The Genus *Juniperus* in Mexico and Guatemala: Numerical and Chemosystematic Analysis

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Abstract—The variation leaf constituents (mostly terpenoids) was analyzed from each of the taxa of *Juniperus* in Mexico and Guatemala by numerical taxonomic methods. These results were compared with those of a previous study utilizing morphological characters. In general, the two agree on the major groups. Differences between more closely related species were more apparent with the chemical data, whereas more distantly related taxa sometimes appeared to be more closely related chemically due to the presence of a single component such as α-pinene in high concentration in each of the oils. Four large groups are apparent: the deppeanan; flaccidan; monticolan; and the one-seeded (pinchotii) complex. Some taxa (*J. durangensis, J. standleyi, J.jaliscana*) are still of uncertain affinities. This study confirms the morphological data indicating that *J. patoniana* Martínez should be reduced to a variety of *J. deppeana* (*J. deppeana* var. *patoniana* (Martínez) Zanoni, *comb. et stat. nov*). No samples typical of *J. mono-sperma* were found in Mexico and *J. monosperma* var. *gracilis* (*sensu* Martínez) was found to not be closely allied with *J. monosperma* from the USA but has some uncertain affinities with species of the one-seeded complex. These relationships need to be examined in more detail. *J. blancoi* appears to be closely related to *J. scopulorum*. This information on the junipers of Mexico and Guatemala should prove invaluable to future studies on the evolution of the *Juniperus* in North America.

Introduction

Recent work [1] on the taxonomic use of volatile leaf oils, mainly terpenoid compounds, has shown that such data are useful in understanding relationships among the species of numerous genera of conifers, where morphological information is often of limited use. Much of the research on *Juniperus* in North America has been at the population level for detection of hybridization and introgression [2–12]. However, von Rudloff [1] showed that *J. scopulorum, J. horizontalis,* and *J. virginiana* are very similar chemically and Vasek and Scora [13] obtained some proximate measures of chemical similarities between *J. californica, J. occidentalis,* and *J. osteosperma*. The relationships between the other *Juniperus* species, especially the related taxa from Mexico and Guatemala, have not been examined using volatile leaf oil data.

Our previous studies on relationships among the junipers of Mexico and Guatemala were based upon morphological characters as well as ecological and geographic distributional data [14–17]. Results using statistical and numerical taxonomic methods substantiated many of the earlier concepts of Martínez [18] about the relationships between the various species, although several discrepancies were uncovered. However, these studies showed that morphological characters were not sufficient to delineate the relationships in all groups of Mexican and Guatemalan junipers. Consequently, it was decided to examine other characters, especially the volatile leaf terpenoids, which, besides providing a wealth of data (each compound can be regarded as a single character) are also quantifiable.

Recent investigations have shown the presence of several new species of *Juniperus* in Mexico; *J. ashei, J. pinchotii,* and *J. scopulorum* [3, 4, 17]. It was deemed highly desirable to include these taxa in the present study, particulary because it was expected that they would elucidate certain affinities anticipated on morphological grounds. In addition, relationships within several of the previously suggested morphological groups needed to be re-examined, particularly in the one-seeded junipers. Further evidence was also needed to confirm conclusions of the affinities of *J.*

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blancoi, J. patoniana, J. erythrocarpa, and *J. saltillensis.*

Results

Twenty-one OTUs (operational taxonomic units) used in the morphological analyses [16] (Fig. 1 and Table 1) weie taken and a similarity matrix was computed using character weighting of $\sqrt{F}-1$ for the data obtained here and in previous studies. The phenogram obtained from morphological data alone (Fig. 2) shows the presence of several groups of OTUs. These include the junipers commonly with one seed per cone: CO, EE, EW, GA, MLT, MG and SL. The deppeanan junipers include DD, DP, DR and DZ. The flaccidan junipers include FF and FP. The other OTUs do not form clusters of any consequence (BL, DU, JA, MC, MM, MO and ST). This phenogram (Fig. 2) is similar to

that of Zanoni and Adams [16] where 24 OTUs and character weighting of $\sqrt{F}-1$ were used, except for the clustering of the forms of *J. monticola* (MC, MM, and MO) in the previous study.

Groups of OTUs formed using terpenoid characters (Fig. 3) include MC, MM, MO; EE, EW, MG and SL; CO, FF and FP; and DD, DP, DR and DZ. The other OTUs (BL, DU, GA, JA, MLT, MS and ST) do not cluster closely with any group.

The high similarity of CO to FF and FP is due to the fact that each OTU has a high percentage of α-pinene, and consequently the other compounds have low percentage values. These OTUs (CO, FF and FP) are mathematically more similar when compared to each other, especially because the range of variation of a particular compound is smaller in CO, FF

FIG. 1. COLLECTION LOCALITIES OF SPECIMENS USED IN THE TERPENOID ANALYSES. Numbers indicate number of trees sampled at each locality. See Table 1 for the OTU names.

TABLE 1. SOURCES OF SPECIMENS USED FOR TERPENOID ANALYSIS. All locations are in Mexico, unless noted. Site mnemonics are indicated only for site OTUs used in Figs. 6–8

