Phylogenetic relationships and evolution of growth form in Cactaceae (Caryophyllales, Eudicotyledoneae)

ARTICLE *in* AMERICAN JOURNAL OF BOTANY · JANUARY 2011

Impact Factor: 2.46 · DOI: 10.3732/ajb.1000129 · Source: PubMed

6 AUTHORS, INCLUDING:

Arturo de Nova
Universidad Autónoma de San Luis Potosí
16 PUBLICATIONS **226** CITATIONS

Luís Enrique Eguiarte
Universidad Nacional Autónoma de México
177 PUBLICATIONS **5,062** CITATIONS

Susana Magallon
Universidad Nacional Autónoma de México
42 PUBLICATIONS **2,089** CITATIONS

American Journal of Botany

American Journal of Botany 98(1): 44–61. 2011.

PHYLOGENETIC RELATIONSHIPS AND EVOLUTION OF GROWTH FORM IN CACTACEAE (CARYOPHYLLALES, EUDICOTYLEDONEAE)[1]

TANIA HERNÁNDEZ-HERNÁNDEZ[2], HÉCTOR M. HERNÁNDEZ[2], J. ARTURO DE-NOVA[2], RAUL PUENTE[3], LUIS E. EGUIARTE[4], AND SUSANA MAGALLÓN[2,5]

[2]Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, 3er Circuito de Ciudad Universitaria, Del. Coyoacán, México D.F. 04510 México; [3]Desert Botanical Garden, 1201 N. Galvin Parkway, Phoenix, Arizona 85008 USA; and [4]Instituto de Ecología, Universidad Nacional Autónoma de México, 3er Circuito de Ciudad Universitaria, Del. Coyoacán, México D.F. 04510 México

- *Premise of the study*: Cactaceae is one of the most charismatic plant families because of the extreme succulence and outstanding diversity of growth forms of its members. Although cacti are conspicuous elements of arid ecosystems in the New World and are model systems for ecological and anatomical studies, the high morphological convergence and scarcity of phenotypic synapomorphies make the evolutionary relationships and trends among lineages difficult to understand.
- *Methods*: We performed phylogenetic analyses implementing parsimony ratchet and likelihood methods, using a concatenated matrix with 6148 bp of plastid and nuclear markers (*trnK/matK*, *matK*, *trnL-trnF*, *rpl16*, and *ppc*). We included 224 species representing approximately 85% of the family's genera. Likelihood methods were used to perform an ancestral character reconstruction within Cactoideae, the richest subfamily in terms of morphological diversity and species number, to evaluate possible growth form evolutionary trends.
- *Key results*: Our phylogenetic results support previous studies showing the paraphyly of subfamily Pereskioideae and the monophyly of subfamilies Opuntioideae and Cactoideae. After the early divergence of *Blossfeldia,* Cactoideae splits into two clades: Cacteae, including North American globose and barrel-shaped members, and core Cactoideae, including the largest diversity of growth forms distributed throughout the American continent. Para- or polyphyly is persistent in different parts of the phylogeny. Main Cactoideae clades were found to have different ancestral growth forms, and convergence toward globose, arborescent, or columnar forms occurred in different lineages.
- *Conclusions*: Our study enabled us to provide a detailed hypothesis of relationships among cacti lineages and represents the most complete general phylogenetic framework available to understand evolutionary trends within Cactaceae.

Key words: Cactoideae; Opuntioideae; Pachycereeae; parsimony ratchet; *ppc*; RAxML; *rpl16*; Trichocereeae; *trnK/matK*; *trnL-trnF*.

Cactaceae (Caryophyllales, Eudicotyledoneae, Angiospermae; Cantino et al., 2007) is a well-known plant family possessing several adaptations for aridity. The family is remarkable due to the evolution of extreme succulence in most of its members, its conspicuous presence in New World dry regions, and its outstanding diversity of growth forms. Several cacti species are commercialized as ornamental plants on a worldwide scale, and some of them are important as a food source, giving this family a strong economical relevance (e.g., Nobel, 1994, 2002; De Kock, 2001; Stintzing and Carle, 2005; Feugang et al., 2006). In addition to being distinctive elements of arid and semiarid biomes, Cactaceae species play fundamental ecological roles (e.g., Parker, 1989; Mandujano et al., 1996; Drezner and Balling, 2002; Godínez-Álvarez et al., 2003). Different cacti species have been studied as models in plant anatomical and physiological studies (e.g., Mauseth, 1999, 2004, 2006, 2007; Shishkova et al., 2008), and some cacti lineages are persistently invasive in different biomes in Africa, Australia, and elsewhere (Nobel, 1994).

The family Cactaceae includes over 1450 species belonging to ca. 127 genera (Barthlott and Hunt, 1993; Hunt et al., 2006). Its greatest species richness is concentrated primarily in Mexico, with secondary centers in the southwestern Andean region and in eastern Brazil. The morphological features that characterize members of Cactaceae are the presence of short shoots modified into areoles, a shoot apical meristem organized into four zones, and in nearly all cacti, inferior ovaries covered by bracts or areoles (Gibson and Nobel, 1986; Nyffeler, 2002). At the molecular level, the family is characterized by the inversion of a chloroplast genetic region including *atpE*, *atpB*, and *rbcL* genes (Downie and Palmer, 1994), and its monophyly has been supported in molecular phylogenetic

---

[1] Manuscript received 8 April 2010; revision accepted 11 November 2010.

The authors are deeply thankful to R. Kiesling and S. Arias, who kindly revised the manuscript. R. Kiesling hosted a collecting trip to Argentina and verified identifications of several South American sampled cacti. C. Gómez-Hinistrosa provided valuable help in species identification and voucher preparation. The authors thank S. Cevallos, R. González, L. Márquez, R. Medina, M. Olson, V. Patiño, J. Reyes, R. Tapia, and J. Verleyen for help in different aspects of this project. They also thank three anonymous reviewers for helpful comments on the manuscript. The Instituto de Biotecnología, UNAM (Macroproyecto de Tecnologías de la Información y la Computación) and Centro Nacional de Supercómputo, IPYCYT (San Luis Potosí, México) provided computing resources. The Consejo Nacional de Ciencia y Tecnología (CONACYT), México, and the Posgrado en Ciencias Biológicas (UNAM) funded the doctoral studies of T.H.-H. This research was partially funded by CONACYT-2004-C01-46475 and UNAM PAPIIT IN202310 research grants.

[5] Author for correspondence (e-mail: s.magallon@ibiologia.unam.mx)

doi:10.3732/ajb.1000129

studies based on different loci (e.g., Applequist and Wallace, 2001; Cuénoud et al., 2002; Nyffeler, 2002).

Taxonomic studies since the 19th century have recognized Pereskioideae, Opuntioideae, and Cactoideae as distinct subfamilies within Cactaceae (Anderson, 2001; Metzing and Kiesling, 2008). The genus *Maihuenia* has been typically considered as a member of Pereskioideae; however, its placement in a monogeneric subfamily has been suggested on the basis of its unique ecological and morphological attributes (Anderson, 2001) and molecular phylogenetic analyses (Wallace, 1995a, b). In a recent molecular phylogeny of Cactaceae (Nyffeler, 2002), species of *Pereskia* and *Maihuenia* were found to form an early-diverging grade within Cactaceae, with Cactoideae and Opuntioideae as well-supported clades.

Species of *Pereskia* have been recognized as morphologically plesiomorphic within Cactaceae (Gibson and Nobel, 1986; Leuenberger, 1986; Metzing and Kiesling, 2008), displaying characters such as broad, flattened leaves with C_3 photosynthesis, arborescent growth form, areoles with leaf production, dense and fibrous wood, simple cortex lacking cortical bundles, poorly developed stem epidermal and hypodermal layers, nonsucculent tissues, and occupation of relatively mesic environments (Leuenberger, 1986; Mauseth and Landrum, 1997). In a molecular-based analysis of relationships among early Cactaceae lineages, Edwards et al. (2005) found that members of *Pereskia* were grouped in two distinct, early-diverging lineages that formed consecutive sister groups of a clade formed by (*Maihuenia*, Cactoideae) and Opuntioideae.

Subfamilies Opuntioideae and Cactoideae have long been recognized as monophyletic on the basis of morphological and molecular data (Barthlott and Hunt, 1993; Nyffeler, 2002; Wallace and Dickie, 2002; Griffith and Porter, 2009). Members of Opuntioideae share a number of structural synapomorphies such as areoles with glochids (small, deciduous, barbed spines), polyporate pollen grains with peculiar exine structures (Leuenberger, 1976), and seeds surrounded by a funicular cover—frequently described as a bony aril (Barthlott and Voit, 1979). In addition, studies based on molecular data show that Opuntioideae is characterized by a deletion in the *accD* region of the chloroplast genome (Wallace, 1995b; Wallace and Dickie, 2002; Griffith and Porter, 2009).

Subfamily Cactoideae has been strongly supported as monophyletic in molecular phylogenies (Nyffeler, 2002). Members of this subfamily are characterized by tubercles or ribs on the stems, reduced or suppressed leaves subtending each areole (Wallace and Gibson, 2002), and the loss of an intron in the *rpoC1* chloroplast gene (Wallace and Cota, 1994). On the basis of detailed morphological observations, Buxbaum (1958) and Endler and Buxbaum (1982) subdivided Cactoideae in nine tribes, with several subtribes and "lines" (Appendix S1, see online at http://www.amjbot.org/cgi/content/full/ajb.1000129/DC1), and Barthlott and Hunt (1993) derived a tribal arrangement for Cactoideae following previous classifications and morphological data (e.g., Buxbaum, 1958; Britton and Rose, 1919–1923); however, this classification has been recently modified (Anderson, 2001; Appendix S1). Morphological phylogenetic reconstruction and taxonomic classification for Cactoideae has proven to be difficult due to frequent character state convergence and lack of clear synapomorphies for tribes; hence, molecular-based phylogenetic analysis might represent a useful alternative for recognizing main lineages and their relationships (Applequist and Wallace, 2002). Recent molecular phylogenetic results for Cactoideae conflict with traditional morphological classifications (Applequist and Wallace, 2002; Nyffeler, 2002). Except for Cacteae, the traditionally defined tribes have been found to be para- or polyphyletic (e.g., Applequist and Wallace, 2002; Nyffeler, 2002; Wallace and Gibson, 2002).

Cactoideae includes the largest number of genera and species and the largest diversity of growth forms among Cactaceae subfamilies (see Barthlott and Hunt, 1993; Anderson, 2001). Its members diversified throughout the American continent, becoming adapted to different environments and evolving a large variety of forms and habits (Gibson and Nobel, 1986). For example, dry, tropical forests in central Mexico host a large radiation of columnar and arborescent cacti, and these growth forms also occur in some lineages from several regions of South America. Epiphytes with cylindrical or flattened stems inhabit humid, tropical forests of Central and South America, and a variety of globose or spherical species that grow independently or form clumps occur in the arid and semiarid regions of South and North America. In North America, they range in size between a few millimeters above the ground to gigantic barrel cacti over 2 m tall (Gibson and Nobel, 1986).

Efforts have been made to describe and explain the profuse diversity of forms within Cactoideae. According to Gibson and Nobel (1986), cacti stem forms within the subfamily can be barrel-shaped, globose, or cylindrical. Barrel-shaped forms include species with globular or spherical stems and a maximum height of 0.5–2 m, whereas globose cacti possess spherical stems less than 0.5 m tall (Gibson and Nobel, 1986). The cylindrical (or columnar, according to the authors) form includes species whose stems are 10 or more times longer than wide (Gibson and Nobel, 1986). According to their branching patterns, cylindrical-stemmed species can be classified in different growth forms (Arias and Terrazas, 2006). If the cylindrical stem is unbranched, the form can be classified as a simple columnar; arborescent forms are tree-like with a trunk branching above its base, and shrubby forms are characterized by the absence of a main trunk or the presence of basitonic branching (Anderson, 2001; Arias and Terrazas, 2006; Buxbaum, 1951). Sometimes, cacti with a cylindrical stem grow in a prostrate, clambering, scrambling, or climbing form (Anderson, 2001) due to the lack of mechanical support of the stem. Instead of growing erect, these cacti grow over the soil or are supported by other plants or neighboring vegetation. When cacti grow on other plants, they are classified as epiphytes (Anderson, 2001).

An important adaptation in the stem of species of Cactoideae is the presence of a folded surface, which allows them to swell without tearing and to absorb large quantities of water when available (Mauseth, 2006). The stem surface in Cactoideae species can be folded longitudinally into ribs or both longitudinally and transversely into tubercles. Whereas ribs allow the inner cortex to expand and shrink radially without damaging the shoot surface, tubercles allow the entire shoot to shorten or lengthen as water content changes (Mauseth, 2006).

This complexity and large diversity of forms and habits present in Cactaceae—and particularly within Cactoideae—have complicated the achievement of a consensus on the classification of cacti growth forms, and with the lack of stable phylogenetic hypotheses for relationships among lineages, have hindered our understanding of the evolution of the family's diversity of forms.

The main goal of our study is to reconstruct the phylogenetic relationships within Cactaceae based on an expanded and more comprehensive taxonomic sample across the family, and molecular sequence data from the plastid and nuclear genomes. We compare our results with published studies to evaluate the monophyly of previously proposed groups (particularly within

Cactoideae), to provide independent evidence to show relationships among them, and to identify lineages where more work is needed. Our taxonomic sample includes 224 species belonging to 108 genera of Cactaceae (of ca. 127; Barthlott and Hunt, 1993; Hunt et al., 2006), representing all traditionally recognized subfamilies, including an increased sample of the North American tribes Cacteae and Pachycereeae, and several South American lineages. We performed phylogenetic analyses with parsimony ratchet and maximum likelihood methods, based on four protein-coding and -noncoding plastid loci, and one low-copy nuclear locus. Although one of the most arresting characteristics of the Cactaceae family is the extraordinary diversity of structure of its members (Mauseth, 2006), the extensive convergence of growth form among distantly related lineages has made understanding the evolution of cacti structure difficult. Although authors have postulated hypotheses based on observations (Britton and Rose, 1919–1923; Buxbaum, 1951; Gibson and Nobel, 1986; Barthlott and Hunt, 1993; Mauseth, 2006), a formal analysis of character evolution is pending. We evaluated possible trends in the evolution of growth form within subfamily Cactoideae, by scoring and reconstructing ancestral character states at the main supported nodes using likelihood methods. Based on an extensive sampling of lineages, our results provide a complete framework to understand evolutionary relationships within Cactaceae and provide insights into the evolution of growth form diversity within Cactoideae.

MATERIALS AND METHODS

Taxa and data—Our taxonomic sampling includes 224 species belonging to 108 genera of Cactaceae, which represent ca. 85% of the generic diversity and 15.4% of the species diversity of the family. Species sampling followed Barthlott and Hunt (1993) and Anderson (2001), aiming to achieve a nearly complete generic representation and to increase the representation of North American taxa belonging to tribes Cacteae and Pachycereeae, as well as several South American lineages. We included at least one species for each genus within Cacteae and Pachycereeae, but more than one for larger genera such as *Mammillaria*, *Echinocereus*, *Pachycereus*, and *Opuntia*. Mexican taxa were collected mainly from natural populations, but several endangered species were sampled from the living collection at the Jardín Botánico, Universidad Nacional Autónoma de México (UNAM). Tissues of several South American taxa were obtained from the living collection at the Desert Botanical Garden (DBG, Phoenix, Arizona), with species identifications subsequently verified. Tissue of *Blossfeldia liliputana* was collected from natural populations. Four additional species of Anacampserotaceae, the closest relatives of Cactaceae (Nyffeler, 2007; Nyffeler and Eggli, 2010), were included as outgroups. Vouchers of collected specimens are deposited at the National Herbarium of Mexico (MEXU, UNAM) (Appendix 1).

Phylogenetic analyses were based on the nucleotide sequences of five loci from the plastid and nuclear genomes. Plastid markers are one protein-coding gene (*matK*), an intron (*rpl16*), and two intergenic spacers (*trnL-trnF* and *trnK-matK*), which have been used successfully in previous phylogenetic analyses of Cactaceae (Nyffeler, 2002; Arias et al., 2003, 2005; Edwards et al., 2005). The nuclear marker is the fourth intron of the phosphoenolpyruvate carboxylase (*ppc*) gene and is here used for the first time to infer phylogenetic relationships in Cactaceae.

Table 1. Primers designed and used to amplify the plastid fragments *rpl16* and *trnK/matK*.

