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A phylogenetic analysis of *Pfeiffera* and the reinstatement of *Lymanbensonia* as an independently evolved lineage of epiphytic *Cactaceae* within a new tribe *Lymanbensonieae*

Dedicated to Beat Ernst Leuenberger (1946–2010)

Abstract

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Pfeiffera is a genus of epiphytic, terrestrial and epilithic cacti. Its acceptance, circumscription and closest relatives have been debated. In the context of a phylogenetic survey of epiphytic cacti, we have studied relationships in *Pfeiffera,* sampling eight of nine species and using sequence data from three group II introns *(trnK, rpl16, trnG)*, four intergenic spacers *(psbA-trnH, trnQ-rps16, rps3-rpl16, trnS-trnG)* and the rapidly evolving gene *matK* of the plastid genome. Phylogenetic analyses revealed *Pfeiffera* to be polyphyletic, comprising two unrelated lineages, both highly supported. One clade includes the type species, *P. ianthothele;* the second contains two *Pfeiffera* and an erstwhile *Lepismium* species. Our results justify generic status for this newly found clade. Since it includes the type species of the earlier-proposed monotypic genus *Lymanbensonia,* we suggest the reinstatement of the latter in an amplified circumscription. The necessary new combinations for *Pfeiffera brevispina* and *Lepismium incachacanum* are provided. Our results further support the establishment of a separate tribe *Lymanbensonieae,* formally proposed here, to contain *Lymanbensonia* and *Calymmanthium.* The phylogenetic results imply that epiphytism evolved more frequently in *Cactaceae* than hitherto assumed and further show that morphological convergences in the family can be extreme. An integrated approach using morphology and sequence data is therefore needed to establish sound generic limits in the *Cactaceae*.

Additional key words: *Rhipsalideae, Echinocereeae, Lepismium, Calymmanthium,* molecular phylogenetics, epiphytism, convergence

1. Introduction

Epiphytes account for a large portion of tropical plant diversity. An estimated 25 000 angiosperms, representing almost 10 % of all species in approx. 70 families, are epiphytes, making epiphytism one of the most frequently evolved life forms in flowering plants (Kress 1989). Even in *Cactaceae,* a family usually associated with arid areas, the epiphytic habit also occurs within 10 % of the family's species, making *Cactaceae* one of the larger epiphyte groups. There are currently eleven accepted epiphytic genera with about 150 species (Hunt 2006).

Epiphytic cacti have been known since Linnaean times, but assumptions concerning how frequently epiphytism has evolved differed and thus the number of epiphytic lineages accepted. The early works of A. P. de Candolle (1828) and Schumann (1899) contained, in effect, two epiphytic lineages, while Britton & Rose (1923) recognised three and Berger (1926) even four. In contrast, Backeberg (1959, 1966) and Buxbaum (1962) placed all the epiphytic genera in one single group. More recently, epiphytism has been regarded as having evolved independently in the tribes *Rhipsalideae* DC. and *Hylocereeae* (Britton & Rose) F. Buxb. (Barthlott 1979; Barthlott & Hunt 1993). Lately, the genus *Pfeiffera* Salm-Dyck was identified as a third independent epiphytic lineage (Nyffeler 2002).

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Table 1. Changing circumscriptions of *Pfeiffera* and allied genera.

	Salm-Dyck 1850	Schumann 1889	Vaupel 1925–26	Britton & Rose 1923, Berger 1926	Backeberg 1959	Kimnach 1983, 1984	Barthlott 1987	Hunt 2006
Pfeiffera	1 sp.	1 sp.	= *Rhipsalis*	1 sp.	1 sp.	= *Rhipsalis*	= *Lepismium*	9 spp.
Acantho-rhipsalis	not yet described	= *Rhipsalis*	= *Rhipsalis*	3 spp.	5 spp.	= *Rhipsalis*	= *Lepismium*	= *Pfeiffera*
Lepismium	1 sp.	= *Rhipsalis*	= *Rhipsalis*	1 sp.	17 spp.	= *Rhipsalis*	14 spp.	6 spp.
Lymanben-sonia	not yet described	= *Rhipsalis*	= *Rhipsalis*	= *Acantho-rhipsalis*	= *Acantho-rhipsalis*	1 sp.	= *Lepismium*	= *Pfeiffera*

Pfeiffera has long been one of the most controversial genera of epiphytic cacti. Its acceptance and circumscription as well as hypotheses about its affinities have received the attention of many systematists. The genus was first described by the prince J. Fürst zu Salm-Dyck (1845) as a monotypic genus separated from *Cereus* Mill., including only *P. cereiformis* Salm-Dyck (= *P. ianthothele* (Monv.) F. A. C. Weber).

Salm-Dyck (1850) and Schumann (1899) assigned *Pfeiffera* to the tribe *Rhipsalideae,* Britton & Rose (1923) placed it in their subtribe *Rhipsalidanae* and Berger (1926: 42) proposed *Pfeiffera* as the independent lineage *"Pfeifferae"*. Although Backeberg (1959, 1966) later considered the *Hylocereeae* the only epiphytic lineage, which included the *Rhipsalideae,* he followed Berger's view and treated *Pfeiffera* as isolated, while Buxbaum (1962) placed *Pfeiffera* in the *Hylocereeae* subtribe *Rhipsalinae*. Besides this disagreement about its putative closest relatives, there was no consensus as to whether *Pfeiffera* should be recognised at all. Generic concepts changed several times within the *Rhipsalideae.* While some authors recognised eight genera, others combined most taxa into *Rhipsalis* Gaertn., as summarised in Table 1. The most recent treatments merged *Pfeiffera* along with *Acanthorhipsalis* Britton & Rose and *Lymanbensonia* Kimnach in *Lepismium* Pfeiff. as part of the *Rhipsalideae* (Barthlott 1987; Barthlott & Taylor 1995).

New hypotheses concerning *Pfeiffera* came from the molecular phylogenetic study of *Cactaceae* by Nyffeler (2002) based on *trnK/matK* and *trnL-F.* Three *Lepismium* species sampled [*L. ianthothele* (Monv.) Barthlott, *L. miyagawae* (Barthlott & Rauh) Barthlott and *L. monacanthum* (Griseb.) Barthlott] formed a maximal supported clade distant from the *Rhipsalideae* and instead close to the *Pachycereeae, Leptocereeae* and *Hylocereeae*. This newly found epiphytic lineage contained *Pfeiffera ianthothele,* the type species of *Pfeiffera*. Based on this evidence from molecular data, Nyffeler (2000, 2002) argued that the resurrection of *Pfeiffera* was needed, and this proposal was adopted in the New Cactus Lexicon (Hunt 2006).

Pfeiffera currently contains nine creeping to erect epiphytic, terrestrial or epilithic species, ranging from southern Ecuador to northern Argentina, the main distribution centre being the eastern Andes of Bolivia. The genus is mainly characterised by mesotonic branching, stems with 3–8 ribs or flattened, usually not producing adventitious roots. Spines are often well developed, the flowers are whitish to intensely coloured and the pericarpels and fruits are spiny. However, some of these characters also occur in other *Rhipsalideae* genera, especially *Lepismium*. The main differences, as currently understood, are the spiny stems and fruits in *Pfeiffera,* whereas spines are usually lacking or reduced and the fruits are naked in the *Rhipsalideae*.

The finding that *Pfeiffera* is an independent lineage from the *Rhipsalideae* was unexpected, since its prior inclusion in *Lepismium* had not been questioned (Nyffeler 2000). But apart from the sampling of three species in the phylogenetic study of Nyffeler (2002), the current circumscription of *Pfeiffera* has not been further evaluated by using DNA data.

Changing generic concepts are, however, typical for *Cactaceae.* They have always been much influenced by subjective views of the different authors and their respective ideas to emphasise morphological similarities or differences. *Cactaceae* genera are currently again in flux and even relationships which seemed clear have to be questioned following DNA analyses. There is increasing evidence that most tribes and genera based on morphology are not monophyletic (e.g. Arias & al. 2005, Butterworth & Wallace 2004, Edwards & al. 2005, Nyffeler 2002, Ritz & al. 2007). Although *Cactaceae* are an important component of the New World's flora and a popular family in horticulture, their phylogenetic relationships remain insufficiently understood.

Phylogenetic trees for the *Cactaceae* have been challenging to resolve so far, due to low sequence divergence even in generally variable genomic regions such as *trnK/matK* or *trnL-F* or *rpl16.* A combination of two or three chloroplast regions still does not yield complete species-level resolution (e.g. Butterworth & Wallace 2004, Ritz & al. 2007). A robust phylogeny thus requires multiple data sets and all current studies further point to the fact that a combination of several fast-evolving regions (at least 5000–6000 nt per taxon) is needed to obtain full resolution between closely related species (Erixon & Oxelman 2008; Löhne & al. 2007; Tesfaye & al. 2007).

To address phylogenetic relationships in *Pfeiffera,* we have selected eight fast evolving chloroplast regions: the

trnK/matK region comprising the *trnK* group II (G2) intron and the *matK* gene, the *psbA-trnH* intergenic spacer (IGS), the *trnQ-rps16* IGS, the *rpl16* G2 intron along with the *rps3-rpl16* IGS, and the *trnS-trnG* region with the *trnS-trnG* IGS and the *trnG* G2 intron. All are well-established markers for phylogenetic studies on a low taxonomic level (Borsch & Quandt 2009; Shaw & al. 2005; Shaw & al. 2007). Besides, the *psbA-trnH* IGS, the *rpl16* intron and *trnK/matK* have already been used for tree reconstruction within *Cactaceae* (Arias & al. 2003; Butterworth & al. 2002; Butterworth & Wallace 2004, 2005; Edwards & al. 2005).

This study is part of an ongoing project dealing with the phylogenetic relationships and systematics of epiphytic cacti. Besides the necessity to establish a sound generic concept for *Pfeiffera,* it still has to be clarified to what extent the morphological similarities between *Pfeiffera* and the *Rhipsalideae* are in fact convergences due to adaptations to the epiphytic habit.

The aims of this study are (1) to evaluate the current circumscription of *Pfeiffera* and (2) to infer relationships between its species. In the long run, insights into the phylogeny and character evolution of *Pfeiffera* as a lineage independent from the *Rhipsalideae* will also help to better understand the evolution of epiphytism in *Cactaceae*.

2. Material and methods

Plant material and taxon sampling — The main source for plant material were the Botanical Gardens of the University of Bonn, where one of the most comprehensive collections of epiphytic cacti in the world has been established during several decades by W. Barthlott. We sampled eight out of nine *Pfeiffera* species recognised by Hunt (2006), but were not able to include *P. crenata* (Britton) P. V. Heath, which is only known from few collections and seems not to be in cultivation anywhere. In total, 14 *Pfeiffera* accessions were sampled and most species were represented by at least two specimens from different collection sites or with differing morphology. Sequences of *trnK/matK* for 41 additional species were taken from GenBank. Details concerning locality data, voucher information and EMBL accession numbers for all taxa sequenced are given in Appendix 1.

Isolation of genomic DNA — Material was collected from living plants. Most of the water-storing tissue was removed as soon as possible after collection and the remaining cortex tissue was dried in silica gel using a drying chamber for one or two days at 50 °C. The high amount of mucilage in cactus tissues often causes problems during isolation, but this treatment significantly lessened the amount of mucilage and the subsequent isolation steps were straightforward. The dried plant material was homogenised using a mixer mill (Retsch MM200, Haan, Germany), then incubated for 20 minutes at 65 °C with 700 µl of extraction buffer containing 2 % CTAB, 1 % PVP, 100 mM Tris (pH 8), 20 mM EDTA, 1.4 M NaCl and 2 vol % mercaptoethanol. Further steps followed the procedure described by Borsch & al. (2003), but only two extractions instead of three were carried out. Concentration and purity of the DNA (A260/A280 as well as A260/A230 ratio) were measured using a spectrophotometer (NanoDrop, peqLab, Erlangen, Germany). Total genomic DNA was stored at -30 °C and a working dilution with a standard concentration of 10 ng/µl was made to be used for PCR.

Amplification and sequencing — All primers used in this study are listed in Appendix 2. The *trnK/matK* region was amplified in overlapping halves using the primer pair trnK-F and ROSmatK655R for the 3' fragment and ACmatK500F and trnK2R for the 5' fragment. Amplification conditions followed Müller & Borsch (2005).

The *psbA-trnH* IGS was amplified with the newly designed primers CApsbA and CAtrnH using a touchdown program with an initial denaturation step of 2 min at 95 °C, followed by 5 cycles of 30 sec at 95 °C, 1 min at 59 °C, 1 min at 72 °C, followed by 30 cycles of 30 sec at 95 °C, 1 min at 55 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C.

The *rps3-rpl16* IGS and the *rpl16* intron were co-amplified using the newly designed primers CArps3F, annealing to the *rps3* exon, and CArpl16R, annealing to the *rpl16* 3' exon. Amplification conditions were: an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 sec at 95 °C, 1 min at 55 °C, 90 sec at 72 °C and a final extension step of 15 min at 72 °C.

Amplification conditions for the *trnQ-rps16* IGS using the primer pair trnQ2 and rps16x1 were: an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 sec at 95 °C, 1 min at 55 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C.

The *trnS-G* region (*trnS-G* IGS and *trnG* G2 intron) was amplified using the primers trnS and trnG. Amplification conditions were: initial denaturation for 2 min at 95 °C, 35 cycles of 30 sec at 95 °C, 1 min at 58 °C, 2 min at 72 °C with a final extension step of 15 min at 72 °C.

All PCR products were stained with 100× SybrGreen nucleic acid stain and electrophoresed on a 2 % agarose gel, excised and purified using the Gel/PCR DNA Fragment Extraction Kit (Avegene) according to manufacturer's instructions and sequenced via Macrogen Inc. (Seoul, South Korea). The *trnK/matK* region was sequenced with the four amplification primers; additional internal sequencing primers were only rarely needed. At least three primer reads were needed to obtain the complete sequence of the *trnS-G* region; the reads of the amplification primers had to be complemented by reads from either trnG2S or trnG2G and a fourth read from CAtrnSG-40R was often required due to a frequently occurring poly-T stretch in the *trnG* intron. The *rps3-rpl16* spacer and the *rpl16* intron were sequenced with the amplification primers and the additional internal se-

quencing primer CArpl16-400R, because a large poly-A stretch occurred around pos. 400 in the *rpl16* intron. The *psbA-trnH* and *trnQ-rps16* spacers were sequenced with one of the amplification primers. The read of the second was often needed due to homo-polynucleotide stretches. Pherograms were edited and sequences were assembled using PhyDe v.995 (Müller & al. 2005+).

