

DIVERSIFICATION WITHIN *LYCIANTHES MOZINIANA* (CAPSICEAE, SOLANACEAE) DIVERSIFICACIÓN EN *LYCIANTHES MOZINIANA* (CAPSICEAE, SOLANACEAE)

MARCO ANTONIO ANGUIANO-CONSTANTE,¹ ELLEN DEAN,² GUADALUPE MUNGUÍA-LINO,^{3,4,5}
 DANIEL SÁNCHEZ-CARBAJAL,^{3,4,5} EDUARDO RUIZ-SANCHEZ,^{3,5} AND AARÓN RODRÍGUEZ^{3,5*}

¹ Doctorado en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas (BEMARENA), Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México.

² UC Davis Center for Plant Diversity, Plant Sciences, Davis, CA, USA.

³ Laboratorio Nacional de Identificación y Caracterización Vegetal (LaniVeg), Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México.

⁴ Cátedras CONACYT-Universidad de Guadalajara, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México.

⁵ Herbario Luz María Villarreal de Puga, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, Jalisco, México.

*Author for correspondence: aaron.rodriquez@academicos.udg.mx

Abstract

Background: *Lycianthes moziniana* (Capsiceae, Solanaceae) is an endemic and widespread species in Mexico. Morphological and molecular evidence suggested three varieties (*L. moziniana* var. *margaretiana*, *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana*).

Hypotheses: *Lycianthes moziniana* is integrated by differentiated lineages corresponding to the three varieties identified.

Studied species: *Lycianthes moziniana* (Dunal) Bitter.

Study sites and dates: Mexico from 2017 to 2021.

Methods: We measured 11 morphological characters based on 207 herbarium specimens. Discriminant Canonical Analysis (DCA), Mahalanobis Square Distance (MD) and Canonical Classificatory Analysis (CCA) were used to uncover the differences among varieties. For geographic and climatic preferences and divergence, we used 288 records and four climatic layers.

Results: Two canonical functions explained 100 % of the morphological variation and seven characters had the highest discriminatory power. In addition, MD was statistically significant. Meanwhile, the CCA recovered three groups at 77 %. The morphological evidence demonstrated the distinctiveness of *L. moziniana* var. *moziniana*. In contrast, *L. moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* were morphologically similar. The identity test showed significant differences among Ecological Niche Models (ENMs). The evidence indicates geographic and climatic isolation among the varieties. *Lycianthes moziniana* var. *moziniana* is widespread and has intermediate overlap with the other two varieties. *Lycianthes moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* were isolated in different biogeographical provinces and showed the lowest overlap.

Conclusions: Morphological and geographic data indicate that incipient speciation is occurring.

Keywords: Geographical barriers, morphology, MTZ, niche overlap, species delimitation.

Resumen

Antecedentes: *Lycianthes moziniana* (Capsiceae, Solanaceae) es endémica y tiene amplia distribución en México. Su apariencia morfológica y la evidencia molecular sugieren tres variedades (*L. moziniana* var. *margaretiana*, *L. moziniana* var. *moziniana* y *L. moziniana* var. *oaxacana*).

Hipótesis: *Lycianthes moziniana* se integra por linajes independientes que corresponden a las tres variedades.

Especie de estudio: *Lycianthes moziniana* (Dunal) Bitter.

Sitio de estudio y fechas: México desde 2017 hasta 2021.

Métodos: Medimos 11 caracteres de 207 especímenes de herbario. Las diferencias se evaluaron con Análisis Canónico Discriminante (ACD), Distancia al Cuadrado de Mahalanobis (DM) y Análisis Clasificatorio Canónico (ACC). Para las preferencias y divergencias geográficas y climáticas, utilizamos 288 registros y cuatro capas.

Resultados: Dos funciones canónicas explicaron el 100 % de la variación morfológica y siete caracteres mostraron poder discriminatorio. Los valores de DM fueron estadísticamente significativos. En tanto, el ACC recuperó tres grupos por encima del 77 %. La evidencia morfológica demostró la distinción de *L. moziniana* var. *moziniana*. En contraste, *L. moziniana* var. *margaretiana* y *L. moziniana* var. *oaxacana* fueron morfológicamente similares. La prueba de identidad mostró diferencias significativas entre los Modelos de Nicho Ecológico (MNE). La evidencia reveló el aislamiento geográfico y climático. *Lycianthes moziniana* var. *moziniana* está muy extendida y tiene superposición intermedia con las otras dos variedades. En tanto, *Lycianthes moziniana* var. *margaretiana* y *L. moziniana* var. *oaxacana* mostraron el menor traslape.

Conclusiones: Los datos morfológicos y geográficos sugieren que el proceso de especiación en *Lycianthes moziniana* es incipiente.

Palabras clave: Barreras geográficas, delimitación de especies, morfología, ZTM, superposición de nicho.

This is an open access article distributed under the terms of the Creative Commons Attribution License CCBY-NC (4.0) international.

<https://creativecommons.org/licenses/by-nc/4.0/>



In biogeography, speciation is an important process and a phylogeographical analysis is an effective way to reconstruct evolutionary history. It also provides more data to decide whether spatial genetic entities merit species status (Avice 2000, Huang 2020). Integrative taxonomy uses different data types to see if there is a match with a taxonomic hypothesis (Dayrat 2005, Padiál *et al.* 2010). In this way, taxa have been revealed and circumscribed using genetic clustering, morphometric analyses, and niche divergence (Spriggs *et al.* 2019, Zhang *et al.* 2021, Wang *et al.* 2022). Integrating DNA sequences, information on ecology, geographic distribution, morphology and reproductive characters can elucidate evolutionary history and clarify the boundaries of difficult taxonomic entities.

The Mexican Transition Zone (MTZ) provides a complex background for plant diversification (Mastretta-Yanes *et al.* 2015). During its formation, the orography and climate have isolated plant populations. Phylogeographic analyses of plant groups that occur in the MTZ have revealed high genetic diversity and significant phylogeographic structure (Rodríguez-Gómez *et al.* 2018, Peñaloza-Ramírez *et al.* 2020, Romero-Soler *et al.* 2022). Empirical evidence has shown that isolation reduces genetic connectivity among populations and promotes morphological variation (Ruiz-Sanchez & Specht 2013, 2014, Gutiérrez-Ortega *et al.* 2020a, Morales-Saldaña *et al.* 2022). This often promotes plant speciation a notion supported by the description of new taxa (Ruiz-Sanchez 2015, Ruiz-Sanchez *et al.* 2019, Gutiérrez-Ortega *et al.* 2020b, 2021).

Lycianthes (Dunal) Hassler (Capsiceae, Solanaceae) comprises herbs, vines, shrubs, trees, and epiphytes. The plants develop poricidal anthers and the calyx lacks lobes. However, many species have 5-10 appendages that emerge just below the calyx rim (Dean *et al.* 2020). The genus includes 187 taxa (152 species, 10 subspecies, and 25 varieties) that are native to the Americas, Asia, and Oceania. It is the third most diverse genus of Solanaceae (Hunziker 2001, Dean *et al.* 2020, Anguiano-Constante *et al.* 2021b). Molecular evidence has indicated that the genus may be paraphyletic, relative to *Capsicum* L. (Olmstead 2013, Särkinen *et al.* 2013, Spalink *et al.* 2018). *Lycianthes* is a diverse lineage within the MTZ with 41 species in the region (Anguiano-Constante *et al.* 2021b).

Lycianthes series *Meizonodontae* was described by Bitter (1919) who recognized 12 taxa, eight species and four varieties. Dean (1995, 2004) published the second monograph of the series, recognizing 10 taxa, eight species and two varieties [*L. acapulcensis* (Baill) D'Arcy, *L. ciliolata* (M. Martens & Galeotti) Bitter, *L. dejecta* (Fernald) Bitter, *L. hintonii* E. Dean, *L. moziniana* var. *margaretiana* E. Dean, *L. moziniana* (Dunal) Bitter var. *moziniana*, *L. moziniana* var. *oaxacana* E. Dean, *L. peduncularis* (Schltdl.) Bitter, *L. starbuckii* E. Dean and *L. rzedowskii* E. Dean]. The series is a monophyletic group of geophytic herbs that develop sympodial units with one or two leaves and inflorescences with solitary flowers. The calyces bear 10 appendages, and the flowers have one long stamen and four short stamens (Figure 1, Dean 2004). Mexico is the center of diversification of the group, and all the species have populations within the MTZ. Eight taxa (six species and two varieties) are endemic to Mexico (Anguiano-Constante *et al.* 2018).

