

PHYLOGENY OF OPUNTIOIDEAE (CACTACEAE)

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Opuntioideae are a diverse subfamily of Cactaceae with significant diversity of form and habitat and wide distribution throughout the New World. Nuclear ribosomal ITS and chloroplast *trnL-trnF* region DNA sequence-based phylogenetic analysis of Opuntioideae derived from Bayesian methods is presented. This analysis supports the following relationships: Opuntioideae are monophyletic; the subfamily is made up of four major lineages (*Maihueniopsis*-like species, *Pterocactus*, other terete-stemmed species, and flat-stemmed species). The two deepest lineages (including most species of *Maihueniopsis* and all *Pterocactus*) are marked by diminutive, succulent, often geophytic habit and are leafless at maturity. These analyses suggest that Opuntioideae have geographic origin in west-central South America. Generic circumscription is considered in the context of the recovered relationships. *Maihueniopsis* is recovered in multiple lineages. Many currently accepted genera may be para- or polyphyletic.

Keywords: Bayesian analysis, biogeography, Cactaceae, character evolution, classification, *Opuntia*.

Online enhancement: appendix table.

Introduction

Subfamily Opuntioideae of the Cactaceae contains 220–350 species of stem succulents native to the Western Hemisphere (Britton and Rose 1919; Barthlott and Hunt 1993; Anderson 2001). Opuntioideae is one of three traditionally recognized subfamilial groups (Cactoideae, Opuntioideae, and Pereskioideae; Cereae, Pereskiaee, and Opuntieae sensu Britton and Rose 1919). Opuntioideae are often viewed as more derived than the leafy, nonsucculent Pereskioideae and less derived than the functionally leafless Cactoideae; Opuntioideae mostly possess ephemeral leaves (Britton and Rose 1919; Hunt 1967; Barthlott and Hunt 1993; Hershkovitz and Zimmer 1997). Recent studies support the view that the Opuntioideae (along with *Maihuenia*) are relatively derived and are sister to the Cactoideae (Nyffeler 2002) or that they are more derived and are sister to Cactoideae + *Maihuenia* (Edwards et al. 2005). Similar studies present contrasting or ambiguous relationships (Wallace 1995; Martin and Wallace 2000; Crozier 2004; Griffith 2004b). Authors have proposed recognition of only two groups of cacti, based on segregating the Opuntioideae from other species (Sclerospermae and Malacospermae, sensu Vaupel 1925; Opuntioideae and a broadly circumscribed Cactoideae, sensu Hunt and Taylor 1986). Many treatments avoid a strong stand on subfamilial relationships (Benson 1982; Gibson and Nobel 1986; Wallace 1995; Anderson 2001).

Opuntioideae are circumscribed by two distinct synapomorphies: small deciduous, barbed spines (glochids) borne on areoles and a bony aril surrounding a campylotropous ovule (Gibson and Nobel 1986; Stuppy 2002). Cactoideae and Peres-

kioideae are often circumscribed by lack of glochids and leaves in Cactoideae and by presence of leaves and lack of glochids in Pereskioideae (Benson 1982; Barthlott and Hunt 1993; Anderson 2001). These circumscriptions rely on negative synapomorphies (Chernyshev 2003). Apart from the two synapomorphies, diverse aspects of vegetative and reproductive morphology have been used to circumscribe the numerous taxa of Opuntioideae (Daston 1946; Barthlott and Hunt 1993; Powell 1998; Anderson 2001; Stuppy 2002; Powell and Weedon 2004, among many others).

Generic and specific classification of Opuntioideae has been challenging. Lack of specimens (Benson 1982), paucity of exomorphic characters (Benson 1982; Gibson and Nobel 1986), natural and clonally reproducing hybrids (Benson and Walkington 1965; Pinkava 2002), and recognition of dubious taxa (Benson 1982; Iliff 2002) are cited obstacles. Frequent phenotypic plasticity among opuntioideae lineages can result in convergence (often via reduction), confounding generic circumscription (Barthlott and Hunt 1993; Stuppy 2001; Wallace and Gibson 2002). Although 21 opuntioideae genera have been validly published (Iliff 2002), most treatments divide a large, heteromorphous *Opuntia* from a few morphologically distinct, cohesive satellite genera such as *Pterocactus* and *Pereskiaopsis* (Britton and Rose 1919; Benson 1982; Barthlott and Hunt 1993). At least one early treatment recognized the artificiality of *Opuntia* sensu lato (Backeberg and Knuth 1935).

Recent morphological (Stuppy 2002) and molecular (Wallace and Dickie 2002) investigation suggest that *Opuntia* sensu lato (sensu Benson 1982) is polyphyletic. Those studies influenced recent classification (Anderson 2001) that divides the subfamily into 15 genera (table 1). Anderson's treatment of genera is widely accepted (Hunt 2002a), although underpinning studies employ preliminary sampling. The genus *Microopuntia* (Daston

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Table 1
Placement of Genera in Four Representative Treatments of Opuntioideae

