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Systematics / Sistemática

Systematic study and niche differentiation of the genus *Aporocactus* (Hylocereeae, Cactoideae, Cactaceae)

Estudio sistemático y diferenciación de nicho del género *Aporocactus* (Hylocereeae, Cactoideae, Cactaceae)

Isaura Rosas-Reinhold [1,2], Daniel Sánchez[3,4], and Salvador Arias[1*]

[1] Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, CDMX, México.
[2] Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, CDMX, México.
[3] CONACYT-Laboratorio Nacional de Identificación y Caracterización Vegetal, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, México.
[4] Herbario Luz María Villarreal de Puga, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Zapopan, México.
*Author for correspondence: sarias@ib.unam.mx

Abstract

Background: *Aporocactus* is an epiphytic or saxicolous genus that is endemic to Mexico and has a distribution restricted to cloud forests and pine-oak forests. As with many cacti, *Aporocactus* presents taxonomic conflicts, especially regarding species delimitation, since five species in this genus have been described and accepted by some authors, while others accept only two species.
Questions: How many species comprise *Aporocactus?* What are their relationships? Do these species show differences in their climatic preferences?
Studied species: The five putative species in *Aporocactus* were investigated.
Study site and dates: This study was conducted in 2015 and 2016. The collection sites were in Hidalgo, Puebla, Querétaro, Veracruz, and Oaxaca states, Mexico.
Methods: In this study, phylogenetic analyses were performed using chloroplast DNA markers from different *Aporocactus* populations and related genera, and ecological niche modeling techniques were also employed.
Results: The phylogenetic analyses indicated that *Aporocactus* is composed of only two species: *A. flagelliformis* and *A. martianus*. Additionally, the phylogenetic analyses corroborated that *Aporocactus* is an early diverging group related to *Weberocereus* and *Selenicereus*. Finally, niche modeling and niche identity testing indicated that the niches of the two species of *Aporocactus* are significantly differentiated and niches are more different than would be expected by chance.
Conclusions: Despite being a genus with only two species, *Aporocactus* represents a useful model for investigating such topics as the ecology of pollination, genetic populations, and flower development to characterize the evolution of these specialized cacti.
Keywords: cpDNA phylogeny, epiphytic cacti, niche differentiation, rat-tail cactus, species delimitation

Resumen

Antecedentes: *Aporocactus* es un género epifito o saxícola, endémico de México, con una distribución restringida a bosque mesófilo y de pino-encino. Como otras cactáceas, *Aporocactus* presenta conflictos taxonómicos, especialmente en la delimitación de especies, con cinco nombres descritos y aceptadas por algunos autores, pero otros solo aceptan dos especies.
Preguntas: ¿Cuántas especies incluye *Aporocactus?* ¿Cuáles son sus relaciones filogenéticas? ¿Las especies muestran diferencias en sus preferencias climáticas?
Especies estudiadas: Cinco especies putativas de *Aporocactus.*
Lugar de estudio y fechas: Estudio realizado entre 2015 y 2016. Los sitios de colecta fueron los estados de Hidalgo, Puebla, Querétaro, Veracruz y Oaxaca, México.
Métodos: El estudio incluyó análisis filogenético utilizando marcadores de ADN de cloroplasto de diferentes poblaciones de *Aporocactus* y géneros relacionados, así como técnicas de modelado de nicho ecológico.
Resultados: El análisis filogenético mostró que *Aporocactus* está compuesto por dos especies: *A. flagelliformis* y *A. martianus*; los análisis filogenéticos corroboraron que *Aporocactus* diverge tempranamente y que está relacionado con *Weberocereus* y *Selenicereus*. Finalmente, el modelado y la prueba de identidad de nicho indicaron que los nichos de ambas especies de *Aporocactus* están significativamente diferenciados y son más diferentes de lo que se esperaría por azar. Esto indica que las especies muestran un conservadurismo de nicho.
Conclusiones: Se reconocen solo dos especies para *Aporocactus*, el cual representa un modelo interesante para estudiar la ecología de la polinización, genética de poblaciones, desarrollo floral, entre otros temas, con el fin de comprender la evolución de estas cactáceas especializadas.
Palabras clave: cactácea epífita, cactus cola de rata, diferenciación de nicho, delimitación de especies, filogenia de cpDNA

The genus *Aporocactus* Lem. is an epiphytic or saxicolous cactus that is endemic to Mexico and is distributed across the states of Guanajuato, Hidalgo, Puebla, Queretaro, Veracruz, and Oaxaca; these species occupy the canopies of mature trees in cloud forests and *Pinus-Quercus* forests (Bravo-Hollis 1978, Guzmán *et al*. 2007). *Aporocactus* is a very popular cultivated plant in Mexican gardens and is known as the "flor de látigo, floricuerno, junco, rattail cactus" because of its stems. However, as with many members of Cactaceae, *Aporocactus* exhibits taxonomic issues that have hindered its taxonomic stability. *Aporocactus* was created by Lemaire (1860) to group species with cylindrical stems that hang more than a metre and zygomorphic pink flowers. Lemaire (1860) included three species in the genus: *A. flagelliformis* Lem. (= *Cactus flagelliformis* L.), *A. baumannii* Lem. (= *C. baumannii* Lem.), and *A. colubrinus* (= *C. colubrinus* Otto ex. C.F. Först.), and this author included *C. leptophis* D.C. as a synonym of *A. flagelliformis*. However, *A. baumannii* and *A. colubribus* were transferred by Lemaire to the South American genus *Cleistocactus* Lem., which also presents repent stems and zygomorphic pink flowers. Later, Lemaire (1868) transferred *Cereus flagriformis* Zucc. ex Pfeiff. to *Aporocactus*. In the preceding century, Britton & Rose (1920) recognized the genus *Aporocactus* as delineated by Lemaire (1860, 1861) and accepted five species: *A. flagelliformis*, *A. leptophis* (*C. leptophis* De Candolle 1829), *A. flagriformis*, *A. martianus* (*C. martianus* Zuccarini 1832), and *A. conzattii* Britton & Rose. Similarly, Bravo-Hollis (1978) recognized the genus *Aporocactus* and the five referred species. The International Organization for Succulent Plant Study (IOS) drastically reduced this number of species, recognizing *Aporocactus* as having only two species (Hunt & Taylor 1986). Hunt (1989) argued that "the northern (Hidalgo) species has markedly zygomorphic purplish pink flowers, the southern (Oaxaca) nearly regular scarlet flowers and somewhat stiffer stems", which correspond to *A. flagelliformis* and *A. martianus*, respectively. The other three names were assigned synonyms of the two aforementioned species. The recognition of species in *Aporocactus* presents a number of problems and a degree of complexity, since all of the existing descriptions were generated based on a few morphological characters (Linneo 1753, Lemaire 1860, De Candolle 1829, Zuccarini 1832, Britton & Rose 1920). However, most of the morphological characters indicated by these authors are continuous, without discrete variation; therefore, it is difficult to recognize the number of species using only morphological characters, with the possible exception of floral symmetry.

Another level of complexity has been the generic position and phylogenetic relationships of this genus. Barthlott (in Taylor & Hunt 1991) included *Aporocactus* in *Disocactus* Lindl. as a subgenus because the diurnal magenta and reddish flowers are similar to those exhibited by some species of *Disocactus*. Barthlott (in Taylor & Hunt 1991), Anderson (2001), Bauer (2003), and Hunt *et al*. (2006) maintain this criterion under the argument that *Disocactus* includes all diurnal and colourful flowers, as is also observed in *Aporocactus*. The studies of Cruz *et al*. (2016) and Korotkova *et al*. (2017) have demonstrated that *Aporocactus* is a monophyletic group that does not belong to *Disocactus* and that these genera are not directly related. In those phylogenies, the position of *Aporocactus* inside the tribe Hylocereeae has not been determined. Also, the recent work by Martínez-Quezada *et al*. (2020) using molecular markers, morphology, and stem anatomical features helped to elucidate the position of *Aporocactus*. However, the sisterhood with the clade formed by *Selenicereus* and *Weberocereus* is supported by the presence of adventitious roots, a character that is present in other genera of the tribe, and the Bayesian analyses using the same dataset did not confirm this relationship.

