UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

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Instituto de Biología

Sistemática

Delimitación taxonómica de *Mammillaria haageana* (Cactaceae)

TESIS

Que para optar por el grado de

DOCTOR EN CIENCIAS BIOLÓGICAS

PRESENTA

RAFAEL CRISTIAN CERVANTES SALCEDO

Tutor principal: Dr. Ángel Salvador Arias Montes

Jardín Botánico, Instituto de Biología, UNAM

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CIUDAD UNIVERSITARIA, CDMX. OCTUBRE, 2023

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **31 de julio de 2023** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **CERVANTES SALCEDO RAFAEL CRISTIAN** con número de cuenta **301206448** con la tesis titulada "**DELIMITACIÓN TAXONÓMICA DE MAMMILLARIA HAAGEANA (CACTACEAE)",** realizada bajo la dirección del **DR. ÁNGEL SALVADOR ARIAS MONTES,** quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 18 de septiembre de 2023

COORDINADOR DEL PROGRAMA

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Índice

Resumen

Los complejos de especies consisten en parientes filogenéticos muy cercanos, donde las similitudes morfológicas dificultan la distinción entre ellos utilizando métodos taxonómicos tradicionales. El trabajo de enfoca en la delimitación de especies del complejo *Mammillaria haageana*, un grupo que presenta una gran diversidad morfológica, que hace que su taxonomía sea complicada. *Mammillaria haageana* es parte de *M.* ser. *Supertextae*, las relaciones filogenéticas dentro de la serie y su relación con otros taxones de *Mammillaria* están lejos de comprenderse por completo. Por lo tanto, en el Capítulo I se intenta dilucidar las relaciones filogenéticas con dos marcadores del cloroplasto y utilizando representantes de todos los taxones del grupo. Los análisis filogenéticos muestran que *M.* ser. *Supertextae* comprende un grupo monofilético bien respaldado, que divergió hace aproximadamente 2.1 millones de años y *M.* ser. *Polyacanthae* se recupera como su grupo hermano; sin embargo, las relaciones dentro de *M.* ser. *Supertextae* no se pudieron resolver. En el Capítulo II, se integran los datos genómicos, morfológicos y ecológicos, para establecer los límites taxonómicos en el complejo *M. haageana*, y estudiar las relaciones evolutivas con el resto de las especies de *M.* ser. *Supertextae*. Los análisis genéticos, así como la evidencia morfológica y ecológica, llevan a proponer que el complejo *M. haageana* se compone de seis entidades distintas (*M. acultzingensis*, *M. conspicua*, *M. haageana*, *M. lanigera*, *M. meissneri* y *M. san-angelensis*), principalmente como resultado de una especiación ecológica. Una propuesta taxonómica reciente considera a estos taxones como una sola especie; por lo que se propone el reconocimiento a nivel de especie de las seis entidades mencionadas. Nuestros resultados también muestran un alto nivel de sorteo incompleto de linajes, lo que es especialmente probable en especies recientemente divergentes, como las que comprenden *M.* ser. *Supertextae*. Las hipótesis de especies propuestas aquí, pueden ser útiles en futuras evaluaciones de riesgo de extinción y estrategias de conservación.

Abstract

Species complexes consist of very close phylogenetic relatives, where morphological similarities make it difficult to distinguish among them using traditional taxonomic methods. Here, we focus on species delimitation in the *Mammillaria haageana* complex, a group that presents great morphological diversity, which makes its taxonomy a puzzle. *Mammillaria haageana* is part of *M. ser. Supertextae*, the phylogenetic relationships within the series, and its relationship to other *Mammillaria* taxa are far from fully understood. Therefore, in Chapter I it was attempted to elucidate phylogenetic relationships with two chloroplast markers and using complete terminal sampling. Phylogenetic analyses showed that *M.* ser. *Supertextae* comprises a well-supported monophyletic group that diverged about 2.1 million years ago and *M.* ser. *Polyacanthae* is recovered as its sister group; however, the relationships within *M.* ser. *Supertextae* cannot be resolved. In Chapter II, we integrate genomic, morphological, and ecological data to establish taxonomic limits in the *M. haageana* complex, and studied evolutionary relationships with the other species of the *M.* ser. *Supertextae*. Genetic analyses, as well as morphological and ecological evidence, led us to propose that the *M. haageana* complex is composed of six distinct entities (*M. acultzingensis*, *M. conspicua*, *M. haageana*, *M. lanigera*, *M. meissneri*, and *M. san-angelensis*), mainly as a result of ecological speciation. A recent taxonomic proposal considered these taxa as a single species, but we propose their recognition at the species level. Our results also show a high level of incomplete lineage sorting, which is especially likely in recently diverged species such as those comprising *M.* ser. *Supertextae*. The species hypotheses proposed here may be useful in future extinction risk assessments and conservation strategies.

Introducción general

Dentro de los objetivos principales de la sistemática se encuentran reconocer las relaciones filogenéticas entre las especies, así como establecer los límites entre éstas (Wiens, 2007). La delimitación de especies es una actividad necesaria, ya que ayuda a la comprensión de los mecanismos y procesos evolutivos, así como al conocimiento sobre la biodiversidad y su conservación (Zachos, 2016). Sin embargo, no es una tarea fácil, debido a las dificultades que esta actividad presenta. Por ejemplo, uno de los primeros problemas a los que se enfrenta al delimitar especies, es que las definiciones de especie se entremezclan con los conceptos de especie, lo cual ha sido una cuestión de debate, que ha abarcado varias disciplinas (Rannala, 2015; Sangster, 2018). Se ha tratado de abordar este problema utilizando diferentes propiedades de las especies (p. ej. aislamiento reproductivo, zona adaptativa distinta, monofilia, etc.); sin embargo, cada propiedad implica un concepto diferente (Pušić et al., 2017). Por su parte, De Queiroz (2005, 2007) considera que existe un elemento de consiliencia entre todos los conceptos de especie: la mayoría consideran a las especies como linajes evolutivos. En sistemática, las especies representan hipótesis para las cuales se puede buscar evidencia mediante el estudio de múltiples propiedades; de acuerdo con De Queiroz (2005, 2007) la ausencia de una cierta propiedad no proporciona evidencia que contradiga la hipótesis de una especie determinada (Haelewaters et al., 2018). En este sentido, la taxonomía integrativa puede considerarse la contrapartida operativa del concepto unificado de especie, al utilizar diferentes tipos de evidencias o propiedades (p. ej. morfología, ecología, ADN, etc.); bajo este marco las hipótesis disminuyen su tasa de error (Schlick-Steiner et al., 2009).

Delimitación de especies en Cactaceae

La familia Cactaceae es originaria del continente americano, con varias especies del género *Opuntia* Mill. naturalizadas en el Viejo Mundo y Australia; el género *Rhipsalis* Gaertn. procedente de América tropical se extiende actualmente en África, incluyendo Madagascar y en Sri Lanka (Arias et al., 2012; Bravo-Hollis, 1978; Hunt et al., 2006). En la familia se han reconocido cerca de 150 géneros y 1851 especies (Korotkova et al., 2021). Las principales características morfológicas que caracterizan a los integrantes de Cactaceae son la presencia de unas yemas axilares altamente especializadas llamadas areolas, una yema terminal organizada en cuatro zonas y la mayoría tienen un ovario ínfero cubierto por brácteas o aréolas (Hernández-Hernández et al., 2011). La monofilia de Cactaceae ha sido soportada por estudios basados en marcadores del cloroplasto (Cuénoud et al., 2002; Nyffeler, 2002) y mediante transcriptomas (Walker et al., 2018). Se ha calculado que el tiempo de divergencia de la familia fue hace 35-32.11 millones de años (Ma) para el grupo troncal, mientras que para el grupo corona se estima en 28.6-26.88 Ma (Arakaki et al., 2011; Hernández-Hernández et al., 2011). Dentro de la familia, los problemas de delimitación de especies se ven reflejados en la incongruencia entre el número de especies reconocidas por diferentes taxónomos. Por ejemplo, en los géneros más diversos de la familia se citan distintos números de especies: *Mammillaria* Haw., 163 spp. (Hunt et al., 2006) ó 320 spp. (Reppenhagen, 1992)*; Echinocereus* Link & Otto, 44 spp. (Taylor, 1985) ó 71 spp. (Blum et al., 1998); y *Opuntia,* 75 spp. (Hunt et al., 2006) ó 189 spp. (Anderson, 2001). Se considera que estas diferencias son resultado de una carencia de estudios que permitan documentar y caracterizar a las especies a través de métodos objetivos (p. ej. morfométricos, filogenéticos, ecológicos, etc.; Sánchez et al., 2013). Los estudios de delimitación de especies han sido, principalmente a través de métodos morfométricos basados en estadística multivariada (Martínez-Quezada et al., 2019; Sánchez et al., 2013; Tapia et al., 2016); sin embargo, los caracteres utilizados suelen ser ambiguos, lo que

dificulta la separación en grupos resueltos taxonómicamente (Butterworth & Wallace, 2004; Copetti et al., 2017). Por otra parte, el uso de marcadores del cloroplasto se ha utilizado principalmente para establecer las relaciones evolutivas al interior de Cactaceae (Cruz et al., 2016; Sánchez et al., 2014; Vázquez-Sánchez et al., 2013), y existen muy pocos estudios dirigidos a la delimitación de especies (Aquino et al., 2019). La falta de marcadores nucleares y su utilizazion en la delimitación de especies en Cactaceae, se debe a que los marcadores existentes proporcionan un menor número de sitios informativos en comparación con los marcadores de cloroplasto y no exhiben polimorfismos, como se ha demostrado para la región del espaciador interno escrito (ITS) del ADN ribosomal (Cruz et al., 2016).

Con el avance de la secuenciación masiva se esperaría un aumento en estudios de filogenómica en cactáceas; sin embargo, existen muy pocos trabajos. Por ejemplo, se secuenciaron cuatro genomas correspondientes a la tribu *Pachycereeae*, donde sólo *Carnegiea gigantea* (Engelm.) Britton & Rose se secuenció con una alta cobertura; mientras que las tres especies restantes con una baja cobertura (*Lophocereus schottii* (Engelm.) Britton & Rose, *Pachycereus pringlei* (S.Watson) Britton & Rose y *Stenocereus thurberi* (Engelm.) Buxb.). Los resultados mostraron que el 37% de los árboles de genes que tenían al menos el 90% de soporte de bootstrap estaban en conflicto con el árbol de especies. Esta discordancia puede estar en función de los tiempos generacionales largos que presentan estas especies y al tamaño efectivo de la población que pueden ser moderadamente grandes, lo que conlleva a un extenso sorteo incompleto de linajes (Copetti et al., 2017). Sin embargo, hasta el momento no se han realizado estudios de delimitación de especies en la familia utilizando secuenciación masiva y métodos de coalescencia.

El complejo *Mammillaria haageana*

Las diferentes clasificaciones que se han propuesto para *Mammillaria* se han basado en el conocimiento de los taxónomos que las han planteado. Dentro de *Mammillaria* se recococen ocho subgéneros (Hunt et al., 2006); *Oehmea, Dolichothele, Phellosperma, Chilita, Krainzia, Cochemiea, Mammillopis* y *Mammillaria*. Para el subgénero *Mammillaria* se proponen tres secciones con base en la prescencia de laticíferos que producen un látex lechoso: *Hydrochylus,* ausencia de laticíferos; *Subhydrochylus,* produce látex semi-lechoso; *Mammillaria*, produce látex lechoso (Mauseth, 1978). Aunque se han realizado esfuerzos para poner a prueba estas hipótesis mediante análisis fenéticos (Lüthy, 1995) y filogenéticos (Breslin et al., 2021; Butterworth & Wallace, 2004), sólo se han hecho para corroborar la monofilia de *Mammillaria* y conocer si se recuperan las categorías infragenéricas (Tabla 1). Dentro de los trabajos que se han realizado, las especies que pertenecen a *M.* ser. *Supertextae* se agrupan con caracteres morfológicos y moleculares; sin embargo, solo se han incluido hasta cinco especies de las nueve reconocidas por Korotkova et al. (2021) y solo se contempla un individuo por especie.

Tabla 1.- Clasificación para el subgénero *Mammillaria* de acuerdo con Hunt et al. (2006).

Subgénero		
Mammillaria		
Sección		
Hydrochylus	*Subhydrochylus*	*Mammillaria*
Series		
Lasiacanthae	*Heterochlorae*	*Leucocephalae*
Stylothelae	*Rhodanthae*	*Mammillaria*
Proliferae	*Polyacanthae*	*Polyedra*
Sphacelatae	***Supertextae***	
Decipientes		
Leptocladodae		

Los análisis previos han mostrado que *M. haageana* Pfeiff. es la especie hermana de *M. albilanata* Backeb.; para esta última especie se han reconocido cuatro subespecies que se distribuyen en Colima (*M. albilanata* subsp. *reppenhagenii* (D.R.Hunt) D.R.Hunt), Guerrero

(*M. albilanata* subsp. *albilanata*), Chiapas (*M. albilanata* subsp. *tegelbergiana* (H.E.Gates ex G.E.Linds.) D.R.Hunt) y Oaxaca (*M. albilanata* subsp. *oaxacana* D.R.Hunt). *Mammillaria haageana* y *M. albilanata* subsp. *oaxacana* comparten distribución en el sur de Oaxaca y se ha documentado que es un problema establecer los límites taxonómicos entre estos dos taxones (Arias et al., 2012). Por lo tanto, el objetivo principal de este estudio es establecer los límites taxonómicos de *M. haageana* entre las especies de *M.* ser. *Supertextae* y al interior de la especie. Para esto se incluirán la mayoría de las especies de *M.* ser. *Supertextae* reconocidas por Korotkova et al. (2021) y se pretende realizar un esfuerzo de colecta para abarcar la distribución geográfica de *M. haageana* y *M. albilanata* subsp. *oaxacana*. Mediante dos marcadores del cloroplasto (*rpl16* y *psbA-trnH*) se pondrá a prueba la monofilia de *M.* ser. *Supertextae* y se determinará la posición de *M. haageana* dentro de la serie. Los resultados obtenidos se utilizarán para seleccionar un conjunto de muestras y diseñar una estrategia de secuenciación, utilizando el método de genotipificación por secuenciación (GBS, por sus siglas en inglés): para establecer los límites taxonómicos en el complejo *M. haageana*. Dado que las hipótesis sobre delimitación de especies son más robustas al incluir diferentes líneas de evidencia (Schlick-Steiner et al., 2009), se realizará un análisis morfométrico para identificar los caracteres morfológicos que puedan ser útiles en la identificación de taxones y se explorarán las variables ambientales que expliquen la distribución actual del complejo *M. haageana*

Capítulo I

Evaluating the monophyly of *Mammillaria* series *Supertextae* (Cactaceae)

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Evaluating the monophyly of *Mammillaria* series *Supertextae* (Cactaceae)

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Abstract

Mammillaria (Cactaceae) taxonomy has been historically problematic due to the morphological variability and sympatry of the species. This has led to several proposals for infrageneric classification, including subgeneric, section and series categories. *Mammillaria* ser. *Supertextae* is one of 15 series and is made up of a variable set of species that are mainly distributed in southern Mexico and Central America. However, the phylogenetic relationships within *M.* ser. *Supertextae* and its relationship to other *Mammillaria* taxa are far from fully understood. Here we attempt to elucidate these relationships using complete terminal sampling and newly obtained chloroplast marker sequences and comparing them to *Mammillaria* species sequences from GenBank. Our phylogenetic analyses showed that *M.* ser. *Supertextae* comprises a well-supported monophyletic group that diverged approximately 2.1 Mya and has *M.* ser. *Polyacanthae* as its sister group; however, relationships within *M.* ser. *Supertextae* remain unresolved. The topology obtained within *M.* ser. *Supertextae* must also be interpreted under the distribution shared by these taxa, but it is difficult to differentiate ancestral polymorphisms from possible introgression, given the short time elapsed and the markers used. Our results show that the infrageneric units of *M. haageana* and *M. albilanata* can be considered independent evolutionary units. We also suggest that the relationship between *M. haageana* and *M. albilanata* is convoluted because their distribution overlaps (mainly towards southern Mexico), with genetic differences that possibly indicate they represent more than two taxonomic entities. One possible explanation is that there could still be gene flow between these taxa, and we might be witnessing an ongoing speciation process.

Keywords
Bayesian inference, Cactaceae, chloroplast DNA, *Mammillaria haageana*, molecular phylogeny, *M.* ser. *Supertextae*, taxonomy

Introduction

Mammillaria Haw. (Cactaceae, Cactoideae, Cacteae) is the most diverse genus within the cactus family, with a broad range of recognized species, ranging from 163 (Hunt et al. 2006) to 181 (Pilbeam 1999) up to 320 species (Reppenhagen 1992). *Mammillaria* is characterized by tuberculate stems, definite dimorphic areoles not connected by a groove, flowers that arise from the base of the tubercles and not apically, and seeds with testa cell walls that are par-concave and undulated (Bravo-Hollis and Sánchez-Mejorada 1991; Lüthy 1995; Anderson 2001; Hunt et al. 2006). This genus, together with *Coryphantha* (Engelm.) Lem., *Escobaria* Britton & Rose, *Neolloydia* Britton & Rose, and *Ortegocactus* Alexander, is integrated into the Mammilloid clade (Butterworth et al. 2002). However, it has been proposed that *Mammillaria* is a polyphyletic group. Breslin et al. (2021), using plastid genomes, confirmed previous studies showing *Mammillaria* is nonmonophyletic, as currently circumscribed, so they proposed that the Mammilloid clade be circumscribed in three monophyletic genera, *Mammillaria* s.s., *Coryphantha* and *Cochemiea* s.l., as previously suggested by Vázquez-Sánchez et al. (2013). Furthermore, the taxonomy of *Mammillaria* has historically been difficult due to large morphological variability, phenotypic plasticity, sympatric distribution of species, and suspected hybridization events.

Within *Mammillaria*, there are 15 recognized series (Hunt et al. 2006), one of which is *M.* ser. *Supertextae* D.R. Hunt, distributed from western Mexico to Central America and even the Caribbean islands (Pilbeam 1999). *Mammillaria* ser. *Supertextae* is a clear example of the species delimitation problem within the genus, illustrated by the number of accepted species ranging from 8 to 27, although the most recent taxonomic proposal recognizes only 9 taxa (Table 1). Morphometric and molecular studies have attempted to assess the proposed infrageneric relationships for *Mammillaria*, but none have been specifically directed at *M.* ser. *Supertextae.* Lüthy (1995) performed a detailed morphological analysis of the genus with a phenetic approach, in which he included four species of *M.* ser. *Supertextae* (*M. albilanata* Backeb., *M. dixanthocentron* Backeb. ex Mottram, *M. haageana* Pfeiff., and *M. huitzilopochtli* D.R. Hunt), showing that *M.* ser. *Supertextae* is characterized by the presence of extracellular crystals; however, the trait is not exclusive to *M.* ser. *Supertextae*, as the *M.* ser. *Leucocephalae* Lem. ex Schumann also shows this characteristic. Butterworth and Wallace (2004) conducted a molecular study using two chloroplast markers (*rpl*16 and *psb*A-*trn*H), including five species of *M.* ser. *Supertextae* (same as Lüthy but including *M. supertexta* Mart. ex Pfeiff.); although the species were grouped together, the support values were low (BS = 63, PP = 0.99), and a phylogenetic relationship could not be established with the remainder of *Mammillaria*. More recently, the complete sequencing of the chloroplast genome of eight *Mammillaria*

Table 1. Historical account of taxonomic classifications of *M.* ser. *Supertextae* D.R. Hunt (= *Elegantes*).

Backeberg (1961)	Bravo-Hollis and Sánchez-Mejorada (1991)	Reppenhagen (1992)	Lüthy (1995)	Hunt et al. (2006)
M. crucigera Mart.	*M. huitzilopochtli* D.R.Hunt	*M. elegans*	*M. albilanata*	*M. albilanata*
M. celsiana Lem.	*M. lanata* Orcutt	*M. meissneri*	*M. columbiana*	*M. crucigera*
M. elegans DC.	*M. albilanata* Backeb.	*M. haageana*	*M. eriacantha*	*M. columbiana*
M. supertexta Mart. ex Pfeiff.	*M. supertexta*	*M. conspicua*	*M. haageana*	*M. dixanthocentron*
M. dyckiana Zucc. ex Pfeiff.	*M. crucigera*	*M. monticola* Repp.	*M. supertexta*	*M. flavicentra*
M. dealbata A.Dietr.	*M. dixanthocentron* Backeb.	*M. lanigera* Repp.	*M. crucigera*	*M. haageana*
M. haageana Pfeiff.	*M. vaupelii* Tiegel	*M. donatii*	*M. dixanthocentron*	*M. halbingeri*
M. acanthoplegma Lehm.	*M. haageana*	*M. albidula* Backeb.	*M. huitzilopochtli*	*M. huitzilopochtli*
M. meissneri Ehrenbg.	*M. collina* J.A.Purpus	*M. lanata*		*M. supertexta*
	M. donatii Berge ex K.Schum.	*M. tlalocii* Repp.		
	M. san-angelensis Sánchez-Mej.	*M. huitzilopochtli*		
	M. martinezii Backeb.	*M. crucigera*		
	M. fauxiana Backeb.	*M. flavicentra*		
	M. conspicua J.A.Purpus	*M. dixanthocentron*		
	M. halbingeri Boed.	*M. supertexta*		
	M. flavicentra Backeb.	*M. reppenhagenii*		
	M. tegelbergiana G.E.Linds.	*M. albilanata*		
	M. reppenhagenii D.R.Hunt	*M. igualensis* Repp.		
	M. ruestii Quehl	*M. tegelbergiana*		
	M. yucatanensis Orcutt	*M. igniota* Repp.		
		M. halbingeri		
		M. noureddineana Repp.		
		M. columbiana Salm-Dyck		
		M. ruestii		
		M. yucatanensis		
		M. chilapensis Repp.		
		M. eriacantha Link & Otto ex Pfeiff.		

species confirms that four *M.* ser. *Supertextae* taxa (*M. crucigera* Mart., *M. supertexta*, *M. huitzilopochtli*, *M. haageana* subsp. *san-angelensis* (Sánchez-Mej.) D.R. Hunt) represent a clade (Solórzano et al. 2019; Hinojosa-Alvarez et al. 2020).

To disentangle the evolution of *Mammillaria*, we decided to focus on elucidating the phylogenetic relationships of *M.* ser. *Supertextae*. We included all taxa proposed by Hunt et al. (2006), except for *M. halbingeri* Boed., as, according to Reppenhagen (1992), the species was not reported again. We also included 12 localities of *M. haageana* and seven of *M. albilanata*. All these species together constitute one taxonomic complex within *M.* ser. *Supertextae* (Arias et al. 2012). We chose two chloroplast markers, the *rpl*16 intron and the intergenic spacer *psb*A-*trn*H. In Cactaceae, both markers have been used to resolve phylogenetic relationships (Korotkova et al. 2010; Sánchez et al. 2014; Cruz et al. 2016; Barrios et al. 2020), including *Mammillaria* (Butterworth and Wallace 2004; Vázquez-Sánchez et al. 2013; Hernández-Hernández et al. 2014); therefore, there are many sequences available in GenBank that can be used to expand the sampling of terminals, including sister groups and outgroups essential for testing the monophyly (Korotkova et al. 2017) of *M.* ser. *Supertextae*. The main objective of this study was to test the monophyly of *M.* ser. *Supertextae* and estimate its divergence time by broadening the sample of terminals within the series.

