Morphological, Molecular, and Ecological Divergence in *Pinus douglasiana* and *P. maximinoi*

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Communicating Editor: Michael J. Moore

**Abstract**—*Pinus douglasiana* and *P. maximinoi* (Pinus subsection Ponderosae) are closely-related New World pines with vague taxonomic boundaries where their natural ranges overlap in western Mexico. They are distinguished from each other by the width of their leaves and thickness of their cone scale apophyses. They are also sometimes confused with two other close relatives, *Pinus pseudostrobus* and *P. yecorensis*. We integrated morphological, molecular, and ecological data to clarify the taxonomic limits among these four species. Following previous studies, we evaluated 16 quantitative leaf and seed cone characters. *Pinus douglasiana*, *P. maximinoi*, and *P. pseudostrobus* formed non-discrete groups in multivariate space. The absence of leaf hypodermal intrusions, a persistent peduncle, and the shape of the seed cone are useful for differentiating *P. pseudostrobus* and *P. yecorensis* from *P. douglasiana* or *P. maximinoi*, and the latter two can usually be distinguished by needle width or cone scale apophysis thickness. Most individuals identified as *P. douglasiana*, and *P. maximinoi* shared haplotypes for a plastid ycf1 fragment that is relatively variable for the genus, while *P. yecorensis* has a closely related, exclusive haplotype. A distinct haplogroup included all individuals of *P. pseudostrobus* and the remaining individuals of *P. douglasiana* and *P. maximinoi*. Leaf width and cone scale thickness of *P. douglasiana* and *P. maximinoi* are correlated with elevation. According to potential distribution models, *P. yecorensis* is distributed in drier areas than *P. douglasiana* or *P. maximinoi*, while *P. pseudostrobus* occurs in more temperate areas, commonly at higher elevations. *Pinus douglasiana* and *P. maximinoi* can be considered as incipient species undergoing divergent evolution characterized by incomplete morphological, molecular, and ecological divergence.

**Keywords**—Morphometry, species distribution modeling, pine, *Pinus pseudostrobus*, *Pinus yecorensis*, polymorphism.

*Pinus* L. (Pinaceae) is a monophyletic and conspicuous genus comprising ca. 119 species of large (up to 80 m in height), long-lived, monocious, and perennial trees distributed almost exclusively in the Northern Hemisphere (Mirov 1967; Richardson and Rundel 1998; Farjon 2010). Pines possess distinctive secondary needle-like leaves arranged singly or in fascicles (Farjon 2005, 2010). Leaf and seed cone characters are usually used to recognize pine species because they are more variable than other structures (Farjon and Styles 1997). Despite often being of great economic and ecological importance, uncertainty still exists on the geographic limits of natural ranges for some pine species, especially in Mexico (Shaw 1909; Critchfield and Little 1966; Perry 1991; Eckenwalder 2009; Farjon 2010; Debreczy and Rácz 2011). Clearer interspecific limits would substantially improve the use, management, and conservation of pines (Mirov 1967; Challenger and Soberón 2008).

**Pinus** subsection *Ponderosae* Loudon includes between approximately 14 and 16 species distributed from western Canada and the U. S. A. to Mexico and Central America (Little and Critchfield 1969; Gernandt et al. 2005; Eckenwalder 2009; Farjon 2010; Hernández-León et al. 2013). Divergent opinions surround the circumscription of species in this group (e.g. Price et al. 1998; Eckenwalder 2009; Farjon 2010; Debreczy and Rácz 2011). Morphometric studies have been carried out to better define the specific limits of variable taxa such as *P. ponderosa* P. Lawson & C. Lawson (Callaham 2013), *P. hartwegii* Lindl. (Matos 1995), and *P. douglasiana* Martínez and *P. maximinoi* H. E. Moore (Stead 1983a). Studies that integrate more diverse sources of data are needed for these and other pines, as are objective criteria for deciding whether or not to recognize taxa.

**Pinus tenuifolia** Bentham. was described from material collected near Guatemala City, Guatemala (Bentham 1842); the name is a later homonym of *P. tenuifolia* Salisb. (1796), which in turn is considered a synonym of *P. strobus* L. (Farjon and Styles 1997). Shaw (1909) treated this taxon as a variety of *P. pseudostrobus* Lindl. (*P. pseudostrobus* var. *tenuifolia* Shaw), and described its distribution as from northwestern and central Mexico to Nicaragua. Martínez (1943) segregated *P. douglasiana* from *P. maximinoi* based on populations occurring in central and western Mexico with thicker, stiffer needles and thicker seed cone scale apophyses. Leaf and cone characters of *P. maximinoi* and *P. douglasiana* were included by Stead (1983a) in subsequent principal components and canonical discriminant analyses that illustrated the differences in leaf and cone size between these taxa. He concluded that they were legitimate species (Stead 1983a, 1983b). According to Farjon and Styles (1997), no other consistent differences have been identified between *P. douglasiana* and *P. maximinoi*. Although Martínez (1948) and Wittak and Perry (1979) stated that *P. douglasiana* and *P. maximinoi* can be distinguished by rough versus smooth branchlet surfaces, others such as Perry (1991) and Farjon and Styles (1997) have concluded that this character is inconsistent. Notwithstanding the morphological differences in size, Silba (1990) demoted *P. maximinoi* to a variety of *P. douglasiana* (*P. douglasiana* var. *maximinoi* [H. E. Moore] Silba).