Lycianthes moziniana is endemic to Mexico and has a wide range. Based on morphological data, the species is divided into three varieties (Dean 2004, Anguiano-Constante *et al.* 2021a, Figure 1). *Lycianthes moziniana* var. *moziniana* develops lanceolate leaves with obtuse to cuneate bases and rounded to acute apices. At anthesis, the calyx appendages are lax but appressed in the mature and green fruit. Lastly, the abaxial side of the corolla lobes are pubescent. In contrast, *L. moziniana* var. *margaretiana* has ovate to elliptic leaves with cuneate to attenuate bases and acute to acuminate apices. The calyx appendages spread out both at anthesis and fruiting, the mature fruits show red or purple maculae, and the abaxial side of the corolla lobes are glabrous. Finally, *L. moziniana* var. *oaxacana* has ovate leaves with obtuse to cuneate bases and acute to acuminate apices. At anthesis, the calyx appendages are lax but then spread out as the green fruit matures and corolla lobes are glabrous abaxially (Dean 2004). *Lycianthes moziniana* has high genetic diversity, and a significant phylogeographic structure (Anguiano-Constante *et al.* 2021a). All three varieties are well distributed along the MTZ within oak forest, pine forest, pine-oak forest and xerophilous scrub. Nevertheless, the varieties have an allopatric geographical range.

Within the MTZ, each variety of *Lycianthes moziniana* grows in a different biogeographic province. *Lycianthes moziniana* var. *margaretiana* inhabits in the Sierra Madre Oriental (SMOr), *L. moziniana* var. *moziniana* grows in the Transmexican Volcanic Belt (TVB) and the Sierra Madre Occidental (SMOc) and *L. moziniana* var. *oaxacana* is found in the Sierra Madre del Sur (SMS, Anguiano-Constante *et al.* 2021a, 2021b, Dean *et al.* 2020). The phylogeographic

Lycianthes moziniana diversification

structure of *L. moziniana* (Anguiano-Constante *et al.* 2021a) suggested that geographic isolation has promoted genetic structure and morphological differentiation. In the present study, we measured morphological data and evaluated climatic variants among *L. moziniana* varieties to test the hypothesis that they represent incipient species lineages.

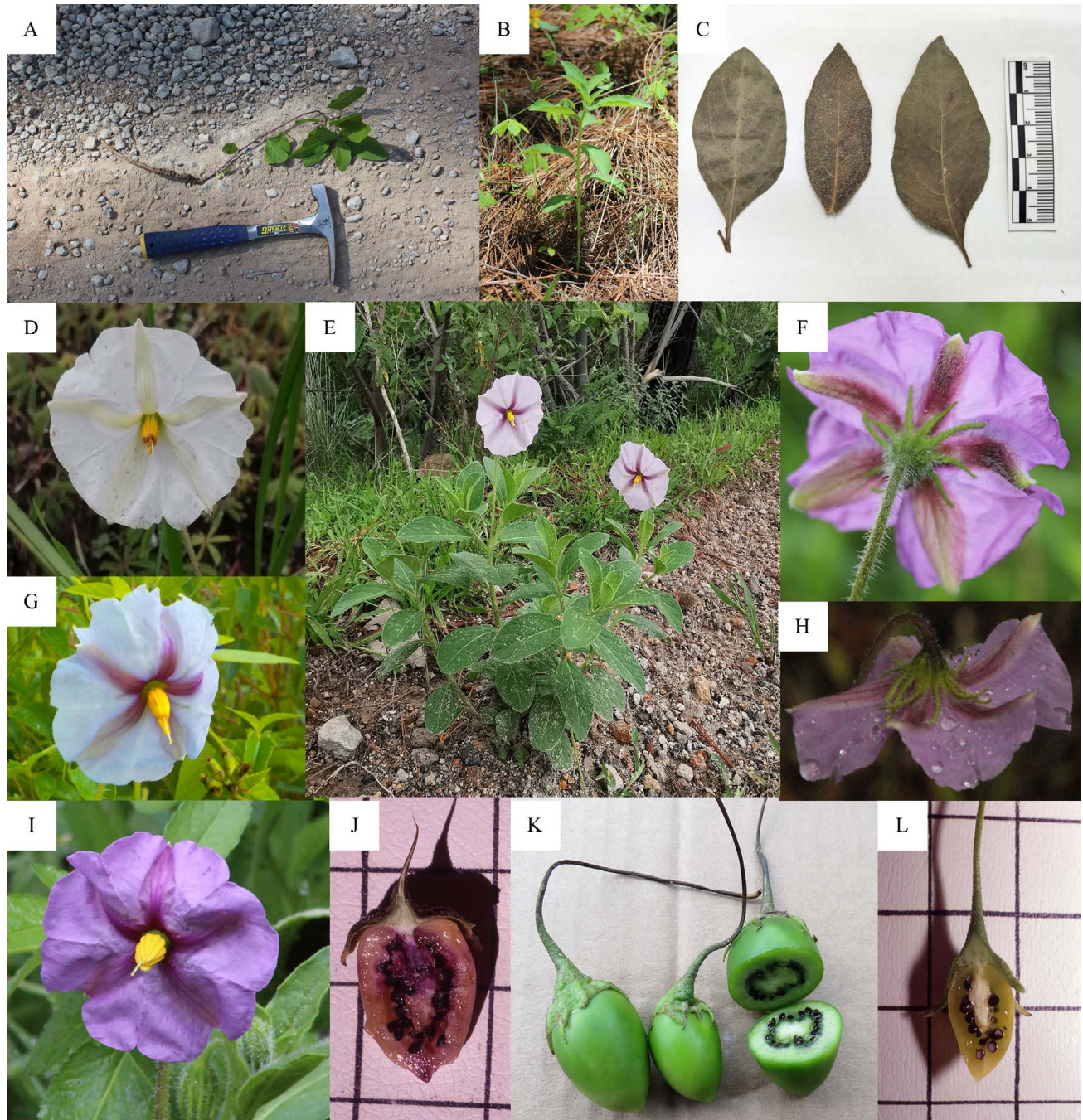


Figure 1. *Lycianthes moziniana*; A. Root of *L. moziniana* var. *margaretiana*; B. Habit of *L. moziniana* var. *moziniana*; C. Leaf of *L. moziniana* var. *margaretiana*, *L. moziniana* var. *moziniana*, *L. moziniana* var. *oaxacana* (left to right); D. white corolla of *L. moziniana* var. *moziniana*; E. Habit of *L. moziniana* var. *moziniana*; F. Abaxial side of the corolla of *L. moziniana* var. *moziniana*; G. Lilac corolla of *L. moziniana* var. *moziniana*; H. Abaxial side of the corolla of *L. moziniana* var. *oaxacana*; I. Purple corolla of *L. moziniana* var. *moziniana*; J. Fruit of *L. moziniana* var. *margaretiana*; K. Fruit of *L. moziniana* var. *moziniana* and L. Fruit of *L. moziniana* var. *oaxacana*. Photographs A, B, C, F, G, H and I by Marco Antonio Anguiano Constante; D by María de la Luz Pérez García; E by Pablo Carrillo Reyes, J and L by Ellen Dean; K by Guadalupe Munguía Lino.

Materials and methods

Morphological data. We measured 207 individuals of *Lycianthes moziniana* from 21 populations. These included 102 individuals collected between 2017-2021. A voucher specimen for each population was deposited in the *Luz María Villarreal de Puga Herbarium* at the University of Guadalajara (IBUG, Thiers 2023). Additionally, we included 105 specimen images from ANSM, CIIDIR, DAV, GBH, HUAA, HUAP, MEXU, NY, QMEX, TEX, US and XAL (Thiers 2023). The taxa sampling included *L. moziniana* var. *margaretiana* (four populations and 35 individuals), *L. moziniana* var. *moziniana* (13 populations and 150 individuals) and *L. moziniana* var. *oaxacana* (four populations and 22 individuals).

Morphological analyses. Herbarium specimens were photographed with a Canon EOS Rebel T5 camera. Both, photos and herbaria specimen images were measured using ImageJ (imagej.net). Using various ImageJ functions, we obtained three leaf values (petiole length, leaf length and leaf width) from the second sympodium unit (Dean 2004). Also, we included pedicel length, calyx diameter and length, calyx appendages length, corolla diameter, long stamen length and short stamens length, style length, calyx in fruit length and diameter, and fruit length and diameter. In addition, the ratio values of leaf length/width, calyx length/diameter, calyx in fruit length/diameter, and mature fruit length/diameter were calculated.

We used 11 characters to test the differences among varieties. Then, the characters were transformed with natural logarithms to fit normality and parametric assumptions. With the transformed values and the three varieties as the classificatory variable, we conducted a Discriminant Canonical Analysis (DCA) with the *candics* package in RStudio version 1.3.1093 (R Studio Team 2020). Mahalanobis Square Distance (MD) between the centroids of the Canonical Classificatory Analysis (CCA) were calculated in SAS (SAS 2013).