Aporocactus occupies an atypical ecological niche for cacti. An ecological niche is defined as the set of abiotic and biotic conditions where a species can persist indefinitely (Hutchinson 1957). The fundamental niche of a species is determined by the set of abiotic conditions that defined its physiological range of tolerance in absence of biotic interactions, while the realized niche of a species refers to the space of the fundamental niche where the species actually occurs and limited by biotic interactions (Hutchinson 1957, Soberón & Arroyo-Peña 2017). It is considered that among closely related species, ecological niches have low differentiation, which is a phenomenon known as niche conservatism (Peterson *et al*. 1999). However, in some empirical studies, niche conservatism is not observed (Ortiz-Medrano *et al*. 2016), since spatial and temporal climatic variation can influence evolutionary processes. *Aporocactus* represents a small monophyletic group, and regardless of the number of species, this genus constitutes an interesting taxon to explore the climatic variables that define the niche of each species and inquire whether the niche has been conserved or diverged

during speciation. The approaches proposed by Warren *et al*. (2008) to test whether the observed ecological niche models vary significantly from each other or the from the 'background' niche in which they occur have been used to suggest niche conservatism or divergence in some taxa (Pyron *et al*. 2015). The aim of this research is to conduct a study to delimit the species that conform to *Aporocactus*, to propose a hypothesis that supports the phylogenetic relationships of the genus in Hylocereeae, and to suggest climate similarity or difference in *Aporocactus*.

Materials and methods

Plant material and taxon sampling. Plant material of *Aporocactus* species was collected from wild locations across the states of Hidalgo, Querétaro, Oaxaca, Puebla, and Veracruz in the springs of 2015 and 2016. Sampling included the type localities for the published names (when included in the protologue). For each locality, a section of stem was collected, and a fragment was subsequently herborized and deposited in MEXU; the second fragment was cultivated in the tempered greenhouse in the Botanical Garden of the Institute of Biology at UNAM (JB-IBUNAM), where a tissue sample was obtained, dried and stored in silica gel at -20 °C for subsequent DNA extractions. We included 50 taxa from Hylocereeae as ingroups, 21 of which corresponded to different localities of *Aporocactus* (Appendix 1), and the remaining 35 taxa corresponded to the genera *Acanthocereus* (Engelm. ex A. Berger) Britton & Rose, *Disocactus* Lindl., *Epiphyllum* Haw., *Pseudorhipsalis* Britton & Rose, *Selenicereus* (A. Berger) Britton & Rose, and *Weberocereus* Britton & Roses from the same tribe. The outgroup consisted of seven species from seven genera pertaining to the sister tribes: *Bergerocactus* Britton & Rose, *Cephalocereus* Pfeiff., *Stenocereus* (A. Berger) Riccob., *Echinocereu*s Engelm., *Deamia* Britton & Rose, *Myrtillocactus* Console, *Marshallocereus* Backeb., and *Leptocereus quadricostatus* Britton & Rose. Sampled taxa in each analysis are described below.

Isolation, amplification and sequencing of DNA. For the isolation of total genomic DNA, most of the water-storing tissue was removed from the stems before the remaining cortex tissue was dehydrated in silica gel. The dried plant material was homogenized using a mixer mill (Retsch MM200, Haan, Germany) and extracted using the EZ-10 mini-prep kit for plant genomic DNA (Bio Basic, Inc., Ontario, Canada) following the manufacturer's protocol. The incubation time in the lysis buffer was increased to 120 min at 65 °C due to the tissue type. The concentration and purity of DNA (A260/A260 and A260/A230 ratios) were measured using a spectrophotometer (NanoDrop, peqLab, Erlangen, Germany). The original genomic DNA was stored at -20 °C and working dilutions with a standard concentration of 10 ng/µl were prepared for subsequent analysis in PCR assays. PCR amplification was performed for the *rpl16* intron (Hernández-Hernández *et al*. 2011), *trnL-trnF* intron (Taberlet *et al*. 1991), *psbA-trnH* intergenic spacer (Sang *et al*. 1997, Tate & Simpson 2003) and *trnQ-rps16* intergenic spacer (Korotkova *et al.* 2010, Shaw *et al*. 2007). The total volume for the standard sample was 25 µl, which consisted of 2.5 µl of 10X buffer, 0.5 µl dNTPs at 200 µM concentration, 1 µl of BSA, 0.75 µl of MgCl2, 0.3 µl F primer, 0.3 µl R primer, 1.25 µl of DNA Platinum Taq Polymerase (Invitrogen™) at 5 U/µl, 0.6 µl of total genomic DNA and 19.025 µl of H_2O. The markers that employed internal primers for sequencing were adjusted to a total volume of 50 µl. The PCR programmes used for each marker were as follows: 1) *trnQ-rps16*, denaturation at 95 °C × 2', denaturation at 95 °C × 1', annealing at 55 °C × 1', extension at 72 °C × 1', and extension at 72 °C × 7', for 35 cycles. 2) *rpl16/trnL-trnF*, denaturation at 95 °C × 2', 94°C × 1', annealing at 54 °C × 1', extension at 72 °C × 1' 30'', and extension at 72 °C × 7', for 30 cycles. 3) *psbA-trnH*, denaturation at 95 °C × 2, denaturation at 95 °C × 30'', annealing at 55 °C × 1', extension at 72 °C × 1', and extension at 72 °C × 10', for 30 cycles. The sequencing of the molecular markers was performed in the Laboratory of Genomic Sequencing of Biodiversity and Health from the Biology Institute at the National Autonomous University of Mexico (UNAM).

Sequence alignment. The sequences from *Aporocactus* samples were quality-checked, assembled and edited using Sequencher® v. 4.8 (Gene Codes, Ann Arbor Michigan USA). The sequences for the species of the genera *Acanthocereus*, *Disocactus*, *Epiphyllum*, *Pseudorhipsalis*, *Strophocactus*, *Bergerocactus*, *Cephalocereus*, *Deamia*, and

Marshallocereus were obtained from the database of the Laboratory of Systematics of Cactaceae from the Botanical Garden/Institute of Biology, UNAM (Arias *et al.* 2005, Cruz *et al.* 2016, Sánchez *et al*. 2014, Hernández-Hernández *et al*. 2011, Tapia *et al.* 2017) (Appendix 1). Additionally, we included the *rps3-rpl16* and *trnK-matK* sequences from Korotkova *et al*. (2017) to complete the matrix (Appendix 1). Individual sequences were cross-checked for possible assembly failures and subsequently stacked and subjected to primary alignment using the software BioEdit (Hall 1999) and the integrated application ClustalW v.1.74 (Thompson *et al.* 1994). Furthermore, individual marker matrices were realigned and corrected by eye using Mesquite® software v. 3.03 (Maddison & Maddison 2016).

Phylogenetic analyses. A phylogenetic analysis for delimiting species was performed by using four cpDNA markers (*psbA-trnH*, *trnQ-rps16*, *rpl16*, and *trnL-F*), including 21 samples of *Aporocactus* and 16 species from eleven genera of Hylocereeae. On the other hand, a phylogenetic analysis for recovered genus relationships used six cpDNA markers and included 35 species from 15 genera. For both analyses, the cpDNA matrix consisted of six markers: *psbA-trnH*, *trnQ-rps16*, *rpl16*, *trnL-F*, *trnk-matk*, and *rps3-rpl16*. The parameters of the Bayesian analyses were identical for both analyses and were performed in MrBayes v. 3.2.1 (Huelsenbeck & Roquist 2001, Ronquist *et al*. 2012). The General Time Reversible model (GTR+I+G) was selected as the best substitution model using the Bayesian Information Criterion (BIC), as implemented in jModeltest v. 2.0 (Darriba *et al*. 2014). The analyses consisted of 10 million generations, sampling of parameters and trees every 1,000 generations, and a burning of 25 % of the resulting trees. The convergence of the chains was evaluated visually from the resulting parameter archive of MrBayes using Tracer v. 1.6 (Rambaut *et al.* 2018).

