

## Phylogenetic Analysis of *Pachycereus* (Cactaceae, Pachycereeae) based on Chloroplast and Nuclear DNA Sequences

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**ABSTRACT.** The phylogenetic relationships of *Pachycereus* (Cactaceae) species and relatives from subtribe Pachycerinae were studied using DNA sequence data. The plastid *rpl16* intron, *trnL* intron, *trnL-F* intergenic spacer, and nuclear rDNA internal transcribed spacer region (ITS) were sequenced for 30 species, representing the four genera of subtribe Pachycerinae (*Carnegiea*, *Cephalocereus*, *Neobuxbaumia*, and *Pachycereus*) as well as three additional outgroup genera from subtribe Stenocereinae. Phylogenetic analyses support neither the monophyly of *Pachycereus* as currently circumscribed nor Pachycerinae unless *Stenocereus aragonii* and *S. eichlamii* are included within it. However, these results suggest that the subtribe can be divided into three major clades. The first includes *Pachycereus hollianus* and *P. lepidanthus*, which is sister to a large clade combining species from the *Pachycereus* and *Cephalocereus* groups. Within this large clade *Cephalocereus* and *Neobuxbaumia* together with *Pachycereus fulviceps* are sister to the remaining species of *Pachycereus* as well as *Stenocereus aragonii*, *S. eichlamii*, and *Carnegiea gigantea*. Our results suggest that *Pachycereus* is paraphyletic and that several other genera (*Backebergia*, *Lemaireocereus*, *Lophocereus*, and *Pseudomitrocereus*) may be resurrected to accommodate these new phylogenetic insights. A number of morphological and anatomical characters support these relationships, indicating that future analyses combining both molecular and morphological characters will be particularly useful in resolving relationships within this group of columnar cacti.

As currently circumscribed, *Pachycereus* (A. Berger) Britton and Rose is a genus of 13 species (Barthlott and Hunt 1993; Gama-López and Arias 1998; Hunt 1999; Anderson 2001) found in arid, warm, and subhumid regions of Mexico and Central America, as well as in the extreme southwestern United States. The highest species richness is found in the southern part of Mexico, including the Balsas Depression, Tehuacan-Cuicatlan Valley, and Southern Mountains. Most species of *Pachycereus* are broadly ramified columnar trees with dense floral bracts and trichomes, fleshy fruits covered by spines, bristles, and trichomes, and oval to broadly oval seeds with glossy black testa. Along with three other genera (*Neobuxbaumia*, *Cephalocereus*, and *Carnegiea*), *Pachycereus* is classified within Pachycerinae (Pachycereeae, Cactoideae, Cactaceae), a subtribe defined by the presence of alkaloids in the stem, seeds with smooth testa, and occurrence of calcium oxalate crystals in the dermal tissue (Gibson and Horak 1978).

The circumscription of *Pachycereus* has been inconsistent (Table 1). Britton and Rose (1909) recognized 10 different species in their revision of the genus. Buxbaum (1961, 1963), however, recognized only six based on floral, fruit, seed, and seedling morphology. He also transferred *Lemaireocereus hollianus* to *Pachycereus*, but excluded *Pachycereus gaumeri* and *Pachycereus lepidanthus* from the genus. Gibson and Horak (1978) also recognized six species of *Pachycereus* that share the presence of alkaloids, large, glossy, black seeds, and stems that

darken quickly after cutting. These authors transferred *Stenocereus weberi* and *Stenocereus marginatus* to *Pachycereus* because they contain abundant alkaloids and lack funicular pearl cells. Later, Gibson (1982) and Gibson et al. (1986) suggested that *Pachycereus hollianus* should be excluded from *Pachycereus* based on fruit morphology, since this species has only scarce, ungrouped spines, and seeds with cuticular ornamentations. Moreover, Gibson et al. (1986) suggested that *Stenocereus aragonii* could be closely related to *Pachycereus* because of its similarity in size and seed cuticular ornamentation. Heath (1992) formally proposed the combination *Pachycereus aragonii* for this species.

Certain species of *Pachycereus* have been separated from the genus *sensu stricto* and placed in the distinct genera *Anisocereus*, *Backebergia*, *Lophocereus*, *Pseudomitrocereus*, and *Pterocereus* (Buxbaum 1961, 1963; Bravo-Hollis 1978; Gibson and Horak 1978; Gibson 1982) as summarized in Table 1. However, in the most recent treatments of the genus, Barthlott and Hunt (1993) and Hunt (1999) broadly recognized 12 species, although without providing any evidence to support their proposal. The same generic delimitation was presented by Anderson (2001). All of these taxa were known when Britton and Rose (1909) published their revision of *Pachycereus*; only one additional species, *P. tepamo* Gama-López and Arias (1998), of western Mexico has been described since that time.

According to Buxbaum (1961) the genera *Pseudo-*

TABLE 1. Classification of the species included in *Pachycereus* according to various authors. Alternative generic names are: 1 *Anisocereus*; 2 *Backebergia*; 3 *Escontria*; 4 *Lemaireocereus*; 5 *Lophocereus*; 6 *Marginatocereus*; 7 *Neobuxbaumia*; 8 *Pseudomitrocereus*; 9 *Pterocereus*; 10 *Stenocereus*; 11  $\times$  *Pachycerocereus*.

Britton & Rose (1909)	Buxbaum (1963)	Bravo-Hollis (1978)	Gibson & Horak (1978)	Barthlott & Hunt (1993); Hunt (1999)
<i>P. pringlei</i>	<i>P. pringlei</i>	<i>P. pringlei</i>	<i>P. pringlei</i>	<i>P. pringlei</i>
<i>P. grandis</i>	<i>P. grandis</i>	<i>P. grandis</i>	<i>P. grandis</i>	<i>P. grandis</i>
<i>P. pecten-aboriginum</i>	<i>P. pecten-aboriginum</i>	<i>P. pecten-aboriginum</i>	<i>P. pecten-aboriginum</i>	<i>P. pecten-aboriginum</i>
<i>P. catous</i>	<i>P. hollianus</i> <sup>4</sup>	<i>P. hollianus</i> <sup>4</sup>	<i>P. hollianus</i> <sup>4</sup>	<i>P. hollianus</i> <sup>4</sup>
<i>P. marginatus</i> <sup>6</sup>	<i>P. orcutti</i> <sup>11</sup>	<i>P. marginatus</i> <sup>6</sup>	<i>P. marginatus</i> <sup>6</sup>	<i>P. marginatus</i> <sup>6</sup>
<i>P. chrysomallus</i> <sup>8</sup>	<i>P. teluantepecanus</i>		<i>P. weberi</i> <sup>10</sup>	<i>P. weberi</i> <sup>10</sup>
<i>P. columna-trajantii</i>			<i>P. fulviceps</i> <sup>8</sup>	<i>P. fulviceps</i> <sup>8</sup>
<i>P. orcutti</i> <sup>11</sup>			<i>P. gatesii</i> <sup>5</sup>	<i>P. gatesii</i> <sup>5</sup>
<i>P. queretaroensis</i> <sup>10</sup>			<i>P. gaumeri</i> <sup>9</sup>	<i>P. gaumeri</i> <sup>9</sup>
<i>P. titan</i>			<i>P. lepidanthus</i> <sup>1,3</sup>	<i>P. lepidanthus</i> <sup>1,3</sup>
			<i>P. militaris</i> <sup>2</sup>	<i>P. militaris</i> <sup>2</sup>
			<i>P. schottii</i> <sup>7</sup>	<i>P. schottii</i> <sup>7</sup>

*mitrocereus* and *Heliabravoa* (= *Polaskia*) are closely related to *Pachycereus* based on such shared floral characters as a receptacular tube with thick walls and a short perianth with trichomes and bristles. Gibson and Horak (1978) considered *Pachycereus sensu stricto* to be closely related to *Lophocereus* because of their similar alkaloid types, as well as unique vegetative characteristics shared between *Lophocereus schottii* and *Pachycereus marginatus*.

