

PHYLOGENETIC RELATIONSHIPS IN THE CACTUS FAMILY (CACTACEAE) BASED ON EVIDENCE FROM *TRNK/MATK* AND *TRNL-TRNF* SEQUENCES¹

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Cacti are a large and diverse group of stem succulents predominantly occurring in warm and arid North and South America. Chloroplast DNA sequences of the *trnK* intron, including the *matK* gene, were sequenced for 70 ingroup taxa and two outgroups from the Portulacaceae. In order to improve resolution in three major groups of Cactoideae, *trnL-trnF* sequences from members of these clades were added to a combined analysis. The three exemplars of *Pereskia* did not form a monophyletic group but a basal grade. The well-supported subfamilies Cactoideae and Opuntioideae and the genus *Maihuenia* formed a weakly supported clade sister to *Pereskia*. The parsimony analysis supported a sister group relationship of *Maihuenia* and Opuntioideae, although the likelihood analysis did not. *Blossfeldia*, a monotypic genus of morphologically modified and ecologically specialized cacti, was identified as the sister group to all other Cactoideae. The tribe Cacteae was found to be sister to a largely unresolved clade comprising the genera *Calymmanthium*, *Copiapoa*, and *Frailea*, as well as two large and well-supported clades. *Browningia* sensu stricto (excluding *Castellanosia*), the two tribes Cereae and Trichocereae, and parts of the tribes Notocactae and Rhipsalideae formed one clade. The distribution of this group is largely restricted to South America. The other clade consists of the columnar cacti of Notocactae, various genera of Browningieae, Echinocereae, and Leptocereae, the tribes Hylocereae and Pachycereae, and *Pfeiffera*. A large portion of this latter group occurs in Central and North America and the Caribbean.

Key words: biogeography; Cactaceae; *matK*; phylogeny; *trnK* intron; *trnL-trnF*.

Cacti are among the most conspicuous and characteristic plants of warm and arid areas of the New World. Their distribution ranges from southern Patagonia in Argentina to Alberta and British Columbia in Canada and encompasses various habitats, including bare, hot deserts, sandy coastal stretches, scrublands, dry deciduous forests, high alpine steppes, and even tropical rain forests (Barthlott and Hunt, 1993). Centers of diversity are the arid regions of North and South America, notably the southwestern United States and Mexico, East Brazil, and the eastern and western slopes of the South American Andes. Only a single epiphytic species, *Rhipsalis baccifera* (J. S. Muell.) Stearn, has a distribution range that naturally extends beyond the New World to southern Africa, Madagascar, and Sri Lanka (Barthlott, 1983; Barthlott and Taylor, 1995).

The cactus family is remarkable for its great diversity in growth forms. Cacti form xerophytic trees or shrubs with conspicuous persistent leaves (*Pereskia* Mill.) or most often branched or unbranched, columnar to globular stem succulents. Cacti can be scandent, epiphytic, or epilithic and have either slender, terete stems or flattened, leaflike cladodes. The unusual vegetative morphology is the result of the following major modifications of the general structure of a perennial dicotyledonous flowering plant (Goebel, 1889; Rauh, 1979): (1)

the leaves are highly reduced or lost, (2) the stems remain green and photosynthetically active for several years with retarded bark formation, (3) cortex and pith are transformed into a succulent water-storage tissue, (4) short side-branches are modified into clusters of spines called areoles, and (5) branching is often highly reduced.

Cacti have fascinated botanists and plant enthusiasts for centuries (Rowley, 1997), and many are grown today as pot plants for their unusual habits and large, showy flowers. The family comprises ~1500–1800 species in slightly >100 genera (Barthlott and Hunt, 1993). The current classification scheme is based on the recent work of an ad hoc Working Party under the auspices of the International Organization for Succulent Plant Study (Hunt and Taylor, 1986, 1990). This group of cactus taxonomists was charged with proposing a consensus on the generic classification of Cactaceae that would provide a compromise between widely divergent views in numbers of genera to be recognized, ranging from ~42 (Mottram, 1990) to 233 (Backeberg, 1966).

The cactus family is characterized by the following unique morphological features: (1) short shoots that are modified into areoles, (2) shoot apical meristems that are organized into four distinct zones, and (3) ovaries that are “sunken” in the receptacles, which in turn are covered with bracts and areoles (Boke, 1941; Gibson and Nobel, 1986; Leuenberger, 1986). Due to their highly modified vegetative and floral morphology, taxonomists generally regarded the cacti as a very distinct group and placed it in its own order, Cactales (Opuntiales according to Engler, 1892; e.g., Hutchinson, 1973; Benson, 1979). There was disagreement about the closest relatives of the cacti until studies of embryology (e.g., Schnarf, 1931), plant pigment chemistry (e.g., Mabry, Taylor, and Turner, 1963), and sieve-element plastids (e.g., Behnke, 1972) suggested a close relationship of the family Cactaceae to the core Caryophyllales. Molecular studies have confirmed this infer-

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ence and have identified a distinct clade consisting of Portulacaceae, Basellaceae, Cactaceae, and Didieraceae (e.g., Manhart and Rettig, 1994). Additionally, recent studies based on increased taxon sampling have suggested that the three latter families are in fact nested in paraphyletic Portulacaceae (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 1999).

The family Cactaceae is generally classified into three subfamilies: Pereskioideae, Opuntioideae, and Cactoideae (Schumann, 1899a; Barthlott and Hunt, 1993). Recently, however, it was suggested that *Maihuenia* (F. A. C. Weber) K. Schum., traditionally placed with *Pereskia* in the subfamily Pereskioideae, should be considered as a subfamily of its own (Wallace, 1995a; Anderson, 2001). Indeed, *Maihuenia* and *Pereskia* have been placed together essentially because they lack distinct synapomorphies. The subfamily Opuntioideae is characterized by the bony aril of the seeds and the presence of glochids (barbed hairs) in the areoles, while the subfamily Cactoideae is distinct in its lack of leaves (with a few exceptions, e.g., *Corryocactus brevistylus* (K. Schum.) Britton & Rose), the characteristic hilum-micropylar region of the seeds (Barthlott and Voit, 1979), and an intron loss in the chloroplast gene *rpoC1* (Wallace and Cota, 1995).

Cactoideae is by far the most diverse and species-rich subfamily, comprising >80% of the cactus species. The present tribal classification of Cactoideae (Barthlott and Hunt, 1993) is largely based on the work by Franz Buxbaum (Buxbaum, 1958; Endler and Buxbaum, 1974) and has received only minor modifications through the reshuffling of a few problematic genera and the lumping together of some tribes (check Table 6 for a summary of four recent tribal classifications for the taxa considered in this study). Recently, Anderson (2001) moved a few genera to new or different tribes (i.e., *Calymmanthium* F. Ritter to Calymmantheae; *Uebelmannia* Buining to Cereae; *Acanthocereus* (A. Berger) Britton & Rose, *Corryocactus* Britton & Rose, *Echinocereus* Engelm., and *Lep-tocereus* (A. Berger) Britton & Rose to Pachycereae; *Harrisia* Britton to Trichocereae) based on results from recent systematic studies (Taylor and Zappi, 1989; Wallace 1995a, b, 1998), but otherwise adhered to the general framework of Buxbaum's tribal classification of Cactoideae.

In order to address questions about cactus evolution, it is of great importance to establish a detailed and robust hypothesis about the phylogenetic relationships of the major lineages in this group. For example, Buxbaum (1956; see also Buchheim, 1964; Buxbaum, 1980) proposed that there is a consistent pattern of increased specialization and morphological reduction in the evolution of various characters of the stems, flowers, fruits, and seeds. This idea of directionality, or trend, in cactus evolution influenced many subsequent ideas about phylogenetic relationships (e.g., Buxbaum, 1967; Barthlott and Hunt, 1993). However, these generalizations about the evolutionary history in cacti can only be rigorously tested with the help of a detailed phylogenetic hypothesis. For instance, Porter, Kinney, and Heil (2000) investigated the homoplasy of morphological characters and the evolution of paedomorphosis in *Sclerocactus* Britton & Rose based on an explicit phylogenetic framework using sequence data. The present study aims at providing a starting point for a phylogeny of the major relationships within Cactaceae using molecular sequences from the chloroplast genome for later comparative analyses of growth-form evolution.

The *trnK* intron (referred to here as *trnK/matK*) consists of

the *matK* gene and two flanking introns. In the Cactaceae this region comprises almost 2600 base pairs (bp). While the *matK* gene, which encodes a protein structurally related to the maturases (Neuhaus and Link, 1987), has been used in a number of infrafamilial studies (e.g., Johnson and Soltis, 1994; Plunkett, Soltis, and Soltis, 1996; Kron, 1997), the adjacent non-coding introns of ~1050 bp have only recently been used for phylogenetic analyses (e.g., Hiroshi, Thien, and Kawano, 1999; Hu et al., 2000; Lavin et al., 2000). In addition, sequences of the *trnL* intron and the *trnL-trnF* intergenic spacer (together referred to here as *trnL-trnF*) were generated and added to a combined analysis in order to improve resolution in three large subclades of Cactoideae. The *trnL-trnF* sequences are rich in indels and have been used previously to resolve relationships among closely related genera and tribes (e.g., Böhle et al., 1994; Bayer and Starr, 1998).

MATERIALS AND METHODS

Taxon sampling—Sampling of all major clades of Cactaceae (i.e., *Pereskia*, *Maihuenia*, *Opuntioideae*, and all nine tribes of Cactoideae) was largely based on the diagram of the “presumed phylogenetic relationships within the family Cactaceae” of Barthlott and Hunt (1993: fig. 34). Exemplars were chosen in order to evenly represent cactus diversity. Later, additional taxa were added based on questions arising from a preliminary analysis. A list of the 72 taxa included in the present study has been archived on the Botanical Society of America website (<http://ajbsupp.botany.org/>). While *trnK/matK* was sequenced for all 72 exemplars, *trnL-trnF* sequences were added to a combined analysis for 41 taxa to further resolve relationships within three large clades of Cactoideae. Species of *Grahamia* Gill. and *Talinum* Adans. (Portulacaceae) were used as outgroup taxa (Hershkovitz and Zimmer, 1997).

