Phylogeny and evolution of the epiphytic Rhipsalideae (Cactaceae)

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Table of Contents

Chapter 1

General Introduction 1

1.1 Evolution and systematics of the Cactaceae 1

1.2 Subject to debates: Cactaceae classification 2

1.3 Current understanding of phylogenetic relationships in Cactaceae 3

1.4 Epiphytic Cactaceae lineages 4

1.4.1 Origin of the epiphytic cacti, their phylogenetic position and putative closest relatives and overview on earlier taxonomic treatments 5

1.4.2 The tribe Rhipsalideae DC. 7

1.5 Phylogeny inference at species level 11

1.6 Marker choice for species-level studies 12

1.7 Plant DNA barcoding 13

1.8 Background, aims and outline of this study 14

1.8.1 Study outline 15

Chapter 2

A phylogenetic analysis of *Pfeiffera* and the reinstatement of *Lymanbensonia* as an independently evolved lineage of epiphytic Cactaceae within a new tribe Lymanbensonieae 17

2.1 Introduction 18

2.2 Material and Methods 21

2.2.1 Plant material and taxon sampling 21

2.2.2 Isolation of genomic DNA 21

2.2.3 Amplification and sequencing 21

2.2.4 Alignment, coding of length mutational events 22

2.2.5 Outgroup definition 23

2.2.6 Phylogenetic analyses 23

2.3 Results 24

2.3.1 Success of amplification, sequencing and alignability 24

2.3.2 Sequence characteristics 24

2.3.3 Inversions 26

2.3.4 Position and circumscription of *Pfeiffera* 26

2.3.4.1 Trees for *Pfeiffera* inferred from single markers 29

2.3.4.2 Relationships within *Pfeiffera* inferred from the combined dataset 31

2.4 Discussion 32

2.4.1 Phylogenetic signal and mutational dynamics of the markers used 32
2.4.2 Circumscription of *Pfeiffera* and reinstatement of *Lymanbensonia* 32
2.4.3 The putative closest relatives of *Pfeiffera* 33
2.4.4 The placement of *Lymanbensonia* 34
2.4.5 Relationships within *Pfeiffera* 35
2.4.6 Relationships within *Lymanbensonia* 37
2.4.7 Generic concepts and morphological characters of *Pfeiffera* and associated genera 37
2.4.8 Biogeographical patterns 40
2.5 Conclusions and future work 42
2.6 Taxonomic conclusions 43

Chapter 3

How much does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalideae (Cactaceae) 51

3.1 Introduction 52
3.2 Material and Methods 56
3.2.1 Plant material and taxon sampling 56
3.2.2 Isolation of genomic DNA 56
3.2.3 Amplification and sequencing 57
3.2.4 Sequence alignment, coding of length mutational events 57
3.2.5 Phylogenetic analyses 58
3.2.6 Comparison of marker performance / phylogenetic structure *R* 58
3.2.7 Definition of operational taxonomic units (OTUs) 59
3.2.8 Testing of OTU identification success 59
3.3 Results 60
3.3.1 Sequence characteristics 60
3.3.2 Microstructural mutations 60
3.3.3 Trees from the single loci 63
3.3.4 Trees from the combined plastid data set 63
3.3.5 Phylogenetic structure *R* 64
3.3.6 Success of OTU identification 64
3.4 Discussion 68
3.4.1 Major relationships within Rhipsalideae 68
3.4.2 Relationships within main Rhipsalideae lineages, circumscription of genera and subgeneric classification 69
3.4.2.1 *Schlumbergera* 69
3.4.2.2 *Hatiora* 71

3.4.2.3 *Rhipsalidopsis* 71

3.4.2.4 *Lepismium* 71

3.4.2.5 *Rhipsalis* 72

Subg. *Calamorhipsalis* K.Schum. (incl. subg. *Epallagogonium* K.Schum. p.p.) 72

Subg. *Erythrorhipsalis* Berger 73

Subg. *Epallagogonium* K.Schum. 74

Subg. *Goniorhipsalis* K.Schum. (incl. subg. *Epallagogonium* p.p., subg. *Rhipsalis* p.p.) 74

Subg. *Phyllarthrorhipsalis* Buxb. (including subg. *Rhipsalis* p.p.) .. 74

Subgenus *Rhipsalis* 76

The Old World *Rhipsalis* 77

3.4.3 The potential of markers for OTU identification within Rhipsalideae 78

3.4.4 Phylogenetic utility of the regions used 81

3.4.5 Comparison of phylogenetic utility and species identification potential of the markers used 85

3.4.6 An improved classification system for Rhipsalideae 85

Chapter 4

Morphology and character evolution of the Rhipsalideae 87

4.1 Introduction 88

4.1.1 Characters applied as diagnostic for taxonomic groups in the Rhipsalideae.... 88

4.1.2 State of knowledge on morphological characters 90

4.2 Material and Methods 92

4.2.1 Taxon sampling 92

4.2.2 Morphological data 92

4.2.3 Analysis of character evolution 92

4.2.4. Modifications of the matrix for BayesTraits analyses 93

4.3 List of morphological characters and their states 94

4.4 Results and discussion Character evolution in the Rhipsalideae 99

4.4.1 Synapomorphies of the Rhipsalideae 99

4.4.2 Apomorphic versus highly homoplastic characters 99

4.4.3 Evolution of characters associated with the epiphytic life-form 110

4.4.4 Evolution of floral traits and assumed pollination syndromes 114

4.4.5 Morphological intermediates in the Rhipsalideae 116

4.4.6 Morphological characterization of the clades inferred by sequence data corresponding to genera 117

Chapter 5

Towards understanding the historical phylogeography of *Rhipsalis baccifera*, the most widespread cactus 121

5.1 Introduction 122
5.2 Material and Methods 124
5.2.1 Plant material and taxon sampling 124
5.2.2 Isolation of genomic DNA, amplification and sequencing 124
5.2.3 Phylogenetic analyses and haplotype network construction 124
5.3 Results 125
5.3.1 Sequence characteristics, phylogenetic analyses and haplotype network construction 125
5.4 Discussion 127
5.5 Conclusions and outlook 128

Chapter 6

Development of microsatellite loci for *Rhipsalis baccifera* using 454 sequencing 129

6.1 Introduction 129
6.2 Material and Methods 130
6.2.1 Plant material and taxon sampling 130
6.2.2 Chromosome count 130
6.2.3 Isolation of genomic DNA 130
6.2.4 Genomic library construction 131
6.2.5 454 sequencing 132
6.2.6 Screening for repetitive motifs and primer design 132
6.3 Results 133
6.3.1 Chromosome counts 133
6.3.2 454 sequencing and microsatellite loci found 133
6.4 Discussion 133

References 139

Summary 153

Appendices 157

Appendix 1. Plant material used in this study 157
Appendix 2. PCR amplification protocols 167
Appendix 3. Sequence parts excluded from the phylogenetic analyses 169
Appendix 4. List of indels coded from the combined Rhipsalideae dataset 170
Appendix 5. Success of single partitions and the combined dataset in OTU identification 174

Appendix 6. Comparison of trees inferred from parsimony analyses of single markers and the complete dataset for the Rhipsalideae 176

Appendix 7. Trees from maximum parsimony analyses of the single markers 177

Appendix 8 Matrix of morphological characters.

