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Molecular Phylogenetics of the Leafy Cactus Genus *Pereskia* (Cactaceae)

CHARLES A. BUTTERWORTH[1,3] and ROBERT S. WALLACE[2]

[1]Department of Botany, Iowa State University, Ames, Iowa 50011;
[2]Department of Ecology, Evolution and Organismal Biology, Iowa State University, Ames, Iowa 50011;
[3]Author for correspondence. Current Address: Desert Botanical Garden, 1201 North Galvin Parkway, Phoenix, Arizona 85008 (cbutterworth@dbg.org)

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ABSTRACT. Members of *Pereskia* exhibit some presumably plesiomorphic characters for the Cactaceae including shrubby habit, non-succulent or partially succulent leaves, and in some species, nearly superior ovaries. In addition, the members show a transition from perigynous flowers with half-inferior ovaries to those species having true receptacular epigyny (the predominant condition in the Cactaceae). To examine interspecific relationships within *Pereskia* we utilized cpDNA restriction-site data and sequences from two non-coding regions of the plastid genome—the *psbA-trnH* intergenic spacer and the *rpl16* intron. Maximum parsimony and Bayesian analyses identified three major clades: a clade containing the widespread *P. aculeata* and the Andean species, a clade containing six species found primarily in southeastern Brazil, Paraguay, Uruguay, Argentina, and Bolivia, and a third clade centered in southern Central America and the Caribbean. The relationship between these three clades and the rest of the Cactaceae remains unresolved, but our data do suggest that *Pereskia* may be paraphyletic. The sister taxon relationship for the yellow flowered species of *Pereskia* (*P. aureiflora*, *P. guamacho*) was also confirmed, despite their widely disjunct distribution.

For many, a typical cactus is a green, leafless stem-succulent plant with numerous spines. However, members of the genus *Pereskia* Miller are broad-leaved trees and shrubs. They are clearly members of the cactus family due to the presence of spine-bearing areoles, a floral cup with leaf-bearing nodes, and numerous perianth segments. Unlike other members of the family, the ovary in *Pereskia* ranges from superior to fully inferior. This feature, coupled with aspects of habit, physiology, and morphology have led some researchers to conclude that *Pereskia* species represent some of the most primitive members of the cacti (Gibson and Nobel 1986). Species of *Pereskia* are distributed throughout the northern two-thirds of South America (from northern Argentina) to Mesoamerica and the Caribbean. Backeberg (1942) concluded that the distribution of *Pereskia* indicates that the genus and the cactus family arose in Mesoamerica and the Caribbean.

Pereskia was first described as *Peireskia* by Plumier (1703) and Linnaeus (1753) used the name at species rank as *Cactus pereskia*. However, in the following year, Miller (1754) elevated the name to genus level in the first valid use of *Pereskia* at that rank. Berger (1926) believed that variation in the ovary position in *Pereskia* was sufficiently significant to warrant the description of subgenus *Rhodocactus* Berger, which was itself raised to genus level by Backeberg and Knuth (1935). The remaining species in the genus *Pereskia* were divided between two subgenera by Backeberg (1956), who placed the small-leaved Andean species in subgenus *Neopeireskia* Backeberg. More recently, authors such as Bravo-Hollis (1978) and Leuenberger (1986) have disregarded the genus *Rhodocactus*, preferring to recognize a more widely circumscribed genus *Pereskia*. The CITES Cactaceae Checklist (Hunt 1999) and Anderson (2001) accept 17 species and two subspecies.

The only recent monograph of *Pereskia* is that of Leuenberger (1986), in which he gives a detailed morphological, anatomical, and developmental account of the genus. He also presents an infrageneric treatment of the genus in which he puts forward an evolutionary and biogeographic hypothesis for the genus based upon a number of anatomical and morphological characters. Without being explicit, Leuenberger (1986) presents seven infrageneric groups based on a small suite of morphological characters (summarized in Table 1). The lack of clear-cut synapomorphies for *Pereskia* suggest that this genus represents a grade of ''basal'' taxa, and that an exploration of variation in the genus is important to our understanding of early evolution in cacti as a whole. This paper investigates evolutionary relationships in *Pereskia* and informal infrageneric groupings developed by Leuenberger (1986) by developing a phylogeny using a combination of sequence data from two chloroplast regions, *rpl16* intron and the *psbA-trnH* intergenic spacer (IGS), and chloroplast DNA (cpDNA) restriction site variation.

