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Molecular systematics of the Cactaceae

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Abstract

Bayesian, maximum-likelihood, and maximum-parsimony phylogenies, constructed using nucleotide sequences from the plastid gene region *trnK-matK*, are employed to investigate relationships within the Cactaceae. These phylogenies sample 666 plants representing 532 of the 1438 species recognized in the family. All four subfamilies, all nine tribes, and 69% of currently recognized genera of Cactaceae are sampled. We found strong support for three of the four currently recognized subfamilies, although relationships between subfamilies were not well defined. Major clades recovered within the largest subfamilies, Opuntioideae and Cactoideae, are reviewed; only three of the nine currently accepted tribes delimited within these subfamilies, the Cactaeae, Rhipsalidoideae, and Opuntieae, are monophyletic, although the Opuntieae were recovered in only the Bayesian and maximum-likelihood analyses, not in the maximum-parsimony analysis, and more data are needed to reveal the status of the *Cylindropuntieae*, which may yet be monophyletic. Of the 42 genera with more than one exemplar in our study, only 17 were monophyletic; 14 of these genera were from subfamily Cactoideae and three from subfamily Opuntioideae. We present a synopsis of the status of the currently recognized genera.

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Gibson et al. (1986) noted that the cacti are famous for their beautiful flowers and many bizarre vegetative features, but infamous for their formidable nomenclatural and systematic problems. In the same year the Cactaceae Working Party of the International Organisation for Succulent Plant Study (IOS) published its first report (Hunt, 1986) and began to address these formidable problems. The Working Party, which later became the International Cactaceae Systematics Group, considered the coexistence of numerous conflicting taxonomies and the associated proliferation of names in usage to be untenable. They sought a stable framework through consensus lists of genera which would serve as working hypotheses until stable, well-supported phylogenies became available. Molecular systematics was seen as having potential to provide stability and clarity at the

generic level, but it was recognized that considerable work was needed. At higher levels, the International Cactaceae Systematics Group were less cautious in adopting novel classifications suggested by molecular phylogenies, and supported the erection of a fourth subfamily, the *Maihuenioideae*, based on molecular studies (Wallace, 1995). In contrast, the subfamilies *Blossfeldioideae* and *Rhipsalidoideae*, erected and resurrected respectively by Crozier (2004) based on molecular phylogenies, have not found accepted usage.

The most recent consensus on generic and species limits for the Cactaceae is presented in the “New Cactus Lexicon” (Hunt, 2006). Although Hunt (2006) stated that it was not his intention to present a definitive or fully worked out higher-level classification, he does present a synopsis of subfamilies, tribes, genera, and groups. These groupings, which differ in some respects from those presented by, for example, Endler and Buxbaum (1974), Gibson and Nobel (1986), Barthlott and Hunt (1993), and Anderson (2001), represent a current working taxonomy which we review in the light

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of our phylogeny. Hunt (2006) recognizes four subfamilies, Cactoideae, Opuntioideae, Pereskioideae, and Maihuenioideae. The Cactoideae is the largest subfamily, representing seven tribes: Cacteeae (25 genera), Cereeeae (15), Echinocereeeae (25), Hylocereeeae (six), Notocacteeae (seven), Rhipsalideae (four), and Trichocereeeae (23). Opuntioideae is the next largest subfamily with two tribes, Opuntieae and Cyllindropuntieae, comprising ten and seven genera, respectively. Two other subfamilies, Pereskioideae and Maihuenioideae, each comprise a single genus.

To date, the most densely sampled phylogeny that represented the whole of the family sampled 70 taxa (Nyffeler, 2002); we add sequences to increase the density of sampling for the family approximately eightfold. We have sampled 86 of the 124 genera (69%) recognized in the New Cactus Lexicon (Hunt, 2006), including representatives of every subfamily and tribe. Our phylogenies are reconstructed from a single region only and therefore may provide less resolution

and support than other studies employing multiple gene regions. Nyffeler (2002) sampled both the *trnK-matK* region we employ here and the *trnL-trnF* region. Another significant study is that of Edwards et al. (2005), which sampled five regions including *trnK-matK* for all the species of Pereskioideae and Maihuenioideae, plus four exemplars of the Cactoideae and eight of the Opuntioideae. To present the most complete overview of the systematic relationships of the family, we review these and other studies (listed in Table 1) in the light of our findings, noting where published studies provide more robust or alternative hypotheses of relationship to those we present here. The structure of our discussion reflects the taxonomic biases of our sampling. We present significant additional data, relative to Nyffeler (2002), for four tribes, namely Cacteeae and Echinocereeeae of the Cactoideae and the two tribes of the Opuntioideae, the Opuntieae and Cyllindropuntieae. The systematics of these tribes is reviewed in detail.

Table 1
Previously published molecular phylogenies based on sequence data for the family Cactaceae

Publication	Taxonomic focus; sampling	Gene regions sampled; genome
Porter et al. (2000)	<i>Sclerocactus</i> and <i>Toumeyia</i> ; 23 exemplars, 22 taxa	<i>trnL-trnF</i> , <i>trnS-trnfM</i> intergenic spacers; plastid
Butterworth et al. (2002)	Tribe Cacteeae; 62 ingroup taxa plus exemplars of four other genera	<i>rpl16</i> intron; plastid
Wallace and Dickie (2002)	Opuntioideae; 33 taxa plus two outgroup taxa	<i>rpl16</i> intron; plastid
Griffith (2002)*	Genus <i>Grusonia</i> ; 36 taxa	rDNA internal transcribed spacer (ITS); nuclear
Hartmann et al. (2002)	Genus <i>Lophocereus</i> †; four ingroup taxa plus exemplars for four other genera	<i>trnL-F</i> , <i>trnC-D</i> , and <i>trnS-trnfM</i> intergenic spacers; all plastid
Nyffeler (2002)	Family Cactaceae; 70 ingroup taxa plus exemplars of two genera from Portulacaceae	<i>trnK-matK</i> region and <i>trnL-F</i> intergenic spacer, both plastid
Arias et al. (2003)	Genera <i>Carnegiea</i> , <i>Cephalocereus</i> , <i>Neobuxbaumia</i> , and <i>Pachycereus</i> plus three outgroup genera	<i>rpl16</i> intron, <i>trnL</i> intron, <i>trnL-F</i> intergenic spacer; plastid. rDNA internal transcribed spacer (ITS); nuclear
Griffith (2004)*	Genus <i>Opuntia</i> ; 49 sequences for 38 species	rDNA internal transcribed spacer (ITS); nuclear
Butterworth and Wallace (2004)	Genus <i>Mammillaria</i> ; 113 ingroup taxa plus 12 exemplars of other genera	<i>rpl16</i> intron and <i>psbA-trnH</i> intergenic spacer; both plastid
Arias et al. (2005)	Tribe Echinocereeeae especially genus <i>Peniocereus</i> ; 98 ingroup (Echinocereeeae) taxa plus seven outgroup genera	<i>rpl16</i> intron and <i>trnL-F</i> intron and intergenic spacer; both plastid
Butterworth and Wallace (2005)	Genus <i>Pereskia</i> ; 18 ingroup taxa plus seven taxa representing other subfamilies and one non-cactus	<i>rpl16</i> intron and <i>psbA-trnH</i> intergenic spacer; both plastid
Edwards et al. (2005)	Genus <i>Pereskia</i> ; 17 ingroup taxa plus 20 taxa representing all other subfamilies of cacti and five non-cactus outgroup	Phytochrome C gene (nuclear), <i>rbcL</i> gene, <i>trnK-matK</i> region, <i>psbA-trnH</i> intergenic spacer (all plastid), <i>cox3</i> gene (mitochondrial)
Harpke and Peterson (2006)	Genus <i>Mammillaria</i> ; 21 taxa plus two outgroup taxa	rDNA internal transcribed spacer (ITS); nuclear
Ritz et al. (2007)	BCT clade <i>sensu</i> Nyffeler (2002); 77 taxa including outgroup	<i>atpB-rbcL</i> , <i>trnK-rps16</i> , and <i>trnL-F</i> intergenic spacers; all plastid
Griffith and Porter (2009)*	Subfamily Opuntioideae; 110 taxa plus ten outgroup taxa	rDNA internal transcribed spacer (ITS) and <i>trnL-F</i> intergenic spacer; nuclear and plastid, respectively

Full publication details are supplied in the references section of this paper. The taxonomic focus and species and genome sampling strategies are indicated.

*Studies which present a topology, but sequence data are not made publically available.

†Congeneric with *Pachycereus* in Hunt (2006).

Table 2
Taxonomic distribution of samples

Taxon	Number of plants sampled	Proportion of species sampled	Proportion of genera sampled
Maihuenioideae	2	2/2 (100%)	1/1 (100%)
Pereskioideae	17	13/17 (76.5%)	1/1 (100%)
Opuntioideae	109	89/186 (47.8%)	12/17 (70.6%)
Cylindropuntieae	53	45/91 (49.5%)	8/10 (80%)
Opuntieae	56	44/95 (46.3%)	4/7 (57.1%)
Cactoideae	538	435/1233 (35.3%)	74/105 (70.5%)
Cactaceae	363	275/356 (77.3%)	23/25 (92%)
Echinocereaceae	117	105/203 (51.7%)	20/25 (80%)
Notocactaceae	19	17/27 (62.9%)	6/7 (85.7%)
Cereeae	14	13/151 (8.6%)	7/15 (46.6%)
Hylocereaceae	10	10/63 (15.9%)	4/6 (66.6%)
Trichocereaceae	10	10/280 (3.5%)	8/23 (34.7%)
Rhipsalideae	5	5/53 (9.4%)	4/4 (100%)

The number of plants our study sampled from Hunt's (2006) higher taxa, and the proportion of species and genera this sampling represents, following Hunt's treatment (Hunt, 2006), are indicated.

Methods

New sequences for the *trnK-matK* region of the chloroplast genome were generated from 580 plants, and 86 sequences from previous studies were used (Nyffeler, 2002; Edwards et al., 2005). New field collections provided 126 samples. Herbarium specimens, existing living collections, and specimens from commercial nursery collections were used to supplement the field-collected samples. Fresh material was dried in silica gel prior to DNA extraction. Note that throughout we follow the taxonomy of the New Cactus Lexicon (Hunt, 2006), using only species names accepted in this source. The taxonomic distribution of samples represented in our final matrix is summarised in Table 2.

Recent studies have shown the Cactaceae to be a well-supported monophyletic group most closely related to family Anacamserotaceae (formerly tribe Anacampseroteae; Nyffeler and Egli, 2010) and the genera *Portulaca* and *Talinum* of the family Portulacaceae (Nyffeler, 2007). We include sequences previously published by Nyffeler (2002) to represent these groups, *Grahamia bracteata* of the Anacamserotaceae, *Portulaca oleracea*, and *Talinum paniculatum*.

Total genomic DNA was isolated using DNeasy plant mini kits (Qiagen, Crawley, UK), following the manufacturer's recommendations. A slightly revised protocol was followed for problematic herbarium specimens (Drabkova et al., 2002). The *trnK-matK* region was amplified using the *trnK* primers 3914F and 2R (Johnson and Soltis, 1994). Primers 23F, 31R, 41R, 44F and 52F (Nyffeler, 2002) were used in initial amplifications where the condition of the material precluded long-range PCR. All PCR amplifications were performed using standard concentrations of reagents (Nyffeler,

2002). PCR temperature profiles had an initial denaturation of 4 min at 94 °C, then 34–40 cycles of denaturation for 30 s at 94 °C, annealing for 1 min at 50–56 °C, extension for 1.5 min at 72 °C, and with a final extension of 7 min at 72 °C. Annealing temperatures were reduced for problematic specimens to a minimum of 50 °C. PCR products were sent to MacroGen Inc. (Seoul, South Korea; <http://dna.macrogen.com>) for purification and sequencing using the primers detailed above. Primers 41R and 52F were used as internal sequencing in both directions; we refer to the reverse complement of the published primers as 41F and 52R. Raw sequences were assembled using SeqMan™ II, in the Lasergene® software package (DNASTAR, Inc., Madison, WI, USA). Sequences were aligned automatically with ClustalW 1.83 (Thompson et al., 1994) and adjusted by hand.