Region	Primer name: Sequence
rpl16	rpl161F: 5′-GCTATGCTTAGTGTGTGACTCGTT-3′
	rpl163R: 5′-CTTCTATTTGTCTAGGCGTGATCC-3′
trnK/matK	matK3F: 5′-GGATGAAGCAAGGAATTCGTCTAC-3′
	matK4R: 5′-TTCATTGCACACGGCTTTCCCTA-3′
	matK9R: 3′-TAGCCAACGATCCAACCAGAG-5′
	matK13F: 3′-GGGCACAAGCACAAGAAGAAT-5′

DNA extraction, PCR amplification, and sequencing—Genomic DNA was extracted from silica-gel-dried tissue following the procedures of the DNeasy Plant Mini kit (Qiagen, Alameda, California, USA) with slight modifications (information available upon request). We designed the amplifying and sequencing primers for *rpl16* and *trnK/matK* loci (Table 1). To amplify the *trnL-trnF* intergenic region, we used primers trnL-c and trnL-f (Taberlet et al., 1991). The *ppc* locus was amplified using primers PPCX4F and PPCX5R (Olson, 2002). PCR amplification reactions were performed using an initial denaturation step at 94°C for 5 min, followed by 34 cycles at 94°C for 1 min, different annealing temperatures (depending on the primers) for 1 min, 72°C elongation temperature for different time durations depending on the length of the product, and a finishing step at 72°C for 4 min. Detailed conditions of each reaction are shown in Table 2. For several species, gel electrophoresis of PCR products of *ppc* yielded two bands. These two copies were extracted using the QIAquick gel extraction protocol (Qiagen), then sequenced at The Genome Center, Washington University (http://genome.wustl.edu/) and Macrogen, Seoul, Korea (http://www.macrogen.com).

Phylogenetic analyses—Data sets were assembled by combining the newly obtained sequences with those downloaded from Genbank for the *trnL-trnF*, *trnK-matK*, *matK*, and *rpl16* regions (Appendix 1). For each locus, sequences were preliminarily grouped in several data sets following tribal classifications of Barthlott and Hunt (1993) and Anderson (2001), automatically aligned using the program MUSCLE (Edgar, 2004), and manually refined with the program BioEdit version 5.0.6 (Hall, 1999). Preliminary alignments were subsequently joined into a single matrix using the profile alignment option in MUSCLE, followed by a second round of manual refinement with BioEdit (data was submitted to TreeBASE, http://www.treebase.org, submission number S11087). Incomplete sequence fragments at the 5′ and 3′ ends of each aligned data matrix were excluded from subsequent analyses (516 base pairs [bp] in total). The final concatenated matrix is 6148 bp long.

Preliminary heuristic parsimony analyses were conducted independently for each locus, except for *trnK-matK* and *matK*, which were analyzed jointly (results for independent analyses are not shown but are available upon request). We performed 1000 replicates implementing a random-order-entry starting tree and tree-bisection-reconnection (TBR) branch swapping with option MULTREES, using the program PAUP* version 4.0b10 (Swofford, 2002). Strict consensus trees for each locus were obtained, and after we observed that the differences among them, especially among chloroplast markers, were not strongly supported, the five loci were combined in a single data set. This combined data set was analyzed with the parsimony ratchet (Nixon, 1999) in PAUP* using PAUPRat (Sikes and Lewis, 2001). Parsimony uninformative and invariant sites were excluded, and 20 independent runs of 500 iterations each were conducted. Most parsimonious (MP) trees were filtered from the set of resulting trees on each iteration and used to construct a strict consensus tree. Clade support was assessed with 1000 replicates of a nonparametric bootstrap analysis following a parsimony ratchet, implemented in the program TNT version 1.1 (Goloboff et al., 2000).

Table 2. PCR conditions for the amplification of the different genomic regions used in this study.

Genomic region	Primers: forward/reverse (μL of each)	Annealing temp. (°C)	Elongation time (min)	Final volume (μL)	PCR buffer (μL)	$MgCl_2$ (μL)	DNTPs (μL)	*Taq* (μL)
rpl16	rpl161F/rpl163R (1.25)	63	2	25	2.5	0.75	0.5	0.125
trnK/matK (long)	matK3F/matK4R (1.625)	66.5	4	65	6.5	1.95	1.625	0.4
trnK/matK (short)	matK3F/matK9RmatK13F/matK4R (1.25)	65/63	2.25	50	5	1.5	1.25	0.312
trnL-trnF	trnL-c/trnL-f (2.5)	62	2	50	5	1.5	1	0.25
ppc	PPCX4F/PPCX5R (1)	60	3	30	2.5	0.75	0.5	0.125

Notes: Primer concentration: 10 mmol/L; *Taq* concentration: 1 mmol/L (5 units/μL).

We estimated the likelihood (−lnL), the proportion of invariant sites and the alpha parameter of the gamma distribution for each of the four noncoding loci (*trnK-matK*, *trnL-trnF*, *rpl16*, and *ppc*), for the coding *matK*, and for codon position partitions in *matK* using the program Modeltest version 3.7 (Posada and Crandall, 1998). We selected best fitting models for each mentioned data set using the Akaike information criterion (AIC). To determine adequate data partitioning in our concatenated matrix for further phylogenetic analyses, we visually examined the parameters and the models obtained for each region and codon positions as a proxy to their molecular evolutionary dynamics. These examinations indicated that the data could be appropriately divided into five partitions: (1) chloroplast intergenic *trnK-matK* (best fit model: K81uf+I+G); (2) chloroplast-coding *matK* (best fit model: TVM+I+G); (3) chloroplast intergenic *trnL-trnF* (best fit model: GTR+I+G); (4) chloroplast *rpl16* intron (best fit model: TIM+I+G); and (5) the nuclear *ppc* region (best fit model: TVM+I+G).

Maximum likelihood (ML) phylogenetic analyses were performed with the program RAxML version 7.0.4 (Stamatakis, 2006) for the matrix consisting of the five concatenated loci, for the plastid matrix (four concatenated loci: *trnK-matK*, *matK*, *trnL-trnF*, and *rpl16*), and for the nuclear matrix (one locus: *ppc*). For the five loci matrix and the plastid matrix, we implemented an independent general time reversible model (GTR) and a gamma distribution for site rates for each data partition, as already described. We set 25 rate categories for the gamma distribution in both cases, because an exploratory analysis in RAxML showed this number of categories lead to an improvement of likelihood values. We performed 500 independent searches starting from different MP initial trees. The ML tree was selected from the entire set of resulting trees on each search. Clade support was assessed with 1000 replicates of a nonparametric bootstrap analysis for the five-loci matrix, the plastid matrix, and the nuclear *ppc*, also conducted with RAxML.

Ancestral character reconstruction within subfamily Cactoideae—To address the evolution of growth form within subfamily Cactoideae, we used the ML phylogeny obtained for the five-loci matrix to perform a reconstruction of ancestral character states with the program BayesMultistate implemented in BayesTraits (Pagel, 1999; Pagel et al., 2004; program available at http://www.evolution.rdg.ac.uk), under an ML approach. This method reconstructs the most probable character states at each tested node, maximizing the probability of the character states observed in terminal taxa. It allows the incorporation of phylogenetic branch lengths by implementing a continuous-time Markov model of character evolution, the number of rate parameters depending on the number of character states (Pagel, 1994, 1999; Pagel et al., 2004).

We scored four different characters describing the growth form of species within subfamily Cactoideae, following discussions in Buxbaum (1951), Loup (1983), Gibson and Nobel (1986), Anderson (2001) and Arias and Terrazas (2006). Character states for Cactoideae species included in this study were obtained from published descriptions and photographs (Kattermann, 1994; Schulz and Kapitany, 1994; Leuenberger and Eggli, 2000; Porter et al., 2000; Anderson, 2001; Taylor and Zappi, 2004; Hunt et al., 2006). Scored characters were (1) main growth form, (2) stem support, (3) stem features, and (4) habit. For main growth form, we distinguished six possible character states: arborescent (with a trunk branching above the base), shrubby (absence of a main trunk or with a basitonic branching), columnar (not branched), globose solitary (plants with spherical stems shorter than 0.5 m, growing singly), globose caespitose (plants with spherical stems shorter than 0.5 m, growing in clumps), and barrel (plants with spherical stems taller than 0.5 m). As noticed by Gibson and Nobel (1986, p. 141), in the flat-stemmed epiphytes of the subfamily Cactoideae, the pith is small and encircled by a vascular cylinder like that found in narrow-stemmed, ribbed, terrestrial cacti; hence, we classified epiphytes (for example, species in *Epiphyllum*, *Rhipsalis*, or *Hylocereus*) as having a shrubby growth form. For the stem support character, we distinguished two possible states: erect or nonerect. For stem features, we distinguished two character states: ribbed or tubercled. For habit, we distinguished two character states: terrestrial or epiphytic. Table S4.1 in Appendix S4 (see online Supplemental Data) shows the matrix with the scored characters for Cactoideae species included in this study.

RESULTS

The final five loci data matrix includes 6148 bp, of which 3400 (55.3%) are invariant and 1597 (26.2%) are parsimony informative. Table 3 summarizes the information content of each genomic region. The alignment of *trnK-matK* plus *matK* was the longest and provided the majority of parsimony informative sites.

Table 3. Characteristics and information for alignments of sequences from the different loci used in this study.

Region	Source	Length (bp) [a]	Constant sites	Parsimony informative	Percentage info. sites (%) [b]
trnK/matK	chloroplast	2514	1616	534	33.4
trnL-trnF	chloroplast	1530	809	406	25.4
rpl16	chloroplast	1546	766	458	28.6
ppc	nucleus	558	231	227	14.2

[a] Length of aligned individual matrixes after removing 5′ and 3′ low quality regions.

[b] Percentage of parsimony informative sites from the total concatenated matrix.

In spite of the need to visually inspect and edit each alignment carefully, particularly for some highly variable regions within *trnL-trnF*, our alignment strategy yielded an adequate global alignment in a reasonable time. The final five-loci matrix was only partially complete, either due to unavailable plant tissues or unsuccessful laboratory techniques. For some taxa, only fragmentary sequences could be obtained (Appendix 1).

The parsimony ratchet analysis for the five-loci matrix resulted in 83 most parsimonious trees of 10476 steps each (CI = 0.5137, RI = 0.7521). The strict consensus is shown in Figure S2a–d in Appendix S2. The ML analysis identified a most likely tree of lnL = −55205.24 (Figs. 1–4). The ML bootstrap values (bML) were generally higher than those obtained with parsimony (bMP; Figs. 1–4); however parsimony and ML analyses failed to find strong support for derived nodes, particularly within core Cactoideae (see Discussion). Parsimony and ML trees differed mostly in relationships close to the tree tips within this clade. Analyses with maximum likelihood methods provided better results than parsimony ratchet in terms of tree resolution and computing time.

The ML phylogeny obtained for the plastid matrix (*trnL-trnF*, *trnK-matK*, *matK*, *rpl16*) is shown in Appendix S3, and its topology and support values are highly similar to the ones obtained with the five-loci matrix, probably due to the overwhelming amount of plastid data in relation to the amount of nuclear data. The nuclear region *ppc* was difficult to amplify for several species with our experimental laboratory strategy, and we were only able to obtain sequences for a portion of our sample including 118 taxa. The ML phylogeny obtained for *ppc* is also shown in Appendix S3. Although some main clades present in the five loci and in the plastid resulting trees were also recovered with the *ppc* nuclear region (see Discussion), several relationships were not recovered, and support values obtained with *ppc* were very low, particularly for deep nodes (Appendix S3). The percentage of variable sites in *ppc* (Table 3), as well as the low support values for deeper nodes and better support values for derived nodes in the *ppc* ML phylogeny (Appendix S3), indicate that this marker by itself is not adequate to infer relationships among main Cactaceae clades. However, in spite of the technical difficulties in its amplification and isolation, the *ppc* marker might be useful to infer relationships within derived cacti lineages. Phylogenetic relationships are here discussed on the basis of the ML tree obtained for the five-loci matrix, with comparisons to the MP strict consensus obtained from the same matrix (Appendix S2), and with references to the ML nuclear and plastid trees (Appendix S3).

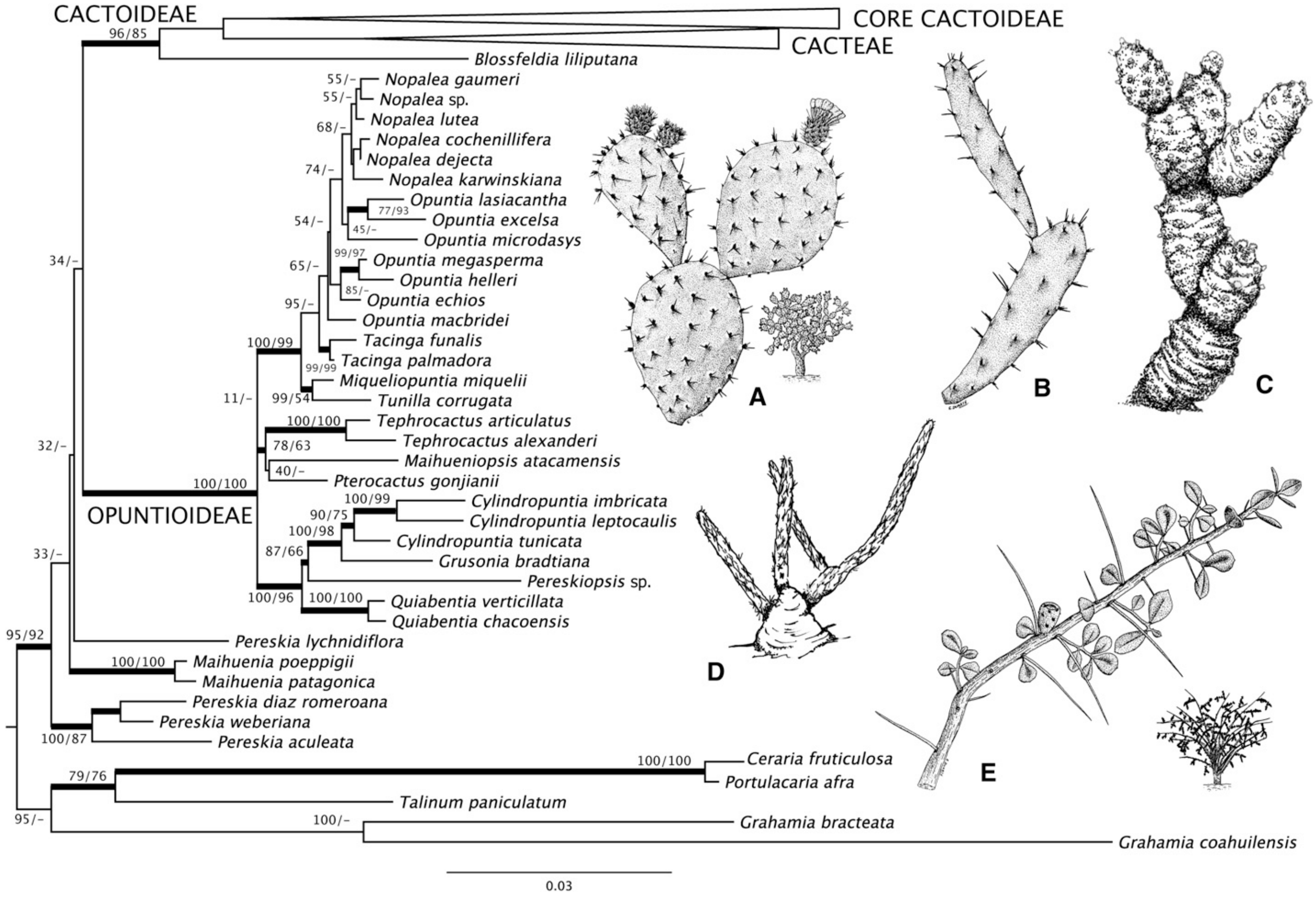

Fig. 1. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16*, and *ppc* (five-loci data matrix) showing early-diverging lineages. Bootstrap percentage values derived from maximum likelihood and parsimony analyses (bML/bMP) are shown above branches. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Opuntia velutina;* (b) *Nopalea;* (c) *Tephrocactus*; (d) *Pterocactus*; and (e) *Pereskiopsis rotundifolia*. Figures (a) and (e) were taken and modified from Arias et al. (1997).

To infer the ancestral character states and evolutionary trends of growth form and habit within Cactoideae, we used the ML phylogeny for the five-loci matrix. Methods used provide a probability value for each character state at each tested node. We reconstructed the ancestral characters of main clades and clades with high support values, identifying the most probable character state at each selected node. A complete table with evaluated nodes, likelihood scores, and probability values is shown in Appendix S4 and a representation of results is shown in Fig. 5.

DISCUSSION

Phylogenetic analyses—Previous phylogenetic studies have revealed difficulties in resolving phylogenetic relationships within Cactaceae (Nyffeler, 2002; Arias et al., 2003, 2005). Although the consensus tree obtained with the resultant MP phylogenies for the five-loci matrix (Appendix S2) provided solid hypotheses about relationships among main lineages, it usually lacked resolution (resulted in polytomies) among derived lineages within Cactoideae, particularly in the South American lineages within the core Cactoideae clade (discussed later).

