescent (at the apex only). **Stamens** not excurrent, attached near the orifice; filaments ca 4 mm long; anthers yellow, ca 1.5 mm long. **Style** apically pilose; upper branches ca 1.3 mm long, the lower branches recurved or sigmoid, 2-3

times as long as the upper lobe. **Nutlets** ovoid, glabrous, ca 2.5 mm long, 1.6 mm wide.

TYPE: **MEXICO. MICHOACAN: Mpio. Coahuayana,** "Waterfall, roadside forest, 12.2 mi (19.9 km) W of turnoff to Villa Victoria; 26.2 mi (41.9 km) E of Coahuayana on road to Coalcoman." ca 880 m, 11 Sep 1985, *Clark P. Cowan 5646* (with Luckow, Kearns, Jacobson & T. Cowan). (Holotype: TEX; isotypes: CAS, F, GH, MEXU, US.)

The species is named for its leaves, which resemble those of the genus *Acer*. The collector himself, by annotation on the edge of a newspaper containing the holotype, noted that the species was probably new and, if so, that he intended to provide the name *S. longipetiolata*. The latter name would be reasonably applicable, but that provided here readily distinguishes the species from all other members of *Salvia* in Mexico.

I have not been able to position the species with any certainty in the sections erected by Epling (1939) but would provisionally include this within or near his monotypic sect. Sphacelioides (containing *S. subhastata* Epling), largely on the basis of habit and floral characters. It is likely that Epling would have positioned the species in a newly erected section, to judge from his published sectional categories, many of these monotypic or small, presumably erected to accommodate odd-ball or very distinct species, such as the present novelty.

ACKNOWLEDGEMENTS

I am grateful to my colleague Guy Nesom for the Latin diagnosis and for reviewing the manuscript.

LITERATURE CITED

Epling, C. 1939. A revision of *Salvia*: subgenus Calosphace. Repert. Spec. Nov. Regni Beih. 110: 1-383.

Fig. 1. *Salvia acerifolia* (holotype).

A NEW SPECIES OF *SALVIA* (LAMIACEAE) FROM GUERRERO, MEXICO

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

Routine identification of Mexican plants has revealed the following novelty:

SALVIA CLARKCOWANII B.L. Turner, **sp. nov. Fig. 1**

Salvia purpurea Ort. similes sed differt foliis majoribus petiolis longioribus (plerumque 4-10 cm longis vs 1-4 cm), calycibus valde purpureis ad apices expansis, et corollas glabris vel paene glabris.

Perennial shrublets or shrubs 1-3 m high. **Stems** minutely pubescent with down-swept hairs. **Leaves** mostly 9-18 cm long; petioles 3-10 cm long; blades broadly ovate to subcordate, glandular-punctate beneath, minutely pubescent on both surfaces, mostly along the major veins, their margins serrate. **Spikes** terminal, arranged in pseudocorymbose panicles 6-15 cm high, 5-10 cm across. **Flowers** 4-10 to a node, the pedicels mostly 3-4 mm long. **Floral bracts** persistent, lanceolate to ovate, 1-2 mm long, 0.5-1.0 mm wide. **Calyces** (flowering) 7-9 mm long, 2-lipped, the upper lip 3-veined and somewhat shorter than the bifid lower lip, at maturity their apices becoming purple and expanded, minutely pubescent with both short hairs and globular glands. **Corollas** purple, arcuate in bud; tubes 20-25 mm long, not papillose within; upper lip 6-10 mm long, glabrous, ca twice as long as the lower. **Stamens** excurrent from the upper lip for 4-8 mm; filaments 10-16 mm long; anthers yellow, ca 2.5 mm long. **Styles** long and slender, extending ca 10 mm beyond the upper lip, glabrous or nearly so, the upper branch 2-3 times as long as the lower. **Nutlets** ovoid, brown, glabrous, ca 2 mm long, 1.5 mm wide.

TYPE: **MEXICO. GUERRERO: Mpio. Atoyac de Alvarez**, 24.3 km al NE de El Paraiso, 1560 m, 19 Dec 1984, *Clark P. Cowan 4941* (Holotype: TEX; isotypes: CAS, GH, MEXU).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. GUERRERO: Mpio. Apaxtla de Castrejon,** "1.5 km al SE de Puerto de El Gallo, Carr. a Atoyac." ca 2320 m, 22 Feb 1985, *Tenorio 8051* (TEX); **Mpio. Atoyac de Alvarez**, 10 km SW of Puerto del Gallo, ca 2100 m, *Martinez S. et al. 6191* (TEX).

The present taxon was given the unpublished name *S. nicolsoniana* by Ramamoorthy. I independently discerned its novelty upon examination of type material; indeed, Cowan himself noted on the margin of a newspaper containing type material, that the collection was undescribed, but he had not "decided on [a] name." I prefer to name the taxon for the collector himself, who first collected the taxon and recognized its novel status. Clark Cowan was a graduate student at The University of Texas, Austin for several years during which time he collected extensively in Mexico. He also collaborated with the present author on a systematic study of the genus *Stemodia* (Turner and Cowan 1993). He is currently involved with botanical studies on Santa Rosa Island, California, working out of the University of California, Santa Barbara.

Salvia clarkcowanii appears to belong to the sect. Purpureae of Epling (1939). While compared in the Latin diagnosis with the widespread, commonly encountered, *S. purpurea,* it is perhaps closest to *S. eizi-matudae* Ramamoorthy of Chiapas, Mexico, the taxa having similar foliage, calyces, and corollas.

ACKNOWLEDGEMENTS

I am indebted to my friend and colleague Guy Nesom for the Latin diagnosis and helpful comments on the article itself.

LITERATURE CITED

Epling, C. 1939. A revision of *Salvia*: subgenus Calosphace. Repert. Spec. Nov. Regni Beih. 110: 1-383.

Turner, B.L. and C.C. Cowan. 1993. Taxonomic overview of *Stemodia* (Scrophulariaceae) for North America and the West Indies. Phytologia 74: 61-103.

Fig. 1. Holotype of *Salvia clarkcowanii*.

STEVIA SCHIBLII (ASTERACEAE: EUPATORIEAE), A NEW SPECIES FROM OAXACA, MEXICO

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

Routine identification of Mexican comps has revealed the following novelty:

STEVIA SCHIBLII B.L. Turner, **sp. nov.** Fig. 1

Steviae elatiori H.B.K. similes sed foliis comparate paucis glabris plerumque basalibus (vs numerosis, valde pubescentibus, vix basalibus) et lobis corollae minoribus (1-2 mm longis vs 2-3 mm longis).

Perennial herbs 30-60 cm high (label data), arising from slender rhizomes. **Leaves** mostly opposite and basal, obovate, 3-6 cm long, 0.5-2.5 cm wide; petioles 2-25 mm long, tapered upon by the blades, the latter 3-nerved from the base, essentially glabrous with rounded serrations along the upper half. **Capitulescence** a much-branched, expanded panicle, 10-45 cm high, 10-30 cm wide, glandular-pubescent throughout, the ultimate peduncles mostly 10-25 mm long. **Involucres** 5-6 mm high having linear-lanceolate bracts with narrowly acute apices. **Corollas** white, 4.5-5.0 mm long, the lobes ca 2 mm long, sparsely pubescent with multiseptate hairs, these scattered among a more numerous vestiture of short glandular hairs. **Achenes** black, ca 3.5 mm long, sparsely pubescent, the pappus a crown of white scales ca 0.3 high, occasionally 1 or 2 of the achenes bearing slender awns 3-5 mm long.

TYPE: **MEXICO. OAXACA:** Mpio. Santiago Textitlan, debajo de Cerro Colmillo, "Bosque de pino, suelo negro," ca 1535 m, 28 Aug 2006, *Idalia Trujillo Olazo 343* (Holotype: TEX).

ADDITIONAL COLLECTIONS EXAMINED: **MEXICO. OAXACA:** Mpio. Santiago Textitlan, "Comunidad de Llano Yerba. Donde se junta el arroyo de Agua Amarilla y el arroyo de Piedra Blanca."disturbed pine-oak forests, ca 1319 m, 16 Aug 2006, *Martinez 1882* (TEX); Buenavista, paraje Cacalosuche, pine-oak forests, ca 1320 m, 31 Aug 2006, *Salinas 434* (TEX).

In my treatment of *Stevia* for the Comps of Mexico (Phytologia Memoirs 11: 170-197. 1997), the present novelty will key to the widespread, highly variable, *S. elatior* of the series Podocephalae. It is readily distinguished from that species by its more delicate habit, relatively fewer, glabrous, mostly basal leaves, and smaller corolla lobes.

The species is named for Leo Schibli (1958-2004), Swiss-born conservationist and specialist in geographic information systems He was a founding member of SERBO (Society for the Study of Biotic Resources of Oaxaca), and was devoted to fostering awareness and appreciation of the rich biological heritage of Oaxaca.

ACKNOWLEDGEMENTS

I am grateful to Guy Nesom for the Latin diagnosis, and to him and Emily Lott for reviewing the manuscript. Emily also provided information relating to the eponym.

Fig. 1. *Stevia schiblii* (holotype)

KARYOTYPE ANALYSES OF FOUR *ASTRAGALUS* L. (FABACEAE) SPECIES FROM TURKEY

Esra Martin, Ahmet Duran, Muhittin Dinç and Semiha Erişen
Selçuk University, Education Faculty, Department of Biology Education, Konya, Turkey
esramartin@gmail.com

Mehmet Babaoğlu
Selçuk University, Faculty of Agriculture, Department of Field Crops, Konya, Turkey

ABSTRACT

Astragalus L. is a perennial herbaceous genus belonging to the family Fabaceae. Chromosome morphology was defined for the first time in four species of *Astragalus* namely; *A. antalyensis* A.Duran & Podlech, *A. nezaketae* A.Duran & Aytaç, *A. cariensis* Boiss. & *A. schizopterus* Boiss. All species contained diploid chromosome numbers of $2n = 16$. However, polyploid cells ($4x = 32$) were also observed in *A. schizopterus* and *A. antalyensis*. *Astragalus nezaketae* species had two double satellite chromosomes. Karyotypes of species were made using an Image Analysis System. Chromosome numbers are all first reports, except for that of *A. schizopterus*.

KEY WORDS: *Astragalus*, Leguminosae, Image Analysis System, karyotype, Turkey

---

Astragalus L. is one of the largest genera of vascular plants in the world, with an estimated number of 3000 species. Many species are narrow endemics. However, a few are widespread, mainly in the Northern Hemisphere, Central Asia, and Western North America (Podlech, 1986; Maassoumi, 1998; Açık et al., 2004). It is also the largest genus in Turkey, where it is represented by nearly 455 species in 61 sections (Chamberlain and Matthews, 1970; Davis et al., 1988; Duran and Aytaç, 2005). In Turkey, the genus *Astragalus* is represented best in the steppe environment of high mountains (Chamberlain and

Matthews 1970; Podlech, 1999). In the Irano-Turanian phytogeographic region of Turkey, which is one of the centers of diversity of the genus (Ghahremaninejad and Behçet, 2003) 210 endemic taxa occur, which is a rate of endemism of about 47% (Duman and Akan, 2003). The species examined in the present study are economically valuable and all are endemic to Turkey.

Karyological knowledge of *Astragalus* consists of chromosome counts of more than fifty species (Spellenberg, 1976; Cartier, 1976, 1979; Davis et al., 1988; Çobanoğlu and Altan, 1989; Şahin et al., 1990; Tünbel, 1993; Kandemir et al., 1994, 1996; Engin et al., 1994; Civelek et al., 1997; Aytaç, 1997; Ekici and Aytaç, 2001; Wang et al., 2002; Hamzaoğlu, 2003; Ekici et al., 2005). Such reports indicate the existence of only one basic chromosome number (x = 8) in the genus. Although chromosome counts are reported for many species in *Astragalus*, few workers have described karyotypes of its species. The lack of karyological studies in Astragalua is probably due to the small length of the chromosomes.

In this contribution, we report mitotic metaphase chromosome numbers and karyotypes of four species of *Astragalus* belonging to sections *Caprini* DC., *A. antalyensis*; *Incani* DC., *A. nezaketae*; *Proselius* Bunge, *A. cariensis* Boiss. and *A. schizopterus* Boiss. (Chamberlain and Matthews, 1970; Podlech, 1999; Duran and Aytaç, 2005). The systematic importance of the results is discussed when appropriate.

MATERIAL AND METHODS

Plant materials of the genus *Astragalus* namely *A. antalyensis*, *A. nezaketae*, *A. cariensis* and *A. schizopterus* representing three sections namely; *Caprini*, *Incani* and *Proselius* were collected from different localities in Turkey as detailed in Table 1. Voucher specimens have been deposited at the herbaria of Selçuk University. All taxa except A. *schizopterus,* are local endemics (Chamberlain and Matthews, 1970; Podlech, 1999; Duran and Aytaç, 2005).

Karyomorphological observations were made on mitotic metaphase cells of root-tips obtained from germinated seeds. Root tips

were pretreated for 16 h in α-monobromonaphthalene at 4°C and washed with distilled water and finally fixed in Carnoys solution (3:1 absolute ethanol : glacial acetic acid, overnight. The root tips were hydrolysed for 10 min in 1 N HCl at room temperature, washed and stained in 2% (w/v) aceto-orcein for 2 h. Stained root tips were then squashed in a drop of 45% acetic acid and permanent slides were made by mounting in Depex. Chromosome measurements were made in at least five well-spread metaphases, bearing the same chromosome contraction. The total lengths (μm) and the arm ratio values were used for comparisons of the karyotypes. Ideograms were designed by using an Image Analysis System (BsPro200).

RESULTS

Chromosome morphologies, total chromosome lengths, arm ratios and centromeric indices are summarized in Table 2. A chromosome number of 2n = 16 was determined for all species. Results of the study are given below.

Section: *Caprini*

Astragalus antalyensis

Chromosome numbers were determined 2n = 2x = 16 = 7m+1sm with a basic number of x = 8 (Fig. 1). The metaphase karyotype consisted of 7 median chromosomes and 1 submedian chromosome. The species had one double satellite metaphase chromosome. Chromosomes varied from 1.36 μm to 2.82 μm. Total haploid chromosome length was 16.71 μm. The ideogram is given in Fig. 5.

Section: *Incani*

Astragalus nezaketae

The chromosome number of this species was determined as 2n = 2x = 16 = 6m+2sm (Fig. 2) The metaphase karyotype consisted of 6 median chromosomes, and 2 submedian chromosomes. The species had two satellite metaphase chromosomes. Chromosomes size ranged from

2.54 μm to 3.35 μm. Total haploid chromosome length was 23.42 μm. The ideogram is given in Fig. 5.

Section: *Proselius*

Astragalus cariensis Boiss.

The chromosome number of this species was determined as 2n = 2x = 16 = 1m+6sm+1st (Fig. 3). The metaphase karyotype consisted of 1 median chromosome, 6 submedian chromosomes and 1 terminal point chromosome. Chromosome sizes ranged from 3.16 μm to 4.69 μm. Total haploid chromosome length was 33.67 μm. The ideogram is given in Fig. 5.

Astragalus schizopterus Boiss.

The chromosome number of this species was determined as 2n = 2x = 16 = 8m (Fig. 4). The metaphase karyotype consisted of 1 median chromosome, 6 submedian chromosomes and 1 terminal point chromosome. Chromosome sizes ranged from 1.81 μm to 3.97 μm. Total haploid chromosome length was 20.90 μm. The ideogram is given in Fig. 5.

DISCUSSION

The chromosome numbers of four species of the genus *Astragalus* were determined based on an analysis of somatic metaphases. The chromosome number 2n = 2x = 16 was found for all species, establishing a basic chromosome number of x = 8. The total haploid chromosome lengths ranged from 16.71 to 33.69 μm with average chromosome lengths from 1.36 to 4.69 μm. The median (m) and submedian (sm) chromosomes are found to form the main part of chromosome complement, while the terminal point (T) chromosomes were rare or absent. Tetraploidy (2n = 4x = 32) is reported here for the first time in *A. antalyensis* and *A. schizopterus*. SAT chromosomes were analyzed for two species (*A. antalyensis* and *A. nezaketae*).

Somatic chromosome number and morphology of *A. antalyensis* are first reported in this study. Some tetraploid cells (2n =

4x = 32) were observed, in accordance with some previous reports (Çobanoğlu and Altan, 1989; Cartier, 1976, 1979). Diploid chromosome number of 2n = 16 is in line with studies made in various taxa of the *Astragalus* (Cartier, 1976, 1979; Çobanoğlu and Altan, 1989; Şahin et al., 1990; Kandemir et al., 1994, 1996; Engin et al., 1994; Civelek et al., 1997; Aytaç, 1997). However, chromosome lengths were the shortest (1.36 μm) in this species among other species studied. In addition, the highest centromeric index (5.09) was found in this species. This species also contained one double satellite on its mitotic chromosomes, separating it from other species.

Chromosome number (2n = 16) and karotype of *A. nezaketae* are first reported in this study. The species contains two double satellites on its chromosomes. Polyploid cells were not observed. The species contains the same somatic chromosome number (2n = 16) as that of *A. ovabaghensis* from the section *Alopecuroidei* DC. (Akan and Aytaç, 2004).

The other endemic species, *A. cariensis*, also had 2n = 16. The longest chromosome was measured as 4.69 μm. Total haploid chromosome length was 33.67 μm and the arm ratio was 2.33. This species had the lowest (3.93) centromeric index among the species examined. Satellite chromosome or polyploidy were not observed in this species.

The chromosome number of *A. schizopterus*, which is not endemic to Turkey, was reported to be 2n = 16 (Index to Plant Chromosome Numbers; Missouri Botanical Garden, http: //mobot.mobot.org/W3T/Search/ipcn.html) with some tetraploid somatic cells. The karyotype formula was different from yet other taxa which consisted of only median chromosome pairs.

In general, the four *Astragalus* species from three sections did not show variation in chromosome number. However, chromosome morphologies varied significantly. For instance, the karyotype of *A. cariensis* was mainly composed of median, submedian and terminal point chromosome pairs, whereas no terminal point chromosomes were observed in other taxa. Similarly, only median chromosome pairs were found in *A. schizopterus*, this contrasting with yet other taxa.

Astragalus cariensis and *A. schizopterus* belonging to the same section (*Proselius*) presented clearly different karyotypes (1m+6sm+1st and 8m). The size of chromosomes were also different in both species. The lack of polyploidy in *A. cariensis* was another distinctive feature of this species.

Astragalus ovalis Boiss. & Balansa from the section *Ammodendron* Bunge has a karyotype formula of 2n = 16 = 8m (Ekici et al., 2005) with total chromosome lengths varying between 1.11 µm and 1.63 µm (haploid chromosome length: 10.79 µm, arm ratios: 1.04 - 1.31). The karyotype formula of *A. schizopterus* is similar to *A. ovalis.* However, chromosome lengths of *A. schizopterus* were longer (1.81-3.97 µm) than those of *A. ovalis.* This was also the case in arm ratio (1.42). The other variation was the existence of tetraploid cells (2n = 4x = 32) in *A. schizopterus.* Ekici and Aytaç (2001) reported that the diploid chromosome number of *A. dumanii* M.Ekici and Aytaç which belongs to the section *Hololeuce* Bunge were also 2n = 16, in line with our reported chromosome number.

A revision of the section *Dasyphyllium* Bunge of the genus *Astragalus* (Fabaceae) in Turkey has been carried out. Its chromosome number was reported to be (2n) = 16 (Aytaç, 1997), similar to the findings in the present study.

Spellenberg (1976) presented 126 original reports of chromosome numbers for 101 taxa of North American *Astragalus*. His study supports earlier work demonstrating the basic division between Old World species, including their close New World relatives (x = 8, euploidy common), and strictly New World lineages (x = 11, 12, 13, 14, 15, euploidy rare). It is proposed, therefore, that the New World species originated from a tetraploid of 2n = 32 or a hypotetraploid, evolutionary radiation in some lineages concomitant with descending aneuploidy. In other lineages with little or no change in number was recorded (Spellenberg, 1976). In another study Wang et al. (2002) reported that the karyotype of *A. complanatus* was K (2n) = 16 = 10m + 6 sm.

Chromosome numbers of 2n = 16 have been reported for other endemic *Astragalus* species in Turkey namely; *A. panduratus* Bunge, *A. barba-jovis* DC. var. *barba-jovis* and *A. plumosus* Willd. var. *nitens* (Freyn and Bornm) Chamb. and Matthews (Tünbel, 1993) and *A. barba-jovis* DC. var. *barba-jovis* (Kandemir et al., 1996).

ACKNOWLEDGEMENTS

We express our thanks to TUBITAK (Project no. TOVAG–106O136), Scientific Investigation Project to Coordinate of Selçuk University (project no: 05401046) and for financial support.

LITERATURE CITED

Açık, L. M. Ekici. and A. Çelebi. 2004. Taxonomic relationships in *Astragalus* sections *Hololeuce* and *Synochreati* (Fabaceae): evidence from RAPD-PCR and SDS-PAGE of seed proteins. Ann. Bot. Fennici. 41: 305–317.

Akan, H. and Z. Aytaç. 2004. *Astragalus ovabaghensis* (Fabaceae), a new species from Turkey. Ann. Bot. Fennici. 41: 209–212.

Aytaç, Z. 1997. The Revision of the Section *Dasyphyllium* Bunge of the Genus *Astragalus* L. of Turkey. Turk. J. Bot. 21: 31–57.

Cartier, D. 1976. In Löve, Á. (ed.), IOPB Chromosome number reports, LIII. Taxon 25: 492–494.

Cartier, D. 1979. Premiéres prospections caryologiques du gene *Astragalus* dans la flore du bassin mēditerranéen oriental. Rev. Cytol. Biol. Vég. Bot. 2: 169–181.

Chamberlain, D. F. and V. A. Matthews. 1970. *Astragalus* L. In: Davis, P. H. (ed.), Flora of Turkey and the East Aegean Islands. Edinburgh Univ. Press, Edinburgh. 3: 49–254.

Civelek, Ş. F. Kapkın, A. Şahin. and N. Gür. 1997. *Astragalus aucheri* Boiss.'nin (Sect. *Xiphidium* Bunge) kromozom sayı, morfolojik,

anatomik ve pollen özellikleri bakımından araştırılması. F. Ü. Fen ve Müh. Bilimleri Dergisi 9: 33–34.

Çobanoğlu, D. and Y. Altan. 1989. *Astragalus decurrens* Boiss.'in (Fabaceae) morfolojik ve sitolojik özellikleri. Turk. J. Bot. 13: 34–44.

Davis, P. H. R. R. Mill. and K. Tan. 1988. *Astragalus* L. In: Davis, P. H., Mill, R. R. and Tan, K. (eds.), Flora of Turkey and the East Aegean Islands (Supplement). Edinburgh Univ. Press, Edinburgh. 10: 114–124.

Duman, H. and H. Akan. 2003. New species of *Astragalus* (sect. *Alopecuroidei*: Leguminosae) from Turkey. Bot. J. Linnean Soc. 143: 201–205.

Duran, A. and Z. Aytaç. 2005. *Astragalus nezaketae* (Fabaceae), a new species from Turkey, Ann. Bot. Fennici. 42: 381–385.

Ekici, M. and Z. Aytaç. 2001. *Astragalus dumanii* (Fabaceae), a new species from Anatolia, Turkey. Ann. Bot. Fennici. 38: 171–174.

Ekici, M. D.Yüzbaşıoğlu. and Z. Aytaç. 2005. Morphology, Pollen, Seed Structure and Karyological Study on *Astragalus ovalis* Boiss. and Balansa (Sect. *Ammodendron*) in Turkey. Intl. J. Bot. 1: 74–78.

Engin, A. N. Tünbel. and H. Korkmaz. 1994. *Astragalus pandaratus* Bunge'nin (Fabaceae) morfolojik ve sitolojik özellikleri. Turk. J. Bot. 18: 375–382.

Ghahremaninejad, F. and L. Behçet. 2003. *Astragalus subhanensis* (Fabaceae), a new species from Turkey. Ann. Bot. Fennici. 40: 209–211.

Hamzaoğlu, E. 2003. *Astragalus hamzae* (Fabaceae), a new species from Central Anatolia, Turkey. Ann. Bot. Fennici. 40: 291–294.

Kandemir, N. A. Engin. and H. Korkmaz.1994. *Astragalus plumosus* Willd. var. *nitens* (Freyn and Bornm.) Chamb. and Matthews'in

(Fabaceae) morfolojik, anatomik ve sitolojik özellikleri. XII. Ulusal Biyoloji Kongresi, Edirne II: 279–285.

Kandemir, N. H. Korkmaz. and A. Engin. 1996. The Morphological and Anatomical Properties of *Astragalus barba-jovis* DC. var. *barba-jovis* (Fabaceae). Turk. J. Bot. 20: 291–299.

Maassoumi, A. A. 1998. *Astragalus* L. in the Old World, Check–List. Islamic Rep. Iran Ministry of Jahas–e Sazandgi Res. Inst. Forest and Rangelands, Tehran.

Podlech, D. 1986. Taxonomic and phytogeographical problems in *Astragalus* of the Old World and South West Asia. Proc. Roy. Soc. 89: 37–43.

Podlech, D. 1999. New *Astragali* and *Oxytropis* from North Africa and Asia, including some new combinations and remarks on some species. Sendtnera 6: 135–171.

Şahin, A. D. Çobanoğlu. and H. Evren. 1990. Bazı *Astragalus* L. türlerinde kromozom sayısı ve özellikleri. X. Ulusal Biyoloji Kongresi Botanik Bildirileri, Erzurum 1: 75–83.

Spellenberg, R. 1976. Chromosome numbers and their cytotaxomic significance for North American *Astragalus* (Fabaceae). Taxon 25: 463–476.

Tünbel, N. 1993. Bazı *Astragalus* L. (Fabaceae) türleri üzerinde morfolojik, anatomik ve karyolojik bir araştırma. Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı. Yüksek Lisans Tezi, Samsun.

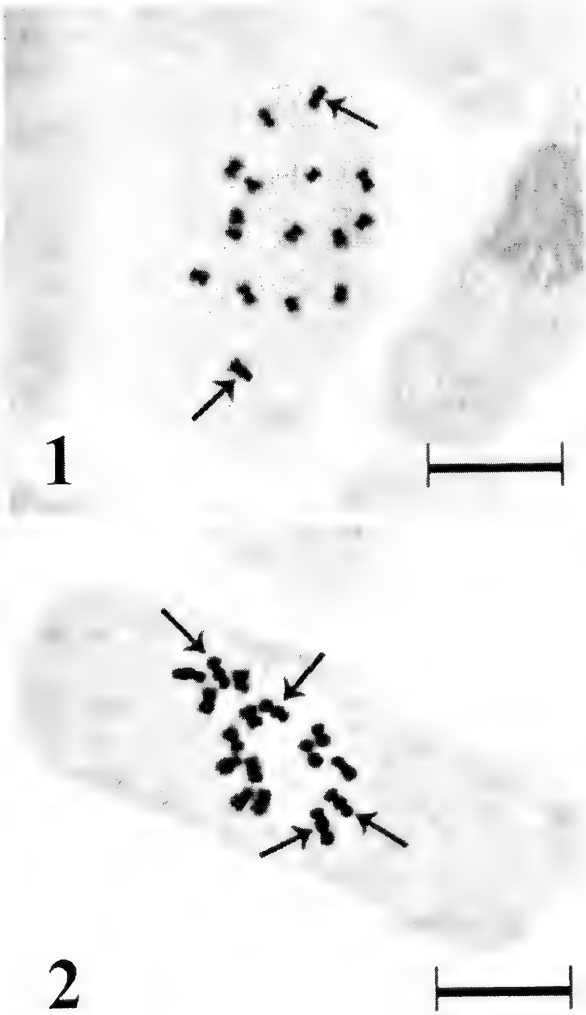
Wang, B. Y. Bai, L. Chen, M. Sha, H. Zheng. and H.Yin. 2002. Karyotype analysis of *Astragalus complanatus* chromosome. Zhong Yao Cai 25: 541-543.