Unfortunately, molecular data for members of tribe Pachycereeae are scarce. Among the few studies published to date is an analysis of chloroplast DNA restriction sites for *Ferocactus* and some members of Pachycereeae by Cota and Wallace (1997). Their study suggested that *Pachycereus* may be paraphyletic because *P. hollianus* was shown to be more closely related to *Neobuxbaumia* than to *P. marginatus*, which showed a relationship with *Lophocereus*. A similar result was obtained in a phylogenetic analysis of Pachycereeae based on morphological and anatomical characters (Terrazas and Loza-Cornejo 2002). In that study, *P. fulviceps* was found to be more closely related to *Cephalocereus* and *Neobuxbaumia* than to the other five species of *Pachycereus* sampled. More recently, Nyffeler (2002) demonstrated that *Pachycereus* is closely related to *Escontria* and *Echinocereus* based on molecular data. However, that study was focused on family-level relationships and very few taxa from Pachycereeae were sampled. Hartman et al. (2002) included 10 taxa of Pachycereeae in their molecular analysis, and they raised the hypothesis that *P. schottii* and *P. gatesii* are more closely related to *P. marginatus*.

The primary purpose of this study is to estimate the phylogenetic relationships among species of *Pachycereus* and genera of Pachycereinae using nucleotide DNA sequences from both plastid (*trnL* intron, *trnL-F* intergenic spacer, and *rpl16* intron) and nuclear (rDNA internal transcribed spacers, ITS) sources of nucleotide variation. Data obtained from these DNA regions will allow us to (a) examine the monophyly and circumscription of *Pachycereus*, (b) evaluate whether *Anisocereus*, *Backebergia*, *Lemaireocereus*, *Lophocereus*, *Marginatocereus*, *Pseudomitrocereus*, and *Pterocereus* should be recognized separately from *Pachycereus*, and (c) determine the intergeneric relationships within the subtribe Pachycereinae.

## MATERIALS AND METHODS

**Taxon Sampling.** The 12 species of *Pachycereus* recognized by Barthlott and Hunt (1993) and Anderson (2001), the new species *P. tepamo*, and representative species of all genera in subtribe Pachycereinae (*Carnegiea*, *Cephalocereus*, *Neobuxbaumia*) were sampled along with appropriate outgroup taxa (*Escontria chiotilla*, *Myrtillocactus schenckii*, *Stenocereus chrysocarpus*, *S. fricii*, *S. martinii*, and *S. stellatus*). These three taxa belong to the subtribe Stenocereinae and it has been shown that they are part of the sister group to subtribe Pachycereinae (Cota and Wallace 1997; Cornejo and Simp-

TABLE 2. Species for which sequences were obtained, with source and voucher information and Genbank accession numbers for the sequences. (CHAPA Herbario Hortorio, Colegio de Postgraduados; HBG Huntington Botanical Garden; MEXU Herbario Nacional de México; NYBG New York Botanical Garden).

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<i>Carnegiea gigantea</i> (Engelm.) Britton & Rose: NYBG s.n., cult., Arizona; <i>trnL-F</i> AY181619, <i>rpl16</i> AY181591, ITS AY181566.
<i>Cephalocereus columna-trajani</i> (Karw. ex Pfeiffer) Schum.: Arias 1377, Puebla, CHAPA; <i>trnL-F</i> AY181648, <i>rpl16</i> AY181599, ITS AY181565. <i>Cephalocereus senilis</i> (Haworth) Pfeiffer: Terrazas 529, Hidalgo, CHAPA; <i>trnL-F</i> AY181638, <i>rpl16</i> AY181616, ITS AY181585.
<i>Escontria chiotilla</i> (Weber ex Schum.) Rose: Terrazas 370, Puebla, CHAPA; <i>trnL-F</i> AY181622, <i>rpl16</i> AY181608, ITS AY181576.
<i>Myrtillocactus schenckii</i> (J. Purpus) Britton & Rose: Terrazas 500, Puebla, CHAPA; <i>trnL-F</i> AY181633, <i>rpl16</i> AY181607, ITS AY181574.
<i>Neobuxbaumia euphorbioides</i> (Haworth) Buxb. ex Bravo: Hamann s.n., cult., Tamaulipas; <i>trnL-F</i> AY181635, <i>rpl16</i> AY181595, ITS AY181562. <i>Neobuxbaumia mezcalaensis</i> (Bravo) Backeb.: Terrazas 533, Guerrero, CHAPA; <i>trnL-F</i> AY181645, <i>rpl16</i> AY181600, ITS AY181567. <i>Neobuxbaumia multiareolata</i> (Dawson) Bravo et al.: Terrazas 531, Guerrero, CHAPA; <i>trnL-F</i> AY181644, <i>rpl16</i> AY181597, ITS AY181564. <i>Neobuxbaumia polylopha</i> (DC.) Backeb.: Terrazas 530, Hidalgo, CHAPA; <i>trnL-F</i> AY181644, <i>rpl16</i> AY181597, ITS AY181564. <i>Neobuxbaumia scoparia</i> (Poselger) Backeb.: Hamann s.n., cult., Oaxaca; <i>trnL-F</i> AY181625, <i>rpl16</i> AY181596, ITS AY181563. <i>Neobuxbaumia tetetzo</i> (Weber ex Coulter) Backeb.: Arias 1376, Puebla, CHAPA; <i>trnL-F</i> AY181632, <i>rpl16</i> AY181592, ITS AY181559.
<i>Pachycereus fulviceps</i> (Lemaire) Hunt: Arias 1371, Puebla, CHAPA; <i>trnL-F</i> AY181621, <i>rpl16</i> AY181602, ITS AY181569. <i>Pachycereus gatesii</i> (M.E. Jones) Hunt: Hamann s.n., cult., B.C.S.; <i>trnL-F</i> AY181637, <i>rpl16</i> AY181601, ITS AY181568. <i>Pachycereus gaumeri</i> Britton & Rose: Arias 1360, Yucatán, MEXU; <i>trnL-F</i> AY181626, <i>rpl16</i> AY181606, ITS AY181573. <i>Pachycereus grandis</i> Rose: Terrazas 534, Puebla, CHAPA; <i>trnL-F</i> AY181646, <i>rpl16</i> AY181605, ITS AY181572. <i>Pachycereus hollianus</i> (Weber) Buxb.: Arias 1373, Puebla, CHAPA; <i>trnL-F</i> AY181623, <i>rpl16</i> AY181603, ITS AY181570. <i>Pachycereus lepidanthus</i> (Eichlam) Britton & Rose: Cseh s.n., cult., Guatemala; <i>trnL-F</i> AY181639, <i>rpl16</i> AY181598, ITS AY181575. <i>Pachycereus marginatus</i> (DC.) Britton & Rose: Arias 1372, Puebla, CHAPA; <i>trnL-F</i> AY181627, <i>rpl16</i> AY181618, ITS AY181587. <i>Pachycereus militaris</i> (Audot) Bravo: Arias 1339, Michoacán, CHAPA; <i>trnL-F</i> AY181628, <i>rpl16</i> AY181609, ITS AY181577. <i>Pachycereus pecten-aboriginum</i> (Engelm.) Britton & Rose: Terrazas 535, Guerrero, CHAPA; <i>trnL-F</i> AY181624, <i>rpl16</i> AY181615, ITS AY181583. <i>Pachycereus pringlei</i> (Watson) Britton & Rose: Arias 1348, Baja California Sur, CHAPA; <i>trnL-F</i> AY181642, <i>rpl16</i> AY181589, ITS AY181584. <i>Pachycereus schottii</i> (Engelm.) Hunt: Terrazas 474, Baja California Sur, CHAPA; <i>trnL-F</i> AY181620, <i>rpl16</i> AY181613, ITS AY181581. <i>Pachycereus tepamo</i> S. Gama & S. Arias: Arias 1150, Michoacán, MEXU; <i>trnL-F</i> AY181647, <i>rpl16</i> AY181593, ITS AY181560. <i>Pachycereus weberi</i> (Coulter) Backeb.: Terrazas 532, Guerrero, CHAPA; <i>trnL-F</i> AY181631, <i>rpl16</i> AY181614, ITS AY181582.
<i>Stenocereus aragonii</i> (Weber) Buxb.: HBG 66864, cult., Nicaragua; <i>trnL-F</i> AY181630, <i>rpl16</i> AY181611, ITS AY181579. <i>Stenocereus chrysocarpus</i> Sánchez-Mej.: Arreola 1578, Michoacán, CHAPA; <i>trnL-F</i> AY181643, <i>rpl16</i> AY181617, ITS AY181586. <i>Stenocereus eichlamii</i> (Britton & Rose) Buxb.: Arias 1363, Yucatán, MEXU; <i>trnL-F</i> AY181629, <i>rpl16</i> AY181610, ITS AY181578. <i>Stenocereus fricii</i> Sánchez-Mejorada: Terrazas 384, Michoacán, CHAPA; <i>trnL-F</i> AY181634, <i>rpl16</i> AY181612, ITS AY181580. <i>Stenocereus martinezii</i> (J.G. Ortega) Buxb.: Arreola 1654, Sinaloa, CHAPA; <i>trnL-F</i> AY181641, <i>rpl16</i> AY181604, ITS AY181571. <i>Stenocereus stellularis</i> (Pfeiff.) Riccob.: Arias 1375, Puebla, MEXU; <i>trnL-F</i> AY181640, <i>rpl16</i> AY181590, ITS AY181588.