In the search for alternatives to MP, we explored ML phylogenetic methods. RAxML is a heuristic, parallelized program that uses ML as the optimization criterion to infer phylogenetic relationships. It implements a novel algorithmic optimization of the likelihood function to estimate large phylogenies in short times (Stamatakis, 2006). Experimental studies on large real-world data sets have shown that RAxML can find trees with higher likelihood values in less time and with less computer memory requirements than other programs, for example, GARLI or PHYML (Hordijk and Gascuel, 2005). Stamatakis (2006) also reports a better performance of RAxML in comparison to MrBayes; nonetheless, he recognizes the comparison is not fair because it considers the algorithm of the latter as an ML heuristic. We mostly base our discussion of phylogenetic relationships and evolution of lineages within Cactaceae on the obtained ML tree for the five-loci matrix (Figs. 1–4). Previous molecular phylogenetic studies have provided insights about relationships among Cactaceae (Nyffeler, 2002) and within its subclades (e.g., Butterworth et al., 2002; Arias et al., 2003, 2005). The larger taxon sampling and sequence data included in this study, particularly of the North American tribes Pachycereeae and Cacteae, allows an independent assessment of relationships among major lineages within Cactaceae, as well as greater resolution within particular clades. The obtained phylogenetic

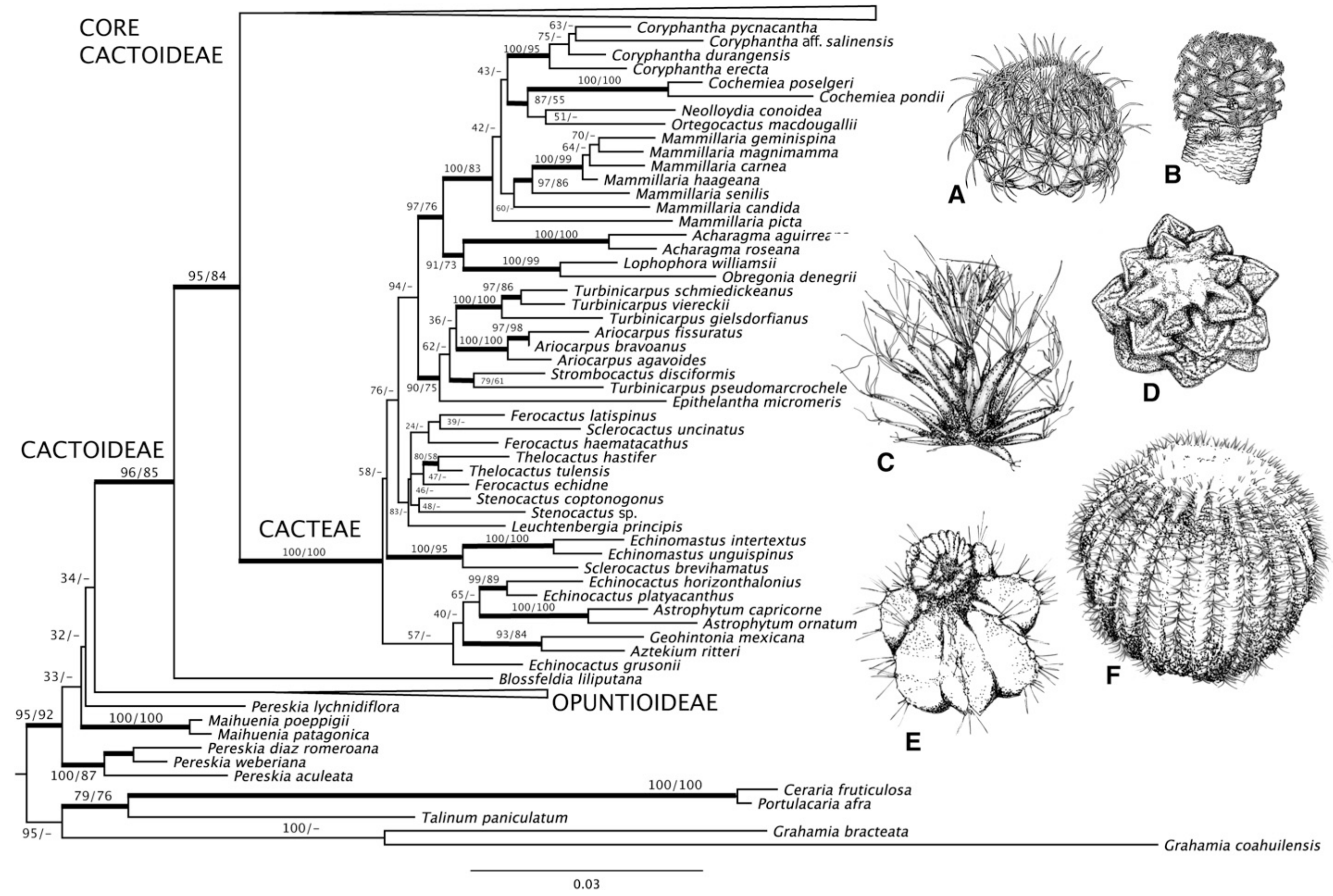

Fig. 2. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16*, and *ppc* (five-loci data matrix) showing the Cacteae clade and relationships within it. Bootstrap percentage values derived from the ML and MP analyses, respectively (bML/bMP) are above or next to clades. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Coryphantha pycnacantha*, (b) *Mammillaria hernandezii*, (c) *Leuchtenbergia principis*, (d) *Ariocarpus fissuratus*, (e) *Astrophytum ornatum*, and (f) *Echinocactus grusonii*. Figures (a) and (b) were taken and modified from Arias et al. (1997).

hypothesis represents a framework to evaluate evolutionary trends within the family, and we used it to trace the evolution of growth forms within subfamily Cactoideae, which possesses the largest diversity of forms within Cactaceae.

***Evolutionary relationships: Early-diverging* Pereskia *and* Maihuenia**—*Pereskia* and *Maihuenia* have usually been placed within subfamily Pereskioideae, because they lack many derived characters of Cactaceae (e.g., Cactoideae members, Edwards et al., 2005). *Maihuenia*, however, has also been placed within Opuntioideae (Britton and Rose, 1919–1923; Butterworth and Wallace, 2005) or within its own subfamily, Maihuenioideae (Anderson, 2001). Our study confirms the paraphyly of *Pereskia* (Fig. 1), whose members have been found to form an early-diverging grade within Cactaceae in molecular phylogenies (Butterworth and Wallace, 2005; Edwards et al., 2005). *Maihuenia* is a cushion- or mat-forming shrub from the southern Andes and Patagonia. Species of *Maihuenia* have persistent leaves, and their relationship with either *Pereskia* or Opuntioideae has been suggested (Leuenberger, 1997; Anderson, 2001). In contrast to *Pereskia*, *Maihuenia* has anatomical adaptations to xeric environments in cold high-Andean habitats (Leuenberger, 1997; Mauseth, 1999). Previous molecular phylogenies have either placed *Maihuenia* as sister of Cactoideae (Edwards et al., 2005) or of Opuntioideae (Nyffeler, 2002) or showed no support for any relationship to either of the two subfamilies (Butterworth and Wallace, 2005). In the five-loci ML tree, *Maihuenia* is weakly supported as the sister of ((Opuntioideae, Cactoideae) *P. lychnidiflora*) (Fig. 1). Our results suggest the placement of *Maihuenia* among the branches of the *Pereskia* grade (Fig. 1; Fig. S2.a in online Appendix S2).

Subfamily Opuntioideae—The monophyly of Opuntioideae has long been recognized (Barthlott and Hunt, 1993; Griffith and Porter, 2009). Although the inclusion of all described genera within a single genus, *Opuntia*, has been proposed (Hunt, 1999), they are morphologically diverse (Fig. 1) and occupy a wide range of habitats through the American continent. Our study confirms the monophyly of Opuntioideae with the five-loci matrix and with the nuclear marker (100 bML/100 bMP; Fig. 1 and Fig. S3.a in online Appendix S3). The five-loci ML phylogeny shows that it is composed of three main clades: one including the cylindrical-stemmed *Quiabentia*, *Grusonia*, *Pereskiopsis* (Fig. 1e) and *Cylindropuntia* (100 bML/96 bMP); another with species characterized by rather spherical stems, including *Maihueniopsis*, *Tephrocactus* (Fig. 1c), and *Pterocactus*

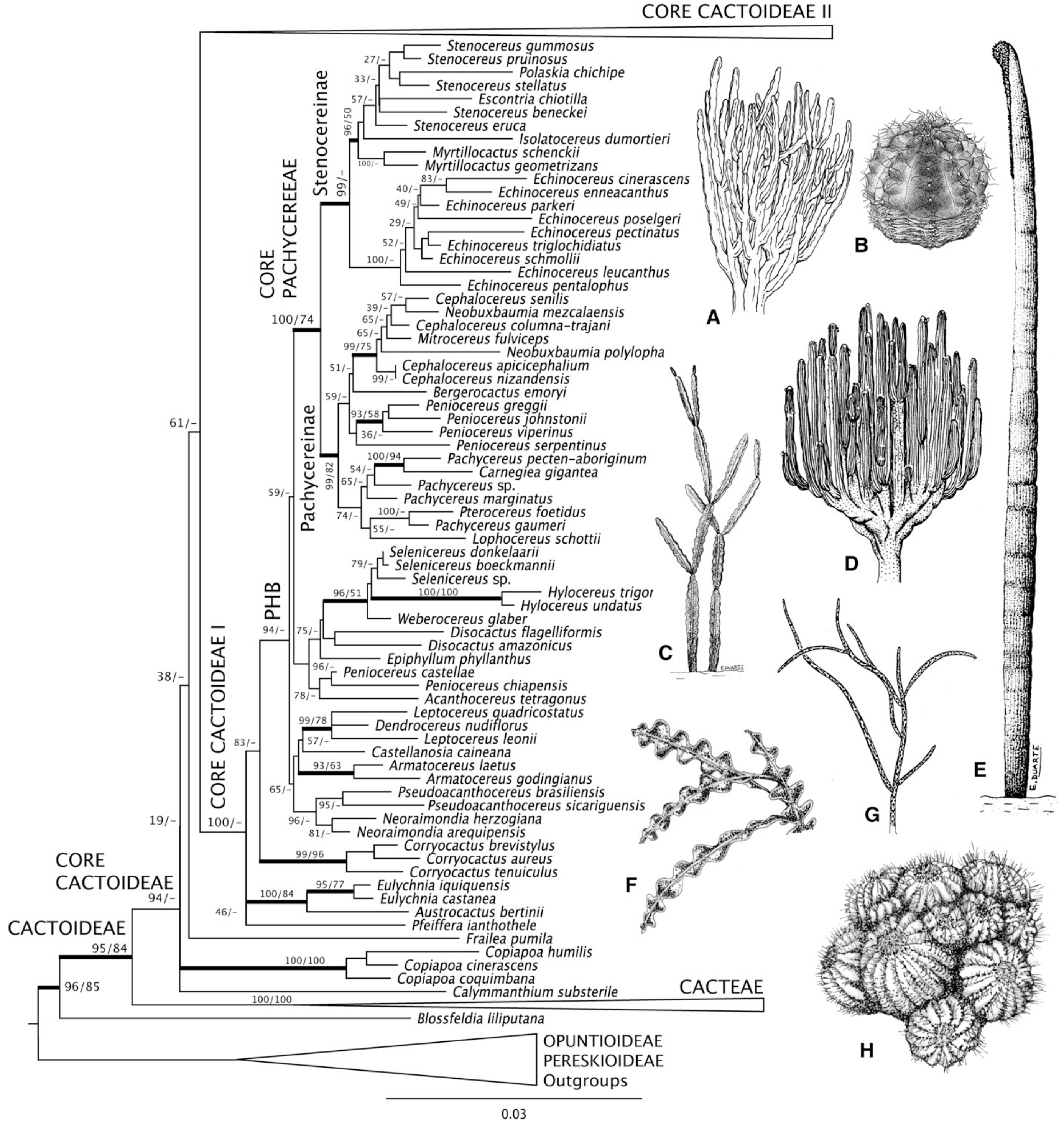

Fig. 3. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16* and *ppc* (five-loci data matrix) showing the core Cactoideae I clade and relationships within it. Bootstrap percentage values derived from the ML and MP analyses respectively (bML/bMP) are above or next to clades. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Stenocereus pruinosus*, (b) *Echinocereus pulchellus*, (c) *Acanthocereus subinermis*, (d) *Pachycereus weberi*, (e) *Cephalocereus columna-trajani*, (f) *Epiphylum anguliger*, (g) *Pterocereus viperinus*, and (h) *Copiapoa cinerea*. Figures (a) to (e) and (g) were taken and modified from Arias et al. (1997).

(Fig. 1d) (78 bML/63 bMP); and a third, which contains *Miqueliopuntia* and *Tunilla,* with cylindrical to spherical stems, as early-divergent members, and also *Opuntia* and *Nopalea*, with flattened stems (cladodes; Figs. 1a, 1b) (100 bML/99 bMP). These results differ slightly from those of Griffith and Porter (2009) who, on the basis of ITS and *trnL-trnF* sequences and a denser taxonomic sampling, found *Pterocactus* to be separated from (*Maihueniopsis*, *Cumulopuntia*).

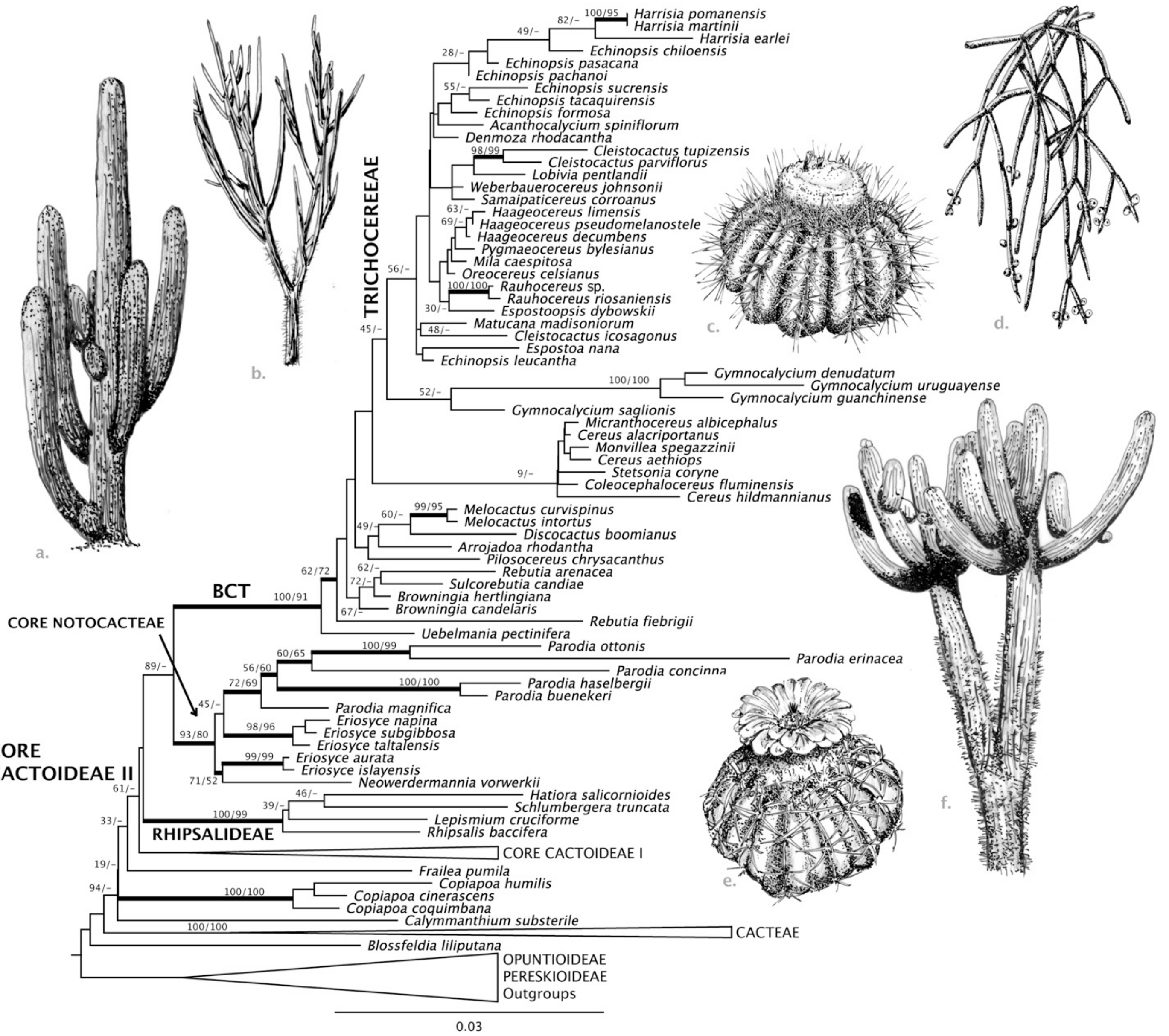

Fig. 4. Maximum likelihood tree for Cactaceae derived from the concatenated analyses of *trnK-matK*, *matK*, *trnL-trnF*, *rpl16*, and *ppc* (five-loci data matrix) showing the core Cactoideae II clade and relationships within it. Bootstrap percentage values derived from the ML and MP analyses, respectively (bML/bMP) are above or next to clades. Thick lines indicate the most strongly supported clades. Figures (not on scale) represent growth forms of (a) *Echinopsis atacamensis*, (b) *Harrisia eriophora*, (c) *Melocactus intortus*, (d) *Rhipsalis baccifera*, (e) *Parodia erinaceae*, and (f) *Browningia candelaris*.