MATERIALS AND METHODS

We sampled 18 of the 19 taxa (17 spp. and 2 subsp.) of *Pereskia* as currently recognized in the CITES Cactaceae Checklist (Hunt 1999). We also included taxa from all other subfamilies of the Cactaceae, including a representative of the genus *Pereskiopsis* Britton and Rose, which is very similar in appearance to members of *Pereskia* due to the presence of persistent leaves. However, it is commonly included in the Opuntioideae due to the presence of glochids, seeds with a bony aril, and typically ''opuntioid'' flowers (Barthlott and Hunt 1993). Dickie (1996) also demonstrated the position of *Pereskiopsis* in the Opuntioideae using chloroplast DNA sequence data. For a non-cactus outgroup, we chose *Talinum paniculatum* from the Portulacaceae. Hershkovitz and Zimmer (1997) and Applequist and Wallace (2001) clearly demonstrated that members of the Portulacaceae form the sister group to the Cac-

TABLE 1. Infrageneric groups, species names, and character correlations in *Pereskia* according to Leuenberger (1986). Notes: [1]Brachyblast leaves absent in *P. quisqueyana*; [2]Some multiseriate trichomes present in *P. quisqueyana*; [3]Leuenberger (1986) did not observe fruit in *P. quisqueyana*; [4]Tuberous roots in *P. guamacho*, fibrous roots in *P. aureiflora*; [5]Fibrous roots in *P. zinniiflora*.

Group	Sclereids	Brachyblast leaves	Trichomes	Pollen	Fruit umbilicus	Roots	Stem stomata	Periderm formation
Group 1. *P. aculeata*	simple-fusiform	absent	some multiseriate	6–9 colpate	small	fibrous	absent	early
Group 2. *P. lychnidiflora*	simple-fusiform	present	some multiseriate	6–9 colpate	small	fibrous	absent	early
Group 3. *P. horrida* *P. diaz-romeroana* *P. weberiana*	simple-fusiform	absent	all uniseriate	3 colpate	small	tuberous	present	retarded
Group 4. *P. bleo*	aggregated-fusiform	absent	all uniseriate	9–12 colpate	large	fibrous	absent	early
Group 5. *P. stenantha* *P. bahiensis* *P. grandifolia* *P. sacharosa* *P. nemorosa*	aggregated-fusiform	present	all uniseriate	12–15 colpate	small	fibrous	absent to present	retarded
Group 6. *P. guamacho* *P. aureiflora*	stone cells	present	some multiseriate	12–15 colpate	small	tuberous & fibrous[4]	absent	retarded
Group 7. *P. zinniiflora* *P. portulacifolia* *P. quisqueyana*	stone cells	present[1]	all uniseriate[2]	12–15 colpate	medium[3]	tuberous[5]	absent	early

TABLE 2. List of taxa included in this study. B = Berlin Botanical Garden, HNT = Huntington Botanical Garden, ISU = Iowa State University. Data are in the following sequence: taxon name, botanic garden accession number or collector name and number, Genbank accession number for *rp116* intron, *psbA-trnH* IGS. Vouchers for all taxa are deposited in the herbarium of Iowa State University (ISC).

Subfamily Pereskioideae

Pereskia aculeata Miller, B 259-04-81-80, AY851589, AY851605; *Pereskia aureiflora* Ritter, B 166-54-83-20, AY851595, AY851569; *Pereskia bahiensis* Guerke, B 166-86-83-10, AY851605, AY851579; *Pereskia bleo* (Kunth) De Candolle, B 277-01-80-80, AY851600, AY851574; *Pereskia diaz-romeroana* Cardenas, ex Hort ISU, AY851592, AY851566; *Pereskia grandifolia* Haworth subsp. *grandifolia*, B 047-01-78-84, AY851603, AY851577; *Pereskia grandifolia* Haworth subsp. *violacea* (Leuenberger) Taylor & Zappi, B 036-01-77-30, AY851604, AY851578; *Pereskia guamacho* Weber, B 001-16-74-70, AY851596, AY851570; *Pereskia horrida* (Kunth) De Candolle subsp. *horrida*, B 039-04-77-30, AY851590, AY851564; *Pereskia horrida* (Kunth) De Candolle subsp. *rauhii* (Backeberg) Ostolaza, B 039-03-77-30, AY851591, AY851565; *Pereskia lychnidiflora* De Candolle, B 003-12-78-10, AY851594, AY 851568; *Pereskia nemorosa* Rojas, B 039-05-77-30, AY851601, AY851575; *Pereskia portulacifolia* (L.) Haworth, B 376-01-86-10, AY851598, AY851572; *Pereskia quisqueyana* Liogier, B 259-05-82-30, AY851599, AY851573; *Pereskia sacharosa* Grisebach, B 133-10-82-30, AY851602, AY851576; *Pereskia stenantha* Ritter, B 166-81-83-20, AY851606, AY851580; *Pereskia weberiana* Schumann, B 037-01-77-30, AY851593, AY851567; *Pereskia zinniiflora* De Candolle, B 200-01-80-30, AY851597, AY851571

Subfamily Opuntioideae

Opuntia polyacantha Haworth, *J. F. Weedin 1790*, AY851611, AY851585; *Opuntia subulata* (Muehlenpfordt) Englemann, *R. S. Wallace s.n.*, AY851612, AY851586; *Pereskiopsis porteri* (Brand. ex Weber) B & R., B 169-03-84-30, AY851607, AY851581; *Pterocactus kuntzei* Schumann, *F. Katterman 621* AY851613, AY851587

Subfamily Maihuenioideae

Maihuenia poeppigii (Pfeiffer) Shumann, *F. Katterman s.n.*, AY851609, AY851583

Subfamily Cactoideae

Calymmanthium substerile Ritter, HNT 46555, AY851614, AY851588; *Leptocereus quadricostatus* (Bello) Britton & Rose, ex Hort ISU, AY851608, AY851582

Outgroup—Portulacaceae

Talinum paniculatum (Jacq.) Willd., ex Hort ISU, AY851610, AY851584

taceae. Table 2 lists the sources for living material used in this study as well as GenBank accession numbers for all sequences generated. Voucher material is deposited in the herbarium at Iowa State University (ISC).