Sequencing methods—Genomic DNA was isolated from ~10–20 mg dried stem cortex tissue using the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA). In order to overcome problems with excessive mucilage, the manufacturer's extraction protocol was modified as follows: (1) spinning for 1 min at 628 rad/sec (6000 rpm) after step 3 and only using supernatant for step 4, and (2) spinning for 5 min at 1256 rad/sec (12000 rpm) after step 4 and only using supernatant for step 5. The two chloroplast markers were amplified using primers trnK-3914F and trnK-2R for *trnK/matK* (Johnson and Soltis, 1994) and primers trnL-c and trnF-f for *trnL-trnF* (Taberlet et al., 1991). The polymerase chain reaction (PCR) amplifications were performed in 30- μ L reactions containing 10 μ L PCR buffer, 2.5 mmol/L MgCl₂, 1 mmol/L dNTPs, 0.5 μ mol/L of each primer, and 2.5 units of Taq DNA polymerase (QIAGEN). Amplifications were carried out with an initial denaturation step at 94°C for 4 min, followed by 34 cycles of 94°C for 30 sec, 48°C for 60 sec, and 72°C for 90 sec, and finished with a final elongation step at 72°C for 7 min. For *trnL-trnF*, the annealing temperature was kept at 52°–54°C. The PCR products were run out on 1% Tris/Boric acid/EDTA (TBE) agarose minigels, and bands were cut out and cleaned using the QIAquick Gel Extraction Kit (QIAGEN). Double-stranded PCR products were directly sequenced using the ABI Prism Big Dye Terminator Cycle Sequencing Reaction Kit (Perkin-Elmer Applied Biosystems, Foster City, California, USA) and run on an ABI 377 automated sequencer using Long Ranger gels (FMC Bioproducts, Rockland, Maine, USA). Sequencing of both strands was accomplished for *trnK/matK* using amplification primers, primer matK-4F (Manos and Steele, 1997), and five newly designed internal primers (Table 1). For *trnL-trnF*, both strands were sequenced using external primers, internal primer trnL-d (Taberlet et al., 1991), and a newly designed primer trnL-edF (Table 1).

Sequence alignment and phylogenetic analyses—Sequences were checked and assembled using Sequencher, version 3.0 (Gene Codes, Ann Arbor, Michigan, USA). The limits of the different regions (i.e., 5' *trnK* noncoding intron, *matK* gene, 3' *trnK* noncoding intron, *trnL* intron, and *trnL-trnF* intergenic spacer) were determined by comparison with published sequences of *Sinapis*

TABLE 1. Primer sequences used for direct sequencing. The relative position of the 5' end is given in comparison to the *trnK* intron sequence of *Sinapis alba* (GenBank GBAN-X04826) and the *trnL-trnF* sequence of *Arabidopsis thaliana* (GenBank GBAN-AP000423).

Primer name	Sequence (5' to 3')	Relative position	Direction
trnK-23F	CTA ACC ATC TTG CTT TGT TAT CC	545	forward
trnK-31R	GAT ACA TAG TGC GAT MCA GTC AAA MC	664	reverse
trnK-41R	ATG GAT TTT TGD GRA GTA ATM AGA C	1532	reverse
trnK-44F	TAT ATC AAT CGR TTA TCA ARG C	1694	forward
trnK-52F	GGT ACG GAG TCA AAT GGT AGA A	1768	forward
trnK-71R	CTA ATG GGA TGT CCT AAT AC	1886	reverse
trnL-edF	GGA GCA GAA TGA AGA TAG AG	435	forward

alba L. for *trnK/matK* (Neuhaus and Link, 1987; GenBank GBAN-X04826) and of *Arabidopsis thaliana* (L.) Heynh. for *trnL-trnF* (Sato et al., 1999; GenBank GBAN-AP000423). (The prefix GBAN- has been added to link the online version of American Journal of Botany to GenBank, but is not part of the actual GenBank accession number.) Sequences of *trnK/matK* were easily aligned manually with the insertion of distinct gaps. However, the alignment of the *trnL-trnF* sequences proved to be cumbersome; an initial automated alignment with ClustalW 1.74 (Thompson, Higgins, and Gibson, 1994) using standard parameters was then manually adjusted. Informative gaps were coded as additional binary or multistate characters (Baum, Sytsma, and Hoch, 1994). Gaps of variable lengths due to runs of just one type of nucleotides were not considered (Schwarzbach and Ricklefs, 1998). For the combined data set, question marks were used as placeholders for those exemplars that lacked the *trnL-trnF* sequence. All sequences were submitted to GenBank (accession numbers GBAN-AY015273–GBAN-AY015344 for *trnK/matK* and GBAN-AY015345–GBAN-AY015426 for *trnL-trnF*) and the aligned data matrix and the consensus trees are available from TreeBase (<http://www.herbaria.harvard.edu/treebase>).

All analyses were conducted with PAUP* 4.0 (Swofford, 2000) on a Celeron 300 MHz with 128 MB RAM. Adjusted pairwise sequence differences were calculated for the *trnK/matK* data set. Six data sets were analyzed with unweighted maximum parsimony: (1) *trnK/matK* sequences including coded gaps, (2) *trnK/matK* sequences excluding coded gaps, (3) *matK* gene sequences including coded gaps, (4) *trnK* noncoding intron sequences including coded gaps, (5) combined *trnK/matK* and *trnL-trnF* sequences including coded gaps, and (6) combined *trnK/matK* and *trnL-trnF* sequences excluding coded gaps. Due to the high number of trees yielded from these analyses, an inverse constraint search approach (Olmstead and Palmer, 1994; Catalán, Kellogg, and Olmstead, 1997) was used with two search strategies: (1) 500 random-taxon-addition replicates with tree bisection-reconnection (TBR) branch swapping with each replicate restricted to 60 sec of swapping, and (2) a simple taxon addition with TBR branch swapping allowed to proceed for 24 h. The strict consensus trees yielded by these analyses were then applied to inverse constraint searches to check for trees equal or shorter in length that contradict the consensus topology. If no such trees were found, it was assumed that the strict consensus tree adequately summarized the available evidence of the analyzed data.

Support for individual branches was determined with parsimony bootstrapping (Felsenstein, 1985) and decay analyses (Bremer, 1988; Donoghue et al., 1992) for the following four different data sets, all including coded gaps: (1) *trnK/matK*, (2) *matK* gene only, (3) *trnK* noncoding intron only, and (4) combined *trnK/matK* and *trnL-trnF*. For the bootstrap analysis each data set was analyzed with 500 replicates and TBR branch swapping restricted to 100 trees per replicate. The inverse constraint search strategy was used to determine the decay index for each clade of the four strict consensus trees with branch swapping restricted to 30 min.

A number of constraint parsimony searches based on 500 random-taxon-addition replicates (search strategy 1) were conducted to determine the cost of rearranging the phylogeny to fit a number of alternative hypotheses as listed in Table 5. The rationale for these constrained analyses is explained in detail in the Discussion part. Random samples of 100 most-parsimonious trees yielded by these constraint searches were compared to a similar number of unconstrained trees using a Wilcoxon signed-rank test (Templeton, 1983; hence-

forth referred to as "Templeton test") as implemented in PAUP* 4.0 (Swofford, 2000). The highest *P* value of the 10 000 comparisons was determined, and statistical significance of the tests is reported after sequential Bonferroni adjustment (Rice, 1989).

The present molecular data set is characterized by a pronounced heterogeneity in substitution rates. It has repeatedly been pointed out that large differences in branch lengths constitute a potential source of bias for parsimony analyses (Felsenstein, 1978; Sanderson et al., 2000). The placement of *Blossfeldia* Werderm. as sister to the rest of subfamily Cactoideae was a highly unexpected finding warranting detailed investigation. In order to investigate the possible cause of this puzzling result, the following three different analyses were conducted (Huelsenbeck, 1997): (1) a parsimony analysis based on the *trnK/matK* data set with coded gaps included but excluding the two exemplars of *Blossfeldia*, (2) a maximum likelihood analysis (i.e., a phylogenetic inference method putatively less prone to "long-branch attraction"; e.g., Kuhner and Felsenstein, 1994; Huelsenbeck, 1995), and (3) a Monte-Carlo simulation analysis with parametric bootstrapping (Huelsenbeck, Hillis, and Jones, 1996).

A maximum likelihood analysis of the *trnK/matK* data set (excluding coded gaps) was conducted using the HKY85 model of molecular evolution (Hasegawa, Kishino, and Yano, 1985). A successive approximation approach (e.g., Sullivan, Holsinger, and Simon, 1996) was used to accommodate for the computationally intensive estimation of model parameters and tree topology. First, two different starting trees were generated: (1) a maximum likelihood tree using the HKY85 model with standard values for the model parameters (base frequencies was empirical; transition/transversion ratio was 2; among-site rate variation was equal) and (2) a minimum evolution tree based on this same model but allowing for substitution rate heterogeneity following a discrete approximation of a gamma (Γ) distribution. For the resulting two different topologies, parameters for the HKY85 + Γ model were estimated. Then heuristic maximum likelihood searches with simple taxon addition and TBR branch swapping were conducted based on these estimated parameters. This successive approximation process of alternately estimating model parameters and tree topologies was repeated until both analyses converged to the same single result and parameter estimates ceased to change.

The single tree obtained from the maximum likelihood analysis was constrained to place *Blossfeldia liliputana* Werderm. as sister to *Parodia microsperma* (F. A. C. Weber) Speng. (see below for justification of this hypothesis), and branch lengths and model parameters were estimated using the HKY85 + Γ model. Then 500 data sets of similar size and substitution rates were simulated based on the estimated parameters using Seq-Gen 1.1 (Rambaut and Grassly, 1997) and analyzed for the difference in tree length between unconstrained and constrained searches (maximum parsimony, simple taxon addition, TBR branch swapping limited to 100 trees) that forced *Blossfeldia liliputana* to be sister to *Parodia microsperma*. The distribution of tree length differences from the simulated data sets was compared with the tree length difference estimated from the analysis of the real data using a significance level of *P* < 0.05.