Cactoideae—Members of subfamily Cactoideae possess succulent stems, usually with ribs or tubercles (Wallace and Gibson, 2002; Figs. 2–4). The common ancestry of all of its members has been supported by morphological, anatomical, and molecular data (Wallace, 1995b; Wallace and Cota, 1996; Terrazas and Arias, 2003). Previous molecular phylogenies (Nyffeler, 2002) and our results (Fig. 2; Fig. S2.b–d in online Appendix S2) confirm this monophyly with high support values (94 bML/85 bMP). Cactoideae encompasses the greatest species richness and growth form diversity within Cactaceae, including ca. 80% of the species in the family (Anderson, 2001; Hunt et al., 2006). It has been subdivided into several tribes, but morphological studies have proven insufficient to clarify relationships within them and even within genera, presumably because of convergent evolution at the species level (Applequist and Wallace, 2002; Nobel, 2002). According to our five-loci ML tree, Cactoideae consists of two large sister clades, Cacteae and core Cactoideae; and *Blossfeldia* as sister to this pair (Fig. 2). Also on the basis of molecular phylogenetic studies, Crozier (2004) suggested the subdivision of Cactoideae into two subfamilies, Cactoideae and Rhipsalidoideae (discussed later), and the placement of *Blossfeldia* within its own subfamily, Blossfeldioideae.

Blossfeldia—As in previous studies (Nyffeler, 2002; Butterworth, 2006), we found *Blossfeldia*, a monotypic genus, as the strongly supported sister of the rest of Cactoideae (96

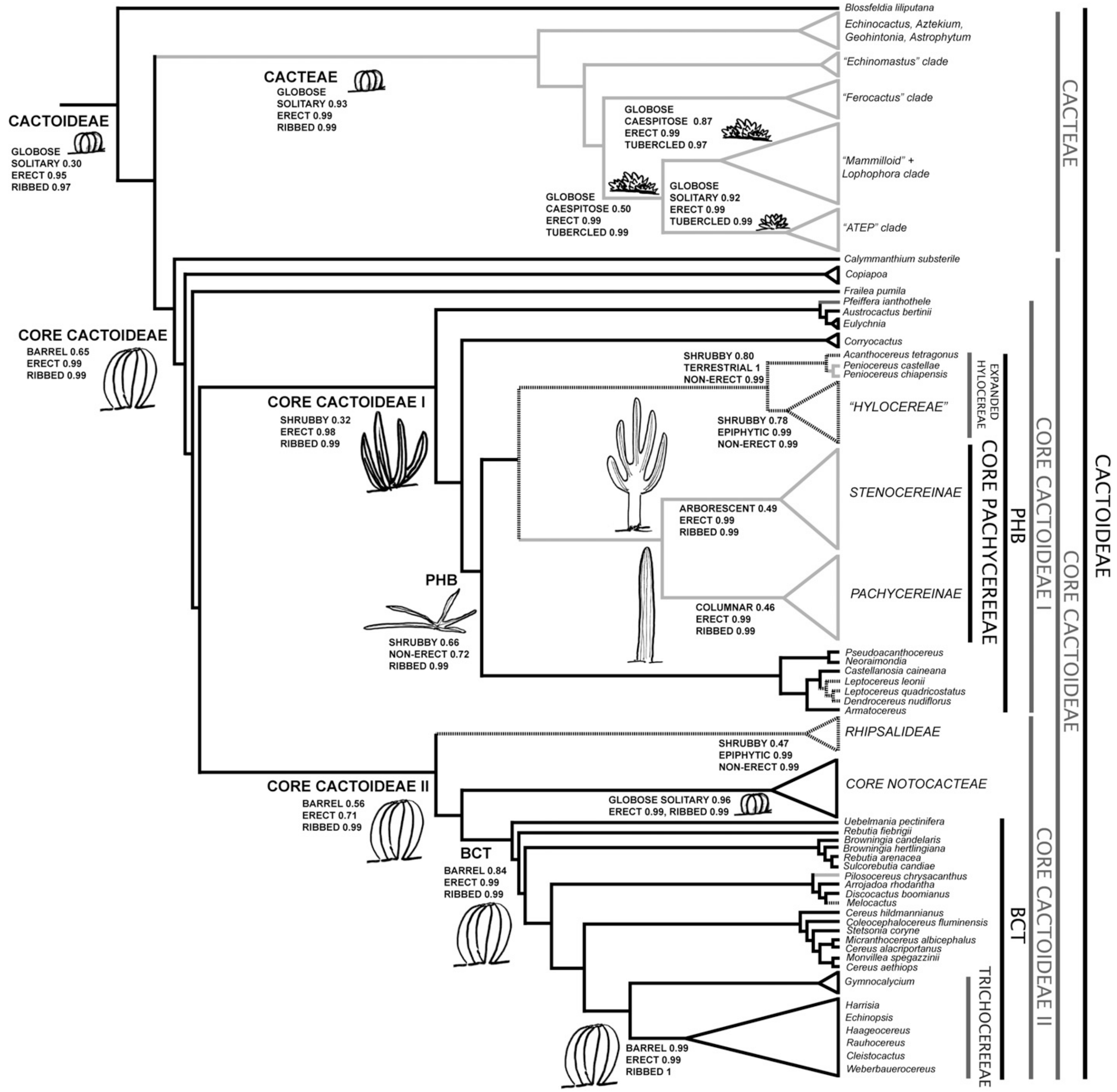

Fig. 5. Maximum likelihood reconstruction of ancestral characters for growth form and habit within Cactoideae. Character states found to have the highest probability, and probability values are next to each node. Figures represent the combination of the most probable character states found at tested nodes. Dark lines indicate lineages distributed mainly in South America, gray lines indicate linages distributed mainly in North America, and dashed lines indicate linages distributed mainly in Central America and the Caribbean.

bML/85 bMP for its inclusion in Cactoideae and 95 bML/84 bMP for the monophyly of all other Cactoideae; Fig. 2). *Blossfeldia*, the smallest cactus, is widely distributed in Argentina and Bolivia, where it grows in rock crevices (Anderson, 2001). It has morphological and ecological features rarely found in other cacti and has been generally recognized as a clearly distinct genus within tribe Notocacteae (Gibson and Nobel, 1986; Barthlott and Hunt, 1993; Anderson, 2001) or even proposed to belong to its own subfamily, Blossfeldioideae (Croizer, 2004). Nyffeler (2002) reported for the first time the intriguing early-diverging position of *Blossfeldia* within Cactoideae. This report led to a controversy in the literature trying to explain *Blossfeldia*'s morphologically derived characters (Gorelick, 2004; Butterworth, 2006). Gorelick (2004) stated a series of hypotheses to explain Nyffeler's results. He noticed that *Blossfeldia liliputana* is a hexaploid species and suggested that this could indicate its possible hybrid origin. In this case, chloroplast sequences would not show the evolutionary history of *Blossfeldia* because they are maternally inherited. Our analyses based on the five-loci data set and on independent loci, including the nuclear *ppc* (Fig. S3.a in Appendix S3), confirm *Blossfeldia* as the sister to all other Cactoideae (Fig. 2).

Cacteae—Cacteae is the only traditionally recognized tribe of Cactoideae that is strongly supported as monophyletic in our ML analyses (100 bML/100 bMP, Fig. 2). This tribe comprises all the North American globose, barrel-shaped, and short-cylindrical cacti (Fig. 2) and reaches its greatest morphological diversity and species richness in Mexico, particularly in the southeastern Chihuahuan Desert (Hernández, et al., 2001; Hernández and Gómez-Hinostrosa, 2005). Previous molecular phylogenies also support the monophyly of Cacteae (Butterworth et al., 2002; Nyffeler, 2002). Buxbaum (1958, p. 195) referred to it as tribe Echinocacteae, described it as "a clear-cut phylogenetic unit", and divided it into subtribes based mainly on seed morphology. Crozier (2004) even proposed to modify the taxonomic status of tribe Cacteae by transforming it into subfamily Cactoideae and placing the remaining tribes in subfamily Rhipsalidoideae.

Our five-loci ML tree resolved four main lineages within Cacteae with well-supported relationships (Fig. 2). Within these lineages, we recognized some clades similar to those reported by Butterworth et al. (2002) in a molecular MP phylogenetic analysis based on *rpl16*. However, our results show different relationships among them. Butterworth et al. (2002) found a clade they referred to as "*Aztekium*" (*Aztekium*, *Geohintonia*) as earliest diverging within Cacteae (Butterworth et al., 2002). We consistently recovered the same "*Aztekium*" clade (93 bML/84 bMP); however, in our results, it is associated with *Echinocactus* and *Astrophytum*, and both clades together form a poorly supported clade sister of all remaining Cacteae (Fig. 2). An association between *Echinocactus* and *Astrophytum* was also found in our nuclear *ppc* analyses, as the earliest-divergent clade within Cacteae (Fig. S3.a in Appendix S3). Although in the five-loci ML tree *E. grusonii* appears to be the earliest-diverging branch within the *Aztekium* clade, this position is not recovered in the plastid ML phylogeny (Fig. S3.b in Appendix S3). The *Aztekium* clade includes solitary globose to barrel cacti with strongly ribbed stems.

The next clade to diverge within tribe Cacteae includes *Sclerocactus brevihamatus* and *Echinomastus* (100 bML/95 bMP), although its earliest-diverging position from remaining Cacteae members is poorly supported (58 bML). This clade includes solitary globose plants with low ribs. The next clade to diverge is only moderately supported in our ML analyses (81 bML) and is similar to the "*Ferocactus*" clade found by Butterworth et al. (2002). This clade includes *Stenocactus* spp., *Thelocactus* spp., *Leuchtenbergia principis*, *Ferocactus* spp., and *Sclerocactus uncinatus*, and a similar association, although poorly supported, was found also in our *ppc* analyses (Fig. S3.a in Appendix S3). Species within this clade are solitary or clustering and possess stems with ribs, tuberculated ribs (*S. uncinatus*), or long tubercles (*L. principis*). The position of *S. uncinatus* as sister to *Ferocactus latispinus* and *F. haematacanthus* species was recognized in our five-loci ML and MP trees (Fig. 2; Fig. S.2.b in Appendix S2). *Sclerocactus uncinatus* and *S. uncinatus* subsp. *crassihamatus* are synonyms of *Glandulicactus uncinatus* and *G. crassihamatus*, respectively. Some authors (e.g., Barthlott and Hunt, 1993; Hunt, 1999; Anderson, 2001) do not recognize *Glandulicactus* as a distinct genus. Considering vegetative and floral morphology, and molecular phylogenies, this genus has been suggested to be closer to *Ferocactus* and *Thelocactus* than to *Sclerocactus* (Ferguson, 1991; Butterworth et al., 2002). Our results, although weakly supported, suggest that *Ferocactus* is polyphyletic and that *Sclerocactus uncinatus* is closely related to members of *Ferocactus* (Fig. 2; Fig. S2.b in Appendix S2).

The genus *Sclerocactus* has been subject to much controversy. Our analyses included two of its species (*S. brevihamatus* and *S. uncinatus*), but they did not group together, suggesting that this genus is also polyphyletic. On the basis of molecular and morphological data, Porter (1999) concluded that *Sclerocactus* should be subdivided and that *Echinomastus* should be recognized as distinct. In spite of our limited intrageneric sampling, our results support Porter (1999) and Porter et al. (2000) in proposing that *Sclerocactus* should be revised and that *Echinomastus* and *Glandulicactus* should be recognized as distinct taxa, supporting the inclusion of *S. brevihamatus* within genus *Echinomastus* (Anderson, 2001).

A sister pair consisting of (*Ariocarpus*, *Strombocactus*, *Turbinicarpus*, and *Epithelantha*) and the "Mammilloid" clade (Butterworth et al., 2002) is strongly supported in the five-loci ML tree (94 bML), but weakly so in the MP tree (Fig. 2). A clade similar to the former was recognized by Butterworth et al. (2002; their "ATEP" clade), except for the absence of *Strombocactus* and the presence of *Pediocactus*. The "Mammilloid" clade (Butterworth et al., 2002) includes (*Acharagma, Lophophora*, *Obregonia*) plus (*Coyphantha*, *Mammillaria*, (*Cochemiea, Neolloydia, Ortegocactus*)). Previous studies, as well as our results, suggest that *Mammillaria* is polyphyletic (see position of *M. picta* in Fig. 2, and Butterworth and Wallace, 2004). An association among *Acharagma*, *Lophophora*, and *Obregonia* was also found by Butterworth and collaborators (the "*Lophophora*" clade; Butterworth et al., 2002) but in their study, this group was distantly related from the "Mammilloid" clade. Although *Cochemiea*, a genus from the Baja California Peninsula in Mexico, has been placed within *Mammillaria* in recent taxonomic reports (Hunt et al., 2006) and MP phylogenetic analyses (Butterworth and Wallace, 2004), here we confirm that it is independent from *Mammillaria*, suggesting that it should be regarded as taxonomically distinct (Barthlott and Hunt, 1993; Anderson, 2001). The "Mammilloid" clade represents one of the most spectacular radiations of Cactaceae in the arid and semiarid regions of North America. This clade comprises small globose members with tuberculated stems and dimorphic areoles (Butterworth et al., 2002) and includes the richest genera within Cacteae, namely *Mammillaria* (ca. 145 species), *Coryphantha* (55 species) and *Escobaria* (23 species; Hunt, 1999). The derived position of the "Mamilloid" clade within Cacteae may be consistent with a recent diversification.

Core Cactoideae—The strongly supported clade here referred to as core Cactoideae (94 bML, Fig. 3) is another species-rich clade within Cactaceae including species distributed throughout the American continent (Nyffeler, 2002). Core Cactoideae has been previously recognized with molecular data (Nyffeler, 2002; Croizer, 2004). It includes members of tribes Pachycereeae, Hylocereeae, Browningieae, Trichocereeae, Cereeae, Rhipsalideae, and Notocacteae, which, as in previous results (Nyffeler, 2002; Arias et al., 2005; Ritz et al., 2007), were found here to be para- or polyphyletic. Core Cactoideae encompasses a vast diversity of growth forms (Figs. 3, 4), including gigantic arborescent forms (such as species of *Echinopsis*, *Pachycereus*, or *Neobuxbaumia*), solitary or clumped globose forms (such as species of *Parodia* and *Copiapoa*, respectively), and hanging epiphytes with cylindrical spineless or flattened two-ribbed stems (for example, different members of *Rhipsalis* or *Hylocereus*) distributed throughout America.

***Early-divergent genera* Copiapoa, Calymmanthium, *and* Frailea**—*Copiapoa*, *Calymmanthium*, and *Frailea* are weakly supported as the three earliest-diverging branches within core Cactoideae in the five-loci ML tree (Fig. 3). This position was not found in the corresponding MP tree, where *Frailea* grouped with members of Rhipsalideae, and *Calymmanthium* grouped with *Pfeiffera* (Fig. S2.c in Appendix S2). The position of these genera was uncertain in the study of Nyffeler (2002).

Copiapoa is endemic to the Atacama Desert in northern Chile and includes 26 species of globose to short, cylindrical cacti that can form massive clumps and are highly adapted to aridity (Schulz and Kapitany, 1994; Anderson, 2001). The monophyly of *Copiapoa* is well supported (100 bML/100 bMP). Its position as sister to all other core Cactoideae was also found in our MP analyses (Fig. 3; Fig. S2.c in Appendix S2), but not in the *ppc* ML tree (Fig. S3.a in Appendix S3); however, its relationships may be reconsidered given the weak support of the branches diverging after it, especially in MP analyses (see also Nyffeler, 2002).

Calymmanthyum is a monotypic, arborescent genus endemic to northern Peru, with unique anatomical and morphological features, for example, a uniseriate to biseriate collenchymatous hypodermis with relatively thin walls, with no mucilage cells in either cortex or pith, and flowers with an inner perianth partially shielded by an outer floral tube (Anderson, 2001; Nobel, 2002; Wallace, 2002). It has been suggested that it retains numerous plesiomorphic features within Cactoideae, for example, few ribbed juvenile shoots (Nobel, 2002; Wallace, 2002; Wallace and Gibson, 2002). The position of *Calymmanthium* in the five-loci ML tree is weakly supported and unresolved within Cactoideae in the *trnK/matK*, *matK* and *ppc* independent analyses. The genus *Frailea* was established by Britton and Rose (1919–1923) to include eight species of dwarf globular cacti from southern Brazil and northeast Argentina. Anatomical studies show that *Notocactus*, *Blossfeldia*, and *Frailea* share common features with *Parodia* (see references in Eggli and Nyffeler, 1998), but our results, as well as those of Nyffeler (2002), suggest that *Frailea* is an unrelated, independent lineage. Its position in the ML tree (Fig. 3) is weakly supported.

Core Cactoideae I—Our ML tree shows a pair of large sister clades that diverge after *Frailea*, here referred to as core Cactoideae I and core Cactoideae II. Core Cactoideae I is composed of *Pfeiffera ianthothele*; a strongly supported *Eulychnia* and *Austrocactus* clade (100 bML/84 bMP); and a clade containing Pachycereeae, Hylocereeae, and Browningieae members, here termed the PHB clade (85 bML; Fig. 3).

Austrocactus and *Eulychnia*, together with *Corryocactus*, were considered early-diverging members of Notocacteae (Buxbaum, 1969). *Austrocactus* comprises five or six species of globose to short cylindrical cacti from southern Argentina and Chile, thought to be closely related to *Corryocactus* (Anderson, 2001). *Eulychnia* on the other hand includes arborescent, candelabriform, sometimes gigantic, species (e.g., *E. breviflora*; Anderson, 2001) that inhabit the coastal deserts of Chile and southern Peru. The highly supported (*Austrocactus*, *Eulychnia*) clade is a good example of the high divergence of growth forms that can be found among closely related taxa within core Cactoideae. In our five-loci ML and MP phylogenies, the (*Austrocactus*, *Eulychnia*) clade is sister to the remaining members of core Cactoideae I (Fig. 3; Fig. S2.c in Appendix S2).

