

# A NEW VARIETY OF *PINUS GREGGII* (PINACEAE) IN MEXICO

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## ABSTRACT

A new variety of *Pinus greggii* is described. Variety *australis* is endemic to east central Mexico and represents the southern disjunct region of the natural range of the species. Variety *greggii* represents the northern population of the species. Characters that segregate the varieties are presented.

## RESUMEN

Se describe una nueva variedad de *Pinus greggii*. La variedad *australis* es endémica del centro-este de México, y abarca la distribución natural austral de la especie. La variedad *greggii* representa la población localizada en el norte de su distribución natural. Se incluyen características que distinguen las dos variedades.

*Pinus greggii* Engelm. occurs in two disjunct regions in Mexico (Fig. 1) separated by approximately 300 km, or four degrees latitude. Northern and southern populations of *P. greggii* grow in distinct environments. Northern populations occur in the northern Mexico states of Coahuila and Nuevo Leon, at elevations ranging from 1900 to 2600 meters above sea level. The average annual temperature at the northern sites is 14° C, and they receive an average annual precipitation of 650 mm (Donahue & Lopez Upton 1996). Topsoils at northern *P. greggii* sites are predominately neutral or slightly alkaline (Donahue 1993).

Southern populations of *P. greggii* occur in the central Mexico states of Hidalgo, Puebla, Queretaro, San Luis Potosi and Veracruz at elevations ranging from 1100 to 2400 meters above sea level. The average annual temperature at the southern sites is 17° C, and they receive an average annual precipitation of 800–1600 mm (Donahue & Lopez Upton 1996). Topsoils at the southern *P. greggii* sites are predominately acidic (Donahue 1993).

A comprehensive study of the species began after differences in growth rate, needle length and needle color became apparent in genetic trials planted