Alignment, coding of length mutational events — Sequences were aligned manually using PhyDE v.0995 (Müller & al. 2005+) according to the rules for the alignment of non-coding regions as outlined by Kelchner (2000) and Löhne & Borsch (2005). All positions excluded due to uncertain homology (= mutational hotspots) are listed in Tables S1–S2 (online edition, Appendix 3). Inversions were placed separately during alignment and reverse-complemented prior to phylogenetic analyses (Quandt & al. 2003; Borsch & Quandt 2009). Secondary structures of hairpins associated with inversions were calculated using RNAstructure 5 (Mathews & al. 1996+). Indels were coded according to the Simple Indel Coding method of Simmons & Ochoterena (2000) using the indel coder option of SeqState 1.40 (Müller 2005b).

Outgroup definition — To infer generic limits within *Pfeiffera,* a first analysis was run with only *trnK/matK* sequences for all taxa of the *Rhipsalideae* and *Pfeiffera* in a data matrix covering all major lineages of the *Cactoideae* with *Opuntia quimilo* K. Schum. and *Pereskia bleo* (Kunth) DC. as outgroup taxa. Thereupon, a second analysis with *trnK/matK* was performed with the same taxon set but only four *Rhipsalideae* species (a detailed phylogeny of the *Rhipsalideae* will be published elsewhere). Finally, analyses including all markers in combination and each marker alone were performed to determine species-level relationships within *Pfeiffera* and species newly found to be related in the preceding analysis. *Browningia hertlingiana* (Backeb.) Buxb., *Echinopsis aurea* Britton & Rose, *Rhipsalis pentaptera* A. Dietr., *Lepismium cruciforme* (Vell.) Miq., *Calymmanthium substerile* F. Ritter and *Eulychnia breviflora* Phil. served as outgroup taxa.

Phylogenetic analyses — Maximum parsimony (MP) analyses were performed using the parsimony ratchet as implemented in PRAP (Müller 2004). Ratchet settings were 200 iterations with 25 % of the positions randomly upweighted (weight = 2) during each replicate and 10 random addition cycles. The number of steps for each tree and the consistency, retention and rescaled consistency indices (CI, RI and RC) were calculated using PAUP* v. 4.0b10 (Swofford 1998). Node support was inferred using jackknifing (JK) with the optimal parameters as described by Müller (2005a). A total number of 10 000 JK replicates was performed using the TBR branch swapping algorithm with 36.788 % of characters deleted and one tree being held during each replicate.

Bayesian Inference (BI) was carried out using MrBayes 3.1 (Huelsenbeck & Ronquist 2001) based on the GTR+Γ+I model as evaluated using jModeltest (Posada 2008). Four simultaneous runs of Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses, each with four parallel chains, were performed for five million generations, saving one tree every 1000th generation, starting with a random tree. Other MCMC parameters were left with the program's default settings. The burn-in was determined using Tracer v1.5 (Rambaut & Drummond 2007) and set at generation 50 000; the remaining trees were summarised in a majority rule consensus tree. All trees were imported into the tree editor TreeGraph2 (Stöver & Müller 2010) for final layout.

3. Results

Success of amplification, sequencing and alignability — All regions were easily amplified and all PCR products were obtained for *psbA-trnH*, *trnQ-rps16, rpl16* and *trnK/matK;* the amplification of *trnS-G* failed only in *Browningia hertlingiana, Copiapoa coquimbana* and *Calymmanthium substerile*. Apart from these taxa, all sequences could be obtained and sequencing problems caused by frequent homo-polynucleotide stretches in all regions but *trnK/matK* could be solved by reads from the additional internal sequencing primers annealing to both strands. Sequencing was most laborious for the *trnS-G* region, where often four reads were necessary.

Alignment was straightforward for *trnK/matK, rpl16, psbA-trnH* and *trnQ-rps16*. The *trnS-G* spacer was more difficult to align due to high frequency of length mutations. Considering probable mechanisms leading to length mutations and following the alignment rules for rapidly evolving non-coding chloroplast DNA, all sequences could be aligned unambiguously except a part of the *trnS-G* spacer with satellite-like repeats, where homology assessment was not possible. The data matrices are available at TreeBase (www.treebase.org).

Sequence characteristics —The *Cactaceae trnK/matK* dataset comprised 2555 aligned characters, with individual sequences ranging from 2383 to 2484. Two poly-As and one poly-T, on average six nt per sequence (0.2 % of the total dataset), were excluded from the *trnK* intron as parts of uncertain homology. The final matrix contained 2539 aligned characters, of which 2101 were constant, 256 uninformative and 182 informative. The *trnK* intron and the *matK* gene provided c. 17 % variable and 7 % informative characters each. The addition of indels yielded further 52 characters, 13 of them informative.

The final concatenated dataset, consisting of the complete sequences of spacers, introns and the *matK* gene, comprised 7556 aligned characters with individual sequences ranging from 4321 to 6761 nt with an average length of 6264 nt per taxon. The detailed sequence characteristics are given in Table 2. In total, 16 regions of un-

Table 2. Sequence statistics of individual regions and the combined dataset for *Pfeiffera*.

	trnK intron	***matK***	***trnS-G* spacer**	***trnG* intron**	***rps3-rp/16* spacer**	***rpl16* intron**	***psbA-trnH***	***trnQ-rps16***	**combined**
Dataset including hotspots									
Position in the alignment	1–718 2253–2486	719–2252	2487–4474	4497–5216	5217–5368	5378–6540	6541–6951	6952–7556	1–7556
Aligned length	952	1534	1988	720	152	1163	411	605	7556
Length range	854–929	1521–1530	1021–1540	668–687	136–152	778–1121	231–358	208–556	4321–6761
Mean length (SD)	910 (4)	1528 (2)	1425 (119)	683 (4)	142 (4)	1000 (91)	336 (4)	350 (95)	6264 (616)
Length range of all hotspots	0	0	26–126	9–15	0	6–15	12–30 (23)	5–22	37–191
Mean length of all hotspots (SD)	0	0	88 (36)	10 (1)	0	11 (2)	23 (5)	14 (4)	147 (47)
% GC	33	32.9	34.6	32.2	26.6	28.3	24.1	35.5	31.3
Inversions	0	1	1	0	0	0	1	0	3
Dataset excluding hotspots									
Position in the alignment	1–679 2222–2452	680–2209	2441–4096	4097–4760	4761–4912	4913–6056	4761–4912	6409–6982	1–6982
Aligned length	919	1530	1718	664	152	1151	352	579	6982
Length range	811–896	1521–1530	980–1506	619–636	136–152	770–1108	194–328	203–543	4243–6592
Mean length (SD)	889 (4)	1528 (2)	1328 (118)	632 (4)	142 (4)	1000 (90)	312 (31)	337 (95)	6046 (572)
% variable characters	6.3	5.8	21.1	9	20	13.2	19.6	9.6	12.6
% informative characters	2.9	3	9.3	4	10	5.5	9	4.8	5.7
Number of coded indels	14	6	49	14	5	39	17	21	165

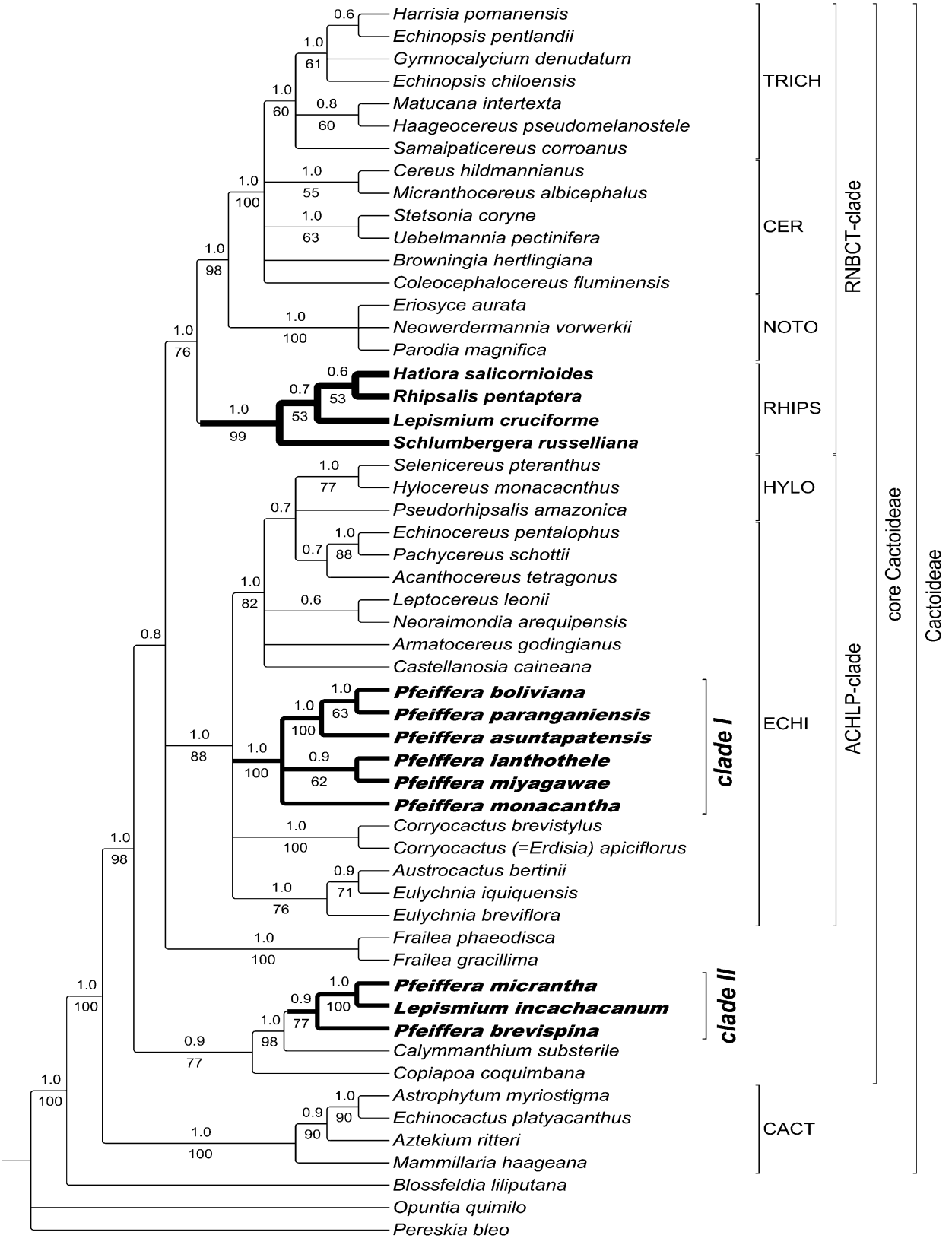

Fig. 1. Overview tree of the *Cactoideae* based on *trnK/matK* with coded indels. Tree topology as inferred from Bayesian Inference (50-majority-rule consensus tree). – Numbers above branches are Bayesian posterior probabilities, numbers below branches are jackknife support values from 10 000 replicates. Tree annotation above tribal level follows Nyffeler (2002), tribal classification follows the Hunt (2006). The clades containing species classified as *Pfeiffera* are highlighted in bold, the *Rhipsalideae* are highlighted in dark grey. Abbreviations indicating tribes: CACT: *Cacteae,* ECHI: *Echinocereae,* HYLO: *Hylocereae,* RHIPS: *Rhipsalideae,* NOTO: core *Notocacteae,* CER: *Cereeae,* TRICH: *Trichocereae*.

certain homology (mutational hotspots), the incomplete beginnings and endings as well as the exons were excluded (Table S1, online edition, Appendix 3). The mutational hotspots were homo-polynucleotide stretches and a satellite-like region in the *trnS-G* spacer. All hotspots taken together comprised on average c. 150 nt in length, ranging from 37 to 191 nt, which corresponds to approximately 2 % of the whole dataset. The largest hotspots were observed in the *trnS-G* spacer.

After exclusion of sequence parts of uncertain homology, 6982 aligned characters remained within the matrix, with an average length of 6046 nt. Thereof, 486 characters were parsimony-uninformative and 398 parsimony-informative. The addition of indels provided further 143 characters, of which 50 were informative. The *trnS-G* spacer provided the highest percentage of variable and informative characters, followed by *psbA-trnH* whereas the *trnK* intron and the *matK* gene were the two least variable regions. The highest length variation was observed in the *trnS-G* spacer, where 49 of the total 143 coded indels occurred, while *trnK/matK* and *psbA-trnH* showed least length mutations.

Inversions — Three inversions were observed. The sequence motif "GCTCTT" at positions 4268–4273 in the combined alignment in the *trnS-G* spacer is inverted to "AAGAGT" in *Pfeiffera ianthothele*. A second inversion occurred in the *psbA-trnH* spacer at positions 6880–6894 ("ACTTTTCATAATTAG" in *Lepismium cruciforme,* "CTAATTATGAATAGT" in other taxa). A four nt inversion with a motif of either "AAAA" / "TTTT" or "CAAA" / "TTTG" was observed within the *matK* gene, about 780 positions downstream from the start codon throughout the *Cactaceae* dataset.

Position and circumscription of *Pfeiffera* — The parsimony ratchet of the *trnK/matK Cactaceae* dataset with simple indel coding resulted in a strict consensus tree of 242 trees with 697 steps; CI: 0.792, RI: 0.833, RC: 0.660, HI: 0.208 (not shown). The topologies obtained from MP and BI did not differ considerably; the BI tree provides higher support values. The BI tree with additional JK support values is shown in Fig. 1.

