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BASAL CACTUS PHYLOGENY: IMPLICATIONS OF *PERESKIA* (CACTACEAE) PARAPHYLY FOR THE TRANSITION TO THE CACTUS LIFE FORM[1]

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The cacti are well-known desert plants, widely recognized by their specialized growth form and essentially leafless condition. *Pereskia*, a group of 17 species with regular leaf development and function, is generally viewed as representing the "ancestral cactus," although its placement within Cactaceae has remained uncertain. Here we present a new hypothesis of phylogenetic relationships at the base of the Cactaceae, inferred from DNA sequence data from five gene regions representing all three plant genomes. Our data support a basal split in Cactaceae between a clade of eight *Pereskia* species, centered around the Caribbean basin, and all other cacti. Two other *Pereskia* clades, distributed mostly in the southern half of South America, are part of a major clade comprising *Maihuenia* plus Cactoideae, and Opuntioideae. This result highlights several events in the early evolution of the cacti. First, during the transition to stem-based photosynthesis, the evolution of stem stomata and delayed bark formation preceded the evolution of the stem cortex into a specialized photosynthetic tissue system. Second, the basal split in cacti separates a northern from an initially southern cactus clade, and the major cactus lineages probably originated in southern or west-central South America.

Key words: biogeography; Cactaceae; inferior ovary; *Pereskia*; phylogeny; phytochrome C; stem-based photosynthesis; trnK/matK.

The Cactaceae contain 1500–1800 species renowned for their remarkable morphological and physiological adaptations to drought (Barthlott and Hunt, 1993). Although they are found in a range of environments, they are especially conspicuous components of the New World's arid regions and represent one of the world's most spectacular desert radiations. The great majority of cactus diversity is found in two major lineages, the Opuntioideae and Cactoideae. Most members of these groups are what might be regarded as "typical" cacti: they are stem succulents with only vestigial or ephemeral leaves and a well-developed photosynthetic stem cortex with CAM carbon metabolism, specialized "collapsable" xylem cells that aid in water storage (Schleiden, 1845; Mauseth et al., 1995), deeply recessed inferior ovaries, and specialized short shoots (areoles) with very reduced internodes that produce spines, new long shoots, glochids (in Opuntioideae), and flowers. The remaining cacti consist of two small genera, *Pereskia* and *Maihuenia*. These two were at one time united into a third subfamily, Pereskioideae, but this grouping is supported only by the shared absence of many of the typical cactus characters noted above, and in recent years *Maihuenia* has been placed in its own subfamily, Maihueniodeae. Multiple phylogenetic studies support the monophyly of the opuntioid and cactoid subfamilies, but their relationships to *Maihuenia* and *Pereskia* have remained unresolved. This is due both to limited sampling of *Pereskia* (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; Nyffeler, 2002), as well as an inability of the molecular data to resolve basal cactus nodes (Wallace, 1995). To date, no studies have confirmed or rejected the monophyly of *Pereskia*.

Pereskia species are often interpreted as "relictual cacti," and are used as a general model of the ancestral condition from which the highly specialized morphology and physiology of the core cacti arose (Rauh, 1979; Gibson and Nobel, 1986; Mauseth and Landrum, 1997). *Pereskia* species are widely distributed in the Caribbean and Central and South America in a range of drier forest habitats. They have been described as having superior to inferior ovaries, broad, flattened leaves with C3 photosynthesis, areoles with leaf production, dense, fibrous wood, a simple cortex without cortical bundles, poorly developed stem epidermal and hypodermal layers, nonsucculent tissues, and as inhabiting relatively mesic environments (Mauseth and Landrum, 1997). This generalized depiction of *Pereskia* species has led botanists to believe that the stem succulent cacti are derived from woody, nonsucculent trees with C3 photosynthesis, as opposed to other growth forms (e.g., an herbaceous, succulent CAM plant) (Griffith, 2004).

While the "*Pereskia* model" has been useful, it also downplays some potentially important ways in which *Pereskia* species differ from one another. An alternative perspective of *Pereskia* is supported by other studies that have emphasized the substantial ecological, morphological, and anatomical diversity found within *Pereskia* (Schumann, 1898; Berger, 1926, 1929; Boke, 1954; Backeberg, 1958; Bailey, 1962; Bailey, 1963a, b, c, d; Boke, 1963, 1966, 1968; Leuenberger, 1986; Martin and Wallace, 2000). In his monograph of the genus, Leuenberger (1986) delineated several species groups within *Pereskia* that

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TABLE 1. *Pereskia* species groups and distribution of traits, as outlined by Leuenberger (1986).

Species	Delayed bark formation	Stem stomata	No areole leaf production	Tuberous roots	Inferior ovary
Pereskia aculeata	—	X	X	—	—
Pereskia grandifolia	X	X	—	—	—
Pereskia bahiensis	X	X	—	—	—
Pereskia sacharosa	X	X	—	—	—
Pereskia stenantha	X	X	—	—	—
Pereskia nemorosa	X	—	—	—	—
Pereskia horrida	X	X	X	X	—
Pereskia diaz-romeroana	X	X	X	X	—
Pereskia weberiana	X	X	X	X	—
Pereskia bleo	—	—	X	—	X
Pereskia quisqueyana	—	—	X	X	X
Pereskia portulacifolia	—	—	—	X	X
Pereskia marcanoi	—	—	—	X	X
Pereskia zinniiflora	—	—	—	X	X
Pereskia lychnidiflora	—	—	—	—	—
Pereskia guamacho	—	—	—	X	—
Pereskia aureiflora	—	—	—	—	—

are marked by particular combinations of vegetative and reproductive features (Table 1), but gave no indication of how these subgroups might be related to one another, nor did he explicitly argue that *Pereskia* is monophyletic. Our ability to infer early events in the evolutionary history of the cacti rests squarely on resolving two outstanding problems in cactus phylogeny: (1) whether *Pereskia* is monophyletic, and (2) how *Pereskia* species are related to the rest of the cacti.

To address these questions, we obtained new DNA sequences from all species of *Pereskia* and *Maihuenia* and from representatives of Opuntioideae, Cactoideae, and selected Portulacaceae. We selected five gene regions representing the three plant genomes: the nuclear gene phytochrome C, *rbcL, trnK/matK,* and *psbA-trnH* IGS from the chloroplast genome, and the mitochondrial gene *cox*3. Based on phylogenetic analyses of these data, we present a new hypothesis of basal relationships in Cactaceae and highlight how these findings bear on our understanding of early cactus evolution. We focus specifically on historical biogeography and the earliest steps in the transition to stem-based photosynthesis.

MATERIALS AND METHODS

Sequence generation—Total genomic DNA was isolated from fresh or dried leaf or cortical tissue using a slightly modified procedure of the DNeasy Plant Mini kit (Qiagen, Valencia, California USA) as described in Nyffeler (2002). In some instances, extractions were so mucilaginous that the precipitation step was repeated.

Five distinct gene regions representing all three plant genomes were amplified and sequenced using standard primers found in the literature (*rbcL*: Bousquet et al., 1992; *trnK/matK*: Johnson and Soltis, 1994; Nyffeler, 2002; *psbA-trnH* IGS: Sang et al., 1997; *phyC*: Mathews and Donoghue, 1999; *cox*3: Duminil et al., 2002) with the exception of *phy*C, for which two new internal sequencing primers were designed (phyC454R: 5′ GSACCCATGTTTGCC 3′ and phyC701F: 5′ GGATTCAAACTGTGC 3′). Fragments were amplified in 25 μL reactions (1 μL genomic DNA, 0.2 μL AmpliTaq polymerase [Applied Biosystems, Foster City, California USA], 2.5 μL, 2.5 mmol dNTP, 2.5 μL 10× buffer, 2.5 μL 10 mmol $MgCl_2$, 2.5 μL 10 mmol primer, 1–2 μL BSA, 9.5–11.5 μL ddH_20) using an automated thermal cycler and standard PCR protocols, with PCR cycling times as follows for the different gene regions: *rbcL*: 95°C for 5 min, 40 cycles of 94°C for 1 min, 48–52°C for 1 min, 72°C for 2 min, final extension of 72°C for 10 min; *psbA-trnH*: 95°C for 2 min, 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min, final extension of 72°C for 7 min; *cox*3: 98°C for 5 min, 40 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 1 min 30 s, final extension of 72°C for 5 min; *phyC*: 94°C for 5 min, then a "touchdown" protocol with a progressive drop in annealing temperature every 3 cycles (94°C for 1 min, 68°C–65°C–62°C–59°C–56°C for 2 min, 72°C for 2 min), 25 cycles of 94°C for 1 min, 53°C for 1 min, 72°C for 1 min, final extension of 72°C for 15 min; *trnK/matK*: 95°C for 4 min, 40 cycles of 94°C for 30 s, 48–52°C for 1 min, 72°C for 1 min 30 s, final extension of 72°C for 7 min. PCR products were cleaned using a PCR Purification kit (Qiagen) and directly sequenced, with the exception of the phytochrome C region. *PhyC* PCR product was cloned using the Invitrogen TOPO TA Cloning Kit for Sequencing (Carlsbad, California USA), and 2–6 clones of appropriate size (~1.1 kb) per taxon were sequenced. Except as noted later, clones from each taxon were nearly identical and formed monophyletic groups in a parsimony analysis of all clones (tree not shown), so we conclude that there is one copy of *phyC* and that slight differences between clones were due to PCR error or multiple alleles. One clone for each species was randomly chosen for inclusion in the phylogenetic analysis. Two distinct copies of *phyC* were recovered from *Pereskiopsis* and *Quiabentia*, but in each case the additional copy was easily distinguished by its great divergence from the other *phy*C sequences.

Dye terminator cycle sequencing reactions for all regions were performed in 20 μL volumes (1.5 μL 5× buffer, 1–3 μL PCR product, 2 μL BigDye v.3, 0.5 pmol primer, 13.3–15.3 μL ddH_2O) using the following protocol: 96°C for 30 s, 26 cycles of 96°C for 10 s, 50°C for 5 s, 60°C for 4 min. Reactions were then cleaned using EdgeBiosystem plates (Gaithersburg, Maryland, USA) and run on either an MJResearch BaseStation51 (MJ Research, South San Francisco, California, USA) or ABIPrism 3700 automated sequencer (Applied Biosystems).