Chapter 1

General Introduction

1.1 Evolution and systematics of the Cactaceae

Cactaceae are one of the most important plant families of the New World's arid and seasonally moist tropical regions. Cactaceae are also one of the most popular plant families in horticulture and have been the subject of interest of many botanists and as plant enthusiasts since the 18th century.

The Cactaceae are morphologically distinct and doubtless supported as monophyletic by morphological synapomorphies and molecular data (Barthlott & Hunt 1993, Gibson & Nobel 1986, Hernández-Hernández & al. 2011, Nyffeler 2002, Wallace & Gibson 2002). They belong to the order Caryophyllales in which they are part of a clade that contains most of the succulent families of the order: Cactaceae, Anacampserotaceae, Basellaceae, Didiereaceae, Halophytaceae, Montiaceae, Portulacaceae, and Talinaceae (Cuénoud & al. 2002, Schäferhoff & al. 2009). The sister group of the Cactaceae is the former Portulacaceae tribe Anacampseroteae, now separated as an own family Anacampserotaceae (Nyffeler 2007, Nyffeler & Eggli 2010a).

The Cactaceae are almost exclusively distributed in the New World, besides one *Rhipsalis* species and introduced *Opuntia* species in the Mediterranean, South Africa and Australia. The Cactaceae are found in the dry areas of North and South America with centres of diversity in north-eastern Mexico, the eastern Andes of Bolivia and Argentina and in south-eastern Brazil. No fossils are known for the Cactaceae and consequently, an age estimate based on fossil record is not possible. Fossils of more distantly related Caryophyllales taxa allowed inferring 19,1 – 3,1 Mya as the age of the family (Ocampo & Columbus 2010).

The Cactaceae are a morphologically very diverse family. They have evolved a variety of growth-forms ranging from tree-like, large columnar forms to shrubby forms or succulent climbers and to small globular forms. But among the perhaps most interesting life-forms and habits are cacti that grow as epiphytes in tropical rainforests. It may be surprising that epiphytic habit is found in Cactaceae because the family is mostly associated with arid areas. But epiphytism has even evolved several times independently within the family (Barthlott 1979, Barthlott & Hunt 1993, Hernández-Hernández & al. 2011, Wallace & Gibson 2002).

1.2 Subject to debate: Cactaceae classification

Although the monophyly of the Cactaceae has hardly ever been questioned, the establishment of taxonomic units within the Cactaceae has been always difficult and controversial. Beginning with Schumann's (1899) first comprehensive monograph of the family, many classification systems have been proposed in the last centuries (Backeberg 1958-1962, Britton & Rose 1919-1923, Buxbaum 1962, Gibson & Nobel 1986, Barthlott 1988, Barthlott & Hunt 1993). These classifications were often rather subjective and therefore largely incompatible with each other. The difficulties in Cactaceae taxonomy are a well known problem. Because Cactaceae often look very similar, there was hardly ever a consensus how Cactaceae genera should be delimited. While Schumann favoured using large hold-all-genera, (Britton & Rose 1919-1923) split many of these and increased the number of genera from 21 to 124 and (Backeberg 1958-1962) split even further and increased to 233 genera. Following authors again tried to reduce the number of genera. The most recent classifications (Anderson 2001, Barthlott & Hunt 1993) were largely based on the consensus initiatives of a working party of the International Organisation for Succulent Plant Study (IOS) (Hunt & Taylor 1986, Hunt & Taylor 1990). This consensus initiative has stabilized Cactaceae names for a while, but after most of the tribes and genera investigated by molecular phylogenetic studies were found as not monophyletic (see below), the Cactaceae classification is again in flux.

Currently the Cactaceae are subdivided into four subfamilies: "Pereskioideae" (accepted as paraphyletic), Maihuenioideae, Opuntioideae, and Cactoideae, the latter containing about 80% of all species. There are 9 tribes, 124 genera and 1430 accepted species. This refers to the current reference work for the family, the "New Cactus Lexicon" (Hunt 2006). The classification therein is to some extent based on hitherto available results of phylogenetic studies, with some tribes (e.g. Notocacteae or Hylocereeae) accepted as poly –or paraphyletic. A revised classification that acknowledges all recent molecular phylogenetic findings has been proposed recently (Nyffeler & Eggli 2010b). Here, revised tribal circumscriptions and recognition of additional subtribes are suggested, with some of then accepted as paraphyletic. The authors of most recent phylogenetic study (Hernández-Hernández & al. 2011) also suggest own names for the clades found. So as a result, the tribal circumscription is incompatible among these three systems.

Defining species limits in Cactaceae was also difficult. Individual populations of one species can vary considerably in their morphology due to phenotypic plasticity and responses to the environmental conditions. Consequently, interpretation of morphological characters is often troublesome. To make things worse, many species have been described based on only few individuals. As a result, Cactaceae have been heavily over-

described - approximately 15000 binomials exist (Anderson 2001). This is ten times higher than the number of currently accepted species (Hunt 2006).

1.3 Current understanding of phylogenetic relationships in Cactaceae

The development on ideas on phylogenetic relationships in Cactaceae before the application of molecular data has been reviewed in detail by Barthlott (1988) and Metzing & Kiesling (2008). The at present most comprehensive phylogenetic hypotheses for the family are based on datasets of the plastid regions *trnK/matK*, *rpl16* intron and *trnL-F* and the nuclear gene *ppc* (Hernández-Hernández & al. 2011) or on *trnK/matK* (Bárcenas & al. 2011). Earlier studies were based on *trnK/matK* and *trnL-F* (Nyffeler 2002) and *rbcL* (Wallace & Gibson 2002). However, all these studies yielded insufficient phylogenetic signal because of low sequence variability or insufficient data. Many nodes, especially in the Cactoideae are weakly supported. Even a large taxon sampling (666 species, Bárcenas & al. 2011) did not significantly improve the resolution and support.

The relationships as currently understood based on these studies and further studies of single tribes and genera are summarised in the following and in Fig. 1.1. The first branching Cactaceae lineages are formed by subfamily Pereskioideae (only *Pereskia*). It is a basal grade with Mesoamerican and Caribbean species found as the first branching lineage followed the South American, especially Andean species (Butterworth & Wallace 2005, Edwards & al. 2005). The next branching lineages are the Opuntioideae and Maihuenioideae. Both are well supported as monophyletic but their exact position is not yet clear. The Cactoideae are well supported as monophyletic. The monotypic peculiar genus *Blossfeldia* is found as sister to the rest of the subfamily. *Blossfeldia* is morphologically and ecologically very different from the other Cactoideae so this placement was unexpected and has been repeatedly questioned (Gorelick 2004). But nevertheless, all currently available data do support this position and a new tribe Blossfeldieae has been proposed (Butterworth 2006). Leaving aside *Blossfeldia*, the tribe Cacteae is sister to the rest of the subfamily, termed core Cactoideae. The Cacteae are so far the only "traditional" tribe entirely confirmed as monophyletic by molecular data (Butterworth & al. 2002). Within Cactoideae, there are some isolated genera (*Copiapoa*, *Calymmanthium*, *Frailea*) and two main clades – the core Cactoideae I and II.