Pfeiffera was not supported as monophyletic but split into two unrelated clades. Apart from the high statistical support, this branching order was supported by numerous indels in the dataset (Table S3, online edition, Appendix 3). The first clade, termed clade I in the following, was supported by 100 % JK, 1.00 Posterior Probability (PP) and comprised *P. boliviana* (Britton) D. R. Hunt, *P. paranganiensis* (Cárdenas) P. V. Heath, *P. asuntapatensis* (M. Kessler, Ibisch & Barthlott) Ralf Bauer, *P. miyagawae* Barthlott & Rauh*, P. monacantha* (Griseb.) P. V. Heath and *P. ianthothele.* This clade appeared isolated within the *Echinocereeae*/ACHLP clade. Clade II was supported by 77 % JK, 0.92 PP and comprised *P. micrantha* (Vaupel) P. V. Heath, *P. brevispina* D. R. Hunt and *Lepismium incachacanum* (Cárdenas) Barthlott. This clade was distant from *Pfeiffera* as depicted above and sister to *Calymmanthium substerile* (98 % JK, 1.00 PP) and *Copiapoa coquimbana* (77 JK, 0.93 PP). The grouping was isolated within the core *Cactoideae* in the parsimony tree and found to be sister to the rest of core *Cactoideae* in the BI tree.

Trees for *Pfeiffera* inferred from single markers — The trees inferred from single regions and the comparison of these, along with the number of variable and informative characters, are given in Fig. S1 and Table S4 (online edition, Appendix 3). The parsimony trees inferred from single markers were slightly incongruent and not fully resolved.

Relationships within *Pfeiffera* inferred from the combined dataset — A strict consensus of five trees was found by the parsimony ratchet without coded indels and only one shortest tree was found when indel characters were included (tree length: 1359, CI:0.845, RI:0.837, RC:0.708; tree not shown). Full resolution at species level with high or maximum support was obtained for all clades. The topologies obtained from MP and BI based only on substitutions, differed only in the resolution within *Pfeiffera paranganiensis* and *P. boliviana,* while MP and BI trees inferred from substitutions and indels were fully congruent, the Bayesian trees providing higher support values. Fig. 2 shows the Bayesian topology with additional JK support values.

Two supported subclades within clade I = *Pfeiffera* s.str. were found, the *P. ianthothele* clade, supported by 73 % JK, 1.00 PP and containing *P. ianthothele, P. monacantha* and *P. miyagawae,* and the *P. boliviana* clade, with 100 % JK, 1.00 PP and comprising *P. boliviana, P. asuntapatensis* and *P. paranganiensis*. The accessions of each species formed maximum supported clades, except *P. boliviana* and *P. paranganiensis,* which could not be separated by substitutions. Only after the addition of indels, the *P. paranganiensis* accessions formed a clade (51 % JK, 0.98 PP), whereas *P. boliviana* was still not found as monophyletic.

4. Discussion

Phylogenetic signal and mutational dynamics of the markers used — This study presents the largest plastid dataset generated for a genus of *Cactaceae* so far: approximately 7000 nt have been sequenced per sample. All markers showed low homoplasy levels; with Consistency Indices of 0.8 to 0.9 (Table S4). The single marker providing best species-level resolution was the *trnS-G* IGS or the combination of the *trnS-G* IGS and the *trnG* intron (Table S4). A large microsatellite-like region in the *trnS-G* IGS could further be suitable for population-level studies or species identification. High resolution was obtained from *rpl16* as well, whereas

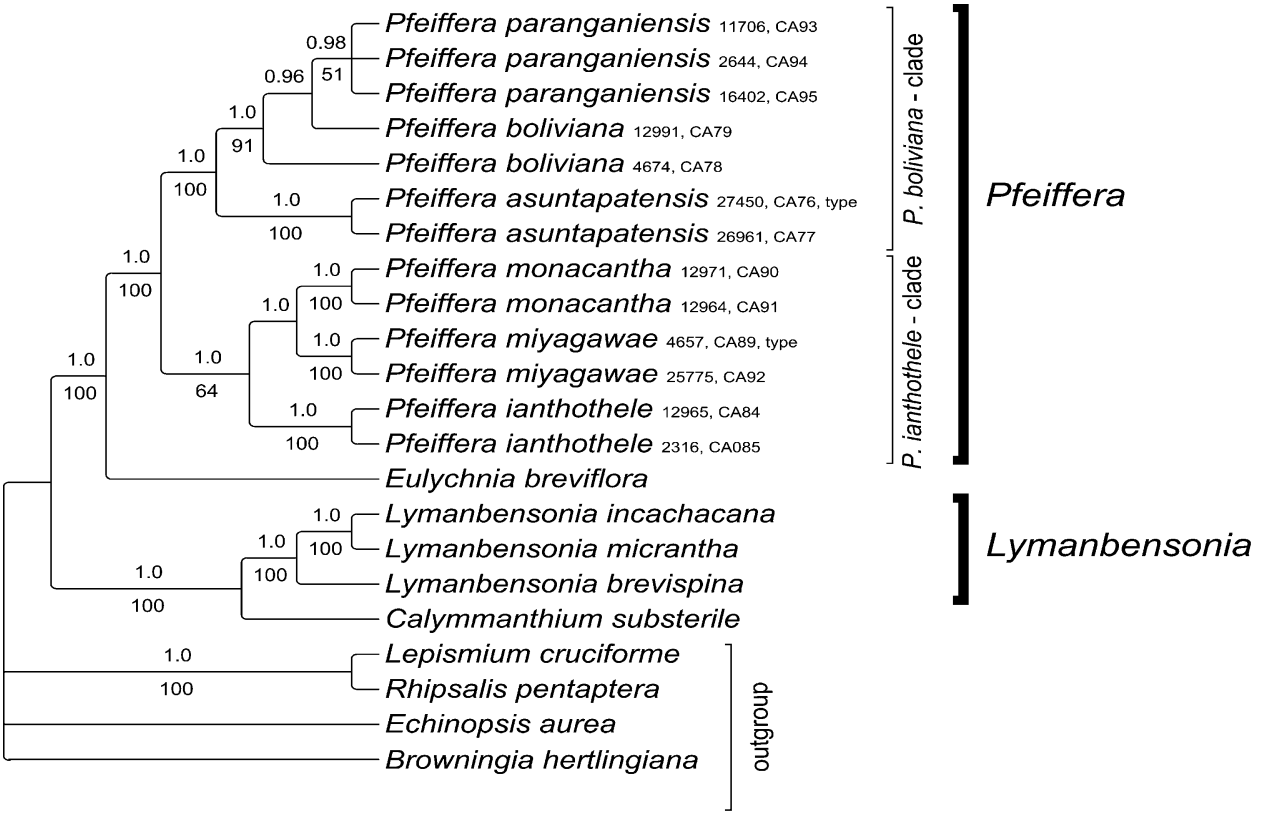

Fig. 2. Majority-rule consensus tree based on combined chloroplast dataset *(trnK/matK, trnS-G, rpl16, psbA-trnH, trnQ-rps16)* and coded indels showing relationships in *Pfeiffera.* Numbers above branches are Bayesian posterior probabilities, jackknife support values from 10 000 replicates are given below the branches. For each *Pfeiffera* sampled, the accession number from Bonn Botanical Gardens and the CA-isolate number are given next to the name.

psbA-trnH and *trnQ-rps16* yielded the lowest resolution. This is in line with earlier experiences with *psbA-trnH:* although it is frequently used in phylogenetics, several problems such as frequent indels and inversions and generally poor phylogenetic performance have been encountered (Borsch & Quandt 2009) along with usually long homo-polynucleotide stretches causing difficulties in sequencing (Devey & al. 2009). The *trnQ-rps16* spacer did not prove to be a highly effective species-level marker, contrary to the proposal of Shaw & al. (2007).

The inversion in the *matK* CDS was found to be homoplastic. An inferred secondary structure shows the inversion to affect only the terminal loop of a hairpin (not shown). Such hairpin-associated inversions have already been shown to switch between closely related species and even at population level (Quandt & al. 2003; Quandt & Stech 2004). A translation of the *matK* CDS reveals that only one amino acid is changed due to the inversion. Since *matK* is one of the fastest evolving genes in the plastid genome (Hilu & Liang 1997; Johnson & Soltis 1995), with a high proportion of substitutions even at the 1st and 2nd codon positions, changes in amino acids are relatively frequent.

Circumscription of *Pfeiffera* and reinstatement of *Lymanbensonia* — The current circumscription of *Pfeiffera* (Hunt 2006) was not confirmed. Instead, *Pfeiffera* was found to be polyphyletic and the clade containing *P. micrantha, P. brevispina* and *Lepismium incachacanum* is depicted as an entirely new lineage, distinct from the epiphytic tribes *Rhipsalideae* and *Hylocereeae,* as well as from *Pfeiffera* s.str., i.e. clade I, that contains the type species. Although the close relationship of the three species, as revealed by our data, was implied by authors who placed them either in *Rhipsalideae* or in *Pfeiffera,* such a position distant from all other epiphytic lineages has never been postulated and this clade is a new and unexpected finding. Since it contains *P. micrantha,* the type species of *Lymanbensonia,* a monotypic genus proposed by Kimnach (1984), we consider it appropriate to recognise this genus in an expanded circumscription. New combinations for these are provided below and, as a consequence, *Pfeiffera* will be restricted to six species: *P. ianthothele, P. monacantha, P. miyagawae, P. paranganiensis, P. boliviana* and *P. asuntapatensis*.

The putative closest relatives of *Pfeiffera* — *Pfeiffera* (in the restricted sense we propose) appears in the position already found by Nyffeler (2002), isolated within the *Echinocereeae*. The clade itself gets high support, but relationships within the *Echinocereeae* are not resolved and the tribe sensu Hunt (2006) is paraphyletic to the *Hy-*

locereeae. This placement distant from the *Rhipsalideae* and the putative close relationships to *Corryocactus* Britton & Rose confirms earlier assumptions about the affinities of *Pfeiffera*. Berger (1926) first suggested the monotypic *Pfeiffera* being an independent lineage "*Pfeifferae*". He justified his view by the branched funiculi, which differ from those of the *Rhipsalideae,* and the lack of adventitious roots. Berger thus first assumed the epiphytic habit of *Pfeiffera* and the morphological similarity to the *Rhipsalideae* to result from convergent evolution. He admitted that the closest relatives of *Pfeiffera* were not clear to him, but he suggested *Erdisia* Britton & Rose. The terrestrial genera *Corryocactus* and *Erdisia* (currently included in *Corryocactus* as the *C. squarrosus* group), shrubby slender-stemmed cacti from Peru, Bolivia and Chile, have subsequently been proposed as the nearest relatives of *Pfeiffera*, because of similarities in habit and flower morphology. Backeberg (1959, 1966) followed Berger's view and placed *Pfeiffera* as "Sippe *Pfeifferae*" within tribe *Cereeae* subtribe *Austrocereinae,* which mainly contained columnar ("cereoid") cacti. He believed *Erdisia* and *Corryocactus* to be closely related and suggested these genera to constitute a morphological "link" to *Pfeiffera,* while *Pfeiffera* itself would be "transitional" from the corryocactoid ancestors to *Acanthorhipsalis* and the *Rhipsalideae*. Contrary, Buxbaum (1962, 1971) regarded *Pfeiffera* as close to *Rhipsalis* and consequently placed it into *Hylocereeae* subtribe *Rhipsalinae,* which corresponds to its placement in the *Rhipsalideae* by preceding authors. Although Buxbaum had placed all epiphytes along with several terrestrial columnar cacti in one single tribe *Hylocereeae,* he could not propose any close relatives of the *Rhipsalinae* and assumed them to be isolated, while *Corryocactus* was placed within the *Leptocereeae* Buxb. In line with Berger's earlier views, Barthlott (1988) and Barthlott & Hunt (1993) suggested that the *Rhipsalideae* including *Pfeiffera* evolved from the terrestrial cacti similar to *Corryocactus* and *Erdisia,* these genera consequently being the next relatives. Hunt (2006) further suggested a close relationship of *Pfeiffera, Corryocactus*/*Erdisia* and probably also *Austrocactus* Britton & Rose and *Eulychnia* Phil.

Along with *Pfeiffera, Acanthorhipsalis* has been regarded as the most "ancestral" group within the *Rhipsalideae*. The first hypothesis on the origin of the epiphytic *Cactaceae* dates back to Ganong (1898), who developed ideas on *Cactaceae* phylogenetics derived from comparative studies of anatomy as well as seedling and embryo morphology. He illustrated his conclusions in a tree-like manner with the "trunk" of the tree representing the whole family and the "branches" showing relationships of the genera and their origin from one another. This illustration can be considered to be the first phylogenetic tree for the *Cactaceae* (Metzing & Kiesling 2008). It shows the epiphytes with *Pfeiffera* as the basal most lineage derived from columnar "cereoid" genera. Berger (1926) published the first true cladogram for the *Rhipsalideae,* which he assumed to consist of three main lineages with *Acanthorhipsalis* being the oldest and most ancestral genus within one of them. Buxbaum (1967) suggested that *Pfeiffera* and *Acanthorhipsalis* represent the ancestral morphological condition within the *Rhipsali(di)nae* and his scheme showed *Pfeiffera* as most basal followed by *Acanthorhipsalis*. Although Barthlott (1987) included *Pfeiffera* and *Acanthorhipsalis* in *Lepismium,* he also suggested the whole grouping to be sister to the other *Rhipsalideae*. Nevertheless, our data as well as the earlier results of Nyffeler (2002) undoubtedly suggest the exclusion of *Pfeiffera* and *Acanthorhipsalis* from *Lepismium* and the *Rhipsalideae*.