Ecological niche modeling. We constructed ecological niche models (ENMs) to predict the current distribution of suitable habitat of the recognized species of *Aporocactus*. Geographic coordinates of occurrence of each species were obtained from field collection, MEXU herbarium specimens, and unambiguous records from Naturalista (www.naturalista.mx). We discarded duplicate records, records with doubtful identity or geographic location and records from cultivated plants. The accessible area (M area, Soberón & Peterson 2005) was defined by the genus range based on the biogeographical provinces proposed by Morrone *et al*. (2017) and the distribution of pine-oak vegetation and cloud forest associate to those provinces (Rzedowski 1990). Bioclimatic variables were used at an ~1 km^2 spatial resolution compiled by Cuervo-Robayo *et al*. (2014). We masked those climate layers to the extent of the M area. To avoid collinearity, we discarded one of the bioclimatic variables that was highly correlated with another (Spearman correlation values > 0.79) for the study area. Nine variables were used in the final analysis (BIO2, BIO4, BIO10, BIO11, BIO13, BIO14, BIO15, BIO18, and BIO19). For each species, we constructed an ENM using MAXENT v. 3.4.1 (Phillips *et al*. 2017) through package "dismo" in R v. 4.0.4 (R Core Team 2020). We thinned occurrence points to 1 km^2 to avoid spatial autocorrelation. We built different models with 10,000 random background points and evaluated them with spatial-cross validation. We used no campling and different parametrization for Maxent, combining regularization multipliers in intervals of 0.5 ranging from 0.5 to 5, and feature class combinations of Linear, Quadratic, Hinge and Product: L, H, LQ, LH, LQH, and LQHP. We performed the evaluation process with the spatial cross validation procedure "random k-fold" (number of folds = 4) using the R package ENMeval v. 2.0.1 (Kass *et al*. 2021) with R. Model selection was made based on the Akaike information criteria corrected for small sample sizes (ΔAICc), that reflects a comparison of the goodness-of-fit and parsimonious model (Muscarella *et al.* 2014). We projected the models using the Maxent "cloglog" transformation. Finally, we evaluated variable importance with Maxent´s variable jackknife test (Phillips *et al.* 2006). Final models were constructed with ten cross-validation replicates without extrapolation.

Niche identity and similarity. The differences between the niches of the species recognized in *Aporocactus* were evaluated by using niche overlap, niche identity, and niche similarity analyses in ENMtools (Warren *et al*. 2010). Niche overlap was calculated through Schoener's index (D) and Hellinger's-based I index, which measures the similarity between predictions of habitat suitability (ENM) of one or more pairs of species (Warren *et al*. 2008, 2010).

The niche identity test indicates whether the ENMs produced by two species are identical. The test pools the georeferenced data points for a pair of species, randomizes the taxon identities of these data points, and extracts two new samples with the same sizes as the two original samples. This process is replicated and generates a null distribution of overlap scores, which is compared with the empirical niche overlap scores (Warren *et al*. 2010). The background similarity test compares the ENM of taxon "A" to an ENM created from *n* random points drawn from the geographic range of taxon "B", which generates a null distribution of overlap scores (Warren *et al*. 2008, 2010). This method is subsequently repeated in the other direction for both taxa in the comparison (B vs. A background). Finally, the test compares the empirical niche overlap of two taxa to a null distribution of overlap scores generated. A total of 100 replicates were run for the niche identity test and background similarity test to assess the differences between the habitat suitability scores defined in the ENMs for both species.

Results

Species delimitation analysis. Four molecular markers were amplified for the ingroup and the outgroup species (Appendix 1). The matrix for the species delimitation analysis was 3,354 bp in length from four concatenated molecular markers (*psbA-trnH*, *rpl16*, *trnL-F*, and *trnQ-rps16*). Phylogenetic analysis for species delimitation recovered the genus *Aporocactus* as a monophyletic group (posterior probability (pp) = 1, Figure 1). Two main clades were observed for *Aporocactus*. One clade included 13 samples from the states of Queretaro, Hidalgo, and Veracruz, which represented the putative taxa *A. flagelliformis*, *A. flagriformis,* and *A. leptophis*. None of those taxa was recovered as a monophyletic group. This clade was well supported (pp = 1) by 11 substitutions: three in *psbA-trnH*, two in *rpl16*, and six in *trnL-F*. The second clade was composed of eight terminals from Oaxaca and Veracruz and included the putative taxa *A. martianus* and *A. conzattii*. This second clade was well supported (pp = 1) by four molecular sites: one in *rpl16* and three in *trnL-F* (positions 1,769, 2,000, 2,456). Additionally, the three samples of *A. conzattii* were recovered in a monophyletic group (pp = 1).

Phylogenetic relationships analysis. The alignment to infer the phylogenetic relationships of *Aporocactus* was 6,920 bp in length from six concatenated DNA markers (*psbA-trnH*, *rpl16*, *trnL-F*, *trnQ-rps16, trnk-matk,* and *rps3-rpl16*). The analysis to infer the phylogenetic relationships of *Aporocactus* recovered three principal clades with good support: the hylocereoid clade (pp = 0.9), the phyllocactoid clade (pp = 1), and the *Acanthocereus* clade (pp = 1) (Figure 2). *Aporocactus* was resolved as a well-supported monophyletic group (pp = 1) in the hylocereoid clade and was positioned in an early divergent group sister to *Selenicereus* and *Weberocereu*s (pp = 0.9). In this analysis, the genera *Disocactus*, *Epiphyllum*, and *Pseudorhipsalis* were nested in the phyllocactoid clade, while *Acanthocereus* was recovered as the earliest diversified lineage in Hylocereeae (Figure 2). The relationship between hylocereoid and phyllocactoid clades in this analysis had low support (pp = 0.7).

Distribution, ecological niche modeling, and niche comparison. Based on Figure 1, the *A. flagelliformis* clade was determined to be primarily distributed in the Sierra Madre Oriental (Morrone *et al*. 2017) through Querétaro, Guanajuato, Hidalgo, northern Puebla, and central Veracruz; while the *A. martianu*s clade occupies primarily Sierra Madre del Sur (Morrone *et al.* 2017) from central Veracruz to southern Puebla and Oaxaca (Figure 3A). The distribution limits of both clades of *Aporocactus* were observed to converge in central Veracruz state, where Sierra Madre Oriental and Sierra Madre del Sur intersect with the Mexican Transvolcanic Belt (Morrone *et al*. 2017). Both species were determined to be clearly distributed in pine-oak forests and cloud forests in those biogeographical regions. Accordingly, these clades were recognized as different species: *A. flagelliformis* and *A. martianus*. Those clades were determined to be congruent with the current taxonomy of the genus (see discussion).

Selected ecological niche model (ENM) for *A. flagelliformis* presented LQ features and regularization multiplier of 0.5 ($\Delta AIC \approx 0$, Table S1 and Figure S1). The ENM showed the AUC value = 0.947 (S2). Projected ENM of *A. flagelliformis* added as suitable areas a number of pine-oak and cloud forests in Nuevo León, Tamaulipas, southern

Veracruz, and Oaxaca (Figure 3B). The variable with the highest percent contribution in the *A. flagelliformis* ENM was BIO18 (precipitation of warmest quarter) (24.3 %), followed by BIO14 (precipitation of driest month) (19.3 %), and BIO4 (temperature seasonality) (16.5 %). Variables with the highest permutation importance were BIO4 (36.8 %) and BIO18 (18.6 %). In the case of *A. martianus*, selected ENM presented LQH features and regularization multiplier of 2 (ΔAIC ≈ 0, Table S2 and Figure S2). This ENM showed an AUC value = 0.928 (S4). Projected ENM of *A. martianus* added some areas of pine oak forest in northern Puebla and Veracruz and northern Guerrero as suitable areas for the species (Figure 3C). The variables with the highest contribution to the ENM of *A. martianus* were BIO2 (mean diurnal range) (43 %) and BIO18 (28.8 %). The variable with the highest permutation importance was BIO2 (62.5 %).