The core Cactoideae I comprises North and South American columnar genera (*Austrocactus*, *Corryocactus*, Leptocereeae, Pachycereeae) and two epiphytic groups: *Pfeiffera* and the Hylocereeae. Relationships within the core Cactoideae I are poorly resolved and low supported and as a result, there is no consistent naming for the subclades. Hunt (2006) divides the whole group in Hylocereeae (as traditionally defined) and Echinocereeae. Hernández-Hernández & al. (2011) suggest the expanded Hylocereeae and the core Pachycereeae as the two main lineages, leaving several genera such as *Corryocactus* and *Eulychnia* unassigned to any tribe. Nyffeler & Eggli

(2010b) propose a tribal name Phyllocacteae for the whole grouping with three subtribes Corryocactinae (paraphyletic), Hylocereinae and Pachycereinae.

The core Cactoideae II comprises the South American tribes Rhipsalideae, Notocacteae p.p., Cereeae (including Browningieae p.p.), and Trichocereeae. The Rhipsalideae are found as sister to the rest of the whole clade. The Notocacteae as earlier defined by Barthlott & Hunt (1993) have been shown to be highly polyphyletic, comprising four independent lineages. They are now restricted to *Parodia*, *Eriosyce*, *Rimacactus*, *Yavia* while *Neowerdermannia*, *Frailea*, *Copiapoa* and *Blossfeldia* are excluded. The rest of the core Cactoideae is made up by Cereeae and Trichocereeae sensu Anderson (2001). These are mainly South American, partly also Caribbean, arborescent, columnar and globular cacti. Within both tribes, there is evidence that most of the genera as currently circumscribed are not monophyletic, e.g. *Echinopsis* (B. Schlumpberger, pers. comm.), *Browningia* (Applequist & Wallace 2002) as well as *Cintia*, *Sulcorebutia* and *Weingartia* (Ritz & al., 2007). Again, as the traditional tribal and generic names do not apply to the groupings found, alternative names have been suggested. Hunt (2006) even accepts the Notocacteae as traditionally defined, i.e. including *Blossfeldia*, *Frailea* and *Copiapoa* and further suggests two tribes Cereeae and Trichocereeae. Hernández-Hernández & al. (2011) name only the core Notocacteae and the Trichocereeae in their trees and Nyffeler & Eggli (2010b) suggest a single tribe Cereeae in a broad circumscription with three subtribes.

To summarise; the major Cactaceae lineages have been identified in molecular phylogenetic studies. But at the same time, it was found that most of tribes and genera as traditionally defined are not monophyletic. So although by now many studies have yielded insights into cactus phylogeny and provided a first basis for a classification which reflects phylogenetic relationships, phylogenetic relationships in Cactaceae remain insufficiently understood.

1.4 Epiphytic Cactaceae lineages

There are about 150 epiphytic species, about 10% of the whole family. Currently epiphytism is assumed to have evolved independently at least three times (Fig. 1.1). The tribes Rhipsalideae DC. and Hylocereeae (Britton & Rose) F.Buxb. are the two largest epiphytic groups with distinct distribution centres: The Hylocereeae are predominantly Mesoamerican and Caribbean, with a diversity center in southern Mexico. The Rhipsalideae are mainly South American and centred in the coastal rainforests of South-Eastern Brazil (Barthlott 1983, Taylor & Zappi 2004). The genus *Pfeiffera* (Echinocereeae) ranges from southern Ecuador to Peru to Northern Argentina and is centred in Bolivia. There may be even more independent origins of epiphytism: a part of *Selenicereus* (Hylocereeae) is assumed to be a distinct epiphytic lineage and currently separated as *Strophocactus* (Bauer 2003, Nyffeler & Eggli 2010b) and there are some

further species which grow occasionally as epiphytes (*Echinopsis arboricola*) and some *Cereus* and *Cleistocactus* spp.).

1.4.1 Origin of the epiphytic cacti, their phylogenetic position and putative closest relatives and overview on earlier taxonomic treatments

The first epiphytic cactus was described already 1753 in "Species Plantarum" as *Cactus phyllanthus* L. (≡ *Epiphyllum phyllanthus* (L.) Haw.). The first *Rhipsalis* was described 1768 as *Cassytha filiformis* Mill. (≡ *Rhipsalis baccifera* (Mill.) Stearn.). But Miller had not recognised the plant he described was a cactus. He thought he had found a new *Cassytha* species, a filiform parasitic Lauraceae indeed resembling *Rhipsalis*.

All authors, starting with the works of Salm-Dyck (1850) and Schumann (1899) assumed that the epiphytes were derived from columnar terrestrial cacti. But the actual closest relatives could not be identified easily. Usually columnar, shrubby genera, most often *Eulychnia*, *Erdisia* or *Corryocactus* have been suggested, as discussed in more detail in Chapter 2. At the moment the terrestrial relatives of the epiphytic cacti are not fully identified. *Acanthocereus* and *Peniocereus* p.p. are related to the Hylocereeae (Arias & al. 2005) while the Rhipsalideae are sister to the Notocacteae p.p., Browningieae, Trichocereeae and Cereeae. *Pfeiffera* is part of a clade where also *Corryocactus* and *Eulychnia* belong to, but their interrelationships are unresolved (Hernández-Hernández & al. 2011, Nyffeler 2002).

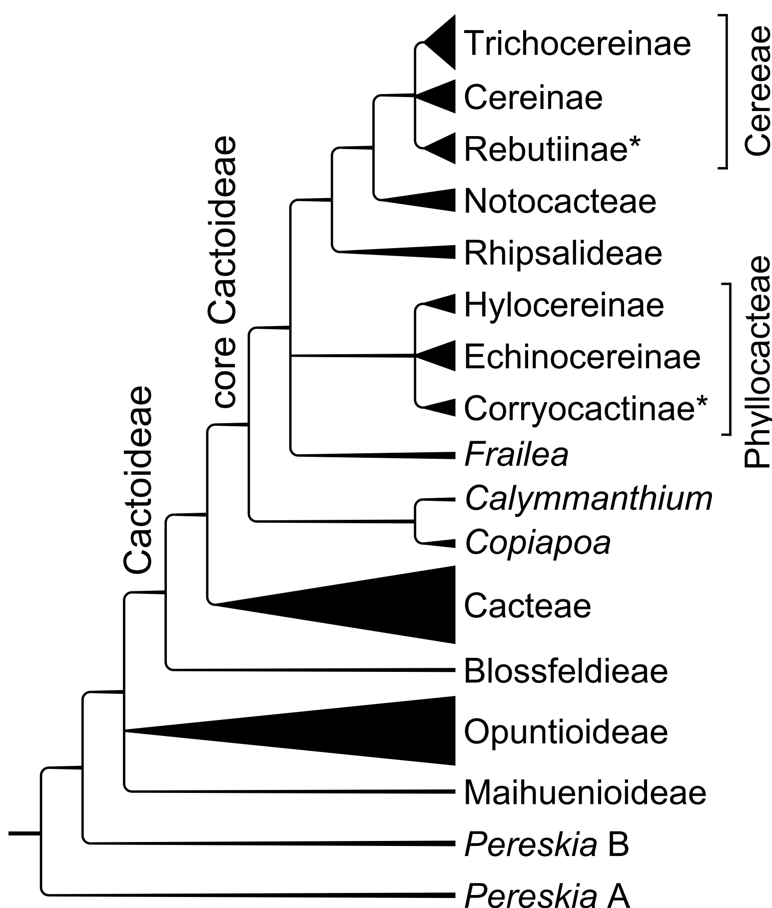

Figure 1.1 Current knowledge on phylogenetic relationships in Cactaceae, showing lineages that contain epiphytic genera. Summarised after Nyffeler 2002, Edwards & al. 2005, Hernández-Hernández & al. 2011. The tribal classification follows Nyffeler & Eggli (2010). Tribes marked with an asterisk are accepted as paraphyletic.