The placement of *Lymanbensonia* — In its revised circumscription, *Lymanbensonia,* along with the terrestrial genera *Copiapoa* Britton & Rose and *Calymmanthium,* is unexpectedly found to form the sister group of the core *Cactoideae* (0.85 PP). The apparently close relationship of *Copiapoa* and *Calymmanthium* has already been found by Nyffeler (2002), although unsupported and none of the *Lymanbensonia* species had been sampled. *Copiapoa* is a genus of globular to short-cylindric terrestrial cacti native to the coastal deserts of northern Chile. It has traditionally been a member of the *Notocacteae* Buxb., where it is still included and considered isolated (Hunt 2006). But the *Notocacteae* are polyphyletic (four lineages) and the closest relatives of *Copiapoa* have remained an open question since the study of Nyffeler (2002). *Calymmanthium* is a monotypic genus containing only *C. substerile* F. Ritter, an arborescent cactus native to Peru. Its affinities have been obscure and it has been placed along with other columnar cactus genera in the *Leptocereeae* (Buxbaum 1962) or *Browningieae* F. Buxb. (Barthlott & Hunt 1993). The first *rbcL* sequence data for *Cactaceae* showed *Calymmanthium* to be isolated within the subfamily *Cactoideae* (Wallace 1995; Wallace & Gibson 2002) and it was furthermore suggested to be the most basal member of *Cactoideae,* with columnar cacti being derived from a *Calymmanthium*-like ancestor (Wallace & Gibson 2002). A plesiomorphic state for the species of *Lymanbensonia* and *Pfeiffera* (as newly defined here) and *Calymmanthium* has been assumed by Wallace & Gibson (2002 [as *Lepismium*]) and these taxa were consequently placed in the *Echinocereeae* (Hunt 2006). However, our findings reveal a polyphyly of this tribe, since part of *Pfeiffera* and *Calymmanthium* have to be excluded.

Since *Calymmanthium* was considered isolated within *Cactoideae,* Wallace (in Hunt 1998) already suggested placing it in a separate tribe but this remained just a proposal and the tribal name has not been validated. Our results support the establishment of a new tribe which includes *Calymmanthium* and *Lymanbensonia* and we favour the name *Lymanbensonieae,* since *Lymanbensonia* is the larger genus; the tribal name is formally proposed below. Although the merging of both genera under the

Fig. 3. *Pfeiffera* – A: *P. paranganiensis* (*Ritter 343,* cult. ZSS); B: *P. miyagawae* (type collection *Miyagawa s.n.*, 1974, iso HEID 32857, cult. BG Bonn 4657); C–D: *P. ianthothele,* cult. BG Bonn 2316; C: fruits; D: flowering stems; E: *P. boliviana,* flower colour variation, left: BG Bonn 4675 *(Kimnach 2546)*, right: BG Bonn 4674 without locality data). – Photos: W. Barthlott.

older name *Calymmanthium* could also be a solution, *Calymmanthium* is morphologically so different that we suggest keeping it separate. A remaining question is whether *Copiapoa,* which, as already stated, is currently included in the *Notocacteae* but appears to be sister to the *Lymanbensonieae,* needs to be included in this tribe. But since *Copiapoa* is morphologically so different from *Calymmanthium* and *Lymanbensonia,* we hesitate to include it until there is more evidence for a close relationship.

Relationships within *Pfeiffera* — When Hunt (2006) transferred part of *Lepismium* to *Pfeiffera,* he did not adopt the subgeneric classification of Barthlott & Taylor (1995). *Lepismium* subg. *Pfeiffera* (Salm-Dyck) Barthlott, subg. *Acanthorhipsalis* (K. Schum) Barthlott and subg. *Lymanbensonia* (Kimnach) Barthlott were treated by Hunt (2006) as unranked infrageneric groups within *Pfeiffera*. Our analyses indicate these groups as polyphyletic: the *Lymanbensonia* group has to be excluded and expanded, the *Pfeiffera* group has additionally to include *P. monacantha,* while the *Acanthorhipsalis* group is highly polyphyletic, a part of it belonging in *Lymanbensonia*.

Our analyses find two clades within *Pfeiffera*. One, informally termed *P. boliviana* clade, includes *P. asuntapatensis, P. boliviana* and *P. paranganiensis* (100 % JK, 1 PP), which were part of the *Acanthorhipsalis* group. All species of the *P. boliviana* clade are endemic to Bolivia and can be characterised by flattened stems, usually without spines (except *P. paranganiensis*) and naked pericarpels and fruits. *P. boliviana* is found as sister to *P. paranganiensis,* and the two species have been regarded as sister species already by Barthlott & Taylor (1995). The two *P. boliviana* accessions sampled are resolved as distinct, indicating that this species might not be monophyletic. It is variable, especially in flower shape and colour (Fig. 3E), showing the highest colour variation within *Pfeiffera*. The need for further population-level

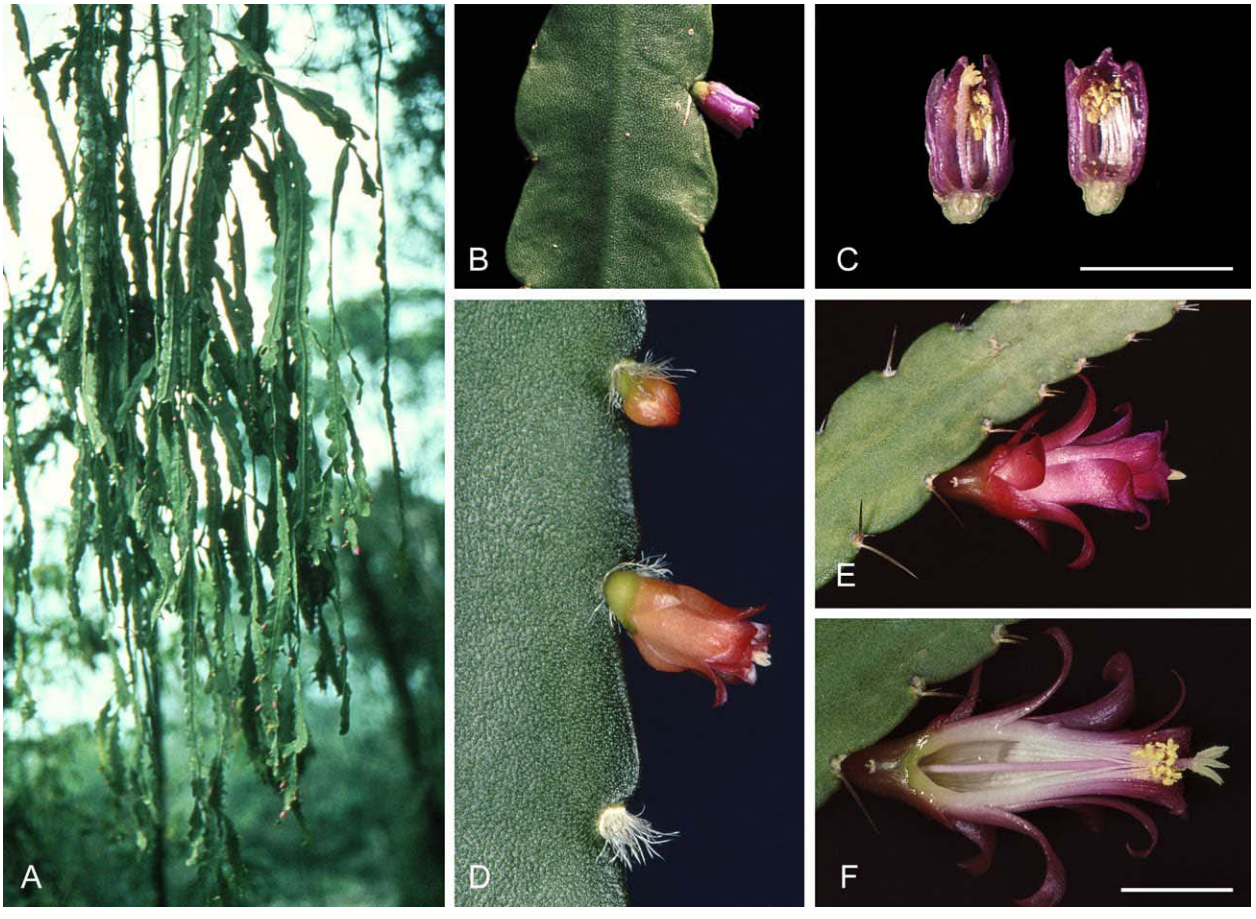

Fig. 4. *Lymanbensonia* – A–C: *L. crenata* (*Kirschnek s.n.* 1981, voucher BONN) – A: plant in habitat; B: flower; C: flower section; D: *L. incachacana* (*Miyagawa 2,* cult. BG Bonn 2639, without locality data, voucher BONN); E–F: *L. micrantha* (*Vargas s.n.,* voucher HNT, cult. BG Bonn 13602, ex UCBG 59.1196, ISI 1164.); E: flower; F: flower section showing the well developed receptacle tube. – Scale bars: C , F = 1 cm. – Photos A: E. Kirschnek, B–F: W. Barthlott.

and taxonomic studies has been pointed out by Ibisch & al. (2000).

The second *Pfeiffera* lineage is termed *P. ianthothele* clade and comprises two species distributed from southern Bolivia to northern Argentina (*P. ianthothele, P. monacantha*) and the Bolivian *P. miyagawae*. While the parsimony topology suggests *P. ianthothele* and *P. miyagawae* as sister species (low support), the BI topology finds *P. miyagawae* as sister to *P. monacantha* with high confidence. *P. miyagawae* and *P. ianthothele* share ribbed stems, well developed spines and spiny fruits and pericarpels; these had been the characteristics of *Pfeiffera* in the original sense. *P. monacantha* has mostly naked pericarpels and fruits but bristles are occasionally developed. Nevertheless, the close relationship of *P. monacantha* and *P. miyagawae* is probable and was indeed suggested following the discovery of *P. miyagawae* (Barthlott & Rauh 1987).

Relationships within *Lymanbensonia* — Our analyses find *Pfeiffera brevispina* as sister to *Lymanbensonia micrantha* (≡ *P. micrantha*), which provides the type of the generic name *Lymanbensonia,* and *Lepismium incachacanum* (97 % JK, 1.00 PP, Fig. 1; 100 % JK, 1.00 PP, Fig. 2). This position is plausible regarding the plant's morphology, since it has the largest body size within this grouping and white flowers, while the other two species have red or magenta flowers. *P. brevispina* was placed next to *Lepismium monacanthum* (≡*P. monacantha*) by Barthlott & Taylor (1995), while Kimnach (1984) suggested a close relationship to *Rhipsalis crenata* (≡*P. crenata*). The position of *Lepismium incachacanum* within this grouping is unexpected; it had not been transferred to *Pfeiffera* by Hunt (2006) but placed as sister to *Lepismium cruciforme,* following Barthlott & Taylor (1995). Both taxa share flattened stems with woolly flower-bearing areoles deeply sunken into the stems (Fig. 4D). The morphological similarities between the two species are indeed high, but evidently have to be regarded as convergences. Furthermore, *L. incachacanum* also differed within *Lepismium* by having orange to red flowers, while all other *Lepismium* species usually have white or whitish flowers (except *L. cruciforme,* which often has deep pink flowers). In contrast, the intensely red or magenta coloured flowers with a scarcely expanded perianth of *L. incachacana* make it fit well into *Lymanbensonia*. It is resolved next to *Lymanbensonia micrantha,* an easily recognisable

species with magenta flowers and a well developed receptacle tube (Fig. 4E–F). Barthlott & Taylor (1995) placed *Lepismium micranthum* within *L.* subg. *Lymanbensonia* (Kimnach) Barthlott as sister to *L. crenatum* (=*Pfeiffera crenata* sensu Hunt 2006), a species that is only known from few collections (Ibisch & al. 2000); the plant cultivated in the Bonn Botanical Gardens (Bolivia, near Corvico, *Kirschnek s.n.,* 1981, BONN, in spirit, Fig. 4A–C) was probably the only cultivated specimen worldwide. Unfortunately the plant did not survive and we could not sample it here. But the study of the plant's morphology, especially the floral morphology, convincingly supports its placement within *Lymanbensonia*.

Generic concepts and morphological characters of *Pfeiffera* and associated genera — *Pfeiffera* and its associated genera are a good example of changing generic concepts within *Cactaceae* as the result of a lack of consensus on the number of genera to be recognised and the characters on which they should be based.

Acanthorhipsalis was originally established by Schumann (1899) as a subgenus of *Rhipsalis,* including only *R. monacantha*. Britton & Rose (1923) raised many of Schumann's subgenera and sections to generic rank and recognised *Acanthorhipsalis* as a genus with *A. monacantha, A. crenata* and *A. micrantha* (which they chose as type, erroneously overlooking Schumann's type, *A. monacantha*). The main characters separating *Acanthorhipsalis* from *Rhipsalis* were the spiny areoles and the receptacle tube. Kimnach (1983) argued that *Acanthorhipsalis* should not be recognised as a genus because of intergrading characters with other *Rhipsalideae*. He consequently combined *Acanthorhipsalis, Lepismium* and *Pfeiffera* into a much expanded *Rhipsalis* but nevertheless proposed a new monotypic genus *Lymanbensonia* solely based on the prominent receptacle tube (Kimnach 1984). This view was not adopted. Barthlott (1987) aimed at establishing a new generic concept for *Lepismium,* which was significantly different from that of Backeberg (1959, 1966), and included *Lymanbensonia* along with *Acanthorhipsalis, Pfeiffera* and part of *Rhipsalis* in *Lepismium*. The genus in this new sense was considerably heterogeneous and defined by mesotonic branching, an often spiny and angled pericarpel, and often spiny stems. The main differential character to separate this redefined *Lepismium* was its mesotonic branching, contrasting with the acrotonic branching of the other *Rhipsalideae* genera.

After *Lepismium* had been shown to be polyphyletic (Nyffeler 2002) and a reinstatement of *Pfeiffera* was deemed necessary, Hunt (2006) transferred *Lepismium* subg. *Acanthorhipsalis,* subg. *Pfeiffera,* subg. *Lymanbensonia* and subg. *Houlletia* p.p. *(L. bolivianum* and *L. paranganiense)* in a newly circumscribed *Pfeiffera,* leaving *Lepismium* as a reduced and more uniform genus. This concept of *Pfeiffera* sensu Hunt with 10 species was again considerably different from those of preceding authors, who had accepted *Pfeiffera* as monotypic until the inclusion of *P. miyagawae*. The "cereoid" habit of *P. ianthothele* was now evidently not so unique as had been thought but shared by *P. miyagawae,* most likely representing the plesiomorphic condition within the genus.

