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# Phylogenomics in Cactaceae: A case study using the chollas sensu lato (Cylindropuntieae, Opuntioideae) reveals a common pattern out of the Chihuahuan and Sonoran deserts

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**PREMISE:** Although numerous phylogenetic studies have been conducted in Cactaceae, whole-plastome datasets have not been employed. We used the chollas to develop a plastome dataset for phylogeny reconstruction to test species relationships, biogeography, clade age, and morphological evolution.

**METHODS:** We developed a plastome dataset for most known diploid members of the chollas (42 taxa) as well as for other members of Cylindropuntieae. Paired-end, raw reads from genome skimming were reference-mapped onto a de novo plastome assembly of one species of cholla, *Cylindropuntia bigelovii*, and were used to build our plastome dataset, which was analyzed using various methods.

**RESULTS:** Our plastome dataset resolved the phylogeny of the chollas, including most interspecific and intraspecific relationships. Tribe Cylindropuntieae arose ~18 mya, during the early Miocene in southern South America, and is supported as sister to the South American clade Tephrocacteae. The (*Micropuntia* (*Cylindropuntia* + *Grusonia*)) clade most likely originated in the Chihuahuan Desert region around 16 mya and then migrated into other North American desert regions. Key morphological characters for recognizing traditional taxonomic series in *Cylindropuntia* (e.g., spiny fruit) are mostly homoplasious.

**CONCLUSIONS:** This study provides the first comprehensive plastome phylogeny for any clade within Cactaceae. Although the chollas s.l. are widespread throughout western North American deserts, their most recent common ancestor likely arose in the Chihuahuan Desert region during the mid-Miocene, with much of their species diversity arising in the early to mid-Pliocene, a pattern strikingly similar to those found in other western North American desert groups.

**KEY WORDS** biogeography; Cactaceae; *Cylindropuntia*; deserts; genome skimming; *Grusonia*; *Micropuntia*; morphological evolution; *Pereskiopsis*; phylogeny.

Putative ages for the formation of the North American desert regions (i.e., Chihuahuan, Great Basin, Mojave, and Sonoran) are still contentious (Wilson and Pitts, 2010). However, there are data suggesting an early to mid-Miocene formation (~15 mya) for the Chihuahuan Desert, while the Great Basin, Sonoran, and Mojave deserts may have formed later. Results of other studies suggest that the now separated Chihuahuan, Sonoran, and Mojave deserts were once part of a large dry region (called "Mojavia"; Morafka, 1977), although their division into distinct deserts may have occurred much later, in the late Pliocene (~2 mya), as a result of Neogene mountain building. The Great Basin Desert may have begun developing in the late Miocene (~8 mya) along with the uplift of the Sierra Nevada range (Axelrod, 1940; reviewed in Wilson and Pitts, 2010). Regardless of the lack of consensus about the timing of desert formation, it is clear that most western North American desert floras have increased in extent and diversified substantially since the Plio-Pleistocene epochs (Raven, 1963; Wilson and Pitts, 2010, and references therein). Included within the floristic components that have evolved and invaded

those arid lands are the Cactaceae, which are represented by members of numerous clades in those areas (Engelmann, 1856; Britton and Rose, 1919; Benson, 1982; Anderson, 2001; Parfitt and Gibson, 2003; Pinkava, 2003a, b; Powell and Weedin, 2004; Hunt et al., 2006; Guerrero et al., 2018). The timing of the diversification of certain clades of cacti (Arakaki et al., 2011; Hernández-Hernández et al., 2014) also is in line with some putative ages for desert formation in western North America (see Wilson and Pitts, 2010).

Tribe Cylindropuntieae s.s. (*sensu* Doweld, 1999; *non* Nyffeler and Eggli, 2010, which included some members of tribe Tephrocacteae) form a clade (Wallace and Dickie, 2002; Wallace and Gibson, 2002; Crozier, 2005; Griffith and Porter, 2009; Arakaki et al., 2011; Hernández-Hernández et al., 2011; Bárcenas, 2016) of mostly cylindrical-stemmed succulents (Fig. 1A–J) that occur broadly throughout the Americas, from southern South America to the western United States. Griffith and Porter (2009) resolved Cylindropuntieae as sister to Tephrocacteae in part, which was recovered as polyphyletic in their analysis, and Wallace and Dickie (2002), Crozier (2005), Edwards et al. (2005), Bárcenas et al. (2011), and Hernández-Hernández et al. (2011, 2014) recovered the clade as unresolved with members of tribes Opuntieae and Tephrocacteae. Arakaki et al. (2011) recovered Opuntieae as sister to Tephrocacteae but with very weak support (bs = 27; fig. S3 in their analyses), and Walker et al. (2018) and Wang et al. (2018), using transcriptome data, likewise resolved Tephrocacteae as sister to Opuntieae, also a poorly supported position (pp = 0.71) and based on limited taxon sampling. Ritz et al. (2012) recovered *Pereskiopsis diguetii* (F.A.C.Weber) Britton & Rose (i.e., Cylindropuntieae) as sister to a Tephrocacteae + Opuntieae clade, albeit with no support. Thus, relationships among those three clades in Opuntioideae are contentious. The chollas (*Cylindropuntia* [Engelm.] F.M.Knuth) and dog chollas or club chollas (*Grusonia* Rchb. ex Britton & Rose, *Micropuntia* Daston), as they are colloquially named (Fig. 1B–J), are an iconic group of cacti that occur broadly throughout parts of western North America. Here, we refer to the three genera (*Cylindropuntia*, *Grusonia*, and *Micropuntia*) informally as "chollas s.l." Most species occur in the Chihuahuan, Great Basin, Mojave, or Sonoran deserts, including Baja California. However, two species also occur in the Greater Antilles: *Cylindropuntia caribaea* (Britton & Rose) F.M.Knuth on Hispaniola and *C. hystrix* (Griseb.) Areces on Cuba. Likewise, *C. leptocaulis* (DC.) F.M.Knuth, the Christmas cholla or tasajillo, is widespread in the Chihuahuan and Sonoran deserts and can be found in central and eastern Texas (Benson, 1982; Pinkava, 2003a; Powell and Weedin, 2004), and *C. tunicata* (Lehm.) F.M.Knuth has been introduced widely into parts of South America (Ritter, 1980; Madsen, 1989; Anderson, 2001; Pinto and Kirkberg, 2009; Hunt, 2014; Ostolaza, 2014; Rodriguez et al., 2018). *Cylindropuntia* is the largest genus in the tribe, with ~39 species (Anderson, 2001; Pinkava, 2003a; Baker and Cloud-Hughes, 2014; Rebman, 2015); *Grusonia* (including *Corynopuntia* F.M.Knuth) is the second largest, with ~22 taxa (Pinkava, 2003b; Rebman, 2009; Donati, 2010, 2012, 2017a, b; Fenstermacher, 2016); and *Micropuntia* is usually circumscribed as one species, *M. pulchella* (Engelm.) M.P.Griff. (Griffith, 2002).

Recent phylogenetic analyses of the chollas s.l. have shown that *Grusonia* s.s. (i.e., *G. braditiana* [J.M.Coult.] Britton & Rose; Fig. 1C) is sister to what has, on occasion, been circumscribed as *Corynopuntia* (Fig. 1D) and that *Micropuntia pulchella* (Fig. 1B) is not resolved within *Grusonia* (Griffith, 2002; Griffith and Porter, 2009; Bárcenas, 2016), although it had been placed there traditionally (see Anderson, 2001; Griffith, 2002; Pinkava, 2003b; Bárcenas, 2004). Earlier analyses resolved *Grusonia* either nested within *Cylindropuntia* or likewise with *G. bradtiana* as sister to *Cylindropuntia* + other *Grusonia* (Griffith, 2002; Griffith and Porter, 2009), although resolution and clade support were lacking in those analyses. *Micropuntia* has been shown to be either sister to the rest of the chollas (*Grusonia* s.l. + *Cylindropuntia*; Griffith and Porter, 2009) or sister to *Pereskiopsis* forming a clade sister to the rest of the chollas (Bárcenas et al., 2011; Bárcenas, 2016). Likewise, previous work has shown that *Marenopuntia* Backeb. (i.e., *Grusonia marenae* [S.H. Parsons] E.F. Anderson) is nested within *Grusonia* (Griffith and Porter, 2009; Bárcenas, 2016). However, most work in Cylindropuntieae has resulted in poorly resolved (or poorly supported) clade and species relationships, and biological data (e.g., ploidy data) have not been taken into consideration during phylogeny reconstruction.

*Cylindropuntia* spp. have been introduced into countries including Australia, South Africa, and Spain, where they have become considerably invasive weeds (Mathenge et al., 2010; Deltoro et al., 2013; Jones et al., 2016). Biological control of those invasive species requires understanding species limits and their appropriate insect parasitoids, such as scale insects (*Dactylopius* spp.; Zimmerman and Granata, 2002; Paterson et al., 2011), and would also be aided by understanding the detailed phylogenetic relationships of those species (Mathenge et al., 2009, 2010; Jones et al., 2016). Likewise, a well-resolved phylogeny of *Cylindropuntia* and relatives would serve greatly in coevolutionary studies with cactophagous insects, such as pyralid moths, for which certain chollas are the host species (e.g., Simonsen, 2008; L.C. Majure, personal observation). Furthermore, providing firm divergence-time estimates for the major clades and species within Cylindropuntieae could aid in our understanding of their use and potential dispersal by Pleistocene mammals such as *Nothrotheriops shastense* Hoffstetter (the extinct shasta ground sloth), and *Neotoma* spp. (packrats or woodrats; Thompson et al., 1980; Jansen, 1986; Van Devender, 1987; Betancourt et al., 1990), as well as their broad prehistoric and historical use by humans (Diguet, 1928; Bravo-Hollis and Sánchez-Mejorada, 1991; Felger and Moser, 1991; Minnis, 1991; Reinhard and Hevly, 1991; Hodgson, 2001; Riley, 2012). Lastly, the development of a robust diploid phylogeny will enable us to more rigorously test parentage of putative auto-/allopolyploids in the group (see Baker and Pinkava, 1987, 1999, 2018; Mayer et al., 2000, 2011; Pinkava, 2002; Baker and Cloud-Hughes, 2014).

The purpose of the present study was to better understand major clade and species relationships, biogeographic history, age, and morphological evolution of the chollas s.l. within North American deserts and to determine the phylogenetic utility of plastome data in Cactaceae. Considering that no phylogenetic studies in Cactaceae have used whole plastome data for specific clades, we sequenced nearly entire plastomes for tribe Cylindropuntieae as a test case, with a focus on diploid chollas. Using our plastome topology, we reconstructed ancestral areas for the chollas and estimated their putative ages. We also carried out ancestral state reconstructions for key morphological characters that have been used traditionally to recognize species groups.