Materials and methods

The present study included a total of 123 taxa, 111 species of *Mammillaria*, 5 closely related genera (*Escobaria*, *Pelecyphora* Ehrenb., *Coryphantha*, *Neolloydia*, and *Ortegocactus*) and three external groups (*Ferocactus haematacanthus* (Salm-Dyck) Borg ex Backeb., *Ferocactus latispinus* (Haw.) Britton & Rose, and *Stenocactus lloydii* Berger). We selected two chloroplast loci: the intron *rpl*16 and the intergenic spacer region *psb*A-*trn*H. We downloaded 95 sequences of the genus *Mammillaria* (Butterworth and Wallace 2004; Hernández-Hernández et al. 2011; Fehlberg et al. 2013) from GenBank (see Appendix 1). For *M.* ser. *Supertextae*, we obtained two sequences: *M. albilanata* subsp. *tegelbergiana* (H. E. Gates ex G. E. Linds.) D.R. Hunt and *M. columbiana* Salm-Dyck subsp. *columbiana* from Vázquez-Sánchez et al. (2013). For the remaining 28 taxa of *M.* ser. *Supertextae*, we generated new sequence data.

DNA was extracted from 40 mg of silica-dried (24 h) stems. The samples were stored at -80 °C, and 12 hours later, they were triturated in a TissueLyser II (Qiagen, Venlo, Netherlands) at 29 rpm for 25 s twice. Extraction was performed with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, and the elution volume was 35 µl twice in Milli-Q water. The *rpl*16 intron and *psb*A-*trn*H intergenic spacer were amplified using standard PCR protocols. The *rpl*16 region was amplified using the primers from Hernández-Hernández et al. (2011), *rpl*161F (5′-GCTATGCTTAGTGTGTGACTCGTT-3′) and *rpl*163R (5′-CTTCTATTTGTCTAGGCGTGATCC-3′), by initially denaturing the DNA for 5 min at 94 °C, followed by 28 cycles of 1 min at 94 °C, 50 s at 55 °C, and 2 min at 72 °C, and a final extension of 4 min at 72 °C. The *psb*A-*trn*H intergenic spacer was amplified with primers from Korotkova et al. (2010), *CApsbA* (5′-CCGTGCTAACCTTGGTATGG-3′) and *CAtrnH* (5′-CCGCGAATGGTGGATTCACAAT-3′). PCR conditions were 2 min at 94 °C; followed by 29 cycles of 30 s at 94 °C, 30 s at 52 °C; and 1 min at 72 °C; and a final extension of 7 min at 72 °C. Amplifications were performed using 0.6 U of Platinum *Taq* polymerase according to the manufacturer's protocol (Invitrogen, Carsbad, California, USA), 4 mM mixed dNTPs (Invitrogen, Thermo Fisher Scientific, Waltham, USA), 1.5 mM MgCl2, 16 mg/mL BSA, 0.25 µM each primer, and 30–50 ng of genomic DNA in a reaction volume of 25 µL. The PCR products were sequenced in an Applied Biosystems Sequencer Model 3730xL at the Laboratorio de Biología Molecular de la Biodiversidad y de la Salud, Instituto de Biología, UNAM.

The sequences were aligned in GUIDANCE2 (v. 2.02, Sela et al. 2015) using MAFFT (v. 7.407, Katoh and Standley 2013). The algorithm was implemented with 100 iterations (–msaProgram MAFFT –MSA_Param "\–globalpair \--maxiterate 100" –bootstraps 100). The resulting matrix was imported into PHYDE (v. 0.9971, Müller et al. 2019) to manually edit ambiguously aligned sites. Both genes were concatenated, and data partitions were determined with the program PARTITIONFINDER (v. 2, Lanfear et al. 2017) using the Bayesian information criterion and greedy search. The model with the best fit for both markers was TVM + I + G. Indels and inversion were

manually coded with MESQUITE (v 3.6, Maddison and Maddison 2019) using the simple coding method of Simmons and Ochoterena (2000).

Bayesian inference (BI) analysis was performed using MRBAYES (v. 3.2.1, Ronquist et al. 2012). The analysis was run with four Markov chain Monte Carlos (MCMC) (nchains = 4) and ten million generations (ngen = 10000000), sampling trees every 100 generations (samplefreq = 100) and discarding the first 25% as burn-in. All parameters were monitored with TRACER (v. 1.7.1, Rambaut 2018) until they had effective sample sizes (ESS) of greater than 200. Maximum likelihood (ML) analysis was conducted using RAXML (v. 8.2.12, Stamatakis 2014) with molecular and binary partitioning, calculating the conditional likelihood of no invariant data and considering CAT for the heterogeneity of rate. The correction was made with the Lewis (2001) method (-m ASC_MULTICAT –asc-corr=lewis -#1000). To evaluate the monophyly of the subgenera and series recognized by Hunt et al. (2006) in the tree, the Monophy package (v. 1.3, Schwery and O'Meara 2016) of the R program (v. 4.0.3, R Core Team 2018) was used.

To estimate divergence times, we used the credibility interval around the estimated age of the Mammilloid clade (5.83–12.56 Mya; Hernández-Hernández et al. 2014). We inferred a time-calibrated phylogenetic tree using a BI approach implemented in BEAST (v. 2.6.1, Bouckaert et al. 2019). Analysis of the concatenated matrix used the uncorrelated lognormal relaxed clock (Drummond et al. 2006) for a total of 20 million generations of MCMC, sampling once every 10000 trees and discarding 15% as burn-in using TREEANNOTATOR v. 2.6.0.

Results

The overall sequence matrix for the two genes included 2257 bp and 8 encoded indels. We excluded 1045 bp in *rpl*16 due to uncertain homology. The final length of the aligned matrix for *rpl*16 was 897 bp and 315 bp for *psb*A-*trn*H, with 168 and 69 potentially informative sites, respectively. The BI and ML analyses produced trees with similar topologies (Fig. 1). *Mammillaria* ser. *Supertextae* was recovered as a monophyletic group (PP = 1, BS = 84), supported by a transversion in *psb*A-*trn*H. *Mammillaria* ser. *Polyacanthae* was also recovered as a monophyletic group (PP = 1, BS = 91), supported by 2 transitions in *psb*A-*trn*H. The sister relationship between *M.* ser. *Supertextae* and *M.* ser. *Polyacanthae* (PP = 0.99, BS = 80) is supported by a deletion in *rpl*16.

A polytomy formed within *M.* ser. *Supertextae*, where four clades were formed (S1, S2, S3, and S4), three of which are defined by specific polymorphisms (i.e., clade S1 a transversion in *rpl*16, clade S3 an inversion in *rpl*16). In three clades, at least one terminal of *M. albilanata* subsp. *oaxacana* was confirmed (S1: CC044 and CC046; S2: CC040; S3: CC036); in addition, part of its geographic distribution was common to *M. haageana* (Fig. 2A, B). The 12 terminals of *M. haageana* are distributed into two clades. S1 is formed by six terminals of *M. haageana* (CC024, CDMX, CC023, CC025, CC027, and CC045), two terminals of *M. albilanata* subsp. *oaxacana* referred

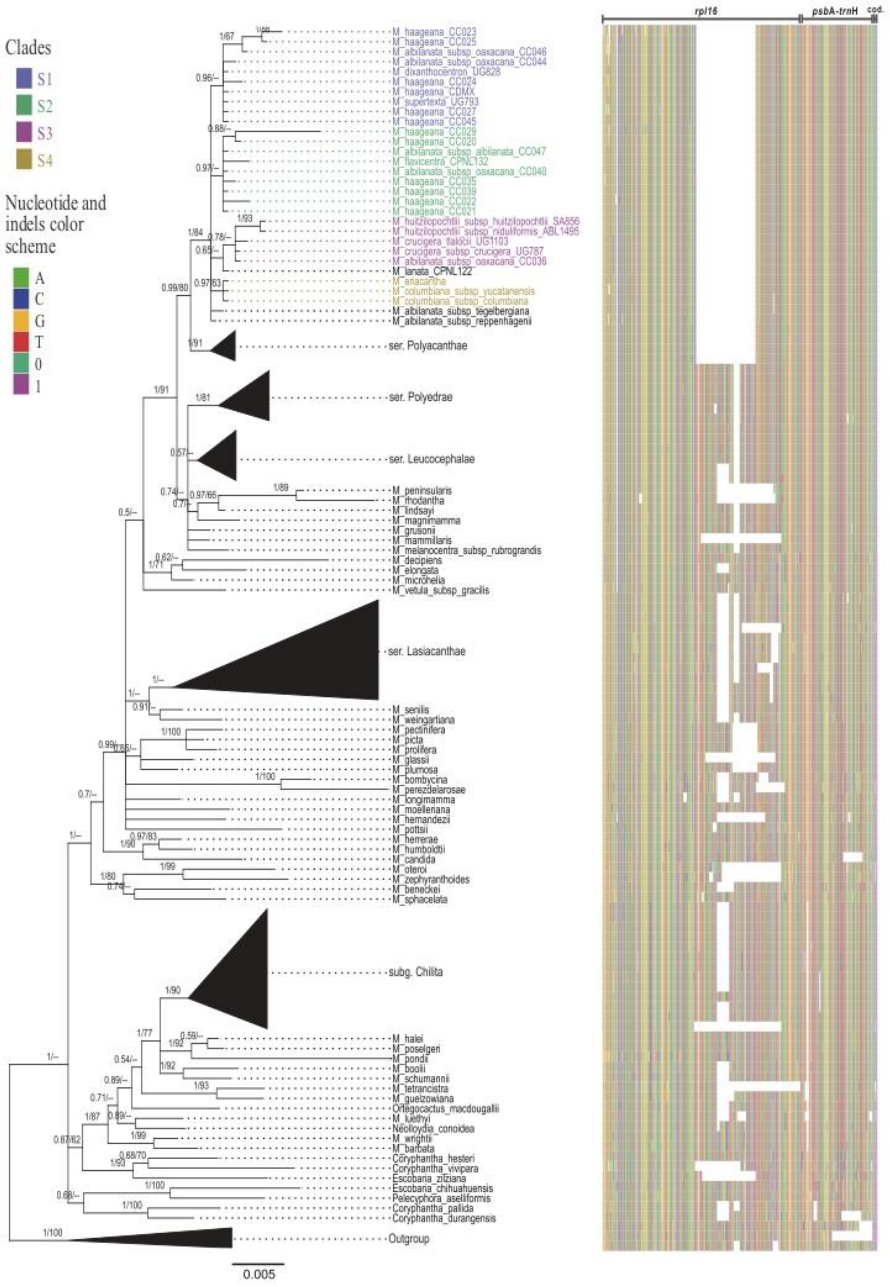

Figure 1. Phylogenetic tree of 123 taxa based on two chloroplast markers using IB. The values on the branches correspond to posterior probability (right) and ML bootstrap (left), and a dash (-) represents values of BS < 60. The left section shows a matrix with variable sites for *rpl*16 and *psb*A-*trn*H, as well as the coding of indels and inversions. Within *M.* ser. *Supertextae*, the clades are marked with colors.

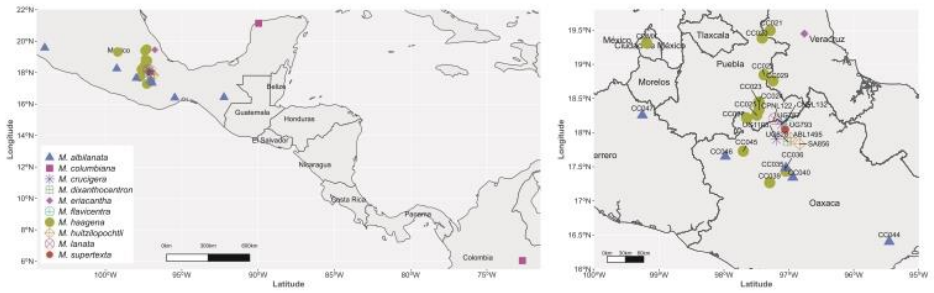

Figure 2. Left distribution of *M.* ser. *Supertextae* species sample collection localities; Right inset with southern Mexico shown in greater detail; sampling with emphasis on *M. haageana* and *M. albilanata* are shown, as well as the collection codes (Appendix 1).

to above, *M. dixanthocentron*, and *M. supertexta*, all of which display a transversion in *rpl*16. The second group consists of six terminals of *M. haageana* (CC020, CC021, CC022, CC029, CC035, and CC039), *M. flavicentra*, one terminal of M. *albilanata* subsp. *oaxacana*, and *M. albilanata* subsp. *albilanata* (CC047), all of which had a duplication of 15 bp in *rpl*16. Despite the rather small sampling of polymorphisms, these observations highlight the taxonomic problem in distinguishing *M. haageana* from *M. albilanata*, together with other sister species. The clade S3 formed by *M. huitzilopochtli* subsp. *huitzilopochtli*, *M. huitzilopochtli* subsp. *niduliformis*, *M. crucigera* subsp. *crucigera*, *M. crucigera* subsp. *tlalocii*, and one terminal of *M. albilanata* subsp. *oaxacana* (CC036) shows an inversion in *rpl*16 of 46 bp, and its sister group was *M. lanata*. Clade S4 is made up of two species, one of which corresponds to *M. columbiana*, which is distributed from Yucatan, Mexico to Colombia and Venezuela (Fig. 2A); the second species is *M. eriacantha*, which is distributed in Veracruz, Mexico (Fig. 2A, B).

The estimated crown age for *M.* ser. *Supertextae* was approximately 2.1 Mya (95% HPD = 0.91–3.47) in the Neogene-Quaternary transition (Fig. 3), whereas the *M.* ser. *Polyacanthae* crown age was estimated to be approximately 1 Mya (95% HPD = 0.15–2.22) in the mid-Pleistocene (Fig. 3). The divergence between these two groups was approximately 2.8 Mya (95% HPD = 1.46–4.73) in the late Pliocene; however, this clade has low support.

Discussion

The concatenation of two matrices (*rpl*16 and *psb*A-*trn*H) and extensive sampling (eight of nine species, according to Hunt et al. 2006; Table 1) helped to recover *M.* ser. *Supertextae* as a monophyletic group, consistent with previous molecular phylogenetic studies that included only five (Butterworth and Wallace 2004) and three taxa (Solórzano et al. 2019) of the series. The phylogenetic position of *M. eriacantha* has been

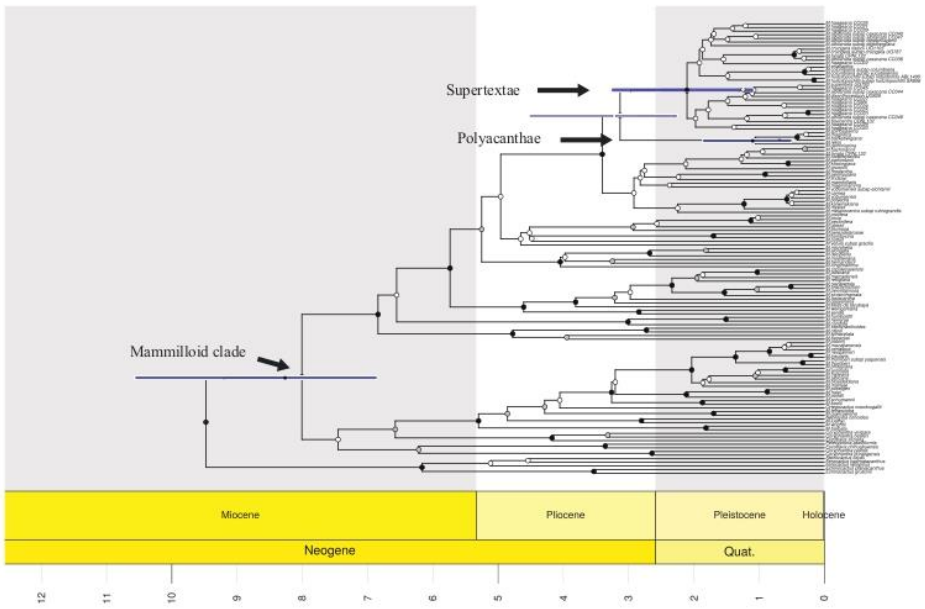

Figure 3. Divergence time estimated using BEAST based on concatenated matrix *rpl*16 and *psb*A-*trn*H. The circles on the nodes represent the PP supports: white < 0.75, gray < 0.95 and black ≥ 0.95. We show the mean divergence times (MDTs) and 95% highest posterior density (HPD) intervals (blue line) to the Mammilloid clade (MDT = 8, HPD = 5.83–11.61), *Supertextae* (MDT = 2.1, HPD = 0.91–3.47), *Polyacanthae* (MDT = 1, HPD = 0.15–2.22), and the sister group relationship (MDT = 2.8, HPD = 1.46–4.73).

uncertain, and it was placed within *M.* ser. *Polyacanthae* due to the size of its flower (Bravo-Hollis and Sánchez-Mejorada 1991; Hunt et al. 2006). Remarkably, we show that *M. eriacantha* is nested within *M.* ser. *Supertextae*, as previously proposed by Reppenhagen (1992) and Lüthy (1995) based on the presence of extracellular crystals. Our results also show that *M.* ser. *Polyacanthae* is the sister series of *M.* ser. *Supertextae* and that they are part of the *Mammillaria* sect. *Subhydrochylus* (Hunt 1987). Within *M.* ser. *Supertextae*, phylogenetic relationships were not resolved; however, both *M. haageana* and *M. albilanata* appeared in more than one clade.

Intron *rpl*16 was demonstrated to be the most variable and informative marker compared to the intergenic spacer *psb*A-*trn*H, which is consistent with previous studies in Cactaceae (Korotkova et al. 2010; Vázquez-Sánchez et al. 2013; Cruz et al. 2016). The molecular characteristics that define the monophyly of *M.* ser. *Supertextae* and *M.* ser. *Polyacanthae* were found in *psb*A-*trn*H, while a deletion in *rpl*16 supports the sister relationship. Although the deletion in *rpl*16 is partial in *M.* ser. *Supertextae* and *M.* ser. *Polyacanthae*, a total deletion has been reported in members of *M.* ser. *Stylothelae* (Butterworth et al. 2007), showing that deletions in *rpl*16 in *Mammillaria* may be a strong characteristic for the identification of infrageneric groups. It remains to be defined whether other polymorphisms, such as the inversion of another chloroplast gene,

trnF-GAA, are also diagnostic of these clades within and between series, as recently reported for *M. crucigera*, *M. huitzilopochtli*, and *M. supertexta* (Solórzano et al. 2019).

The Mammilloid clade originated approximately 8.62 Mya (95% HPD = 5.83–12.56; Hernández-Hernández et al. 2014), and within *Mammillaria*, it is likely that *M.* ser. *Supertextae* is a recently divergent group that originated in the Neogene-Quaternary transition approximately 2.1 Mya (95% HPD = 0.91–3.47). In some geographic regions, the *M.* ser. *Supertextae* species have undergone tectonic, erosive, alluvial and volcanic changes for millions of years; during the Pleistocene, these processes continued, giving rise to the current geomorphology (Siebert and Carrasco-Núñez 2002; Medina-Sánchez et al. 2020). Paleontological and molecular evidence suggests that glacial climate cycles that occurred during the last 2.5 Mya affected the distribution, diversity, and genetic structure of plant and animal populations (Gámez et al. 2014; Scheinvar et al. 2016; Cornejo-Romero et al. 2017). The hypotheses suggest that during the Pleistocene, these species sought refuge during adverse environmental conditions and expanded again when conditions improved (Scheinvar et al. 2016). Our results show that within *M.* ser. *Supertextae*, four clades are formed, two of which have distinctive climatic and topographic characteristics: Clade S1 (*M. haageana*, *M. albialanta*, *M. dixanthocentron* and *M. supertexta*), with species that are distributed in warm zones mainly at altitudes ranging from 447 to 2318 meters in thorn and tropical deciduous forests; and Clade S2 (*M. haageana*, *M. albilanata* and *M. flavicentra*), with species that are distributed in temperate zones at altitudes that range mainly from 1285 to 2518 meters in pine-oak forests. The environmental, geological and topographic differences between closely related species produced during climatic changes suggest differential selection pressures and local adaptation, which could have driven the speciation process (Mastretta-Yanes et al. 2015; Aquino et al. 2021), as has been suggested for *Mammillaria pectinifera* (Cornejo-Romero et al. 2014), *Cephalocereus columna-trajani* (Cornejo-Romero et al. 2017) and the genus *Epithelantha* (Aquino et al. 2021). *Mammillaria haageana* and *M. albilanata* represent a complex that extends widely in southern Mexico. Our results show that the infrageneric units of *M. haageana* and *M. albilanata* can be considered independent evolutionary units. It is possible that the variation in these inhabited environments promotes divergence in these taxa, although more in-depth studies are needed to understand and corroborate the hypotheses raised here.

The chloroplast marker sequences that we used (*rpl*16 and *psb*A-*trn*H) were not sufficient to establish the relationships among the taxa within *M.* ser. *Supertextae*. This was not surprising, as chloroplast markers have been used to resolve relationships at the species level; however, they have limitations when the species are closely related (Yan et al. 2018). This is because recently diversified groups may generate complicated genetic patterns, such as incomplete lineage sorting and hybridizations and/or introgressions (Li et al. 2016; Goetze et al. 2017), which may be true for *M.* ser. *Supertextae*. In other taxa (e.g., *Petalidium* Nees, Acanthaceae; Tripp et al. 2017), these problems have been addressed using multiple-locus methods to infer genetic trees, although they require nuclear markers that are not linked with levels of sequence variation according to phy-

logenetic questions (Eaton and Ree 2013). To date, no effective nuclear markers have been developed for Cactaceae, and existing markers provide fewer informative sites than chloroplast markers (Cruz et al. 2016). Recently, proposals for nuclear markers have been generated through mining strategies to test hybridization in *Opuntia* species (Granados-Aguilar et al. 2020). Currently, several methodologies have been designed that allow biological questions to be answered using a reduced representation of the genome (Anderson et al. 2017; Choquet et al. 2019; David et al. 2019). This confers advantages when working with nonmodel species such as *M.* ser. *Supertextae*, since genomic markers can be genotyped in many individuals at low cost, and in most cases, it is not necessary to have a priori information such as a reference genome (da Fonseca et al. 2016).