Table 1. The localities, collector and chromosome numbers of *Astragalus* specimens examined.

Taxa	2n	Locality	Vouchers
A. antalyensis	16	C3 Antalya: Akseki Çukurköy Yaylası,Teketaşı mevki, 1900 m, 09.08.2006, eğimli taşlı yamaçlar	A.Duran 7361 and M.Dinç
A. nezaketae	16	B7 Erzincan: Üzümlü, Keşiş Dağı, Merdo'nun kayası mevki, kalker taşlı yerler, 2450 m, 27.07.2006.	M.Dinç 2813 and A.Duran
A. cariensis	16	Muğla: eski Kale Yolu, Yılanlı Dağı, vericiler civarı, 1360 m, 23.07.2006, *P. nigra* ve *Juniperus* açıklığı.	A.Duran 7303 and M.Dinç
A. schizopterus	16	Burdur: Dirmil-Gölhisar, 7. km, 1175 m, 25.07.2006, açık yerler.	A.Duran 7334 and M.Dinç

Table 2. Chromosome comparison in the four studied taxa of *Astragalus*. AR: arm ratio; CI: centromeric index; THC: total length of haploid complement; M: median; SM: submedian; T: terminal point.

Taxa	2n	Ploidy level	Chromosome sizes (µm)	AR	CI	THC (µm)	M	SM	T
Astragalus									
antalyensis	16	4x	1.36-2.82	1.49	5.09	16.71	7	1	-
nezaketae	16		2.54-3.35	1.57	4.92	23.42	6	2	-
cariensis	16		3.16-4.69	2.33	3.93	33.67	1	6	1
schizopterus	16	4x	1.81-3.97	1.42	5.18	20.90	8	-	-



Figures 1-2. Somatic metaphase chromosomes in *Astragalus* species. (1). *A. antalyensis* (2n = 16), (2). *A. nezaketae* (2n = 16). Scale Bar: 10 μm.

Figures 3-4. Somatic metaphase chromosomes in *Astragalus* species. (3). *A. cariensis* (2n = 16), (4). *A. schizopterus* (2n = 16). Scale Bar: 10 µm.

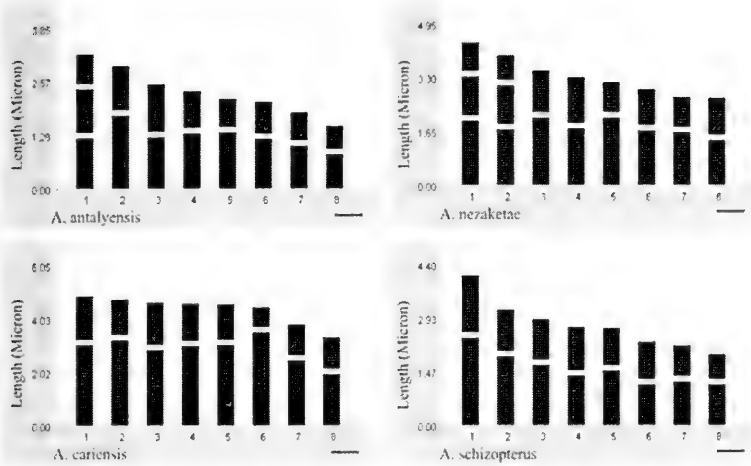


Figure 5. Ideograms in *Astragalus* taxa. Scale bar: 10 µm.

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

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

ABSTRACT

A new species, **Tridax serboana** B.L. Turner, is described from Oaxaca (Mpio. Santiago Yosondua), Mexico. The taxon is compared to *T. dubia* Rose, but is sufficiently different so as to be considered as possibly generically distinct. Most noteworthy is its annual habit, elongate peduncles bearing but single heads, and markedly conical receptcales. A figure of the holotype is provided.

KEY WORDS: *Tridax*, Asteraceae, Mexico, Oaxaca

Routine identification of Mexican Asteraceae has revealed the following novelty:

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

Tridaci dubiae Rose similes sed differt habitu anno, pedunculis elongates capitula solitarios ferentibus, receptaculis valde conicis (5-6 mm altis, 2 mm latis vs 1.0 mm altis, 2 mm latis, et cetera).

Annual herbs 20-30 cm high. **Stems** tap-rooted, moderately pubescent with multiseptate glandular hairs ca 1.0 mm long. **Leaves** (primary), opposite, sessile, linear-lanceolate, irregularly dentate, 3-4 pairs per stem, 3-5 cm long, 1-2 cm wide. **Heads** ca 7 mm high, 6 mm wide, borne single on peduncles 4-9 cm long; involucral bracts biseriate, 5-6 mm long, pubescent like the stems. **Receptacle** conical, 5-6 mm high, ca 2 mm wide, the pales flabellate, scarious. **Ray florets** 5, pistillate, fertile; corollas yellow, 3-lobed, the tubes ca 2 mm long, densely pilose; rays ca 5 mm long, 5 mm wide, the base of ligules with two

short lobes ca 0.5 mm high. **Disk florets** 20-30; corollas yellowish-green, ca 3 mm long, the tubes ca 0.4 mm long, lobes ca 0.5 mm long, the latter bearing short peculiar greenish hairs on their outer surfaces. **Achenes** of ray florets ca 3 mm long, densely appressed pubescent; pappus of ca 24 fimbriate scales ca 0.5 mm high; achenes of the disk florets similar but less pubescent.

TYPE: **MEXICO. OAXACA:** Distrito Tlaxiaco, Mpio. Santiago Yosondua. "Imperio Santiago Yosondua. Vegetacion secondaria con pino y encino." ca 2248 m, 25 Aug 2005, *Maurita Mendoza Osorio 143* (Holotype: TEX).

Tridax serboana would appear to belong to the sect. Tridax, as conceived by Powell (1965), and will key in his treatment to or near *T. dubia* Rose. The latter is a species of dubious affinities, as discussed by Powell and suggested by its epithet. More detailed examination of these two taxa might suggest a basal position in *Tridax*. Indeed, with DNA analysis, *T. serboana* and *T. dubia* might prove worthy of generic status.

ACKNOWLEDGEMENTS

I am grateful to Guy Nesom for the Latin diagnosis, and to him and Emily Lott for reviewing the manuscript.

LITERATURE CITED

Powell, A.M. 1965. Taxonomy of *Tridax* (Compositae). Brittonia 17: 47-96.

Fig. 1. *Tridax serboana* (holotype).

RECENSION OF *SALVIA* SECT. *FARINACEAE* (LAMIACEAE)

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

ABSTRACT

A preliminary recension of the Mexican species of *Salvia* sect. *Farinaceae* is rendered. Fourteen species are recognized as occurring in the complex in this area, four of these new to science: *S. gypsophila* B.L. Turner from Nuevo Leon; *S. jacalana* B.L. Turner from Hidalgo; *S. richardsonii* B.L. Turner from Tamaulipas; and *S. zaragozana* B.L. Turner from Nuevo Leon. A key to the species is provided, along with photographs of the types concerned and maps showing the distribution of all of the included species.
KEY WORDS: *Salvia*, Lamiaceae, sect. *Farinaceae*, Mexico.

Preoccupation with the identification of species of *Salvia* native to Mexico has occasioned the following treatment of the sect. *Farinaceae* (sensu Epling 1939).

SALVIA* SECT. *FARINACEAE

Perennial herbs to 3 m high; leaves linear to obovate or sub-cordate; stems glabrous to variously pubescent; spikes terminal, interrupted or not, the flowers mostly arranged 2-6 at a node; floral bracts lanceolate to ovate, soon deciduous, rarely not; calyx two-lipped, the upper lip with 3 or 5-7 major veins, or the lips sometimes truncate, or nearly so; corollas blue, or with lines of white, the tube invaginate or not; upper lip shorter than lower; stamens arising from the corolla throat, not extruding from the lip; style pilose apically, the upper branch much longer than the lower.

As treated here, and by Epling (1939), sect. *Farinaceae* is a very heterogeneous assemblage. For example, the latter author keyed the section in three places within his key to Mexican and Central American sectional groupings, including in this species having 3-veined

as well as 5-veined upper calyx lobes, a character of major importance by his evaluation. In the account that follows, I have largely followed his arrangement, except that I would exclude from the section *S. amissa* Epling, *S. platycheila* A. Gray, and *S. similis* Brandeg., relating these to the closely related sect. *Tomentosae*. So treated, I recognize 14 species in the complex, 4 of these described as new.

KEY TO SPECIES

1. Upper lip of calyx 5-7 veined.....**(9)**
1. Upper lip of calyx 3-veined.....**(2)**

2. Leaves not especially bicolored.....**(4)**
2. Leaves markedly bicolored.....**(3)**

3. Pubescence of calyx appressed; mid-stem leaves with petioles 10 mm long or less; Gue.....**S. jacobii**
3. Pubescence of calyx spreading; mid-stem leaves with petioles 11 mm long or more; Nue.....**S. gypsophila**

4. Petioles of mid-stem leaves mostly 2-5 cm long; Coa.....
.....**S. pseudopallida**
4. Petioles of mid-stem leaves mostly 1-2 cm long.....**(5)**

5. Corolla tube clearly 2-lipped (**8**)
5. Corolla tube more or less truncate, weakly 2-lipped (**7**)

7. Corolla tube ca 5 mm long; Coa, Sierra Paila.....**S. lanicalyx**
7. Corolla tubes 6-9 mm long; widespread.....**S. farinosa**

8(**5**). Leaves linear-lanceolate, widest at or near the middle; Tam.....
.....**S. richardsonii**
8. Leaves lanceolate, widest well below the middle; Nue.....
.....**S. zaragozana**

9(**1**). Mid-stem leaves mostly 6-30 mm wide.....**(11)**
9. Mid-stem leaves mostly 1-3(4) mm wide...**(10)**

10. Stems glandular-pubescent; Jal.....**S. heterotricha**

10. Stems not glandular-pubescent; widespread.....**S. reptans**

11. Blades of leaf lanceolate, markedly rugose on both surfaces; Mic.....**S. nigriflora**
11. Blades of leaf otherwise.....**(12)**

12. Herbs 40 cm high or more; blades lanceolate to oblanceolate, 4-5 times as long as wide.....**(14)**
12. Herbs to 30 cm high; blades ovate to oval, 1-3 times as long as wide; Cps.....**(13)**

13. Blades of leaf ovate to oval, 1-2 times as long as wide.....**S. duripes**
13. Blades of leaf lanceolate, 2-3 times as long as wide.....**S. oblongifolia**

14. Leaves glabrous beneath or nearly so; Nue, Tam, Dur.....**S. jaimehintoniana**
14. Leaves pubescent beneath, especially along the veins; Hid.....**S. jacalana**

SALVIA DURIPES Epling & Mathias, Brittonia 8: 309. 1957. **Map 1**

Cps, pine forests, near Monte Cristo and Margaritas, 1500-1600 m; Jun.

Perennial herbs 10-30 cm high, the stems pilose with hairs 1-2 mm long; leaves thin, remotely serrate, the blades mostly 1-2 times as long as wide, widest near the middle.

Salvia duripes is known to me only by the two collections cited by Epling and Mathias in their original description. It appears to be a well-marked taxon, what with its small habit and thin broad leaves.

SALVIA FARINACEA Benth., Lab. Gen. et Sp. 274. 1833. **Map 2**
Salvia earlei Woot. & Standl.
Salvia trichostyla Bisch.

Nue and southwestern U.S. (N. Mex. and Tex), shrubby areas, oak-pine woodlands and grasslands in mostly calcareous soils, 1400-2100 m; Apr-Sep.

Relatively simple-stemmed perennial herbs to 1 m high, the calyces usually bluish and densely farinose.

Mexican material belongs to the typical var. **farinacea**; var. *latifolia* Shinners, having broader, usually dentate, leaves occurs in sandy soils of southeastern Texas.

SALVIA GYPSOPHILA B.L. Turner, **sp. nov. Fig. 1, Map 3**

Salviae rubropunctatae B. Rob. & Fernald similis sed differt labio supero calycis 3-venoso (vs. 5-7-venoso) et tubo corollae trichomatibus non ramosis (vs. ramosis).

Stiffly erect perennial herbs 40-100 cm high. **Stems** minutely white-pubescent. **Leaves** at mid-stem broadly lanceolate, more or less rugose above, 5-9 cm long, 2.5-3.0 cm wide; petioles 1.5-3.5 cm long; margins crenulate. **Spikes** terminal, 4-20 cm long, 2.0-2.5 cm wide (the corollas excluded). **Floral bracts** broadly ovate, apiculate, soon deciduous. **Calyx** 2-lipped, the lips 2-3 mm long; upper lip 3-veined, the tube pubescent with unbranched trichomes. **Corollas** "blue" or "bluish-purple"; tubes 8-10 mm long, the upper lip 4-5 mm long, lower lip 8-10 mm long. **Stamens** attached near the throat, mostly included within the lip. **Style** pilose, the upper branch 2-3 times as long as the lower. **Nutlets** ovoid, mottled brown-grey, glabrous, ca 2 mm long, 1.3 mm wide.

TYPE: **MEXICO. NUEVO LEON: Mpio. Aramberri,** "On exposed gypsum hills...about 7 miles north of La Escondida, 24 Sep 1973, *J.L. Reveal & N.D. Atwood 3421* (holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. NUEVO LEON: Mpio. Aramberri:** Las Norias, 1975 m, 26 Oct 1978, *Hinton et al. 17470* (TEX); La Escondida, 1775 m, 3 Aug 2003, *Hinton et al. 23136* (TEX); near San Rafael, 17 Aug 1989, *Peterson 1376* (TEX).

Most previous workers have misidentified *S. gypsophila* as *S. rubropunctata*; the latter is a species of northwestern Mexico belonging

to the sect. *Tomentella*, having a very different calyx with branched hairs, the upper lip 5-7 veined.

SALVIA HETEROTRICHA Fernald, Proc. Amer. Acad. Arts 35: 500. 1900.
Map 2
Salvia heterotricha var. *multinervia* Fernald

Nay and southwestern Jal, dry gravelly or sandy soils in oak woodlands, ca 5500 ft; Jul-Aug.

Perennial herbs to 50 cm high, the leaves linear; best recognized by its glandular-pubescent stems and calyces. The type of var. *multinervia* is from near Tepec, Nayarit, having more venose leaves, this taxon not recognized by subsequent workers.

SALVIA JACOBII Epling, Bull. Torrey Bot. Club 67: 522. 1940.
Map 2

Known only from western Gue (Mina Distr.) in pine-oak forests, 2500-3100 m; Nov-Mar.

Reportedly perennial herbs or sub-shrubs 1-2 m high; among related species, readily recognized by its bicolored, relatively rugose, lanceolate leaves.

The species is named for James Hinton, who first collected the species. Jacob is Latin for James; Epling was an expert in Latin and preferred the translation concerned.

SALVIA JACALANA B.L. Turner, **sp. nov. Fig. 2, Map 4**

Salviae jaimehintonianae Ramamoorthy similis sed differt caulibus dense albopubescentibus trichomatibus patentibus (vs glabris vel paene glabris) et foliis pubescentibus subter secus venas (vs glabris).

Perennial **rhizomatous herbs** to 80 cm high. **Stems** moderately to densely pubescent with stiffly spreading white hairs, the vestiture ca

0.6-0.7 mm high. **Leaves** at mid-stem lanceolate to oblanceolate, 7-8 cm long, 1.0-1.5 cm wide, pinnately venose, the veins of lower surfaces pubescent like the stems; petioles 2-8 mm long, grading into the blades. **Spikes** ca 30 cm long, much-interrupted below, less so above, flowers 6 or more to a node. **Floral bracts** linear-lanceolate, 10-11 mm long, 2-3 mm wide, soon deciduous. **Calyces** ca 7 mm long, 2-lipped, the upper lip ca 1.5 mm long, 5-7 veined. **Corollas** blue, 14-15 mm long; tube ca 6 mm long; upper lip ca 4 mm long; lower lip reflexed, 8-10 mm long. **Stamens** not excurrent, the anthers purple. **Style** pilose apically, the dorsal branch 3 times longer or more than the ventral branch. Mature **nutlets** absent.

TYPE: **MEXICO. HIDALGO: Mpio. Jacala,** "6.5 air km E-NE of Jacala, between Cuesta Colorado and El Pinalito on Mex 85. At a sharp bend in road. Limestone boulders covered with cacti and many ferns in woodland of pine and oak." 1700 m, 13 Jul 1991. *M Mayfield, A. Hemple & A. Jack 820* (holotype: TEX).

ADDITIONAL SPECIMEN EXAMINED: **MEXICO. HIDALGO: Mpio. Jacala**, along highway 85, immediately N of La Zorra, 7.1 mi NE of Jacala, 1720 m, 21 Aug 1981, *Peterson 650* (TEX).

Salvia jacalana, named for the municipio in which collected, is clearly closely related to *S. jaimehintoniana*, and was placed under the fabric of that species in my original description. I did, however, call attention to its unique vestiture, and I feel that this, and its isolation from the body mass of the species, warrants its specific status as proposed herein. After the foregoing was written, I chanced upon an additional collection (*Peterson 650*, cited above) that was annotated by Jay B. Walker of WIS as "Salvia tarayensis K. M. Patterson," presumably an unpublished name coined by Ms. Peterson; I prefer my name to hers, the origin of which I am ignorant. According to label data on her collection, the taxon was "plentiful" at the site concerned.

SALVIA JAIMEHINTONIANA Ramamoorthy ex B.L. Turner, Phytologia 79: 97. 1995. **Map 2**
Salvia azurea subsp. *mexicana* Epling

Dur, Nue and Tam, pine-oak forests and oak woodlands, 1700-2800 m; May-Aug.

In my original description of this taxon, I called attention to its relationship to *S. azurea*, as also noted by Epling (1940), a species confined to the U.S.A. In the present paper, I have elevated the single collection from Hidalgo to the status of species (*S. jacalana*, cf. above). I have retained the isolated Durango specimen called to the fore in my original treatment as belonging to *S. jaimehintoniana*, but this too might ultimately prove to be worthy of formal recognition, considering its geographic isolation.

SALVIA LANICALYX Epling, Repert. Spec. Nov. Regni Veg. Beih. 110: 190. 1939. **MAP 1**

Coa, Sierra de Paila, calcareous soils in pine-oak woodlands, 1300-1600m; Oct.

Simple-stemmed perennial herbs to 1 m high; calyces small, weakly 2-lipped and densely grey-lavender pubescent, the corollas quite short, having tubes ca 4.5 mm long, the upper lip ca 3 mm long.

According to its author, the species resembles *S. farinacea* but has smaller flowers and a distinctive pubescence. It seems confined to the Sierra de Paila, site of numerous local endemics.

SALVIA NIGRIFLORA Epling, Bull. Torrey Bot. Club 67: 529. 1940. **Map 1**

Mic, Coalcoman Distr. in pine-oak or oak forests,1300-2400 m; Jul-Oct.

Perennial herbs to 1 m high, readily recognized by its strongly corrugated leaves and relatively large deep blue corollas.

SALVIA OBLONGIFOLIA Mart. & Gal., Bull. Acad. Brux. 11: 279. 1844. **Map 1**

Oax and Cps, pine-oak forests, 2100-2500 m; Jul-Oct.

A poorly collected taxon; known to me only by reports in the literature and phototypes. It appears to be a perennial herb with ovate leaves, the flowers borne upon elongate much-interrupted spikes.

SALVIA PSEUDOPALLIDA Epling, Bull. Torrey Bot. Club 67: 522. 1940. **Map 3**

Central and northern Coa, oak woodlands and gullies, 1400-2100 m; Jul-Sep.

Stiffly erect, simple-stemmed, rhizomatous perennials to 1 m high, the leaf blades broadly ovate and borne upon elongate petioles, the flowers dark blue, and relatively large (the tubes 10-12 mm long).

Known to Epling only by the type, several additional collections of this distinct species have come to the fore, all of these from the Serranias del Burro, in Mpio. Villa Acuna of northern Coahuila (LL, TEX).

SALVIA REPTANS Jacq., Hort. Schoenbr. 3, 38. 1798. **Map 4**
Salvia angustifolia Cav. 1797; non *S. angustifolia* Salisb. 1796
Salvia angustifolia var. *glabra* A. Gray
Salvia leptophylla Benth.
Salvia leptophylla var. *glabra* (A. Gray) Epling
Salvia linearis Sesse & Moc.
Salvia linifolia Mart. & Gal.
Salvia virgata Ort.

Chi, Coa, San, Agu, Zac, Gua, Que, Hid, Jal, Mic, Mex, Pue, Oax, Cps and Guatemala, plains and pine-oak woodlands, 500-2000 m; Jun-Sep.

As suggested by the above synonymy, *S. reptans* is a widespread variable species. Epling (1939) accepted the name *S. leptophylla* for this taxon, not having seen the type of the earlier *S. reptans*, this omission corrected by Ramamoorthy (2001) and others. Epling discussed in some detail regional variation in this complex, noting that in northern Mexico and the southwestern U.S. populations are glabrous with longer leaves than is characteristic of the populations in central Mexico. Indeed, he recognized the more northern populations as var. *glabra*, as did Asa Gray before him. The complex

is in need of critical study both in the herbarium and in the field. My superficial examination of collections at LL.TEX, suggest that at least 3 or more infraspecific taxa might be warranted: a northern var. *glabra*, and a more southern Chiapasan complex, the latter surprisingly similar to the more northern populations. Central Mexico is largely occupied by the typical var. *reptans*, the latter perhaps including yet other cryptic taxa, some of these alluded to by Epling (1940).

SALVIA RICHARDSONII B.L. Turner, **sp. nov. Fig. 3, Map 3**

Salviae zaragozanae B.L. Turner similis sed foliis midcaulinis longioribus (9-12 cm longis vs 6-8 cm) laminis lineari-lanceolatis (vs ovatis) latissimis ad vel prope medium et bracteis floralibus longioribus plus minusve persistentibus (vs deciduis).

Rhizomatous perennial herbs 0.5-1.2 m high. **Mid-stems** minutely appressed-pubescent to glabrate. **Leaves** at mid-stem mostly 9-12 cm long, 1.5-2.5 cm wide; petioles 1-2 cm long, grading into the blades, the latter linear-lanceolate, widest at or near the middle. **Peduncles** 6-8 cm long. **Spikes** 6-12 cm long, ca 2 cm wide. **Floral bracts** lanceolate to ovate-lanceolate, more or less persistent, 1.5-2.0 cm long, ciliate with multiseptate trichomes 1-2 mm long. **Calyx** 8-9 mm long, pilose with multiseptate, white to lavender, trichomes 1-2 mm long, 2-lipped, the upper lip 3-veined. **Corolla** "blue," 9-10 mm long; tube 6-7 mm long; upper lip 3-4 mm long; lower lip 4-5 mm long. **Anthers** attached near the throat, not excurrent. **Style** pilose, the upper branch 2-3 times as long as the lower. **Nutlet** ovoid, glabrous, tan, ca 2.5 mm long, 1.5 mm wide.

TYPE: **MEXICO. TAMAULIPAS: Mpio. Gomez Farias,** Rancho Del Cielo, "Between La Perra and Indian Springs," 26 Nov 1968, *Alfred Richardson 1050* (holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. TAMAULIPAS: Mpio. Gomez Farias:** Rancho Del Cielo, "Between Julilo and La Perra," 29 Aug 1968, *Richardson 849* (TEX); Rancho Del Ciielo, "Above Olla de Nubes," 26 Nov 1968, *Richardson 1046* (TEX).

Salvia richardsonii is known only from cloud forests at ca 6800 ft in the well-studied Rancho Del Cielo of Tamaulipas (Johnston et al. 1989). It is clearly related to its closest cohorts *S. gypsophila* and *S. zaragozana* of southern Nuevo Leon. The distribution of these several taxa is shown in Map 1.

The species name was first proposed, but never published, by my colleague, Dr. Ramamoorthy. Richardson is an academic son of mine, having obtained his Ph.D. under my direction working on the genus *Tequilia* (Boraginaceae). He lives in southernmost Texas and is the author of "Plants of Southernmost Texas" (1990, Gorgas Science Foundation), "Plants of the Rio Grande Delta" (1990, Univ. of Texas Press), and "Wildflowers and Other Plants of Texas Beaches and Islands" (2002, Univ. of Texas Press).

SALVIA ZARAGOZANA B.L. Turner, **sp. nov. Fig. 4, Map. 3**

Salviae gypsophilae B.L. Turner similis sed differt foliis subter appressi-pilosis (non gossypinis) venatione perspicue visibili et calycibus aliquantum majoribus non dense floccosis.

Perennial herbs 0.6-1.2 m high, forming small colonies, presumably by rhizomes. **Stems** pubescent with recurved white hairs. **Leaves** ovate, 3-8 cm long, 1.5-2.5 cm wide; petioles 2-10 mm long; blades moderately appressed-pilose above and below, the margins crenulate. **Peduncles** 6-8 cm long. **Spikes** interrupted below, 15-30 cm long, ca 3 cm wide, bearing 8 or more florets at a node, the pedicels 3-10 mm long. **Floral bracts** broadly ovate-lanceolate,10-15 mm long, soon deciduous. **Calyx** 8-10 mm long, pubescent throughout with multiseptate purplish trichomes 1-2 mm long; lips 3-4 mm long, the upper lip seemingly 3-veined. **Corollas** 16-18 mm long, "purple" or "purplish-blue;" tube ca 12 mm long; upper lip 5-6 mm long; lower lip 6-8 mm long. **Stamens** attached near the corolla throat; anthers purple. **Style branches** pilose; stigmas purple, the lower branch ca 1.5 mm long, the upper branch twice as long and variously curved or sigmoid. **Nutlets** ovoid, ca 2 mm long, 1.5 mm wide, variously mottled or not.

TYPE: **MEXICO. NUEVO LEON: Mpio. Zaragoza,** Cerro El Viejo, pine-oak woodlands, 2375 m, 5 Oct 1992, *Hinton et al. 22382* (holotype TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. NUEVO LEON: Mpio. Zaragoza,** same locality as type, 2025 m, 16 Oct 1992, *Hinton et al. 22529* (TEX); 1830 m, 16 Oct 1992, *Hinton et al. 22589* (TEX).

