Molecular Systematics of Tribe Cacteae (Cactaceae: Cactoideae): A Phylogeny Based on rpl16 Intron Sequence Variation

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ABSTRACT. Parsimony analysis of plastid *rpl16* sequences from 62 members of Tribe Cacteae, and four outgroup taxa yielded 1296 equally parsimonious trees of length 666. Strict consensus evaluation of these trees established a highly pectinate topology, which delimited clades within the tribe that correspond to several previously considered generic groups. *Aztekium* and *Geohintonia*, which manifest ribs in their stem morphology were shown to represent an early divergence in the tribe, forming a sister group to remaining members of the tribe. Clades containing other genera having ribbed stems also are basal to those that develop tubercles. The most derived clade forms a distinct group of typically small stemmed species with tubercular stem morphology. Within *Mammillaria*, species formerly placed in the genus *Cochemiea* and members of the Series Ancistracanthae formed a well-supported, sister clade to the remaining members of *Mammillaria*. Length variation of the intron in two members of *Mammillaria* series Stylothelae was also observed.

Buxbaum (1958b) first described the tribe Cacteae (as the Echinocacteae) as a 'clear-cut phylogenetic unit' in which he included all of the short-columnar or globose cacti with spineless flowers native to North America, with the notable exception of the genus *Astrophytum* Lemaire, which he considered part of the Notocacteae. The tribe is in considerable taxonomic flux, and poor generic delineation means that the exact number of genera is uncertain. Twenty-three genera are recognized in the CITES Cactaceae checklist (Hunt 1999), although at least 34 other genera have been described. Hunt (1999) accepts 314 species (plus 224 provisionally accepted species) in the tribe, whereas Anderson (2001) recognizes 26 genera and 384 species. The geographic range of the Cacteae extends from western Canada (*Escobaria vivipara* (Nuttall) Buxbaum) to Colombia, Venezuela, and the Caribbean (*Mammillaria colombiana* Salm-Dyck and *M. mammillaris* (L.) Karsten), with maximal diversity in Mexico.

Characterized as globular or depressed to short columnar cacti, members of the Cacteae range in size from dwarf (*Turbinicarpus* (Backeberg) Backeberg and Buxbaum and some *Mammillaria* Haworth) to gigantic (*Ferocactus* Britton and Rose and *Echinocactus* Link and Otto). Stems may be either ribbed, as in *Echinocactus*, or tuberculate as in *Coryphantha* (Engelmann) Lemaire. Zimmerman (1985) states that ribs and tubercles are mutually exclusive terms, although a number of intermediates are found. He recommends the use of the term podarium, suggesting that in reality ribs are series of podaria joined end-to-end. Tubercles, however, represent free or distinct podaria. This terminology allows for intermediacy between ribs and tubercles. The size and shape of tubercles range from long and leaf-like (as in *Leuchtenbergia* Hooker, *Obregonia* Fric, and some species of *Ariocarpus* Scheidweiler) to broad with shallow axils, as in *Turbinicarpus*.

Areoles may be borne on the ribs, or in the case of the tuberculate members, may occur at or near the tubercle apex, or form a groove on the upper surface as in *Coryphantha* and some species of *Escobaria* Britton and Rose. In a number of genera the tubercles are differentiated into spine-bearing areoles at the tubercle apex and floriferous or vegetative areoles in the axils of the tubercles. Buxbaum (1958b) suggested that species with differentiated tubercles such as *Mammillaria* are derived within the tribe. Actinomorphic or, rarely, zygomorphic (*Mammillaria* subgenus *Cochemiea* Brandegee) diurnal flowers arise from the areoles. The pericarpel (in cacti defined as ovary wall plus stem tissue external to the ovary wall) ranges from scaly and woolly to petaloid. Fruits in the Cacteae are fleshy to juicy berries, and the seeds are borne on short, simple funiculi. Since the 1920's, a number of researchers have revised the Cacteae, variously interpreting its classification based on differing concepts of broadly-defined or narrowly circumscribed genera. Table 1 lists genera accepted in a number of key treatments of the Cacteae.

Britton and Rose (1919–1923) did not recognize the Cacteae as a discrete entity. Within their tribe Cereeae (equals subfamily Cactoideae), they divided the barrel cacti into two subtribes, Echinocactinae and Coryphanthinae. Their subtribe Echinocactinae included all ribbed barrel cacti from both North and South America, which manifest a generally low growing, globular habit. The North American barrel cacti that share the character of possessing tubercles were placed into the subtribe Coryphanthinae, although some taxa with true tubercles or modified tubercles, such as *Pediocactus* Britton and Rose, *Ariocarpus*, and *Lophophora* Coulter were placed within the ribbed subtribe Echinocactinae. It is evident that Britton and Rose (1919–1923) realized that mutually exclusive suites of morphological char-

TABLE 1. Comparisons of previous treatments for members of the Cacteae.

This paper	Anderson (2001)	Hunt (1999)	Barthlott & Hunt (1993)	Backeberg (1970)	Buxbaum (1958b)	Britton & Rose (1919–1923)
Acharagma	Escobaria	Escobaria	Escobaria	Escobaria, Gymnocactus	Escobaria	Not Described
Ariocarpus	Ariocarpus	Ariocarpus	Ariocarpus	Ariocarpus, Neogomesia, Roseocactus	Ariocarpus	Ariocarpus
Astrophytum	Astrophytum	Astrophytum	Astrophytum	Astrophytum	Astrophytum	Astrophytum
Aztekium	Aztekium	Aztekium	Aztekium	Aztekium	Aztekium	Not Described
Coryphantha	Coryphantha	Coryphantha	Coryphantha	Coryphantha, Cumarinia, Lepidocoryphantha	Coryphantha, Cumarinia	Coryphantha
Echinocactus	Echinocactus	Echinocactus	Echinocactus	Echinocactus	Echinocactus	Echinocactus
Encephalocarpus	Pelecyphora	Pelecyphora	Pelecyphora	Encephalocarpus	Encephalocarpus	Pelecyphora
Epithelantha	Epithelantha	Epithelantha	Epithelantha	Epithelantha	Epithelantha	Epithelantha
Escobaria	Escobaria	Escobaria	Escobaria	Escobaria, Neobesseya	Escobaria, Neobesseya	Escobaria
Ferocactus	Ferocactus	Ferocactus	Ferocactus	Ferocactus	Ferocactus	Ferocactus
Geohintonia	Geohintonia	Geohintonia	Geohintonia	Not Described	Not Described	Not Described
Glandulicactus	Sclerocactus	Sclerocactus	Sclerocactus	Glandulicactus	Hamatocactus	Hamatocactus
Homalocephala	Echinocactus	Echinocactus	Echinocactus	Homalocephala	Homalocephala	Homalocephala
Leuchtenbergia	Leuchtenbergia	Leuchtenbergia	Leuchtenbergia	Leuchtenbergia	Leuchtenbergia	Leuchtenbergia
Lophophora	Lophophora	Lophophora	Lophophora	Lophophora	Lophophora	Lophophora
Mammillaria	Cochemiea, Mammillaria, Mammilloydia	Mammillaria, Mammilloydia	Mammillaria, Mammilloydia	Bartschella, Cochemiea, Dolichothele, Krainzia, Mamillopsis, Mammillaria, Phellosperma, Solisia	Cochemiea, Dolichothele, Leptocladodia, Mamillopsis, Mammillaria, Mammilloydia, Oehmea, Pseudomammillaria	Bartschella, Cochemiea, Dolichothele, Mamillopsis, Neomammillaria, Phelosperma, Solisia
Neolloydia	Neolloydia	Neolloydia	Neolloydia	Neolloydia	Neolloydia	Neolloydia
Obregonia	Obregonia	Obregonia	Obregonia	Obregonia	Obregonia	Not Described
Ortegocactus	Ortegocactus	Ortegocactus	Ortegocactus	Ortegocactus	Not Described	Not Described
Pediocactus	Pediocactus	Pediocactus	Pediocactus	Navajoa, Pediocactus, Pilocanthus, Utahia	Pediocactus, Utahia	Pediocactus, Utahia
Pelecyphora	Pelecyphora	Pelecyphora	Pelecyphora	Pelecyphora	Pelecyphora	Pelecyphora
Sclerocactus	Sclerocactus	Sclerocactus	Sclerocactus	Coloradoa, Echinomastus, Gymnocactus, Sclerocactus, Toumeya	Ancistrocactus, Coloradoa, Echinomastus, Sclerocactus	Ancistrocactus, Sclerocactus, Toumeya
Stenocactus	Stenocactus	Stenocactus	Stenocactus	Echinofossulocactus	Echinofossulocactus	Echinofossulocactus
Strombocactus	Strombocactus	Strombocactus	Strombocactus	Strombocactus	Strombocactus	Strombocactus
Thelocactus	Thelocactus	Thelocactus	Thelocactus	Echinomastus, Thelocactus	Thelocactus	Echinomastus, Thelocactus
Turbinicarpus	Turbinicarpus	Turbinicarpus	Neolloydia	Gymnocactus, Turbinicarpus	Rapicactus, Toumeya	Neolloydia

acters could not be used to delineate subtribes within their tribe Cereeae, accepting that boundaries between subtribes Echinocereanae, Echinocactinae and Coryphanthinae were not clearly defined.