The taxonomic proposals of *M.* ser. *Supertextae* species have been mainly based on interpretations according to the author's experience (Table 1), and their relationships have not been specifically tested under phylogenetic methods. Our methods are intended to be systematic (explicit criteria) and reproducible. Under this scheme, the results obtained show that within *M. haageana* and *M. albilanata*, there are genetic differences possibly indicating that these species comprise more than one taxonomic entity. Nevertheless, when distinguishing between *M. haageana* and *M. albilanata*, the task becomes difficult because both share similar distributions and habitats (mainly in southern Mexico; Fig. 2), and the morphological differences have not been well defined (Arias et al. 2012). A possible hypothesis is that there could still be gene flow between these taxa, and we might be witnessing an ongoing speciation process.

Conclusion

By including most of the species recognized by Hunt et al. (2006), our results show that *M.* ser. *Supertextae* is monophyletic, and we corroborate that *M. eriacantha* is part of the series as previously proposed. We find that *M.* ser. *Polyacanthae* is the sister series, as proposed by (Hunt 2011). The results also showed that *M.* ser. *Supertextae* is a recently diverged group.

This is a first approximation to understand the evolutionary processes within *M.* ser. *Supertextae*. Future work should test sequencing techniques that allow genomic markers to be genotyped in many individuals since it is possible that conflicts in the phylogeny were the result of reticulate evolution. Furthermore, disentangling this problem will require a comprehensive pool of approaches regarding morphology and ecology, opening an avenue to develop *M.* ser. *Supertextae* as a model for studying complex evolutionary processes in *Mammillaria*.

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References

Anderson EF (2001) The Cactus Family. Timber Press, Oregon, 776 pp.

Anderson BM, Thiele KR, Krauss SL, Barrett MD (2017) Genotyping-by-sequencing in a species complex of Australian hummock grasses (*Triodia*): Methodological insights and phylogenetic resolution. PLoS ONE 12(1): e0171053. https://doi.org/10.1371/journal.pone.0171053

Aquino D, Moreno-Letelier A, González-Botello MA, Arias S (2021) The importance of environmental conditions in maintaining lineage identity in *Epithelantha* (Cactaceae). Ecology and Evolution 00: 1–235. https://doi.org/10.1002/ece3.7347

Arias S, Guzmán-López L, Guzmán-Cruz U, Vázquez-Benítez B (2012) Cactaceae. In: Medina R, Sánchez G, García A, Arias S (Eds) Flora del Valle de Tehuacán-Cuicatlán. Universidad Nacional Autónoma de México 95: 1–235.

Backeberg C (1961) Die Cactaceae (Vol. 5). VEB Gustav Fischer Verlag, Jena, 911 pp.

Barrios D, González-Torres LR, Arias S, Majure LC (2020) Phylogeny and taxonomy of the Antillean endemic genus *Leptocereus* (Cactaceae) inferred from chloroplast markers and morphological evidence. Plant Systematics and Evolution 306(3): e63. https://doi.org/10.1007/s00606-020-01693-5

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, Maio ND, Matschiner M, Mendes FK, Müller NF, Ogilvie HA, du Plessis L, Popinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu C-H, Xie D, Zhang C, Stadler T, Drummond AJ (2019) BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Computational Biology 15(4): e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bravo-Hollis H, Sánchez-Mejorada H (1991) Las Cactáceas de México (Vol. 3). Universidad Nacional Autónoma de México, 643 pp.

Breslin P, Wojciechowski MF, Majure LM (2021) Molecular phylogeny of the Mammilloid clade (Cactaceae) resolves the monophyly of *Mammillaria*. Taxon tax.12451. https://doi.org/10.1002/tax.12451

Butterworth C, Wallace RS (2004) Phylogenetic studies of *Mammillaria* (Cactaceae) insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. American Journal of Botany 91(7): 1086–1098. https://doi.org/10.3732/ajb.91.7.1086

Butterworth C, Cota-Sánchez H, Wallace RS (2002) Molecular systematics of tribe Cacteae (Cactaceae: Cactaceae): A phylogeny based on *rpl16* intron sequence variation. Systematic Botany 27(2): 257–270.

Butterworth CA, Butterworth KM, Maurice WAF, Maurice BF (2007) A localized loss of the chloroplast *rpl16* intron in *Mammillaria* series *Stylothelae* (Cactaceae) delineates members of the *M. crinita* group. Bradleya 25(25): 187–192. https://doi.org/10.25223/brad.n25.2007.a13

Choquet M, Smolina I, Dhanasiri AKS, Blanco-Bercial L, Kopp M, Jueterbock A, Sundaram AYM, Hoarau G (2019) Towards population genomics in non-model species with large genomes: A case study of the marine zooplankton *Calanus finmarchicus*. Royal Society Open Science 6(2): e180608. https://doi.org/10.1098/rsos.180608

Cornejo-Romero A, Medina-Sánchez J, Hernández-Hernández T, Rendón-Aguilar B, Valverde PL, Zavala-Hurtado A, Rivas-Arancibia SP, Pérez-Hernández MA, López-Ortega G, Jiménez-Sierra C, Vargas-Mendoza CF (2014) Quaternary origin and genetic divergence of the endemic cactus *Mammillaria pectinifera* in a changing landscape in the Tehuacán Valley, Mexico. Genetics and Molecular Research 13(1): 73–88. https://doi.org/10.4238/2014.January.8.6

Cornejo-Romero A, Vargas-Mendoza CF, Aguilar-Martínez GF, Medina-Sánchez J, Rendón-Aguilar B, Valverde PL, Zavala-Hurtado JA, Serrato A, Rivas-Arancibia S, Pérez-Hernández MA, López-Ortega G, Jiménez-Sierra C (2017) Alternative glacial-interglacial refugia demographic hypotheses tested on *Cephalocereus columna-trajani* (Cactaceae) in the intertropical Mexican drylands. PLoS ONE 12(4): e0175905. https://doi.org/10.1371/journal.pone.0175905

Cruz MÁ, Arias S, Terrazas T (2016) Molecular phylogeny and taxonomy of the genus *Disocactus* (Cactaceae), based on the DNA sequences of six chloroplast markers. Willdenowia 46(1): 145–164. https://doi.org/10.3372/wi.46.46112

da Fonseca RR, Albrechtsen A, Themudo GE, Ramos-Madrigal J, Sibbesen JA, Maretty L, Zepeda-Mendoza ML, Campos PF, Heller R, Pereira RJ (2016) Next-generation biology: Sequencing and data analysis approaches for non-model organisms. Marine Genomics 30: 3–13. https://doi.org/10.1016/j.margen.2016.04.012

David KT, Wilson AE, Halanych KM (2019) Sequencing disparity in the genomic era. Molecular Biology and Evolution 36(8): 1624–1627. https://doi.org/10.1093/molbev/msz117

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. PLoS Biology 4(5): e88. https://doi.org/10.1371/journal.pbio.0040088

Eaton DAR, Ree RH (2013) Inferring phylogeny and introgression using RADseq data: An example from flowering plants (*Pedicularis*: Orobanchaceae). Systematic Biology 62(5): 689–706. https://doi.org/10.1093/sysbio/syt032

Fehlberg SD, Allen JM, Church K (2013) A novel method of genomic DNA extraction for Cactaceae. Applications in Plant Sciences 1(3): apps.1200013. https://doi.org/10.3732/apps.1200013

Gámez N, Escalante T, Espinosa D, Eguiarte LE, Morrone JJ (2014) Temporal dynamics of areas of endemism under climate change: A case study of Mexican *Bursera* (Burseraceae). Journal of Biogeography 41(5): 871–881. https://doi.org/10.1111/jbi.12249

Goetze M, Zanella CM, Palma-Silva C, Büttow MV, Bered F (2017) Incomplete lineage sorting and hybridization in the evolutionary history of closely related, endemic yellow-flowered

Aechmea species of subgenus *Ortgiesia* (Bromeliaceae). American Journal of Botany 104(7): 1073–1087. https://doi.org/10.3732/ajb.1700103

Granados Aguilar XC, Arias S, Granados Mendoza C, Cervantes CR, Montes JR (2020) Unraveling reticulate evolution in *Opuntia* (Cactaceae) from southern Mexico. Frontiers of Plant Science 11: e606809. https://doi.org/10.3389/fpls.2020.606809

Hernández-Hernández T, Hernández HM, De-Nova JA, Puente R, Eguiarte LE, Magallón S (2011) Phylogenetic relationships and evolution of growth form in Cactaceae (Caryophyllales, Eudicotyledoneae). American Journal of Botany 98(1): 44–61. https://doi.org/10.3732/ajb.1000129

Hernández-Hernández T, Brown JW, Schlumpberger BO, Eguiarte LE, Magallón S (2014) Beyond aridification: Multiple explanations for the elevated diversification of cacti in the New World succulent biome. The New Phytologist 202(4): 1382–1397. https://doi.org/10.1111/nph.12752

Hinojosa-Alvarez S, Arias S, Ferrand S, Purugganan MD, Rozas J, Rosas U, Wegier A (2020) The chloroplast genome of the pincushion cactus *Mammilllaria haageana* subsp. *san-angelensis*, a Mexican endangered species. Mitochondrial DNA. Part B, Resources 5(3): 2038–2039. https://doi.org/10.1080/23802359.2020.1757523

Hunt DR (1987) A new review of *Mammillaria* names. British Cactus and Succulent Society, Oxford, 128 pp.

Hunt DR (2011) Focus on the *Supertextae*. Huitzilopochtlia 6: 47–51.

Hunt DR, Taylor NP, Glass H (2006) The new Cactus Lexicon: Descriptions and Illustrations of the Cactus Family (Vol. 1). DH Books, Milborne Port, 375 pp.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Korotkova N, Zabel L, Quandt D, Barthlott W (2010) A phylogenetic analysis of *Pfeiffera* and the reinstatement of *Lymanbensonia* as an independently evolved lineage of epiphytic Cactaceae within a new tribe Lymanbensonieae. Willdenowia 40(2): 151–172. https://doi.org/10.3372/wi.40.40201

Korotkova N, Borsch T, Arias S (2017) A phylogenetic framework for the Hylocereeae (Cactaceae) and implications for the circumscription of the genera. Phytotaxa 327(1): 1–46. https://doi.org/10.11646/phytotaxa.327.1.1

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Systematic Biology 50(6): 913–925. https://doi.org/10.1080/106351501753462876

Li Q-Q, Zhou S-D, Huang D-Q, He X-J, Wei X-Q (2016) Molecular phylogeny, divergence time estimates and historical biogeography within one of the world's largest monocot genera. AoB Plants 8: plw041. https://doi.org/10.1093/aobpla/plw041

Lüthy JM (1995) Taxonomische untersuchung der gattung *Mammillaria* Haw. PhD Thesis, Universität Bern, Bern.

Maddison WP, Maddison DR (2019) Mesquite, version 3.6: A modular system for evolutionary analysis. University of Arizona, Tucson. http://www.mesquiteproject.org

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(9): 1586–1600. https://doi.org/10.1111/jbi.12546

Medina-Sánchez J, McLaren SJ, Ortega-Ramírez J, Valiente-Banuet A (2020) Tectono-stratigraphic basin evolution in the Tehuacán-Mixteca highlands, south western México. Heliyon 6(3): e03584. https://doi.org/10.1016/j.heliyon.2020.e03584

Müller J, Müller K, Neinhuis C, Quandt D (2019) PhyDE, version 0.9971: Phylogenetic data editor. http://www.phyde.de/download.html [Accessed on 20.03.2019]

Pilbeam J (1999) *Mammillaria*. Nuffield Press, Oxford, 376 pp.

R Core Team (2018) R: A language and environment for statistical computing. Foundation for Statistical Computing, Vienna. https://www.R-project.org/ [Accessed on 11.03.2019]

Rambaut A (2018) Tracer, version 1.7.1: MCMC trace analysis tool. University of Oxford. http://tree.bio.ed.ac.uk/software/tracer/ [Accessed on 02.05.2019]

Reppenhagen W (1992) Die gattung *Mammillaria*. Monographie (Vol. 2). Druckerei Steinhart. Titisee-Neustadt, Germany, 442 pp.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Sánchez D, Arias S, Terrazas T (2014) Phylogenetic relationships in *Echinocereus* (Cactaceae, Cactoideae). Systematic Botany 39(4): 1183–1196. https://doi.org/10.1600/036364414X683831

Scheinvar E, Gámez N, Castellanos-Morales G, Aguirre-Planter E, Eguiarte LE (2016) Neogene and Pleistocene history of *Agave lechuguilla* in the Chihuahuan Desert. Journal of Biogeography 44(2): 322–334. https://doi.org/10.1111/jbi.12851

Schwery O, O'Meara BC (2016) MonoPhy: A simple R package to find and visualize monophyly issues. PeerJ. Computer Science 2: e56. https://doi.org/10.7717/peerj-cs.56

Sela I, Ashkenazy H, Katoh K, Pupko T (2015) GUIDANCE2: Accurate detection of unreliable alignment regions accounting for the uncertainty of multiple parameters. Nucleic Acids Research 43(W1): W7–W14. https://doi.org/10.1093/nar/gkv318

Siebert L, Carrasco-Núñez G (2002) Late-Pleistocene to precolumbian behind-the-arc mafic volcanism in the eastern Mexican Volcanic Belt; implications for future hazards. Journal of Volcanology and Geothermal Research 115(1): 179–205. https://doi.org/10.1016/S0377-0273(01)00316-X

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49(2): 369–381. https://doi.org/10.1093/sysbio/49.2.369

Solórzano S, Chincoya DA, Sanchez-Flores A, Estrada K, Díaz-Velásquez CE, González-Rodríguez A, Vaca-Paniagua F, Dávila P, Arias S (2019) De novo assembly discovered novel structures in genome of plastids and revealed divergent inverted repeats in *Mammillaria* (Cactaceae, Caryophyllales). Plants 8(10): e392. https://doi.org/10.3390/plants8100392

Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Tripp EA, Tsai Y-HE, Zhuang Y, Dexter KG (2017) RADseq dataset with 90% missing data fully resolves recent radiation of *Petalidium* (Acanthaceae) in the ultra-arid deserts of Namibia. Ecology and Evolution 7(19): 7920–7936. https://doi.org/10.1002/ece3.3274

Vázquez-Sánchez M, Terrazas T, Arias S, Ochoterena H (2013) Molecular phylogeny, origin and taxonomic implications of the tribe Cacteae (Cactaceae). Systematics and Biodiversity 11(1): 103–116. https://doi.org/10.1080/14772000.2013.775191

Yan M, Xiong Y, Liu R, Deng M, Song J (2018) The application and limitation of universal chloroplast markers in discriminating east Asian evergreen oaks. Frontiers of Plant Science 9: e569. https://doi.org/10.3389/fpls.2018.00569

Appendix 1

List of GenBank accession numbers and vouchers for newly published sequences for all sequences used in the analyses. Data are arranged in the following order: taxon name in bold (in alphabetical order); voucher data (country, estate, locality, collecting date, collector, collecting number, *rpl*16, *psb*A-*trn*H).

Coryphantha durangensis Britton & Rose, HM041405, AY545338; ***C. hesteri*** Y. Wright, AY545234, AY545342; ***C. pallida*** Britton & Rose, AY545232, AY545340; ***C. vivipara*** Britton & Rose, KC196809, KC196847; ***Escobaria chihuahuensis*** Britton & Rose, AY545233, AY545341; ***E. zilziana*** (Boed.) Backeb., AY545236, AY545344; ***Ferocactus haematacanthus*** (Salm-Dyck) Borg ex Backeb., HM041431, MH129870; ***F. latispinus*** Britton & Rose, HM041432, MH129871; ***Mammillaria albicans*** A. Berger, AY545238, AY545346; ***Mammillaria albilanata*** subsp. ***albilanata*** Backeb., México, Guerrero, 10 km east of Huitzuco, 31 October 2018, Cristian Cervantes, CC047, MT995687, MT995715; ***Mammillaria albilanata*** subsp. ***oaxacana*** D. R. Hunt, México, Oaxaca, Santiago Huauclilla-Santa Catarina Tlaxila road near the river, 1 October 2017, Cristian Cervantes, CC036, MT995698, MT995726; México, Oaxaca, 1.1 km from the junction of highway 135 Teotitlán de Flores Magón-San Fransisco Telixtlahuaca heading to San Sebastián Sedas, 2 October 2017, Cristian Cervantes, CC040, MT995689, MT995717; México, Oaxaca, Santa Maria Jalapa de Marquéz towards the microwave antenna, 22 June 2018, Cristian Cervantes, CC044, MT995678, MT995706; México, Oaxaca, 3.8 Km from Santo Domingo Tonalá to San Agustín Atenango, 24 June 2018, Cristian Cervantes, CC046, MT995677, MT995705; ***Mammillaria albilanata*** subsp. ***reppenhagenii*** (D. R. Hunt) D. R. Hunt, México, Jalisco, Tolimán, -, -, Hilda Arreola, s.n., MT995702, MT995730; ***Mammillaria albilanata*** subsp. ***tegelbergiana*** (H. E. Gates ex G. E. Linds.) D. R. Hunt, México, Chiapas, Comitán de Dominguez – 3.1 km from Comitán, 19 January 2007, Salvador Arias, SA1630, -, -; ***M. armillata*** K. Brandegee,

AY545240, AY545349; ***M. bachmannii*** Boed., AY545241, AY545350; ***M. backebergiana*** Franc. G. Buchenau, AY545242, AY545351; ***M. barbata*** Engelm, AY545243, AY545352; ***M. beneckei*** Ehrenb., AY545244, AY545353; ***M. blossfeldiana*** Boed., AY545245, AY545354; ***M. bombycina*** Quehl & Quehl, AY545246, AY545356; ***M. boolii*** G. E. Linds., AY545247, AY545357; ***M. brachytrichion*** Lüthy, AY545248, AY545358; ***M. cadereytensis*** R. T. Craig, AY545249, AY545359; ***M. candida*** Scheidw., AY545250, AY545360; ***M. carnea*** Zucc. ex Pfeiff., HM041449, AY545363; ***M. cerralboa*** Orcutt, AY545254, AY545364; ***M. columbiana*** subsp. ***columbiana*** Salm-Dyck, Venezuela, Mérida, -, 2012, Teresa Terrazas, TT957, -, -; ***M. columbiana*** subsp. ***yucatanensis*** (Britton & Rose) D. R. Hunt, México, Yucatán, Municipio Dzemul, 2004, CICY, G. Carnevali & I. M. Ramírez, 7449, MT995701, MT995729; ***M. crucigera*** subsp. ***crucigera*** Mart., México, Oaxaca, 2 km from San Antonio Nahuahautipan, 9 August 1990, Ulises Guzmán, UG787, MT995697, MT995725; ***M. crucigera*** subsp. ***tlalocii*** (Repp.) D. R. Hunt, México, Oaxaca, 8 km from the rural road Santa María Tecomavaca-Santa María Ixcatlán, 9 November 1994, Ulises Guzmán, UG1103, MT995696, MT995724; ***M. decipiens*** Scheidw., AY545255, AY545369; ***M. dixanthocentron*** Backeb. ex Mottram, México, Oaxaca, km 107 highway 135 between Tecomavaca and Cuicatlán, 24 October 1990, Ulises Guzmán, UG828, MT995679, MT995707; ***M. elongate*** DC., AY545258, AY545373; ***M. eriacantha*** Link & Otto ex Pfeiff., México, Veracruz, km 305 of the Xalapa-Veracruz highway between Plan del Río and Cerro Gordo, 21 January 2012, Salvador Arias, SA2169, MT995700, MT995728; ***M. flavicentra*** Backeb. ex Mottram, México, Oaxaca, Teotitlan de Flores Magón 10 Km on the way to Huautla, 13 July 1995, Patricia Novoa, CPNL132, MT995688, MT995716; ***M. formosa*** Scheidw., AY545259, AY545376; ***M. fraileana*** (Britton & Rose) Boed., AY545260, AY545377; ***M. gasseriana*** Boed., AY545261, AY545378; ***M. geminispina*** DC., AY545262, AY545379; ***M. glassii*** R. A. Foster, AY545263, AY545380; ***M. grusonii*** Runge, AY545266, AY545383; ***M. guelzowiana*** Werderm., AY545267, AY545384; ***M. haageana*** Pfeiff., México, Puebla, 1 km over the gap from the junction with the Puebla-Xalapa highway, 15 May 2017, Cristian Cervantes, CC020, MT995686, MT995714; México, Veracruz, 7 km from Perote, 15 May 2017, Cristian Cervantes, CC021, MT995693, MT995721; México, Puebla, 1.5 km south of Esperanza, 16 May 2017, Cristian Cervantes, CC022, MT995692, MT995720; México, Puebla, 7 km west of Tehuacan, 16 May 2017, Cristian Cervantes, CC023, MT995675, MT995703; México, Puebla, near the Helia Bravo Botanical Garden, 16 May 2017, Cristian Cervantes, CC024, MT995680, MT995708; México, Puebla, 9.5 km from junction 125 to Huajolotitlán towards Los Reyes Metzontla, 16 May 2017, Cristian Cervantes, CC025, MT995676, MT995704; México, Oaxaca, km 59.5 Highway 125 then join the road to San Sebastián Frontera, 17 May 2017, Cristian Cervantes, CC027, MT995683, MT995711; México, Veracruz, In La Organera area near the Tecamalucan town, 29 September 2017, Cristian Cervantes, CC029, MT995685, MT995713; México, Oaxaca, 26 Km on the Huauclilla-El Parian dirt road, 1 October 2017, Cristian Cervantes, CC035, MT995690, MT995718; México, Oaxaca, 11.5 km from