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son 1997; Gibson and Horak 1978; Terrazas and Loza-Cornejo 2002).

To support our selection of outgroup taxa, a preliminary analysis using distantly related genera as outgroup taxa from other subfamilies (*Pereskia sacharosa* Grisebach and *Opuntia subulata* (Muehlenpfordt) Engelm.), along with a pair of taxa considered putatively related to the tribe (*Leptocereus arboreus* Britton & Rose and *L. quadricostatus* (Bello) Britton & Rose), was applied to the less variable *trnL-F* data. The results of this analysis are not shown, but the species of *Escontria*, *Myrtillocactus*, and *Stenocereus* listed above were sister taxa to subtribe Pachycereinae, while *S. aragonii* and *S. eichlamii* fell within Pachycereinae.

Most of the tissue from the 30 taxa sampled was collected from epidermis of fresh stems in native populations and dried in silica gel. A few samples were obtained from cultivated material in the living collections of The New York Botanical Garden and Huntington Botanical Garden (see voucher information in Table 2).

**DNA Sequencing.** Total DNA was extracted using the Fast-Prep™ (Qbiogene Inc., Carlsbad, California, USA) and glassmilk method from approximately 0.5 cm<sup>2</sup> dried tissue as described by Struwe et al. (1998). Aliquots were then stored at -20°C. Target regions were amplified in 50 µL volumes using standard polymerase chain reaction (PCR) protocols that included the addition of BSA, TMAI, and/or betaine (in the case of ITS). We achieved the highest quality amplifications of *trnL-F* and *rpl16* using the following thermal cycling profile for 30 cycles: 95°C for 50 seconds, 60°C for 50 seconds, 72°C for 90 seconds. In the case of ITS, a lower annealing temperature of 53°C was preferred. The *trnL-F* intron and spacer region was amplified using primers c and f as designed by Taberlet et al. (1991). These same primers, along with

primers d and e (Taberlet et al. 1991), were used for cycle sequencing. The *rpl16* intron, *rpl16* exon 1, part of the *rpl16* exon 2, and the *rpl16-rps3* intergenic spacer were amplified as a unit using primers *rpl16-1216F* and *rps3-42R* (or sometimes *rpl16-18R*; see Asmussen 1999). These same primers, along with *rpl16-584F* and *rpl16-957F* were used for cycle sequencing. To amplify and sequence the ITS region (including ITS1, 5.8S, and ITS2) we used primers ITS4 and ITS5 (White et al. 1990). In all cases, the resulting PCR products were purified using QIAquick™ spin columns (Qiagen Inc., Valencia, California, USA) according to manufacturer's protocols (32 cycles: 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 3 minutes). Cycle sequencing reactions were performed using a combination of purified PCR template, primer, dRhodamine Ready Reaction mix (Applied Biosystems Inc., Foster City, California, USA), and *halfTerm* (GenPak, Inc., Stony Brook, New York, USA) for 20 cycles. These reactions resulted in complete forward and reverse strands of the target regions for nearly all sequences. Centri-Sep sephadex columns (Princeton Separations, Inc., Adelphia, New Jersey, USA) were used according to the manufacturer's instructions to remove excess dye terminators and primer from the cycle sequencing products. These were subsequently dehydrated in a vacuum centrifuge, resuspended in a mixture of formamide and loading dye, and loaded onto a 5% denaturing polyacrylamide gel. Samples were run for nine hours on an Applied Biosystems ABI 377XL automated DNA sequencer, and resulting chromatograms were edited using Sequencher 3.0 (GeneCode Corp., Ann Arbor, Michigan, USA). Sequences for each individual data matrix were aligned manually with little difficulty. These are available from the first author.