Total genomic DNA was isolated using a modified organelle pellet method suitable for mucilaginous material (Wallace 1995; Wallace and Cota 1996), briefly summarized as follows: fresh, chlorenchymatous plant tissue was homogenized in a 0.35M sorbitol buffer and filtered through Miracloth™ (EMD Biosciences Inc., San Diego, California). The organelles were pelleted, supernatant removed, and pellets were then suspended in 2x CTAB (Doyle and Doyle 1987) for 1 hr at 60°C. After partitioning against $CHCl_3$: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.

With the exception of four taxa (*Calymmanthium substerile*, *Opuntia polyacantha*, *O. subulata*, and *Pterocactus kuntzei*), all samples were cut with a battery of 18 restriction endonucleases (*Ava*I, *Bam*HI, *Ban*I, *Ban*II, *Bcl*I, *Bgl*II, *Bst*NI, *Cla*I, *Dra*I, *Eco*O109, *Eco*RI, *Eco*RV, *Hinc*II, *Hind*III, *Nci*II, *Nsi*I, *Xba*I, and *Xmn*I). The digested DNA fragments were separated using agarose gel electrophoresis in TAE buffer. Following electrophoresis, the DNA fragments were bidirectionally transferred (Smith and Summers 1980) to nylon membranes (Zetabind AMF-Cuno, Meridian, Connecticut). The fragments were then hybridized with nick-translated [^{32}P] plasmid probes according to Jansen and Palmer (1987). Recombinant plasmid subclones for the chloroplast genome of *Nicotiana tabacum* L. (Shinozaki et al. 1986) were used to assess restriction site variation according to Palmer (1986). Restriction site variation was identified relative to the condition observed in the outgroup taxon *Talinum*. These were scored as binary characters (0, 1) for absence or presence. Cells with missing data were scored as 'N'.

Polymerase chain reaction (PCR) amplification of the *rpl16* intron and the *psbA-trnH* intergenic spacer was conducted in 100 µl reactions using GeneAmp™ PCR Core Reagents (Perkin Elmer). Primers and PCR reaction conditions used for amplification and sequencing are detailed in Butterworth and Wallace (2004).

After agarose electrophoresis confirmation of amplification, the amplicons were cleaned and concentrated in Microcon 100 spin microconcentrators (Amicon Inc.) following the manufacturer's directions. The products were quantified using an ultraviolet spectrophotometer and diluted to 50 µg ml^{-2} for use in sequencing reactions.

Sequence data for the *rpl16* intron and *psbA-trnH* IGS were obtained 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 Big Dye to terminator ready reaction solution gave acceptable reads. Electrophoresis and automated sequence readings were conducted using Perkin Elmer/Applied Biosystems automatic sequencing units (ABI Prism 377) at the Iowa State University DNA Sequencing and Synthesis Facility. For the *rpl16* intron, sequences typically were 650 or more nucleotides in length. Due to extensive poly-A and poly-T regions in Domain I at the 5′ end, 150–200bp of the *rpl16* 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. For a number of taxa, poly-A regions thwarted attempts to sequence approximately 100 bp of the middle region of the *psbA-trnH* IGS.

Sequence alignment was carried out using AutoAssembler (Applied Biosystems 1995) and Se-Al (Rambaut 1995). Sequences were aligned manually. Insertions/deletions considered to be phylogenetically informative (Graham et al. 2000) were coded as binary characters (presence/absence) and added to the end of the data matrix. Areas where alignments were of doubtful homology were excluded from the analyses. A total of 1,698 (3.6%) of the cells were scored as missing data. The complete data matrix is available from TreeBASE (study accession no. S1343; matrix accession no. M2364) or from the corresponding author.

Incongruence Length Difference (ILD) tests (Farris et al. 1995) were undertaken to assess congruence and hence combinability of the three datasets (cpDNA restriction site, *rpl16* intron and *psbA-trnH* IGS). The ILD tests was conducted in PAUP* 4.0b2 (Swofford 1999) for 100 replicates, saving 1,000 most-parsimonious trees for each replicate.

Parsimony analyses were done using the heuristic search option in PAUP*. 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 1,000 most-parsimonious trees for each replicate, 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 1998). Bootstrap values were estimated for 1,000 replicates using a heuristic search with the same parameters as above.