Genus	Britton and Rose 1919	Backeberg and Knuth 1935	Benson 1982	Anderson 2001 ^a
<i>Austrocylindropuntia</i>	In <i>Opuntia</i>	In <i>Tephrocactus</i> and <i>Cylindropuntia</i>	In <i>Opuntia</i>	Recognized
<i>Brasiliopuntia</i>	In <i>Opuntia</i>	Recognized	In <i>Opuntia</i>	Recognized
<i>Consolea</i>	In <i>Opuntia</i>	Recognized	In <i>Opuntia</i>	Recognized
<i>Corynopuntia</i>	In <i>Opuntia</i>	Recognized	In <i>Opuntia</i>	In <i>Grusonia</i>
<i>Cumulopuntia</i>	In <i>Opuntia</i>	In <i>Tephrocactus</i>	In <i>Opuntia</i>	Recognized
<i>Cylindropuntia</i>	In <i>Opuntia</i>	Recognized	In <i>Opuntia</i>	Recognized
<i>Grusonia</i>	Recognized	Recognized	In <i>Opuntia</i>	Recognized
<i>Maihuenia</i>	Recognized	In <i>Pe[i]reskioideae</i>	In <i>Pereskiaea</i>	In <i>Maihuenioideae</i>
<i>Maihueniopsis</i>	In <i>Opuntia</i>	Recognized	In <i>Opuntia</i>	Recognized
<i>Miqueliopuntia</i>	In <i>Opuntia</i>	In <i>Cylindropuntia</i>	In <i>Opuntia</i>	Recognized
<i>Nopalea</i>	Recognized	Recognized	In <i>Opuntia</i>	In <i>Opuntia</i>
<i>Opuntia</i>	Recognized	Recognized	Recognized	Recognized
<i>Pereskioipsis</i>	Recognized	Recognized (as <i>Pe[i]reskiopsis</i>)	Recognized	Recognized
<i>Pterocactus</i>	Recognized	Recognized	Recognized	Recognized
<i>Quiabentia</i>	In <i>Pereskia</i>	Recognized	Recognized	Recognized
<i>Tacinga</i>	Recognized	Recognized	Recognized	Recognized
<i>Tephrocactus</i>	In <i>Opuntia</i>	Recognized	In <i>Opuntia</i>	Recognized
<i>Tunilla</i>	In <i>Opuntia</i>	In <i>Opuntia</i>	In <i>Opuntia</i>	Recognized

^a Many students of cacti now recognize *Nopalea* in addition to these genera (Hunt 2002a).

1946) is included in *Grusonia* sensu Anderson, although *Micro-puntia* was not included in previous analysis. Microgenera not examined (e.g., *Pseudotephrocactus* Fric and Scheele) potentially represent distinct lineages that deserve recognition (Griffith 2002). Testing current classification via broadened sampling is one aim of this study. Here we test of the hypothesis of monophyly of tribal classifications (Wallace and Dickie 2002), using data from both nuclear ribosomal and chloroplast genes. Molecular data may also offer an independent test of monophyly of groups recognized in recent generic classifications (Barthlott and Hunt 1993; Anderson 2001; Stuppy 2002).

Characters circumscribing some Opuntioideae may not reflect natural groups. For example, *Puna*, unified by geophytism (Kiesling 1982; Ferguson and Kiesling 1997), appears unnatural based on seed anatomical (Stuppy 2001) and molecular investigation (Wallace and Dickie 2002). *Maihueniopsis* and *Cumulopuntia* intergrade morphologically (Kiesling 1984) and are distinguished by distribution of areoles (Iliff 2002; Stuppy 2002), although seed characters may be useful (Stuppy 2002). Putative homoplasy may result from convergence, possibly via paedomorphic reduction of characters, a proposed process among cacti (Boke 1944; Buxbaum 1956; Anderson 1986; Mauseth and Landrum 1997; Mauseth 2006a). Or numerous lineages may have radiated from an ancestral, relatively undifferentiated form similar to *Maihueniopsis* or *Puna*, and these taxa may unite multiple extant lineages circumscribed by symplesiomorphies. Molecular investigation may illuminate possible paedomorphic convergence and/or radiation of multiple lineages from an ancestral morphotype.

Molecular investigation of relationships among Opuntioideae can offer other insights. Similarities among Opuntioideae in North and South America (*Austrocylindropuntia* and *Cylindropuntia*; *Micro-puntia* and *Pterocactus*) prompted biogeographic hypotheses (Daston 1946; Buxbaum 1969). These biogeographic relationships merit further and more inclusive testing.

To address these questions, we have sequenced nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *trnL-trnF* region DNA from specimens of Opuntioideae representing all genera recognized in recent treatments, as well as additional species of uncertain placement. These sequences have been analyzed using Bayesian analyses.

Material and Methods

Plants

We examined 110 specimens of Opuntioideae. Ingroup sampling was based on current generic circumscriptions (Anderson 2001; Stuppy 2002), with liberal inclusion of taxa to test accepted generic boundaries (table 2). Outgroup sampling was based on the phylogenetic relationships of the Cactaceae suggested by previous studies (Wallace 1995; Hershkovitz and Zimmer 1997; Applequist and Wallace 2001; Nyffeler 2002; Edwards et al. 2005). Two specimens of *Pereskia* were examined directly, and GenBank accessions from eight additional taxa in Cactaceae and Portulacaceae were also used for outgroups. Plants were obtained from documented living collections at DES, RSA, K, HNT, SRSC, Mesa Garden (Belen, NM), and Texas A&M University (Kingsville) or from specimens field-collected by the authors. Determinations of all acquired plants were verified by comparison with published descriptions (Britton and Rose 1919; Anderson 2001; Iliff 2002). Table A1, available in the online edition of the *International Journal of Plant Sciences*, lists sources of plants studied.