## MATERIALS AND METHODS

### Taxon sampling, DNA extraction, and sequencing

Considering our knowledge of ploidy in this group (Baker and Pinkava, 2018), we took a diploids-only approach to phylogeny


**FIGURE 1.** Members of tribe Cylindropuntieae. (A) *Quiabentia verticillata* showing large, bifacial, functionally photosynthetic leaves, and erect, cylindrical stems (cultivated at Museo de Historia Natural, Lima, Peru). (B) *Micropuntia pulchella* showing large tuberous taproots, glochid-clothed lower stems, and short clavate, tuberculate stems (White Pine County, Nevada; *Puente 5289*). (C) *Grusonia bradtiana* showing erect, ribbed stems and large leaves on upper, new growth (cultivated at Desert Botanical Garden). (D) *G. parishii* showing strongly flattened spines, spreading growth form, and glochid-covered fruit (Mohave County, Arizona; *Majure 5407*). (E) *Cylindropuntia spinosior* with floral buds, showing long, unifacial (cylindrical) leaves, glochid-like spines on the pericarpel, and extrafloral nectaries from the areoles (cultivated at Desert Botanical Garden). (F) *C. leptocaulis* showing mature, smooth, red fruit (Yavapai County, Arizona). (G) *C. fulgida* showing chaining fruit, glochid-like spines on some immature fruit, and purple-pink flowers (Yavapai County, Arizona; *Majure 5376*). (H) *C. spinosior* showing yellow, tuberculate, and spineless fruit (Pima County, Arizona; *Majure 6697*). (I) *C. multigeniculata* showing spiny fruit (Clark County, Nevada). (J) *C. bigelovii* showing arborescent growth form and easily detaching stem segments at the base of plant (La Paz County, Arizona; *Majure 5424*). Photos taken by L. C. Majure.

reconstruction of the chollas s.l. to try to minimize potential topological misinterpretations by incorporating allopolyploids, and thus taxa of potentially reticulate origin, into phylogenetic analyses (*sensu* Majure et al., 2012). Nearly all known diploid, putative non-hybrid taxa of *Cylindropuntia*, *Grusonia*, and *Micropuntia* (i.e., chollas s.l.) were sampled, including 32 *Cylindropuntia*, eight *Grusonia* s.l., and two accessions of *Micropuntia pulchella*. We also included one sample of *C. bigelovii* (Engelm.) F.M.Knuth from the triploid part of its range (presumably representing autopolyploidy). Three *Pereskiopsis* spp. and one *Quiabentia* sp. were sampled from the Desert Botanical Garden's living collection (https://www.dbg.org/research-conservation/living-collections/). We also sampled members of the closely related tribes Opuntieae (*Opuntia arechavaletae* Speg., *O. quitensis* F.A.C.Weber, and *O. austrina* Small) and Tephrocacteae (*Maihueniopsis camachoi* [Espinoso] F.Ritter, *Tephrocactus alexanderi* [Britton & Rose] Backeb., and *T. articulatus* [Otto] Backeb.). DNA was extracted using a standard CTAB incubation, followed by chloroform/isoamyl alcohol and silica column-based purification steps, as described in Neubig et al. (2014) from silica-dried epidermal or tepal material or from fresh epidermal tissue. In brief, tissues were homogenized using a mortar and pestle, combined with 1.2 mL of CTAB buffer and 10 µL of proteinase K, and incubated for 2 h at 55°C. A 24:1 solution of chloroform/isoamyl alcohol was then added and the mixture was vortexed and spun for 10–15 min. The supernatant was placed directly into an EconoSpin Spin Column for DNA with 400 µL of Qiagen Buffer PB (binding buffer) and 20 µL of 3M NaAc and spun for 1 min, then cleaned once with Qiagen Wash Buffer PE (spinning each time for 1 min). DNA was resuspended in 300 µL of TE (Tris-EDTA) buffer (pH 8.0), and DNA quantity was analyzed on a Qubit 2.0 Fluorometer. Whole genomic DNA was sent to Rapid Genomics LLC (http://rapid-genomics.com/home/; Gainesville, Florida, USA) for library preparation (including shearing) and sequencing via a genome skimming method. Genome skimming involves shallow sequencing of whole genomic DNA using NGS sequencing technology, such as Illumina, which preferentially sequences highly repetitive sequences and thus is very useful for retrieving plastome and nrDNA sequences (Straub et al., 2012; Malé et al., 2014; Ripma et al., 2014; Weitemier et al., 2014; Zeng et al., 2018). All taxa were sequenced on the Illumina HiSeq X platform using paired-end reads (yielding 150 bp reads). Sixty samples were included per lane. Data for two other accessions of *Quiabentia* and *Pereskiopsis*, as well as the outgroup taxa *Pereskia aculeata* Mill., *P. sacharosa* Griseb. (*Pereskia* s.s.; *sensu* Edwards et al., 2005), *Leuenbergeria bleo* (Kunth) Lodé, *Maihuenia poeppigii* Speg., *Weingartia kargliana* Rausch, *Blossfeldia liliputana* Werderm. (Cactaceae), and *Portulaca oleracea* L. (Portulacaceae s.s.) were downloaded from GenBank (Bethesda, Maryland, USA), which were data generated by Arakaki et al. (2011) and Moore et al. (2017) (see Appendix 1). Both newly generated data and data downloaded from GenBank were reference mapped onto our plastome of *C. bigelovii* (see below).

### Data processing and phylogenomic analysis

For a select group of samples, raw reads were imported into the Galaxy portal (University of Florida instance; http://galaxy.rc.ufl.edu). Reads were cleaned using fastq groomer (Blankenberg et al., 2010), and paired-end reads were interlaced with fastQ interlacer and trimmed using fastQ quality trimmer (Blankenberg et al., 2010) with a sliding window of 5 and quality score of 20. Trimmed reads were then assembled using velvet (Zerbino and Birney, 2008) with a hash length of 81 and coverage cutoff of 50%. Velvet assemblies of select taxa were then uploaded into Geneious version 11.1.5 (Biomatters, Auckland, New Zeland) and further assembled using the Geneious *de novo* assembler. *Cylindropuntia bigelovii* (*Baker 18286*) yielded a nearly complete plastid genome from six contigs (~124,000 bp). Subsequently, we *de novo* assembled the raw reads from that same accession using the Geneious assembler, using the default settings and 25% of the raw reads, which yielded a more complete plastome (125,158 bp) from two contigs (the two contigs were then joined at the *trnF-ndhJ* IGS). This plastome was annotated in Geneious using a database based on the *Portulaca oleracea* (NC-036236; Liu et al., 2018) plastid genome downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The fully annotated cholla plastome from *C. bigelovii* was then used as a reference for mapping raw reads from all taxa sampled, including those sequences downloaded from GenBank. We did not perform de novo assemblies on all taxa sequenced for use in alignment building, as taxa within Opuntioideae have been shown to have variable gene order and content, and therefore assemblies cannot always be aligned appropriately (L.C. Majure, unpublished data). Thus, only the large single copy subunit (LSC), small single copy subunit (SSC), inverted repeat (IRb), and *ndhF-ycf1* gene suite (including *rpl32*) were extracted from the consensus sequences of mapped raw reads, concatenated and aligned using the Mafft (Katoh and Standley, 2016) plugin in Geneious, and then checked manually. We removed portions of the alignment from subsequent analysis in regions flanking the IRb (one copy; see below), LSC, SSC, and *ndhF-ycf1* gene suite, because those areas were not consistently alignable, based on what we interpret to be inherent chloroplast genome structural differences among different species across Opuntioideae (Cylindropuntieae, Opuntieae, and Tephrocacteae), which also has been found in numerous *Opuntia* spp. (L.C. Majure, unpublished data).

The plastome alignment (130,132 bp including indels) was analyzed with maximum likelihood (ML) using the RAxML (Stamatakis, 2014) plugin in Geneious, undertaking 100 bootstrap pseudoreplicates under the GTR+Γ model of molecular evolution. We also analyzed our dataset using maximum parsimony (MP) in PAUP* (Swofford, 2003). Our starting tree was generated via random stepwise addition with 100 replicates, and we used tree bisection–reconnection for our branch-swapping algorithm. We then carried out 100 bootstrap pseudoreplicates using the fast-heuristic search, treating all gaps as missing data.

### Divergence time estimates

Divergence time estimates for the family Cactaceae published previously by Arakaki et al. (2011) and Hernández-Hernández et al. (2014) were 35 mya and 32.11 mya for the stem ages and 28.6 and 26.88 mya for crown ages, respectively. Because of the similarity between these two studies for the crown age of Cactaceae, we used the age from Arakaki et al. (2011) to be conservative. We used the plastome dataset (unpartitioned) with a GTR+Γ site model with a yule model prior (uniform distribution), a relaxed clock log normal model, and 200 million generations (chain length). Divergence time estimates were undertaken in BEAST version 2.4.8 (Drummond and Rambaut, 2007; Suchard and Rambaut, 2009) on the CIPRES Science Gateway (Miller et al., 2010). Four separate runs under each of two scenarios were made: (1) with just the crown age constrained

at 28.6 mya with a log normal distribution (in real space) with a standard deviation of 0.02; and (2) with the crown age constrained to 28.6 mya (in the same manner) and *Leuenbergeria* constrained as sister to the rest of the family (Appendices S1–S2). The logs of these runs were examined in Tracer version 1.7 (Rambaut et al., 2018) to estimate convergence of parameter estimates before the resulting trees from the four runs were combined, with burn-in removed, in LogCombiner version 2.4.7. A maximum clade credibility tree with mean heights was calculated from these trees in TreeAnnotator and visualized in FigTree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

### Biogeographic analysis

Biogeographic areas were based broadly on Brown and Lowe (1980) for North American dry regions, where applicable. Some notable exceptions were the Great Plains and Coastal Plain regions, which are areas not covered by those authors. Biogeographic areas for chollas s.l. were designated as the (1) North/Central American Seasonally Dry Tropical Forest (including the Baja Cape area), (2) California Coastal Scrub/Chaparral, (3) Chihuahuan Desert, (4) Colorado Plateau, (5) Great Basin Desert, (6) Great Plains, (7) Mojave Desert, (8) Sinaloan Thorn Scrub, (9) Sonoran Desert, and (10) the Vizcaino region of the Baja Peninsula (Brown and Lowe, 1980; Brown et al., 2007). Biogeographic delimitations for outgroups were broadly defined as the Chaco formation, Coastal Plain, South America dry desert formation (including high-elevation, dry Andean plains), and South American Seasonally Dry Neotropical Forests (including Peruvian Interandean valleys and Caatinga), based on Pennington et al. (2000). We analyzed putative historical biogeographic scenarios with the program RASP (Yu et al., 2015) using the statistical dispersal-extinction-cladogenesis model (S-DEC; Beaulieu et al., 2013; Yu et al., 2015), which incorporates all the biogeographic range likelihoods at a given node. Those likelihoods of biogeographic ranges are then evaluated using Akaike weights to determine the relative probability of putative ancestral ranges, thus comprehensively accounting for uncertainty in ancestral area reconstruction (Yu et al., 2015), rather than just providing a node likelihood as in the (non-statistical) DEC model.

### Morphological evolution

Character mapping was carried out in Mesquite (Maddison and Maddison, 2017) using MP and ML. For ML, we used the Mk1 model of evolution, which allows for an equally probable rate of change from one state to another. We coded nine morphological characters across our dataset: (1) growth form, (2) stem shape, (3) leaf size, (4) leaf functionality, (5) leaves unifacial vs. bifacial, (6) spine sheath presence, (7) inner tepal color, (8) fruit fleshiness, and (9) fruit with or without spines. Opuntioideae consist mostly of trees and shrubs (Fig. 1A–B, J), typical of Cactaceae, and were coded as such here. Specifically, we coded the growth forms as erect shrubs, spreading shrubs, geophytic shrubs, trees, and herbs (for our outgroup *Portulaca oleracea* L.). We coded key characters of the chollas, such as the presence of spine sheaths, and photosynthetically functional leaves in tribe Cylindropuntieae, which are found in both *Quiabentia* and *Pereskiopsis*. We also coded characters that have been used in taxonomic treatments, such as fruit with or without spines, fruit fleshy or dry at maturity, and inner tepal color (Appendix S3).