A Bayesian analysis was also undertaken on the combined dataset using MrBayes version 3 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) in a 4 chain (three hot, one cold) Markov chain Monte Carlo run for a million cycles with sampling every 100th cycle. ModelTest (Posada and Crandall 1998) was used to determine an appropriate model of sequence evolution and recommended the F81 (Felsenstein 1981) model plus a gamma distribution—F81+Γ. Following the Bayesian analysis, tree posterior probabilities were graphed to allow an estimate of the number of trees to be discarded as ''burn-in.'' The majority-rule consensus was created from the trees produced by the Bayesian analysis after the first 168 trees had been discarded as ''burn-in.''

Results

The cpDNA restriction site data yielded 206 characters and the chloroplast DNA sequence data contributed 992 and 586 characters for the *rpl16* intron and *psbA-trnH* IGS respectively. The total, aligned data matrix, including binary coded indels, had 1,789 characters. After exclusion of 20 nucleotides from *psbA-trnH* that were of doubtful homology, the dataset consisted of 1,769 characters, of which 201 were parsimony informative (RFLP = 88, *psbA-trnH* IGS = 63, *rpl16* intron = 50). The g-statistics for the datasets were −0.96 and −1.45 for the cpDNA restriction site and sequence data respectively, indicating strong phylogenetic signals (Hillis and Huelsenbeck 1992).

The results of the ILD tests clearly indicated that there was sufficient congruence between all datasets to justify combining data (RFLP vs. *rpl16* = 0.05; RFLP vs. *psbA-trnH* = 0.34; *rpl16* vs. *psbA-trnH* = 0.63). Only phylogenies derived from the combined dataset are presented here.

The initial heuristic search in PAUP using the combined data found six most parsimonious trees of 638 steps with a consistency index (CI) of 0.84 (rescaled CI = 0.70), homoplasy index (HI) of 0.16 (rescaled HI = 0.30), and retention index of 0.81. The strict consensus of the six most-parsimonious trees is shown in Fig. 1. In terms of the number of clades recovered by the analyses, the strict consensus tree (Fig. 1) is 80% resolved (i.e., resolution index = 0.80; Butterworth and Wallace 2004).

The general topology of the strict consensus tree from the parsimony analysis (Fig. 1) shows that the Patagonian species *Maihuenia poeppigii* along with members of subfamily Opuntioideae form a polytomy with the remaining genera of the Cactaceae. Although forming a polytomy, sampled members of subfamily Opuntioideae (*Pereskiopsis*, *Opuntia* and *Pterocactus*) are strongly supported as a single clade with a bootstrap (BS) of 100% and a decay value of 20 steps.

Of the remaining members of the Cactaceae, a single, albeit moderately supported, clade (BS = 74%, decay = 1) contains all species of *Pereskia* sampled and two genera of subfamily Cactoideae (*Calymmanthium* and *Leptocereus*); *Pereskia* does not form a monophyletic group in this phylogeny. A well-supported clade (BS = 100%, decay = 11) contains the widespread *P. aculeata* and the Andean species (*P. diaz-romeroana*, *P. weberiana* and both subspecies of *P. horrida*). Although statistical support is moderate (BS = 74%, decay = 0), a single clade unites the Caribbean species of *Pereskia* (*P. zinniiflora*, *P. portulacifolia*, *P. quisqueyana*) with species distributed in Colombia (*P. bleo*) and Venezuela (*P. guamacho*). Also included in this clade is the yellow flowered *P. aureiflora* from southeastern Brazil, which is sister to *P. guamacho*, the only other yellow-flowered species of *Pereskia* (BS = 78, decay = 2). The remaining species form two moderately to well-supported clades: 1) *P. lychnidiflora*, *Leptocereus quadricostatus*, and *Calymmanthium substerile*, and 2) the Grandiflora Group, *P. bahiensis*, *P. stenantha*, *P. grandifolia*, *P. sacharosa*, and *P. nemorosa*.

Although the phylogeny recovered by the Bayesian analysis (Fig. 1) is not as well resolved as that from the parsimony analysis (due to the large polytomy towards the base of the tree), it is largely congruent with the most parsimonious tree (Fig. 1). In the Bayesian tree, *Maihuenia* forms the sister species to all remaining members of the Cactaceae with a posterior probability (PP) of 0.84. Those clades recovered by the maximum parsimony analysis but not recovered by the Bayesian analysis all had little to no bootstrap support. Similarly, most of the well supported (BS ≥ 90) clades from the MP analysis were well supported by the Bayesian analysis (PP ≥ 95%).

Discussion

***Intergeneric Relationships of* Pereskia.** Karl Schumann (1898) placed *Pereskia* in the subfamily Pereskioideae, which also included the genus *Maihuenia* (Philippi ex Weber) Schumann. A number of subsequent workers (Backeberg 1970; Barthlott and Hunt 1993; Gibson and Nobel 1986; Hunt and Taylor 1986) continued this classification. However, Britton and Rose (1920) placed *Maihuenia* in their tribe Opuntieae rather than with *Pereskia*. Wallace (1995) reported that preliminary cpDNA restriction site data for *Pereskia* indicated that the placement of *Maihuenia* within sub-