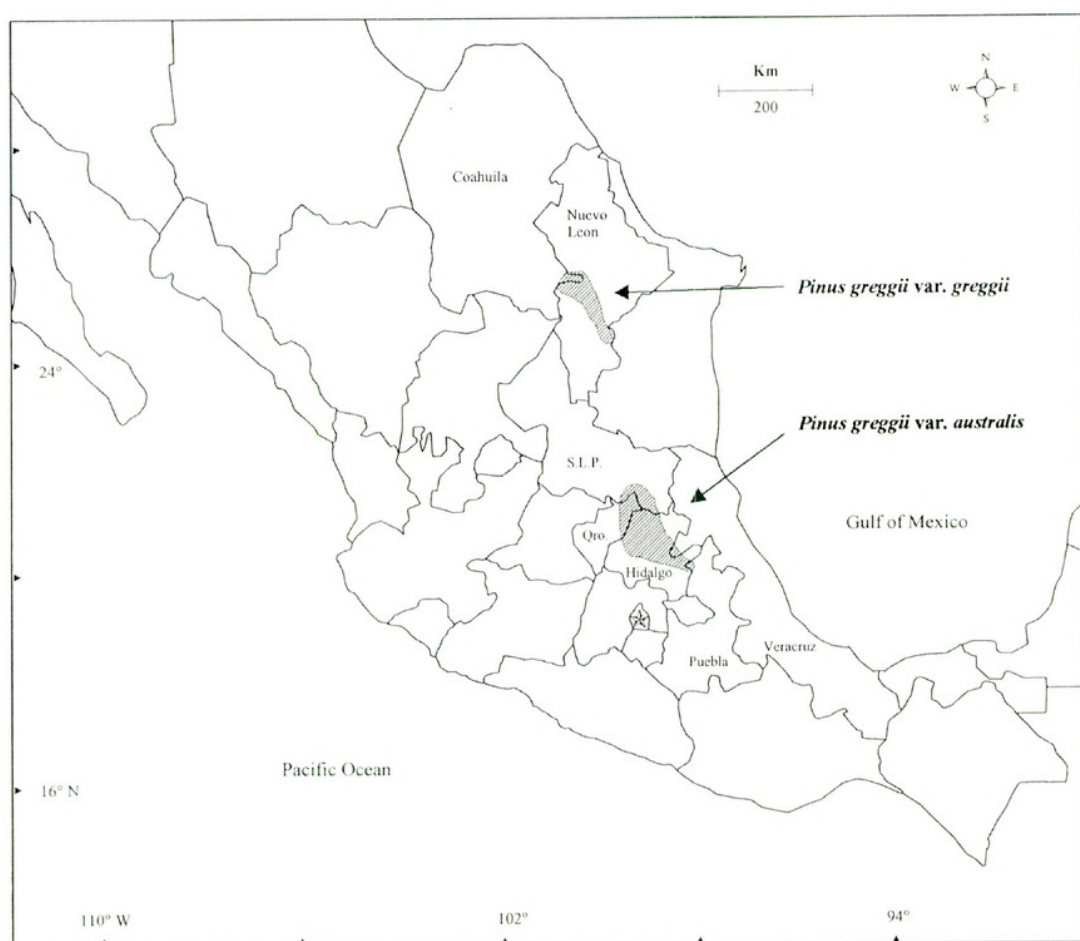


FIG. 1. Geographic distribution of *Pinus greggii* in Mexico.

in Brazil, Chile, Colombia, South Africa (Dvorak et al. 1996) and Mexico (Lopez Ayala 1998; Alba Landa et al. 1998), where trees from northern and southern populations were planted side-by-side. The differences observed in these field trials suggested that two distinct, unrelated taxa had been planted by mistake. Studies of geographic variation in leaf, cone and seed morphology, seed production and terpene chemistry were performed to determine what differences existed, and to quantify them. Results from our comprehensive study indicated that only *P. greggii* was included in the trials, but several character differences exist between northern and southern populations suggesting recognition taxonomically.

#### MATERIALS AND METHODS

Field sampling was done in six northern populations and six southern populations from a wide range of geographic locations and elevations (Table 1). Leaf specimens were collected from 172 trees, and cones from 177 trees of *Pinus greggii*. The samples were collected from the upper 1/3 of the crown from healthy dominant and co-dominant trees in the stands, at least 100 meters apart. Leaf and cone specimens were collected from five distinct as-



TABLE 1. Summary of the sampling of *Pinus greggii* sites included in the leaf, cone and seed morphology, seed production and terpene chemistry studies.

	Latitude-Longitude	Elevation (m)	Number of trees sampled		
			a	b	c
Southern populations					
Cerro Perico, Hidalgo	20°44' N – 99°02' W	1830-1970	14	15	24
Laguna Atezca, Hidalgo	20°49' N – 98°46' W	1250-1420	15	15	16
Laguna Seca, Hidalgo	21°02' N – 99°10' W	1670-1830	12	12	0
El Madroño, Queretaro	21°16' N – 99°10' W	1650-1730	15	15	17
San Joaquín, Queretaro	20°56' N – 99°34' W	2310-2380	15	15	15
Valle Verde, Queretaro	21°29' N – 99°12' W	1150-1250	15	15	0
	total		86	87	72
Northern populations					
Cerro Perico, Hidalgo	20°44' N – 99°02' W	1830-1970	14	15	24
Cañon Los Lirios, Coahuila	25°22' N – 100°29' W	2260-2460	14	15	0
Mesa del Rosario, Coahuila	25°26' N – 100°28' W	1920-2325	12	12	13
Santa Anita, Coahuila	25°27' N – 100°34' W	2515-2620	18	18	20
La Tapon, Nuevo Leon	24°43' N – 100°10' W	2090-2350	15	15	24
Las Placetas, Nuevo Leon	24°55' N – 100°11' W	2370-2520	13	15	22
Loma El Oregano, Coahuila	25°22' N – 100°55' W	2310-2350	14	15	19
	total		86	90	98

a = leaf characters b = cone & seed characters c = terpene analysis

pects in the crown, and leaves were sampled only from first-order branches, prior to elongation. The five cones collected per tree were used in both the morphology and seed production studies. Stem-xylem oleoresin was collected from 170 trees for analysis of terpene chemistry using standard procedures. Details of the sampling procedure and chemical analysis for the terpene study are given in Donahue et al. (1995).