RESULTS

Descriptive data on *trnK/matK* and *trnL-trnF* sequences— Except for the last 42 bp at the 3' end, the full *trnK/matK*

TABLE 2. Informative indels coded as additional binary and multistate characters. Multistate characters were used for gaps of varying length, but only the full extent of the gap, relative to the aligned data matrix, is reported here. Taxa with derived character states, relative to the outgroup taxa, are listed together with the codings for the multistate characters (in parentheses).

Sequence	Gap position	Taxa
5' <i>trnK</i> intron	150–153	<i>Corryocactus</i>
	163–173	BCT (1); HLP, core Notocactaceae, core Rhipsalideae (2)
	184–185	<i>Austrocactus</i> , <i>Eulychnia</i>
	212–220	<i>Blossfeldia</i> (1); <i>Eriosyce napina</i> , <i>E. subgibbosa</i> (2); <i>Frailea</i> , <i>Parodia alacriportana</i> , <i>P. haselbergii</i> , <i>Frailea</i> (3)
	279–282	<i>Corryocactus</i>
	364–369	<i>Frailea gracillima</i> , <i>Echinocereus pentalophus</i>
	385–388	<i>Copiapoa</i>
	628–635	<i>Eriosyce aurata</i> , <i>E. islayensis</i>
	653–654	<i>Blossfeldia</i> (1); all other Cactoideae (2)
	<i>matK</i>	1024–1029
1091–1096		<i>Echinopsis pentlandii</i> , <i>Harrisia pomanensis</i>
1377–1388		BCT (1); <i>Parodia maassii</i> , <i>P. microsperma</i> , <i>P. ottonis</i> (2)
1566–1571		<i>Maihuenia</i>
1885–1890		<i>Copiapoa</i>
2224–2229		core Notocactaceae
2372–2378		Cactaceae (1), <i>Copiapoa</i> (2)
3' <i>trnK</i> intron <i>trnL</i> intron	2700–2738	<i>Eriosyce aurea</i> , <i>E. islayensis</i> , <i>Parodia maassii</i> , <i>P. microsperma</i> , <i>P. ottonis</i>
	2857–2913	<i>Acanthocereus tetragonus</i> , <i>Disocactus amazonicus</i> , <i>Selenicereus boeckmannii</i> (1); <i>Echinopsis chiloensis</i> , <i>E. pentlandii</i> , <i>Harrisia pomanensis</i> (2)
	2968–2988	<i>Gymnocalycium</i> , <i>Stetsonia</i> , core Trichocereae, <i>Uebelmannia</i>
	3003–3035, 3046–3053	<i>Parodia magnifica</i> , <i>P. maassii</i> , <i>P. microsperma</i> , <i>P. ottonis</i>
	3065–3078	BCT, <i>Parodia</i>
	3096–3118	<i>Parodia</i>
	3153–3162	<i>Echinopsis chiloensis</i> , <i>Harrisia pomanensis</i>
	3229–3233	<i>Gymnocalycium denudatum</i> , <i>Parodia maassii</i> , <i>P. microsperma</i> , <i>P. ottonis</i>
	3262–3266	<i>Parodia microsperma</i> , <i>P. ottonis</i>
	3295–3296	<i>Eriosyce</i>
	3460–3477	<i>Parodia maassii</i> , <i>P. microsperma</i> , <i>P. ottonis</i> (1); <i>Parodia alacriportana</i> , <i>P. haselbergii</i> (2); BCT excluding Trichocereae (3); Trichocereae (4); HLP (5); <i>Austrocactus</i> (6)
	3621–3630	<i>Cereus alacriportanus</i> , <i>Micranthocereus albicephalus</i> , <i>Coleocephalocereus fluminensis</i> , <i>Stetsonia coryne</i>

gene, corresponding to positions 239–2770 of the transfer RNA gene for lysine of *Sinapis alba*, was sequenced for 72 exemplars. The *trnK/matK* matrix comprised 2577 aligned sites, of which 1989 were constant and 270 (10.5%) were informative. In addition, 16 informative indels were coded as binary or multistate characters (Table 2). The two partitions, consisting of the *trnK* noncoding intron region (1029 aligned sites plus ten coded gaps) and the *matK* coding region (1548 aligned sites plus six coded gaps), contributed a comparable amount of informative characters (10.9% for the *trnK* introns, 11.2% for the *matK* gene). The ratio of terminal taxa (72) to informative characters (286, including coded gaps) was 1:3.97.

Adjusted pairwise sequence differences ranged from almost 6% between *Grahamia bracteata* Gill. and *Parodia microsperma* to no difference between *Cereus alacriportanus* Pfeiff. and *Micranthocereus albicephalus* (Buining & Brederoo) F. Ritter. In general, sequence differences among taxa of the tribes Cereeae, Trichocereae, and the genus *Eriosyce* Phil. were very low (<1.5%). The adjusted sequence difference between the two outgroups, *Grahamia bracteata* and *Talinum paniculatum* (Jacquin) Gaertn. (4.54%), was almost as big as the biggest difference between *Talinum paniculatum* and any of the cacti sampled for this study (adjusted difference to *Parodia microsperma* 4.58%). The largest pairwise sequence difference within Cactaceae was between *Pereskopsis diguetii* (F. A. C. Weber) Britton & Rose and *Parodia microsperma* with 4.09%.

Complete sequences of the *trnL* intron and the *trnL-trnF*

intergenic spacer were added to a combined *trnK/matK* and *trnL-trnF* data matrix for 41 exemplars of three major clades of Cactoideae (see below; no sequences were available for *Browningia chlorocarpa* (Kunth) W. T. Marshall, *Hylocereus peruvianus* Backeb., *Matucana intertexta* F. Ritter, *Pfeiffera miyagawae* Barthlott & Rauh, and *P. monacantha* (Griseb.) P. V. Heath). The aligned *trnL-trnF* matrix comprised 1123 sites, of which 903 were constant and 96 (8.5%) were informative. Length variation in *trnL-trnF* sequences between different taxa was remarkable (gaps ranging 2–57 bp) and required the insertion of 12 informative indels, which were coded as binary or multistate characters (Table 2). The addition of *trnL-trnF* sequences to a combined data set increased the ratio of terminal taxa to informative characters from 1:2.41 for the *trnK/matK* data set to 1:5.05 for the combined data set (comparison based on 41 exemplars for which *trnK/matK* and *trnL-trnF* were available). Further sequence information of the different partitions of the *trnK/matK* and *trnL-trnF* data sets are given in Table 3.

Parsimony analyses—All six heuristic parsimony analyses produced strict consensus trees that were the same regardless of which of the two search strategies were employed. Inverse constraint searches based on the strict consensus trees only yielded trees that were one step longer than the original trees, suggesting that the strict consensus trees, even if based on just a subsample of the total number of trees, adequately summarized the available evidence (Downie, Katz-Downie, and Wat-

TABLE 3. Sequence information about the different portions of the *trnK/matK* and *trnL-trnF* data.

Sequence	3' <i>trnK</i> intron	<i>matK</i> gene	5' <i>trnK</i> intron	<i>trnL</i> intron	<i>trnL-trnF</i> spacer
Length of aligned matrix (sites)	765	1548	264	681	442
Length of sequences (bp)	677–708	1512–1536	239–252	399–654	141–384
Number of informative gaps	9	6	1	8	4
Number of constant sites	598	1197	194	551	352
Number of informative sites (% of total sites)	70 (9.2%)	167 (10.8%)	33 (12.5%)	68 (10%)	28 (6.3%)
GC content	0.33–0.34	0.32–0.33	0.34–0.36	0.26–0.32	0.32–0.35

son, 2000). Descriptive information about the six different parsimony analyses are listed in Table 4. The strict consensus of 61 354 most-parsimonious (mp) trees from the *trnK/matK* analysis, including coded gaps, yielded from search strategy 1 (500 random-taxon-addition replicates) is given in Fig. 1. This analysis resolved 39 clades, with bootstrap support values ranging from 47 to 100% (Fig. 1; values above the branches). The three clades that were not supported by the analysis with coded gaps excluded are marked with asterisks.

The strict consensus trees from the individual analyses of the *matK* and *trnK* partitions were largely congruent with the tree shown in Fig. 1 (except for *Neowerdermannia vorwerkii* (Fric) Backeb., which is sister to a clade of *Parodia maassii* (Heese) A. Berger, *P. microsperma*, and *P. ottonis* (Lehm.) N. P. Taylor in the *matK* data set, and *Schlumbergera truncata* (Haw.) Moran, which is sister to *Rhipsalis floccosa* Pfeiff. in the *trnK* data set). Bootstrap support values for the two partitions are mapped onto the strict consensus tree of the *trnK/matK* analysis (Fig. 1; values below the branches). The *matK* partition, which contributed slightly >60% of the informative sites but just 37.5% of the informative gaps to the total *trnK/matK* data set, resolved a few more clades (32 clades vs. 29 clades) with generally higher bootstrap values (20 clades vs. 15 clades) than the *trnK* partition. Overall, however, the information provided by the two partitions was complementary.

The combined analysis of *trnK/matK* and *trnL-trnF* sequences (search strategy 1) yielded 62 850 mp trees, and the strict consensus tree of this analysis is given in Fig. 2. The tree topology is largely congruent with the one derived from the *trnK/matK* analysis. The only taxa with incongruent positions in the two analyses were *Harrisia pomanensis* (F. A. C. Weber) Britton & Rose (sister to *Echinopsis pentlandii* (Hooker) A. Dietrich based on the *trnK/matK* data; sister to *Echinopsis chiloensis* (Colla) Friedrich & G. D. Rowley based on the combined data) and *Parodia microsperma* (sister to *P. maassii* based on the *trnK/matK* data; sister to *P. ottonis* based on the combined data). The addition of *trnL-trnF* data resulted in improved resolution, increasing the number of clades on the strict consensus tree from 39 to 51. In addition, bootstrap sup-

port values and decay indices increased consistently for the different subclades of these three groups.