Data Inclusion

Generic limits within Opuntioideae have historically been determined by morphological characters. Therefore, this study included only DNA sequence data, in order to provide an in-

Table 2
Sampling Structure

Genus	Accepted species	Sampled species
<i>Austrocylindropuntia</i> Backeb.	10	4
<i>Brasiliopuntia</i> A. Berger	1	1
<i>Consolea</i> Lem.	9	6
<i>Cumulopuntia</i> F. Ritter	20	5
<i>Cylindropuntia</i> (Engelm.) F. M. Knuth	42	12
<i>Grusonia</i> Hort. Nicolai. ex K. Schum. ^a	17 (1, 15, 1)	8 (1, 6, 1)
<i>Maihueniopsis</i> Speg. ^b	18 (15, 3)	9 (6, 3)
<i>Miqueliopuntia</i> Fric ex F. Ritter	1	1
<i>Opuntia</i> Mill. ^c	191 (182, 9)	36 (30, 6)
<i>Pereskioopsis</i> Britton & Rose	8	2
<i>Pterocactus</i> K. Schum.	9	6
<i>Quiabentia</i> Britton & Rose	2	1
<i>Tacinga</i> Britton & Rose	7	2
<i>Tephrocactus</i> Lem.	6	4
<i>Tumilla</i> D. R. Hunt & J. Iliff	9	2

Note. Species accepted and sampled following Anderson (2001).

^a Parentheses indicate numbers of *Grusonia*, *Corynopuntia*, and *Micropuntia* species, respectively, treated as segregates in this study.

^b Parentheses indicate numbers of *Maihueniopsis* and *Puna* species, respectively, treated as segregates.

^c Parentheses indicate number of *Opuntia* and *Nopalea* species, respectively, treated as segregates.

dependent test of morphologically determined classification (Felsenstein 1985; MacLeod 2001; Pisani et al. 2002).

DNA Sequencing

A protocol for DNA extraction from mucilaginous cacti (Griffith and Porter 2003) was used for all extractions. Amplification of templates for sequencing was performed with the primers *trnE*, *trnF* (Taberlet et al. 1991), ITS5, and ITS4 (White et al. 1990). Thermal cycling parameters for the nrITS and *trnL-trnF* regions follow Porter (1996) and Porter et al. (2000), respectively. PCR products were screened after amplification, and only templates with single bands were used. Templates were purified by precipitation in polyethylene glycol (Morgan and Soltis 1993) and washed once in 100 μ L 80% ethanol. Purified templates were then sequenced directly with six primers: ITS5, ITS4, ITS3, ITS2 (White et al. 1990), *trnE*, and *trnF* (Taberlet et al. 1991). For cycle sequencing, Big Dye chemistry (Applied Biosystems) was used according to the manufacturer's specifications. An Applied Biosystems 3100 genetic analyzer gathered all DNA sequence data.

Sequence Editing and Alignment

Raw sequences were assembled into contigs and edited using Sequencer, version 4.1 (Gene Codes). Sequences were screened for fungal contamination via comparison with previously published sequences (Altschul et al. 1990). The ends of the nrITS and *trnL-trnF* regions were determined via comparison with published sequences for *Gilia* (Polemoniaceae; for nrITS; Porter 1996) and *Sclerocactus* (Cactaceae; for *trnL-trnF*; Porter et al.

2000). The program Se-AL (Rambaut 1996) assisted with manual alignment of consensus sequences, which were converted to a NEXUS file used for subsequent analyses. Indels were not coded and were treated as missing data.

Phylogenetic Estimation

The aligned data matrix was analyzed using Bayesian analysis (Rannala and Yang 1996; Douady et al. 2003). Bayesian analyses were implemented with Mr. Bayes, version 3.1.2 (Huelsenbeck and Ronquist 2001). For the analysis, the sequence data was partitioned by gene into two sets representing the nrITS and *trnL-trnF* sequence data, and a site-specific gamma model was employed (Huelsenbeck 2002). For all Bayesian analyses, a random starting tree was used, and four Markov chain Monte Carlo (MCMC; Metropolis et al. 1953) replicates were run for 50,000 generations (nrITS or *trnL-trnF* only) or 500,000 generations (combined analysis), sampling every 100 generations. Metropolis-coupled MCMC analyses were performed using the Metropolis-Hastings-Green algorithm (Huelsenbeck and Ronquist 2001), as implemented by Mr. Bayes. The log likelihood for each sampled tree was viewed and graphed using Microsoft Excel. Trees from the burn-in period (Huelsenbeck and Ronquist 2001) were visually identified and eliminated, and a majority rule consensus tree was recovered using PAUP (Swofford 1998) for remaining trees sampled. The Bayesian analyses were replicated 10 times, and the results from each replication were compared for consistency. The posterior probabilities of specific clades being recovered given the data (Miller et al. 2002) were determined as frequencies of each clade occurring in the majority rule consensus tree (obtained by PAUP). These values represent estimations of confidence in the relationships recovered by the Bayesian analysis (Huelsenbeck and Ronquist 2001).

Results

The recovered sequences varied from 560 to 619 (575–583 in ingroup) nucleotides for nrITS and from 427 to 438 nucleotides for *trnL-trnF*. Aligned sequences resulted in matrices of 708 (nrITS) and 474 (*trnL-trnF*) base pairs. The nrITS alignment had 208 variable sites (29.8%) in the ingroup, and the *trnL-trnF* alignment gave 80 variable sites (16.8%). The highest likelihood values recovered via Bayesian analyses were $-3,222.0021$ for the nrITS data and $-1,450.9329$ for the *trnL-trnF* data. Bayesian analysis replicates for the combined data had a burn-in period of 100,000 generations, leaving $\sim 400,000$ generations of explored tree space/model space for computing the posterior probability distribution of recovered clades. The partition homogeneity test did not present significant evidence for incongruity between the two regions ($P = 0.07$). The combined-data Bayesian consensus tree is shown in figure 1. All 10 replicates of the Bayesian analysis showed an identical basic topology.