Using an underlying principle of determining taxonomic groups based on inferred phylogenetic relatedness, Buxbaum (1958b) described the North American barrel cacti (with minor exceptions) at the rank of tribe (Cacteae), and defined this group by bringing together Schumann's earlier tribe Echinocacteae (Schumann 1899) and Britton and Rose's subtribes Echinocactanae and Coryphanthanae. With the exception of *Astrophytum* (which he placed into the tribe Notocacteae), Buxbaum (1958b) recognized 36 genera in the tribe and regarded this group of North American barrel cacti as a distinct phylogenetic unit. Within his tribe Cacteae, four subtribes were defined based upon seed morphology: 1) the Echinocactinae, with a smooth, hard, black testa with conspicuous perisperm; 2) the Thelocactinae, with a verrucose, mostly black testa becoming secondarily smooth or 'spotted'; 3) the Ferocactinae, with a pitted or reticulate testa; and 4) the Coryphanthinae, with a smooth, brown testa.

In contrast to Buxbaum's phylogenetically-based classification, Backeberg's (1970) classification of the cacti used a complex system of infrafamilial ranks including semitribes, subtribes, groups, and subgroups. This classification was never intended to be phylogenetic. Britton and Rose's tribe Cereeae was split, largely based on geographic origins of the plants, into the North and South American semitribes Boreocereeae and Austrocereeae, respectively. Ignoring Buxbaum's (1958b) tribe Cacteae, Backeberg created the subtribe Boreocactinae to accommodate the North American barrel cacti, which was further divided into two groups based on flower position: 1. The Boreoechinocacti has flowers borne from undifferentiated (vegetative vs. flowering) areoles (the Boreoechinocacti were still further divided into two subgroups, the Euboreoechinocacti and the Mediocoryphanthae) and 2. The Mammillariae, which has differentiated areoles (e.g. flowers borne in tubercle axils), with three subgroups, Coryphanthiae, Mediomammillariae, and Eumammillariae. In total, Backeberg's subtribe Boreocactinae included 48 genera, consistent with his philosophy of recognizing many genera with few species in each. In modern taxonomic treatments, many of these ''microgenera'' have been united into more broadly defined groups; for example *Ariocarpus* was expanded by Anderson (1960, 1962) to include *Roseocactus* Berger and *Neogomesia* Castañeda, and the genera *Porfiria* Bödecker, *Krainzia* Backeberg, *Phellosperma* Britton and Rose, *Dolichothele* (Schumann) Britton and Rose, *Bartschella* Britton and Rose, *Mamillopsis* Morren ex Britton and Rose, and *Cochemiea* (Brandegee) Walton were sub-

sumed into the genus *Mammillaria* by Hunt (1971, 1977a, b; 1981).

Besides the treatment by Buxbaum (1958b), there has been only one other attempt at a phylogenetic evaluation of the tribe Cacteae. In his unpublished Ph.D. thesis, Zimmerman (1985) presented a cladistic study of the tribe based on an analysis of morphological characters, the majority of which are derived from the study of floral structures. Zimmerman suggested that the Pachycereeae and the Notocacteae probably represent the closest outgroups to tribe Cacteae, and that the tribe likely had its origins in South America, sharing a sister-group relationship with the Notocacteae. Influenced by Buxbaum's (1958b) treatment, both Barthlott (1977) and Zimmerman (1985) questioned the placement of *Astrophytum* in the Cacteae, noting significant differences in seed morphology. Zimmerman concluded that, with the possible exception of this genus, the Cacteae formed a monophyletic unit. Furthermore, Zimmerman (1985) placed *Astrophytum* within a clade together with *Echinocactus* and *Homalocephala* Britton and Rose that shows a sister-group relationship to other members of the tribe. Despite problems associated with morphological plasticity in the tribe, Zimmerman made a number of insightful conclusions, for example that *Escobaria*, *Ortegocactus*, *Mammillaria*, and *Coryphantha* sensu stricto are derived from a *Mammillaria*-like rather than a *Ferocactus*-like ancestor.

In their treatment of the genera of the Cactaceae, Barthlott and Hunt (1993) united a number of Cacteae genera, recognizing 22 genera in total. *Homalocephala* was included within *Echinocactus*, and the genera *Oehmea* Buxbaum, *Cochemiea*, *Dolichothele*, and *Mamillopsis* were subsumed within *Mammillaria*. Hunt (pers. comm.) doubts that the Cacteae are monophyletic, reasoning that because the globular growth form has arisen independently in several cactus lineages in South America, it has likely also arisen in North American lineages independently.

The primary goals of this investigation were to test monophyly of the tribe, resolve intergeneric relationships in the Cacteae, and to assess monophyly in previously proposed Cacteae genera using chloroplast *rpl16* intron sequence data. Further, we wished to ascertain relevant outgroup taxa for an ongoing study of the genus *Mammillaria*.

MATERIALS AND METHODS

Taxonomic Sampling.   A total of 66 taxa were sampled (Table 2), including 62 representative taxa from the tribe Cacteae. Two species from tribe Notocacteae and one species from tribe Pachycereeae were also included with members of tribe Cacteae as the ingroup. *Calymmanthium substerile* (tribe Browningieae) was used as the outgroup based upon its basal position within the subfamily Cactoideae (Wallace 2001). Additional phylogenetic analyses of chloroplast DNA variation (Butterworth and Wallace, unpublished) were conducted in which representative taxa were examined from throughout the subfamily, and demonstrated that tribe

TABLE 2. Species sampled for *rpl*16 study. CANTE = CANTE Botanic Garden, Mexico; UCONN = University of Connecticut; DES = Desert Botanic Garden, Arizona; ISC = Ada Hayden Herbarium, Iowa State University; HNT = Huntington Botanic Garden, California; HUMO = Universidad Autónoma del Estado de Morelos, Mexico; and UNAM = Universidad Autónoma de Mexico, Mexico City.