Excluding coded gaps from the parsimony analyses of the *trnK/matK* and the combined *trnK/matK* and *trnL-trnF* data sets resulted in slightly reduced but congruent strict consensus trees (Table 4, trees not shown). When *Blossfeldia* was excluded from the analysis of the *trnK/matK* data set (70 instead of 72 exemplars), the mp trees were 39 steps shorter, more or less in accordance with the estimated branch lengths of 35–39 steps for that particular clade. In this analysis, relationships among the major clades remained unaltered, except that the clade consisting of *Maihuenia* and subfamily Opuntioideae collapsed. A majority rule consensus tree revealed that in this case 96% of the mp trees favored a grade with *Maihuenia* sister to the Cactoideae and Opuntioideae sister to these two clades.

Maximum likelihood analysis—The successive approximation approach used for the maximum likelihood analysis of the *trnK/matK* data set (excluding coded gaps) based on the HKY85 + Γ model converged from the two suboptimal starting trees, the initial maximum likelihood tree ($-\log L = 9876.224$) and the minimum evolution tree ($-\log L = 9891.006$), to the same optimal tree and the same estimated parameters within two successive approximation cycles. The resulting phylogram is given in Fig. 3 and the estimated parameters are $-\log L = 9875.797$, transition/transversion ratio = 0.646543, nucleotide parts are A = 0.30996, C = 0.16795, G = 0.17639, T = 0.34570 and gamma shape parameter alpha = 0.37489. The maximum likelihood tree, when compared to the strict consensus tree of the maximum parsimony analysis, favored slightly different relationships in the tribe Cactaceae and *Echinopsis* Zucc., while providing no indication for distinct clades formed by *Maihuenia* + Opuntioideae and *Calymanthium* + *Copiapoa* Britton & Rose (see below for more details).

Constraint parsimony analyses—The findings from the ten different constraint analyses based on the *trnK/matK* data set

TABLE 4. Information about the parsimony analyses of the six different data sets.

Sequence data ^a	<i>trnK/matK</i>		<i>matK</i> only	<i>trnK</i> only	combined <i>trnK/matK</i> + <i>trnL-trnF</i>	
Data set	1 (incl. gaps)	2 (excl. gaps)	3 (incl. gaps)	4 (incl. gaps)	5 (incl. gaps)	6 (excl. gaps)
Tree length	938	911	546	382	1273	1221
Number of mp trees (search strategy in parenthesis)	61 354 (1), 60 067 (2)	34 348 (1), 59 478 (2)	15 074 (1), 17 237 (2)	53 517 (1), 34 344 (2)	62 850 (1), 67 037 (2)	58 314 (1), 154 239 (2)
CI	0.77	0.77	0.76	0.80	0.76	0.76
CI' (excluding uninformative characters)	0.63	0.62	0.63	0.66	0.62	0.61
RI	0.85	0.84	0.86	0.85	0.84	0.83
Number of resolved clades	39	36	32	29	51	43

^a Abbreviations: mp = most parsimonious; CI = consistency index; RI = retention index.

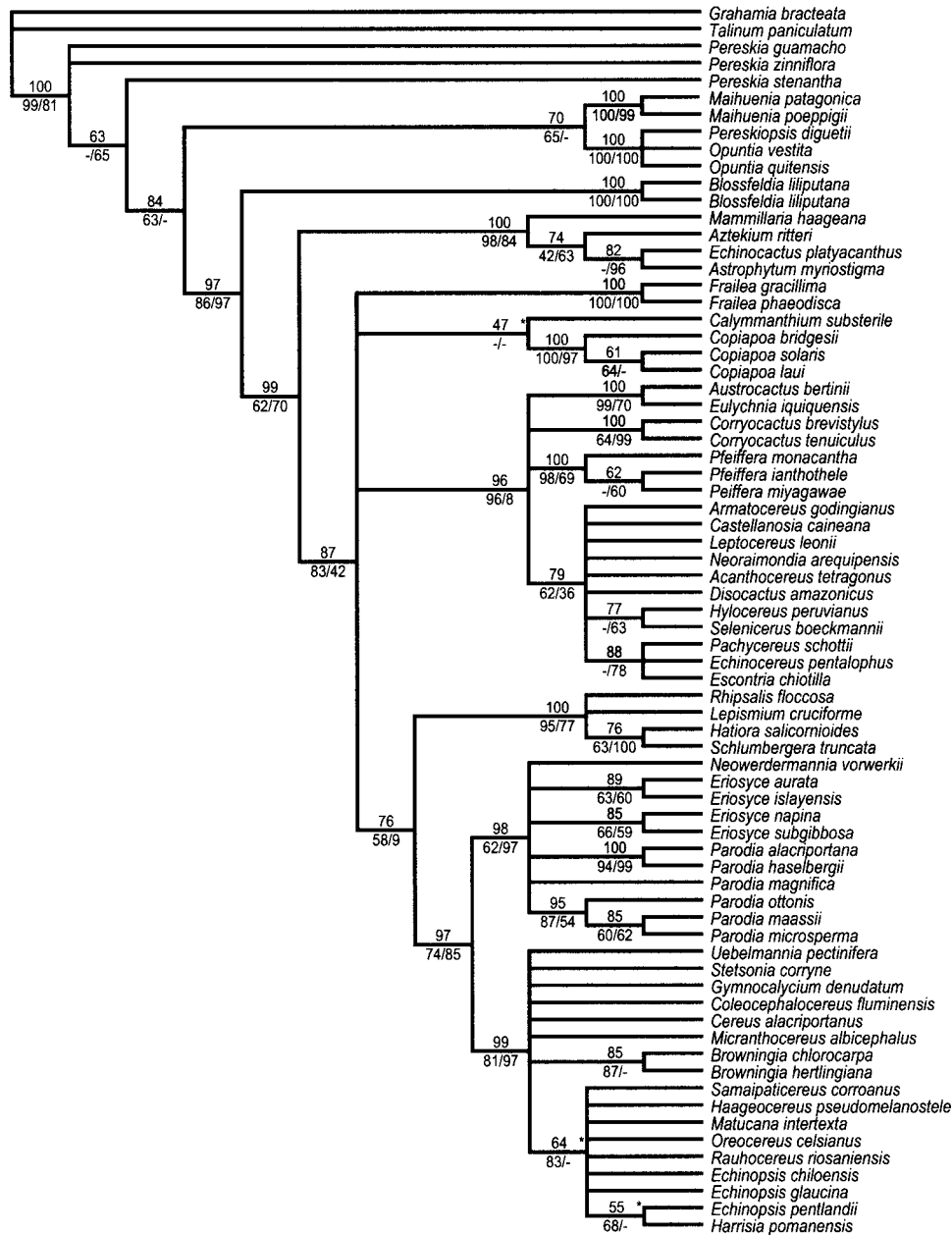


Fig. 1. Strict consensus of 61 354 most-parsimonious trees of length 938 (CI = 0.77, RI = 0.85) for Cactaceae derived from the analysis of the *trnK/matK* data set (search incomplete). Bootstrap values for the *trnK/matK* data set are given above the branches; those for the *matK* and the *trnK* partitions are below the branches separated by a forward slash. A dash indicates <5% support for that particular clade and data set. Clades not supported when coded gaps were excluded from the analysis are marked with an asterisk.

are summarized in Table 5. A monophyletic genus *Pereskia* (one additional step; $P = 1.0$), a sister group relationship between *Copiapoa* and core Notocactae (seven additional steps; $P = 0.49$), a sister group relationship between *Pfeiffera* Salm-Dyck and core Rhipsalideae (eight additional steps; $P = 0.57$), and a clade consisting of *Austrocactus* Britton & Rose, *Coryocactus*, *Eulychnia* Phil., and core Notocactae (12 additional steps; $P = 0.24$) cannot be rejected based on Templeton tests of 10 000 random pairwise comparisons sampled for each constraint search after sequential Bonferroni adjustment.

Parametric bootstrapping—The tree lengths yielded from the 500 simulated data sets with *Blossfeldia liliputana* forced

to be sister to *Parodia microsperma* ranged from 842 to 1045 steps (mean = 944.35; SD = 39.24). The constrained analyses of these simulated data sets generally resulted in mp trees of the same length or trees just one to three steps longer (number of simulated data sets with corresponding tree length difference in steps: no difference in 398 data sets, one step difference in 85 data sets, two steps difference in 14 data sets, three steps difference in 3 data sets) compared to unconstrained analyses of the same data sets, which showed the two tested taxa nested together in a small clade. The difference in tree lengths yielded from the real data set, however, comprised 34 steps. This large discrepancy in tree lengths derived from simulated data sets compared to those from the real data set made