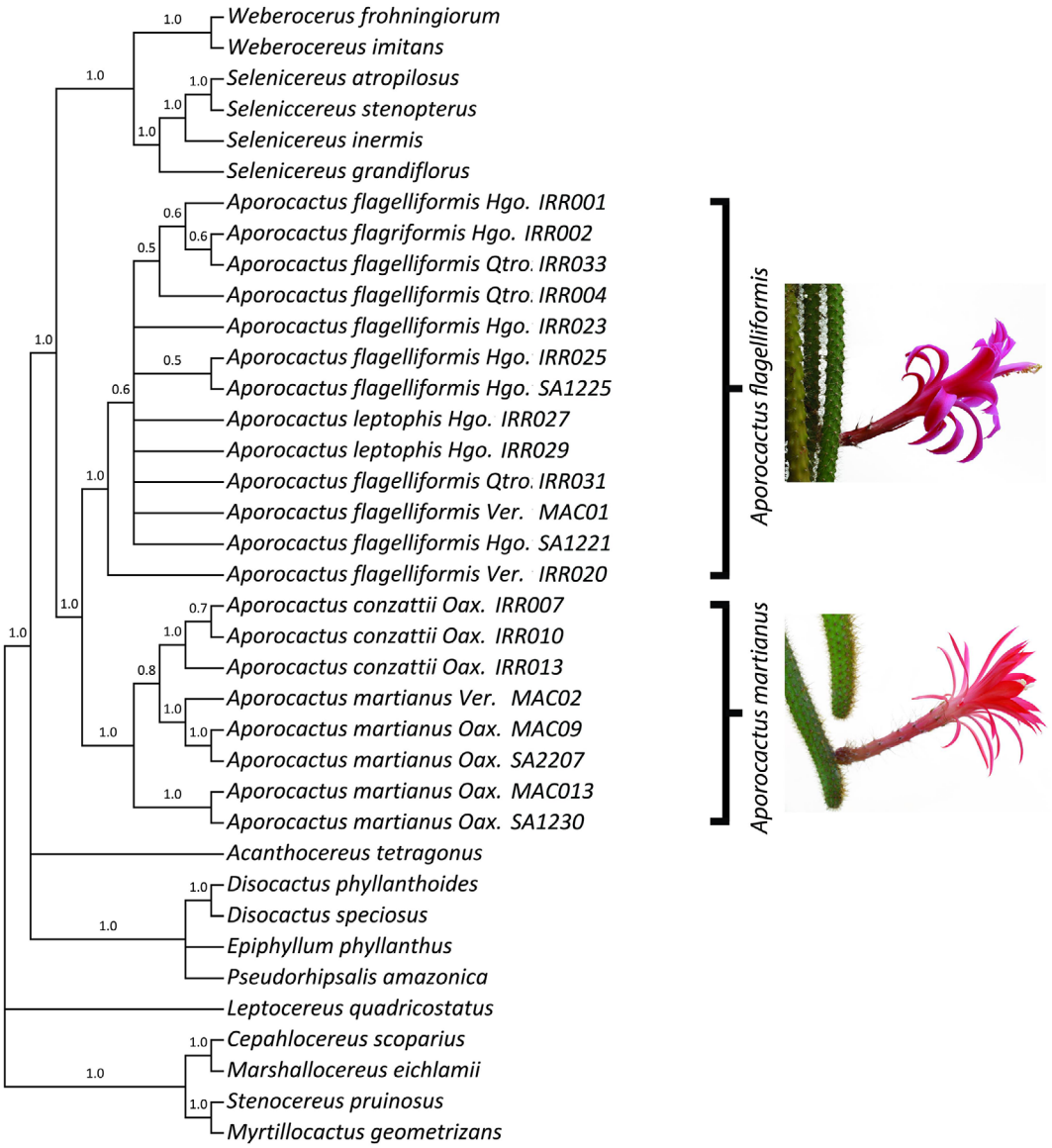

Figure 1. Species delimitation in *Aporocactus*. Cladogram of the majority rule consensus tree from the Bayesian analysis of the concatenated *trnQ-rps16*, *trnL-trnF*, *psbA-trnH*, and *rpl16* markers. Numbers above branches are the Bayesian posterior probability values.

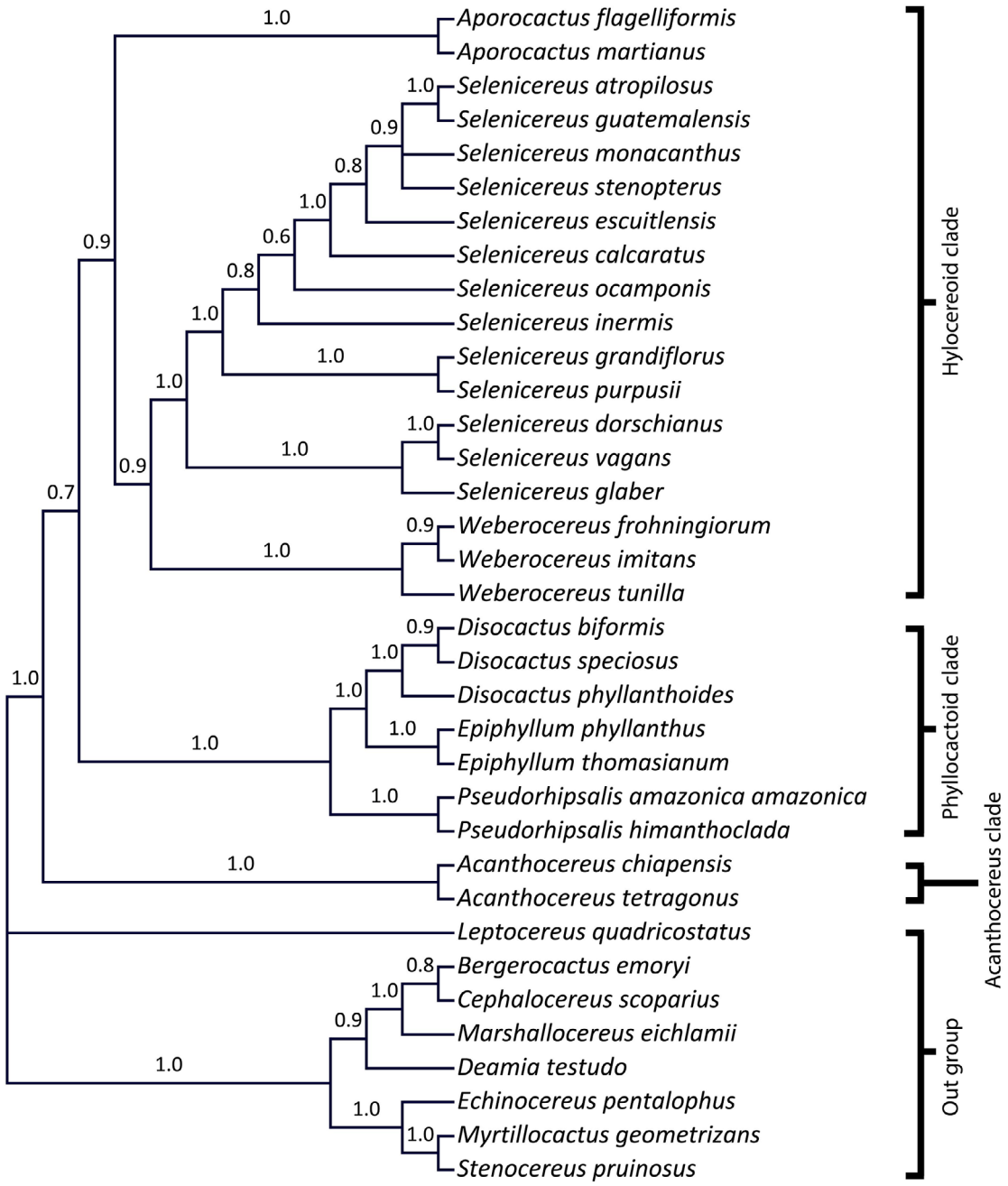

Figure 2. Phylogenetic relationships in *Aporocactus*. Cladogram of the majority rule consensus tree from the Bayesian analysis of the concatenated *trnk-matk*, *rps3-rpl16*, *trnQ-rps16*, *trnL-trnF*, *psbA-trnH*, and *rpl16* markers. Numbers above branches are the Bayesian posterior probability values.