Climatic range, distribution models and niche differentiation. The geographic distribution was obtained from herbarium specimens, electronic databases, and literature. We examined data from the following 34 herbaria (either in person or using digital images): ANSM, CFNL, CICY, CIIDIR, CIMI, CIQRO, DAV, F, GBH, GUADA, HCIB, HGOM, HUAA, HUAP, HUAZ [not in Index Herbariorum (Thiers 2023), Herbario de la Universidad Autónoma de Zacatecas], IBUG, IEB, INEGI, MEXU, MO, NY, OAX, P, QMEX, SERBO, TEX, UC, UCR, UNL, UNSIJ [not in Index Herbariorum (Thiers 2023), Universidad de la Sierra Juárez, Oaxaca], US, WIS, XAL and ZEA (acronyms according to Thiers 2023). In addition, three electronic databases were consulted: Global Biodiversity Information Facility (GBIF, www.gbif.org), Missouri Botanical Garden (Tropicos www.tropicos.org) and Southwest Environmental Information Network (SEINet, www.swbiodiversity.org). Information contained in Dean (2004), Dean *et al.* (2020), Martínez *et al.* (2020) and Nee (1986) was added. We built a database of 420 records with 32 records of *Lycianthes moziniana* var. *margaretiana*, 349 of *L. moziniana* var. *moziniana* and 39 of *L. moziniana* var. *oaxacana*. The spatial autocorrelation of distribution records was eliminated manually in QGIS Las Palmas v. 2.18.3 (QGIS Development Team 2017). In other words, from the projected records points and the climatic layers, a single point for each pixel was selected. The final database contained 348 records of *L. moziniana* var. *margaretiana* (25), *L. moziniana* var. *moziniana* (288) and *L. moziniana* var. *oaxacana* (35).

We obtained 19 climatic variables from WORLCLIM Database based on the CCSM4 model (Hijmans *et al.* 2005, Otto-Bliesner *et al.* 2006) with a resolution of 30 arc seconds (www.worldclim.org). Using the presence points, we extracted the values for the climatic variables. Then, to select the variables, the correlation of the variables was tested for each variety with Person's correlation using a 0.05 level of significance in the *corrplot* R package in RStudio. Later, we calculated the variance inflation factor for correlated variables and excluded the highly correlated variables using the threshold of 0.9 in the *usdm* R package in R Studio. Finally, the layers were trimmed out to the accessible area (M) (Soberón & Peterson 2005, Barve *et al.* 2011). The M represents the MTZ of Morrone *et al.* (2017) plus a buffer of 50 km.

We executed an Ecological Niche Model (ENM) for each variety. The analysis included 25 records of *Lycianthes moziniana* var. *margaretiana*, 288 records of *L. moziniana* var. *moziniana*, and 35 records of *L. moziniana* var. *oaxa-*

cana in MAXENT v. 3.3.3K (Phillips *et al.* 2006). Each model was replicated 10 times. Samples collected between 2017-2021 were used to validate the models. These included four records for *L. moziniana* var. *margaretiana*, four presence points of *L. moziniana* var. *oaxacana* and 15 records for *L. moziniana* var. *moziniana*. The rest of the parameters had the default configuration. Finally, the models were evaluated using under the curve values.

The niche overlap among varieties was estimated with the values D (niche equivalency) and I (niche similarity) in ENMTools (Warren *et al.* 2008, 2010). These indices consisted of values between 0 and 1, indicating no overlap and full overlap among ENMs, respectively. This test was carried on among all binary combinations among the three taxa (*Lycianthes moziniana* var. *moziniana* and *L. moziniana* var. *margaretiana*, *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana* and *L. moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana*). Identity test was carried on with the average layer of ENMs constructed in MAXENT using four variables (BIO4, BIO7, BIO15 and BIO18) shared among the three varieties and the occurrences files mentioned above and 100 replicates. The test calculated the overlap between ENM of one taxon and the ENMs constructed using random occurrence point samples of the other taxon. To evaluate the differentiation among niches, we used the measured niche overlap as empirical estimation and compared with the distribution of overlaps from pseudo replicates.

Results

Morphological analyses. Discriminant Canonical Analysis recovered three groups. Two canonical functions explained 100 % of the morphological variation, 78.6 and 21.4 %, respectively (Figure 2H). Canonical structure coefficients showed that seven morphological characters (petiole length, leaf ratio, long stamen length, short stamens length, style length, calyx in fruit and fruit ratios) had the highest discriminatory power in the DCA (Table 1). On the first function (Can1), petiole length, leaf ratio and style length explained the variation. On the second (Can2), the short stamens length, the long stamen length, calyx in fruit and fruit ratios explained the variation (Table 1). The petiole length was important for *Lycianthes moziniana* var. *margaretiana*. Four characters (leaf ratio, style length, and the short and long stamens length) grouped the individuals of *L. moziniana* var. *moziniana* and two characters (calyx in fruit and fruit ratio) grouped the individuals of *L. moziniana* var. *oaxacana* (Figure 2).

The MD among all a priori groups were statistically significant ($P < 0.05$, Table 2). Between pairwise varieties comparisons, the centroids of *Lycianthes moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* clusters were closer together. Followed by *L. moziniana* var. *margaretiana* and *L. moziniana* var. *moziniana*. In contrast, the furthest clusters were *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana*. The confusion matrix of CCA, including the seven characters with highest discriminatory scores, had 82 % recovery of groups corresponding to *L. moziniana* var. *margaretiana*, 80 % recovery of *L. moziniana* var. *moziniana* and 77 % of *L. moziniana* var. *oaxacana* (Table 3).

Climatic range, distribution models and niche differentiation. For each variety, the climatic variable selection was different. The analysis recovered five variables (BIO4, BIO7, BIO9, BIO15 and BIO18) for *Lycianthes moziniana* var. *margaretiana*, eight variables (BIO4, BIO7, BIO12, BIO14, BIO15, BIO17, BIO18 and BIO19) for *L. moziniana* var. *moziniana* and six variables (BIO4, BIO7, BIO9, BIO13, BIO15 and BIO18) for *L. moziniana* var. *oaxacana*. The ENMs of each variety showed high predictive power with AUC values 0.953, 0.912 and 0.984, respectively (Figure 3C). The jackknife test selected BIO15 and BIO4 as the most important variables of *L. moziniana* var. *margaretiana*. In contrast, *L. moziniana* var. *moziniana* was associated with BIO4, BIO14 and BIO7, and the presence of *L. moziniana* var. *oaxacana* was determined by BIO4 and BIO9.

The niche identity test showed significant differences among ENMs. The empirical niche overlap index in all comparisons was significantly lower than the expected by chance. The empirical niche overlap between *Lycianthes moziniana* var. *margaretiana* and *L. moziniana* var. *moziniana* was $I = 0.6098$ and $D = 0.3227$ (Figure 4A). The same values between *L. moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* were $I = 0.2868$ and $D = 0.1186$ (Figure 4B). Lastly, similarity and equivalency values between *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana* summed $I = 0.5279$ and $D = 0.2749$ (Figure 4C).