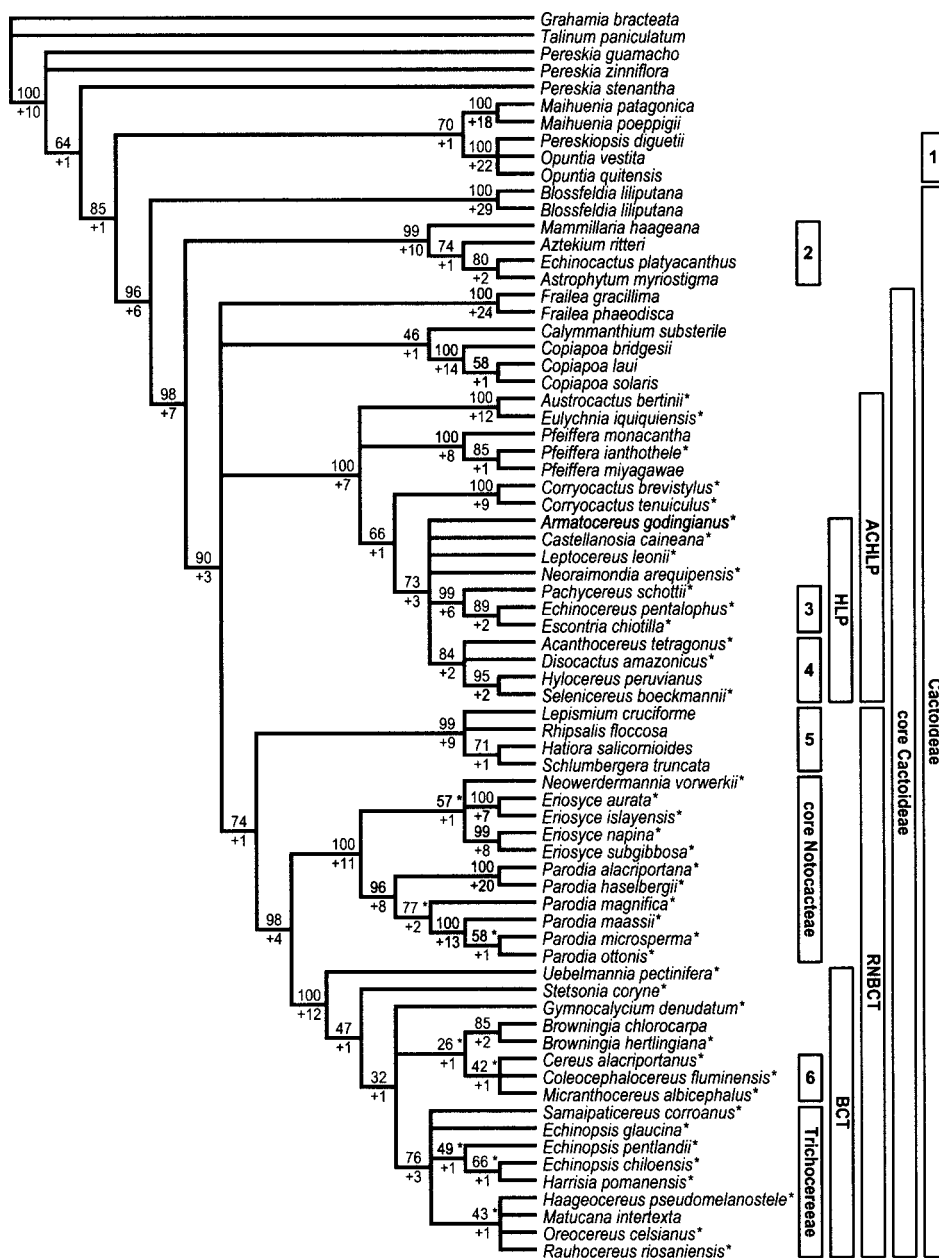


Fig. 2. Strict consensus of 62 850 most-parsimonious trees of length 1273 (CI = 0.76, RI = 0.84) for Cactaceae derived from the analysis of the combined *trnK/matK* and *trnL-trnF* data set (search incomplete). Bootstrap values are given above the branches, decay indices below the branches (please consider that only taxa marked with an asterisk included *trnL-trnF* sequences). Clades not supported when coded gaps were excluded from the analysis are marked with an asterisk. Clade names are: 1, Opuntioideae; 2, Cactaceae; 3, Pachycereeae; 4, Hylocereeae; 5, core Rhipsalideae; 6, Cereeae.

it safe to reject the hypothesis that branch length heterogeneity is a reasonable explanation for the unexpected result of *Blossfeldia* being sister to all other Cactoideae (Huelsenbeck, Hillis, and Jones, 1996).

DISCUSSION

Phylogenetic signal, partially combined data sets, and misleading factors—The *trnK/matK* data set, consisting of 2577 aligned sites and 286 informative characters, proved to be fairly successful in resolving relationships among the major clades in the family Cactaceae. The amount of inconsistent signal (homoplasy) is low in this data set as judged based on the

modified consistency indices (Table 4) of the different parsimony analyses (Sanderson and Donoghue, 1989; Givnish and Sytma, 1997). However, the large number of exemplars, compared to the number of informative characters available (ratio about 1 : 4) and the uneven allocation of this information among the clades (see branch length differences in Fig. 3) are likely responsible for the large number of trees yielded from the parsimony analyses. For this reason, it was not possible to conduct TBR branch swapping to completion, and an inverse constraint search approach was chosen to assess the validity of the strict consensus trees based on the incomplete parsimony analyses.

In order to improve resolution within three larger clades of

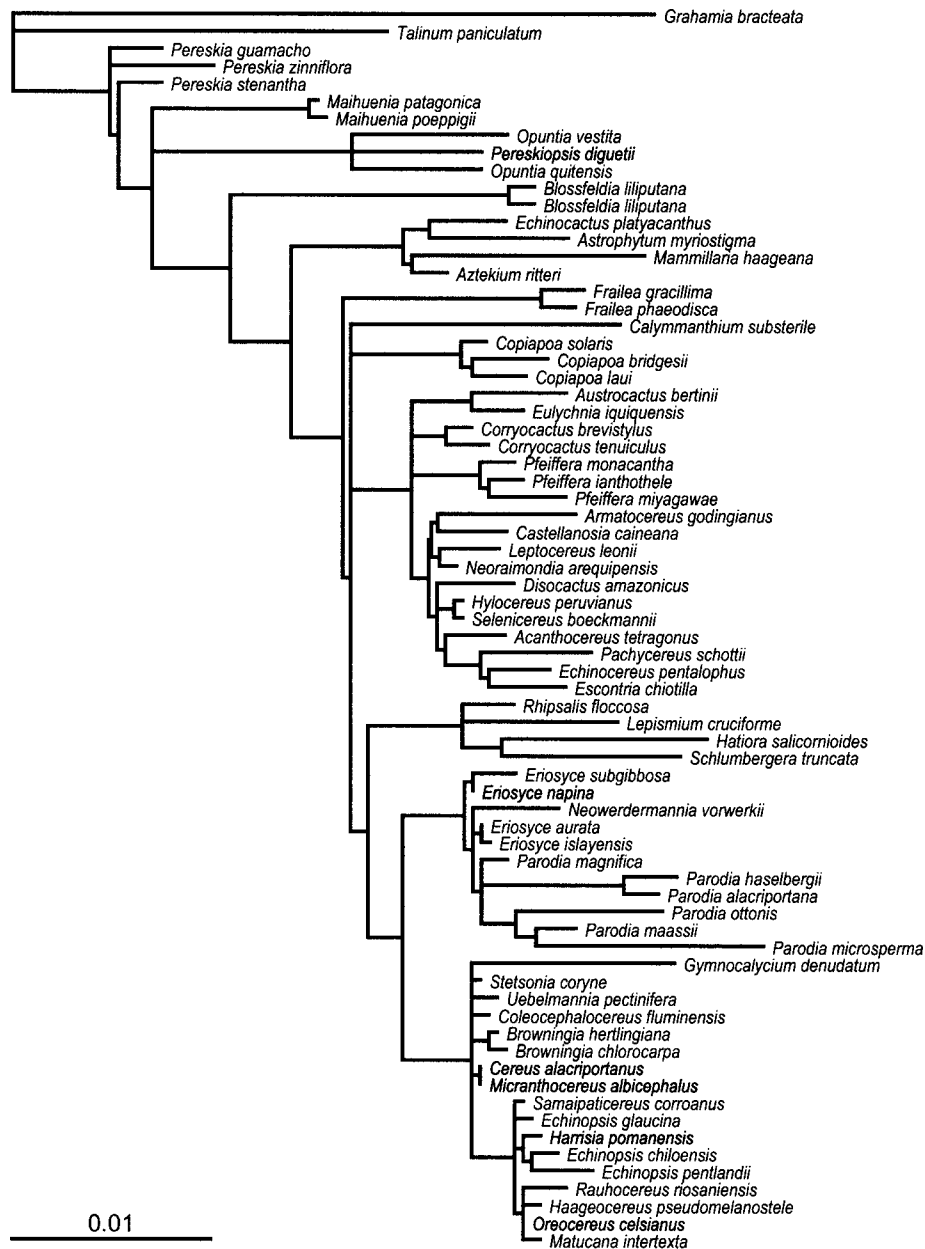


Fig. 3. Maximum likelihood tree ($-\log L = 9875.797$) for Cactaceae derived from a successive approximation analysis (alternate estimation of tree topology and model parameters) of the *trnK/matK* data set based on the HKY85 + Γ model of molecular evolution. The bar indicates the expected number of substitutions per site.

Cactoideae, I added sequences of the *trnL* intron and the *trnL-trnF* intergenic spacer to a combined analysis of chloroplast markers. The additional information helped to resolve relationships among rather closely related taxa within three large clades of Cactoideae.

Researchers have recently raised a number of concerns about potential sources for misleading results in molecular systematic analyses (e.g., Doyle, 1992; Sanderson and Doyle, 1992; Swofford et al., 1996; Wendel and Doyle, 1998). For the present study, I verified a number of unexpected findings, such as the position of *Blossfeldia*, *Browningia* Britton & Rose, *Copiapoa*, *Frailea* Britton & Rose, and *Pfeiffera*, with sequencing of additional congenic exemplars in order to decrease the possibility of contamination and mistakes. Further-

more, the parsimony and the maximum likelihood analyses yielded very similar topologies, which indicated that inconsistencies of one or the other algorithm in reconstructing the phylogenetic relationships are not an obvious explanation for some of the unexpected results. This was, in addition, confirmed for the special case of *Blossfeldia* with a Monte-Carlo simulation analysis as described by Huelsenbeck, Hillis, and Jones (1996).

Relationships among the major clades in Cactaceae—The monophyly of the family Cactaceae, as indicated by a number of unique morphological features (e.g., Gibson and Nobel, 1986) and molecular data (Wallace, 1995b; Wallace and Cota, 1995; Hershkovitz and Zimmer, 1997), is also strongly sup-

TABLE 5. Information on constrained parsimony analyses. *P* values were determined based on Templeton tests of random samples of 100 trees from the unconstrained and constrained parsimony analyses. Alternative tree topologies not rejected at the significance level $P < 0.05$ after sequential Bonferroni adjustment (maximum *P* value multiplied by $1 + k$ [number of tests; in this case, 10] - *i* [rank of the test given the uncorrected *P* values]) are marked with an asterisk.