Niche analyses indicated that empirical niche overlap between *A. flagelliformis* and *A. martianus* was low for de *D* index ($D = 0.261$); and moderate for the *I* index ($I = 0.654$). The identity test indicated that the ENM between the two species was significantly different ($D_{H0} = 0.772 \pm 0.038$ vs. $D_{H1} = 0.261$ and $I_{H0} = 0.947 \pm 0.017$ vs. $I_{H1} = 0.654$) (Figure 4A). The background similarity test comparing *A. flagelliformis* ENM in the *A. martianus* background and vice versa showed that the observed values of empirical niche similarity ($D = 0.261$, $I = 0.654$) were lower than expected under the null distribution (Figure 4B, C), indicating that the niches of the two species were significantly different than expected by chance in the available background environments.

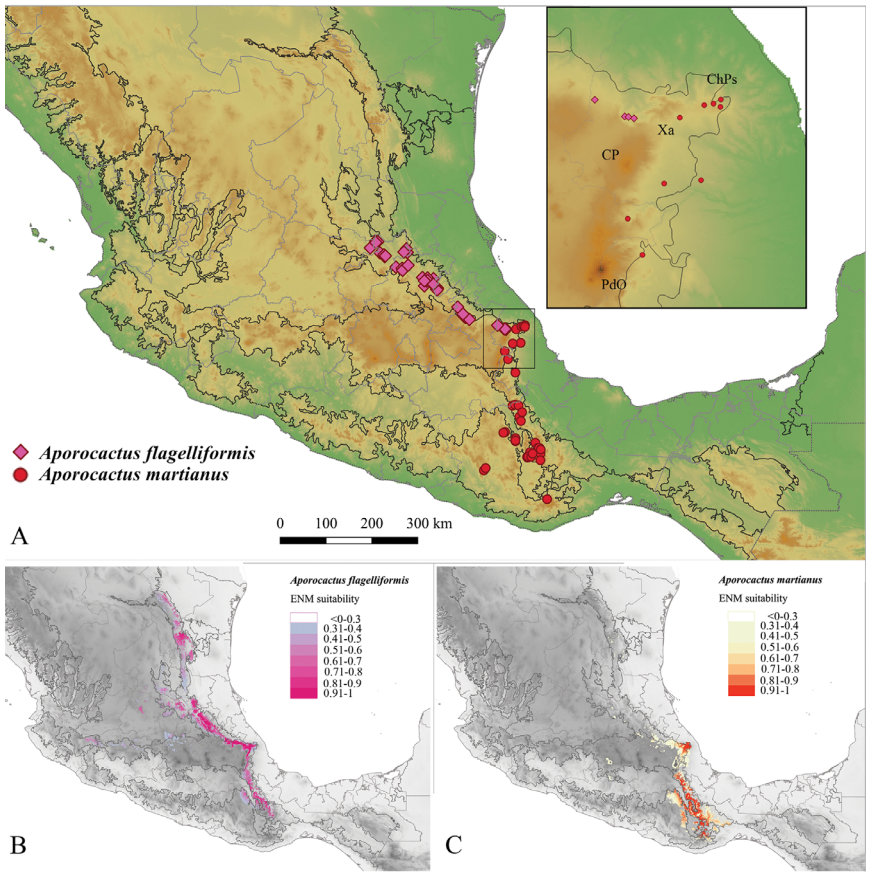

Figure 3. Actual and potential distribution of *Aporocactus*. A) Actual distribution of the genus *Aporocactus*, PdO: Pico de Orizaba, CP: Cofre de Perote, Xa: Xalapa volcanic field, CHPs: Chiconquiaco-Palma Sola. B) ENM of *Aporocactus flagelliformis*. C) ENM of *Aporocactus martianus*.

Discussion

Species delimitation in Aporocactus. Considering monophyly as a property to recognize species, as well as the geographic distribution and floral morphology of each clade, our results indicated that the two clades in *Aporocactus* represent two different species (separately evolving metapopulation lineages, De Queiroz 2007). The first clade is formed by the samples initially identified as *A. flagelliformis*, *A. flagriformis*, and *A. leptophis*, but no internal group is formed based on these putative names or by their geographic origin; therefore, in this study, we recognize that samples comprise one species. It is worth mentioning that samples corresponding to the name *A. leptophis* and *A. flagriformis* were collected in their respective type localities (Zimapán, probably los Mármoles, Hidalgo and San José del Oro, Hidalgo, respectively). However, those have morphological features corresponding to the variation reported for *A. flagelliformis*. All samples included in this clade from Querétaro, Hidalgo, and northern Veracruz present zygomorphic flowers and magenta tepals (Figure 5A, B, C, D). *Aporocactus flagelliformis* (L.) Lem. (≡*Cactus flagelliformis* L.) is the first published name of the three samples mentioned above, and according to the principle of priority (Art. 11, Turland *et al*. 2018), it is the correct name for this species. The second clade includes the samples previously identified as *A. conzattii* and *A. martianus* (Figure 1). All specimens were distributed from central Veracruz to Oaxaca and exhibited actinomorphic symmetry with red tepals (Figure 5E, F, G, H, I, J). In this case, the name *Aporocactus martianus* (Zucc.) Britton & Rose (≡*Cereus martianus* Zucc.) has priority. This result is in keeping with the proposal of Hunt (1989), who discussed the recognition of a northern species with zygomorphic purplish pink flowers and a southern species with regular scarlet flowers, assigning names on base to the ancient name. Hunt (1989) considered

A. flagriformis and *A. leptophis* as stem and flower variations of *A. flagelliformis* and considered that *A. conzattii* is a re-description of *A. martianus*. Notably, a subclade was recovered with the samples of *A. conzattii* (Figure 1) from the Sierra Madre de Oaxaca at the Sierra Madre del Sur province. However, no particular character was observed in those samples of *A. conzattii* (Figure 5G), and this group probably represents the population genetic structure of *A. martianus*. We did not observe any infraspecific entity in *A. martianus*. Our results agree with the current taxonomy of *Aporocactus*, which recognizes two species for the genus (see Taxonomic treatment section in Korotkova *et al*. 2017). Wide variation in flower colour and size was observed, ranging from pink to magenta and from 4 to 7 cm in *A. flagelliformis* and from light red to deep red and from 7 to 12 cm in *A. martianus* (Figure 5).

Phylogenetic relationships of Aporocactus*.* The results supported the monophyly of the genus *Aporocactus* (Cruz *et al*. 2016, Korotkova *et al*. 2017) and rejected the hypothesis of some authors that *Aporocactus* is a member of *Disocactus* because of the similarity in the shape, colour, and diurnal anthesis of these plants, which are presumably pollinated by hummingbirds (Barthlott in Taylor & Hunt 1991, Bauer 2003, Hunt *et al*. 2006). These results indicated that *Aporocactus* and *Disocactus* are independent lineages in different clades and suggest that diurnal anthesis in bright-coloured flowers appeared independently at least two times in Hylocereeae. In the sister tribe Echinocereeae, hummingbird pollination syndrome independently evolved in *Morangaya pensilis* (K. Brandegee) G.D. Rowley, *Echinocereus* section *Triglochidiati* Bravo, *Stenocereus alamosensis* (J.M. Coult.) A.C. Gibson & K.E. Horak and *S. kerberi* (K. Schum.) A.C. Gibson & K.E. Horak (Sánchez *et al*. 2014). Martínez-Quezada *et al*. (2020) postulated that *Aporocactus* has two anatomical synapomorphies in the stem: 1) a delay in fibre development in the wood and 2) cortical bundles with secondary growth. In field work, we observed that *Aporocactus* plants do not develop wood, as occurs in other genera, such as *Disocactus* or *Selenicereus*; instead, in the base of the oldest stem in *Aporocactus*, the roots release them and promote vegetative propagation.

Aporocactus was recovered as a sister to *Selenicereus* + *Weberocereus* in the hylocereoid clade. This result was significant, since Korotkova *et al*. (2017) did not recover these relationships by using cpDNA markers only. We noted that the addition of cpDNA markers in the present study results in a more resolved phylogeny. This sisterhood (*Aporocactus* (*Selenicereus* and *Weberocereus*)) was also achieved by Martínez-Quezada *et al*. (2020) by using the cpDNA markers from Korotkova *et al*. (2017) and a complement of morpho-anatomical characters. Martínez-Quezada *et al*. (2020) suggest that the hemiepiphytic condition and the presence of adventitious roots along the stem represent the synapomorphies of this clade. Nevertheless, other members of Hylocereeae, such as *Disocactus* and *Epiphyllum* (phyllocactoid clade), can develop this type of root frequently in different stages of growth (juvenile, adult); rather, this root represents a homoplasy, which in combination with other characters is useful to diagnose the hylocereoid clade. It is important to highlight that in the absence of more DNA sequences, the addition of morphological characters can be useful for obtaining a more resolved topology, as observed in other cacti (Sánchez *et al*. 2018, Vargas-Luna *et al*. 2018, Martínez-Quezada *et al*. 2020).