Contiguous sequences were constructed and edited using Sequencher v.4.2 (Gene Codes Corp., Ann Arbor, Michigan, USA), and homologous sequences for each region were aligned in MacClade v. 4.06 (Maddison and Maddison, 2003). In most cases, alignment was unambiguous. However, the *psbA-trnH* IGS region varied considerably in length between taxa, and attempts to align outgroup sequences with Cactaceae sequences for this region gave dubious results. We therefore coded outgroup taxa as missing for this region. Aligned data matrices for all analyses are available from the first author upon request, and are also available from TreeBASE (http://www.treebase.org).

Phylogenetic analyses—We arranged the data into two major partitions, nuclear (*phyC*) and chloroplast/mitochondrial (*rbcL, psbA-trnH* IGS, *trnK/*

TABLE 2. Sequence information for the different gene partitions.

Sequence	*phyC*	*psbA/trnH* IGS	*trnK/matK*	*rbcL*	*cox3*	Combined
Genome	Nuclear	Chloroplast	Chloroplast	Chloroplast	Mitochondrial	—
Number of taxa in data set	34	29	36	36	36	38
Length of aligned matrix	1133	468	2527	1431	592	6151
Number of constant sites	733	323	2017	1254	563	4890
Number of informative sites	211	63	237	79	13	603
% informative sites	18.6%	13.5%	9.4%	5.5%	2.2%	9.8%

matK, cox3), and analyzed them separately and in combination. We combined the cpDNA and mtDNA markers into one partition as both of these organelles are typically maternally inherited. Also, there is little variation in the mtDNA marker (13 informative sites; see Table 2), and each informative site supports a bipartition that is also supported by the cpDNA markers. We tested for incongruence between the partitions using a partition homogeneity test (the ILD test of Farris et al., 1994), as implemented in PAUP* version 4.b10 (Swofford, 2002). We then performed parsimony (MP), maximum likelihood (ML), and Bayesian phylogenetic analyses on all three data sets (nuclear, cpDNA/mtDNA, combined) using similar methods for each data set. All MP and ML analyses were performed using PAUP* version 4.b10; Bayesian analyses used MrBayes v. 3.0b4 (Huelsenbeck and Ronquist, 2001).

MP analyses—Heuristic searches were performed using a starting tree built by stepwise addition with 1000 random addition replicates and tree-bisection-reconnection (TBR) branch swapping. To assess confidence in clades, bootstrap tests were performed using 1000 bootstrap replicates with 10 random addition replicates per bootstrap.

ML analyses—Models of molecular evolution were chosen for each data set using Modeltest version 3.05b (Posada and Crandall, 1998). Heuristic searches were performed using a starting tree built by stepwise addition with 100 random addition replicates and TBR branch swapping, and 500 bootstrap replicates with five random addition replicates per bootstrap were performed to estimate support values for clades. In the combined data set, only 350 bootstrap replicates were performed.

Bayesian analyses—Models of molecular evolution were chosen for each data set and each gene region using Modeltest version 3.05b (Posada and Crandall, 1998). For any data set containing more than one gene region (i.e., all analyses with the exception of the nuclear data set), we ran a mixed-model analysis, allowing each gene region to evolve under its own best-fit model. All Bayesian analyses were performed assuming default prior parameter distributions set in MrBayes v.3.04b. Posterior probabilities of trees were approximated using the MCMC algorithm with four incrementally heated chains ($T = 0.2$) for 10 000 000 generations and sampling trees every 1000 generations. To estimate appropriate burn-ins, posterior parameter distributions were viewed using Tracer v.1.01 (Rambaut and Drummond, 2003). Discarding the first 100 trees (100 000 generations) was adequate in ensuring stationarity.

Hypothesis testing and sensitivity analyses—To test for alternative positions of the root of Cactaceae, two parametric bootstrap tests were performed (Goldman et al., 2000). Our first test constrained *Pereskia* to be monophyletic, and in this case, our outgroups rooted along the branch to the Opuntioideae. In our second test, we constrained the position of the root along the branch to the Andean and southern South American *Pereskia* clades (see Discussion, *Pereskia* paraphyly and ovary position). This scenario is consistent with one first proposed by Berger (1926), in which his *Eupereskia*, a subgenus corresponding roughly to our Andean and southern South American *Pereskia* clades, is sister to all other cacti.

In each case, a ML heuristic search was performed on the original combined data set with tree topology T_0 constrained to the alternative hypothesis, and the test statistic $\delta = L_{ML} - L_T$ was calculated. Using SeqGen v.1.2.7 (Rambaut and Grassly, 1997), we simulated 100 replicate data sets using the best-fit model of evolution for the original data set and tree topology T_0 with its branch lengths. For each simulated data set D^i, two ML heuristic searches were performed, one unconstrained (L_{ML}) and one constrained to topology $T_0(L_T)$, and the test statistic $\delta^i = L^i_{ML} - L^i_T$ was calculated. The alternative hypothesis T_0 was rejected if the original δ fell outside the 95th percentile of the distribution of δ^i.

In performing these analyses, we were concerned that our original data set contained missing data, while our simulated data sets did not; the simulated data sets might therefore contain more phylogenetic information than our own. For this reason, we trimmed our combined data set into a smaller matrix with minimal missing data: in all, we removed six taxa (*Ceraria fruiticulosa*, *Portulacaria afra*, *Pereskiopsis porteri*, *Quiabentia verticillata*, *Pereskia diaz-romeroana*, and *Pereskia bahiensis*), the *psbA-trnH* IGS region, and the 5′ *trnK* intron. This resulted in a data set of length 5060 characters, 450 of which are informative. Modeltest chose the same model (GTR + G + I) with slight differences in base frequencies, the substitution rate matrix, and the gamma distribution. The ML tree for this data set is topologically identical to the ML tree for the larger data set. With this smaller data set, we re-ran the parametric bootstrap tests as described.

Outgroup jackknifing—To assess the degree to which outgroup taxon sampling might affect the position of the root of Cactaceae, we generated a set of random combinations of our seven outgroups (both in the number and identity of taxa) and ran MP analyses using each set. To generate the outgroup combinations, a small Perl script was written that is executable in Mac OSX terminal window or Linux (available from the first author or as a free download called "hotgroup" at http://www.yphy.org/phycom). For this analysis, we generated 100 random outgroup replicates and ran each under a MP heuristic search with five random addition replicates and TBR branchswapping. Best trees from each search were saved to a tree file, and outgroup number, identity, and root placement was recorded for all trees.

RESULTS

Taxon sampling and missing data—While our combined data set includes 38 taxa, not all taxa were sequenced for all five gene regions (Table 2; Appendix). We were unable to amplify the *phyC* region from two of our outgroup taxa, *Ceraria fruticulosa* and *Portulacaria afra*. In the *trnK/matK* data set, 36 of 38 taxa are sequenced for the *matK gene* and flanking 3′ *trnK* intron, while a subset of those taxa are also sequenced for the flanking 5′ *trnK* intron. Also, two *Pereskia* species (*Pereskia bahiensis* and *Pereskia diaz-romeroana*) were only sequenced for the *trnK/matK* region; this region provided substantial information regarding the placement of these taxa within two strongly supported *Pereskia* clades. Finally, two taxa in our data set are chimeric: *Cereus* comprises *C. alacriportanus* (*trnK/matK*) and *C. fernambucensis* (all other regions), and *Pereskiopsis* A comprises *P. diguetii* (*trnK/matK*) and *P. aquosa* (all other regions).

Data partition combinability—The ILD test rejected the null hypothesis that our two data partitions were derived from the same data pool ($P = 0.045$). Visual inspection of trees from analyses of the individual partitions revealed two distinct

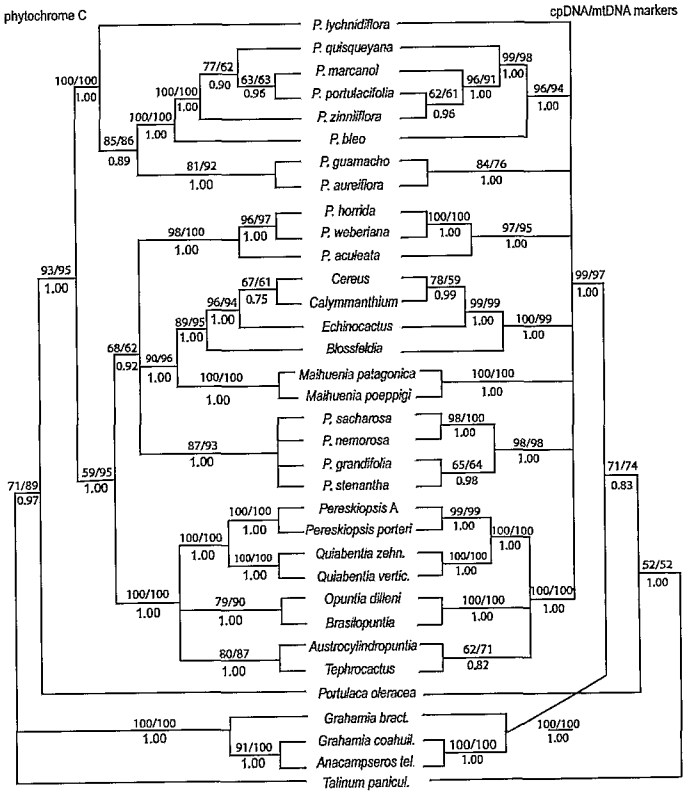

Fig. 1. Majority-rule consensus trees built from most parsimonious trees found in 1000 heuristic parsimony searches of each of the two data partitions. Four taxa were pruned from the cpDNA/mtDNA tree that were not sequenced for *phy*C. Numbers along branches represent support values for clades. Numbers above branches are (MP bootstraps/ML bootstraps); numbers below branches are Bayesian posterior probabilities. Branches with less than 50% bootstrap support values are collapsed. For full taxon names please refer to the Appendix.

areas of incongruence (Fig. 1), and removal of either the representatives of the Anacampseroteae (= *Grahamia bracteata*, *Grahamia coahuilensis*, and *Anacampseros telephiastrum*) outgroup taxa or the clade comprising *P. zinniiflora*, *P. portulacifolia*, *P. quisqueyana*, and *P. marcanoi* from the ILD analysis resulted in $P > 0.05$. As accurate resolution of either tree region is not the primary purpose of this study, we did not investigate partition congruence issues further, and pooled all markers together for a combined analysis. To be certain that incongruence in one part of the tree was not affecting our analysis in other parts of the tree, we removed the aforementioned taxa from the data set and re-ran our MP analyses. The resulting trees for the remaining taxa were topologically identical to those recovered from analyses of the full data set.