Some pine species may be in a formative stage (Farjon and Styles 1997; Perry et al. 1998). Molecular studies suggest that Mexican and Central American taxa of *Pinus* subsection *Ponderosae* diversified relatively recently. A plastid DNA study of the group found only minor differences between *P. douglasiana* and *P. maximinoi*, with some *P. maximinoi* individuals also sharing haplotypes with *P. pseudostrobus* Lindl., demonstrating the lack of genealogical monophyly in *P. maximinoi* (Gernandt et al. 2009). Plastid sequences typical of *P. pseudostrobus* are more closely related to two other Mexican and Central American species of the *Montezumae Group*, *P. montezumae* Lamb. and *P. hartwegii* (Gernandt et al. 2009). Hybridization, introgression, or incomplete lineage sorting could explain the sharing of plastid haplotypes among pine species (Delgado et al. 2007; Syring et al. 2007; Willyard et al. 2009). A subsequent molecular clock-based estimate based on plastid DNA indicated that *P. douglasiana*
and *P. maximinoi* shared a common ancestor in the Pleistocene (Hernández-León et al. 2013).

*Pinus douglasiana* and *P. maximinoi* are also difficult to distinguish in the field and herbarium from two closely related species in subsection *Ponderosa*, *P. pseudostrobus* and *P. yecorensis* Debreczy and Rác. These four taxa differ in leaf and cone characters (Table 1). Farjon and Styles (1997) recognized two varieties of *P. pseudostrobus* var. *pseudostrobus* and var. *apulcensis*. *Pinus pseudostrobus* var. *pseudostrobus* is the most similar in appearance to *P. douglasiana* and *P. maximinoi* (Table 1). *Pinus yecorensis* was described more recently (Debreczy and Rác 1995). It was treated as a doubtful name, possibly a variety of *P. pseudostrobus* by Farjon and Styles (1997), but recognized as a legitimate species in a recent treatment of the trees of Sonora (Felger et al. 2001). Individuals from the type locality have sequences that belong to the same haplogroup as (Felger et al. 2001). Individuals from the type locality have *P. pseudostrobus* 1995). It was treated as a doubtful name, possibly a variety of *yecorensis* appearance to *P. maximinoi* (Table 1).

Farjon and Styles (1997), Debreczy and Rácz (1995), and Gernandt et al. (2009). In this work only hypodermal intrusions that come in contact with the endodermis are referred to as such.

Pines, like many trees, have high intraspecific variation and low mutation rates per unit time (Petit and Hampe 2006). Pines are further characterized by high outcrossing rates, huge effective population sizes, and weak barriers to gene flow, all of which obscure their interspecific limits (Ledig 1998; Delgado et al. 2007; Wilyard et al. 2007). Reproductive isolation, monophyly, diagnosability, and ecological differences are contingent attributes, not an unavoidable result of speciation processes; however, the presence of these attributes often helps differentiate species (de Queiroz 1998; Sites and Marshall 2004).

Our main objective is to integrate three different lines of evidence that could help understand the process of speciation and divergence in recently formed sister taxa; morphology, molecules, and climate. Synthesis of this information is needed to understand complex morphological variation in *Pinus*. Given the deficiency of fixed qualitative differences for this group, we quantify morphological variation and evaluate it using statistical techniques.

### MATERIALS AND METHODS

**Plant Material**—Field work was conducted from 2010–2013. To capture the greatest possible morphological variation, field collections were made from representative sites throughout the natural range of *P. douglasiana* and *P. maximinoi* in Mexico. Branches and seed cones of the study species were collected from the bottom of the crown. Material was pressed, dried, and deposited in the National Herbarium of Mexico (MEXU). Herbarium specimens from Guatemala, Honduras, and Nicaragua was examined, but no fieldwork was conducted in these countries. A total of 287 individuals were included (Appendix 1). Our sampling for *P. yecorensis* was limited, but we included it in comparisons whenever possible given its presumed close relationship with *P. douglasiana* and *P. maximinoi*. Locality data for 16 collections of *P. yecorensis* and three collections of *P. pseudostrobus* were taken from online databases (Appendix 1).

For the molecular study two or three fresh leaves were conserved in a freezer at -20°C. To obtain anatomical data, ~1.0 cm segments from the medial part of fresh leaf samples were preserved in a FAA solution. For herbarium material, ~1.0 cm segments from the medial part of a leaf were rehydrated in boiling water and then immersed ~24 hrs in FAA. Transverse sections were made manually. The sections were cleared in 50% sodium hypochlorite for 2 min, rinsed several times with distilled water, dehydrated in a series of alcohol rinses (50, 75, 96, and 100%), stained with fast green in 100% alcohol, and mounted on a slide with resin. Because the data set was incomplete, not all collections were included in all analyses. The number and details of the individuals included in each analysis are specified below.

**Preliminary Taxonomic Identification**—A preliminary identification was made of each individual based upon field observations and microscopic examination of the leaves (Table 1). The persistence of the cone peduncle and presence of hypodermal intrusions in the leaf were the

### Table 1. Main diagnosable characters for *Pinus douglasiana*, *P. maximinoi*, and closely related taxa. Information based on Farjon and Styles (1984), Farjon and Styles (1997), Debreczy and Rác (1995), and Gernandt et al. (2009). In this work only hypodermal intrusions that come in contact with the endodermis are referred to as such. *P. maximinoi* can lack hypodermal intrusions at its southernmost distribution. Persistent peduncles are those that remain on the branch after the mature cone falls from the tree.