## RESULTS

### Plastome sequencing, assembly, and phylogeny

The number of raw reads per accession sequenced ranged from 8 million to 22 million. The quantity of plastid reads mapped to our reference plastome of *C. bigelovii* ranged from 3.5% to 13.02% of the raw reads.

One copy of the inverted repeat was mostly absent (a small portion of the IRa including the *rrn5* gene was present) from our plastome assembly of *Cylindropuntia bigelovii*, and a ~6000 bp inversion of the *trnV-UAC–rbcL* gene suite also was present. Most *ndh* genes, however, appeared to be present and functional in our plastome of *C. bigelovii*. The *ycf2* gene is apparently pseudogenized in *Cylindropuntia*, with only a 1995 bp segment recovered in our *C. bigelovii* plastome, and *ycf1* also was truncated and translocated adjacent to *rpl32*, instead of adjacent to *ndhF*.

High support was shown for Tephrocacteae as sister to Cylindropuntieae (bs = 88/100, ML and MP respectively; Fig. 2) and Opuntieae as sister to that clade (bs = 100/100). *Quiabentia* and *Pereskiopsis* were resolved as subsequent sisters to the MCG (*Micropuntia* + (*Cylindropuntia* + *Grusonia*)) clade (i.e., chollas s.l.; bs = 100/100), and *Micropuntia pulchella* was resolved as sister to the *Grusonia* + *Cylindropuntia* clade (bs = 95/100), confirming its placement outside of the *Grusonia* clade and not closely related to *Pereskiopsis*. *Pereskiopsis aquosa* was sister to the remaining members of the *Pereskiopsis* clade (bs = 100/100). No major structure (i.e., subclades) was seen in the *Grusonia* s.l. clade, but the clade was strongly supported (bs = 100/100), and *G. bradtiana* was resolved as sister to the rest of the clade.

Six major clades of *Cylindropuntia* were resolved and were well supported (bs = 100/100). Those species with multiple accessions (including intraspecific taxa) were resolved as monophyletic, except for *C. californica* (Torr. & A.Gray) F.M.Knuth. *Cylindropuntia californica* var. *parkeri* (J.M.Coult.) Pinkava was resolved as sister to *C. ganderi* rather than closely related to the other two *C. californica* accessions (*C. californica* var. *californica* and *C. californica* var. *rosarica* [G.E.Linds.] Rebman); and *C. alcahes* (F.A.C.Weber) F.M.Knuth s.l., *C. californica* var. *californica*, and *C. californica* var. *rosarica*, although forming a well-supported clade with *C. munzii* (C.B.Wolf) Backeb. (bs = 100/100), were unresolved at the species level. The *Bigelovii* clade was sister to the rest of *Cylindropuntia*. The large *Graveolens* clade included a morphologically heterogeneous group of species, including small, ephemeral shrubs such as *C. davisii* (Engelm. & J.M.Bigelow) F.M.Knuth and large trees like *C. fulgida* (Engelm.) F.M.Knuth. The Christmas cholla, *C. leptocaulis* (de Candolle) F.M.Knuth (i.e., the *Leptocaulis* clade), was found to be phylogenetically unrelated to any other taxa and was sister to the *Imbricata* + (*Acanthocarpa* + *Californica*) clade. The well-supported (bs = 100/100) and morphologically homogeneous *Imbricata* clade was resolved with *C. spinosior* (Engelm.) F.M.Knuth embedded within it. The *Acanthocarpa* clade, which consists principally of taxa from the Sonoran Desert, was sister to the *Californica* clade, which consists of species mostly restricted to the Baja Peninsula and Californian Coastal Scrub/Chaparral. *Cylindropuntia acanthocarpa* (Engelm. & J.M.Bigelow) F.M.Knuth formed a well-supported clade (bs = 100/100) with *C. acanthocarpa* var. *acanthocarpa* sister to *C. acanthocarpa* var. *ramosa* (Peebles) Backeb. + *C. acanthocarpa* var. *thornberi* (Thornber & Bonker) Backeb.

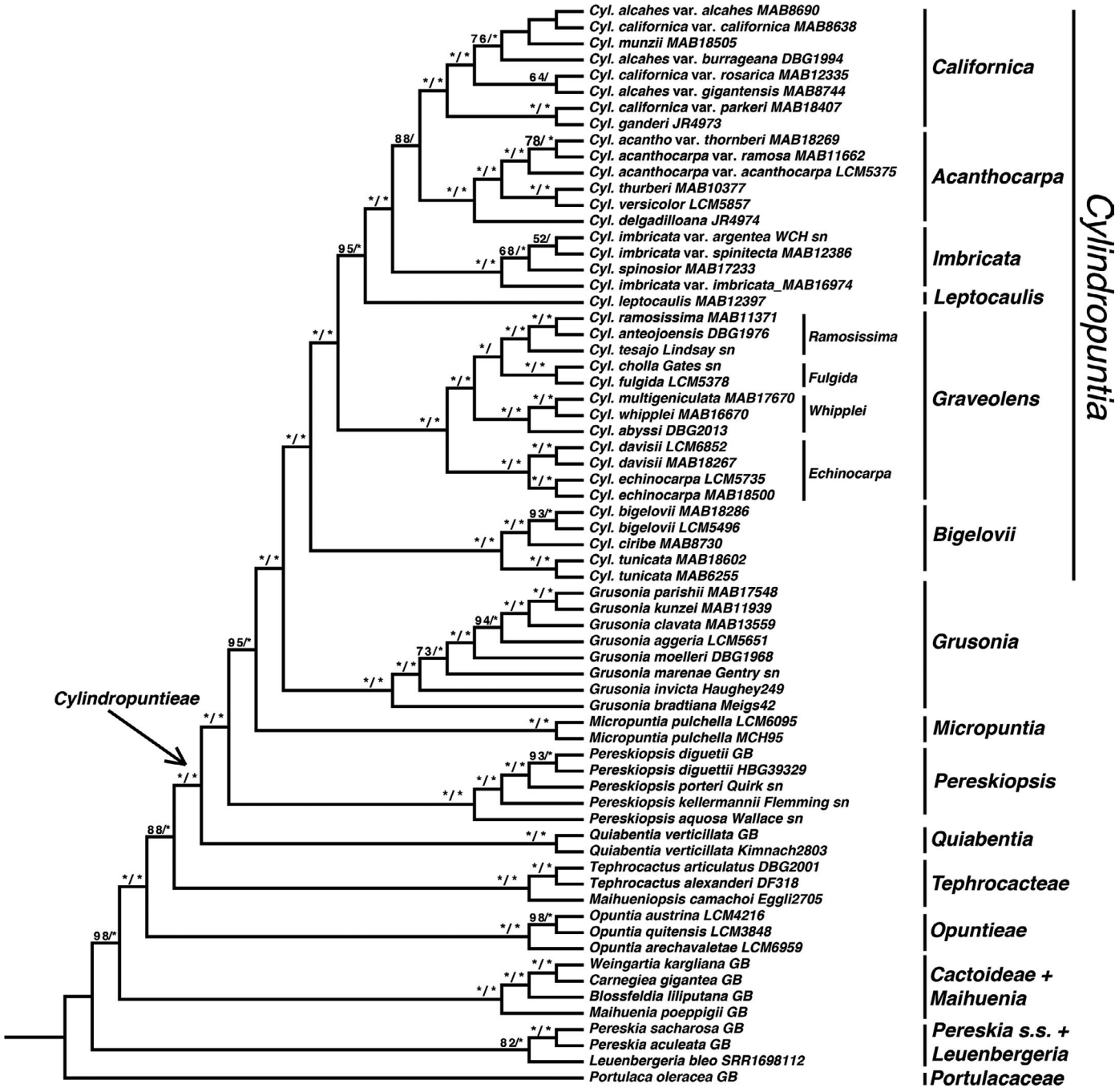


**FIGURE 2.** Most likely topology based on our maximum likelihood analysis of plastome data showing six major clades of *Cylindropuntia*, the *Grusonia*–*Cylindropuntia* sister relationship, and the sister relationship of *Micropuntia pulchella* to the rest of the chollas. Tephrocacteae was resolved as sister to Cylindropuntieae and Opuntieae as sister to that clade. Bootstrap support (ML/MP) of 100% is indicated by an asterisk; values are shown for bootstrap support <100%, but not for bootstrap support <50%.

### Divergence time estimation

Based on our BEAST analyses, subfamily Opuntioideae was estimated to have diverged in the early Miocene, 22.2 (19.3–25.1) mya (Appendix S1), an estimate older than the estimated origin of subfamily Cactoideae at 14.6 (8.8–20.4) mya (Fig. 3). Tribe Cylindropuntieae was found to have diverged in the early to mid-Miocene, 17.9 (14.8–20.8) mya. The MCG clade diverged later in the Miocene, at 15.7 (12.8–18.6) mya, and the *Grusonia* + *Cylindropuntia* clade is estimated to have diverged 12 (9.3–14.5) mya. The *Cylindropuntia* clade diverged in the latter part of the Miocene, 11 (8.5–13.3) mya, and the *Grusonia* clade diverged in the early Pliocene, 5.6 (3.3–8.2) mya. *Quiabentia* (6.8 [2.8–11.4] mya), *Pereskiopsis* (5.6 [2.7–8.9] mya), and *Micropuntia* (4.2 [1.3–7.60 mya] all have divergence times dating to the