Separate partition analyses—The different gene regions sequenced vary widely in the amount of phylogenetic information they contain, with the nuclear marker *phyC* offering markedly greater resolution at the deeper nodes within Cactaceae (Table 2; Fig. 1).

cpDNA/mtDNA markers—The 1000 MP heuristic searches resulted in 26 shortest trees with length 1173 and CI = 0.83 (CI excluding autapomorphies = 0.69). The 100 heuristic searches using maximum likelihood and the HKY85 model of evolution resulted in a single tree with score $-\ln L$ = 14937.27192 (Fig. 2). Bootstrap support was weak for deeper nodes within the tree using either MP or ML (unresolved in Fig.1, support values <50%). There is, however, strong MP, ML, and Bayesian support for Cactaceae, Cactoideae, and Opuntioideae, as well as several clades of *Pereskia* that correspond well with Leuenberger's (1986) species groups (Fig. 1). *Pereskia guamacho* and *P. aureiflora* are sister to one another, as indicated by Leuenberger. A group of five *Pereskia* species (*P. grandifolia*, *P. sacharosa*, *P. nemorosa*, *P. stenantha*, and *P. bahiensis*), distributed in the cerrhado, caatinga,

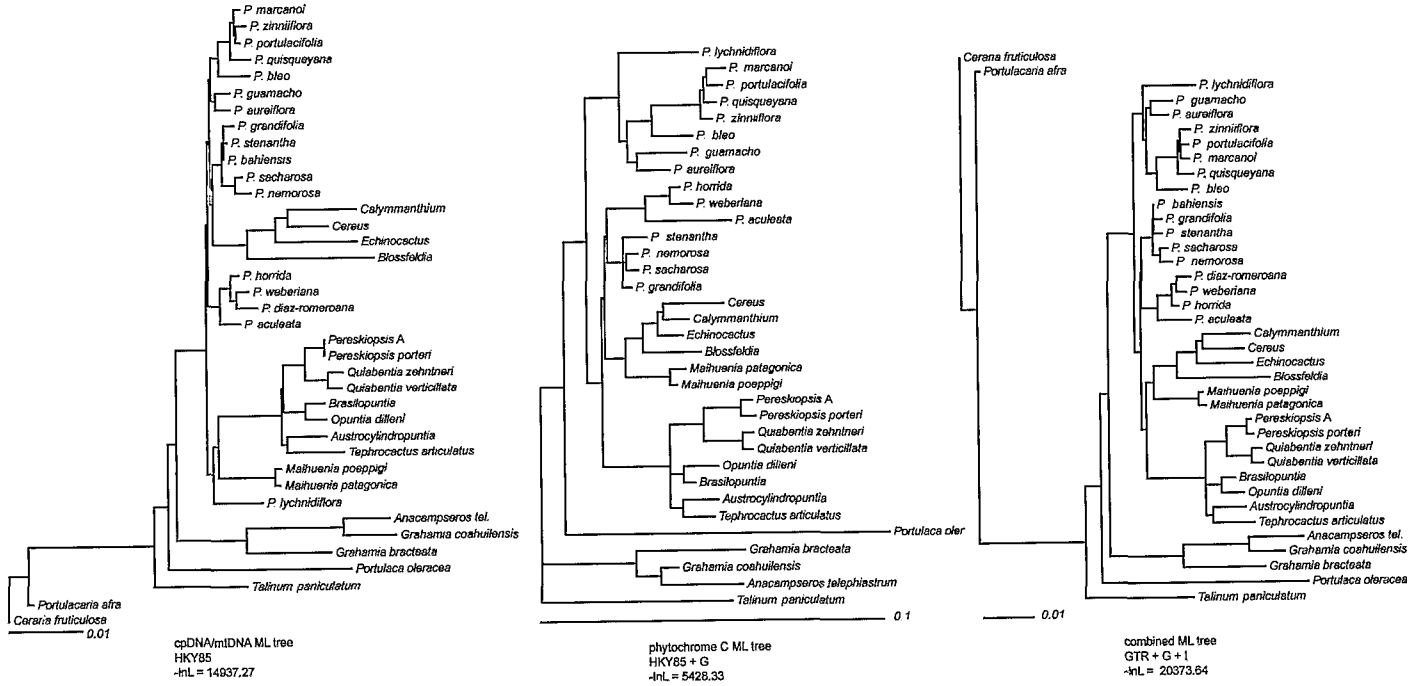

Fig. 2. MLE phylogram trees from separate data partitions and combined analysis. For full taxon names please refer to the Appendix.

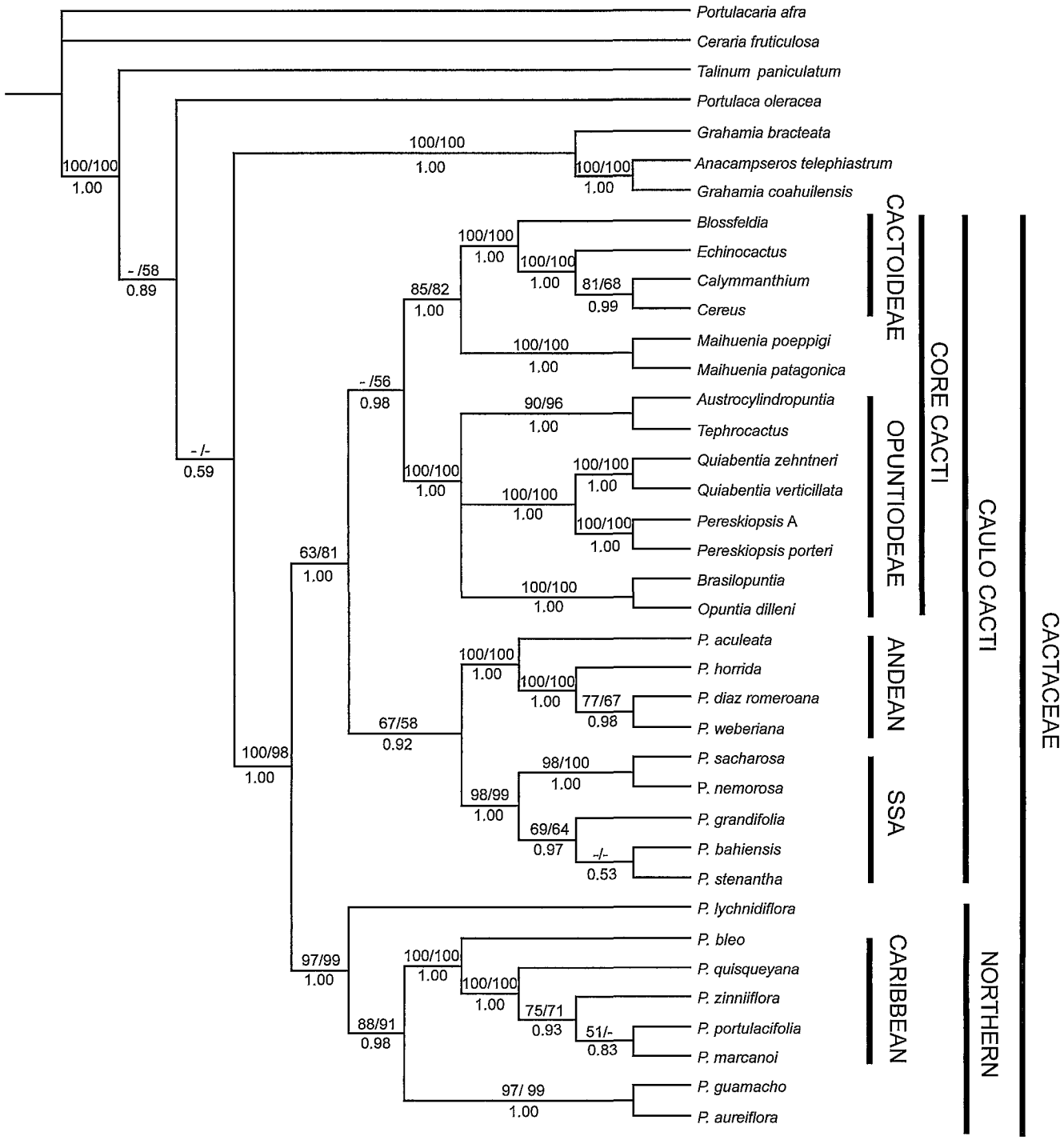

Fig. 3. Bayesian consensus tree for combined analysis. Numbers along branches represent support values for clades; numbers above branches are (MP bootstraps/ML bootstraps). Numbers below branches are Bayesian posterior probabilities. Bootstrap values less than 50% are represented by dashes.

and chaco woodland ecosystems of southern South America, is well supported and is hereafter referred to as the SSA (= southern South America) clade (Fig. 3). There is also strong support for uniting the four *Pereskia* species restricted to the Caribbean islands of Hispaniola and Cuba. The cpDNA/mtDNA data set strongly suggests that the species endemic to Hispaniola (*P. quisqueyana*, *P. portulacifolia*, and *P. marcanoi*) are not monophyletic; instead, *P. zinniiflora*, an endemic to the island of Cuba, is the sister species of *P. portulacifolia*. The Caribbean island endemics are subtended by *P. bleo*, which has been described as a riparian species, and is distributed in forests of Panama and Colombia. We will refer to (*Pereskia bleo* (*P. quisqueyana*, *P. marcanoi*, *P. portulacifolia*, *P. zinniiflora*)) as the Caribbean clade (Fig. 3). *Pereskia aculeata*, a geographically widespread and ecologically diverse species with a semi-scandent habit, is placed as sister to a group of three morphologically similar species (*P. horrida*, *P. diaz-romeroana*, and *P. weberiana*) that live in the dry

Inter-Andean valley regions of Peru and Bolivia. Leuenberger united the three Andean species but could not place *P. aculeata*. We will refer to (*P. aculeata* (*P. horrida*, *P. diaz-romeroana*, and *P. weberiana*)) as the Andean clade (Fig. 3).