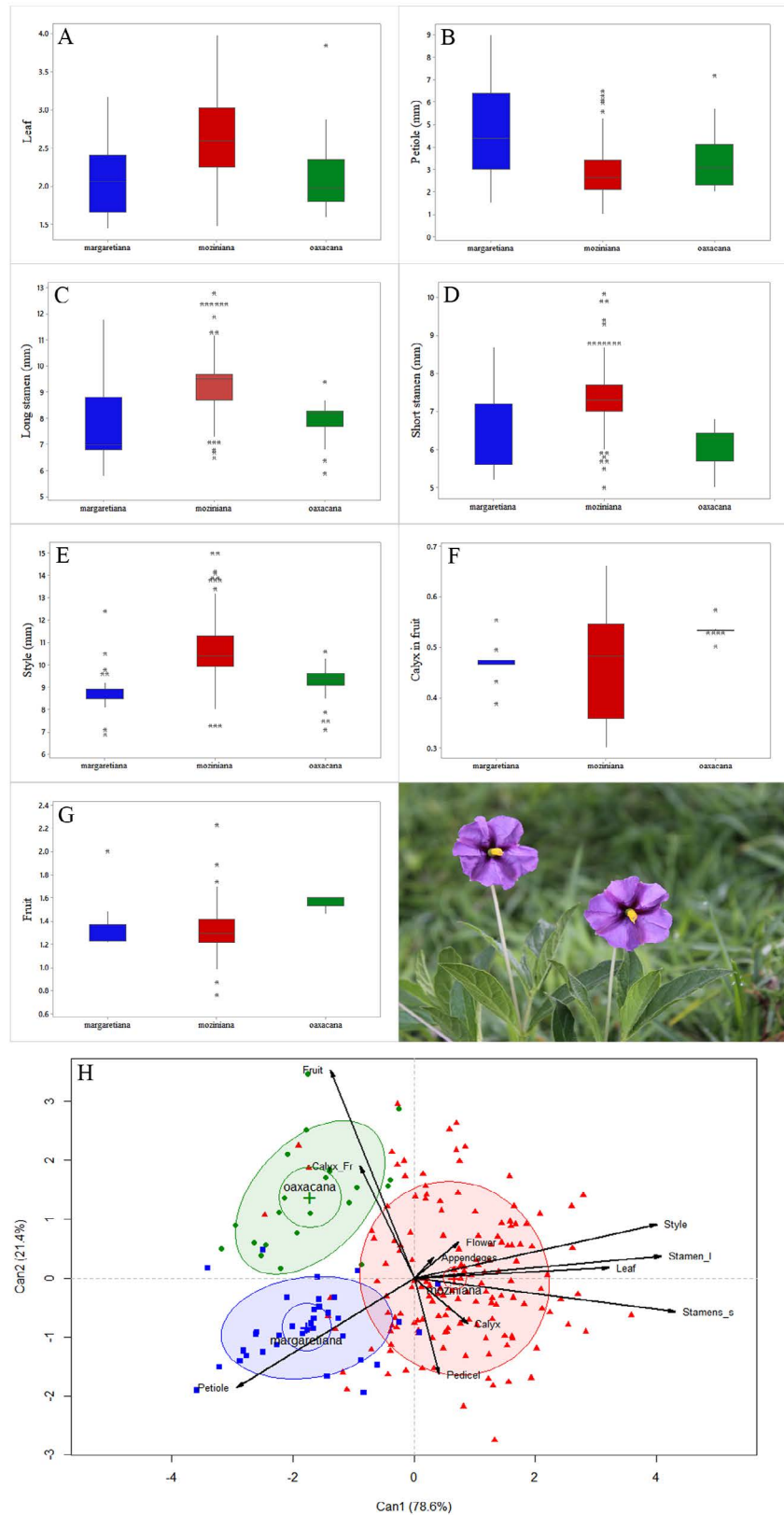


Figure 2. Characters with the highest discriminatory power from Canonical Discriminant Analysis. Boxplot A. leaf ratio; B. petiole length; C. long stamen length. D. short stamens length; E. style length; F. calyx in fruit ratio; G. fruit ratio and H. Discriminant Canonical Analysis.

Table 1. Canonical structure coefficient. Asterisks designate the contribution of the character to each canonical function.

	Can1	Can2
Petiole length	-0.51738775*	-0.29220325
Leaf ratio	0.45948258*	0.14329004
Pedicle length	0.01830623	-0.23569747
Appendages length	0.12728664	-0.05119904
Calyx ratio	0.08766814	-0.30766801
Corolla diameter	0.04012253	0.39952381
Long stamen length	-0.05491305	0.41944009
Short stamens length	0.50252134	-0.59021056*
Style length	0.41346526*	0.29213365
Calyx in fruit ratio	-0.25101036	0.57326853*
Fruit ratio	-0.18517747	0.778596*
Variation explained	78.6 %	21.4 %

Discussion

The morphological and geographical analyses recovered three groups within *Lycianthes moziniana* (Figures 2, 3). On the other hand, ENM comparisons within each variety showed low niche overlap (Figure 4). This evidence revealed the geographic and climatic isolation of the varieties. These results were supported also by the morphological and ITS sequences differences observed by Dean (2004) and the genetical structure found in populations distributed in different biogeographic provinces (Anguiano-Constante *et al.* 2021a). The combined evidence suggests that each variety has been isolated long enough to generate genetic structure and morphological differences. We argue that all three are separately evolving metapopulations and in the process of speciation (de Queiroz 2007).

Morphological analyses (DCA, MD and CCA) recovered three statistically supported groups that correspond to the three varieties (Figure 2 and Tables 2, 3). These analyses have been useful for delimiting species boundaries in other groups (Aquino *et al.* 2019, Gándara *et al.* 2021, Gutiérrez-Ortega *et al.* 2021, Sánchez *et al.* 2020). Interestingly, the DCA explained the variation of *Lycianthes moziniana* in two canonical functions (Figure 2). Seven out of 11 examined diagnostic characters (petiole length, leaf ratio, short stamens length, long stamen length, style length, calyx in fruit and fruit ratios) were significantly different. Five of the seven were reproductive characters and could be closely related to pollinators and dispersal agents. The genetic variation along with geographic isolation has promoted morphological fixation and differentiation of populations.

Foliar characters changed among varieties. Petiole length in *Lycianthes moziniana* var. *margaretiana* was longer [0.1-10 (\pm 4.7 mm)] than *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana*. In the last two taxa, the leaves were sessile or the petiole length was less than 7 mm (\pm 2.8 and \pm 3.4 mm). The leaves of *L. moziniana* var. *moziniana* tended to be lanceolate in shape and narrower [2.2-9.2 (\pm 4.7) \times 0.7-4 (\pm 1.9 cm)], in comparison to the wider ovate to elliptic leaves of *L. moziniana* var. *margaretiana* [2.5-10 (\pm 6.2) \times 1.7-6 (\pm 3.7 cm)] and *L. moziniana* var. *oaxacana* [3-7 (\pm 4.8) \times 1-4.6 (\pm 2.3 cm)]. The leaf variation is indicative of adaptations to different climatic conditions. Temperature and precipitation have been recovered in other analyses as the main climatic factors modulating morphological variation (Rodríguez-Gómez *et al.* 2018, Maya-García *et al.* 2020, Martínez-García *et al.* 2022).

In the same way, flower characters differed among varieties. *Lycianthes moziniana* var. *moziniana* showed more variation in stamen length [long 6.5-12.8 (\pm 9.4 mm), short 5-10 (\pm 7.3 mm)] and style length [7.3-15 (\pm 10.6 mm)] than *L. moziniana* var. *margaretiana* [long 5.8-11.8 (\pm 7.8 mm), short 5.2-8.7 (\pm 6.2 mm) and style length 6.9-12.4 (\pm 8.7 mm)]. *Lycianthes moziniana* var. *oaxacana* [long 5.9-9.4 (\pm 7.9 mm), short 5-6.8 (\pm 6 mm), and style 7.1-10.6 (\pm 9.1 mm)] had the shortest stamens and style. In the case of the length of the calyx in fruit, the results did not overlap between *L. moziniana* var. *margaretiana* [7.5-10.9 (\pm 8.9) mm long \times 18.3-19.8 (\pm 19 mm diameter)] and *L. mozini-*