OTU	Taxon	Site OTUs	Source
ASH	*J. ashei*		Texas, USA
BL	*J. blancoi*		El Salto, Durango
			Las Adjuntas, Durango
CO	*J. comitana*		La Trinitaria, Chiapas
DD	*J. deppeana*	DDR	Rio Frio, Puebla
	var. *deppeana*	DDE	El Chico, Hidalgo
		DDL	Los Lirios, Coahuila
DP	*J. deppeana*	DP	Puerto Buenos Aires, Durango
	var. *patoniana*		El Salto, Durango
DR	*J. deppeana*	DRE	W of Durango, Durango
	var. *robusta*	DRC	Casa Colorado, Chihuahua
DU	*J. durangensis*		Puerto Buenos Aires, Durango
DZ	*J. deppeana*	DZ1	Sombrerete, Zacatecas
	var. *zacatecensis*	DZ2	Ojo de Agua, Zacatecas
EE	*J. erythrocarpa*		Los Lirios, Coahuila
EW	*J. erythrocarpa*		La Zarca Durango
			Santa Lucia, Chihuahua
FF	*J. flaccida*	FFM	Morelos, Oaxaca
	var. *flaccida*	FFH	Huasca, Hidalgo
		FFS	San Roberto, Nuevo Leon
FP	*J. flaccida*	FPA	Amozoc, Puebla
	var. *poblana*	FPO	San Dionisio Ocotepec, Oaxaca
GA	*J. gamboana*		Comitan, Chiapas
JA	*J. jaliscana*		Cuale, Jalisco
MC	*J. monticola*	MCP	Cerro Potosi, Nuevo Leon
	f. *compacta*	MCI	Volcan Iztaccihuatl, Mexico
		MCT	Nevado de Toluca, Mexico
MG	*J. monosperma*		Dr. Arroyo, Nuevo Leon
	var. *gracilis*		
MLT	*? J erythrocarpa*		La Trinidad, Nuevo Leon
MM	*J. monticola*	MME	El Chico, Hidalgo
	f. *monticola*	MMT	Tantoco, D.F.
MO	*J. monticola*	MO	Pico de Orizaba, Puebla
	f. *orizabensis*		
MS	*J. monosperma*		Trinidad, Colorado, USA
PIN	*J. pinchotii*		Texas and New Mexico, USA
SC	*J. scopulorum*		Masonville, Colorado, USA
			Guadalupe Mts., Texas, USA
SL	*J. saltillensis*		San Roberto, Nuevo Leon
			Los Lirios, Coahuila
ST	*J. standleyi*		Huehuetenango, Guatemala
P9	*J. erythrocarpa*		Alpine, Texas, USA
P12	*J. erythrocarpa*		Sierra Blanca, Texas, USA

and FP when compared to the range of variation of the compound among all other OTUs in the analysis. The junipers with one seed did not form a group as they did in the morphological analysis (Fig. 2), suggesting that the morphological data does not always reflect the same patterns of similarity as the chemical data.

The composite analysis of morphological and terpenoid data is shown in Fig. 4. The similarity measures (S) were derived by combining equally the similarity matrices of the morphological similarity (M) and of the terpenoid similarity (T), ($S = (M+T)/2$). As expected [19], the combined use of morphological and terpenoid characters would accentuate the groupings which were found when the data were considered separately. Groups of OTUs in this category include the deppeanan

junipers (DD, DP, DR, DZ) and the flaccidan junipers (FF and FP). The junipers with one
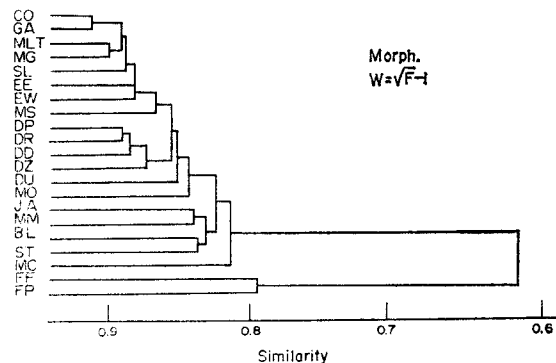

FIG. 2. PHENOGRAM OF 21 OTUs, USING MORPHOLOGICAL DATA. OTU names are given in Table 1.
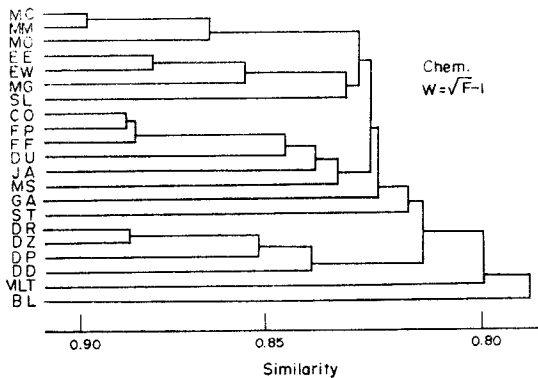
FIG. 3. PHENOGRAM OF 21 OTUs, USING TERPENOID DATA.
OTU names are given in Table 1.

FIG. 5. PHENOGRAM OF 25 OTUs, USING TERPENOID DATA.

seed were reassembled to include CO, EE, EW, GA, MG, MLT, MS and SL. This group formed a unit in the morphological analysis (Fig. 2), but was split in the analysis of terpenoid (Fig. 3). The *J. monticola* forms (MC, MM, MO) clustered together (Fig. 4), as they had done in the terpenoid analysis (Fig. 3). It is interesting to note that the taxa BL, DU, JA and ST exhibit no particular pattern of grouping in any of the analyses (Figs. 2–4).

It was thought that if more taxa (or OTUs) were added to the similarity matrices, more groups and/or better defined groups might emerge. Terpenoid data from four other OTUs (*J. ashei* (ASH), *J. scopulorum* (SC), *J. erythrocarpa* from Alpine, Texas (population P12 of Adams [3]) and *J. pinchotii* (PIN) were therefore added to that from the 21 OTUs used for the previous analysis and the results are given in Fig. 5. The pinchotii complex (EE, EW, PIN and P12) cluster together as does *J. mono-*

sperma var. *gracilis* (MG, *sensu* Martínez) with *J. pinchotii* (PIN) and *J. erythrocarpa* (EE, EW and P12). *J. ashei* (ASH) and *J. saltillensis* (SL) are also quite similar and, as expected, the deppeanan taxa (DD, DP, DR and DZ) and the *J. monticola* forms (MC, MM, MO) form clusters as before. The flaccidan junipers, FF and FP, still cluster with *J. comitana* (CO) (cf. Fig. 3) and *J. blancoi* (BL) and *J. scopulorum* (SC) form a group. The OTUs, DU, GA, JA, MLT, MS and ST all show low levels of similarity to the others.

