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Phylogeny and biogeographic history of *Astrophytum* (Cactaceae)

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Abstract—Despite efforts for a complete phylogeny of Cactaceae, generic circumscription and species level relationships remain unsolved. Within *Astrophytum* one species is assigned to a different genus (*Digitostigma*) and two are often treated as a single species (*A. myriostigma* and *A. coahuilense*). *Astrophytum* is endemic to the Chihuahuan Desert, but may also be found in adjacent regions. We reconstructed the phylogeny to date the origin of clades and infer the biogeographic patterns of *Astrophytum* in order to understand the relationships between group members. Three sequences of chloroplast DNA (*rbcL* gene, *trnL-trnF* intergenic spacer, and *trnK-matK* region) were used with Bayesian and Maximum likelihood methods to generate a phylogeny. Divergence times for each node were estimated using an uncorrelated lognormal clock (BEAST) and inferred ancestral distribution using RASP. *Astrophytum* is monophyletic, with six species grouped in two main clades. *Astrophytum caput-medusae* (recognized as *Digitostigma*) is derived within *Astrophytum*. Individuals of *A. myriostigma* and *A. coahuilense* are grouped in independent clades, supporting their recognition as distinct species. Data suggest posterior diversification in the Late Miocene, during a transition to drier climates in North America. Occurrences of *Astrophytum* species outside of the Chihuahuan Desert are attributable to two recent independent events.

Keywords—Cactaceae, Chihuahuan Desert.

Cactaceae is an exceptionally diversified family of angiosperms whose ca. 2,000 species are mainly distributed in arid and semiarid regions of America (Anderson 2001; Hernández-Hernández et al. 2011). Molecular evidence indicates that major diversification events within the family occurred rapidly in the late Miocene (Arakaki et al. 2011; Hernández-Hernández et al. 2014), probably influenced by recent drastic climate changes in these regions. Rapid diversification events make inference of molecular phylogenies difficult, due to incomplete lineage sorting or to the absence of sequence variation. Although remarkable efforts to resolve the Cactaceae phylogeny have been conducted (Butterworth et al. 2002; Nyffeler 2002; Arakaki et al. 2011; Bárcenas et al. 2011; Hernández-Hernández et al. 2011; Vázquez-Sánchez et al. 2013), a complete understanding of infrageneric relationships for most genera are still pending.

According to Bravo-Hollis and Sánchez-Mejorada (1991) and Anderson (2001), *Astrophytum* Lem. s. s. includes species with a consistent morphology: usually simple, globose to shortly-columnar stems, often covered with fine white tufts of trichomes, and 4–10 ribs, the funnel-form flowers are borne on the stem tips, having a pericarpel and receptacular tube with yellowish trichomes and pointed scales. Fruits are globose, semifleshy, with trichomes and dry scales, and they are irregularly dehiscent. Seeds are hat shaped, brownish black, and have a sunken hilum. The number of accepted species in *Astrophytum* is variable, but some of the most comprehensive reviews for Cactaceae recognize four species (e.g. Britton and Rose 1921; Bravo-Hollis and Sánchez-Mejorada 1991; Anderson 2001; Table 1). The latest treatment of Cactaceae (Hunt et al. 2006) recognizes two more species. First, *A. caput-medusae*

(Velasco & Nevárez) D. R. Hunt is considered an “aberrant” species (Hunt 2003, 2005a). It was originally described as a monospecific genus, *Digitostigma caput-medusae* Velasco & Nevárez, based on differences in growth form (long tubercles), plus thickened root and dimorphic areoles (Velasco and Nevárez 2002). The other species is *A. coahuilense* (Moeller) Kaysser, which has been considered conspecific with *A. myriostigma* Lem. by Bravo-Hollis and Sánchez-Mejorada (1991) and Anderson (2001). Hunt et al. (2006), using the previous subgeneric classification of Backeberg (1950), proposed three infrageneric entities based on a combination of morphological features: *Astrophytum* subgenus *Astrophytum* (*A. myriostigma* Lem. and *A. ornatum* (DC.) Britton & Rose), characterized by uniformly yellow tepals and apically dehiscent fruits; *Astrophytum* subgenus *Neoastryphytum* Backeb. (*A. asterias* Lem., *A. capricorne* F. A. C. Weber, and *A. coahuilense*), distinguished by bicolored tepals (yellow with orange or red throat), and fruits with irregular or basal dehiscent; and *Astrophytum* subgenus *Stigmatodactylus* D. R. Hunt (*A. caput-medusae*), with tuberculate stems, bicolored tepals, and irregularly dehiscent fruits (Table 1; Fig. 1). *Astrophytum* s. l. (Hunt et al. 2006) is then accepted as a genus including six species, with a distribution from the southern United States of America (Texas) to central Mexico (Querétaro and Hidalgo) (Anderson 2001).

Extensive efforts to infer phylogenetic relationships within Cactaceae and the tribe Cactaeae have consistently supported a close relationship between *Astrophytum* s. l. and some members of *Echinocactus* Link & Otto, which in turn has been recovered as a polyphyletic genus (Butterworth et al. 2002; Nyffeler 2002; Arakaki et al. 2011; Bárcenas et al. 2011;

TABLE 1. Critical taxonomic classifications of *Astrophytum* species included according to taxonomic revisions (subgeneric classification: A = *Astrophytum* subgenus *Astrophytum*; N = *Astrophytum* subgenus *Neoastrophytum*; S = *Astrophytum* subgenus *Stigmatodactylus*).