Taxon	Source/Voucher	GenBank No.
Tribe Cacteae		
Acharagma aguirreana (Glass & Foster) Glass	Mesa Garden—ISC	AF267915
Acharagma roseana (Boed.) Glass	DES 1990-0791-0201—ISC	AF267916
Ariocarpus agavoides (Castañeda) Anderson	C. Glass 6889—CANTE	AF267918
Ariocarpus retusus Scheidw.	C. Glass 6923—CANTE	AF267919
Astrophytum capricorne (Dietrich) Br. & R.	HNT 69033—ISC	AF267920
Astrophytum myriostigma Lem.	HNT 69032—ISC	AF267921
Aztekium hintoni Glass & Fitz Maurice	C. Glass 6647—CANTE	AF267922
Aztekium ritteri (Boed.) Boed.	C. Staples s.n.—ISC	AF267923
Coryphantha pallida Br. & R.	H. Cota 8050—HUMO	AF267926
Echinocactus grusonii Hildm.	R. Wallace s.n.—UCONN	AF267927
Echinocactus horizonthalonius Lem.	M. Mendes 186—CANTE	AF267928
Echinocactus ingens Zucc.	HNT 59498—ISC	AF267929
Encephalocarpus strobiliformis (Werderm.) Berger	HNT 60211—ISC	AF267930
Epithelantha bokei L. D. Benson	DES 1993-0717-0101—ISC	AF267931
Escobaria zilziana (Boed.) Backeb.	DES 1989-0137-0102—DES	AF267932
Ferocactus cylindraceus (Engelm.) Orcutt	Ecker (Slausson) 110—ISC	AF267933
Ferocactus flavovirens (Scheidw.) Br. & R.	H. Cota 8051—HUMO	AF267934
Ferocactus glaucescens (DC.) Br. & R.	HNT 10339—ISC	AF267979
Ferocactus histrix (DC.) Lindsay	H. Cota 8037—CANTE	AF267935
Ferocactus latispinus (Haw.) Br. & R.	H. Cota 8039—CANTE	AF267936
Ferocactus robustus (Link & Otto) Br. & R.	H. Cota 8045—HUMO	AF267974
Ferocactus wislizenii (Engelm.) Br. & R.	L Slauson 112—ISC	AF267937
Geohintonia mexicana Glass & Fitz Maurice	C. Glass 6648—CANTE	AF267938
Glandulicactus crassihamatus (Weber) Backeb.	C. Glass 5201—CANTE	AF267939
Glandulicactus uncinatus (Galeotti) Backeb.	C. Glass 6846—CANTE	AF267917
Homalocephala texensis (Hoppfer) Br. & R.	HNT 67080—ISC	AF267940
Leuchtenbergia principis Hook.	HNT s.n.—ISC	AF267941
Lophophora diffusa (Croizat) Bravo	Mesa Garden—ISC	AF267942
Lophophora williamsii (Lem.) J. M. Coult.	D. Martinez s.n.—HUMO	AF267943
Mammillaria beneckei Ehrenb.	DES 1993-0550-0101—DES	AF267944
Mammillaria candida Schweidw.	DES 1957-5907-0101—ISC	AF267945
Mammillaria decipiens Schweidw.	HNT 68830—ISC	AF267946
Mammillaria glassii Foster	HNT 60162—ISC	AF267952
Mammillaria haageana Pfeiffer	H. Cota 8053—HUMO	AF267953
Mammillaria halei Brandegee	HNT 72646—ISC	AF267947
Mammillaria jaliscana (Br. & R.) Boed.	Lau 1050—ISC	AF267948
Mammillaria karwinskiana Mart.	H. Cota s.n.—ISC	AF267949
Mammillaria longimamma DC.	DES 1992-0049-0203—DES	AF267950
Mammillaria magnifica Buchenau	HTN—ISC	AF267951
Mammillaria plumosa Weber	HTN 28166—ISC	AF267954
Mammillaria poselgeri Hildm.	DES 1983-0746-1018—ISC	AF267955
Mammillaria senilis Salm-Dyck	Mesa Garden—ISC	AF267956
Mammillaria voburnensis Scheer	Lippold s.n.—UCONN	AF267957
Mammillaria yaquensis Craig	HNT 7715—ISC	AF267958
Neolloydia conoidea (DC.) Br. & R.	Lippold s.n.—ISC	AF267959
Obregonia denegrii Fric	R. Wallace s.n.—ISC	AF267960
Ortegocactus macdougallii Alexander	R. Wallace s.n.—ISC	AF267961
Pediocactus simpsonii (Engelm.) Br. & R.	C. Butterworth 60—ISC	AF267962
Pelecyphora aselliformis Ehrenb.	DES 1961-6848-0101—DES	AF267963
Sclerocactus brevihamatus (Engelm.) D. R. Hunt	DES 1989-0315-0101—DES	AF267964
Sclerocactus spinosior (Engelm.) Woodruff & L. Benson	Hughes 2—ISC	AF267965
Sclerocactus whipplei (Engelm. & Bigelow) Br. & R.	DES 1993-0925-0103—DES	AF267966
Stenocactus crispatus Berger	HNT 46450—HNT	AF267980
Stenocactus lloydii Berger	ex Hort. UCONN—UCONN	AF267977
Stenocactus vaupelianus (Werdem.) F. M. Knuth	DES 1948-1289-0101—DES	AF267978
Strombocactus disciformis (DC.) Br. & R.	H. Sánchez-Mejorada 3603—UNAM	AF267967
Thelocactus conothelos (Reg. & Klein) F. M. Knuth	Lippold s.n. (ex Hort)—UCONN	AF267968
Thelocactus hastifer (Werderm. & Boed.) F. M. Knuth	Peter Sharp s.n.	AF267973
Thelocactus macdowellii (Rebut ex Quehl) C. Glass	HNT s.n.—ISC	AF267969
Turbinicarpus gielsdorfianus (Werdermann) John & Riha	HNT 50008—ISC	AF267970

TABLE 2. Continued.

Taxon	Source/Voucher	GenBank No.
Turbinicarpus pseudomacrochele (Backeb.) F. Buxb. & Backeb.	Brach's Nursery—ISC	AF267971
Turbinicarpus schmiedickianus var. *schwartzii* (Shurly) Glass & Foster	Ex Martiny s.n.—ISC	AF267972
Tribe Browningieae		
Calymmanthium substerile Ritter	HNT 46555—ISC	AF267924
Tribe Notocacteae		
Corryocactus brachypetalus (Vaupel) Br. & R.	HNT 18015—ISC	AF267925
Parodia haselbergii (Haage ex Rümpler) Brandt	ex Hort.—UCONN	AF267975
Tribe Pachycereeae		
Bergerocactus emoryi (Engelm.) Br. & R.	HNT 16514—ISC	AF267976

Cacteae was well supported as a monophyletic group. Specimens were obtained from a number of sources and maintained in the greenhouse prior to DNA extraction. Institutions in which voucher specimens are deposited are also listed in Table 2.

DNA Extraction and Purification. Total genomic DNA of representative Cacteae samples was isolated using one of two methods:

1. Modified organelle pellet method suitable for mucilaginous material. Genomic DNA samples were prepared using previously published methods (Wallace 1995; Wallace and Cota 1996) for extraction of nucleic acids from highly mucilaginous plants, briefly summarized as follows: fresh, chlorenchymatous stem tissue was homogenized in 0.35M sorbitol buffer, filtered through Miracloth® (Calbiochem). The organelles were pelleted, supernatant removed, and pellets were then suspended in 2x CTAB (Doyle and Doyle 1987) for 1 h at 60°C. After partitioning against CHCl₃:octanol, 24:1. DNA was isopropanol-precipitated and resuspended for further purification using isopycnic ultracentrifugation in cesium chloride/ethidium bromide gradients, followed by dialysis against TE.

2. Nucleon Phytopure® plant and fungal DNA extraction kit for 1g samples (Amersham Life Science). DNA was extracted from living stem tissue according to the manufacturer's recommendations and stored at −20°C in TE buffer.

Amplification and Sequencing. Polymerase chain reaction (PCR) amplification of the *rpl16* intron was conducted in 100 μl reactions using GeneAmp® PCR Core Reagents (Perkin Elmer), and the amplification primers RP71F and RP1661R (Applequist and Wallace 2000). Each reaction included 20 ng of each primer and 5 μl of unquantified DNA template. The PCR reactions were conducted in a MJ Research PTC-100 thermal cycler using the following temperature cycling parameters: l) initial melting at 95°C for 5 min; 2) 24 cycles of the following protocol: 95°C melt for 2 min, 50°C annealing for 1 min, ramp temperature increase of 15°C at 0.125°C per sec, 65°C extension for 4 min; and a final extension step at 65°C for 10 min.

Agarose electrophoresis in TAE was used to confirm the presence of 1.1 kb to 1.3 kb PCR amplification products. The amplicons were cleaned and concentrated in Microcon 100 spin microconcentrators (Amicon Inc.) following the manufacturer's directions. The products were then quantified in an ultraviolet spectrophotometer and diluted to 50μg/ml for use in sequencing reactions.