A clade similar to the PHB was previously recognized by Nyffeler (2002), who referred to it as the AHCLP clade. The PHB clade (94 bML) includes members of tribe Pachycereeae, Hylocereeae, and three genera formerly included in Browningieae: *Castellanosia, Neoraimondia*, and *Armatocereus*. *Corryocactus*, which includes shrubby to arborescent columnar species from Peru, western Bolivia and northern Chile, is sister to the PHB clade (85 bML; Fig. 3). Whereas its tribal placement has been the subject of controversy (Anderson, 2001), its position as the sister branch to the PHB clade (Fig. 3) was also found in previous molecular phylogenies (Nyffeler, 2002; Wallace, 2002; Wallace and Gibson, 2002).

A weakly supported clade containing *Armatocereus*, *Neoraimondia*, *Castellanosia* (Browningieae), and *Dendrocereus*, *Leptocereus*, and *Pseudoacanthocereus* (formerly in Pachycereeae) was recognized in our five-loci ML and MP analyses (Fig. 3; Fig. S2.c in Appendix S2). *Castellanosia*, *Neoraimondia*, *Armatocereus*, and *Leptocereus* were unresolved in the AHCLP clade of Nyffeler (2002). The geographical distribution of these genera makes their association intriguing. *Leptocereus* and *Dendrocereus* share a Caribbean distribution (Anderson, 2001), but whereas the former contains sprawling, cylindrical forms, the latter contains arborescent species with woody trunks. Their sister species, *Castellanosia caineana* from lowland Bolivia, is also arborescent. *Castellanosia* was considered as part of *Neoraimondia* (Mauseth and Kiesling, 1998; Kiesling and Mauseth, 2000); however, in our five-loci ML tree, their representative species do not form a clade (Fig. 3). *Neoraimondia* includes arborescent candelabriform cacti up to 15 m tall, from the Peruvian coast and the Peruvian and Bolivian Andes (Anderson, 2001). *Armatocereus* is a massive shrubby genus with segmented, cylindrical stems, mainly from the Andes of Colombia, Ecuador and Peru (Anderson, 2001). *Pseudoacanthocereus* is distributed in Brazil and Venezuela and includes sprawling to upright shrubs (Anderson, 2001). The finding of a South American/Caribbean clade as early divergent within the PHB clade supports previous suggestions regarding the possibility that the closest relatives of North American columnar Pachycereeae and the primarily epiphytic Hylocereeae are South American (Wallace, 2002).

Pachycereeae is the second largest tribe of North American cacti and represents a diversification that gave rise to columnar and arborescent forms that can reach gigantic sizes, such as the saguaro (*Carnegiea gigantea*) and cardón (*Pachycereus pringlei*) from northern Mexico and southwest USA. In their anatomical, chemical, and morphological study of Mexican columnar cacti, Gibson and Horak (1978) and Gibson (1982) recognized two major lineages within Pachycereeae: subtribe Pachycereinae (*Pachycereus*, *Neobuxbaumia*, *Cephalocereus*, *Carnegiea*, *Mitrocereus*, and *Lophocereus*) and subtribe Stenocereinae (*Escontria*, *Myrtillocactus*, *Polaskia*, and *Stenocereus*). Our results are congruent with this hypothesis because we recovered clades similar to the described subtribes. The North American columnar genera are well supported as a monophyletic group (100 bML/74 bMP), here referred to as core Pachycereeae. The deepest split within this clade separates the clades we here refer to as Pachycereinae (99 bML/82 bMP) and Stenocereinae (99 bML; Fig. 3) following Gibson and Horak (1978) and Gibson (1982). However, in addition, and as in previous studies (Arias et al., 2005), we found a strongly supported *Echinocereus* clade as sister to the remaining members of Stenocereinae (Fig. 3).

Peniocereus had been typically considered as closely associated with Pachycereeae or Echinocereeae (Barthlott and Hunt, 1993; Anderson, 2001), and molecular and morphological studies found this genus to be paraphyletic (Arias et al., 2005; Gómez-Hinostrosa and Hernández, 2005) with some of its species more closely associated to Hylocereeae. Our results confirm these previous results in that *Peniocereus* subgen. *Pseudoacanthocereus* (*P. castellae* and *P. chiapensis*) is closely related to *Acanthocereus tetragonus* and to tribe Hylocereeae, forming a clade here named "expanded Hylocereeae", whereas other species of *Peniocereus* (e.g., *P. greggii*, *P. johnstonii*, *P. serpentinus*, and *P. viperinus*) are closer to Pachycereinae.

Hylocereeae is one of only two tribes within Cactoideae that includes epiphytes (see below). Its members are scandent or epiphytic shrubs with flattened or few-ribbed stems, mainly distributed in Central America. It was considered to be related to Echinocereeae (*Harrisia*, *Acanthocereus*) and Cereeae (Barthlott and Hunt, 1993). As recognized by Arias et al. (2005), we found that all included species of Hylocereeae form a weakly supported monophyletic group within the PHB clade (Fig. 3).

Core Cactoideae II—Core Cactoideae II includes all remaining South American members of core Cactoideae as well as members of tribe Rhipsalideae. This clade is recognized, but weakly supported, in our five-loci ML and MP analyses (Fig. 4; Fig. S2.d in Appendix S2). Species included within this clade were scarcely sampled in our nuclear *ppc* analyses and did not form the same association as in the phylogenetic results obtained with the plastid or the five-loci matrices (Fig. S3a and b in Appendix S3).

Rhipsalideae, mostly distributed in eastern Bolivia and southeastern Brazil (Barthlott and Hunt, 1993), is another Cactoideae tribe that includes epiphytes and lithophytes. A clade comprising members of tribe Rhipsalideae (100 bML/99 bMP) was found in our five-loci ML tree as the sister of the remainder of core Cactoideae II, but with weak support (Fig. 4). Nyffeler (2002) found an equivalent relationship, but with stronger support, suggesting a close relationship between some Rhipsalideae members and the remaining South American tribes (i.e., Cereeae, Notocacteae, Trichocereeae, Browningieae) within core Cactoideae II. Nevertheless, in our five-loci MP tree, the Rhipsalideae clade is the sister of (core Cactoideae I, core Cactoideae II) (Fig. S2.c in Appendix S2) and in the nuclear *ppc* analyses, a clade of (*Hatiora salicornoides*, *Rhipsalis baccifera*) is sister to representative species of core Cactoideae I (Fig. S3.a in Appendix S3).

Pfeiffera, regarded as "transitional" between *Corryocactus* and Rhipsalideae (Gibson and Nobel, 1986), is the only traditional member of Rhipsalideae that did not group with other members of this tribe. Instead, *Pfeiffera* was found to be closely related to North American members of core Cactoideae (Fig. 3), as previously recognized by Nyffeler (2002). This position needs to be confirmed with sequences obtained from additional specimens and other species, given that we used Nyffeler's data for our single representative of this genus. Besides *Pfeiffera,* the epiphytic habit hence seems to have evolved independently in two distantly related lineages of Cactaceae: in Hylocereeae, within core Cactoideae I; and in Rhipsalideae, within core Cactoideae II.

A clade containing *Eriosyce*, *Parodia*, and *Neowerdermannia vorwerkii* (93 bML/80 bMP) was here recognized (Fig. 4). Nyffeler (2002) found an equivalent clade and referred to it as core Notocacteae. *Eriosyce* includes 33–35 species of globular to elongated, rarely columnar cacti distributed in central Chile, southern Peru, and northwest Argentina (Anderson, 2001). It was previously allied to *Austrocactus*, *Eulychnia*, *Copiapoa*, *Corryocactus*, and *Neowedermannia* (Kattermann, 1994). *Parodia* includes ca. 29 accepted and 76 provisional species, which are small to moderately sized, solitary or clustering, generally with globose stems, and inhabit the east slope of the Andes in southern Brazil, Uruguay, and Paraguay to the northern half of Argentina (Eggli and Nyffeler, 1998, 2007). *Neowerdermannia* consists of two globose species disjunctly distributed in southwestern Chile (*N. chilensis*), and Bolivia and northern Argentina (*N. vorwerkii*; Kattermann, 1994). It has been previously associated with *Gymnocalycium* (Anderson, 2001). Our results, together with those of Nyffeler (2002), indicate that Notocacteae sensu Anderson (2001) and Barthlott and Hunt (1993) is a polyphyletic assemblage with *Austrocactus* and *Eulychnia* closely related to Pachycereeae; *Copiapoa* as an early-diverging independent lineage within core Cactoideae; and *Eriosyce, Parodia*, and *Neowedermannia* forming a clade within core Cactoideae II.

A clade that includes all sampled members of tribe Trichocereeae and some members of Cereeae and Browningieae conforms to another major clade within core Cactoideae II (Fig. 4). A similar association has been referred to as the BCT clade (Nyffeler, 2002; Wallace, 2002) and includes several South American columnar or arborescent species, with *Uebelmannia* as its earliest-diverging branch (Fig. 4; see also Nyffeler, 2002). Our results found this BCT clade to be strongly supported (100 bML/91 bMP), and it was also recovered with high support values in the nuclear *ppc* analyses (Fig. S3.a in Appendix S3). After *Uebelmannia*, several taxa formerly assigned to Browningieae and Cereeae, as well as *Discocacuts*, *Rebutia*, and *Sulcorebutia* (Trichocereeae), form an early-diverging grade within BCT (Fig. 4). *Browningia* and *Stetsonia* (Browiningieae) are shrubby or large arborescent forms (Fig. 4e), mainly from the central Andean region (northern Chile, Bolivia, and Peru), and presumably display plesiomorphic floral attributes (Wallace, 2002). In contrast to the western South America diversity-centered taxa (i.e., *Gymnocalycium*, *Parodia*, *Eriosyce*), former members of Tribe Cereeae (Appendix S1) are most diverse in eastern South America. This tribe includes cylindrical-shaped species ranging from large, robust trees to thin-stemmed shrubs, but also includes globular forms such as *Melocactus* (Fig. 4c). Tribe Trichocereeae consists of long-stemmed cylindrical cacti with a wide variety of habits, including sprawling, shrubby, large arborescents, or candelabriforms, and even globoses (possibly reduced; Wallace, 2002) forms. In our five-loci ML analysis, members of Trichocereeae, except for *Discocacuts*, *Rebutia*, and *Sulcorebutia*, belong to a weakly supported clade nested within BCT (Fig. 4). Our analyses indicate that *Echinopsis* sensu lato is polyphyletic and requires to be taxonomically revised.

Evolutionary trends of growth form and habit within Cactoideae—The extraordinary vegetative diversity within Cactaceae (Mauseth, 2006) difficults the proposal of a simple set of categories for the growth forms and habits it encompasses (Anderson, 2001). Subfamily Cactoideae is probably the richest in terms of species number and diversity of growth forms (see Barthlott and Hunt, 1993; Anderson, 2001). Within this subfamily, the Cacteae clade includes globose cacti with ribbed or tubercled stems, which range in sizes from few centimeters (*Escobaria*) to a couple of meters above the ground (*Echincactus platyacanthus*), while the predominant growth forms in the core Cactoideae clade are scandent, shrubby, or arborescent cacti with cylindrical stems. The arborescent and the columnar growth form evolved in derived clades both in core Cactoideae I and core Cactoideae II, and both clades also include globose forms, apparently caused by stem reduction.

To trace the evolution of growth forms and habits within subfamily Cactoideae, we reconstructed the ancestral character states using ML methods. According to our results (Fig. 5; Appendix S4), the ancestor of Cactoideae was a solitary, globose cactus with a ribbed stem. However, the probability value for the globose, solitary character state at this node is not high (0.30, see probability values obtained for each character in Table S4.2 in Appendix S4). The ancestral growth form of the Cacteae clade was also inferred to be ribbed, solitary globose, with a probability of 0.99 and 0.93 respectively (Fig. 5; Table S.4.2 in Appendix S4). Nevertheless, the ancestral condition for the ("Mamilloid" clade and "*Lophophora*" clade) plus the ATEP clade appears to be clustering or caespitose, globose, with tubercled stems, which evolved to a solitary stem within the ATEP clade (Fig. 5).

According to our results, in contrast to the globose ancestral condition in Cacteae, the ancestor of core Cactoideae was probably a ribbed barrel (Fig. 5; Table S4.2 in Appendix S4). This condition probably changed in the ancestral node for core Cactoideae I, where the shrubby character state had the highest probability (Fig. 5; Table S4.2 in Appendix S4). The main clade within core Cactoideae I is the PHB clade. The prevailing growth forms observed among early-diverging lineages within the PHB clade are arborescent or shrubby (e.g., *Armatocereus*, *Neoraimondia*), or more frequently, sprawling epiphytes or lithophytes (e.g., *Selenicereus*, *Hylocereus*, *Pseudoacanthocereus*, *Disocactus*, *Peniocereus*) distributed in Central and South America. The columnar or candelabriform forms within this clade acquired their most conspicuous expression in highly nested clades, such as in core Pachycereeae, suggesting a trend toward the evolution of large columnar and arborescent growth forms. Our reconstruction suggests shrubby cacti with nonerect stems as ancestral to the PHB clade. This nonerect, shrubby condition apparently evolved into an arborescent ancestor in the Stenocereinae clade and a columnar ancestor in the Pachycereinae clade (both with probabilities of 0.4, see Fig. 5). Our results suggest that the large columnar and arborescent North American cacti probably evolved from a Central or South American shrubby, nonerect ancestor (but see below).

As shown in Table S4.1 of Appendix S4, the ancestor of the majority of the evaluated nodes was most probably terrestrial, including the "expanded Hylocereeae" clade. Our results confirmed that the epiphytic condition is derived and originated independently in two evolutionary lineages: the Rhipsalideae clade and the Hylocereeae sensu stricto clade (Fig. 5; Table S4.1 of Appendix S4).

The globose, solitary, ribbed condition found to be most probable at the base of the Cacteae clade appeared again in the ancestral node of Core Notocacteae, possibly as a reduction of a barrel-shaped ancestor (Fig. 5). However, the ribbed, barrel-shaped condition prevailed as most probable in the BCT and Trichocereeae ancestral nodes tested. Our results indicate that, on the contrary of the evolution of columnar and arborescent North American cacti, the columnar and arborescent South American cacti (for example, *Echinopsis chiloensis* or *Browningia candelaris*) evolved from a barrel-shaped ancestor (Fig. 5; Table S4.2 of Appendix S4). Unfortunately, the poor support values and taxonomic sampling within the BCT and the Trichocereeae clades in our study do not allow a more detailed reconstruction and comparison.

Within core Cactoideae II, and particularly in the BCT clade, distinct trends in growth form evolution are not apparent, possibly due to the high variability and convergence of the character within lineages. The absence of clearly distinct trends may be also the consequence of insufficient taxonomic representation, particularly of South American lineages.

Conclusions—The phylogenetic relationships presented in this study corroborate previous results and provide further resolution of evolutionary relationships and tendencies within Cactaceae, in the context of a denser generic representation, molecular sequence data from the plastid and nuclear genomes, and parsimony and maximum likelihood phylogenetic analyses. The onset of diversification within Cactaceae gave rise to a phylogenetic grade that includes *Pereskia* and possibly *Maihuenia*. Following that early grade, the subfamilies Opuntioideae and Cactoideae, each strongly supported as monophyletic, form a sister pair.

Opuntioideae consists of a clade of flat-stemmed *Opuntia* and *Nopalea*, a clade including genera with spherical stems, such as *Pterocactus* and *Maihueniopsis*, and a clade of cylindrical or spherical stemmed genera, such as *Cylindropuntia* and *Quiabentia*. Within Cactoideae, the placement of the monotypic *Blossfeldia* as the sister to (Cacteae, core Cactoideae) is confirmed. This relationship was obtained both in analyses of the five-loci matrix, the plastid matrix, and the individual loci, including a nuclear marker. Cacteae, strongly supported as monophyletic, is a cohesive lineage that comprises the globose and barrel-shaped cacti from North America. Our ancestral character reconstruction analyses showed a possible trend within this clade from a globose ribbed ancestor toward tubercled, caespitose, or solitary cacti present in derived nodes. Our results indicate possible cases of paraphyly and polyphyly in the circumscriptions of several genera within Cacteae (*Mammillaria*, *Sclerocactus*, *Echinocactus*, *Ferocactus*, *Turbinicarpus*).

Core Cactoideae is a well-supported clade comprising species of North and South America that display a wide variety of growth forms. Its earliest-diverging lineages appear to be South American globose forms (*Copiapoa*, *Frailea*), but their relationships are weakly supported. Our results suggest a barrel-shaped, ribbed condition as the ancestor of core Cactoideae, which changed to a shrubby ancestor in the core Cactoideae I clade. The predominant growth forms within core Cactoideae I are shrubs or trees with cylindrical stems, possibly derived from a South American ancestor. Our results suggest that the North American columnar or arborescent growth forms within the core Cactoideae I clade evolved from a shrubby, nonerect (sprawling, prostrated, clambering) ancestor, while arborescent South American lineages of the Trichocereeae clade evolved from a barrel-shaped ancestor. The barrel condition possibly became reduced toward a globose form in core Notocacteae and other lineages within the BCT clade. Our results show the high diversity and frequent convergence in the evolution of growth form in this major clade.