Britton and Rose (1922)	Backeberg (1950, 1961)	Sadovsky and Schütz (1979)	Bravo-Hollis and Sánchez-Mejorada (1991)	Anderson (2001)	Hunt et al. (2006)
<i>A. asterias</i>	(N) <i>A. asterias</i>	<i>A. asterias</i>	<i>A. asterias</i>	<i>A. asterias</i>	(N) <i>A. asterias</i>
<i>A. capricorne</i>	(N) <i>A. capricorne</i>	<i>A. capricorne</i>	<i>A. capricorne</i>	<i>A. capricorne</i>	(N) <i>A. capricorne</i>
<i>A. myriostigma</i>	(N) <i>A. coahuilense</i>	<i>A. coahuilense</i>	<i>A. myriostigma</i>	<i>A. myriostigma</i>	(S) <i>A. caput-medusae</i>
<i>A. ornatum</i>	(A) <i>A. mysriostigma</i>	<i>A. crassispinum</i>	<i>A. ornatum</i>	<i>A. ornatum</i>	(N) <i>A. coahuilense</i>
	(A) <i>A. ornatum</i>	<i>A. myriostigma</i>			(A) <i>A. myriostigma</i>
	(N) <i>A. senile</i>	<i>A. niveum</i>			(A) <i>A. ornatum</i>
		<i>A. senile</i>			

Hernández-Hernández et al. 2011; Vázquez-Sánchez et al. 2013). The *Echinocactus* - *Astrophytum* clade may represent an early divergence within tribe Cactaceae (Butterworth et al. 2002; Arakaki et al. 2011; Bárcenas et al. 2011; Hernández-Hernández et al. 2011; Vázquez-Sánchez et al. 2013), although recent studies have helped clarify relationships between *Astrophytum* and Cactaceae members, the circumscription of *Astrophytum* and infrageneric relationships within the genus remain uncertain. Phylogenetic analysis reported by Bárcenas et al. (2011) show that *Astrophytum* s. s. species, including *A. caput-medusae*, make up a monophyletic group. However, this inference was based on sequences from a single locus with poor resolution for interspecific relationships and *A. coahuilense* was not included in their study. Based on combined analysis of chromosomes and RAPD markers, Das (2008) found similarity between taxa with spineless stems (*A. asterias* and *A. myriostigma*), and between taxa with spines present on stems (*A. capricorne* and *A. ornatum*). More recently, Vázquez-Sánchez et al. (2013) reported a phylogenetic analysis for the tribe Cactaceae using five chloroplast DNA regions. Their results are congruent with Bárcenas et al. (2011), showing a possible sister relationship between *A. caput-medusae* and *Astrophytum* s. s., but also suggesting that *A. capricorne* is the sister species of *A. asterias*, while *A. myriostigma* is the sister of *A. ornatum*. *Astrophytum* species have discrete distributions within the Chihuahuan Desert and two adjacent areas. One of the adjacent areas is the Meridional subregion of the Chihuahuan Desert (Hernández and Gómez-Hinostrosa 2011), which is also considered as a peripheral desert (Hafner and Riddle 2011; Gándara and Sosa 2014), referred to here as semidesert region. The other is the lowland plain to the east of the Sierra Madre Oriental known as Tamaulipan mezquital or Tamaulipan thornscrub (Reid et al. 1990; Hernández and Gómez-Hinostrosa 2011).

The aim of our study is to improve the phylogenetic and biogeographic knowledge of the cactus genus *Astrophytum* s. l. using plastid DNA sequences. For this study we used nucleotide sequences of three plastid regions: (1) a fragment of the coding *rbcL* gene, (2) the *trnL-trnF* region (including the *trnL* intron and an intergenic spacer), and (3) the *trnK-matK* intergenic spacer. We inferred phylogenetic trees using maximum likelihood (ML) and Bayesian inference (BI) methods to clarify the taxonomic status of the genus. To test the infrageneric classification proposed by Hunt et al. (2006), we included three to five samples per species, including *A. coahuilense*, which has not been sequenced previously. Additionally, we performed a relaxed molecular clock analysis to provide a time frame to analyze the evolutionary history of the genus. Finally, we reconstructed ancestral distribution areas to infer the biogeographic history of *Astrophytum* and to

contribute to the understanding of the natural history of the Chihuahuan Desert.

MATERIALS AND METHODS

Taxon Sampling—Samples of *Astrophytum asterias*, *A. capricorne*, *A. caput-medusae*, *A. myriostigma*, *A. ornatum*, and some of the species used as outgroups (*Ariocarpus agavoides* E. F. Anderson, *Echinocactus parryi* Engelm., *Geohintonia mexicana* Glass & W. A. Fitz Maur., *Leuchtenbergia principis* Hook., *Lophophora williamsii* (Lem.) J. M. Coult., *Mammillaria candida* Scheidw., and *Strombocactus disciformis* Britton & Rose), were collected by excising the epidermis tissue of fresh stems in native populations, or from the living collection of the Jardín Botánico, Universidad Nacional Autónoma de México (UNAM). The tissue was placed in plastic zip-close bags with 20 g of silica gel. Samples of *A. coahuilense* were obtained from plants from a registered management unit (i.e. authorized nursery SEMARNAT-UMA-MX-VIV-CO//214-MOR/03, Mexico). Attempts to extract DNA from collected tissue of *A. asterias* were unsuccessful, so extractions were performed from fresh tissue of seedlings germinated in laboratory conditions. Additionally, based on previously published phylogenies (Arakaki et al. 2011; Bárcenas et al. 2011; Hernández-Hernández et al. 2011), public sequences from other Cactaceae species were included as outgroups (Appendix 1).

DNA Isolation, PCR Amplification and Sequencing—DNA was isolated from 15–50 mg of fresh or dried tissue using a CTAB protocol (Vázquez-Lobo 1996). For each sample the three chloroplast loci *rbcL*, *trnL-F*, and *trnK-matK* were amplified and sequenced. Amplifications were conducted using 1U GoTaq (Promega, Wisconsin), 1X GoTaq Flexi buffer (Promega, Wisconsin), 2.5mM MgCl₂, 0.4mM dNTP mix (GE healthcare), and 0.4 μM forward and reverse primers. Sequences of primers used for amplifications and sequencing are specified in Table 2. Amplifications were performed with an ABI 2720 Thermal Cycler (Applied Biosystems, California) under the following conditions: 5 min at 95°C; followed by 35 cycles of 1 min at 94°C, 1 min at 56–60°C (depending on particular primers; Table 2), and 1–2 min (depending on the length of target region) at 72°C; and 5 min at 72°C for final extension. When PCR was unsuccessful in amplifying long regions (*trnK-matK* and *trnL-trnF*), shorter segments were amplified using nested primers (*matK3F-trnK2R* and *matKF-matKR* for *trnK-matK*; C-E and D-F for *trnL-trnF*; Table 2). PCR products were sequenced at the High-Throughput Genomics Unit at the University of Washington (www.htseq.org).