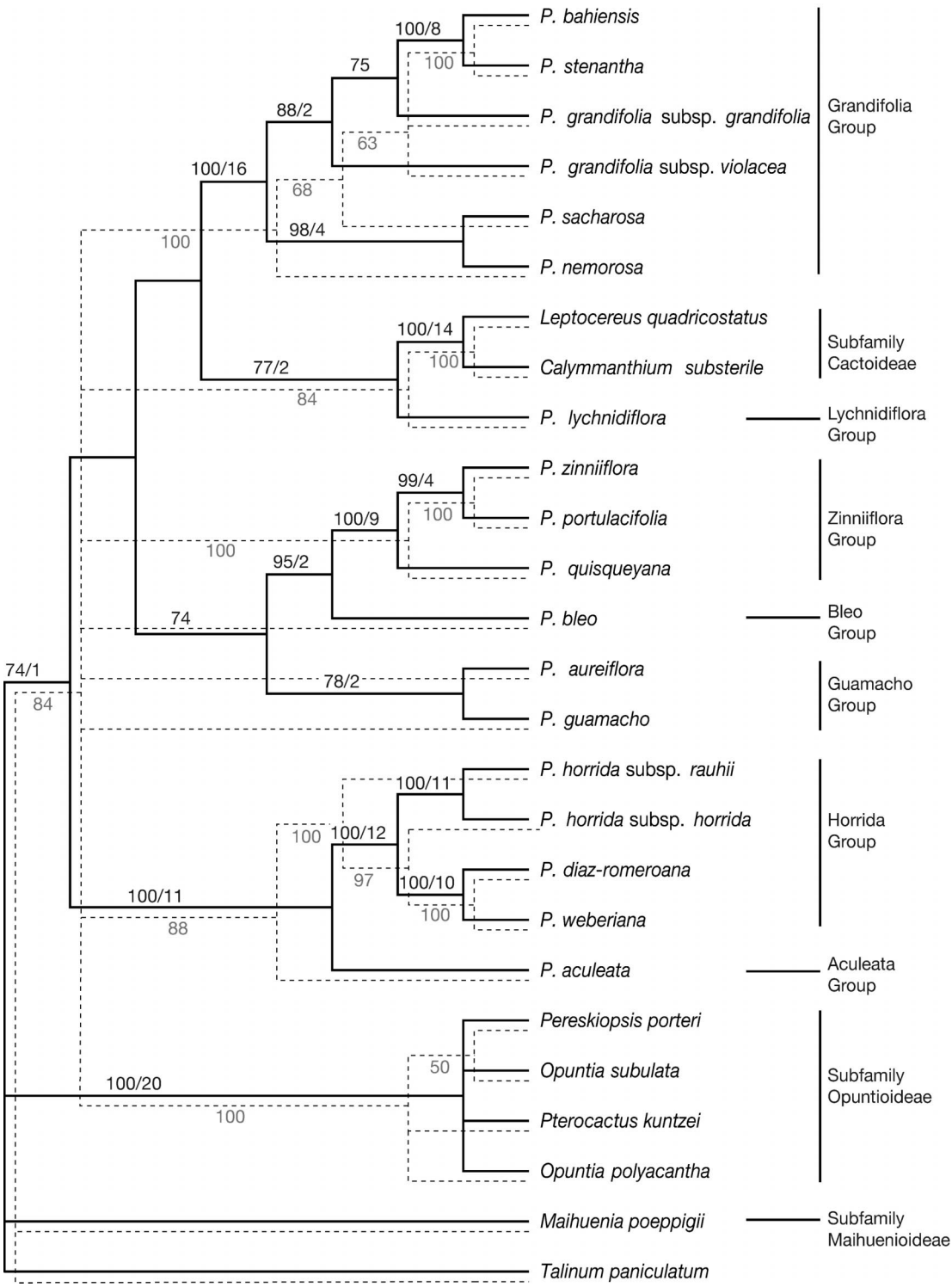

FIG. 1. Strict consensus (solid lines) tree of six most parsimonious trees for the combined cpDNA RFLP, *rpl16* intron, and *psbA-trnH* IGS data. Bootstrap values greater than 50% for 100 replicates are given above the branches followed by decay values. The Bayesian tree is shown as dashed lines with Bayesian posterior probabilities shown below the branches. Infrageneric groupings within *Pereskia* referred to in the text (following Leuenberger, 1986) are shown to the right of the cladogram.

family Pereskioideae needed reevaluation. Fearn (1996) circumscribed subfamily Maihuenioideae to include only the genus *Maihuenia*. The data presented here moderately support (BS = 74%, decay = 1) the exclusion of *Maihuenia* from both subfamilies Pereskioideae and Opuntioideae.