Two cohesive deep lineages appear in the combined analyses: (1) a clade of *Maihueniopsis* and *Cumulopuntia* is sister to all other Opuntioideae, and (2) a clade of *Pterocactus* is sister to the remaining Opuntioideae. The remaining specimens are included in two lineages: (1) a clade marked by flat-stemmed morphology (containing the genera *Brasiliopuntia*, *Consolea*, *Miqueliopuntia*, *Nopalea*, *Opuntia*, *Tacinga*, and *Tumilla*) and (2) a clade containing

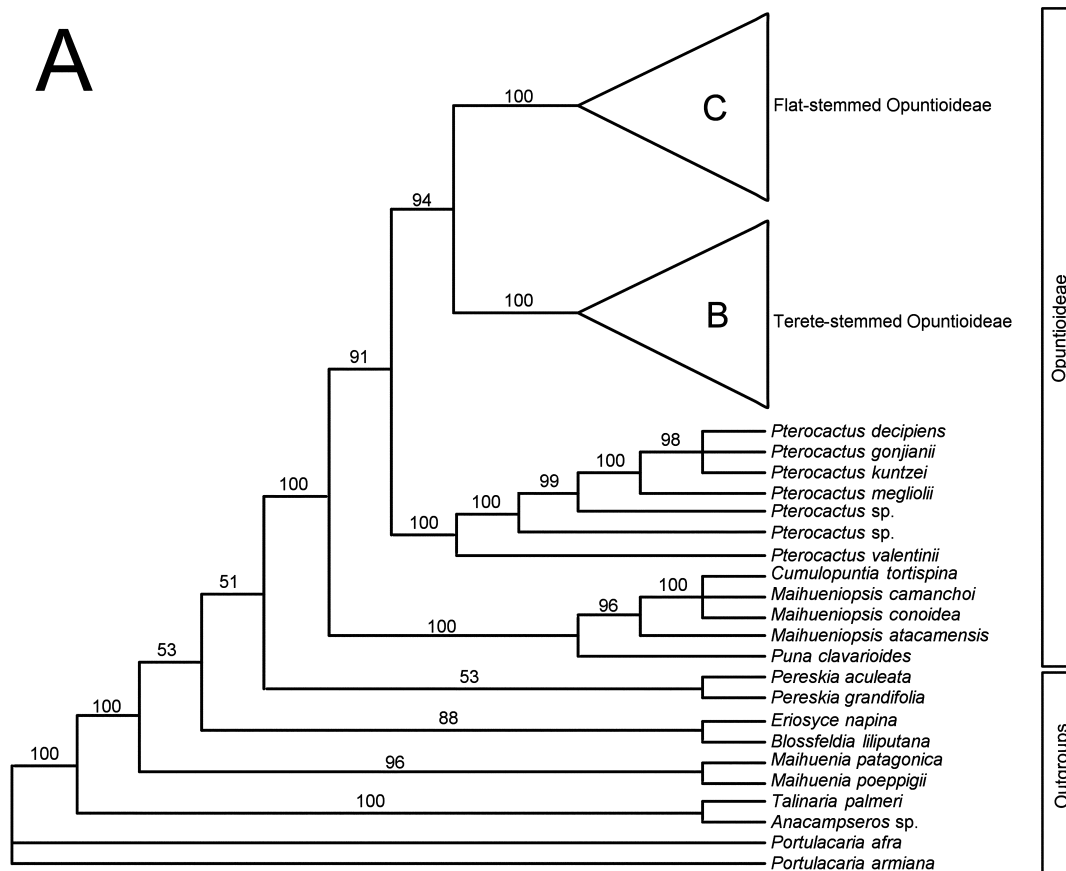


Fig. 1 Bayesian analysis of combined nrITS and *trnL-trnF* data. Numbers above branches represent the percentage of explored high-likelihood trees possessing that clade. Four major clades of Opuntioideae were recovered. A, Overall structure; detail of outgroups and two deepest lineages; B, terete-stemmed Opuntioideae; C, flat-stemmed Opuntioideae.

the remaining terete-stemmed opuntioids (*Austrocylindropuntia*, *Corynopuntia*, *Cylindropuntia*, *Grusonia*, *Micropuntia*, *Pereskiaopsis*, *Quiabentia*, and *Tephrocactus*, *Cumulopuntia*, and *Maihueniopsis*, in part). Specimens currently treated as *Cumulopuntia* and *Maihueniopsis* are found in multiple lineages, with *Maihueniopsis* appearing widely polyphyletic (fig. 1). A number of genera appear to circumscribe natural monophyletic groups. These relationships are discussed below.

Discussion

Relationships among the Opuntioideae

The combined nuclear and chloroplast DNA sequence analyses strongly support Opuntioideae as monophyletic (100% Bayesian posterior probability). This reinforces earlier evidence from plastid gene deletions (Wallace 1995; Wallace and Gibson 2002) and chloroplast sequences (Wallace and Dickie 2002) and is additional evidence that glochids and seed arils represent real synapomorphies for this group. Four major lineages are supported by this study: two early-divergent lineages giving rise to *Maihueniopsis*-like plants and the genus *Pterocactus* and two more recent lines marked by terete- or flat-stemmed morphology. These clades are discussed in this section.