Phytochrome C—Our *phyC* fragments correspond to the exon 1 region of Mathews and Donoghue (2000). Our MP heuristic search recovered 48 trees with length 665 and CI = 0.75 (CI excluding autapomorphies = 0.63). ML recovered a single best tree with −lnL = 5428.33231 (Fig. 2). MP, ML, and Bayesian analyses recover all of the aforementioned clades supported by the chloroplast–mitochondrial data set. Additionally, *phyC* resolves a basal split in the Cactaceae between one clade of *Pereskia* and all other cacti. The basal *Pereskia* clade comprises *P. lychnidiflora*, *P. guamacho* + *P. aureiflora*, and the Caribbean clade. This basal split is strongly supported by ML bootstraps and Bayesian posterior probabilities, but less so by parsimony (Fig. 1). This clade comprises all of the *Pereskia* species with distributions centered loosely around the Caribbean basin. We will refer to this newly identified *Pereskia* lineage as the Northern *Pereskia* clade (Fig. 3).

The Andean and SSA *Pereskia* clades are united with the opuntioid, cactoid, and *Maihuenia* lineages. *Maihuenia* and Cactoideae, in turn, are placed in a sister relationship. Within the Cactoideae, *Blossfeldia liliputana* is placed as sister to the other cactoids included in this study. This supports the controversial finding of Nyffeler (2002) that *B. liliputana*, a diminutive, globular cactus of northern Argentina and Bolivia, is the basal member of Cactoideae. While the sampling of Cactoideae in this study is clearly too limited to hypothesize major cactoid relationships, it is encouraging that *phyC*, a nuclear gene, is so far in agreement with the results of Nyffeler (2002), which was based on only chloroplast markers.

Two areas of incongruence between our nuclear and cpDNA/mtDNA data sets are worth noting (Fig. 1). The first concerns the arrangement of taxa in the *Portulaca*/*Talinum*/Anacampseroteae outgroups, with the chloroplast sequences suggesting a Cactaceae-Anacampseroteae sister relationship, and *phyC* suggesting that *Portulaca* is the nearest living relative of Cactaceae. Because *Portulacaria* and *Ceraria* were not included in our *phyC* analysis, we thought this may be a result of differential outgroup taxon sampling. Re-analyzing our cpDNA/mtDNA data set without *Portulacaria* and *Ceraria*, however, did not change the branching order of these outgroups.

The second incongruence lies within the well-supported *Pereskia* clade restricted to the Caribbean islands of Cuba and Hispaniola. Our chloroplast data places the Cuban endemic *P. zinniiflora* within a clade comprising the Hispaniolan endemics *P. quisqueyana*, *P. portulacifolia*, and *P. marcanoi*. The nuclear data, on the other hand, firmly resolve the Hispaniolan endemics as a monophyletic group sharing a sister relationship with the Cuban *P. zinniiflora*. Relationships among these four taxa deserve additional attention. The three Hispaniolan species currently exist as a handful of small, rather isolated populations, though it is not impossible to imagine gene flow occurring between them.

Combined analyses—Combining all gene regions into one data set yielded a well-supported hypothesis of basal cactus phylogeny (Figs. 2, 3). The 1000 heuristic MP searches resulted in 12 trees of length 1846 and CI = 0.80 (CI excluding autapomorphies = 0.66). The 100 heuristic ML searches found a single ML tree with −lnL = 20373.64. MP, ML, and Bayesian analyses all resulted in an identical tree topology, consisting of 3 major clades: the Northern *Pereskia* clade, the Opuntioideae, and *Maihuenia* + Cactoideae. *Pereskia* is paraphyletic, with the Northern *Pereskia* clade placed as the sister of all remaining cacti. The Andean and SSA *Pereskia* lineages in turn form a weakly supported clade that appears to share a sister relationship with the "core cacti" (Fig. 3), consisting of the opuntioid, cactoid, and *Maihuenia* lineages. We will refer to ((Andean, SSA)(Opuntioideae (*Maihuenia*, Cactoideae))) as the caulocactus clade (caulo = stem, referring to their specialized stems, see Discussion, early events in the evolution of stem-based photosynthesis).

For the most part, the *phyC* and combined trees are in agreement, with the major exception being the formation of the core cacti lineage in the combined analysis. Bayesian support for the core cacti is strong, ML bootstrap support is less so, and the MP bootstrap value is less than 50%.

Testing for alternative rootings—Our conclusion of a paraphyletic *Pereskia* is dependent upon the assumption that the outgroup taxa are attaching along the correct branch in Cactaceae. Problems with incorrect outgroup attachment become more likely when chosen outgroups are distantly related to the ingroup (Sanderson and Doyle, 2001; Graham et al., 2002; Soltis and Soltis, 2004). While we feel that our sample of outgroup taxa probably includes the closest living relatives of the cacti (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; R. Nyffeler, unpublished data), there is considerable sequence divergence among the outgroups, as evidenced by the long branch lengths in Fig. 2. As described earlier, we investigated this potential problem in two ways. First, we used parametric bootstrapping to compare two alternative rooting hypotheses. Second, we conducted an outgroup jackknifing experiment to see how different combinations of available outgroups might alter the placement of the root.

Results from our parametric bootstrapping tests strongly reject a monophyletic *Pereskia* (original data set, $P < 0.01$, reduced data set, $P < 0.01$), as well as a rooting along the branch to the Andean + SSA clade (original, $P < 0.01$, reduced, $P < 0.01$). In addition, results from our outgroup jackknifing experiment provide some evidence that rooting along the Northern *Pereskia* clade is not an artifact of outgroup taxon sampling. Of 100 random replicate outgroup samplings, six outgroup combinations resulted in alternative rootings along either the opuntioid or cactoid branches. In each of these six cases, three or fewer outgroup taxa were present, and *Ceraria fruticulosa* and/or *Portulacaria afra* were always included. These two taxa are the most distantly related outgroups in our analysis (Hershkovitz and Zimmer, 1997; Applequist and Wallace, 2001; R. Nyffeler, unpublished data). Within the *Talinum*/*Portulaca*/Anacampseroteae lineages, nearly all taxa rooted along the branch to the Northern *Pereskia* clade, whether they were alone or present in any other combination. The exception was *Anacampseros telephiastrum*, which rooted to the Northern *Pereskia* clade when it was the only outgroup, but rooted along the opuntioid or cactoid branches when it was present with *C. fruticulosa* or *P. afra*.

DISCUSSION

Pereskia paraphyly and ovary position—The tree shown in Fig. 3, in which *Pereskia* is paraphyletic, is strongly supported

by our analyses. However, most of the basal structure of our tree is contributed by the *phyC* gene, so it may be more appropriate at this stage to consider this to be a gene tree rather than the cactus species tree. While the implications of *Pereskia* paraphyly should be considered at this stage, we would like to see our results confirmed with additional genes before embarking on a new nomenclature for Cactaceae.

The first hypothesis of *Pereskia* paraphyly dates back to Berger (1926, "Schema 1"), who split the genus into *Eupereskia* and *Rhodocactus* and united *Rhodocactus* with Opuntioideae, Cactoideae, and *Maihuenia*. His decision to split *Pereskia* in this way was based on his interpretation of the *Pereskia* ovary, with *Eupereskia* having a distinctly superior ovary with basal placentation and *Rhodocactus* having parietal placentation and a more developed receptacle surrounding the ovary. From later work, it appears that Berger had access to five *Pereskia* species (*P. sacharosa*, *P. aculeata*, *P.grandifolia*, *P. bleo*, and *P. lychnidiflora*) (Berger, 1929). His two *Pereskia* genera correspond with the Northern (= *Rhodocactus*) and Andean + SSA (= *Eupereskia*) lineages, with the exception of *P. grandifolia*, which he united with the Northern species. However, his idea of family level relationships (*Eupereskia* (*Rhodocactus*, Cactoideae, Opuntioideae, *Maihuenia*)) was rejected in our parametric bootstrap tests.

Subsequent *Pereskia* classifications followed on this theme, though they have proposed slight rearrangements of taxa and have differed in the assignment of genus or subgenus rank (Backeberg and Knuth, 1936; Backeberg, 1958). All these schemes have been based primarily on differences in ovary position because it was generally agreed that the variability of the *Pereskia* gynoecium held the key to understanding the evolution of the "sunken ovary" of the core cacti. In spite of this interest, however, the *Pereskia* gynoecium did not receive careful study until much later (Boke, 1963, 1964, 1966, 1968; Leins and Schwitalla, 1988), and earlier misinterpretations are likely responsible for the confusing history of *Pereskia* classification. Leuenberger (1986) stressed the complex nature of the ovary in *Pereskia*, noting that "the transitional nature of ovary and ovule position, subject to distortions during ontogeny of flower and fruit, makes it an unreliable character" (p. 51). *Pereskia sacharosa*, for example, has been described as having "fully superior" and "half inferior" ovaries, with both "basal" and "basal-parietal" placentation, depending on the author. Leuenberger concluded that most *Pereskia* species could be considered half superior or half inferior, with truly inferior ovaries found only in the Caribbean clade (Fig. 4A). It appears that the evolutionary transition to the "cactus-type" inferior ovary that botanists had long hoped to find in *Pereskia* does exist, but as an independent event within the Caribbean clade. Careful comparative studies within the Northern *Pereskia* clade might be helpful in understanding this transition, and it would also be useful to assess the degree of similarity of the Caribbean ovary to the inferior ovary of the core cacti.