### Leaf and Cone Morphology

For the morphology study ten leaf characters were analyzed: number of needles per fascicle, fascicle sheath length, needle length and width, number of ventral and dorsal surface stomatal lines, number of stomata per 3 mm length on dorsal surface, number of medial and internal resin canals and total number of resin canals. Ten needles per tree were measured for length, width and number per fascicle (1720 needles total). Five needles per tree were analyzed for the resin canal and stomata information (860 needles total). Five fascicle sheaths per tree were measured for length (860 sheaths total). Leaf width and stomatal counts were taken at the mid-point of the leaf, as well as the resin canal assessment, using standard microtechniques.

The ten cone characters measured were cone length and width, cone scale apophysis height and width, seed length and width, seed wing length and width, seed coat thickness and seed weight. Five cones per tree were mea-



sured for length and width. Cone length was measured in a straight line from the base to the top; cone curvature was excluded. Cone width was the average of two perpendicular measurements taken at the widest point of the cone. Cone scale apophysis height and width were measured on four sides, perpendicular to each other, located at the widest part of the cone. Seed wing width was measured at the widest point of the wing.

The leaf and cone data were analyzed using statistical procedures of the Statistical Analysis System (Donahue & Lopez Upton 1996). First a multivariate stepwise discriminant analysis was performed on the data to determine which traits would be most useful in separating trees from the two regions. A canonical discriminant analysis was conducted on the variables selected by the stepwise discriminant analysis to look at patterns of differentiation among populations, and calculate spatial Mahalanobis distances.

Analyses of variation by region were done on each individual variable to determine which varied significantly between the northern and southern groups of populations. Next a multivariate analysis of variation was conducted on the subset of variables that were significantly different between the two regions. Means and coefficients of variation were calculated and a correlation analysis was conducted on all morphological traits.

### **Seed Production**

For the seed production study, five cones per tree were assessed (885 cones total). The extracted seeds were counted and classified as filled, empty, first-year aborted, second-year aborted, or insect-damaged. The number of fertile cone scales was counted to calculate seed potential and determine seed efficiency. Of the seed production criteria, the number of fertile cone scales is least affected by environmental variation. Population and individual tree means and coefficients of variation were computed for each seed-yield trait (Lopez Upton & Donahue 1995). An analysis of variance was conducted on the individual tree means with a nested model: trees, populations and populations within region (north and south regions). Waller-Duncan comparisons were performed on population means to detect differences among them, and to look for trends across regions.

### **Terpene Chemistry**

The terpene chemistry analysis was performed on one resin sample from each of 170 trees by gas chromatography (Donahue et al. 1995). The chemical components were identified by comparison with known chemical standards, and also compared to results from gas chromatography/mass spectrometry analyses run on one sample from each of nine populations in the study. The chemical compositions were reported as "percent of terpenes." This included all the monoterpenes and longifolene, a sesquiterpene, which was of particular interest. Since myrcene and carene were not separated, a combined



amount was reported for both. Population means were calculated for six terpenes. To characterize individual trees as "high" or "low" types for specific terpenes, frequency distributions were examined to help establish threshold values as criteria for determining the point of separation for the concentration classes. Threshold values were established at points where the trees' frequency distributions might be separated into two modes.