Sequence data were obtained using the sequencing primers RP1516R and RP637R (Applequist and Wallace 2000) at concentrations of 5 pmol in chain-termination reactions using the ABI Prism Big Dye® Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). We found that dilutions of 1:4 of terminator ready reaction solution gave acceptable reads.

Electrophoresis and automated sequence reading were conducted using Perkin Elmer/Applied Biosystems automatic sequencing units (ABI Prism 377) at the Iowa State University Nucleic Acid DNA Facility. Sequences typically were 650 or more nucleotides in length. In a small number of taxa, a poly-T region approximately 400bp from the RP1516R priming site caused extremely poor reads upstream of the RP637R priming site. To overcome this problem, a new primer, RP543F, (5′-TCAAGAAGCGATGGGAAC-GATGG-3′) was designed to run forward from just downstream of the RP637R priming site, overlapping the unreadable section of sequence. Due to extensive poly-A and poly-T regions in Domain I at the 5′ end, 150–200bp of the intron sequence could not be obtained using the automated method. Kelchner and Clark (1997) demonstrated low levels of sequence divergence in this region and because it is of limited phylogenetic usefulness, further attempts at obtaining a full length intron sequence were discontinued.

Phylogenetic Analysis. Sequence alignment was carried out using AutoAssembler (Applied Biosystems 1995) and Se-Al (Rambaut 1995). Following an initial Clustal W alignment, sequences were further aligned manually (e.g., Golenberg et al. 1993). Insertions/deletions considered to be phylogenetically informative were coded in binary (presence/absence) and added to the end of the data matrix. There were two regions (totalling 61 nucleotides) where alignments were of doubtful homology. These regions were excluded from the analyses. All analyses were carried out using PAUP* 4.0b2 (Swofford 1999). To test the *rpl16* intron dataset for phylogenetic signal the g-statistic for 10,000 random trees was calculated. According to Hillis and Huelsenbeck (1992) the distribution of lengths of random trees for all topologies provides a 'sensitive' measure of phylogenetic signal within the dataset. Matrices that contain a strong phylogenetic signal show distributions that approach a left-skewed gamma distribution as opposed to a more normal distribution for matrices containing random noise.

Parsimony analyses were done using the heuristic search option. All substitutions and indels were equally weighted. An initial heuristic search using TBR branch swapping saving multiple parsimonious trees (MULTREES ON) was conducted. Random addition searches of 1,000 replicates, saving 100 most parsimonious trees at each step, were undertaken to search for islands of shorter trees. Estimates of decay (Bremer 1988) were obtained using converse constraint trees as implemented using Autodecay (Eriksson and Wikström 1995). Bootstrap values were estimated using the 'fast bootstrap' method for 1,000 replicates. A neighbor-joining analysis was also undertaken using the F81 substitution model.

RESULTS

Sequence length ranged from 650bp in *Mammillaria glassii* and 673bp in *M. magnifica* and *M. haageana* to 935bp in *Ferocactus glaucescens*. Aligned sequence length for the *rpl16* dataset was 1057bp. The full dataset (including binary-coded indels) totaled 1069 characters. After exclusion of indels and the two regions of doubtful homology, the dataset was 953 characters long, of which 177 were parsimony-informative. The g-

statistic for the *rpl16* dataset is 0.506. This value falls well within the 99% confidence interval (C.I.) for datasets of over 25 taxa and 500 characters (Hillis and Huelsenbeck 1992) and therefore indicates significant phylogenetic structure within the dataset. The data matrix of aligned *rpl16* sequences is available from the authors.

A heuristic search using PAUP* found 1296 most parsimonious trees with length of 666 steps. There appears to be considerable homoplasy in the *rpl16* dataset with a C.I. of 0.632 (0.494 excluding uninformative characters). However, a low C.I. may be expected, due in part to the nature of large datasets, thus the retention index (R.I.) gives a more suitable indication of support. In the case of the *rpl16* dataset, the R.I. (excluding uninformative characters) is 0.699. A random addition search of 100 replicates did not find any islands of shorter trees. The strict consensus tree (Figure 1) supports monophyly of the Cacteae, with a decay value of 9 and a bootstrap value of 100%.

Within the Cacteae, the general tree topology resolves a number of clades nested pectinately within each other: 1. ''Aztekium Clade'' consisting of *Aztekium* Bödecker and *Geohintonia* Glass and Fitz Maurice (bootstrap 100%, decay 7); 2. ''Echinocactus Clade''—*Astrophytum*, *Echinocactus*, and *Homalocephala* (bootstrap 62%, decay 2); 3. *Sclerocactus* Britton and Rose (bootstrap 89%, decay 4); 4. ''Lophophora Clade''—*Acharagma* (Taylor) Glass, *Lophophora*, and *Obregonia* (bootstrap 87%, decay 5); 5. *Strombocactus disciformis* forms a single lineage; 6. ''ATEP Clade''—A weakly supported clade (bootstrap <50%, decay 1) unites *Ariocarpus*, *Turbinicarpus*, *Epithelantha*, and *Pediocactus*; 7. ''Ferocactus Clade''—consisting of *Ferocactus*, *Ancistrocactus* Britton and Rose, *Leuchtenbergia*, *Echinocactus grusonii*, *Thelocactus* (Schumann) Britton and Rose, and *Glandulicactus* Backeberg (this clade is poorly resolved and poorly supported with bootstrap <50% and decay 1); 8. *Stenocactus* (Schumann) Hill (bootstrap 100%, decay 4); 9. ''Mammilloid Clade'' including *Pelecyphora* Ehrenberg, *Encephalocarpus* Berger, *Escobaria*, *Coryphantha*, *Neolloydia* Britton and Rose, *Ortegocactus*, and *Mammillaria* (this terminal clade is well-supported with bootstrap 60%, and decay 3).

Analysis of the *rpl16* data using a neighbor-joining algorithm with the F81 substitution model resulted in an initial tree that was topologically quite congruent with the maximum parsimony tree. There were, however a number of exceptions. *Mammillaria glassii* forms a sister-group to all of the remaining members of the Cacteae in the neighbor-joining tree. This incongruence is caused by differences in sequence length of *Mammillaria glassii* of only 650bp due to a large deletion spanning the region with most informative characters. Other topological differences between the maximum-parsimony and neighbor-joining trees were observed in the placement of members of the *Lophophora* and *Echinocactus* clades of the maximum parsimony tree, which form a single clade in the neighbor-joining tree.

DISCUSSION

Phylogenetic Relationships in the Cacteae. MONOPHYLY OF THE CACTEAE. The phylogeny presented in this paper supports a monophyletic origin for members of the Cacteae as currently circumscribed; no direct relationship was shown with *Bergerocactus emoryi* (Pachycereeae), and *Parodia haselbergii* and *Corryocactus brachypetalus*, members of the morphologically similar South American tribe Notocacteae. A number of synapomorphic substitutions resulted in a decay value of 9 with 100% bootstrap support, providing robust support for the Cacteae clade. The monophyly of tribe Cacteae was further tested using *rpl16* intron sequences in which *Austrocylindropuntia* Backeberg (subfamily Opuntioideae) and *Maihuenia poepegii* (Otto ex Pfeiffer) Philippi ex Schumann (subfamily Maihuenioideae) were used as outgroups for comparisons with each genus of the Cacteae used in this study, together with representatives of all other tribes in the subfamily Cactoideae (Butterworth and Wallace, unpublished data). Support for monophyly of the tribe Cacteae was also very strong (98% bootstrap) with this test.