Bayesian analysis

Models of sequence evolution were selected for each region using MrModeltest (Nylander, 2004). The data were partitioned into coding (*matK*) and non-coding (*trnK1*, *trnK2*) regions by comparison with published sequences. The aligned sequences were analysed using MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck, 2003). The analysis used two runs of four chains for 10 000 000 iterations, sampling every 1000th generation. A consensus tree was created using the "sumt" command, to determine the posterior probabilities of clades.

Maximum-likelihood analysis (ML)

An unpartitioned alignment was analysed using RaxML-VI v.7.0.4. as implemented through the Phylobench Phylogenetic Software Benchmarking Suite (Stamatakis, 2006). Branch support was assessed with 100 non-parametric bootstrap replicates under the GAMMA+P-Invar model and bootstrap values were plotted on the ML tree with the best likelihood.

Maximum-parsimony analysis (MP)

NONA was spawned in WINCLADA ver. 1.00.08 (Nixon, 2002), with the following options in place. One hundred replicates were performed holding one tree per replicate and implementing TBR branch swapping without max*. A consensus tree was calculated using the Nelson option in WINCLADA. Branch support was assessed using bootstrapping implemented in PAUP* (ver. 4.0b10; Swofford, 2003), using SPR branch swapping and a heuristic search strategy with 100 replicates, two random addition sequences per replicate, and holding two trees per replicate.

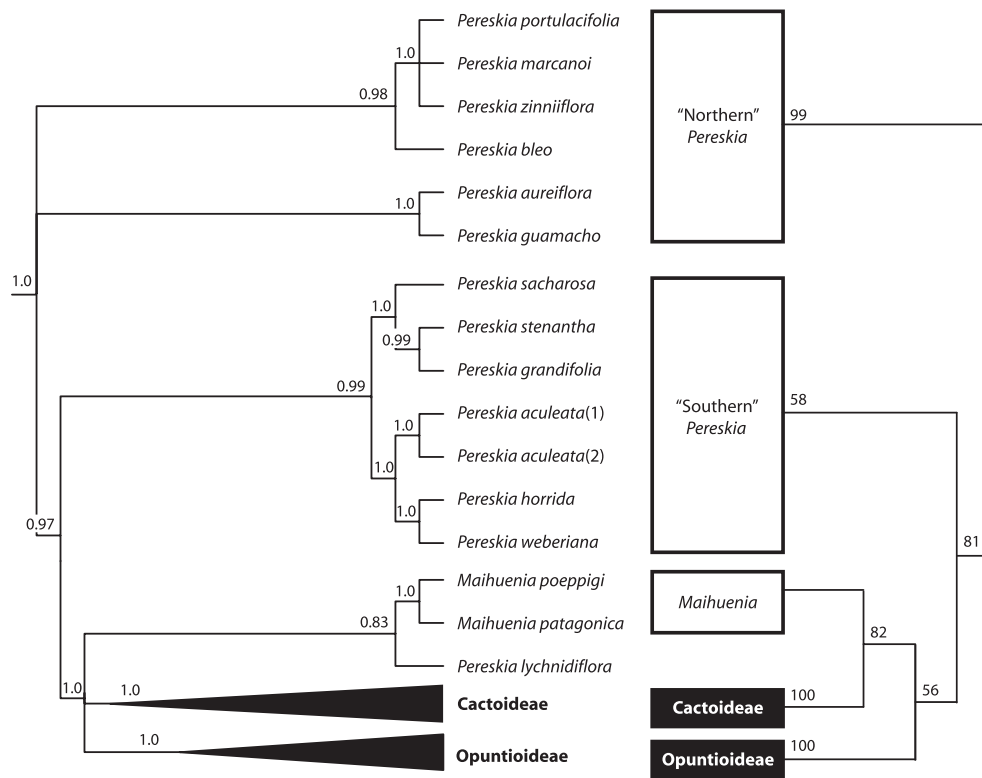


Fig. 1. Intrafamilial relationships. The relationships recovered in this study are indicated by the Bayesian Majority Rule tree (tree on the left), shown with posterior probabilities recovered in the Bayesian analysis. Edwards et al.'s (2005) summary topology is shown for comparison purposes, with ML bootstrap values indicated. The monophyletic subfamilies Opuntioideae and Cactoideae are collapsed in both trees. Numbers in parentheses after the species name refer to collections in Appendix S1.

Results

The phylogenetic analysis was performed on 666 aligned sequences representing 538 identified species of Cactaceae, seven samples that could be identified only to genus, two putative interspecific hybrids and three outgroups. The seven samples identified only to genus were one exemplar of genus *Escobaria* and six exemplars of genus *Stenocactus*. The aligned matrix was 2916 bp, of which 1037 bp were constant and 1181 bp were parsimony-informative. The GTR+G model was selected by Modeltest for each of the coding and non-coding regions. Burn-in was reached by 3 000 000 replicates. The full alignment, Bayesian, ML and MP phylogenies can be accessed at TreeBASE (<http://www.treebase.org/>). Six trees are presented (Figs 1–6); each has selected clades collapsed, but taken together the set of trees shows the relationships of all accessions sampled as recovered in the Bayesian analysis. The tree describing the relationships within *Pereskia* and between subfamilies (all subfamilies collapsed except Pereskioideae) is presented in Fig. 1. The relationships recovered by Edwards et al. (2005) are also redrawn in this figure. Trees are also presented for the Cactoideae

(Fig. 2; all other subfamilies and tribe Cactae collapsed), the Opuntioideae (Figs 3 and 4; Fig. 3 showing only Opuntieae, Fig. 4 showing only *Cylindropuntieae*) and the Cactae (Figs 5 and 6). Tables 3 and 4 show the status of genera represented by multiple accessions in our study. The Bayesian, ML and MP trees were largely congruent, although there were notable differences in topology. The Bayesian tree places two taxa in what appear to be anomalous placements, relative to the ML and MP trees. First, a sample of *Opuntia*, *O. atrispina*, is placed outside of the Opuntieae and within the *Cylindropuntieae* in the Bayesian analysis. This accession is placed, as would be expected, within the Opuntieae in the ML analysis. The MP analysis does not recover the Opuntieae as a group, but *O. atrispina* groups with other Opuntieae and not with the *Cylindropuntieae*. Another notable conflict relates to one accession of *Coryphantha elephantidens* (Miranda 1194). In the Bayesian analysis this accession is recovered as sister to the whole of the Cactae, but in the ML and MP trees it is nested with another accession of the same species within the Mammilloid clade. The sequence from *Coryphantha elephantidens* accession Miranda 1194 is a partial sequence only

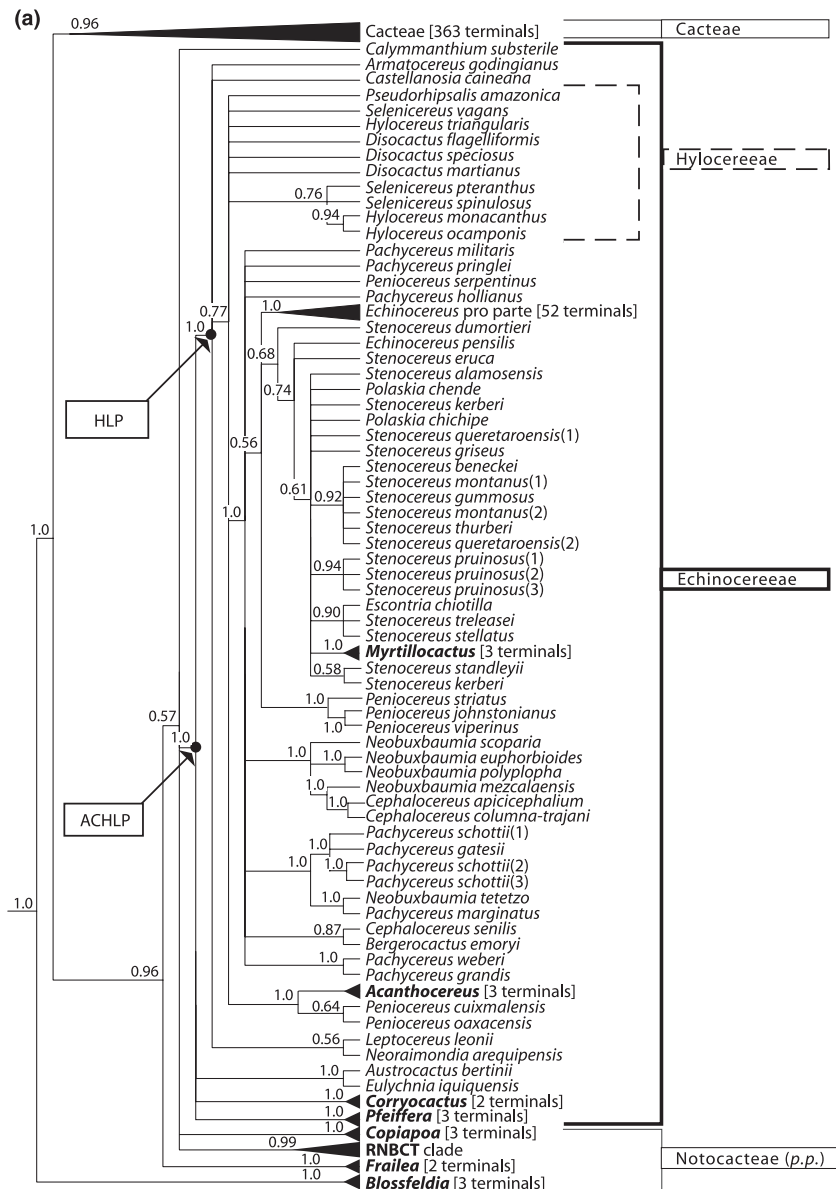


Fig. 2. (a) Relationships within Cactoideae indicated by the Bayesian Majority Rule tree. Monophyletic genera in bold [*Acanthocereus* (three terminals), *Copiapoa* (three), *Corryocactus* (two), *Frailea* (two), *Myrtillocactus* (three), and *Pfeiffera* (three)], the monophyletic tribe Cacteae (363 terminals), the three specimens sampled of the monophyletic genus *Blossfeldia*, and the RNBCT clade are collapsed. One species of *Echinocereus*, *E. pensilis*, is placed outside of the main group, but the 52 terminals of the monophyletic main group are collapsed. Numbers in parentheses after the species name refer to collections in Appendix S1 and numbers in square brackets after the names of higher taxa refer to the number of terminals for each collapsed clade. Arrows are used to indicate the ACHLP and HLP clades, and boxes indicate the tribes *sensu* Hunt. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis. (b) The RNBCT clade. Monophyletic genera in bold [*Browningia* (two terminals), *Pilosocereus* (seven), *Rhypsalis* (two)] are collapsed. The BCT clade is arrowed, and boxes indicate the tribes *sensu* Hunt. Numbers in parentheses after the species name refer to collections in Appendix S1 and numbers in square brackets after the names of higher taxa refer to the number of terminals for each collapsed clade. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis.