Core Maihueniopsis lineage. The clade that is sister to all other Opuntioideae (in this study) consists of four samples from the genus *Maihueniopsis* and one specimen of *Cumulopuntia*. Neither one of these genera appears monophyletic (see below). Recent studies propose a basal lineage for the Opuntioideae composed of *Austrocylindropuntia* (Wallace and Dickie 2002; Wallace and Gibson 2002); this genus is excluded from the deepest clade recovered here. The phylogenetic position of *Puna* (*Maihueniopsis*) *clavarioides* in this deepest lineage is notable; this species is sister to all other core *Maihueniopsis* plants, and it is unusual relative to other Opuntioideae in its geophytism and complete lack of glochids (Stuppy 2002). The core *Maihueniopsis* clade is very well supported (100% Bayesian posterior probability; fig. 1A).

Pterocactus lineage. All *Pterocactus* specimens examined fall into this well-supported (100% Bayesian posterior probability), monophyletic group. This lineage is also morphologically cohesive; the seeds of *Pterocactus* possess a distinct winged aspect unlike any other opuntioideid seed (Schumann 1897; Stuppy 2002). This genus has long been recognized as distinct and cohesive (table 1; fig. 2).

Terete-stemmed lineage. One of two strongly supported (100%) highly diverse clades of the remaining Opuntioideae is marked by terete-stemmed morphology. Plants circumscribed

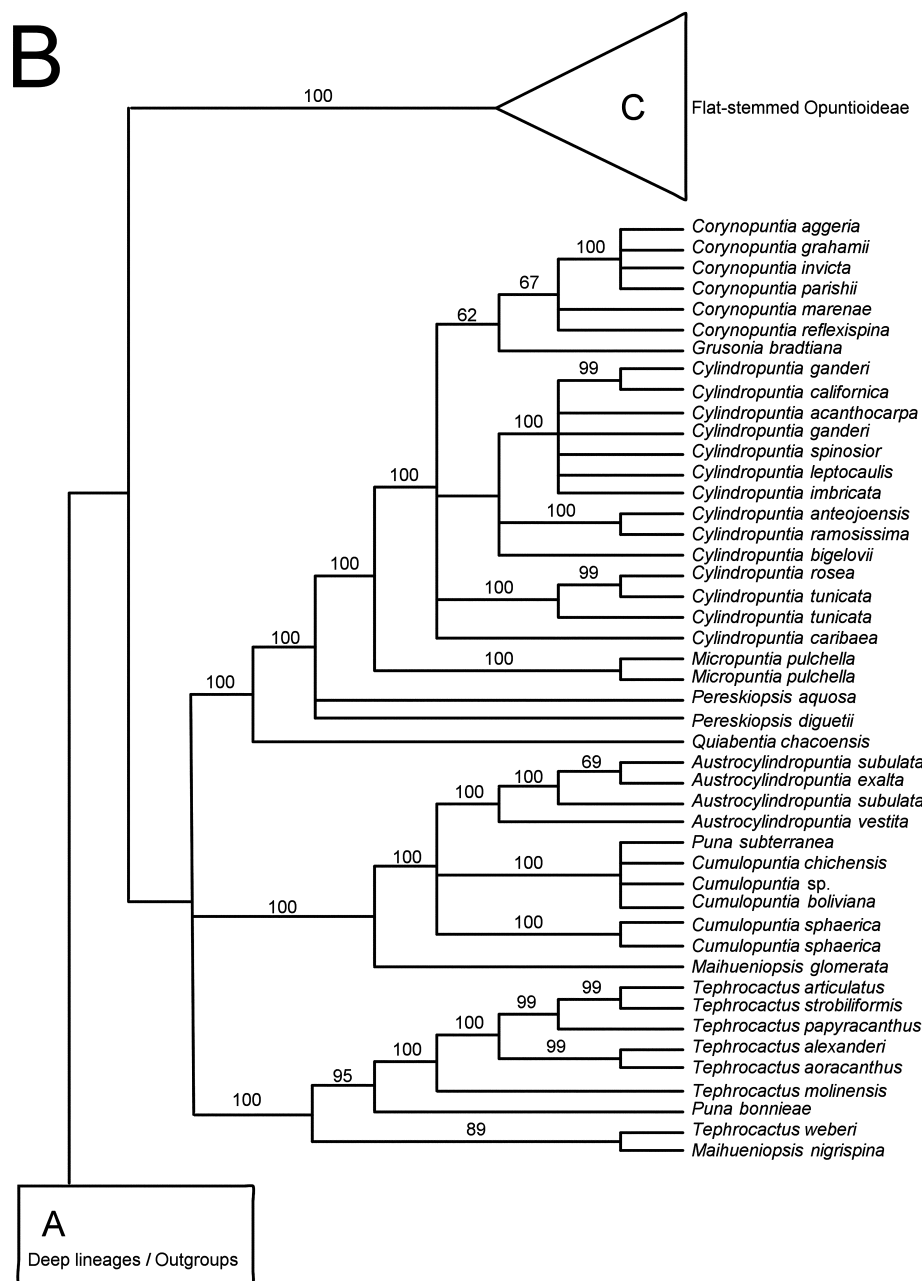


Fig. 1 (Continued)

as *Austrocylindropuntia*, *Corynopuntia*, *Grusonia*, *Micropuntia*, *Pereskiopsis*, *Quiabentia*, and *Tephrocactus* make up this group. Specimens of *Cumulopuntia* and *Maihueniopsis* are also included here, in addition to those that belong to clade 1 (above). The relatively derived position of *Pereskiopsis* is of note because this genus retains relictual anatomical features (Mauseth 2005).