**Phylogenetic Analysis.** Sequences were analyzed using the

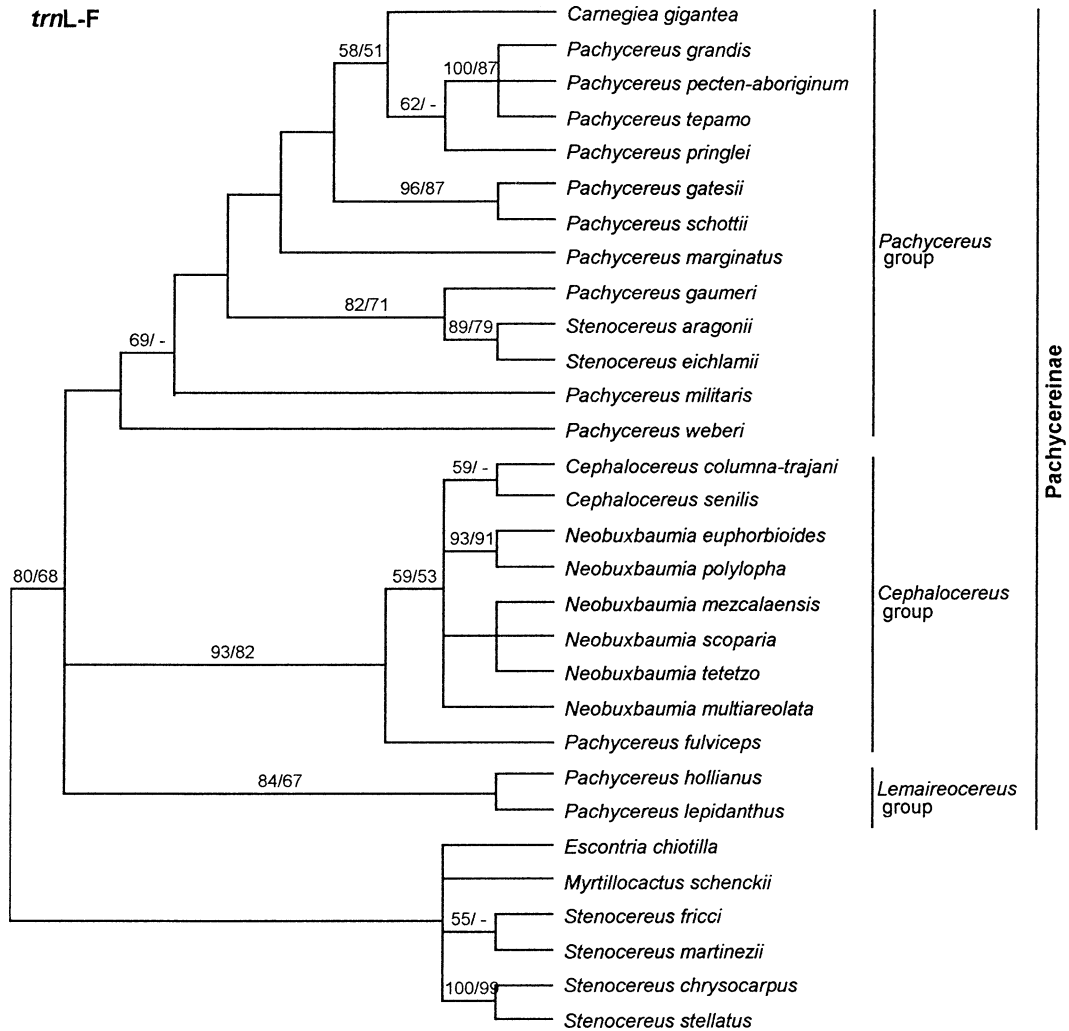


FIG. 1. Strict consensus of 10 equally parsimonious trees for Pachycereinae and outgroup taxa based on plastid *trnL* intron and *trnL-F* spacer sequences. Bootstrap/jackknife percentages ( $\geq 50\%$ ) are given above branches.

parsimony criterion in PAUP\* 4.0b8 (Swofford 1998), with gaps treated as missing data. Trees of maximum parsimony were found by executing a heuristic search of 1,000 random addition replicates using equal weights and TBR branch swapping, but keeping only 10 trees per replicate in order to discover possible islands of maximum parsimony. All trees obtained in the first round of searching were then used as starting trees for a second heuristic search using the same parameters, but this time saving all shortest trees. Support values for the relationships discovered in each analysis were calculated by performing both bootstrap (bts) and jackknife (jck) analyses. One thousand heuristic search replicates were executed using the TBR branching swapping algorithm and keeping 10 trees within each replicate.

In the case of ITS, the sequence of *Escontria chiotilla* is highly divergent from the others in the study, and its alignment to them was ambiguous. For this reason, *Escontria* was deleted from the ITS matrix. Instead, *Stenocereus chrysocarpus*, *S. fricci*, *S. martinezii*, *S. stellatus*, and *Myrtillocactus schenckii* were designated as outgroup taxa. To assess the level of congruence between the data sets, we employed the incongruence length difference (ILD) test (Farris et al. 1995), implemented in PAUP\* as the partition homogeneity test. One thousand heuristic search replicates were performed using the TBR branching swapping and keeping 10 trees within each

replicate. The data set is available on TreeBASE (study accession number = S900; matrix accession number = M1478).

## RESULTS

**trnL-F.** Sequence length ranged from 1130bp in *Cephalocereus columna-trajani* and 1189bp in *Pachycereus pecten-aboriginum*. Aligned sequence length for the *trnL-F* dataset was 1196bp, and after exclusion of the terminal regions the dataset was 1117 characters long. Figure 1 shows the strict consensus of 10 equally parsimonious trees recovered in the analysis. Within Pachycereinae three major clades are evident, and each contains species of *Pachycereus*. *Pachycereus hollianus* and *P. lepidanthus* are strongly supported sister species (84% bts/67% jck) and are herein referred to as the *Lemaireocereus* group. Another species of *Pachycereus*, *P. fulviceps*, is supported as sister to a clade containing all

TABLE 3. Summary of results for individual and combined data matrices.

	<i>trnL-F</i>	<i>rpl16</i>	<i>trnL-F + rpl16</i>	ITS	<i>trnL-F + rpl16 + ITS</i>
No. taxa	30	30	30	29	29
No. characters	1117	1222	2339	619	2958
No. variable characters (% total)	103 (9.2%)	110 (9.0%)	213 (9.1%)	104 (16.8%)	307 (10.3%)
No. informative characters (% total)	40 (3.5%)	42 (3.4%)	82 (3.5%)	51 (8.2%)	132 (4.4%)
No. trees	10	25	10	1344	375
Tree length	115	137	254	149	415
CI (with autapomorphies)	0.948	0.869	0.888	0.745	0.812
RI (with autapomorphies)	0.946	0.881	0.886	0.820	0.835
No. clades supported by bootstrap/jackknife $\geq$ 50%	15/11	11/7	18/17	7/6	17/16

TABLE 4. ILD test of pairwise matrix comparisons.  $p < 0.01$  is considered to be significantly incongruent.

Pairwise comparison	$p$
<i>trnL-F + rpl16</i>	0.889
<i>trnL-F + ITS</i>	0.143
<i>rpl16 + ITS</i>	0.084
plastid + ITS	0.030

species of *Neobuxbaumia* together with *Cephalocereus senilis* and *C. columna-trajani*. This clade of nine species is herein referred to as the *Cephalocereus* group and receives 93% bts/82% jck support. Ten *Pachycereus* species plus *Carnegiea* and two *Stenocereus* species are found within the third clade, here named informally as the *Pachycereus* group. In general, there is poor resolution among the taxa and limited support for relationships, with the exception of three subclades. Four *Pachycereus* species (*P. grandis*, *P. pecten-aboriginum*, *P. pringlei*, and *P. tepamo*) form a monophyletic group, with *Carnegiea gigantea* sister to them. *Pachycereus gatesii* and *P. schottii* are sister species, as is *Stenocereus eichlamii* with *S. aragonii*, with *Pachycereus gaumeri* as sister to this pair. A summary of this matrix and tree statistics for this gene region and the others is presented in Table 3.