Evolutionary relationships in Cactaceae, mainly in lineages within subfamily Cactoideae, have been difficult to elucidate only on the basis of morphological attributes, possibly because of substantial convergent evolution. Our phylogenetic results further document the complexities in resolving evolutionary relationships within Cactaceae using molecular data, even in the context of a substantial representation at the generic level. These molecular results provide solid information at the subfamilial level; however, relationships at derived phylogenetic levels generally lack strong support, particularly those of less-intensely studied South American lineages. A full understanding of phylogenetic relationships and evolutionary tendencies within Cactaceae will require a further increase in the taxonomic sampling, in the context of greater concerted efforts to improve data matrices by filling existing gaps.

LITERATURE CITED

Anderson, E. F. 2001. The cactus family. Timber Press, Portland, Oregon, USA.

Applequist, W. L., and R. S. Wallace. 2001. Phylogeny of the Portulacaceous cohort based on *ndhF* sequence data. *Systematic Botany* 26: 406–419.

Applequist, W. L., and R. S. Wallace. 2002. Deletions in the plastid *trnT-trnL* intergenic spacer define clades within Cactaceae subfamily Cactoideae. *Plant Systematics and Evolution* 231: 153–162.

Arias, S., T. Gama, and L. U. Guzmán. 1997. Flora del Valle de Tehuacán-Cuicatlán, Fascículo 14. Cactaceae A.L. Juss. Instituto de Biología, Universidad Nacional Autónoma de México, México, D.F., Mexico.

Arias, S., and T. Terrazas. 2006. Análisis cladístico del género *Pachycereus* (Cactaceae) con caracteres morfológicos. *Brittonia* 58: 197–216.

Arias, S., T. Terrazas, H. J. Arreola-Nava, M. Vázquez-Sánchez, and K. M. Cameron. 2005. Phylogenetic relationships in *Peniocereus* (Cactaceae) inferred from plastid DNA sequence data. *Journal of Plant Research* 118: 317–328.

Arias, S., T. Terrazas, and K. M. Cameron. 2003. Phylogenetic analysis of *Pachycereus* (Cactaceae, Pachycereeae) based on chloroplast and nuclear DNA sequences. *Systematic Botany* 28: 547–557.

Barthlott, W., and D. R. Hunt. 1993. Cactaceae. *In* K. Kubitzki [ed.], The families and genera of vascular plants. Springer-Verlag, New York, New York, USA.

Barthlott, W., and G. Voit. 1979. Mikromorphologie der Samenschalen und Taxonomie der Cactaceae: Ein raster-elektronenmikroskopischer Überblick. *Plant Systematics and Evolution* 132: 205–229.

Britton, N. L., and J. N. Rose. 1919–1923. The Cactaceae, 4 vols. Carnegie Institution, Washington, D.C., USA.

Butterworth, C. A. 2006. Resolving "Nyffeler's puzzle"—The intriguing taxonomic position of *Blossfeldia. Haseltonia* 12: 3–10.

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

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

Butterworth, C. A., and R. S. Wallace. 2005. Molecular phylogenetics of the leafy cactus genus *Pereskia* (Cactaceae). *Systematic Botany* 30: 800–808.

Buxbaum, F. 1951. Morphology of cacti, section I. Roots and stems. Abbey Garden Press, Pasadena, California, USA.

Buxbaum, F. 1958. The phylogenetic division of the subfamily Cereoideae, Cactaceae. *Madroño* 14: 177–216.

Buxbaum, F. 1969. Die entwicklungswege der Kakteen in Sudamerika. *In* E. J. Fittkau, J. Illies, H. Klineg, G. H. Schwabe, and H. Sioli [eds.], Biogeography and ecology in South America, 583–623. W. Junk, The Hauge, Netherlands.

Cantino, P. D., J. A. Doyle, S. W. Graham, W. S. Judd, R. G. Olmstead, D. E. Soltis, P. S. Soltis, and M. J. Donoghue. 2007. Towards a phylogenetic nomenclature of Tracheophyta. *Taxon* 56: 822–846.

Crozier, B. 2004. Subfamilies of Cactaceae Juss., including Blossfeldioideae subfam. nov. *Phytologia* 86: 52–64.

Cuénoud, P., V. Savolainen, L. W. Chatrou, M. Powell, R. J. Grayer, and w. Chase. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL, atpB* and *matK* DNA sequences. *American Journal of Botany* 89: 132–144.

De Kock, G. C. 2001. The use of *Opuntia* as a fodder source in arid areas of southern Africa. *FAO Plant Production and Protection Paper* 169: 101–105.

Downie, S. R., and J. D. Palmer. 1994. A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. *Systematic Botany* 19: 236–252.

Drezner, T. D., and R. C. Balling Jr. 2002. Climatic controls of saguaro (*Carnegiea gigantea*) regeneration: A potential link with El Niño. *Physical Geography* 23: 465–475.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.

Edwards, E. J., R. Nyffeler, and M. J. Donoghue. 2005. Basal cactus phylogeny: Implications of *Pereskia* (Cactacae) paraphyly for the transition to the cactus life form. *American Journal of Botany* 92: 1177–1188.

Eggli, U., and R. Nyffeler. 1998. Proposal to conserve the name *Parodia* against *Frailea* (Cactaceae). *Taxon* 47: 475–476.

Eggli, U., and R. Nyffeler. 2007. *Parodia allosiphon* (Marchesi) N.P. Taylor (Cactaceae-Cactoideae)—A cladistically 'basal' species with a restricted distribution range (Notes towards a checklist of Cactaceae of Uruguay, 2). *Bradleya* 25: 37–44.

Endler, J., and F. Buxbaum. 1982. Die Pflanzenfamilie der Kakteen. 3rd ed. Lehrmeister-Bücherei Nr. 872, Alberch Philler, Minden, Germany.

Ferguson, D. J. 1991. In defense of the genus *Glandulicactus* Backeb. *Cactus and Succulent Journal* 63: 87–91.

Feugang, J. M., P. Konarski, D. Zou, F. C. Stintzing, and C. Zou. 2006. Nutritional and medicinal use of Cactus pear (*Opuntia* spp.) cladodes and fruits. *Frontiers in Bioscience* 11 (suppl. 2): 2574–2589.

Gibson, A. C. 1982. Phylogenetic relationships of Pachycereeae. *In* J. S. F. Barker and W. T. Stramer [eds.], Ecological genetics and evolution: The cactus–yeast–*Drosophila* model system, 3–16. Academic Press, Sydney, Australia.

Gibson, A. C., and K. E. Horak. 1978. Systematic anatomy and phylogeny of Mexican columnar cacti. *Annals of the Missouri Botanical Garden* 65: 999–1057.

Gibson, A. C., and P. S. Nobel. 1986. The cactus primer. Harvard University Press, Cambridge, Massachusetts, USA.

Godínez-Álvarez, H., T. Valverde, and P. Ortega-Baes. 2003. Demographic trends in the Cactaceae. *Botanical Review* 69: 173–203.

Goloboff, P., S. Farris, and K. C. Nixon. 2000. TNT (Tree analysis using New Technology) (beta) [computer program]. Published by the authors, Tucumán, Argentina.

Gómez-Hinostrosa, C., and H. M. Hernández. 2005. A new combination in *Peniocereus* (Cactaceae). *Revista Mexicana de Biodiversidad* 76: 129–135.

Gorelick, R. 2004. Resolving the phylogenetic placement of *Blossfeldia liliputana* (Cactaceae): Reticulate evolution, chloroplast inheritance, and graft-chimeras. *Bradleya* 22: 9–14.

Griffith, M. P., and J. M. Porter. 2009. Phylogeny of Opuntioideae (Cactaceae). *International Journal of Plant Sciences* 170: 107–116.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Hernández, H. M., and C. Gómez-Hinostrosa. 2005. Cactus diversity and endemism in the Chihuahuan Desert Region. *In* J. L. Cartron, R. Felger, and G. Ceballos [eds.], Biodiversity and conservation in northern Mexico, 264–275. Oxford University Press, New York, New York, USA.

Hernández, H. M., C. Gómez-Hinostrosa, and R. Bárcenas. 2001. Diversity, spatial arrangement, and endemism of Cactaceae in the Huizache area, a hot-spot in the Chihuahuan Desert. *Biodiversity and Conservation* 10: 1097–1112.

Hordijk, W., and O. Gascuel. 2005. Improving the efficiency of SPR moves in phylogenetic tree search methods based on maximum likelihood. *Bioinformatics* 21: 4338–4347.

Hunt, D. 1999. CITES Cactaceae checklist, 2nd ed. Royal Botanic Gardens, Kew and International Organization for Succulent Plant Study, London, UK.

Hunt, D., N. P. Taylor, and G. Charles. 2006. The new cactus lexicon. dh Books, Milborne Port, UK.

Kattermann, F. 1994. *Eriosyce* (Cactaceae). The genus revised and amplified. *In* D. Hunt and N. Taylor [eds.], Succulent plant research, vol. 1. Royal Botanic Gardens, Kew, UK.

Kiesling, R., and J. Mauseth. 2000. History and taxonomy of *Neoraimondia herzogiana* (Cactaceae). *Haseltonia* 7: 47–52.

Leuenberger, B. E. 1976. Die pollenmorphologie der Cactaceae und ihre bedeutung fur die systematik. *Dissertationes Botanicae* 31: 1–321.

Leuenberger, B. E. 1986. *Pereskia* (Cactaceae). *Memoirs of the New York Botanical Garden* 41.

Leuenberger, B. E. 1997. *Maihuenia*: Monograph of a Patagonian genus of Cactaceae. Reprint of *Botanische Jahrbucher für Systematik, Pflanzengeschichte und Pflanzengeographie* 119.

Leuenberger, B. E., and U. Eggli. 2000. The genus *Eulychnia* (Cactaceae) in Chile: Notes on the taxonomy, types, and other old specimens. *Haseltonia* 7: 63–76.

Loup, C. 1983. Premières données sur l'architecture des Cactaceae. Diplôme d'etudes, Université des Sciences et Techniques du Languedoc, Montpellier, France.

Mandujano, M. D. C., C. Montaña, and L. E. Eguiarte. 1996. Reproductive ecology and inbreeding depression in *Opuntia rastrera* (Cactaceae) in the Chihuahuan Desert: Why are sexually derived recruitments so rare? *American Journal of Botany* 83: 63–70.

Mauseth, J., and R. Kiesling. 1998. Comparative anatomy of *Neoraimondia roseiflora* and *Neocardenasia herzogiana* (Cactaceae). *Haseltonia* 5: 37–50.

Mauseth, J. D. 1999. Anatomical adaptations to xeric conditions in *Maihuenia* (Cactaceae), a relictual, leaf-bearing cactus. *Journal of Plant Research* 112: 307–315.

Mauseth, J. D. 2004. Wide-band tracheids are present in almost all species of Cactaceae. *Journal of Plant Research* 117: 69–76.

Mauseth, J. D. 2006. Structure–function relationships in highly modified shoots of Cactaceae. *Annals of Botany* 98: 901–926.

Mauseth, J. D. 2007. Tiny but complex foliage leaves occur in many "leafless" cacti (Cactaceae). *International Journal of Plant Sciences* 168: 845–853.

Mauseth, J. D., and J. V. Landrum. 1997. Relictual vegetative anatomical characters in Cactaceae: The genus *Pereskia. Journal of Plant Research* 110: 55–64.

Metzing, D., and R. Kiesling. 2008. The study of cactus evolution: The pre-DNA era. *Haseltonia* 14: 6–25.

Nixon, K. C. 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.

Nobel, P. S. 1994. Remarkable agaves and cacti. Oxford University Press, New York, New York, USA.

Nobel, P. S. 2002. Cacti: biology and uses. University of California Press, Berkeley, California, USA.

Nyffeler, R. 2002. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trnK/matK* and *trnL-trnF* sequences. *American Journal of Botany* 89: 312–326.

Nyffeler, R. 2007. The closest relatives of cacti: Insights from phylogenetic analyses of chloroplast and mitochondrial sequences with special emphasis on relationships in the tribe Anacampseroteae. *American Journal of Botany* 94: 89–101.

Nyffeler, R., and U. Eggli. 2010. Disintegrating Portulacaceae—A new familial classification of the suborder Portulacineae (Caryophyllales) based on molecular and morphological investigations. *Taxon* 59: 227–240.

Olson, M. E. 2002. Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). *Systematic Botany* 27: 55–73.

Pagel. M. 1994. Detecting correlated evolution on phylogenies: A general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society of London, B, Boiological Sciences* 255:37–45.

Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* 48: 612–622.

Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53: 673–684.

Parker, K. C. 1989. Nurse plant relationships of columnar cacti in Arizona. *Physical Geography* 10: 322–335.

Porter, J. M. 1999. *Sclerocactus* and *Pediocactus*: A summary of the molecular evidence. *Cactus Consensus Initiatives* 7: 5–6.

Porter, J. M., M. S. Kinney, and K. D. Heil. 2000. Relationships between *Sclerocactus* and *Toumeya* (Cactaceae) based on chloroplast *trnL-trnF* sequences. *Haseltonia* 7: 8–23.

Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. *Bioinformatic* 14: 817–818.

Ritz, M. C., L. Martins, R. Mecklenburg, V. Goremykin, and F. H. Hellwig. 2007. The molecular phylogeny of *Rebutia* (Cactaceae) and its allies demonstrates the influence of paleogeography on the evolution of South American mountain cacti. *American Journal of Botany* 94: 1321–1332.

Schulz, R., and A. Kapitany. 1994. *Copiapoa* in their environment, Chañaral to El Cobre. Southbank Book, Melbourne, Australia.

Shishkova, S., T. L. Rost, and J. G. Dubrovsky. 2008. Determinate root growth and meristem maintenance in angiosperms. *Annals of Botany* 101: 319–340.

Sikes, D. S., and P. O. Lewis. 2001. Beta software, version 1. PAUPRat: PAUP* implementation of the parsimony ratchet [computer program]. Distributed by the authors, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut, USA.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Stintzing, F. C., and R. Carle. 2005. Cactus stems (*Opuntia* spp.): A review on their chemistry, technology, and uses. *Molecular Nutrition & Food Research* 49: 175–194.

Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10 for Macintosh and 4.0b10 for UNIX. Sinauer, Sunderland, Massachusetts, USA.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

Taylor, N., and D. Zappi. 2004. Cacti of eastern Brazil. Royal Botanic Gardens, Kew, UK.

Terrazas, T., and S. Arias. 2003. Comparative stem anatomy in the subfamily Cactoideae. *Botanical Review* 68: 444–473.

Wallace, R. S. 1995a. A family-wide phylogeny, subfamilial and tribal relationships, and suggestions for taxonomic realignments. *IOS Bulletin* 6: 13.

Wallace, R. S. 1995b. Molecular systematic study of the Cactaceae: Using chloroplast DNA variation to elucidate cactus phylogeny. *Bradleya* 13: 1–12.

Wallace, R. S. 2002. The phylogeny and systematics of columnar cacti: An overview. *In* T. H. Fleming, and A. Valiente-Banuet [eds.], Columnar cacti and their mutualists: Evolution, ecology and conservation. University of Arizona Press, Tucson, Arizona, USA.

Wallace, R. S., and J. H. Cota. 1996. An intron loss in the chloroplast gene *rpoC1* supports a monophyletic origin of the subfamily Cactoideae of the Cactaceae. *Current Genetics* 29: 275–281.

Wallace, R. S., and S. L. Dickie. 2002. Systematic implications of chloroplast DNA sequence variation in subfam. Opuntioideae (Cactaceae). *Succulent Plant Research* 6: 9–24.

Wallace, R. S., and A. C. Gibson. 2002. Evolution and systematics. *In* P. S. Nobel [ed.], Cacti, biology and uses. University of California Press, Los Angeles, California, USA.

Appendix 1. Taxa, voucher information, and GenBank accessions used in the present study. *Abbreviations*—lc: from a living collection; DBG AZ: Desert Botanical Garden, Phoenix, AZ; JB UNAM: Jardín Botánico de la Universidad Nacional Autónoma de México, MEXU: Herbario Nacional de México, CORD: Herbario de la Universidad de Córdoba, Argentina; na: sequence not available. GenBank numbers in boldface indicate sequences that we obtained for this study.