Sequence Alignment and Phylogenetic Analyses—Electropherograms were visually inspected using 4Peaks (Griekspoor and Groothuis 2006). Sequences were assembled and aligned manually in Se-AL v2.0 (Rambaut 2002). Given the lack of availability of GenBank sequences for a single *Cylindropuntia* species for all three sequence regions in our study, sequences from two different species were concatenated and used to represent the genus. Models of nucleotide substitution for different regions were selected using the Akaike information criterion (AIC) as implemented in jModeltest 2.0 (Posada 2008). Given that the *trnK-matK* region consisted of an intron and a coding sequence, the two were evaluated independently. A concatenated matrix for the three regions (*rbcL*, *trnL-trnF*, and *trnK-matK*) was used to infer relationships within the genus *Astrophytum* using BI and ML methods. The selected models and the proportion of invariant sites were used to set the priors of BI analyses conducted using MrBayes 3.2 (Ronquist et al. 2012) and BEAST 1.7 (Drummond et al. 2012). For BI analysis with MrBayes, two searches of four chains were run through 3,000,000 generations, sampling one tree every 1000 generations, and starting with a random tree. Searches were stopped after both runs

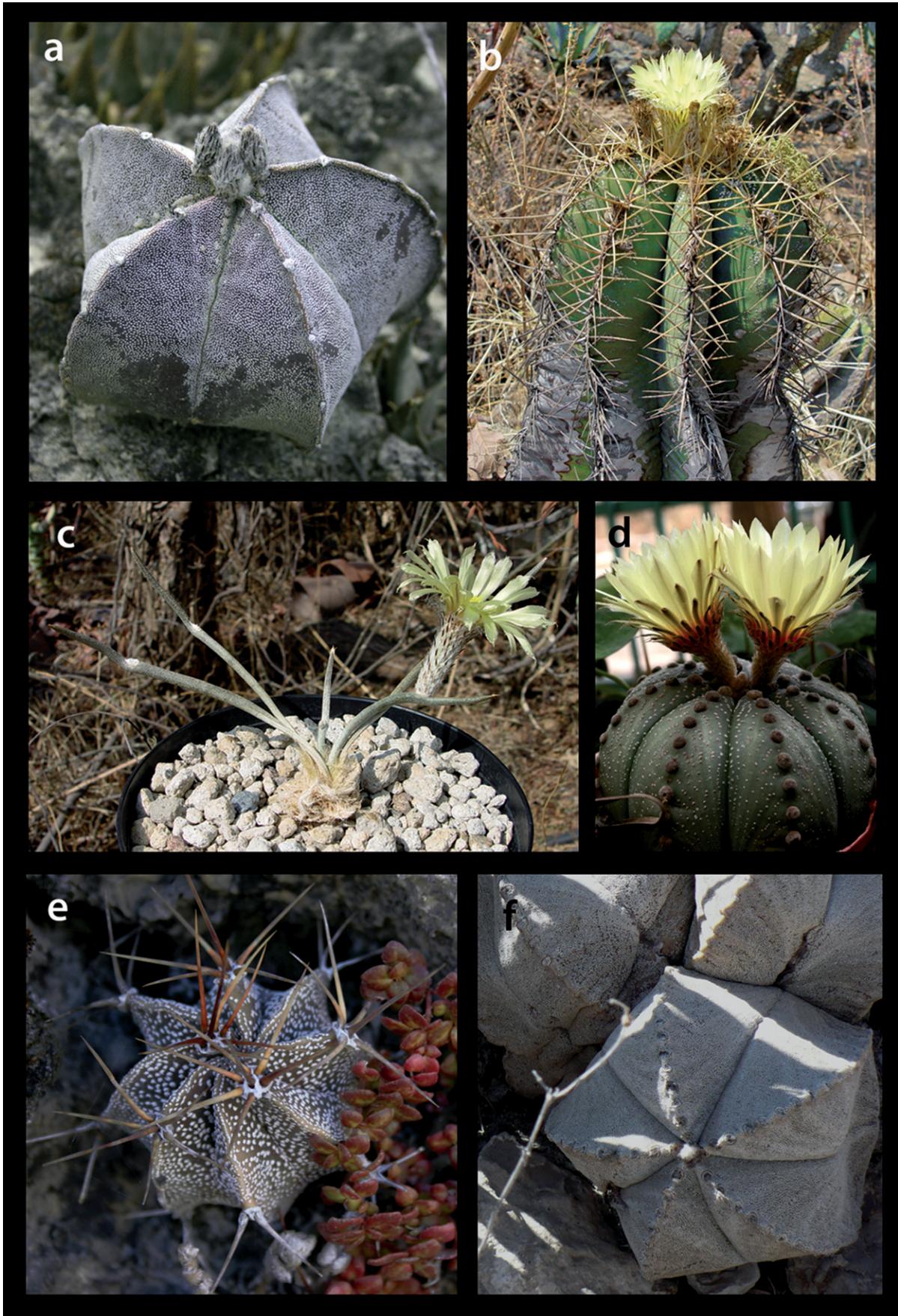


FIG. 1. *Astrophytum* species. A. *A. myriostigma* habitat. B. *A. ornatum* habitat. C. *A. caput-medusae* cultivated at the Jardín Botánico, UNAM. D. *A. asterias* cultivated. E. *A. capricorne*. F. *A. coahuilense*.

TABLE 2. Target region, sequences, and annealing temperatures for the primers used in PCR and sequencing reactions. ¹Primers additionally used for sequencing or to amplify shorter regions are labeled as "nested." ²References: a, this work; b, Taberlet et al. (1996); c, Hernández-Hernández et al. (2011).

Amplified region ¹	Primer name	5'-3' primer sequence	Author ²	(T°C)	Relative position
<i>rbcL</i>	rbcLF	GTGTTGGATTAAAGCAGGTG	a	58	1–21
	rbcLR	ACGGTCTCTCCAACGCATAAATG	a	58	600–624
<i>trnL-trnF</i>	C	CGAAATCGGTAGACGCTACG	b	60	1–20
	F	ATTTGAAGTGGTGACACGAG	b	60	1163–1182
nested <i>trnL-trnF</i>	E	GGTCAAGTCCCTCTATCCC	b	60	702–721
	D	GGGGATAGAGGGACTTGA	b	60	705–722
<i>trnK-matK</i>	matK3F	GGATGAAGCAAGGAATTCGTCTAC	c	56	1–24
	matKR	CGTACAGTACTTTTGTGTTTACG	a	56	2,006–2,028
nested <i>trnK-matK</i>	matKF	CCCATTTCATCTGGAAATCTTG	a	56	1,136–1,156
	trnKCR	GACTGTATCGCACTATGTATC	a	56	852–872
	trnKC2R	GACTATTCCAATTACGATACT	a	56	1,224–1,244

converged, and 25% of the trees were discarded as burn-in. The topology of the best-scoring tree and values for 100 bootstrap replicates using the ML criterion were obtained using default values in RAXML 7.7.1 software in the BlackBox web-service (Stamatakis et al. 2008). Divergence times for each node were coestimated with phylogeny using an uncorrelated log-normal clock, as implemented in BEAST (Drummond et al. 2012), through 3 runs of 10,000,000 generations, sampling one tree every 10,000 generations and constraining the age of the Cactaceae crown group to a range between 8.33–17.27 mya (Hernández-Hernández et al. 2014). For the final tree we use a conservative burn-in of 50%.