Taxa constrained to form a monophyletic group	No. of extra steps	Rank (<i>i</i>)	Highest <i>P</i> value found in 10 000 comparisons	<i>P</i> value after sequential Bonferroni adjustment
<i>Pereskia</i>	1	10	1.0	1.0
<i>Browningia</i> + <i>Castellanosia</i>	25	3	0.0003	0.0024*
<i>Browningia</i> + HLP clade	23	5	0.0012	0.0072*
<i>Lepismium</i> + <i>Pfeiffera</i>	19	6	0.0041	0.0205*
<i>Pfeiffera</i> + core Rhipsalideae	8	8	0.19	0.57
<i>Austrocactus</i> + <i>Blossfeldia</i> + <i>Copiapoa</i> + <i>Corryocactus</i> + <i>Eulychnia</i> + <i>Frailea</i> + core Notocactaceae	26	4	0.0006	0.0042*
<i>Austrocactus</i> + <i>Corryocactus</i> + <i>Eulychnia</i> + core Notocactaceae	12	7	0.06	0.24
<i>Copiapoa</i> + core Notocactaceae	7	9	0.2451	0.4902
<i>Blossfeldia</i> + <i>Frailea</i> + <i>Parodia</i>	28	2	0.0002	0.0018*
<i>Blossfeldia</i> + <i>Parodia microsperma</i>	34	1	0.0001	0.001*

ported by the present *trnK/matK* data set (bootstrap [BS] = 100%, decay index [DI] = 10). The comparison of pairwise sequence distances in the *trnK/matK* data set confirmed previous observations (Herskovitz and Zimmer, 1997) that the cacti are remarkable for their small genetic differences, even among morphologically very different taxa, such as *Pereskiaopsis diguetii* and *Parodia microsperma*, when compared to a small sample of taxa from the sister group in Portulacaceae.

Relationships as inferred by the combined analysis of *trnK/matK* and *trnL-trnF* data (Fig. 2) are summarized in a cladistic classification scheme for the genera investigated in this study (Table 6). A number of clade names, derived from tribal and generic names, are listed together with four more recent traditional tribal classifications for the Cactoideae. All clades, with the exception of the tribe Cereeae, recognized for this classification scheme received bootstrap support values of 70% or higher.

Well-supported major clades within Cactaceae are the genus *Maihuenia* (*trnK/matK* data: BS = 100%, DI = 18; Fig. 1), and the subfamilies Opuntioideae (*trnK/matK* data: BS = 100%, DI = 22) and Cactoideae (*trnK/matK* data: BS = 97%, DI = 7), while the three exemplars of *Pereskia* do not form a monophyletic group, but a basal grade, in the present analysis. Bootstrap support for a paraphyletic genus *Pereskia* is low (*trnK/matK* data: BS = 63%, DI = 1) and is only indicated by the *trnK* noncoding intron partition. Furthermore, a constraint analysis forcing the exemplars of *Pereskia* to form a distinct clade yielded mp trees just one step longer. Hence, neither paraphyly nor monophyly of *Pereskia* can be ruled out based on the present data. The genus *Maihuenia*, traditionally placed in subfamily Pereskioideae because it lacks the synapomorphies of Opuntioideae and Cactoideae, is ecologically specialized (Leuenberger, 1997; Mauseth, 1999) and has a restricted distribution in southern Argentina and Chile. Recently, the genus was placed in a monotypic subfamily Maihuenioideae (Wallace, 1995b; Anderson, 2001). Opuntioideae and Cactoideae, in contrast, are very well-characterized by a number of structural and molecular synapomorphies. Different taxa of both subfamilies occur over the entire distribution area of the cacti and often are found at the same localities; however, Cactoideae comprises about seven times the number of species of Opuntioideae (estimate based on Barthlott and Hunt, 1993).

The present study supported a clade consisting of *Maihuenia*, Opuntioideae, and Cactoideae (*trnK/matK* data: BS = 84%,

DI = 1; signal provided by the *matK* partition only) sister to *Pereskia*. In addition, *Maihuenia* and Opuntioideae form a rather weakly supported monophyletic group in parsimony analyses (*trnK/matK* data: BS = 70%, DI = 1; signal provided by the *matK* partition only). However, there is indication that this finding is affected by including *Blossfeldia* to the parsimony analysis. The strict consensus tree of the *trnK/matK* analysis excluding *Blossfeldia* showed a polytomy consisting of *Maihuenia*, Opuntioideae, and Cactoideae. Furthermore, the maximum likelihood analysis did not provide support for this sister group relationship. Summary trees of other molecular analyses (Wallace, 1995b; Martin and Wallace, 2000) identified the same major clades of Cactaceae, though these trees suggested different relationships among them.

Relationships in Cactoideae—The relationships in the subfamily Cactoideae, by far the most diverse clade of Cactaceae, are the focal point of the present study. The findings revealed by this molecular analysis conflict in a number of cases with traditional, well-established ideas about relationships within this clade, but they also provide strong evidence to resolve various old debates about the placement of certain enigmatic taxa.

Blossfeldia—Certainly the most controversial result of the present study is the placement of *Blossfeldia liliputana*. This monotypic genus of tiny globular cacti from the eastern slopes of the Andes of northern Argentina and southern Bolivia (Leuenberger and Eggli, 1999) forms the sister group to the rest of the subfamily Cactoideae (*trnK/matK* data: BS = 99%, DI = 7; Fig. 1). The same relationship is also favored by the maximum likelihood analysis (Fig. 3). *Blossfeldia* occurs in crevices of more or less vertical cliffs and is ecologically and morphologically specialized. The plants lack any xeromorphic structures that might prevent them from desiccation. Under water stress, the plants may lose up to 80% of their initial mass and then recover within a few weeks to their original vital condition (Barthlott and Porembski, 1996). The tiny seeds with prominent strophiola are very similar to those of *Parodia microsperma*, wherefore a close relationship between these two genera was widely accepted (Buxbaum, 1967; Taylor, 1989). However, this alternative hypothesis was strongly opposed by the results of a Monte-Carlo simulation analysis and a Templeton test (Table 5), as was a less stringent hypothesis

TABLE 6. Cladistic classification of genera sampled for the present study in comparison with four recent suprageneric classification schemes of Cactoideae proposed by Endler and Buxbaum (1974), Gibson and Nobel (1986), Barthlott and Hunt (1993), and Anderson (2001).

Taxon	Endler and Buxbaum (1974)	Gibson and Nobel (1986)	Barthlott and Hunt (1993)	Anderson (2001)
<i>Pereskia</i> ^a	Pereskioideae	Pereskioideae	Pereskioideae	Pereskioideae
[unnamed clade]				
[unnamed clade]				
<i>Mathuenia</i>	Pereskioideae	Pereskioideae	Pereskioideae	Maihuenioideae
[clade: Opuntioideae]				
<i>Opuntia</i>	Opuntioideae	Opuntioideae	Opuntioideae	Opuntioideae
<i>Peresklopsis</i>	Opuntioideae	Opuntioideae	Opuntioideae	Opuntioideae
[clade: Cactoideae]				
<i>Blossfeldia</i>	Notocactaeae	Notocactaeae	Notocactaeae	Notocactaeae
[unnamed clade]				
[clade: Cactaeae]				
<i>Astrophytum</i>	Notocactaeae	Cactaeae	Cactaeae	Cactaeae
<i>Aztekium</i>	Cactaeae	Cactaeae	Cactaeae	Cactaeae
<i>Echinocactus</i>	Cactaeae	Cactaeae	Cactaeae	Cactaeae
<i>Mammillaria</i>	Cactaeae	Cactaeae	Cactaeae	Cactaeae
[clade: core Cactoideae]				
<i>Calymmanthium</i>	Leptocereaeae	Leptocereaeae	Browningieae	Calymmantheae
<i>Copiapoa</i>	Notocactaeae	Notocactaeae	Notocactaeae	Notocactaeae
<i>Frailea</i>	Notocactaeae	Notocactaeae	Notocactaeae	Notocactaeae
[clade: ACHLP]				
<i>Austrocactus</i>	Notocactaeae	Notocactaeae	Notocactaeae	Notocactaeae
<i>Corryocactus</i>	Notocactaeae	Notocactaeae	Notocactaeae	Pachycereaeae
<i>Eulychnia</i>	[uncertain]	Notocactaeae	Notocactaeae	Notocactaeae
<i>Pfeiffera</i>	Hylocereaeae	Notocactaeae	Rhipsalideae	Rhipsalideae
[clade: HLP]				
<i>Armatocereus</i>	Leptocereaeae	Leptocereaeae	Browningieae	Browningieae
<i>Castellanosia</i>	Browningieae	Browningieae	Browningieae	Browningieae
<i>Leptocereus</i>	Leptocereaeae	Leptocereaeae	Echinocereaeae	Pachycereaeae
<i>Neoraimondia</i>	Leptocereaeae	Leptocereaeae	Browningieae	Browningieae
[clade: Hylocereaeae]				
<i>Acanthocereus</i>	Hylocereaeae	Hylocereaeae	Echinocereaeae	Pachycereaeae
<i>Disocactus</i>	Hylocereaeae	Hylocereaeae	Hylocereaeae	Hylocereaeae
<i>Hylocereus</i>	Hylocereaeae	Hylocereaeae	Hylocereaeae	Hylocereaeae
<i>Selenicereus</i>	Hylocereaeae	Hylocereaeae	Hylocereaeae	Hylocereaeae
[clade: Pachycereaeae]				
<i>Echinocereus</i>	Echinocereaeae	Echinocereaeae	Echinocereaeae	Pachycereaeae
<i>Escontria</i>	Pachycereaeae	Pachycereaeae	Pachycereaeae	Pachycereaeae
<i>Pachycereus</i>	Pachycereaeae	Pachycereaeae	Pachycereaeae	Pachycereaeae
[clade: RNBCT]				
[clade: core Rhipsalideae]				
<i>Hatiora</i>	Hylocereaeae	Notocactaeae	Rhipsalideae	Rhipsalideae
<i>Lepismium</i>	Hylocereaeae	Notocactaeae	Rhipsalideae	Rhipsalideae
<i>Rhipsalis</i>	Hylocereaeae	Notocactaeae	Rhipsalideae	Rhipsalideae
<i>Schlumbergera</i>	Hylocereaeae	Notocactaeae	Rhipsalideae	Rhipsalideae
[unnamed clade]				
[clade: core Notocactaeae]				
<i>Eriosyce</i>	[uncertain]	Notocactaeae	Notocactaeae	Notocactaeae
<i>Neowerdermannia</i>	Notocactaeae	Notocactaeae	Notocactaeae	Notocactaeae
<i>Parodia</i>	Notocactaeae	Notocactaeae	Notocactaeae	Notocactaeae
[clade: BCT]				
<i>Browningia</i>	Browningieae	Browningieae	Browningieae	Browningieae
<i>Gymnocalycium</i>	Notocactaeae	Notocactaeae	Trichocereaeae	Notocactaeae
<i>Stetsonia</i>	Cereaeae	Cereaeae	Browningieae	Browningieae
<i>Uebelmannia</i>	Notocactaeae	Notocactaeae	Notocactaeae	Cereaeae
[clade: Cereaeae ^b]				
<i>Cereus</i>	Cereaeae	Cereaeae	Cereaeae	Cereaeae
<i>Colecephalocereus</i>	Cereaeae	Cereaeae	Cereaeae	Cereaeae
<i>Micranthocereus</i>	Trichocereaeae	Cereaeae	Cereaeae	Cereaeae
[clade: Trichocereaeae]				
<i>Echinopsis</i>	Trichocereaeae	Trichocereaeae	Trichocereaeae	Trichocereaeae
<i>Haageocereus</i>	Trichocereaeae	Trichocereaeae	Trichocereaeae	Trichocereaeae
<i>Harrisia</i>	Hylocereaeae	Hylocereaeae	Echinocereaeae	Trichocereaeae
<i>Matucana</i>	Trichocereaeae	Trichocereaeae	Trichocereaeae	Trichocereaeae
<i>Oreocereus</i>	Trichocereaeae	Trichocereaeae	Trichocereaeae	Trichocereaeae
<i>Rauhocereus</i>	Browningieae	[not mentioned]	[not mentioned]	Trichocereaeae
<i>Samaipaticereus</i>	Leptocereaeae	Leptocereaeae	Trichocereaeae	Trichocereaeae