Early events in the evolution of stem-based photosynthesis—While there are no obvious reproductive characters uniting the Andean and SSA *Pereskia* clades with the major cactus lineages, our phylogenetic hypothesis provides some insight into early events in the development of the stem as the primary photosynthetic organ of the cacti. Several traits from Table 1 could potentially be important in the evolutionary transition to a functionally leafless state. For instance, leaves occur in two places in cacti. In all cacti, during new stem growth a leaf (usually microscopic and/or ephemeral) is borne subtending the areole, while in some *Pereskia* species, leaves are also borne directly on the areoles (Gibson and Nobel, 1986). This is reminiscent of the short-shoot systems of many desert plants, which are utilized to produce quick flushes of leaves without first investing in new stem tissue. Because some *Pereskia* species and all core cacti lack this trait, a reasonable scenario in the transition to leaflessness would be first to lose the ability to flush leaves from the areole. While this may still be the case (as likely in the Cylindropuntieae; see Wallace and Dickie, 2002), extant *Pereskia* species provide us with no clear record of it. Areole leaf production appears to have a complicated history, with potentially two losses (in the caulocactus and Caribbean clades) and then two subsequent gains (within the Caribbean and SSA clades), or three gains (in the Northern, Caribbean, and SSA clades) and one loss (within the Caribbean clade) (Fig. 4B).

In contrast, there are two stem characters that clearly unite the Andean and SSA *Pereskia* clades with the core cacti. Most members of the caulocactus clade produce stomata on their stem epidermis and have the delayed bark formation characteristic of the stem photosynthesizing cacti (Fig. 4C, D). A notable exception is *Maihuenia*. While Mauseth (1999) discussed stomata on fragmented epidermis in the areolar pits of both *Maihuenia* species, they lacked stomata in all other stem regions, so we interpret *Maihuenia* stems here to be essentially astomatous (Leuenberger, 1997, also describes them as lacking stem stomata). The placement of *Maihuenia* in our trees implies that both stem stomata and delayed bark formation have been lost in this lineage. *Maihuenia* today consists of two highly specialized cushion plant species living in cold, dry regions of Patagonia and south-central Chile (Leuenberger, 1997; Mauseth, 1999). It is not immediately clear why these traits have been lost, though their cushion plant growth habit (often forming very dense stands of stems with leaves crowded at tips) seems unlikely to promote the use of the stem as a photosynthetic organ.

All members of the Northern *Pereskia* clade lack stem stomata and delayed bark formation, though stomata are very rarely found on the stems of *P. portulacifolia* and *P. guamacho* (E. Edwards, personal observation). It is currently impossible to ascertain whether these traits were lost in the Northern clade or gained in the caulocactus clade because we are still uncertain as to the true sister taxa to Cactaceae, and there is little mention of these traits in the Portulacaceae literature (Carolin, 1987, 1993; Eggli and Ford-Werntz, 2001; but see Hershkovitz, 1993, for comment on stem stomata in *Talinum*). Nevertheless, we can say that stem stomata and delayed bark formation were present in the cactus lineage prior to the divergence of opuntioid and cactoid lineages and that the evolution of these two characters appears to have been correlated.

It is worth considering how the presence of these traits might influence the functioning of stems and leaves in *Pereskia*. Several greenhouse studies have investigated the photosynthetic behavior of a variety of *Pereskia* species (Rayder and Ting, 1981; Nobel and Hartsock, 1986, 1987; Martin and Wallace, 2000) and minimal C3 stem photosynthesis has been recorded only in *P. horrida* (Martin and Wallace, 2000). It is difficult to extrapolate these findings to what might happen in a natural environment, however, and ecological observations of wild *Pereskia* species are currently quite limited in scope. Projects characterizing the ecological physiology of the different *Pereskia* lineages are currently underway (Edwards et

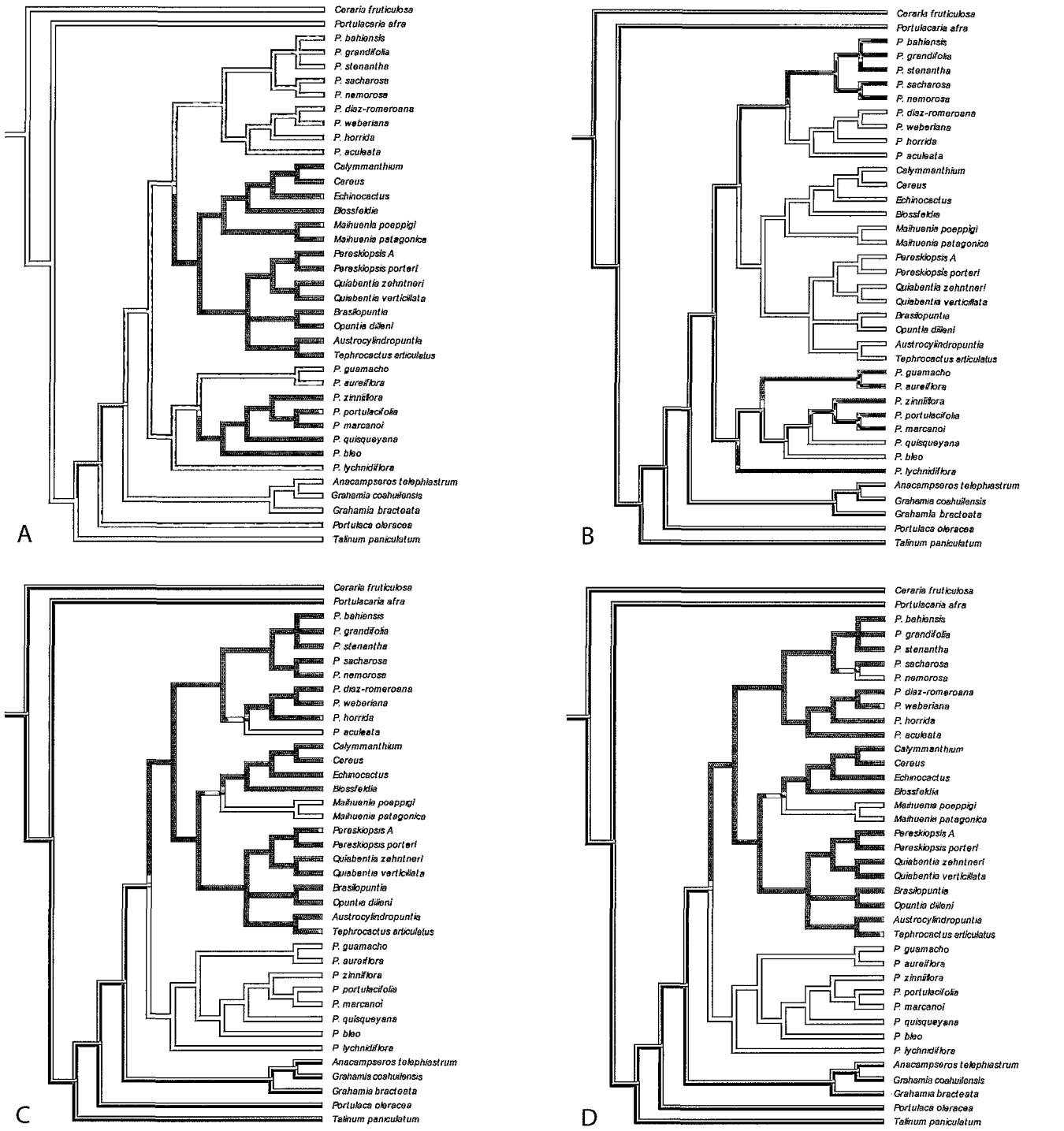

Fig. 4. Reconstruction of ancestral character states using unordered parsimony. A. Ovary position (white = superior, gray = half inferior, black = inferior). B. Areole leaf production (white = no leaves on areoles, black = leaves on areoles). C. Delayed bark formation (white = precocious bark formation, black = delayed). D. Stem stomata (white = no stem stomata, black = stem stomata). In B, C, and D, outgroups are coded as unknown.

al., 2004) and should be helpful in discerning any functional differences associated with these anatomical changes.

In the meantime, however, it seems that further modifications of the cortical tissue are needed to make much photosynthetic use of the stem. In a survey of 28 cacti, including seven *Pereskia* species representing the Northern, Andean, and SSA clades, Sajeva and Mauseth (1991) emphasized what they considered to be key developments in the evolution of the cactus stem. These include a hypodermal layer comprised of collenchymatous cells with thickened walls, substantial intercellular air space to aid in CO_2 diffusion, and the arrangement of cortical chlorenchyma tissue into "spongy mesophyll" and "palisade" layers that appear similar to those in photosynthetic leaves. While their sampling did not include any opuntioids, other researchers have found similar cortical structure in Opuntioideae (Nobel, 1988; North et al., 1995). No *Per-*