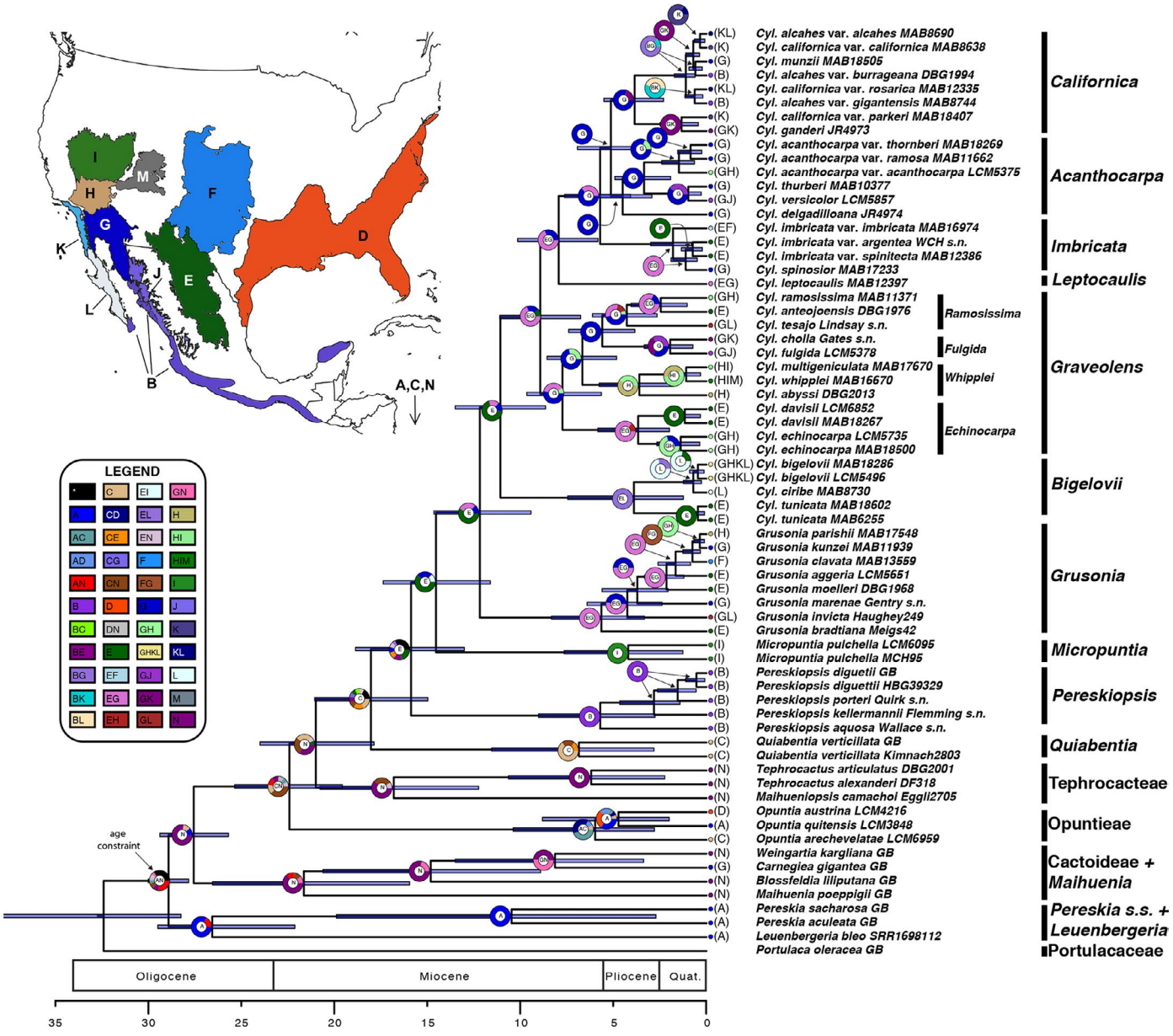


**FIGURE 3.** Maximum clade credibility tree showing estimated divergence time of tribe Cylindropuntieae based on analysis in BEAST. The age of Cactaceae was constrained to 28.6 mya (based on Arakaki et al., 2011). The chollas s.l. (*Micropuntia* + (*Cylindropuntia*+*Grusonia*)) originated during the mid-Miocene according to our analyses. Rectangles correspond to geographic areas or combinations thereof based on (A) South American Seasonally Dry Neotropical Forest, (B) Central/North American Seasonally Dry Tropical Forest, (C) Chaco, (D) Coastal Plain, (E) Chihuahuan Desert, (F) Great Plains, (G) Sonoran Desert, (H) Mojave Desert, (I) Great Basin Desert, (J) Sinaloan Thorn Scrub, (K) California Coastal Scrub/Chaparral, (L) Vizcaino Region, (M) Colorado Plateau, and (N) South America dry desert formation, which are also presented in the map, except for areas A, C, and N, which are not shown. Circles on the phylogeny correspond to our biogeographic analysis using S-DEC (see text), with the most likely ancestral area given in the center.

late Miocene or mid- to late Pliocene (Fig. 3). It is noteworthy that the two most speciose clades within *Cylindropuntia*, the *Graveolens* clade and the clade composed of the *Leptocaulis*, *Imbricata*, *Californica*, and *Acanthocarpa* clades, are older than the rest of the clades in Cylindropuntieae, including *Grusonia*, *Micropuntia*, *Pereskiopsis*, and *Quiabentia*. The oldest age estimates of individual *Cylindropuntia* spp., where we sampled more than one accession per species, ranged from 0.46 to 1.78 mya. The BEAST analyses constraining *Leuenbergeria* as sister to the rest of Cactaceae consistently yielded younger ages for most clades than those presented above (Appendix S2).

## Biogeography

Our S-DEC analysis indicated that the Chihuahuan Desert was the most likely ancestral area for the MCG clade, as well as for the *Grusonia* + *Cylindropuntia* clade (Fig. 3). The *Grusonia* clade was reconstructed as having originated in the Chihuahuan/Sonoran

Desert region, with eventual movements north into the Mojave Desert and the Great Plains and south into the Vizcaino Desert. The *Cylindropuntia* clade most likely originated in the Chihuahuan Desert before moving into the Sonoran Desert. The *Graveolens* + *Californica* clade most likely originated in the Chihuahuan/Sonoran Desert region, and the *Acanthocarpa* + *Californica* clade was reconstructed as Sonoran in origin. The *Graveolens* clade most likely originated in the Sonoran Desert. The *Whipplei* clade most likely originated in the Mojave Desert and from there moved north into the Colorado Plateau and the Great Basin Desert. The *Fulgida* + *Ramosissima*, *Acanthocarpa*, and *Californica* clades also most likely originated in the Sonoran Desert. The *Acanthocarpa* clade then moved north into the Mojave Desert from the Sonoran (*C. acanthocarpa* var. *acanthocarpa*; for a discussion on the circumscription of intraspecific taxa in *C. acanthocarpa*, see Baker et al., 2018), as well as south into the Sinaloan Thorn Scrub (*C. thurberi* [Engelm.] F.M.Knuth). The *Californica* clade moved west into the California Coastal Scrub/Chaparral and south into the Vizcaino and the North/Central American Seasonally Dry Tropical Forest regions (i.e., Baja Cape area), and the *Fulgida* clade moved south into the Sinaloan Thorn Scrub and west into the California Coastal Scrub/Chaparral. The *Ramosissima* clade moved south into the Vizcaino (*C. tesajo* [Engelm. ex Coult.] F.M.Knuth) and Chihuahuan deserts (*C. anteojoensis* [Pinkava] E.F.Anderson) and north into the Mojave Desert (*C. ramosissima* [Engelm.] F.M.Knuth). Our reconstruction suggested a Chihuahuan/Sonoran Desert origin for the *Imbricata* and *Echinocarpa* clades and a Chihuahuan/Vizcaino origin for the *Bigelovii* clade. Altogether, the Sonoran Desert was the area with the highest numbers of speciation events among all the desert regions ($n = 18$).

### Morphological Evolution

The growth form of the most recent common ancestor (MRCA) of the chollas s.l. was reconstructed as an erect shrub, a retained plesiomorphy (Fig. 4A). There was a noticeable shift to spreading shrubs in *Grusonia* (certain members of the *Corynopuntia* group); the erect, shrubby growth form of *G. bradtiana* (Coult.) Britton & Rose, *G. invicta* (Brandegee) E.F.Anderson, and *G. marenae* (S.H.Parsons) E.F.Anderson are retained plesiomorphies. The shift to an arborescent growth form in *Cylindropuntia*, from the erect shrubby growth form, occurred independently several times, in all six major clades (e.g., *C. fulgida* and *C. versicolor* [Engelm. ex Coult.] F.M.Knuth). The cylindrical stem shape of the group is a retained plesiomorphy, having switched to flattened stems in tribe Opuntieae, a putative synapomorphy for that clade (Appendix S4A). The presence of spine sheaths is synapomorphic for the *Cylindropuntia* + *Grusonia* clade (Appendix S4B) according to ML (87%), although sheaths are restricted to the spine apex in *Grusonia* and are sometimes minute, as in *G. invicta*, *G. marenae*, and *G. bradtiana*, which form a grade of subsequent sisters to the rest of the clade.

Photosynthetically functional leaves are homoplasious, having evolved separately in *Pereskiopsis* and *Quiabentia*, although our ML reconstruction suggests that functional leaves in *Maihuenia* are a retained plesiomorphy (81.4%) subsequently lost twice, once in subfamily Cactoideae and once in Opuntioideae (Appendix S5A). Under ML, there is an 84% likelihood that bifacial leaf blades represent homoplasy in Cylindropuntieae and that they evolved separately in both *Quiabentia* and *Pereskiopsis*. Thus, unifacial leaf blades are ancestral for the Cylindropuntieae (Appendix S5B). Large (i.e., macroscopic) leaves on vegetative growth (i.e., stems) are plesiomorphic in Opuntioideae according to our reconstruction, and "small" (mostly microscopic) leaves are thus a shared, derived feature (i.e., synapomorphy) of subfamily Cactoideae (Appendix S7).

Yellow or yellow-green inner tepals are plesiomorphic in Cylindropuntieae. Magenta-pink tepals have evolved at least five times, and orange-red tepals have evolved at least four times (Appendix S6A). Spiny, dry fruit at maturity—two characters that are highly correlated and that were used to separate species groups traditionally—are highly homoplasious across the chollas, represented by *Micropuntia pulchella*, *Grusonia bradtiana*, *G. invicta*, and *G. marenae*, as well as by some members of the *Acanthocarpa* clade, certain members of the *Graveolens* clade, and certain members of the *Californica* clade (Fig. 4B; Appendix S6B). From our analyses, it is most likely that the common ancestor of Cylindropuntieae had fleshy, spineless fruit; this also holds for the MRCA of *Cylindropuntia*. However, spiny fruit in *Grusonia* most likely represent the plesiomorphic condition, with spineless fruit being derived in the clade (Fig. 4B).

## DISCUSSION

### Plastome assembly and phylogeny

As has been found in other cacti (Sanderson et al., 2015), one copy of the inverted repeat was mostly absent from *Cylindropuntia bigelovii* assembled in this study (except for a small portion of the IRa including the *rrn5* gene). A ~6000 bp inversion of the *trnV-UAC–rbcL* gene suite also was present, which has been found in *Pereskia* and other cacti, including saguaro (Downie and Palmer, 1994; Wallace, 1995; Sanderson et al., 2015), as well as in several members of Amaranthaceae (Downie and Palmer, 1994). However, unlike the saguaro genome (Sanderson et al., 2015), *ndh* genes appeared to be present and functional in our *C. bigelovii* plastome. Another notable difference between the saguaro genome and *Cylindropuntia* is the placement of *ycf1* adjacent to *rpl32*, instead of beside *ycf2* as in the saguaro plastid genome (Sanderson et al., 2015) or adjacent to *ndhF* as in most typical angiosperm plastomes (see *Portulaca oleracea*; Liu et al., 2018). All other assembled Opuntioideae to date show the same placement of *ycf1* (L. C. Majure, unpublished data).