AZTEKIUM CLADE. The Aztekium Clade forms the sister-group to the remaining taxa of the Cacteae. Plants in this clade typically are globose to subglobose, rarely short columnar reaching 20 cm by 10 cm in size. Stem morphology is ribbed, the ribs in *Aztekium* having characteristic transverse wrinkles. Spines are notable by their absence from mature areoles; even in young areoles they are highly reduced and very brittle. *Aztekium* and *Geohintonia* presently are restricted to a small area of eastern Nuevo Leon in NE Mexico. Hunt and Taylor (1992) suggested that *Geohintonia* may represent an intergeneric hybrid involving *Aztekium* and possibly *Echinocactus horizonthalonius*. Corriveau and Coleman (1988) demonstrated biparental inheritance of chloroplast DNA in *Rhipsalidopsis* Britton and Rose, and *Zygocactus* Schumann but maternal inheritance in *Echinocereus* Engelmann and *Opuntia* Miller. If *Geohintonia* is descended from an ancient intergeneric hybrid, and its plastid organelles are maternally inherited, then the maternal parent of the ancient hybrid was closely related to *Aztekium*, probably *A. hintonii* which is sympatric with *Geohintonia*. However, if the chloroplasts of *Geohintonia* show biparental inheritance then *Aztekium* could represent the descendant of either the pollen or ovule donor. The relationship between *Aztekium* and *Strombocactus* Britton and Rose has been cause for discussion. In a preliminary list of accepted genera by the working party of the International Organization for Succulent Plant Study (IOS) (Hunt and Taylor 1986) and a follow-up report (Hunt and Taylor 1990), the
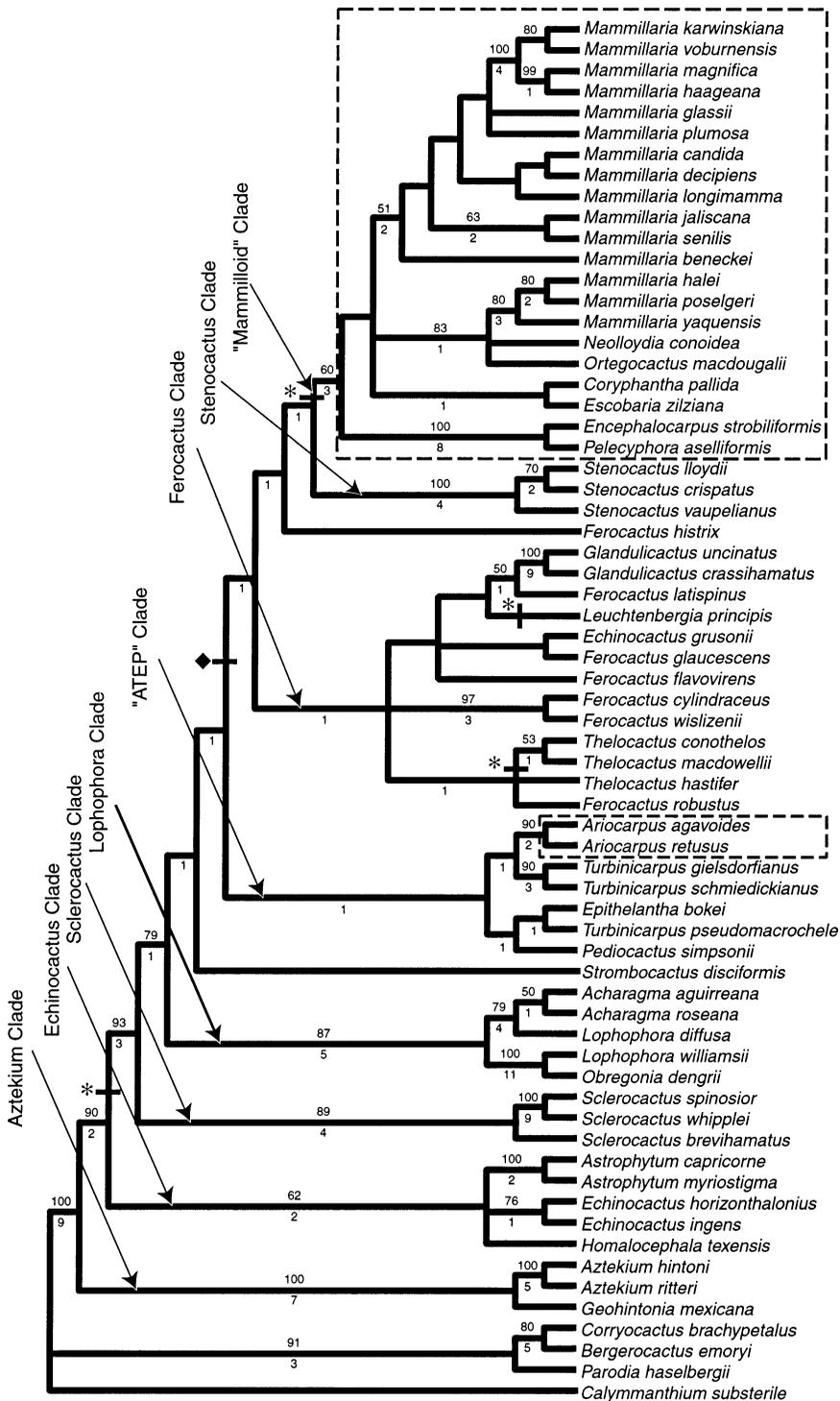
FIG. 1. Strict consensus of 1296 most parsimonious trees for *rpl16* intron sequences. Length = 666 steps, C.I. = 0.632, C.I. (excluding uninformative characters) = 0.494, R.I. (excluding uninformative characters) = 0.699. Bootstrap values over 50% for 1000 replicates are given above the branches. Decay values are shown below the branches. * = switch from ribbed to tubercular stems. ♦ = switch from tubercular to ribbed stems. Boxes indicate clades with dimorphic areoles.
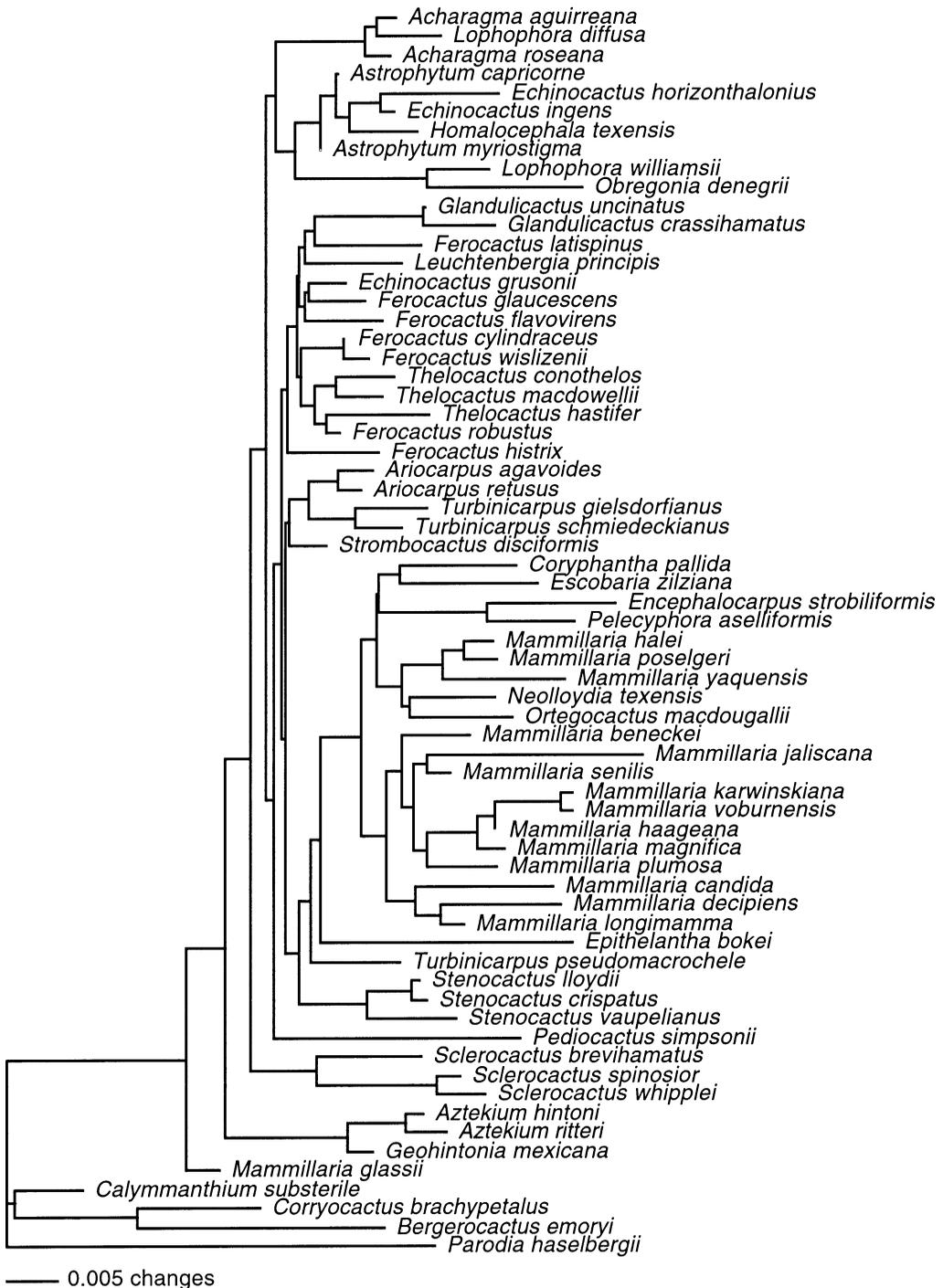
FIG. 2.   Neighbor-joining tree for the tribe Cacteae based on F81 distances.