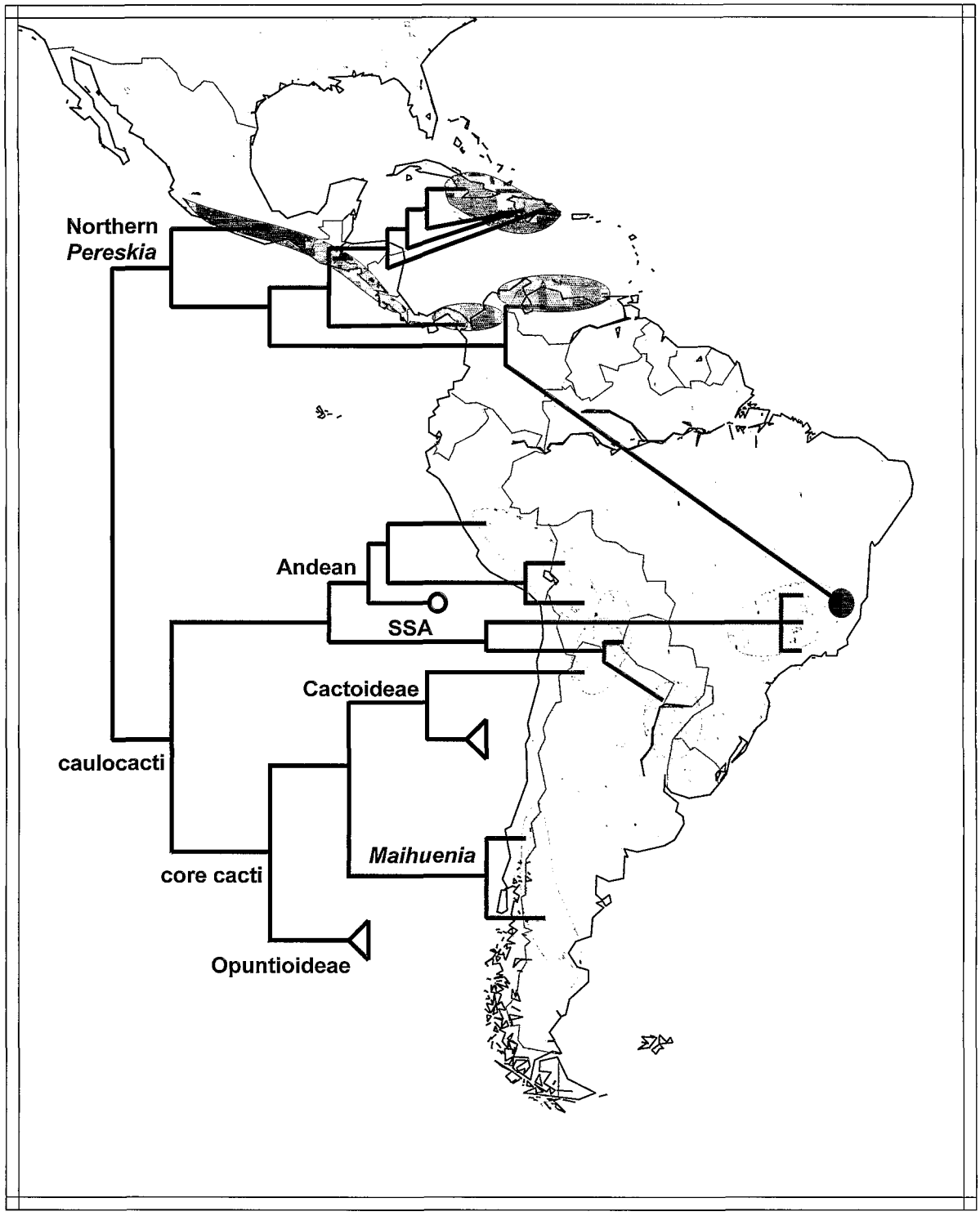

Fig. 5. Geographic distribution of basal cacti. Species ranges correspond to shaded area, except for the geographically widespread taxa: *Pereskia aculeata* (a circle), the Opuntioideae (a triangle), and all Cactoideae minus *Blossfeldia* (a triangle).

eskia species included in the Sajeva and Mauseth (1991) study had their cortical tissue differentiated into such layers, and further, all of their stems had relatively small volumes of intercellular air space. It appears from our phylogenetic results that the evolution of stem stomata and delayed bark formation preceded the key cortex modifications that allowed the stem to function as an efficient photosynthetic organ (cf. "developmental enablers" in Donoghue, 2005). This order of events may not be unique to cacti: in a recent survey of noncactus plants with stem-based photosynthesis, Mauseth (2004) found that the majority of investigated species with delayed bark formation, potentially photosynthetic stems, and photosyn-

thetic leaves did not have a specialized cortex, while nearly all of the aphyllous species with stems as their primary photosynthetic organ also had a well-developed palisade cell layer in their outer cortical tissue.

Historical biogeography of the cacti—There have been several hypotheses regarding the geographical origin of the cacti, all largely based on where the presumably basal members of Cactaceae currently reside. Buxbaum (1969) cited both the Caribbean and central South America as likely areas, due to the presence of *Pereskia* and of opuntioid and cactoid lineages that he considered "ancestral." Leuenberger (1986) concluded that northwestern South America was a more reasonable location, primarily because he imagined a late Cretaceous origin of the group and because centering its origin as far away from Africa as possible might explain the poor representation of cacti there. Our data set, as well as other recent molecular phylogenetic studies (Hershkovitz and Zimmer, 1997; Nyffeler, 2002), suggest that the cacti are not that old, however, because sequence divergence between the major cactus clades is limited. More recently, Wallace and Gibson (2002) hypothesized a central Andean origin for the family. They assumed that the Andean *Pereskia*, certain Opuntioideae, and the cactoid *Calymmanthium* are basal cactus lineages, and all reside in Peru, Bolivia, and northern Argentina.

Our ability to infer the geographic origin of Cactaceae is limited by insufficient knowledge of its closest relatives, but within the cacti there is a striking geographic structure to our hypothesis of basal relationships (Fig. 5). The basal split in Cactaceae suggests an initial separation within the cacti into a primarily northern and a primarily southern clade. The Northern *Pereskia* clade is comprised of species scattered in Central and northern South America, Cuba, and Hispaniola, with the exception of *P. aureiflora* of eastern Brazil. Within the caulocactus clade, *Maihuenia* is restricted to central/southern Chile and Argentina, the Andean *Pereskia* clade (with the exception of *P. aculeata*, the only geographically widespread *Pereskia* species) is found only in Peru and Bolivia, and the SSA clade is scattered throughout drier forest regions of eastern Brazil, Paraguay, Uruguay, Argentina, and Bolivia. *Blossfeldia* is confirmed here as a basal cactoid lineage, and grows only in northern Argentina and Bolivia. While our sampling and resolution are insufficient within Opuntioideae to make inferences about the geographic distribution of its basal members, recent work of others has placed its earliest diverging lineages in Chile and Argentina (Griffith, 2004).

Our results imply that both Opuntioideae and Cactoideae originated in the southern half of South America, possibly in the central Andean region of Peru, Bolivia, and northern Argentina. Andean orogeny has been viewed as an important cause of diversification for other plant lineages (Raven and Axelrod, 1974; Burnham and Graham, 1999). Early uplift in the central Andean region (~25–20 mya) is hypothesized to have occurred under a fluctuating arid/semi-arid climate regime (Hartley, 2003), which presents a likely scenario for early cactus diversification. The placement of Cacteae, an exclusively North American lineage with its center of diversity in Mexico, as being among the earliest diverging lineages of Cactoideae (represented here with *Echinocactus*, but confirmed with broader sampling in Nyffeler, 2002), suggests relatively early movements out of this region and across the continent.

Conclusions—*Pereskia*, long thought to represent the "ancestral" cactus, appears to be paraphyletic, with the Andean and SSA *Pereskia* clades more closely related to the core cacti. This result allows us to make several new inferences regarding early cactus evolution. First, it appears that the specialized "sunken" inferior ovary of the cacti has evolved twice independently, once in the core cacti, and once in the Caribbean *Pereskia* clade. Second, most members of the two *Pereskia* clades that are united with the core cacti delay bark formation and produce stem stomata. As these *Pereskia* clades lack other stem specializations found in the core cacti (e.g., differentiation of cortical tissue into specialized cell types, large intercellular air spaces, well developed hypodermis), we infer that the expression of stem stomata and delayed bark formation were among the first changes associated with the transition to stem-based photosynthesis. Finally, our phylogenetic hypothesis implies an initial north–south geographic split during early cactus evolution and that both Opuntioideae and Cactoideae originated in southern or west central South America.

While these results are promising, they also point to areas that need work. First and foremost, we would like to see our results tested with additional genes before reclassifying the Cactaceae. Clearly, the true position of the cacti within the portulacaceous alliance needs to be settled, as this is critical to interpretations of early cactus character evolution. Perhaps most important, however, is the need for careful study of *Pereskia* ecology. The specialized physiology and water use strategies of the core cacti have been well characterized (Nobel, 1988), while virtually no studies have been conducted on *Pereskia* in the wild (but see Diaz, 1984; Diaz and Medina, 1984). The cactus life form represents one of the more extreme cases of ecological and morphological specialization in plants, and knowledge that (and precisely how) *Pereskia* is paraphyletic provides a unique opportunity to examine the earliest steps in its evolution. Discovering more about how members of the different *Pereskia* lineages function in their environments would complement what we already know about their anatomy and morphology, allowing for an integrated approach to understanding this remarkable evolutionary transition.

LITERATURE CITED

APPLEQUIST, W. L., AND R. S. WALLACE. 2001. Phylogeny of the portulacaceous cohort based on *ndh*F sequence data. *Systematic Botany* 26: 406–419.

BACKEBERG, C. 1958. Die Cactaceae. Handbuch der Kakteenkunde. G. Fischer, Jena, Germany.

BACKEBERG, C., AND F. M. KNUTH. 1936. Kaktus-ABC. Nordisk Forlag, Copenhagen, Denmark.

BAILEY, I. W. 1962. The xylem of *Pereskia sacharosa* and *Pereskia aculeata*. *Journal of the Arnold Arboretum* 43: 376–388.

BAILEY, I. 1963a. The xylem of *Pereskia grandifolia* and *Pereskia bleo*. *Journal of the Arnold Arboretum* 44: 222–231.

BAILEY, I. 1963b. The xylem of pereskias from Peru and Bolivia. *Journal of the Arnold Arboretum* 44: 127–137.

BAILEY, I. 1963c. The xylem of pereskias from southern Mexico and Central America. *Journal of the Arnold Arboretum* 44: 211–221.

BAILEY, I. 1963d. The xylem of *Pereskia colombiana*, *Pereskia guamacho*, *Pereskia cubensis*, and *Pereskia portulacifolia*. *Journal of the Arnold Arboretum* 44: 390–401.