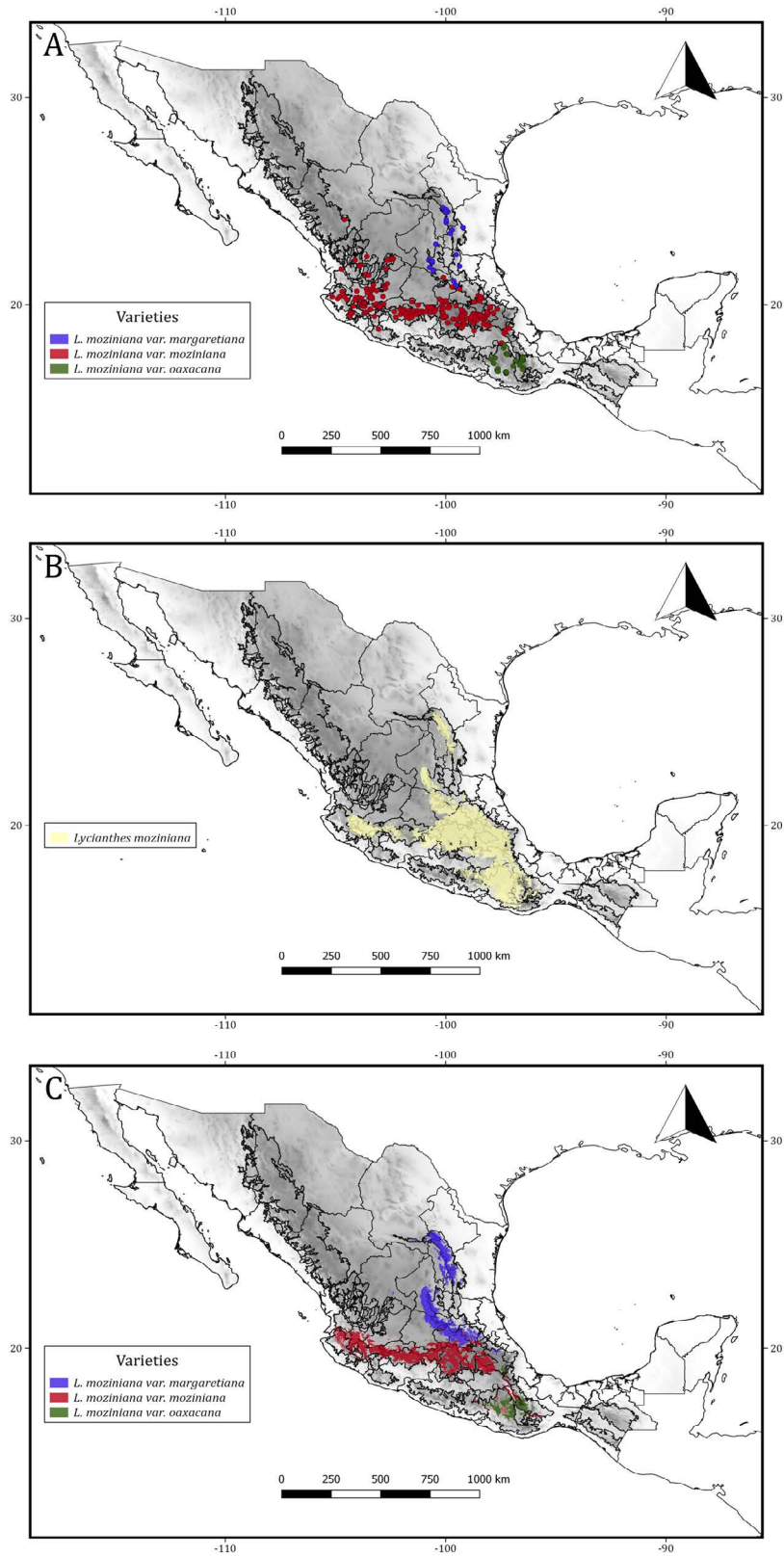


Figure 3. Distribution of *Lycianthes moziniana*; A. Actual distribution of the varieties of *L. moziniana*; B. Potential distribution of *L. moziniana*; C. Potential distribution of each variety of *L. moziniana*.

ana var. *oaxacana* [9.2-9.7 (± 9.4) × 16.9-18.4 (± 17.7 mm)]. *Lycianthes moziniana* var. *moziniana* [3.3-10.7 (± 6.5) × 7.8-33.8 (± 13.7 mm)] had the shortest length of the calyx in fruit (Figure 2A-F).

Finally, the fruit shape in *Lycianthes moziniana* var. *moziniana* was narrow [0.7-2.4 (± 1.6 cm)] and long [1.1-3.2 (± 2.1 cm)]. In contrast, *L. moziniana* var. *margaretiana* [1.9-2.2 (± 2) × 2.3-4.4 (± 2.7 cm)] and *L. moziniana* var. *oaxacana* [2-2.2 (± 2) × 3-3.4 (± 3.2 cm)] had ovoid fruits (Figure 2G). Dean (2004) found that the combination of fruit shape and appendages position in the fruiting calyx were important for delimiting the varieties. In fruit, the appendages of the calyx were appressed in *L. moziniana* var. *moziniana* and spreading in the other two varieties. The ovoid fruit in *L. moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* was green but the former presented purple blotches. Moreover, the fruit in *L. moziniana* var. *moziniana* was green, round or ovoid. The morphological variation observed in the three varieties has been affected by gene flow, geographic distance, pollination ecology and climatic conditions (Rodríguez-Peña & Wolfe 2023). Thus, these events have determined the independent evolutionary trajectories among lineages (Jacobo-Arteaga *et al.* 2022).

The MD demonstrated significant differences among varieties (Table 2). *Lycianthes moziniana* var. *moziniana* was the most distant, relative to *L. moziniana* var. *oaxacana*, and *L. moziniana* var. *margaretiana*. This was congruent with the taxonomic treatments of Dean (2004) and Dean *et al.* (2020). However, *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana* hybridized under greenhouse conditions (Dean 2004) and *L. moziniana* var. *moziniana* and *L. moziniana* var. *margaretiana* were sympatric in Sierra de Álvarez, San Luis Potosí. The same analysis recovered *L. moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* close to each other, but they were isolated by the TVB. None of the varieties show genetic flow among them (Anguiano-Constante *et al.* 2021a).

Table 2. Mahalanobis Square Distance by varieties of *Lycianthes moziniana* (* indicates $P < 0.05$).

	<i>L. m. margaretiana</i>	<i>L. m. moziniana</i>	<i>L. m. oaxacana</i>
<i>L. m. margaretiana</i>	0	6.64*	4.87*
<i>L. m. moziniana</i>		0	7.56*
<i>L. m. oaxacana</i>			0

Table 3. Canonical Classificatory Analysis by varieties of *Lycianthes moziniana*.

Taxon	<i>L. m. margaretiana</i>	<i>L. m. moziniana</i>	<i>L. m. oaxacana</i>	Total
<i>L. m. margaretiana</i>	29	3	3	29/35
<i>L. m. moziniana</i>	16	120	14	120/150
<i>L. m. oaxacana</i>	4	1	17	17/22
Total	82 %	80 %	77 %	

The varieties grew in different biogeographic provinces, but in similar vegetation types. *Lycianthes moziniana* var. *margaretiana* is restricted to the SMOr, *L. moziniana* var. *oaxacana* to SMS and *L. moziniana* var. *moziniana* inhabit TVB and SMOc (Anguiano-Constante *et al.* 2021a, Figure 3A). In addition, we obtained a different predicted set of climatic variables for each variety. Nevertheless, the temperature seasonality (BIO4) was important for the three varieties. This was congruent with the rest of the species included in the *Lycianthes* series *Meizonodontae* (Anguiano-Constante *et al.* 2018). Other important variables were precipitation seasonality (BIO15) for *L. moziniana* var. *margaretiana*, temperature annual range (BIO7) for *L. moziniana* var. *moziniana* and mean temperature of driest quarter (BIO9) for *L. moziniana* var. *oaxacana*. Precipitation and temperature seasonality are important climatic variables for the evolution of geophytes (Sosa & Loera 2017, Howard *et al.* 2019). Thus, climatic variables in these geophytic taxa, could be an important cause of divergence.

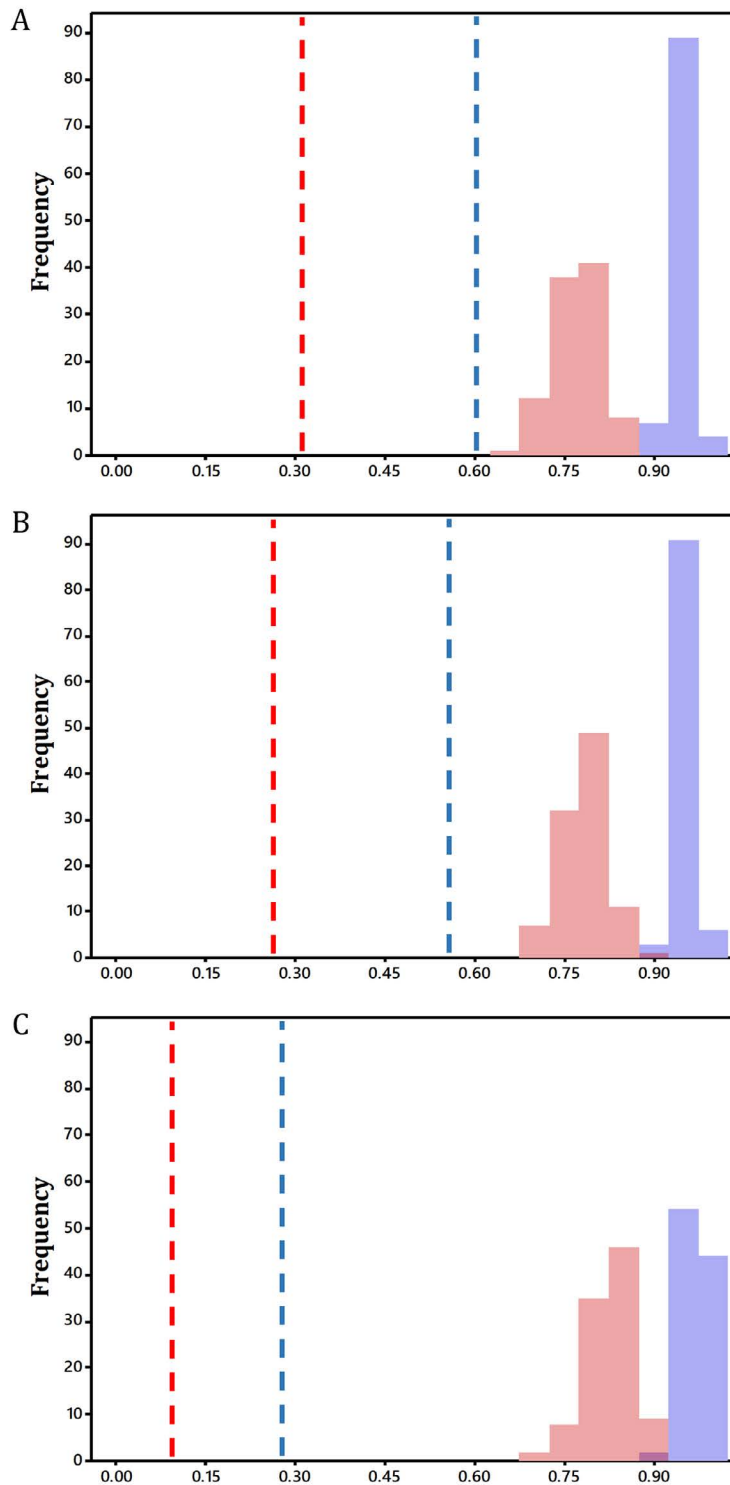


Figure 4. Niche conservatism inference in *Lycianthes moziniana*; A. Niche identity test between *L. moziniana* var. *moziniana* and *L. moziniana* var. *margaretiana*; B. Niche identity test between *L. moziniana* var. *moziniana* and *L. moziniana* var. *oaxacana*; C. Niche identity test between *L. moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana*. The dotted line shows the empirical value of *J* index in blue and *D* index in red. The bars represent the null distribution of *J* index in blue and *D* index in red.