Biogeographical Inferences—Ancestral distribution areas for *Astrophytum* were inferred using the Bayesian Binary MCMC (BBM) analysis and the statistical dispersal-vicariance analysis (S-DIVA; Yu et al. 2010) implemented in RASP (Yu et al. 2015). Given the wide distributions of several included species from the Cactaceae, and the uncertainty regarding relationships of major clades within the tribe, only *Astrophytum* and *Echinocactus* were considered for inferring the ancestral distribution of the genus, and based on Hernández-Hernández et al. (2011) and Arakaki et al. (2011) the *Aztekium* clade was used as an outgroup (including *Geohintonia* and *Aztekium* Boed.). For this purpose an ultrametric sub-tree was con-

structed in BEAST, only including one sequence for each *Astrophytum* species and the two associated clades: *Echinocactus* species (*E. parryi*, *E. platyacanthus* Link & Otto, and *E. horizontalonius* Lem.) and the *Aztekium* clade. The age for the *Astrophytum* crown group (5.7 mya) obtained in the total analyses was used to constrain ancestral areas inference. The distributions for included species were assigned to five areas following proposed subregions of the Chihuahuan Desert (Hafner and Riddle 2011): A) the Tehuacan-Cuicatlan Valley, B) the southern semiarid slopes of the Sierra Gorda (semidesert and Mezquital), C) a Chihuahuan Desert area delimited to the north by the Sierra de Parras and extending to the western slopes of the Sierra Madre Oriental and to the south to the Sierra Gorda (Zacatecan subregion), D) the northern Chihuahuan Desert corresponding to Mesa del Norte or northern plateau (Coahuilan subregion), and E) the eastern lowland plains of the Sierra Madre Oriental from Tamaulipas to southeast Texas (Tamaulipan mezquital or thornscrub) (Fig. 2). Areas B and C belong to the region called Mesa Central (Mexican central plateau), although different authors consider the southern region (semidesert and Mezquital) independent from the Chihuahuan Desert (Hafner and Riddle 2011; Gándara and Sosa 2014). This region has been influenced by the volcanic activity of

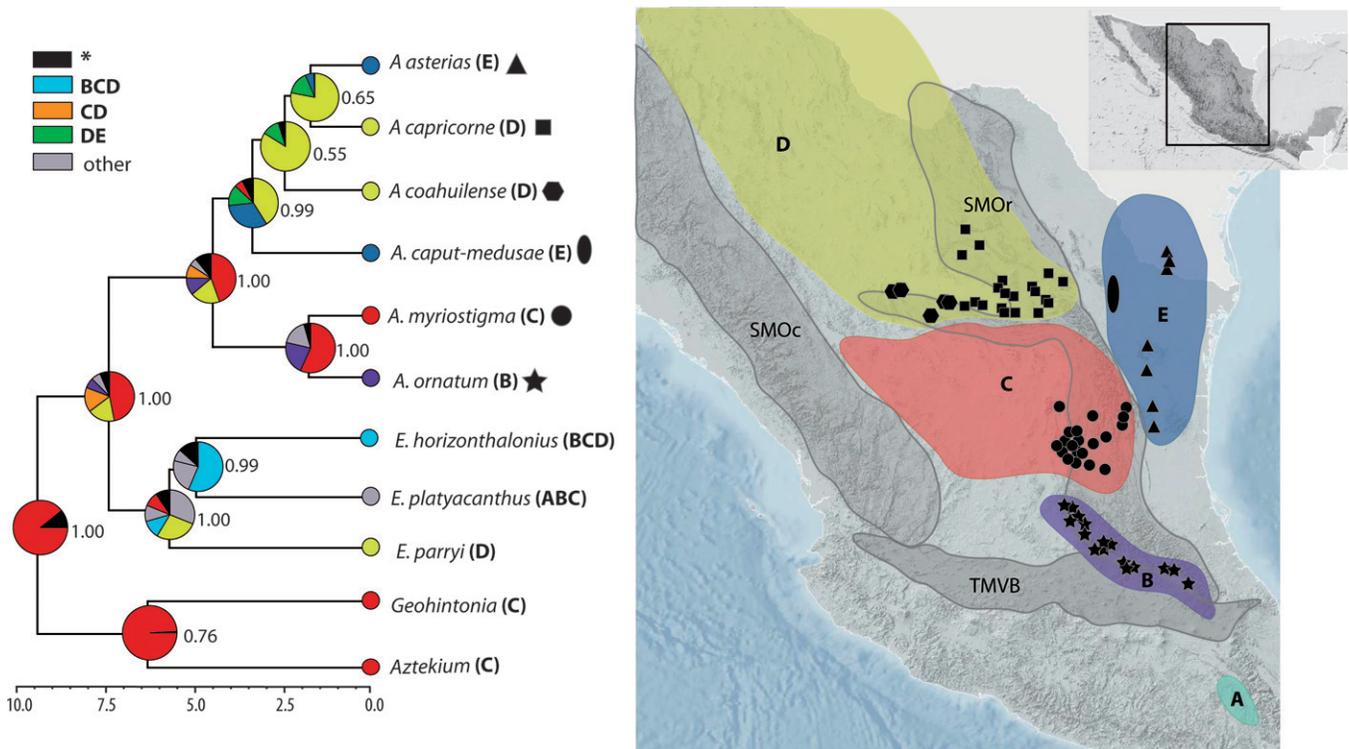


FIG. 2. Inference of the *Astrophytum* ancestral distribution using BMM. Pie charts for each node indicate probabilities for each alternative ancestral range. Probability of inference for each node is shown. A color key for each region is indicated on the map. Upper left corner color key corresponds to some of the likely distributions that include more than one region. Black color indicates the alternative distribution ranges with lowest probability (*). Ruler indicates time in millions of years. SMOOr = Sierra Madre Oriental, SMOc = Sierra Madre Occidental, TMVB = Trans Mexican Volcanic Belt.