Leuenberger (1986) believed that the presence of leaves as primary photosynthetic organs and variable ovary position indicated that *Pereskia* represents the most ''primitive'' genus within Cactaceae. Gibson and Nobel (1986) also referred to *Pereskia* as possessing the most ''primitive'' features in the Cactus family. The occurrence of Crassulacean Acid Metabolism (CAM) cycling in species of *Pereskia* and *Maihuenia* (Martin and Wallace 2000) rather than obligate CAM further supports this hypothesis. Furthermore, facultative CAM metabolism has been observed in *P. guamacho* (E. Edwards, pers. comm.). However, the phylogeny presented in this paper contradicts Leuenberger's (1986) hypothesis that *Pereskia* represents the most ''primitive'' cacti, suggesting that the genus *Maihuenia* forms the sister-group to all other cacti. If our phylogeny is a good representation of evolution in Cactaceae, and if the presence of persistent leaves in *Pereskia* is a plesiomorphic condition for the family, then the loss of leaves and the acquisition of a stem succulent habit (found in the subfamilies Opuntioideae and Cactoideae) must have occurred independently in each of these cactus lineages.

The phylogeny shown in Fig. 1 demonstrates that subfamily Cactoideae (represented in this study by *Calymmanthium substerile* and *Leptocereus quadricostatus*) is nested within *Pereskia*, rendering it paraphyletic. The genus *Pereskiopsis* (subfamily Opuntioideae) is clearly not a member of *Pereskia*. This genus, along with *Quiabentia* Britton and Rose (not sampled for this study) is unusual within the Opuntioideae due to the presence of persistent leaves. However, all members of the Opuntioideae (including *Pereskiopsis* and *Quiabentia*) have glochids and seeds possessing a bony aril—features which are not found elsewhere in the Cactaceae (Anderson 2001).

***Infrageneric Relationships within* Pereskia.** Leuenberger's (1986) subgeneric groupings within *Pereskia* (outlined in Table 1) receive considerable support in the MP phylogeny presented in Fig. 1. The clade containing *P. aculeata* and the Horrida Group is well supported in our phylogeny (BS = 100%, decay = 11). Leuenberger (1986) treated *P. aculeata* by itself, whereas the other members of this clade were placed in the Horrida Group. Although the natural range of *P. aculeata* is not clear—it is found throughout eastern South America and into the Caribbean (Leuenberger 1986), the members of the Horrida Group have very distinct distributions in the Andes of Peru and Bolivia. This strictly Andean clade is well supported (BS = 100%, decay = 12; Fig. 1), and is morphologically and anatomically characterized by reduced leaf size, tricolpate pollen, tuberous roots, stem stomata, delayed periderm formation, and lack of brachyblast leaves (Leuenberger 1986). Both subspecies of *P. horrida* (treated as *P. humboldtii* by Leuenberger, 1986) are found in northern Peru in the Río Marañón drainage system (Leuenberger 1986). Further south, *Pereskia weberiana* is found in the dry open forests of the Río Beni drainage area (Leuenberger 1986). *Pereskia diaz-romeroana* is distributed in Central Bolivia. *Pereskia weberiana* and *P. diaz-romeroana* form a well-supported clade (BS = 100%, decay = 10; Fig. 1). Leuenberger (1986) states that although these species are very closely related, they can be distinguished because *P. weberiana* lacks the long hairs on the receptacular and fruit areoles. *Pereskia diaz-romeroana* also has purplish-red stamens, as opposed to white-pink in *P. weberiana*.

The clade consisting of Guamacho, Bleo, and Zinniiflora Groups are from northern South America and the Caribbean. All members of this clade (with the exception of *P. bleo*) possess stone cells (short, roughly isodiametric sclereids). All other species of *Pereskia* (including *P. bleo*) possess either simple or aggregated fusiform sclereids (Leuenberger 1986). Within this clade, *Pereskia aureiflora* (Brazil) and *P. guamacho* (Colombia and Venezuela) comprise the Guamacho Group which corresponds to one of Leuenberger's (1986) species groups. This group is noteworthy as it contains the only species with yellow flowers and provides possible evidence of an independent, long-distance dispersal event from northern South America into Brazil.

The Brazilian and Argentinean species of *Pereskia* found in the Grandiflora Group (Fig. 1) were united into a single group by Leuenberger (1986) and form a well supported clade (BS = 100%, decay = 16; Fig. 1). These species have aggregated fusiform sclereids and 12–15 colpate pollen with the exception of *P. grandifolia*, which has 9–15 colpi (Leuenberger 1986). *Pereskia nemorosa* and *P. sacharosa* have been confused in the past. *Pereskia nemorosa* is distributed in southern Brazil, Paraguay, northeastern Argentina, and Uruguay whereas *P. sacharosa* is generally found further west in southeastern Brazil, Bolivia, Paraguay, and northwestern Argentina. *Pereskia nemorosa* also has the largest flowers in the genus, besides the presence of staminodal hairs which are lacking in its closest relatives (Leuenberger 1986). The natural distribution of *P. grandifolia* is uncertain—it is commonly planted throughout eastern South America, the Caribbean, Central America, and Florida. Specimens of *P. grandifolia* subsp. *violacea* were misidentified as *P. bahiensis* by a number of authors including Leuenberger (1976), Barthlott (1979), and Rauh (1979). Subsequently Leuenberger (1986) formally described *P. grandifolia* var. *violacea* (as distinct from *P. bahiensis*) noting that while herbarium specimens of

var. *violacea* are almost impossible to distinguish from var. *grandifolia*, there are notable differences in the coloration of bracts and flower buds in live material. Taylor and Zappi (1997) changed the rank of this taxon to subspecies. Our phylogeny (Fig. 1) clearly separates *P. bahiensis* from *P. grandifolia* var. *violacea*, supported by 12 unique mutational differences in our dataset (nine RFLP gains, three single point mutations in the *psbA-trnH* IGS and *rpl16* intron). Furthermore, only a single unique RFLP gain supports the clade containing *P. grandifolia* var. *grandifolia*, *P. bahiensis*, and *P. stenantha*.