The three varieties shared four climatic variables (BIO4, BIO7, BIO15 and BIO18). Except for the precipitation of the warmest quarter (BIO18), all of them were important predictors for the ENMs. However, the niche overlap analysis among varieties showed significant differences. *Lycianthes moziniana* var. *moziniana* overlapped the most with *L. moziniana* var. *margaretiana* ($I = 0.6098$ and $D = 0.3227$), followed by *L. moziniana* var. *oaxacana* ($I = 0.5279$ and $D = 0.2749$, [Figure 4A, B](#)). This result makes sense, because *L. moziniana* var. *moziniana* has a wide range relative to the other two varieties. A similar case was observed in *Pinus montezumae* Lamb. and *P. pseudostrobus* Lindl. (Pinaceae) which were distributed in the SMO_r, SMS and TVB, and had intermediate niche overlap and the ability to hybridize (Manzanilla-Quñones *et al.* 2019).

Lycianthes moziniana var. *margaretiana* and *L. moziniana* var. *oaxacana* had the lowest niche overlap ($I = 0.2868$ and $D = 0.1186$, [Figure 4C](#)). The morphological evidence suggested significant differences ([Figure 2](#) and [Table 2](#)). The isolation between the taxa could be responsible for this divergence. Several Mexican plants show a similar pattern when their populations are distributed in the SMO_r and SMS (Suárez-Mota *et al.* 2015, Alvarado-Sizzo *et al.* 2018, Rosas-Reinhold *et al.* 2022). This pattern suggests that both taxa diverged from an ancestral population and isolation generated and maintained the genetic and morphological differences.

Most likely, differences observed in *Lycianthes moziniana* resulted from geological, topographic, and climatic variation. These conditions isolated the populations, and each evolved separately, forming, and fixing the variation. The Mexican biogeographic provinces have different origins and ages (Mastretta-Yanes *et al.* 2015). Also, the climatic conditions have changed drastically in the last 100 Myr (Cevallos-Ferriz *et al.* 2012). All together contributed to the current biodiversity of Mexico and were directly related to genetic and morphological variation of the taxa. Important patterns have been documented. First, the geographic barriers along the MTZ promoted the speciation in several Mexican plant groups (Alvarado-Sizzo *et al.* 2018, Gutiérrez-Ortega *et al.* 2020a, Romero-Soler *et al.* 2022). Second, the climate refugia created genetic variation in *Ephedra compacta* Rose (Loera *et al.* 2017), *Psittacanthus sonorae* (S. Watson) Kuijt (Ornelas *et al.* 2018), *Podocarpus* spp. (Ornelas *et al.* 2019) and *L. moziniana* (Anguiano-Constante *et al.* 2021a). Lastly, our results suggest that the geographic barriers play an important role for the differentiation among *Lycianthes moziniana* varieties.

Current climatic and soil conditions of the SMO_r, SMS and TVB are different. Even though, they have similar vegetation types, the taxonomic composition is different. These facts might promote the morphological differences observed in *Lycianthes moziniana* varieties. The TVB soils are volcanic in origin, with annual temperature ranging from 12 to 18 °C and 200 to 4,500 mm of annual precipitation (Hernández-Cerda & Carrasco-Anaya 2007). In contrast, the SMO_r has limestone soils derived from sedimentary rocks, with an annual temperature of 12 to 25.5 °C and 1,000 to 4,000 mm of annual precipitation (Hernández-Cerda & Carrasco-Anaya 2004). The SMS and the TVB have similar soil types, temperature values and precipitation ranges. In the SMS, the temperature varies between 18 and 20 °C and precipitation ranges from 400 to 4,000 mm (Hernández-Cerda *et al.* 2016). These may have facilitated ecological responses and contributed to the morphological variation among varieties.

In conclusion, the three varieties displayed an incipient process of speciation. The morphological and geographic differences among the varieties support this conclusion. The morphological evidence was statistically significant and showed that *Lycianthes moziniana* var. *moziniana* was more different relative to the others. *Lycianthes moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* were morphologically similar. Furthermore, the climatic and geographical data showed low niche overlap among varieties. *Lycianthes moziniana* var. *moziniana* had a widespread range and had intermediate overlap with the other two varieties. *Lycianthes moziniana* var. *margaretiana* and *L. moziniana* var. *oaxacana* were isolated in different biogeographical provinces and showed the lowest overlap. Previous studies showed DNA sequence variation (Dean 2004, Anguiano-Constante *et al.* 2021a). For these reasons, we consider that each variety is a separated evolving metapopulation. Further plastome analyses, could help to decide if each variety merits species status.

Acknowledgments

We are grateful to the curators of the herbaria consulted ANSM, CFNL, CICY, CIIDIR, CIMI, CIQRO, DAV, F, GBH, GUADA, HCIB, HGOM, HUAA, HUAP, HUAZ, IEB, INEGI, MEXU, OAX, QMEX, SERBO, UNL, UNSIJ, XAL and ZEA. Likewise, we thank the staff of F, MO, NY, P, TEX, UC, UCR, US, WIS, Tropicos, GBIF and SEINET for access to specimen images and data. George Yatskievych (TEX), Amber Horning (LL), Andrew Sanders (UCR), Ubaldo Melo Samper Palacios (MEXU), Alison Colwell (DAV), Teri Barry (DAV) and Beatriz Velázquez (QMEX) provided digitalized images of specimens. Juan Ortiz Brunel, María de la Luz Pérez García, Luis Manuel Cardona Cruz and Jorge David López Pérez assisted during fieldwork. This research was supported by the Consejo Nacional de Ciencia y Tecnología (CONACyT) with a scholarship (855486/2020-000026-02NACF-19054) to the first author. This study was also supported by the Laboratorio Nacional de Identificación y Caracterización Vegetal (LaniVeg) and the Universidad de Guadalajara which provided economic support for visiting herbaria and carrying out field work.

Literature cited

- Alvarado-Sizzo H, Casas A, Parra F, Arreola-Nava HJ, Terrazas T, Sánchez C. 2018. Species delimitation in the *Stenocereus griseus* (Cactaceae) species complex reveals a new species, *S. huastecorum*. *Plos One* **13**: e0190385. DOI: <https://doi.org/10.1371/journal.pone.0190385>
- Anguiano-Constante MA, Munguía-Lino G, Ortiz E, Villaseñor JL, Rodríguez A. 2018. Riqueza, distribución geográfica y conservación de *Lycianthes* serie *Meizonodontae* (Capsiceae, Solanaceae). *Revista Mexicana de Biodiversidad* **89**: 516-529. DOI: <https://doi.org/10.22201/ib.20078706e.2018.2.2340>
- Anguiano-Constante MA, Zamora-Tavares MP, Ruiz-Sanchez E, Rodríguez A, Munguía-Lino G. 2021a. Population differentiation and phylogeography in *Lycianthes moziniana* (Solanaceae: Capsiceae), a perennial herb endemic of the Mexican Transition Zone. *Biological Journal of the Linnean Society* **132**: 358-373. DOI: <https://doi.org/10.1093/biolinnean/blaa198>
- Anguiano-Constante MA, Dean E, Starbuck T, Rodríguez A, Munguía-Lino G. 2021b. Diversity, species richness distribution and centers of endemism of *Lycianthes* (Capsiceae, Solanaceae) in Mexico. *Phytotaxa* **514**: 39-60. DOI: <https://doi.org/10.11646/phytotaxa.514.1.3>
- Aquino D, Cristian-Cervantes R, Gernandt DS, Arias S. 2019. Species delimitation and phylogeny of *Epithelantha* (Cactaceae). *Systematic Botany* **44**: 600-615. DOI: <https://doi.org/10.1600/036364419X15620113920635>
- Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge: Harvard University Press. ISBN: 0-674-66638-0
- Barve N, Barve V, Jiménez-Valverde A, Lira-Noriega A, Maher SP, Peterson AT, Soberón J, Villalobos F. 2011. The crucial role of the accessible area in ecological niche modeling and species distribution modeling. *Ecological Modelling* **222**: 1810-1819. DOI: <https://doi.org/10.1016/j.ecolmodel.2011.02.011>
- Bitter G. 1919. Die Gattung *Lycianthes*. *Abhandlungen Herausgegeben vom Naturwissenschaftlichen Verein zu Bremen* **24**: 292-520.
- Cevallos-Ferriz SRS, González-Torres EA, Calvillo-Canadell L. 2012. Perspectiva paleobotánica y geológica de la biodiversidad en México. *Acta Botanica Mexicana* **100**: 317-350. DOI: <https://doi.org/10.21829/abm100.2012.39>
- Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**: 407-415. DOI: <https://doi.org/10.1111/j.1095-8312.2005.00503.x>
- De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56**: 879-886. DOI: <https://doi.org/10.1080/10635150701701083>
- Dean E. 1995. *Systematics and ethnobotany of Lycianthes series Meizonodontae*. PhD thesis. University of California. Berkeley.