the Trans Mexican Volcanic Belt (TMVB) and species distribution patterns within some genera of cacti suggest that a barrier could exist or could have existed between these two areas (Hernández and Gómez-Hinostrosa 2011). For the BBM analysis 50,000 generations were run in 10 simultaneous chains, sampling one state every 100 generations over 9,000 ultrametric trees obtained using BEAST. The selected input model was Fixed JC+G (Jukes-Cantor + Gamma) and the maximum number of areas was set to 5 with a null root distribution. The same trees were used for the S-DIVA analysis with default values and constraining the search to 4 maximum areas at each node.

RESULTS

Sequences and Phylogeny—The resulting three-locus matrix consisted of 41 taxa and 3,195 nucleotides with 2,772 invariable sites and 423 variable sites (matrix is available at TreeBASE, <http://treebase.org/treebase-web/home.html>: M30148). There were only a small number of cases for which we could not amplify any part of a sequence region: *trnK-matK* for *A. capricorne* 5; and *trnL-trnF* for *A. coahuilense* 2 and *Turbinicarpus beguinii*. Some PCR amplifications were not successful for the *trnK-matK* region, but partial sequences of the *matK* gene were obtained. The models selected through the AIC implemented in jModeltest for the *rbcL*, *trnK* intron, *matK*, and *trnL-trnF* loci were HKY+I, F81, TPM3uf+I, and TPM1uf, respectively.

Our topologies indicate that the genus *Astrophytum* comprises the six species recognized by Hunt et al. (2006) and partially hypothesized by Bárcenas et al. (2011) and Vázquez-Sánchez et al. (2013). The resulting topology recovered two main clades of *Astrophytum* with a consistency index of 0.87 (Fig. 3) (tree is available at TreeBASE, <http://treebase.org/treebase-web/home.html>: Tr85662). The first clade includes *A. ornatum* with three samples and *A. myriostigma* with four samples. The second clade includes *A. caput-medusae* (three samples) as the sister species of *A. coahuilense* (three samples), *A. asterias* (four samples), and *A. capricorne* (five samples). All three samples of *A. coahuilense* are grouped into a monophyletic group, and are nested in a different clade from that of the *A. myriostigma* samples (Fig. 3). The topologies retrieved from the ML and BI analyses were identical for relationships within *Astrophytum* with high support for BI, although relationships among different genera were not well supported with different methods (Fig. 3 and Fig. S1). An *Echinocactus* clade (*E. platyacanthus*, *E. horizontalonius*, *E. parryi*) is consistently retrieved as the sister group of *Astrophytum*, whereas the *Aztekium* clade (in this case represented by *Geohintonia mexicana* and *Aztekium ritteri*) appears, with low support, as the sister group to the remaining Cactaceae genera (Fig. 3).

Node Age Estimation and Inferences of Ancestral Distribution—Inferences for the ancestral distribution of *Astrophytum* were different between the two methods. For most nodes, BBM assigned a major probability to only one area (Fig. 2) whereas using the S-DIVA approach, multiple alternative ancestral areas were inferred with the same (or similar) probability (Fig. S2). Using the BBM method and dates estimated using BEAST, the most probable ancestral area of distribution for the stem group of the genus was the central plateau of the Chihuahuan Desert (Zacatecan subregion) (area C; Fig. 2) about 7.7 mya (2.9–12.6 mya) (Fig. 3). In this suggested scenario, an ancestral species extended its distribution to the north reaching the Coahuilan subregion (D) and a later vicariant event would have led to the divergence between species distributed on the northern and central plateaus (areas C and D; Fig. 2) 5.96 mya (1.9–10.6 mya, Fig. 3). Species in the

northern plateau reached the eastern lowlands of the SMOR and diverged in two independent events, the first 4.5 mya (1.2–8.75 mya) and the second 2.5 mya (0.4–5.7 mya) in the splits of both *A. caput-medusae* and *A. asterias*, respectively (Figs. 2, 3). Meanwhile, about 3.7 mya (0.5–7.7 mya), the common ancestor of *A. myriostigma* and *A. ornatum* reached the southern region of the SMOR with a later vicariant event leading to the origin of these two species (Figs. 2, 3). Analyses with S-DIVA did not allow inference of a single ancestral area for the genus; rather, the method inferred two alternative ancestral distributions with similar probabilities and covering multiple areas (Fig. S2), and for this reason we decided to only discuss results based on the Bayesian inference of ancestral distributions.

DISCUSSION

Membership and Relationships within *Astrophytum*—*Astrophytum* is a monophyletic genus that includes six described species with discrete distributions. With regard to the infra-generic classification of Hunt et al. (2006), only *Astrophytum* subgenus *Astrophytum* is recovered as a monophyletic group (*Astrophytum* subgenus *Euastrrophytum* sensu Backeberg 1950), comprising *A. myriostigma* and *A. ornatum* (Table 1, Fig. 3). Both species share invariable yellow color of inner tepals, and apical dehiscence of fruit (Fig. 1). The remaining species form a clade that includes species of the *Astrophytum* subgenus *Neoastrrophytum* (sensu Backeberg 1950) and *A. caput-medusae*, sharing bicolored inner tepals, and red fruit with basal (or nearly basal) dehiscence. Our phylogenetic inferences support the membership of *A. caput-medusae* within *Astrophytum* subgenus *Neoastrrophytum*, and it shares the two reproductive characters noted. The debate over recognizing a monotypic genus *Digitostigma* (Velazco and Névarez 2002) or incorporating it into *Astrophytum* (Hunt 2003, 2005a; Hunt et al. 2006) is here addressed. Changes in vegetative structures should not be underestimated as potential synapomorphies, but we argue that vegetative structures should be evaluated in conjunction with those reproductive features that provide a strong phylogenetic signal. Moreover, changes in vegetative structures between related species may be more common. Vázquez-Sánchez et al. (2013) recognize that *Leuchtenbergia principis*, with a stem consisting of long podaria, is a member of a well-defined *Ferocactus* clade, where members possess spherical stems and no podaria. In fact, Butterworth et al. (2002) and Hernández-Hernández et al. (2011) also suggests that long podaria on the stem are relatively common within Cactaceae and represent a derived condition, as occurs in *A. caput-medusae*.