Group Analyses

The terpenoid analyses have shown several groups of OTUs to be fairly consistent in their similarities. Analyses of each of these groups separately should be useful in understanding what constitutes an apparently natural group, without the analysis being influenced by data from other taxa which do not show close affinities. Analyses have therefore been carried out on the deppeanan group, the flaccidans, the monticolans, the one-seed junipers (erythrocarpa–pinchotii–monosperma) and the blancoi–scopulorum group.

Deppeanan Junipers

Juniperus deppeana is represented by three varieties: var. *deppeana* (DD) with three site collections, DDE, DDL and DDR; var. *robusta* (DR) with two site collections DRE and DRC; and var. *zacatecensis* with two site collections DZ1 and DZ2. The three site collections of var. *deppeana* shows a high degree of similarity to each other and cluster together (Fig. 6), whereas, those of the other two varieties,
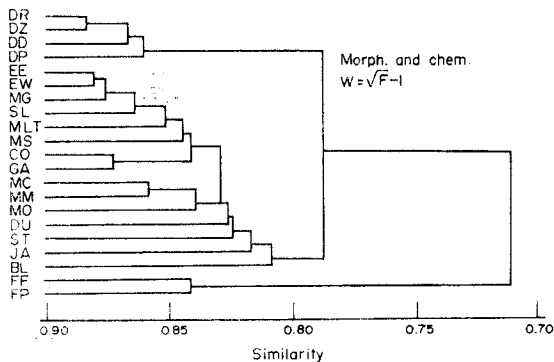

FIG. 4. PHENOGRAM OF 21 OTUs, USING MORPHOLOGICAL AND TERPENOID DATA. OTU names are given in Table 1. Similarities were obtained by averaging the morphological and chemical similarity for each taxon pair and then clustering.
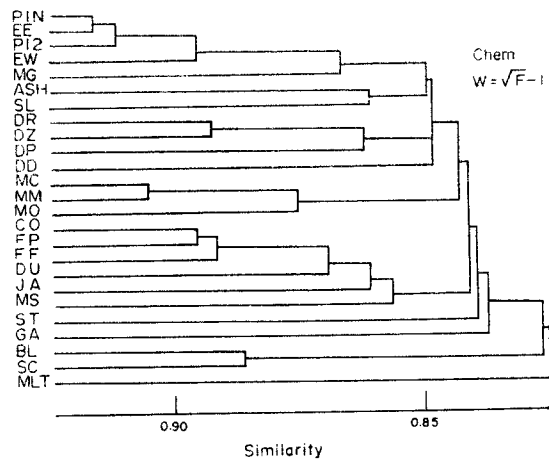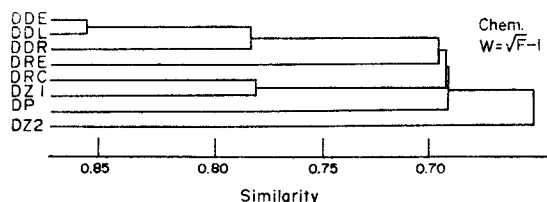
FIG. 6. PHENOGRAM OF THE DEPPEANAN JUNIPERS BY SITE, USING TERPENOID DATA. Note that DP (*J. patoniana*) fits well within the varieties of *J. deppeana*. *J. deppeana* var. *robusta* and *zacatecensis* are split by sites.

robusta and zacatecensis do not form such groups. *J. patoniana* (DP) clusters among the site collections of the western deppeanan junipers (DR and DZ), being most similar to DZ1.

Flaccidan Junipers
Juniperus flaccida var. *flaccida* (FF) is represented by three site collections FFH, FFM, and FFS, and *J. flaccida* var. *poblana* (FP) by two site collections, FPA and FPO. These are high similarities among FFH, FFM and FFS which result in their clustering together (Fig. 7) but the OTUs FPA and FPO are less alike.

J. monticola Junipers
The morphological analysis (Fig. 2) of the three forms of *Juniperus monticola* (MM, MC and MO) did not exhibit a distinctive clustering, although there was some similarity. The terpenoid data (Figs. 3 and 5) and the combination of morphological and terpenoid data (Fig. 4) indicated a higher degree of similarity. Figure 8 summarizes the analysis of the six site collections of *J. monticola* (see Table 1). No distinctive patterns are apparent, the three forms do not give rise to clusters of any significance. *J. monticola* f. *compacta*, MCP, shows a very low level of similarity to the other OTUs.

One-Seeded Junipers (Erythrocarpa–Pinchotii–Monosperma)
The OTUs EE, EW, PIN, P9 and P12 are a part of the *Juniperus erythocarpa–J. pinchotii* complex of the southwestern United States and northern Mexico. These OTUs show high
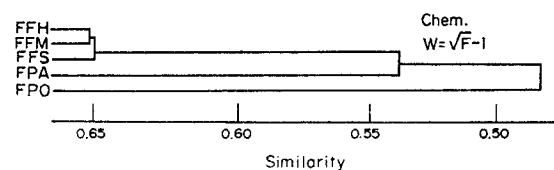

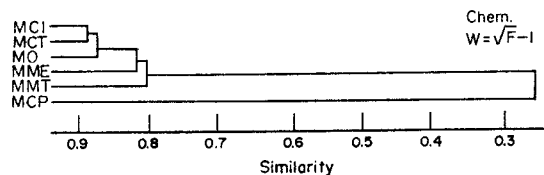FIG. 7. PHENOGRAM OF THE FLACCIDAN JUNIPERS BY SITE, USING TERPENOID DATA.