Since our study has found part of *Pfeiffera* and *Lepismium* to be part of the unrelated *Lymanbensonia,* the morphological characters again need to be re-evaluated. Characters that were regarded as of common ancestry within *Lepismium,* including *Pfeiffera, Lymanbensonia* and *Acanthorhipsalis,* have to be interpreted as defining a distinct genus. The morphology of *Pfeiffera* and *Acanthorhipsalis* in comparison to the *Rhipsalideae* has evidently been misinterpreted.

There are characters shared by *Lepismium, Pfeiffera* and *Lymanbensonia,* such as mesotonic branching, indeterminate stem segments, lack of terminal composite areoles and lateral flowers. Flattened as well as angular stems occur in all three genera. Some *Lepismium* and *Pfeiffera* species are indeed very similar, but most *Lepismium* are so distinct that they can be recognised as such and not mixed up with any *Pfeiffera* or *Lymanbensonia*. Only *Lepismium lorentzianum* and *L. cruciforme* can be confused in the vegetative stage. Furthermore, there are several characters that do separate *Lymanbensonia, Pfeiffera* and *Lepismium,* as summarised in Table 3. The main differences are the habit and flower shape as well as the fruits. A further character, already pointed out by Berger (1926), are the branched and long-stalked funiculi of *Pfeiffera*. This was one of the main characters which led Berger to the conclusion that *Pfeiffera* does not belong to the *Rhipsalideae* and which has recently been pointed out again as a potential character to separate *Pfeiffera* from *Lepismium* (Nyffeler 2000). Although not yet studied in all *Pfeiffera* species, our examinations showed that three out of six species *(P. miyagawae, P. ianthothele* and *P. monacantha)* do have branched or at least stalked funiculi, whereas *Lymanbensonia* and the *Rhipsalideae* have funiculi with a short stalk. Furthermore, while some species of *Pfeiffera* are facultative epiphytes and some of *Lymanbensonia* grow as terrestrials, *Lepismium* species are obligate epiphytes or sometimes lithophytes, but never terrestrial. *Pfeiffera* and *Lymanbensonia* always have spines or at least dense bristles or wool, while stem spines are usually not developed in *Lepismium*. The flowers of *Lepismium* have a different shape and are mostly white or whitish (except *L. cruciforme*), while coloured flowers predominate in *Pfeiffera* and *Lymanbensonia*. *Lepismium* can be further characterised by the dark purple or red to almost black fruits and the naked fruit surface.

The similarities result from convergent morphological shifts, which seem to be always associated with epiphytism as summarised by Gibson & Nobel (1986) and Wallace & Gibson (2002). Flattened stems result from the reduction of ribs, spination is reduced to various degrees and the reduced ribs do not provide enough sup-

Table 3. Summary of main characters differentiating *Pfeiffera, Lymanbensonia* and *Lepismium.*

	Pfeiffera	***Lymanbensonia***	***Lepismium***
Life form	predominantly epiphytic	terrestrial or epiphytic	obligate epiphytic, rarely also epilithic
Habit	erect, shrubby, pendent	erect, shrubby, pendent	pendent
Branching	mesotonic	mesotonic	mesotonic
Stems	flattened or ribbed	predominantly flattened	terete, ribbed or flattened
Stem spination	mostly well developed	mostly well developed	usually not developed, only bristles or wool
Composite terminal areoles	absent	absent	absent
Flower position	lateral	lateral	lateral
Flower colour	intensely coloured (orange, yellowish) or white/whitish	intensely coloured, orange to red and deep magenta, white only in *L. brevispina*	white or whitish-cream, varies from white to yellow and pink in *L. cruciforme*
Flower shape	funnel-shaped, tepals fully expanded	narrowly campanulate, tepals not entirely expanded, spreading at the apex	flowers somewhat erumpent and pendent, campanulate, tepals expanded to c. 45° relatively to pericarpel (fully expanded only in *L. houlletianum*)
Pericarpel form	tuberculate or not tuberculate (= smooth); conical; angled	not tuberculate (= smooth); terete or conical; not conspicuously angled	not tuberculate (= smooth); conical or almost terete; mostly angled
Pericarpel spination	developed (or at least bristles), or pericarpel naked	not developed	not developed
Fruits	spiny, bristly or naked, translucent, veiny	naked, opaque, not veiny	usually naked or with hairs, opaque, not veiny
Fruit colour	orange-red, pinkish, whitish, olive-green, brownish	red-brown, white to pinkish, greenish	dark purple to black, red, brown
Distribution	eastern Andes of Bolivia to northern Argentina	southern Ecuador to southern Peru and eastern Andes of Bolivia	southeastern Brazil to northern Argentina and southern Bolivia

port for the plant, so pendent habit results. Reduction in flower size compared to that of terrestrial cacti and shifts to insect or bird pollination are also regarded as characteristics of all epiphytic cacti. Finally, all produce small berry-like fruits dispersed by birds. The convergent evolution of such a specialised life form as epiphytism makes the distinction difficult when only macro-morphological characters are regarded. The micro-morphology of seeds and pollen as well as anatomical characters might provide further informative characters.

Biogeographical patterns — *Pfeiffera, Lymanbensonia* and *Lepismium* have separate distribution areas. *Pfeiffera* is distributed from the eastern Andes of Bolivia to northern Argentina, while *Lymanbensonia* ranges from southern Ecuador (Loja) to southern Peru and the eastern Andes of Bolivia but does not reach northern Argentina. The sister taxon, *Calymmanthium substerile,* is endemic to the north of Peru and found sympatrically with *L. brevispina* (Kimnach 1984). *Lepismium,* together with other *Rhipsalideae* has its distribution centre in southeastern Brazil, but ranges to Paraguay, northern Argentina and eastern Andes of Bolivia. Neither *Pfeiffera* nor *Lymanbensonia* occur in Brazil, so it has to be assumed that the widely distributed *Lepismium* probably originated in southeastern Brazil with the other *Rhipsalideae* and reached the Andes later, whereas *Pfeiffera* and *Lymanbensonia* evolved in the Andes of Bolivia or Peru.

Conclusions and future work — Of all eight regions used, *trnK/matK, trnS-G* and *rpl16* have proved to be most effective, with the *trnS-G* spacer providing the highest number of variable and informative characters. These three regions seem especially promising for future applications for species-level studies within *Cactaceae*. In contrast, the *psbA-trnH* and *trnQ-rps16* spacers provided low resolution and support and produced inconsistent topologies. Only the concatenated dataset of *trnK/matK, trnS-G, rps3-rpl16*, *rpl16* intron, *trnQ-rps16* and *psbA-trnH* provided full resolution between all species in our study. Consequently, in order to resolve relationships between closely related species, combined datasets of several markers selected for their high phylogenetic structure are needed, as emphasised by Borsch & Quandt (2009) and Erixon & Oxelman (2008). Our results suggest that the *psbA-trnH* and *trnQ-rps16* spacers are not only outperformed by the

other markers in terms of phylogenetic structure but also in terms of providing significant amounts of characters to discriminate species. The rather low species discrimination power of *psbA-trnH* was observed in other studies, too, e.g. of *Fabaceae* (Edwards & al. 2008). Even if proposed as barcoding marker (Kress & al. 2005), the *psbA-trnH* spacer may not be an efficient region to sequence at all. Further studies are needed to test the relation between species discrimination power and phylogenetic structure of genomic regions in various taxa.

Molecular phylogenetic trees show that morphological convergences can be frequent in the *Cactaceae.* It is therefore not surprising that species of *Lymanbensonia, Acanthorhipsalis, Lepismium* and *Pfeiffera* have been regarded as closely related, since they are indeed morphologically similar. All share leaf-like flattened or angled stems, well-developed or reduced spines, woolly areoles, small coloured or whitish flowers and berry-like coloured fruits. Other shared characters, such as mesotonic branching or indetermined stem segments, are probably either plesiomorphic or homoplastic.

Generic classification based on single or few morphological characters consequently cannot predict actual relationships. For phylogenetic studies in the *Cactaceae,* the morphology-based taxonomic units consequently may be misleading to guide taxon sampling. The best solution therefore would be including all morphologically deviant groups and species in the given study.

Finally, our results provide evidence that epiphytism evolved more frequently in *Cactaceae* than hitherto assumed. There are in fact four geographically distinct lineages containing epiphytic species: the Mesoamerican *Hylocereeae,* the predominantly Brazilian *Rhipsalideae,* the Bolivian/Argentinean *Pfeiffera* and the newly found Peruvian/Bolivian *Lymanbensonia*. Terrestrial relatives of an epiphytic group of *Cactaceae* have been identified in the case of *Lymanbensonia,* while the closest relatives of *Pfeiffera,* the *Hylocereeae* and *Rhipsalideae,* are still not known with confidence and remain among the open questions in *Cactaceae* phylogenetics. Although *Corryocactus* incl. *Erdisia* and *Eulychnia* have been found putatively close to *Pfeiffera,* their exact position is unresolved and generic limits of *Corryocactus* need further evaluation. Future studies should aim at finding the next relatives and identifying morphological shifts and putative preadaptations for the evolution of the epiphytic habit, thus providing further insights into the evolution of epiphytism in the *Cactaceae*.

5. Taxonomic conclusions: new circumscriptions of *Pfeiffera (Echinocereeae)* and *Lymanbensonia (Lymanbensonieae)* with a key to their species

Echinocereeae (Britton & Rose) Buxb.

Members. — *Acanthocereus* Britton & Rose (1 sp.), *Armatocereus* Backeb. (7 spp. + 2 infraspec.), *Austrocactus* Britton & Rose (3 spp.), *Bergerocactus* Britton & Rose (1 sp.), *Carnegiea* Britton & Rose (1 sp.), *Castellanosia* Cárdenas (1 sp.), *Cephalocereus* Pfeiff. (3 sp.), *Corryocactus* Britton & Rose (12 sp.), *Dendrocereus* Britton & Rose (2 sp.), *Echinocereus* Engelm. (67 spp. + 39 infrasp. taxa), *Escontria* Rose (1 sp.), *Eulychnia* Phil. (4 spp. + 1 infrasp. taxa), *Jasminocereus* Britton & Rose (1 sp.), *Leptocereus* Britton & Rose (11 sp.), *Myrtillocactus* Console (4 spp.), *Neobuxbaumia* Backeb. (8 spp.), *Neoraimondia* Britton & Rose (2 sp.), *Pachycereus* Britton & Rose (13 spp.), *Peniocereus* Britton & Rose (20 sp.), *Pfeiffera* Salm-Dyck (6 spp.), *Polaskia* Backeb. (2 sp.), *Pseudoacanthocereus* F. Ritter (2 sp.), *Stenocereus* Riccob. (24 spp. + 1 infrasp. taxon), *Strophocactus* Britton & Rose (3 spp.)

Description. — Plants terrestrial or epiphytic *(Pfeiffera)* or scandent *(Strophocactus)*, treelike, shrubby or columnar, stems ribbed or winged, rarely flat. Flowers large or small, usually spiny or bristly, especially the pericarpel, the tube often short, perianth coloured or white.

Distribution and habitat. — Found in the Caribbean region, Mexico, southwestern USA, Brazil, Peru, Bolivia, Chile, western and southern Argentina.

Pfeiffera Salm-Dyck, Cact. Hort. Dyck. 1844: 40. 1845. – Type: *Pfeiffera cereiformis* Salm-Dyck, nom. illeg. ≡ *Cereus ianthothele* Monv.

= *Acanthorhipsalis* (K. Schum.) Britton & Rose, Cactaceae 4: 211. 1923 ≡ *Rhipsalis* subg. *Acanthorhipsalis* K. Schum., Gesamtbeschr. Kakt.: 615. 1898. – Type: *Rhipsalis monacantha* Griseb. [incorrectly designated by Britton & Rose, Cactaceae 4: 212. 1923 as *Acanthorhipsalis micrantha* (Vaupel) Britton & Rose].

Accepted species: 6 (+2 infraspec.)

Note. — The name *Acanthorhipsalis* can no longer be maintained for a potential subgenus because its type species *Pfeiffera monacantha* belongs to the same clade as *P. ianthothele*. *Acanthorhipsalis* therefore remains just a generic synonym. If subgenera are to be recognised for *Pfeiffera,* a new name would have to be found, but we suggest that subgenera are not needed for this small genus.

Etymology. — Named after Ludwig G. K. Pfeiffer (1805–1877), German physician and botanist.

Description. — *Life form* predominantly epiphytic, rarely epilithic or terrestrial; epiphytic habit mostly obligatory; facultative in *Pfeiffera paranganiensis;* data deficient for *P. miyagawae;* plants usually erect at first, then spreading, pendent; sometimes shrubby *(P. miyagawae). Adventitious roots* lacking, *branching* mesotonic. *Stems* 3–8 ribbed (mostly 3–4) or flattened; of indeterminate growth, old stem segments not deciduous. *Branch segments* narrowly oblong, cladode margins mostly crenate or crenulate. *Areoles* superficial, 1.5–4 cm apart, composite terminal

areoles absent, bristles and trichomes often present, areoles densely woolly in *P. asuntapatensis*. *Spines* usually well developed, whitish or yellowish, up to 10 per areole (usually 1–6). *Pericarpel* sharply differentiated from perianth, tuberculate (occasionally in *P. monacantha*) or not tuberculate (=smooth), cup-shaped (± conical); angled, spiny or at least with tiny bristly/woolly areoles, or naked. *Hypanthium* (receptacle tube) not developed. *Flowers* usually solitary, rarely 2 per areole, lateral, and also subterminal in *P. boliviana* and *P. miyagawae,* actinomorphic, funnel-shaped or broad-campanulate, mostly 1–2 cm in diameter; tepals fully expanding, white or intensely coloured (yellow, orange, red). *Funiculi* with long stalks, occasionally branched (examined in *P. ianthothele, P. miyagawae* and *P. monacantha*). *Stamens* numerous, c. 40–100, filaments and anthers white or whitish/cream. *Fruits* globose or subglobose, ± translucent, veiny, coloured (orange-red, pinkish, whitish, olive-green, brownish), spiny or naked.

Distribution and habitat. — Distributed from Bolivia (La Paz, Cochabamba, Santa Cruz, Chuquisaca and Tarija) to northern Argentina (Jujuy, Salta, and Tucumán); centred in the eastern Andes of Bolivia.