Distribution of Aporocactus*.* The known distribution of *Aporocactus* (Figure 3A) was restricted to the old pine-oak and cloud forests. As suggested by Hunt (1989), *A. flagelliformis* represents the northern species through the Sierra Madre Oriental and extends to central Veracruz in the Transmexican Volcanic Belt. Traditionally, the distribution of *A. martianus* was only reported in Oaxaca at the Sierra Madre del Sur; however, our results showed that this species is also distributed in central Veracruz, at the limit of the Transmexican Volcanic Belt. Although the distribution of both species converges in central Veracruz, a detailed analysis of this region indicated that *A. flagelliformis* and *A. martianus* present an allopatric distribution. Our results suggested that speciation of the ancestral *Aporocactus* lineage was influenced by the formation of the modern Transmexican Volcanic Belt in the eastern part during the late Pliocene-Quaternary (2.0-0.1 ma) (Rodríguez *et al*. 2010). A similar biogeographic pattern is also observed in other epiphytic sister species, namely, *Disocactus phyllanthoides* and *D. ackermannii* (Cruz *et al*. 2016). Even the vicariant consequence of the Transmexican Volcanic Belt can be observed in sister species, such as *Cephalocereus senilis* and *C. columna-trajani* (Tapia *et al*. 2017), in the lower western parts of the Sierra Madre Oriental and Sierra Madre del Sur.

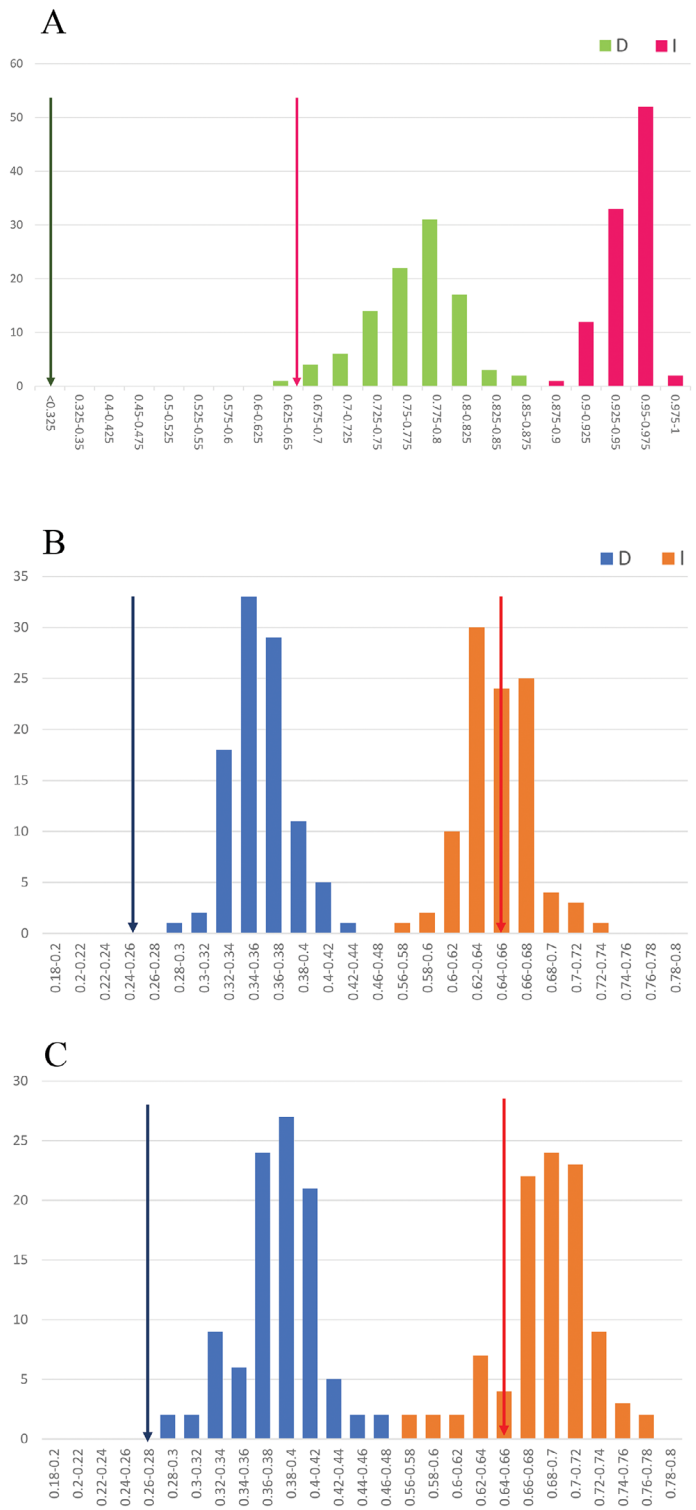

Figure 4. Niche conservatism inference in *Aporocactus*. A) Niche identity test, green bars: D index frequency from null distribution, pink bars: I index frequency from null distribution, green arrow: empirical niche overlap D index, pink arrow: empirical niche overlap I index**.** B) Niche similarity test of *Aporocactus flagelliformis* as focus species and *A. martianus* as background. C) Niche similarity test of *Aporocactus martianus* as focus species and *A. flagelliformis* as background. For B) and C), blue bars: D index frequency from null distribution, orange bars: I index frequency from null distribution, blue arrow: empirical niche overlap D index, orange arrow: empirical niche overlap I index.