Magdalena Jaltepec heading to Santiago Tilangongo, 1 Octuber 2017, Cristian Cervantes, CC039, MT995691, MT995719; México, Oaxaca, 7.5 km from Corral de Piedra to Santa Maria Tutla, 22 June 2018, Cristian Cervantes, CC045, MT995684, MT995712; México, CDMX, in the Reserva Ecológica del Pedregal de San Ángel (REPSA), 6 December 2018, -, -, MT995681, MT995709; ***M. halei*** K. Brandegee, AY545269, AY545386; ***M. hernandezii*** Glass & R. A. Foster, AY545270, AY545387; ***M. herrerae*** Werderm., AY545271, AY545388; ***M. huitzilopochtli*** subsp. ***huitzilopochtli*** D. R. Hunt, México, Oaxaca, 7 km northwest of San Juan Bautista Cuicatlán, 5 August 1990, Salvador Arias, SA856, MT995694, MT995722; ***M. huitzilopochtli*** subsp. ***niduliformis*** (A.B.Lau) Pilbeam, México, Oaxaca, Río Santo Domingo up the junction Rio Salado, 12 March 1983, A. B. Lau, ABL1495, MT995695, MT995723; ***M. humboldtii*** Ehrenb., AY545273, AY545390; ***M. insularis*** H. E. Gates ex Shurly, AY545275, AY545392; ***M. jaliscana*** Boed., AY545276, AY545393; ***M. karwinskiana*** Mart., AY545277, AY545394; ***M. klissingiana*** Boed., AY545278, AY545395; ***M. lanata*** Orcutt, México, Puebla, Rio Hondo cerca del puente Calapa autopista Tehuacán-Oaxaca, 19 November 1994, Patricia Novoa, CPNL122, MT995699, MT995727; ***M. lasiacantha*** Engelm., AY545279, AY545396; ***M. lindsayi*** R. T. Craig, AY545280, AY545398; ***M. longimamma*** DC., AY545281, AY545399; ***M. luethyi*** G. S. Hinton, AY545282, AY545400; ***M. magnifica*** Franc. G. Buchenau, AY545283, AY545401; ***M. magnimamma*** Haw., AY545284, AY545402; ***M. mainiae*** K. Brandegee, AY545285, AY545403; ***M. mammillaris*** H. Karst., AY545286, AY545404; ***M. mazatlanensis*** K. Schum., AY545287, AY545407; ***M. melanocentra*** subsp. ***rubrograndis*** (Repp. & A. B. Lau) D. R. Hunt, AY545288, AY545408; ***M. mercadensis*** Patoni, AY545289, AY545410; ***M. microhelia*** Werderm., AY545291, AY545411; ***M. moelleriana*** Boed., AY545292, AY545412; ***M. mystax*** Mart., AY545294, AY545414; ***M. nazasensis*** (Glass & R. A. Foster) Repp., AY545295, AY545416; ***M. neopalmeri*** R. T. Craig, AY545296, AY545417; ***M. oteroi*** Glass & R. A. Foster, AY545297, AY545418; ***M. parkinsonii*** Ehrenb., AY545298, AY545419; ***M. patonii*** Werderm. in Backeb., AY545299, AY545420; ***M. pectinifera*** F. A. C. Weber, AY545300, AY545421; ***M. peninsularis*** Orcutt, AY545301, AY545422; ***M. pennispinosa*** Krainz, AY545302, AY545423; ***M. perezdelarosae*** Bravo & Scheinvar, AY545303, AY545424; ***M. phitauiana*** Werderm. in Backeb., AY545305, AY545426; ***M. picta*** Meinsh., HM041452, AY545427; ***M. plumosa*** F. A. C. Weber in Bois, AY545307, AY545428; ***M. polyedra*** Mart., AY545308, AY545429; ***M. pondii*** Greene, HM041399, AY545431; ***M. poselgeri*** Hildm., HM041400, AY545432; ***M. pottsii*** Scheer ex Salm-Dyck, AY545312, AY545433; ***M. prolifera*** (Mill.) Haw., AY545313, AY545434; ***M. rekoi*** Vaupel, AY545314, AY545435; ***M. rettigiana*** Boed., AY545315, AY545436; ***M. rhodantha*** Link & Otto, AY545316, AY545437; ***M. schumannii*** Hildm., AY545317, AY545438; ***M. senilis*** Lodd. ex Salm-Dyck, AY545318, AY545440; ***M. sinistrohamata*** Boed., AY545319, AY545441; ***M. sphacelate*** Mart., AY545320, AY545442; ***M. spinosissima*** Lem., AY545321, AY545443; ***M. stella-detacubaya*** Heese, AY545322, AY545444; ***M. supertexta*** Mart. ex Pfeiff., México, Oaxaca, 0.5 km east of San Juan de los Cues, 9 August 1990, Ulises Guzmán, UG793,

MT995682, MT995710; ***M. tetrancistra*** Engelm., KC196805, KC196840; ***M. thornberi*** subsp. ***thornberi*** Orcutt, AY545324, AY545447; ***M. thornberi*** subsp. ***yaquensis*** (R. T. Craig) D. R. Hunt, AY545325, AY545448; ***M. vetula*** subsp. ***gracilis*** (Pfeiff.) D. R. Hunt, AY545327, AY545449; ***M. voburnensis*** subsp. ***voburnensis*** Scheer, AY545328, AY545450; ***M. voburnensis*** subsp. ***eichlamii*** (Quehl) D. R. Hunt, AY545329, AY545451; ***M. weingartiana*** Boed., AY545330, AY545452; ***M. wrightii*** Engelm., AY545331, AY545454; ***M. zacatecasensis*** Shurly, AY545332, AY545455; ***M. zephyranthoides*** Scheidw., AY545333, AY545457; ***Neolloydia conoidea*** Britton & Rose, HM041462, AY545458; ***Ortegocactus macdougallii*** Alexander, HM041484, AY545459; ***Pelecyphora aselliformis*** C. Ehrenb., AY545336, AY545460; ***Stenocactus lloydii*** A. Berger, AY545337, AY545461.

Capítulo II

Phylogenetic discordance and integrative species delimitation in the *Mammillaria haageana* species complex (Cactaceae)

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Phylogenetic discordance and integrative species delimitation in the *Mammillaria haageana* species complex (Cactaceae)

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ABSTRACT

Species complexes consist of very close phylogenetic relatives, where morphological similarities make it difficult to distinguish between them using traditional taxonomic methods. Here, we focused on the long-standing challenge of species delimitation in the *Mammillaria haageana* complex, a group that presents great morphological diversity that makes its taxonomy a puzzle. Our work integrates genomic, morphological, and ecological data to establish the taxonomic limits in the *M. haageana* complex, and we also studied the evolutionary relationships with the remainder of the *M.* ser. *Supertextae* species. Our genetic analyses, as well as morphological and ecological evidence, led us to propose that the *M. haageana* complex is made up of six distinct entities (*M. acultzingensis*, *M. conspicua*, *M. haageana*, *M. lanigera*, *M. meissneri*, and *M. san-angelensis*), mainly as a result of ecological speciation. A recent taxonomic proposal considered these taxa as a single species; therefore, we propose their recognition at the species level. Our results also show a high level of incomplete lineage sorting rather than reticulation, which is especially likely in recently diverged species such as those comprising *M.* ser. *Supertextae*. The species hypotheses proposed here may be useful in future extinction risk assessments and conservation strategies.

1. Introduction

Establishing taxonomic boundaries between species can be challenging when lineages are closely related and/or have recently diversified (Perez et al., 2022; Salicini et al., 2011). Complex evolutionary histories can generate discordant genome-wide signals commonly produced by incomplete lineage sorting (ILS) and reticulation (e.g. hybridization and/or introgression; Goetze et al., 2017; Li et al., 2016). Recent and complex evolutionary histories can result in lineages that have large and overlapping morphological variation, making the distinction of taxonomic units a challenge for Systematic Biologists; therefore, these types of species complexes can be neglected by taxonomists. The development of massive sequencing platforms, particularly methods for reduced representation shotgun sequencing (Campbell et al., 2018), can be useful tools in phylogenetic inference and species delimitation. Genotyping-by-sequencing (GBS; Elshire et al., 2011) has been shown to be useful for resolving species complexes (Anderson et al., 2017), setting taxonomic limits (Hashemzadeh Segherloo et al., 2021; Pérez-Escobar et al., 2020), and evaluating genetic population structure (Otto et al., 2017). The genetic information obtained by GBS can be integrated together with multiple lines of evidence (e.g., morphology and ecology), improving the strategy to define the limits among species within complexes (Padial et al., 2010; Sturaro et al., 2018).

Cactaceae is a large family that includes approximately 150 genera and 1,851 species (Korotkova et al., 2021). *Mammillaria* is the most diverse genus in the family, with a variable number of species depending on the author; for example, Reppenhagen (1992) considered 320 species and, recently, Korotkova et al. (2021) recognized 143 species. A few studies have shown that *Mammillaria s.l.* is a polyphyletic group (Butterworth et al., 2002; Butterworth and Wallace, 2004). Nonetheless, Breslin et al. (2021) circumscribed *Mammillaria s.s.* to reflect a monophyletic group. This shows that *Mammillaria* taxonomy has been historically difficult because of the wide interspecific morphological

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variation (Callejas-Chavero et al., 2021; Rosas et al., 2022). Based on the morphology, three infrageneric categories have been proposed for *Mammillaria*: subgenus, section, and series. *Mammillaria* ser. *Supertextae* is one of the 16 series recognized within *Mammillaria* (Hunt et al., 2006), and the number of species that are included in *M.* ser. *Supertextae* may vary between 4 and 27, depending on the authors (Table 1; Cervantes et al., 2021). This genus has a wide distribution from western Mexico to Colombia and Venezuela (Pilbeam, 1999). *Mammillaria haageana* is a member of the *M.* ser. *Supertextae*. Similar to many other cactus species, it has a complex taxonomic history, resulting in many interpretations about its circumscription and nonproven intraspecific units of varieties or subspecies (Table 1). Some authors recognize six to seven *M. haageana* subspecies (Guzmán et al., 2003; Pilbeam, 1999), while others do not recognize intraspecific taxa and only suggest continuous and wide morphological intraspecific variation (Hunt et al., 2006). This latter simplified idea is currently adopted in the Cactaceae global synthesis (Korotkova et al., 2021). Some taxa within the complex, such as *M. haageana* subsp. *san-angelensis*, have been included in a risk category in the Mexican federal legislation on wild flora and fauna (NOM-059-SEMARNAT-2010; Semarnat, 2010), so the taxonomic uncertainty of the complex may present a great challenge for conservation driven by species because extinction risk assessments and conservation strategies can only be as reliable as their underlying taxonomy (Scherz et al., 2019).

Recently, our phylogenetic analyses showed that *M. haageana* is composed of two clades that have distinct climatic and topographic characteristics, suggesting that *M. haageana* could be composed of more than one species (see Fig. 1 in Cervantes et al., 2021). Within Clade S2, *M. haageana* shares distribution to the south of Oaxaca with *M. albilanata* subsp. *oaxacana*, where it is difficult to set taxonomic boundaries between the two taxa (Arias et al., 2012). Our analyses also showed that the *M. haageana* complex is a recently diversified lineage (2.1 mya; Cervantes et al., 2021) whose evolution and speciation are not well understood. Although traditional morphological and conventional molecular markers have traditionally been used for species delimitation in plants (Belton et al., 2014), animals (Schwarzfeld and Sperling, 2014) and fungi (Stielow et al., 2011), their usefulness decreases considerably when dealing with taxonomically challenging species complexes such as the *M. haageana* complex. In this context, the objective is to assess species boundaries using an integrative framework incorporating genomic data (GBS data analysis, phylogenetic network, and coalescent-based analyses), complex phenotypes (morphometric analysis) and environmental variables (ecological niche models). Based on our results, we propose an adjustment to the taxonomic classification of the clade to disentangle long-standing discussions about *M. haageana* delimitation, which is explained with evidence of the genomic, morphological and distributional variation of this puzzling cactus lineage.

Table 1
Circumscription changes in the *M. haageana* complex according to some taxonomic proposals.

Bravo-Hollis and Sánchez-Mejorada (1991)	Pilbeam (1999)	Reppenhagen (1992)	Guzmán et al. (2003)	Korotkova et al. (2021)
M. haageana *M. vaupelii* *M. collina* *M. donatii* *M. san-angelensis* *M. conspicua*	*M. haageana* subsp. *haageana* subsp. *conspicua* subsp. *elegans* subsp. *san-angelensis* subsp. *schmollii* subsp. *acultzingensis*	*M. haageana* *M. meissneri* *M. donatii* *M. conspicua* var. *vaupelii* *M. albidula* *M. elegans* var. *lupina* var. *longicaudata* var. *teyuca*	*M. haageana* subsp. *haageana* subsp. *acultzingensis* subsp. *conspicua* subsp. *elegans* subsp. *meissneri* subsp. *san-angelensis* subsp. *vaupelii*	*M. haageana*

2. Materials and methods

2.1. Taxon sampling, DNA extraction, and GBS library preparation

According to our previous work (Cervantes et al., 2021), *M. haageana* contains more than one independent evolutionary lineage. Samples of *M. haageana* were putatively identified (based on morphological and/or geographical characteristics) as *M. haageana*, *M. meissneri*, *M. conspicua*, *M. acultzingensis,* and *M. san-angelensis*. Samples were collected from May to October 2017 and June 2018 covering the *M. haageana* complex, including the distribution of *M. albilanata* subsp. *oaxacana*. A total of 26 localities were visited (Supplementary Table 1). Five living plants were collected per location and deposited in the Cactaceae Collection of Botanical Garden IB-UNAM. Other samples of the *M.* ser. *Supertextae* were included, mainly those from the Tehuacán-Cuicatlán Valley (Fig. 1; *M. supertexta*, *M. lanata*, *M. huitzilopochtli*, *M. dixanthocentron*, *M. crucigera*, and *M. flavicentra*).

Total DNA was extracted from 75 individuals (Supplementary Table 1), 72 of *M. ser. Supertextae* from the field and from the Cacti living collection at Botanical Garden IB-UNAM, and three more samples from species of *M. duoformis* (*M.* ser. *Polyacanthae*), species sister to *M.* ser. *Supertextae* (Cervantes et al., 2021), *M. magnimamma* (*M.* ser. *Mammillaria*), and *M. mystax* (*M.* ser. *Polyedrae*); these last three samples were used as an outgroup. The stem tissue was dried in silica gel for 24 hrs and stored at -80 °C before DNA extraction. Then, 40 mg of the dried tissue was ground using TissueLyser II (Qiagen, Venlo, Netherlands) at 29 rpm for 25 min two times. DNA extraction was performed using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The elution volume was 35 µl twice using Milli-Q water. Sample quality assessment was performed with a Nanodrop 2000 (Nanodrop, Wilmington, Delaware, USA). DNA quantification was performed using Qubit® v. 2.0 (Invitrogen, Carlsbad, California, USA). GBS sequencing was performed at the Biotechnology Center, University of Wisconsin-Madison in paired-end mode using the enzymes *NsiI* (5 '… ATGC ▽ T… 3') and *MspI* (5 '… C ▽ CGG… 3).

2.2. GBS data analysis

The raw reads (Supplementary Fig. 1) were treated with ipyrad v. 0.7.30 (Eaton et al., 2020). Sequences were filtered for adapters/primers and cut, eliminating the first 10 bp from the 130 positions where low quality was observed. In order to avoid introducing errors in the dataset or clustering paralogous loci (Mastretta-Yanes et al., 2015; McCartney-Melstad et al., 2019), we tested different minimum coverage values (mindepth_statistical), different cluster threshold values (clust_threshold) and the minimum number of samples (min_samples_locus; Supplementary Table 2). The results obtained during parameter optimization were evaluated using the following four metrics: number of single nucleotide polymorphisms (SNPs), number of loci, heterozygosity, genetic distance, and phylogenetic resolution (Supplementary Fig. 2). Removing distant species should increase the amount of data recovered and the accuracy of the analyses (Pante et al., 2015); therefore, we performed assembly on a dataset containing only samples from the *M. haageana* complex: *M. haageana*, *M. meissneri*, *M. conspicua*, *M. acultzingensis,* and *M. san-angelensis*; *M. albilanata* subsp. *albilanata*, *M. albilanata* subsp. *oaxacana*, *M. supertexta*, and *M. huitzilopochtli* were included as outgroups (Supplementary Table 3). The above dataset was used for coalescence-based analyses and species delimitation analyses. A total of 18 runs were performed in ipyrad (Supplementary Table 2–3).

2.3. Population structure analysis and phylogenetic network

To study the population structure, data filtering was performed using VCFtools v. 0.1.17 (Danecek et al., 2011), preserving only the genotypes

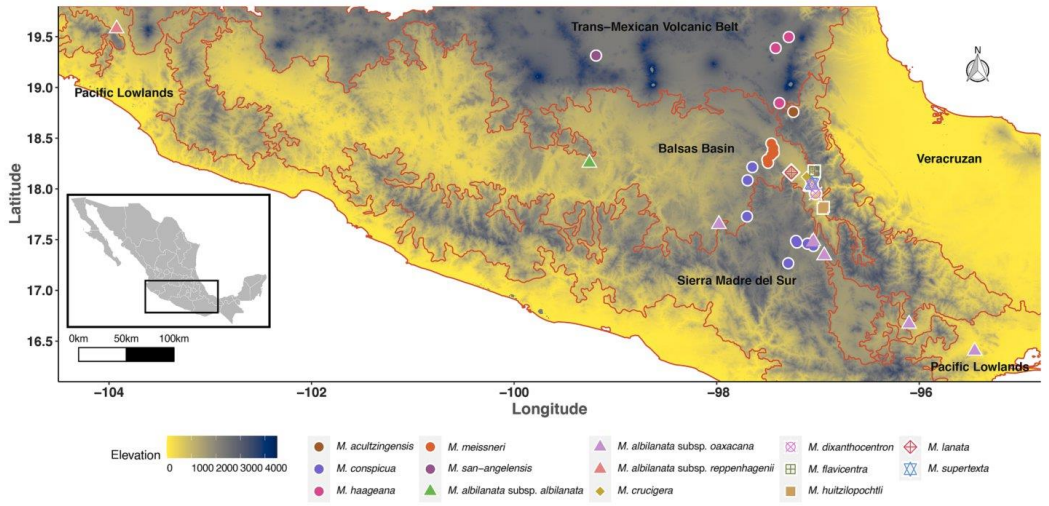

Fig. 1. Geographic distribution of samples used in this study. The red divisions on the map correspond to the biogeographic provinces according to Morrone et al. (2017). Yellow and blue show the areas with the lowest and highest elevations, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that are present in at least 70% of the individuals and had an allele frequency (MAF) ≦ 0.1. To explore the genetic structure, we used the package adegenet v. 2.1.3 (Jombart and Ahmed, 2011). A principal component analysis (PCA) was performed, and a discriminant analysis of principal components (DAPC) was used to determine the number of clusters. The function "find.clusters" from adegenet was used to find the number of genetic clusters (K) in the nonrelated SNP dataset. For the K values, we used the Bayesian Information Criterion (BIC) method. A cross validation was performed using the "xvalDapc" function to establish the number of principal components (PCs) in the analysis. Before the phylogenetic network was obtained, genetic distances were calculated as a pairwise proportion of alleles that were not identical by state (pairwise heterozygosity) using the function "ibs.dist" in the package snpStats v. 1.40.0. (Solé et al., 2006). The Neighbor-Net method using SplitsTree4 v. 4.18.1 (Huson and Bryant, 2006) was used to create the phylogenetic network.

2.4. Phylogenetic analysis

Maximum likelihood (ML) and Bayesian inference (BI) analyses were performed using the concatenated matrix. The ML analysis was performed using RAxML v. 8.2.12 (Stamatakis, 2014), using a fast bootstrap (BS) with 1,000 replicates and the substitution model GTR+GAMMA. The BI analysis was performed using ExaBayes v. 1.5.1 (Aberer et al., 2014). The analysis consisted of four independent Markov Chain Monte Carlo (MCMC) runs with two chains in parallel for 1.5 million generations. The default parameters and the substitution model GTR+GAMMA were used. The MCMC output was visualize with Tracer v. 1.7.1 (Rambaut et al., 2018). Most consensus trees were calculated using ExaBayes after 25% burn-in.

2.5. Coalescent-based analyses

A species tree was inferred under a multispecies coalescent model in SVDquartets (Chifman and Kubatko, 2014) implemented in PAUP* v. 5.0 (Swofford, 2002). For this method, we used a concatenated alignment with 830 nuclear loci (Supplementary Table 3) in the format NEXUS. A maximum of 1,000,000 randomly chosen quartets were estimated, and we performed bootstrapping using 1,000 replicates. Additionally, we inferred a species tree in ASTRAL-III v. 5.7.3 (Mirarab and Warnow, 2015; Zhang et al., 2018) from the 584 best trees. Nuclear genes were individually aligned in MAFFT v. 7.0 (Katoh and Standley, 2013), and the trees were reconstructed in RAxML (Stamatakis, 2014) under the GTR model with the gamma parameter (G) and 1,000 bootstrap searches. We concatenated the best trees as input files to ASTRAL. We estimated the local posterior probability (LPP) of the branches with the -t flag and parameter 3. Coalescent units were examined through branch lengths to quantify ILS. We also estimated quartet conflicts (QC) using the -t flag and parameter 8 in ASTRAL. Quartet conflicts are a measure that evaluates discordance at the level of gene trees. SVDquartets and ASTRAL species trees were imported into FigTree v. 1.4.3 for further editing (Rambaut, 2017). Phylogenetic networks were inferred under a multispecies coalescent model in PhyloNet v. 3.6.7 (Than et al., 2008; Than et al., 2009). Three independent reticulation events were performed with maximum pseudolikelihood criteria (InferNetwork_MPL) based on 584 trees previously reconstructed in RAxML. Inside of the NEXUS file with 584 gene trees, we added a command line to estimate 100 independent searches to avoid sampling in local optimums (-x 100) and optimizing both branch length and inheritance probabilities using a likelihood framework (-o) in the 5 returned phylogenetic networks. The network with the lowest likelihood was selected and displayed graphically with Dendroscope v. 3.0 (Huson and Scornavacca, 2012). Additionally, we used the HyDe program v. 1.0 (Hybridization Detection; Blischak et al., 2018; Chifman and Kubatko, 2015) to test each individual within putative hybrid lineages recovered by PhyloNet. A file with assignment of individuals to species (map.txt) and concatenated alignment with 830 nuclear loci was the input file to run HyDe. We used two Python scripts: individual_hyde.py to test each putative hybrid lineage using a list of specified triples (P1|hybrid|P2) and bootstrap_hyde.py to conduct 500 bootstrap searches. We filtered the results from hybridization analysis to only include significant results of γ ($0 < \gamma < 1$). Both γ distribution and bootstrap values were plotted to examine uniform admixture (hybridization) or nonuniform admixture (not all individuals contain introgressed alleles).

2.6. Morphometric analysis

For the morphometric analysis, only samples from the *M. haageana* complex were included, excluding *M. san-angelensis* due to a lack of biological material. The analysis included the number of radial spines and the length of the radial and central spines of every sample collected (approximately 50 records by locality). Measurements were performed using ImageJ v. 1.48b (Abràmoff et al., 2004). Our analyses also include geometric morphometric floral data of tepal shape and pigmentation from Rosas et al. (2022). Imputation was performed using MICE v. 3.13.0 to account for missing floral data (Buuren and Groothuis-Oudshoorn, 2011). A classificatory analysis was performed using the random forest method (RF; Breiman, 2001). The database was divided to generate a training set (75%) and a validation set (25%). To find the best model, we evaluated ntree = 100:1000, each 100 trees and mtry = 1:10 to select the best model. The multidimensional scale was applied using randomForest v. 4.6–14 (Liaw and Wiener, 2002).