The taxonomic status of *A. coahuilense* has changed since it was originally described as a subspecies of *Echinocactus myriostigma* (Möller 1927) and later placed as a synonym of *A. myriostigma* by Hunt (1999) and Bravo-Hollis and Sánchez-Mejorada (1991). Characters such as stem form and absence of spines were cited by Bravo-Hollis and Sánchez-Mejorada (1991) as evidence that *A. coahuilense* and *A. myriostigma* represent a single species. In contrast, differences in flower color and fruit dehiscence were reevaluated by Hunt (2005b), who at that time advocated for their recognition as separate species. Our results support this latter proposal, and illustrates the high taxonomic value of reproductive structures as well as geographical range to support species delimitation in the genus. Therefore our results indicate that characters in common

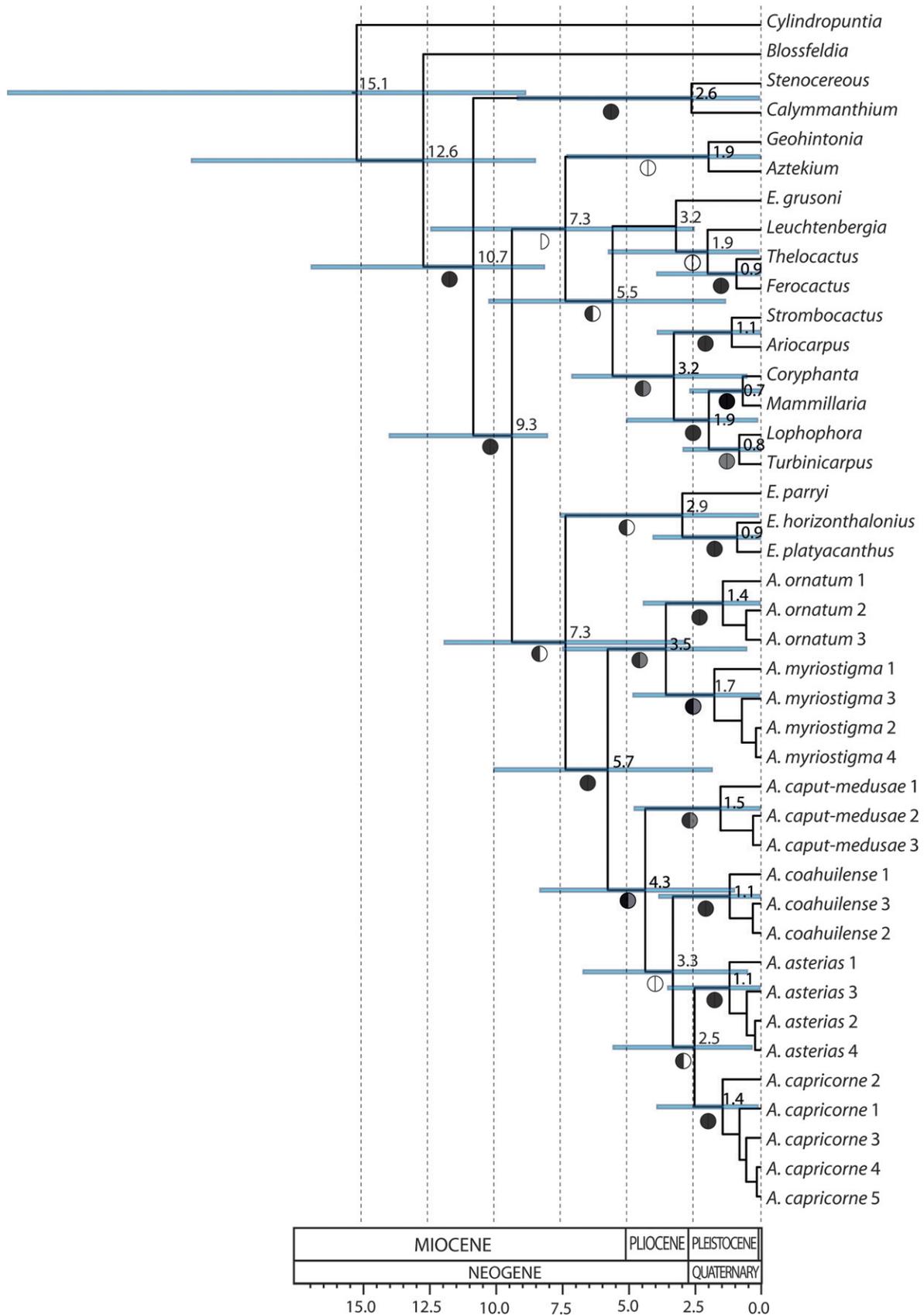


FIG. 3. Phylogeny of *Astrophytum* species and divergence time estimates. Topology and node ages were estimated using BEAST. Blue bars on nodes represent the 95% HPD confidence interval for age estimates. Circles under branches show support values for branches obtained in the consensus tree from BI using MrBayes (left half) and ML using RAxML (right half). Absence of circle indicates that branches were not retrieved in the corresponding analysis. For each circle, black represents 0.95–1.0 posterior probability (left) or 86–100% bootstrap values (right); gray 0.91–0.94 or 71–85% and white < 0.9 or < 70%. Support values within species samples are not shown.

between *A. myriostigma* and *A. coahuilense* are homoplastic. In contrast to Das (2008), who inferred a phylogeny using RAPD markers and karyotype, in our results *Astrophytum* species are neither grouped by the presence of spines (*A. capricorne* and *A. ornatum* appeared in different clades in our study) or chromosome affinities (*A. asterias* and *A. myriostigma*), suggesting that evolution of these traits occurred independently among clades. It is also possible that samples identified as *A. myriostigma* by Das (2008) were in fact *A. coahuilense*. Even though chloroplast topology is congruent with the species distribution, Das' (2008) result suggests that nuclear markers should be included in future research.