<table>
<thead>
<tr>
<th>Species</th>
<th>Needle characters</th>
<th>Seed cone characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypodermal intrusions</td>
<td>Needle length (cm)</td>
</tr>
<tr>
<td><em>Pinus maximinoi</em></td>
<td>Usually present*</td>
<td>20–35</td>
</tr>
<tr>
<td><em>P. douglasiana</em></td>
<td>Present</td>
<td>22–35</td>
</tr>
<tr>
<td><em>P. pseudostrobus var. pseudostrobus</em></td>
<td>Absent</td>
<td>(18–)20–30(–35)</td>
</tr>
<tr>
<td><em>P. pseudostrobus var. apulcensis</em></td>
<td>Absent</td>
<td>(18–)20–30(–35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. yecorensis</em></td>
<td>Absent</td>
<td>28–35</td>
</tr>
</tbody>
</table>
principal characters used to distinguish *P. pseudostrobus* from *P. douglasiana* or *P. maximinoi* (Martínez 1948; Mittak and Perry 1979; Stead and Styles 1984; Perry 1991; Farjon and Styles 1997). Length and thickness of the cone peduncle in *P. pseudostrobus* are variable. Forty-five new collections from northwestern Mexico, principally the western extreme of the Trans-Mexican Volcanic Belt (TMVB; Jalisco and Michoacán) and the Sierra Madre Occidental (Sinaloa, Sonora) have homogeneous seed cone morphology, deciduous cone peduncles typical of *P. douglasiana* and *P. maximinoi*, and predominantly lack leaf hypodermal intrusions. We refer to them here as *Pinariss aff. douglasiana*. Some individuals identified as *P. pseudostrobus* lack leaf hypodermal intrusions, but have both persistent and deciduous peduncles and have flat cone apophyses that resemble *P. douglasiana* and *P. maximinoi* in pedermal intrusions; this taxon has subtle differences from *P. douglasiana* such as stout branches and short and more spherical cones with stout peduncles (Gernandt et al. 2009).

**Analysis of Quantitative Variation**—A total of 219 individual collections were included in the morphological analyses. Only collections with leaves that were mature in size and appearance were measured; similarly, only collections with open seed cones were measured. Data were obtained for herbarium specimens (mainly from Guatemala, Honduras, and Nicaragua) although it was not always possible to obtain all measurements. The herbarium specimens were included in the analyses given the desirability of evaluating their variation.

**DNA Sequencing, Edition, and Analysis**—

**DNA Isolation and Amplification**—DNA was isolated from 40 new sequences from *P. maximinoi* and *P. pseudostrobus*. We also chose it for further characterization with the *ycf1* fragment were identical for five of eight individuals in a previous study (Gernandt et al. 2009). How-

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Fig. 1. Cone and leaf variables. A. Fascicle. B. Fascicle sheath. C. Cross sectional anatomy of a secondary leaf. D. Stomatal lines on the dorsal face of a leaf. E. Cone. F. Cone scale. G. Lateral view of a cone scale (See methods for an explanation of abbreviations).
missing or low quality base calls. Sequences were used to assemble a matrix 803 bp in length, without indels and without missing data. The haplotype of each individual is available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.931h7. A haplotype network was built to estimate gene genealogies with the statistical parsimony method implemented in TCS 1.21 (Templeton et al. 1992; Clement et al. 2000).

Species Distribution Modeling—The potential distributions of \textit{P. douglasiana}, \textit{P. maximinoi}, \textit{P. pseudostrobus} var. \textit{pseudostrobus}, and \textit{P. yezoensis} were modeled using MaxEnt 3.1 (Phillips et al. 2006) with the default settings. Climatic and elevational variables, with a spatial resolution of about \(\sim 1\) km\(^2\), were used for the respective distribution modeling. Bioclimatic data for Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, and Panama were obtained from WorldClim 1.4 (Hijmans et al. 2005). A predictive model based on presence-only data was generated using geographic coordinates of collections from the natural distribution of the study taxa. Only one occurrence record per species per grid cell (\(\sim 1\) km\(^2\)) was included.

Records with locality descriptions but lacking coordinates were georeferenced using Google Earth 5.1.3509.4636 (beta) and the Mexican website of the Instituto Nacional de Geografía e Informática (INEGI 2014). For \textit{P. douglasiana} and \textit{P. maximinoi} records, 17 and 39 points, respectively (Appendix 1) correspond to individuals that we identified with multivariate analyses or herbarium collections. The type locality of \textit{P. douglasiana} in Cerro Juanacata near the town of Jala in Nayarit, Mexico (Martínez 1943, 1948) was not included in the modeling of \textit{P. douglasiana} because we failed to locate the species when we visited this area. In agreement with the conclusions of Stead and Styles (1984), we did not find the typical form of \textit{P. maximinoi} in Sonora and Sinaloa. Points for \textit{P. maximinoi} from cold and high altitude localities in the eastern TMVB (Vigés-Xalapa, Veracruz and Nevado de Toluca, State of Mexico) were not included because the collections that we examined from these sites lack leaf hypodermal intrusions and were thus redetermined as \textit{P. pseudostrobus}. Three additional georeferenced points for \textit{P. pseudostrobus} were obtained from the online botanical database, Tropicos (Missouri Botanical Garden 2015). Locality data were obtained for \textit{P. yezoensis} from six MEXU specimens, 16 records from the website of the Consortium of Intermountain Herbaria (2014), and one locality from the original species description (Debrezcy and Racz 1965). According to our statistical analyses, the distribution points of \textit{P. maximinoi} and \textit{P. douglasiana} included for modeling do not include sites where the two species are in sympatry. To visualize zones of sympatry, the potential distribution of these species was displayed on the same map. To test each resulting model, a partition of the occurrence localities was made setting the random test option in MaxEnt at 25\%. Likewise, the scores of the area under the receiver operating curve (AUC) for the training data were considered as statistical tests to evaluate the performance of maximum entropy modeling. It has been reported that scores greater than 0.90 are indicative of a good performance (Phillips et al. 2006; Baldwin 2009). The inferred potential distribution was trimmed considering the logistic threshold value associated with maximum training sensitivity plus specificity value and the resulting models were displayed graphically in ArcMap 10.0 (Environmental Systems Research Institute, Inc., Redlands, California).

To evaluate the relationship between climate and morphological variation we used k-means to compared the groups formed with the climatic variables, the morphological variables, and the preliminary clustering criteria. The same, 19 climatic variables used for modeling and the 16 quantitative morphological variables were standardized for the analysis. One collection per site was included. Sites lacking morphological information were excluded. The morphological data correspond to individual collections identified as \textit{P. aff. douglasiana} (n = 9), \textit{P. douglasiana} (n = 12), \textit{P. maximinoi} (n = 20), \textit{P. pseudostrobus} (n = 7), and \textit{P. yezoensis} (n = 2). The tree's bioclimatic information for each of the climatic variables (layers with a resolution of 0.5 sec) and the sites included in the modeling were extracted using the raster package (Hijmans and van Etten 2012). A table was constructed to compare the resulting classifications.