Pereskia stenantha and *P. bahiensis* form a species pair in our phylogeny (BS = 100%, decay = 6), supported by five unique RFLP gains and a single, unique nucleotide substitution in the *rpl16* intron. Leuenberger (1986) noted that these two species are almost identical vegetatively. However, the flowers of *P. stenantha* are unique in the genus due to their urceolate corolla and larger nectary, indicative of hummingbird pollination; Ritter (1979) suggested this may be due to rapid evolution in this taxon. *Pereskia bahiensis* is very similar in appearance to *P. grandifolia* but according to Leuenberger (1986) it has shorter, fleshier leaves, smaller seeds and inflorescences containing fewer flowers.

***Biogeography of* Pereskia.** The distribution of *Pereskia* in Central and South America ranges from the Mexican state of Guerrero through Central America, along the eastern edges of the Andes into Argentina, eastward into the West Indies and southward to Brazil, Uruguay, and Paraguay. Leuenberger (1986) states that the genus is doubtfully native in Florida and is absent from the Pacific side of the Andes. There are a number of areas of endemism—the Greater Antilles, Brazil, and eastern Andes of Bolivia and Peru.

Based on the presence of assumed ''primitive'' characters in the genus, Backeberg (1942) postulated that ancestors of the cactus family would be found in the region of the West Indies and Central America, followed by dispersal of cacti to the arid zones of North and South America. Leuenberger (1986) does not totally accept the reasoning of Backeberg, preferring a center of origin for the Cactaceae that is located on the northwestern South American continent. He states that the dispersal of the major groups of cacti to North America and the Caribbean, and the fact that only one species of cactus has dispersed to Africa, supports his hypothesis. Applequist and Wallace (2001) investigated the biogeography of the ''Portulacaceous Cohort'' based upon chloroplast *ndhF* sequence data. Their data showed that the majority of species in the sister-clade to the Cactaceae have modern-day distributions in North and South America, giving credence to a New World origin for the Cactaceae.

Based upon our phylogeny (Figs. 1, 2), Leuenberger's (1986) hypothesis of the origin of *Pereskia* in northwestern South America appears reasonable. However, in the absence of fossil data for cacti and a better understanding of divergence times for the family and its subfamilies, a more detailed discussion of the location of origin for the Cactaceae and *Pereskia* can only be speculation. This is certainly the case considering the phylogenetic placement (albeit only moderately supported, Fig. 1) of *Maihuenia*, whose extant members with a geographic distribution in southern South America are far from the hypothesized center of origin for the cactus family. If Leuenberger's theory of the origin of *Pereskia* is accurate, then our data support an early divergence of *Pereskia* with migration of one lineage along the Andes to the region of present day Bolivia and Peru (Fig. 2). This region currently harbors *P. horrida*, *P. diaz-romeroana*, and *P. weberiana*, which, along with the widespread *P. aculeata*, form the sister-clade to all other species within the genus. A major migration into Brazil, Paraguay, Bolivia, Uruguay and Argentina is indicated by the clade containing *P. grandifolia*, *P. stenantha*, *P. bahiensis*, *P. nemorosa* and *P. sacharosa* (Fig. 2). The species from northern South America (*P. guamacho* and *P. bleo*) are closely related to the Caribbean species (*P. quisqueyana*, *P. zinniiflora* and *P. portulacifolia*); they form a well-supported clade indicative of a single migration into the Caribbean (Fig. 2).

Evolution of the Earliest Cactus Lineages. The placement of *Maihuenia* in our phylogeny (Fig. 1) is problematic with respect to the concept of *Pereskia* representing the earliest lineages of the cacti. To date, relationships among the basal lineages of the cacti have not been fully resolved. Hershkovitz and Zimmer (1997) placed the Cactaceae as a monophyletic assemblage within members of the Portulacaceae. Although their taxon sampling for the Cactaceae was limited to five species (one species of *Pereskiopsis*, two of *Pereskia*, and two of *Maihuenia*), their phylogeny indicated that *Pereskia* was paraphyletic due to the inclusion of *Maihuenia* and *Pereskiopsis*, the latter belonging to subfamily Opuntioideae. Wallace (1995) presented a phylogeny of the Cactaceae based upon *rbcL* sequences in which a ''basal'' polytomy within the family is formed between members of the Opuntioideae, *Pereskia*, *Maihuenia*, and the Cactoideae. Nyffeler (2002) used *trnK/matK* and *trnL-trnF* sequences for 70 members of the Cactaceae. While his data further supported the concept of monophyletic subfamilies Cactoideae and Opuntioideae, members of *Pereskia* formed a basal grade, and both species of *Maihuenia* formed a monophyletic group in a weakly supported sister-group relationship to the Opuntioideae. Work is currently in progress to evaluate relationships between the ancestral lineages of the cacti through the use of DNA sequence data (E. Edwards, pers. comm.).