^a *Pereskia* did not form a monophyletic group in the present analysis.^b Statistical support for this clade was very low.

of a clade consisting of *Blossfeldia*, *Frailea*, and *Parodia* Speg. (Templeton test, $P = 0.0018$).

Several DNA extractions from different samples were prepared in order to overcome the problem of contamination or mistake. However, all the different sequences yielded the same result as reported here. Further studies are needed to investigate whether the present chloroplast gene tree might significantly differ from the organismal phylogeny or whether in fact this result reflects the correct relationships of *Blossfeldia* in the cactus phylogeny.

Cactaceae—The circumscription of the tribe Cactaceae has never been challenged in recent classification schemes (Table 6), and it emerges as the only suprageneric group undisputed by the present molecular analysis with high support values (*trnK/matK* data: BS = 100%, DI = 10; unique 4 bp indel in 3' *trnK* partition). This clade of mostly short cylindrical to globular or globular-caespitose cacti comprises ~20 genera and 500 species (estimate based on Barthlott and Hunt, 1993) and is predominantly found in Mexico and the southwestern United States, with a number of species of *Mammillaria* Haw. extending into Central America, northern South America, and the Caribbean. This tribe is well circumscribed by its seeds, which have hilum and micropyle disjunct and only rarely conjunct (Barthlott and Voit, 1979). The tribe Cactaceae is sister to a large unresolved clade, here referred to as core Cactoideae (*trnK/matK* data: BS = 87%, DI = 3).

Core Cactoideae—The core of Cactoideae consists of the ACHLP clade, the RNBCT clade and three orphan genera (Fig. 2). *Calymmanthium* (columnar, densely branched cacti from northern Peru; 1 sp.) forms a very weakly supported clade (*trnK/matK* data: BS = 47%, DI = 1; Fig. 1) with *Copiapoa* (globular or subcolumnar cacti from the Atacama Desert, Chile; ~20 sp.) when coded gaps are included in the analysis. A similar tendency of these two morphologically very different genera to form a clade was also found in preliminary analyses of ITS sequences (R. Nyffeler, unpublished data). On the other hand, this clade collapsed when coded gaps were excluded from the analysis, and a constraint analysis (Table 5) showed that the present data do not conflict with the traditional conception of a close relationship of *Copiapoa* with other globular Notocactaceae (Templeton test, $P = 0.49$). *Frailea* is a genus of tiny globular cacti from southeastern South America. It has often been thought to be closely related to either *Blossfeldia* (Barthlott, 1988) or *Parodia* (Eggl and Nyffeler, 1998; R. Nyffeler, unpublished data). However, the present data conflicts with various alternative hypotheses, i.e., a sister group relationship with *Blossfeldia*, *Parodia* sensu stricto (s.s.), or with any other globular Notocactaceae (Table 5).

The ACHLP clade, HLP clade, Hylocereeae, and Pachycereeae—The ACHLP clade (name derived from the initials of the genera *Austrocactus* and *Corryocactus* and the tribes Hylocereeae, Leptocereae, and Pachycereeae) comprises ~25 genera and 250 species (estimate based on Barthlott and Hunt, 1993) with predominantly columnar or scandent habit and two distinct groups of epiphytes (*Pfeiffera* and the tribe Hylocereeae). The combined analysis with additional *trnL-trnF* sequences (Fig. 2) helped to further resolve relationships within this large clade. There are four distinct subclades in the ACHLP clade. *Austrocactus* + *Eulychnia* and *Corryocactus* are columnar cacti from the western slopes of southern South

America (Chile, Peru, Bolivia; a few species of *Austrocactus* also occur in southern Argentina) and were previously thought to be “basal” members of the tribe Notocactaceae (Buxbaum, 1967, 1969). *Pfeiffera* comprises a handful of epiphytic and epilithic species from the eastern Andes of Bolivia and northwestern Argentina. This genus was traditionally included in the tribe Rhipsalideae and was regarded as “transitional” between *Corryocactus* and the other members of Rhipsalideae (e.g., Gibson and Nobel, 1986). The fourth clade, here called HLP clade, consists of taxa previously referred to either the tribes Browningieae, Echinocereae, Hylocereeae, Leptocereae, or Pachycereeae (Table 6). Relationships within this group are not well resolved, with the exception of the two clades comprising the members of the tribe Pachycereeae + *Echinocereus* (*trnK/matK* data: BS = 88%, DI = 2 [Fig. 1]; combined data: BS = 99%, DI = 6 [Fig. 2]) and those of the tribe Hylocereeae + *Acanthocereus* (combined data: BS = 84%, DI = 2). The close relationship of *Acanthocereus* with the Hylocereeae is favored by the *trnL-trnF* data, while the maximum likelihood analysis (Fig. 3) of the *trnK/matK* data indicates a closer relationship of this genus with Pachycereeae. In addition, the maximum likelihood analysis also revealed a distinct clade consisting of *Acanthocereus*, *Echinocereus*, and the two tribes Hylocereeae and Pachycereeae. Both clades occur to a large extent in Central America and Mexico, though they are generally found in different habitats. The tribe Pachycereeae comprises large columnar cacti (only *Bergerocactus* Britton & Rose and in particular *Echinocereus* form densely branched, low-growing shrubs) and occur in rather arid scrub and desert habitats. In contrast, the tribe Hylocereeae includes scandent or mostly epiphytic cacti, often with very large showy flowers, and is generally found in tropical forests. *Acanthocereus*, though recently excluded from the tribe Hylocereeae (Barthlott, 1988; Barthlott and Hunt, 1993; Anderson, 2001), mainly differs from that group by forming terrestrial shrubs with slender branches, but is otherwise very similar. *Armatocereus* Backeb., *Neoraimondia* Britton & Rose, and *Castellanosia* Cárdenas are large columnar cacti from South America (Bolivia, Colombia, Ecuador, Paraguay, and Peru), the former two genera largely from the western Andes of South America. *Castellanosia* was generally thought to be very closely related to *Browningia* s.s. and was recently included in the latter genus (Barthlott and Hunt, 1993; Hunt, 1999), although this is not supported by the present analysis. The genus *Leptocereus* is found in the Caribbean and forms small trees or shrubs and grows in dry forests.

The RNBCT clade—The other large clade of the core Cactoideae consists of the core Rhipsalideae (R), the core Notocactaceae (N), and the Browningieae-Cereae-Trichocereae (BCT) clade. This clade is weakly supported (*trnK/matK* data: BS = 76%, DI = 2 [Fig. 1]), and information is largely contributed by the *matK* partition. Similar relationships in this RNBCT clade are also identified by the maximum likelihood analysis. Core Rhipsalideae is sister to the latter two clades. The clade consisting of core Notocactaceae and the BCT group received high support values (*trnK/matK* data: BS = 97%, DI = 3 [Fig. 1]; combined data: BS = 98%, DI = 4 [Fig. 2]) from both *trnK/matK* partitions as well as the *trnL-trnF* data.

Core Rhipsalideae—The epiphytic cacti of the tribe Rhipsalideae, with the exception of *Pfeiffera* and some species of *Acanthorhipsalis* Kimmach, which are associated in the present

analysis with taxa of the ACHLP clade, form a well-supported clade (*trnK/matK* data: BS = 100%, DI = 9; signal provided by both partitions). However, a monophyletic tribe Rhipsalideae in the traditional circumscription (including the genus *Pfeiffera* sensu lato [s.l.]) cannot be rejected (Templeton test, $P = 0.57$; Table 5). In any case, the present analysis revealed that sinking the genus *Pfeiffera* in *Lepismium* Pfeiff., as recently proposed by Barthlott and Taylor (1995), leaves the latter at best paraphyletic. A monophyletic genus *Lepismium* in the broad sense is opposed by the present *trnK/matK* data (Templeton test, $P = 0.0205$; Table 5).