Salvia zaragozana is clearly closely related to *S. gypsophila*, described herein. I take the latter to be a localized edaphic endemic readily recognized by its strongly bicolored leaves and densely lanose calyx. Field work might ultimately show that the two are but varieties, but this is not suggested by the material at hand.

According to label data, *S. zaragozana* occurs as "small colonies" in pine-oak woodlands on Cerro El Viejo from 1830-2375 m; *S. gypsophila* occurs at lower elevations in oak woodlands. The taxon is named for the municipality to which it seems confined.

ACKNOWLEDGEMENTS

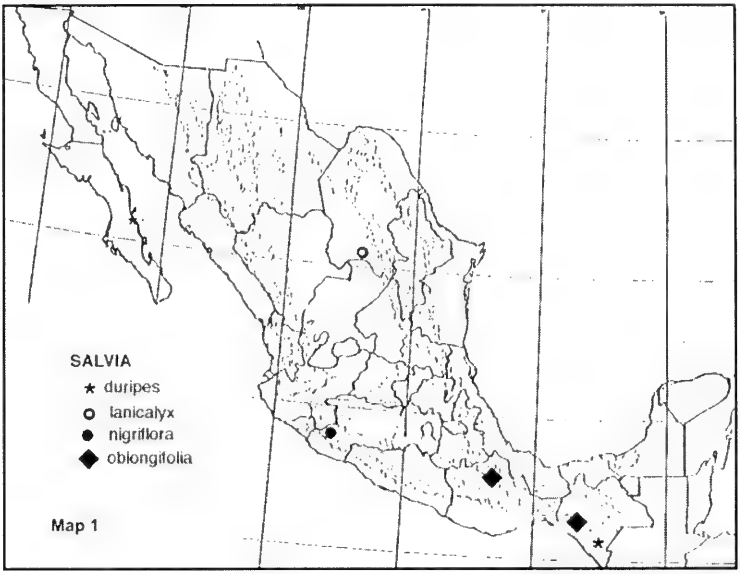
I am grateful to my colleague Guy Nesom for the Latin diagnoses and for reviewing the paper itself.

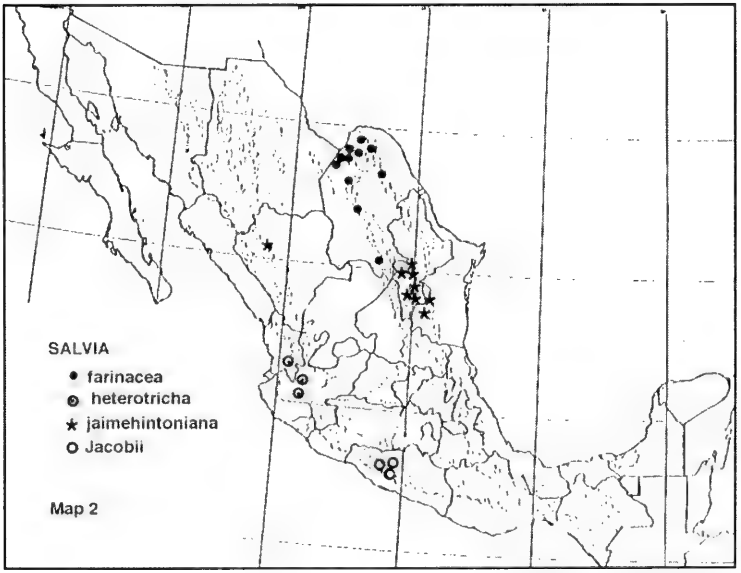
LITERATURE CITED

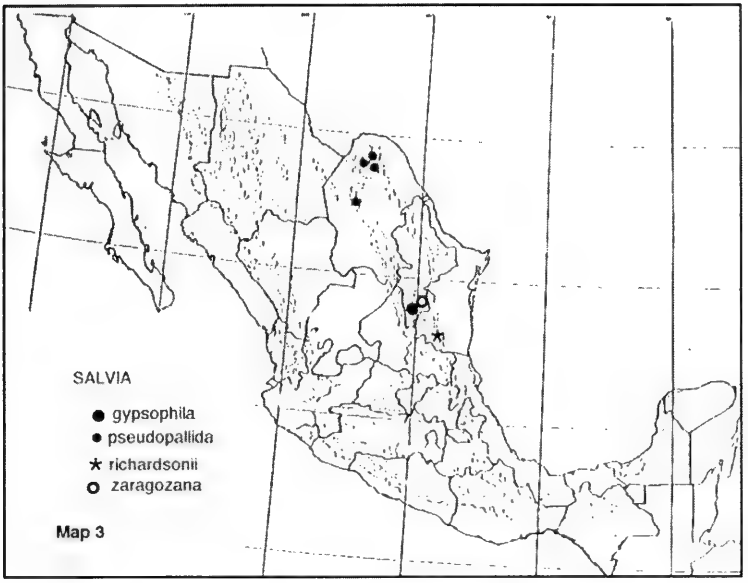
Epling, C. 1939. A revision of *Salvia* subgenus *Calosphace*. Repert. Spec. Nov. Regni Veg. Beih. 110: 1-383.

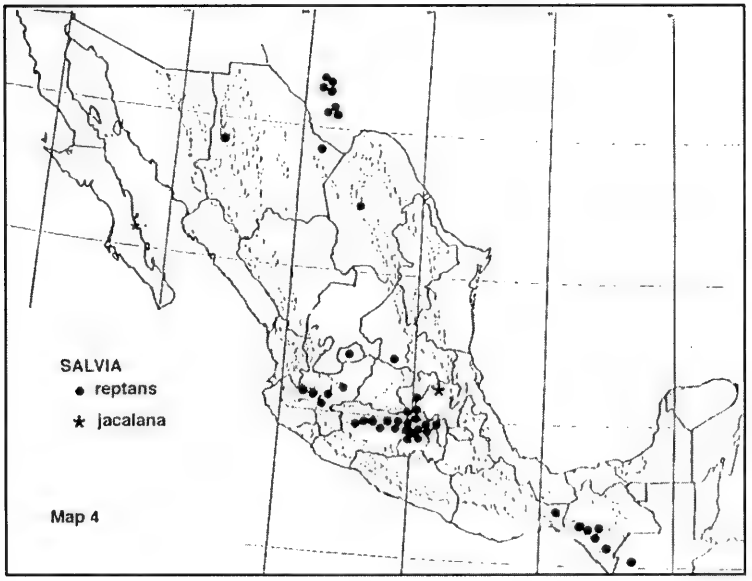
Johnston, M.C., K. Nixon, G.L. Nesom, and M. Martinez. 1989. Listado de plantas vasculares conocidas de la Sierra de Guatemala, Gomez Farias, Tamaulipas, Mexico. BIOTAM 1(2): 21–33. [reprinted in Biotam 10(1): 25-40. 1998.]

Ramamoorthy, T.P. 2001. *Salvia*, in Fl. Fanerogamica del Valle de Mexico. pp. 632-644.


SALVIA
★ duripes
○ lanicalyx
● nigriflora
◆ oblongifolia
Map 1


SALVIA
• farinacea
⊙ heterotricha
★ jaimehintoniana
○ Jacobii
Map 2


SALVIA
gypsophila
pseudopallida
richardsonii
zaragozana
Map 3


SALVIA
reptans
jacalana
Map 4

RHYNCHOSPORA NIVEA (CYPERACEAE) AND *SAURURUS CERNUUS* (SAURURACEAE): NEW TO MEXICO

Walter C. Holmes
Department of Biology, Baylor University
Waco, Texas 76798-7388 U.S.A
walter_holmes@baylor.edu

Jason R. Singhurst
Wildlife Diversity Program
Texas Parks and Wildlife Department
3000 South IH-35, Suite 100
Austin, Texas 78704 U.S.A.
jason.singhurst@tpwd.state.tx.us

ABSTRACT

Rhynchospora nivea and *Saururus cernuus* are reported as new to Mexico. Both species were collected on the Río Bravo del Norte in Coahuila, while *Saururus* is additionally reported from Volcán Jorulla, Michoacán. The entire distribution of each species is also discussed.

RESUMEN

Se cita *Rhynchospora nivea* y *Saururus cernuus* como nueva a México. Ambas especies se han recogidos en el Río Bravo del Norte en Coahuila, minetras que *Saururus* también se cita del Volcán Jorulla, Michoacán. Se discute la distribución entera de cada especie también.

KEY WORDS: Cyperaceae, *Rhynchospora*, Saururaceae, *Saururus*, Mexico, Coahuila, Michoacán, Texas.

The following species are described as being new to the flora of Mexico. All specimens from Coahuila and Val Verde Co., Texas occurred on the shoreline of the Río Bravo del Norte (Rio Grande), which forms the border between Mexico and the United States at that point.

Rhynchospora nivea Boeckl. (Cyperaceae), Snowy White-top Sedge, is considered by Thomas (1984) and Kral (2002) as endemic to Texas and Oklahoma, United States. Mention of the occurrence of the species in Arkansas by Britton (1880), Correll & Johnston (1970), and NCRS USDA (2007) may be based upon a specimen (*Leavenworth s.n.*, NY) that is labeled as being from Arkansas. The collector, Melines C. Leavenworth, a U.S. Army surgeon, never visited Arkansas (see McKelvey 1956), but was twice posted to what is now Oklahoma. It is likely that the specimen originated from near, but not within, what is now Bryan Co., Oklahoma and was collected in 1836 during Leavenworth's second posting to the state. Turner et al. (2003) mapped the species as occurring in 22 counties of mainly central Texas, with the distribution extending southwest to Val Verde Co. The species occurs in seeps, edges of streams and similar wet areas, all on limestone.

The specimen, collected at the Coahuila site, described below, occurred in silt and gravels naturally deposited in a limestone seep. This site is about 10 km from the nearest occurrence of the species in Val Verde Co., Texas. Associated plants included *Justicia americana, Eleocharis cellulosa, E. montevidensis, Saururus cernuus, Fuirena simplex, Eupatorium serotinum, Pluchea camphorata, Cladium mariscus, Juncus acuminatus, Ludwigia peploides,* and *Bacopa monnieri.*

SPECIMEN EXAMINED: **MÉXICO. COAHUILA. Muni. de Acuña**. Bank of the Río Bravo del Norte, 13.7 km NW of the International Bridge in Ciudad Acuña, 7.1 km S of Mex. Hwy 2; 29° 25' 50.02" N, 101° 02' 44.47" W, ca. 300 m., 17 Jul 2007, *Singhurst 14756* (BAYLU, IEB, TEX).

Saururus cernuus L. (Saururaceae), Lizard's-tail, considered endemic to Canada and the United States, is distributed from Ontario and Quebec south to Rhode Island and Connecticut, west to Michigan and southward to Florida and Texas (Buddell & Thieret, 1997). In Texas, *Saururus* is generally restricted to the Pineywoods and Post Oak Savannah vegetational areas of the eastern part of the state. The species is known to occur naturally as far south and west as Ottine in Gonzales Co., which is near the southwest limit of the Post Oak Savannah. Reports from Tarrant and Travis counties, both west of the Post Oak

Savannah, are based upon cultivated populations or plants recently escaped from a cultivated population (see Diggs et al., 1999 and Turner et al., 2003). The Coahuila and Val Verde Co. records are approximately 375 km west of the Gonzales Co. location.

There were approximately 350 plants at the Coahuila station and about 250 plants growing at the nearby Texas location, which is directly across the river. At both locations, *Saururus* occurred along the banks of the Río Bravo del Norte where water from seeps flowed into the river from fairly low limestone bluff contacts. This produced, at the site of inflow, sediment rich spongy to mucky mud flats intermixed with silt and gravel. Associated plants included *Bacopa monnieri*, *Cladium mariscus*, *Eleocharis cellulosa*, *E. montevidensis*, *Eupatorium serotinum*, *Fuirena simplex*, *Juncus acuminatus*, *Justicia americana*, *Ludwigia peploides*, *Panicum virgatum*, *Paspalum urvillei*, *Pluchea camphorata*, and *Rhynchospora nivea*.

Another population of *Saururus* was observed, but not collected, in August, 2005 on the Mexican side of the river approximately 13 miles downstream from the present Coahuila station.

Saururus appears to be another of the more eastern aquatic plants that have been reported in the south central portion of Texas and adjacent Coahuila. The rivers of the lower Edwards Plateau area of Texas, especially the Nueces and the Río Bravo del Norte itself, serve as wetland corridors for westward movement of these aquatic plants. Other species with a similar distribution are *Mikania scandens, Hibiscus moscheutos, Eleocharis cellulosa, Hydrocotyle ranunculoides, Cyperus strigosus, Myriophyllum heterophyllum*, and *Ludwigia octovalvis*.

The Michoacán collection of *Saururus* is an interesting and rather unusual record. The specimen, *Eggler 208*, was determined by C. V. Morton (US) in 1954. His annotation included the comment "first collection from Mexico." The collection site is 1150 km south of the Coahuila/Texas border records reported here. Although elevation is not specified on the label, the volcano base is about 800 m in elevation while the summit is near 1190 m. This is the highest elevation that this species has been found, which is considerably greater than the 500 m

cited by Buddell & Thieret (1997). Even more curious is that the species must be considered to be a fairly recent arrival at the location. Volcán Jorulla was formed in 1759 (Eggler 1959). Eggler conducted a study on the invasion of volcanic deposits by plants in which he cites the families of plants represented at Jorullo. Unfortunately, Saururaceae was not mentioned. No additional collections of *Saururus* are known from this site.

SPECIMENS EXAMINED: **MÉXICO. COAHUILA. Mun. de Acuña**. Bank of the Río Bravo del Norte, 13.7 km NW of the International Bridge in Ciudad Acuña, 7.1 km S of Mex. Hwy 2, 29° 25' 50.02" N, 101° 02' 44.47" W, ca. 300 m, 17 Jul 2007, *Singhurst 14754* (BAYLU + elsewhere); **MICHOACÁN. Mun. La Huacana**. Volcán Jorullo, 1951, *Eggler 208* (US). **UNITED STATES. TEXAS. Gonzales Co.** *Fraxinus* bog, Ottine, 22 May 1927, *Bogusch 1898* (TEX); Ash bog, Ottine, 5 May 1928, *Bogusch 3229* (TEX); **Travis Co.** Pond on U. of T. campus, Austin, 24 May 1944, *Tharp 44160* (TEX); **Val Verde Co**. 4.6 miles S of Lake Amistad National Recreational Area, 320 meters N of where Eightmile Creek feeds into the Rio Grande, east side of river, ca. 300 m, 17 Jul 2007, *Singhurst 14755* (BAYLU, TEX).

ACKNOWLEDGMENTS

This study was made possible, in part, through use of herbarium specimens at TEX and US, both of whose helpfulness is greatly appreciated. We are grateful to Ann Bradburn (NO) who searched for additional collections from Michoacán made by Eggler, who was employed by Neucomb College of Tulane University at that time. We also thank the United States Border Patrol, Del Rio, Texas, for assistance. Gina Gollub, a student worker at BAYLU, assisted with preparation of the manuscript.

LITERATURE CITED

Britton, N. L. 1888. New or noteworthy North American phanerogams. Bull. Torrey Bot. Club 15: 97-104.

Buddell, G. F., II and J. W. Thieret. 1997. Saururaceae. In: Flora of North America Editorial Committee. Flora of North America, Vol. 3. Magnoliophyta: Magnoliidae and Hamamelidae. Oxford University Press, New York. pp. 258-271.

Correll, D. S. and M. C. Johnston. 1970. Manual of the vascular plants of Texas. Texas Research Foundation, Renner.

Diggs, G. M., Jr., B. L. Lipscomb, and R. J. O'Kennon. 1999. Shinners & Mahler's illustrated flora of north central Texas. Sida, Bot. Misc. 16, Botanical Research Institute of Texas, Fort Worth.

Eggler, W. A. 1959. Manner of invasion of volcanic deposits by plants, with further evidence from Parícutin and Jorullo. Ecol. Monog. 29: 267-284.

Kral, R. 2002. *Rhynchospora.* In: Flora of North America Editorial Committee. Flora of North America, Vol. 23. Magnoliophyta: Commelinidae (in part): Cyperaceae. Oxford University Press, New York. pp. 200-239.

McKelvey, S. D. 1955. Botanical exploration of the trans-Mississippi west, 1790-1850. Arnold Arboretum of Harvard University, Jamaica Plain, Massachusetts.

Thomas, W. W. 1984. The systematics of *Rhynchospora* section *Dichromena.* Mem. New York Bot. Gard. 37: 1-116.

Turner, B .L., H. Nichols, G. Denny, and O. Doron. 2003. Atlas of the vascular plants of Texas. Vol. 1. Sida, Bot. Misc. 24. Botanical Research Institute of Texas, Fort Worth.

USDA, NRCS. 2007. The PLANTS Database (http://plants.usda.gov, 4 October 2007). National Plant Data Center, Baton Rouge, LA 70874-4490 USA.

TAXONOMY OF *JUNIPERUS COMMUNIS* IN NORTH AMERICA: INSIGHT FROM VARIATION IN nrDNA SNPs

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

ABSTRACT

Plants of *Juniperus communis* L. var. *communis*, *J. c.* var. *depressa* Pursh, *J. c.* var. *jackii* Rehdr, *J. c.* var. *saxatilis* Pall. were sampled and SNPs from nrDNA were examined. Based on these data and previous data, a new variety of *J. communis* is recognized: ***Juniperus communis*** var. ***charlottensis*** R. P. Adams, **var. nov.** It occurs in muskeg bogs on Queen Charlotte Island, British Columbia and in the Ketchikan, Alaska area. *Juniperus communis*, in North America, is treated as five varieties: vars. *charlottensis*, *depressa*, *jackii*, *megistocarpa*, and *saxatilis*. Keys and distribution maps are presented.

KEY WORDS: *Juniperus communis*, *J. communis* var. *charlottensis*, var. *depressa*, var. *jackii*, var. *megistocarpa* and var. *saxatilis*, Cupressaceae, geographic variation, nrDNA, SNPs.

Juniperus communis is the only *Juniperus* that occurs in both the eastern and western hemispheres. In North America, *J. communis* has been treated (Adams, 2004) as composed of as many as four varieties. More recently, Adams and Nguyen (2007) treated the north American *J. communis* as three varieties: *J. c.* var. *depressa, J. c.* var. *jackii*, and *J. c.* var. *megistocarpa* (Fig. 1). Their classification was based on RAPDs data and leaf morphology.

Analysis of Arctic populations of *J. communis* (Adams et al., 2003) revealed that these populations clustered by continent, with the populations in Greenland and Iceland showing the highest affinities to populations from Europe and not to those from North America (Fig. 1).

Adams et al. (2003) concluded that the post-Pleistocene populations on Greenland and Iceland came from Europe and not North America. Unfortunately, none of the Pacific northwestern putative *J. c.* var. *saxatilis* was included in the study.

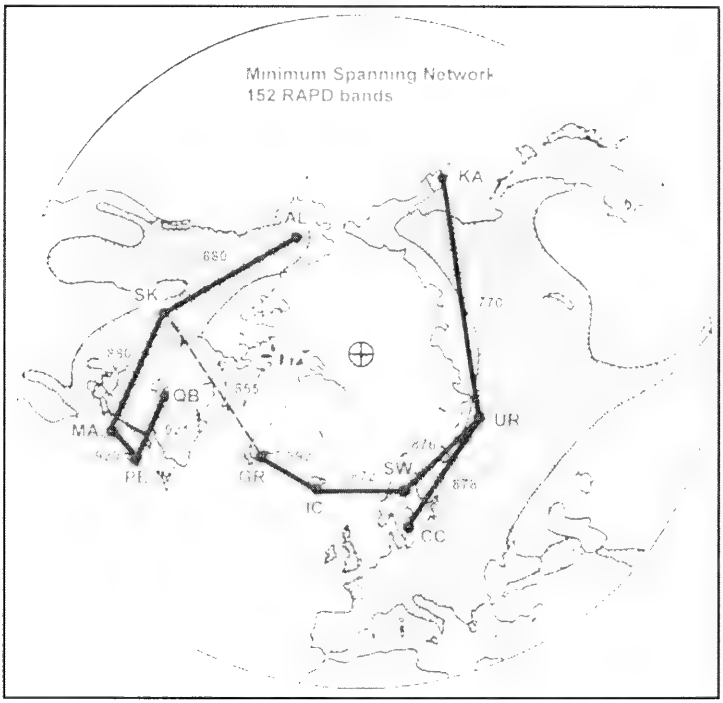


Figure 1. Minimum spanning network showing that the North American *J. communis* var. *depressa* and var. *megistocarpa* populations link together and all the *J. communis* populations from the e. hemisphere link together (Adams et al., 2003). The dashed line is the minimum link between eastern and western hemisphere populations.

Analysis of the currently named *J. communis* varieties (Adams and Pandey, 2003), resolved these taxa into six major groups: *J. c.* var. *communis* and var. *saxatilis* Pall. from Europe; *J. c.* var. *depressa*, N.

America; *J. c.* var. *megistocarpa*, Quebec; *J. c.* var. *nipponica* (Maxim.) E. H. Wilson, Japan; and putative *J. c.* var. *saxatilis*, Kamchatka, Russia. However, Adams and Pandey (2003) did not include *J. c.* var. *jackii*, or putative *J. c.* var. *saxatilis* from the Pacific northwest, US/Canada in their analysis.

Ashworth, et al. (1999, 2001) used DNA fingerprinting to examine *J. communis* plants identified as *J. c.* var. *depressa*, *J. c.* var. *jackii*, *J. c.* var. *montana* Aiton (= *J .c.* var. *saxatilis,* see Adams, 2004) collected from California, Oregon, Nevada and Utah in the southwest and west coast of the United States. They did not get a clear pattern separating these taxa, and concluded that their samples represented a single varietal taxon. However, it not clear if they utilized replicated samples to remove spurious variation in RAPD bands.

Adams and Nguyen (2007) collected additional samples of putative *J. c.* var. *saxatilis* from the Pacific northwest, *J. c.* var. *jackii* from NW California and *J. c.* var. *depressa* from the southernmost locations in North America (Mt. Charleston, Nevada and Mt. Satula, North Carolina).

The major trend (figure 2) among the taxa was the separation of the eastern hemisphere plants (*J. communis* var. *communis, J. c.* var. *saxatilis,* and putative *J. c.* var. *saxatilis*, Kamchatka) from the western hemisphere plants *(J. c. var. depressa*, *J. c.* var. *jackii*, *J. c.* var. *megistocarpa*, and putative var. *saxatilis*). The resolution (figure 2) of *J. c.* var. *jackii* (and plants from Mt. Hood) was in contrast to the report by Ashworth, et al. (1999, 2001). The Banff, Alberta individuals (putative hybrids) were intermediate between the coastal, short, curved-leaved plants (putative var. *saxatilis*) and *J. c.* var. *depressa* (figure 2). *Juniperus c.* var. *megistocarpa* was distinct from *J. c.* var. *depressa.*

However, examination of additional *J. communis* specimens from California, Oregon, Washington, British Columbia and Alaska revealed that plants with short, curved leaves and a stomatal band 1.5 to 2 times as wide as the green side bands are common in the Pacific Northwest to Alaska and even eastward into Idaho at Redfish Lake and Banff, Alberta. To further elucidate the patterns of variation in *J. communis*, an analysis involving sequencing the ITS of nrDNA was conducted. The purpose of the present study is to present new data and

combine this information with previous results to help resolve the very complex patterns of variation in *J. communis* from North America.

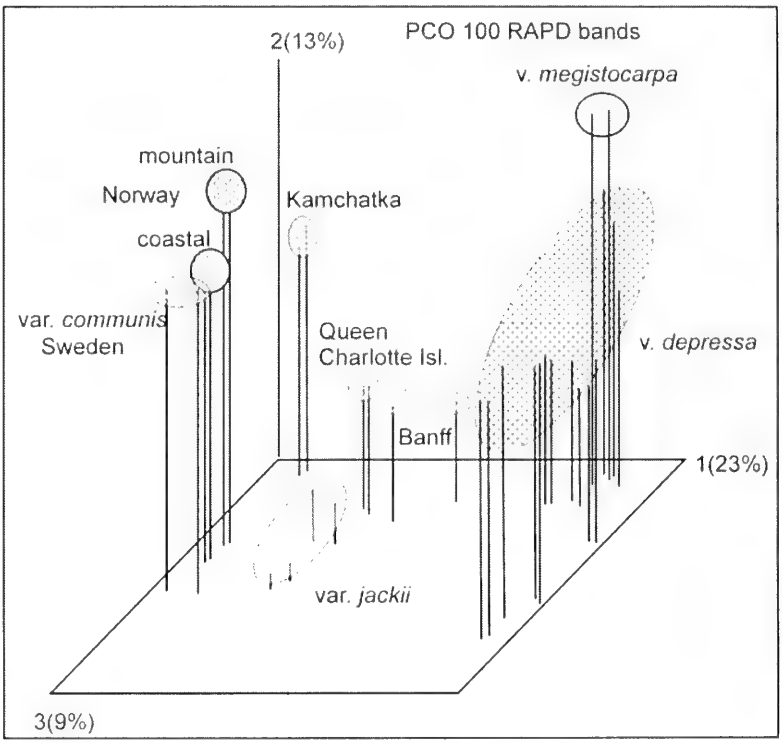


Figure 2. PCO based on 100 RAPD bands (Adams and Nguyen, 2007).

MATERIALS AND METHODS

Specimens used in the present study: *J. communis* var. *communis*: *Adams 11173-11175*, Norway; *J. c.* var. *depressa*: *Adams 7582-7584,* Denali National Park, AK, USA; *Adams 9394-9395*, Hudson Bay, Quebec, Canada (ex N. Dignard); *Adams 10225-10229*, on granite, Mt. Satula, NC, USA; *Adams 7802-7804*, Victor, CO, USA; *Adams 11230-11232*, Vancouver Island, BC, Canada (ex. A. Ceska); *Adams 11128-11132*, Ellery Lake Dam, CA, USA; *Adams 11233-*

11235, 11237-11238, Coastal Range, n BC, Canada (ex Ken Marr); *Adams 11236*, Goat Lake, Olympic National Park, WA, USA (ex Ken Marr); *Adams 11074*, sand dunes, Whidbey Island, WA, USA; *Adams 8572-8574*, MA, USA; *Adams 10225-10229*, on Granite, Mt. Satula, NC, USA, *J. c.* var. *jackii*: *Adams 10287-10291*, serpentine, Del Norte Co., CA; USA, *Adams 10300-10303,* on volcanic rock, Mt. Hood, Wasco Co., OR, USA; *J. c.* var. *saxatilis*: *Adams 11206, 11207* Norway; *Adams 8685 - 8787*, Hokkaido, Japan, *Adams 10890-10893*, Redfish Lake, Idaho; *Adams 10304-10308*, Queen Charlotte Island, BC, Canada; *Adams 9181-9183* Esso, Kamchatka, Peninsula, Russia (ex. J. W. Leverenz). Voucher specimens are deposited at the Baylor University herbarium (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 using the Qiagen DNeasy mini kit (Qiagen Inc., Valencia CA).