\section*{Results}

\subsection*{Comparison of Quantitative Character Means—} The comparison of \textit{P. douglasiana} and \textit{P. maximinoi} in western and southern Mexico, respectively, with individuals from Michoacán and the State of Mexico in the TMVB indicated that this group is statistically different from typical \textit{P. douglasiana} or \textit{P. maximinoi} from other localities. Variances among groups in the TOAU character after transforming the data were statistically different (Levene test \(F_{2, 142} = 4.75; p = 0.010\)). A non-parametric test (Kruskal-Wallis test) showed significant differences among groups (\(x^2 = 103.5887; df = 2; p < 0.01\); see Fig. 2A). Although statistically different, the mean value of TOAU from the Michoacán and State of Mexico collections was closer to the value of \textit{P. douglasiana} than to \textit{P. maximinoi} from Chiapas, Guerrero, and Oaxaca (3.72, 4.45, and 2.29 mm, respectively). For WON, collections from Michoacán and the State of Mexico had needles that were wider on average than \textit{P. maximinoi} from Chiapas, Guerrero, and Oaxaca but thinner than \textit{P. douglasiana} from Jalisco and Sinaloa (Fig. 2B). The group variances were not statistically different (Levene test; \(F_{2, 142} = 2.82; p = 0.063\)) but the residuals were not distributed normally (Shapiro-Wilk normality test, \(W = 0.97, p = 0.0060\)). A non-parametric test (Kruskal-Wallis test) showed significant differences among groups (\(x^2 = 83.3541; df = 2; p < 0.01\)), and a nonparametric pairwise comparisons (Wilcoxon rank sum test) showed that all groups were different \((p < 0.01)\). In this comparison of the WON variable, unidentified individuals from Michoacán and the State of Mexico were more similar to \textit{P. maximinoi} (Fig. 2B). However, based on the high similarity of TOAU with \textit{P. douglasiana} we decided to treat tentatively these individuals as this species.

\subsection*{Multivariate Data—} The PCA-1 and PCA-2 did not result in discrete groups in multivariate space among species. For the PCA-1 (inclusion of \textit{P. douglasiana} and \textit{P. maximinoi} only), the sum of variation explained by the first and second principal components (PC1 and PC2) was less than 66\% (Table 2). According to the analysis to distinguish gaps in multivariate space, the overlap in the ellipsoidal region of tolerance at a \gamm 0.95 and \beta 0.90 is such that no gaps were detected and therefore no discrete groups were found (Fig. 2E). All coefficients of PC1 (PCA-1) had negative values (Table 2), indicating that differences between individuals of \textit{P. douglasiana} and \textit{P. maximinoi} explained by PC1 were mostly in size and not form. Most variables, but mainly NEC, TOAU, and TOS, contributed to this size difference (Table 2). In the dispersion graph (Fig. 2C), collections from Michoacán and the State of México grouped with individuals from Jalisco and Sinaloa, which supported their identity as \textit{P. douglasiana}. For PCA-2 (Fig. 2D), the sum of variation explained by PC1 and PC2 was less than 61\% (Table 3). With the exception of number

<table>
<thead>
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<th>Variable</th>
<th>PC1 (50.04%)</th>
<th>PC2 (14.98%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOA</td>
<td>-0.284</td>
<td>-0.150</td>
</tr>
<tr>
<td>Int</td>
<td>-0.089</td>
<td>0.220</td>
</tr>
<tr>
<td>LOC</td>
<td>-0.202</td>
<td>-0.396</td>
</tr>
<tr>
<td>LOCS</td>
<td>-0.226</td>
<td>-0.431</td>
</tr>
<tr>
<td>LOS</td>
<td>-0.239</td>
<td>-0.014</td>
</tr>
<tr>
<td>Ncan</td>
<td>-0.282</td>
<td>-0.015</td>
</tr>
<tr>
<td>NEC</td>
<td>-0.284</td>
<td>0.224</td>
</tr>
<tr>
<td>NSLD</td>
<td>-0.128</td>
<td>0.160</td>
</tr>
<tr>
<td>NSLV</td>
<td>-0.278</td>
<td>0.209</td>
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<tr>
<td>TOAU</td>
<td>-0.294</td>
<td>0.013</td>
</tr>
<tr>
<td>TON</td>
<td>-0.271</td>
<td>0.271</td>
</tr>
<tr>
<td>TOS</td>
<td>-0.297</td>
<td>0.219</td>
</tr>
<tr>
<td>WOA</td>
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<td>-0.266</td>
</tr>
<tr>
<td>WOG</td>
<td>-0.228</td>
<td>-0.432</td>
</tr>
<tr>
<td>WON</td>
<td>-0.274</td>
<td>0.289</td>
</tr>
</tbody>
</table>
Fig. 2. Statistical comparisons of morphometric variables. A. Comparison of TOAU for individuals from the TMVB (VB; n = 21; mean = 3.78; sd = 0.45), *P. douglasiana* (Pdo; n = 55; mean = 4.32; sd = 0.93), and *P. maximinoi* (Pmi; n = 69; mean = 2.3; sd = 0.55) from other localities; the three groups were significantly different ($p_{2, 142} < 0.01$). B. Comparison of WON among individuals from the TMVB (VB; n = 21; mean = 815.2; sd = 68.6), *P. douglasiana* (Pdo; n = 55; mean = 959.8; sd = 127.9), and *P. maximinoi* (Pmi; n = 69; mean = 715.4; sd = 103.4) from other localities; the three groups showed significant differences from each other ($p_2 < 0.05$). C. Scatter plot of PCA-1, *P. douglasiana* (black circles; n = 76); *P. maximinoi* (open circles; n = 62); Specimens of *P. maximinoi* without intrusions (rhombs n = 7). D. Scatter plot of PCA2; *P. aff. douglasiana* (open triangles; n = 24), *P. douglasiana* (black circles; n = 76), *P. maximinoi* (open circles; n = 62), *P. pseudostrobus* var. *pseudostrobus* (open squares; n = 24), *P. yeorensis* (black triangles, n = 4). E. The proportion of individuals ($\beta$) of *P. douglasiana* (dashed line) and *P. maximinoi* (solid line) at different values of $\alpha$ (which define tolerance regions) and a constant confidence level ($\gamma$) of 0.95. The graph shows that at a given ($\alpha$) there never exists the possibility of finding tolerance regions that include at least 90% of the individuals for each species. The dashed line shows the limit for $\beta = 0.90$. F. Linear regression showing the relationship between morphology and elevation *P. douglasiana* (black circles); *P. maximinoi* (open circles).
of leaf intrusions, the positive values of the PC1 variables indicated that there were differences in size among taxa (Table 3). The variables HOA, NEC, and TOAU contributed most to this size difference. The negative and positive coefficient values for PC2 indicated that there were differences in form among individuals, principally between _P. pseudostrobus_ and _P. douglasiana_ and _P. maximinoi_ (Table 3). These differences indicated an inverse relationship among the cone variables and leaf variables, mainly the number of leaf hypodermal intrusions.