generic status of *Aztekium* was accepted, although among members of the working party, opinion was divided as to whether *Aztekium* and *Strombocactus* were congeneric or convergent. Anderson and Skillman (1984) concluded that *Aztekium* and *Strombocactus* should each be recognized at the generic level, citing

a number of differences in vegetative, floral, pollen and seed morphology. The phylogeny presented in this paper strongly supports (bootstrap 100%, decay 7) a clade containing *Aztekium* and *Geohintonia* and does not support a close relationship between *Aztekium* and *Strombocactus*.

ECHINOCACTUS CLADE. The clade comprising *Astrophytum*, *Echinocactus horizonthalonius*, *E. ingens* and *Homalocephala* are globose to shortly columnar cacti with ribbed stems. Areoles are large, and in some species of *Astrophytum*, spines are lacking. Flowers are shortly funnelform to campanulate, the wooly pericarpel having numerous spine-tipped bracts. These cacti are distributed throughout Mexico and SW United States. A close relationship between genera of this clade is also supported by chloroplast restriction-site data (Wallace 1995). Previous authors (Bravo-Hollis and Sánchez-Mejorada 1991; Ferguson 1992; Barthlott and Hunt 1993) have considered *Homalocephala* as congeneric with *Echinocactus.* The *rpl16* data (this paper) does not fully resolve the relationships between *Astrophytum*, *Echinocactus*, and *Homalocephala* that were shown by Wallace (1995), instead displaying a trichotomy, such that with the inclusion of *Homalocephala*, the genus *Echinocactus* may be paraphyletic. These data also corroborate the conclusion of Cota and Wallace (1997) that *Echinocactus grusonii* is more closely related to members of the genus *Ferocactus* than to other species in the *Echinocactus* clade.

SCLEROCACTUS CLADE. Porter et al. (2000) attempted to define generic boundaries for the morphologically diverse genus *Sclerocactus* using chloroplast *trnL-trnF* sequence data. Although sampling from other genera of the tribe was not as broad as in the study presented here, sampling from within the genus *Sclerocactus* clearly contradicted the hypothesized close relationship between *Sclerocactus* and *Pediocactus*, suggested by previous authors (Arp 1972 as cited in Porter et al. 2000; Benson 1982). Our phylogeny places members of *Sclerocactus* in a well-supported clade (bootstrap 89%, decay 4) and shows no affinity between *Sclerocactus* and the genus *Pediocactus*; thus our results are consistent with those of Porter et al. (2000). The working party of the IOS (Hunt and Taylor 1986, 1990) and Barthlott and Hunt (1993) treat the genus *Glandulicactus* as a synonym of *Sclerocactus*. Ferguson (1991 and pers. comm.) disagrees with the placement of *Glandulicactus* within *Sclerocactus*, based on vegetative and floral morphology. Our data support Ferguson's view (see section on *Ferocactus* Clade).

LOPHOPHORA CLADE. Although there is strong support for this clade (87% bootstrap, 5 decay steps), few morphological features unite this clade. All members have napiform or carrotlike tap-root systems, although these features are also found in other members of the tribe.

The two species of *Acharagma* have been a source of taxonomic confusion. Described originally in the genus *Echinocactus*, *E. roseanus* was transferred into the genus *Gymnocactus* Backeberg by Glass and Foster (1970), who later also described *G. aguirreanus* (Glass and Foster 1972). However, Anderson and Ralston (1978) felt

that these two species were better placed in the genus *Turbinicarpus*, contrary to the views of Glass and Foster (1977), who felt that despite high degrees of similarity in distribution, appearance, and flower, fruit and seed morphology, the larger size and generally heavier spination of species of *Gymnocactus* warranted recognition as a separate genus. In a review of *Escobaria*, Taylor (1986) placed *G. roseanus* and *G. aguirreanus* as sole members of the section *Acharagma* of *Escobaria*. Unlike other members of the genus, the axillary areole and tubercular groove is absent in these two species. Furthermore, the flowers are borne in a zone adjacent to the spine-bearing areoles, in contrast to the more typical position in the axils of the tubercles. Glass (1998) elevated Taylor's section *Acharagma* to the rank of genus following Zimmerman's provisional generic treatment in which *Acharagma* was placed in a large clade containing *Ferocactus*, *Coryphantha*, *Mammillaria*, *Ortegocactus*, and *Escobaria*, mainly based on foveolate seeds (Zimmerman 1985). However, Zimmerman (1985) acknowledged that *Acharagma* only has weakly derived character states and so his placement of the genus was uncertain. The *rpl16* intron data suggest the removal of these two species from *Escobaria*, placing them in a well-supported (bootstrap 87%, decay 5) clade containing *Obregonia* and *Lophophora*, the latter shown to be polyphyletic based on this topology.

STROMBOCACTUS. This monotypic genus from the states of Queretero and Hidalgo in central Mexico forms a sister lineage to the ''ATEP'', *Ferocactus*, *Stenocactus* and ''Mammilloid'' clades according to the phylogeny presented in this paper. On the basis of seed morphology, Buxbaum (1958a) suggested that the genus *Strombocactus* ought to include the then monotypic genus *Aztekium*. This was in spite of the tuberculate stem anatomy of *Strombocactus* which contrasts the ribbed anatomy of *Aztekium*. Buxbaum (1958a) explained this by suggesting a progression from the hardened tubercles of *Strombocactus* to the formation of ribs in *Aztekium*. Anderson and Skillman (1984), using morphological and anatomical data, concluded that *Strombocactus* and *Aztekium* each deserved recognition at the genus level. No direct relationship between *Strombocactus* and *Aztekium* is demonstrated in our *rpl16* phylogeny.

''ATEP'' CLADE. This clade's acronym-based name is derived from its included genera—*Ariocarpus*, *Turbinicarpus*, *Epithelantha* Weber ex Britton and Rose, and *Pediocactus*, and has poor support in our phylogeny (bootstrap <50%, decay 1 step). Stem morphologies are tuberculate, and in *Ariocarpus* dimorphic areoles are present, this feature an example of convergence with members of the ''Mammilloid'' clade. *Turbinicarpus* is a genus of around sixteen species of small, inconspicuous cacti from north-central Mexico. Due to poor seed dispersal mechanisms, species of *Turbinicar-*

pus are highly localized (Glass and Foster 1977). A number of species of *Turbinicarpus* have been allied or subsumed into other genera such as *Gymnocactus* (Backeberg 1970) and *Neolloydia* (Anderson 1986; Hunt and Taylor 1990; Barthlott and Hunt 1993). However, in the CITES Cactaceae checklist, Hunt chose to accept generic status for species of *Turbinicarpus* leaving only two species in *Neolloydia* (Hunt 1992; 1999). The phylogeny presented here supports the exclusion of *Turbinicarpus* from *Neolloydia s. str.* as *N. conoidea* (type species for the genus) is strongly positioned within the "Mammilloid" clade.