FIG. 8. PHENOGRAM OF JUNIPERUS MONTICOLA BY SITE, USING TERPENOID DATA. The forms of *monticola* are fairly uniform except for MCP (*monticola* f. *compacta*) from the quite disjunct locality on Cerro Potosi, N.L.

similarity to each other. *J. pinchotii* (PIN) from Texas is most similar to *J. erythrocarpa* (EE) of eastern Mexico. The other *J. erythrocarpa* OTUs, EW, P9 and P12, are most similar to EE. OTUs that show similarity to *J. pinchotii* and *J. erythrocarpa* include MG, MLT and MS. These OTUs show a lower degree of similarity to EE and PIN, and are most similar to EE, MG and P9, respectively. The similarity of *J. monosperma* var. *gracilis* (*sensu* Martínez [18]) MG to *J. monosperma* from USA (MS) is markedly lower than its similarity to the pinchotii complex.

Discussion
Comparison of similarities based on morphological data (Fig. 2) to similarities based on terpenoid data (Fig. 3) showed several patterns of groups of OTUs. The analysis (Fig. 4) based on the combination of both sets of data emphasized the similarities that are present separately in the morphological analysis and in the terpenoid analysis.

The deppeanan junipers, including *J. deppeana* var. *deppeana* (DD) var. *robusta* (DR), var. *zacatecensis* (DZ), and *J. patoniana* (DP), consistently cluster as a unit (Figs. 2–5). This group was shown to possess distinctive morphological characteristics, including stem bark of quadrangular plates (except for *J. patoniana*'s rectangular plates) and large, fibrous cones with several large seeds, which are not duplicated in other junipers of North America. Close similarities based on terpenoid data confirm the unity of these taxa. The analysis of separate site collections of the deppeanan junipers (Fig. 6) revealed little structuring of sites, other than for *J. deppeana* var. *deppeana* from the eastern Sierra Madre Oriental of Mexico. The remaining OTUs DP, DRE, DRC, DZ1 and DZ2 did not show significant clustering patterns. The high variability among the trees within a collection site of these junipers from the western Sierra Madre Occidental contributed to distinctive clusters. *J. patoniana* (DP) is closer to the OTUs of *J. deppeana* vars. *robusta* and

zactecensis. The cohesion of the OTUs of *J. deppeana* var. *deppeana* and the diverse nature of the western Mexico OTUs (DP, DR, DZ) suggest that these may have been undergoing differentiation in many directions and that chemotypes may not correspond to the morphological patterns found. It was recommended by Zanoni and Adams [15], that *J. patoniana* be relegated to the status of variety. The present study reaffirms this recommendation, that *J. patoniana* Martínez be recognized as *J. deppeana* var. *patoniana* (Martínez) Zanoni *comb. et stat. nov.* based on *J. patoniana* Martínez [18]. The distinguishing morphological character of *J. deppeana* var. *patoniana* is the stem bark pattern of long, rectangular strips, that are sometimes interlaced.

Juniperus flaccida var. *flaccida* (FF) and var. *poblana* (FP) are readily recognized morphologically by the large cones with many small seeds and flaccid branches. Evidence from the combination of morphological and terpenoid data (Fig. 4) confirms the uniqueness of this species. Examination of site collection OTUs (Fig. 7) showed the coherence of the specimens of *J. flaccida* var. *flaccida* (FFH, FFM and FFS). The two collections of the specimens of *J. flaccida* var. *poblana* (FPA and FPO) did not cluster together and were similar to FFM. This finding is interesting in that the three sites of Morelos, Oaxaca (FFM), San Dionisio Ocotepec, Oaxaca (FPO), and Amozoc, Puebla (FPA), are close to each other. Nevertheless, these collections represent two morphological varieties of *J. flaccida*, supporting earlier conclusions [16, 18] that the geographic distributions of the varieties are not exclusive. The range of *J. flaccida* var. *poblana* occurs in the southern portion of the range of *J. flaccida* var. *flaccida*. The clustering of *J. comitana* (CO) with *J. flaccida* (FF and FP) in the terpenoid data appears to be related to the high similarities related to the larger terpenoid peaks in common to these OTUs.

The three forms of *J. monticola*, f. *compacta* (MC), f. *monticola* (MM), and f. *orizabensis* (MO), form an elusive group in the analyses. Morphological similarities (Fig. 2) among the forms are a little lower than similarities to other OTUs; the forms do not cluster as a unit. However, when analyzed using terpenoid data (Figs. 3 and 5), the OTUs MC, MM and MO do cluster together. Apparently, the similarities of the terpenoids bring these forms together in the combined analysis (Fig. 4), although the clustering does not occur at high levels of

similarity. In the analysis of *J. monticola* forms in the 21 and 25 OTU terpenoid analyses (Figs. 3 and 5), similarities of MC, MM and MO were not very high as compared to other groups of OTUs, suggesting considerable divergence in terpenoid patterns among them. The analysis of the terpenoid data of *J. monticola* by site collections (Fig. 8) reveals several interesting features. *J. monticola* f. *compacta* from sites MCI and MCT are most similar to each other. *J. monticola* f. *orizabensis* (MO) and *J. monticola* f. *monticola* (MME and MMT) are also very similar to each other, the first being most similar to MCT, MCI and MCI. *J. monticola* f. *compacta* (MCP) from Cerro Potosi, Nuevo Leon, is by far, the most divergent collection, being most similar (0·247) to MMT. The divergence of MCP is related to the considerably different chemotype of the Cerro Potosi trees.