SNPs obtained from DNA sequencing

ITS (nrDNA) amplifications were performed in 50 µl reactions using 10 ng of genomic DNA, 3 units Qiagen Taq polymerase, 5 µl 10x buffer [final concentration: 50 mM KCl, 10 mM Tris-HCl (pH 9), 0.01% gelatin and 0.1% Triton X-100), 1.75 mM $MgCl_2$, 20 µl Q solution (2X final), 400 µM each dNTP, 1.8 µM each primer and 4% (by vol.) DMSO].

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. In each case the band was excised and purified using a Qiagen QIAquick gel extraction kit.

The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

The aligned data sets were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Analyses of 1119 bp of nrDNA (ITS) sequences revealed 23 SNPs among the taxa including 2 and 4 bp deletions in *J. c.* var. *communis* and var. *saxatilis* from Norway as well as *J. c.* var. *jackii* (NW CA and Mt. Hood, OR). The 2 bp and 4 bp deletions were each coded as single deletion events in making comparisons.

Factoring the association matrix yielded five eigenroots before they began to asymptote, implying that six groups were present. These eigenroots accounted for 48.9, 15.8, 12.0, 6.9, and 5.7% of the variation among the 58 individuals analyzed. PCO of the eigenvectors (Fig. 3) clearly shows that the differentiation of *J. c.* var. *jackii* from NW CA and Mt. Hood, OR accounts for 49% of the variance among the 58 individuals. *Juniperus c.* var. *jackii* had 5 bp differences, plus a 4 bp deletion and a 2 bp deletion. Interestingly, the 4 bp and 2 bp deletions were shared with *J. c.* vars. *communis* and *saxatilis* from Norway. The Queen Charlotte Island junipers are separated by 2 bp (Fig. 3). These

junipers grow in muskeg bogs that are very atypical of *J. communis*. An individual plant of *J. communis* found growing on a sand dune on Whidbey island has 3 mutations that separate it from all other individuals (Fig. 3). The leaves and habit of the plant are similar to *J. c.* var. *depressa*. All of the other samples (27 individuals) of *J. c.* var. *depressa* (N. A.) and v. *saxatilis* (Asia) form a fifth group.

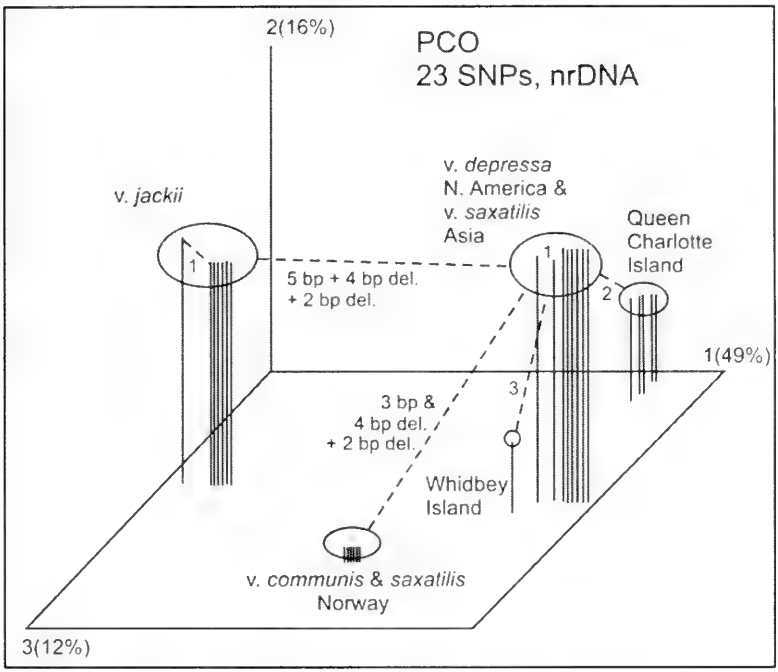


Figure 3. PCO of 58 individuals based on 23 SNPs. Identical bars, closely spaced indicate no variation among these individuals. The bars in the largest group are symbolic, as that group contains 27 individuals!

Because six groups were indicated by the presence of five significant eigenroots, additional variation is hidden in the PCO in figure 3. Therefore, the *J. c.* var. *jackii* individuals (NW CA and Mt. Hood, OR) were removed and the PCO re-run. This resulted in four eigenroots accounting for 35.5, 23.7, 13.4 and 11.0% of the variance

before asymptoting. This implies the presence of five groups as seen in figure 4. Each of the groups is separated by 2 - 3 bp (plus 4 bp and 2 bp deletion in the Norway group). The large group (27 individuals) of *J. c.* var. *depressa* and *J. c.* var. *saxatilis* is relative uniform, with only 1 bp difference between members of that group. All of the individuals that morphologically appear to be *J. c.* var. *saxatilis* (short, curved leaves, with a stomatal band that is twice as wide the green leaf margins) are within the large group. Therefore, the addition of *'saxatilis'* plants from NW North America to this analysis did not change the PCO in comparison with the RAPDs data (Fig. 2).

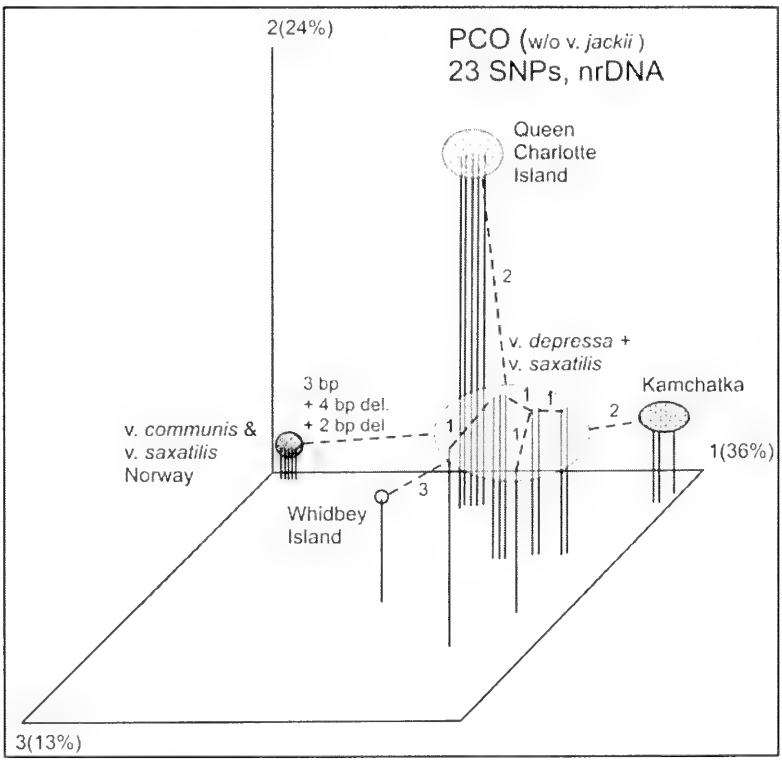


Figure 4. PCO of 23 SNPs of nrDNA without *J. c.* var. *jackii* individuals.

However, it is interesting that *J. c.* var. *saxatilis* from Japan is within this group, in contrast to a previous RAPDs study (Adams and Nguyen, 2007). Differentiation in the nrDNA sequence was not as great as found in RAPDs. However, the pattern of considerable differentiation in the RAPDs of the *J. c.* var. *jackii* and Queen Charlotte Island plants (Fig. 2) is clearly concurrent with the nrDNA (Figs. 3, 4).

An unusual aspect of this study of nrDNA was the association of mutations across widely spaced distances. Figure 5 shows the first group with six widely distributed individuals with identical nrDNA.

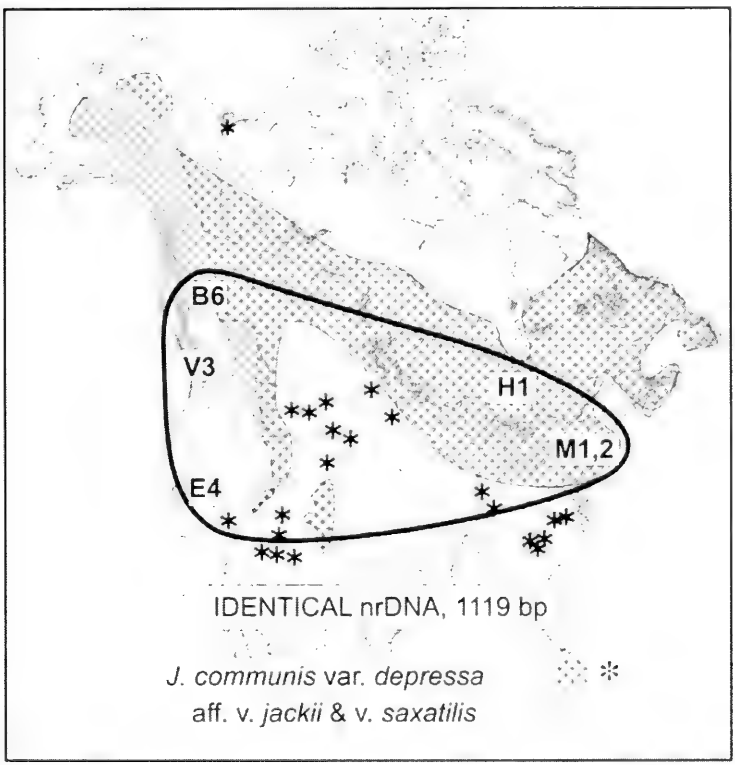


Figure 5. Six individuals, widely spaced, with identical nrDNA.

Each of the six plants shows some morphological affinity to var. *depressa*, but has one or two nucleotide differences that separate them from var. *depressa*.

Figure 6 depicts the largest group of individuals (20 samples) with identical nrDNAs. The southernmost populations were generally very uniform. This nrDNA pattern is that characteristic of *J. c.* v. *depressa*. But the California population at Ellery Dam (E1, 2, 3), had a 4th plant that grouped with more northerly plants

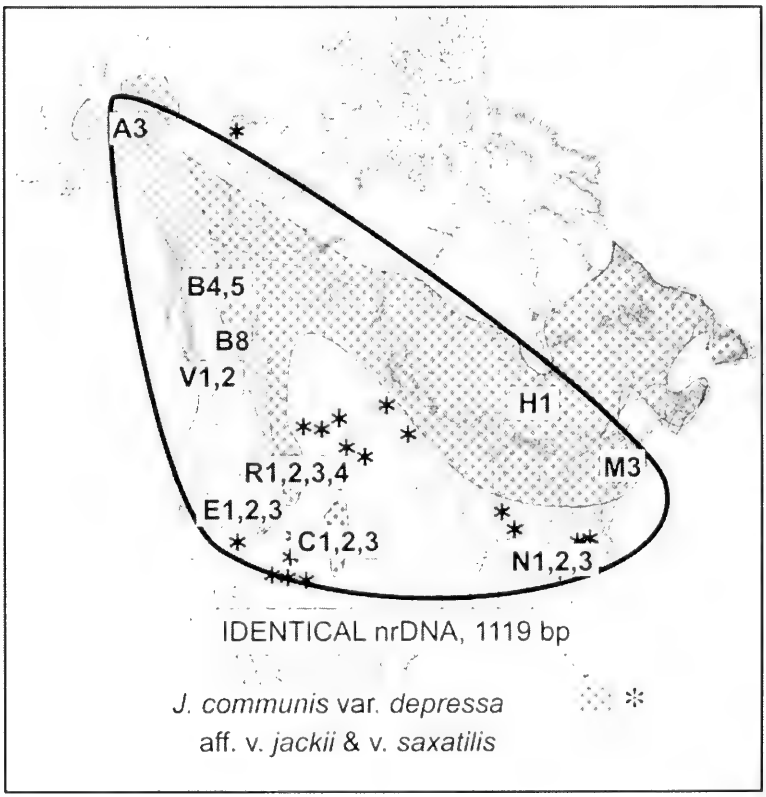


Figure 6. A group of 20 individuals, widely distributed, yet with identical nrDNA sequences.

(Fig. 5). The North Carolina collections (Mt. Satula, N1,2,3) had another individual (N4) that proved identical to *J. c.* var. *saxatilis* from Japan (Fig. 7).

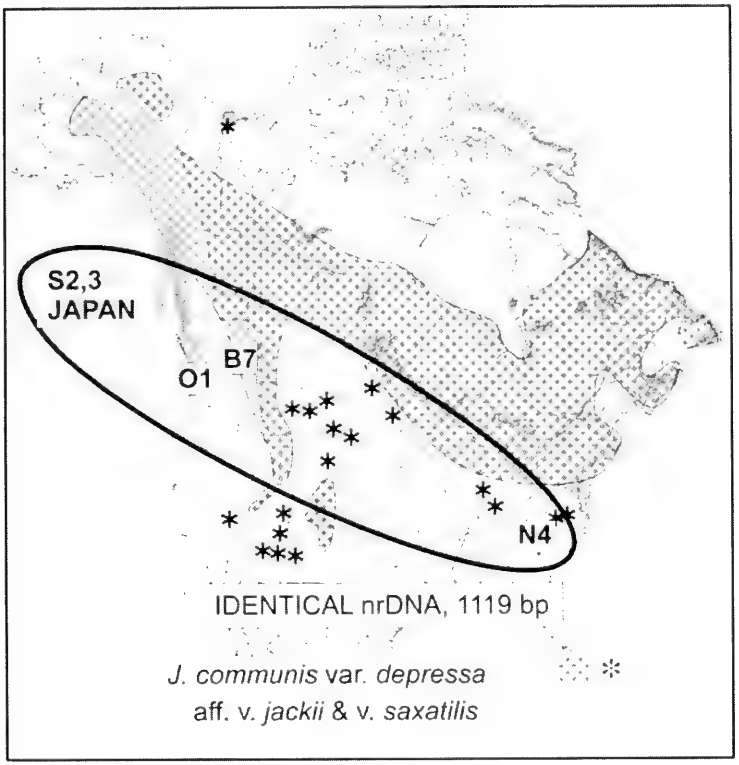


Figure 7. Five individuals with identical nrDNA sequences. Notice that one of the plants from North Carolina (N4) is identical to *J. c.* var. *saxatilis* plants from Japan.

These widely spaced nrDNA identities may reflect a mixing of genotypes after the Pleistocene glaciations. However, it is surprising that such genotypes have persisted in such widely disjunct populations.

TAXONOMIC TREATMENT

The recognition of infraspecific taxa of *J. communis* in North America is a difficult problem. Adams and Nguyen (2007) recognized *J. c.* var. *depressa*, *J. c.* var. *jackii* and *J. c.* var. *megistocarpa* based on leaves, female cones, and RAPDs data. Although the Queen Charlotte Island plants were quite distinct in their RAPDs and their habitat, it was felt that an analysis of plants from the British Columbia mainland were needed before a decision regarding their taxonomic status could be made.

The present study has shown that the Queen Charlotte Island junipers are distinct in their nrDNA from the junipers on the mainland of British Columbia. These junipers grow in a muskeg bog that is atypical of *J. communis*. Undoubtedly, this isolated population has evolved some physiological genes that enable it to cope with this environment. Thus, in addition to divergence in RAPDs and nrDNA, there is also divergence in its physiology. It seems, therefore, appropriate to recognize the plants growing on muskegs on Queen Charlotte Island (and elsewhere) as a new variety:

Juniperus communis var. ***charlottensis*** R. P. Adams, **var. nov.** TYPE: Canada, Queen Charlotte Island, 9 km s of Masset, along hwy 16, in muskeg bog, 53° 55.511'N, 132° 06.471'W, 61m, 8-July-2007, *R. P. Adams 10306* (HOLOTYPE: BAYLU, PARATYPES: *R. P. ADAMS 10304, 10305, 10307, 10308* (BAYLU).

Junipero communi var. *jackii* similis sed differt strobilis majoribus globosisque (vs. elongati-subglobosis in var. *jackii*).

This new variety is similar to *J. communis* var. *jackii* but differs in having seed cones that are larger and globose (vs. elongated-subglobose). It was first discovered on Queen Charlotte Island, but examination of specimens from the Ketchikan, Alaska area and islands adjacent to Queen Charlotte Island (see below) revealed that it grows on muskegs in several coastal areas. At present, the habitat seems conserved, so it does not appear to be threatened nor endangered.

ADDITIONAL SPECIMENS EXAMINED: **UNITED STATES, ALASKA, Chichagof Island**, about 0.5 mile NW of Whitestone Harbor, on muskeg, 9 June 1981, *Mary Clay Muller 4257* (ALA), **Mitkof Island, Petersburg**, between downtown and the airport, on muskeg, 12 Feb. 1981, *Mary Clay Muller 4199* (ALA), **Ketchikan** area, near Ward Lake, on muskeg, 15 June 1963, *B. J. Neiland 749* (ALA), **Misty Fjords National Monument**, **Smeaton Bay** area, Bakewell Trail, on muskeg, 17 July 1980, *D. E. Bramlet B-131* (ALA), **Cleveland Peninsula, Frosty Bay**, on muskeg with sphagnum and sedges, 21 July 1982, *J. Ver Hoef* 642 (ALA), **Nakat Inlet**, 2 miles s of head inlet, in Nootka cedar/shore pine on muskeg, 8 July 1993, *J. DeLapp & M. Duffy 93-362* (ALA), **Misty Fjords National Monument, Princess Bay**, e side, in muskeg on exposed rock, 17 July 1993, *J. DeLapp & M. Duffy 93-548* (ALA). **CANADA, BRITISH COLUMBIA, Khtada swamp**, 60 miles sw of Terrace, 18 June 1966, *G. Mendel 196* (V), **Calvert Island, Kwakshua**, on muskeg, 14 July 1939, *I. McT. Cowan ns* (V), **Queen Charlotte Island, Langara Island**, sphagnum bog, 21 May 1952, *F. L. Beebe ns* (V), **Dewdney Island**, w of Pemberton Bay, on rock outcrop in bog, 10 July 1984, *R. T. Ogilvie & Hans Roemer* 8471067 (V), **Vancouver Island, Green Mountain**, 35 degree slope, rocky, 1431 m, 16 July 2002, *R. Hebda, N. Hebda, L. Kennedy 02-57* (V).

The distribution of var. *charlottensis*, as presently known, is shown in figure 8. It is interesting that this variety seems to be confined to muskeg bogs that are low-lying, near the ocean. All of the specimens examined grew on muskeg or sphagnum bogs, except the unusual specimen from Green Mountain, Vancouver Island that grew on rocks at 1431 m; whether this specimen is truly var. *charlottensis* is questionable.

The area of distribution of var. *charlottensis* was glaciated during the Wisconsin (Flint, 1971). Because the variety seems restricted to muskeg bogs, it is difficult to determine a refugium for this taxon during the late Pleistocene Wisconsin glacial maximum.

In contrast to our previous study (Adams and Nguyen, 2007, Fig. 2, above), the present study included putative 'var. *saxatilis*' plants from NW US and W Canada. The nrDNA SNPs analyses indicate

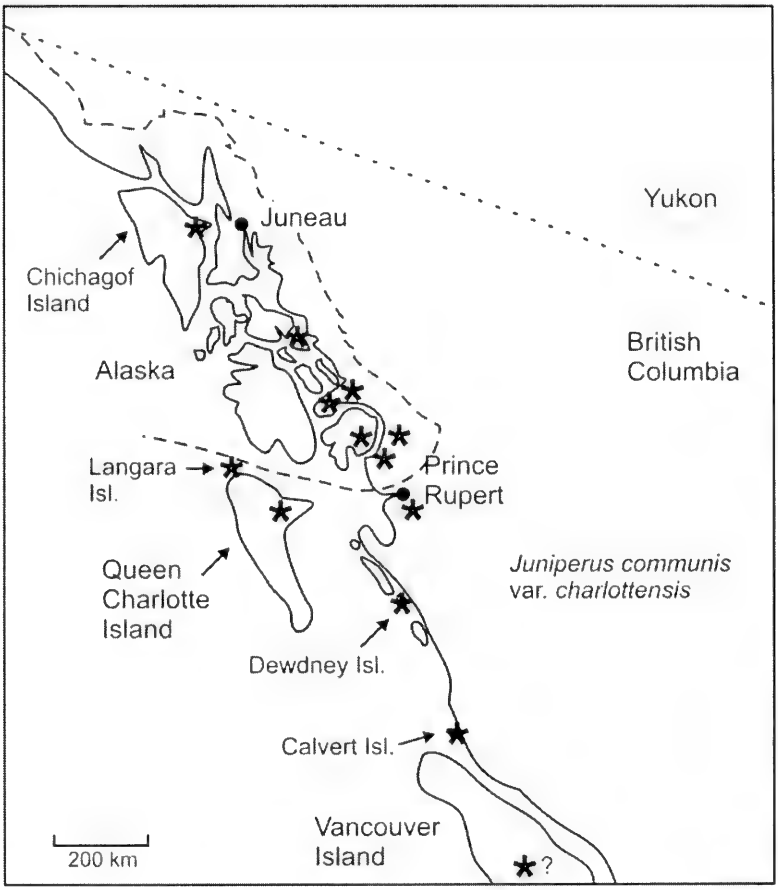


Figure 8. Distribution of *Juniperus communis* var. *charlottensis*. The star with a ? on Vancouver Island is the only location that putative var. *charlottensis* does not grow in a muskeg bog, but in a rocky area.

(Figs. 3, 4) that the putative 'var. *saxatilis*' plants from North America and var. *saxatilis* from the eastern hemisphere are very similar.

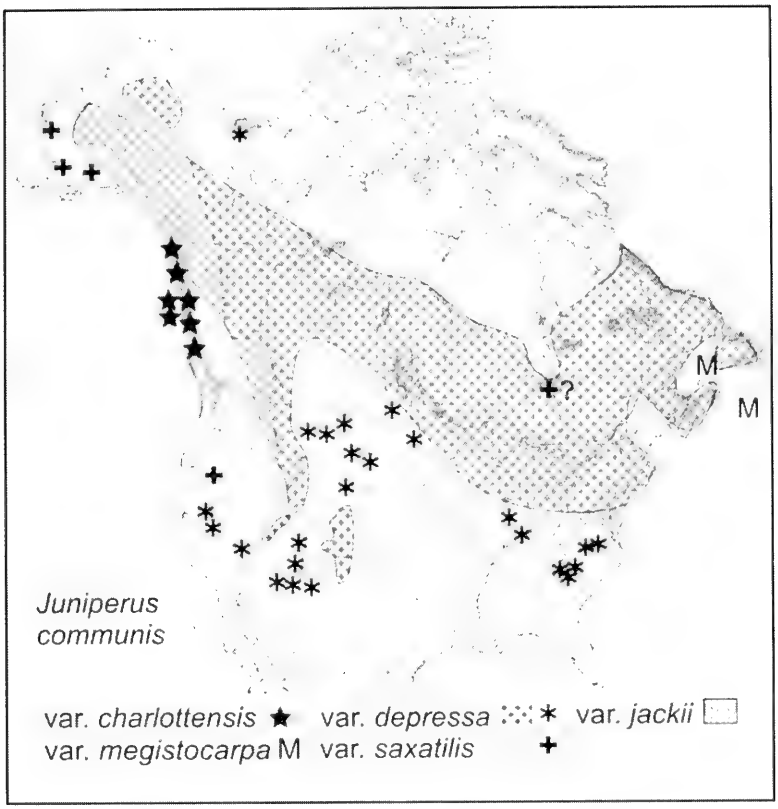


Figure 9. Distribution of *Juniperus communis* varieties in North America. The asterisk and plus symbols represent outlying individuals.

At present, it appears that five (5) varieties of *J. communis* merit recognition in North America: var. *charlottensis*, var. *depressa*, var. *jackii*, var. *megistocarpa*, and var. *saxatilis*. The distributions of the varieties, as presently understood, are shown in figure 9.

Clearly, infraspecific variation in *J. communis* is very complex. The present study has not completely resolved the complex variation. Additional, more detailed populational analysis is being conducted to more fully understand the patterns of variation.

Key to *J. communis* varieties in North America:

1. Glaucous stomatal band twice (or more) as wide as each green-leaf margin; spreading, mat-like shrub (or occasionally upright); leaves upright, sometimes almost imbricate, closely set, curved, 5 - 10 (12) mm long.....3.
1. Glaucous stomatal band about as wide to 1.5 x as wide as each green-leaf margin; prostrate or low shrub with ascending branchlet tips (or occasionally a spreading shrub), leaves upturned, rarely spreading, linear to curved, 10-20+ mm long.....2.

2. Seed cones 6 – 9 mm diam., smaller than leaf-length; widespread in North America.....var. **depressa**
2. Seed cones 10 – 13 mm diam., larger than leaf length, known only from southeastern Canada.....var. **megistocarpa**

3. Mature seed cones length greater than leaf-length; on muskeg bogs, Calvert Island to Queen Charlotte Island, and north to Chichagof Island, Alaska.....var. ***charlottensis***
3. Mature seed cones length less than or equal to leaf-length, Coastal range of w. Canada and U. S., on serpentine, lava and other rock substrates.....4.

4. Mature seed cones elongated-subglobose, stomatal band 3 to 4 times as wide as each green-leaf margin.....var. **jackii**
4. Mature seed cones globose, stomatal band 2 times as wide as each green-leaf margin.....var. **saxatilis**

ACKNOWLEDGEMENTS

Thanks to Guy Nesom for the Latin diagnosis and Billie Turner and Andrea Schwarzbach for reviewing the manuscript. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 2004. The junipers of the world: The genus *Juniperus*. Trafford Publ., Victoria, BC.

Adams, R. P. and S. Nguyen. 2007. Post-Pleistocene geographic variation in *Juniperus communis* in North America. Phytologia 89(1): 43-57.

Adams, R. P. and R. N. Pandey. 2003. Analysis of *Juniperus communis* and its varieties based on DNA fingerprinting. Biochem. Syst. Ecol. 31: 1271-1278.

Adams, R. P., R. N. Pandey, J. W. Leverenz, N. Dignard, K. Hoegh and T. Thorfinnsson. 2003. Pan-Arctic variation in *Juniperus communis*: History Biogeography based on DNA fingerprinting. Biochem. Syst. Ecol. 31: 181-192.

Ashworth, V. E. T. M., B. C. O'Brien and E. A. Friar. 1999. Fingerprinting *Juniperus communis* L. cultivars using RAPD markers. Madrono 46: 131-141.

Ashworth, V. E. T. M., B. C. O'Brien and E. A. Friar. 2001. Survey of *Juniperus communis* L. varieties from the western United States using RAPD fingerprints. Madrono 48: 172-176.

Flint, R. F. 1971. Glacial and quaternary geology. John Wiley, NY.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874

Veldman D. J., 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

KEYS TO THE FLORA OF FLORIDA: 19, *PHYSALIS* (SOLANACEAE)

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

ABSTRACT

Physalis (Solanaceae) is represented in Florida by 13 species. Of these, 8 are native and 5 are believed introduced. Though some are rare, none is considered endangered. Two species (*P. arenicola*, *P. walteri*) are each formed of two varieties. **Physalis walteri** var. **glabra** is recognized as a new combination. *Physalis pubescens* var. *integrifolia* is here assigned specific rank, as **Physalis integrifolia**, a new combination. *Physalis grisea*, *P. integrifolia*, *P. lanceifolia* and *P. turbinata* are newly reported for Florida. Two species reported for Florida are excluded. An amplified key is given to the Florida taxa.