Key to the species of *Pfeiffera*

1. Branch segments 3–8 ribbed; stem spination well developed; pericarpel and fruits spiny or at least with bristles 2
– Branch segments flattened; stem spination usually inconspicuously developed; pericarpel and fruits naked 3
2. Flowers orange 4
– Flowers white . . . . . (3) *P. ianthothele*
3. Flowers intensely red-magenta to orange . . . . . (1) *P. asuntapatensis*
– Flowers yellowish, whitish or cream, not intensely red 5
4. Flowers large, c. 4 cm in diameter, intensely orange, shimmering, pericarpel with prominent, long, dark spines . . . . . (4) *P. miyagawae*
– Flowers smaller, c. 2 cm in diameter, waxy-orange, pericarpel naked or with few bristles . . . . . (5) *P. monacantha*
5. Stem pendulous, spines absent or weak; mature fruit globose, pale pinkish to whitish . . . . . 2. *P. boliviana*
– Stem erect at first, spines developed; mature fruit depressed-globose, angled, olive-brown . . . . . (6) *P. paranganiensis*

(1) *Pfeiffera asuntapatensis* (M. Kessler & al.) Ralf Bauer in Cactaceae Syst. Init. 20: 6. 2005 ≡ *Lepismium asuntapatense* M. Kessler & al. in Bradleya 18: 13–14. 2000. – Holotype: Bolivia, La Paz, Prov. J. Bautista Saavedra M. Pauji-Yuyo, between Apolo and Charazani, 1300 m, 6.6.1997, *Kessler 9800* (LPB; isotypes: GOET, K); cultivated at Bot. Gard. Bonn acc. 27450.

(2) *Pfeiffera boliviana* (Britton) D. R. Hunt in Cactaceae Syst. Init. 14: 18. 2002 ≡ *Hariota boliviana* Britton in Mem. Torrey Bot. Club 3(3): 40. 1893. – Lectotype (designated by Barthlott & Taylor 1995: 46): Bolivia, La Paz, 1890, *Bang 601* (K; isolectotype: US); lectoparatype: *Rusby 2048* (US, NY).

(3) *Pfeiffera ianthothele* (Monv.) F. A. C. Weber in Bois, Dict. Hort. 2: 944. 1898 ≡ *Pfeiffera cereiformis* Salm-Dyck, Cact. Hort. Dyck. 1844: 41. 1845, nom. illeg. ≡ *Cereus ianthothele* Monv., Hort. Universel 1: 218. 1839 [as *"Cereus ianthothelus"*]. – Holotype: 'Montevideo' cult. in Hort. Monville, not known to have been preserved; neotype (designated by Barthlott & Taylor 1995: 45): Argentina, Salta, 15.1.1929, *Venturi 8169* (K).

(4) *Pfeiffera miyagawae* Barthlott & Rauh in Cact. Succ. J. (Los Angeles) 59: 63–64. 1987. – Holotype: "Bolivia, Cochabamba, between Cochabamba and Santa Cruz, yungas of Alto Beni, near Mataral, 600 m", 19.10.1974, *Miyagawa s.n.* (HEID 32854; isotypes: BONN, HNT, ZSS); cultivated at Bot. Gard. Bonn acc. 4657.

Note. — This species had been long known only from the type collection, but the type locality as given in the first description has been suspected to be incorrect (Ibisch & al. 2000). It has been only recently re-collected in Bolivia, dept. La Paz, prov. Sud Yungas, south of La Asunta, 31.10.2003, 750 m, *Krahn 1044* (BONN), cult. Bot. Gart. Bonn, acc. 25775. It seems now very likely that the type collection was also made at the same locality near La Asunta, not near Mataral; further comments by Bauer (2005).

(5) *Pfeiffera monacantha* (Griseb.) P. V. Heath in Calyx 4: 158. 1994 ≡ *Rhipsalis monacantha* Griseb. in Abh. Königl. Ges. Wiss. Göttingen 24: 140. 1879. – Holotype: Argentina, Salta, San Andrés (west of San Ramón de la Nueva) Orán, 25.9.1873, *Lorentz & Hieronymus 453* (GOET; isotype: US 603291).

Key to the subspecies

1. Stem segments angled or flattened, spines 1–2 or more, pericarpel angled, often spiny . . . . . subsp. *monacantha*
– Stem segments flattened, spines absent, pericarpel not spiny . . . . . subsp. *kimnachii*

subsp. ***kimnachii*** (Doweld) Ralf Bauer in Cactaceae Syst. Init. 19: 8. 2005 ≡ *Acanthorhipsalis monacantha* subsp. *kimnachii* Doweld in Sukkulenty 4(1–2): 41. 2002 ≡ *Rhipsalis monacantha* var. *espinosa* Kimnach in Cact. Succ. J. (Los Angeles) 67(1): 38. 1995. – Holotype: Bolivia, dept. Cochabamba, road from Cochabamba-Chapare highway to Tablas, 1974, *Aguilar s.n.* in *Kimnach 2757,* cult. Huntington Bot. Gard. 51587 (HNT; isotypes: HEID, US).

(6) *Pfeiffera paranganiensis* (Cárdenas) P. V. Heath in Calyx 4: 158 [as *"paraganiensis"*]. 1994 ≡ *Acanthorhipsalis paranganiensis* Cárdenas in Cactus (Paris) 34: 126. 1952. – Holotype: Bolivia, Cochabamba, Ayapaya, Parangani, 10.1947, *Cárdenas 4856* (LIL 531577; isotype US).

Lymanbensonieae N. Korotkova & Barthlott, **trib. nov.** [– *Calymmanthieae* Lakomski in Swiat Kakt. 38(1–2): 66. 2003, nom. inval., without Latin diagnosis (ICBN Art. 36.1)]. – Type: *Lymanbensonia* Kimnach

Plantae aut epiphyticae pendulae caulibus foliaceis vel terrestres erectae caulibus ascendentibus *(Lymanbensonia)* aut plantae fruticosae erectae caulibus columnaribus usque ad 8 m altae *(Calymmanthium)*. Flores rubro-roseae vel albae, pericarpelli non spinosi. Habitat in Bolivia et Peru usque ad Equadoriam australem.

Description. — Plants epiphytic, pendent with leaf-like flattened stems or terrestrial, erect *(Lymanbensonia)* or shrubby, erect columnar plants up to 8 meters high *(Calymmanthium)*. Flowers mostly pink to red or white, pericarpels not spiny. Occurring in Bolivia, Peru, extending to southern Ecuador.

Members. — *Calymmanthium* F. Ritter (1 sp.), *Lymanbensonia* Kimnach (4 spp.).

Lymanbensonia Kimnach in Cact. Succ. J. (Los Angeles) 56: 101. 1984. – Type: *Lymanbensonia micrantha* (Vaupel) Kimnach

= *Acanthorhipsalis* Kimnach in Cact. Succ. J. (Los Angeles) 55: 179. 1983, nom. illeg.

Accepted species: 4

Note. — In his revision of *Acanthorhipsalis* (K. Schum.) Britton & Rose, Kimnach (1983) excluded all species from the genus but *A. micrantha*. Noticing that by excluding the type species *A. monacantha* he had created an illegitimate homonym, he afterwards proposed a new genus *Lymanbensonia* for *A. micrantha* (Kimnach 1984).

Etymology. — Named after Lyman Benson (1903–1993), American botanist.

Description. — Life form predominantly terrestrial or obligatorily or facultatively epiphytic; plants usually erect at first, then spreading, pendent. *Adventitious roots* lacking. *Branching* mesotonic. *Stems* flattened, angled at first in *L. micrantha,* of indeterminate growth, old stem segments not deciduous. *Branch* segments narrowly oblong (broadly oblong in *L. incachacana*); cladode margins crenate or crenulate. *Areoles* superficial (sunken in *L. incachacana*), composite terminal areoles absent, bristles and trichomes often present. *Spines* usually well developed, yellowish-whitish or grey, 1–10 per areole. *Pericarpel* ± sharply differentiated from perianth, not tuberculate (= smooth); terete or cup-shaped, not conspicuously angled, not spiny. *Flowers* usually solitary, rarely 2 per areole, lateral, actinomorphic, 1.2–3 cm long, narrowly tubular bell-shaped; tepals not fully expanding, spreading at apices, intensely coloured (red, pink, orange, magenta) or white in *L. brevispina*. *Hypanthium* (receptacle tube) not conspicuously developed, except in *L. micrantha*. *Stamens* c. 20–50, filaments and anthers white or whitish/cream. Fruits globose or subglobose, red-brown, white to pinkish, greenish, opaque, naked. *Funiculi* simple, with short stalk (examined so far only in *L. micrantha*).

Distribution and habitat. — Ranges from southern Ecuador (Loja) to central and southern Peru (Amazonas, Junín, Puno) and the eastern Andes of Bolivia (La Paz; Cochabamba, Santa Cruz).

Key to the species of *Lymanbensonia*

1. Flower-bearing areoles and pericarpel deeply sunken into the stem, areoles with dense tufts of bristles and wool . . . . . (3) *L. incachacana*
– Flower-bearing areoles not deeply sunken, areoles not densely woolly 2
2. Flowers white . . . . . (1) *L. brevispina*
– Flowers orange, pink or magenta 3
3. Flowers 3–4 cm long, receptacle tube well developed . . . . . (4) *L. micrantha*
– Flowers smaller, receptacle tube not developed . . . . . (2) *L. crenata*

(1) *Lymanbensonia brevispina* (Barthlott) Barthlott & N. Korotkova, **comb. nov.**

≡ *Lepismium brevispinum* Barthlott in Bradleya 5: 99. 1987 [– *Acanthorhipsalis brevispina* F. Ritter, Kakteen Südamerika 4: 1260. 1981, nom. inval.]. – Holotype: [icon] F. Ritter, Kakteen Südamerika 4: 1529, fig. 1114.

= *Pfeiffera brevispina* D. R. Hunt in Cactaceae Syst. Init. 14: 18. Oct 2002 ≡ *Acanthorhipsalis brevispina* Ritter ex Doweld in Sukkulenty 4(1–2): 34. late 2002/ early 2003 ["2001"], nom. illeg. [– *Acanthorhipsalis brevispina* F. Ritter, Kakteen Südamerika 4: 1260. 1981, nom. inval.]. – Holotype: Peru, Amazonas, east of Balsas, *Ritter 1419* (U).

= *Rhipsalis riocampanensis* Madsen & Z. Aguirre in Nordic J. Bot. 23: 26–29. 2004.

Note. — The nomenclature of this species is complicated. When F. Ritter first described it as *Acanthorhipsalis brevispina* F. Ritter, he deposited a type specimen at U, but did not cite it in the protologue. The name hence is invalid (ICBN Art. 37.1, McNeill & al. 2006). Barthlott (1987) intended to validate the name for this taxon when transferring it to *Lepismium,* designating Ritter's illustration as the type, not the specimen. As an illustration was at that time not permitted as type, the name *L.*

brevispinum Barthlott had been invalid when first published in 1987 but became valid after a change in ICBN Art. 37.4 (McNeill & al. 2006). Prior to that, Hunt (in Hunt & Taylor 2002) provided a valid name for Ritter's taxon under *Pfeiffera* as *P. brevispina,* based on the original Ritter specimen. The earlier combinations *Rhipsalis brevispina* (F. Ritter) Kimnach in Cact. Succ. J. (Los Angeles) 55(4): 181. 1983 and *Pfeiffera brevispina* (F. Ritter) P. V. Heath in Calyx 4: 158. 1994 are both invalid, because they were based on Ritter's invalid name. Independently, Ritter's original name *Acanthorhipsalis brevispina* was validated by Doweld, but, as currently known, published later than Hunt's name (Hunt 2003: 3; Eggli & Zappi 2003: 10); this renders Doweld's name illegitimate. As the name *P brevispina* D. R. Hunt is not based on the same type as *Lepismium brevispinum,* it constitutes a new name and not a transfer of the latter. Consequently, *L. brevispinum* Barthlott as the older name has priority over *P. brevispina* D. R. Hunt and the latter is the correct name of this taxon only in *Pfeiffera,* because a transfer of *L. brevispinum* to *Pfeiffera* is blocked due to the identical epithet.

(2) *Lymanbensonia crenata* (Britton) Doweld in Sukkulenty 4: 34. Dec 2002/early 2003 ["2001"] ≡ *Hariota crenata* Britton in Bull. Torrey Bot. Club 18: 35. 1891. – Holotype: Bolivia, La Paz, Yungas, 1885, *Rusby 2047* (US).

(3) *Lymanbensonia incachacana* (Cárdenas) Barthlott & N. Korotkova, **comb. nov.** ≡ *Rhipsalis incachacana* Cárdenas in Cactus (Paris) 34: 125. 1952. – Holotype: Bolivia, Cochabamba, Incachaca, 6.1950, *Cárdenas 4855* (LIL 511565).

(4) *Lymanbensonia micrantha* (Vaupel) Kimnach in Cact. Succ. J. (Los Angeles) 56: 101. 1984 ≡ *Pfeiffera micrantha* (Vaupel) P. V. Heath in Calyx 4: 158. 1994 ≡ *Acanthorhipsalis micrantha* (Vaupel) Britton & Rose, Cactaceae 4: 211. 1923 ≡ *Cereus micranthus* Vaupel in Bot. Jahrb. Syst. 50 (Beibl. 111): 19. 1913. – Holotype: Peru, Puno, near Sandía, 31.7.1902, *Weberbauer 1353* (B, destroyed; isotype: US).

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Appendix 1. Plant material used in this study

Samples obtained from living collections (mainly from the Bonn Botanical Gardens) first list the garden accession number and then country and locality data in square brackets, collector and collection number in italics and the herbarium abbreviation in parentheses. In the case of collections originally made in the field, the plants sampled from cultivation represent the same material and the voucher cited refers to the original field collection. Further vouchers have been made in the course of this study and will be deposited in B. Each sample has a unique DNA isolate code (CA-XXX), given after the voucher information. For sequences generated from other material than the isolates listed here, the respective publication is indicated. Tribal classification and accepted species names follow Hunt (2006), except for *Pfeiffera, Lymanbensonieae* and *Lymanbensonia*.