### Herbarium specimens examined

Of the specimens collected for and examined in the comprehensive study, the following vouchers, which are now considered variety *australis*, are located in the indicated herbaria:

**MEXICO. Hidalgo:** Laguna Atezca, 26 May 1993, *Donahue & Lopez Upton* F85 (A) F84 (F) F77, F90 (MICH) F76, F80, F83, F86, F89 (MO) F75, F81, F82 (NCSC) F78, F87 (TEX). Laguna Seca, 24 May 1993, *Donahue & Lopez Upton* D56 (A) D50 (F) D47 (MICH) D49, D51, D57, D55, D53 (MO) D54 (NCSC) D46 (TEX). **Queretaro:** Mpio. Landa de Matamoros, Ejido El Madroño, 19 May 1993, *Donahue & Lopez Upton* B27 (A) B20 (F) B19 (MICH) B18, B28, B26, B22 (MO) B23 (NCSC) B17 (TEX); San Joaquin, 17 May 1993, *Donahue & Lopez Upton* A1, A13 (A) A2, A11 (F) A5, A9 (MICH) A6, A8, A10, A12, A15 (MO) A14 (NCSC) A3, A7 (TEX); Valle Verde, 22 May 1993, *Donahue & Lopez Upton* C44 (A) C43 (F) C38 (MICH) C35, C37, C39, C42, C45 (MO) C36 (TEX).

The following vouchers from the comprehensive study, are considered variety *greggii*, and are located in the indicated herbaria:

**MEXICO. Coahuila:** Loma El Oregano, 5 Jun 1993, *Donahue & Lopez Upton* I158 (A) I156 (F) I150 (MICH) I153, I155, I157 (MO) I152, I166 (NCSC) I146 (TEX); Los Lirios, 4 Jun 1993, *Donahue & Lopez Upton* H 139 (A) H136 (F) H132, H144 (MICH) H134, H138, H140, H143, H145, H148, H151 (MO) H135 (NCSC) H131, H142 (TEX); Mesa del Rosario, 3 Jun 1993, *Donahue & Lopez Upton* G129 (A) G107, G114 (F) G104, G112 (MICH) G102, G105, G108, G111, G115 (MO) G106, G113 (NCSC) G101, G110 (TEX); Santa Anita, 3 Jun 1993, *Donahue & Lopez Upton* G129 (A) G123 (MICH) G120, G122, G124, G127, G130 (MO) G119, G126 (NCSC) G121 (TEX). **Nuevo Leon:** La Taponia, 7 Jun 1993, *Donahue & Lopez Upton* K184 (A) K128 (F) K179, K188 (MICH) K177, K180, K183, K186, K190 (MO) K181 (NCSC) K178, K187 (TEX); Las Placetas, 6 Jun 1993, *Donahue & Lopez Upton* J170 (A) J167 (F) J164 (MICH) J162, J165, J168, J171, J174 (MO) J163, J175 (TEX).

In addition to the 860 specimens from the comprehensive study, the authors have also examined the following additional herbarium material:

**MEXICO. Coahuila:** Mountain sides, San Antonio near Saltillo, 30 Aug 1848, *J. Gregg* 402 (MO). **Hidalgo:** 8 km al este de Cardonal, Mpio. Ixmiquilpan, 21 Oct 1979, *D. Cibrian* T. 2687 (CHAP); Rio Malila, Mpio. Molango. 1 Sep 1983 *J., Lopez Garcia* 359 (CHAP); Molango. 7 Jan 1978, *J. Perez Fitz* 1258 (CHAP). **NUEVO LEON:** Cerro del Potosi, 20 km al oeste de Galeana, 2 Nov 1973, *Castillo y Villar* 1257 (CHAP); Cerro del Potosi, Mpio. Galeana, 4 Mar 1978, *T. Eguiluz* 1261 (CHAP). **Queretaro:** Minas Viejas, Mpio. Zimapan, 14 Jan 1978, *A. Cabrera* A. 1259 (CHAP); Carr. Jalpan-Xilita Km. 230, 2 Mar 1978, *T. Eguiluz* 1260 (CHAP); Zona arqueologica Las Ranas, Mpio. San Joaquin, 20 Feb 1987, *J. Rzedowski* 42515 (CHAP).

During the course of several years of field study, the authors visited a number of sites and examined material that was not archived in herbaria.