The results of the k-means clustering analysis (with k = 3) for the 16 quantitative morphological variables demonstrated a notable similarity between the individuals from the TMVB with those of _P. douglasiana_ in Jalisco and Sinaloa (19 of 21 of these individuals grouped with _P. douglasiana_). The results for k = 4 (Table 4) considering all the individuals (_P. aff. douglasiana_, _P. douglasiana_, _P. maximinoi_, _P. pseudostrobus_, and _P. yecorensis_) and excluding the variable PPED demonstrated that all groups were similar, although there was a notable distinction for _P. douglasiana_ and _P. maximinoi_. The most distinct taxon was _P. maximinoi_, with 41% of its individuals separated in a completely exclusive group. Including the variable PPED increased the congruence with the groups formed with the preliminary identifications. In general, there was a high similarity between the _P. aff. douglasiana_ individuals and those of _P. douglasiana_, _P. maximinoi_, and _P. pseudostrobus_. Unfortunately, the results from analyses that include PPED must be treated with caution because it was coded as a binary (presence or absence) variable.

Elevation and needle and cone size were positively correlated in the regression analysis ($R^2 = 0.5275$; $F_{1, 141} = 159.52$; $p < 0.01$). Residuals showed a normal distribution according to the Shapiro-Wilk normality test ($W = 0.9942$, $p = 0.84$). _Pinus douglasiana_, with needles and cone scale apophysis that are thicker than _P. maximinoi_, is distributed at higher elevations (Fig. 2F).

**Molecular Variation**—Eight haplotypes were found. In the haplotype network (Fig. 3), 88% of the individuals identified as _P. douglasiana_ had the same haplotype, which was also shared by 72% of the individuals identified as _P. maximinoi_. All individuals classified as _P. aff. douglasiana_ had the typical haplotype of _P. maximinoi_ and _P. douglasiana_. No _P. pseudostrobus_ individuals had the haplotype typical of _P. douglasiana_ or _P. maximinoi_. Exclusive haplotypes of _P. yecorensis_ connected to the typical haplotype of _P. douglasiana_ and _P. maximinoi_. One individual identified as _P. maximinoi_ from Oaxaca and another from Guatemala (Guatemala), had haplotypes typical of _P. pseudostrobus_. The latter individual from Guatemala lacked leaf hypodermal intrusions.

**Potential Distribution**—Scores of the area under the receiver operating curve (AUC) for the training data were greater than 0.90 in all models. The AUC score was 0.990 for _P. douglasiana_, 0.980 for _P. maximinoi_, 0.977 for _P. pseudostrobus_ var. _pseudostrobus_, and 0.989 for _P. yecorensis_. These high scores indicated that model performance was good (Phillips et al. 2006). The training omission rate and test omission associated with the logistic threshold had a value of 0.0 for all models.

The potential distribution for _P. douglasiana_ was mainly confined to central and western Mexico (Fig. 4A). The results indicated that the major discontinuities in its distribution corresponded to areas of disruption between the SMOC and mountain ranges of the TMVB, but in general, its distribution

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**Table 3.** Coefficients of the first and second component (PC1 and PC2) of the second principal component analysis (PCA-2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1 (44.89%)</th>
<th>PC2 (15.89%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOA</td>
<td>0.295</td>
<td>−0.172</td>
</tr>
<tr>
<td>Int</td>
<td>−0.016</td>
<td>0.402</td>
</tr>
<tr>
<td>LOC</td>
<td>0.234</td>
<td>−0.373</td>
</tr>
<tr>
<td>LOCS</td>
<td>0.249</td>
<td>−0.308</td>
</tr>
<tr>
<td>LON</td>
<td>0.225</td>
<td>0.136</td>
</tr>
<tr>
<td>LOS</td>
<td>0.271</td>
<td>0.066</td>
</tr>
<tr>
<td>Ncan</td>
<td>0.223</td>
<td>−0.049</td>
</tr>
<tr>
<td>NEC</td>
<td>0.282</td>
<td>0.244</td>
</tr>
<tr>
<td>NSLD</td>
<td>0.169</td>
<td>−0.111</td>
</tr>
<tr>
<td>NSLV</td>
<td>0.255</td>
<td>0.095</td>
</tr>
<tr>
<td>TOAU</td>
<td>0.287</td>
<td>0.038</td>
</tr>
<tr>
<td>TON</td>
<td>0.276</td>
<td>0.298</td>
</tr>
<tr>
<td>TOS</td>
<td>0.269</td>
<td>0.332</td>
</tr>
<tr>
<td>WOA</td>
<td>0.293</td>
<td>−0.246</td>
</tr>
<tr>
<td>WOC</td>
<td>0.255</td>
<td>−0.330</td>
</tr>
<tr>
<td>WON</td>
<td>0.261</td>
<td>0.316</td>
</tr>
</tbody>
</table>