Outgroups (*Cactaceae* dataset): ***Opuntia quimilo*** K. Schum., [Argentina], *Leuenberger 3558* (B 159-94-86-10), *trnK/matK* AY015279 (Nyffeler 2002). ***Pereskia bleo*** (Kunth) DC., BGBM 277-01-88-80; *Schwerdtfeger 12678* (B-Gartenherbar), *trnK/matK* AY875359 (Edwards & al. 2005).

Cacteae: Astrophytum myriostigma Lem., [Mexico], *Brack 264* (ZSS 19865), *trnK/matK* AY015288 (Nyffeler 2002). ***Aztekium ritteri*** (Böd.) Böd., [Mexico], *Anderson 1684* (ZSS 862607), *trnK/matK* AY015290 (Nyffeler 2002). ***Echinocactus platyacanthus*** Link & Otto, [hort. ZSS, without locality data] (ZSS 921686), *trnK/matK* AY015287 (Nyffeler 2002). ***Mammillaria haageana*** Pfeiff., [hort. ZSS, without locality data], (ZSS 941125), *trnK/matK* AY015289 (Nyffeler 2002).

Lymanbensonieae: Calymmanthium substerile F. Ritter, ZSS 893442 [hort. ZSS, without locality data] presumably *F. Ritter collection* from c. 1960 (no voucher), CA133, *trnS-G –*, *trnK/matK* AY015291 (Nyffeler 2002), *rpl16* FN673676 (this study), *psbA-trnH* FN669004 (this study), *trnQ-rps16* FN677924 (this study). ***Lymanbensonia brevispina*** (D. R. Hunt) Barthlott & N. Korotkova, [Peru, Prov. Amazonas, east of Balsas] *Charles GC1065.02* (photo voucher), CA131, *trnS-G* FR716737 (this study), *trnK/matK* FR716759 (this study), *rpl16* FR716770 (this study), *psbA-trnH* FR716780 (this study), *trnQ-rps16* FR716790 (this study). ***L. incachacana*** (Cárdenas) Barthlott & N. Korotkova, BG Bonn 2639 [Bolivia, Prov. Sud-Yungas] *Miyagawa 2* (BONN, photos), CA086, *trnS-G* FR716738 (this study), *trnK/matK* FN669728 (this study), *rpl16* FN673634 (this study), *psbA-trnH* FN669038 (this study), *trnQ-rps16* FN677881 (this study). ***L. micrantha*** (Vaupel) Kimnach, BG Bonn 13602 ex UCBG 59.1196, ISI 1164 [Peru, Dept. Puno, near Sándia], *Vargas s.n.* (HNT, B), CA073, *trnS-G* FR716739 (this study), *trnK/matK* FN669722 (this study), *rpl16* FN673628 (this study), *psbA-trnH* FN669039 (this study), *trnQ-rps16* FN677877 (this study).

Cereeae: Browningia hertlingiana (Backeb.) Buxb, [Peru], Knize 334 (ZSS 19869), *trnK/matK* AY015315 (Nyffeler 2002); [BG Bonn 2416 ex. ZSS, without locality data], no voucher, CA001, *trnS-G –*, *rpl16* FN673555 (this study), *psbA-trnH* FN995427 (this study), *trnQ-rps16* FN677806 (this study). ***Cereus hildmannianus*** Pfeiff., [Brazil], *Eggli & al. 2493* (ZSS 941313), *trnK/matK* AY015313 (Nyffeler 2002). ***Colecephalocereus fluminensis*** (Miquel) Backeb., [Brazil], *Supthut 8893* (ZSS 881544), *trnK/matK* AY015318 (Nyffeler 2002). ***Micranthocereus albicephalus*** (Buining & Brederoo) F. Ritter, [Brazil], *Taylor & al. 1490a* (ZSS 911583), *trnK/matK* AY015314 (Nyffeler 2002). ***Stetsonia coryne*** (Förster) Britton & Rose [Argentina], *Leuenberger & Eggli 4361* (ZSS 941689), *trnK/matK* AY015320 (Nyffeler 2002). ***Uebelmannia pectinifera*** Buining, [Brazil], *Horst & Uebelmann 550* (ZSS 874114), *trnK/matK* AY015319 (Nyffeler 2002).

Echinocereeae: Acanthocereus tetragonus (L.) Hummelink, [Mexico], *Escalante s.n.* (ZSS 892219), *trnK/matK* AY015295 (Nyffeler 2002). ***Armatocereus godingianus*** (Britton & Rose) Backeb., [Ecuador] *Supthut 89103* (ZSS 901109), *trnK/matK* AY015296 (Nyffeler 2002). ***Austrocactus bertinii*** (Herincq) Britton & Rose, [Argentina] *Nyffeler & Eggli 352* (ZSS 961153), *trnK/matK* AY015300 (Nyffeler 2002). ***Castellanosia caineana*** Cárdenas, [Bolivia] *Ritter 843* (B 31606), *trnK/matK* AY015298 (Nyffeler 2002). ***Corryocactus apiciflorus*** (Vaupel) Hutchison, [hort. ZSS, without locality data] (ZSS 19926), *trnK/matK* AY015303 (Nyffeler 2002). ***C. brevistylus*** (K. Schum.) Britton & Rose, [Chile], *Eggli 2748a* (B 122-23-97-10), *trnK/matK* AY015302 (Nyffeler 2002). ***Echinocereus pentalophus*** (DC.) Lem., [Mexico], *Donikyan 91/109* (ZSS 912367), *trnK/matK* AY015307 (Nyffeler 2002). ***Eulychnia breviflora*** Phil., BG Bonn 26764 [without locality data] (no voucher), CA137, *trnS-G* FR716740 (this study), *trnK/matK* FN669772 (this study), *rpl16* FN673680 (this study), *psbA-trnH* FN669003 (this study), *trnQ-rps16* FN677928 (this study). ***E. iquiquensis*** (K. Schum.) Britton & Rose, [Chile], *Eggli 2887* (ZSS 18409), *trnK/matK* AY015301 (Nyffeler 2002). ***Leptocereus leonii*** Britton & Rose [Cuba], *Areces s.n.* (ZSS 931856), *trnK/matK* AY015297 (Nyffeler 2002). ***Neoraimondia arequipensis*** (Meyen) Backeb., [Peru], *Ostolaza 94966* (ZSS 19861), *trnK/matK* AY015299 (Nyffeler 2002). ***Pachycereus schottii*** (Engelm.) D. R. Hunt, [hort. MG, without locality data] (ZSS 19859), *trnK/matK* AY015309 (Nyffeler 2002). ***Pfeiffera asuntapatensis*** (M. Kessler & al.) Ralf Bauer, BG Bonn 27450 [Bolivia, La Paz] *Kessler 9800* (holo: LPB, iso: GOET, K), CA076, *trnS-G* FR716742 (this study), *trnK/matK* FR716760 (this study), *rpl16* FR716771 (this study), *psbA-trnH* FR716781 (this

study), *trnQ-rps16* FR716791 (this study); BG Bonn 26961 [Bolivia, La Paz], *Krahn 970* (B), CA077, *trnS-G* FR716741 (this study), *trnK/matK* FR716761 (this study), *rpl16* FR716772 (this study), *psbA-trnH* FR716782 (this study), *trnQ-rps16* FR716792 (this study). ***P. boliviana*** (Britton) D. R. Hunt, BG Bonn 4674 [without locality data] (B), CA078, *trnS-G* FR716743 (this study), *trnK/matK* FR716762 (this study), *rpl16* FR716773 (this study), *psbA-trnH* FR716783 (this study), *trnQ-rps16* FR716793 (this study); BG Bonn 12991 [Bolivia, Santa Cruz] *Ibisch 93.438* (B), CA079, *trnS-G* FR716744 (this study), *trnK/matK* FR716763 (this study), *rpl16* FR716774 (this study), *psbA-trnH* FR716784 (this study), *trnQ-rps16* FR716794 (this study). ***P. ianthothele*** (Monv.) F. A. C. Weber, BG Bonn 12965 [Bolivia, Santa Cruz] *C. & P. Ibisch 93.884* (LPB, FR), CA084, *trnS-G* FR716748 (this study), *trnK/matK* FR716764 (this study), *rpl16* FR716775 (this study), *psbA-trnH* FR716785 (this study), *trnQ-rps16* FR716795 (this study); BG Bonn 2316 [without locality data] (B), CA085, *trnS-G* FR716749 (this study), *trnK/matK* FR716765 (this study), *rpl16* FR716776 (this study), *psbA-trnH* FR716786 (this study), *trnQ-rps16* FR716796 (this study). ***P. miyagawae*** Barthlott & Rauh, BG Bonn 4657 [locality given as "Bolivia, Cochabamba; near Mataral" is incorrect] *Miyagawa 1974 s.n.* (HEID 32857 holo: BONN, ZSS, HNT iso), CA089, *trnS-G* FR716750 (this study), *trnK/matK* FN669731 (this study), *rpl16* FN673637(this study), *psbA-trnH* FN995429 (this study), *trnQ-rps16* FN677885 (this study); BG Bonn 25775 [Bolivia, La Paz, prov. Sud Yungas] Krahn 1044 (B, BONN), CA092, *trnS-G* FR716751 (this study), *trnK/matK* FN669734 (this study), *rpl16* FN673640(this study), *psbA-trnH* FN995432 (this study), *trnQ-rps16* FN677888 (this *study).* ***P. monacantha*** (Griseb.) P. V. Heath, BG Bonn 12971 [Bolivia, Dept. Tarija,] *C. & P. Ibisch 93.1228* (FR), CA090, *trnS-G* FR716752 (this study), *trnK/matK* FN669732 (this study), *rpl16* FN673638 (this study), *psbA-trnH* FN995430 (this study), *trnQ-rps16* FN677886 (this study); BG Bonn 12964 [Bolivia, Santa Cruz] *C. & P. Ibisch 93.874* (BOLV, LPB, FR), CA091, *trnS-G* FR716753 (this study), *trnK/matK* FN669733 (this study), *rpl16* FN673639 (this study), *psbA-trnH* FN995431 (this study), *trnQ-rps16* FN677887 (this study). ***P. paranganiensis*** (Cárdenas) P. V. Heath, BG Bonn 11706 [Bolivia, between Morochata and Parangani] *Augustin s.n.* (B), CA093, *trnS-G* FR716754 (this study), *trnK/matK* FR716767 (this study), *rpl16* FR716777 (this study), *psbA-trnH* FR716787 (this study), *trnQ-rps16* FR716797 (this study); BG Bonn 2644 [Bolivia, La Paz, Lambate] *Miyagawa 7* (B), CA094, *trnS-G* FR716755 (this study), *trnK/matK* FR716768 (this study), *rpl16* FR716778 (this study), *psbA-trnH* FR716788 (this study), *trnQ-rps16* FR716798 (this study); BG Bonn 16402 ex HBG 15931, UCBG 56.1257, ISI 1102 [Bolivia, La Paz, Prov. Inquisivi] *Cárdenas s.n.* (HNT), CA095, *trnS-G* FR716756 (this study), *trnK/matK* FR716769 (this study), *rpl16* FR716779 (this study), *psbA-trnH* FR716789 (this study), *trnQ-rps16* FR716799 (this study).

Hylocereeae: Hylocereus monacanthus (Lem.) Britton & Rose, [Peru], *Rauh 35393* (ZSS 912367), *trnK/matK* AY015310 (Nyffeler 2002). ***Pseudorhipsalis amazonica*** (K. Schum.) Ralf Bauer, [Venezuela], *Supthut 8750* (ZSS 874339), *trnK/matK* AY015312 (Nyffeler 2002). ***Selenicereus pteranthus*** Britton & Rose, [Cuba], *Rauh 70036* (ZSS 891255), *trnK/matK* AY015311 (Nyffeler 2002).

Notocacteae: Parodia magnifica (F. Ritter) F. H. Brandt, hort. MG [without locality data], (ZSS 19873), *trnK/matK* AY015332 (Nyffeler 2002). ***Eriosyce aurata*** (Pfeiff.) Backeb., hort. Z [without locality data] (ZSS 19925), *trnK/matK* AY015336 (Nyffeler 2002). ***Neowerdermannia vorwerkii*** (Fric) Backeb., [Argentina], *Leuenberger & Eggli 4549* (ZSS 18843), *trnK/matK* AY015340 (Nyffeler 2002).

Rhipsalideae: Hatiora salicornioides (Haw.) Britton & Rose, BG Bonn 4637 [without locality data] (B), CA048, *trnS-G* --, *trnK/matK* FN669698 (this study), *rpl16* FN673603 (this study), *psbA-trnH* FN669030 (this study), *trnQ-rps16* FN677853 (this study). ***Lepismium cruciforme*** (Vell.) Miq., BG Bonn 5760 [Brazil, Paraná] W. Barthlott 90-27 (B), CA010, *trnS-G* FR716747 (this study), *trnK/matK* FN669662 (this study), *rpl16* FN673565 (this study), *psbA-trnH* FN669012 (this study), *trnQ-rps16* FN677815 (this study). ***Rhipsalis pentaptera*** A. Dietr., BG Bonn 4517 [without locality data] (B), CA065, *trnS-G* FR716757 (this study), *trnK/matK* FN669714 (this study), *rpl16* FN673620 (this study), *psbA-trnH* FN669103 (this study), *trnQ-rps16* FN677869 (this study). ***Schlumbergera russelliana*** (Hook.) Britton & Rose, BG Bonn 2636 [Brazil, close to Teresopolis] *Ehlers s.n.* (BONN, photos), CA036, *trnS-G* FR716758 (this study), *trnK/matK* FN669686 (this study), *rpl16* FN673591(this study), *psbA-trnH* FN669021 (this study), *trnQ-rps16* FN677841 (this study).