Table 2 summarizes details of those additional sites where trees of *Pinus greggii* were observed and information gathered for this work.

#### RESULTS AND DISCUSSION

The results from the morphology study given in Table 3 showed that based on population means, the southern populations had significantly longer needles, more stomata per unit of leaf length, lighter seeds and five times greater frequency of internal resin canals than northern populations (Donahue & Lopez Upton 1996). Although the number of leaves per fascicle did not differ significantly between northern and southern trees, four-needle fascicles did occur more frequently in the north. The total number of resin canals did not differ between north and south, however trees from southern populations had five times more internal resin canals. The values shown for the northern and southern "regions" in Table 3 are means of the six individual population means that were part of that region. The values shown for (range) indicate the range of those six population means.

While both the northern and southern populations had the same number of lines of stomata on ventral and dorsal surfaces, the number of stomata per 3 mm length of leaf was greater in southern trees. On average, southern trees had narrower needles and wider seed wings. Northern trees had thicker seedcoats and heavier seeds. In the morphology study, northern and southern population means for needle length were significantly different, without overlapping values.

In the seed production study, no traits were significantly different between north and south, however the number of fertile cones scales showed a clear trend. Cones from southern trees had 28% more fertile cone scales than those from northern trees. The number of fertile cone scales is considered to be under more genetic control than the other criteria.

Table 4 gives a summary of the terpene chemistry results.  $\beta$ -phellandrene was found to be the single most abundant terpene in the chemistry analysis of *Pinus greggii* (51%) (Donahue et al. 1995). Southern populations had significantly higher proportions of  $\alpha$ -pinene and myrcene and lower proportions of limonene and longifolene than northern populations. Northern populations had almost no variation in the frequency of high and low concentration types of trees; all trees within a population were either "high" or "low". In this respect, trees from southern populations had varying numbers of high and low concentrations of  $\alpha$ -pinene, myrcene and limonene. Results of this study indicated that genes that control these terpenes appeared to be fixed in the northern populations. Longifolene is the sole terpene that distinguishes trees from northern and southern populations. Southern trees had a near-zero content of longifolene, while northern trees had an average content of 5%.

TABLE 2. List of additional sites observed but not included in the comprehensive study.

State	Locality	Latitude	Longitude	Elevation
Northern populations				
Coahuila	Agua Fria	25° 26' N	100° 30' W	2400
Coahuila	Cañon de Caballos	25° 15' N	100° 55' W	2410
Coahuila	Cerro El Potosi	24° 53' N	100° 13' W	2430-2500
Coahuila	Jame	25° 21' N	100° 35' W	2450
Coahuila	Puerto Chapultepec	25° 15' N	100° 56' W	2410
Coahuila	Puerto Los Conejos	25° 28' N	100° 34' W	2380-2700
Coahuila	Puerto San Juan	25° 25' N	100° 33' W	2630-2680
Nuevo Leon	La Chona	24° 17' N	99° 58' W	2300
Nuevo Leon	Ojo de Agua	24° 54' N	100° 12' W	2200
Southern populations				
Hidalgo	Cieneguilla	20° 44' N	99° 02' W	1860
Hidalgo	El Piñon	20° 56' N	99° 12' W	1830
Hidalgo	Elochoxitlan	20° 45' N	98° 47' W	1710-1860
Hidalgo	Jalamelco	20° 47' N	98° 42' W	1800-1950
Hidalgo	Minas San Francisco	20° 48' N	99° 20' W	1950-2100
Hidalgo	Molango	20° 50' N	98° 44' W	1400
Hidalgo	Pemuxtitla	20° 49' N	98° 46' W	1400
Hidalgo	Xochicoatlan	20° 50' N	98° 43' W	1840
Puebla	Patoltecoya	20° 13' N	98° 03' W	1440
Veracruz	Carrizal Chico	20° 26' N	98° 20' W	1580

TABLE 3. Means by region and (ranges of means within region) and p-values for the characteristics which varied significantly by geographic location.