---

**Table 4.** Comparison between clustering criteria. Clustering with the k-means criterion with k = 4, with and without the variable describing the persistence of the cone peduncle (PPED), and the initial classification. Based on 16 continuous leaf and cone variables and PPED. * Individuals lacking leaf hypodermal intrusions.
Fig. 4. Real and potential distributions for the study group. A. *Pinus douglasiana* (n = 24), *P. maximinoi* (n = 41), and *P. yecorensis* (n = 20). B. *Pinus pseudostrobus* var. *pseudostrobus* (n = 16).
was fragmented. Its potential distribution also included areas of sympatry in northern Mexico with *P. yecorensis* and western Mexico with *P. maximinoi*. The results also indicated some small areas with favorable conditions for *P. douglasiana* scattered in the mountain ranges of southern Mexico, namely the Sierra Madre del Sur, Sierra Norte de Oaxaca, and Sierra Madre de Chiapas. Throughout these areas it could occur in sympatry with *P. maximinoi* or *P. pseudostrobus* (Fig. 4A). Variables that contributed most to distribution modeling of *P. douglasiana* were elevation (24.6%) and temperature seasonality (23%). Considered alone, the mean temperature of the warmest quarter (19.0°C on average) was the most useful for explaining the distribution.

The potential distribution of *P. maximinoi* was located principally along the coastal slopes that extend to the Pacific Ocean in Mexico (Fig. 4A). In Central America, areas of distribution of *P. maximinoi* were predicted in small mountain ranges. Areas of potential distribution of *P. maximinoi* were fragmented and disrupted mainly among the mountain ranges of western and southern Mexico (Fig. 4A). Elevation contributed most to the distribution model (31.5%), followed by annual precipitation (22.9%). Considered alone, temperature seasonality was the most useful for explaining the distribution, contributing 11.9% to the model.

The potential distribution of *P. pseudostrobus* var. *pseudostrobus* was wide and occurred mainly in cold-temperate to warm-temperate zones (Fig. 4B). Its potential distribution occurred throughout the major mountain ranges, from northern Mexico to northern Nicaragua. The variables that most contributed to model performance were temperature seasonality (36%), elevation (27.1%), and mean temperature of the warmest quarter (17.7°C; 16.2%). Considered alone, the maximum temperature of the warmest month (25.6°C on average) was the most useful for explaining the potential distribution of this taxon.

The potential distribution of *P. yecorensis* was in the northeastern SMOC, where dry climates prevail (Fig. 4A). Precipitation seasonality contributed most to the distribution model for this species (31%), followed by precipitation of the warmest quarter (478.26 mm on average; 23.8%), and elevation (19.6%). Temperature seasonality and mean temperature of the driest quarter, each considered alone, were the most useful for explaining its potential distribution.

For the k-means analysis, taking into account the climatic variables we found a 100% correspondence between the groups formed with those based on the same method and morphology (without including the variable PPED; Table 5). Groups formed with the k-means analysis and morphology (without including the variable PPED; Table 5).

| Table 5. Comparison of the classification taking into account the climatic variables and the k-means method versus the classification of groups that takes into account morphological variables, k-means, and the initial classification. |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| **climatic**        | **k-means**         | **1** | **2** | **3** | **4** | **Initial classification** | **P. aff. douglasiana** | **P. douglasiana** | **P. maximinoi** | **P. pseudostrobus** | **P. yecorensis** |
| 1                   | 0                   | 6     | 0     | 0     | 0     | 1                   | 2                     | 2                   | 0                   | 1                   | 1                   |
| 2                   | 0                   | 0     | 20    | 0     | 0     | 2                   | 2                     | 0                   | 19                  | 0                   | 0                   |
| 3                   | 13                  | 0     | 0     | 0     | 0     | 1                   | 6                     | 0                   | 0                   | 0                   | 0                   |
| 4                   | 0                   | 0     | 0     | 0     | 11    | 1                   | 6                     | 3                   | 0                   | 1                   | 1                   |

**Discussion**

**Taxonomic Limits**—Despite their morphological similarities, *Pinus douglasiana* and *P. maximinoi* have been considered distinct species in most recent taxonomic treatments (e.g. Price et al. 1998; Eckenwalder 2009; Farjon 2010; Debreczy and Rácz 2011). An exception is the demotion of *P. maximinoi* to *P. douglasiana* var. *maximinoi* by Silba (1990). The quantitative characters helped to differentiate partially *P. douglasiana* and *P. maximinoi*, but the two taxa share many characters and we were unable to identify any that were consistently diagnostic. The individuals from the TMVB with intermediate leaf width and cone scale apophysis thickness were more similar to *P. douglasiana*, but our decision to classify them as this taxon could be considered subjective due to the overlapping range of morphological variation with respect to typical individuals of *P. maximinoi*.