Trichocereeae: Echinopsis aurea Britton & Rose, BG Bonn 24068 [without locality data] (no voucher), CA104, *trnS-G* FR716745 (this study), *trnK/matK* FN669743 (this study), *rpl16* FN673649 (this study), *psbA-trnH* FN669005 (this study), *trnQ-rps16* FN995670 (this study). ***E. chiloensi***s (Colla) Friedrich & G. D. Rowley, [Chile], KG17-87 (ZSS 19874), *trnK/matK* AY015322 (Nyffeler 2002). ***E. pentlandii*** (Hook.) A. Dietrich, [hort. MG, without locality data], (ZSS 19858), *trnK/matK* AY015323 (Nyffeler 2002). ***Gymnocalycium denudatum*** (Link & Otto) Mittler, [hort. MG, without locality data], (ZSS 19870), *trnK/matK* AY015317 (Nyffeler 2002). ***Haageocereus pseudomelanostele*** (Werderm. & Backeb.) Backeb., [hort. MG, without locality data] (ZSS

19862), *trnK/matK* AY015329 (Nyffeler 2002). ***Harrisia pomanensis*** (F. A. C. Weber) Britton & Rose, [Argentina], *Leuenberger & Eggli 4710* (ZSS 18994), *trnK/matK* AY015324 (Nyffeler 2002). ***Matucana intertexta*** F. Ritter, [Peru], Knize 1153 (ZSS 751672), *trnK/matK* AY015327 (Nyffeler 2002). ***Samaipaticereus corroanus*** Cárdenas, [hort. ZSS, without locality data] (ZSS 903741), *trnK/matK* AY015321 (Nyffeler 2002).

Isolated and unplaced genera: ***Blossfeldia liliputana*** Werderm., [Bolivia], Jucker 443 (ZSS 952518) *trnK/matK* AY015284 (Nyffeler 2002). ***Copiapoa coquimbana*** (Karw. ex Rümpler) *Britton & Rose,* BG Bonn 14730 ex. ZSS 761603/c, [Chile, El Molle], *Knidze s.n.* (BONN, photo), CA126, *trnS-G* –, *trnK/matK* FN995677 (this study), *rpl16* FN673557 (this study), *psbA-trnH* FN669002 (this study), *trnQ-rps16* FN677918 (this study). ***Frailea gracillima*** (Lem.) Britton & Rose, [Brazil], Hofacker 382 (ZSS 19927), *trnK/matK* AY015285 (Nyffeler 2002). ***Frailea phaeodisca*** (Speg.) Speg., [Brazil], Hofacker 25 (ZSS 893932), *trnK/matK* AY015286 (Nyffeler 2002).

Appendix 2. Primers used in this study (A: amplification, S: sequencing)

***trnK/matK* (*trnK* intron and *matK* gene)**

primer name	sequence (5'–3')	application	direction	Reference
trnK-F	GGGTTGCTAACTCAATGGTAGAG	A, S	F	Wicke & Quandt 2009
trnK-2R	AACTAGTCGGATGGAGTAG	A, S	R	Johnson & Soltis 1995
ROSmatK-655R	GGATTCGTATTCACATACAT	A, S	R	Worberg 2009
ROSmatK-530F	AGATGCCTCTTCTTTGC	A, S	F	Worberg 2009
ACmatK500F	TTCTTCTTTGCATTTATTACG	A, S	F	Müller & Borsch 2005
ACmatK650R	GGATTCATATTCACATACATRG	S	R	Müller & Borsch 2005
ACmatK1300F	ATAAAGTATATACTTCGAC	S	F	Müller & Borsch 2005
trnK-71R	CTAATGGGATGTCCTAATAC	S	R	Nyffeler 2002
CAtrnK-270R	GAGCTTATCTTCGTAATTTG	S	R	this study

***trnS-trnG (trnS-G* spacer and *trnG* intron)**

primer name	sequence (5'–3')	application	direction	Reference
trnS	AACTCGTACAACGGATTAGCAATC	A, S	F	Shaw & al. 2007
trnG	GAATCGAACCCGCATCGTTAG	A, S	R	Shaw & al. 2007
trnG2G	GCGGGTATAGTTTAGTGGTAAAA	S	F	Shaw & al. 2005
trnG2S	TTTTACCACTAAACTATACCCGC	S	R	Shaw & al. 2005
CAtrnSG-650F	AGGAGGAGAGATAATAAACG	S	F	this study
CAtrnSG-400F	CAAAGTAATGCTAAAATTCTG	S	F	this study
CAtrnSG-40R	GGAATAGTAATCAAACCGG	S	R	this study

***rps3-rp/16* spacer and *rp/16* intron**

primer name	sequence (5'–3')	application	direction	Reference
CArps3F	GATTATTGCGCCTATCCG	A, S	F	this study
CArpl16R	CCGATAAGATAATCCCTTCA	A, S	R	this study
CArpl16-400R	GAACTTTGTTCTTGAGCC	S	R	this study

psbA-trnH

primer name	sequence (5'–3')	application	direction	Reference
CApsbA	CCGTGCTAACCTTGGTATGG	A, S	F	this study
CAtrnH	CCGCGAATGGTGGATTCACAAT	A, S	R	this study

trnQ-rps16

primer name	sequence (5'–3')	application	direction	Reference
trnQ2	CCAAGTGGTAAGGCGTCGGG	A, S	F	this study
rps16xl	GTTGCTTTCTACCACATCGTTT	A, S	R	Shaw & al. 2007

Appendix 3. Supplementary material

Table S1. Positions excluded from the *Cactaceae* dataset

Position	Region	Comment
579–582	*trnK* intron	poly-A
728–730	*trnK* intron	poly-T
2359–2363	*trnK* intron	poly-A

Table S2. Positions excluded from the combined *Pfeiffera* dataset

Position	Region	Comment
1–36	*trnK* intron	excluded incomplete beginning
2320–2322	*trnK* intron	poly-A
2736–2745	*trnS-G* spacer	poly-A
3086–3096	*trnS-G* spacer	poly-A
3641–3936	*trnS-G* spacer	satellite-like region with multiple repeats, no correct homology assessment possible
4475–4496	*trnG* 5'exon	excluded as uninformative
4719–4733	*trnK* intron	poly-T
4816–4824	*trnK* intron	poly-T
5180–5216	*trnK* intron	excluded incomplete ending
5639–5377	*rpl16* 5'exon	excluded as uninformative
5412–5418	*rpl16* intron	poly-A
5563–5565	*rpl16* intron	poly-T
5596–5608	*rpl16* intron	poly-A
6650–6663	*psbA-trnH*	poly-T
6701–6720	*psbA-trnH*	poly-T, poly-A
6927–6931	*psbA-trnH*	poly-A
7201–7211	*trnQ-rps16*	poly-A
7373–7383	*trnQ-rps16*	poly-T
7420–7434	*trnQ-rps16*	poly-A

Table S3. Synapomorphic indels of *Pfeiffera* and *Lymanbensonia.* – No. of indels refers to the numbering of all indels in the dataset.

Region	**No.**	**extension**	**Sequence motif**
trnK intron	3	134–137	"CAAA" in all other taxa, missing in *Lymanbensonia* and *Calymmanthium*
	10	535	"A" insertion in *Pfeiffera asuntapatense*
	11	546–557	12 nt deletion in *Pfeiffera ianthothele*
matK	17	1461–1463	3 nt deletion in *Pfeiffera monacantha*
trnS-G spacer	20	2474–2491	gap in *Lymanbensonia* (missing data for *Calymmanthium*)
	21	2500–2793	insertion in *Lymanbensonia*
	30	2813–2821	"AAAGGATTT" insertion in *Lymanbensonia incachacana* and *L. micrantha*
	33	2909–2915	gap in *Pfeiffera*
	37	3008–3029	gap in *Lymanbensonia* (missing data for *Calymmanthium)*
	42	3045–3081	multiple "AAATTCG" repeat, 1× in *Lymanbensonia brevispina,* 6× in *L. incachacana* and *L. micrantha* (missing data for *Calymmanthium*)
	45	3081–3143	gap in *Lymanbensonia* (missing data for *Calymmanthium*)
	65	3984	gap in *Lymanbensonia incachacana* and *L. micrantha*
trnG intron	70	4148	„G" insertion in *Pfeiffera*
rps3-rpl16 spacer	82	4763–4767	gap in *Lymanbensonia*
rpl16 intron	94	5228–5235	"TCTTTGAA" insertion of unknown origin in *Lymanbensonia* and *Calymmanthium*
	110	5732–5736	gap in *Pfeiffera*
	112	5784–5792	gap in *Pfeiffera*
	116	5869–5880	gap in *Lymanbensonia*
	120	5924–5955	gap in *Lymanbensonia incachacana* and *L. micrantha*
psbA-trnH	130	6166–6204	gap in *Pfeiffera ianthothele*
	133	6201	gap in *Lymanbensonia incachacana* and *L. micrantha*
trnQ-rps16	146	6530–6763	large deletion in *Pfeiffera*
	159	6842–6854	gap in *Pfeiffera ianthothele*
	163	6963–6971	gap in *Lymanbensonia incachacana* and *L. micrantha* (missing data for *L. brevispina*)

Table S4. Comparison of trees from parsimony analysis of single markers.

	matK	***trnK* intron**	***trnK/matK***	***trnSG* spacer**	***trnG* intron**	***trnS-G***	***rpl16***	***psbA-trnH***	***trnQ-rps16***
Total characters	1530	910	2440	1656	663	2320	1296	352	574
Constant characters	1430	846	2276	1328	608	1937	1122	284	480
Variable, uninformative	54	34	88	185	26	211	89	33	66
Parsmony-informative	46	30	76	143	29	172	85	35	28
Number of shortest trees	9	27	4	2	63	1	6	4	44
Tree length	123	79	203	453	78	537	227	87	121
CI	0.878	0.886	0.877	0.868	0.782	0.845	0.855	0.839	0.884
RI	0.908	0.885	0.896	0.851	0.827	0.834	0.844	0.859	0.856
RC	0.797	0.784	0.786	0.738	0.646	0.705	0.721	0.720	0.757
HI	0.122	0.114	0.123	0.132	0.218	0.155	0.145	0.161	0.116
Pfeiffera monophyletic	100	node not found	100	100	99	100	100	98	75
Lymanbensonia monophyletic	75	99	80	100	node not found	100	57	88	63

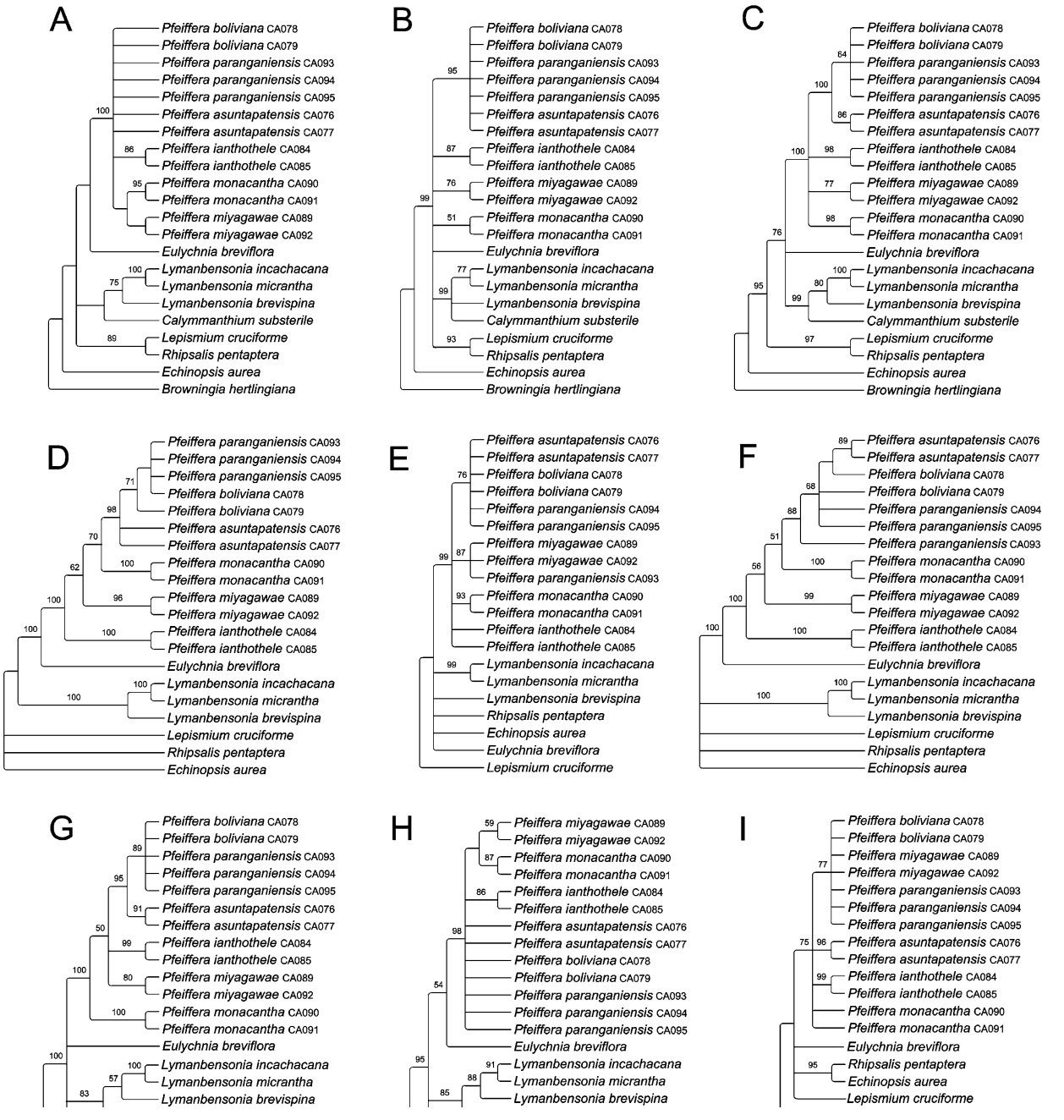

Fig. S1. Trees inferred from single markers – A: *matK;* B: *trnK* intron; C: *trnK/matK;* D: *trnS-G* spacer; E: *trnG* intron; F: *trnS-G;* G: *rpl16;* H: *psbA-trnH;* I: *trnQ-rps16*. – All trees are strict consensus trees found by the parsimony ratchet. Numbers above branches are jackknife support values from 10 000 replicates.