1852 bp long, but the *O. atrispina* sequence is full-length. Although the topologies recovered by the Bayesian, ML and MP analyses are generally congruent, there are some striking differences in resolution and support between the trees. Bayesian trees find Bayesian support values are notably higher than many ML and MP bootstrap values (MLBS and MPBS); for

example, in several instances posterior probabilities of 1.0 pp are matched with bootstrap support values of 70% or less for the same clade; in other cases the discrepancies are more extreme (e.g. 0.96 pp and 34% MLBS, or in another case 1.00 pp, 82% MLBS but < 50% MPBS). The MP consensus tree is often less resolved, and groups recovered in the Bayesian tree and

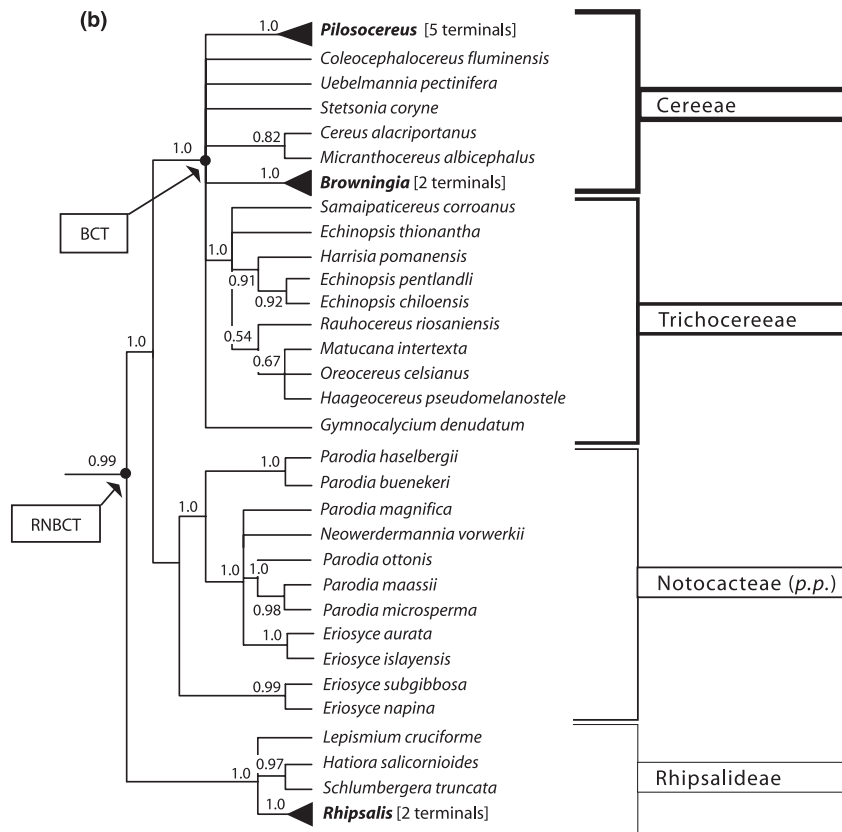


Fig. 2. (Continued)

ML tree, such as the Opuntieae, are not recovered by parsimony.

Discussion

Deep relationships between subfamilies

Basal cactus phylogeny was the focus of detailed study by Edwards et al. (2005) and by Butterworth and Wallace (2005). The work of Edwards et al. (2005) was based on an analysis of five gene regions (although not all taxa were sequenced for all gene regions); Butterworth and Wallace (2005) sampled two plastid regions and included plastid restriction site data in their combined analysis. Edwards et al.'s (2005) study arguably represents the most robust hypothesis to date of the relationships between subfamilies. We present a summary tree (Fig. 1) showing the major groupings recovered here alongside this most robust existing hypothesis of deep relationships, that recovered by Edwards et al. (2005). Edwards et al. (2005) sampled all the known species of *Pereskia* and *Maihuenia*, four representatives of the Cactoideae, eight of the Opuntioideae, plus selected Portulacaceae. They recovered three subfamilies as monophyletic: Opuntioideae,

Cactoideae, and Maihuenioideae. Maihuenioideae was recovered as sister to the Cactoideae with moderate to good support, but the placement of Opuntioideae as sister to the Cactoideae plus Maihuenioideae was weakly supported. The Pereskioideae were the only subfamily which was not monophyletic. Edwards et al.'s (2005) study delimited two Pereskioideae groups, one comprising eight Northern species and the other nine Southern species, including the widespread *P. aculeata* in the Southern group. Their Southern group was recovered as sister to the core cacti (Maihuenioideae, Cactoideae, and Opuntioideae) and the group comprising the core cacti plus the Southern Pereskioideae was designated the Caulocacti. The Northern Pereskioideae were sister to the Caulocacti, rendering the subfamily and genus paraphyletic. Butterworth and Wallace (2005) recover the same major groups, but find little or no support for relationships between them. Notably, they place *P. lychnidiflora* as sister to the Cactoideae.

Edwards et al. (2005) included the *trnK-matK* region in their gene region sampling strategy, and all of our Pereskioideae and Maihuenioideae data are sourced from their publication. Any differences between our reconstructed relationships and theirs might be attributed to the increased sampling of the Cactoideae and Opuntioideae

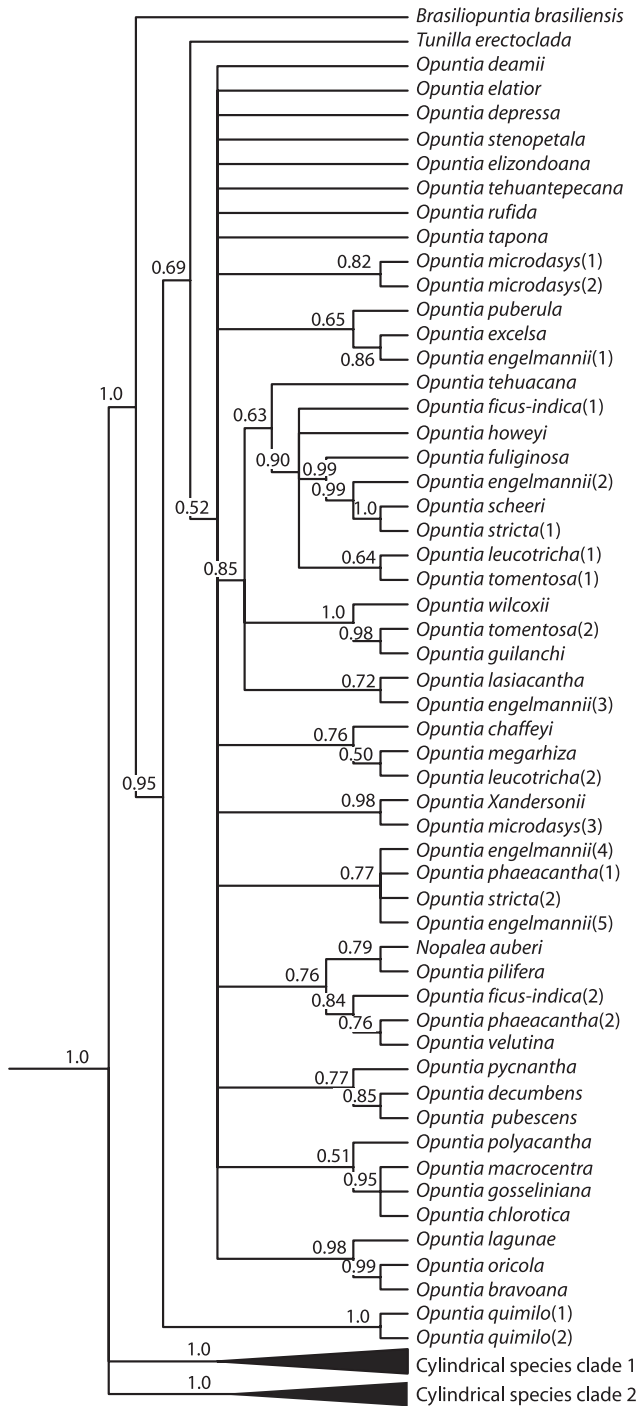


Fig. 3. The Opuntioideae showing relationships within Opuntieae indicated by the Bayesian Majority Rule tree. Numbers in parentheses after the species name refer to collections in Appendix S1. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis.

in our study, or more probably to the relative paucity of data from the *trnK-matK* region alone in comparison with the five-gene analysis. Close examination of our

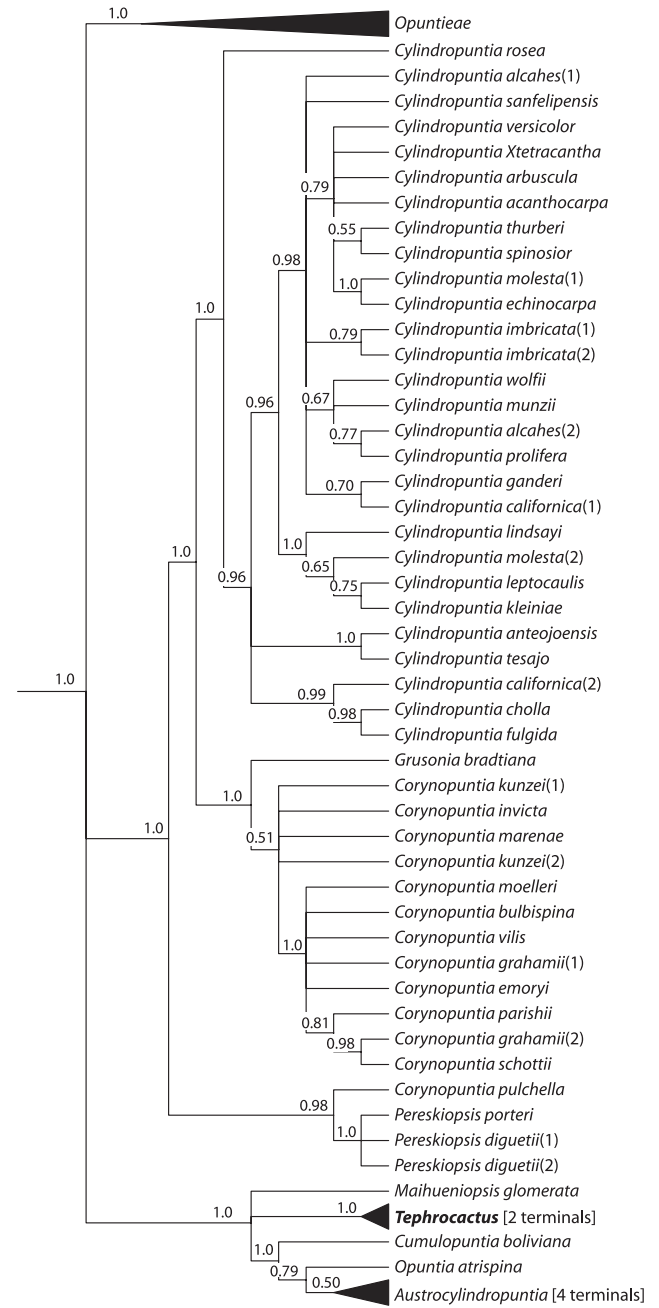


Fig. 4. The Opuntioideae showing relationships within Cylindropuntieae indicated by the Bayesian Majority Rule tree. Numbers in parentheses after the species name refer to collections in Appendix S1 and numbers in square brackets after the names of genera refer to the number of terminals for each collapsed clade. Names of monophyletic genera are in bold. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis. Note the unexpected placement of *Opuntia atrispina* as sister to *Austrocyliandropuntia*. This species was placed in the Opuntieae in the RAxML analysis.

topologies against the combined and partitioned topologies of Edwards et al. (2005) shows that our reconstruction of deep relationships suffers from a lack