Species such as *J. monticola*, which are extremely widespread, from Cerro Potosi, Nuevo Leon south to Pico de Orizaba, Puebla/Vera Cruz and west to Nevado de Colima, Colima, and represent extremely isolated locations (often on mountains at 12,000 ft elevation) would be expected to have excellent opportunities to develop morphological and/or chemically divergent populations. With very little or no gene flow existing between the populations, differentiation would be similar to that of insular populations, with the gene pools being related to the original colonizing plants and their subsequent responses to natural selection. *J. standleyi* (ST) and *J. durangensis* (DU) were considered to be closely related to *J. monticola* by Martínez [18]. The morphological analyses (Fig. 2) indicate that if these taxa are related, it is at a rather low level of similarity and the terpenoid data analyses (Fig. 3 and 5) concur. *J. monticola* and *J. standleyi* have probably been geographically isolated from each other since the Tertiary, just as the other plants of the highlands of Chiapas and western Guatemala [21] have been isolated from those growing in central Mexico. This long term isolation would be sufficient for speciation, if these taxa are assumed to have a common ancestor. *J. durangensis* may have come from the ancestor. However, there are differences in habitats of *J. monticola* and *J. standleyi* (alpine and subalpine) and *J. durangensis* (pine–oak woodland) which appear to be significant. It may be noted that *J. monticola* is known from one locality, El Chico, Hidalgo, which has pine–oak woodlands perhaps

ecologically similar to those in the Sierra Madre Occidental where *J. durangensis* is found.

In the morphological and terpenoid analyses of the 21 OTUs (Figs. 2–4), *J. blancoi* (BL) exhibited a low similarity to *J. jaliscana* (JA). The terpenoid analysis of 25 OTUs (Fig. 5) indicated that *J. blancoi* was more similar to *J. scopulorum* (SC) as anticipated on morphological grounds [16]. Similarities of cone and leaf characteristics indicated affinities of *J. blancoi* to the smooth (entire) leaf margin junipers which include *J. scopulorum.* The work of Engelmann [22] and Gaussen [23, 24] indicates that an apparently natural group, the "Integrae" [23] exists among the sabinoid junipers in Europe, Asia, and North America. Included in the North American "Integrae section Virginioides" [23] are the smooth leaf margin junipers referred to by Gaussen, including *J. barbadensis, J. silicicola, J. urbaniana, J. blancoi, J. gracilis, J. bermudiana, J. prostrata, J. horizontalis, J. saxicola, J. gracilior, J. lucayana, J. ekmanii, J. virginiana* and *J. scopulorum.* (The taxonomic monograph by Gaussen [24], is imperfect in that species recognized by Gaussen include many epithets that are relegated to synonymy.) Preliminary terpenoid investigations on *J. virginiana* indicate it is closely similar to *J. blancoi* and *J. scopulorum.* Von Rudloff [1] has also demonstrated the similarity of *J. horizontalis* to this group of North American junipers. Martínez [18] considered *J. jaliscana* to be closely related to *J. blancoi* but morphological and terpenoid analyses do not concur his view. *J. jaliscana* does not appear too closely related to the other junipers in North America.

The morphological group called the one-seeded junipers, *J. comitana* (CO), *J. erythrocarpa* (EE, EW), *J. gamboana* (GA), *J. monosperma* var. *gracilis* (MG), MLT from La Trinidad, Nuevo Leon, *J. monosperma* var. *monosperma* from USA (MS) and *J. saltillensis* (SL), do not hold together as a unit in
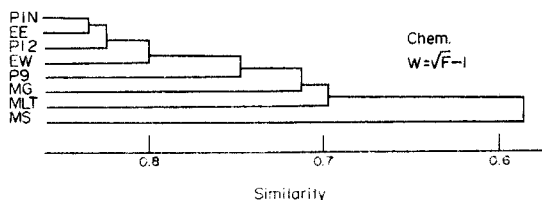

FIG. 9. PHENOGRAM OF THE ERYTHROCARPA, PINCHOTII AND MONOSPERMA JUNIPERS, USING TERPENOID DATA. Note the cluster of pinchotii complex populations (PIN, EE, EW, P9) and the tailing-in of MG and MLT while MS (*J. monosperma* from the USA) is quite distinct.

the terpenoid analyses (Figs. 3 and 5). Based on this data, one group of three OTUs is apparent: *J. erythrocarpa* from both eastern (EE); and western Mexico (EW); and *J. monosperma* var. *gracilis* (MG). *J. saltillensis* (SL) which enters this group in the 21 OTU analysis (Fig. 3), is more similar to *J. ashei* (ASH) than to MG in the 25 OTU analysis (Fig. 5). The remaining one-seeded junipers, *J. comitana* (CO), *J. gamboana* (GA), MLT, and *J. monosperma* var. *monosperma* from USA (MS), are scattered throughout the terpenoid analyses phenograms (Figs. 3 and 5), not showing a high degree of similarity to any other group of OTUs.

If the morphological group of one-seeded junipers is a natural assemblage, the divergence in terpenoids for these OTUs could be related to a response to environmental influences. Differentiation of terpenoid and phenolic content within species and closely related taxa have been observed in pines [25, 26] and many other plant species. The value of chemical diversity in relation to resistance to fungal infection [27], interplant relationships (allelopathy) [28], and interaction between plants and animals [29] has become apparent in recent years, showing that there are many adaptational responses other than morphological changes.

The habitats of the one-seeded junipers in northern Mexico and the southwestern United States represent some of the most xeric sites in which any of the junipers in that region exist. Similarity or conservation of morphological vegetative characteristics might be expected in such severe habitats. The chemical similarity of *J. ashei* (ASH) and *J. saltillensis* (SL) suggests that morphological expression may be altered while terpenoid similarity is conserved. However, if these taxa are part of the one-seeded juniper group, they must belong to a segregate within it.

J. erythrocarpa from eastern Mexico (EE), *J. monosperma* var. *gracilis* (MG), and MLT from La Trinidad, Nuevo, Leon are all found in the foothills southeast of Saltillo, Coahuila. The similarity of these OTUs chemically and morphologically is of particular interest, considering the identity of these OTUs and their relationship had not been resolved [16]. The relationship of these OTUs to *J. monosperma* var. *monosperma, J. pinchotii,* and *J. erythrocarpa* can be examined now with reference to the work by Adams [2, 3].