Population (region)	Needle Length (cm)	Needle Width (mm)	# of Stomata	# of Internal Resin Canals	Wing Width (mm)	Seed Weight <sup>1</sup>	Seed Coat Thickness (mm)
Northern	11.3 (10.4-11.8)	1.28 (1.19-1.35)	35.6 (34.1-36.8)	0.09 (0.0-0.18)	5.95 (5.55-6.37)	1.73 (1.61-1.87)	0.146 (0.138-0.156)
Southern	12.5 (12.0-13.3)	1.18 (1.09-1.24)	38.8 (36.2-41.3)	0.48 (0.28-0.91)	6.4 (6.06-6.65)	1.47 (1.18-1.58)	0.138 (0.128-0.145)
Significance level	0.002	0.016	0.01	0.002	0.012	0.005	0.037

<sup>1</sup>weight of 100 seeds (gms)

TABLE 4. A comparison of terpene composition (%) of southern and northern populations of *Pinus greggii*.

<i>Pinus greggii</i>	α-pinene	β-pinene	Myrcene/ Carene	Limonene	β-phellandrene	Longifolene
Southern populations	17	0.8	15	10	58	0.3
Northern populations	5	0.7	3	41	54	5



In summary, trees from northern populations of *P. greggii* can be distinguished from southern ones using needle length, number of stomata, number of internal resin canals, seed wing width, seed weight, seed coat thickness, number of fertile cone scales and terpene composition. Of these, needle length, number of stomata, number of internal resin canals and percent content of longifolene had non-overlapping population means in the studies.

### Leaf Characteristics

Subsequent to the comprehensive study, the authors and colleagues made informal observations of leaf color in native stands in Mexico, and trials planted outside the species natural distribution both within and outside of Mexico. Differences in leaf color were reported in exotic trials (Dvorak et al. 1996), where color was noted as dark green for northern populations and pale green for southern. Using Munsell® color charts for plant tissues, which assign a code based on the hue, value and chroma of an object, an attempt was made to characterize the differences seen on live trees in native stands in Coahuila and Queretaro, Mexico, and planted trials in Veracruz, Mexico, and Louisiana, USA. Leaf color was found to be variable both within and among trees, but variation could be generalized as differences between hue classes. Northern populations were predominately hue class "5 GY", value & chroma combinations 4/6, 4/8 and 5/6, while southern populations were predominately hue class "7.5 GY", value & chroma combinations 4/4, 4/6 and 5/6 (light green vs yellowish green). In addition to color differences it was observed that needles of northern trees are stiffer, more erect than those of southern trees. Figure 2 is a photograph of two branches that illustrate the differences in leaf color, length and rigidity between var. *greggii* and var. *australis*. The eighteen-inch long branches were collected from the lower third portion of the crown of three-year-old trees planted in Singer, Louisiana, USA. The trees were planted in field plots located adjacent to each other, and are typical of the rest of the trees (approximately 400) planted in the same plots. They are also representative of the differences in foliage characteristics seen in the twelve native populations cited in this study.

Distinguishing characters for the two varieties are summarized in Table 5. Most of the quantitative characters reflect the statistical results presented in Table 3, except for leaf length. Table 3 shows mean leaf lengths, whereas Table 5 gives a range of values that takes into consideration individual leaves and trees from our study.

Other characteristics distinguish southern from northern populations of *P. greggii*. In genetic field trials planted in Brazil, Colombia and South Africa, progeny from southern populations grew significantly taller than northern progeny, and maintained their differences in foliage color and stiffness. Southern trees planted in Brazil and Colombia were 100% taller than northern trees at



TABLE 5. Summary of the characters that distinguish northern and southern populations of *Pinus greggii*.