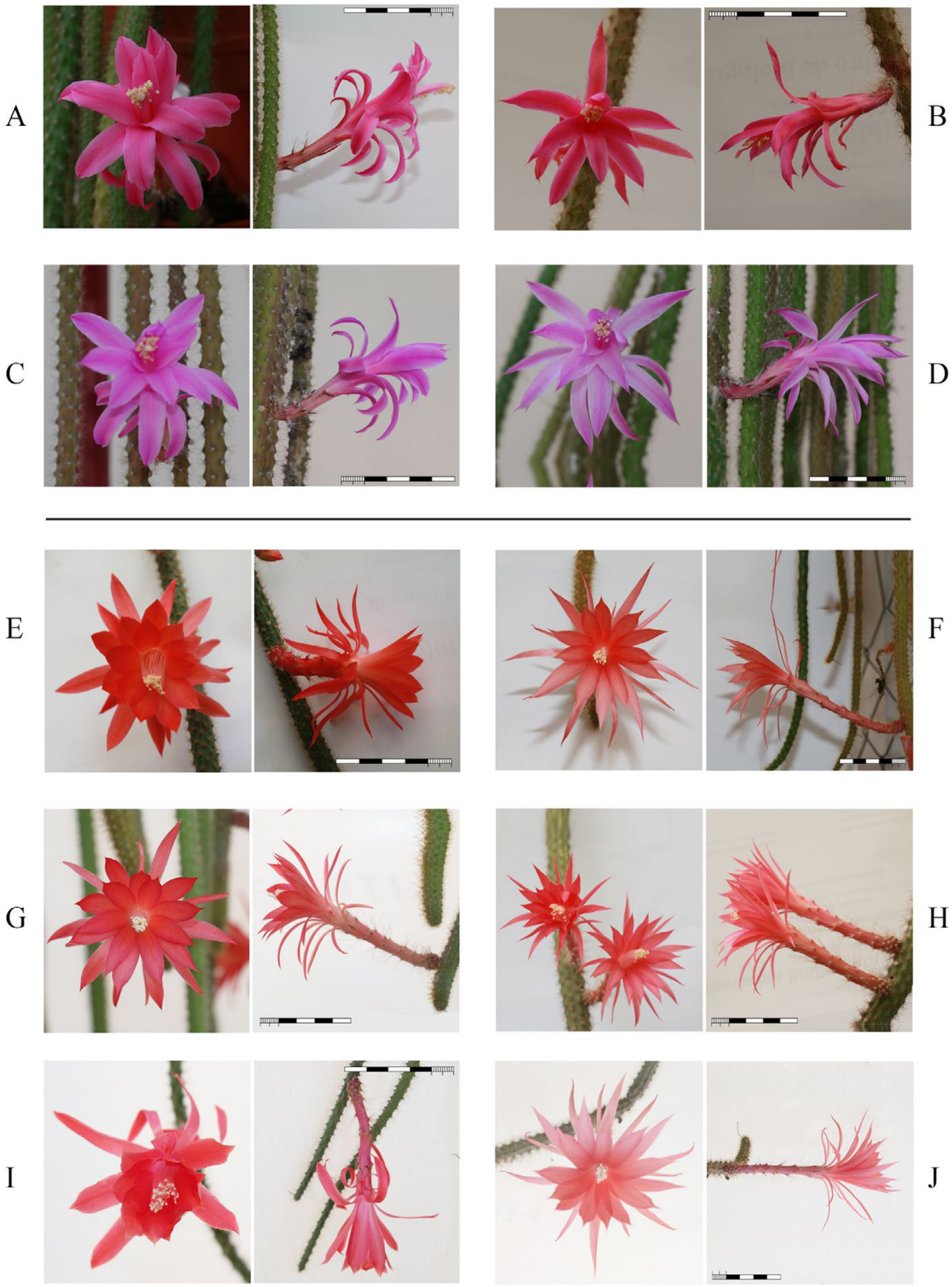

Figure 5. *Aporocactus* flowers and their variation in color and sizes. A-D) *Aporocactus flagelliformis*, pink to magenta flowers, all zygomorphic [A, *S. Arias* 1225, Hidalgo; B, *I. Rosas* 006, Querétaro; C, *I. Rosas* 022, Veracruz; D, *I. Rosas* 024, Hidalgo]. E-J) *Aporocactus martianus*, light red to deep red flowers, with short to long receptacular tube, actinomorphic [E, *M. A. Cruz* 09, Oaxaca; F, *I. Rosas* 17, Oaxaca; G, *I. Rosas* 14, Oaxaca; H, *I. Rosas* 15, Oaxaca; I, *M. A. Cruz* 02, Veracruz; J, *I. Rosas* 08, Oaxaca].

Niche modeling and niche conservatism. Temperature and precipitation are the main factors determining the altitudinal and longitudinal plant distribution (Archibold 1995). It has been proposed that precipitation and humidity variations have a more prominent effect on epiphytic plants (Hernández-Ruíz *et al*. 2016, Zotz 2016). Even in the globular cactus *Thelocactus*, precipitation (precipitation in the wettest quarter) constrains the ENM for most species (Mosco 2017). This pattern coincides for the ENM of *Aporocactus flagelliformis*, in which precipitation of the warmest month (BIO18) and the driest month (BIO14), and the temperature seasonality defined the model. Also, for *A. martianus*, the mean diurnal range (BIO2) and precipitation of the warmest month (BIO18) defined the model. Temperature seasonality is considered important in growth and other phenological processes (Menzel & Sparks 2006). The latter factor is critical for the conservation of *Aporocactus* and other epiphytic cacti in the context of climate warming. Although other regions with high suitability of distribution for *A. flagelliformis* were recovered, it is necessary to corroborate their presence in particular zones (*e.g.*, Sierra Madre Oriental at San Luis Potosí) or to investigate biological factors limiting the actual distribution (*e.g.*, pollinator availability).

Analyses suggested that the niche overlap is low and niches of the two species of *Aporocactus* are not identical and are significantly differentiated. Species of *Aporocactus* have specific environmental constraints and do not occupy niches that are similar as possible given what is available. Epiphytic plants in cloud forests are especially sensitive to climate changes (Foster 2001), floristic and climatic differences have been documented for cloud forests in Hidalgo, Querétaro, and central Veracruz versus cloud forests in southern Veracruz and Oaxaca (Ruíz-Jiménez *et al*. 2012). Comparative analysis of niche overlap and niche similarity has been addressed in other close related Mexican plants and cacti, and lead some authors to consider the existence of niche conservatism on those lineages (Suárez-Mota *et al*. 2015, Mosco 2017, Gutiérrez-Ortega *et al*. 2020), however our results suggest niche divergence in these sister species. A critical review by Münkemüller *et al*. (2015) suggests that studies investigating niche conservatism should compare alternative evolutionary models, including multiple-optima OU models. A comparative niche evolution analysis, as previous authors recommend, including a wider sampling of the tribe Hylocereeae, will allow to corroborate phylogenetic niche conservatism and niche shift in the Mesoamerican epiphytic lineages of cacti. For now, base on the difference of the ecological niches, we suggest the possibility of niche divergence in *Aporocactus*, as is expected for allopatric species (Peterson *et al.* 1999, Warren *et al.* 2008). Finally, the primary differences between both species of *Aporocactus* are established by the floral morphology; therefore, it is likely that the primary factor driving the evolution of these lineages is their association with pollinators. For many years, epiphytic cactus species have received scarce attention. Although *Aporocactus* is a small genus, it may represent an interesting model for research on such topics as the ecology of pollination, population genetics, and flower development to characterize the evolution of those specialized cacti.

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Supplementary material

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Author Contributions: IRR and SA designed the study. IRR and SA collected the field samples. IRR performed the DNA extractions. IRR and DS performed the phylogenetic analyses and performed the biogeographic analyses. DS performed the ecological niche modeling analyses. IRR and DS wrote the first draft of the manuscript, and SA revised and critically evaluated the manuscript. All the authors approved the final version of the manuscript.

Appendix 1. Taxa included in plastid *rpl16, trnL-F, psbA-trnH, trnQ-rps16, trnk-matK* and *rps3-rpl16* phylogenetic analyses. The sequences lacking for a locus/specimen GenBank accession are marked with dash (—), N.A.: no data.

Taxon	**Source, Voucher**	**GenBank accession number**					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Acanthocereus chiapensis Bravo	MX: Chiapas, *Guzmán 999*, MEXU	KU598005	KU598057	KU597952	KU598110	HM041754.1	_
Acanthocereus oaxacensis (Britton & Rose) Lodé	MX: Oaxaca, *Arias 2185*, MEXU	KU598008	KU598060	KU597955	KU598113	_	_
Acanthocereus tetragonus (L.) Hummelinck	MX: Chiapas, *Guzmán 1002*, MEXU	KU598021	KU598074	KU597969	KU598127	HM041645.1	_
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *Rosas 01*, MEXU	MZ836110	MZ836080	MZ836172	MZ836141	LT745632	LT745515.1
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *Rosas 02*, MEXU	MZ836118	MZ836081	MZ836181	MZ836150	–	–
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *Rosas 04*, MEXU	MZ836119	MZ836082	MZ836182	–	–	–
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *Rosas 023,* MEXU	MZ836112	MZ836084	MZ836174	MZ836143	–	–
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *Rosas 025,* MEXU	MZ836113	–	MZ836175	MZ836144	–	–
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *I Rosas 027,* MEXU	MZ836114	MZ836085	MZ836176	MZ836145	–	–

Taxon	Source, Voucher	GenBank accession number					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *I Rosas 029,* MEXU	MZ836115	MZ836086	MZ836177	MZ836146	–	–
Aporocactus flagelliformis (L) Lem.	MX: Hidalgo, *S. Arias 1221,* MEXU	MZ836120	MZ836090	MZ836183	MZ836151	–	–
Aporocactus flagelliformis (L) Lem.	MX: Querétaro, *I Rosas 031,* MEXU	MZ836116	MZ836087	MZ836178	MZ836147	–	–
Aporocactus flagelliformis (L) Lem.	MX: Querétaro, *I Rosas 032,* MEXU	MZ836127	MZ836088	MZ836179	MZ836148	–	–
Aporocactus flagelliformis (L) Lem.	MX: Querétaro, *I Rosas 033,* MEXU	MZ836117	MZ836089	MZ836180	MZ836149	–	–
Aporocactus flagelliformis (L) Lem.	MX: Veracruz, *I Rosas 020,* MEXU	MZ836111	MZ836083	MZ836173	MZ836142	–	–
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *I Rosas 07,* MEXU.	MZ836121	MZ836091	MZ836184	MZ836152	LT745634	LT745517.1
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *I Rosas 010,* MEXU	MZ836122	MZ836092	MZ836185	MZ836153	–	–
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *I Rosas 013,* MEXU	MZ836123	MZ836096	MZ836186	MZ836154	–	–
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *S. Arias 1225,* MEXU	MZ836124	MZ836093	MZ836187	MZ836155	–	–