BARTHLOTT, W., AND D. R. HUNT. 1993. Cactaceae. *In* K. Kubitzki, J. G. Rohwer, and V. Bittrich [eds.], The families and genera of vascular plants, 161–197. Springer Verlag, Berlin, Germany.

BERGER, A. 1926. Die entwicklungslinien der Kakteen. G. Fisher, Jena, Germany.

BERGER, A. 1929. Illustrierte handbucher sukkulenter pflanzen: Kakteen. Ulmer, Stuttgart, Germany.

BOKE, N. H. 1954. Organogenesis of the vegetative shoot in *Pereskia*. *American Journal of Botany* 41: 619–637.

BOKE, N. H. 1963. Anatomy and development of the flower and fruit of *Pereskia pititache*. *American Journal of Botany* 50: 843–858.

BOKE, N. H. 1964. The cactus gynoecium: a new interpretation. *American Journal of Botany* 51: 598–610.

BOKE, N. H. 1966. Ontogeny and structure of the flower and fruit of *Pereskia aculeata*. *American Journal of Botany* 53: 534–542.

BOKE, N. H. 1968. Structure and development of the flower and fruit of *Pereskia diaz-romeroana*. *American Journal of Botany* 55: 1254–1260.

BOUSQUET, J., S. H. STRAUSS, AND P. LI. 1992. Complete congruence between morphological and *rbc*L-based molecular phylogenies in birches and related species (Betulaceae). *Molecular Biology and Evolution* 9: 1076–1088.

BURNHAM, R. J., AND A. GRAHAM. 1999. The history of neotropical vegetation: new developments and status. *Annals of the Missouri Botanical Garden* 86: 546–589.

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

CAROLIN, R. 1987. A review of the family Portulacaceae. *Australian Journal of Botany* 35: 383–412.

CAROLIN, R. 1993. Portulacaceae. *In* K. Kubitzki [ed.], Families and genera of vascular plants, 544–555. Springer-Verlag,Heidelberg, Germany.

DIAZ, M. 1984. Estudios fisoecologicos de 4 especies de cactaceas en condiciones naturales. Masters thesis, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

DIAZ, M., AND E. MEDINA. 1984. Actividad CAM de cactaceaes en condiciones naturales. *In* E. Medina [ed.], Eco-Fisiologia de Plantas CAM. Centro Internacional de Ecologia Tropical,Caracas, Venezuela.

DONOGHUE, M. J. 2005. Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. *Paleobiology* 31: 77–93.

DUMINIL, J., M. H. PEMONGE, AND R. J. PETIT. 2002. A set of 35 consensus primer pairs amplifying genes and introns of plant mitochondrial DNA. *Molecular Ecology Notes* 2: 428–430.

EDWARDS, E. J., M. DIAZ, AND M. J. DONOGHUE. 2004. Water relations of *Pereskia guamacho*, a cactus with leaves. Botany 2004: Annual meeting of the Botany Society of America, Snowbird, Utah USA, available at website http://www.2004.botanyconference.org/engine/search/index.php?func=detail&aid=663.

EGGLI, U., AND D. FORD-WERNTZ. 2001. Portulacaceae. *In* U. Eggli [ed.], Illustrated handbook of succulent plants: dicotyledons, 370–432. Springer-Verlag, Berlin, Germany.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.

GIBSON, A. C., AND P. S. NOBEL. 1986. The cactus primer. Harvard University Press, Cambridge, Massachusetts, USA.

GOLDMAN, N., J. P. ANDERSON, AND A. G. RODGRIGO. 2000. Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* 49: 652–670.

GRAHAM, S. W., R. G. OLMSTEAD, AND S. C. H. BARRETT. 2002. Rooting phylogenetic trees with distant outgroups: a case study from the commelinoid monocots. *Molecular Biology and Evolution* 19: 1769–1781.

GRIFFITH, M. P. 2004. What did the first cactus look like? An attempt to reconcile the morphological and molecular evidence. *Taxon* 53: 493–499.

HARTLEY, A. J. 2003. Andean uplift and climate change. *Journal of the Geological Society, London* 160: 7–10.

HERSHKOVITZ, M. A. 1993. Revised circumscriptions and subgeneric taxonomies of *Calandrinia* and *Montiopsis* (Portulacaceae) with notes on phylogeny of the portulacaceous alliance. *Annals of the Missouri Botanical Garden* 80: 333–365.

HERSHKOVITZ, M. A., AND E. A. ZIMMER. 1997. On the evolutionary origins of the cacti. *Taxon* 46: 217–232.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

JOHNSON, L. A., AND D. E. SOLTIS. 1994. *mat*K DNA sequences and phylogenetic reconstruction in Saxifragaceae. *Systematic Botany* 19: 143–156.

LEINS, P., AND S. SCHWITALLA. 1988. Placentation in Cactaceae. *In* P. Leins, S. C. Tucker, and P. K. Endress [eds.], Aspects of floral development. J. Cramer, Berlin-Stuttgart, Germany.

LEUENBERGER, B. E. 1986. *Pereskia* (Cactaceae). *Memoirs of the New York Botanical Garden* 41: 1–141.

LEUENBERGER, B. E. 1997. *Maihuenia*—monograph of a Patagonian genus of Cactaceae. *Botanische Jahrbucher* 119: 1–92.

MADDISON, D. R., AND W. P. MADDISON. 2003. MacClade v. 4.06. Sinauer Associates, Sunderland, Massachusetts, USA.

MARTIN, C. E., AND R. S. WALLACE. 2000. Photosynthetic pathway variation in leafy members of two subfamilies of the Cactaceae. *International Journal of Plant Sciences* 161: 639–650.

MATHEWS, S., AND M. J. DONOGHUE. 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. *Science* 286: 947–950.

MATHEWS, S., AND M. J. DONOGHUE. 2000. Basal angiosperm phylogeny inferred from duplicate phytochromes A and C. *International Journal of Plant Sciences* 161: S41–S55.

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

MAUSETH, J. D. 2004. The structure of photosynthetic succulent stems in plants other than cacti. *International Journal of Plant Sciences* 165: 1–9.

MAUSETH, J. D., AND J. V. LANDRUM. 1997. Relictual vegetative anatomical characters in Cactaceae: the genus *Pereskia*. *Journal of Plant Research* 110: 55–64.

MAUSETH, J. D., Y. UOZUMI, B. J. PLEMONS, AND J. V. LANDRUM. 1995. Structural and systematic study of an unusual tracheid type in cacti. *Journal of Plant Research* 108: 517–526.

NOBEL, P. S. 1988. Environmental biology of agaves and cacti. Cambridge University Press, New York, New York, USA.

NOBEL, P. S., AND T. L. HARTSOCK. 1986. Leaf and stem CO_2 uptake in the 3 subfamilies of the Cactaceae. *Plant Physiology* 80: 913–917.

NOBEL, P. S., AND T. L. HARTSTOCK. 1987. Drought-induced shifts in daily CO_2 uptake patterns for leafy cacti. *Physiologia Plantarum* 70: 114–118.

NORTH, G. B., T. LIN MOORE, AND P. S. NOBEL. 1995. Cladode development for *Opuntia ficus-indica* (Cactaceae) under current and doubled CO_2 concentrations. *American Journal of Botany* 82: 159–166.

NYFFELER, R. 2002. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trn*K/*mat*K and *trn*L-*trn*F sequences. *American Journal of Botany* 89: 312–326.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

RAMBAUT, A., AND A. J. DRUMMOND. 2003. Tracer v. 1.0.1, Available from website http://evolve.zoo.ox.ac.uk/.

RAMBAUT, A., AND N. C. GRASSLY. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. *Bioinformatics* 13: 235–238.

RAUH, W. 1979. Kakteen an ihren standorten. Parey, Berlin, Germany.

RAVEN, P. H., AND D. I. AXELROD. 1974. Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden* 61: 539–673.

RAYDER, L., AND I. P. TING. 1981. Carbon metabolism in 2 species of *Pereskia* (Cactaceae). *Plant Physiology* 68: 139–142.

SAJEVA, M., AND J. D. MAUSETH. 1991. Leaf-like structure in the photosynthetic, succulent stems of cacti. *Annals of Botany* 68: 405–411.

SANDERSON, M. J., AND J. A. DOYLE. 2001. Sources of error and confidence in estimating the age of angiosperms from *rbc*L and 18s rDNA data. *American Journal of Botany* 88: 1499–1516.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.

SCHLEIDEN, M. J. 1845. Beiträge zur anatomi der Kacteen. *Mémoires de l' Académie Impéeriale des Sciences de St-Pétersbourg* 4: 335–380.

SCHUMANN, K. 1898. Gesamtbeschreibung der Kakteen. Neumann, Neudamm, Germany.

SOLTIS, D. E., AND P. S. SOLTIS. 2004. *Amborella* not a "basal angiosperm"? Not so fast. *American Journal of Botany* 91: 997–1001.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts, USA.

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

WALLACE, R. S., AND S. L. DICKIE. 2002. Systematic implications of chloroplast DNA sequence variation in the Opuntioideae. *In* D. R. Hunt [ed.], Studies in the Opuntioideae (Cactaceae). David Hunt, Sherborne, UK.

WALLACE, R. S., AND A. C. GIBSON. 2002. Evolution and systematics. *In* P. S. Nobel [ed.], Cacti: biology and uses, 1–21. University of California Press, Berkeley, California, USA.

APPENDIX. Taxa used in this study, GenBank accession numbers for the five regions studied, source (wild, or if cultivated, location and accession number), and voucher information. The following abbreviations are used for herbaria and botanical gardens: YU = Yale University, B = Berlin Botanical Garden, ZSS = Sukkulenten-Sammlung, Zürich, Z = Zürich Botanical Garden, NTG = National Tropical Garden-Kampong, Miami, JBN = Jardín Botánico Nacional, Dominican Republic, DBG = Desert Botanical Garden, Phoenix, ISC = University of Iowa.

Taxon; GenBank accession: *phyC, rbcL, cox*3, *psbA-trnH, trnK/matK*; Source; Voucher specimen.