There were either one or several taxonomic groups containing epiphytes recognised in the earlier classification systems. But often no clear ideas were provided whether they were related or not. One can only assume that the authors implied an independent origin by placing epiphytic genera in different tribes or subtribes. But basically, and regardless of their ranks, the Mesoamerican, large flowered groups and the South American small flowered groups were usually treated separately, although sometimes only as subtribes within one single tribe. But the remarkable similarities of the Hylocereeae and Rhipsalideae in vegetative morphology, and even concerning pollen and seed characters have led to long ongoing misinterpretations of the morphology and caused much taxonomic confusion.

In most works on the Cactaceae, two or more groups containing epiphytes can be found. This is also the case in the earliest works. The first author to recognise a group of epiphytic cacti as a higher level taxon was A. P. de Candolle (1828) who divided the Cactaceae in the Opuntiaceae and Rhipsalideae. The latter included only *Rhipsalis* while the other epiphytic species were placed in Opuntiaceae. Salm-Dyck (1850) added *Lepismium* and *Pfeiffera* to the Rhipsalideae and placed *Epiphyllum* and *Phyllocactus* in the Phyllocacteae. Schumann (1899) subdivided the Cereoideae (= Cactoideae) in three tribes: Mammillariae, Rhipsalideae and Echinocacteae, which contained the epiphytic genera *Epiphyllum* and *Phyllocactus*. Britton & Rose (1919-1923) recognised a total of three subtribes of epiphytic cacti. Their Hylocereanae contained part of the modern Hylocereeae, i.e the climbing cacti with ribbed stems bearing spiny areoles. The large flowered taxa with leaf-like flattened stems were classified as Epiphyllanae, which corresponds to today's flat-stemmed Hylocereeae and the large flowered Rhipsalideae. The Rhipsalidanae in contrast included all the small flowered epiphytic taxa. Except for stating that Epiphyllanae were not closely related to the Rhipsalidanae, Britton & Rose did not provide any further ideas on the relationships of the epiphytic groups; their works generally lack phylogenetic ideas and they used a more phenetic approach instead. Berger (1926), comparable to Britton & Rose, suggested three epiphytic groups, not given a formal rank. But he emphasised that the South American Rhipsalideae were distinct from the Mesoamerican groups he treated as Epiphylleae and Hylocereae. He furthermore for the first time regarded the genus *Pfeiffera* as an additional distinct lineage.

The idea of at least two distinct epiphytic Cactaceae lineages was rejected in the two subsequent Cactaceae classifications. Backeberg (1958-1962, 1966) and similarly Buxbaum (1962, in Krainz) simply placed all epiphytic cacti in a single tribe Hylocereeae; Buxbaum's circumscription even included terrestrial genera. Hunt (1967) used a similar approach and combined all epiphytic cacti in an unranked polyphyletic group (Group B within his subtribe Cereinae).

Later, Barthlott (1979; 1988) again brought forward the idea of an independent origin of epiphytic cacti in South and Central America. He emphasized that the South

American Rhipsalideae were not related to the predominantly Mesoamerican and Caribbean Hylocereeae. Barthlott also moved *Pseudorhipsalis* from Rhipsalideae, where it has been put in until then, into the Hylocereeae. It was the first time the morphological similarities between *Rhipsalis* and *Pseudorhipsalis* were recognised to be a result of convergent evolution. Barthlott's treatment can be considered as the first modern treatment of the epiphytic tribes. It has been kept by Barthlott & Hunt (1993) and became widely accepted. The view that epiphytism had evolved two times independently within Cactaceae was widely favoured in the late 20th century. But phylogenetic studies revealed that epiphytism evolved even more often in the family (see above).

Currently the Hylocereeae and the Rhipsalideae are accepted as tribes (Hunt 2006). The Rhipsalideae are circumscribed as in Barthlott & Hunt (1993), only the delimitation of *Lepismium* changed (see Chapter 2). The Hylocereeae could either be classified either as a tribe (Hunt 2006), or as subtribe Hylocereinae within Phyllocacteae (Nyffeler & Eggli 2010b). Both suggestions favour the traditional circumscription of the Hylocereeae, as for example the one of Barthlott & Hunt (1993). Another proposal is to expand the Hylocereeae to include their terrestrial closest relatives (Hernández-Hernández & al. 2011).

1.4.2 The tribe Rhipsalideae DC.

This study focuses especially on the Rhipsalideae, which is one of the two largest groups of epiphytic cacti. The Rhipsalideae are centred in the Mata Atlântica, few species are also found in the Andes of Bolivia and Argentina, as well as in western Peru, Ecuador and Colombia (Fig. 1.2). *Rhipsalis* is the largest and most widely distributed genus of the Rhipsalideae and also the main South American epiphytic Cactaceae genus. It is found in neotropical forests from eastern Mexico, to southern Florida and the Caribbean to northern Argentina and Uruguay. The Rhipsalideae are predominantly epiphytes, rarely lithophytes and characterised by angled, filiform terete or thin flattened stems; many species are only slightly succulent. Most species of *Rhipsalis* and *Lepismium* have small white or whitish flowers. Large coloured flowers occur in *Schlumbergera* and in *Hatiora* subg. *Rhipsalidopsis*. The fruits are usually small, berry like, either coloured or whitish and bird-dispersed. An overview of the morphology of the Rhipsalideae is shown in Figure 1.3.

Rhipsalideae were the first epiphytic Cactaceae group to be recognised at suprageneric level (de Candolle 1828). They originally contained only *Rhipsalis*; the other genera were not yet described. Later, generic limits became controversial and therefore unstable. Some authors favoured a broadly defined *Rhipsalis* while others created segregate genera. As a result, there were two, 10 or even 14 genera accepted; see Table 1.1 for an overview. The most comprehensive and up-to-date treatment of the Rhipsalideae was provided by Barthlott & Taylor (1995) and is largely based on the earlier proposals of Barthlott (1987). Barthlott & Taylor have compiled a commented

checklist in which they recognised four genera with 58 species and 28 infraspecific taxa: *Lepismium* (14 spp.), *Rhipsalis* (33 spp.), *Hatiora* (5 spp.) and *Schlumbergera* (6 spp.). Only minor changes to this classification have been made in the New Cactus Lexicon (Hunt 2006) mainly to include newly described *Rhipsalis* species *R. ormindoi, R. olivifera* and *R. agudoensis*. The only major difference in Hunt's treatment is the resurrection of *Pfeiffera* and its exclusion from the Rhipsalideae resulting in a new circumscription of *Lepismium*. The tribe currently comprises four genera: *Lepismium* (6 spp.), *Rhipsalis* (35 spp.), *Hatiora* (6 spp.) and *Schlumbergera* (6 spp.).