CHARACTER	var. <i>greggii</i>	var. <i>australis</i>
Leaves		
position	erect	frequently drooping
rigidness	stiff	flexible
color	light green	yellowish green
length (cm)	7–12	10–15
stomata number <sup>1</sup>	34–36	36–41
internal resin canals	lacking	sometimes 1 or 2
Seeds		
wing width (mm)	5.5–6.4	6.0–6.7
Terpenes (%)		
a-pinene	low	high
limonene	high	low
longifolene	high	low
myrcene	low	high

<sup>1</sup>per 3 mm leaf length

three years of age, and in South Africa they were 17 to 30 % taller than northern trees (Dvorak et al. 1996). Similar results were observed in field trials in Mexico, where southern populations grew 106% taller at two years of age (Alba Landa et al. 1998) and 118% taller at nearly three years of age (Lopez Ayala et al. 1999). Trees from southern populations showed greater resistance to pine pitch canker disease caused by *Fusarium subglutinans* f.sp. *pini* than northern trees in a greenhouse screening trial (Hodge & Dvorak 2000). Although it has not been demonstrated that the cause is genetic, flowering and pollination of *P. greggii* in Mexico occurs in April–May in southern populations, and in May–June in northern populations (Lopez Upton & Donahue 1995).

Recent taxonomic works on Mexican pines by Perry (1991) and Farjon and Styles (1997) did not recognize a distinction between the two populations either because they did not observe a large enough sample size to detect the differences, or attributed them to climatic effects. The differences in internal leaf characteristics and terpene composition cited here are least likely caused by environmental changes, while differences in leaf size and color remained when the two populations were planted side by side in the same environment.

CONCLUSION

Based on the results of the aforementioned studies and observations, the authors believe that trees from southern populations located in the states of Hidalgo, Puebla, Queretaro, San Luis Potosi and Veracruz constitute a separate taxon treated as follows:

***Pinus greggii* Engelm. ex Parl. var. *australis* Donahue & Lopez, var. nov.** (Figs. 1, 2). TYPE: MEXICO. QUERETARO: Mpio. Landa de Matamoros, El Madroño, 21° 16' N, 99° 10' W, 1690 m, 19 May 1993, *Donahue & Lopez Upton B30* (HOLOTYPE: MO).

Varietas *australis* ab *Pinus greggii* var. *greggii* distinguibilis est foliis elongatis, flavovirentibus, cum stomatibus plus per 3 mm longitudine, interdum canalibus resiniferis internis uno vel duobus, et terpene compositis myrcene altis et longifolene demissis, et endemicis ad Mexico centralis.

The variety *australis* has leaves in fascicles of 3, 10–15 cm long, 1.0–1.3 mm wide, with 36–41 stomata per 3-mm leaf length. Resin canals are 3–4(–6) in number, predominantly medial, and occasionally 1–2 internal. Cone length is 8–13(–14) cm and cone widths are 3–5 cm. Seeds are 5–7 mm in length and 3–4 mm wide. Seed wing length is 11–16 mm, and width is 6–8 mm.

PARATYPES: MEXICO. Queretaro: Mpio. Landa de Matamoros, El Madroño, 21° 16' N, 99° 10' W, 1690 m, 19 May 1993, *Donahue & Lopez Upton B27* (A) *B20* (F) *B19* (MICH) *B18* (MO) *B23* (NCSC) *B17* (TEX).

The variety *greggii* has leaves in fascicles of 3, 7–12 cm long, 1.2–1.4 mm wide, with 34–37 stomata per 3 mm leaf length. Resin canals are (2–)3–4(–6) in number, medial. Cone length is 8–12 cm and cone widths are 3–5 cm. Seeds are 5–8 mm in length and 3–4 mm wide. Seed wing length is 13–16 mm, and width is 5–7 mm.

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FIG. 2. A comparison of leaf characteristics of (A) *Pinus greggii* var. *greggii* from La Tapon, Nuevo Leon, and (B) *Pinus greggii* var. *australis* from El Madroño, Queretaro. The trees are growing in Louisiana, USA, produced from seed from the corresponding native stands in Mexico.

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