In accordance with previously inferred phylogenies for Cactaceae, in our results *Astrophytum* shares an immediate common ancestor with some *Echinocactus* species that make up the *Echinocactus* clade (Butterworth et al. 2002; Bárcenas et al. 2011; Hernández-Hernández et al. 2011; Vázquez-Sánchez et al. 2013). In the BI consensus tree the relationships between the *Aztekium* clade and the remaining sampled genera are not resolved (Fig. 3). Other inferences with a wider taxon sampling, based either on the *trnK-matK* region (Bárcenas et al. 2011), the *trnL-trnF*, *trnK-matK* regions, *rpl16* intron, and the fourth intron of the phosphoenolpyruvate carboxylase (*ppc*) gene (Hernández-Hernández et al. 2011) or the *trnK-matK* region and phytochrome C (*PHYC*) gene (Arakaki et al. 2011), retrieved the *Aztekium* clade (*Aztekium* and *Geohintonia*) as the sister group of the *Echinocactus* clade. These previous studies coincide at least in one of the plastid regions used in our study, and Bárcenas et al. (2011) only included plastid sequences. Therefore the lack of resolution for basal relationships within Cactaceae in our analyses could be related more to a smaller taxon sampling than to a different phylogenetic signal.

Origin and Diversification of *Astrophytum*—Based on our estimates, *Astrophytum* stem group origin and divergence from *Echinocactus* occurred during the Late Miocene, (7.7 mya, 2.9–12.6 mya; Figs. 2, 3), during the transition to more arid conditions in North America (Fig. 2). Posterior diversification events within the genus occurred during the Pliocene. *Astrophytum* distribution is associated with the Sierra Madre Oriental (SMOr), although development of this mountain system ended during the Middle Eocene (40 mya; Cevallos-Ferriz and González-Torres 2005), therefore distribution patterns for the genus should be influenced by more recent events, such as climate changes.

Considering different types of evidence, authors have estimated contrasting ages for the Chihuahuan Desert, ranging from 15 my (Middle Miocene) to 10,000 yr or younger (Wilson and Pitts 2010). A clear trend to aridity in Central North America during the Pliocene has been documented (Eronen et al. 2012), although young fossil assemblages of species of cooler and wetter environments are found in many portions of the Chihuahuan Desert (Wilson and Pitts 2010). Considering that the Late Miocene and Pliocene were characterized by episodic drastic climatic fluctuations with a global cooling trend (Zachos et al. 2001), the establishment of the complete range and conditions of the modern Chihuahuan Desert may have occurred very recently, although evidence suggests that different subregions have been established since the Late Miocene (Arakaki et al. 2011, Gándara and Sosa, 2014). This scenario of climate fluctuation could explain the vicariant diversification pattern in *Astrophytum* species, as occurs in many other cactus species (Hernández et al. 2010)

characterized by poor seed dispersal where long distance colonization events are unlikely (Hernández et al. 2007). Under certain climatic conditions, a species can increase its distribution due to expansion of suitable environments, and posterior changes would cause a reduction of these environments leading to fragmentation and isolation. Diversification driven by isolation events was found in phylogroups of *Lophocereus schottii* (Engelm.) Britton & Rose in the Baja California peninsula and Sonora (Nason et al. 2002), where the separation of the peninsula from the continent promoted the genetic divergence among groups. In addition, they also infer that populations of this species expanded into the north of Baja California to form a single southern refuge during the contraction and expansion cycles of the Pleistocene.

According to our results, the first divergence within *Astrophytum* occurred at the end of Late Miocene (5.7 mya, 1.7–10 mya; Figs. 2, 3), giving rise to a clade that includes species distributed over the central plateau (*A. myriostigma* and *A. ornatum*) and another with species distributed over the northern plateau or the eastern lowlands of the SMOr (*A. coahuilense*, *A. capricorne*, *A. asterias*, and *A. caput-medusae*). Members of these two clades are separated geographically by a mountain system that belongs to the SMOr (Sierra de Parras and Sierra de Guadalupe; Hafner and Riddle 2011). Inferences of the ancestral distribution for the genus with the BBM method indicated that the ancestral distribution area with the highest probability is the Zacatecan subregion (C) at the central plateau ($p = 46.18\%$; Fig. 2) with a posterior expansion to the Coahuilan subregion at the northern plateau (D). The high species richness of cacti in the Zacatecan subregion (Hernández-Hernández et al. 2011) along with our inference and other recent evidence (e.g. Gándara and Sosa 2014), suggest that environmental conditions for this subregion were established since the Late Miocene.

Posterior divergence between the central plateau species occurred around 3.7 mya (0.52–7.7 mya), coinciding with an activity peak in the TMVB (Adams et al. 2006). This pattern of divergence for species distributed in the boundary between the SMOr and the TMVB (area B, Fig. 2) is observed in other cactus genera, such as *Lophophora*, *Sclerocactus*, and *Thelocactus* (Hernández and Gómez-Hinostroza 2011), suggesting that intense volcanic activity and raising of the TMVB during the Pliocene climate fluctuations could function as a barrier to gene flow, allowing diversification of species in this region.

Remarkably, the two species distributed in the Tamaulipan mezquital (*A. caput-medusae* and *A. asterias*), an area with unusual environmental conditions for Cactaceae species, do not form a monophyletic group. This pattern suggests two independent expansion events leading to colonization of new environments and posterior isolation. During the Pliocene, two important conditions are worth mentioning that may explain speciation patterns for this area. On one hand, the reactivation of the San Marcos fault affected the shape and elevation of the northern slopes of the SMOr (Aranda-Gómez et al. 2005), isolating Tamaulipan mezquital from Chihuahuan Desert; and on the other hand, it is proposed that from the Early to Middle Pliocene, climate in this area (and the entire Gulf of Mexico), contrary to the global trend, was cooler and wetter due to the influence of a climate phenomenon that resembles El Niño (Molnar and Cane 2007). These two conditions could be considered favorable for changes in distribution of species and fragmentation.

Taxonomy of *Astrophytum* becomes complicated when stem characters are considered. We corroborated that absence of spines is a homoplastic character between *A. coahuilense* and *A. myriostigma*, and that these two species are separated phylogenetically. Moreover, evolutionary changes in stem form, such as the transition from globose to tubercular stems, are common for Cactaceae (Butterworth et al. 2002, Hernández-Hernández et al. 2011). Therefore, tubercles commonly are derived characters with low taxonomic value; such is the case for *A. caput-medusae*, which is a derived species within the genus. Inferred divergence times for *Astrophytum* species are congruent with a fluctuating climatic history in the region during the Miocene and Pliocene and are similar to other inferences for the families Cactaceae (Arakaki et al. 2011; Hernández-Hernández et al. 2014) and Agavaceae (Good et al. 2006), suggesting that much of the plant diversity of arid regions of North America has a recent origin. Finally, inferences regarding the ancestral distribution of *Astrophytum* point to the Zacatecan subregion, where high diversity levels for cacti are found (Hernández and Gómez-Hinostrosa, 2011), indicating that this Chihuahuan Desert subregion was established at the end of the Miocene.