Flat-stemmed lineage. The remaining Opuntioideae form a very well-supported (100% Bayesian posterior probability) clade marked by flat cladodes (as in prickly pears). All specimens of *Brasiliopuntia*, *Consolea*, *Miqueliopuntia*, *Nopalea*, *Opuntia* sensu stricto, and *Tumilla* that were examined group in this lineage. In addition, one *Maihueniopsis* species, *Maihueniopsis ovata*,

is included in this clade. The occurrence of this specimen in this lineage prompted resequencing of two accessions to verify this outcome, and all replications gave this result (fig. 1C). Placement of *Maihueniopsis* in this phylogeny is addressed below.

Biogeographical Overview

Phylogenetic relationships recovered by this study support a west-central South American origin for the Opuntioideae, consistent with previous studies (Buxbaum 1969; Gibson and Nobel 1986; Wallace and Dickie 2002; Wallace and Gibson 2002; Edwards et al. 2005). The two early-divergent lineages are

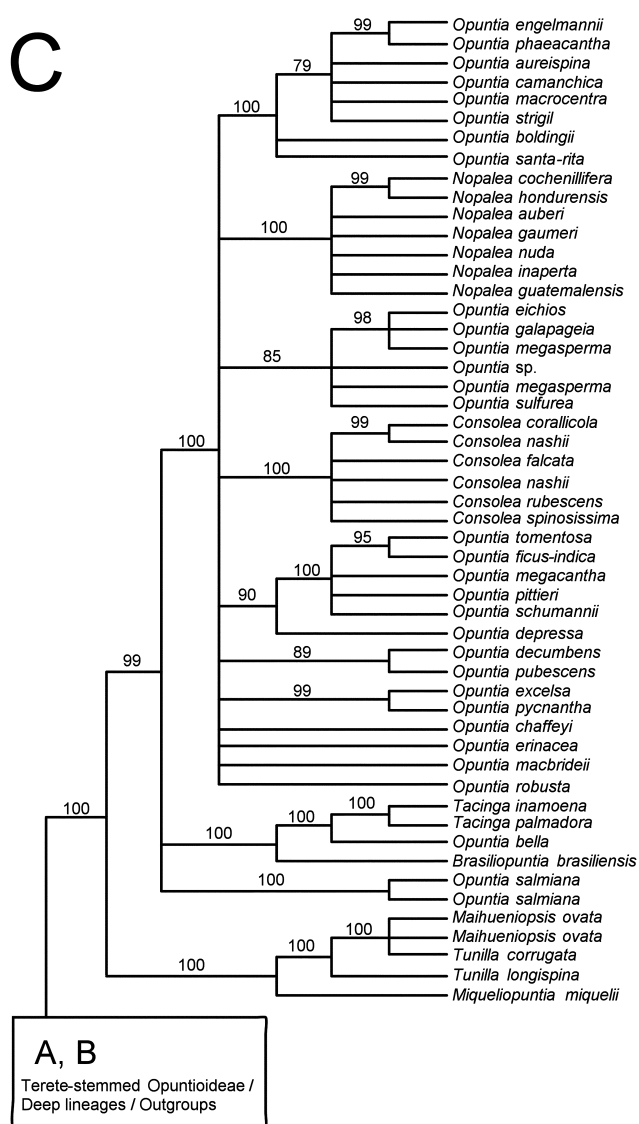


Fig. 1 (Continued)

extant only in Argentina, Bolivia, Chile, and Peru (Britton and Rose 1919; Anderson 2001; Iliff 2002). Only late-diverging members of terete- and flat-stemmed lineages of Opuntioideae are represented in North America, with particularly high diversification of taxa in the Chihuahuan Desert region (Gómez-Hinostrosa and Hernández 2000; Hernández et al. 2001; Powell and Weedon 2004).

Classification of the Opuntioideae

Lower-level classification has been variable. Classifications of Britton and Rose (1919), Backeberg and Knuth (1935), and Benson (1982) differ in significant ways (table 1). Based on contemporary philosophy, earlier circumscriptions are not marked by monophyly (fig. 2). Recent tribal classification of Opuntioideae based on cpDNA (Wallace and Dickie 2002) recognized five monophyletic tribes: Opuntieae, Cyliandropuntieae, Austrocylindropuntieae, Tephrocactae, and Pterocactae. These five tribes

are morphologically cohesive, and four tribes (all except Tephrocactae) are essentially monophyletic in this study as well. Hunt (2002b) proposed recognition of these tribal groups as genera, pending more inclusive examination.

Generic circumscription in this group requires careful study. Currently, the most predictive morphological approach has been analysis of seed characters (Stuppy 2001, 2002). The work presented here, although the broadest sampling of Opuntioideae to date, may not fully tease out all the complicated relationships in this group, particularly with regard to South American genera. For that task, comprehensive multigenic sampling of all taxa will be desirable, based on the degree of polyphyly inferred from the analyses of this study. Molecular phylogenetic analyses can illuminate cactus evolution, but classification based solely on limited molecular sampling may lend undue instability to classification, particularly in this group, with many stakeholders (Hendriksen 1993; Hoffman 1993; Rowley 1997; see also Hannon 1993; Wallace 1993; Gorelick 2002). That caveat notwithstanding, some observations on generic circumscription can be made via the following analyses.

The genera Grusonia, Micropuntia, and Corynopuntia. This work provides additional support for recognizing the monotypic *Grusonia* circumscription of Britton and Rose (1919) over more recent treatments that include *Micropuntia* and *Corynopuntia* (Anderson 2001; Wallace and Dickie 2002; Pinkava 2003). *Micropuntia* (Daston 1946) is well supported (100%) as sister to *Cylindropuntia*, *Grusonia* sensu stricto, and *Corynopuntia*. *Micropuntia*, therefore, is best treated as a segregate genus. *Grusonia*, *Micropuntia*, and *Corynopuntia* are morphologically distinct and cohesive (Griffith 2002).