Figure 9 uncovers several interesting

features. *J. pinchotii* (PIN) was shown to be very similar in terpenoid composition to *J. erythrocarpa*, the "rose-fruited variant of *J. pinchotii*" [3]. The data of PIN was taken from 15 populations used by Adams [2]. The OTUs P9 (Sierra Blanca, Texas) and P12 (Alpine, Texas) were part of the *J. erythrocarpa* complex [3]. EE (*J. erythrocarpa* from eastern Mexico) was composed of trees of population 2 (Los Lirios, Coahuila) [3], and additional trees collected in 1974. EW is *J. erythrocarpa* from western Mexico, near population 1 [3]. The similarity of several of the *J. monosperma* var. *gracilis* (MG) and MLT trees, and the geographic proximity to EE prompted the inclusion of these OTUs. *J. monosperma* var. *monosperma* from USA was included because of the assumed relation to *J. monosperma* var. *gracilis* (MG) [18].

The chemical similarity of *Juniperus pinchotii* (PIN) and *J. erythrocarpa* (EE, EW, P9, and P12) is apparent (Fig. 9). The lack of distinctive chemotype of *J. erythrocarpa* accounts for the "tailing in" of the *J. erythrocarpa* OTUs. The data of Adams [2, 3] also exhibited a similar "tailing in" of *J. erythrocarpa* into the rather uniform populations of *J. pinchotii. J. monosperma* var. *gracilis* (MG), MLT and *J. monosperma* var. *monosperma* from USA (MS) show similarities to the *J. erythrocarpa–J. pinchotii* complex through EE, MG and P9, respectively. Note that *J. monosperma* var. *monosperma* (MS) show the lowest similarity (0·589 to MG) values of any OTU in this analysis (Fig. 9). It is clear that the taxon MG (called *J. monosperma* var. *gracilis* by Martínez [18]) has strong affinities to the pinchotii complex, whereas it is less closely allied with *J. monosperma* from the US. Further work is necessary to resolve this question.

J. comitana (CO) and *J. gamboana* (GA) were found to be quite similar morphologically (Fig. 2) within the one-seeded juniper group. Terpenoid data (Fig. 3 and 5) showed that *J. comitana* was more similar to *J. flaccida* var. *poblana* (FP) and *J. flaccida* var. *flaccida* (FF) than to the other junipers. *J. gamboana* (GA) was most similar to *J. comitana* (CO), and then to *J. flaccida* (FF and FP) (see Fig. 3, Fig. 5). The relationships of *J. comitana* and *J. gamboana* are not clear. The composite analysis (Fig. 4) places these two taxa in the one-seeded junipers, but the overall similarities of CO and GA are reduced due to their chemical similarities to *J. flaccida* (FF and FP) which are

not in the one-seeded group. The geographic isolation of *J. comitana* and *J. gamboana* (as in the case of *J. standleyi*) reduces the value of distributional data as evidence for possible taxonomic relationships, especially if this isolation dates from the Tertiary [21].

Hall [30] stated that *J. saltillensis* "belongs in association with those species of higher altitudes and more mesic habitats, *J. monticola* Mart., *J. standleyi* Mart., and the poorly known species, *J. jaliscana* Mart., and *J. blancoi* Mart. *J. saltillensis* is more xeric in habitat than its closer relatives and has on the average fewer seeds per berry-cone. It may be considered a taxon which links the polyspermous, mesic species with the xeric monospermous ones." Zanoni and Adams [16] concluded from morphological studies of all Mexican and Guatemalan junipers that *J. saltillensis* was more similar to the one-seeded junipers of northern Mexico. Ecological preferences of *J. saltillensis* were considered more similar to the one-seeded junipers than to the species listed by Hall [30]. The terpenoid composition of *J. saltillensis* was found to be similar to *J. ashei* (Fig. 5). Comparison of the female cones of *J. ashei* and *J. saltillensis* showed that they are similar. A heavy, glaucous coat on the cones give them a whitish-blue cast; the cone surfaces are dark brownish-blue. The seed hilum scar in *J. ashei* is usually light tan, and there are shallow grooves on the single seed. *J. saltillensis* has usually one seed per cone (1·23 seeds/cone average) with occasionally deeper resin grooves on the seed with a tan to chestnut color hilum scar. Seeds in both species are chestnut brown. The main differences in the two species are in the foliage. The overall similarity of *J. ashei* and *J. saltillensis* may indicate close relationship.

Phylogenetic Aspects
The junipers are considered to have been present in North America by the mid-Tertiary [35]. Diversification and speciation of the early junipers occurred since that time as climatic and geologic surface features began to assume their modern characteristics. It may be inferred that many habitats of the junipers were not existant prior to the late Tertiary; orogenesis of the Sierra Madre Oriental and the Sierra Madre Occidental was still proceeding at that time [36]. Volcanic activity occurred as recently as the Pleistocene, and glaciers are reported on several older volcanoes during that period [36].
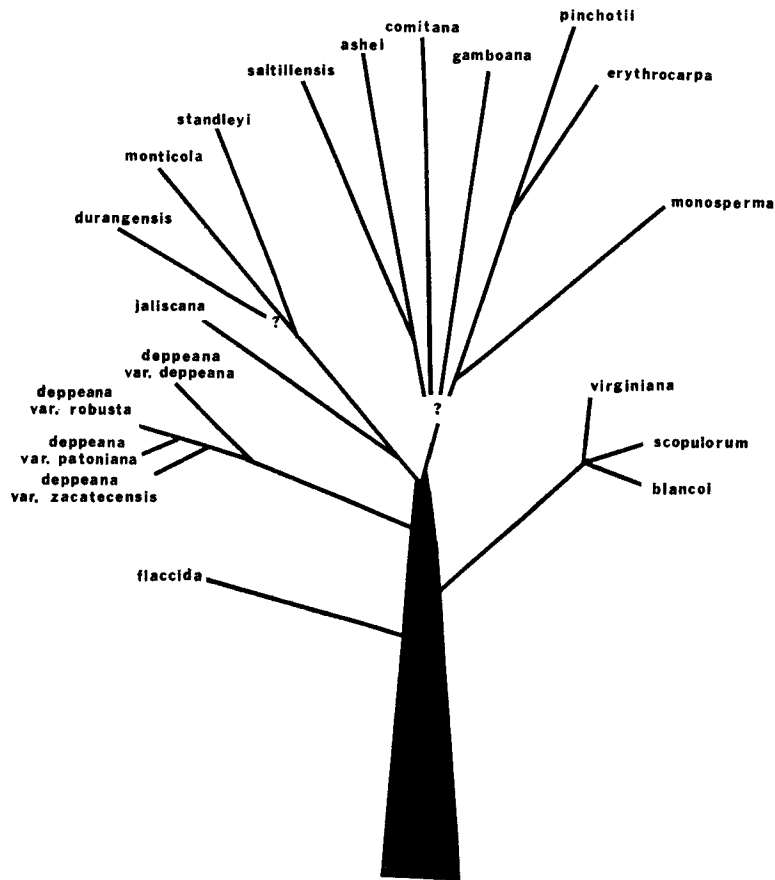
FIG. 10 SUGGESTED PHYLOGENY OF THE SPECIES OF *JUNIPER* OF MEXICO, GUATEMALA AND SOUTHWESTERN UNITED STATES
The main trunk represents the ancestral stock of the junipers of the world.