- Dean E. 2004. A taxonomic revision of *Lycianthes* series *Meizonodontae* (Solanaceae). *Botanical Journal of the Linnean Society* **145**: 385-424. DOI: <https://doi.org/10.1111/j.1095-8339.2004.00296.x>
- Dean E, Poore J, Anguiano-Constante MA, Nee MH, Kang H, Starbuck T, Rodríguez A, Conner M. 2020. The genus *Lycianthes* (Solanaceae, Capsiceae) in Mexico and Guatemala. *Phytokeys* **168**: 1-333. DOI: <https://doi.org/10.3897/phytokeys.168.51904>
- Gándara E, Ortiz-Brunel JP, Castro-Castro A, Ruiz-Sanchez E. 2021. Morphological variation in *Bessera* (Asparagaceae: Brodiaeoidae) allows for the recognition of two new species. *Phytotaxa* **512**: 257-271. DOI: <https://doi.org/10.11646/phytotaxa.512.4.2>
- Gutiérrez-Ortega JS, Pérez-Farrera MA, Vovides AP, Salas-Morales SH, Chemnick J. 2020a. *Dioon oaxacensis* (Zamiaceae): a new cycad species from the arid central valleys of Oaxaca (Mexico). *Phytotaxa* **474**: 51-61. DOI: <https://doi.org/10.11646/phytotaxa.474.1.5>
- Gutiérrez-Ortega JS, Salinas-Rodríguez MM, Ito T, Pérez-Farrera MA, Vovides AP, Martínez JF, Molina-Freaner F, Hernández-López A, Kawaguchi L, Nagano AJ, Kajita T, Watano Y, Tsuchimatsu T, Takahashi Y, Murakami M. 2020b. Niche conservatism promotes speciation in cycads: the case of *Dioon merolae* (Zamiaceae) in Mexico. *New phytologist* **227**: 1827-1884. DOI: <https://doi.org/10.1111/nph.16647>
- Gutiérrez-Ortega JS, Pérez-Farrera MA, Chemnick J, Gregory TJ. 2021. A reassessment of *Dioon merolae* (Zamiaceae) leads to the description of *Dioon salas-moralesae*, a new cycad species from southeastern Oaxaca, Mexico. *Phytotaxa* **528**: 93-110. DOI: <https://doi.org/10.11646/phytotaxa.528.2.3>
- Hernández-Cerda ME, Carrasco-Anaya G. 2004. Climatología. In: Luna-Vega I, Morrone J, Espinosa D, eds. *Biodiversidad de la Sierra Madre Oriental*. Ciudad de México: Universidad Nacional Autónoma de México, pp. 63-108. ISBN: 970-32-1526-2.
- Hernández-Cerda ME, Carrasco-Anaya G. 2007. Rasgos climáticos más importantes. In: Luna I, Morrone JJ, Espinosa D, eds. *Biodiversidad de la Faja Volcánica Transmexicana*. México, Universidad Nacional Autónoma de México, pp. 57-72. ISBN: 970-32-4871-3.
- Hernández-Cerda ME, Azpra-Romero E, Aguilar-Zamora V. 2016. Condiciones climáticas en la Sierra Madre del Sur. In: Luna-Vega I, Espinosa D, Contreras-Medina R, eds. *Biodiversidad de la Sierra Madre del Sur: una síntesis preliminar*. Ciudad de México: Universidad Nacional Autónoma de México, pp. 91-106. ISBN: 607-02-7906-9.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965-1978. DOI: <https://doi.org/10.1002/joc.1276>
- Howard CC, Folk RA, Beaulieu JM, Cellinese N. 2019. The monocotyledonous underground: global climatic and phylogenetic patterns of geophyte diversity. *American Journal of Botany* **106**: 1-14. DOI: <https://doi.org/10.1002/ajb2.1289>
- Huang JP. 2020. Is population subdivision different from speciation? From phylogeography to species delimitation. *Ecology and Evolution* **10**: 6890-6896. DOI: <https://doi.org/10.1002/ece3.6524>
- Hunziker A. 2001. Genera Solanacearum: the genera of Solanaceae illustrated arranged according to a new system. ARG Gantner, Córdoba, 500 pp. ISBN: 390-41-4477-4.
- Jacobo-Arteaga LE, Medina-Rodríguez MD, Hernández-Hernández B, Piña de la Rosa IA, Cacho NI. 2022. Leaf morphospace in *Euphorbia tithymaloides* (Euphorbiaceae) was likely shaped by evolutionary contingencies rather than climate. *Plant Ecology and Evolution* **155**: 315-331. DOI: <https://doi.org/10.5091/plecevo.91487>
- Loera I, Ickert-Bond SM, Sosa V. 2017. Pleistocene refugia in the Chihuahuan Desert: the phylogeographic and demographic history of the gymnosperm *Ephedra compacta*. *Journal of Biogeography* **44**: 2706-2716. DOI: <https://doi.org/10.1111/jbi.13064>
- Manzanilla-Quñones U, Delgado-Valerio P, Hernández-Ramos J, Molina-Sánchez A, García-Magaña JJ, Rocha-Granados MC. 2019. Similaridad del nicho ecológico de *Pinus montezumae* y *P. pseudostrobus* (Pinaceae) en México: implicaciones para la selección de áreas productoras de semillas y de conservación. *Acta Botanica Mexicana* **126**: e1398. DOI: <https://doi.org/10.21829/abm126.2019.1398>