Overlapping variation in continuous characters has been observed previously in *Pinus* subsection Ponderosae (Matos 1995). Many species in the subsection may be of recent origin (Hernández-León et al. 2013), and many are partially sympatric (Farjon and Styles 1997), which may have delayed their divergence (Matos and Schaal 2000; Delgado et al. 2007; Willyard et al. 2009). Stead (1983a), sampling fewer populations in western Mexico, found groups that were better defined than the groups found in this study (Fig. 2D). Although our results are partially congruent with his, our PCA results are not completely comparable. In this study we did not sample as intensively within populations; we usually sampled fewer than 25 individuals at each locality, and did not always obtain mature cones. We also only considered continuous variables in our analyses. In contrast, Stead (1983a) sampled as many as 25 individuals per population, and coded the presence of a peduncle and the roughness of the branchlets as qualitative characters. There are other notable aspects that highlight the differences between our results and those of Stead (1983a, 1983b). These are related with the fortunate fact that current techniques allow the consideration of molecular characters. For example, we identified individuals as *P. aff. douglasiana* that aside from having an external morphology similar to *P. douglasiana* and occasionally to *P. maximinoi*, had the typical haplotype of these two species. Individuals of *P. aff. douglasiana* are similar to *P. yecorensis* in lacking leaf hypodermal intrusions and a persistent peduncle. Their morphological variation is heterogeneous, but at present we treat them as *P. douglasiana*. In this respect we differ from Stead (1983a; 1983b) in interpreting the presence of leaf hypodermal intrusions as an inconsistent character for diagnosing *P. douglasiana* and *P. maximinoi*. Furthermore, the individuals treated as *P. aff. douglasiana* may be hybrids formed between *P. douglasiana* and *P. pseudostrobus*. These were collected from...
the TMVB and SMOC in areas between 1,400 and 2,400 m elevation where *P. douglasiana* and *P. pseudostrobus* may be in contact. Although *P. aff. douglasiana* included forms that were morphologically intermediate, hybrids do not always take intermediate forms (Rieseberg 1995). Detailed studies are needed to explore the relationship between *P. aff. douglasiana* and other closely related species such as *P. deoviana* or *P. pseudostrobus*.

Whang and Pak (2001) reported that the stomatal apparatus of leaf cuticles are rectangular in *P. maximinoi* but elliptic in *P. douglasiana*, epidermal cells of *P. douglasiana* have vertical end walls while those of *P. maximinoi* are vertical and oblique, that the shape of the anticlinal walls in *P. douglasiana* are straight and sinuous while those of *P. maximinoi* are mainly straight, and that *P. douglasiana* has 7-9 epidermal cell rows between stomatal rows while *P. maximinoi* has 12-14. These characters and others merit further study.

**Interspecific Gene Flow**—Most individuals of *P. douglasiana* and *P. maximinoi* that we included have the same haplotype, and the same occurs with *P. pseudostrobus*. The typical haplotype of *P. pseudostrobus* is most closely related to haplotypes of *P. montezumae* and *P. hartwegii*, while the typical haplotype of *P. douglasiana* and *P. maximinoi* are more closely related to *P. deoviana* and other species in the Devoniana clade (Gernandt et al. 2009). Hybridization, introgression or incomplete lineage sorting could explain why other individuals of *P. maximinoi* and *P. douglasiana* share plasidic haplotypes with *P. pseudostrobus*. Hybridization and introgression are well documented in closely related species of *Pinus* (reviewed by Ledig 1998; recent examples in North American pines include Matos and Schaal 2000; Delgado et al. 2007; Liston et al. 2007). A molecular study of multiple unlinked loci would be helpful for determining whether these patterns are due to hybridization and introgression or incomplete lineage sorting in these taxa.

**Geographic Distribution**—The modeling of potential distribution of *P. douglasiana*, *P. maximinoi*, *P. pseudostrobus*, and *P. yecorensis* was satisfactory, but we should treat these results with caution given that the optimal conditions were not completely fulfilled in the performance of the modeling (Elith et al. 2011). Ecological niche models offer great promise for the development of hypotheses regarding the distribution of species, but they are subject to a series of limitations (Baldwin 2009). A molecular study of multiple unlinked loci would be helpful for determining whether these patterns are due to hybridization and introgression or incomplete lineage sorting in these taxa.

**Size** —The modeling of potential distribution of *P. douglasiana*, *P. maximinoi*, *P. pseudostrobus*, and *P. yecorensis* was satisfactory, but we should treat these results with caution given that the optimal conditions were not completely fulfilled in the performance of the modeling (Elith et al. 2011). Ecological niche models offer great promise for the development of hypotheses regarding the distribution of species, but they are subject to a series of limitations (Baldwin 2009). A molecular study of multiple unlinked loci would be helpful for determining whether these patterns are due to hybridization and introgression or incomplete lineage sorting in these taxa.
although its distribution remains poorly understood, some ecological differences with respect to *P. douglasiana* and *P. maximinoi* have been identified, such as inhabiting a drier climate. Only *P. yeorensis* has both exclusive plastid haplotypes and a distinct combination of leaf and seed cone characters. It might occur in parapatry with *P. douglasiana* so it is possible to expect limited gene flow between the two. Detailed population studies of morphological and molecular variation in *P. yeorensis* are needed to determine its taxonomic status more conclusively.

If *P. douglasiana*, *P. maximinoi*, and *P. pseudostrobus* have achieved reproductive isolation, monophyly, or diagnosability, we have been unable to demonstrate it. Most individuals collected across a wide distribution range and identified as *P. maximinoi* or *P. douglasiana* share a haplotype (Fig. 3), and studies to date have concluded that variation in peduncle persistence, presence of leaf hypothermal intrusions, and the number and position of resin canals seem to unite more than separate *P. douglasiana* and *P. maximinoi*. We advocate treating *P. maximinoi* and *P. douglasiana* as separate species, as our data suggest that they are in the early stages of ecological and morphological divergence. Further study of these taxa using a wider diversity of morphological and molecular markers may help us to understand early patterns and processes of speciation in recently diverged conifers.

Acknowledgments. This work is in partial fulfillment of the requirements of the Posgrado en Ciencias Biológica, UNAM, whose support of the first author is gratefully acknowledged. The first author also thanks CONACYT for providing a graduate scholarship. Funding for field and laboratory work was provided by UNAM-DGAPA-PAPIIT (IN228209). The authors thank Mark Olson, Martín García, other colleagues of the Instituto de Biología, UNAM, and Victoria Sosa for feedback in the design of the study and for their support. We also thank Ann Willyard, James Smith, Michael Moore, and two anonymous reviewers for their valuable comments on a previous version of this manuscript.