Our topological results were mostly congruent with what has been found previously in tribe Cylindropuntieae (Griffith, 2002; Griffith and Porter, 2009; Bárcenas, 2016), although with much greater resolution and support (Fig. 2). Although there has been much discussion regarding clade interrelationships in Opuntioideae, our dataset supports (bs = 88/100) Cylindropuntieae as sister to Tephrocacteae, and Opuntieae as sister to that clade (bs = 100/100). *Quiabentia* is supported as sister to the rest of Cylindropuntieae, with *Pereskiopsis* sister to the chollas s.l. (*Cylindropuntia*, *Grusonia*, and *Micropuntia*). *Pereskiopsis aquosa* (F.A.C.Weber) Britton & Rose was sister to the rest of the species sampled here, confirming Arias's (1996) ideas regarding character evolution in that group, from the arborescent growth form in *P. aquosa* to the erect shrub (or modified scandent shrub; Arias, 1996) growth form in the rest of the species. The placement of *Micropuntia* has been debated at length (Daston, 1946; Griffith, 2002; Bárcenas, 2004, Bárcenas, 2016); however, our dataset shows it highly supported as sister to the *Grusonia* + *Cylindropuntia* clade, as shown in Griffith and Porter (2009). Our data show no

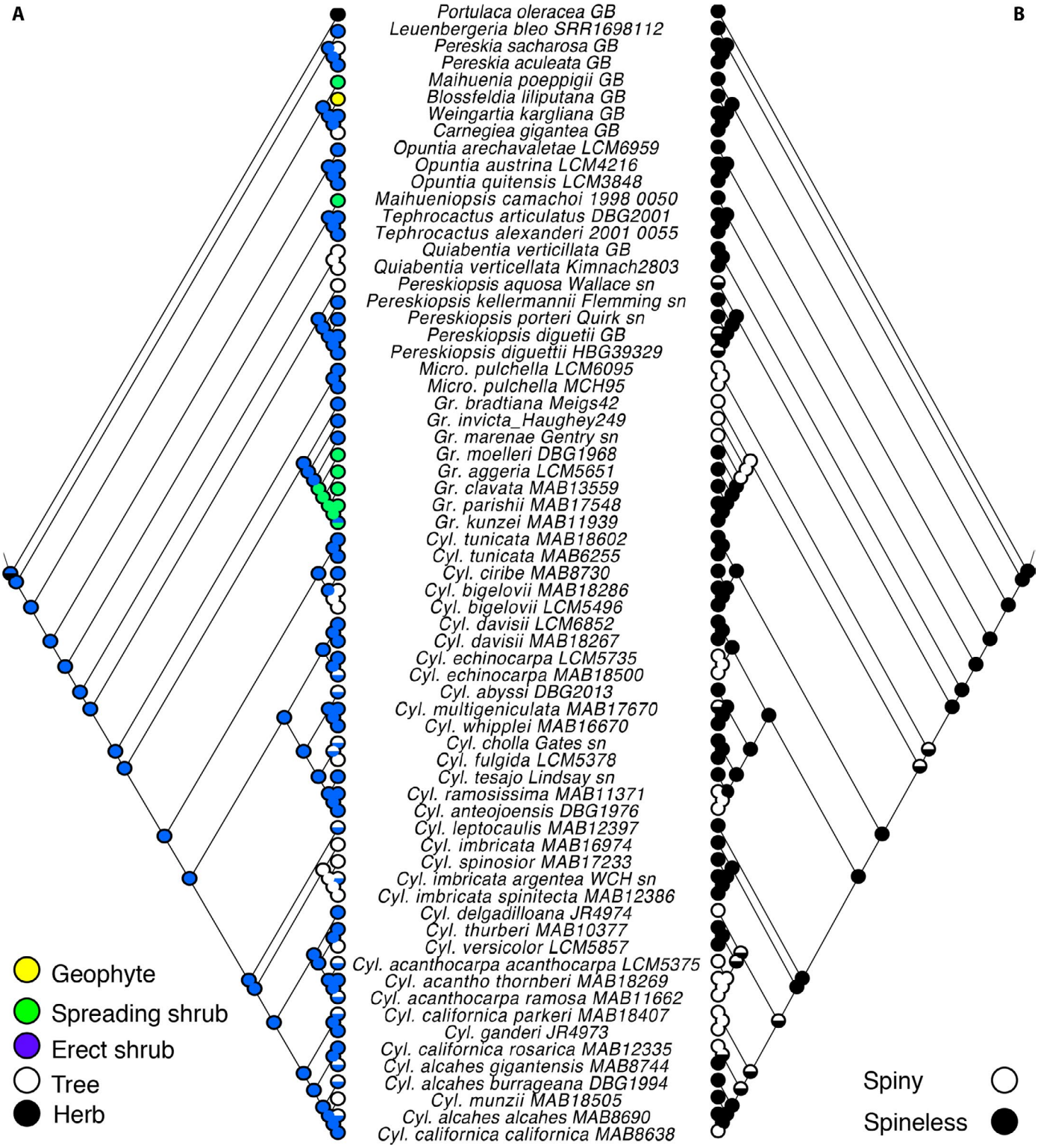


**FIGURE 4.** Ancestral state reconstruction using maximum parsimony in Mesquite. (A) An erect, shrubby growth form (blue circles) is plesiomorphic for the Cylindropuntieae. Spreading shrubs (green circles) are derived in *Grusonia*, and trees (white circles) have formed numerous times in the clade. (B) Spiny fruit (white circles) evolved multiple times in Cylindropuntieae, most likely from a spineless-fruited ancestor (black circles).

indication that *Micropuntia* is sister to *Pereskiopsis*, as in Bárcenas (2016), a position that could have been the result of homoplasy or symplesiomorphy in those data at that level.

As in Bárcenas (2016), our data also resolve the *C. anteojoensis*, *C. ramosissima*, and *C. tesajo* (*Ramosissima*) clade as sister to the *C. cholla* (F.A.C.Weber) F.M.Knuth + *C. fulgida* (*Fulgida*) clade, a

relationship not before proposed on the basis of morphology. Our data show that the *C. echinocarpa* (Engelm. & Bigelow) F.M.Knuth + *C. davisii* (*Echinocarpa*) clade and the *C. abyssi* (Hester) Backeb. + (*C. multigeniculata* [Clokey] Backeb. + *C. whipplei* [Engelm. & Bigelow] F.M.Knuth) (*Whipplei*) clade also form part of this clade (i.e., the *Graveolens* clade; Fig. 2), species previously not sampled in phylogenetic analyses (Griffith and Porter, 2009; Bárcenas, 2016). Baker (2016) hypothesized, on the basis of morphological analyses, that *C. whippeli* and *C. multigeniculata* were close relatives, and our data confirm that relationship. Likewise, Benson (1982) considered *C. multigeniculata* a variety of *C. whipplei*, thus suggesting a very close relationship between the two taxa. Our data do not support a hybrid origin of *C. multigeniculata* from *C. echinocarpa* and *C. whipplei*, as suggested by Pinkava (1999) and as treated by Hunt et al. (2006). Morphological studies by Baker (2016) and Baker and Cloud-Hughes (2014) also evidenced a non-hybrid origin for *C. multigeniculata*. Likewise, our data do not support a putative hybrid derivation of *C. abyssi* from either *C. acanthocarpa* or *C. bigelovii* as has been suggested (Pinkava, 2003a; Hunt et al., 2006), given that *C. abyssi* is not closely related to either of the two latter species.

Although *C. davisii*, which until now had not been analyzed phylogenetically, has been considered closely related to *C. tunicata* (Lem.) F.M.Knuth (Benson, 1982), we found that the species was sister to *C. echinocarpa*. Interestingly, Britton and Rose (1919) and Powell and Weedin (2004) made a similar observation regarding relationships when they considered that *C. davisii* could be closely related to *C. whipplei*, a species recovered in the *Graveolens* clade with *C. davisii* and that exhibits characters similar to those of *C. echinocarpa*. Baker (2016) mentioned the possibility of the diploid *C. echinocarpa* arising as a result of hybridization between *C. acanthocarpa* and *C. multigeniculata*, but our data do not support that hypothesis. The characters linking these species (e.g., spiny, dry fruit) are apparently a result of homoplasy (Fig. 4B).

Hunt et al. (2006) suggested that *C. delgadilloana* Rebman & Pinkava may not be distinct from *C. californica* var. *rosarica*; however, our results show that *C. delgadilloana* is not closely related to *C. californica* but rather is well supported (bs = 100) as sister to the *Acanthocarpa* clade (Fig. 2). Benson (1982) and Hunt et al. (2006) regarded *C. munzii* as a possible hybrid between *C. bigelovii* and *C. acanthocarpa*, and Parfitt and Baker (1993, 2002) considered it a nothospecies, probably of hybrid origin between *C. bigelovii* and *C. echinocarpa*. However, Baker et al. (2012) removed the nothospecies designation and downgraded this putative hybrid origin to a mere possibility. Our analyses show that *C. munzii* is not closely related to either of those species but rather is firmly nested in the *Californica* clade, most closely related to *C. alcahes* and *C. californica*.

*Cylindropuntia versicolor* was resolved as sister to *C. thurberi* in our analysis, a relationship supported by morphology; the two have even been considered conspecific by some (e.g., *Opuntia thurberi* Engelmann subsp. *versicolor* [Engelmann ex J.M. Coulter] Felger; Felger and Lowe, 1970). However, Bárcenas (2016) recovered *C. versicolor* as sister to *C. acanthocarpa* and *C. thurberi* as sister to *C. spinosior*, the latter of which was not well supported in those analyses (pp = 81). We consider that the topological differences could be a result of the use of putative hybrid material for phylogeny reconstruction in the Bárcenas (2016) topology. *Cylindropuntia versicolor* is well known to hybridize with *C. spinosior* and other species (Grant and Grant, 1971; Pinkava, 2003a). Likewise, the incorporation of polyploids in those analyses, as well as the lack of resolution, could be another explanation for the topological differences. We also consider that the material used by Bárcenas (2016) of *C.* cf. *spinosior* may have been misidentified, given that *C. spinosior* is clearly very closely related to *C. imbricata* on the basis of morphology (e.g., purple inner tepals; see Appendix S6) and our plastome topology, where it is nested within *C. imbricata* (Fig. 2).

Bárcenas (2016) recovered *Grusonia kunzei* (Rose) Pinkava as sister to *G. marenae*, although our data show *G. kunzei* to be sister to *G. parishii* (Orcutt) Pinkava (Fig. 2). *Grusonia wrightiana* (E.M.Baxter) E.M. Baxter has long been confused with *G. kunzei* as a result of a nomenclatural problem (Felger et al., 2014), so material used in Bárcenas (2016) may be referrable to *G. wrightiana* instead of *G. kunzei*, hence the topological differences. We did not sample *G. wrightiana* to test this hypothesis, because it is tetraploid (Baker and Pinkava, 2018); however, the placement of *G. kunzei* with *G. parishii* in our analyses is in line with morphology, as the two very closely resemble one another (Felger et al., 2014). Griffith and Porter (2009) resolved *G. invicta* in a subclade with *G. aggeria* (Ralston & Hilsenb.) E.F.Anderson, *G. parishii*, and the polyploid *G. grahamii* (Engelm.) H.Rob., although our topology resolves it as sister to the rest of the *Grusonia* clade after *G. bradtiana*, which also is suggested by the topology of Bárcenas (2016), albeit unresolved. The use of ITS in Griffith and Porter (2009) may have resulted in that topological difference. More comparative phylogenetic work will be necessary to test incongruent topologies between plastome and nrDNA datasets and to determine the significance of those incongruences.

### Divergence time estimation

Although subfamily Opuntioideae has considerably fewer species than subfamily Cactoideae, our divergence time estimates suggest that the clade could be nearly 8 Ma older. The ~16 Ma age for the MCG clade roughly corresponds to the perceived timing of the origin of the Chihuahuan Desert (~15 mya; Morafka, 1977), the putative ancestral area for the clade, in the mid-Miocene, suggesting that the diversification of the clade corresponds with the expansion and opening of desert niches. Those clades with a high probability of originating in the Sonoran Desert (e.g., *Californica* + *Acanthocarpa* and *Ramosissima* + *Fulgida*) are sometimes younger than related clades, which is consistent with a more recent expansion of the Sonoran Desert (Axelrod, 1979). However, the bulk of diversity among the Cylindropuntieae has a relatively young age in general (late Miocene or early Pliocene), suggesting that more recent and extreme aridification of western North America and the expansion of desert areas (Wilson and Pitts, 2010) played a major role in shaping the diversity of the clade. Most species complexes, such as *C. acanthocarpa*, *C. bigelovii*, *C. californica*, and *C. imbricata* (Haw.) F.M.Knuth, have a Pleistocene origin, suggesting that the events surrounding the past ~2 Ma, including increasing aridification in places such as the Sonoran Desert (Wilson and Pitts, 2010), have given rise to further diversity in the clade. That the bulk of speciation events in Cylindropuntieae occurred in the Sonoran Desert also lends evidence to this idea.