The genus Puna. Kiesling (1982) established this genus, now widely included in *Maihueniopsis* (Anderson 2001; Hunt 2002a), although *Puna bonnieae* has been rightly placed in *Tephrocactus* (Stuppy 2001). All three described species are included in our analyses, and the uniting characters (fleshy geophytic storage organ, crowding of areoles near distal end of cladodes; Kiesling 1982) appear to be iterative. *Puna clavarioides* groups with *Maihueniopsis* (fig. 1A), and the third species, *Puna subterranea*, exhibits a close relationship with *Cumulopuntia* (fig. 1B).

The genus Cumulopuntia. Ritter first recognized this genus in 1980 (Iliff 2002). In modern treatments, 20 species are recognized (Anderson 2001, after Iliff 2002). Of six specimens, five are included in a well-supported (100% Bayesian posterior probability) clade with *Austrocylindropuntia* and one specimen of *Puna* (the “core *Cumulopuntia*” lineage). One specimen is well supported as sharing recent ancestry with *Maihueniopsis* species (*Cumulopuntia tortispina*; fig. 1A). *Cumulopuntia* will require close scrutiny in future treatments. As noted above, Kiesling (1984) states that *Cumulopuntia* and *Maihueniopsis* appear to intergrade morphologically; this observation is reflected in the mutual polyphyly seen here.

The genera Consolea and Nopalea. These genera are morphologically distinct and cohesive (Anderson 2001; Rebman 2002), and this is reflected in our molecular analyses. *Consolea* and *Nopalea* each exhibit specialized floral morphologies that readily segregate each from *Opuntia*. Both genera form strongly supported monophyletic groups (100% Bayesian posterior probability for each; fig. 1C). Although the resolution within and among *Opuntia* s.s. is not complete, *Consolea* and *Nopalea*

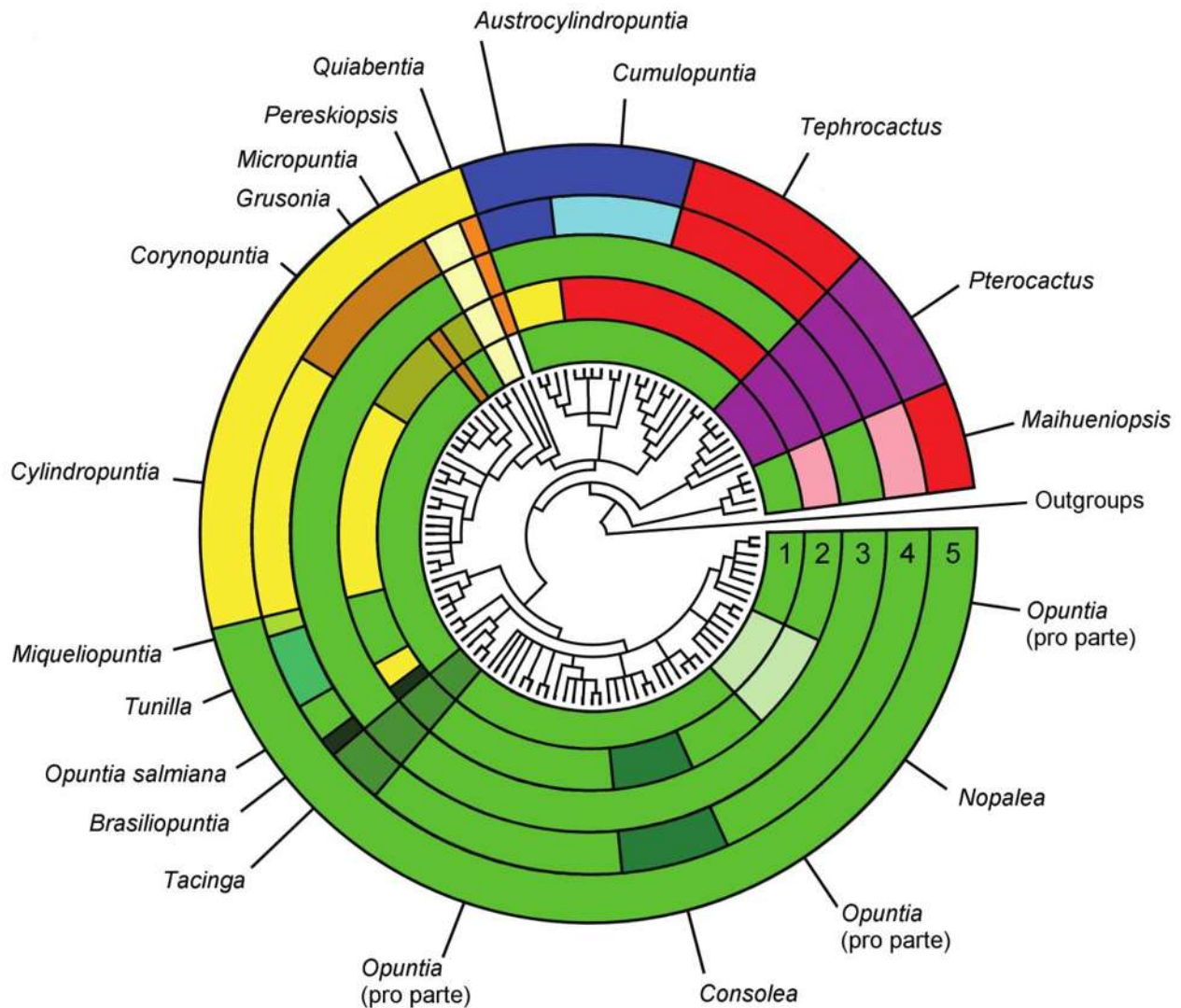


Fig. 2 Reciprocal comparison of Opuntioideae classifications (table 1) and the current phylogeny. Identities of clades are marked along the outside of the circle, and classifications (by treatment) are held together by color. Classifications are as follows: 1 = Britton and Rose (1919); 2 = Backeberg and Knuth (1935); 3 = Benson (1982); 4 = Anderson (2001); 5 = Hunt (2002*b*, as provisional genera) or Wallace and Dickie (2002, as tribes). The genus *Pterocactus*, with the unique synapomorphy of winged seed, has been consistently recognized as a segregate genus. Other generic limits have differed over the last century.