***rpl16*.** Sequences length ranged from 1287bp in *Pachycereus marginatus* to 1510bp in *Neobuxbaumia polylopha*. Aligned sequence length for the *rpl16* dataset was 1570bp. After exclusion of two terminal regions, the dataset was 1222 characters long. A strikingly similar tree topology with comparable levels of support was obtained with analysis of the *rpl16* data. The analysis resulted in 25 equally parsimonious trees divided into the same three major subclades: the *Lemaireocereus* group, the *Cephalocereus* group, and the *Pachycereus* group as found in the *trnL-F* trees. Within the *Pachycereus* group resolution among taxa is poor with the following exceptions: *Stenocereus eichlamii* is sister to *S. aragonii* (100% bts/100 jck), *Pachycereus gatesii* is sister to *P. schottii* (90% bts/98 jck), and *P. grandis*, *P. pecten-aboriginum*, *P. pringlei*, *P. tepamo*, and *P. weberi* are recovered as a monophyletic group (68% bts/51% jck). Within the *Cephalocereus* group, which is weakly supported as sister to the *Pachycereus* group, both species of *Cephalocereus* are strongly supported as sister taxa (100% bts/95% jck).

***trnL-F + rpl16*.** The ILD test did not detect significant incongruence between the independent plastid data matrices (Table 4), thus they were combined in a subsequent parsimony analysis. Ten equally parsimonious trees were found, and the same three major subclades recovered by the separate *trnL-F* and *rpl16* data are strongly supported as monophyletic (Fig. 2). In

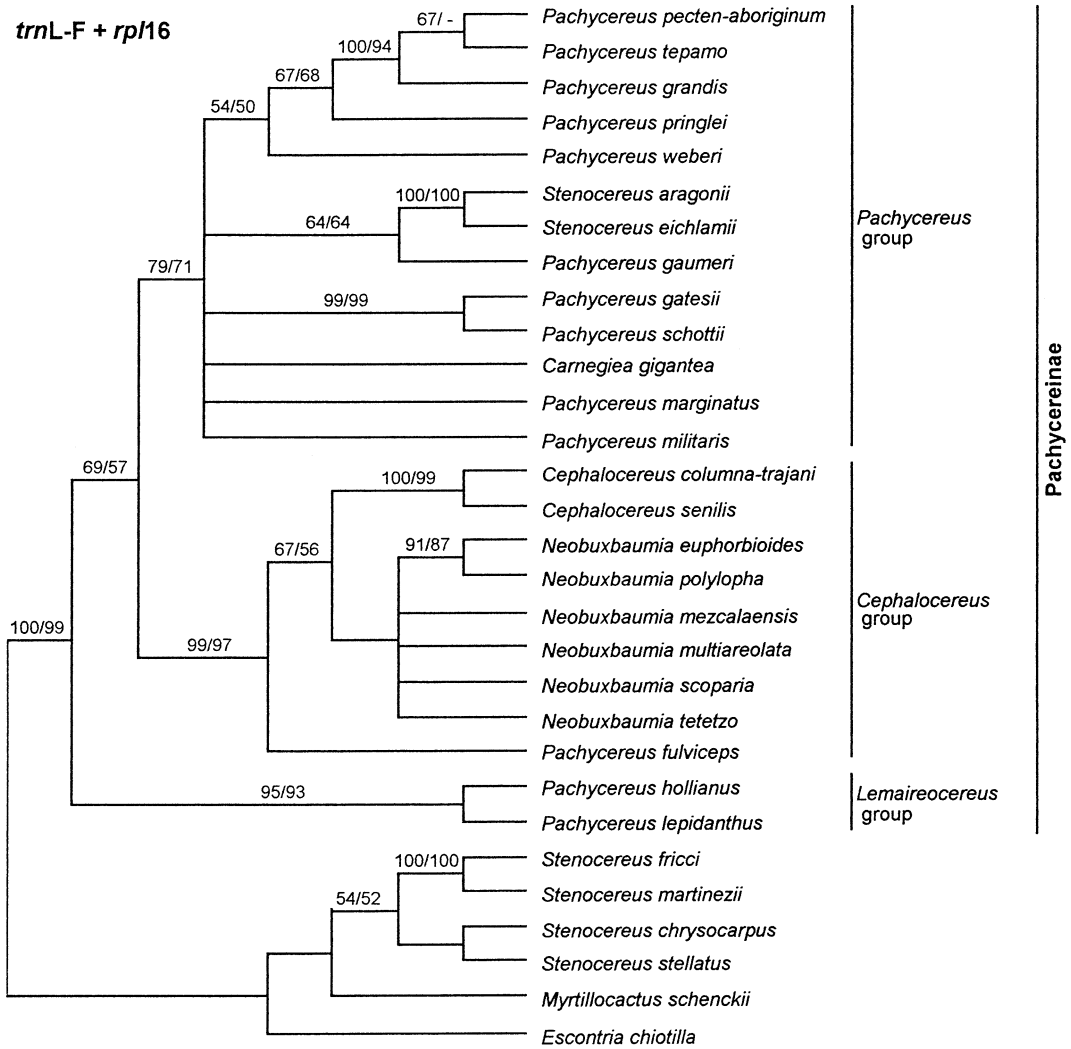


FIG. 2. Strict consensus of 10 equally parsimonious trees for Pachycereinae and outgroup taxa based on combined plastid sequences from the *trnL* intron, *trnL-F* spacer, and *rpl16* region. Bootstrap/jackknife percentages ( $\geq 50\%$ ) are given above branches.

general, resolution and support values increased with in both the *Pachycereus* and *Cephalocereus* groups.

**ITS.** Sequences length ranged from 779bp in *Pachycereus weberi* and 1023bp in *P. marginatus*. Aligned sequence length was 1065bp, and after exclusion of the two terminal regions the dataset was 619 characters long. Analysis of the ITS sequences resulted in 1344 equally parsimonious trees. The strict consensus tree (Fig. 3) is similar to those obtained with the plastid data, with a few exceptions. The *Lemaireocereus* group is not monophyletic in all trees, although the two species continue to be isolated from the rest of Pachycereinae. *Pachycereus militaris* is sister to the remainder of the subtribe rather than being a member of the *Pachycereus* group, *P. fulviceps* does not associate with the *Cephalocereus* group (but its position is not supported

by either the bootstrap or jackknife), and a clade of some *Pachycereus* species (e.g., *P. schottii*, *P. gatesii*, *P. gaumeri*, *P. marginatus*) are separated from the other members of the *Pachycereus* group (but their position in the tree is not supported either). In general, this data matrix has a higher amount of homoplasy relative to the other matrices (Table 3), and this factor may be responsible for some of the incongruent (but unsupported) relationships compared to the plastid data.