### Biogeography

The chollas show biogeographic patterns that are strikingly similar to those of other groups of arid-adapted plants from western North American desert regions that have been studied phylogenetically, including other cacti. Vázquez-Sánchez et al. (2013) showed that

most of the major clades of tribe Cacteae (Cactoideae) originated in the Mexican Plateau, which includes the Chihuahuan Desert. Hernández-Hernández et al. (2014) found further evidence for the origin of Cacteae in the Chihuahuan Desert region, and both studies arrived at a mid-Miocene origin for the clade. Within the prickly pears, the North American *Opuntia* clade is the most diverse in the group, and the *Macrocentra* clade has its roots in the Chihuahuan Desert as well (Majure et al., 2012). The large subclade *Eddya* of the genus *Tiquilia* (Boraginaceae) is restricted to the Chihuahuan Desert, while other members of the *Tiquilia* subclade are found either in other North American deserts or in arid regions of South America (Moore and Jansen, 2006), suggesting a potential Chihuahuan Desert origin for the entire clade. Analyses by De-Nova et al. (2018) of *Fouquieria* likewise suggest a Chihuauhan Desert origin for that group during the mid Miocene. Likewise, the Chihuahuan Desert was reconstructed in our analyses as the most likely ancestral area for the chollas s.l. (the MCG clade) and from there moving into the Great Basin and Sonoran deserts. The consistent split between the Sonoran and Chihuahuan deserts in the chollas, exemplified by Sonoran–Chihuahuan desert species pairs—for example, *C. anteojoensis*–*C. ramosissima* (see also Pinkava, 1976), *C. bigelovii/ciribe*–*C. tunicata*, *C. davisii*–*C. echinocarpa*, and *C. imbricata*–*C. spinosior*—illustrates the significant role the Chihuahuan Desert likely played in the diversification of the clade.

The Sonoran Desert likewise played a very large role in the development of the Cylindropuntieae clade, with several clades originating there (e.g., *Acanthocarpa* and *Fulgida* + *Ramosissima* clades), along with the highest amount of speciation ($n$ = 18) compared to all other biogeographic regions, based on our diploid sampling. The movement from the Sonoran Desert into the California coastal scrub and chaparral, and eventually into the Vizcaino region and Baja Cape, created the perfect window of opportunity for the diversification of the *Californica* clade. The potential for movement from the Sonoran Desert into the Mojave Desert and Colorado Plateau appears evident with the *Whipplei* clade. *Cylindropuntia echinocarpa* most likely moved into the Mojave Desert from the Sonoran Desert, although it will be necessary to test this hypothesis with increased sampling.

The South–North American disjunction in tribe Cylindropuntieae is seen in numerous clades of arid-adapted flowering plants, such as *Cryptantha* (Boraginaceae; Hasenstab-Lehman and Simpson, 2012), *Castela* (Simaroubaceae; Thomas, 1990), *Fagonia* (Zygophyllaceae; Beier et al., 2004), *Grindelia* (Asteraceae; Moore et al., 2012), *Hoffmannsseggia* (Fabaceae; Simpson et al., 2004), *Larrea* (Zygophyllaceae; Lia et al., 2001), *Opuntia* (Cactaceae; Majure et al., 2012; Majure and Puente, 2014), *Parkinsonia* (Fabaceae; Hawkins et al., 2007), *Prosopis* (Fabaceae; Bessega et al., 2006), *Senecio* (Asteraceae; Coleman et al., 2003), and many other groups (see Raven, 1963; Simpson et al., 2017). Many of those disjunctions appear to have originated from north to south, whereas others, like Cylindropuntieae, have moved from south to north (for further examples of this pattern, see Raven, 1963; Wen and Ickert-Bond, 2009; and Simpson et al., 2017).

The South–North American disjunct pattern, and juxtaposed south to north movement, in species distribution as seen in tribe Opuntieae (Majure et al., 2012) is also seen in tribe Cylindropuntieae, with *Quiabentia* from the southern South American Chaco formation and the Caatinga of Brazil sister to the rest of the clade. Likewise, the *Pereskiopsis* spp. in North American Seasonally Dry Tropical Forest are sister to the rest of the more arid-adapted members of the clade (*Cylindropuntia*, *Grusonia*, *Micropuntia*), mirroring the pattern seen in the Seasonally Dry Tropical Forest species of the *Nopalea* clade of *Opuntia* s.s. that are sister to a more xeric-adapted clade (i.e., *Basilares* clade; Majure et al., 2012). Nevertheless, there have been movements out of these xeric zones back into more humid, tropical areas, such as with *C. fulgida* and *C. thurberi* moving into the Sinaloan Thorn Scrub from the Sonoran Desert, and *C. alcahes* moving into the Seasonally Dry Tropical Forest of the Baja Cape area (Fig. 3).

It is likely that populations of *Cylindropuntia* spp. have moved large distances since the Last Glacial Maximum. Van Devender (1987) recorded fragments of *C. whipplei* from packrat midden samples taken from southwestern Arizona in the Sonoran Desert and dated to 14,120 yr before present. Baker (2016), upon closer inspection of those samples, identified them as the close relative *C. multigeniculata*, which now occurs much farther north in the Mojave Desert—the putative ancestral area of *C. multigeniculata* (Fig. 3). Therefore, it seems clear that southern populations of *C. multigeniculata* migrated northward at the end of the Pleistocene, or at least that the southern populations went extinct at some point.

Dispersability of members of Cylindropuntieae has not been studied in detail, although we know from packrat midden fossils and paleontological and ethnobotanical work that mammals have played a role in the dispersal of species over extended periods. Thompson et al. (1980) found remnants of *C. imbricata* in coprolite samples of the now extinct Shasta ground sloth (*Nothrotheriops shastense* Hoffstetter) dating to ~11,330 yr before present. Likewise, chollas (*C. echinocarpa*, *C. leptocaulis*, *C. multigeniculata*, and *C. whipplei*) have been found in fossilized packrat middens dating from 30,000–10,650 yr before present (Phillips, 1977; Thompson et al., 1980; Van Devender, 1987) and are commonly used in recent times as lining material for packrat dens to sway would-be predators, as well as for a food, water, and a shelter resource (Humphrey and Mehrhoff, 1958; Finley, 1990; Vaughan, 1990).

Janzen (1986), although focusing primarily on *Opuntia*, suggested that Pleistocene megafauna played a significant role in the consumption and subsequent dispersal of members of that clade. It is just as likely that those large herbivores played a major role in the dispersal of *Cylindropuntia*. For instance, the fleshy yellow fruit of *C. imbricata*, which contrasts sharply with the dark, lead-green vegetative background (i.e., the stem of the species), is a perfect candidate for large mammal dispersal through fruit consumption and subsequent deposition of seeds upon defecation. The pendant, chaining, fleshy fruits of large tree-like forms of *C. fulgida* also suggest frugivory by large mammals. Likewise, overgrazing in rangelands of the southwestern United States often results in high-density populations of *Cylindropuntia* (Humphrey and Mehroff, 1958), illuminating the effect large migrating herbivores could have on the dispersal of members of this clade, whether it be through consumption of the fruit or deposition and subsequent establishment of vegetative segments through disturbance (Allen et al., 1991). There are numerous *Cylindropuntia* spp. that propagate vegetatively (Rebman and Pinkava, 2001) from easily disarticulating joints (e.g., *C. bigelovii*, *C. fulgida*, and *C. tunicata*), and those species could be moved substantial distances from parent plants by large herbivores. Cattle and other rangeland fauna commonly disperse stem segments of *Cylindropuntia*, which increases the range of those cholla species (Toumey, 1895; Johnson, 1918; Humphrey and Mehroff, 1958);

this may have led to the introduction of *Cylindropuntia* into South America and the Greater Antilles.

### Morphological evolution

The large, bifacial leaves of *Quiabentia* and *Pereskiopsis* are unique in Opuntioideae and most likely evolved independently from those of *Leuenbergeria* and *Pereskia*, the two successive sisters to the rest of Cactaceae. Mauseth (2017) suggested that the large bifacial leaves in *Pereskiopsis* and *Quiabentia* were retained ancestral characters, as have other authors (Arias, 1996; Nobel and Bobich, 2002), which is not supported by our ancestral character state analysis here. However, greater taxon sampling, including more outgroup taxa, will be necessary to fully test this hypothesis. Interestingly, anatomical work by Bailey (1960) suggested that the leaves and stems of *Quiabentia* and *Pereskiopsis* were quite distinct from, and more derived, than "*Pereskia* s.l."; venation in both *Pereskiopsis* and *Quiabentia* is palmate or pseudopalmate rather than mostly pinnate as in "*Pereskia* s.l." (Bailey, 1960). That both taxa display derived rather than ancestral characters is further supported here. The independent evolution of large, flat photosynthetic leaves would be beneficial in the Seasonally Dry Tropical Forest where both taxa are found, habitats that are quite similar to those occupied by the large-leaved *Leuenbergia* spp. and *Pereskia* spp. (Edwards et al., 2005; Edwards and Donoghue, 2006; Leuenberger, 2008). Edwards and Donoghue (2006) suggested that the loss of photosynthetically functional leaves occurred numerous times in Opuntioideae. However, considering our ancestral state reconstruction here (albeit based on very limited sampling across the subfamily), it appears more likely that the acquisition of photosynthetically functional leaves occurred multiple times, with the loss of photosynthetically functional leaves occurring once in the common ancestor of Opuntioideae. A greater phylogenetic sampling will be necessary to test this across the entire family, including more members of Cactoideae. This interpretation also is based on the assumption that the ephemeral leaves of other Opuntioideae (non-*Austrocylindropuntia*, *Pereskiopsis*, or *Quiabentia*) are not significant with regard to photosynthesis, given that they are reduced and ephemeral. However, currently there are insufficient data on which to base this assumption. It has been shown that certain Opuntioideae (*Opuntia* and *Quiabentia*) are facultatively CAM plants (Koch and Kennedy, 1980; Nobel and Bobich, 2002; Ocampo and Columbus, 2010; Winter et al., 2011), thus opening the opportunity for greater production via something reminiscent of the $C_3$ pathway. Therefore, the production of relatively large leaves could aid during those times of rapid growth under a $C_3$ pathway and may then be more photosynthetically useful than presumed on the basis of their size and duration. Indeed, Martin and Wallace (2000) found that a number of Opuntioideae were either $C_3$–CAM intermediates or CAM-cycling species. Their study included *Cylindropuntia spinosior*, and they found that the ephemeral leaves of that species actually were responsible for most of the daytime uptake of $CO_2$. It is also curious that these ephemeral and supposedly nonfunctional leaves are completely covered in stomata (Eggli, 1984; L. C. Majure, personal observation). The actual photosynthetic capacity of these reduced leaves and photosynthetic pathways of taxa within Opuntioideae need to be examined in much greater detail for a clear understanding of functionality and correlations with other physiological traits.