Taxon	Source, Voucher	GenBank accession number					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *Arias 1230,* MEXU	MZ836125	MZ836094	MZ836188	MZ836156	–	–
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *Arias 2207,* MEXU.	MZ836126	MZ836095	MZ836189	MZ836157	–	–
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *Cruz 02,* MEXU	KU597983	KU598035	KU597930	KU598088	_	_
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *Cruz 09,* MEXU	KU597986	KU598038	KU597933	KU598091	_	_
Aporocactus martianus (Zucc.) Britton & Rose	MX: Oaxaca, *Cruz 13,* MEXU	KU597989	KU598041	KU597936	KU598094	_	_
Aporocactus martianus (Zucc.) Britton & Rose	MX: Veracruz, *Cruz 01*, MEXU	KU597980	KU598032	KU597927	KU598085	_	_
Bergerocactus emoryi (Engelm.) Britton & Rose	MX: Baja Cal., *Arias 1307*, CHAPA	DQ099994	DQ099925	KF783478	KF783697	HM041654.1	_
Cephalocereus sco-parius (Poselg.) Britton & Rose	MX: Oaxaca, *Hamman N.A*. (cult.)	AY181596	AY181625	KY624675	KY624747	_	_
Disocactus biformis (Lindl.) Lindl.	GT: Sacatepéquez, *Véliz 19901*, BIGU	KU598016	KU598069	KU597964	KU598122	LT745639	_
Deamia chontalensis (Alexander) Doweld	MX: Oaxaca, Yañez 03, MEXU	MH107788	MH107803	MH107793	–	LT745733	_

Taxon	Source, Voucher	GenBank accession number					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Disocactus phyllanthoides (DC.) Barthlott	MX: Veracruz, *Arias 2201*, MEXU	KU598025	KU598078	KU597973	KU598131	LT745651	LT745535.1
Disocactus speciosus (Cav.) Barthlott	MX: Jalisco, *Morales 01*, MEXU	KU597992	KU598044	KU597939	KU598097	LT745654	LT745538.1
Echinocereus pentalophus (DC.) Lem.	MX: Querétaro, *Arias 1737*, MEXU	KF783558	KF783628	KF783509	KF783699	KF783558.1	_
Deamia testudo (Karw. ex Zucc.) Britton & Rose	MX: Oaxaca, *Yáñez 001*, MEXU	KY624648	KY624662	KY624695	KY624765	LT745735	–
Epiphyllum phyllanthus (L.) Haw.	SR: *Hammel 22377*, INB	KU598015	KU598068	KU597963	KU598121	LT745667	LT745550.1
Epiphyllum thomasianum (K.Schum.) Britton & Rose	PA: Cocle, *Hammel 22020*, INB	KU598018	KU598071	KU597966	KU598124	LT745672	LT745556.1
Leptocereus quadricostatus Britton & Rose	PR: Cabo Rojo, *Arias 1464*, MEXU	KF783620	KF783690	AY851582	KF783768	_	_
Myrtillocactus eichlamii Britton & Rose	MX: Yucatan, *Arias 1363*, MEXU.	AY181610	AY181629	KY624690	KY624760	_	_
Myrtillocactus geometrizans Console.	MX: Querétaro, *Terrazas 557*, CHAPA	DQ100012	DQ099943	KY624694	KY624764	_	_

Taxon	Source, Voucher	GenBank accession number					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Pseudorhipsalis amazonica (Rol.-Goss.) Britton & Rose.	PA: Colon, *Hammel 24524*, INB	KU597994	KU598046	KU597941	KU598099	LT745699	LT745582.1
Pseudorhipsalis himantoclada (Rol.-Goss.) Britton & Rose	CR: San José, *Hammel 22076*, INB	KU597998	KU598050	KU597945	KU598103	LT745703	LT745586.1
Selenicereus atropilosus Kimnach	MX: Jalisco, *Arreola 1473*, MEXU.	KU598029	KU598082	KU597977	KU598135	LT745709	LT745592.1
Selenicereus calcaratus (F.A.C. Weber) D.R. Hunt	CR: San José, *Hammel 18394*, INB	MZ836128	MZ836097	MZ836190	MZ836158	LT745674	LT745558.1
Selenicereus dorschianus Bauer	MX: Jalisco, *Arias 2218*, MEXU	MZ836129	MZ836098	MZ836191	MZ836159	LT745712	LT745595.1
Selenicereus escuintlensis (Kimnach) D.R. Hunt	GT: Escuintla, *Véliz 20047*	MZ836130	MZ836099	MZ836192	MZ836160	–	–
Selenicereus glaber (Eichlam) S.Arias & N.Korotkova	MX: Chiapas, *Bravo 5614*, MEXU	KU598031	KU598084	KU597979	KU598137	LT745738	LT745621.1
Selenicereus grandiflorus (L.) Britton & Rose	MX: Veracruz, *Guzmán 1365*, MEXU	DQ100039	DQ099970	KU597971	KU598129	LT745713	LT745596.1
Selenicereus guatemalensis (Eichlam ex Weing.) D.R.Hunt.	GT: Guatemala, *Arias 1161*, MEXU	MZ836131	–	MZ836193	MZ836161	–	–

Taxon	Source, Voucher	GenBank accession number					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Selenicereus inermis (Otto ex Pfeif.) Britton & Rose	CR: Puntarenas, *Hammel 24274*, INB	MZ836132	MZ836100	MZ836194	MZ836162	LT745721	LT745604.1
Selenicereus monacanthus (Lem.) D.R.Hunt	CR: Heredia, *Hammel 26600*, INB	MZ836133	MZ836101	MZ836195	MZ836163	LT745682	LT745566.1
Selenicereus ocamponis (Salm-Dyck) D.R.Hunt	MX: Guerrero, *Gama 104*, MEXU	MZ836134	MZ836102	MZ836196	MZ836164	LT745688	LT745572.1
Selenicereus purpusii (Weing.) S.Arias & N.Korotkova	MX: Oaxaca, *Guzmán* 1095, MEXU	MZ836135	MZ836103	MZ836197	MZ836165	–	–
Selenicereus stenopterus (F.A.C.Weber) D.R.Hunt	CR: Heredia, *Hammel 22282*, INB	MZ836136	MZ836104	MZ836198	MZ836166	LT745729	LT745577.1
Selenicereus vagans (K.Brandegee) Britton & Rose	MX: Sinaloa, *Arias 1832*, MEXU	MZ836137	MZ836105	MZ836199	MZ836167	LT745730	LT745614.1
Stenocereus pruinosus (Otto ec. Pffeif.) Buxb.	MX:Puebla, Arias 750, MEXU	KF783618	KF783688	KF783554	KF783765	_	_
Weberocereus frohningiorum Bauer	CR: San José, *Hammel 22419*, INB	MZ836138	MZ836106	MZ836200	MZ836168	LT745737	LT745620.1

Taxon	**Source, Voucher**	**GenBank accession number**					
		rpl16	*trnL-F*	*psbA-trnH*	*trnQ-rps16*	*trnk-matk*	*rps3-rpl16*
Weberocereus imitans (Kimnach & Hutchison) Buxb.	CR: San José, *Hammel 26140*, INB	MZ836139	MZ836107	MZ836201	MZ836169	LT745740	LT745623.1
Weberocereus tunilla subsp. biolelly (F.A.C. Weber) Bauer	CR: Alajuela, Hammel 25603, INB	MZ836140	MZ836108	MZ836202	MZ836170	LT745746	LT745629.1
Weberocereus tunilla subsp. tunilla (F.A.C. Weber) Britton & Rose.	CR: Cartago, Hammel 22442, INB	–	MZ836109	MZ836203	MZ836171	LT745745	LT745628.1