There are only few hypotheses on Rhipsalideae relationships available. Schemes showing the assumed relationships between are found only in the works of Berger (1926), Buxbaum (1967) and Barthlott (1987b). The inclusion of Rhipsalideae taxa in overall Cactaceae phylogenies recovered the tribe as monophyletic, leaving aside those species excluded from *Lepismium*. Some more detailed hypotheses based on sequence data of *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH* and ITS have been recently published, focussing on *Schlumbergera* and *Hatiora* (Calvente & al. 2011). The Rhipsalideae were confirmed as monophyletic and also the genera could be found as monophyletic, but only based on the plastid data – ITS produced a large polytomy. The findings of this study are discussed in more detail in Chapter 3.

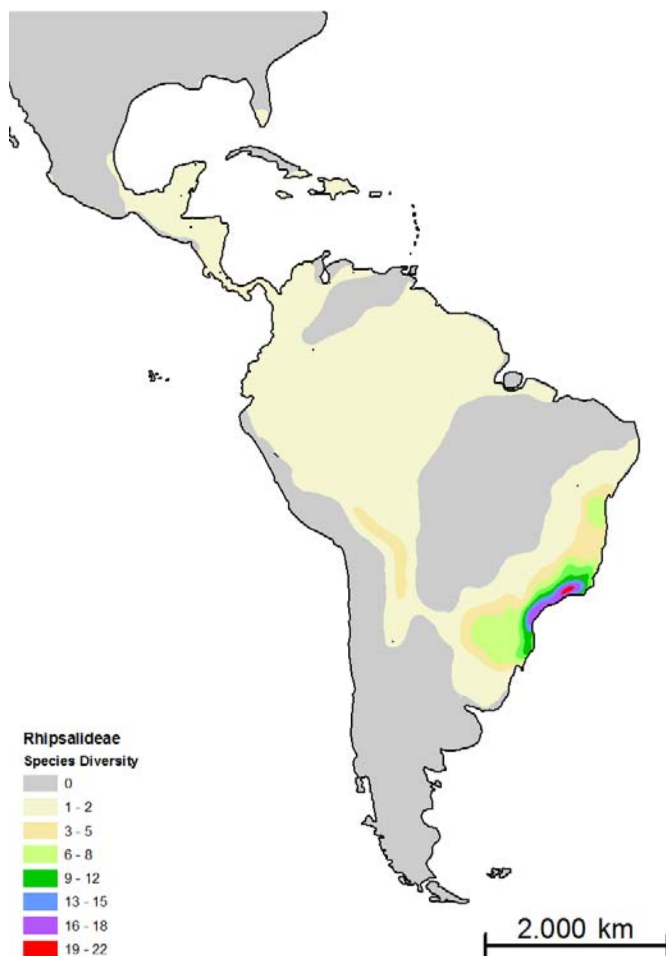

Figure 1.2 Distribution and diversity of the Rhipsalideae. The map shows species numbers per 2500 km^2. Data from Barthlott et al. in prep, largely taken from (1983), Taylor & Zappi (2004), Taylor pers.obs.

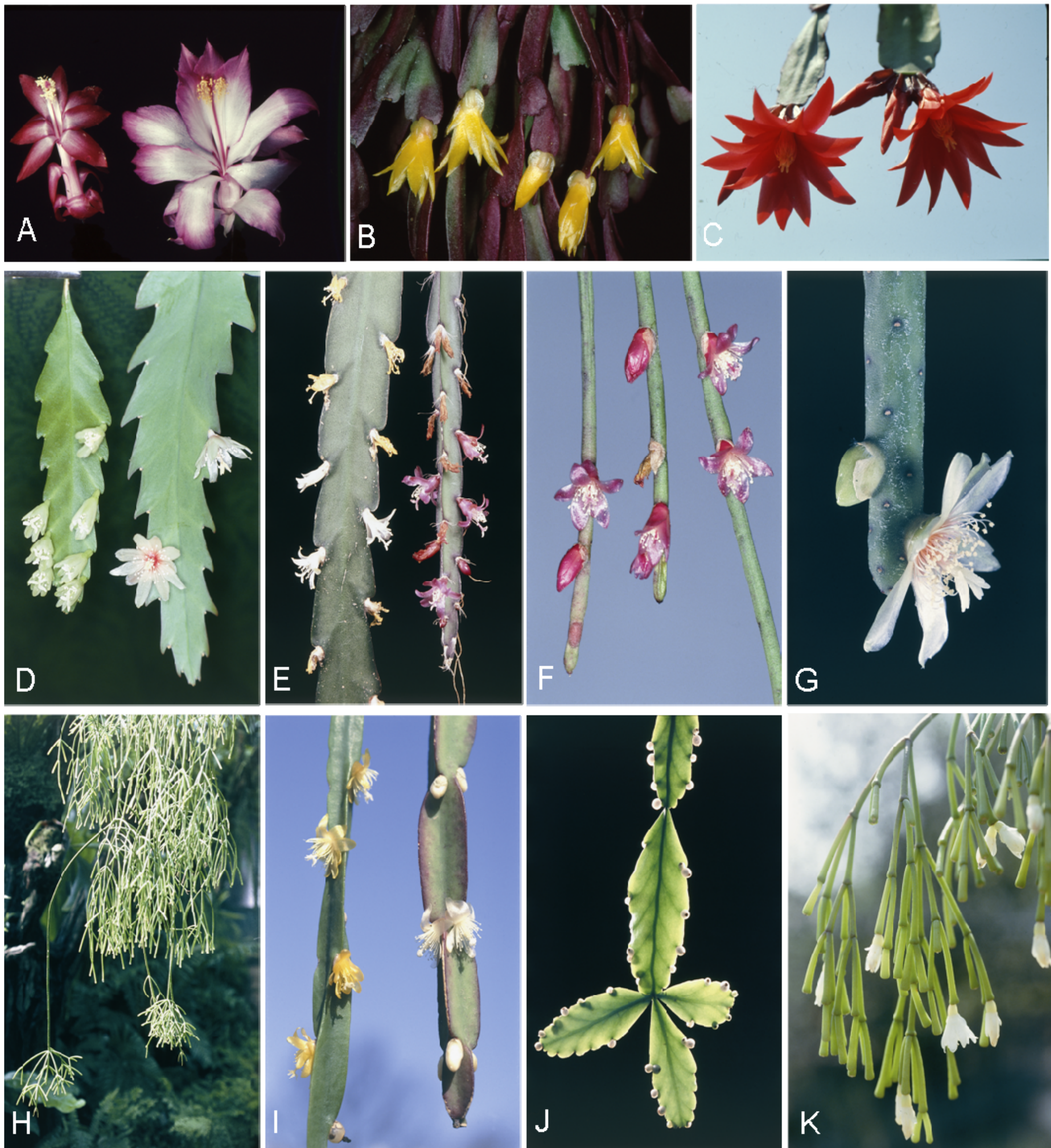

Figure 1. 3 Morphological variation in the Rhipsalideae. A: left: *Schlumbergera truncata*, right: *Schlumbergera orssichiana*, clonotype from *Orssich s.n.* collection, cult. BG Bonn 5584; B: *Hatiora epiphylloides* subsp. *epiphylloides;* C: *Rhipsalidopsis gaertneri*; D: *Lepismium houlletianum*; left: f. *regnellii*, right: f. *houlletianum*; E: two forms of *Lepismium cruciforme*; F: *Rhipsalis hoelleri*, type collection *Orssich s.n.,* 1987, cult. BG Bonn 4841, G: *Rhipsalis neves-armondii f. megalantha*, cult. BG Bonn 12176, H**:** *Rhipsalis teres*; I: *Rhipsalis paradoxa*, left: subsp. *septentrionalis*, *Braun s.n.*, right: subsp. *paradoxa*; J: *Rhipsalis occidentalis*, K: *Rhipsalis clavata*. All photos: W. Barthlott.