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- APPENDIX 1. List of sampled taxa, locality, voucher information and Gen Bank Accession numbers (*rbcL*, *trnK-matK*, *trnL-trnF*). Localities for sequences obtained directly from GenBank are not included. Two sources of tissue for the different species were used: either the tissue was taken from a plant from natural populations or from living collections at the Botanical Garden, UNAM (the latter are marked with * after taxa name). *A. coahuilense* was the only species that was collected from a nursery.
- Astrophytum asterias* 1: González, Tamaulipas, México, 878 MA, Herbario UAT, KC776996, KC776968, KC776942; *Astrophytum asterias* 2: González, Tamaulipas, México, 879 MA, Herbario UAT, KC776997, KC776969, KC776943; *Astrophytum asterias* 3: Vaquerías, Tamaulipas, México, 918 MA, Herbario UAT, KC776994, KC776966, KC776940; *Astrophytum asterias* 4: Vaquerías, Tamaulipas, México, 919 MA, Herbario UAT, KC776995, KC776967, KC776941; *Astrophytum capricorne* 1: Cuatrociénegas, Coahuila, México, C. Gómez 1907, MEXU, KC777019, KC776990, KC776962; *Astrophytum capricorne* 2: Cuatrociénegas, Coahuila, México, C. Gómez 1907, MEXU, KC777020, KC776991, KC776963; *Astrophytum capricorne* 3: Cuatrociénegas, Coahuila, México, C. Gómez 1907, MEXU, KC777016, KC776988, KC776959; *Astrophytum capricorne* 4: San Vicente, Cuatrociénegas, Coahuila, México, C. Gómez 1907, MEXU, KC777017, KC776989, KC776960; *Astrophytum capricorne* 5: Cuatrociénegas, Coahuila, México, C. Gómez 1907, MEXU, KC777018, —, KC776961; *Astrophytum coahuilense* 1: Municipio Viesca, Coahuila, México, 2298 H.M. Hernández, MEXU: 649020, KC777013, KC776985, KC776957; *Astrophytum coahuilense* 2: Cerro bola, Coahuila, México, 2298 H.M. Hernández, MEXU: 649020, KC777014, KC776986, —; *Astrophytum coahuilense* 3: Cerro bola, México, Coahuila, 2298 H.M. Hernández, MEXU: 649020, KC777015, KC776987, KC776958; *Astrophytum caput-medusae* 1*: Nuevo León, México, México, S. Arias 1862a, MEXU, KC776998, KC776970, KC776944; *Astrophytum caput-medusae* 2*: Nuevo León, México, S. Arias 1862b, MEXU, KC776999, KC776971, KC776945; *Astrophytum caput-medusae* 3*: Nuevo León, México, S. Arias 1862c, MEXU, KC777000, KC776972, KC776946; *Astrophytum myriostigma* 1: Santa Ana, San Luis Potosí, México, S. Arias 1730, MEXU, KC777007, KC776979, KC776953; *Astrophytum myriostigma* 2: Chihue, Tamaulipas, México, 0063 MA, Herbario UAT, KC777004, KC776976, KC776950; *Astrophytum myriostigma* 3: Chihue, Tamaulipas, México, 1874 MA, Herbario UAT, KC777005, KC776977, KC776951; *Astrophytum myriostigma* 4: Chihue, Tamaulipas, México, 1901 MA, Herbario UAT, KC777006, KC776978, KC776952; *Astrophytum ornatum* 1: Cadereyta, Querétaro, México, S. Arias 1699, MEXU, KC777001, KC776973, KC776947; *Astrophytum ornatum* 2: Cadereyta, Querétaro, México, S. Arias 1699, MEXU, KC777002, KC776974, KC776948; *Astrophytum ornatum* 3: Cadereyta, Querétaro, México, S. Arias 1699, MEXU, KC777003, KC776975, KC776949; *Ariocarpus agavoides*: Tula, Tamaulipas, México, H. Sánchez-Mejorada 3628, MEXU, KC777009, KC776981, KC776955; *Aztekium ritteri*: GenBank —, —, AY015290, —; *Blossfeldia liliputana*: GenBank —, AY875232, AY015283, HM041234; *Calymmanthium substerile*: GenBank —, AY875230, AY015291, DQ099926; *Coryphantha durangensis*: GenBank —, —, HM041671, HM041251; *Cylindropuntia tunicata*: GenBank —, —, —, HM041327; *Cylindropuntia spinosior*: GenBank —, JN796947, FN997455, —; *Echinocactus horizonthalonius*: GenBank —, —, HM041678, HM041258; *Echinocactus grusonii*: GenBank —, —, AY015287, HM041257; *Echinocactus parryi* *: Chihuahua, México, S. Arias 1791, MEXU, KC776993, KC776965, KC776939; *Echinocactus platyacanthus*: GenBank —, AY875215, AY015287, HM041259; *Ferocactus haematacanthus*: GenBank —, —, HM041696, HM041277; *Geohintonia mexicana*: GenBank —, KC787557, HM041280, HM041699; *Leuchtenbergia principis* *: Nuevo León, México, H. Sanchez-Mejorada 3826, MEXU, KC776992, KC776964, KC776938; *Lophophora williamsii* *: San Luis Potosí, México, S. Arias 1849, MEXU, KC777011, KC776983, HM041292; *Mammillaria candida* *: Guanajuato, México, J. Reyes, MEXU, KC777008, KC776980, KC776954; *Thelocactus hastifer*: Querétaro, México, S. Arias 81, MEXU, —, HM041877, HM041367; *Turbincarpus beguinii* *: Nuevo León, México, S. Arias 1852, MEXU, KC777010, KC776982, —; *Stenocereus aragonii*: GenBank —, JQ591000, JQ587179, AY181630; *Strombocactus disciformis* *: Querétaro, México, S. Arias 1738, MEXU, KC777012, KC776984, KC776956.