Literature Cited


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Querétaro, Cerro Zacatón, Cerca de Múlpillas, Santos 1341 (MEXU), -100.88, 19.7169444; Morelia, Cerca de Agua Zarca, Santos 1371 (MEXU), -101.12, 19.6052861.

Oaxaca: Ixtlán de Juárez, Southwest of border of Rancho Teja, Debreczy 37636 (MEXU), -; Santa Catarina Juquila, El Pedimento, López 81, 82 (MEXU), -; San Juan Lachao, Al sur de Lachao, López 30 (MEXU), KJ152826, -; San Juan Lachao, Al sur de Lachao, López 30 (MEXU), ARIZ, -, -100.69, 17.8218333; Oaxaca, El Cerro Anole, Rodríguez 76716-3-26, 76716-1-22, 76716-1-34, 76716-2-1, 76716-4-13, 76716-2-21, 76716-1-4 (MEXU), -; Santa Catarina Ixtpeje, Al Noreste de Yuvila, Yescas 72698-2-9 (MEXU), -; Sinaloa: Concordia, La Llantera, López 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 216, 219 (MEXU), -105.8517778, 23.5658889; Concordia, Puente el Carrizo, López 224 (MEXU), -; Sinaloa: Concordia, El Carrizo, CBFF 31A (MEXU), -105.8286611, 23.5275; Concordia, 2 Km al este de la Lobera, CBFF 6 (MEXU), -105.8380556, 23.48.

**P. maximinoi** H. E. Moore, GUATEMALA. Guatemala: San Juan Sacatepéquez, Alrededores de la granja avícola, Armus 8 (MEXU), -; El progreso: Morazan, Finca Bucaral, Sacatepéquez, Alrededores de la granja avicola, San Pedro Sula, Road from cofradia to Buenos Aires, Viñas 1341 (MEXU), -, -97.08333333, 14.03333333; Valle de los Angeles, Valle de Los Angeles, Chilpancingo, López 43, 44, 45, 46, 47 (MEXU), FJ580236, -, -92.26636111, 17.2051667; Sonora: Cabo Corrientes, Carretera hacia el Tuito, López 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 (MEXU), -96.45747222, 15.97369444; San Mateo Pilas, Camino a San Mateo Pilas, López 95, 96, 97 (MEXU), -96.45747222, 15.97369444; San Mateo Pilas, Camino a San Mateo Pilas, López 101, 102 (MEXU), -96.50318889, 16.0185; San Miguel del Puerto, El Faro, Salas 276 (MEXU), -96.11472222, 15.99830333; Santa Ana del Valle, El este de Teotitlán del Valle, Salas 436 (MEXU), -96.11472222, 15.99830333; San Pedro Sojoltepec, San Carlos, Los Nahuatlacas, López 77. 3.6 Km N de San Miguel Chongo en Road to San Pedro Sojoltepec, Saynes 233 (MEXU), -96.97694444, 16.03688899; Santa María Yavesia, Al norte del Cerro Yati, Trejo 3059 (MEXU), -96.41853889, 17.18510278. NUEVA GUINEA. Jinfoleta: Jinfoleta, Road Meta-galpa to Jinfoleta, Stead 5129 (MEXU), -; Municipio no stated, N slope of volcano Yali, Douglas, 15073 (MEXU), 17.03988899; San Carlos, Agua Amarilla, Martin 75 (MEXU), -93.5147, 28.458333; Alamos, Chiribos, Martin 75 (MEXU), -93.017117, 27.3; Yecora, San Carlos, Chihuahua. **P. pseudostubos** Lindley, GUATEMALA. Alta Verapaz: Chacop, Suerese de Chiceit, Stand 338 (MO), -90.225, 15.775. MÉXICO. Chiapas: Teopisca, 3 km al sur de Teopisca, López 106 (MEXU), -, -; Huixtlan, Rancho Merced Bazon, Martínez 27 (MEXU), -; Las Rosas, Al norte del poblado Las Rosas, Higman 14 (ARIZ), -92.38, 16.4. **State of Mexico**: Ocuilan, Picacho, Gernandt 769 (MEXU), FJS80236, -94.4503556, 19.01166667; Soltepec, México en road to Las Banderas electrical station, Gernandt 434 (MEXU), FJS80198, -; Tenamalcaltepec, Cerca de Meson Viejo, López 136 (MEXU), -99.87041667, 19.17625; Texcaltitlán, Cerca del Poblado el Chapaneal, López 138 (MEXU), -99.96257278, 18.95247222; Temascaltepec, Albarradas, López 150 (MEXU), -99.988, 12.18398899. **State of Morelos**: Cuale, Morelos, Gernandt 815 (MEXU), -99.21, 20.072222; Oaxaca: Santa Catarina Ixtpeje, Al norte de la Carretera Hoyo, 2.5 Km N de La Llantera, Gernandt 321 (MEXU), -; -; San Juan Mixtpec, Cienega de la Ardiilla, Reyes 4862 (MEXU), -; -; Santa María Yavesia, Al norte del cerro Yati, Trejo 3077 (MEXU), -96.38645, 17.2051667; Veracruz: Las Vegas, East of Las Vegas in malpais, Gernandt 1178, 1179 (MEXU), KJ152827, -97.07244444, 16.92602778; Tepocate, Antigua Carretera Xalapa-Coatepec, Gernandt 1180 (MEXU), -96.94722222, 15.90483333; Acajete, Gravel road between Cieno Palos and Las Vegas, Gernandt 1181 (MEXU), -; -; Veracruz, Las Vegas, East of Las Vegas along highway to Xalapa, Gernandt,1185, (MEXU), KJ152828, -; -; Las Vegas, East of Las Vegas along highway to Xalapa, Gernandt 1186 (MEXU), -; -; Huatusco, Al norte del poblado Huatusco, Nev 28907 (MO), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics, Gernandt 354 (MEXU), -90.01, 19.2, U.S.A. California, placer, Institute of Forest Genetics.