KEY WORDS: *Physalis*, Solanaceae, Florida flora.

> The North American flora hardly contains a more difficult genus for its size than *Physalis*. -- Asa Gray, Proc. American Academy 10: 62. 1874.

The cautionary warning of Asa Gray has not deflected American scholars from efforts to understand the genus *Physalis* (Solanaceae) in North America. From the limited materials then available, Gray (1874) recognized 17 species. Per Axel Rydberg took up the task (Mem. Torrey Bot. Club 4: 297-374. 1896), with regional treatments contributed to J. K. Small's *Flora* (1903) and *Manual* (1933). A detailed, lifelong study by U. T. Waterfall (Rhodora 60: 107-114, 128-142, 152-173. 1958; ibid. 69: 82-120, 203-239, 319-329. 1967) documented 93 species and numerous varieties. In the 1940s, Margaret Y. Menzel began an extensive series of greenhouse crossing experiments (Proc. Amer. Philos. Soc. 95: 132-183. 1951; Yearb.

Amer. Philos. Soc. 1957: 262-266. 1957). In 1984, Janet R. Sullivan drafted a floristic review of the southeastern species (Rhodora 106: 305-326. 2004). The Florida species have been addressed by two highly useful guides: Andre F. Clewell (1985), who reported 8 species in the Florida panhandle; and Richard P. Wunderlin & Bruce F. Hansen (2003), who recognized 9 species from throughout the state.

The present synopsis treats 13 species of *Physalis* from within the state. The increase over the numbers reported by Clewell and Wunderlin & Hansen is caused, not by new introductions, but by variations that have long been known elsewhere (Mexico, mostly) and previously overlooked in Florida, now treated at specific rank.

The Florida species of *Physalis* are best understood when partitioned into sections (not necessarily nomenclaturally valid), as recognized by Menzel (1951). The "Carpenterianae" consists of a single species, the coastal plain endemic, *P. carpenteri*. This is separated morphologically by a unique clustering of flowers (rather than single) at each node, a condition described by Sullivan (2004) as perhaps representing a "telescoped axillary branch." Its habitat is similarly unique in that it seems restricted to small sites of disturbed soil, often on the loose materials pushed out from burrows of the gopher tortoise (*Gopherus polyphemus*). It has been suggested (Erdman West, pers. comm., 1962) that the tortoise would encounter, and surely feed upon, the fruits of this plant, then deposit its seeds on suitable loose soil surrounding the burrow. The food-source relationship of the tortoise with the Gopher Apple (*Licania michauxii*) has been documented (Castanea 64: 263-265. 1999), an alliance not yet shown with *P. carpenteri*. But the historic range of the gopher tortoise closely corresponds to the known range of the *Physalis*, permitting a long co-adaptation and perhaps co-evolution of these two rare species.

The "Viscosae" in Florida consists of at least two quite distinct, largely coastal species, with a probable hybrid intermediate in both morphology and range (Waterfall, 1958: 134-135; Menzel, 1951: 174). Species of this group have been carefully analyzed (Sullivan, Syst. Bot. 10: 426-444. 1985) and are now well understood. All are

sparsely to densely pubescent with stellate or radially branched hairs. *Physalis angustifolia*, with narrow, glabrous leaves, is quite frequent along the panhandle coastal dunes, then is disjunct to shores of the southernmost peninsula and keys. *Physalis walteri*, with broad, densely pubescent leaves, is more widespread, locally common from coastal Virginia into peninsular Florida.

The geographic distance between the two areas of *Physalis angustifolia* is almost precisely occupied by plants obviously related to these two species, an apparent hybrid, but with broad, glabrous leaves. Sullivan (1985: 439) found plants from these populations to be interfertile with similar flavonoid profiles. She followed Rydberg (1897) in weighing pubescence above leaf shape (rather than Waterfall, 1958), and thus placed the intermediate with *P. angustifolia* (rather than with *P. maritima*). Because of the nearly identical flavonoid profiles, Sullivan chose to leave the intermediate taxon unnamed.

But field experience is not content with this null assignment. For a long span of the Florida gulf coast, between Wakulla County in the eastern panhandle to Collier County in the southwest peninsula, a distance of 500 km., plants of the *angustifolia* - *maritima* complex are almost entirely of the intermediate form. These differences, sufficient to be recognized by any school child, merit nomenclatural recognition.

This intermediate form has long been known as *Physalis elliottii*, but Kunze's type for that name seems closer to typical *P. maritima* (Waterfall, 1958: 134). A name that clearly represents the intermediate is Waterfall's ponderous *Physalis viscosa* ssp. *maritima* var. *elliottii* forma *glabra* (1958: 135). Since Sullivan used Waterfall's "*glabra*" as her label (not in a formal nomenclatural sense) for plants of the intermediate form, it is appropriate that Waterfall's terminal epithet be retained. There is no prior varietal epithet for the taxon. The required new combination follows.

> *Physalis walteri* Nuttall var. **glabra** (Waterfall) D. B. Ward, comb. et stat. nov. Basionym: *Physalis viscosa* L. ssp.

maritima (Nutt.) Waterfall var. *elliottii* (Kunze) Waterfall forma *glabra* Waterfall, Rhodora 60: 135. 1958.

The "Lanceolatae" in Florida, as elsewhere, are difficult to identify with confidence. Plants believed to be typical *Physalis virginiana* are found occasionally, but its var. *subglabrata*, common northward, and the other varieties recognized by Waterfall (1958: 152-156) seem not to reach the state. *Physalis arenicola* with two poorly distinguished varieties occur throughout the peninsula.

Physalis heterophylla, a species assumed by most writers to be a Florida species, was termed by Waterfall (1958: 140) "an extremely variable assemblage." Yet northeastern plants -- the type was from Pennsylvania -- are easily and consistently distinguished from Florida plants bearing that name. Rydberg (1903: 986) recognized that difference, and assigned the name *P. sinuata*. Rydberg's type (NY), a Chapman collection borrowed for this study, was of a plant with broadly ovate leaves and coarse low rounded teeth, well matching plants found in dry pinelands of the upper peninsula. The Florida plants exhibit a feature that has not been mentioned and which understandably disappears upon pressing -- the blades tend to be borne in a vertical plane. Though the distribution and characteristics of the Florida plants are scarcely now better known than by Rydberg, it has seemed prudent to call attention to their differences, by the use of his epithet.

The "Angulatae" in Florida are nearly all the broad-leaved typical *Physalis angulata*, found commonly through the state; its leaves are broadly ovate, coarsely dentate, and its flowers are of moderate size with green or brown centers, and bluish stamens. But in an area of the Everglades centered around Flamingo, plants have been known for many years with lanceolate sinuate-margined leaves, much smaller flowers with yellow centers, and yellow stamens. Authors have struggled with these anomalous plants, from Rydberg (1933: 1109) who noted them as an unnamed form under *P. angulata*, to Sullivan (2004: 318) who remarked on similarities with the western *P. acutifolia* (Miers) Sandw. Their true relationship, however, seems to be with a

Mexican plant, *P. lanceifolia*, not previously recognized to occur in Florida. The differences with *P. angulata* are quite sufficient to retain specific rank, rather than reduced by Waterfall (1967: 219) to *P. angulata* var. *lanceifolia*. Again, the distribution and characteristics of the Florida plants are poorly understood, and it may be found that variations within *P. angulata* are a consequence of introgression with this Mexican relative.

The "Pubescentes" in Florida seem entirely to be introduced. All are annuals, and have migrated readily with cultivation. Five species are present within the state. The near-glabrous *P. cordata* is most readily recognized. But *P. pubescens* and its variants are commonly treated as a complex that is either undivided or composed of inconsequential varieties. Recently, a seeming extreme disjunct -- from Central America to northeastern U.S. -- was resolved by recognition of the northern plant as distinct (M. Martinez, Taxon 42: 103-104. 1993), as *P. grisea*; it is now known in Florida as a weed of cultivated fields. Two other uncommon Florida plants, usually overlooked as *P. pubescens*, are worthy of specific distinction. *Physalis turbinata*, though distinguished largely on its larger size, seems not to overlap *P. pubescens*, and is recognized here, new to the Florida flora. *Physalis integrifolia*, readily identified by its translucent leaves and also new to the Florida flora, requires a transfer from its present varietal status, as follows.

Physalis integrifolia (Dunal) D. B. Ward, comb. et stat. nov. Basionym: *Physalis hirsuta* Dunal var. *integrifolia* Dunal in DeCandolle, Prodr. 13(1): 445. 1852.

This summary of the Florida species of *Physalis* has taken an inordinate span of time. Soon after receiving U. T. Waterfall's 1958 synopsis of the species north of Mexico -- and then quite without field knowledge of my own -- I spread out the FLAS materials with the expectation I could quickly sort the jumble of erratically identified Florida specimens. Though many sheets fell into place, a number resisted assignment. I was cheered on by my mentor and beloved friend, Erdman West (FLAS), who had collected many of the puzzling

specimens and was frustrated in being unable to name them. I was pushed to gather more field data by Margaret Y. Menzel (FSU) who was then winding down her extensive greenhouse crossing experiments. Dr. Waterfall resolved some of my questions by his detailed 1967 report of the Mexican and West Indian species; in 1970, after much correspondence, I visited him in his attic herbarium in Stillwater and received further generous help. In the 1980s Patricia K. Holmgren (NY) loaned me a series of critical early Florida specimens. Janet R. Sullivan entered the field with her analysis of the *Physalis viscosa* allies, then with her synopsis of the southeastern species. Milo Pyne, then a student in North Carolina, corresponded regarding my interests and his careful (but yet unpublished) documentation of the Carolina species. In 2007 Jimi Sadle, botanist of the Everglades National Park, came upon plants of *Physalis lanceifolia* and stimulated me to completion and publication of these observations. I am grateful to all these folks.

PHYSALIS L. Ground Cherries[1]

1. Flowers several in each upper leaf axil; corolla yellow to greenish-yellow with green or brownish blotches in throat; fruiting calyx nearly globose, small (to 1 cm. dia.), scarcely larger than enclosed berry; leaf blades broadly ovate, at least some strongly unequal at base (one side extending along petiole 5-10 mm. further than the other); plants erect, to 1.5 m. tall, from perennial vertical taproot. Moist to dry hammocks, usually on disturbed and exposed mineral soil (gopher mounds, tree throws). Panhandle and north Florida (s. to Dixie, Alachua counties); rare. Summer.
CARPENTER'S GROUND CHERRY.
Physalis carpenteri Ridd. ex Rydb.

1. Flowers solitary in leaf axils; fruiting calyx conspicuously inflated, larger than enclosed berry; leaf blades approximately symmetrical (each side extending equally along petiole).

2. Plants pubescent at least in part with hairs stellate (radially branched, with 3-4 arms), either abundantly covering foliage or, if leaves glabrous, restricted to tips and edges of sepals; perennial, from deeply buried rhizomes.

3. Leaves linear, 10-20 times longer than wide, glabrous; plants erect. Vegetated dunes and sandy roadsides, always near the coast. Panhandle (Escambia to Wakulla County), disjunct to south peninsula (Collier to Dade County, and Florida Keys); frequent. All year.
Physalis angustifolia Nutt.

3. Leaves ovate, elliptic, obovate, or spatulate, 2-10 times longer than wide, stellate pubescent (except var. *glabra*); plants erect to many-stemmed and sprawling. Sandy soils, dunes. All year. **Physalis walteri** Nutt.

a. Leaves stellate pubescent. Coasts and interior of the peninsula, disjunct and perhaps adventive in panhandle (Jackson, Wakulla counties); frequent. [*Physalis elliottii* Kunze; *Physalis maritima* Curtis; *Physalis viscosa* L. ssp. *maritima* (Curtis) Waterfall] A variant with leaves 5-7 cm. broad, occurring along the lower east coast (St. Lucie southward), has been called forma *latifolia* Waterfall. var. **walteri**

a. Leaves glabrous. West coast of peninsula (Levy to Monroe County), inland (Hendry County); infrequent. [*Physalis elliottii*, misapplied.] Clearly an intergrade with *P. angustifolia* in leaf pubescence and shape. var. **glabra** (Waterfall) D. B. Ward

2. Plants glabrous or pubescent, but if pubescent, with simple hairs; stellate hairs lacking or few and inconspicuous; annual or perennial.

4. Perennial, from slender horizontal rhizomes (often deeply buried and broken off in collecting); anthers yellow, 2.5-5.0 mm. long.

5. Pedicels and upper stems with retrorse, stiff, short hairs; leaves narrowly ovate, sinuately toothed. Sandy roadsides, disturbed pinelands. Panhandle (Leon, Lafayette counties); rare. Spring.
Physalis virginiana Mill.

5. Pedicels and upper stems with spreading soft lax hairs (some long, to 3-5 mm.).

6. Foliage and stems abundantly viscid-pubescent (often encrusted with adhering sand); leaf blades borne in

vertical plane, broadly ovate with wavy margins; rhizomes cord-like (2-3 mm. dia.), often deeply buried. Sandy pinelands. Upper peninsula; infrequent. Spring. [*Physalis heterophylla* Nees var. *villosa* Waterfall] **Physalis sinuata** Rydb. in Small

6. Foliage and stems not viscid or sparingly so; leaf blades borne in horizontal plane, narrowly ovate with even margins; rhizomes slender (1.5 mm.), commonly shallow. Waste areas, sandy fields. Peninsula (Suwannee R. southward); frequent. Spring.
Physalis arenicola Kearney

a. Pubescence short, fine, more or less glandular.
var. **arenicola**

a. Pubescence long (1.5-1.0 mm.), non-glandular. [*Physalis ciliosa* Rydb.]
var. **ciliosa** (Rydb.) Waterfall

4. Annual, from much-branched, mostly vertical roots; anthers bluish (rarely yellow), 1.0-2.5 mm. long.

7. Blades coarsely dentate, with long-acuminate teeth; fruiting calyx 10-ribbed (the intermediate veins equal in prominence to the midveins of the calyx lobes); corolla dark yellow or greenish in center; plant robust (to 1 m. tall), weedy.

8. Leaves ovate to ovate-lanceolate; flowers ±10 mm. dia.; anthers 2.0-2.5 mm. long. Moist shady riverbottoms, thickets, ditchbanks. Throughout; frequent (less so in panhandle). All year. **Physalis angulata** L.

8. Leaves lanceolate to linear-lanceolate; flowers ±8 mm. dia.; anthers 1.0-2.0 mm. long. Open tropical hammocks. Southwest peninsula (Monroe County), sometimes northward; infrequent. All year. [*Physalis angulata* Nees var. *lanceifolia* (Nees) Waterfall]
Physalis lanceifolia Nees

7. Blades variously sub-entire to serrate, teeth rounded to acute; fruiting calyx 5-ribbed (with each rib centered on a calyx lobe midvein); corolla with dark (usually black) center; plants weak-stemmed, often sprawling (to 0.4 m. tall).

9. Plants glabrous (or with a few hairs on the calyx); leaf blades with acute regular teeth. Gardens, disturbed areas, often weedy. Panhandle and north Florida where frequent, to central and south peninsula (Dade) where rare. Summer. [*Physalis pubescens* L. var. *glabra* (Michx.) Waterfall; *Physalis turbinata*, misapplied] * **Physalis cordata** Mill.

9. Plants pubescent, long-haired or with intermixed long hairs and stalked glands, at least on petioles and stems; leaf blades with irregular blunt teeth (except at times *P. grisea*), or entire.

10. Surface of leaf gray-mealy, only sparingly pubescent, glands absent or not apparent; blades toothed, often sharply so, to the base. Cultivated fields, where weedy. West-central peninsula (Citrus=Inverness); rare. Summer. [*Physalis pruinosa*, misapplied; *Physalis pubescens* L. var. *grisea* Waterfall] * **Physalis grisea** (Waterfall) M. Martinez

10. Surface of leaf copiously pubescent, at least along veins below, not mealy, stalked glands usually conspicuous.

11. Leaf blade flaccid and translucent, usually with a few teeth, 3-4 on each side, or entire. Mucklands, low fields. Throughout; rare. Summer. [*Physalis pubescens*, misapplied; *Physalis pubescens* L. var. *integrifolia* (Dunal) Waterfall] * **Physalis integrifolia** (Dunal) D. B. Ward

11. Leaf blade opaque, usually toothed nearly to base with 5-8 teeth on each side.

12. Leaves 2-4 cm. broad; fruiting calyx 20-30 mm. long. Waste areas, cultivated fields, mucklands. Southern peninsula; frequent. All year. [*Physalis floridana* Rydb. in Small] * **Physalis pubescens** L.

12. Leaves 4-8 cm. broad; fruiting calyx 30-40 mm. long. Cleared fields, pinelands, prairies. South-central peninsula (Lee, Polk, Highlands counties); rare. Spring-summer. [*Physalis*

barbadensis Jacq.; *Physalis pubescens*, misapplied]

* **Physalis turbinata** Medic.

Excluded names:

Physalis heterophylla Nees

Northern. Reported for Florida (Small, 1933), and for several north Florida counties (Clewell, 1985; Wunderlin, 1998). Spms. so named are apparently all *P. sinuata* or *P. virginiana*.

Physalis longifolia Nutt.

Northern. Reported for Leon Co. (Wunderlin & Hansen, 2003). The several spms. so named (FSU) are better referred to *P. virginiana*.

---

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

TAXONOMIC STUDY OF *JUNIPERUS EXCELSA* AND *J. POLYCARPOS* USING SNPs FROM nrDNA AND cp trnC-trnD, PLUS ESSENTIAL OILS AND RAPD DATA.

Robert P. Adams
Biology Department, Baylor University, Box 727,
Gruver, TX 79040, USA
email Robert_Adams@baylor.edu

Julie A. Morris
Department of Biological Sciences, University of Texas at Brownsville
Brownsville, TX, 78520, USA.

Andrea E. Schwarzbach
Department of Biological Sciences, University of Texas at Brownsville
Brownsville, TX, 78520, USA.

ABSTRACT

SNPs from nrDNA and cp trnC-trnD were analyzed for *J. excelsa*, *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* and compared to terpene and RAPDs data. These data, taken together, support the continued recognition of *J. excelsa* and *J. polycarpos* as separate species as well as the three varieties of *J. polycarpos*: var. *polycarpos*, var. *seravschanica* and var. *turcomanica.*

KEY WORDS: *Juniperus excelsa, J. polycarpos, J. p.* var. *seravschanica*, *J. p.* var. *turcomanica*, Cupressaceae, nrDNA, trnC-trnD, SNPs, essential oils, terpenes, DNA fingerprinting, systematics.

The taxonomy of *J. polycarpos* K. Koch. from Armenia, Kazakhstan, Pakistan and Turkmenistan has been examined using leaf oil compositions and DNA fingerprinting (Adams, 1999; Adams, 2001). The compositions of the volatile leaf oils (Adams, 2001) are given in Table 1. Notice the large amounts of α-pinene in the plants from Armenia, Kazakhstan and Turkmenistan. Myrcene is a large component in the oils from Kazakhstan and Pakistan (*J. p.* var. *seravschanica*). Several compounds distinguish *J. excelsa*: decadienal

isomer (KI 1312), trans-cadina-1(6),4-diene, cubebol, 1-epi-cubenol, and KI 1666 (Table 1). Compounds that distinguish *J. polycarpos* (including *J. p.* var. *turcomanica* and *J. p.* var. *seravschanica* for this discussion) are: hexyl 3-methyl butanoate, δ-elemene, γ-cadinene, elemol, germacrene B, germacrene D-4-ol, α & β-eudesmols and KI 1688 (Table 1). Several diterpenes are unique to *J. procera* (Table 1) and show its separation from *J. excelsa* and the other junipers.

The trend in the volatile leaf oils is seen in figure 1. The leaf

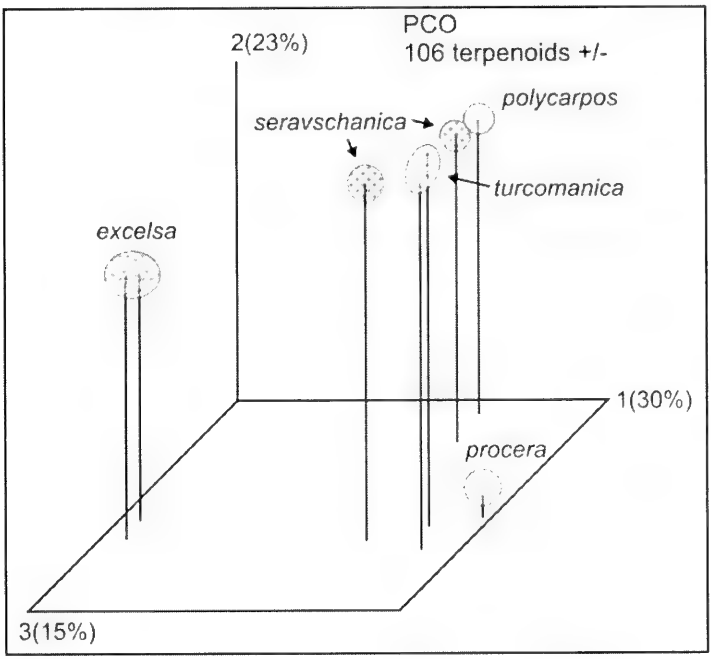


Figure 1. PCO of *J. excelsa*, *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica*, *J. p.* var. *turcomanica* and *J. procera* based on 106 leaf terpenoids scored as present (+) or absent (-). Based on Adams (2001) data.

terpenoids clearly separate *J. excelsa* and *J. procera* from *J. polycarpos*. The varieties of *J. polycarpos* are not well resolved. This is seen in the raw data in table 1.

PCO analysis based on RAPDs (Adams, 2001) shows (Fig. 2) a very similar pattern to that seen with terpenoids. Notice that again, *J. excelsa* and *J. procera* are very well resolved from *J. polycarpos* (Fig. 2). There appears to be more slightly more separation of the *J. polycarpos* varieties in the RAPDs data (Fig. 2) than in the terpenoids (Fig. 1) but the overall trend is very similar.

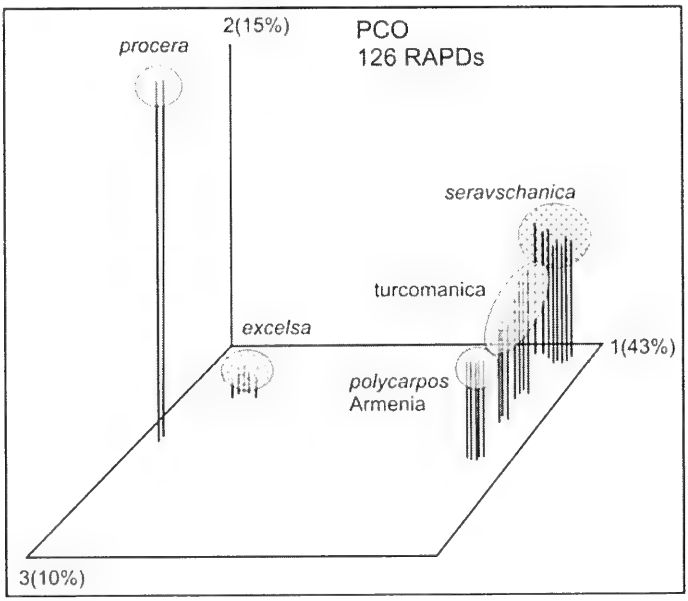


Figure 2. PCO based on 126 RAPDs. Notice that 43% of the variance separated *J. excelsa*/J. *procera* from *J. polycarpos/turcomanica/ seravschanica* (axis 1) and 15% of the variance separates *J. procera* from all other taxa on axis 2. Based on Adams (2001) data.

The pattern is simplified by removing *J. procera* from the RAPDs data, as seen in figure 3. *Juniperus excelsa* is still well

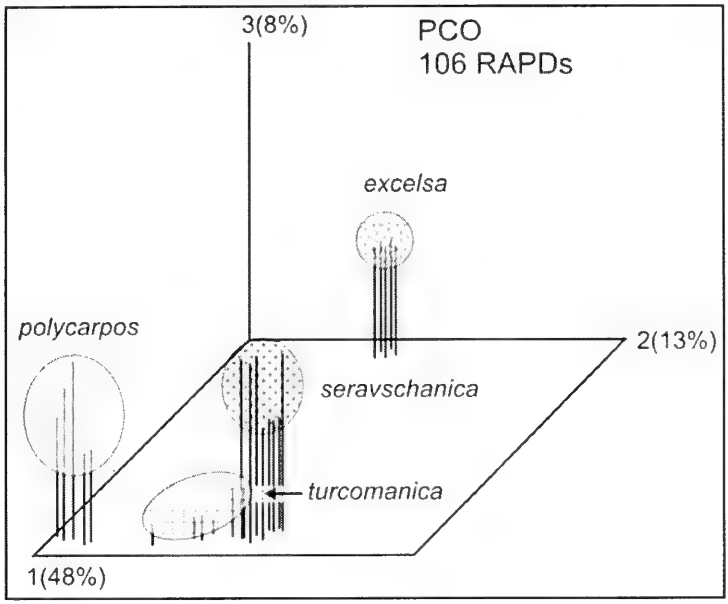


Figure 3. PCO of J. excelsa, *J. polycarpos*, *J. p.* var. *seravschanica* and *J. turcomanica* using 106 RAPDs. Based on Adams (2001) data.

resolved. However, there is now some separation between *J. polycarpos*, *J. p.* var. *seravschanica* and *J. turcomanica* (Fig. 3).

Removing *J. excelsa* from the data set and re-analyzing the RAPDs data gave a clearer picture of the pattern among the *J. polycarpos* varieties (or populations). This resulted in four groups, each well resolved. The *J. p.* var. *seravschanica* populations are resolved into the Pakistan and Kazakhstan sites.

Should these entities be recognized as varieties of *J. polycarpos* or do they merely represent geographical interspecific variation?

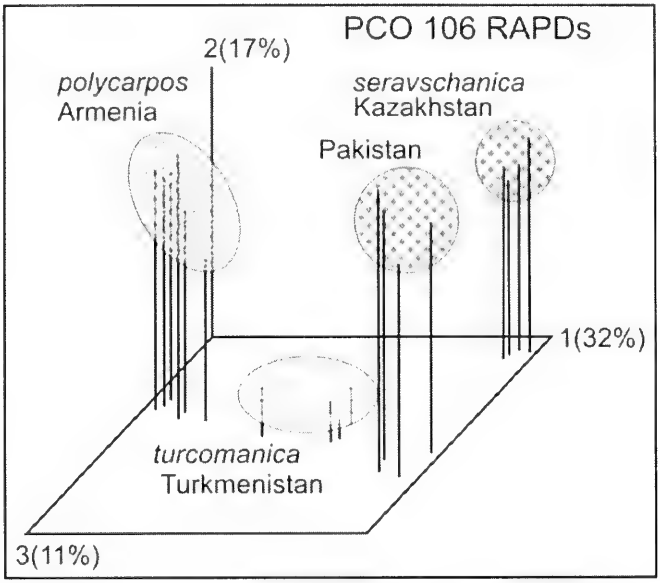


Figure 4. PCO of four taxa of *J. polycarpos* based on RAPDs. Based on Adams' (2001) data.