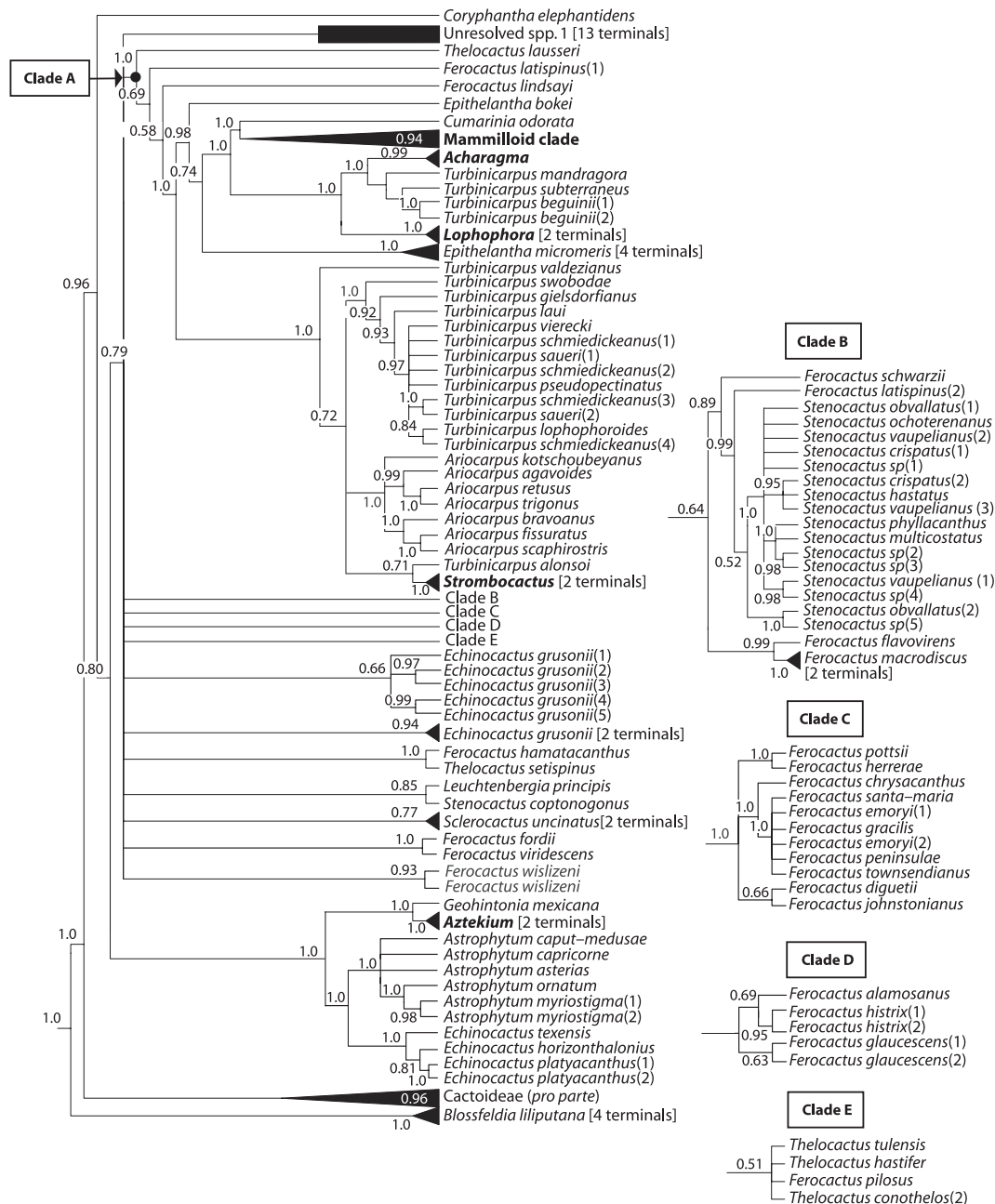


Fig. 5. Relationships within the Cactaceae indicated by the Bayesian Majority Rule tree. Monophyletic genera in bold [*Acharagma* (two terminals), *Aztekium* (two terminals), *Astrophytum* (six terminals), *Ariocarpus* (seven terminals) and *Lophophora* (two terminals)] and the monophyletic Mammilloid clade are collapsed. The names of 13 unresolved species [*Echinocactus grusonii*(6), *Echinocactus grusonii*(7), *Echinocactus grusonii* (8), *Echinocactus polycephalus*, *Ferocactus echidne*, *Ferocactus robustus*, *Thelocactus bicolor*(1), *Thelocactus bicolor*(2), *Thelocactus bicolor*(3), *Thelocactus conothelos*(1), *Thelocactus hexaedrophorus*, *Thelocactus macdowellii*, *Thelocactus rinconensis*] are omitted for clarity. The clades discussed in the text—clades A to E—are indicated; the positions of clades B to E are indicated, but the topologies within these clades are indicated in insets. Numbers in parenthesis after the species name refer to collections in Appendix S1 and numbers in square brackets after the names of higher taxa refer to the number of terminals for each collapsed clade. Names of monophyletic genera are in bold. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis.

of informative characters. We sample 538 and 108 Cactoids and Opuntioids, respectively, as opposed to four and eight by Edwards et al. (2005), and find strong support for monophyletic subfamilies Opuntioideae

(1.0 pp, 94% MLBS, 96.5% MPBS) Cactoideae (1.0 pp, 97% MLBS, 97.5% MPBS), and Maihuenioideae (1.0 pp, 100% MLBS, 100% MPBS), but little support for relationships between these well-supported

groups and for the placement of the species of *Pereskia*. Bayesian and ML topologies are the same for the deep relationships indicated in Fig. 1, and show two well-supported northern *Pereskia* clades forming a basal trichotomy with a third clade comprising the remainder of the cacti. *P. lychnidiflora*, a Northern *Pereskia*, is placed as sister to *Maihuenia* as one of three clades comprising the entire family less the other *Pereskia*. The MP analysis also recovers a *P. lychnidiflora*/*Maihuenia* clade, but places it as sister to the Opuntioideae. This anomalous placement of *P. lychnidiflora*, apart from other *Pereskia*, is also recovered in Edwards et al.'s (2005) cpDNA/mtDNA partition, a scheme contradicted by their total-evidence analysis. However, our recovery of a strongly supported group comprising Opuntioideae, Cactoideae, Maihuenioideae, and *P. lychnidiflora* is a notable conflict with their cpDNA/mtDNA partition. Our placement of *P. lychnidiflora* also conflicts with the findings of Butterworth and Wallace (2005), who place *P. lychnidiflora* as sister to the Cactoideae but place the Opuntioideae and Maihuenioideae in a basal trichotomy with the single outgroup employed in their analysis. Edwards et al.'s (2005) hypothesis of relationships between subfamilies remains the most robust to date. Our significant contribution is with respect to the relationships within Cactoideae and Opuntioideae.

Relationships within subfamily Cactoideae

The most comprehensive study across the whole of subfamily Cactoideae to date is that of Nyffeler (2002); here we review relationships within the subfamily, assessing whether the tribal delimitations of Hunt (2006) hold up, and how additional sampling affects the relationships indicated by Nyffeler (2002), as well as how our topologies compare with other significant but less widely sampled studies (Table 1). Most notable of these are the publications of Butterworth et al. (2002), Butterworth and Wallace (2004), Arias et al. (2005), and Ritz et al. (2007). Butterworth et al.'s (2002) study is the most densely sampled to date of the Cactaceae, sampling 62 representatives. Butterworth and Wallace (2004) focus on *Mammillaria* and its closest relatives. Arias et al. (2005) concentrated on the tribes Echinocereae and Hylocereae (98 taxa). Harpke and Peterson (2006) concentrated on *Mammillaria*, whilst Ritz et al. (2007) mainly sampled the tribes Trichocereae and Cereae (87 taxa). Nyffeler sampled 62 representatives across all the tribes of the Cactoideae. We include 538 sequences for the Cactoideae in this analysis, including all of Nyffeler's published sequences.

Status of Nyffeler's higher-level groupings. Nyffeler (2002) referred to four novel higher-level clades recovered by his analysis, the BCT (*Browningia*, Cereae, and Trichocereae) clade which was nested within the

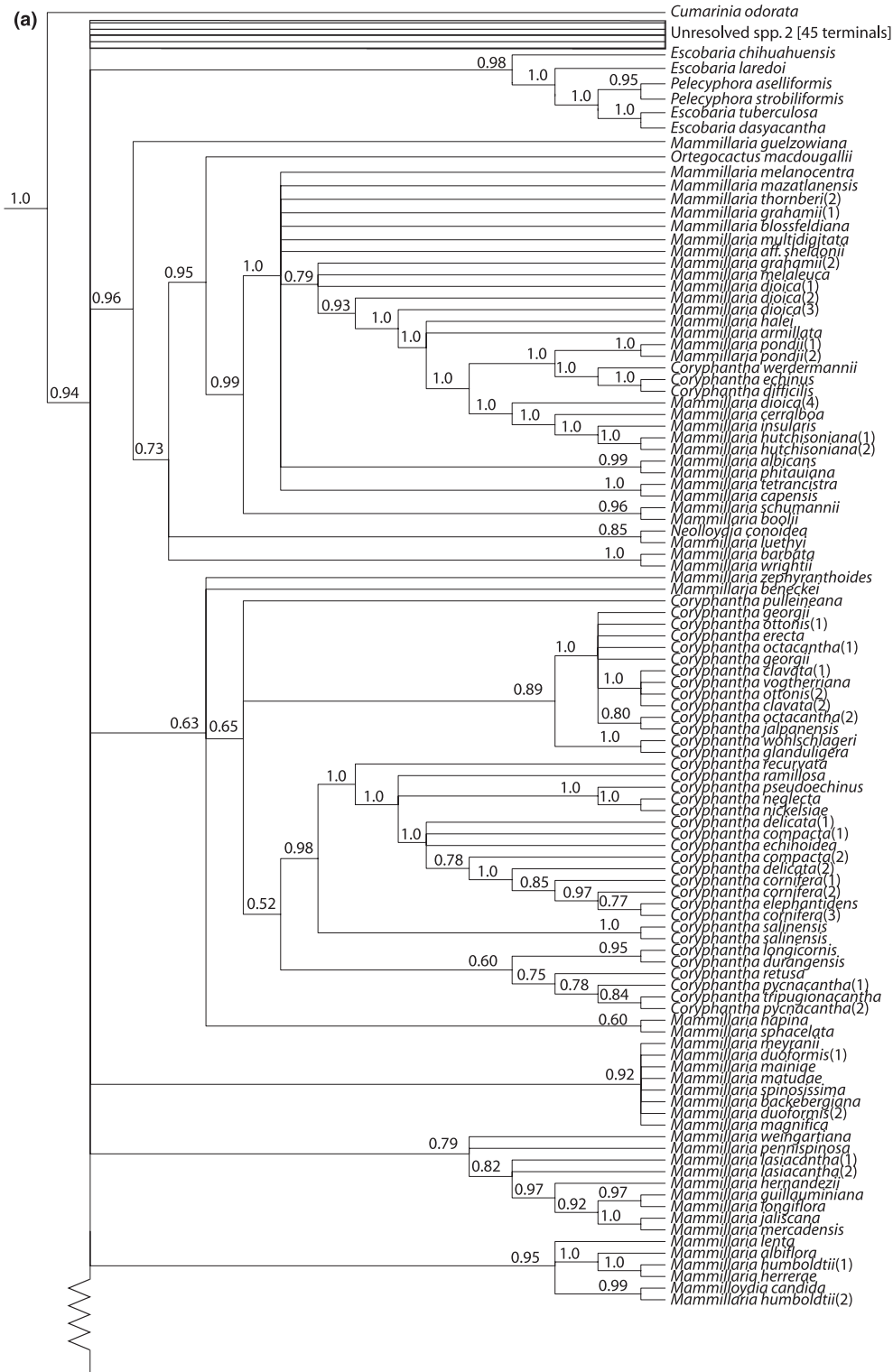
RNBCT (core Rhipsalideae, core Notocactae, plus BCT) clade, and the HLP (Hylocereae, Leptocereae, Pachycereae) clade which was nested within the ACHLP (*Austrocactus*, *Corryocactus*, plus HLP) clade. He found moderate support (74% BS) for the RNBCT, but strong support for the BCT (100% BS). His RNBCT clade was placed in a polytomy with the ACHLP clade, *Frailea*, and a clade comprising *Copiapoa* and *Calymmanthium*. In contrast, we find *Frailea* to be sister to the remainder of these taxa (with only 0.57 pp, 36% MLBS, < 50% MPBS) in our Bayesian tree, but place *Calymmanthium*, *Copiapoa*, the ACHLP, and the RNBCT clades in a polytomy. In the ML topology *Frailea*, *Copiapoa*, *Calymmanthium*, the ACHLP, and the RNBCT clade are all placed in a polytomy.

The BCT clade is recovered with strong support (1.0 pp, 99% MLBS, 98% MPBS), as it was for Nyffeler (2002). Nyffeler was able to unequivocally assign 17 species and 14 genera to the BCT clade. We are able to increase the sampling of this clade to 23 species representing 15 genera by placing six identified species of *Pilosocereus* as within the BCT. Like Nyffeler, we also find the BCT clade nested within a monophyletic RNBCT clade. The RNBCT clade is supported with 0.99 posterior probability, 78% MLBS, and 65.5% MPBS. We present only a slightly more informative scheme of relationships than Nyffeler (2002), as we place *Pilosocereus*, but our sampling does not approach that of Ritz et al. (2007). They sample 87 taxa accessions representing eight outgroup taxa and 79 presumed members of the BCT clade. Thus their analysis provides the strongest hypothesis of the relationships within the BCT clade to date.