Table 1. 1 Subdivision of Rhipsalideae and circumscription of genera. ([1]Cereoideae is a synonym of the modern name Cactoideae. [2]*Hariota* was the original name, it was abandoned because of numerous nomenclatural uncertainties and replaced by the anagram "*Hatiora*" by Britton & Rose. [3]Britton & Rose did not recognise subfamilies. [4]Berger does not provide tribal ranks but writes of groups – "Gruppe". [4]*Pseudorhipsalis* had been already moved to the Hylocereeae by Barthlott (1979).

	Salm-Dyck 1850	**Schumann 1898**	**Britton & Rose 1923**	**Vaupel 1925, 1926**	**Berger 1926**	**Backeberg 1959**	**Barthlott & Taylor 1995**	**Hunt 2006**
	Cacteae rotatae, Squamatae	subfamily Cereoideae[1]	Tribe 3: Cereeae[3]	Malacosperma e-Chorineurae	subfamily Cereoideae	subfamily Cereoideae	Subfamily Cactoideae	Subfamily Cactoideae
	Trib. V Rhipsalideae	**III Gruppe Rhipsalideae**	**Subtribe Rhipsalidanae**		**Group[4] Rhipsalideae**	**Tribe Hylocereeae, subtribe Rhipsalidinae**	**Tribe Rhipsalideae**	**Tribe Rhipsalideae**
Rhipsalis Gaertn. 1788	9 spp.	47 spp.	57 spp.	84 spp.	57 spp.	60 spp.	33 spp.	35 spp.
Lepismium Pfeiffer 1835	1 sp.	= *Rhipsalis*	1 sp.	= *Rhipsalis*	1 sp.	17 spp.	14 spp.	6 spp.
Pfeiffera Salm-Dyck 1845	1 sp.	1 sp.	1 sp.	= *Rhipsalis*	1 sp. (transferred to Pfeifferae)	1 sp. (transferred to Cereeae-Austrocereinae	= *Lepismium*	9 spp. (trasferred to Echinocereeae
Hatiora Britton & Rose 1915 (= ***Hariota*** DC. 1834)	= *Rhipsalis*	2 spp. (as *Hariota*[2])	3 spp.	= *Rhipsalis*	3 spp.	4 spp.	5 spp.	6 spp.
Erythrorhipsalis A.Berger 1920			1 sp.	= *Rhipsalis*	1 sp.	1 sp.	= *Rhipsalis*	= *Rhipsalis*
Acanthorhipsalis Britton & Rose 1923			3 spp.	= *Rhipsalis*	3 spp.	5 spp.	= *Rhipsalis*	= *Pfeiffera*
Pseudorhipsalis Britton & Rose 1923			2 spp.	= *Rhipsalis*	2 spp.	3 spp.	in Hylocereeae[4]	in Hylocereeae
Rhipsalidopsis Britton & Rose 1923			1 sp.	= *Rhipsalis*	1 sp.	1 sp.	= *Hatiora*	= *Hatiora*
Schlumbergera Lemaire 1858		= *Phyllocactus* (in Echinocacteae	2 spp. (in Epiphyllanae)	= *Epiphyllum*	2 spp.	2 spp.	6 spp.	6 spp.
Zygocactus K.Schum 1890		= *Epiphyllum* (in Echinocacteae)	1 sp. (in Epiphyllanae)	= *Epiphyllum*	1 sp.	1 sp.	= *Hatiora*	= *Hatiora*
Epiphyllanthus A.Berger 1905			3 spp. (in Epiphyllanae)	= *Epiphyllum*	3 spp.	3 spp.	= *Hatiora*	= *Hatiora*
Epiphyllopsis A.Berger 1929						1 sp.	= *Schlumbergera*	= *Schlumbergera*
Pseudozygocactus Backeb. 1938						1 sp.	= *Schlumbergera*	= *Schlumbergera*
Lymanbensonia Kimnach 1984							= *Lepismium*	= *Pfeiffera*

1.5 Phylogeny inference at species level

Species-level phylogenies are among the most interesting but probably also among the most difficult-to-address issues in systematics. This is because lots of sequence data are usually necessary to provide enough resolution between closely related, recently diverged species. But species are regarded as the fundamental units of biological diversity. Therefore reliable species phylogenies are essential to understand the evolutionary history of the study group. This most commonly implied the study of character evolution or biogeographic patterns.

The term "species" is maybe among the most debated terms in biology - there are numerous attempts to define what a species actually is. The term species also immediately leads to the consideration of the species concept that was used to define it. The most commonly applied concept is the biological species concept as formulated by Ernst Mayr (1942). He defined species as "*groups of interbreeding natural populations that are reproductively isolated from other such groups*". But Mayr himself stated at the same time that "*it is at least doubtful whether this applies equally to plant species*" (Mayr 1942). Following authors have repeatedly questioned whether plant species are "real" at all. Rieseberg & Brouillet (1994) assumed that most of the plant species are not monophyletic due to the modes of speciation and that "*species concepts that insist in monophyly are inadequate for a significant proportion of plant species*". They furthermore state that each species can be expected to pass through stages of polyphyly to paraphyly and, after a sufficient time, finally to monophyly. Each of these stages, the "phylogenetic status" of a given species should consequently be determinable and therefore monophyly would therefore not be the only criterion for species recognition (Rieseberg & Brouillet 1994).

So there is increasing evidence that many angiosperm species will not be found as monophyletic in gene trees. There are several reasons for this. Introgression, hybridization, reticulate evolution and incomplete lineage sorting lead to incongruence of gene trees and species trees (e.g. Jakob & Blattner 2006, Rieseberg & Willis 2007). Consequently not all species relationships can be resolved as dichotomous trees (e.g. Erixon & Oxelman 2008, Minder & Widmer 2008). Lots of species have indeed been shown as not monophyletic. This applies to even morphologically well recognisable species which are nevertheless not found as exclusive lineages based on sequence datasets or AFLP markers. Some recent examples include species of *Camassia*, Asparagaceae (Fishbein & al. 2010), *Stephanomeria*, Asteraceae (Ford & al. 2006) *Ruppia*, Ruppiaceae (Ito & al. 2010), *Pinus*, Pinaceae (Syring & al. 2007) or *Ipomopsis*, Polemoniaceae (Wood & Nakazato 2009).