Farjon (2005, p. 291) treated *J. polycarpos* as a variety of *J. excelsa* (*J. excelsa* var. *polycarpos* (K. Koch) Takht.) and treated *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as synonyms of *J. excelsa* var. *polycarpos*. Clearly, neither the terpenoids nor RAPDs support Farjon's merging of *J. polycarpos* and *J. excelsa*.

Farjon (2005, p. 343) states his philosophy as "I consider species based on the chemistry of terpenes and/or RAPD analysis as based on inconclusive evidence" although he allows that "DNA sequence data certainly can (which is the main reason for their 'superiority')" (Farjon, 2005, p. 232).

The goal of sequencing a single gene is now easily accomplished but these data are proving to be more difficult to interpret than perhaps imagined. For example, Syring et al. (2007) examined *Pinus* species utilizing sequences from three nuclear genes (AGP6, cesA1, LEA-like). They found that none of the three genes,

analyzed separately, placed the multiple accessions of *P. strobus*, *P. monticola* and *P. chiapensis* into monophyletic clades. We suggest that additional data from multiple genome sites such as RAPDs as well as phenotypic data such as leaf terpenoids can help to complement single gene sequences. In cases where sequence data alone results in multiple alternative scenarios, those additional data might be able to help discriminate between them.

Recent DNA sequence phylogenetic research of *Juniperus* (Schwarzbach et al., in prep.) has shown (Fig. 5) that *J. polycarpos* (Armenia, cf. *J. p.* var. *polycarpos*, above) is 100% supported as being separate from *J. procera* and *J. excelsa* and not forming a monophyletic group. The thesis of Farjon (2005) that *J. polycarpos* is a variety of *J. excelsa* is not supported by these data. In fact, the sequencing data is in full agreement with both the terpenoid and RAPD

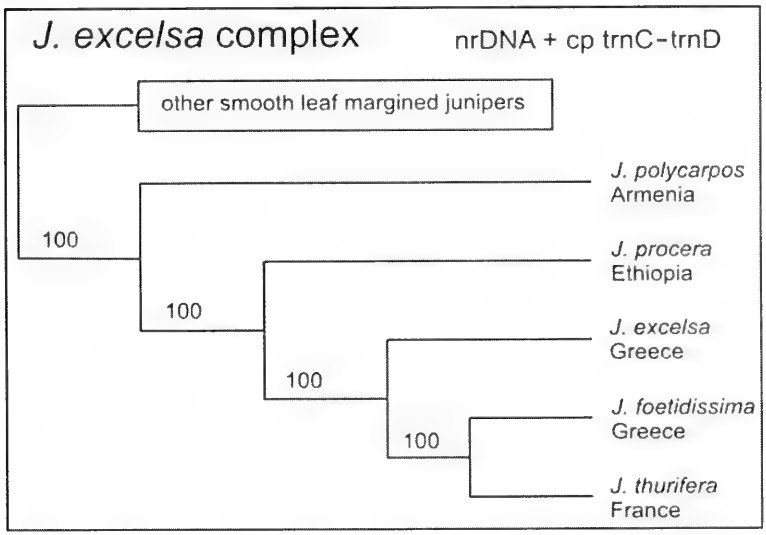


Figure 5. Bayesian tree based on nrDNA and cp trnC-trnD sequences. *Juniperus polycarpos* is resolved from *J. procera, J. excelsa, J. foetidissima* and *J. thurifera*. Numbers above the branch points are posterior probabilities on a percent basis.

data (above) that show *J. excelsa*, *J. polycarpos*, and *J. procera* being well differentiated. In fairness to Farjon (2005), it should be conceded that if only morphological data are utilized, then one could readily make a case for the merging of *J. excelsa* and *J. polycarpos* (and perhaps *J. procera*). However, morphology can be misleading when used solely for species circumscriptions, as has been shown in several other studies. Cryptic speciation has been found in *J. deltoides* R. P. Adams (Adams et al. 2005) and *J. maritima* R. P. Adams (Adams 2007), to name but two cryptic juniper species.

Schwarzbach et al. (in prep) have analyzed one accession per species for *J. excelsa*, *J. polycarpos*, and *J. procera*. As a result, the relationships of the species were established in a basic framework. However, the sampling in this previous study did not allow any assessments of intraspecific variation or the monophyly of the taxa involved. The purpose of the present paper is to re-examine the taxonomy of *J. excelsa*, *J. polycarpos* and its varieties using SNPs from sequence data (nrDNA and cpDNA trnC-trnD) by adding multiple accessions for each taxon.

MATERIALS AND METHODS

Specimens used in this study: *J. excelsa*, Adams , 8785-8786 - 7 km w of Lemos, Greece; Adams 9433-9435, 40 km n of Eskisehir, Turkey; *J. p.* var. *seravschanica*, Adams 8224-8226, 2 km s Dzhabagly, Kazakhstan (not Kyrgystan as previously reported, Adams, 1999); *J. p.* var. *seravschanica*, Adams 8483-8486, Quetta, Balochistan, Pakistan; *J. turcomanica*, Adams 8757-8760, Kopet Mts., Turkmenistan. Voucher specimens for all collections are deposited at 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 as per manufacturer's instructions.

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD 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 or K (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. All 12 (A-L) of the Epi-Centre's buffers were screened and buffer K gave the cleanest, most-abundant amplification for both ITSA/ITSB and buffer E was best for trnC-trnD (CD10F/CD3R) primers. However, buffers D, F, G, H, and J were nearly as good as buffer E or K.

Primers (5'-3'): for nrDNA: ITSA and ITSB primers from Blattner (1999), for trnC-trnD: CD10F and CD3R, see Adams et al. (2008). The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). The nrDNA primers (ITSA, ITSB) produced a band of approx. 1120 bp. The internal trnC-trnD primers, CD10F-CD3R produced a band of approx. 850 bp. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS region) resulted in 1210 bp of data. Aligning sequences for *J. excelsa* (5 individuals, Greece, Turkey), *J. polycarpos* var. *polycarpos* (4 individuals, Armenia); *J. p.* var. *seravschanica* (4 individuals, Kazakhstan, 4 individuals, Pakistan) and *J. p.* var. *turcomanica* (4 individuals, Turkmenistan) revealed 14 SNPs among these individuals. PCO of these individuals gave three significant eigenroots accounting for 74.4%, 9.1% and 6.7% of the variance among individuals. PCO ordination shows (Fig. 6) two major groups to be present: *J. excelsa* - *J. p.* var. *turcomanica* and *J. p.* var. *polycarpos* and var. *seravschanica*.

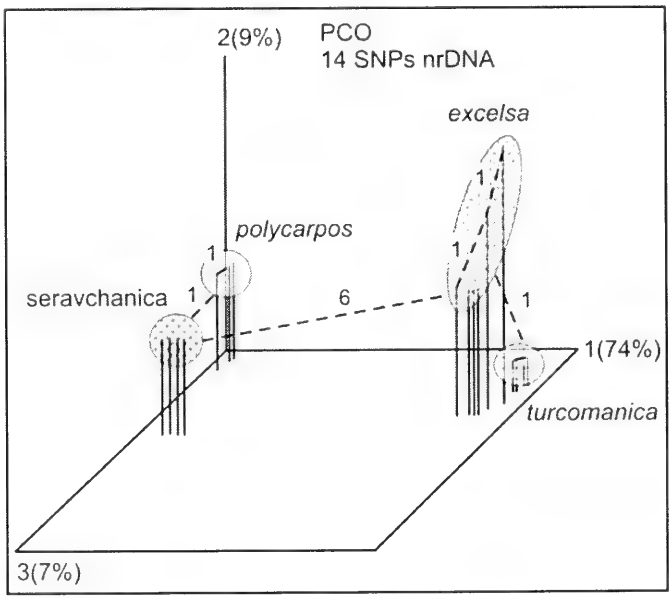


Figure 6. PCO based on 14 nrDNA SNPs. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Equally spaced lines denote identical DNA sequences.

Sequencing the partial trnC-trnD sequence resulted in 877 bp of data when utilizing the same genomic DNA as above. Aligning these 21 sequences revealed 14 SNPs. Factoring the association matrix resulted in 3 eigenroots that accounted for 49.1%, 43.9% and 3.3% of the variance among these individuals in their partial trnC-trnD SNPs. Ordination of these individuals reveals (Fig. 7) three groups: *J. excelsa*; *J. polycarpos* - *J. p.* var. *turcomanica*; and *J. seravschanica*. These groups are separated by 7 and 8 SNPs. Notice (Fig. 7) that there was no variation among individuals of any taxon except *J. excelsa*.

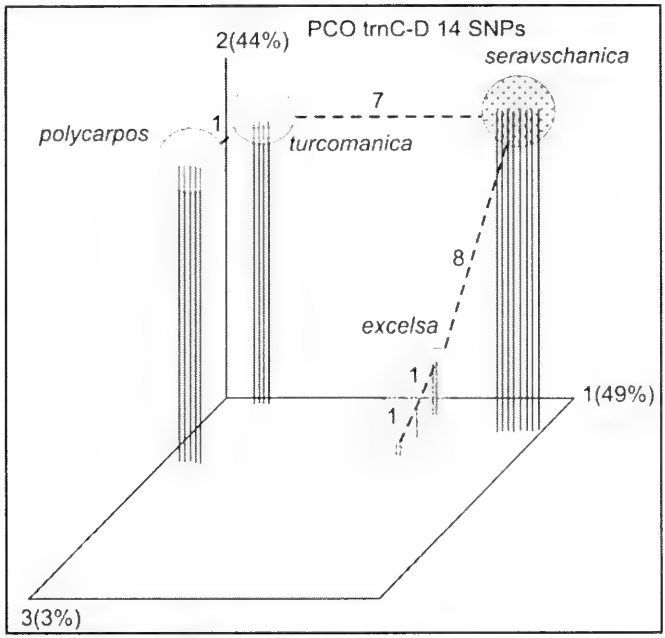


Figure 7. PCO ordination based on 14 SNPs from trnC-trnD sequences. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Equally spaced lines denote identical DNA sequences.

Combining the 14 nrDNA SNPs and 14 trnC-trnD SNPs for a PCO analysis resulted in 3 eigenroots of 52%, 30% and 12%. Ordination (Fig. 8) shows 4 well-defined groups, each separated by 7 to 9 SNPs. It is interesting that the 8 samples of *J. p.* var.

seravschanica (4 from Kazakhstan and 4 from Pakistan) had identical sequences for both nrDNA (1210 bp) and cp trnC-trnD (877 bp). *Juniperus p.* var. *polycarpos* and *J. p.* var. *turcomanica* both had a single SNP within their samples. In contrast, *J. excelsa* had 2 SNPs within its samples.

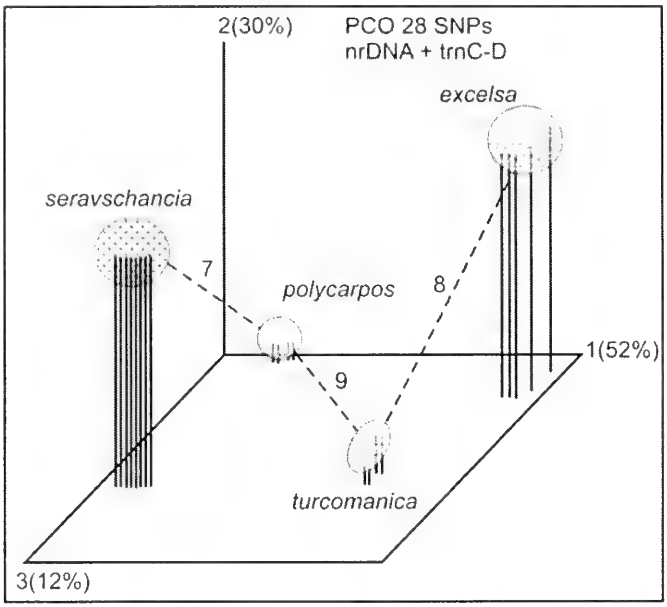


Figure 8. PCO using SNPs from both nrDNA and trnC-trnD. Note the four well defined groups. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Equally spaced lines denote identical DNA sequences.

Graphic summaries of morphology, terpenes (+/- basis), RAPDs, nrDNA, trnC-trnD and combined nrDNA + trnC-trnD reveal (Fig. 9) general agreement between morphology, terpenes, RAPDs and combined nrDNA + trnC-trnD classifications. The combined nrDNA + trnC-trnD SNPs showed the largest differences between *J. polycarpos* var. *polycarpos*, *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* of any data set. Using only nrDNA or trnC-trnD SNPs would lead to very

different taxonomies in this study. The concordance of terpenes, RAPDs and combined nrDNA + trnC-trnD classifications seems to

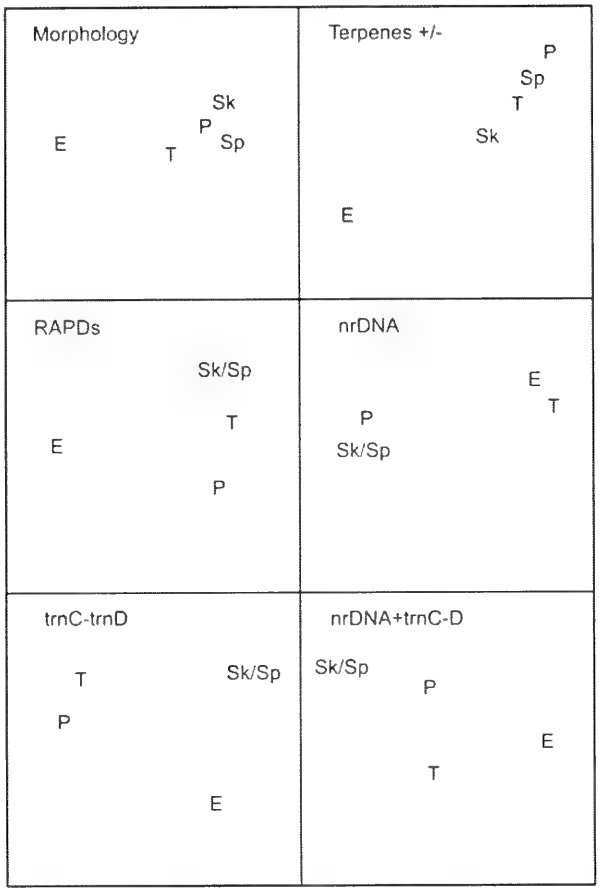


Figure 9. Graphic summaries of morphology, terpenes, RAPDs, nrDNA, trnC-trnD, and combined nrDNA + trnC-trnD data. E = *J. excelsa*, P = *J. p.* var. *polycarpos*, T = *J. p.* var. *turcomanica*, Sk = *J. p.* var. *seravschanica*, Kazakhstan, Sp = *J. p.* var. *seravschanica*, Pakistan.

provide the strongest evidence that *J. polycarpos* is composed of three genetically distinct (but scarcely distinct in morphology) taxa, supporting the continued recognition of these taxa at the variety level.

ACKNOWLEDGEMENTS

Thanks to Tonya Yanke for lab assistance and Naresh Pandey for assistance in running RAPDs. Thanks to A. D. Dembitsky, Kazakhstan; M. Dourikov, Turkmenistan; Vrezh Manakyan, Armenia; Y. Turospekov, Kazakhstan; for assistance on field trips, and A. H. Buzdar, for supplying samples from Balochistan, Pakistan. This research was supported in part with funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 1999. Systematics of multi-seeded eastern hemisphere Juniperus based on leaf essential oils and RAPD DNA fingerprinting. Biochem. Syst. Ecol. 27: 709-725.

Adams, R. P. 2001. Geographic variation in leaf essential oils and RAPDs of *Juniperus polycarpos* K. Koch in central Asia. Biochem. Syst. Ecol. 29: 609 - 619.

Adams, R. P. 2004. Junipers of the World: The genus *Juniperus*. Trafford Publ., Vancouver, B. C.

Adams, R. P. 2007. *Juniperus maritima*, the seaside juniper, a new species from Puget Sound, North America. Phytologia 89(3): 263-283.

Adams, R. P., J. A. Morris, R. N. Pandey and A. E. Schwarzbach. 2005. Cryptic speciation between *Juniperus deltoides* and *J. oxycedrus* (Cupressaceae) in the Mediterranean. Biochem. Syst. Ecol. 33: 771-787.

Adams, R., P., A. E. Schwarzbach and J. A. Morris. 2008. The evolution of Caribbean *Juniperus* (Cupressaceae): Terpenoids, RAPDs, and DNA SNPs data Phytologia 90(1): 103-119.

Farjon, A. 1992. The taxonomy of the multiseed junipers (*Juniperus* sect *Sabina*) in southwest Asia and east Africa. Edinb. J. Bot. 49: 251-283.

Farjon, A. 2005. A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew. Richmond, Surrey, UK.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Syring, J., R. F. del Castillo, R. Cronn and A. Liston. 2007. Multiple nuclear loci reveal the distinctiveness of the threatened, neotropical *Pinus chiapensis*. Syst. Bot. 32: 703-717.

Table 1. Comparisons of the percent total oil for leaf essential oils for *J. excelsa* - Greece (EG), *J. excelsa*, Tbilisi Botanic Garden (ET), *J. polycarpos* var. *polycarpos*: Armenia, L. Sevan (AS); *J. p.* var. *turcomanica*, Turkmenistan, Kopet Mts. (TK), Alma Ata Botanic Garden (ex. Ashgabad, Turkmenistan, TA); *J. p.* var. *seravschanica*: Kazakhstan, Talasskiy Mtns. (KT), Pakistan, Quetta (PQ), and *J. procera*, east Africa (PR). Components that tend to separate the species are highlighted in boldface. From Adams (2001).

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
926	tricyclene	0.1	0.1	0.1	0.1	0.2	0.3	0.1	t
931	**α-thujene**	**-**	**-**	**t**	**t**	**t**	**0.6**	**0.4**	**t**
939	**α-pinene**	**22.5**	**26.5**	**68.4**	**68.8**	**59.7**	**44.4**	**15.5**	**12.5**
953	α-fenchene	0.2	t	t	t	t	-	0.2	0.1
953	camphene	0.5	1.0	0.2	0.1	0.5	0.5	0.2	0.1
957	thuja-2,4(10)-diene	0.1	-	t	t	t	-	-	-
975	verbenene	t	-	-	-	-	-	-	-
976	sabinene	t	0.1	0.2	0.1	0.4	0.9	0.5	t
978	1-octen-3-ol	-	-	-	-	-	-	-	0.3
980	β-pinene	0.6	1.0	0.5	0.6	1.8	2.2	1.2	1.2
991	**myrcene**	**1.9**	**2.2**	**1.2**	**1.5**	**3.7**	**19.2**	**20.7**	**1.2**
1005	α-phellandrene	0.1	0.1	-	-	t	0.1	0.1	-
1011	δ-3-carene	2.3	0.4	t	t	t	-	3.5	6.1
1018	α-terpinene	0.1	0.1	t	t	t	0.1	0.1	t
1026	p-cymene	0.4	0.2	0.1	0.1	0.2	0.1	0.7	t
1028	sylvestrene	-	-	-	-	-	-	-	0.1
1031	**limonene**	**22.6**	**5.5**	**1.2**	**1.5**	**1.8**	**4.4**	**9.0**	**0.2**
1031	β-phellandrene	t	-	-	-	0.1	0.5	1.0	0.8
1032	1,8-cineole	-	t	-	-	-	-	-	t
1050	(E)-β-ocimene	t	-	t	t	t	t	0.2	t
1062	γ-terpinene	0.6	0.9	0.2	0.3	0.6	1.4	1.3	t
1068	cis-sabinene hydrate	-	-	-	-	-	0.1	0.2	-
1068	fenchone	-	-	t	t	t	t	t	-
1088	terpinolene	0.9	1.1	0.4	0.5	1.3	1.5	1.7	1.1
1097	trans-sabinene hydrate	-	-		-	t	-	-	-
1098	linalool	-	0.1	0.1	t	t	0.5	0.7	0.5
1103	isopentyl-isovalerate	-	-	t	t	t	-	-	-
1110	1,3,8-p-menthatriene	-	-	-	-	-	-	-	t
1112	endo-fenchol	0.2	-	-	-	t	-	-	-
1114	trans-thujone	-	-	t	t	0.2	0.1	-	-
1121	cis-p-menth-2-en-1-ol	0.1	-	-	-	-	t	-	t
1125	chrysanthenone	-	-	t	t	t	-	-	-
1125	α-campholenal	0.1	0.2	0.2	0.1	0.2	t	0.1	t

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
1134	cis-limonene oxide	-	-	-	-	-	-	-	0.1
1139	trans-pinocarveol	0.2	0.3	0.1	0.1	t	-	t	t
1143	camphor	0.5	0.2	t	0.3	1.7	t	t	t
1143	cis-sabinol*	-	-	0.4	0.3	-	-	0.2	0.2
1143	trans-verbenol	-	0.5	-	-	t	t	-	t
1159	p-mentha-1,5-dien-8-ol	-	-	-	-	-	-	-	0.1
1160	trans-pinocamphone	-	-	t	-	-	-	-	-
1163	pinocarvone	-	0.1	t	-	-	-	-	-
1165	borneol	-	t	-	t	t	t	t	0.2
1167	δ-terpineol	-	-	t	t	-	t	t	t
1173	cis-pinocamphone	-	-	t	-	-	-	-	-
1177	terpinen-4-ol	0.2	0.2	0.1	t	0.2	0.4	0.3	0.1
1178	naphthalene	t	t	0.1	0.4	t	t	t	-
1180	m-cymen-8-ol	-	-	-	-	-	-	-	0.1
1183	p-cymen-8-ol	-	-	t	-	-	-	-	t
1185	trans-p-mentha-1(7), 8-dien-2-ol	0.1	-	-	-	-	-	-	-
1189	α-terpineol	t	0.1	t	t	0.2	0.1	t	0.5
1191	hexyl butyrate	-	-	-	-	0.1	-	-	-
1204	verbenone	0.1	0.1	0.1	0.1	t	-	-	-
1217	trans-carveol	0.1	0.1	t	t	-	-	-	-
1220	endo-fenchyl acetate	0.3	0.1	-	-	-	-	-	-
1242	**hexyl 3-methyl butanoate**	**-**	**-**	**0.1**	**0.2**	**0.4**	**-**	**t**	**-**
1257	4Z-decen-1-ol	-	-	-	-	0.2	-	-	-
1274	unknown,79,91,105, 147,FW162	-	-	-	-	-	-	-	0.3
1285	bornyl acetate	0.4	0.9	0.2	0.2	0.7	1.0	0.6	0.4
1286	linalool oxide acetate (pyranoid)	0.2	0.1	-	-	-	-	-	-
1290	trans-sabinyl acetate	-	-	-	-	-	-	0.1	-
1312	**decadienal isomer?**	**3.3**	**5.6**	**-**	**-**	**-**	**-**	**-**	**-**
1319	2E,4E-decadienal	-	-	-	-	t	t	t	-
1339	**δ-elemene**	**-**	**-**	**t**	**0.1**	**t**	**t**	**t**	**-**
1376	α-copaene	-	0.2	-	t	-	-	-	-
1383	β-bourbonene	0.1	-	-	-	-	-	-	-
1381	hexyl n-hexanoate	-	-	-	0.1	0.7	-	-	-
1389	β-cubebene	0.1	0.1	-	0.1	-	-	-	-
1409	α-cedrene	-	t	-	-	-	-	-	-
1409	1,7-di-epi-β-cedrene	1.6	0.7	1.3	-	-	0.2	1.4	-
1418	(E)-caryophyllene	-	0.1	0.3	0.4	-	0.1	0.2	0.5

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
1418	β-cedrene	0.9	0.5	-	-	-	0.1	0.2	-
1429	cis-thujopsene	0.3	0.2	0.2	-	-	0.2	0.4	-
1446	cis-muurola-3,5-diene	0.2	0.6	-	0.2	-	t	-	-
1454	α-humulene	0.2	0.2	-	0.1	-	-	-	0.7
1458	E-β-farnesene	0.2	0.1	0.1	-	-	-	0.1	-
1461	cis-muurola-4(14),5-diene	-	-	0.1	0.2	-	-	0.1	-
1466	β-acoradiene	0.1	t	0.1	-	-	-	t	-
1473	**trans-cadina-1(6),4-diene**	**0.4**	**0.8**	**-**	**-**	**-**	**-**	**-**	**-**
1477	γ-muurolene	-	t	-	0.2	0.1	t	-	-
1480	germacrene D	0.9	1.7	0.2	0.8	0.8	0.1	0.2	0.3
1491	trans-murrola-4(14),5-diene	0.4	1.4	-	0.1	0.1	t	-	-
1493	epi-cubebol	-	1.3	-	t	0.2	t	t	-
1499	γ-amorphene	-	-	-	-	-	-	t	-
1499	α-muurolene	0.2	0.1	-	0.3	0.2	0.2	0.2	-
1499	bicyclogermacrene	-	-	t	-	-	-	t	-
1502	cuparene	-	-	-	-	-	-	t	-
1503	germacrene A	-	-	-	0.1	0.1	-	-	-
1509	β-bisabolene	-	-	t	-	-	-	t	-
1512	α-alaskene	0.3	t	0.1	-	-	-	0.4	-
1513	**γ-cadinene**	**-**	**-**	**0.2**	**1.1**	**0.9**	**0.4**	**0.4**	**-**
1513	**cubebol**	**0.8**	**2.6**	**-**	**-**	**-**	**-**	**-**	**-**
1521	cis-calamenene	t	-	-	-	-	-	-	-
1524	δ-cadinene	0.7	1.5	0.3	1.4	1.2	1.0	0.7	-
1532	E-γ-bisabolene	0.2	-	0.1	-	-	-	-	-
1532	trans-cadina-1(2),4-diene	t	0.2	-	t	-	t	-	-
1538	α-cadinene	-	-	-	0.2	0.2	0.1	0.1	-
1549	**elemol**	**-**	**-**	**t**	**0.2**	**1.9**	**0.5**	**0.5**	**4.3**
1556	**germacrene B**	**-**	**-**	**0.7**	**1.6**	**1.8**	**0.4**	**0.6**	**-**
1574	**germacrene D-4-ol**	**-**	**t**	**0.4**	**3.0**	**1.5**	**0.5**	**2.0**	**0.1**
1581	caryophyllene oxide	-	-	-	-	-	-	-	0.5
1587	sesquiterpene, FW220?	1.9	1.7	0.8	-	-	1.0	1.7	-
1596	**cedrol**	**28.1**	**30.8**	**19.0**	**t**	**-**	**14.6**	**26.4**	**-**
1606	humulene epoxide II	t	-	-	-	-	-	-	0.5
1606	β-oplopenone	t	-	t	0.2	0.2	-	-	-
1607	4E-tridec-6-yne*	-	0.6	-	-	-	-	-	-
1627	**1-epi-cubenol**	**1.6**	**2.2**	**t**	**-**	**-**	**-**	**-**	**-**
1630	γ-eudesmol	-	-	-	t	0.5	-	-	1.4

KI	Compound	EG	ET	AS	TK	TA	KT	PQ	PR
1640	epi-α-cadinol	t	0.3	0.1	0.7	0.9	0.5	0.4	-
1640	epi-α-muurolol	t	0.5	t	0.7	0.9	0.1	0.1	-
1645	α-muurolol	t	0.2	-	0.2	0.4	t	t	-
1649	**β-eudesmol**	**-**	**-**	**t**	**t**	**0.8**	**t**	**0.1**	**2.3**
1652	**α-eudesmol**	**-**	**-**	**t**	**t**	**0.5**	**0.1**	**t**	**3.8**
1653	α-cadinol	t	0.2	0.2	1.6	3.2	0.9	0.8	-
1666	bunesol	-	-	t	t	0.4	-	0.1	1.3
1666	**unknown(57,41,85, 79,136)**	**0.6**	**2.4**	**-**	**-**	**-**	**-**	**-**	**-**
1685	eudesma-4(15),7-dien-1-β-ol	-	-	-	-	-	-	-	0.2
1688	**sesquiterpene alcohol, FW 222**	**-**	**-**	**0.3**	**0.9**	**3.6**	**0.4**	**-**	**-**
1688	cadinol isomer	-	-	-	t	1.2	-	-	-
1789	8-α-acetoxyelemol	-	-	-	-	-	-	-	3.5
1809	unknown(43,79,71,99, 136,252)	-	0.6	-	-	t	-	-	-
1930	rosa-5,15-diene	-	-	-	-	-	-	-	0.4
1961	sandaracopimara-8(14),15-diene	t	-	-	-	-	-	-	0.3
1989	manoyl oxide	t	0.2	0.1	0.1	-	-	0.3	0.5
2054	abietatriene	t	0.4	t	t	0.3	-	t	1.3
2080	**abietadiene**	**0.3**	**2.2**	**0.3**	**0.7**	**-**	**-**	**0.2**	**15.4**
2103	diterpene, 41,79,191, 257,FW286?	-	-	-	-	-	-	-	2.6
2147	abieta-8(14),13(15)-diene*	-	-	-	-	-	-	-	0.3
2181	diterpene,41,91, 271, 257,FW286	-	-	-	-	-	-	-	0.8
2278	sempervirol	-	-	-	-	-	-	-	0.6
2288	4-epi-abietal	0.1	1.2	0.6	1.1	1.7	-	1.0	1.8
2293	diterpene,41,55,255, 269,FW284?	-	-	-	-	-	-	-	1.0
2302	abieta-7,13-dien-3-one	-	0.1	-	-	0.2	-	-	-
2302	**trans-totarol**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**21.4**
2325	trans-ferruginol	-	0.3	-	-	-	-	-	3.4

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

BOTANICAL ANECDOTES: CIRCUMSTANCES SURROUNDING THE COLLECTION OF *DICHONDRA MICRANTHA* (CONVOLVULACEAE) IN LASALLE COUNTY, TEXAS

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

ABSTRACT

A pathetically humorous account of events relating to the collection of *Dichondra micrantha* in LaSalle County in the spring of 1954 by the late B.C. Tharp and M. C. Johnston is rendered by a detached observer. It is further noted that most botanical excursions are likely to be surrounded by interesting vignettes, the publication of which might serve to enliven the botanical literature. Authors familiar with such are urged to place on record the happenstances concerned.