Our sampling of the HLP and ACHLP clades represents a significant increase relative to that of Nyffeler (2002). The HLP clade *sensu* Nyffeler included 11 genera, four placed in one clade, *Acanthocereus*, *Disocactus*, *Hylocereus*, and *Selenicereus*, three—*Pachycereus*, *Echinocereus*, and *Escontria*—in another and four unresolved genera, *Armatocereus*, *Castellanosia*, *Leptocereus*, and *Neoraimondia*. His ACHLP clade included four more genera, *Austrocactus*, *Eulychnia*, *Pfeiffera*, and *Corryocactus*. All of the genera placed in the ACHLP clade by Nyffeler (2002) are placed within a single clade in our analysis, too. Considering the ACHLP and HLP groups, the four genera outside the HLP clade but placed within the ACHLP in Nyffeler's analysis, *Austrocactus*, *Eulychnia*, *Pfeiffera*, and *Corryocactus*, also lie outside of a well-supported larger clade in our analysis. We find the broadly circumscribed ACHLP, including these four ACHLP genera outside of the HLP, receives posterior probability of 1.0 (87% MLBS, 75.5% MPBS), as does the nested clade (HLP) which excludes these four genera, *Austrocactus*, *Eulychnia*, *Pfeiffera*, and *Corryocactus* (1.0 pp, 66% MLBS, < 50% MPBS). Whereas Nyffeler placed 11 genera

within the nested HLP group we find a total of 30 genera in that group. The relationships of *Austrocactus*, *Eulychnia*, *Pfeiffera*, and *Corryocactus* to the HLP are slightly different in our analysis compared with Nyffel-

er's. Nyffeler (2002) placed *Corryocactus* as sister to the HLP, but with low support. We find *Austrocactus* and *Eulychnia* to be sisters (1.0 pp, 100% MLBS, 100% MPBS). *Austrocactus*/*Eulychnia*, *Pfeiffera*, and



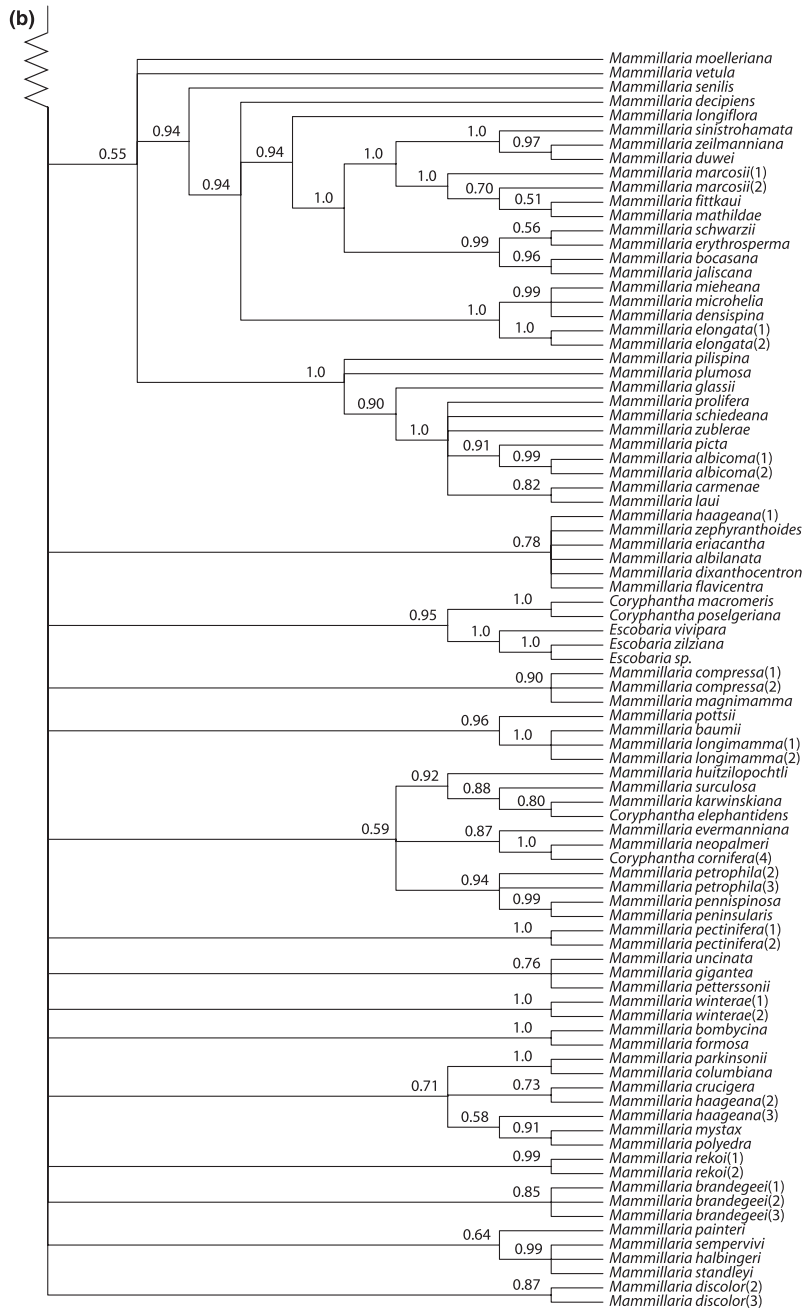


Fig. 6. Relationships within the Mammilloid clade indicated by the Bayesian Majority Rule Tree and shown as two subtrees. (a) The Mammilloid clade, part 1. Numbers in parenthesis after the species name refer to collections in Appendix S1. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis. The names of 45 unresolved species [*Coryphantha maiz-tablasensis*, *Coryphantha robustispina*, *Mammillaria aureilanata*, *Mammillaria bocensis*, *Mammillaria brandegeei*, *Mammillaria carnea*, *Mammillaria carretii*, *Mammillaria coahuilensis*, *Mammillaria discolor*, *Mammillaria geminispina*, *Mammillaria grusonii*, *Mammillaria guerreronis*, *Mammillaria hahniana*(1), *Mammillaria hahniana*(2), *Mammillaria heyderi*(1), *Mammillaria heyderi*(2), *Mammillaria knippeliana*, *Mammillaria marksiana*, *Mammillaria morganiana*, *Mammillaria muehlenpfordtii*, *Mammillaria nunezii*, *Mammillaria orcuttii*, *Mammillaria perbella*, *Mammillaria petrophila*, *Mammillaria petterssonii*, *Mammillaria polythele*, *Mammillaria rhodantha*, *Mammillaria roseoalba*, *Mammillaria saboae*, *Mammillaria sanchez-mejoradae*, *Mammillaria sartorii*, *Mammillaria scrippsiana*, *Mammillaria sonorensis*, *Mammillaria standleyi*(1), *Mammillaria standleyi*(2), *Mammillaria standleyi*(3), *Mammillaria standleyi*(4), *Mammillaria supertexta*, *Mammillaria tayloriorum*, *Mammillaria theresae*, *Mammillaria thornberi*(1), *Mammillaria uncinata*, *Mammillaria variaculeata*, *Mammillaria wagneriana*, *Mammillaria xaltianguiensis*] placed at the base of the tree are omitted for clarity. (b) The Mammilloid clade, part 2. Numbers in parenthesis after the species name refer to collections in Appendix S1. Values shown on branches are the posterior probabilities recovered in the Bayesian analysis.

Table 3
The status of genera placed in the Cactoideae by Hunt (2006)

Genus	No. of species sampled/no. of species in total	Status of genus†
<i>Acharagma</i>	2/2	Monophyletic (1.0 pp, 100% MLBS, 89% MPBS)
<i>Ariocarpus</i>	7/7	Monophyletic (1.0 pp, 67% MLBS, 56% MPBS)
<i>Astrophytum</i>	5/6	Monophyletic (1.0 pp, 99% MLBS, 98% MPBS)
<i>Aztekium</i>	2/2	Monophyletic (1.0 pp, 91% MLBS, 68% MPBS)
<i>Browningia*</i>	2/8	Monophyletic (1.0 pp, 92% MLBS, 89% MPBS)
<i>Cephalocereus</i>	3/3	Not monophyletic in any tree
<i>Copiapoa*</i>	3/21	Monophyletic (1.0 pp, 100% MLBS, 100% MPBS)
<i>Corryocactus*</i>	2/12	Monophyletic (1.0 pp, 99% MLBS, 100% MPBS)
<i>Coryphantha</i>	33/42	Not monophyletic in any tree. Core <i>Coryphantha</i> (0.65 pp, 10% MLBS: not recovered in parsimony tree) comprises 25 species. Taxa with supported relationships away from the core <i>Coryphantha</i> group in all trees are <i>C. macromeris</i> and <i>C. posegeriana</i> (1.0 pp, 100% MLBS, 98% MPBS as sisters, allied to <i>Escobaria</i>) and <i>C. difficilis</i> , <i>C. echinus</i> , and <i>C. werdermannii</i> (monophyletic, 1.0 pp, 100% MLBS, 99% MPBS) placed with <i>Mammillaria pondii</i> and allies, and <i>C. robustispina</i> , placed within an unresolved group of <i>Mammillaria</i> species. Species with multiple accessions which are split between the core <i>Coryphantha</i> and other placements include <i>C. cornifera</i> (in Bayesian and MP trees only—all four accessions are within the core clade in the ML analysis) and <i>C. elephantidens</i> . Bayesian, ML and MP trees differ in some placements
<i>Disocactus*</i>	3/11	Not monophyletic in any tree
<i>Echinocactus</i>	5/6	Not monophyletic in any tree, core <i>Echinocactus</i> (1.0 pp, 86% MLBS, 57.5% MPBS) comprises <i>E. horizonthalonius</i> , <i>E. platyacanthus</i> , <i>E. texensis</i>
<i>Echinocereus</i>	48/67	Not monophyletic in any tree. Core <i>Echinocereus</i> (1.0 pp, 95% MLBS, 57% MPBS) excludes <i>E. pensilis</i>
<i>Echinopsis*</i>	3/77	Not monophyletic in any tree
<i>Epithelantha</i>	2/2	Not monophyletic in or Bayesian or ML trees. Monophyletic in the MP tree (63% MPBS)
<i>Eriosyce*</i>	4/32	Not monophyletic in any tree
<i>Escobaria*</i>	7/19	Not monophyletic in any tree
<i>Ferocactus</i>	25/28	Not monophyletic in any tree, the largest monophyletic group recovered, a core <i>Ferocactus</i> (1.0 pp, 38% MLBS, < 50% MPBS), includes a subset of species from Sonora and Baja California and its islands: <i>F. chrysacanthus</i> , <i>F. diguettii</i> , <i>F. emoryi</i> , <i>F. gracilis</i> , <i>F. herrerae</i> , <i>F. johnstonianus</i> , <i>F. peninsulae</i> , <i>F. potsii</i> , <i>F. santa-mariae</i> , <i>F. townsendianus</i>
<i>Frailea*</i>	2/12	Monophyletic (1.0 pp, 100% MLBS, 100% MPBS)
<i>Hylocereus*</i>	3/14	Not monophyletic in any tree
<i>Lophophora</i>	2/3	Monophyletic (1.0 pp, 84% MLBS, 89% MPBS)
<i>Mammillaria</i>	141/163	Not monophyletic in any tree
<i>Myrtillocactus</i>	3/4	Monophyletic (1.0 pp, 82% MLBS, 75% MPBS)
<i>Neobuxbaumia</i>	5/8	Not monophyletic in any tree
<i>Pachycereus</i>	9/13	Not monophyletic in any tree
<i>Parodia*</i>	6/58	Not monophyletic in any tree
<i>Pelecypora</i>	2/2	Monophyletic (1.0 pp, 61% MLBS, 70% MPBS)
<i>Peniocereus*</i>	6/20	Not monophyletic in any tree
<i>Pfeiffera*</i>	3/9	Monophyletic (1.0 pp, 100% MLBS, 98% MPBS)
<i>Pilosocereus*</i>	6/41	Monophyletic (1.0 pp, 83% MLBS, 72% MPBS)
<i>Polaskia</i>	2/2	Not monophyletic in any tree
<i>Rhipsalis*</i>	2/35	Monophyletic (1.0 pp, 98% MLBS, 93% MPBS)
<i>Selenicereus*</i>	3/12	Not monophyletic in any tree
<i>Stenocactus</i>	8/10	Bayesian and MP analysis recovers core <i>Stenocactus</i> (0.52 pp, 82% MPBS) including all species except <i>S. coptonogonus</i> . ML also places <i>S. coptonogonus</i> outside of the <i>Stenocactus</i> clade, but in the core <i>Stenocactus</i> clade is unresolved and has <i>Ferocactus latispinus</i> as sister species
<i>Stenocereus</i>	14/24	Not monophyletic in any tree
<i>Thelocactus</i>	10/14	Not monophyletic in any tree
<i>Turbincarpus</i>	13/16	Polyphyletic, core <i>Turbincarpus</i> (1.0 pp, 92% MLBS, 70% MPBS): <i>T. gielsdorffianus</i> , <i>T. laui</i> , <i>T. lophophoroides</i> , <i>T. pseudomacroleche</i> , <i>T. saueri</i> , <i>T. schmiedickeanus</i> , <i>T. swoboda</i> and <i>T. viereckii</i>

*Each genus for which we sampled more than one species is included in the table, and its status (whether monophyletic or not) is indicated. For monophyletic genera, posterior probabilities and ML and MP bootstrap values are indicated. Genera for which fewer than two-thirds of species are sampled are indicated with an asterisk. In the absence of dense sampling, including a sampling strategy which explicitly targets all major lineages, the status of these poorly sampled genera should be considered uncertain. Note differences between the three trees. In the ML and MP trees, one accession of *C. elephantidens* is placed with a group of *Mammillaria* species, but is sister to the Cactaceae in the Bayesian tree (see text). *C. maiz-tablasensis* is placed within the core *Coryphantha* group in the ML but not in the Bayesian or MP analyses. A group referred to in the table, the Core *Coryphantha*, comprises the following in the Bayesian analysis: *C. clavata*, *C. compacta*, *C. cornifera*, *C. delicata*, *C. durangensis*, *C. echinoidea*, *C. erecta*, *C. georgii*, *C. glanduligera*, *C. jalpanensis*, *C. longicornis*, *C. neglecta*, *C. nickelsiae*, *C. octacantha*, *C. ottonis*, *C. pseudoechinus*, *C. pulleinea*, *C. pycnantha*, *C. ramillosa*, *C. recurvata*, *C. retusa*, *C. salinensis*, *C. tripugionacantha*, *C. vogtherriana*, and *C. wohlschlagerei*.