There are biological reasons leading to species non-monophyly. But the way how species were defined is also important. Although the biological and phylogenetic species concepts are most favoured as species definitions, it can be assumed that most plant species were in fact described as morpho-species. So they are defined based on morphological similarities rather then on reproductive barriers. Consequently, a "good" morphological species is not necessarily a "good" biological or phylogenetic species. Discrepancies between morphology and sequence data are therefore not surprising. Poor taxonomic knowledge or misinterpreted morphology are therefore further reasons for species non-monophyly (Funk & Omland 2003).

1.6 Marker choice for species-level studies

As outlined above, it appears that species non-monophyly can almost be expected throughout the angiosperms and should therefore be considered when designing species-level studies. This would mean that several individuals, especially from widely distributed or morphologically variable species should be included. A solid taxonomic understanding of the study group is also desirable to guide the taxon sampling.

Current sequencing techniques allow the inclusion of more taxa and sequences in a given study. Even large sequence datasets with thousands of nucleotides can be generated in short time and with reasonable effort. But nevertheless, the outcome of the phylogenetic study will depend on the markers used, not just on the pure amount of data generated. Markers that provide good phylogenetic signal are therefore fundamental when attempting to resolve species relationships. Chloroplast introns and spacers are the main source of data for such studies as they have constantly been shown to be the best-performing regions for species-level applications (Borsch & Quandt 2009). Currently, rather few plastid regions are frequently sequenced at species level while others are only rarely used. In order to find and recommend the most informative regions, (Shaw & al. 2005, 2007) have provided comparisons of potentially informative characters (PICs) of different plastid regions resulting in a ranking of the regions according to their levels of variability. But as emphasised by (Borsch & al. 2009, Borsch & Quandt 2009), the phylogenetic structure of markers differs. The reasons are different mutational dynamics, varying levels of conservation as the result of functional constraints and structural features. Consequently, is not just pure sequence variability but the quality of the phylogenetic signal that should be taken into account when choosing markers (Borsch & Quandt 2009, Müller & al. 2006). However, comparisons of phylogenetic structure of markers in the same taxon dataset are still largely lacking.

1.7 Plant DNA barcoding

DNA barcoding was proposed some years ago as a way to identify species by means of a short DNA sequence – the DNA barcode. The underlying hypothesis for DNA barcoding is that species are distinct entities and recognisable by means of DNA sequences unique for them. In the strict sense, DNA barcoding aims at recognising already known species, not at discovering new species.

Contrary to animal groups, for which the mitochondrial COI gene is widely and successfully applied after it was initially proposed by Hebert & al. (2003), there is still no consensus which genomic region(s) to use as barcodes for flowering plants. The *rbcL* gene was among the first proposed regions, and by now, many other markers have been evaluated for plant barcoding purposes (Edwards & al. 2008, Fazekas & al. 2008, Ford & al. 2009, Lahaye & al. 2008, Newmaster & al. 2006, Newmaster & al. 2008, Seberg & Petersen 2009, Zhang & al. 2009). An overview of all barcode markers tested so far was provided by Hollingsworth & al. (2009). Almost all authors agreed that a multi-locus barcode will be needed for plants, with several thousands of nucleotides to be sequenced for reliable species identifications. The plastid loci *rpoC1*, *rpoB*, *rbcL*, *matK*, *psbA-trnH*, *atpF-atpH*, and *psbK-psbI* were among the most frequently used barcodes. Currently the coding regions *matK* and *rbcL* and the *psbA-trnH* spacer are among the most often suggested regions. *MatK* and *rbcL* have been recently adopted as universal plant barcodes by the Plant Working Group of the Consortium for the Barcoding of Life (http://www.barcoding.si.edu/plant_working_group.html). The only plastid intron evaluated as plant barcode so far is the *trnL* intron (Gonzalez & al. 2009, Taberlet & al. 2007). This is rather surprising, because plastid introns are among the most frequently used regions for phylogenetic studies at low taxonomic levels (Borsch & Quandt 2009, Kelchner 2002).

So far available studies reported a species identification success of about 60-70% (e.g. Fazekas & al. 2008, Gonzalez & al. 2009, Kress & Erickson 2007). Therefore, the question is sometimes raised whether plant species are as distinct and can be as easily barcoded as animal species (Fazekas & al. 2009). But at the same time, there are only few botanical barcoding studies where a complete taxonomic group was sampled based on an underlying solid taxonomic understanding of the study group. Furthermore, many plastid markers have not yet been evaluated as barcodes. In many of the studies cited above not the most variable markers were used as barcodes. Therefore the unsatisfying results of the so far available studies may be due to limited marker variability and other barcode markers could give better results.

The problems encountered in phylogeny inference at species level (see above) also apply to DNA barcoding. Non-monophyly of morphospecies is again a potential problem as there will be no unique sequence characterising a species if different chloroplast haplotypes are found in different populations (Fazekas & al. 2009). Barcoding

approaches also immediately raise the point of the species concepts used to define the species to be barcoded. Barcoding success will likely depend on the underlying taxonomy - poorly defined species can be more difficult to barcode. If too many different species are sampled which are in fact one single species described under several names, bacroding success will be poor. Therefore, several authors advocate the application of phylogenetic methods to estimate species boundaries prior to barcoding studies (Meyer & Paulay 2005, Zhang & al. 2009). Especially in those groups with a poor taxonomic understanding, phylogeny inference would help estimating species limits thus enabling a more accurate barcoding approach. Species monophyly may even be not required for successful barcoding, as long as one species does not share any sequence with its closest relatives.

1.8 Background, aims and outline of this study

One of the world's most comprehensive living collections of the Rhipsalideae has been established at the Botanical Gardens Bonn. It is a result of a long ongoing research interest in the Rhipsalideae of the supervisor of this thesis, W. Barthlott. Many additional data and observations are also already available which can back up the molecular phylogenetic findings and the living collection allows further observations.

Only few Cactaceae tribes or genera have been extensively evaluated by DNA data so far, in contrast to other comparatively popular plant families (such as Orchidaceae or Bromeliaceae) where relationships are much better understood. The Rhipsalideae are one of the best-suited Cactaceae groups to study phylogenetic relationships. It is a comparatively small group. Despite the taxonomic uncertainties and disagreements in the past, the works of Barthlott & Taylor (1995) and Taylor & Zappi (2004) have provided a good taxonomic understanding of the group on which the taxon sampling can be based. All phylogenetic studies published so far suggest that multiple datasets have to be generated in order to resolve a Cactaceae species-level tree. This therefore study presents a much higher amount of sequence data generated and may serve as a case study for a resolution of a Cactaceae species-level tree. The markers sequenced were chosen based on the already available experiences in comparable studies and on the success of these markers in other Cactaceae groups. Cactaceae have not been subject to a barcoding study so far although accurate species delimitation and recognition are important issues in Cactaceae as most are CITES-listed (Hunt 1999 and www.cites.org). Having accurate species identification tools is also important because cacti are often difficult to identify due to their phenotypic plasticity.