Core Notocactaceae—The circumscription of the tribe Notocactaceae has always been rather unclear (Table 6). Based on the present analysis, the core group of Notocactaceae consists of the genera *Eriosyce* (including *Neoporteria* Britton & Rose sensu Barthlott and Hunt, 1993), *Neowerdermannia* Fric, and *Parodia*. This clade of ~100 species is very strongly supported (*trnK/matK* data: BS = 98%, DI = 6; combined data: BS = 100%, DI = 11; with a unique 6 bp indel in the *matK* partition), with a large share of information contributed by the *trnK* partition. Core Notocactaceae comprises mostly globular, or in a few cases subcolumnar, often unbranched cacti from southern South America. *Eriosyce* is predominantly West Andean (Chile, Peru, Argentina), *Neowerdermannia* occurs on the Altiplano, and *Parodia* is exclusively East Andean (Argentina, Bolivia, Brazil, Paraguay, Uruguay). Various other genera, like *Austrocactus*, *Blossfeldia*, *Copiapoa*, *Corryocactus*, *Eulychnia*, and *Frailea*, previously always included in the tribe Notocactaceae, seem to be more closely related to other groups of Cactoideae, and a clade including these genera is rejected by the present data (Templeton test, $P = 0.0042$; Table 5).

The BCT clade, Cereeae, and Trichocereae—The BCT clade (name derived from the initials of the tribes Browningieae, Cereeae, and Trichocereae) is sister to the core Notocactaceae and comprises ~30 genera and 400 species (estimate based on Barthlott and Hunt, 1993) of mostly South American columnar and globular cacti. Besides the tribes Cereeae and Trichocereae, *Browningia* s.s. (excluding *Castellanosia*) of the tribe Browningieae, *Harrisia* of the tribe Echinocereae, and *Uebelmannia* of the tribe Notocactaceae fall into this strongly supported clade (*trnK/matK* data: BS = 99%, DI = 8 [Fig. 1]; combined data: BS = 100%, DI = 12 [Fig. 2]; with two indels of 11 and 6 bp in the 5' *trnK* and *matK* partitions, respectively). The combined analysis indicates that this BCT clade consists of a basal grade formed by *Stetsonia* Britton & Rose and *Uebelmannia* and a polytomy comprising *Gymnocalycium* Mittler, Cereeae + *Browningia*, and Trichocereae. It remains unclear whether the three taxa of the Cereeae included in this study, i.e., *Cereus* Mill., *Coleocephalocereus* Backeb., and *Micranthocereus* Backeb., in fact form a clade. As judged from the branch lengths yielded by the maximum likelihood analysis, there is virtually no information present in the *trnK/matK* data set that would help resolving relationships among these taxa. The combined analysis, with the addition of the *trnL-trnF* sequences, resolved a monophyletic clade Cereeae as sister to *Browningia* s.s., but with a very low support values (combined data: BS = 42%, DI = 1 [Fig. 2]). The support for Cereeae + *Browningia* s.s. is even lower (combined data: BS = 26%, DI = 1). In contrast, the tribe Trichocereae, including *Harrisia*, *Rauhocereus* Backeb., and *Samaipaticereus* Cárdenas, receives considerably stronger support

(*trnK/matK* data: BS = 64%, DI = 1; combined data: BS = 76%, DI = 3). However, information for this relationship is only contributed by the *matK* partition of the *trnK/matK* data set. *Harrisia* has traditionally never been associated with the taxa of the tribe Trichocereae although similarities in pollen characters led Leuenberger (1976) to propose a possible relationship of *Harrisia* with *Trichocereus* Riccob. (alias *Echinopsis*), and this was later confirmed by a molecular study (Wallace, 1995b). Buxbaum (1967) argued that *Rauhocereus* and *Samaipaticereus* are basal in the lineage of *Browningia* s.l., while others suggested a closer relationship between those two genera and the tribe Trichocereae (Rauh, 1979; Ritter, 1980, 1981). The parsimony analysis of the combined data and the maximum likelihood analysis both support a close relationship of *Rauhocereus* with the genera *Haageocereus* Backeb., *Matucana* Britton & Rose, and *Oreocereus* (A. Berger) Riccob. These taxa either occur on the West Andean side of Chile and Peru or in the Altiplano. The relationships of *Samaipaticereus*, a monotypic genus from East Andean Bolivia, within the tribe Trichocereae remain unresolved.

Biogeography, origin, and age of the cacti—All major lineages of the cacti, i.e., *Pereskia*, *Maihueunia*, Opuntioideae, and Cactoideae, occur mostly or exclusively in South America. Furthermore, the closest relatives of the cacti from the “portulacaceous cohort” (Appelquist and Wallace, 1999) have their highest diversity on continents of the former Gondwana landmass (Hershkovitz and Zimmer, 1997). This is generally taken as circumstantial evidence that the family Cactaceae originated in South America (e.g., Schumann, 1899b; Buxbaum, 1969; Mauseth, 1990). Hence, various groups of *Pereskia*, Opuntioideae, and Cactoideae invaded Central and North America and the Caribbean from their postulated northwestern South American center of origin (Leuenberger, 1986). The present molecular analysis suggests that three major clades of Cactoideae contributed to the cactus flora of Central and North America (including the Caribbean). The tribe Cactaceae is exclusively North American, with some species of the supposedly derived genus *Mammillaria* occurring in Venezuela and Colombia. The tribes Hylocereae and Pachycereae of the HLP clade are widely distributed in Central and North America. The present data are ambiguous about whether these two tribes, including *Acanthocereus* and *Echinocereus*, in fact represent a single monophyletic group or whether they form two independent lineages within the HLP clade. The BCT clade, whose taxa most often are found in South America south of the equator, contributed only a few species of the genera *Harrisia*, *Melocactus* Link & Otto, and *Pilosocereus* Byles & G. D. Rowley (the latter two genera were not sampled for this study) to the flora of Central and North America. Finally, there is one widely distributed species of *Rhipsalis* Gaertn., which extends its distribution to Central and North America.

It is interesting to note that the South American taxa of the ACHLP clade, whose bulk of species diversity is contributed by the Central and North American Hylocereae and Pachycereae (180 species, or ~70% of the total diversity), mostly occur in West Andean Colombia, Ecuador, Peru, and Chile. Prominent exceptions are *Castellanosia* from East Andean Bolivia and *Pfeiffera* from eastern Bolivia and adjacent northwestern Argentina and eastern Peru. In contrast, the RNBCT clade is almost exclusively South American, with different groups either occurring East or West of the Andes.

The presence of a *Rhipsalis* species in tropical Africa, Mad-

agascar, and Sri Lanka (Barthlott, 1983; Barthlott and Taylor, 1995) led some authors to propose that this distribution indicates an old vicariance between South America and Africa (e.g., Backeberg, 1942) or even an origin of the cacti in the Old World (Croizat, 1952). This would imply that the cacti originated before the split of the two continents during the late Cretaceous and that all other cacti that might have naturally occurred in Africa got extinct. More recently, however, this distribution pattern of *Rhipsalis baccifera*, which is characterized by having very sticky seeds comparable to those of the mistletoe (Barthlott, 1983), has been explained as the result of relatively recent long-distance dispersal by birds (Gibson and Nobel, 1986; Barthlott and Hunt, 1993).

While there is growing consensus concerning the spatial origin of cacti in northern South America, there is a disagreement about the temporal aspect of cactus origin. Traditionally, a late Cretaceous origin of cacti, perhaps 65–90 million years ago (mya) immediately following the breakup of the western part of the Gondwana supercontinent, has been favored (e.g., Gibson and Nobel, 1986; Mauseth, 1990). This time frame would allow explanation of the absence of endemic cacti in the Old World, while maximizing the time for the evolution of the various distinctive morphological features of extant cacti (Hershkovitz and Zimmer, 1997). Based on molecular investigations of ITS sequences, Hershkovitz and Zimmer (1997) proposed a much more recent origin of cacti in mid-Tertiary ~30 mya. The present molecular investigation, though not specifically analyzed for this aspect, adds support to the latter hypothesis. The small amount of sequence divergence found in the present data set of chloroplast markers is indicative of a fairly recent origin of the major radiations in Cactaceae. Unfortunately, the large differences in sequence divergence among different groups of cacti are not conducive to a straightforward molecular clock analysis of the present data set.

Conclusions—The present molecular study helped to resolve a number of old disputes about the relationships of some enigmatic taxa. *Gymnocalycium*, *Stetsonia*, and *Uebelmannia* take a basal position in a large clade of South American cacti consisting of the tribes Browningieae, Cereae, and Trichocereae (BCT clade). *Neowerdermannia* is closely related to *Erioseye* and *Parodia* of the core Notocactae. *Harrisia*, *Rauhocereus*, and *Samaipaticereus* are members of the tribe Trichocereae, while *Acanthocereus* shares affinities to the tribe Hylocereae and *Echinocereus* is nested in the tribe Pachycereae. Furthermore, this study provides for the first time a detailed phylogenetic hypothesis of the major relationships within the subfamily Cactoideae, though the basal position of *Blossfeldia*, as sister to the rest of Cactoideae, is difficult to understand based on structural data. The tribe Cacteeae, composed of Central and North American globular cacti, is sister to a few orphan genera (*Calymmanthium*, *Copiapoa*, *Frailea*) and two major clades of basically South American cacti. The predominantly Central and North American columnar cacti of the two tribes Hylocereae and Pachycereae share close relationships to largely West Andean South American taxa of the former tribes Browningieae and Leptocereae. However, the present study also unearthed a number of new problems for cactus systematics, e.g., the question about the closest relatives of *Blossfeldia*, *Castellanosia*, *Copiapoa*, *Frailea*, and *Pfeiffera*. More comparative sequencing of various other molecular markers, in particular from the nuclear genome, is

needed to test relationships as proposed by the present study of *trnK/matK* and *trnL-trnF* data.

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