group with *Opuntia*. Separate analyses of these nuclear and chloroplast data place *Consolea* differently relative to *Opuntia* (Griffith 2005). Negron-Ortiz (2007) demonstrated chromosomal evidence consistent with hybrid origin of some species of *Consolea*. Morphological (Griffith 2001*b*), biosystematic (Griffith 2001*a*), RAPD (Mayer et al. 2000) and median network analysis (Griffith 2003) have demonstrated reticulation in other Opuntioideae. A separate history for the chloroplast in *Consolea* may indicate the possibility of reticulate evolution (Mason-Gamer and Kellogg 1996). Imposing a tree topology may obscure potential reticulate relationships. Further analysis for reticulation may address this question (Huson and Bryant 2006). Two-thirds of *Consolea* species are known polyploids, while no *Nopalea* species are known polyploids (Pinkava 2002). Based on the analysis in this study, if strict monophyletic ge-

neric classification is desired, *Consolea* and *Nopalea* may be sunk into a slightly wider *Opuntia* (yet not as wide as *Opuntia* sensu Benson).

The placement of Opuntia salmiana. This terete-stemmed South American species has often been *sedes incertae* (Hunt and Taylor 2002). Anderson (2001) places it in *Opuntia*, but *O. salmiana* has been included in *Cylindropuntia* (Knuth 1935), *Austrocyllindropuntia*, and the monotypic *Salmiopuntia* proposed by Fric in 1935 (Ilf 2002), although that name is not validly published. Our study sampled two accessions, and the data support a distinct lineage for *O. salmiana* within the flat-stemmed clade (fig. 1C). With the distinct character combination of terete, smooth stems and tunalike fruits (Anderson 2001), recognition of these plants under a monotypic genus (like *Salmiopuntia* Fric, nom. inval.) may be advised.

The Maihueniopsis problem. This genus is narrowly circumscribed (Stuppy 2002) as forming compact cushions, with small ovate to obconic cladodes and areoles evenly distributed upon the cladode. As noted above, plants from this genus appear in numerous lineages. *Maihueniopsis* appears in four separate clades (fig. 1). *Maihueniopsis camachoii*, *Maihueniopsis conoidea*, and *Maihueniopsis atacamensis* (along with *Puna clavarioides* and *Cumulopuntia tortispina*) form a well-supported monophyletic group that is sister to all other Opuntioideae (the “core *Maihueniopsis*” lineage). Additional *Maihueniopsis* species are found: (a) sister to the core *Cumulopuntia* and *Austrocylindropuntia* (*Maihueniopsis glomerata*), (b) included within the well-supported *Tephrocactus* clade (*Maihueniopsis nigripina*), and (c) in the clade sister to all other flat-stemmed Opuntioideae, with members of the genera *Miqueliopuntia* and *Tunilla* (*Maihueniopsis ovata*). These specimens were resampled and resequenced to verify these results, and the determinations were reverified. Pronounced polyphyly prompts examination of two hypotheses. Firstly, characters used to circumscribe *Maihueniopsis* may be convergent between numerous lineages, as proposed for *Puna* (Stuppy 2001). This convergence could result from paedomorphosis since the external morphology of *Maihueniopsis* is uncomplicated relative to that of other Opuntioideae (Anderson 2001). Otherwise, if these characters do not result from convergence, they may represent persistent relictual characters; in other words, *Maihueniopsis* is circumscribed by symplesiomorphies. In the phylogeny recovered here, *Maihueniopsis* is the deepest lineage in Opuntioideae and otherwise appears in deep positions relative to conladal taxa. Some implications of this result on concepts of character evolution in Cactaceae are discussed elsewhere (Griffith 2004a, 2004b).

Other taxa. Neither *Opuntia* sensu stricto nor *Cylindropuntia*, the two largest and most widespread genera, appears to circumscribe monophyletic groups. *Opuntia bella*, native to Colombia, groups with *Brasiliopuntia* and *Tacinga*. The following genera (excluding those species detailed above) appear to be well-supported, cohesive, and monophyletic, as inferred from this

study: *Austrocylindropuntia*, *Pterocactus*, *Tacinga*, and *Tephrocactus*. Additionally, *Brasiliopuntia* and *Miqueliopuntia* are monotypic genera, and neither appears nested within other genera. *Quiabentia* has two recognized species (Anderson 2001); the one specimen sampled here is not nested within any other genus.

Synoptic view. At the minimum, this study clearly demonstrates the artificiality of *Opuntia* sensu lato and provides initial insight into other potential problems in circumscription. As noted above, classification changes might be best postponed to a point at which we have broad information (of multiple data types) regarding all species of opuntoid cacti. Examination of anatomical characters of the deeper lineages (South American globose and geophytic Opuntioideae) in the context framed by these phylogenetic analyses will provide further insight (Mauseth 2006b). Resolution of relationships in the flat-stemmed Opuntioideae will require further phylogenetic study.

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