**Combined Data.** The ILD test between plastid and nuclear ITS data sets indicated that these are not significantly incongruent (Table 4). Combining all three data sets resulted in 375 equally parsimonious trees. These trees are 415 steps long (CI = 0.812, RI = 0.835) and the strict consensus is shown in Fig. 4. In general the topology is similar to the combined plastid DNA

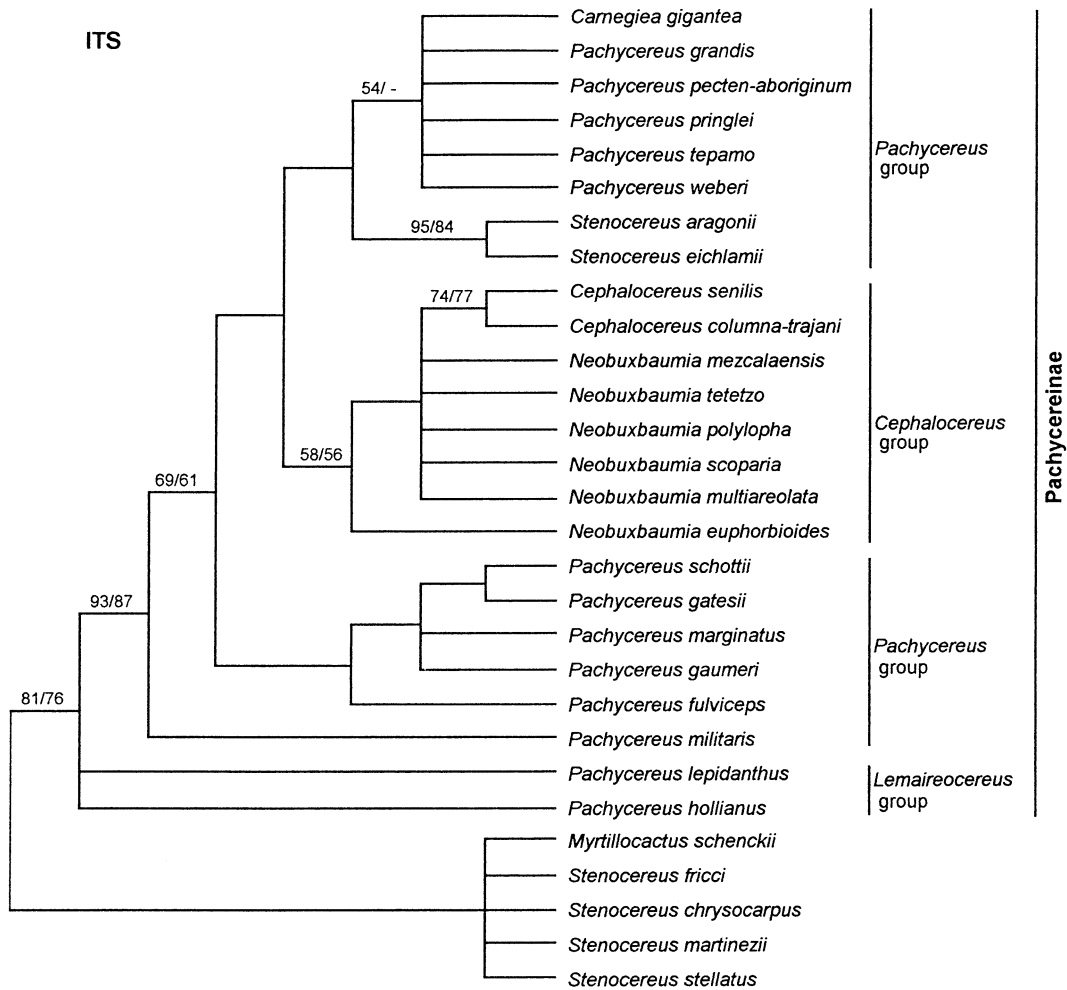


FIG. 3. Strict consensus of 1344 equally parsimonious trees for Pachycereinae and outgroup taxa based on nuclear ITS1, 5.8S, and ITS2 sequences (ITS). Bootstrap/jackknife percentages ( $\geq 50\%$ ) are given above branches.

tree. As in most of the previous analyses, *Pachycereus hollianus* and *P. lepidanthus* (the *Lemaireocereus* group) are monophyletic and sister to the remaining Pachycereinae. *Pachycereus fulviceps* is sister to a clade containing species of *Neobuxbaumia* and *Cephalocereus* (the *Cephalocereus* group), and within this clade *Cephalocereus* is monophyletic. Support for the large *Pachycereus* group decreased compared to the plastid DNA tree (52% bts vs 79% bts), but within this group a few monophyletic subclades are resolved and well supported. These include four species of *Pachycereus* (i.e., *P. marginatus* and its relatives), *P. gatesii* sister to *P. schottii*, and *Stenocereus aragonii* sister to *S. eichlamii*.

#### DISCUSSION

Our analyses do not support the monophyly of *Pachycereus* as circumscribed by Barthlott and Hunt (1993) or Anderson (2001). Similarly, subtribe Pachy-

cereinae can only be considered monophyletic if *Stenocereus aragonii* and *S. eichlamii* are treated within it. The plastid and combined data analysis recover three major subclades (informally referred to as the *Lemaireocereus*, *Cephalocereus*, and *Pachycereus* groups). These are discussed below.

**Lemaireocereus Group.** Separate and combined data analyses consistently pair *Pachycereus hollianus* with *P. lepidanthus*, which form the sister clade to all remaining members of Pachycereinae. *Pachycereus hollianus* is found in southern Mexico, and was previously placed in the genus *Lemaireocereus* (Britton and Rose 1909). These authors pointed out that the flowers of this species differ from most *Pachycereus* species by being shortly infundibuliform with white to pinkish petals. Buxbaum (1961), however, chose to include this species in *Pachycereus* arguing in favor of its similar floral morphology to other species of the genus and its