*Micropuntia pulchella* is unique in tribe Cylindropuntieae for its antrorsely barbed spines on the stem and pericarpel. A similar spine structure in the genus *Gymnocalycium* (Cactoideae, Cactaceae) has been shown to aid in the uptake of water from ambient sources (Liu et al., 2015). Although *Micropuntia* was said to not possess glochids (Daston, 1946), true, retrorsely barbed glochids are present (Robinson, 1974; L. C. Majure, personal observation) both in the stem and pericarpel areoles along the adaxial surface, and retrorsely barbed glochids are present on the lower stem areoles, at the transition of the stem and tuberous central tap root (these were referred to as basal areoles by Bárcenas, 2004). The large, tuberous taproots are found nowhere else in the tribe, although *C. davisii*, *C. imbricata* (Pinkava, 2003a), and *C. californica* var. *parkeri* (Coult.) Pinkava possess branching, tuberous roots (L. C. Majure and R. Puente, personal observation). Although Bárcenas (2004) mentioned the presence of an exfoliating epidermis of the glochid apex in *Micropuntia*, we have not observed any structure equivalent to the spine sheaths of *Cylindropuntia* or *Grusonia* in *Micropuntia*. Likewise, neither *Cylindropuntia* nor *Grusonia* has sheathed glochids, although glochid-like (deciduous) spines are present on the pericarpels of numerous species (e.g., *C. bigelovii*) and possess sheaths, showing their clear homology with non-glochid spines.

The presence of spine sheaths originated with the MRCA of the *Grusonia* + *Cylindropuntia* clade, and apparently they are derived from a deciduous epidermis (Mauseth, 2006). In *Grusonia*, the sheaths are often reduced and may be mostly restricted to the apex of the spine. There is no clear function of the spine sheaths (although they have been shown to have taxonomic value; Baker, 2016), and there appears to be no clear reason why the *Grusonia* clade would produce very reduced sheaths, while *Cylindropuntia* produce sheaths that cover the entire spine. *Quiabentia verticillata* (Vaupel) Backeb. also produces sheath-like structures, although these appear to be derived from hairs, as Ganong (1894) suggested for sheaths in general (see Buxbaum, 1950), and not from an entire epidermal layer. Research is needed to fully understand the development and utility of spine sheaths in this group.

An erect, shrubby growth form is ancestral for Cylindropuntieae (Fig. 4A). Thus, the erect, shrubby growth form of *Grusonia bradtiana*, often cited as a means to separate that species from the rest of *Grusonia*, merely represents a retained plesiomorphy. The erect, shrubby growth form is also seen in *G. marenae* and *G. invicta* (as well as in the polyploid species *G. wrightiana*, not sampled here), with a switch back to an erect, shrubby growth form in *G. kunzei* from the spreading, shrubby habit exhibited by most other *Grusonia*, although *G. kunzei* is polymorphic for growth form, exhibiting both erect and spreading forms.

*Cylindropuntia* spp. that exhibit dry, spiny fruit are often sister to species with fleshy, spineless fruit (Fig. 4; Appendix S6). This can be seen in numerous species pairs, including *C. echinocarpa* + *C. davisii* and *C. whipplei* + *C. multigeniculata*. Likewise, the dry-fruited *C. acanthocarpa* clade is sister to the fleshy-fruited *C. thurberi* + *C. versicolor* clade, and the dry-fruited *C. ramosissima* + *C. anteojoensis* clade is sister to the fleshy-fruited *C. tesajo*.

The *Graveolens* clade formed by *Cylindropuntia echinocarpa* and relatives (Fig. 1), as well as the *Leptocaulis* clade, include species that produce a distinct odor reminiscent of rancid butter or cyanoacrylate (e.g., Super Glue). Although we have not determined the exact chemical origin of that odor, a study is under way and preliminary results suggest that a combination of alkanes, alcohols, and benzyl compounds are responsible (M. Maurer, assisitant research scientist,

Goldwater Enviromental Lab, Arizona State University, personal communication). A similar odor occurs in some species in the genera *Consolea*, *Opuntia*, *Quiabentia*, and *Tacinga* (L. C. Majure, personal observation) and is similar to odors exhibited by *Aloe vera* (L.) Burm. f. Likewise, the sister species in the *Graveolens* clade, *C. cholla* and *C. fulgida*, turn black when their tissue is damaged, a character that is seen in a variety of species in subfamily Cactoideae (e.g., *Leptocereus*, *Pachycereus*, and *Stenocereus*; Gibson and Horak, 1978; L. C. Majure, personal observation) and that may be related to the presence of the glucosidic alcohol lemairin (Mata and McLaughlin, 1980; discussed in Gibson and Nobel, 1986). Thus, it appears likely that the chemical composition of species within specific clades would provide phylogenetically useful data in Cylindropuntieae, as well as within other clades of Cactaceae.

Britton and Rose (1919) subdivided *Cylindropuntia* into different series, which for the most part are not supported by our data. Their series *Echinocarpa*, for example, linked species with spiny fruit, which our data show to be a homoplasious character (Fig. 4B), and thus included members of the *Acanthocarpa* and *Graveolens* clades. Other members of the *Graveolens* clade here (*C. davisii*, *C. whipplei*) were circumscribed under their series *Thurberianae*, which in our topology also contain species of the *Acanthocarpa* clade (*C. thurberi*). Their series *Leptocaules* also included a member of the *Graveolens* clade, *C. tesajo*. Their series *Imbricatae* included members of the *Bigelovii* (*C. tunicata*), *Echinocarpa* (*C. cholla*), *Acanthocarpa* (*C. versicolor*), and *Imbricata* (*C. imbricata*) clades, and series *Fulgidae* included members of the *Imbricata* (*C. spinosior*), *Echinocarpa* (*C. fulgida*), and *Californica* (*C. alcahes*) clades. It is clear that morphology alone, using traditional characters that are subject to rampant homoplasy, is not a sufficient indication of species relationships, and also that certain key morphological characters of the chollas were not considered by Britton and Rose (1919), such as the blackening stems of both *C. cholla* and *C. fulgida* in response to tissue damage, the purple anther filaments of various species, or the dark purple inner tepals of *C. imbricata* s.l. and *C. spinosior*. A much closer inspection of morphological characters will be necessary to determine synapomorphies for the major clades within *Cylindropuntia*.

### Taxonomic implications

Although morphologically very similar, *Pereskiopsis* clearly forms a clade separate from the South American *Quiabentia*. Our results confirm that *Grusonia* s.l. (i.e., including *M. pulchella*) is non-monophyletic, as has been shown by several other authors (Griffith, 2002; Griffith and Porter, 2009; Bárcenas, 2011, 2015). *Micropuntia* clearly forms a clade separate from *Grusonia* s.s. (including *Corynopuntia*) and is sister to *Cylindropuntia* + *Grusonia*. However, our results show no close relationship between *Micropuntia* and *Pereskiopsis*, as was found in the three-locus phylogeny of Bárcenas (2016), where the two taxa were resolved as sisters.

Although certain authors have suggested that *Corynopuntia* be separated from *Grusonia* (Griffith, 2002; Hunt et al., 2006; Donati, 2010, 2014, 2017a, b; Fenstermacher, 2016), there appears to be no substantial reason, morphologically or phylogenetically, to do so. Previous polyphyly issues, other than that of *Micropuntia pulchella* (formerly *Corynopuntia pulchella* or *Grusonia pulchella*), with regard to *Corynopuntia*, *Cylindropuntia*, and *Grusonia*, were mostly based on the lack of phylogenetic resolution (Griffith, 2002; Griffith and Porter, 2009), thereby recovering *Grusonia* nested within *Cylindropuntia*. Unfortunately, the use of poorly supported topologies for taxonomic classification (Hunt et al., 2006; Nyffeler and Eggli, 2010) has led to further taxonomic confusion in this group. Thus, the more reasonable way forward in these situations would be to await more robust phylogenetic hypotheses for these groups, instead of making name changes and cluttering the taxonomic literature even further (e.g., Rowley, 2006; Bulot and Solichon, 2009) before we have a clear understanding of relationships.

*Grusonia* + *Corynopuntia* (i.e., *Grusonia* s.l. as circumscribed in Bárcenas, 2016 and here) form a very well-supported clade (bs = 100; see Fig. 2), and there are no clear morphological characters to separate the two groups. Absence of glochids on the pericarpel areoles and presence of areoles in ribs have been cited as characters separating *G. bradtiana* (Fig. 1C) from the rest of the species (Griffith, 2002); however, upon close inspection *G. bradtiana* does have glochids in the adaxial portion of the pericarpel areoles (although they are the same color as the spines and thus more cryptic; see also Donati, 2014), and the presence of areoles in ribs is a variable character (see also Robinson, 1973; Bárcenas, 2004). Baxter (1932) included *G. bradtiana*, *G. wrightiana*, and *Cylindropuntia santamaria* (E.M.Baxter) Rebman in his concept of *Grusonia*, basing that mostly on the production of areoles in ribs. *Cylindropuntia santamaria* indeed produces areoles in ribs, although the character is more variable in *G. wrightiana*, which tends to produce areoles in the more typical alternate, spiral pattern (L. C. Majure et al., personal observation). Thus, the production of areoles in ribs is not unique to *G. bradtiana* within Cylindropuntieae. *Grusonia bradtiana* produces very few or no glochids in the adaxial portion of the stem areoles, although *G. invicta* likewise does not produce many glochids in the stem areoles on some plants. *Grusonia bradtiana*, like the other *Grusonia* spp., possesses spine sheaths restricted to the apex of the spine (L. C. Majure et al., personal observation), although in *G. bradtiana* the spine sheaths appear to be quickly deciduous, being found mostly on young, developing spines, which may have led Britton and Rose (1919) to suggest that sheaths were lacking altogether. Baxter (1932) and Donati (2014) also suggested that spine sheaths were not present in *G. bradtiana*. *Grusonia invicta* and *G. marenae* likewise produce very short apical spine sheaths, which appear to be quickly deciduous; this makes sense considering the phylogenetic placement of those taxa (Fig. 2). Buxbaum (1950) suggested that spine sheaths are poorly formed or not formed at all in individuals grown in shaded conditions. Considering that much work has been carried out in Cactaceae based on greenhouse-grown plants in suboptimal conditions, the lack of spine sheaths as has been recorded for these species (Baxter, 1932; Donati, 2014) could also be a result of microhabitat. Bárcenas (2004) suggested that the stems of *G. bradtiana* were quadrangular in cross section, but we have not seen quadrangular forms and consider them to be cylindrical, as in other *Grusonia* spp.

Splitting these taxa into two genera would create unnecessary redundancy, wherein *Grusonia* would be monotypic, represented only by *G. bradtiana*. Likewise, *G. marenae* s.l. (including *G. reflexispina* [Wiggins & Rollins] E.F.Anderson) is quite morphologically distinctive, as compared to the rest of the species of the *Corynopuntia* group, which is why it had been placed in its own genus or subgenus (*Marenopuntia*) by some authors. However, morphological distinctiveness based mostly on gestalt, rather than actual shared, derived characters (i.e., synapomorphies), is not a robust method for separating genera, as we have shown that certain "distinctive" characters of *G. bradtiana* and *G. marenae* (e.g., erect, shrubby growth form) actually represent plesiomorphy. So, from an evolutionary perspective, these two taxa are exhibiting ancestral characters while most

other members of *Corynopuntia* exhibit the derived feature of a spreading shrub. Thus, no real information is gained by splitting these taxa into two genera, and we suggest they be circumscribed under *Grusonia* s.l., as advocated by Robinson (1973) and Bárcenas (2004, 2015). Alternatively, *Corynopuntia* could be recognized as a subgenus of *Grusonia*, as has been advocated by some authors (Robinson, 1973; Stuppy, 2002), but this would have to include the erect shrubs *G. invicta* and *G. marenae*. Otherwise, *G. invicta* and *G. marenae* would have to be circumscribed as separate monotypic subgenera, which would merely create redundancy. Because only diploid species were used in our analyses, we cannot make any informed statement with regard to the phylogenetic placement of polyploid species, which could complicate a subgeneric-level classification even further. As an example, Bárcenas (2016) included material of *G. kunzei*, which was resolved as sister to *G. marenae* in his analyses. Unfortunately, *G. kunzei* has long been confused with *G. wrightiana* (Baxter, 1935; Pinkava, 2003b; Felger et al., 2014), so it seems likely that the *G. kunzei* material used by Bárcenas (2016) was actually of the tetraploid and erect shrub *G. wrightiana* (*G. kunzei* is strongly supported as sister to *G. parishii* here; see Fig. 2), meaning that if subgenus *Marenopuntia* were recognized, *G. wrightiana* would likely have to be included. Likewise, we do not know if the polyploid *G. wrightiana* could be derived from hybridization between *G. marenae* and a member of traditional *Corynopuntia*, which would mean that subgeneric delimitations would be even less appropriate and necessarily polyphyletic.