2.7. Environmental analysis

Only samples from the *M. haageana* complex were included, excluding *M. san-angelensis* and *M. acultzingensis*, as only one locality is known. To obtain the environmental analysis, occurrence points were obtained from different sources: a) samples collected in the wild, b) herbaria (MEXU, IBUG, IZTA, FCME, UAMIZ, CORU, SERO and XALU) and c) the Global Biodiversity Information Facility (GBIF). The database was filtered, eliminating the repeated points and points with<1 km of distance to avoid overrepresentation using Wallace v. 1.0.6.2 (Kass et al., 2018). The 19 bioclimatic variables were obtained from WorldClim (Hijmans et al., 2005). Furthermore, elevation data, soil type and geoforms were included (Fischer et al., 2008; Pineda et al., 2014). Variables were in ASCII format with a 30 arc-second resolution. Layer values were extracted from occurrence points using Elevatr v. 0.3.1 (Hollister et al., 2021), Raster v. 3.4.5 (Hijmans and van Etten, 2012), and sp v. 1.1.4 (Pebesma and Bivand, 2005). PCA was performed using the bioclimatic variables, and PERMANOVA was performed with scores from the PCA selecting the first three components. Pearson correlation analysis was performed for all bioclimatic variables, selecting those with more contribution to the data variation and then those with correlation values < -0.85 and > 0.85. ANOVA and Tukey's test were performed to evaluate the differences in elevation between the putative means of taxa. Contingency tables and chi-square tests were used to evaluate the geoforms and soil types.

The potential distribution models were built using Wallace and 6 bioclimatic variables (bio04, bio07, bio08, bio13, bio15, and bio17) selected from the previous analyzes. The studied area was delimited using a polygon that covers the species distribution and adding a 0.2° buffer distance. The pseudo absences were 10,000. Calibration data were computed using the jackknife method (k = n). The models were built using maximum entropy (MaxEnt; Phillips et al., 2006), selecting Linear (L), Quadratics (Q), Hinge (H), and Product (P), using regularization multipliers (RM) from 0 to 10. The best models were selected using the lowest Akaike Information Criterion (AIC) value (Muscarella et al., 2014). Binary maps were generated using the ASCII format with the 10th percentile training presence logistic threshold. To evaluate the similarity or difference between the species niches, the *D* and *I* statistics (Schoener, 1968; Warren et al., 2010) were obtained using ENMTools v. 1.4.4 (Warren et al., 2010).

3. Results

3.1. Different clusters suggest different species in the M. haageana complex

After filtering the dataset with the optimal parameters and including all samples (Supplementary Table 3), 201 SNPs were obtained, which were used to analyze the genetic structure using PCA, DAPC, and a phylogenetic network. The first three PCs explained 46.59% of the genetic variation (Fig. 2), showing that accessions of the *M. haageana* complex do not form a cluster, nor do the accessions of *M. albilanata*. The DAPC results show that the first three axes explain 90.44% of the genetic variation, and nine clusters can be distinguished, which was in concordance with the lowest BIC value (Fig. 2). The phylogenetic network represents the phylogenetic relationships between accessions (Fig. 3); these results were consistent with the clusters found in the DAPC. The accessions of the *M. haageana* complex are found in five of the nine clusters. The genetic group Mcons+Mao4 corresponds to *M. lanigera* (see taxonomic treatment).

Because some accessions of *M. conspicua* and *M. meissneri* were grouped together with *M. lanata*, *M. dixanthocentron*, *M. supertexta*, and *M. crucigera*, while accessions of *M. albilanata* subsp. *oaxacana* were ambiguously assigned, a new sequence assembly was run *de novo* (Supplementary Table 3). In this analysis, only the accessions of Mcons+Mao1 and Mmeiss+Mao1+super clusters were included to obtain a greater number of SNPs and thus to improve the resolution in these clusters. A total of 607 SNPs were obtained after filtering. The results of the new assembly showed that the first three PCs explained 32.48% of the genetic variation (Supplementary Fig. 3). The DAPC results showed that the first three axes explained 99.98% of the genetic variation, and four clusters could be distinguished according to the BIC values (Supplementary Fig. 3). *Mammillaria meissneri* is grouped with species from the Tehuacán-Cuicatlán Valley, while *M. san-angelensis* is grouped with *M. albilanata* subsp. *albilanata* and *M. conspicua* group accessions that were identified a priori as *M. meissneri*. The results also showed that *M. meissneri, M. conspicua, M. san-angelensis,* and Mao1 represent an independent genetic group (Supplementary Fig. 4).

3.2. Mammillaria haageana is not a monophyletic group

To resolve the phylogeny of the *M. haageana* complex, we performed ML and BI analyses. The concatenated matrix included 427 loci (Supplementary Table 3), with an average length per locus of 229.64±46.27 bp and a final length of 97,131 bp. In both ML and BI analyses (Supplementary Fig. 5), *M.* ser. *Supertextae* was recovered as a monophyletic group with similar topologies. In both phylogenetic analyses, accessions of the *M. haageana* complex do not form a clade; this same result is observed in accessions of *M. albilanata*. For that reason, we considered that *M. haageana s.l.* is not a monophyletic group and is composed of different species.

3.3. Phylogenetic incongruence is explained by incomplete lineage sorting (ILS)

Our network showed signals of reticulation (Fig. 3), which is commonly pointed out as a major source of phylogenetic incongruence. This incongruence can be explained by several processes, such as incomplete lineage sorting (ILS), reticulation, and horizontal gene transfer. Nonetheless, the latter has not been explored with genomic data in this group, as to date, there is no evidence of such a phenomenon reported for cacti or other Caryophyllales taxa but is possible that occur commonly in plants (Aubin et al., 2021).

We used a dataset where the average number of base pairs (bp) per individual locus was 221.5 with a range of 124–279 bp in length. The concatenated matrix was 183,877 bp in size, and 2,318 sites were informative. The analysis of SVDquartets shows 75,282 quartets, with 86.5% quartets compatible and 13.5% quartets incompatible. The species tree recovered eight well-supported species (Supplementary Fig. 6). Values of sCF were lower than the bootstrap values and show ILS signals in every relationship. In all nodes, ≈55% of discordance was recovered, with the exception of the *M. acultzingensis* + Mcons+Mao4 clade, where the discordance percentage was higher (37.2%). The ASTRAL tree recovered similar relationships with the exception of the *M. meissneri* +

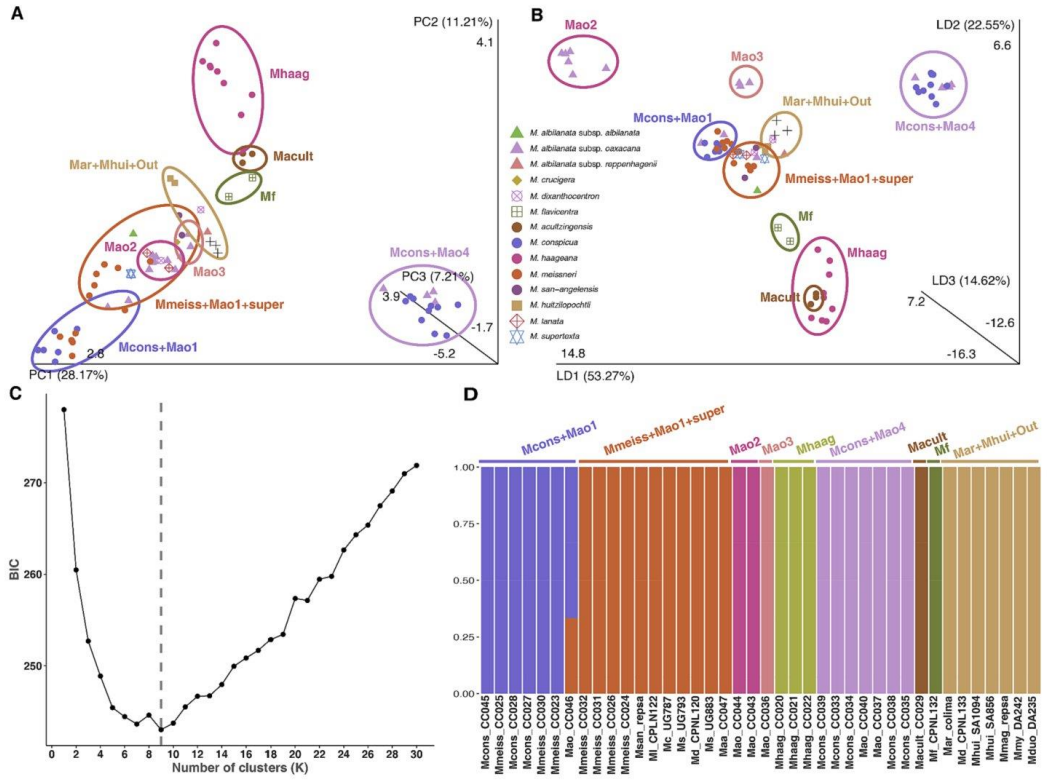

Fig. 2. Analyses of population structure using DAPC and PCA: (A) PCA results displaying the first three components; (B) DAPC scatterplot displaying clusters along the first three discriminant functions; (C) BIC results for estimating the appropriate number of genetic clusters; (D) Bar-graphs showing assignment of individuals to the nine clusters recovered by DAPC.

M. conspicua + *M. supertexta* clade (Supplementary Fig. 6). The levels of ILS in the three groups were high (0.4). The local posterior probability in the branches was well supported, and the coalescent units examined through the length branches were very low. The average discordance through the quality controls shows high levels of conflict in all branches (<40%), indicating that the quality control values were lower than the probability values. The concordance factor values were different between gCF and sCF (Supplementary Fig. 6), while sCF showed values in a range of 20% and 60% concordance, and gCF showed lower concordance values.

For reticulation, a total of three events were evaluated (Supplementary Fig. 7). In the first reticulation event, PhyloNet found introgression from *M. conspicua* into Mcons+Mao4. A second analysis permitting two reticulation events was conducted, and we found almost equal proportion of gene flow from *M. acultzingensis* and Mcons+Mao4 into *M. haageana,* suggesting hybridization. Finally, a third analysis that permitted three reticulation events, the last two events were conserved, and a third event detected the common ancestor of *M. supertexta* and *M. meissneri* as an event possible of hybrid speciation between *M. conspicua* and ancestor of *M. san-angelensis*. This third event is the hypothesis with the least likelihood. Although three different scenarios of reticulation were detected with PhyloNet, HyDe did not consider any event significant, suggesting that not all individuals per reticulation hypotheses contain introgressed alleles (Supplementary Fig. 8). Hence, our analyses suggest that ILS rather than reticulation can be the most important source of the phylogenetic discordance in the *M. haageana* complex, yet we do not rule out other processes that could explain the evolutionary patterns observed.

3.4. Spination patterns (rather than flower attributes) used to differentiate species morphologically

Cactus spines have been one of the morphological features most used for species delimitation in *Mammillaria* (Arias et al., 2012; Bravo-Hollis and Sánchez-Mejorada, 1991; Reppenhagen, 1992). For that reason, a morphometric analysis was performed using these structures in *M. haageana*, excluding *M. san-angelensis* because there are no additional accessions available. Therefore, RF analysis included 14 variables (Supplementary Fig. 9): six related to spines and eight related to flowers from our previously published work (Rosas et al., 2022). The results showed that the best model had 86.36% precision using ntree = 600 and mtry = 6, and the most important variable was the radial spine length G (at angles between 165° and 195°). Using the training model, the misclassification error was low for *M. haageana*, Mcons+Mao4 and *M. acultzingensis*, while the error was higher for *M. meissneri* and *M. haageana* (Supplementary Table 4). However, when testing the model, an optimal classification was recorded for all taxa (Supplementary Table 5). The multidimensional scaling plot shows that there is low

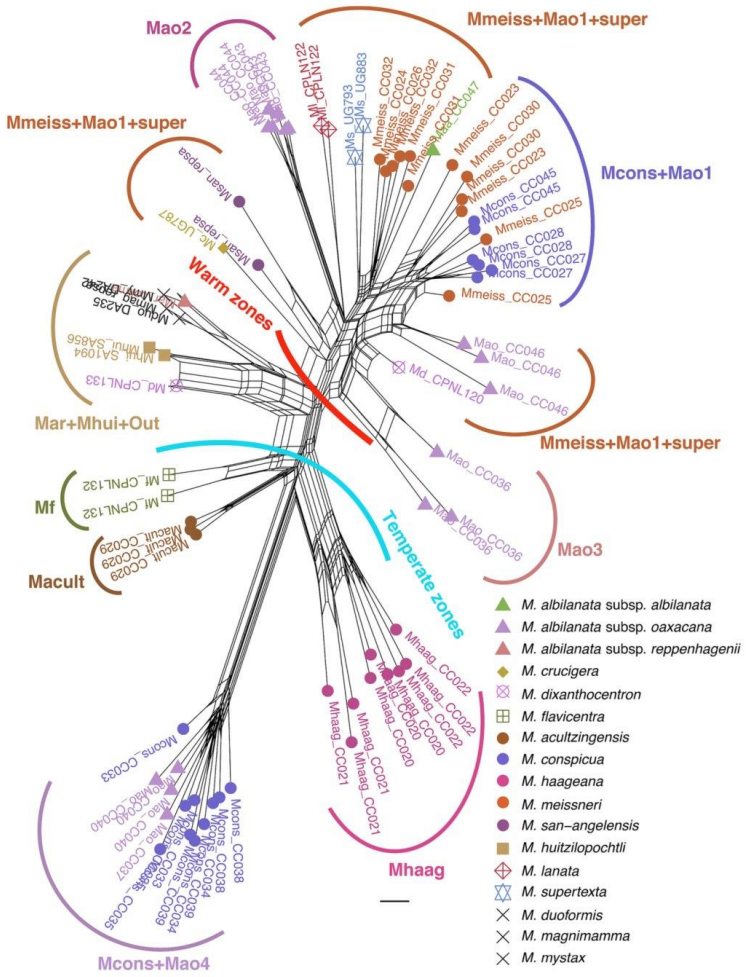

Fig. 3. Phylogenetic network analysis with a dataset containing 207 SNPs using the Neighbor-Net method in SplitsTree4.

morphological proximity between the taxa (Supplementary Fig. 9). Our data show that the spines and the color saturation of the flowers can be used for the identification of these clades; this is the first study that evaluates these characters under morphometric methods.

3.5. The environment can explain the distribution of the clades and species

To determine whether there is a correlation between the distribution and environmental variables, we performed multivariate analyses. First, the species *M. conspicua*, *M. meissneri*, *M. haageana,* and Mcons+Mao4 are distributed in elevational ranges from 1000 to 3000 m.a.s.l. (Fig. 4). The variance analysis showed significant differences ($P < 0.01$) among the species, and the Tukey test showed that means were different between species. Conversely, regarding geoforms and soil types, we found that species have a tendency to be more related to soil type than to geoforms (Fig. 4). The species that are positively associated with andosols are *M. meissneri*, mainly with ochric (To), and *M. haageana* is associated with vitric (Tv) and humic (Th), while Mcons+Mao4 has a negative association with these soil types. In contrast, Mcons+Mao4 is positively associated with karstic cambisols (Bk) and chromic luvisols (Lc). Overall, the results show that *M. conspicua* species do not have any soil preference, but other species do, while there is an elevational cline in the species distribution.

The PCA with 19 bioclimatic variables showed that the first three components retained 89.2% of the total variation between the occurrence records (Supplementary Fig. 10). The mean temperature of the wettest quarter (bio08) was the variable with the greatest contribution in the first component (Supplementary Table 6). In the second component, precipitation of the wettest month (bio13) was the variable with the greatest contribution, and finally, in the third component, temperature annual range (bio07) was the variable with the greatest contribution. The plot with the first two components shows five clusters; the first component separates *M. conspicua*, *M. meissneri*, and Mcons+Mao4,

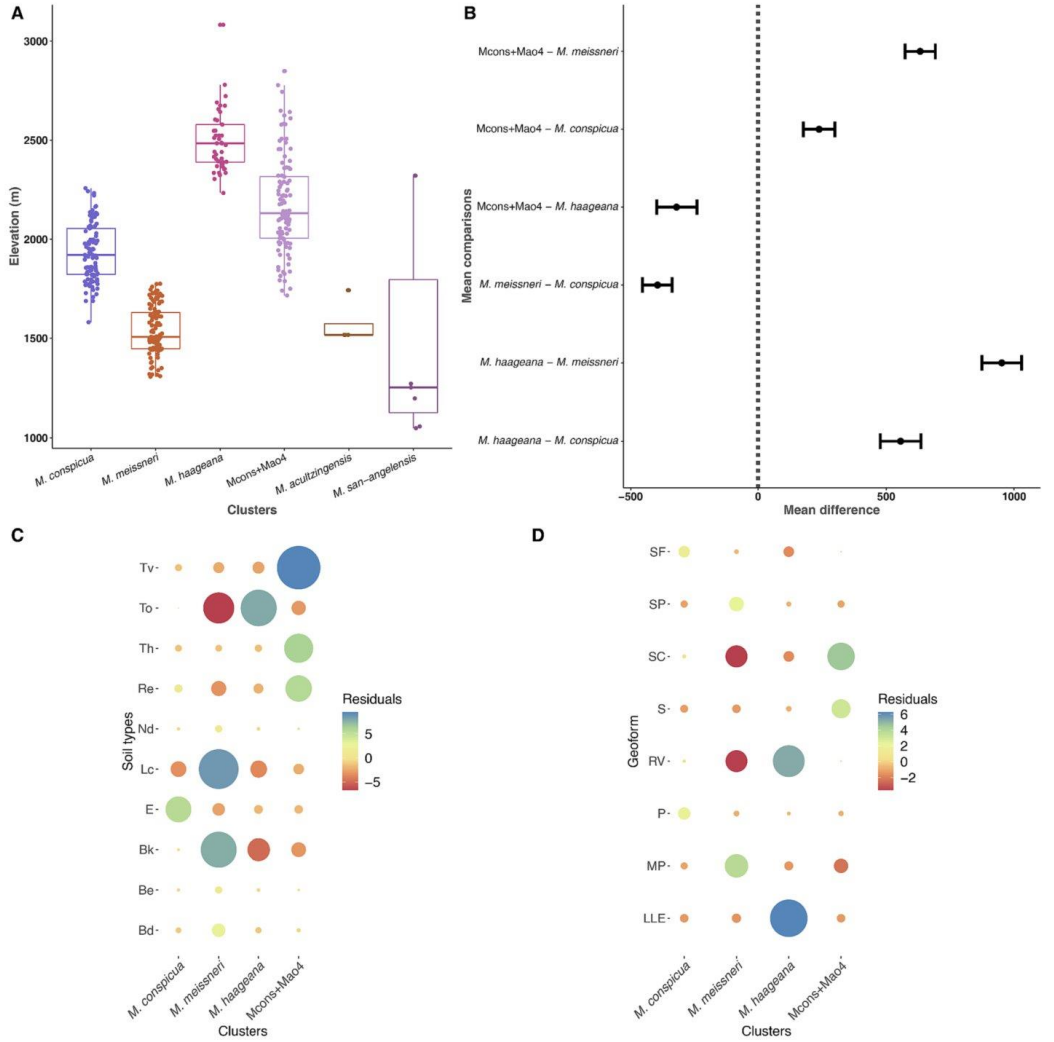

Fig. 4. Results for four species of the *M. haageana* complex regarding preferences for soil types and topographic variables. A) Elevation ranges; B) Tukey's test on elevation ranges; C) plot of residuals from the chi-square test of soil types; D) plot of residuals from the chi-square test of geoforms.

while the second separates *M. haageana* and *M. san-angelensis* from the other species. The third component partially separates *M. acultzingensis* from the remainder of the species. PERMANOVA showed significant differences ($P < 0.01$) in distance between the species. The set of non-correlated bioclimatic variables includes the variables that have the greatest contribution in the three principal components, as well as temperature seasonality (bio04), precipitation seasonality (bio15) and precipitation of the driest quarter (bio17).

The area under the curve (AUC) values of the selected models were equal to 0, and the AUC scores were higher than 0.95 (Supplementary Table 7). Based on our environmental preference and distribution data, we estimated the current potential distribution for *M. meissneri*, *M. haageana*, Mcons+Mao4, and *M. conspicua* (Fig. 5). The potential distribution of *M. haageana* is mainly in the northeast of Puebla and in the part that corresponds to the Trans-Mexican Volcanic Belt in Veracruz. Interestingly, the potential distribution of *M. conspicua* is in the southeast of Puebla surrounding the potential distribution of *M. meissneri*, which is mainly in the center of Zapotitlan Salinas. Both species are in the Tehuacán-Cuicatlan Valley. In contrast, Mcons+Mao4 has a potential distribution mainly to the north of Oaxaca in the Sierra Madre del Sur. The overlapping points between the niches of the species (D and I) are shown in Supplementary Table 8. The sympatric species *M. conspicua* and *M. meissneri* show higher niche overlap, while *M. haageana* shows lower overlap with the other species.

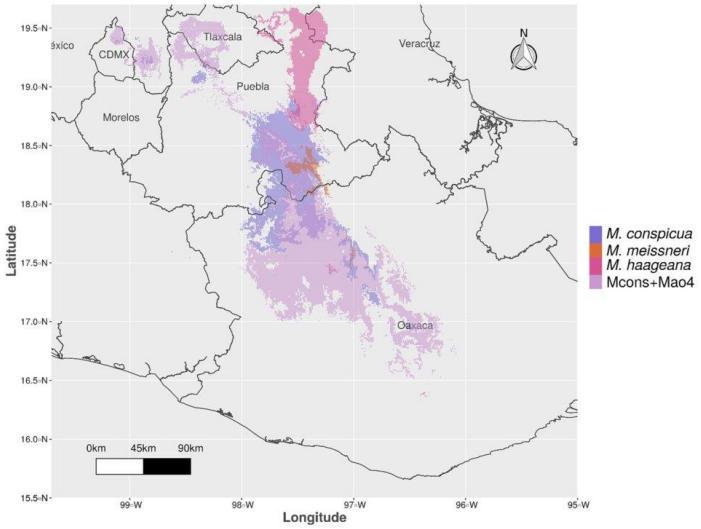

Fig. 5. Current potential distribution of four species of the *M. haageana* complex.

4. Discussion

This is the first study within *Mammillaria* that uses a genome reduced-representation sequencing method and an integrative strategy by including molecular, morphological and ecological data for species delimitation with formal methods. Many hypotheses have been proposed about the taxonomy of the *M. haageana* complex (Table 1), the most recent considering that it is a widely distributed species (Korotkova et al., 2021). However, we previously showed that within the *M. haageana* complex, there can be more than two independent evolutionary lineages (Cervantes et al., 2021). In this study, we used comprehensive sampling, multiple datasets, and extensive analyses; the results showed that the *M. haageana* complex is composed of 6 species (*M. haageana*, *M. conspicua*, *M, lanigera, M. meissneri*, *M. acultzingensis,* and *M. san-angelensis*), which morphologically can be distinguished by their spination patterns and geographical distribution.