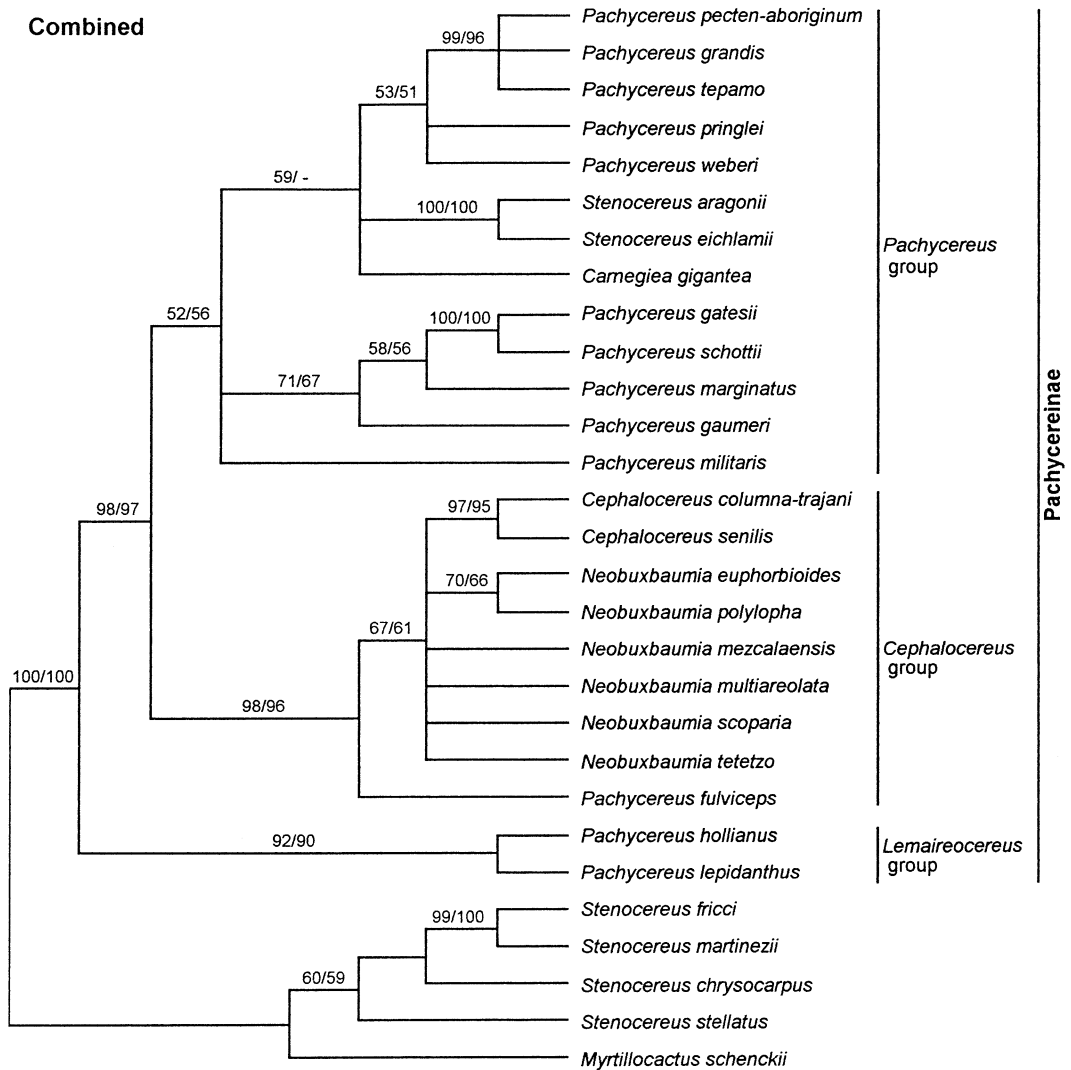


FIG. 4. Strict consensus of 375 equally parsimonious trees for Pachycereinae and outgroup taxa based on combined *trnL-F*, *rpl16*, and ITS sequences. Bootstrap/jackknife percentages ( $\geq 50\%$ ) are given above branches.

lack of funicular pearl cells. This placement was maintained by Bravo-Hollis (1978) and Gibson and Horak (1978), but Gibson (1982) later recognized that *P. hollianus* possesses a unique chemical composition and several seed characters that differ from all other species of *Pachycereus*. Based on this evidence, he suggested that this species could be sister to *Pachycereus*.

*Pachycereus lepidanthus*, a species endemic to Guatemala, is poorly studied. Britton and Rose (1920) included it in *Pachycereus* based only on the original description, although they commented on its floral similarity to *Escontria*. Backeberg (1938) proposed the new genus *Anisocereus* for this species based on distribution and morphological characters (flowers with coriaceous and fleshy bracts in their pericarpel and receptacular tube with trichomes and bristles). He suggested that

*Pachycereus gaumeri* might be its closest relative, since both species share several morphological attributes. However, Buxbaum (1961) transferred it to *Escontria*, and suggested that both species are basal members of the tribe Pachycereeae. Gibson and Horak (1978) and Gibson et al. (1986) also considered this taxon to be primitive, and placed *Anisocereus lepidanthus* at the base of their evolutionary proposal for the tribe Pachycereeae. Our results clearly show that *Pachycereus lepidanthus* and *P. hollianus* can be considered members of the subtribe Pachycereinae, but neither should be classified within *Pachycereus*. Instead, our results support either the recognition of two monotypic genera, *Lemaireocereus* and *Anisocereus* for these species, or simply *Lemaireocereus*, to which *P. lepidanthus* would be transferred.

**Cephalocereus Group.** All of our analyses support



*Cephalocereus* and *Neobuxbaumia* as a monophyletic clade sister to *P. fulviceps*. *Pachycereus fulviceps* is a unique species with a confused taxonomic history. Britton and Rose (1909) chose to include it in *Pachycereus* (= *P. chrysomallus*) based on similar floral structures such as the presence of bracts and dense trichomes that cover the pericarpel and receptacular tube. Backeberg (1938) transferred the species to *Cephalocereus* and classified it within subgenus *Mitrocereus*. Later, he changed the taxonomic rank of subgenus *Mitrocereus* to that of a genus (Backeberg 1942), and referred to distinctive differences between its reproductive and vegetative branches, in addition to having flowers covered by dense, long trichomes and bristles. Moreover, Bravo and Buxbaum (in Buxbaum 1961) proposed a new generic name, *Pseudomitrocereus*, for the same species. Gibson and Horak (1978) pointed out that *Pachycereus fulviceps* shares features with the genus *Neobuxbaumia* such as fruits with white pulp. We have observed that *Cephalocereus* also shares this feature. Gibson (1982) later suggested a relationship between *Pachycereus fulviceps* and *Carnegiea gigantea*, and even considered that they might represent a single genus. A proposal to include *P. fulviceps* in *Carnegiea* was carried out by the International Organization for Succulent Plant Study (Hunt and Taylor 1990), and Heath (1992) formalized the combination *Carnegiea fulviceps*. However, these two taxa have quite different fruits and vegetative anatomy (Terrazas and Loza-Cornejo 2002), and are not closely related as our molecular data point out. More recently, Barthlott and Hunt (1993) and Anderson (2001) included *P. fulviceps* again in *Pachycereus*, but without any evidence or explanation to justify their proposal.

In addition to fruits with white pulp, recent anatomical studies have shown that *Cephalocereus*, *Neobuxbaumia*, and *Pachycereus fulviceps* are all characterized by the presence of crystals in their dermal tissue (Gibson 1982; Terrazas and Loza-Cornejo 2002). These morphological characters corroborate the results of our DNA analyses and favor the exclusion of *P. fulviceps* from *Pachycereus*. We suggest that the monotypic genus *Pseudomitrocereus* should be resurrected for this species. Another possibility is to treat *P. fulviceps*, *Cephalocereus* (3–5 spp.) and *Neobuxbaumia* (9 spp.) as a single genus. Morphological and anatomical studies are currently underway to gain further insight into the relationship among these species.