KEY WORDS: Flatulence, B. C. Tharp, M. C. Johnston, *Dichondra micrantha*, Texas, LaSalle County

---

To me, a long-time herbarium and field botanist at the University of Texas, Austin, almost every plant I ever collected had some sort of history or story connected with it, and must have been so for the thousands of collections assembled by a plethora of collectors both before my time and afterwards (see for example, Turner 2005). Most such nuances are soon forgotten, but these can be imaginatively reconstructed from collecting accounts of some of the more flamboyant collectors who recorded their travels (such as Humboldt, Bonpland and Kunth in the New World, Spruce along the Rio Negro in Brazil, Berlandier, Lindheimer and Charles Wright in Texas and the southwestern U.S., M.E. Jones in Mexico, etc.). Indeed, every plant ever collected for voucher purposes has its own little history, most of this unrecorded by the collector or collectors concerned.

One of the more notable (to me!) such collections was that of the late Lloyd Shinners (1918-1971) who gathered the type of *Aster correllii* Shinners from a precarious crevasse along a mountain ridge in the Guadalupe Mts. of Trans-Pecos, Texas, at the same time falling several hundred feet, this nearly causing his death. He clung to his specimen throughout the fall, subsequently pressing the plant concerned, this expressed in more detail in Turner (1998). Unfortunately, none of this adverse adventure is recorded on the label of the type.

This brings me to the collection of *Dichondra* referred to in the title of this telling. The specimen concerned (TEXAS: LaSalle Co. "Lawn in the city square of Cotulla, *Turner et al. 3479*," 1 Mai [sic] 1954, TEX) is cited by Tharp and Johnston (1961) in their account of the North American species of the genus. While I provided the voucher number and witnessed its collection, I did not otherwise participate in its procurement.

The details surrounding the collection of this particular voucher are still vivid in my memories; indeed, it remains one of the more pathetically humorous collecting incidents of my career, one that always emerges when I see a member of that genus along the roadside, or wherever, bringing a sordid grin to my cheeks and fond memories of Professor B. C. Tharp and his doctoral graduate student, Mr. M. C. Johnston.

At the time of the field sortie, I was 29 years of age, fresh out of my Ph.D. studies at Washington State University, and newly hired as an Instructor to replace the venerable B. C. Tharp (1885-1964), potential tenure of course depending upon my academic approval by the latter. In short, I aimed to please the beloved, long-time taxonomist at Texas on this our first lengthy field trip together. Tharp had requested my presence on the foray, no doubt to ascertain my knowledge of plants in general, and perhaps to get me to know better his last doctoral candidate to work under his immediate supervision, Marshall C. Johnston, who later on became an expert on the family Rhamnaceae, and perhaps better recognized as the coauthor with D. S. Correll on the well known, Flora of Texas.

Tharp and Johnston had planned this trip to south Texas mainly to collect specimens of *Dichondra*, the former having had a long time interest in the group. He was collaborating with his student, hoping of course that the latter might bring the study to fruition given time and circumstance, which he did, as indicated in the above citation.

Having made several stops along the highway from Austin to the border town of Laredo, Texas, Tharp, driving a newly purchased Dodge vehicle, pulled up to the lawn of the City Square in Cotulla, Texas, presumably having seen a mass of prostrate Dichondras in the mowed lawn as we slowed to pass through the small village. We all exited the vehicle, and Tharp and Johnston proceeded to get on their hands and knees, each with a geology field-pick or hammer, digging up long strands of the mat-forming plants which typically root at their nodes (hence their popularity as pent-house "grasses" in cities the world over, the long stubby growths not requiring much lawn care, least of all mowing).

While the two collectors dug away, I was struck with the strange maneuverings of Tharp. Instead of finding his own clumps of *Dichondra* to extricate from the lawn, he began to circle in on the diggings of his student, soon aligning himself a foot or two in front of the fellow, who was presumably unaware of this posturing, hardly noting that the arse of Professor Tharp was now only six inches or so in the front of his face. So positioned, the Professor loosened a mighty fart which startled both me as well as the student. My first reaction was "who on earth would foist off such an affront on a final year doctoral student, least of all in front of an aspiring tenure track professor who hardly knew either."

With such a thought lingering, I was amazed by what then transpired. While Professor Tharp, still on his all fours, chuckled mightily at his spontaneous petard, his student arose suddenly, and assuming the stance of some pompous politician (What came to my mind at the time was the French leader Charles de Gaulle; I can still recollect that memory!) recited a spontaneous verse which I shall never forget, this spoken slowly and with great dignity as a truck full of hay appeared suddenly in the background passing along the town square. I quote:

Farmer Brown came to town
With a load of hay.
Professor Tharp let a fart
And blew it all away.

One might think that such an extraordinary display of acceptance would have been the end of the matter. But not so! Professor Tharp became very indignant with his student's response, becoming livid with anger and noting that Mr. Johnston was very disrespectful of his major professor, and that he did not appreciate this presumed sarcasm. Marshall said nothing in his defense, nor did I.

Tharp was so disgusted with his student's brilliant elocution (in my opinion) that he immediately ended the excursion. On the return trip to Austin, his student received a chain of angry castigations that were mostly reiterative accusations of disrespectfulness. Marshall remained mute, as did I. Indeed, Tharp exceeded the speed limit most of the drive back to Austin; anything either of us might have said would surely have decreased our chance of survival. I was pleased to exit his vehicle alive, and I am sure the student felt the same. Because of Marshall's stance and spontaneous oration of that day, and judgment to keep his cool thereafter, I knew he would be a leader in his field, which he became.

ACKNOWLEDGEMENTS

I am grateful to my literary friend, Jana Kos, and to my colleague of many years now, Dr. M. C. Johnston for reviewing the paper.

LITERATURE CITED

Tharp, B.C. and M.C. Johnston. 1961. Recharacterization of *Dichondra* (Convolvulaceae) and a revision of the North American species. Brittonia 13: 346-360.

Turner, B.L. 1998. Plant Systematics: Beginnings and endings. Aliso: 17: 189-200.

Turner, B.L. 2005. BOTANICAL ANECDOTES: *Bajacalia moranii* (Asteraceae: Tageteae), comments of James Reveal regarding its discovery. Phytologia 87: 56-57.

A NEW VARIETY OF *CALEA MEGACEPHALA* (ASTERACEAE: HELIANTHEAE) FROM OAXACA, MEXICO

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

Identification of Mexican Asteraceae has occasioned description of the following novelty:

Calea megacephala B.L. Rob. & Greenm. var. **pachutlana** B.L. Turner, **var. nov**. Fig. 1.

Caleae megacephalae similes sed differt foliis angustioribus, involucris minoribus (ca 18 mm longis vs 20-23 mm), pedunculis brevioribus (25-30 cm longis vs 40-50 cm), et trichomatibus subter capitula brevioribus (minus quam 1 mm longis vs 1.5-3.0 mm).

TYPE: **MEXICO. OAXACA: Distr. Pachutla, Mpio. Pluma Hidalgo,** ca 9 mi N of Pachutla, southern slopes of Cerro Espina, 23 Aug 1980, *B.L. Turner 80A-29* (TEX).

ADDITIONAL SPECIMENS EXAMINED: **MEXICO. OAXACA: Distr. Pachutla, Mpio. San Miguel del Puerto.** "Comunidad, el Encinal. Bosque de Encino." ca 972 m, 5 Nov 2003, *Pascual 893* (TEX); "Rancho de Jorge Palacio Aragon. Selva mediana subperenifolia." ca 986 m, 2 Oct 2006, *Pascual 1979* (TEX); 800 m NE of Rancho San Agustin, 930 m, 23 Aug 2001, *Sayes V. 2537* (TEX).

The variety is named for the Distrito in which first collected, and perhaps confined.

The type of *Calea megacephala* is from Chiapas, Mexico, first described in 1896 by Robinson and Greenman. Brandegee (1914),

unaware of its existence, redescribed the taxon as belonging to the newly erected monotypic genus *Tonalanthus*, this subsequently called to the fore by Rzedowski (1986). Wussow et al. (1985) retained the species in *Calea*, noting its similarity to a number of caleoid herbaceous taxa from Central and South America, these belonging to the subgenus *Leontophthalamum*, which seems to be the case.

Nevertheless, species of the subgenus *Calea* (typified by the shrubby species, *C. jamaicensis*) are very different from those of the subgenus *Leontophthalamum* (especially in gynecial characters), and it is likely that the latter will ultimately be subsumed under the earliest available generic name, *Tonalanthus*.

Key to varieties:

1. Peduncles 25-35 cm long; hairs beneath head less than I mm long; leaves 4-6 cm wide; vicinity of Pachutla, Oax .....var. **pachutlana**
1. Peduncles 40-50 cm long; hairs beneath head 1.5-3.0 mm long; leaves 6-9 cm wide; Chiapas and closely adjacent Oaxaca .....var. **megacephala**

Populations of the two varieties recognized here are well separated geographically and apparently by elevation (above 1000 m in pine forests; below 1000 m in oak forests), to judge from label data, although the typical var. *megacephala* has been collected at least once in easternmost Oaxaca (as reported by Wussow et al. 1985) near the border of Chiapas (Fig.1, based upon holdings at TEX). Strother (1999) provides an excellent description of var. *megacephala* based upon a number of specimens that I have not examined. If accurately described, it is clear that the two varieties intergrade in at least a few of their characters, but perhaps not in combination. Finally, it should be noted that the only chromosome count reported for *C. megacephala* (n = 19 pairs) was obtained from the type of var. *pachutlana*.

ACKNOWLEDGEMENTS

I am grateful to my colleague Guy Nesom for the Latin diagnosis and for helpful comments on the manuscript itself.

LITERATURE CITED

Rzedowski, J. 1968. Nota sobre la identidad de los generos *Aiolotheca* DC. y *Tonalanthus* T.S. Brandegee (Compositae). Brittonia 20: 166-168.

Strother, J.L. 1999. *Calea*, in Fl. Chiapas 5: 29-34.

Wussow, J.R., L.E. Urbatsch, and G.A. Sullivan. 1985. *Calea* (Asteraceae) in Mexico, Central America, and Jamaica. Syst. Bot. 10: 241-267.

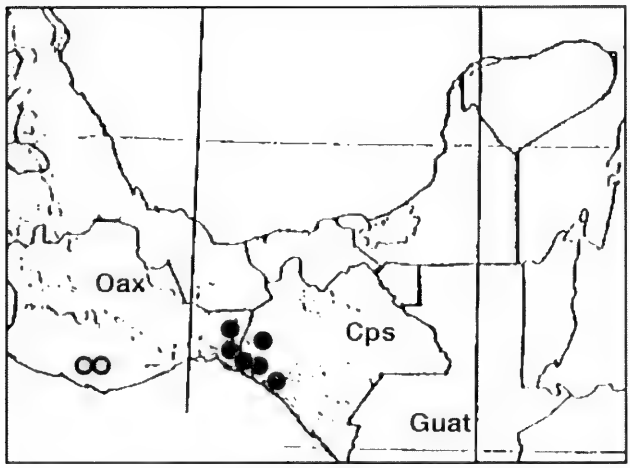


Fig. 1. Distribution of *Calea megaphylla*: var. *megaphylla* (dots); var. *pachutlana* (circles).

TAXONOMIC AFFINTITY OF RUSHFORTH'S BHUTAN JUNIPER AND *JUNIPERUS INDICA* USING SNPs FROM nrDNA AND cp trnC-trnD, TERPENOIDS AND RAPD DATA

Robert P. Adams
Biology Department, Baylor University, Box 727
Gruver, TX 79040, USA
Robert_Adams@baylor.edu

Julie A. Morris
Department of Biological Sciences, University of Texas at Brownsville
Brownsville, TX, 78520, USA.

Andrea E. Schwarzbach
Department of Biological Sciences, University of Texas at Brownsville
Brownsville, TX, 78520, USA.

ABSTRACT

SNPs from nrDNA and cp trnC-trnD were analyzed from *J. indica*, *J. recurva* and Rushforth's juniper from Bhutan and compared with previous terpene and RAPD data. These data, taken together, show that Rushforth's juniper is allied, but distinct from *J. indica* and a new variety is named: ***J. indica*** var. ***rushforthiana*** R. P. Adams from Bhutan.

KEY WORDS: *Juniperus indica, J. indica.* var. *rushforthiana, J. recurva, J. wallichiana*, Cupressaceae, nrDNA, trnC-trnD, SNPs, essential oils, terpenes, DNA fingerprinting, systematics.

The taxonomy of *J. indica* Bertol. and *J. wallichiana* Hook f. & Thomson ex Brandis has been confusing. Farjon (2005, p. 311) cleared up this confusion, stating "This species (*J. indica*, my addition) has long been known as *Juniperus wallichiana* Hook. f. & Thomson but that name was not validly published until it was taken up by Brandis (1874) by which time Bertoloni (1862) had validly published *Juniperus indica* based on the *same collections* (italics mine) made by Hooker & Thomson in Sikkim". So it appears that Bertoloni and

Brandis used the same collections to name *J. indica* and *J. wallichiana*, respectively!

Farjon (2005) designated the illustration of Bertoloni (1862) as the lectotype of *J. indica* and then designated *J. D. Hooker s.n.*, India, Sikkim, Lachen River, Lachen, K as the lectotype for *J. wallichiana*. Farjon (p 313, 2005) concludes that "There is no doubt that this (*illustration of Bertoloni,* my addition) represents the same species and that *J. wallichiana* by its delayed validation had become superfluous." If, as Farjon indicates, both *J. indica* and *J. wallichiana* were based on the same specimens, then they are indeed the same taxon.

In 1997, Keith Rushforth allowed me (RPA) to collect leaf samples from two trees he cultivated at Abbotsmarsh Arboretum, Devon, England. Adams 8140 (= ex seed from Rushforth 0802, Soe, Bhutan) and Adams 8141 (= ex seed from Rushforth 0899, Lingshi, Bhutan) became the source of putative "*J. wallichiana*, Bhutan" for analyses of terpenes (Adams, 1999) and RAPDs (Adams, 2000). However, in view of Farjon's historical research (2005), these samples should be merely labeled "Rushforth's Bhutan juniper". However, an examination of the leaf oil compositions and DNA fingerprinting showed some differences (Adams, 2000) and led to the recognition of both *J. indica* and *J. wallichiana* (Rushforth's juniper) as separate species in the monograph of *Juniperus* (Adams, 2004). In the key, Adams (2004) keyed *J. indica* as "monecious, shrubs and shrubby trees" versus *J. wallichiana* (Rushforth's juniper) as "dioecious, trees with a strong central axis".

Examination of two Hooker f. *n.s.* specimens from Sikkim at Kew revealed an annotation of "syn type". One of these Hooker f. *s. n.* specimens is apparently the lectotype of *J. wallichiana* designated by Farjon (2005) These Hooker f. specimens match the morphology of *J. indica Adams 7625-7627* from Nepal. In addition, my *J. indica* specimens from Nepal were large shrubs and small trees (to 4 m) which agree with the description of *J. indica* (Farjon, 2005). However, the Rushforth junipers from Bhutan, although very similar in morphology, were monecious and were large trees with a strong central axis. This does not fit any known variety of *J. indica.* Farjon (2005) recognized a new variety, *J. indica* var. *caespitosa* Farjon as a

decumbent or ascending shrub 50-100 cm tall. Foliage branches (nearly) erect, very dense with short branchlets. Seed cones when mature (sub) globose to broadly ovoid, (4.5-) 5-8 x 4 - 6.5 mm, blue-black. Its distribution is in NW Nepal, S Xizang (Tibet), and Bhutan. However, my both my *J. indica*, Nepal specimens and Rushforth's Bhutan juniper specimens have seed cones that are 9-12 mm long and turbinate, not (sub)globose and they are shrubs-small trees or large trees, respectively. So neither my *J. indica,* Nepal nor the Rushforth Bhutan collections are *J. indica* var. *caespitosa*.

Recent DNA sequence phylogenetic research of *Juniperus* (Schwarzbach et al., in prep.) has revealed (Fig. 1) that *J. indica* and

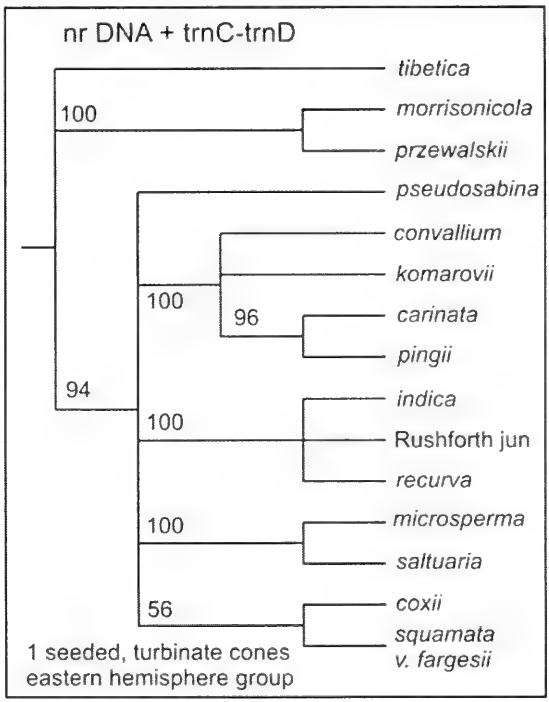


Figure 1. Bayesian tree based on combined nrDNA and cp trnC-trnD sequences from Schwarzbach et al. (in prep.). Notice that *J. indica* and Rushforth's juniper are in a clade with *J. recurva*. Numbers above the branch points are posterior probabilities on a percent basis.

Rushforth's juniper is in a clade with *J. recurva*. The large clade (Fig. 1) contains all the 1-seeded, turbinate-cone *Juniperus* of the eastern hemisphere. Based on this limited sampling, it appears uncertain if Rushforth's juniper is a species distinct from both *J. indica* and *J. recurva*. This result prompted us to broaden the scope of sequencing to look for SNPs in nr DNA and trnC-trnD regions for these three taxa.

Previous work on analyses of the volatile leaf oil compositions of *J. indica* and Rushforth's juniper revealed that their oils are very similar (Adams, 2000). Their oils differ primarily in (*indica*, Rushforth's juniper): α-pinene (2.8%, 9.4%); trans-thujone (16.0, 0.1); trans-sabinyl acetate (15.7, 0.1); trans-murrola-4(14),5-diene (0.9, 3.9); γ-cadinene (0.7, 3.8); 1-epi-cubenol (0.3, 2.4); 8-α-acetoxyelemol (0.0, 0.8); and nezukol (0.0, 4.0). *Juniperus indica* and Rushforth's juniper are very similar in their terpenes (Fig. 2). In fact, most of these differences are in trace components. *Juniperus recurva*,

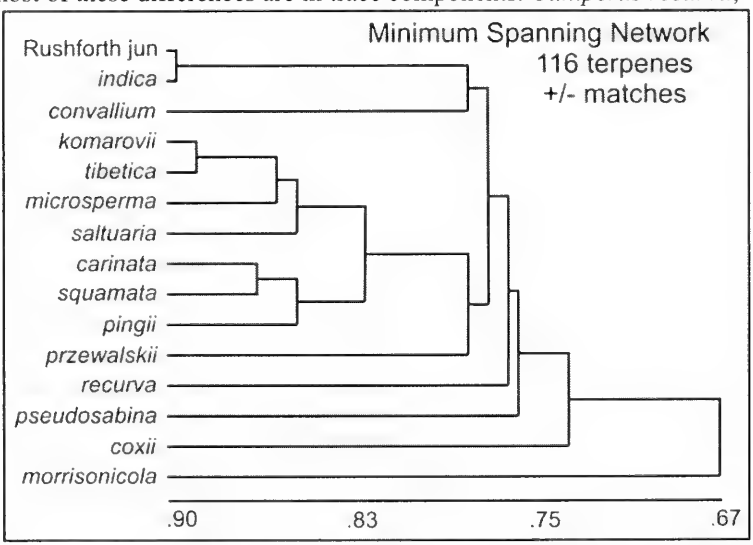


Figure 2. Minimum spanning network of the 1-seeded, turbinate junipers of the eastern hemisphere based on terpenes. Notice that the oils of *J. indica* and Rushforth's juniper are the most similar in this group. From Adams (2000).

although similar in its sequence data (Fig. 1) is quite different in its oils (Fig. 2). Based solely on terpene data, one could treat *J. indica* and Rushforth's juniper as conspecific.

A minimum spanning network based on RAPDs (data from Adams, 2000) shows (Fig. 3) *J. indica* and Rushforth's juniper link loosely, about at the level of other distinct species such as *J. tibetica* and *J. saltuaria*. *Juniperus recurva* is not closely linked to *J. indica* and Rushforth's juniper, in contrast to the sequence data (Fig. 1).

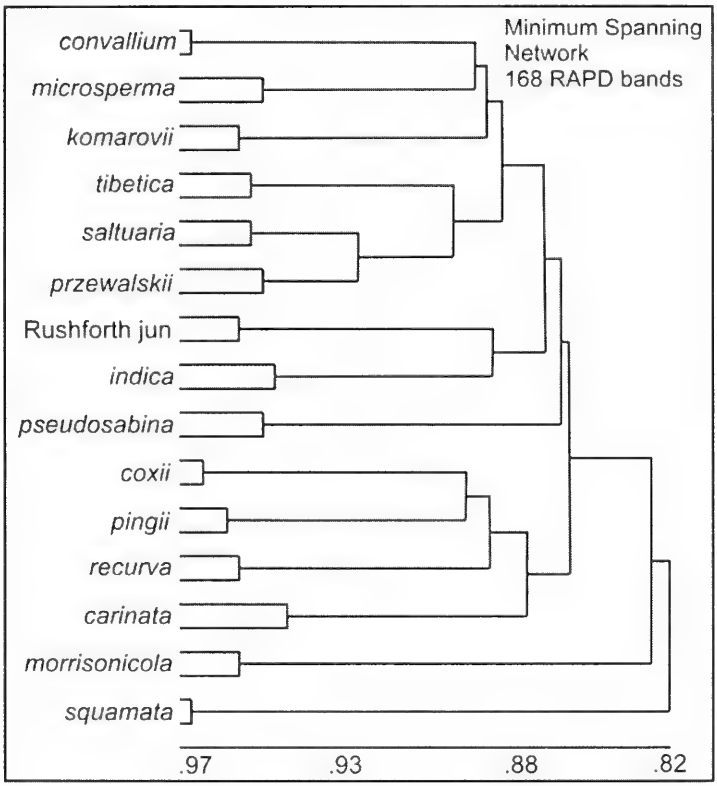


Figure 3. Minimum spanning network based on 168 RAPDs for the 1 seeded, turbinate junipers of the eastern hemisphere. From Adams (2000).

Based on limited morphological (including flowering dates), terpenoid and RAPDs data, Adams (2000, 2004) recognized both *J. indica* and *J. wallichiana* (i.e., *J. wallichiana* for Rushforth's juniper). Of course, unknown to Adams at the time, the name *J. wallichiana* could not be used.

The aforementioned sequence analysis of Schwarzbach et al. (in prep) was based on one accession per species for *J. indica*, *J. recurva* and Rushforth's juniper. As a result, the relationships of the species were established in a basic framework. However, the sampling in this previous study did not allow any assessments on intraspecific variation or the monophyly of the taxa involved. The purpose of this present paper is to re-examine the taxonomy of *J. indica* and Rushforth's juniper using SNPs from sequence data (nrDNA and cpDNA trnC-trnD) by the addition of multiple accessions for each taxon as well as with comparison with the morphologically quite distinct *J. recurva*.