4.1. Mammillaria ser. supertextae is recovered as a monophyletic group

Mammillaria ser. *Supertextae* was recovered as a monophyletic group as previously reported (Cervantes et al., 2021). *Mammillaria huitzilopochtli* and *M. albilanata* subsp. *reppenhagenii* are recovered as early divergent taxa, sister of the rest of *M*. ser. *Supertextae.* The phylogenetic network using all the data were divided into two groups. Group 1 is distributed in temperate zones at elevations ranging mainly from 1,285 to 2,518 m.a.s.l. in pine-oak forests and includes the accessions of *M. haageana*, *M. flavicentra*, *M. acultzingensis*, and the Mcons+Mao4 lineage. Group 2 is distributed in warm zones mainly at altitudes ranging from 447 to 2,318 m.a.s.l. in thorn and tropical deciduous forests and includes accessions of *M. crucigera*, *M. dixanthocentron*, *M. conspicua*, *M. meissneri*, *M. san-angelensis*, *M. lanata*, *M. supertexta*, and three lineages associated with *M. albilanata* subsp. *oaxacana* (Mao1, Mao2, and Mao3). Our results are consistent with those reported by Cervantes et al. (2021), who found that these two groups separated by climatic factors using two chloroplast markers.

4.2. Incomplete lineage sorting rather than reticulation could explain phylogenetic discordance in the M. haageana complex

Phylogenetic discordance may be due to different factors: horizontal gene transfer, incomplete lineage sorting (ILS) and reticulation (Arekar et al., 2018). The most studied are ILS and reticulation; distinguishing between these factors remains difficult even using high-throughput sequencing but can be useful in combination with network analysis (Gagnon et al., 2022). Our results showed that there are reticulation events between the species of the *M. haageana* complex but none were significant (Supplementary Fig. 7 and 8; PhyloNet and HyDe analyses). It is necessary to explore the phenomenon of reticulation more carefully and use other methods to corroborate our hypotheses. Our results could be biased by the limitations of the programs. Phylonet is a non-scalable and computationally demanding method when it comes to increasing the number of species or the number of reticulations (Elworth et al., 2019). Furthermore, it is difficult to allow only one individual per species to be included and to detect an introgressed individual at the population level. Phylonet also lacks evolutionary models in scenarios where reticulation is present (Mirarab et al., 2021). HyDe can generate endless hypotheses (combinations of small subsets of taxa), creating challenges with multiple tests and possible conflicting results on sampled subsets of the same groups (Blair et al., 2020). Additionally, HyDe does not allow incorporating the stochasticity of the coalescing process in a principled manner (Mirarab et al., 2021).

On the contrary, our coalescence analysis showed that ILS is very likely to be the cause of the phylogenetic discordance. ILS is common in lineages with high rates of diversification and recent divergence (Meyer et al., 2017). Such factors are present in *M.* ser. *Supertextae* as it is a recently diverged group that originated approximately 2.1 mya (Cervantes et al., 2021), and it has recently been shown that the clade *Mammillaria s.s.* has high rates of diversification (Breslin et al., 2022). The presence of short internal branches is typical of ILS (Gagnon et al., 2022); this can be observed in clades where *M. conspicua* and *M. meissneri* are found (Supplementary Fig. 5). It was difficult to separate these species from the rest of the species of *M.* ser. *Supertextae* with which they are related in the Tehuacán-Cuicatlán Valley (Fig. 1). To better unravel the relationships and understand the diversification

processes of these species, studies that include a larger sample size are needed, since in this study, the species of the *M. haageana* complex were mainly considered.

4.3. Taxonomic implications in M. haageana complex delimitation

Based on our previous results (Cervantes et al., 2021) and according to previously published taxonomic proposals (Table 1), we summarized operative taxonomic units as hypotheses of initial species and analyzed them with topotypes, which allowed us to define and associate the names of existing taxa with the putative species (Liu et al., 2019). Our analysis showed that the use of genomic, morphological and environmental data helped to identify the putative species. Reduced representation genomic data generally help to elucidate complex phylogenetic relationships and to establish the boundaries between closely related species (Anderson et al., 2017; Hashemzadeh Segherloo et al., 2021; Pérez-Escobar et al., 2020). Conversely, the morphological data of flowers and spines showed apparently continuous variation. Although flower color saturation is variable among species (Rosas et al., 2022), the number and length of radial spines were rather useful as diagnostic characters. This is the first study testing these morphological characters for species delimitation in the *M. haageana* complex. The results of the PCA of 19 bioclimatic variables, the elevational differences, the associations to soil types and the niche models showed a great difference in niche among the species of the *M. haageana* complex. This implies that lineage separation via niche divergence mediated by disruptive selection (ecological speciation) is very likely (Cheng et al., 2021). The climatic and geological fluctuations during the Pleistocene could generate different environmental conditions where each species had different adaptations and phylogenetic divergences were generated (Díaz-Castellón et al., 2012; Mastretta-Yanes et al., 2015; Ortiz-Medrano et al., 2016). Yet we acknowledge that our study has some limitations such as 1) the representation of *M.* ser. *Supertextae* species, 2) the missing data given by the sequencing technology and bioinformatics processes, 3) the possible presence of paralogues, given that the is not reference genome, and 4) the coalescence algorithms. Nevertheless, we believe that taken together, these analyses lead to our main conclusion regarding the recognition of the following species:

Mammillaria haageana (Voucher ID: CC020, CC021, CC022; Supplementary Table 1) differs mainly by the color of the tepals, since it presents lighter saturation tones (Rosas et al., 2022). The type locality of *M. haageana* is Perote, Veracruz, located southeast of the Trans-Mexican Volcanic Belt (Fig. 1). The vegetation is usually a transition from xerophytic scrub to pine-oak forest in an elevational range of 2,338 to 2,647 m.a.s.l., where soil derived from volcanic rock predominates, which is positively associated with this species (Fig. 4).

Mammillaria acultzingensis (Voucher ID: CC029) differs by having longer radial spines (5.6 to 7.9 mm) and fewer radial spines (14 to 18), with all spines presenting a yellow color. This species has only been reported from a single locality in Acultzingo, Veracruz, in the northwestern Sierra Madre del Sur; therefore, it was not possible to include it in the environmental analyses. The vegetation is a xeric scrub at an elevation of 1,460 to 1,686 m.a.s.l.

Mammillaria conspicua (Voucher ID: CC023, CC025, CC027, CC028, CC030, CC045) has 19 to 21 radial spines, and their lengths range from 4 to 5 mm. It is distributed mainly in the Tehuacán Valley, from Puebla to Oaxaca. The vegetation is xerophytic scrub and secondary vegetation in an elevational range of 1,936 to 2,081 m.a.s.l., and it does not present any association with any particular type of soil. According to our identifications a priori, some samples (Voucher ID: CC023, CC025 and CC030) were located as part of *M. meissneri*, but our genetic analyses show that they are part of *M. conspicua*.

Mammillaria meissneri (Voucher ID: CC024, CC026, CC031, CC032) has the shortest spines of 3.5 to 3.9 mm, generally white with a black apex. It is distributed mainly in the Zapotitlán Salinas area in the northwestern Tehuacán-Cuicatlán Valley. The vegetation is xerophytic scrub in an elevational range of 1,541 to 1,660 m.a.s.l. It is positively associated with chromic luvisols and calcic cambisols. Its distribution overlaps with that of *M. conspicua*; however, genetic data indicate that it has more affinity with the rest of the *M.* ser. *Supertextae* species that are distributed toward the southeastern Tehuacán-Cuicatlán Valley.

Mammillaria san-angelensis (Voucher ID: REPSA) is a member of the *M. haageana* complex that is furthest geographically from the rest of the species. It is mainly distributed in Pedregal de San Ángel, CDMX. Only two individuals are known, for which morphological and environmental analyses could not be performed. The genetic data indicate that it is an independent lineage from the rest of the species of the *M. haageana* complex, although it maintains an affinity with *M. albilanata* subsp. *albilanata,* which is distributed around the part of Iguala, Guerrero. Therefore, it is necessary to make a collection effort toward Morelos and Guerrero, since according to Reppenhagen (1992), variations of *M. san-angelensis* can be found in this area. For the moment, we consider that *M. san-angelensis* is independent of *M. albilanata* subsp. *albilanata*, but future studies could clarify the boundaries between these taxa. Our results may be useful for carrying out risk assessments and conservation strategies for species proposed in this study.

The lineage Mcons+Mao4 (Voucher ID: CC033, CC034, CC035, CC037, CC038, CC039, CC040) differs by having radial spine lengths ranging from 4.3 to 5.9 mm. It is distributed in the eastern part of the Sierra Madre del Sur, mainly in the transition from xeric scrub to pine-oak forest in an elevational range from 1,928 to 2,419 m.a.s.l. The lineage Mcons+Mao4 has a positive association with growth in soils formed by volcanic ash that have a vitric horizon in their first meter. The taxonomy has not been clear for this lineage, since the collected samples have been identified as *M. conspicua* or *M. albilanata* subsp. *oaxacana* (Arias et al., 2012). The name of *M. conspicua* has already been assigned to a lineage that is distributed mainly south of the Tehuacán-Cuicatlán Valley. In contrast, *M. albilanata* subsp. *oaxacana* has been described in the region where the samples of the Mcons+Mao4 lineage are distributed. However, *M. albilanata* is composed of more independent lineages; in this case, a nomenclatural reconsideration on name priority for species level is required, in accordance with the ICN (Turland et al., 2018, Art. 11.2; see Taxonomic Treatment).

Mammillaria albilanata includes four subspecies (*M. albilanata* subsp. *albilanata*, *M. albilanata* subsp. *oaxacana*, *M. albilanata* subsp. *reppenhagenii* and *M. albilanata* subsp. *tegelbergiana;* Korotkova et al., 2021); however, our results and those of Cervantes et al. (2021) show that they can be independent lineages, as in the case of *M. albilanata* subsp. *oaxacana*. The distribution of this last lineage has been reported for all of southern Mexico; however, our data indicate that within *M. albilanata* subsp. *oaxacana*, up to four independent lineages can be recognized, such as the case of Mcons+Mao4. Therefore, studies are needed that include the total distribution of what is recognized as *M. albilanata* (Korotkova et al., 2021) to establish the limits within this species. With our data we can only recognize the Mcons+Mao4 lineage as a *M. lanigera*-like species.

Based on the discussion offered here, we propose the following taxonomic treatment, including recognizing six species:

1.- ***Mammillaria acultzingensis*** Linzen, Rogoz. & F.Wolf, Mitteilungsbl. Arbeitskreises Mammillarienfr. 18: 72, 88. 1994. ≡ *Mammillaria haageana* subsp. *acultzingensis* (Linzen, Rogoz. & F.Wolf) D.R.Hunt, Mammillaria Postscripts 6: 9. 1997. Type: Mexico, Veracruz, Acultzingo, 14 Mar 1992, *H. Rogozinski 257* (holotype ZSS 012476, isotype MEXU 01291727!).

2.- ***Mammillaria conspicua*** J.A.Purpus, Monatsschr. Kakteenk. 22: 163. 1912. ≡ *Mammillaria haageana* subsp. *conspicua* (J.A.Purpus) D.R. Hunt, Mammillaria Postscripts 6: 9. 1997. Lectotype (designated by Arias et al., 2012): [illustration] fig. s.n. "Mam[m]illaria conspicua J.A. Purpus", in Monatsschr. Kakteenk. 24: 37, 1914.

= *Mammillaria albidula* Backeb., Cactaceae (Backeberg) 5: 3429. 1961. Neotype (designated by Reppenhagen, 1992): Mexico, Puebla, Colonia San Martin, *W. Reppenhagen 1913* (KL).

= *Mammillaria vaupelii* Tiegel, Möller's Deutsche Gärtn.-Zeitung 48: 412. 1933. ≡ *Neomammillaria vaupelii* (Tiegel.) Y.Itô, Cactaceae: 585. 1981. ≡ *Mammillaria conspicua* var. *vaupelii* (Tiegel) Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 113. 1989. ≡ *Mammillaria haageana* subsp. *vaupelii* (Tiegel) U.Guzmán, Cactaceae Syst. Init. 16: 18. 2003. Type: Mexico, without locality, *S.C. s.n.*, s.f. (not preserved).

3.- ***Mammillaria haageana*** Pfeiff., Allg. Gartenzeitung 4: 257. 1836. ≡ *Cactus haageanus* (Pfeiff.) Kuntze, Revis. Gen. Pl. 1: 260. 1891. ≡ *Neomammillaria haageana* (Pfeiff.) Britton & Rose, Cactaceae 4: 110. 1923. Neotype (designed here): Mexico, Veracruz, Perote, km 84, carretera 140 Acatzingo-Perote, límites con el estado de Puebla, 2,340 m, 27 feb. 1993, *S. Arias & U. Guzmán 1042* (MEXU 838716!).

Notes. According to the protologue, this name was described from Perote, but a type specimen has never been found (Hunt et al., 2006). Subsequently, Scheinvar and Olalde (2008) proposed a neotype based on a specimen from Tlaltizapán, in Morelos state (*S. Arias et al. 924* MEXU 733937). Our results indicate that the plants from this last locality do not match *M. haageana* in morphological description and distribution, so that when they come into conflict with the protologue, a new neotype for *M. haageana* is proposed (Art. 9.19, ICNB, 2018).

= *Mammillaria perote* Pfeiff., Allg. Gartenzeitung 4: 257. 1836.

Notes. The name *Mammillaria perote* is a misinterpretation of the original description of *M. haageana*. In the protologue, Pfeiffer (1836) abbreviates "*Mammillaria*" as "M" and when he mentions the name "perote", alludes to the type locality of *M. haageana*, so *M. perote* should be considered as a *nomen nudum* (Art. 38, ICNB, 2018).

= *Mammillaria collina* J.A.Purpus, Monatsschr. Kakteenk. 22: 162. 1912. ≡*Neomammillaria collina* (J.A.Purpus) Britton & Rose, Cactaceae 4: 111. 1923. ≡ *Mammillaria haageana* var. *collina* (J.A.Purpus) Linzen & al., Mitteilungsbl. Arbeitskreises Mammillarienfr. 15(6): 234. 1992. Lectotype (designated by Arias et al., 2012): [ilustration] fig. s.n. "Mam [m]illaria collina J.A. Purpus". in Fl. Valle Tehuacán-Cuicatlán 95: 93. 2012.

= *Mammillaria kunthii* Ehrenb., Bot. Zeitung (Berlin) 2: 835. 1844. ≡ *Cactus kunthii* (Ehrenb.) Kuntze, Revis. Gen. Pl. 1: 260. 1891. Type: not designated.

=*Mammillaria donatii* Berge ex K.Schum., Gesamtbeschr. Kakt., Nachtr. 1: 135. 1903. ≡*Neomammillaria donatii* (Berge ex K.Schum.) Britton & Rose, Cactaceae 4: 111. 1923. Type: not designated.

4.- ***Mammillaria lanigera*** Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 116. 1987. Type: Mexico, Oaxaca, San Miguel Maninaltepec, 18 Feb 1975,

W. Reppenhagen 944 (holotype ZSS).

= *Mammillaria albilanata* subsp. *oaxacana* D.R. Hunt Mammillaria Postscripts 6: 9. 1997. ≡

Mammillaria ignota Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 126–128. 1987. Nom. inval. Type. México, Oaxaca, Tomillinpaβ [Tomellin pass to Oaxaca], 18 Dec 1980, *W. Reppenhagen 1644* (holotype ZSS 872182! (alcohol), isotype 872183!).

Notes. The name *M. ignota* Repp. was replaced with *M. albilanata* subsp. *oaxacana* by Hunt (1997), because the first is the homonym of another previously published name (*M. ignota* Lawr.) (Art. 11.2, ICNB, 2018).

= *Mammillaria lanigera* Repp., subsp. *juxtlahuacensis* Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 118. 1987. Type: Mexico, Oaxaca, Juxtlahuaca, 19 Oct 1974, *W. Reppenhagen 877* (holotype ZSS 01001! (alcohol)).

= *Mammillaria noureddineana* Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 128. 1987. Type. Mexico, Oaxaca, San Lázaro [Etla], 18 Dec 1980, *W. Reppenhagen 1646* (holotype ZSS 000634).

Notes. Based on our results presented here, *M. lanigera* and *M noureddineana* represent only variation of the same species. The radial spines number in *M. noureddineana* (15–20) and *M. lanigera* (20–22), as well as the central spines number from 4 to 6 in *M. noureddineana* and from 2 to 4 in *M. lanigera* allows us to confirm that the variation represents a continuum (Reppenhagen 1987).

5.- ***Mammillaria meissneri*** Ehrenb., Bot. Zeitung (Berlin) 2(49): 834–835. 1844. ≡ *Mammillaria acanthophlegma* var. *meissneri* (Ehrenb.) Salm-Dyck, Cact. Hort. Dyck., 1849: 9. 1850. ≡ *Mammillaria elegans* var. *meissneri* (Ehrenb.) Hofmann, Repert. Pl. Succ. 40: 5. 1989. ≡ *Mammillaria haageana* subsp. *meissneri* (Ehrenb.) U.Guzmán, Cactaceae Syst. Init. 16: 18. 2003. Neotype (designated by Arias et al., 2012): Mexico, Oaxaca, cerca de San Antonio Nanahuatipan, 9 Nov 1994, *L.U. Guzmán-Cruz and S. Arias 1104* (MEXU 791381!).

= *Mammillaria elegans* var. *schmollii* R.T.Craig, Mammill. Handb.: 283. 1945. ≡ *Mammillaria haageana* var. *schmollii* (R.T.Craig) D.R.Hunt, Cact. Succ. J. Gr. Brit. 41: 63. 1979. ≡ *Mammillaria haageana* subsp. *schmollii* (R.T.Craig) D.R.Hunt, Mammillaria Postscripts 6: 9. 1997. ≡ *Neomammillaria neoelegans* var. *schmollii* (R.T.Craig) Y.Itô, Cactaceae: 583. Lectotype (designated by Arias et al. 2012): [illustration] fig. 254. "Mammillaria elegans var. schmollii x 1" in *Mammillaria* Handb. 283. 1945.).

6.- ***Mammillaria san-angelensis*** Sánchez-Mej., Cact. Suc. Mex. 26: 8. 1981. ≡ *Mammillaria haageana* Pfeiff. subsp. *san-angelensis* (Sánchez-Mej.) D.R.Hunt, Mammillaria Postscripts 6: 9. 1997. Lectotype (designated by Sánchez-Mejorada, 1981): Mexico, Distrito Federal, Pedregal de San Ángel, cerca del Espacio Escultórico de la UNAM, 12 Jan. 1981, *M. Panti s.n.* (MEXU 293929!).

Notes. Sánchez-Mejorada (1981) indicated the specimen "M. Panti 486" as the lectotype, but we consider that the collection number is an error, because the herbarium specimen in MEXU indicates: "M. Panti s. n."

= *Mammillaria dealbata* A.Dietr., Allg. Gartenzeitung 14: 309. 1846. ≡ *Cactus dealbatus* (A.Dietr.) Kuntze, Revis. Gen. Pl. 1: 260. 1891. *Neomammillaria dealbata* (A.Dietr.) Britton & Rose, Cactaceae 4: 110, 1923. ≡ *Mammillaria elegans* var. *dealbata* (A.Dietr.) Borg, Cacti, ed. 1: 336. 1937. Type: not designated.

= *Mammillaria elegans* var. *lupina* Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 108. 1987. Type: Mexico, Morelos, Canon [Cañón] de Lobo[s] bei Cuernavaca, 21 Jan 1981, *W. Reppenhagen* 1670 (holotype ZSS 25112!).

= *Mammillaria elegans* var. *longicaudata* Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 109. 1987. Type: Mexico, Morelos, SE slopes near Las Estacas, 21 Jan 1975, *W. Reppenhagen 918* (holotype ZSS 00557! (alcohol)).

Names excluded from the synonymy of *Mammillaria haageana* and its allies.

Mammillaria elegans var. *teyuca* Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 111. 1987. Type: Mexico, Puebla, [San Pedro] Teyuca, 25 Feb 1975, *W. Reppenhagen 956* (holotype: ZSS). **Notes**. *Mammillaria elegans* var. *teyuca* was described from specimens collected in the Nexapa River Basin (Reppenhagen 1989). We believe that this name should be included in the synonymy of *M. albilanata* since both taxa share a cylindrical stem, the presence of abundant trichomes on the stem and areoles, as well as the number of radial spines (15 to 20 in var. *teyuca* and from 16 to 24 in *M. albilanata*).

Mammillaria monticola Repp., Gattung Mammill. nach dem Heutigen Stand Meines Wissens: 115. 1987. Type. Mexico, Puebla, [Tehuitzingo], Puente Marques, 01 Oct 1974, *W. Reppenhagen 844* (holotype ZSS 24843!). **Notes**. This name should be included in the synonymy of *M. albilanata*. The presence of abundant trichomes on the stem, the length of the radial spines (2–5 mm), the number (1–2) and length (2–3 mm) of the central spines, as well as their distribution over the Balsas River Basin allows us to conclude that this taxon is part of the variation of *M. albilanata* (Reppenhagen 1987).

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CRediT authorship contribution statement

Cristian R. Cervantes: Conceptualization, Investigation, Methodology, Software, Formal analysis, Data curation, Writing – original draft. **José-Rubén Montes:** Methodology, Software, Formal analysis, Data curation, Writing – review & editing. **Ulises Rosas:** Conceptualization, Resources, Supervision, Writing – review & editing. **Salvador Arias:** Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Aberer, A.J., Kobert, K., Stamatakis, A., 2014. ExaBayes: massively parallel Bayesian tree inference for the whole-genome era. Mol. Biol. Evol. 31, 2553–2556. https://doi.org/10.1093/molbev/msu236.

Abràmoff, M.D., Magelhães, P.J., Ram, S.J., 2004. Image processing with Image. J. Biophotonics Int. 11, 36–42.

Anderson, B.M., Thiele, K.R., Krauss, S.L., Barrett, M.D., Sun, G., 2017. Genotyping-by-sequencing in a species complex of Australian hummock grasses (*Triodia*): Methodological insights and phylogenetic resolution. PLoS One 12 (1), e0171053.

Arekar, K., Parigi, A., Karanth, K.P., 2018. Hybridisation or incomplete lineage sorting: The convoluted story about the origin of the capped-golden langur lineage (Cercopithecidae: Colobinae). https://doi.org/10.1101/508929.

Arias, S., Guzmán-López, L., Guzmán-Cruz, U., Vázquez-Benítez, B., 2012. Cactaceae. Flora Val. Tehuacán-Cuicatlán 95, 1–235.

Aubin, E., El Baidouri, M., Panaud, O., 2021. Horizontal Gene Transfers in Plants. Life 11, 857. https://doi.org/10.3390/life11080857.

Belton, G.S., van Reine, W.F.P., Huisman, J.M., Draisma, S.G.A., D. Gurgel, C.F., 2014. Resolving phenotypic plasticity and species designation in the morphologically challenging *Caulerpa racemosa–peltata* complex (Chlorophyta, Caulerpaceae). J. Phycol. 50, 32–54. https://doi.org/10.1111/jpy.12132.

Blair, C., Ané, C., Hahn, M., 2020. Phylogenetic trees and networks can serve as powerful and complementary approaches for analysis of genomic data. Syst. Biol. 69 (3), 593–601.

Blischak, P.D., Chifman, J., Wolfe, A.D., Kubatko, L.S., 2018. HyDe: a python package for genome-scale hybridization detection. bioRxiv 188037. https://doi.org/10.1101/188037.