†Bayesian posterior probability, maximum-likelihood bootstrap support, maximum-parsimony bootstrap support.

Table 4
The status of genera placed in the Opuntioideae by Hunt (2006)

Genus	No. of species sampled/no. of species in total	Status of genus*
<i>Pereskioopsis</i>	2/3	Monophyletic (1.0 pp, 99% MLBS, 97.5% MPBS)
<i>Tephrocactus</i>	2/2	Monophyletic (1.0 pp, 87% MLBS, 88.5% MPBS)
<i>Austrocylindropuntia</i>	3/4	Monophyletic (1.0 pp, 86% MLBS, 74.5% MPBS)
<i>Corynopuntia</i>	10/12	Not monophyletic in any tree
<i>Cylindropuntia</i>	24/28	Monophyletic (1.0 pp, 85% MLBS, 77.5% MPBS)
<i>Opuntia</i>	41/53	Not monophyletic in any tree

Each genus for which we sampled more than one species is included in the table, and its status (whether monophyletic or not) is indicated. For monophyletic genera, posterior probabilities and ML and MP bootstrap values are indicated. In the absence of dense sampling, including a sampling strategy which explicitly targets all major lineages, the status of these poorly sampled genera should be considered uncertain.

*Bayesian posterior probability, maximum-likelihood bootstrap support, maximum-parsimony bootstrap support.

Corryocactus are unresolved at the base of the ACHLP in our Bayesian analysis. In the ML analysis *Austrocactus/Eulychnia* comprise the monophyletic sister group to the ACHLP, whereas in the MP analysis *Austrocactus/Eulychnia* plus *Corryocactus* (a group with < 50% MPBS) is sister to the ACHLP.

Status of Cacteeae sensu Hunt. Arguably there has been no rigorous test of the monophyly of the Cacteeae to date. Butterworth et al. (2002) recovered the Cacteeae in their analysis of the tribe with 100% bootstrap support but only sampled four outgroups. Nyffeler (2002) recovered a monophyletic tribe but only sampled four exemplar species. The support we find for the Cacteeae (1.00 pp, 82% MLBS but < 50% MPBS) tentatively supports morphologists' interpretations that the tribe is a clear-cut phylogenetic unit (Buxbaum, 1958). Aside from the misplacement of one accession of *Coryphantha elephantidens* in the Bayesian tree, all trees place *Blossfeldia* as sister to the sister pair ((Cacteeae)(subfamily Cactoideae less Cacteeae)), a scheme of relationships also recovered by Nyffeler's (2002) analysis, although Nyffeler's (2002) study sampled three exemplars of the tribe as opposed to the 363 samples here. That Nyffeler's (2002) placement of *Blossfeldia* is supported in our more densely sampled phylogeny will be of interest to many workers, especially in the light of the numerous studies of its unique biology (Barthlott and Porembski, 1996; Butterworth, 2006; Mauseth, 2006). Relationships within the tribe are considered below, in relation to the most notable tribal analysis to date, that of Butterworth et al. (2002).

Status of Cereeeae sensu Hunt. Nyffeler (2002) sampled six species which would be placed in the Cereeeae sensu Hunt (2006); our sampling increases that number to 11. Ritz et al. (2007) included a similar level of sampling of the Cereeeae in their study focused on *Rebutia* and Nyffeler's (2002) BCT clade. In our study many of the species sampled remain largely unresolved at the base of a clade which has the Trichocereeeae sensu Hunt (2006) nested within it. Comparing our results with those of

Ritz et al. (2007), we find nothing in conflict with their study.

Status of Trichocereeeae sensu Hunt. Nyffeler (2002) recovered a monophyletic Trichocereeeae comprising nine species sampled from seven genera. Our analysis did not include additional exemplars of the tribe and we recovered the same group. As in Nyffeler's (2002) analysis, the group is part of a wider group comprising elements of the Cereeeae. Notably, in our Bayesian and MP analyses *Gymnocalycium denudatum* is unresolved at the base of the wider clade, and in our ML analysis *Gymnocalycium denudatum* is sister to the remainder of the same group. As Nyffeler's (2002) analysis does not place *Gymnocalycium* within the Trichocereeeae, Hunt's (2006) placement of this genus in tribe Trichocereeeae may not be stable. By far the most densely sampled and robust phylogeny to date for the Trichocereeeae is that of Ritz et al. (2007). Their ingroup comprised 79 samples representing Nyffeler's (2002) BCT clade, and the phylogeny was reconstructed using three plastid regions, the *atpB-rbcL*, *trnL-F*, and *trnK-rps16* intergenic spacers. As in Nyffeler's (2002) analysis the BCT found high support. Ritz et al.'s (2007) study nested three genera placed outside Trichocereeeae by Hunt (2006) within a strongly supported BCT clade. These were *Browningia*, *Cereus*, and *Stetsonia* from Hunt's (2006) Cereeeae.

Status of Rhipsalideae sensu Hunt. We recover the Rhipsalideae sensu Hunt as a monophyletic group with strong support (1.0 pp, 100% MLBS, 100% MPBS) including the four species sampled by Nyffeler plus one additional taxon.

Status of Notocacteeae sensu Hunt. One of the most intriguing and controversial findings of Nyffeler (2002), since supported by Edwards et al.'s (2005) sampling of multiple gene regions, was that *Blossfeldia* is sister to the remainder of the Cactoideae subfamily. Our denser sampling overall (we present no new sequences for the Notocacteeae) does not refute Nyffeler's (2002)

placement of the genus. *Blossfeldia* has traditionally been placed in tribe Notocactaceae, and this classification is upheld by Hunt (2006). However, the placement of *Blossfeldia* in the tribe renders the delimitation of Notocactaceae polyphyletic under any delimitation broader than that of *Blossfeldia* alone. Indeed the tribe seems to be something of a dustbin for isolated genera. As well as *Blossfeldia*, two other early-evolving genera placed outside of the major clades, *Frailea* and *Copiapoia*, are placed in the group. In contrast, a strongly supported (1.0 pp, 100% MLBS, 96% MPBS) core Notocactaceae is recovered which comprises the other three genera sampled here (*Parodia*, *Neowerdermannia*, *Eriosyce*). This core Notocactaceae is exactly the same, comprising the same samples, as the core Notocactaceae recovered by Nyffeler (2002). Only one genus of the Notocactaceae *sensu* Hunt (2006), *Yavia*, remains unsampled.

Status of Echinocereae sensu Hunt. Hunt's (2006) Echinocereae is the second largest of the tribes recognized, comprising 247 species placed in 25 genera. Our study samples 119 of the species and 20 of the genera. Hylocereae is nested in two clades within the group, rendering it polyphyletic. The Echinocereae and Hylocereae taken together are strongly supported as a monophyletic group, equivalent to the ACHLP of Nyffeler (2002) and recovered here with 1.00 pp (87% MLBS, 75.5% MPBS). All of the species of Hylocereae sampled here are recovered within the Echinocereae clade. Relationships within the clade comprising Hylocereae and Echinocereae are discussed below relative to relationships presented by Arias et al. (2005).

Status of Hylocereae sensu Hunt. The ten Hylocereae species representing four genera (three species of *Disocactus*, three species of *Hylocereus*, *Pseudorhypsalis amazonica* and three species of *Selenicereus*) sampled here are all nested within the larger Echinocereae clade. Within that larger clade six species of Hylocereae are unresolved, but four species (*Selenicereus pteranthus*, *Selenicereus spinulosus*, *Hylocereus monacanthus* and *Hylocereus ocamponis*) comprise a moderately to weakly supported (0.76 pp, 65% MLBS, 53% MPBS) monophyletic group; other species sampled for these genera remain outside of this clade.

Relationships within tribe Echinocereae. Nyffeler (2002) did not point to any significant groupings amongst the 11 genera he placed in his HLP clade, but based on a denser sample, which places 30 genera in this group, we are able to do so. We identify the core HLP, a clade comprising 11 genera (*Bergerocactus*, *Cephalocereus*, *Echinocereus*, *Escontria*, *Myrtillocactus*, *Neobuxbaumia*, *Pachycereus pro parte*, *Peniocereus pro parte*, *Polaskia*, *Stenocereus*, and three of four accessions of *Acantho-*

reus tetragonus sampled). The core HLP comprises in large part genera placed in Hunt's tribe Echinocereae and excludes all genera sampled from the Hylocereae *sensu* Hunt (2006), and several genera of the Echinocereae (*Armatocereus*, *Castellanosia*, *Neoraimondia*, *Peniocereus pro parte*, and one of four accessions of *Acanthocereus tetragonus* sampled). Hunt's Echinocereae genera *Austrocactus*, *Corryocactus*, *Eulychnia*, and *Pfeiffera*, as noted above, are also excluded. Our topology for the HLP clade can be compared with that of Arias et al. (2005); although our sampling is greater, combining *rpl16*, *trnL* intron, and *trnL-F* intergenic spacer regions results in slightly better resolution. Arias et al. (2005) identify a well-supported (99% BS) core Echinocereae group comparable with ours. Both analyses find genera or part-genera placed in Echinocereae *sensu* Hunt outside of the core Echinocereae. The Echinocereoid genera, which both analyses place outside of the core group, comprise one group of *Acanthocereus* species, one group of *Peniocereus* species, and *Armatocereus*. Arias et al. (2005) also place *Dendrocereus* and *Pseudoacanthocereus*, genera we did not sample, outside of the core Echinocereae. Other genera sampled by Arias et al. (2005) which we did not include are *Carnegiea* and *Weberocereus*. *Carnegiea* is placed in the core Echinocereae and *Weberocereus* groups with other Hylocereae genera. It is notable that in Arias et al.'s (2005) study, genus *Acanthocereus* is split between two groups. *Acanthocereus tetragonus*, a species we sample several times, is recovered by Arias et al. outside of the core Echinocereae with a group of *Peniocereus* species.

We also compare our findings with those of Hartmann et al. (2002) and Arias et al. (2003). Hartmann et al. (2002) found *Pachycereus schottii* (syn. *Lophocereus schottii*) and *P. gatesii* (syn. *Lophocereus gatesii*) in an unresolved group sister to *P. marginatus* in their study, basing hypotheses in shifts in pollination syndrome on their findings. Alternative sampling in our case places *P. schottii* in a strongly supported clade with *P. gatesii* (1.0 pp, 88% BS) in Bayesian and ML analysis, but strongly supported (1.0 pp, 98% BS) as sister to this clade is a sister pairing of *P. marginatus* and *Neobuxbaumia tetetzo* (1.0 pp, 100% BS), a relationship not recovered in their analysis. This group of four species (*P. schottii*, *P. gatesii*, *P. marginatus*, and *Neobuxbaumia tetetzo*) was also recovered in the MP analysis, although notably one accession of *P. schottii* is unresolved at the base of the clade, while two others are sister to *P. gatesii*. Arias and Terrazas (2009) revised *Pachycereus* to recognize *Pachycereus sensu stricto* (*s.s.*) comprising five species (*P. grandis*, *P. pecten-aboriginum*, *P. pringlei*, *P. tepamo*, and *P. weberi*). The re-delimitation of the genus was based on molecular (Arias et al., 2003, 2005) and morphological (Arias and Terrazas, 2006) analyses. However, *Pachycereus s.s.* was

not recovered in our analyses. We sample three of the species; the narrowly distributed *P. tepamo* is not included in our study, *P. grandis* and *P. weberi* are sisters, and *P. pringlei* is unresolved in our analyses.