A suggested phylogeny of the junipers of Middle America based on morphological, terpenoid, ecological and distributional data is shown in Fig. 10. The main trunk serves to represent the ancestral stock of the junipers. Reference is made only to the American junipers, due to the inadequate data available for the others.

The "Integrae" of Gaussen [23] form a complex of taxa that have an unusually broad ecological and geographic amplitude throughout the Northern Hemisphere. *J. virginiana, J. scopulorum,* and *J. blancoi* are broadly distributed in North America. The existence of species in Europe and Asia with similar characteristics indicates that the origin of the "Integrae" occurred early in the history of this genus, probably preceding the mid-Tertiary.

The characteristics of *J. flaccida* mark this species as anomalous in the sabinoid junipers, suggesting early derivation from ancestral stock. The wide-spread distribution can be related to the prolific seed-producing capacity of this species, 6–14 seeds per cone. However, the recent expansion of the range of *J. flaccida* is probably not directly related to dissemination of seeds by birds. The cones of this species are some of the largest in *Juniperus* and are fibrous. Dispersal by other agents (mammals) is probably more important than in other species with small, soft-fleshed cones.

The alligator juniper, *J. deppeana,* is another distinctive widespread species. Diversification accompanied the development of the sierras in Mexico and the southwestern United States. *J. deppeana* var. *deppeana* is chemically and morphologically more uniform than the other taxa in this species. This variety is restricted to

the Sierra Madre Oriental in eastern Mexico. *J. deppeana* var. *robusta,* var. *patoniana,* and var. *zactecensis* are morphologically distinguishable, although there appear to be no distinctive chemotypes in these varieties. It might be expected that these three taxa were derived from outliers of the ancestral *J. deppeana* in the Sierra Madre Occidental. The variety *zacatecensis* (DZ) is the only western Mexican taxon of *J. deppeana* with a distinctive geographic distribution (in Zacatecas and adjacent Durango). The other varieties *robusta* and *patoniana* appear to be closely related morphological variants with sympatric distributions.

The morphological group of junipers with one seed per cone is chemically varied. Diversity in this group, if it can be considered a natural one, may be related to early divergence of taxa into widely separated regions of southwestern United States and Middle America. *J. comitana* and *J. gamboana* have been separated from the other junipers at least since the late Tertiary, because of the development of the highlands of Chiapas, Mexico and adjacent western Guatemala and the lowland barrier of the Isthmus of Tehuantepec. The chemical and morphological similarity of *J. ashei* and *J. saltillensis* suggest common ancestry, probably prior to the Pleistocene, with expansion of ranges since the Pleistocene, resulting in only a few sympatric populations at the present time. Population studies by Adams [3] suggest that *J. pinchotii,* which has its center of distribution in western Texas, has undergone extensive range expansions in recent times. *J. erythrocarpa* occurs at the southwestern and southern edges of the range of *J. pinchotii,* extending into Arizona, Sonora, Chihuahua, Durango, Coahuila and Nuevo Leon in the *Bouteloua* grasslands. These grasslands of eastern Mexico, western Mexico (Chihuahua and Durango), and northern Sonora and Arizona became dissected from one another during the development of the sierras and the dry northern desert regions in northern Mexico. The considerable variation in populations of *J. erythrocarpa* is not understood at the present time. Possible causes of variation may suggest that the taxon may have resulted from (a) hybridization (between *J. pinchotii* and *J. monosperma* ?); (b) derivation from *J. pinchotii;* or (c) it may be an extremely polymorphic species. Overall similarity of *J. monosperma* and *J. pinchotii* suggests a recent ancestral relationship. The current distribution range of *J. monosperma* is to the west and

northwest of *J. pinchotii.* These two taxa are known to be sympatric only in the Guadalupe Mountains of southeastern New Mexico.

The several-seeded junipers form a diverse group chemically and morphologically. The present isolation of populations at higher altitudes and on mountain tops indicates a broader distribution of *J. monticola* in the past. Part of the distribution of *J. monticola* is interconnected with the recent origin of several of the volcanic sites that are of Pleistocene or post-Pleistocene origin. Similarity of *J. standleyi* and *J. durangensis* implies relationships to *J. monticola;* however, common ancestry may have been in the Tertiary. *J. jaliscana* is most similar to this group than to the other North American junipers. This similarity may be due to the lack of other closely allied species. It is placed in this group assuming considerable divergence has occurred.

A thorough treatment of the phylogeny of the junipers is not possible until additional species have been studied and the nature of convergence of characters is understood.

This study clarifies many of the relationships among the junipers of Mexico and Guatemala. Four major groups have been well resolved: *J. deppeana* and its varieties; *J. flaccida* and its varieties; *J. monticola* and its forms; and various one-seeded junipers allied with *J. pinchotii.* However, several are not closely related (using both morphological and chemical data) to each other or to other taxa.