Bravo-Hollis, H., Sánchez-Mejorada, R., 1991. Las Cactáceas de México, vol. Universidad Nacional Autónoma de México, México D.F, III.

Breiman, L., 2001. Random forests. Mach. Learn. 45, 5–32. https://doi.org/10.1023/A:1010933404324.

Breslin, P.B., Wojciechowski, M.F., Majure, L.C., 2021. Molecular phylogeny of the Mammilloid clade (Cactaceae) resolves the monophyly of *Mammillaria*. Taxon 70 (2), 308–323.

Breslin, P.B., Wojciechowski, M.F., Majure, L.C., 2022. Remarkably rapid, recent diversification of *Cochemiea* and *Mammillaria* in the Baja California, Mexico region. Am. J. Bot. 109, 1472–1487. https://doi.org/10.1002/ajb2.16048.

Butterworth, C.A., Cota-Sanchez, J.H., Wallace, R.S., 2002. Molecular systematics of Tribe Cacteae (Cactaceae: Cactoideae): A phylogeny based on rpl16 intron sequence variation. Syst. Bot. 27, 257–270. https://doi.org/10.1043/0363-6445-27.2.257.

Butterworth, C.A., Wallace, R.S., 2004. Phylogenetic studies of *Mammillaria* (Cactaceae) insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. Am. J. Bot. 91, 1086–1098. https://doi.org/10.3732/ajb.91.7.1086.

Buuren, S., Groothuis-Oudshoorn, C., 2011. MICE: Multivariate imputation by chained equations in R. J. Stat. Softw. 45 https://doi.org/10.18637/jss.v045.i03.

Callejas-Chavero, A., Vargas-Mendoza, C.F., Gomez-Hinostrosa, C., Arriola-Padilla, V.J., Cornejo-Romero, A., 2021. Breeding system in a population of the globose cactus *Mammillaria magnimamma* at Valle del Mezquital. Mexico. Bot. Sci. 99, 229–241. https://doi.org/10.17129/botsci.2654.

Campbell, E.O., Brunet, B.M.T., Dupuis, J.R., Sperling, F.A.H., 2018. Would an RRS by any other name sound as RAD? Methods Ecol. Evol. 9, 1920–1927. https://doi.org/10.1111/2041-210X.13038.

Cervantes, C.R., Hinojosa-Alvarez, S., Wegier, A., Rosas, U., Arias, S., 2021. Evaluating the monophyly of *Mammillaria* series *Supertextae* (Cactaceae). PhytoKeys 177, 25–42. https://doi.org/10.3897/phytokeys.177.62915.

Cheng, S., Zeng, W., Wang, J., Liu, L., Liang, H., Kou, Y., Wang, H., Fan, D., Zhang, Z., 2021. Species delimitation of *Asteropyrum* (Ranunculaceae) based on morphological, molecular, and ecological variation. Front. Plant Sci. 12 https://doi.org/10.3389/fpls.2021.681864.

Chifman, J., Kubatko, L., 2014. Quartet inference from SNP data under the coalescent model. Bioinforma. Oxf. Engl. 30, 3317–3324. https://doi.org/10.1093/bioinformatics/btu530.

Chifman, J., Kubatko, L., 2015. Identifiability of the unrooted species tree topology under the coalescent model with time-reversible substitution processes, site-specific rate variation, and invariable sites. J. Theor. Biol. 374, 35–47. https://doi.org/10.1016/j.jtbi.2015.03.006.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.

Díaz-Castellón, R., Hubbard, B.E., Carrasco-Núñez, G., Rodríguez-Vargas, J.L., 2012. The origins of Late Quaternary debris avalanche and debris flow deposits from Cofre de Perote volcano, México. Geosphere 8, 950–971. https://doi.org/10.1130/GES00709.1.

Eaton, D.A.R., Overcast, I., 2020. ipyrad: Interactive assembly and analysis of RADseq datasets. Bioinformatics 36, 2592–2594. https://doi.org/10.1093/bioinformatics/btz966.

Elshire, R.J., Glaubitz, J.C., Sun, Q.i., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E., Orban, L., 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6 (5), e19379.

Elworth, R. A. L., Ogilvie, H. A., Zhu, J. Nakhleh, L., 2019 Advances in computational methods for phylogenetic networks in the presence of hybridization, pp. 317-360 in Bioinformatics and Phylogenetics, edited by T. Warnow. Springer, Cham.

Fischer, G., Nachtergaele, F., Prieler, S., van Velhuizen, H.T., Verelst, L., Wiberg, D., 2008. Global agro-ecological zones assessment for agriculture (GAEZ 2008). IIASA and FAO, Rome, Italy.

Gagnon, E., Hilgenhof, R., Orejuela, A., McDonnell, A., Sablok, G., Aubriot, X., Giacomin, L., Gouvêa, Y., Bragionis, T., Stehmann, J.R., Bohs, L., Dodsworth, S., Martine, C., Poczai, P., Knapp, S., Särkinen, T., 2022. Phylogenomic discordance suggests polytomies along the backbone of the large genus *Solanum*. Am. J. Bot. 109, 580–601. https://doi.org/10.1002/ajb2.1827.

Goetze, M., Zanella, C.M., Palma-Silva, C., Büttow, M.V., Bered, F., 2017. Incomplete lineage sorting and hybridization in the evolutionary history of closely related, endemic yellow-flowered *Aechmea* species of subgenus *Ortgiesia* (Bromeliaceae). Am. J. Bot. 104, 1073–1087. https://doi.org/10.3732/ajb.1700103.

Guzmán, U., Arias, S., Dávila, P., 2003. Catálogo de cactáceas mexicanas. Universidad Nacional Autónoma de México y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, D.F.

Hashemzadeh Segherloo, I., Freyhof, J., Berrebi, P., Ferchaud, A.-L., Geiger, M., Laroche, J., Levin, B.A., Normandeau, E., Bernatchez, L., 2021. A genomic perspective on an old question: *Salmo* trouts or *Salmo trutta* (Teleostei: Salmonidae)?

Mol. Phylogenet. Evol. 162, 107204 https://doi.org/10.1016/j.ympev.2021.107204.
Hijmans, R.J., Etten, J. van., 2012. raster: Geographic analysis and modeling with raster data. Available online: https://cran.r-project.org/web/packages/raster/index.html (accessed on 9 April 2023).
Hijmans, R., Cameron, S., Parra, J., Jones, P., Jarvis, A., 2005. Very high resolution interpolated climate surfaces of global land areas. Int. J. Climatol. 25, 1965–1978. https://doi.org/10.1002/joc.1276.
Hollister, J., Shah, T., Robitaille, A.L., Beck, M.W., Johnson, M., 2021. elevatr: Access elevation data from various APIs. https://doi.org/10.5281/zenodo.5809645, R package version 0.4.2, https://github.com/jhollist/elevatr/.
Hunt, D.R., Taylor, N.P., Charles, G., 2006. The new cactus lexicon. DH Books, Milbourne Port.
Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267. https://doi.org/10.1093/molbev/msj030.
Huson, D.H., Scornavacca, C., 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. Syst. Biol. 61, 1061–1067. https://doi.org/10.1093/sysbio/sys062.
Jombart, T., Ahmed, I., 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinforma. Oxf. Engl. 27, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521.
Kass, J.M., Vilela, B., Aiello-Lammens, M.E., Muscarella, R., Merow, C., Anderson, R.P., 2018. Wallace: A flexible platform for reproducible modeling of species niches and distributions built for community expansion. Methods Ecol. Evol. 9, 1151–1156. https://doi.org/10.1111/2041-210X.12945.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.
Korotkova, N., Aquino, D., Arias, S., Eggli, U., Franck, A., Gómez-Hinostrosa, C., Guerrero, P.C., Hernández, H.M., Kohlbecker, A., Köhler, M., Luther, K., Majure, L. C., Müller, A., Metzing, D., Nyffeler, R., Sánchez, D., Schlumpberger, B., Berendsohn, W.G., 2021. Cactaceae at Caryophyllales.org – a dynamic online species-level taxonomic backbone for the family. Willdenowia 51, 251–270. https://doi.org/10.3372/wi.51.51208.
Li, Q.-Q., Zhou, S.-D., Huang, D.-Q., He, X.-J., Wei, X.-Q., 2016. Molecular phylogeny, divergence time estimates and historical biogeography within one of the world's largest monocot genera. AoB Plants 8, plw041. https://doi.org/10.1093/aobpla/plw041.
Liaw, A., Wiener, M., 2002. Classification and regression by randomForest. R News 2, 18–22.
Liu, T., Sun, K., Csorba, G., Zhang, K., Zhang, L., Zhao, H., Jin, L., Thong, V.D., Xiao, Y., Feng, J., 2019. Species delimitation and evolutionary reconstruction within an integrative taxonomic framework: A case study on *Rhinolophus macrotis* complex (Chiroptera: Rhinolophidae). Mol. Phylogenet. Evol. 139, 106544 https://doi.org/10.1016/j.ympev.2019.106544.
Mastretta-Yanes, A., Arrigo, N., Alvarez, N., Jorgensen, T.H., Piñero, D., Emerson, B.C., 2015. Restriction site-associated DNA sequencing, genotyping error estimation and de novo assembly optimization for population genetic inference. Mol. Ecol. Resour. 15, 28–41. https://doi.org/10.1111/1755-0998.12291.
McCartney-Melstad, E., Gidiş, M., Shaffer, H.B., 2019. An empirical pipeline for choosing the optimal clustering threshold in RADseq studies. Mol. Ecol. Resour. 19, 1195–1204. https://doi.org/10.1111/1755-0998.13029.
Meyer, B.S., Matschiner, M., Salzburger, W., 2017. Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika Cichlid Fishes. Syst. Biol. 66, 531–550. https://doi.org/10.1093/sysbio/syw069.
Mirarab, S., Warnow, T., 2015. ASTRAL-II: Coalescent-based species tree estimation with many hundreds of taxa and thousands of genes. Bioinforma. Oxf. Engl. 31, i44-52. https://doi.org/10.1093/bioinformatics/btv234.
Mirarab, S., Nakhleh, L., Warnow, T., 2021. Multispecies coalescent: Theory and applications in phylogenetics. Annu. Rev. Ecol. Evol. Syst. 52, 247–268. https://doi.org/10.1146/annurev-ecolsys-012121-095340.
Morrone, J.J., Escalante, T., Rodríguez-Tapia, G., 2017. Mexican biogeographic provinces: Map and shapefiles. Zootaxa 4277, 277–279. https://doi.org/10.11646/zootaxa.4277.2.8.
Muscarella, R., Galante, P.J., Soley-Guardia, M., Boria, R.A., Kass, J.M., Uriarte, M., Anderson, R.P., 2014. ENMeval: An R package for conducting spatially independent evaluations and estimating optimal model complexity for Maxent ecological niche models. Methods Ecol. Evol. 5, 1198–1205. https://doi.org/10.1111/2041-210X.12261.
Ortiz-Medrano, A., Scantlebury, D.P., Vázquez-Lobo, A., Mastretta-Yanes, A., Piñero, D., 2016. Morphological and niche divergence of pinyon pines. Ecol. Evol. 6, 2886–2896. https://doi.org/10.1002/ece3.1994.
Otto, L.-G., Mondal, P., Brassac, J., Preiss, S., Degenhardt, J., He, S., Reif, J.C., Sharbel, T. F., 2017. Use of genotyping-by-sequencing to determine the genetic structure in the medicinal plant chamomile, and to identify flowering time and alpha-bisabolol associated SNP-loci by genome-wide association mapping. BMC Genomics 18. https://doi.org/10.1186/s12864-017-3991-0.
Padial, J.M., Miralles, A., De la Riva, I., Vences, M., 2010. The integrative future of taxonomy. Front. Zool. 7, 16. https://doi.org/10.1186/1742-9994-7-16.
Pante, E., Abdelkrim, J., Viricel, A., Gey, D., France, S.C., Boisselier, M.C., Samadi, S., 2015. Use of RAD sequencing for delimiting species. Heredity 114, 450–459. https://doi.org/10.1038/hdy.2014.105.
Pebesma, E.J., Bivand, R.S., 2005. Classes and methods for spatial data in R. R News 5, 9–13.
Perez, M.F., Bonatelli, I.A.S., Romeiro-Brito, M., Franco, F.F., Taylor, N.P., Zappi, D.C., Moraes, E.M., 2022. Coalescent-based species delimitation meets deep learning: Insights from a highly fragmented cactus system. Mol. Ecol. Resour. 22, 1016–1028. https://doi.org/10.1111/1755-0998.13534.
Pérez-Escobar, O.A., Bogarín, D., Schley, R., Bateman, R.M., Gerlach, G., Harpke, D., Brassac, J., Fernández-Mazuecos, M., Dodsworth, S., Hagsater, E., Blanco, M.A., Gottschling, M., Blattner, F.R., 2020. Resolving relationships in an exceedingly young Neotropical orchid lineage using genotyping-by-sequencing data. Mol. Phylogenet. Evol. 144, 106672 https://doi.org/10.1016/j.ympev.2019.106672.
Phillips, S.J., Anderson, R.P., Schapire, R.E., 2006. Maximum entropy modeling of species geographic distributions. Ecol. Model. 190 (3-4), 231–259.
Pilbeam, J., 1999. Mammillaria. Nuffield Press, Oxford, UK.
Pineda, N., Ochoa, R., Espinosa, L., Palacio, J., Flores, P., Balderas, M., Magaña, D., Canchola, Y., Reyna, M., Reyes, I., García, G., 2014. IX Reunión nacional de geomorfología. Resúmenes. Universidad Autónoma del Estado de México, Toluca.
Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., Susko, E., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67 (5), 901–904.
Rambaut, A., 2017. FigTree-version 1.4.3, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software/figtree/.
Reppenhagen, W., 1987. Die gattung *Mammillaria* nach dem heutigen Stand meines Wissens, 1987. Arbeitskreis für Mammillarienfreunde e. V, St. Veit/Glan, p. 223.
Reppenhagen, W., 1992. Die Gattung Mammillaria. Monographie. Druckerei Steinhart, Titisee-Neustadt, Germany.
Rosas, U., Fuentes-Pérez, E.S., Cervantes, C.R., Sandoval-Zapotitla, E., Santiago-Sandoval, I., Arias, S., Reyes-Santiago, J., 2022. Evolution of flower allometry and pigmentation in *Mammillaria haageana* (Cactaceae). BMC Plant Biol. 22, 52. https://doi.org/10.1186/s12870-021-03386-8.
Salicini, I., Ibáñez, C., Juste, J., 2011. Multilocus phylogeny and species delimitation within the Natterer's bat species complex in the Western Palearctic. Mol. Phylogenet. Evol. 61, 888–898. https://doi.org/10.1016/j.ympev.2011.08.010.
Sánchez-Mejorada, H., 1981. Un nombre nuevo para una cactácea del Anáhuac. Cactáceas y suculentas mexicanas 25, 71–79.
Scheinvar, L., Olalde, G., 2008. Tipificación de algunas cactáceas del estado de Guerrero. Cactáceas y suculentas mexicanas 53, 36–50.
Scherz, M.D., Glaw, F., Hutter, C.R., Bletz, M.C., Rakotoarison, A., Köhler, J., Vences, M., Lötters, S., 2019. Species complexes and the importance of data deficient classification in Red List assessments: The case of *Hylobatrachus* frogs. PLoS One 14 (8), e0219437.
Schoener, T.W., 1968. The Anolis lizards of Bimini: Resource partitioning in a complex fauna. Ecology 49, 704–726. https://doi.org/10.2307/1935534.
Schwarzfeld, M.D., Sperling, F.A.H., 2014. Species delimitation using morphology, morphometrics, and molecules: Definition of the *Ophion scutellaris* Thomson species group, with descriptions of six new species (Hymenoptera, Ichneumonidae). ZooKeys 462, 59–114. https://doi.org/10.3897/zookeys.462.8229.
Semarnat, 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental - Especies nativas de México de flora y fauna silvestres - Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio - Lista de especies en riesgo. D. Of. Fed. 30 Diciembre 2010 Segunda Sección México.
Solé, X., Guinó, E., Valls, J., Iniesta, R., Moreno, V., 2006. SNPStats: a web tool for the analysis of association studies. Bioinforma. Oxf. Engl. 22, 1928–1929. https://doi.org/10.1093/bioinformatics/btl268.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
Stielow, B., Bratek, Z., Orczán, A.K.I., Rudnoy, S., Hensel, G., Hoffmann, P., Klenk, H.-P., Göker, M., Litvintseva, A., 2011. Species delimitation in taxonomically difficult fungi: The case of *Hymenogaster*. PLoS One 6 (1), e15614.
Sturaro, M.J., Rodrigues, M.T., Colli, G.R., Knowles, L.L., Avila-Pires, T.C.S., 2018. Integrative taxonomy of the lizards *Cercosaura ocellata* species complex (Reptilia: Gymnophthalmidae). Zool. Anz. 275, 37–65. https://doi.org/10.1016/j.jcz.2018.04.004.
Swofford, D., 2002. PAUP*. Phylogenetic analysis using parsimony (*and Other Methods). Version 4.0b10. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x.
Than, C., Ruths, D., Nakhleh, L., 2008. PhyloNet: a software package for analyzing and reconstructing reticulate evolutionary relationships. BMC Bioinf. 9, 322. https://doi.org/10.1186/1471-2105-9-322.
Than, C., Nakhleh, L., Li, W.-H., 2009. Species tree inference by minimizing deep coalescences. PLoS Comput. Biol. 5 (9), e1000501.
Turland, N.J., Wiersema J.H., Barrie F.R., Greuter W., Hawksworth D.L, Herendeen P. S, Knapp S., Kusber W.H., Li D.Z., Marhold K., May T.W., McNeill J., Monro A.M., Prado J., Price M.J., Smith G. F., 2018. International code of nomenclature for Algae, Fungi, and Plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten, Hesse: Koeltz Botanical Books.
Warren, D.L., Glor, R.E., Turelli, M., 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography 33, 607–611. https://doi.org/10.1111/j.1600-0587.2009.06142.x.
Zhang, C., Rabiee, M., Sayyari, E., Mirarab, S., 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinf. 19, 153. https://doi.org/10.1186/s12859-018-2129-y.

Discusión general

Las historias evolutivas recientes y complejas, pueden dar como resultado linajes que tienen una variación morfológica y superpuesta, lo que hace que la distinción de unidades taxonómicas sea un desafío para los sistemáticos; por lo tanto, estos tipos de complejos de especies pueden ser ignorados por los taxónomos. Para el complejo *M. haageana* se han propuesto diferentes hipótesis taxonómicas (Tabla 1; Capítulo II); sin embargo, ninguna ha sido puesta a prueba bajo ningún análisis morfométrico, molecular o ambiental. En *Mammillaria*, los estudios morfométricos (Lüthy, 1995) y moleculares (Breslin et al., 2021; Butterworth & Wallace, 2004) se han realizado principalmente para evaluar las relaciones infragenéricas, pero ninguno se ha dirigido específicamente al complejo *M. haageana.* En los trabajos previos se ha incluido a *M. haageana* y de tres a cuatro especies de *M.* ser. *Supertextae*, serie a la cual pertenece. Por lo anterior, se conoce muy poco sobre las relaciones entre las especies del complejo *M. haageana* y el resto de las especies de *M.* ser. *Supertextae.*

En el Capítulo I se reporta como se puso a prueba la monofilia de *M.* ser. *Supertextae* y se estimó el tiempo de divergencia al incluir todos los taxones propuestos por Korotkova et al. (2021). Para ello, se utilizaron dos marcadores del cloroplasto (*rpl*16 y *psb*A-*trn*H) y se realizo un análisis filogenético mediante inferencia bayesiana. Los resultados mostraron que *M.* ser. *Supertextae* se recupera como un grupo monofilético (Figura 1; Capítulo I), consistente con estudios filogenéticos previos que incluyeron cinco especies (Butterworth & Wallace, 2004) y tres taxones (Solórzano et al., 2019) de la serie. Los resultados también muestran que *M.* ser. *Polyacanthae* es la serie hermana de *M.* ser. *Supertextae* como se había sugerido anteriormente (Hunt, 2011). Para *Mammillaria* ser. *Supertextae* se estimo un tiempo de divergencia de aproximadamente 2.1 Ma (95% HPD = 0,91–3,47; Figura 3; Capítulo I) en la transición Neogeno-Cuaternario. Las regiones geográficas donde se distribuyen las especies de *M.* ser.

Supertextae han sufrido cambios climáticos, tectónicos, erosivos, aluviales y volcánicos durante millones de años; durante el Pleistoceno estos procesos continuaron dando lugar al clima y geomorfología actual (Medina-Sánchez et al., 2020; Siebert & Carrasco-Núñez, 2002). Las diferencias ambientales, geológicas y topográficas entre especies estrechamente relacionadas, producidas durante estos cambios, sugieren presiones de selección diferencial y adaptación local, que podrían haber impulsado el proceso de especiación (Aquino et al., 2021; Mastretta-Yanes, et al., 2015), como se ha sugerido para *Mammillaria pectinifera* F.A.C.Weber (Cornejo-Romero et al., 2014), *Cephalocereus columna-trajani* (Karw. ex Pfeiff.) K.Schum. (Cornejo-Romero et al., 2017) y especies del género *Epithelantha* F.A.C.Weber ex Britton & Rose (Aquino et al., 2021).

Ya que las relaciones al interior de *M.* ser. *Supertextae* no pudieron ser resueltas utilizando dos marcadores del cloroplasto, se utilizó la técnica GBS, descrita en el Capítulo II, con el fin de muestrear de forma aleatoria el genoma. La información genética obtenida por GBS se integró junto con dos líneas de evidencia (morfología y ecología) para mejorar la estrategia para definir los límites entre las especies (Padial et al., 2010; Sturaro et al., 2018) del complejo *M. haageana*.

Mediante una red filogenética se muestra que *M.* ser. *Supertextae* se divide en dos grupos (Figura 3; Capítulo II). El grupo de zonas templadas se distribuye en altitudes que oscilan entre 1285 y 2518 m.s.n.m., en bosques de pino-encino e incluye las accesiones de *M. haageana*, *M. flavicentra*, *M. acultzingensis* y el linaje Mcons+Mao4 (*M. lanigera*; ver tratamiento taxonómico del Capítulo II). El grupo de zonas cálidas se distribuye principalmente en altitudes que van desde 447 a 2318 m.s.n.m., en matorrales xerófilos y bosques tropicales caducifolios, e incluye las accesiones de *M. crucigera*, *M. dixanthocentron*, *M. conspicua*, *M. meissneri*, *M.*

san-angelensis, *M. lanata*, *M. supertexta* y tres linajes asociados con *M. albilanata* ssp. *oaxacana* (Mao1, Mao2 y Mao3). Nuestros resultados son consistentes con los reportados en el Capítulo I, donde se encontró que estos dos grupos están separados por factores climáticos, utilizando dos marcadores de cloroplastos.