Relationships within tribe Cactaceae. The tribe Cactaceae has been the focus of a study by Butterworth et al. (2002). Their study sampled approximately 650 bp of the plastid *rpl16* intron for 62 representative taxa for the tribe. Here we present a topology for the Cactaceae which includes 271 species represented by 363 samples; more than half of the species are representatives of genus *Mammillaria* (180 plants, representing 140 *Mammillaria* species). The most complete study of *Mammillaria*, prior to this one, by Butterworth and Wallace (2004), sampled 113 species in this genus for two gene regions, the *rpl16* intron and the *psbA-trnH* intergenic spacer region.

Butterworth et al.'s (2002) analysis recovered a monophyletic core Mammilloid group they called the Mammilloid clade. Their support for this clade was low (60% BS). In our study the smallest clade which includes all our samples of *Mammillaria* finds support in the Bayesian tree of 0.94 pp, but only 53% MLBS and < 50% MPBS. Sister to this clade in all analyses is the monotypic *Cumarinia*. The clade including *Cumarinia*, a genus not sampled by Butterworth et al. (2002), has support of 1.0 pp and 60% MLBS but less than 50% MPBS. We propose that genus *Cumarinia* is considered part of the informal Mammilloid clade. This genus, considered “curious and very distinct” by Hunt (2006), has not previously been placed in a molecular phylogeny. Its placement as sister to the remainder of the Mammilloid clade in our analysis contradicts proposals of affinities with *Coryphantha clavata* ssp. *stipitata* and species of *Mammillaria* with scented fruits, showing these affinities to be the result of convergence. In other respects we recover a comparable Mammilloid clade to Butterworth et al. (2002), providing stronger support for this hypothesis of relationship. We recover seven genera within the clade, *Acharagma pro parte*, *Coryphantha*, *Escobaria*, *Mammillaria*, *Neolloydia*, *Ortegocactus*, and *Pelecyphora*. Butterworth et al. (2002) placed the same genera in their study, except that they placed *Acharagma aguirreanum* elsewhere, and they recognized genus *Encephalocarpus*, subsumed into *Pelecyphora* in Hunt's (2006) treatment. Our placement of *Acharagma aguirreanum* with *Mammillaria* is notable as it conflicts with Butterworth et al.'s (2002) placement of both species of this genus with *Lophophora* and *Obregonia*. In contrast, we split *Acharagma aguirreanum* from *Acharagma roseanum*, placing the latter with *Turbiniocarpus pro parte*. Figure 5 shows the position of the Mammilloid clade within the Cactaceae; relationships within the Mammilloid clade are shown in Fig. 6a,b.

Butterworth and Wallace (2004) focused in more detail on the Mammilloid clade and tested existing generic delimitation and subdivisions of *Mammillaria*. Their analysis showed that *Mammillaria* was polyphyletic. However, noting that the genera *Coryphantha* and *Escobaria* comprise 55 and 23 species, respectively [Hunt (2006) recognizes 42 and 19] and that their sample was of three and four species, they argued that wider sampling of these genera is necessary before redelimitation of the genus *Mammillaria* is possible. Our sampling of the Mammilloid clade does include more species of *Mammillaria* and denser sampling of the other genera placed in the Mammilloid clade. In Butterworth and Wallace's (2004) analysis all eight species representing the genera *Coryphantha*, *Escobaria*, *Neolloydia*, *Ortegocactus*, and *Pelecyphora* were placed in a clade (their clade A) which otherwise corresponds to Hunt's (1981) circumscription of series *Ancistracanthae*. We recover a corresponding clade in our analyses (0.96 pp, 34% MLBS, < 50% MPBS), but with some differences. We place three samples of *Mammillaria* not sampled by Butterworth and Wallace (2004) in the clade; these are *M. dioica*, *M. melaleuca*, and *M. tetrancistra*. One species placed by Butterworth and Wallace (2004) in their clade A was placed elsewhere in our Bayesian analysis, but not in our ML or MP analyses—*M. melanocentra*. Most significantly we find strong support for the inclusion of *Ortegocactus*, *Neolloydia*, and three species of *Coryphantha* (*C. difficilis*, *C. echinus*, *C. werdermannii*) in this clade, but place a core group of *Coryphantha* and our core *Escobaria/Pelecyphora* group elsewhere. We sample 33 species of *Coryphantha* and recover a core *Coryphantha* group in the Bayesian and ML analyses, but with low support (0.65 pp, 10% BS); this group was not recovered in the MP analysis, which is notably less resolved. One of the species (*C. elephantidens*) with multiple accessions has one accession found within the core clade, and other outside of it. A third accession of this species finds an anomalous placement as sister to the remainder of the tribe in the Bayesian analysis, but is placed with the accession outside of the core *Coryphantha* clade but nested within the Mammilloid clade. Nine species of *Coryphantha* which we sample lie outside of the core *Coryphantha* group, and 23 within it in Bayesian and ML analyses. Four of the seven samples of genus *Escobaria* are placed into a monophyletic group which also has the two species of *Pelecyphora* nested in it. There is poor support for *E. chihuahuensis* as sister to the remainder of this group in all analyses, but the remainder of the samples are recovered with strong support (1.0 pp, 97% MLBS, 98% MPBS). The group including the four samples of *Escobaria* and two of *Pelecyphora* finds 98% BS support in the ML analysis and 85% MPBS support. The remaining three samples of *Escobaria* are sister to

two species of *Coryphantha* in an unresolved clade within the Mammilloid clade. The relationships between the “clade A” group, the *Coryphantha* group and the *Escobaria*/*Pelecyphora* group are not resolved in this study, but failure to recover them as part of a supported “clade A” group shows that Butterworth and Wallace (2004) were correct to call for wider sampling of these genera prior to any re-delimitation of *Mammillaria*. In terms of further sub-delimitations of the genus, we find less resolution than Butterworth and Wallace (2004). Many of the species of *Mammillaria* placed outside of the “clade A” group are unresolved within the Mammilloid clade, although there are several small groups of species (poorly to well-supported) that are unresolved relative to each other. Whilst our study provides wider sampling, further gene regions are needed alongside wider sampling to robustly recover relationships within *Mammillaria*.

Relationships we reconstruct within the Cactaceae but outside the Mammilloid clade are generally better supported than those in Butterworth et al.’s (2002) study using the *rpl16* intron. Forty-one of their samples were Cactaceae placed outside the Mammilloid clade; our sampling of the Cactaceae excluding the Mammilloid clade includes 124 accessions. Butterworth et al. (2002) recovered the Mammilloid clade with successive sisters such that a series of clades were nested pectinately within each other. However, only three of the nine internal branches contributing to their pectinate scheme find bootstrap values greater than 50%. Differences between our schemes—given the differences in sampling and the low support afforded to the *rpl16* intron topology—are not unexpected.

Our Bayesian topology finds a large polytomy grouping together 13 unresolved species, six species pairs and six clades of more than two species; these five clades are labelled A to E in Fig. 5. Considering these labelled clades in the Bayesian analysis, Clade A (1.0 pp, 7% MLBS) is the largest, and has the Mammilloid clade nested in it. This clade is not recovered in the parsimony analysis, as *Ferocactus lindsayi*, *Ferocactus latispinus*, and *Thelocactus lausseri* are placed elsewhere. The group excluding these three species but the remainder of Clade A finds 49% MLBS and < 50% MPBS. In Butterworth et al.’s (2002) analysis the clade sister to the Mammilloid clade comprised the three species of *Stenocactus* sampled. Sixteen of our 17 samples of *Stenocactus* also comprise a monophyletic group, but the group is not recovered as sister to the Mammilloid clade. Instead the Mammilloid clade is nested in the largest of our six clades, Clade A, where it is sister to a well-supported clade (1.0 pp, 67% MLBS, 68.5% MPBS) comprising the two species of *Lophophora*, a subset of *Turbincarpus* (four of the 18 samples), and two of the three *Acharagma* samples. This group is nested in turn within a paraphyletic

Epithelantha in both the Bayesian and the ML analyses, although it is sister to the *Epithelantha* clade in the parsimony analysis. All of the samples of *Turbincarpus* are found within Clade A, but not as a monophyletic group. Clade A also contains all the samples of *Ariocarpus* (recovered as a monophyletic group), and both samples of the monotypic *Strombocactus*. *Strombocactus* is recovered as sister to *Turbincarpus alonsoi*, a species with which it grows, and which has been hypothesized to be a hybridizing parent. Differences in the *trnK-matK* sequences of the two species do not support the hypothesis that *Strombocactus* has a hybrid origin.

Thelocactus and *Ferocactus* are notably polyphyletic in our analysis. Butterworth et al.’s (2002) also recovered a polyphyletic *Ferocactus*, although whereas our 12 samples of *Thelocactus* are distributed widely, their three samples of *Thelocactus* were placed in an unsupported monophyletic group. In our analysis the majority of our *Ferocactus* samples are placed in Clade C, a clade composed entirely of *Ferocactus* samples. Of the remaining Ferocacti in our analysis, four species are placed in Clade B with the core *Stenocactus*, although this clade is poorly supported (0.64 pp, 13% BS) in the Bayesian and ML analyses and not recovered in the parsimony analysis, and four species are placed in Clade D, a second clade entirely comprising *Ferocactus* samples. Two species are placed in Clade A (*F. latispinus* and *F. lindsayi*) but a second accession of *F. latispinus* is placed in Clade B. These species are in an unresolved group in the parsimony analysis. *F. pilosus* is grouped with three other *Thelocactus* species in Clade E in the Bayesian analysis, but with poor support (0.51 pp). This is another group not recovered in the parsimony analysis, which is generally less resolved. In the ML analysis *F. wislizeni* and *F. echidne* are part of this clade, although the two accessions of *F. wislizeni* and *F. echidne* are unresolved in the Bayesian analysis. *F. hamatacanthus* groups with *Thelocactus setispinus* (1.0 pp, 59% MLBS, < 50% MPBS). *F. robustus*, a species entirely unresolved in the Bayesian analysis, and *F. fordii* and *F. viridescens* [an unplaced sister pair (1.0 pp) in the Bayesian analysis] are grouped together in the ML analysis, with *Thelocactus hexaedrophorus* and *Thelocactus bicolor*. In the Bayesian analysis the situation for *Thelocactus* is similarly complex, with seven unresolved species, one species sister to the remainder of Clade A, the three species in the poorly supported clade with *F. pilosus*, and one other species in an unresolved species pair with *F. hamatacanthus*. Again, ML gives a more resolved topology, but with little or no support for the relationships recovered and the parsimony analysis is less resolved and finds lower support. Both *Thelocactus* and *Ferocactus* need to be reconsidered in the light of phylogenetic analysis sampling more gene regions.

There is evidence that *Echinocactus* is also polyphyletic. Three species are nested in Clade F (1.0 pp, 82% MLBS, 59% MPBS) as sister to a monophyletic *Astrophytum*. Butterworth et al.'s (2002) place the same Echinocacti in a clade with *Astrophytum*. Our analysis shows that the sister pair *Aztekium* and *Geohintonia* (1.0 pp, 91% MLBS, 81.5% MPBS) are sister to this clade (1.0 pp, 75% MLBS, < 50% MPBS). *Echinocactus grusonii* and *Echinocactus polycephalus*, the other species of *Echinocactus* we sample, are unresolved outside of this group.