MATERIALS AND METHODS

Specimens used in this study: *J. indica*, *Adams/Chaudary, 7625-7627*, between Yangjin Gompa and Langtang Glacier, 4000 m, Nepal; *J. recurva*, *Adams 7215, 7217-7219*, Sing Gompa, 3570 m, Nepal; Rushforth's juniper, *Rushforth/Adams 8140-8141* ex Bhutan, 11400 ft (*8140*, Rushforth 0802) and 13,250 ft (*8141*, Rushforth 0899), seed germinated and plants cultivated UK. Voucher specimens for all collections are deposited at BAYLU, except *Rushforth 0802, 0899* that are deposited at E.

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 DNeasy, Qiagen, Valencia, CA) as per manufacturer's instructions.

SNPs obtained from DNA sequencing

ITS (nrDNA) and trnC-trnD 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 or K (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. All 12 (A-L) of the Epi-Centre's buffers were screened and buffer K gave the cleanest, most abundant amplification for both ITSA/ITSB and buffer E was best for trnC-trnD (CD10F/CD3R) primers. However, buffers D, F, G, H, and J were nearly as good as buffer E or K.

Primers (5'-3'):

ITS: ITSA = GGA AGG AGA AGT CGT AAC AAG G;
ITSB = CTT TTC CTC CGC TTA TTG ATA TG.

ITSA and ITSB primers from Blattner (1999).

trnC-trnD: CDFor: CCA GTT CAA ATC TGG GTG TC
CDRev: GGG ATT GTA GTT CAA TTG GT

CDFor, CDRev primers from Demesure et al. (1995).

CD10F: AAA GAG AGG GAT TCG TAT GGA
CD3R: AAC GAA GCG AAA ATC AAT CA

CD10F and CD3R primers from Andrea Schwarzbach (pers. comm.).

Amplification and sequencing of *J. indica* proved to be difficult using ITSA/ITSB. So two additional primers were designed based on aligned conifer sequences from GenBank:

ITSA-42F GAT TGA ATG ATC CGG TGA AGT Tm 56° C
42 bp upstream from ITSA into the 18S region.

ITSB+57R ATT TTC ATG CTG GGC TCT Tm 52° C
57 bp downstream from ITSB into the 26S region.

The nrDNA primers (ITSA-42F, ITSB+57R) produced a band of approximately 1210 bp. The internal (partial) trnC-trnD primers, CD10F-CD3R produced a band of: 776 bp for Rushforth's juniper, 775 bp for 2 individuals of *J. indica* (1 deletion); and 770 for one individual (the aforementioned deletion, plus a string of 5bp unique deletion), 3 individuals of *J. recurva* contained 775 bp with no variation in their sequences. In contrast, the sample of *J. indica* 7218 had several insertions not found in any sample in this study: a 57 bp, 21 bp, and a 1 bp. In addition, 7218 had a single SNP shared by all plants in this study. Due to these factors, 7218 was omitted from the partial trnC-trnD data analysis.

The following PCR conditions were used: MJ Research Programmable Thermal Cycler, 30 cycles, 94°C (1 min.), 50°C (2 min.), 72°C (2 min.), with a final step of 72°C (5 min.). The PCR reaction was subjected to purification by agarose gel electrophoresis (1.5% agarose, 70 v, 55 min.). In each case the band was excised and purified using a Qiagen QIAquick gel extraction kit. The gel purified DNA band with the appropriate primer was sent to McLab Inc. for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Alignments were made using Clustal W and then manually corrected. Indels were coded with a "-" for the first nucleotide and "I" for succeeding nucleotides such that an indel was treated as a single mutation event. Overall sequences have been deposited in GenBank (Schwarzbach et al., in prep.).

SNPs analyses

Aligned data sets (nrDNA and trnC-trnD) were analyzed by CLEANDNA (Fortran, R. P. Adams) to remove invariant data. Mutational differences were computed by comparing all SNPs, divided by the number of comparisons over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). A minimum spanning network was constructed by selecting the nearest neighbor for each taxon from the pair-wise similarity matrix, then connecting those nearest neighbors as nodes in the network (Adams, 2004).

RESULTS AND DISCUSSION

Sequencing nrDNA (ITS region) resulted in: 1210 bp of data for each *J. indica* sample; 1209 bp for each Rushforth's juniper sample (1 bp deletion); and 1179 bp for each *J. recurva* sample (29 bp deletion only found in *J. recurva*, coded as a single SNP; 1 bp deletion only found in *J. indica*, and 1 bp deletion shared in *J. indica* and Rushforth's juniper samples). Aligning sequences of *J. indica* (3 individuals), *J. recurva* (4 individuals) and Rushforth's juniper (2 individuals) revealed 10 SNPs among these individuals (the 29 bp indel was treated as a single SNP). PCO of these individuals gave three eigenroots accounting for 64.9%, 23.4% and 6.0% of the variance among

individuals. PCO ordination shows (Fig. 4) three groups: *J. indica* (no variation among individuals), *J. recurva* and Rushforth's juniper. *Juniperus indica* and Rushforth's juniper are separated by 3 SNPs, whereas *J. recurva* is separated from *J. indica* by 5 SNPs. One SNP was found between 2 individuals of *J. recurva* (Fig. 4) and one SNP was found between the 2 Rushforth's juniper individuals (Fig. 4). These differences are comparable to those found between Caribbean junipers (Adams et al., 2008) of 3-7 SNPs; the nw US junipers (Adams, 2007) of 4-5 SNPs; and the Mediterranean junipers (Adams et al., 2005) of 6-8 SNPs.

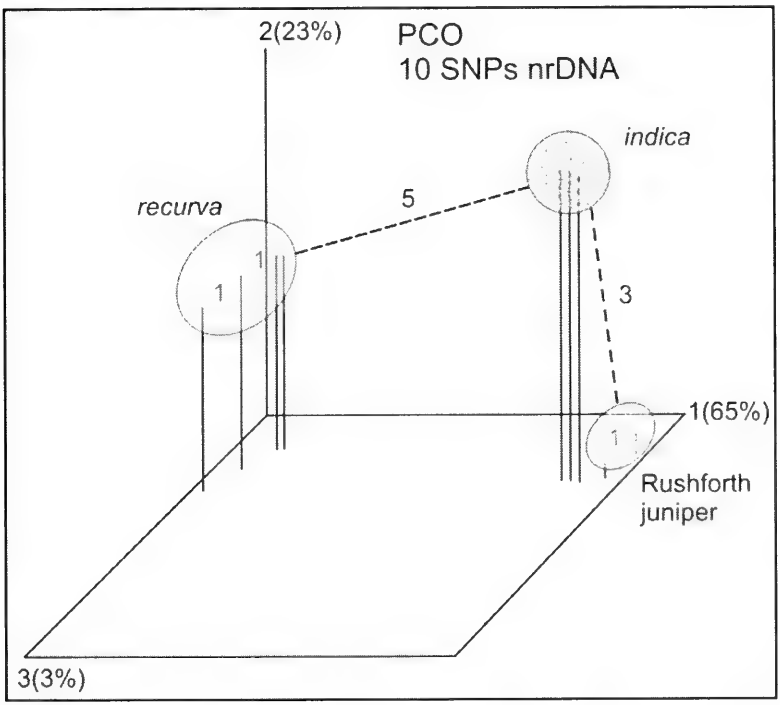


Figure 4. PCO based on 10 nrDNA SNPs. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Closely spaced lines denote identical DNA sequences.

Sequencing and aligning the partial trnC-trnD sequences revealed 5 SNPs. Factoring the association matrix resulted in 3 eigenroots that accounted for 67.1%, 24.7% and 6.0% of the variance among these individuals in their partial trnC-trnD SNPs. Ordination of these individuals revealed (Fig. 5) the three taxa to be equally separated but by only 2 SNPs. There was no variation within *J. recurva* and Rushforth's juniper, but one SNP was present within *J. indica* (Fig. 5).

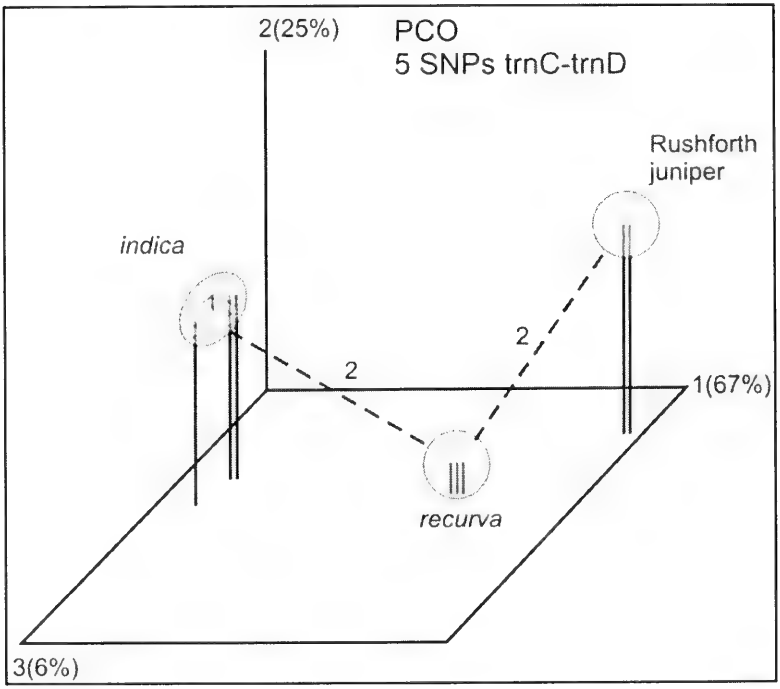


Figure 5. PCO ordination based on 5 SNPs from trnC-trnD sequences. Dashed lines show the minimum linkage between groups. Numbers above the dashed lines are the number of SNP events separating the groups. Closely spaced lines denote identical DNA sequences.

These trnC-trnD SNPs differences are similar to those found between Caribbean junipers (Adams et al., 2008) of 0 - 1 and 5 SNPs.

The trnC-trnD SNPs failed to separate *J. maritima* and *J. virginiana* in the nw US junipers (Adams, 2007), but *J. scopulorum* and *J. virginiana* were separated by 7-9 SNPs.

Combining the 10 nrDNA SNPs and 5 trnC-trnD SNPs for a PCO analysis resulted in 3 eigenroots of 51.2%, 40.6% and 3.0%. Ordination (Fig. 6) shows that *J. indica* and Rushforth's juniper are

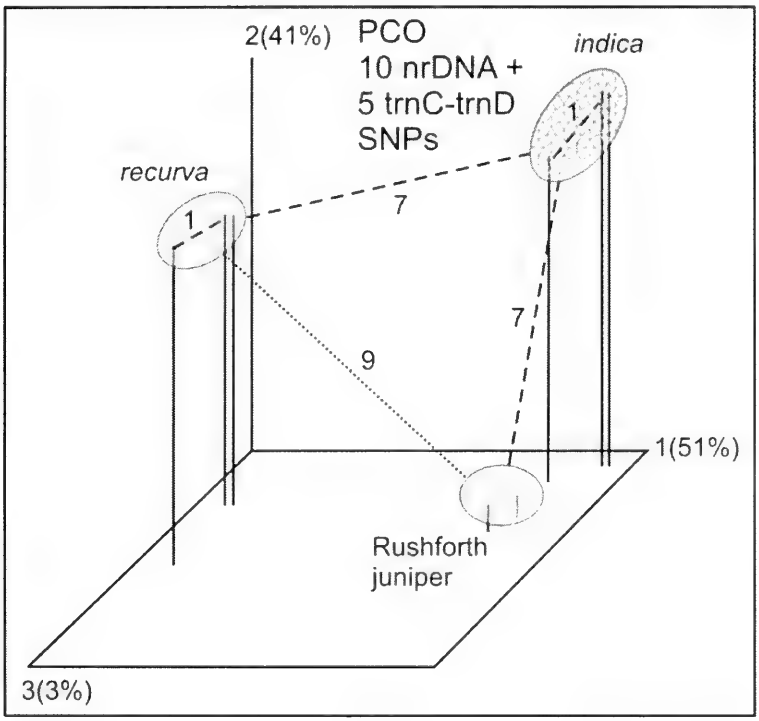


Figure 6. PCO using SNPs from both nrDNA and trnC-trnD. Dashed lines show the minimum linkage between groups. The dotted line shows the linkage between *J. recurva* and Rushforth's juniper is 9 SNPs. Numbers above the dashed lines are the number of SNP events separating the groups. The closely spaced lines denote identical DNA sequences.

separated by 7 SNPs as are *J. indica* and *J. recurva*. In contrast, *J. recurva* and Rushforth's juniper are separated by 9 SNPs. These differences are about the same as found (Adams et al., 2008) between *J. excelsa* and *J. polycarpos* varieties (7 - 9 SNPs).

To summarize the data bearing on the taxonomic status of *J. indica* and Rushforth's juniper: Terpenes - favor a very close relationship (Fig. 2) perhaps at the infraspecific level; RAPDs - indicate more differentiation at the variety or specific level (Fig. 3); combined SNPs from nr DNA plus partial trnC-trnD support *J. indica* and Rushforth's juniper as distinct species (Fig. 6). The morphology supports *J. indica* and Rushforth's juniper being conspecific.

Based on the composite of all these data to date, I propose a new varietal name for Rushforth's Bhutan juniper:

Juniperus indica Bertol. **var. *rushforthiana*** R. Adams, **var. nov.** Rushforth's juniper, Type: Bhutan, Soe, at Soe Tajitang campsite, tree 15 m, 11,400 ft., *Rushforth 0802 (= Adams 8140)* (HOLOTYPE: BAYLU).

Junipero indicae similis sed sexu dioecio; arbores axe centrali valido.

Similar to *J. indica*, except this variety is dioecious, trees with a strong central axis.

Distribution: Bhutan, also, likely to occur in neighboring Xizang (Tibet) at 11,000 ft to timberline.

Other specimens: *Rushforth 0899* (=*Adams 8141*), Bhutan, Lingshi, on path to Yale La, 13,250 ft, coppiced 2 m.

ACKNOWLEDGEMENTS

Thanks to Guy Nesom for providing the Latin diagnosis and B. L. Turner and others for reviews. Thanks to Tonya Yanke for lab assistance and Naresh Pandey for assistance in running RAPDs and to Ram Chaudary, Naresh Pandey, Keith Rushforth and Lakhan Singh for assistance on field trips. This research was supported in part with

funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

LITERATURE CITED

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.

Adams, R. P. 2000. Systematics of the one seeded *Juniperus* of the eastern hemisphere based on leaf essential oils and random amplified polymorphic DNAs (RAPDs). Biochem. Syst. Ecol. 28: 529-543.

Adams, R. P. 2004. Junipers of the World: The genus *Juniperus*. Trafford Publ., Vancouver, B. C.

Adams, R. P. 2007. *Juniperus maritima*, the seaside juniper, a new species from Puget Sound, North America. Phytologia 89(3): 263-283.

Adams, R. P., J. A. Morris, R. N. Pandey and A. E. Schwarzbach. 2005. Cryptic speciation between *Juniperus deltoides* and *J. oxycedrus* (Cupressaceae) in the Mediterranean. Biochem. Syst. Ecol. 33: 771-787.

Adams, R., P., A. E. Schwarzbach and J. A. Morris. 2008. The evolution of Caribbean *Juniperus* (Cupressaceae): Terpenoids, RAPDs, and DNA SNPs data. Phytologia 90(1): 103-119.

Adams, R. P., J. A. Morris and A. E. Schwarzbach. 2008. Taxonomic study of *Juniperus excelsa* and *J. polycarpos* using SNPs from nrDNA and cp trnC-trnD plus essential oils and RAPD data. Phytologia 90(2) 216-233.

Blattner, F. R. 1999. Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. BioTechniques 27: 1180-1186.

Farjon, A. 2005. A monograph of Cupressaceae and *Sciadopitys*. Royal Botanic Gardens, Kew. Richmond, Surrey, UK.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Rushforth, K. D. 1987. Conifers. Christopher Helm, Publ., London.

NODULATING LEGUMINOUS WEEDS OF SOME MAJOR CROPS OF PAKISTAN

Mohammad Athar

California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento CA 95832, USA. atariq@cdfa.ca.gov
and
Shaikh Mohammad Shabbir
Faculty of Agriculture, University of Azad Kashmir, Rawalakot, Azad Kashmir. shaikhmshabbir@gmail.com

ABSTRACT

A survey was conducted to explore the nodulating leguminous weeds of some major crops of Pakistan. A total of 20 weeds are reported from the major crops which consisted of carrot, cotton, maize, potato, rice, sugarcane, tomato and wheat.

KEYWORDS: Nodulation, leguminous weeds, major crops, Pakistan.

INTRODUCTION

Generally, weeds are defined as plants growing where they are not desired. Many weeds grow in areas where they are not well adapted, but may still thrive in the absence of competition. Usually they are favored by vigorous reproductive powers. Most of them are tolerant to adverse conditions of growth such as extreme heat or cold, drought or excessive moisture, saline or water-logged environments and marginal or disturbed soils. Weeds often possess hard seeds, underground root stocks or tubers, and show greater persistence.

Weeds compete with crops for nutrients, water and light. They are often fast-growing and more efficient in utilizing nutrients than are crop plants and therefore have a distinct competitive advantage (Holst et al., 2007). Weeds may be pathogenic or act as an alternative host for insect pests, nematodes, and fungi. Certain weeds secrete substances that inhibit the growth of other plants. In short, weeds are

often harmful to crop plants and may cause serious yield losses. Their effect on crops is not as spectacular as insect pests or plant diseases, but they may lower yields as much as 80% and often by more than 50% if not controlled (Parker & Riches, 1993).

Weeds belong to practically all plant families but certain families, particularly Asteraceae, Brassicaeae and Poaceae constitute the major weed flora throughout the world (Nasir & Ali, 1972; Muenscher, 1980; DiTomosa & Healy, 2007). Leguminous plants enrich the soil with fixed nitrogen and also increase the rhizobia population (Amarger, 2001). However, under certain conditions many legume species usually considered as harmless, may turn into weeds difficult to eradicate. No research has been carried out in Pakistan to determine the role of nodulating legumes as weeds. The present study is aimed at exploring the nodulating weeds of the major crops of Pakistan.

MATERIALS AND METHODS

Periodic field trips were made to various parts of Pakistan during different seasons of the year and major crops were screened for nodulating leguminous weeds. The major crops included cotton, maize, rice, sugarcane and wheat. The vegetables surveyed were carrot, potato, and tomato. In the present report a "leguminous weed" means either a legume of no agricultural significance or one which, though used in agriculture, is growing in some other crop. At least five nodulated plants were collected per field of crops and the frequency of nodulation was determined by counting the average number of nodules per plant. Only positive reports of nodulation were recorded. The flowering period of these weeds was also observed. Special care was taken to distinguish root nodules from other kinds of malformations such as those caused by nematodes, insects or other root-inhabiting parasitic microorganisms (Truchet et al., 1989). Weeds were arranged alphabetically within species and their distribution in various crops of Pakistan is described. The nomenclature and classification follow Nasir & Ali (1972) and Nasir & Rafiq (1995), and author citations follow Brummitt & Powell (1992).

RESULTS AND DISCUSSION

Leguminous weeds found to be nodulated are listed in Table 1. The plants examined included herbs, vines and shrubs. A total of 20 leguminous species are reported as nodulating weeds of different major crops. The crops included carrots, cotton, maize, potato, rice, sugarcane, tomato and wheat. Such weeds were also observed growing in a number of different summer and winter vegetables, as well as in grassy lawns. Most of the weeds were abundantly nodulated, indicating wide spread and large populations of rhizobia (Table 1).

Various soil bacteria have been reported as natural antagonists against parasitic weeds (Mabrouk et al., 2007). As reported in a number of plant microbe-interactions, antagonistic bacteria interact by competition and antibiosis (Buchenauer, 1998). Rhizobia released from legume nodules activate various defense responses, ranging from hypersensitive cell-death of infected cells, to accumulation of enzymes responsible for defense reaction (Mabrouk et al., 2007). Apart from their usual role, rhizobia also fix atmospheric nitrogen. Most of the nitrogen added to the biosphere each year is supplied by nitrogen-fixing plants (Amarger, 2001; Vessey et al., 2004). Although their potential has been established, the exploitation of such novel nitrogen sources will be dependent on the identification of limiting factors and agronomically feasible practices to eliminate them. The general account presented here is an essential first step in quantifying the contribution of these nodulating weeds to the nitrogen cycle of the biosphere.

ACKNOWLEDGEMENTS

Special gratitude is expressed to Joseph H. Kirkbride, USDA-Agricultural Research Service, Beltsville, MD, for checking the nomenclature author citation of these plants.

LITERATURE CITED

Amarger N. 2001. Rhizobia in the field. Adv. Agron. 73: 109-168.

Brummit, K.R. and C.E Powell. (eds.). 1992. *Authors of Plant Names.* Royal Botanic Gardens, Kew, UK.

Buchenauer, H. 1998. Biological control of soil-borne diseases by rhizobacteria. J. Plant Dis. Protect. 105: 329-348.

DiTomosa, J.M. and E.A. Healy. 2007. Weeds of California and other Western States. Vol. 1 & 2. Division of Agriculture and Natural Resources, Publication 3488, University of California, Oakland, CA.

Holst, N., I.A. Rasmussen and L. Bastiaans. 2007. Field weed population dynamics: a review of model approaches and applications. Weed Res. 47: 1-14.

Mabrouk, Y., L. Zourgui, P. Delavault, P. Simier and O. Belhadj. 2007. Some compatible *Rhizobium leguminosrum* strains in peas decrease infections when parasitized by *Orobanche crenata.* Weed Res. 47: 44-53.

Nasir, E. and S.I. Ali. 1972. *An Annotated Flora of West Pakistan.* Stewart Herbarium Gordon College, Rawalpindi and Department of Botany, University of Karachi, Pakistan.

Nasir, Y.J. and R.A. Rafiq. 1995. *Wild Flowers of Pakistan.* Elite Publishers Ltd., Islamabad, Pakistan.

Parker, C. and C.R. Riches. 1993. *Parasitic Weeds of the World: Biology and Control.* CAB International, Wallingford, UK.

Truchet, G., S. Camut, F. de Billy, R. Odorico and J. Vase. 1989. The *Rhizobium*-legume symbiosis: Two methods of discriminating between nodules and other root derived structures. Protoplasma 149: 82-88.

Vessey, J.K., K. Pawlowski and B. Bergman. 2004. Root-based N-fixing symbiosis: Legumes, actinorhizal plants, *Parasponia* sp. and Cycads. Plant and Soil 266: 205-230.

Table 1. List of nodulated leguminous weeds of some major crops of Pakistan.

Species	Nod. Freq.	Flowering Period	Crops
Alysicarpus monilifer (L.) DC	+	Oct	Cotton, maize
Indigofera cordifolia Heyne ex Roth.	+	Aug-Oct	Cotton, maize, potato, tomato, summer veg.
Indigofera hochstetteri Baker	+	Aug-Oct	Cotton, maize, potato
Indigofera linifolia (L. f.) Retz.	+	July-Oct	Cotton, potato, tomato, summer vegetables
Indigofera oblongifolia (L.) DC	+	Sep-Nov	Cotton, maize, potato, tomato, summer veg.
Lathyrus aphaca L.	++	Feb-Apr	Wheat
Melilotus alba Desr.	++	March	Carrot, mustard, onion, pea, sugarcane, wheat
Melilotus indica (L.) All.	++	March	Carrot, mustard, onion, pea, sugarcane, winter vegetable
Medicago laciniata (L.) Mill.	++	March	Wheat
Medicago lupulina L.	+++	Mar-Jun	Wheat
Medicago polymorpha L.	+++	Mar-May	Wheat
Medicago sativa L.	+	May	Carrots, wheat
Sesbania bispinosa (Jacq.) W.F. Wight	+++	Jun-Sep	Cotton, rice, summer vegetable
Sesbania sesban (L.) Merr.	+++	Aug	Maize, potato, rice, summer vegetables
Trigonella monantha C.A. Meyer	++	April	Carrot, coriander, wheat, winter vegetables
Vicia monantha Retz.	++	Feb-Apr	Sugarcane, wheat
Vicia peregrina L.	++	Apr-May	Sugarcane, wheat

Vicia sativa L.	++	Jul-Aug	Sugarcane
Vigna aconitifolia (Jacq.) Marechal	++	Sep-Oct	Cotton, potato, tomato, summer vegetables
Vigna trilobata (L.) Verde.	++	October	Cotton, potato, tomato, summer vegetables

Nodulating status:

\+ Indicates 1 to 5 nodules per plant
++ Indicates 6 tp 10 nodules per plant
+++ Indicates more than 10 nodules per plants

Call for Cover Photos

Each issue we feature a photo of an interesting plant on the cover of Phytologia. When you are drafting a manuscript, consider sending a photo for the cover Phytologia.

Photos can be accepted in all computer graphic formats (ex. jpg, tif, etc.) as well as 35 mm slides or glossy prints. So keep the cover in mind as you are working on your next contribution to Phytologia.

Robert P. Adams
Editor
Phytologia
Robert_Adams@baylor.edu

SUBSCRIPTION INFORMATION

PHYTOLOGIA is published three times a year. Annual domestic individual subscription (3 issues): $30.00. Annual domestic institutional subscription (3 issues): $90.00. Foreign individual subscription $50, Foreign institutional subscriptions $110, send subscription checks to Robert P. Adams, Mgr. Ed., P. O. Box 727, Gruver, TX 79040, USA. Please make checks payable to Phytologia.

INFORMATION FOR AUTHORS

Articles from botanical systematics and ecology, including biographical sketches, critical reviews, and summaries of literature will be considered for publication in *PHYTOLOGIA*. Authors should arrange for two workers in the appropriate field to review the manuscript before submission. Copies of reviews should be forwarded to the editor **with** the manuscript. Additional review may occur for articles proposing significant nomenclatural changes and or revisions to established taxa. Effort will be made to locate a reviewer for author(s) if he/she desires. Language of manuscripts is English. Format of articles should follow past issues of *PHYTOLOGIA* (preferably single spaced and Times New Roman in 10 point font. **Please visit www.phytologia.org for detailed computer file instructions.**

Cost of publication is currently $15.00 US per final page for publication without reprints. PDF files of papers will be made available to authors at www.phytologia.org. Page charges are due with manuscripts. No extra charges are made for line drawings or grey scale photos. Extra fees will be charged for color photos, the charge will be determined on an individual basis.

All manuscripts should be submitted digitally, either by 3.5"disk, CD, DVD or email to Robert_Adams@baylor.edu.
or, if mailed,
Phytologia, Dr. Robert Adams
P. O. Box 727
Gruver, TX 79040, USA

Two new books in the Phytologia Memoirs series were printed in the spring, 2007!

B. L. Turner. The Comps of Mexico: Chapter 8: Liabeae and Vernonieae. Phytologia Memoirs Vol. 12. 144 pgs. 2007 $25.

B. L. Turner and Tom Watson. Taxonomic revision of *Gaillardia* (Asteraceae). Phytologia Memoirs Vol. 13. 118 pgs. 2007. $25.

Available for $25 each. Order from Phytologia (see address inside cover).