J. blancoi is not related to *J. jaliscana* as stated by Martínez [18], but is related to *J. scopulorum,* and probably was derived when *J. scopulorum* was more widespread in distribution into Mexico (during the Tertiary ?). The present southerly distribution [17] of *J. scopulorum* in Arizona, New Mexico, and Texas and the northern Sierra Madre Occidental is a vestige of the former range of this species.

Typical *J. monosperma* (var. *monosperma*) has still not been found, to our satisfaction, in Mexico; *J. monosperma* var. *gracilis* does not exhibit the similarity to *J. monosperma* as indicated by Martínez [18]. Hybridization of junipers, does not appear to be frequent, as one might expect from the reports by Hall [8–12]. A region of possible hybridization is in the Saltillo, Coahuila area involving segregates of the one-seeded (pinchotii) complex. Studies of population variation in *J. deppeana, J. monticola,* and in the one-seeded complex near Saltillo will provide excellent opportunities to study populational differentiation.

Experimental

Plant Material

Fresh foliage (of female plants, whenever possible) on terminal whips were collected in December, 1973, except as noted below. Samples of *Juniperus ashei* (ASH), *J. erythrocarpa* (EE, P9, P12), and *J. pinchotii* (PIN) were collected by Adams [3,4] in November and December, 1970. Samples of *J. jaliscana* (JA) were collected in December, 1972. *J. scopulorum* (SC) from Colorado was collected in December, 1974, and *J. scopulorum* from Texas was collected in January, 1975. All specimens were stored in sealed, plastic sacks and were frozen as soon as possible.

Locations

Localities and numbers of specimens of each operational taxonomic unit (OTU) are shown in Fig. 1 and Table 1. Several localities for each OTU were sampled whenever possible. The inaccessibility of the few localities known for several of the rarer OTUs limited the collections to one site only. Twenty-one OTUs were analyzed, corresponding to those used in the previous morphological study [16]. There OTUs (DZA, LLR and MLB) included in that study were not examined, based on evidence presented there [16] and are referable to *J. deppeana* var. *robusta* (DR), and *J. erythrocarpa* (EE), respectively, in this study. (The OTUs previously referred to as FL and ERW [16] have been assigned new symbols of FF and EE, respectively.) Several additional OTUs are included in certain analyses. These represent either taxa that are now documented for Mexico (*J. ashei*, ASH: *J. pinchotii*, PIN; and *J. scopulorum*, SC), or critical groups in ascertaining the affinities of the one-seeded junipers (populations P9 and P12 of Adams [3]). Nomenclature follows that of Martínez [18] and Zanoni and Adams [20]. Specimens are vouchered at the Colorado State University herbarium (CS).

Analysis

Approximately 50-100 g of foliage from each tree were steam distilled for 2 h using a modified Clevenger-type, circulatory, steam-distillation apparatus [31,32]. The distillate was trapped in Et_2O and stored at $-20°$ in tightly capped vials until analysis by capillary GLC. Gas chromatograph conditions were the same as outlined by Adams [3] except the upper limit temperature held isothermally for an additional 8·2 min at 227·6°. Individual peaks were quantified with an Infotronics CRS 104 digital integrator with automatic key-punching on an IBM 026 card keypunch.

Due to difficulties in resolution of certain compounds by the techniques used here, α-pinene and α-thujene are both included in peak 3, and peak 17 in *J. flaccida* includes γ-terpinene and an unidentified compound. Peaks 82A, 82B, 83, 83A, 83B and 83C were not considered in the data analyses because they were all often covered by a broad (decomposition?) peak.

IR spectra of several compounds were taken on a Perkin-Elmer 727 Infrared Spectrophotometer. Verification of compounds was made by comparison of the spectra with published data where possible. The chemical composition of the volatile oils of the Mexican junipers will be published separately.

Several data runs were made to investigate relationships of the Mexican and Guatemalan junipers. Each run included an analysis of variance (ANOVA) performed on the characters of the OTUs, and a modified Student–Newman–Keuls (SNK) multiple range test [5] for unequal sample sizes was used to detect OTU means which were significantly different at the 0·05 level. Data on terpenoids which were never present in more than 0·5% concentration in any OTU were not considered.

The similarity measure of Adams and Turner [5] which was based on the matching coefficient [33] was utilized to determine similarities among OTUs. Character weighting of $\sqrt{F-1}$ (where F is the ratio of variance among OTUs/variance within OTUs) for each character comparison was used in all similarity computations. This character weighting is similar to $\sqrt{F-1}$ weighting of Zanoni and Adams [16]. The single linkage method [34] was used for cluster analysis.

The data analyses were:

(a) 21 OTUs, using morphological data from a previous study [14], 45 characters were used, (b) 21 OTUs, using 83 terpenoid characters, these OTUs correspond to those used in the morphological analysis, (c) 21 OTUs, using morphological and terpenoid data, (d) the similarity matrices of analyses (a) and (b) were combined to form a new matrix, total characters being 128, (e) 25 OTUs, using chemical data, the OTUs PIN, P12, ASH and SC were added to the 21 OTUs of analysis (b), 84 terpenoid characters were used, (f) *J. deppeana* (DDE, DDL, DDR, DRE, DRC, DZ1, DZ2) and *J. patoniana* (DP) were analyzed as sites, giving 8 OTUs with 50 terpenoid characters, (g) *J. flaccida* (FFH, FFM, FFS, FPA, FPO) analyzed as sites, giving 5 OTUs with 64 terpenoid characters, (h) *J. monticola* (MCI, MCP, MCT, MME, MMT, MO) analyzed as sites, giving 5 OTUs with 42 terpenoid characters, and (i) one-seeded junipers (EE, EW, MLT, MS, PIN, MG, P9, P12) analyzed giving 8 OTUs with 43 terpenoid characters.

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