*Cylindropuntia californica* is clearly non-monophyletic, with *C. californica* var. *parkeri* resolved highly supported as sister to *C. ganderi* (C.B.Wolf) Rebman & Pinkava (Fig. 2). These two taxa intergrade in the northern and western part of the range of *C. ganderi* (Pinkava, 2003a; M. A. Baker, M. Cloud-Hughes, and J. P. Rebman, personal observation), which may further indicate their close relationship. The most useful solution to this scenario is to recognize those two taxa as separate species (see Baker and Pinkava, 2018). *Cylindropuntia californica* var. *californica* and *C. californica* var. *rosarica* are unresolved within the clade containing *C. alcahes* and *C. munzii*. Further work will be necessary to sort out species relationships in the *Californica* clade.

*Cylindropuntia spinosior* is clearly nested within *C. imbricata* and exhibits the same sets of morphological characters as that species (e.g., tuberculate, yellow fruit, and magenta inner tepals, as well as strongly tuberculate stems). The maintenance of *C. spinosior* as a species is certainly justified with regard to peripheral isolate speciation, with *C. spinosior* evolving as a separate morphological entity adapted to the Sonoran Desert diverging from the northern Chihuahuan Desert populations of *C. imbricata* var. *imbricata*. Likewise, *C. spinosior* could easily be considered an intraspecific taxon within *C. imbricata*, given that the taxa share numerous morphological characters (mentioned above), and intergradation occurs between populations of *C. spinosior* and *C. imbricata* var. *imbricata* (M. A. Baker, personal observation). On the basis of these phylogenetic data, as well as morphological characters and introgressive populations between *C. imbricata* and *C. spinosior*, Baker and Pinkava (2018) have recircumscribed *C. spinosior* as a variety of *C. imbricata*.

## FUTURE WORK

Only one accession of most taxa was used for our phylogeny reconstruction. Multiple accessions per species should be used in future work to test finer-scale biogeographic hypotheses. This is especially important for species such as *C. leptocaulis*, which is the sole known diploid representative of its clade and occurs over a very large distribution. More phylogenetic work also will be necessary to clarify relationships within species, such as in the *Imbricata* and *Californica* clades, which were not totally resolved in our analyses (Fig. 2).

Large-scale datasets based on genomic data will be especially useful for broad analyses across subfamily Opuntioideae, as well as other major groups within cacti, and are currently being developed for specific clades. Our phylogeny of Cylindropuntieae clearly indicates that these data will aid in resolving taxa/clades with problematic phylogenetic placements, as well as species-level relationships. On the basis of this robust diploid framework, we can now begin to incorporate homoploid hybrid and allopolyploid taxa into phylogenetic analyses to test for the origins of those species using comparative nuclear and plastid datasets.

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## AUTHOR CONTRIBUTIONS

L.C.M. designed the study, aided in data acquisition, analyzed the data, and wrote the paper. M.A.B. and M.C-H. aided in the study design and data acquisition and helped write the paper. A.S. helped write the paper. K.M.N. analyzed data and helped write the paper.

## DATA AVAILABILITY

The sequences and alignment analyzed in this study are available in Dryad (Majure et al., 2019), and our plastid genome sequence of *Cylindropuntia bigelovii* is available in GenBank (MN121762). Our plastid reads our archived in GenBank (SAMN12504894–SAMN12504949) under BioProject PRJNA558967.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**APPENDIX S1.** Inferred ages and ranges of relevant phylogenetic nodes based on the BEAST analysis constraining only Cactaceae. Ages are in millions of years (Ma). HPD = highest posterior density interval.

**APPENDIX S2.** Inferred ages and ranges of relevant phylogenetic nodes based on the BEAST analysis constraining Cactaceae and *Leuenbergeria* as sister to the rest of Cactaceae. Ages are in millions of years (Ma). HPD = highest posterior density interval.

**APPENDIX S3.** Morphological characters and their states used in our ancestral state reconstruction.

**APPENDIX S4.** ML reconstruction of stem shape (A) and spine sheaths (B). Flattened stems are synapomorphic for tribe Opuntieae. Sheaths are synapomorphic for the *Grusonia* + *Cylindropuntia* clade.

**APPENDIX S5.** ML reconstruction of photosynthetically functional vs. nonfunctional leaves (A) and unifacial vs. bifacial leaf blades (B). Photosynthetically functional leaves (white circles) were lost twice, once in Cactoideae and once in Opuntioideae, although they were regained separately in *Pereskiopsis* and *Quiabentia*. Unifacial leaves most likely evolved once in the common ancestor of the (Maihuenia + Cactoideae (Opuntioideae)) clade and were lost in both *Pereskiopsis* and *Quiabentia*, separately.

**APPENDIX S6.** MP reconstruction of inner tepal color (A) and fruit fleshiness (B). Yellow/greenish-yellow flowers (white circles) are ancestral in Cylindropuntieae, whereas magenta-pink (green) and orange-red (red) flowers have been derived multiple times. Fleshy fruit (white) are ancestral in Cylindropuntieae and dry fruit (black) have been derived on numerous occasions.

**APPENDIX S7.** Reconstruction of leaf size in Cylindropuntieae. Large (macroscopic) leaves of the vegetative shoot are plesiomorphic, whereas small (microscopic) leaves are derived in Cactoideae.

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**APPENDIX 1**. Species used in our analysis of the tribe Cylindropuntieae. Herbarium vouchers are cited with the collector's last name and number followed by the herbarium acronym where deposited, following Thiers (2017), or the author is cited for data generated by others. Those accessions that are maintained as part of the Desert Botanical Garden (DBG) living collection are also given with their DBG accession number.

Cactaceae. Cactoideae. *Blossfeldia liliputana* (Arakaki et al., 2011), *Weingartia kragliana* (Arakaki et al., 2011). ***Maihuenia***. *Maihuenia poeppigii* (Arakaki et al., 2011). Opuntioideae. ***Cylindropuntia***: *C. abyssi* (*Hodgson 24537*-DES, DBG 2013 0007 0101), C. acanthocarpa var. *acanthocarpa* (*Majure 5375*-DES, FLAS), *C. acanthocarpa* var. *ramosa* (*Baker 11662*-ASU), *C. acanthocarpa* var. *thornberi* (*Baker 18269*-ASU), *C. alcahes* var. *alcahes* (*Baker 8690*-ASU, DBG 1993 0579 01), *C. alcahes* var. *burrageana* (*Rebman 2865*-ASU, DBG 1994 0671 10), *C. alcahes* var. *gigantensis* (*Baker 8744*-ASU, DBG 1993 0596 01), *C. anteojoensis* (DBG 1976 0089 01), *C. bigelovii* (*Baker 18286*-ASU); (*Majure 5496*-DES, FLAS, DBG 2015 0705 01), *C. californica* var. *californica* (*Baker 8638*-ASU, DBG 1993 0575 01), *C. californica* var. *parkeri* (*Baker 18407*-ASU), *C. californica* var. *rosarica* (*Baker 12335*, DBG 2011 0167 01), *C. cholla* (*Gates s.n.*-DES, DBG 1939, 0189 0101), *C. ciribe* (*Baker 8730*-ASU, DBG 1993 0591 01), *C. davisii* (*Baker 18267*-ASU; *Majure 6852*-DES, FLAS), *C. delgadilloana* (*Rebman 4974*-ASU, DBG 1999 0052 0101), *C. echinocarpa* (*Baker 18500*-ASU; *Majure 5735*-DES), *C. fulgida* var. *fulgida* (*Majure 5378*-DES, FLAS, DBG 2015 0627 01), *C.*

*ganderi (Rebman 4973*-ASU, DBG 1999 0048 21-2), *C. imbricata* var. *argentea* (*Hodgson s.n.*-DES, DBG 1993 0722 21-1), *C. imbricata* var. *imbricata* (*Baker 16974*-ASU), *C. imbricata* var. *spinitecta* (*Baker 12386*-ASU, DBG 2016 0683 01), *C. leptocaulis* (*Baker 12397*-ASU), *C. multigeniculata* (*Baker 17670*-ASU), *C. munzii* (*Baker 18505*-ASU), *C. ramosissima* (*Baker 11371*-ASU), *C. spinosior* (*Baker 17233*-ASU), *C. tesajo* (*Lindsay s.n.*-DES, DBG 1939 0035 01), *C. thurberi* (*Baker 10377*-ASU), *C. tunicata* (*Baker 6255*-ASU, DBG 1985 0568 0108; *Baker 18602*-ASU), *C. versicolor* (*Majure 5857*-DES, FLAS, DBG 2015 0711 01), *C. whipplei* (*Baker 16670*-ASU). ***Grusonia***: *G. aggeria (Majure 5651*-DES, DBG 2015 0708 01), *G. bradtiana* (*Meigs 42*-DES, DBG 1956 5660 01), *G. clavata* (*Baker 13559*-ASU), *G. invicta* (*Haughey 249*-DES, DBG 1993 0040 0101), *G. kunzei (Baker 11939*-ASU), *G. marenae (Gentry s.n.*-DES, DBG 1966 8491 0103), *G. moelleri* (DBG 1968 9412 02), *G. parishii (Baker 17548*-ASU). ***Leuenbergeria.*** *L. bleo* (Moore et al., 2017). ***Maihueniopsis***: *M. camachoi* (*Eggli & Leuenberger 2705,* DBG1998 0050 10). ***Micropuntia***: *M. pulchella* (*Cloud-Hughes 95*-DES; DBG 2018 0684 01); (*Majure 6095*-DES). ***Opuntia***: *O. arechavaletae (Majure 6959*-DES, FLAS; DBG 2017 0724 01), *O. austrina (Majure 4216;* FLAS), *O. quitensis* (*Majure 3848;* FLAS). ***Pereskiopsis***: *P. aquosa* (*Wallace s.n.*-DES, DBG 1997 0185 01), *P. diguetii* (Arakaki et al., 2011); (HBG39329-HNT9446/ DBG 2018 0128 01), *P. kellermannii* (*Fleming s.n.*-DES, DBG 2016 0542 01), *P. porteri* (*Quirk s.n.*-DES, DBG 1984 0537 0105). ***Quiabentia:*** *Q. verticillata* (Arakaki et al., 2011), *Q. verticillata* (*Kimnach 2803*-DES, DBG 1992 1063 01). ***Tephrocactus*****:** *T. alexanderi* (*Ferguson 318,* DBG 2001 0055 01), *T. articulatus* (*Krattermann 595,* DBG 2001 0014 21). ***Pereskia s.s.*** *P. aculeata* (Arakaki et al., 2011), *P. sacharosa* (Arakaki et al., 2011). Portulacaceae. ***Portulaca.*** *P. oleracea* (Arakaki et al., 2011).