Taxon*—voucher* or plant register (if collected for this study; otherwise, refer to GenBank number); geographic origin (lc, if from a living collection), collection or herbarium; GenBank accessions (*trnK-matK*, *trnL-trnF*, *rpl16*, *ppc*)

Acanthocalycium spiniflorum (Schumann) Backeberg—1977 1079 0101 G, lc, DBG AZ; HM041644, HM041222, HM041377, na; ***Acanthocereus tetragonus*** (Linnaeus) Hummelinck, *T.Hernández 5*, México: Guerrero, MEXU, HM041645, HM041223, HM041378, HM041534; ***Acharagma aguirreana*** (Glass & R.A.Foster) C.E.Glass, *J. Reyes 4442*, lc, JB UNAM, HM041646, HM041224, HM041379, HM041535; ***Acharagma roseana*** (Boedeker) E.F. Anderson, *J. Reyes 4411*, lc, JB UNAM, HM041647, HM041225, HM041380, na; ***Ariocarpus agavoides*** (Castañeda) Anderson, *H. Hernández 2560*, México: San Luis Potosí, MEXU, HM041648, HM041226, HM041381, HM041536; ***Ariocarpus bravoanus*** Hernandez & Anderson, *J. Reyes s.n.*, lc, JB UNAM, na, HM041227, HM041382, HM041537; ***Ariocarpus fissuratus*** (Engelmann) Schumann, *C. Gómez 1758*, México: Coahuila, MEXU, HM041649, HM041228, HM041383, HM041538; ***Armatocereus godingianus*** (Britton & Rose) Backeb., gb, —, —, AY015296, AY015346, na, na; ***Armatocereus laetus*** (Kunth) Backeberg ex A.W.Hill, gb, —, —, na, DQ099923, DQ099992, na; ***Arrojadoa rhodantha*** (Guerke) Britton & Rose, 1993 0548 0101 G, lc, DBG AZ, HM041651, HM041230, HM041385, HM041539; ***Astrophytum capricorne*** (Dietrich) Britton & Rose, *H. Hernández 2095*, México: Coahuila, MEXU, HM041652, HM041231, HM041386, HM041540; ***Astrophytum ornatum*** (De Candolle) Britton & Rose, *T. Hernández H04*, México: Hidalgo, MEXU, HM041653, HM041232, HM041387, HM041541; ***Austrocactus bertinii*** (Cels ex Henricq) Britton & Rose, gb, —, —, AY015300, AY015300, na, na; ***Aztekium ritteri*** (Boedeker) Boedeker, gb, —, —, AY015290, na, AF267923, na; ***Bergerocactus emoryi*** (Engelmann) Britton & Rose, 1969 9558 0101, lc, DBG AZ, HM041654, HM041233, HM041388, HM041542; ***Blossfeldia liliputana*** Werdermann, *T. Hernández p104*, Argentina: Mendoza, MEXU, HM041655, HM041234, HM041389, HM041543; ***Browningia candelaris*** (Meyen) Britton & Rose, 1999 0004 0105, lc, DBG AZ, HM041656, HM041235, HM041390, HM041544; ***Browningia hertlingiana*** (Backeberg) Buxbaum, gb, —, —, AY015315, AY015403-AY015362, na, na; ***Calymmanthium substerile*** Ritter, gb, —, —, AY015291, DQ099926, AF267924-AY851614-DQ099995, na; ***Carnegiea gigantea*** (Engelmann) Britton & Rose, s.n., lc, JB UNAM, HM041657, HM041236, HM041391, HM041545; ***Castellanosia caineana*** (Cárdenas) D.R.Hunt, gb, —, —, AY015298,AY015389–AY015348,na,na; ***Cephalocereus apicicephalium*** Dawson, gb, —, —, na, DQ099927, DQ099996, na; ***Cephalocereus columna-trajani*** (Karwinsky ex Pfeiffer) Schumann, *T. Hernández 22*, México: Puebla, MEXU, HM041658, HM041237, HM041392, HM041546; ***Cephalocereus nizandensis*** (Bravo & Macdougall) Buxbaum, gb, —, —, na, DQ099928, DQ099997, na; ***Cephalocereus senilis*** (Haworth) Pfeiffer, *T. Hernández H12*, México: Hidalgo, MEXU, na, HM041238, HM041393, HM041547; ***Ceraria fruticulosa*** H.Pearson & Stephens, gb, —, —, AY875371, AF094846, na, na; ***Cereus aethiops*** Haworth, 1987 0458 2101 Z, lc, DBG AZ, HM041659, HM041239, HM041394, HM041548; ***Cereus alacriportanus*** Pfeiff., gb, —, —, AY015313, AY015313, na, na; ***Cereus hildmannianus*** Schumann, 1950 2885 0102 G, lc, DBG AZ, HM041660, HM041240, HM041395, HM041549; ***Cleistocactus icosagonus*** (Kunth) Weber ex Roland-Gosselin, 1950 2845 0102 G, lc, DBG AZ, HM041661,HM041241,HM041396,HM041550; ***Cleistocactus parviflorus*** (Schumann) Roland-Gosselin,1994 0226 1006 W, lc, DBG AZ, HM041662, HM041242, HM041397, HM041551; ***Cleistocactus tupizensis*** (Vaupel) Backeberg, 1994 0227 1002 W, lc, DBG AZ, HM041663, HM041243, HM041398, HM041552; ***Cochemiea pondii*** (Greene) Walton, *V. Alvarado 36*,México: Baja California Sur, MEXU, HM041664, HM041244, HM041399, HM041553; ***Cochemiea poselgeri*** (Hildmann) Britton & Rose, *T. Hernández p106*, México: Baja California Sur, MEXU & JB UNAM, HM041665, HM041245, HM041400, HM041554; ***Coleocephalocereus fluminensis*** (Miquel) Backeberg, gb, —, —, AY015318, AY015318, na, na; ***Copiapoa cinerascens*** (Salm-Dyck) Britton & Rose, 2002 0155 0101 W, lc, DBG AZ, HM041666, HM041246, HM041401, HM041555; ***Copiapoa coquimbana*** (Karwinski ex Ruempler) Britton & Rose, 1992 0994 0102 W, lc, DBG AZ, HM041667, HM041247, HM041402, HM041556; ***Copiapoa humilis*** (Philippi) Hutchison, 1992 0237 0101 Z, lc, DBG AZ, HM041668, HM041248, HM041403, HM041557; ***Corryocactus aureus*** (Meyen) Hutchison ex Buxbaum, 2003 0364 0101 G, lc, DBG AZ, HM041669, HM041249, HM041404, HM041631; ***Corryocactus brevistylus*** (Schumann) Britton & Rose, gb, —, —, AY015302, AY015393-AY015352-AY566650, na, na; ***Corryocactus tenuiculus*** (Backeberg) Hutchison ex Buxbaum, gb, —, —, AY015303, AY015394-AY015353, na, na; ***Coryphantha salinensis*** (Poselger) A. Zimmerman ex Dicht & A.Luethy, *T. Hernández 65*, México: Nuevo León, MEXU, HM041670, HM041250, na, HM041558; ***Coryphantha durangensis*** (Schumann) Britton & Rose, *H. Hernández 2300*, México: Coahuila, MEXU, HM041671, HM041251, HM041405, HM041559; ***Coryphantha erecta*** (Pfeiffer) Lemaire, *T. Hernández 97*, México: Guanajuato, MEXU, HM041672, HM041252, HM041406, HM041560; ***Coryphantha pycnacantha*** (Martius) Lemaire, *T. Hernández 47*, México: Oaxaca, MEXU, HM041673, HM041253, HM041407, na; ***Cylindropuntia imbricata*** (Haworth) F.M. Knuth, *T. Hernández 20*, México: Puebla, MEXU, HM041739, HM041320, HM041475, na; ***Dendrocereus nudiflorus*** (Engelmann ex Sauvalle) Britton & Rose, gb, —, —, na, DQ099929, DQ099998, na; ***Denmoza rhodacantha*** (Salm-Dyck) Britton & Rose, 1939 0374 0101 G, lc, DBG AZ, HM041674, HM041254, HM041408, HM041561; ***Discocactus boomianus*** (Buining & Brederoo) N. P. Taylor & Zappi, 1992 0232 0102 G, lc, DBG AZ, HM041675, HM041255, HM041409, HM041562; ***Disocactus amazonicus*** (Schumann) Hunt, gb, —, —, AY015312, AY015400-AY015359, na, na; ***Disocactus flagelliformis*** (Linnaeus) Barthlott, *T. Hernández 44*, México: Oaxaca, MEXU, HM041676, HM041256, HM041410, HM041563; ***Echinocactus grusonii*** Hildmann, *H. Hernández 2442*, México: Queretaro, MEXU, HM041677, HM041257, HM041411, HM041640; ***Echinocactus horizonthalonius*** Lemaire, *T. Hernández 61*, México: San Luis Potosí, MEXU, HM041678, HM041258, HM041412, HM041642; ***Echinocactus platyacanthus*** Link & Otto, *T. Hernández H08*, México: Hidalgo, MEXU, HM041679, HM041259, HM041413, HM041641; ***Echinocereus cinerascens*** (De Candolle) Lemaire, *T. Hernández H21,* México: Hidalgo, MEXU, HM041680, HM041260, HM041414, HM041564; ***Echinocereus enneacanthus*** Engelmann, *C. Gómez 2156*, México: Zacatecas, MEXU, HM041681, HM041261, HM041415, HM041565; ***Echinocereus leucanthus*** Taylor, gb, —, —, na, DQ099932, DQ100001, na; ***Echinocereus parkeri*** Taylor, gb, —, —, na, DQ099933, DQ100002, na; ***Echinocereus pectinatus*** (Scheidweiler) Engelmann, *H. Hernández 3403*, México: Coahuila, MEXU, HM041682, HM041262, HM041416, HM041566; ***Echinocereus pentalophus*** (De Candolle) Lemaire, gb, —, —, AY015307, AY015396-AY015355, na, na; ***Echinocereus poselgeri*** Lemaire, gb, —, —, na, DQ099935, DQ100004, na; ***Echinocereus schmollii*** (Weingart) Taylor, gb, —, —, na, DQ099936, DQ100005, na; ***Echinocereus triglochidiatus*** Engelmann, gb, —, —, na, DQ099937, DQ100006, na; ***Echinomastus intertextus*** (Engelm.) Britton & Rose, 1993 0823 1001, lc, DBG AZ, HM041683, HM041263, HM041417, HM041567; ***Echinomastus unguispinus*** (Engelm.) Britton & Rose, *J. Reyes 5145*, lc, JB UNAM, HM041684, HM041264, HM041418, HM041568; ***Echinopsis chiloensis*** (Colla) Friedrich & Rowley, gb, —, —, AY015322, AY566653-AY015409-AY015368, na, na; ***Echinopsis formosa*** (Pfeiffer) Jacobi ex Salm-Dyck, 1998 0056 1003 W, lc, DBG AZ, HM041685, HM041265, HM041419, na; ***Echinopsis leucantha*** (Gillies ex Salm-Dyck) Walpers, 1995 0358 1001, lc, DBG AZ, HM041686, HM041266, HM041420, na; ***Echinopsis pachanoi*** (Britton & Rose) Friedrich & Rowley, 2003 008 0102 Z, lc, DBG AZ, HM041687, HM041267, HM041421, na; ***Echinopsis pasacana*** (F. A. C. Weber) Navarro, 1952 3603 0104, lc, DBG AZ, HM041688, HM041268, HM041422, na; ***Echinopsis sucrensis*** Cardenas, 1992 0237 0101 Z, lc, DBG AZ, HM041689, HM041269, HM041423, na; ***Epiphyllum phyllanthus*** (Linnaeus) Haworth, *T. Hernández p107*, México: Chiapas, MEXU, HM041690, HM041270, HM041424, HM041569; ***Epithelantha micromeris*** (Engelmann) Weber, *H. Hernández 3402*, México: Coahuila, MEXU, HM041691, HM041271, HM041425, HM041570; ***Eriosyce aurata*** (Pfeiffer) Backeberg, gb, —, —, AY015336, AY015336, na, na; ***Eriosyce islayensis*** (Foerster) Kattermann, gb, —, —, AY015337, AY015337, na, na; ***Eriosyce napina*** (Philippi) Kattermann, gb, —, —, AY015339, AY015339, na, na; ***Eriosyce subgibbosa*** (Haworth) Kattermann, gb, —, —, AY015338, AY015338, na, na; ***Eriosyce taltalensis*** (Hutchison) Kattermann, 2002 0150 0101 W, lc, DBG AZ, HM041692, HM041272, HM041426, na; ***Escontria chiotilla*** (Weber ex Schumann) Rose, gb, —, —, AY015308, AY015397-AY015356-AY181622, AY181608, na; ***Espostoa nana*** Ritter, 1994 0247 1012 W, lc, DBG AZ, HM041693, HM041273, HM041427, na; ***Espostoopsis dybowskii*** (Roland-Gosselin) Buxbaum, 1979 0374 0101 G, lc, DBG AZ, HM041694, HM041274, HM041428, na; ***Eulychnia castanea*** Philippi, 1993 0231 0101 W, lc, DBG AZ, na, HM041275, HM041429, na; ***Eulychnia iquiquensis*** (Schumann) Britton & Rose, gb, —, —, AY015301, AY015301, na, na; ***Ferocactus echidne*** (De Candolle) Britton & Rose, *T. Hernández H09*, México: Hidalgo, MEXU, HM041695, HM041276, HM041430, HM041571; ***Ferocactus haematacanthus*** (Salm-Dyck) Bravo ex Backeberg & F.Knuth, *T. Hernández 72*, México: Nuevo León, MEXU, HM041696, HM041277, HM041431, HM041572; ***Ferocactus latispinus*** (Haworth) Britton & Rose, *T. Hernández 51*, México: Queretaro, MEXU, HM041697, HM041278, HM041432, HM041573; ***Frailea pumila*** (Lemaire) Britton & Rose, 1981 0090 0101 G, lc, DBG AZ, HM041698, HM041279, HM041433, na; ***Geohintonia mexicana*** Glass & Fitz Maurice, *J. Reyes 3226*, lc, JB UNAM, HM041699, HM041280, HM041434, na; ***Grahamia bracteata*** Gill., gb, —, —, AY015273, na, na, na; ***Grahamia coahuilensis*** (S. Watson) G.D. Rowley, gb, —, —, DQ855854, na, na, na; ***Grusonia bradtiana*** (J. M. Coult.) Britton & Rose, *H. Hernández 3342*, México: Coahuila, MEXU, HM041700, HM041281, HM041435, HM041638; ***Gymnocalycium denudatum*** (Link & Otto) Pfeiffer ex Mittler, gb, —, —, AY015317, AY015404-AY015363, na, na; ***Gymnocalycium guanchinense*** A. Berger, 1993 0183 0101 G, lc, DBG AZ, HM041701, HM041282, HM041436, na; ***Gymnocalycium saglionis*** (Cels) Britton & Rose, 1999 033 0101 W, lc, DBG AZ, HM041702, HM041283, HM041437, na; ***Gymnocalycium uruguayense*** (Arechavaleta) Britton & Rose, 1993 0179 0101 G, lc, DBG AZ, HM041703, HM041284, HM041438, na; ***Haageocereus pseudomelanostele*** (Werdermann & Backeberg) Backeberg, gb, —, —, AY015329, AY015415–AY015374, na, na; ***Haageocereus decumbens*** (Vaupel) Backeberg, 1994 0284 1006 W, lc, DBG AZ, HM041704, HM041285, HM041439, na; ***Haageocereus limensis*** (Vaupel) Backeb., 1994 0249 1001 W, lc, DBG AZ, HM041705, HM041286, HM041440, na; ***Harrisia earlei*** Britton & Rose, gb, —, —, na, DQ099939, DQ100008, na; ***Harrisia martinii*** (Labouret) Britton, 1996 1255 0102, lc, DBG AZ, HM041706, HM041287, HM041441, na; ***Harrisia pomanensis*** (Weber) Britton & Rose, gb, —, —, AY015324, AY015411-AY015370, na, na; ***Hatiora salicornioides*** (Haworth) Britton & Rose ex Bailey, *T. Hernández p108*, personal colection, MEXU, HM041707, HM041288, HM041442, HM041636; ***Hylocereus trigonus*** (Haworth) Safford, *H. Hernández 2236*, México: Yucatan, MEXU, HM041708, HM041289, HM041443, na; ***Hylocereus undatus*** (Haworth) Britton & Rose, *T. Hernandez 06 (M002)*, México: Oaxaca, MEXU, HM041709, HM041290, HM041444, HM041574; ***Isolatocereus dumortierii*** (Scheidweiler) Backeberg, *J. A. Barba 1*, México: San Luis Potosí, MEXU, HM041749, na, HM041485, HM041602; ***Lepismium cruciforme*** (Vellozo) Miquel, gb, —, —, AY015344, AJ583237, na, na; ***Leptocereus leonii*** Britton & Rose, gb, —, —, AY015297, AY015388-AY015347, na, na; ***Leptocereus quadricostatus*** (Bello) Britton & Rose, gb, —, —, na, DQ099942, DQ100011, na; ***Leuchtenbergia principis*** Hooker, *H. Hernández 2009*, México: Nuevo León, MEXU, HM041710, HM041291, HM041445, HM041575; ***Lobivia pentlandii*** (Hook.) Britton & Rose, gb, —, —, AY015323, AY015369-AY015410, na, na; ***Lophocereus schottii*** (Engelm) Britt. & Rose, gb, —, —, AY015309, AY181620, AY181613, na; ***Lophophora williamsii*** (Lemaire