One species of *Stenocactus*, *S. coptogonus*, is placed with the monotypic genus *Leuchtenbergia* in Bayesian and ML analyses (0.77 pp, 46% MLBS), although it is unplaced in the parsimony analysis. The placement of this species away from the remainder of the samples of *Stenocactus* is perhaps not remarkable given that *S. coptogonus* is the only member of the genus with broad, straight ribs, whilst the other species have usually very numerous, thin ribs. Its placement with *Leuchtenbergia principis* is more remarkable, as the latter is a strongly tuberculate species, looking more like a leaf-succulent agave than a cactus. Another example of a close relationship between a strongly tuberculate species and ribbed relatives is also revealed here, between *A. caput-medusae* and the remaining *Astrophytum* species, showing transitions between these strongly divergent forms are possible.

Relationships within subfamily Opuntioideae

The Opuntioideae *sensu* Hunt (2006) includes two tribes, Opuntieae and Cyliandropuntieae, comprising ten and seven genera, respectively, and 192 species. In Hunt's treatment, 75 of these species are placed in the largest genus *Opuntia*. The most complete phylogeny available for the subfamily is that of Griffith and Porter (2009). They sampled 110 specimens for two gene regions, the nuclear internal transcribed spacer (*ITS*) and the chloroplast *trnL-trnF*. We present comparable levels of sampling here for the *trnK-matK* gene region, with 122 species, although we select different exemplar species. In both the Bayesian and the ML topologies we show the Opuntioideae comprising three monophyletic groups whose relationships are unresolved as a trichotomy. The first group corresponds to tribe Opuntieae, the second comprises the core Cyliandropuntieae, and the third we refer to as the *Maihueiopsis/Tephrocactus/Austrocylindropuntia/Cumulopuntia* clade (MTAC clade, Fig. 3). Notably, only the core Cyliandropuntieae and the MTAC clade are recovered in our parsimony analysis.

Relationships within tribe Opuntieae. Our Bayesian topology recovers the tribe Opuntieae with strong support (1.0 pp), although one species sampled, *Opuntia*

atrispina, has an anomalous placement outside of the tribe in this analysis. The Bayesian analysis places this species in the core Cyliandropuntieae clade, but ML places it, as expected, within tribe Opuntieae where it is found with moderate support (76% MLBS) as sister to *Opuntia wilcoxii*. Parsimony also recovers the species as sister to *O. wilcoxii*. However, in contrast to the Bayesian tree, support for the Opuntieae is very weak (44%) in the ML phylogeny, and the parsimony analysis fails to recover the tribe at all. We suggest that *Opuntia atrispina* be critically examined and that additional samples should be sequenced to determine the cause of the alternative placements and weak support for the tribe in our ML and parsimony analyses. The species was not sampled by Griffith and Porter (2009).

We sample four of the seven genera placed within tribe Opuntieae. Although three of the four genera sampled have more than one species (four *Nopalea*, five *Tunilla*, and 75 *Opuntia*) we provide multiple exemplars only of *Opuntia* (52 of the 75 species are represented). Relationships within the tribe show some differences from those presented by Griffith and Porter (2009). Here we report the findings of the Bayesian and ML analyses, as the parsimony analysis results in a relatively poorly resolved tree. In our Bayesian analysis *Brasiliopuntia brasiliensis* is strongly supported (0.95 pp) as sister to the remainder of the Opuntieae clade, and *Tunilla erectoclada* and *Opuntia quimilo* are placed outside of a core *Opuntia* clade (all species of *Opuntia* sampled with the exception of two samples of *Opuntia quimilo* and the anomalous *Opuntia atrispina* in the Bayesian analysis). The ML tree places *Brasiliopuntia brasiliensis* as sister to *O. elizondoana* (only 14% BS), and this pair as sister to the rest of the clade (only 17% BS). *Tunilla erectoclada* and *Opuntia quimilo* placed outside of the core *Opuntia* clade in both analyses, but although a core *Opuntia* clade is recovered by both analyses (differing only in the placement of *O. atrispina*) it is not supported (0.52 pp, 11% BS) in either analysis. The fourth genus we sample, *Nopalea*, is nested within the core *Opuntia* clade as sister to *O. pilifera* (0.97 pp, 68% BS) in both ML and Bayesian topologies. Griffith and Porter (2009) also find *Nopalea* to be nested within *Opuntia*, but recover *Tunilla* as earlier branching than *Brasiliopuntia*. They also recover one of the three species of *Maihueiopsis* that they sample in the Opuntieae clade.

Relationships within tribe Cyliandropuntieae. In contrast to the Opuntieae, which is monophyletic in the Bayesian and parsimony analyses, the Cyliandropuntieae *sensu* Hunt (2006) is not a monophyletic group in any analysis. A core group comprising the genera *Cylindropuntia*, *Grusonia*, *Corynopuntia*, and *Pereskiaopsis* is recovered with strong support (1.0 pp, 97% MLBS, 98.5% MPBS), but a second clade (1.0 pp, 74% MLBS, 63% MPBS) comprising the MTAC clade (and the

anomalous *Opuntia atrispina* specimen in the Bayesian analysis) is unresolved relative to this one. Further sampling of gene regions may yet support or refute the Cyliandropuntieae as monophyletic; however, it is notable that the analysis of Griffith and Porter (2009) does not recover a monophyletic Cyliandropuntieae either. Comparing our topology for the Cyliandropuntieae (Fig. 4) with that of Griffith and Porter (2009) reveals some differences which may be attributed to sampling. Griffith and Porter (2009) describe a terete-stemmed lineage and two additional lineages, a *Pterocactus* lineage (comprising only *Pterocactus* species) and a core *Maihueniopsis* lineage. We do not recover either the *Pterocactus* or the *Maihueniopsis* lineage, but do not sample any of the species Griffith and Porter (2009) place in these lineages. However, our core Cyliandropuntieae and our MTAC clade taken together are equivalent to Griffith and Porter's (2009) terete-stemmed clade, a monophyletic group in Griffith and Porter's (2009) study, but not monophyletic in the trees we recover here. Within the Cyliandropuntieae, alternative delimitations of the genera *Grusonia*, *Micropuntia*, and *Corynopuntia* have been suggested by molecular studies. Griffith and Porter's (2009) study provided those authors with additional support for the recognition of a monotypic *Grusonia*, following the treatment of Britton and Rose (1919), in contrast to the treatment by Wallace and Dickie (2002) that sinks *Micropuntia*, *Marenopuntia*, and *Corynopuntia* into *Grusonia*. In phylogenies by Griffith (2002) and Griffith and Porter (2009), *Micropuntia* is sister to a clade comprising *Cyliandropuntia* and *Grusonia* s.s. plus *Corynopuntia*. In this light *Micropuntia* is treated by Griffith as a segregate genus. The treatment proposed by Griffith was not adopted by the "New Cactus Lexicon" (Hunt, 2006), which lists a monotypic *Grusonia* but does not recognise *Micropuntia*, and Bárcenas and Hawkins (2007) describe this latter genus as "highly debatable". The phylogenies recovered here find a placement for *Corynopuntia pulchella* incongruent with that of Griffith (2002) and Griffith and Porter (2009). In the present study, *Corynopuntia pulchella* is recovered as sister to *Pereskioopsis*, and further studies are presently underway.

A revised classification of the Cactaceae?

Suprageneric groupings. It has been demonstrated convincingly that one of four recognized subfamilies, Pereskioideae, is not monophyletic (Butterworth and Wallace, 2005; Edwards et al., 2005), and many of the papers we cite here have demonstrated that several tribes as currently recognized are not monophyletic either. Our study confirms that existing tribal delimitations can provide a poor guide to the relationships of the genera placed in them. Only three are monophyletic—

the Cactaceae, Rhipsalideae, and Opuntieae—although more data may yet reveal a monophyletic Cyliandropuntieae. Phylogeneticists working with cacti [e.g. Edwards et al. (2005) with respect to subfamilial classification; Griffith and Porter (2009) with respect to generic delimitation in the Opuntioideae] argue that additional genes and further sampling is necessary before any reclassification of the cacti. We concur; given the confusion that has arisen due to multiple and conflicting classifications it is essential that any taxonomic revision is well founded. The Maihuenioideae was proposed in the light of new molecular data in 1996, and has been recognized by significant classifications since then (Anderson, 2001; Hunt, 2006), reflecting the willingness of cactologists to be informed by molecular phylogenetics when robust schemes are available. However, a strictly monophyletic classification of the cacti informed by the most recent higher-level analysis (Edwards et al., 2005) would not recognize Maihuenioideae at the same rank as the Cactoideae or Opuntioideae, suggesting subfamilial rank was assigned prematurely.

A strict requirement for monophyly and the retention of traditional nomenclature are not necessarily mutually exclusive, although a requirement for monophyly can result in undesirable proliferation of small, monospecific higher taxa. To base a traditional classification on a phylogeny requires the recognition of a hierarchy of ranked monophyletic groups and the assignment of Linnaean rank to selected ranks in the hierarchy. Nyffeler (2002) summarized relationships inferred by his analysis in a cladistic classification scheme with eight ranks. Although he refrained from assigning Linnaean ranks to his groups, this scheme could serve as a working hypothesis for a future Linnaean classification based on cladistic principles. Our more inclusive analysis presents the possibility of reviewing Nyffeler's (2002) classification, adding additional taxa and clades where our analysis—and those of Butterworth et al. (2002), Arias et al. (2005), Edwards et al. (2005), and Ritz et al. (2007)—show there is sufficient support for a placement. However, our phylogeny is typical of many widely and densely sampled phylogenies (e.g. Bello et al., 2009) in that the basal-most branches and branches at the tips of the trees are relatively poorly resolved, although there are strongly supported groups recovered at intermediate levels. Even considering the basal relationships recovered by Edwards et al. (2005), there is insufficient resolution for a top-down classification, assigning ranks to the sub-familial rank in the first instance. Similarly, there are insufficient data for a generic-delimitation-upwards approach until more fast-evolving gene regions are sampled to resolve species relationships and re-delimit robust genera. It is in the light of these limitations, and the expectation that other analyses in preparation (Ruiz

Maqueda et al., in prep; Nyffeler, pers. comm.) might provide additional sampling, resolution and support, that we refrain from updating Nyffeler's (2002) classification scheme here.

Generic delimitation. According to Hunt (2006), the most notable battles in cactus taxonomy have been fought over the delimitation of genera. The publication of alternative generic delimitations has led to enormous instability in names and to species with synonyms in half a dozen genera or more. Although not all interested parties have supported all of the delimitations presented in the consensus list (e.g. negative reactions amongst amateur enthusiasts to the decisions for *Sulcorebutia* and *Weingartia*, subsumed into *Rebutia*—see <http://www.mfaint.demon.co.uk/cactus/noto/consensus.html>), the consensus list has been thought of as working hypotheses which might be tested in a molecular systematic framework. Indeed, some changes to the list of accepted genera have been made on the basis of molecular evidence.

Our phylogeny is considerably more densely sampled than that of Nyffeler (2002) so would be able to assign more species in any revised version of his cladistic classification. Placements of species at the lowest ranks in the cladistic classification could represent well-supported groupings and might be considered more satisfactory generic groupings than those currently accepted. However, many species are unresolved, and our least inclusive groupings are significantly larger than currently accepted genera. Assigning the rank of genus to these least inclusive groupings could create further instability as future studies using faster evolving genes recognize and name robust subgroupings. Rather than creating further instability in generic names we confine ourselves to presenting a synopsis of the status of genera. Tables 3 and 4 show the status of those genera recognized in Hunt (2006) which we were able to critically evaluate in our study. These tables show that an extraordinarily high proportion of genera are not monophyletic. Subfamily Cactoideae comprises 105 genera, of which 25 are monotypic (Hunt, 2006). We sample two or more exemplars for 36 of these genera, listed in Table 4. Only 14 genera were recovered as monophyletic. However, a monophyletic core could be delimited for many genera in this subfamily. The situation for subfamily Opuntioideae is similar. Of 17 recognized genera three are monotypic. Four of the six genera for which we sample more than one species were monophyletic. These very high proportions of poly- and paraphyletic genera suggest that a cladistic classification of the Cactaceae would significantly alter generic limits. However, although many genera are not monophyletic, many of these follow a pattern of a monophyletic core, with one or two outliers, suggesting relatively robust groups with “fuzzy edges” so that in several cases small

adjustments to classifications (i.e. moving one species outside of the genus) may produce monophyletic groups without significant nomenclatural changes. In terms of future work, our higher level scheme identifies well-supported higher-level monophyletic groups that could be sampled for fast-evolving genes in studies with the objective of delimiting genera.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Specimens included in the study.

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