It must be noted that the data presented in this paper do not resolve relationships robustly enough to al-

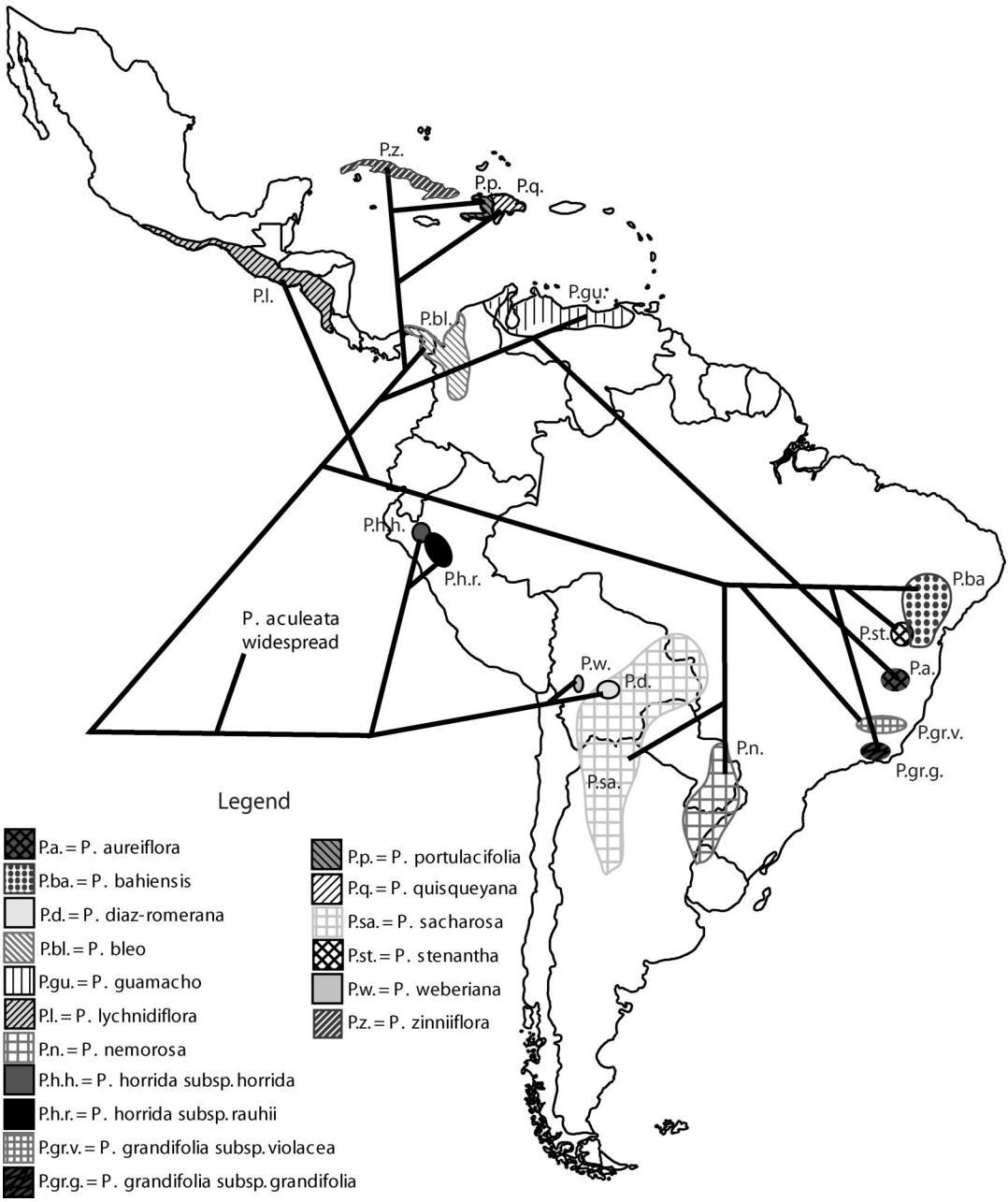

FIG. 2. Distribution and biogeography of *Pereskia*. The cladogram is adapted from the most parsimonious tree shown in Fig. 1 and distributions are for assumed natural populations after Leuenberger (1986).

low confident taxonomic changes to be made. The use of cpDNA restriction sites can be criticized because homologies within the cpDNA restriction site data are assumed. However, because of the use of hybridization probes, false homologies within the cpDNA restriction site dataset are highly unlikely. The markers chosen for our study were also selected because they have been shown to evolve at a rate rapid enough for study at the species level (the original purpose of study). Further morphological studies need to be undertaken and combined with molecular data into a more thorough synthesis of *Pereskia*, Pereskioideae, and Cactoideae.

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