Anacampseros telephiastrum D.C.; AY875311, AY875247, AY875270,—,—; Cult. ZSS 90 1682/10; *Lavranos & Bleck s.n.*, South Africa, ZSS.

Anacampseros telephiastrum D.C.;—,—,—,—, AY875373; Cult. ZSS 90 16523; *Lavranos & Bleck s.n.*, South Africa, ZSS.

Austrocylindropuntia subulata (Muehlenpf.) Backbg.; AY875305, AY875235, AY875281, AY875346, AY875364; Cult. B 153-19-74-80; *Cubr 37075,* garden, B.

Blossfeldia liliputana Werderm.; AY875301, AY875232, AY875282, AY875348, AY875366; Cult. B 160-16-86-20; *Leuenberger & Arroyo 3579,* Argentina, B.

Brasilopuntia brasiliensis (Willd.) A. Berger; AY875304, AY875234, AY875278, AY875343, AY875370; Cult. B 153-76-74-80; *Cubr 29521, 38452,* garden, B.

Calymmanthium substerile Ritter; AY875314, AY875230, AY875250, AY875320, AY015291; Cult. ZSS 89 3442; *Anon. 1437,* garden, ZSS.

Ceraria fruticulosa Pearson & Stephens;—, AY875218, AY875266,—, AY875371; Cult. YU; *Edwards 96,* garden, YU.

Cereus alacriportanus Pfeiff.;—,—,—,—, AY015313; Cult. ZSS 941313; *Eggli et al. 2493,* Brazil, Z.

Cereus fernambucensis Lemaire; AY875293, AY875240, AY875272, AY875328,—; Cult. B 166-88-83-10; *Leuenberger et al. 3107,* Brazil, B.

Echinocactus platyacanthus Link & Otto;—, AY875215, AY875257, AY875327, AY015287; Cult. ZSS 92–16–86; *Anon.,* Mexico, ZSS.

Echinocactus platyacanthus Link & Otto; AY875294,—,—,—,—; Cult. ZSS 90 2985/0; *Lüthy 015,* Mexico, ZSS.

Grahamia bracteata Gill.;—,—,—,—, AY015273; Cult. ZSS 94 1326; *Leuenberger & Eggli 4184,* Argentina, ZSS.

Grahamia bracteata Gill.; AY875308, AY875217, AY875273,—,—; Cult. B 142-32-94-10; *Leuenberger & Eggli 4230b,* Argentina, B.

Grahamia coahuilensis (S. Watson) G.D. Rowley; AY875310, AY875246, AY875280,—,—; Cult. B 262-01-94-40; *Lautner L92/22,* Mexico (Cult.), B.

Grahamia coahuilensis (S. Watson) G.D. Rowley;—,—,—,—, AY875374; Cult. ZSS 90 1259; *Glas & Foster 1934,* Mexico, ZSS.

Maihuenia patagonica (Phil.) Britton & Rose; AY875303, AY875245, AY875277, AY875342, AY015281; Cult. B 030-30-88-10; *Leuenberger & Arroyo 3850,* Argentina, B.

Maihuenia poeppigii (Pfeiff.) K. Schum.; AY875309, AY875216, AY875269, AY875329, AY015282; Cult. B 048-15-93-10; *Leuenberger & Arroyo 4180,* Chile, B.

Opuntia dillenii (Ker-Gawl.) Haw.; AY875302, AY875233, AY875283, AY875341, AY875369; Cult. B 304-11-99-10; *Greuter s.n.* (4 December 1999), Dominican Republic, B.

Pereskia aculeata Miller; AY875312, AY875229, AY875260, AY875323,—; Cult. NTG; *Edwards 5,* garden, YU.

Pereskia aculeata Miller;—,—,—,—, AY875355; Cult. B 376-02-86-20; *Zanoni 36108,* Dominican Republic, B.

Pereskia aureiflora Ritter; AY875297, AY875231, AY875261, AY875322, AY875354; Cult. B 166-54-83-20; *Leuenberger et al. 3054,* Brazil, B.

Pereskia bahiensis Gürke:—,—,—,—, AY875351; Cult. B 166-86-83-10; *Leuenberger et al. 3054,* Brazil, B.

Pereskia bleo (Kunth) D.C.; AY875289, AY875227, AY875265, AY875339,—; Cult. JBN; *Edwards 13,* garden, YU.

Pereskia bleo (Kunth) D.C.;—,—,—,—, AY875359; Cult. B 277-01-88-80; *Schwerdtfeger 12678,* garden, B.

Pereskia diaz-romeroana Cardenas;—,—,—,—, AY875353; Cult. B 039-02-77-30; *Rauh 40627,* Bolivia, B.

Pereskia grandifolia Howarth var. *grandifolia*; AY875298, AY875228, AY875253, AY875325,—; Cult. NTG; *Edwards 2,* garden, YU.

Pereskia grandifolia Howarth var. *grandifolia*;—,—,—,—, AY875362; Cult. B 038-04-77-80; *Schwerdtfeger 12489,* garden, B.

Pereskia guamacho F.A.C. Weber; AY875291, AY875242, AY875254, AY875335,—; Wild; *Edwards 15,* Venezuela, YU.

Pereskia guamacho F.A.C. Weber;—,—,—,—, AY015275; Cult. B 001-16-74-70; *Schwerdtfeger 13066,* garden, B.

Pereskia horrida (Kunth) D.C. var. *horrida*; AY875287, AY875224, AY875258, AY875332, AY875356; Cult. B 256-01-82-30; *Schwerdtfeger 13066,* garden, B.

Pereskia lychnidiflora D.C.; AY875286,—,—, AY875330, AY875358; Cult. B 003-04-78-10; *Leuenberger & Schiers 2508,* Guatemala, B.

Pereskia lychnidiflora D.C.:—, AY875238, AY875255,—,—; Cult. DBG 1990054502; *Zimmerman 2603,* Honduras, DBG.

Pereskia marcanoi Areces; AY875288, AY875221, AY875267, AY875337,—; Wild; *Edwards 9,* Dominican Republic, YU.

Pereskia marcanoi Areces;—,—,—,—, AY875360; Cult. B 259-02-82-30; *Marcano & Cicero,* Dominican Republic, B.

Pereskia nemorosa Rojas Acosta; AY875296, AY875241, AY875276, AY875334,—; Cult. B 305-01-80-70; *Cubr 23639b,* garden, B.

Pereskia nemorosa Rojas Acosta;—,—,—,—, AY875350; Cult. B 039-05-77-30; *Schwerdtfeger 7085a, 15650,* garden, B.

Pereskia portulacifolia (L.) D.C.; AY875315, AY875226, AY875264, AY875338,—; Wild; *Edwards 11,* Dominican Republic, YU.

Pereskia portulacifolia (L.) D.C.:—,—,—,—, AY875361; Cult. B 376-01-86-10; *Zanoni 35204,* Haiti, B.

Pereskia quisqueyana Liogier; AY875292, AY875220, AY875263, AY875336,—; Wild; *Edwards 7,* Dominican Republic, YU.

Pereskia quisqueyana Liogier;—,—;—,—, AY875352; Cult. B 259-05-82-33; *Cicero s.n.*, Dominican Republic, B.

Pereskia sacharosa Grisebach; AY875299, AY875222, AY875256, AY875326, AY875363; Cult. B 133-10-82-30; *Rente s.n.,* Bolivia, B.

Pereskia stenantha Ritter; AY875295, AY875244, AY875271, AY875333,—; Cult. B 166-81-83-20; *Leuenberger 3081,* Brazil, B.

Pereskia stenantha Ritter;—,—,—,—, AY015276; Cult. ZSS 86 4200; *Horst & Uebelmann 759,* Brazil, ZSS.

Pereskia weberiana K. Schumann; AY875313, AY875223, AY875259, AY875331, AY875357; Cult. B 046-03-80-70; *Schwerdtfeger 10206,* garden, B.

Pereskia zinniiflora D.C.; AY875290, AY875237, AY875262, AY875321, AY015277; Cult. ZSS 84 2526; *Anon. 19537,* Cuba (cult.), ZSS.

Pereskiopsis aquosa (F.A.C. Weber) Britton & Rose; AY875300, AY875225, AY875251, AY875324,—; Cult. YU; *Edwards 98,* garden, YU.

Pereskiopsis diguettii (F.A.C. Weber) Britton & Rose;—,—,—,—, AY015280; Cult. ZSS 94 2160; *Lomeli et al. s.n.,* Mexico, ZSS.

Pereskiopsis porteri (Brandegee ex F.A.C. Weber) Britton & Rose; AY875306, AY875243, AY875275, AY875344,—; Cult. B 169-03-84-30; *Hohmann s.n.,* Mexico, B.

Portulaca oleracea L.; AY875317, AY875249, AY875284,—,—; Unk.; *Applequist 7,* Unk., ISC.

Portulaca oleracea L.;—,—,—,—, AY875349; Wild; *Nyffeler s.n.,* Switzerland, Z.

Portulacaria afra Jacq.;—, AY875219, AY875268,—, AY875368; Cult. YU; *Edwards 97,* garden, YU.

Quiabentia verticillata (Vaupel) A. Berger; AY875319, AY875239, AY875279, AY875340,—; Cult. B 154-12-74-80; *Schwerdtfeger 22507,* garden, B.

Quiabentia zehntneri Britton & Rose; AY875307, AY875236, AY875274, AY875345, AY875372; Cult. B 163-09-88-30; *Leuenberger et al. 3078,* Brazil, B.

Talinum paniculatum (Jacq.) Gaertn.; AY875316, AY875214, AY875252,—,—; Cult. YU; *Edwards 6,* garden, YU.

Talinum paniculatum (Jacq.) Gaertn.:—,—,—,—, AY015274; Cult. ZSS 93 1952; *Martinez & Eggli 203,* Mexico, ZSS.

Tephrocactus articulatus (Pfeiff.) Backbg.; AY875318, AY875248, AY875285, AY875347, AY875367; Cult. YU; *Edwards 99,* garden, YU.