**Pachycereus Group.** This clade of 13 taxa is moderately supported as monophyletic by cpDNA data, but is not recovered in the ITS tree (Figs. 2, 3). The combined analysis provides weak support for the clade and resolves several subclades (Fig. 4). The *Pachycereus* group includes the monotypic genus *Carnegiea*, two of the six sampled species of *Stenocereus*, and the remaining 10 species of *Pachycereus*. Five species of

*Pachycereus* (including *P. pringlei*, the type species of the genus) form a monophyletic group in the combined analysis. Three of these taxa (*P. grandis*, *P. pecten-aboriginum*, and *P. pringlei*) invariably have remained in the genus since Britton and Rose (1909) first erected it (Table 1). The fourth species, *P. weberi*, was included in *Lemaireocereus* by Britton and Rose (1909) based on its narrow infundibuliform flowers, and later in *Stenocereus* by Buxbaum (1961) and Bravo-Hollis (1978) based on the low density of shorter trichomes that cover its flowers. However, Backeberg (1960) and Gibson and Horak (1978) included it in *Pachycereus* based on the presence of alkaloids, lack of crystals in the dermal tissue, lack of funicular pearl cells, and presence of smooth, glossy black seeds. The fifth species in this group, *P. tepamo*, was described recently by Gama-López and Arias (1998), who suggested a close relationship of *P. tepamo* with *P. pecten-aboriginum* because of the presence of distinct vegetative and reproductive branches. However, they also considered that *P. tepamo* might be related to *P. weberi* because of their shared branch color. Our molecular data consistently indicate that *P. tepamo* is more closely related to *P. pecten-aboriginum* and *P. grandis* than to *P. weberi*.

*Pachycereus gatesii* and *P. schottii*, native to the Sonoran Desert, are strongly supported sister species in all analyses. Britton and Rose (1909) erected the genus *Lophocereus* including *P. schottii* (= *Cereus schottii*) based on distinctive vegetative (branches with differentiated reproductive and vegetative areas) and floral features (flower size and naked pericarpel and receptacular tube). Buxbaum (1961), Bravo-Hollis (1978), and Gibson and Horak (1978) maintained the position of both species in *Lophocereus*, but Barthlott and Hunt (1993) placed them into *Pachycereus* without supporting evidence. *Pachycereus marginatus* and *P. gaumeri* show a relationship to these species in our ITS and combined trees, confirming the hypothesis of Gibson and Horak (1978), Gibson et al. (1986), and Hartman et al. (2002) that *P. marginatus* might be related to *P. gatesii* and *P. schottii* (i.e., *Lophocereus*), since they share a common growth habit and several alkaloids. Moreover, our field observations suggest that they share the feature of ribs with acute margins, reproductive areoles with flexible spines, and more than one flower per areole. *Pachycereus marginatus* was included in the genus by Britton and Rose (1909), subsequently transferred to *Lemaireocereus* (Berger 1929), later to its own genus *Marginatocereus* (Backeberg 1938, 1942), and then to *Stenocereus* (Buxbaum 1961). Gibson and Horak (1978) argued in favor of its membership in *Pachycereus* based on its large, glossy seeds without ornamentation, lack of silica grains in its dermal tissue, and lack of funicular pearl cells. *Pachycereus gaumeri* was described as a species of *Pachycereus* by Britton and Rose (1920), then transferred to *Pterocereus*, and later treated as a mem-

ber of *Anisocereus* (Table 1; Bravo-Hollis 1978). It has a habit of disorderly growth, branches with few ribs, and flowers covered with large, green foliar bracts. These features make it unique among the species of this clade, and it has frequently been proposed to be the basal taxon in the tribe Pachycereeae (Buxbaum 1961; Gibson and Horak 1978). Our data do not support this idea, but are not fully satisfactory in explaining its position among the species of Pachycerinae either. If this group is maintained as monophyletic by further evidence, their biogeography suggests a vicariant distribution. *Pachycereus schottii* and *P. gatesii* are restricted to the Sonoran Desert, whereas *P. gaumeri* occurs in southeastern dry forests of Mexico, and *P. marginatus* in the Central Plateau.

The position of *Pachycereus militaris* within this subclade is poorly supported, but it is isolated from all other species of *Pachycereus* in most of our trees. *Pachycereus militaris* was previously classified as a monotypic genus, *Backebergia*, by Bravo-Hollis (1953) because of its terminal cephalium. Buxbaum (1961, 1975) pointed out the strong similarities in its habit, flowers, and large seeds with *Pachycereus*. However, the branching pattern of *P. militaris* is more dense and disorderly in comparison with the other *Pachycereus* species, and it exhibits highly differentiated reproductive branches that are recognized as true cephalia (Bravo-Hollis 1978; Terrazas and Arias 1999).

The *Pachycereus* group also contains two species of *Stenocereus*, *S. aragonii* and *S. eichlamii*, that are strongly supported as sister taxa, but their relationship to other taxa is unresolved. Both species are poorly studied and frequently confused in the field with other species of *Stenocereus* (e.g., *S. laevigatus* and *S. pruinosus*) based on architectural similarities. Gibson et al. (1986) pointed out that *S. aragonii* is the only species of *Stenocereus* with large (3 mm) glossy seeds, suggesting a relationship with *Pachycereus*. In fact, Heath (1992) proposed the combination *Pachycereus aragonii*, but without explicit discussion. Preliminary anatomical and morphological results corroborate the shared seed characters, and also confirm the lack of silica grains in the dermal tissue of *S. eichlamii* and other *Pachycereus* species (unpublished data). Since the presence of silica grains is one of the synapomorphies for most other species of *Stenocereus* (Terrazas and Loza-Cornejo 2002), we were not surprised by these molecular results and fully accept the exclusion of these two species from the remaining species of *Stenocereus*. However, further evidence is needed to assess their exact phylogenetic and taxonomic status.

Likewise, we do not wish to prematurely transfer *Carnegiea gigantea* (the well-known "saguaro" cactus of the Sonoran Desert) into *Pachycereus*, although our data weakly indicate that this would be justified. Gibson and Horak (1978) suggested that *Carnegiea* was related

to *Pachycereus*, but they did not present any detailed evidence to support this assertion. They also considered that *Carnegiea* could be related to *Neobuxbaumia* based on their shared habit, vegetative morphology, and floral structure. Based on this assertion, Hunt and Taylor (1990) proposed to broaden the limits of *Carnegiea* by including *Neobuxbaumia*, *Neodavsonia*, and *Pseudomitrocereus* in it. Interestingly, Heath (1992) transferred all *Neobuxbaumia* species to *Carnegiea* based on Gibson and Horak's assessment. This second hypothesis (*Carnegiea* and *Neobuxbaumia* as single genus) is clearly not supported by the molecular data presented here.

The major contribution of this paper is to provide a comprehensive phylogeny of *Pachycereus* and related genera of subtribe Pachycerinae, suggesting that a monophyletic genus *Pachycereus* might include only five species and would be sister to *Carnegiea*. Previous assertions that *Carnegiea* is closely related to *Neobuxbaumia* were based on convergent morphological features. Our results also suggest that *Backebergia*, *Lemairoocereus*, *Lophocereus*, and *Pseudomitrocereus* might have to be resurrected. However, we consider that our DNA data must be analyzed in combination with morphological data before proceeding with any substantial taxonomic or nomenclatural restructuring in the group. Characters such as presence of silica grains, seed and floral morphology, appear to be of particular systematic value. Further investigations, including the collection of fundamental morphological and anatomical evidence from *Pachycereus* and related taxa, are being pursued to attain increased phylogenetic resolution among these cacti. This will be published in a subsequent paper, and should allow for an interesting comparison to be made with the molecular data presented here.

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