- Martinez M, Montero JC, Dean EA, Bye R, Luna-Cavazos M, Medina JM, Rzedowski J. 2020. Familia Solanaceae I: géneros *Acnistus* – *Witheringia* (excepto *Solanaum*). *Flora del Bajío y Regiones Adyacentes* **218**: 1-238. DOI: <https://doi.org/10.21829/fb.310.2020.218>
- Martínez-García AL, Hornung-Leoni CT, Mayer-Goyenechea IG, Bueno-Villegas J, Granados-Mendoza C. 2022. Morphometric analysis provides evidence for two traditionally defined species of the *Tillandsia erubescens* complex (Bromeliaceae). *Plant Ecology and Evolution* **155**: 29-40. DOI: <https://doi.org/10.5091/plecevo.84524>
- Mastretta-Yanes A, Moreno-Letelier A, Piñero D, Jorgensen TH, Emerson BC. 2015. Biodiversity in the Mexican highlands and the interaction of geology, geography and climate within the Trans-Mexican Volcanic Belt. *Journal of Biogeography* **42**: 1586-1600. DOI: <https://doi.org/10.1111/jbi.12546>
- Maya-García R, Torres-Miranda A, Cuevas-Reyes P, Oyama K. 2020. Morphological differentiation among populations of *Quercus elliptica* Née (Fagaceae) along and environmental gradient in Mexico and Central America. *Botanical Sciences* **98**: 50-65. DOI: <https://doi.org/10.17129/botsci.2395>
- Morales-Saldaña S, Valencia-Ávalos S, Oyama K, Tovar-Sánchez E, Hipp AL, González-Rodríguez A. 2022. Even more oak species in Mexico? Genetic structure and morphological differentiation support the presence of at least two specific entities within *Quercus laeta*. *Journal of Systematics and Evolution* **60**: DOI: <https://doi.org/10.1111/jse.12818>
- Morrone JJ, Escalante T, Rodríguez-Tapia G. 2017. Mexican biogeographic province: map and shapefiles. *Zootaxa* **4277**: 277-279. <https://doi.org/10.11646/zootaxa.4277.2.8>
- Nee M. 1986. Solanaceae I. *Flora de Veracruz* **49**: 1-191.
- Olmstead RG. 2013. Phylogeny and biogeography in Solanaceae, Verbenaceae and Bignoniaceae: a comparison of continental and intercontinental diversification patterns. *Botanical Journal of Linnean Society* **171**: 80-102. DOI: <https://doi.org/10.1111/j.1095-8339.2012.01306.x>
- Ornelas JF, Licona-Vera Y, Vásquez-Aguilar AC. 2018. Genetic differentiation and fragmentation in response to climate change of the narrow endemic *Psittacanthus auriculatus*. *Tropical Conservation Science* **11**: 1-15. DOI: <https://doi.org/10.1177/1940082918755513>
- Ornelas JF, Ortiz-Rodríguez AE, Ruiz-Sanchez E, Sosa V, Pérez-Ferrera MA. 2019. Ups and downs: genetic differentiation among populations of the *Podocarpus* (Podocarpaceae) species in Mesoamerica. *Molecular Phylogenetics and Evolution* **138**: 17-30. DOI: <https://doi.org/10.1016/j.ympev.2019.05.025>
- Otto-Bliesner BL, Marshall SJ, Overpeck JT, Miller GH, Hu A. 2006. Simulating Arctic climate warmth and icefield retreat in the last interglaciation. *Science* **311**: 1751-1753. DOI: <https://doi.org/10.1126/science.1120808>
- Padiál JM, Miralles A, Riva IR, Vences M. 2010. The integrative future of taxonomy. *Frontiers in Zoology* **7**: 16. DOI: <https://doi.org/10.1186/1742-9994-7-16>
- Peñaloza-Ramírez JM, Rodríguez-Correa H, González-Rodríguez A, Rocha-Ramírez V, Oyama K. 2020. High genetic diversity and stable Pleistocene distributional ranges in the widespread Mexican red oak *Quercus castanea* Née (1801) (Fagaceae). *Ecology and Evolution* **10**: 4204-4219. DOI: <https://doi.org/10.1002/ece3.6189>
- Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographical distribution. *Ecological Modelling* **190**: 231-259. DOI: <https://doi.org/10.1016/j.ecolmodel.2005.03.026>
- QGIS Development Team. 2017. QGIS Geographic Information System. Open Source Geospatial Foundation Project Available at: <http://qgis.osgeo.org>
- Rodríguez-Gómez F, Oyama K, Ochoa-Orozco M, Mendoza-Cuenca L, Gaytán-Legaria R, González-Rodríguez A. 2018. Phylogeography and climate-associated morphological variation in the endemic white oak *Quercus deserticola* (Fagaceae) along the Trans-Mexican Volcanic Belt. *Botany* **96**: 121-133. DOI: <https://doi.org/10.1139/cjb-2017-0116>
- Rodríguez-Peña RA, Wolfe AD. 2023. Flower morphology variation in five species of *Penstemon* (Plantaginaceae) displaying Hymenoptera pollination syndrome. *Botanical Sciences* **101**: 217-232. DOI: <https://doi.org/10.17129/botsci.3084>

- Romero-Soler KJ, Ramírez-Morillo IM, Ruiz-Sanchez E, Hornung-Leoni CT, Carnevali G. 2022. Historical biogeography and comparative phylogeography of the Mexican genus *Bakerantha* (Bromeliaceae): insights into evolution and diversification. *Botanical Journal of the Linnean Society* **199**: 109-127. DOI: <https://doi.org/10.1093/botlinnean/boab084>
- Rosas-Reinhold I, Sánchez D, Arias S. 2022. Systematic study and niche differentiation of the genus *Aporocactus* (Hylocereeae, Cactoideae, Cactaceae). *Botanical Sciences* **100**: 423-445. DOI: <https://doi.org/10.17129/botsci.2893>
- R Studio Team. 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>
- Ruiz-Sanchez E. 2015. Parametric and non-parametric species delimitation methods result in the recognition of two new Neotropical woody bamboo species. *Molecular Phylogenetic and Evolution* **93**: 261-273. DOI: <https://doi.org/10.1016/j.ympev.2015.08.004>
- Ruiz-Sanchez E, Specht CD. 2013. Influence of the geological history of the Trans-Mexican Volcanic Belt on the diversification of *Nolina parviflora* (Asparagaceae: Nolinoideae). *Journal of Biogeography* **40**: 1336-1347. DOI: <https://doi.org/10.1111/jbi.12073>
- Ruiz-Sanchez E, Specht CD. 2014. Ecological speciation in *Nolina parviflora* (Asparagaceae): Lacking spatial connectivity along of the Trans-Mexican Volcanic Belt. *PLoS ONE* **9**: e98754. DOI: <https://doi.org/10.1371/journal.pone.0098754>
- Ruiz-Sanchez E, Carrillo-Reyes P, Hernandez-Sandoval L, Specht CD. 2019. Two new species of *Nolina* (Nolinoideae: Asparagaceae) endemic to western Mexico. *Phytotaxa* **402**: 187-198. DOI: <https://doi.org/10.11646/phytotaxa.402.4.2>
- Sánchez D, Gómez-Quintero D, Vargas-Ponce O, Carrillo-Reyes P, Dávila-Aranda P. 2020. Species delimitation in the *Echinocereus pulchellus* complex (Cactaceae). *Brittonia* **72**: 433-452. DOI: <https://doi.org/10.1007/s12228-020-09632-x>
- Särkinen T, Bohs L, Olmstead RG, Knapp S. 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. *BMC Evolutionary Biology* **13**: 214. DOI: <https://doi.org/10.1186/1471-2148-13-214>
- SAS. 2013. Statistical Analysis Software. Users' Guide Statistics Version 9.4. SAS Institute Inc. Cary.
- Soberón J, Peterson AT. 2005. Interpretation of models of fundamental ecological niches and species distributional area. *Biodiversity Information* **2**: 1-10. DOI: <https://doi.org/10.17161/bi.v2i0.4>
- Sosa V, Loera I. 2017. Influence of current climate, historical climate stability and topography on species richness and endemism in Mesoamerican geophyte plants. *PeerJ* **5**: e3932. DOI: <https://doi.org/10.7717/peerj.3932>
- Spalink D, Stoffel K, Walden GK, Hulse-Kemp AM, Hill TA, Van Deyze A, Bohs L. 2018. Comparative transcriptomics and genomic patterns of discordance in Capsiceae (Solanaceae). *Molecular Phylogenetics and Evolution* **126**: 293-302. DOI: <https://doi.org/10.1016/j.ympev.2018.04.030>
- Spriggs EL, Eaton DAR, Sweeney PW, Schlutius C, Edwards EJ, Donoghue MJ. 2019. Restriction-site-associated DNA sequencing reveals a cryptic *Viburnum* species on the North American Coastal Plain. *Systematic Biology* **68**: 187-203. DOI: <https://doi.org/10.1093/sysbio/syy084>
- Suárez-Mota ME, Villaseñor JL, López-Mata L. 2015. Ecological niche similarity between congeneric Mexican plant species. *Plant Ecology and Evolution* **148**: 318-328. DOI: <https://doi.org/10.5091/plecevo.2015.1147>
- Thiers B. 2023. Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/science/ih/> (accessed 9 Feb 2023).
- Wang MZ, Fan XK, Zhang YH, Wu J, Mao LM, Zhang SL, Cai MQ, Li MH, Zhu ZSC, Zhao MS, Liu LX, Cameron KM, Li P. 2022. Phylogenomics and integrative taxonomy reveal two new species of *Amana* (Liliaceae). *Plant Diversity* **45**: 54-68. DOI: <https://doi.org/10.1016/j.pld.2022.03.001>
- Warren DL, Glor RE, Turelli M. 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution* **62**: 2868-2883. DOI: <https://doi.org/10.1111/j.1558-5646.2008.00482.x>

- Warren DL, Glor RE, Turelli M. 2010. ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography* **33**: 607-611. DOI: <https://doi.org/10.1111/j.1600-0587.2009.06142.x>
- Zhang MH, Wei R, Xiang QP, Ebihara A, Zhang XC. 2021. Integrative taxonomy of *Selaginella helvetica* group based on morphological, molecular and ecological data. *Taxon* **70**: 1163-1187. DOI: <https://doi.org/10.1002/tax.12565>

Associate editor: Martha González Elizondo

Author contributions: MAAC conceived the idea, collected, revised herbarium specimens, constructed the morphological databases, carried out the analyzes and prepared the illustrations. DSC and ERS assisted in the statistical analysis. ED, GML and AR collected, revised herbarium specimens and contributed to the conception and development of the study. All wrote and edited the draft and approved the final version.