FEROCACTUS CLADE.   This clade contains a number of seemingly disparate genera with few morphological affinities (such as conspicuous pericarpel scales) that unite the entire clade. Members of the genus *Ferocactus* possess a number of morphological synapomorphies including nectar-secreting areolar glands and a ring of hairs that separate the stamens from the tepals. Although morphologically striking due to elongate, glaucous tubercles, the single species of *Leuchtenbergia*—*L. principis* (the "Agave Cactus") is placed in the *Ferocactus* clade. Barthlott and Hunt (1993) describe the flowers of this species as similar to those of *Ferocactus*, and the fruit as being typical of those in subgenus *Ferocactus*—dry, globose to oblong with thick-walls and dehiscing at the base. A close affinity between *Ferocactus* and *Leuchtenbergia* is also demonstrated by the ease with which these genera hybridize. The phylogeny presented here, as well as chloroplast restriction site data (Cota 1997; Cota and Wallace 1997), shows that *Echinocactus grusonii* is more closely related to members of *Ferocactus* (particularly *F. histrix* and *F. glaucescens*) than it is to the remaining species of *Echinocactus* sampled. These species share a number of distinct character traits, including straight or slightly curved, terete central spines as opposed to hooked spines with flat crosssections that are more typical of *Ferocactus*. In our phylogeny, however, *F. histrix* is positioned outside the *Ferocactus* clade. If *F. histrix* is moved and placed sister to *F. glaucescens* and *E. grusonii*, tree-length increases by only three steps. The slight change in tree-length and low decay value (decay = 1) for the branch separating *F. histrix* from the *Ferocactus* clade implies possible homoplasy in our dataset. Previous molecular studies of *Ferocactus* by Cota and Wallace (1997) and Cota (1997) were only able to partially resolve species relationships between *Ferocactus* and its allies, but did recover similar clades within the genus *Ferocactus*.

Glandulicactus uncinatus and *G. crassihamatus* currently are recognized as *Sclerocactus uncinatus* and *S. uncinatus* ssp. *crassihamatus*, respectively, by a number of authors (Hunt 1992; Barthlott and Hunt 1993; Hunt 1999; Anderson 2001). However, Ferguson (1991) argued that this genus did not belong in *Sclerocactus*, citing a number of morphological differences. Instead, he allied members of this genus with *Ferocactus*, *Thelocactus*, and *Leuchtenbergia* based on vegetative and floral morphology. Although the phylogeny presented in this paper does not necessarily support Ferguson's viewpoint that *Glandulicactus* should be recognized at genus level, it does corroborate his conclusions that the members of this genus are more closely related to *Ferocactus* and *Thelocactus* than to *Sclerocactus*.

STENOCACTUS CLADE.   Comprising about 10 species, *Stenocactus* tends to be separable from the related *Ferocactus* clade by two morphological characters: 1) narrow, fin-like ribs as opposed to wide ribs, and 2) areoles in which the large spines are subtended by the smaller spines as opposed to areoles in which the larger spines subtend the smaller spines in *Ferocactus*. However, Taylor (1983) argued that despite these morphological differences, flower, fruit and seed morphology required a broader generic concept that included the members of *Stenocactus* in the genus *Ferocactus*. Our *rpl16* phylogeny suggests that the *Stenocactus* clade (bootstrap 100%, decay 4) is distinct from the *Ferocactus* clade.

"MAMMILLOID" CLADE.   Although support for the "Mammilloid" clade is not particularly strong (bootstrap 60%, decay 3), members share the morphological synapomorphies of tuberculate stem anatomy and dimorphic areoles (the spine-bearing areoles being apical and the flowering areoles being axillary to the tubercles). Within the clade, generic delimitations have traditionally been confused. *Pelecyphora* and *Encephalocarpus* form a well-supported clade (bootstrap 100%, decay 8). These genera have been treated as congeneric (*Pelecyphora*) by some previous authors (Anderson and Boke 1969; Barthlott and Hunt 1993; Anderson 2001), and are recognized as such in the CITES Cactaceae Checklist (Hunt 1992, 1999). Relationships between *Escobaria* and *Coryphantha* are controversial. Berger (1929 cited in Zimmerman 1985) subsumed *Escobaria* into *Coryphantha*. Taylor (1986) cites a number of character traits that distinguish the two genera, including pitted seeds and ciliate outer perianth segments in *Escobaria* versus non-pitted seeds and non-ciliate outer perianth segments in *Coryphantha*. Taylor (1986) suggests that *Escobaria* is more closely related to *Mammillaria* than it is to *Coryphantha*. Indeed, a sister relationship between *Escobaria* and *Coryphantha* is suggested by *rpl16* intron data, although additional data and more intense sampling are required in order to evaluate more robustly their relationships to *Mammillaria*.

Based on *rpl16* sequences, *Mammillaria* is not monophyletic as currently circumscribed due to the placement of *Neolloydia conoidea* and *Ortegocactus macdougallii*. The working party of the IOS (Hunt and Taylor 1986, 1990) chose to include the genus *Turbinicarpus* and *Gymnocactus* within *Neolloydia*. Barthlott and Hunt (1993) followed the same treatment but suggested that

the dimorphic areoles and hence axillary flowers of the type species of *Neolloydia* (*N. conoidea*) were sufficient to justify recognition of *Turbinicarpus* at genus level while continuing to recognize the genus *Neolloydia*. Zimmerman (1985) concludes that *Neolloydia* is distinct from *Coryphantha* and its allies (being more closely related to *Ariocarpus*, *Obregonia*, *Lophophora*, *Strombocactus*, and *Aztekium*), and that the tubercular groove, in this case, is non-homologous with the areolar groove in *Escobaria* and *Coryphantha*. However, Zimmerman failed to cite which species of *Neolloydia* were used in his study, so it is unsure if he used the type—*N. conoidea* or other species referable to *Turbinicarpus*. Our data supports the view of Barthlott and Hunt (1993) that *Neolloydia* in the strict sense (*N. conoidea* and *N. matahuelensis* Backeberg) are not closely related to either *Turbinicarpus* or *Gymnocactus*.

Ortegocactus is a monotypic genus, known only from the state of Oaxaca, Mexico. Although it shares many morphological features with members of the ''Mammilloid'' clade, taxonomists have had difficulty assessing relationships of this species to other members of the clade. Bravo-Hollis and Sánchez-Mejorada (1991) placed *Ortegocactus* in the genus *Neobesseya* Britton and Rose. Zimmerman (1985) concluded that *Ortegocactus* was a member of a phylogenetically distinct clade containing *Coryphantha*, *Escobaria* and *Mammillaria*. The *rpl16* phylogeny suggests that *Ortegocactus* is more closely related to *Mammillaria* than to other members of the ''Mammilloid'' clade, and shows no direct relationship with *Coryphantha* or *Escobaria*.

The clade containing *M. halei*, *M. poselgeri*, and *M. yaquensis* is phylogenetically distinct (bootstrap 83%, decay 1) from the remaining species sampled in *Mammillaria* sensu stricto. Two of these three species (*M. halei* and *M. poselgeri*) are referable to the genus *Cochemiea*. *Mammillaria yaquensis* (series *Ancistracanthae* Schumann) has been allied to other members of *Cochemiea* by Lüthy (1995). That *Cochemiea* (represented by *M. halei* and *M. poselgeri* in this study) are found only in Baja California raises the question of their origin and dispersal from mainland Mexico. The series *Ancistracanthae* are distributed in western Mexico and Baja California reaching as far north as the southern USA (California Arizona and New Mexico). A reasonable hypothesis suggests that ancestral members of the *Ancistracanthae* migrated northwards in mainland Mexico, before migrating south through Baja California. The geologic history of Baja California seems unclear, but there is evidence that the Gulf of California began to separate around 4.5 million yr ago (Atwater 1970) or 5.5 million yr ago according to Riddle et al. (1997). However, the gulf may have been in existence for the last 12 million yr (Gastil et al. 1983). Assuming a north-south migration of ancestral *Ancistracanthae*, the phylogeny presented here suggests a more recent ori-

gin for *Cochemiea*. Hunt (pers. comm.) disputes recognizing *Cochemiea* as distinct, stating that ornithophilous flowers (found only in *Cochemiea*) are derived and contradict a sister-group relationship to other members of *Mammillaria*. Our phylogeny does support ornithophily as being derived and suggests that *Cochemiea* arose from an *Ancistracanthae*-like ancestor.