ex Salm-Dyck) J.Coulter, *T. Hernández 62*, México: San Luis Potosí, MEXU, HM041711, HM041292, HM041446, HM041576; ***Maihuenia patagonica*** (Philippi) Spegazzini, *Las Peñas & Uñates 14*, Argentina: Neuquen, CORD, HM041712, HM041293, HM041447, na; ***Maihuenia poeppigii*** (Pfeiffer) Schumann, gb, —, —, AY015282, AY015282, AY851609 AF191656, na; ***Maihueniopsis atacamensis*** (Philippi) F.Ritter, 2004 0354 0101 W, lc, DBG AZ, HM041713, HM041294, HM041448, HM041577; ***Mammillaria candida*** Scheidweiler, gb, —, —, na, AJ583218, AF267945 AY545250, na; ***Mammillaria carnea*** Zuccarini ex Pfeiffera, *T. Hernández 32*, México: Puebla, MEXU, HM041714, HM041295, HM041449, HM041578; ***Mammillaria geminispina*** Haworth, *T. Hernández 93*, México: Queretaro, MEXU, HM041715, HM041296, HM041450, HM041579; ***Mammillaria haageana*** Pfeiffer, gb, —, —, AY015289, AY545268-AF267953, na, na; ***Mammillaria magnimamma*** Haworth, *T. Hernández H10*, México: Hidalgo, MEXU, HM041716, HM041297, HM041451, HM041580; ***Mammillaria picta*** Meinshausen, *T. Hernández 63*, México: Nuevo León, MEXU, HM041717, HM041298, HM041452, HM041581; ***Mammillaria senilis*** Loddiges ex Salm-Dyck, gb, —, —, na, AJ583212, AY545318-AF267956, na; ***Matucana madisoniorum*** (Hutchinson) Rowley, 1994 0702 0102, Knize 456, lc, DBG AZ, HM041718, HM041299, HM041453, HM041639; ***Melocactus curvispinus*** Pfeiffer, 1995 0053 1008 W, lc, DBG AZ, HM041719, HM041300, HM041454, na; ***Melocactus intortus*** (Miller) Urban, 1985 0172 1004 W, lc, DBG AZ, HM041720, HM041301, HM041455, HM041582; ***Micranthocereus albicephalus*** (Buining & Brederoo) Ritter, gb, —, —, AY015314, AY015314, na, na; ***Mila caespitosa*** Britton & Rose, 1996 0168 1006 W, lc, DBG AZ, HM041721, HM041302, HM041456, na; ***Miqueliopuntia miquelii*** (Monville) Ritter, 2001 0107 0102 FK1098, lc, DBG AZ, HM041722, HM041303, HM041457, HM041643; ***Monvillea spegazzinii*** (F.A.C. Weber) Britton & Rose, 2005 0028 0101 G, lc, DBG AZ, HM041723, HM041304, HM041458, na; ***Myrtillocactus geometrizans*** (Martius) Console, gb, —, —, na, DQ099943, DQ100012, na; ***Myrtillocactus schenckii*** (J.Purpus) Britton & Rose, gb, —, —, na, AY181633, AY181607, na; ***Mytrocereus fulviceps*** (F. A. C. Weber) Backeb., *T. Hernández 45*, México: Oaxaca, MEXU, HM041724, HM041305, HM041459, HM041583; ***Neobuxbaumia mezcalaensis*** (Bravo) Backeberg, *T. Hernández 37*, México: Oaxaca, MEXU, HM041725, HM041306, HM041460, HM041584; ***Neobuxbaumia polylopha*** (De Candolle) Backeberg, *T. Hernández 91*, México: Queretaro, MEXU, HM041726, HM041307, HM041461, HM041585; ***Neolloydia conoidea*** (De Candolle) Britton & Rose, *T. Hernández 85*, México: Tamaulipas, MEXU, HM041727, HM041308, HM041462, HM041634; ***Neoraimondia arequipensis*** (Meyen) Backeberg, gb, —, —, AY015299, AY015390-AY015349, na, na; ***Neoraimondia herzogiana*** (Backeberg) Buxbaum & Krainz, 1954 4849 0102 G, lc, DBG AZ, HM041728, HM041309, HM041463, HM041586; ***Neowerdermannia vorwerkii*** (Fric) Backeberg, gb, —, —, AY015340, AY015340, na, na; ***Nopalea cochenillifera*** (L.) Salm-Dyck, 1997 0395 0101, lc, DBG AZ, HM041729, na, HM041464, HM041587; ***Nopalea dejecta*** Salm-Dyck, 2002 0342 0103 Z, lc, DBG AZ, HM041730, HM041310, HM041465, HM041588; ***Nopalea gaumeri*** Britton & Rose, 1999 0027 0101, lc, DBG AZ, HM041731, HM041311, HM041466, HM041589; ***Nopalea karwinskiana*** (Salm-Dyck) Schum, *T. Hernández 7*, México: Oaxaca, MEXU, HM041732, HM041312, HM041467, HM041590; ***Nopalea lutea*** Rose, 2002 0044 0101 W, lc, DBG AZ, HM041733, HM041313, HM041468, HM041591; ***Nopalea sp***, *T. Hernández P036*, México: Oaxaca, MEXU, HM041734, HM041314, HM041469, HM041635; ***Notocactus concinnus*** (Monv.) A.Berger, 1985 0339 0101, lc, DBG AZ, HM041735, HM041315, HM041470, HM041592; ***Obregonia denegrii*** Fric, *L. White 26*, México: Tamaulipas, MEXU, na, HM041316, HM041471, na; ***Opuntia echios*** Howell, 1994 0007 0103 Z EFA 2560, lc, DBG AZ, HM041736, HM041317, HM041472, HM041593; ***Opuntia excelsa*** Sánchez-Mejorada, *T. Hernández 3*, México: Guerrero, MEXU, HM041737, HM041318, HM041473, HM041594; ***Opuntia helleri*** Schumann ex Robinson, 1995 0316 0101 YD 24743, lc, DBG AZ, HM041738, HM041319, HM041474, HM041595; ***Opuntia lasiacantha*** Hort. Vindob. ex Pfeiffer, *T. Hernández H11*, México: Hidalgo, MEXU, HM041740, HM041321, HM041476, HM041596; ***Opuntia leptocaulis*** De Candolle, *T. Hernández 87*, México: Tamaulipas, MEXU, HM041741, HM041322, HM041477, na; ***Opuntia macbridei*** Britton & Rose, 1990 0601 0101, lc, DBG AZ, HM041742, HM041323, HM041478, HM041597; ***Opuntia megasperma*** Howell, 1994 0019 0201 Z, lc, DBG AZ, HM041743, HM041324, HM041479, HM041630; ***Opuntia microdasys*** (Lehmann) Pfeiffer, *T. Hernández 59*, México: San Luis Potosí, MEXU, HM041744, HM041325, HM041480, HM041598; ***Opuntia palmadora*** Britton & Rose, 1997 0187 0102, lc, DBG AZ, HM041745, HM041326, HM041481, HM041599; ***Opuntia tunicata*** (Lehmann) Link & Otto ex Pfeiffer, 1993 0198 0101 FK 352, lc, DBG AZ, HM041746, HM041327, HM041482, HM041600; ***Oreocereus celsianus*** (Salm-Dyck) Riccobono, 1987 0468 2108 W, lc, DBG AZ, HM041747, HM041328, HM041483, na; ***Ortegocactus macdougallii*** Alexander, *J. Reyes 4007*, lc, JB UNAM, HM041748, HM041329, HM041484, HM041601; ***Pachycereus gaumeri*** Britton & Rose, gb, —, —, na, AY181626, AY181606, na; ***Pachycereus marginatus*** (De Candolle) Britton & Rose, *T. Hernández 14*, México: Puebla, MEXU, na, HM041330, HM041486, HM041603; ***Pachycereus pecten-aboriginum*** (Engelmann) Britton & Rose, *T. Hernández 8*, México: Oaxaca, MEXU, HM041750, HM041331, HM041487, HM041604; ***Pachycereus sp***, *T. Hernández H03*, México: Hidalgo, MEXU, HM041751, HM041332, HM041488, HM041605; ***Parodia buenekeri*** (Buining) Ritter, gb, —, —, AY015331, AY015331, na, na; ***Parodia erinacea*** (Haworth) Taylor, 1965 8091 0101 G, lc, DBG AZ, HM041752, HM041333, HM041489, HM041625; ***Parodia haselbergii*** (Haage ex Ruempler) Brandt, gb, —, —, AY015330, na, AF267975, na; ***Parodia magnifica*** (Ritter) Brandt, 1985 0436 0101 G, lc, DBG AZ, HM041753, HM041334, HM041490, na; ***Parodia ottonis*** (Lehmann) Taylor, gb, —, —, AY015335, AY015380, na, na; ***Peniocereus castellae*** Sánchez-Mejorada, gb, —, —, na, DQ099945, DQ100014, na; ***Peniocereus chiapensis*** Bravo, *C. Gómez 2100*, México: Chiapas, MEXU, HM041754, HM041335, HM041491, HM041606; ***Peniocereus greggii*** (Engelmann) Britton & Rose, *J. Reyes 4688*, lc, JB UNAM, HM041755, HM041336, HM041492, HM041607; ***Peniocereus johnstonii*** Britton & Rose, gb, —, —, na, DQ099951, DQ100020, na; ***Peniocereus serpentinus*** (Lagasca & Rodrigues) Taylor, *T. Hernández 92*, México: Queretaro, MEXU, HM041756, HM041337, HM041493, HM041608; ***Peniocereus viperinus*** (Weber) Kreuzinger, gb, —, —, na, DQ099964, DQ100033, na; ***Pereskia aculeata*** Miller, *T. Hernández p111*, lc, JB UNAM, HM041757, HM041338, HM041494, na; ***Pereskia diaz-romeroana*** Cárdenas, gb, —, —, AY875353, na, AY851592, na; ***Pereskia lychnidiflora*** De Candolle, gb, —, —, AY875358, na, AY851594, na; ***Pereskia weberiana*** Schumann, gb, —, —, AY875357, na, AY851593, na; ***Pereskiopsis sp***, *T. Hernández p109*, México: Chiapas, MEXU, HM041758, HM041339, HM041495, HM041627; ***Pfeiffera ianthothele*** (Monville) Weber, gb, —, —, AY015304, AY015354, na, na; ***Pilosocereus chrysacanthus*** (Weber) Byles & Rowley, *T. Hernández 17*, México: Puebla, MEXU, HM041759, HM041340, HM041496, na; ***Polaskia chichipe*** (Roland-Gosselin) Backeberg, *T. Hernández 16*, México: Puebla, MEXU, HM041760, HM041341, HM041497, HM041615; ***Portulacaria afra*** Jacq., gb, —, —, AY875368, na, na, na; ***Pseudoacanthocereus brasiliensis*** (Britton & Rose) Ritter, gb, —, —, na, DQ099967, DQ100036, na; ***Pseudoacanthocereus sicariguensis*** (Croizat & Tamayo) Taylor, gb, —, —, na, DQ099968, DQ100037, na; ***Pterocactus gonjianii*** Kiesling, 1997 0260 0103, lc, DBG AZ, HM041761, HM041342, HM041498, na; ***Pterocereus foetidus*** Th. MacDoug. & Miranda, *T. Hernández p110*, México: Chiapas, MEXU, HM041762, HM041343, HM041499, HM041616; ***Pygmaeocereus bylesianus*** Andreae & Backeberg, 1994 0519 0101, lc, DBG AZ, HM041763, HM041344, HM041500, na; ***Quiabentia chacoensis*** Backeberg, 1985 0461 0101, lc, DBG AZ, HM041764, HM041345, HM041501, na; ***Quiabentia verticillata*** Vaupel, 1992 1063 0101, lc, DBG AZ, HM041765, HM041346, HM041502, na; ***Rauhocereus riosaniensis*** Backeberg, gb, —, —, AY015326, AY015413-AY015372, na, na; ***Rauhocereus sp***, 93-2010, lc, DBG AZ, HM041766, HM041347, HM041503, na; ***Rebutia arenacea*** Cardenas, 1992 0082 0102 Z, lc, DBG AZ, HM041767, HM041348, HM041504, HM041633; ***Rebutia fiebrigii*** (Guerke) Britton & Rose ex Bailey, 1992 0068 0202, lc, DBG AZ, HM041768, HM041349, HM041505, na; ***Rhipsalis baccifera*** (J.S. Mueller) Stearn, *T. Hernandez 12*, México: Hidalgo, MEXU, HM041769, HM041350, HM041506, HM041619; ***Samaipaticereus corroanus*** Cardenas, gb, —, —, AY015321, AY015408-AY015367, na, na; ***Schlumbergera truncata*** (Haworth) Moran, gb, —, —, AY015343, AJ583238, na, na; ***Sclerocactus brevihamatus*** (Engelmann) Hunt, *T. Hernández 68*, México: Nuevo León, MEXU, HM041770, HM041351, HM041507, HM041620; ***Sclerocactus uncinatus*** (Galeotti) Taylor, *H. Hernández 3597*, México: Coahuila, MEXU, HM041771, HM041352, HM041508, na; ***Selenicereus boeckmannii*** (Otto ex Salm-Dyck) Britton & Rose, gb, —, —, AY015311, AY015399, na, na; ***Selenicereus donkelaarii*** (Salm-Dyck) Britton & Rose ex Bailey, *H. Hernández 2226*, México: Yucatán, MEXU, HM041772, HM041353, HM041509, HM041621; ***Selenicereus sp***, *T. Hernández 04 (G31)*, México: Guerrero, MEXU, HM041773, HM041354, HM041510, HM041632; ***Stenocactus coptonogonus*** (Lemaire) Berger ex Hill, *H. Hernández 1773*, México: San Luis Potosí, MEXU, HM041774, na, HM041511, HM041626; ***Stenocactus sp***, *T. Hernández H19*, México: Hidalgo, MEXU, HM041775, HM041355, HM041512, HM041622; ***Stenocereus beneckei*** *(Ehrenberg) Buxbaum*, 1980 0297 0102 G, lc, DBG AZ, HM041776, HM041356, HM041513, na; ***Stenocereus eruca*** (T.Brandegee) Gibson & Horak, 1939 0425 0111 W, lc, DBG AZ, HM041777, HM041357, HM041514, HM041623; ***Stenocereus gummosus*** (Engelmann) Gibson & Horak, 1966 8584 0101 G, lc, DBG AZ, HM041778, HM041358, HM041515, HM041624; ***Stenocereus pruinosus*** (Otto ex Pfeiffer) Buxbaum, *T. Hernández 43*, México: Oaxaca, MEXU, HM041779, HM041359, HM041516, HM041617; ***Stenocereus stellatus*** (Pfeiffer) Riccobono, *T. Hernández 19*, México: Puebla, MEXU, HM041780, HM041360, HM041517, HM041618; ***Stetsonia coryne*** (Foerster) Britton & Rose, 1939 0402 0103 G, lc, DBG AZ, HM041781, HM041361, HM041518, na; ***Strombocactus disciformis*** (De Candolle) Britton & Rose, *H. Hernández 1522*, México: Queretaro, MEXU, HM041782, HM041362, HM041519, HM041637; ***Sulcorebutia candiae*** (Cardenas) Buining & Donald, 1992 0082 0102 Z, lc, DBG AZ, HM041783, HM041363, HM041520, HM041609; ***Tacinga funalis*** Britton & Rose, ex Zurich. SS HU 748, lc, DBG AZ, HM041784, HM041364, HM041521, na; ***Talinum paniculatum*** (Jacq.) Gaertner, gb, —, —, AY015274, na, AY851610, na; ***Tephrocactus alexanderi*** (Britton & Rose) Backeberg, 2001 0055 0101, lc, DBG AZ, HM041785, HM041365, HM041522, na; ***Tephrocactus articulatus*** (Pfeiffer) Backeberg, 1993 0335 0202, lc, DBG AZ, HM041786, HM041366, HM041523, na; ***Thelocactus hastifer*** (Werdermann & Boedeker) F.Knuth, *C. Gómez 1608*, México: Queretaro, MEXU, HM041787, HM041367, HM041524, HM041610; ***Thelocactus tulensis*** (Poselger) Britton & Rose, *T. Hernández 55*, México: San Luis Potosí, MEXU, HM041788, HM041368, HM041525, na; ***Trichocereus taquimbalensis*** Cardenas, 999 0011 0101 Z, lc, DBG AZ, HM041789, HM041369, HM041526, na; ***Tunilla corrugata*** (Salm-Dyck) D.R.Hunt & J.Iliff, 2003 0022 0102 Z, lc, DBG AZ, HM041790, HM041370, HM041527, HM041628; ***Turbinicarpus gielsdorfianus*** (Werdermann) John & Riha, *J. Reyes 6168*, lc, JB UNAM, HM041791, HM041371, HM041528, HM041611; ***Turbinicarpus pseudomacrochele*** (Backeberg) Buxbaum & Backeberg, *J. Reyes 5016*, lc, JB UNAM, HM041792, HM041372, HM041529, HM041612; ***Turbinicarpus schmiedickeanus*** (Boedeker) Buxbaum & Backeberg, *H. Hernández 2362*, México: San Luis Potosí, MEXU, HM041793, HM041373, HM041530, HM041613; ***Turbinicarpus viereckii*** (Werdmann) John & Riha, *C. Gómez 1368*, México: San Luis Potosí, MEXU, HM041794, HM041374, HM041531, HM041614; ***Uebelmannia pectinifera*** Buining, 1981 0226 0101 G, lc, DBG AZ, HM041795, HM041375, HM041532, na; ***Weberbauerocereus johnsonii*** Ritter, HBG 95204 ISI 2007-9, lc, DBG AZ, HM041796, HM041376, HM041533, na; ***Weberocereus glaber*** (Eichlam) Rowley, gb, —, —, na, DQ099984, DQ100053, na.