La red filogenética también mostró que, dentro de *M.* ser. *Supertextae* existe una evolución reticulada. Las reconstrucciones filogenéticas basadas en múltiples loci son potencialmente útiles en la delimitación de especies; sin embargo, puede existir incongruencia filogenética entre múltiples conjuntos de datos, debido a varios procesos evolutivos que pueden actuar de manera diferente en árboles de genes individuales, incluida la clasificación de linaje incompleto (ILS) y la reticulación (Maddison, 1997; Mao et al., 2019; Murillo-A. et al., 2022; Santos et al., 2017). El primero implica la retención de polimorfismos ancestrales entre especies, mientras que el segundo puede ser causado por el cruzamiento entre individuos de diferentes especies (hibridación), la transferencia de genes entre ellas mediada principalmente por retrocruzamiento (introgresión) y/o la transferencia horizontal de genes (Meyer et al., 2017; Twyford & Ennos, 2012). Los resultados muestran que no hay eventos de reticulación entre las especies del complejo *M. haageana* (Figura S8; Capítulo II), mientras que el análisis de coalescencia muestra que es muy probable que el ILS sea la causa de la discordancia filogenética. El sorteo incompleto de linajes es común en grupos con altas tasas de diversificación y divergencia reciente (Meyer et al., 2008). Dichos factores están presentes en *M.* ser. *Supertextae*, ya que es un grupo de reciente divergencia (aprox. 2.1 Ma), y recientemente se ha demostrado que el clado *Mammilaria* s.s. tiene altas tasas de diversificación (Breslin et al., 2022). La presencia de ramas internas cortas es típica de ILS (Gagnon et al., 2022); esto se puede notar en los clados donde se encuentran *M. conspicua* y *M. meissneri* (Figura S5; Capítulo II).

Los análisis muestran que el uso de datos genómicos, morfológicos y ambientales ayuda a establecer los límites taxonómicos en el complejo *M. haageana*. Los datos genómicos de representación reducida, generalmente, ayudan a dilucidar relaciones filogenéticas complejas y a establecer los límites entre especies estrechamente relacionadas (Anderson et al., 2017; Hashemzadeh Segherloo et al., 2021; Pérez-Escobar et al., 2020). Por otro lado, los datos morfológicos de flores y espinas muestran una variación aparentemente continua. Aunque la saturación del color de las flores (Rosas et al., 2022a), el número y la longitud de las espinas radiales son útiles como caracteres de diagnóstico. Este es el primer estudio que prueba estos caracteres morfológicos para la delimitación de especies en el complejo *M. haageana*. Los resultados del PCA de 19 variables bioclimáticas, las diferencias altitudinales, las asociaciones con tipos de suelo y los modelos de nicho, muestran una gran diferencia entre las especies del complejo *M. haageana*, lo que implica que la separación de linajes a través de la divergencia de nicho, mediada por selección disruptiva (especiación ecológica), es muy probable (Cheng et al., 2021). Las fluctuaciones climáticas y geológicas durante el Pleistoceno pudieron generar diferentes condiciones ambientales, donde cada especie tuvo diferentes adaptaciones y se generaron divergencias filogenéticas (Díaz-Castellón et al., 2012; Mastretta-Yanes, et al., 2015; Ortiz-Medrano et al., 2016). Tomando en conjunto estos análisis, nuestra principal conclusión es el reconocimiento de seis especies dentro del complejo *M. haageana*: *M. acultzingensis, M. conspicua, M. haageana, M. lanigera, M. meissneri,* y *M. san-angelensis.*

Conclusiones generales

Los resultados demostraron que el método de GBS es una herramienta útil para la identificación de SNPs, que mejoran la delimitación de especies del complejo *M. haageana.* Aunque los métodos de representación reducida del genoma proporcionan un gran número de loci, es importante tener en consideración los posibles errores introducidos durante la secuenciación y la identificación de loci ortólogos. Actualmente existen programas que ayudan a disminuir la tasa de errores, por lo cual, es importante generar protocolos de optimización de parámetros en los procesos bioinformáticos como los que se propen en este trabajo.

Al integrar diferentes líneas de evidencia (genómica, morfológica y ecológica), se proporcionó un marco sólido para la delimitación de especies del complejo *M. haageana.* Al tomar en cuenta todas las líneas de evidencia de reconocen seis especies dentro del complejo *M. haageana*: *M. acultzingensis, M. conspicua, M. haageana, M. lanigera, M. meissneri,* y *M. san-angelensis*. Sin embargo, aun falta realizar estudios que ayuden a resolver las relaciones al interior de *M. albilanata*, ya que nuestros resultados sugieren que *M. albilata* puede estar integrada por más de dos linajes evolutivamente independientes. También es importante hacer estudios para comprender las relaciones entre *M. albilanata* subsp. *albilanata* y *M. san-angelensis*, lo cual podría ayudar a establecer posibles estrategias para la conservación de *M. san-angelensis.*

Referencias bibliográficas

Anderson, B. M., Thiele, K. R., Krauss, S. L., & Barrett, M. D. (2017). Genotyping-by-Sequencing in a species complex of Australian hummock grasses (*Triodia*): Methodological insights and phylogenetic resolution. PLOS ONE, 12(1), e0171053. https://doi.org/10.1371/journal.pone.0171053

Anderson, E. F. (2001). The cactus family. Timber Press.

Aquino, D., Cervantes, R. C., Gernandt, D. S., & Arias, S. (2019). Species delimitation and phylogeny of *Epithelantha* (Cactaceae). Systematic Botany, 44(3), 600–615. https://doi.org/10.1600/036364419X15620113920635

Aquino, D., Moreno-Letelier, A., González-Botello, M. A., & Arias, S. (2021). The importance of environmental conditions in maintaining lineage identity in *Epithelantha* (Cactaceae). Ecology and Evolution, 11(9), 4520–4531. https://doi.org/10.1002/ece3.7347

Arakaki, M., Christin, P. A., Nyffeler, R., Lendel, A., Eggli, U., Ogburn, R. M., Spriggs, E., Moore, M. J., & Edwards, E. J. (2011). Contemporaneous and recent radiations of the world's major succulent plant lineages. Proceedings of the National Academy of Sciences, 108(20), 8379–8384. https://doi.org/10.1073/pnas.1100628108

Arekar, K., Parigi, A., & Karanth, K. P. (2018). Hybridisation or incomplete lineage sorting: The convoluted story about the origin of the capped-golden langur lineage (Cercopithecidae: Colobinae) (p. 508929). bioRxiv. https://doi.org/10.1101/508929

Arias, S., Guzmán-López, L., Guzmán-Cruz, U., & Vázquez-Benítez, B. (2012). Cactaceae. Flora Del Valle de Tehuacán-Cuicatlán, 95, 1–235.

Blum, W., Lange, M., Rischer, W., & Rutow, J. (1998). *Echinocereus*. Fa. Proost N. V.

Bravo-Hollis, H. (1978). Las Cactáceas de México. Vol. 1. Universidad Nacional Autónoma de México.

Breslin, P. B., Wojciechowski, M. F., & Majure, L. C. (2022). Remarkably rapid, recent diversification of *Cochemiea* and *Mammillaria* in the Baja California, Mexico region. American Journal of Botany, 109(9), 1472–1487. https://doi.org/10.1002/ajb2.16048

Breslin, P. B., Wojciechowski, M., & Majure, L. (2021). Molecular phylogeny of the Mammilloid clade (Cactaceae) resolves the monophyly of *Mammillaria*. Taxon. https://doi.org/10.1002/tax.12451

Butterworth, C. A., & Wallace, R. S. (2004). Phylogenetic studies of *Mammillaria* (Cactaceae) insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. American Journal of Botany, 91(7), 1086–1098.

Cheng, S., Zeng, W., Wang, J., Liu, L., Liang, H., Kou, Y., Wang, H., Fan, D., & Zhang, Z. (2021). Species delimitation of *Asteropyrum* (Ranunculaceae) based on morphological, molecular, and ecological variation. Frontiers in Plant Science, 12. https://www.frontiersin.org/articles/10.3389/fpls.2021.681864

Copetti, D., Búrquez, A., Bustamante, E., Charboneau, J., Childs, L., Eguiarte, L. E., Lee, S., Liu, T. L., McMahon, M., Whiteman, N. K., Wing, R. A., Wojciechowski, M. F., & Sanderson, M. J. (2017). Extensive gene tree discordance and hemiplasy shaped the genomes of North American columnar cacti. Proceedings of the National Academy of Sciences, 114(45), 12003–12008.

Cornejo-Romero, A., Medina-Sánchez, J., Hernández-Hernández, T., Rendón-Aguilar, B., Valverde, P. L., Zavala-Hurtado, A., Rivas-Arancibia, S. P., Pérez-Hernández, M. A., López-Ortega, G., Jiménez-Sierra, C., & Vargas-Mendoza, C. F. (2014). Quaternary origin and genetic divergence of the endemic cactus *Mammillaria pectinifera* in a changing landscape in the Tehuacán Valley, Mexico. Genetics and Molecular Research, 13(1), 73–88.

Cornejo-Romero, A., Vargas-Mendoza, C. F., Aguilar-Martínez, G. F., Medina-Sánchez, J., Rendón-Aguilar, B., Valverde, P. L., Zavala-Hurtado, J. A., Serrato, A., Rivas-Arancibia, S., Pérez-Hernández, M. A., López-Ortega, G., & Jiménez-Sierra, C. (2017). Alternative glacial-interglacial refugia demographic hypotheses tested on *Cephalocereus columna-trajani* (Cactaceae) in the intertropical Mexican drylands. PLOS ONE, 12(4), e0175905. https://doi.org/10.1371/journal.pone.0175905

Cruz, M. Á., Arias, S., & Terrazas, T. (2016). Molecular phylogeny and taxonomy of the genus *Disocactus* (Cactaceae), based on the DNA sequences of six chloroplast markers. Willdenowia, 46(1), 145–164. https://doi.org/10.3372/wi.46.46112

Cuénoud, P., Savolainen, V., Chatrou, L. W., Powell, M., Grayer, R. J., & Chase, M. W. (2002). Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbc*L, *atp*B, and *mat*K DNA sequences. American Journal of Botany, 89(1), 132–144. https://doi.org/10.3732/ajb.89.1.132

De Queiroz, K. (2005). Different species problems and their resolution. BioEssays, 27(12), 1263–1269. https://doi.org/10.1002/bies.20325

De Queiroz, K. (2007). Species concepts and species delimitation. Systematic Biology, 56(6), 879–886. https://doi.org/10.1080/10635150701701083

Díaz-Castellón, R., Hubbard, B. E., Carrasco-Núñez, G., & Rodríguez-Vargas, J. L. (2012). The origins of Late Quaternary debris avalanche and debris flow deposits from Cofre de Perote volcano, México. Geosphere, 8(4), 950–971. https://doi.org/10.1130/GES00709.1

Edwards, E. J., Nyffeler, R., & Donoghue, M. J. (2005). Basal cactus phylogeny: Implications of *Pereskia* (Cactaceae) paraphyly for the transition to the cactus life form. American Journal of Botany, 92(7), 1177–1188. https://doi.org/10.3732/ajb.92.7.1177

Franck, A. R., Cochrane, B. J., & Garey, J. R. (2013). Phylogeny, biogeography, and infrageneric classification of *Harrisia* (Cactaceae). Systematic Botany, 38(1), 210–223. https://doi.org/10.1600/036364413X662105

Gagnon, E., Hilgenhof, R., Orejuela, A., McDonnell, A., Sablok, G., Aubriot, X., Giacomin, L., Gouvêa, Y., Bragionis, T., Stehmann, J. R., Bohs, L., Dodsworth, S., Martine, C., Poczai, P., Knapp, S., & Särkinen, T. (2022). Phylogenomic discordance suggests polytomies along the backbone of the large genus *Solanum*. American Journal of Botany, 109(4), 580–601. https://doi.org/10.1002/ajb2.1827

Haelewaters, D., De Kesel, A., & Pfister, D. H. (2018). Integrative taxonomy reveals hidden species within a common fungal parasite of ladybirds. Scientific Reports, 8(1), 1–16. https://doi.org/10.1038/s41598-018-34319-5

Hashemzadeh Segherloo, I., Freyhof, J., Berrebi, P., Ferchaud, A.-L., Geiger, M., Laroche, J., Levin, B. A., Normandeau, E., & Bernatchez, L. (2021). A genomic perspective on an old

question: *Salmo trouts* or *Salmo trutta* (Teleostei: Salmonidae)? Molecular Phylogenetics and Evolution, 162, 107204. https://doi.org/10.1016/j.ympev.2021.107204

Hernández-Hernández, T., Hernández, H. M., De-Nova, J. A., Puente, R., Eguiarte, L. E., & Magallón, S. (2011). Phylogenetic relationships and evolution of growth form in Cactaceae (Caryophyllales, Eudicotyledoneae). American Journal of Botany, 98(1), 44–61. https://doi.org/10.3732/ajb.1000129

Hunt, D. R. (2011). Focus on the Supertextae. Huitzilopochtlia, 6, 47–51.

Hunt, D. R., Taylor, N. P., & Charles, G. (2006). The new cactus lexicon. DH Books.

Korotkova, N., Aquino, D., Arias, S., Eggli, U., Franck, A., Gómez-Hinostrosa, C., Guerrero, P. C., Hernández, H. M., Kohlbecker, A., Köhler, M., Luther, K., Majure, L. C., Müller, A., Metzing, D., Nyffeler, R., Sánchez, D., Schlumpberger, B., & Berendsohn, W. G. (2021). Cactaceae at Caryophyllales.org – a dynamic online species-level taxonomic backbone for the family. Willdenowia, 51(2), 251–270. https://doi.org/10.3372/wi.51.51208

Lüthy, J. M. (1995). Taxonomische untersuchung der gattung Mammillaria Haw. [Ph. D. Thesis]. Universität Bern.

Maddison, W.P. (1997). Gene trees in species trees. Syst. Biol. 46, 523–536. https://doi.org/10.1093/sysbio/46.3.523

Majure, L. C., Puente, R., Griffith, M. P., Judd, W. S., Soltis, P. S., & Soltis, D. E. (2012). Phylogeny of *Opuntia* s.s. (Cactaceae): Clade delineation, geographic origins, and reticulate evolution. American Journal of Botany, 99(5), 847–864. https://doi.org/10.3732/ajb.1100375

Mao, K., Ruhsam, M., Ma, Y., Graham, S.W., Liu, J., Thomas, P., Milne, R.I., & Hollingsworth, P.M. (2019). A transcriptome-based resolution for a key taxonomic controversy in Cupressaceae. Annals of Botany, 123, 153–167. https://doi.org/10.1093/aob/mcy152

Martínez-Quezada, D. M., Estela, S.-Z., & Arias, S. (2019). Análisis morfométrico del grupo *Selenicereus vagans* (Cactaceae: Hylocereeae) con base en la anatomía del tallo. Revista Mexicana de Biodiversidad, 90, 1–11. https://doi.org/10.22201/ib.20078706e.2019.90.2924

Mastretta-Yanes, A., Moreno-Letelier, A., Piñero, D., Jorgensen, T. H., & Emerson, B. C. (2015). Biodiversity in the Mexican highlands and the interaction of geology, geography and climate within the Trans-Mexican Volcanic Belt. Journal of Biogeography, 42(9), 1586–1600. https://doi.org/10.1111/jbi.12546

Medina-Sánchez, J., McLaren, S. J., Ortega-Ramírez, J., & Valiente-Banuet, A. (2020). Tectono-stratigraphic basin evolution in the Tehuacán-Mixteca highlands, south western México. Heliyon, 6(3), e03584. https://doi.org/10.1016/j.heliyon.2020.e03584

Meyer, M., Stenzel, U., & Hofreiter, M. (2008). Parallel tagged sequencing on the 454 platform. Nature Protocols, 3(2), 267–278. https://doi.org/10.1038/nprot.2007.520

Meyer, B. S., Matschiner, M., & Salzburger, W. (2017). Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika Cichlid fishes. Syst. Biol. 66, 531–550. https://doi.org/10.1093/sysbio/syw069

Murillo-A., J., Valencia-D., J., Orozco, C.I., Parra-O., C., & Neubig, K.M. (2022). Incomplete lineage sorting and reticulate evolution mask species relationships in Brunelliaceae, an Andean family with rapid, recent diversification. American Journal of Botany, 109, 1139–1156. https://doi.org/10.1002/ajb2.16025

Nyffeler, R. (2002). Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trn*K/*mat*K and *trn*L-*trn*F sequences. American Journal of Botany, 89(2), 312–326.

Ortiz-Medrano, A., Scantlebury, D. P., Vázquez-Lobo, A., Mastretta-Yanes, A., & Piñero, D. (2016). Morphological and niche divergence of pinyon pines. Ecology and Evolution, 6(9), 2886–2896. https://doi.org/10.1002/ece3.1994

Padial, J. M., Miralles, A., De la Riva, I., & Vences, M. (2010). The integrative future of taxonomy. Frontiers in Zoology, 7(16). https://doi.org/10.1186/1742-9994-7-16

Pérez-Escobar, O. A., Bogarín, D., Schley, R., Bateman, R. M., Gerlach, G., Harpke, D., Brassac, J., Fernández-Mazuecos, M., Dodsworth, S., Hagsater, E., Blanco, M. A., Gottschling, M., & Blattner, F. R. (2020). Resolving relationships in an exceedingly young Neotropical

orchid lineage using Genotyping-by-sequencing data. Molecular Phylogenetics and Evolution, 144, 106672. https://doi.org/10.1016/j.ympev.2019.106672

Pušić, B., Gregorić, P., & Franjević, D. (2017). What do biologists make of the species problem? Acta Biotheoretica, 65(3), 179–209. https://doi.org/10.1007/s10441-017-9311-x

Rannala, B. (2015). The art and science of species delimitation. Current Zoology, 61, 846–853. https://doi.org/10.1093/czoolo/61.5.846

Reppenhagen, W. (1992). Die Gattung *Mammillaria*. Monographie: Vol. Band 1 und 2. Druckerei Steinhart. Titisee-Neustadt.

Ritz, C. M., Reiker, J., Charles, G., Hoxey, P., Hunt, D., Lowry, M., Stuppy, W., & Taylor, N. (2012). Molecular phylogeny and character evolution in terete-stemmed Andean opuntias (Cactaceae-Opuntioideae). Molecular Phylogenetics and Evolution, 65(2), 668–681. https://doi.org/10.1016/j.ympev.2012.07.027

Rosas, U., Fuentes-Pérez, E. S., Cervantes, C. R., Sandoval-Zapotitla, E., Santiago-Sandoval, I., Arias, S., & Reyes-Santiago, J. (2022). Correction to: Evolution of flower allometry and pigmentation in *Mammillaria haageana* (Cactaceae). BMC Plant Biology, 22(1), 100. https://doi.org/10.1186/s12870-022-03481-4

Sánchez, D., Arias, S., & Terrazas, T. (2013). Análisis morfométrico de las especies de *Echinocereus* sección *Triglochidiati* (Cactaceae) en México. Brittonia, 65(3), 368–385. https://doi.org/10.1007/s12228-012-9285-6

Sánchez, D., Arias, S., & Terrazas, T. (2014). Phylogenetic relationships in *Echinocereus* (Cactaceae, Cactoideae). Systematic Botany, 39(4), 1183–1196. https://doi.org/10.1600/036364414X683831

Sangster, G. (2018). Integrative taxonomy of birds: The nature and delimitation of species. In D. T. Tietze (Ed.), Bird Species: How They Arise, Modify and Vanish (pp. 9–37). Springer International Publishing. https://doi.org/10.1007/978-3-319-91689-7_2

Santos, L., Alves, A., & Alves, R. (2017). Evaluating multi-locus phylogenies for species boundaries determination in the genus *Diaporthe*. PeerJ 5, e3120. https://doi.org/10.7717/peerj.3120

Schlick-Steiner, B., Steiner, F., Seifert, B., Stauffer, C., Christian, E., & Crozier, R. (2009). Integrative taxonomy: A multisource approach to exploring biodiversity. Annual Review of Entomology, 55, 421–438. https://doi.org/10.1146/annurev-ento-112408-085432

Siebert, L., & Carrasco-Núñez, G. (2002). Late-Pleistocene to precolumbian behind-the-arc mafic volcanism in the eastern Mexican Volcanic Belt; implications for future hazards. Journal of Volcanology and Geothermal Research, 115(1), 179–205. https://doi.org/10.1016/S0377-0273(01)00316-X

Solórzano, S., Chincoya, D. A., Sanchez-Flores, A., Estrada, K., Díaz-Velásquez, C. E., González-Rodríguez, A., Vaca-Paniagua, F., Dávila, P., & Arias, S. (2019). De novo assembly discovered novel structures in genome of plastids and revealed divergent inverted repeats in *Mammillaria* (Cactaceae, Caryophyllales). Plants, 8(10), 392. https://doi.org/10.3390/plants8100392

Sturaro, M. J., Rodrigues, M. T., Colli, G. R., Knowles, L. L., & Avila-Pires, T. C. S. (2018). Integrative taxonomy of the lizards *Cercosaura ocellata* species complex (Reptilia: Gymnophthalmidae). Zoologischer Anzeiger, 275, 37–65. https://doi.org/10.1016/j.jcz.2018.04.004

Tapia, H. J., Arias, S., Yáñez-Espinosa, L., & Terrazas, T. (2016). El uso de espinas del tallo en la identificación de las especies de *Neobuxbaumia* (Cactaceae). Revista Mexicana de Biodiversidad, 87(2), 288–300.

Taylor, N. P. (1985). The genus *Echinocereus*. Kew Magazine Monograph. Timber Press, Portland.

Twyford, A.D., & Ennos, R.A. (2012). Next-generation hybridization and introgression. Heredity 108, 179–189. https://doi.org/10.1038/hdy.2011.68

Vázquez-Sánchez, M., Terrazas, T., Arias, S., & Ochoterena, H. (2013). Molecular phylogeny, origin and taxonomic implications of the tribe Cacteae (Cactaceae). Systematics and Biodiversity, 11(1), 103–116.

Walker, J. F., Yang, Y., Feng, T., Timoneda, A., Mikenas, J., Hutchison, V., Edwards, C., Wang, N., Ahluwalia, S., Olivieri, J., Walker-Hale, N., Majure, L. C., Puente, R., Kadereit, G., Lauterbach, M., Eggli, U., Flores-Olvera, H., Ochoterena, H., Brockington, S. F., & Smith, S. A. (2018). From cacti to carnivores: Improved phylotranscriptomic sampling and hierarchical homology inference provide further insight into the evolution of Caryophyllales. American Journal of Botany, 105(3), 446–462. https://doi.org/10.1002/ajb2.1069

Wiens, J. J. (2007). Species delimitation: New approaches for discovering diversity. Systematic Biology, 56(6), 875–878. https://doi.org/10.1080/10635150701748506

Zachos, F. E. (2016). Species Concepts in Biology: Historical Development, Theoretical Foundations and Practical Relevance. Springer International Publishing. https://doi.org/10.1007/978-3-319-44966-1