Within the main clade of *Mammillaria*, there are a number of species whose inclusion in the genus has been disputed by various cactus taxonomists. *Mammillaria beneckei* was considered by Buxbaum (1951, in Hunt 1977a) as a distinct genus (*Oehmea*) and argued that this was an example of morphological convergence with *Mammillaria*, but that it was actually derived from a *Thelocactus*-type ancestor. Hunt (1977a) subsumed the genus *Oehmea* within *Mammillaria*, giving it subgeneric status based upon the rugose/pitted seed testa, which is also found in other members of the genus. In their work on the Cactaceae, Britton and Rose (1919–1923) gave separate generic status to *Mammillaria senilis* by describing it within the genus *Mamillopsis*. Their justifications for this were based on a number of floral traits that they considered sufficiently different from *Mammillaria* to warrant its generic status. However, Hunt (1971) concluded that vegetative, floral, and seed morphology when taken in the sum of their characters did not support generic status of *Mamillopsis* and that it should only be retained at the subgeneric level within *Mammillaria*. Britton and Rose (1919–1923) also elevated Schumann's (1899) subgenus *Dolichothele* (represented in this study by *M. longimamma* DC) to genus level, separating it from other species of *Mammillaria* due to its very elongate tubercles. As with *Mamillopsis*, Hunt (1971) believed that there were insufficient differences to justify *Dolichothele* at the rank of genus, instead accepting it as a subgenus of *Mammillaria*. Our *rpl16* phylogeny does not support the view of Buxbaum (1951, in Hunt 1977a) regarding the generic status of *Oehmea* because no direct relationship between *Oehmea* and *Thelocactus* is demonstrated. Although sampling from the genus *Mammillaria* is limited, our data also suggest that recognition of *Mamillopsis* and *Dolichothele* at the generic rank is unwarranted as they are nested within other *Mammillaria* species. Further sampling from *Mammillaria* is required and for the present, *Oehmea*, *Mamillopsis*, and *Dolichothele* should be retained in *Mammillaria*.

The relationship of *Mammillaria candida* to other members of the genus has also been a source of past debate. Schumann (1899) considered this species to be within his subgenus *Eumamillaria* (true mammillarias). Buxbaum (1951, in Hunt 1977a) elevated this species to genus status—*Mammilloydia candida* based soley on a tuberculate seed testa morphology. Riha and Riha (1975) disputed Buxbaum's observations, going so far as to suggest that Buxbaum had accidentally observed

seed material that was not from *Mammillaria candida*. Hunt (1977) argued in support of Buxbaum, suggesting that *Mammilloydia candida* was the product of a separate evolutionary lineage than that of the remaining species of *Mammillaria*. However, he considered the retention of subgenus *Mammilloydia* a taxonomic compromise. The International Cactaceae Systematics Group recently has accepted that *M. candida* merits recognition at the generic rank as *Mammilloydia* (Hunt 1999) and it is treated as such in Anderson (2001). Sequence analysis of the *rpl16* intron presented here, and from a more broad sampling of the ''Mammilloid'' clade (Butterworth 2000) indicate that recognition of *M. candida* at the rank of genus would render *Mammillaria* paraphyletic. Further studies on the ''Mammilloid'' clade are in progress to resolve these issues.

Morphological Evolution. EVOLUTION OF TUBERCLES IN THE CACTEAE. Buxbaum (1958a) presented a number of different scenarios in which tubercular stem morphologies may have arisen in the Cactaceae. Within the tribe Cacteae, he described the convergent evolution (in a number of lineages within the tribe) of transversely-arranged tubercles formed from ribs in which the basal portions of the podaria have become enlarged to form tubercles, implying that a ribbed stem morphology represents the primitive condition for the tribe. Gibson and Nobel (1986) also suggest that the primitive condition for the subfamily Cactoideae is likely based on ribbed-stem morphology. From our studies using *rpl16* intron sequence data, it appears that in the Cacteae tubercular stem morphologies represent a derived condition. The question of multiple origins of tubercles or reversals to ribbed stems is debatable. The most parsimonious explanation based on the phylogeny presented in this paper is that tubercular stems have arisen independently in a number of clades, once following the divergence between the *Echinocactus* clade and the remaining Cacteae. A reversal to ribbed stems is implicated in *Ferocactus histrix*, *Stenocactus*, and the *Ferocactus* clade, with secondarily derived tubercular stem morphologies representing a zone of transition between ribs and tubercles. The genus *Ferocactus* has ribbed stems, while *Leuchtenbergia* has very distinct elongate tubercles. *Glandulicactus* has deeply notched ribs and may represent the intermediate condition, and in *Thelocactus*, both ribs and tubercles are present—*Thelocactus hastifer* with distinct, spiraling ribs divided into tubercles, and the sister clade of *T. conothelos* and T. *macdowellii* having indistinct ribs and pronounced tubercles (Anderson 1987). There is also a switch from ribbed to tubercular stems in the ''Mammilloid'' Clade, whose members also share the synapomorphy of having dimorphic areoles (see bellow).

EVOLUTION OF DIMORPHIC AREOLES. The majority of genera in the Cacteae produce flowers from the spine-forming areoles. However, a number of taxa in the tribe have dimorphic areoles in which spines and flowers are borne from different regions or even from separate areoles. To an extent this correlates with tubercular stem morphologies where spines are produced from apical areoles (the axillary areoles becoming reproductive). Buxbaum (1958a) proposed that the evolution of dimorphic areoles in the tribe Cacteae occurred along two distinct lines. The first lineage shows a succession from *Leuchtenbergia*, which has elongated tubercles tipped by undifferentiated areoles, to *Roseocactus* (*Ariocarpus fissuratus*, *A. kotschoubeyanus*) with areoles forming an elongated furrow along the length of the tubercle with separate floral and spine-bearing regions (Anderson 1960), to *Ariocarpus* with separate floral and spine-bearing areoles and some species lacking spine-bearing areoles altogether (Anderson 1960). Buxbaum's second evolutionary lineage occurred from a non-differentiated ''Thelocactus-type'' areole in which growth occurs below the areole causing it to be forced to the tip of the tubercle. In a number of species, lengthening growth divides the growing point forcing the spine producing part towards the tubercle tip, the flower producing region remains in the tubercle axil. Species with this form of dimorphic areole may have a groove running along the adaxial length of the tubercle connecting the vegetative and reproductive areoles. In *Mammillaria*, the groove is absent due to division of the growing point at a very early stage in development. According to our phylogeny, undifferentiated areoles represent the plesiomorphic condition for the Cacteae with the evolution of dimorphic areoles occurring independently in *Ariocarpus* and the ''Mammilloid'' clade.

In summary, the pattern of evolution that we present in the tribe Cacteae, as inferred from the *rpl16* intron phylogeny, suggests that the *Aztekium-Geohintonia* clade, represents a relictual, yet highly specialized lineage. The remaining members of this North American barrel cactus tribe have undergone diversification into several clades, the more derived clades manifesting a shift from plesiomorphic ribbed stems to those that are tuberculate, concomitantly undergoing a general reduction in plant size. Shifts in floral position and areole are also inferred from the phylogeny. These changes occurred in parallel within the Cacteae, further adding to the systematic confusion experienced by many earlier cactologists. Although the inferred evolutionary relationships we present are based on data from a single molecular marker of the plastid genome, the resulting tree topology and clades defined are telling as to the broad-scale, intergeneric relationships within the tribe that have heretofore only received ''support'' through speculative conclusions, accompanied by little empirical analyses.

Here we broadly sample representative members of

the tribe Cacteae in a uniformly comparative fashion, and evaluate the group to determine its primary lineages. The intergeneric relationships inferred now lend themselves to further testing with additional markers and more intensive sampling for the more species-rich genera (e.g., *Mammillaria, Coryphantha, Escobaria, Ferocactus*), as well as reexamining those clades that were not well supported (e.g. the 'ATEP' Clade). These investigations are ongoing at present and will extend the value of the present study through the use of its conclusions in prudent outgroup sampling, identification of morphological evolutionary trends among the taxa, and by establishing a baseline phylogeny for integration with other similar studies being conducted on other tribes in the Cactaceae.

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