The aim of this study is an integrated approach combining molecular datasets and morphological characters to arrive at a comprehensive understanding of the study group. Currently widely discussed topics such as marker performance in phylogeny reconstruction and in DNA barcoding are important elements.

1.8.1 Study outline

Chapter 2 presents a phylogenetic survey of *Pfeiffera*. It is based on the already available evidence that *Lepismium* as it had been circumscribed by Barthlott & Taylor (1995) is polyphyletic and part of it is a lineage distant to the Rhipsalideae (Nyffeler 2002). Therefore, the first aim was to infer the generic limits of *Pfeiffera* and *Lepismium*, thus also inferring the circumscription of the Rhipsalideae. The position of *Pfeiffera*, its circumscription and relationships between its species are evaluated based on a sampling comprising 8 of 9 species and datasets of the rapidly evolving plastid regions *trnK/matK*, *rpl16* intron, *trnG* intron, *psbA-trnH*, *trnQ-rps16*, *rps3-rpl16*, *trnS-trnG* and *matK*.

A detailed molecular phylogenetic study of the Rhipsalideae is presented in Chapter 3. It is among the most comprehensive species-level-study for the Cactaceae and is based on a nearly complete sampling of the group. A dataset of six fast evolving plastid regions *trnK/matK*, *rpl16* intron, *psbA-trnH*, *trnQ-rps16*, *rps3-rpl16* and *matK* was generated. Several accessions of the widely distributed and variable species have been sampled to cover the morphological and geographical variation. This was also used to test whether these species are monophyletic or not. A further goal was to identify the species using sequence data. Finally, the performance of the markers used was to be compared on the one hand with regard to their phylogenetic structure and ability to resolve a species-level tree. And on the other hand with regard to their variability at species level and applicability for DNA based species recognition (DNA barcoding).

A detailed analysis of morphological characters of the Rhipsalideae is presented in Chapter 4. Ancestral states of main vegetative and floral characters are reconstructed using a Bayesian approach and the evolution of these characters is discussed. A focus is put on the characters associated with the epiphytic life form and the floral traits. The characters synapomorphic for the major Rhipsalideae clades are pointed out.

Rhipsalis baccifera is the most widespread cactus. Chapter 5 presents a haplotype network analysis based on the *rps3-rpl16* spacer and the *rpl16* intron as a first step towards a better understanding of the distribution patterns and historical phylogeography of this species.

To obtain further resolution among the populations, microsatellite markers for *Rhipsalis baccifera* have been developed using 454 sequencing to be used in future applications. The approach is outlined in Chapter 6.

Chapter 2

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

Summary

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*, *rps*3-*rpl16*, *trnS-trnG*) and the rapidly evolving gene *matK* of the plastid genome. Phylogenetic analyses revealed *Pfeiffera* polyphyletic, comprising two unrelated lineages, both highly supported. One clade includes the type species, *Pfeiffera 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.

2.1 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).

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 *Pfeiffera cereiformis* Salm-Dyck (= *Pfeiffera 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) proposed *Pfeiffera* as an 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 of 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, and while some authors recognised eight genera, others combined most taxa into *Rhipsalis* Gaertn., as summarised in Table 2.1. 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 maximally 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 using DNA data.

Table 2.1 Changing circumscriptions of *Pfeiffera* and allied genera.

	Salm-Dyck 1850	**Schumann 1889**	**Vaupel 1925-1926**	**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.
Lyman-bensonia	not yet described	= *Rhipsalis*	= *Rhipsalis*	= *Acantho-rhipsalis*	= *Acantho-rhipsalis*	1 sp.	= *Lepismium*	= *Pfeiffera*

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 emphasize 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 as understood based on morphology are not monophyletic, e.g. (Arias & al. 2005, Butterworth & Wallace 2004, Edwards & al. 2005, Nyffeler 2002, Ritz & al. 2007). And, 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 datasets 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 *trnK* 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 low a 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).

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 first to evaluate the current circumscription of *Pfeiffera* and second, 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.

2.2 MATERIAL AND METHODS

2.2.1 Plant material and taxon sampling

The main source for plant material were the Botanical Gardens of the University of Bonn, where one 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 *Pfeiffera 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 the Appendix 1.

2.2.2 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 homogenized 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 0,2 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 10ng/µl was made to be used for PCR.

2.2.3 Amplification and sequencing

All primers used in this study and the detailed amplification conditions are listed in Appendix 2. The *trnK*/*matK* region was amplified in overlapping halves using the primer pair *trnK*-F and ROS*trnK*655R for the 3' fragment and AC*trnK*500F and *trnK*2R for the 5' fragment. Amplification conditions followed Müller & Borsch (2005). The *psbA*-*trnH* IGS was amplified with the newly designed primers CA*psbA* and CA*trnH* using a touchdown program with an initial denaturation step 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 *rps*3-*rpl16* IGS and the *rpl16* intron were co-amplified using newly designed primers CArps3F, annealing to the *rps*3 exon and CA*rpl16*R which anneals to the *rpl16* 3' exon. Amplification conditions were: An initial denaturation step 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 *trnQ*2 and *rps16*x1 were: An initial denaturation step 2 min at 95°C, followed by 35 cycles of 30 se 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 and min at 72°C with a final extension step of 15 min at 72°C. All PCR products were stained with 100x 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 *trnG*2S or *trnG*2G and a fourth read from CA*trnS*G-40R was often required due to a frequently occurring poly-T stretch in the *trnG* intron. The *rps*3-*rpl16* spacer and the *rpl16* intron were sequenced with the amplification primers and the additional internal sequencing primer CA*rpl16*-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+; www.phyde.de).

2.2.4 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 the Appendix 3. Inversions were placed separately during alignment and reverse-complemented prior to phylogenetic analyses (Quandt et al. 2003, Borsch & Quandt 2009). Secondary structures of hairpins associated with inversions were calculated using RNAstructure 5.0 (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).

2.2.5 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. Finally, analyses including all markers in combination and each marker alone were performed to determine species-level relationships within *Pfeiffera* and species newly found as 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.

2.2.6 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 was 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 5 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.

2.3 RESULTS

2.3.1 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, study ID S11122).

2.3.2 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 *trnK* gene provided each ca. 17% variable and 7% informative characters. 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 *trnK* gene and comprised 7556 aligned characters with individual sequences ranging from 4321 to 6761nt with an average length of 6264 nt per taxon. The detailed sequence characteristics are given in Table 2.2. In total, 16 regions of uncertain homology (mutational hotspots) as well as incomplete beginnings and endings as well as the exons were excluded (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.

Table 2.2 Sequence statistics of individual regions and the combined dataset for *Pfeiffera*.

	trnK **intron**	***matK***	***trnS-G*** **spacer**	***trnG*** **intron**	***rps3-rpl16*** **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

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 *trnK* 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.

2.3.3 Inversions

Three inversions were observed. A 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 either “AAAA” / “TTTT” or “CAAA” / “TTTG” was observed within the *trnK* gene, about 780 positions downstream from the *trnK* start codon throughout the Cactaceae dataset.

2.3.4 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. 2.1.

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 2.3). The first clade, termed clade I in the following, was supported by 100% JK/1.00 Posterior Probability (PP) and comprised *Pfeiffera 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.

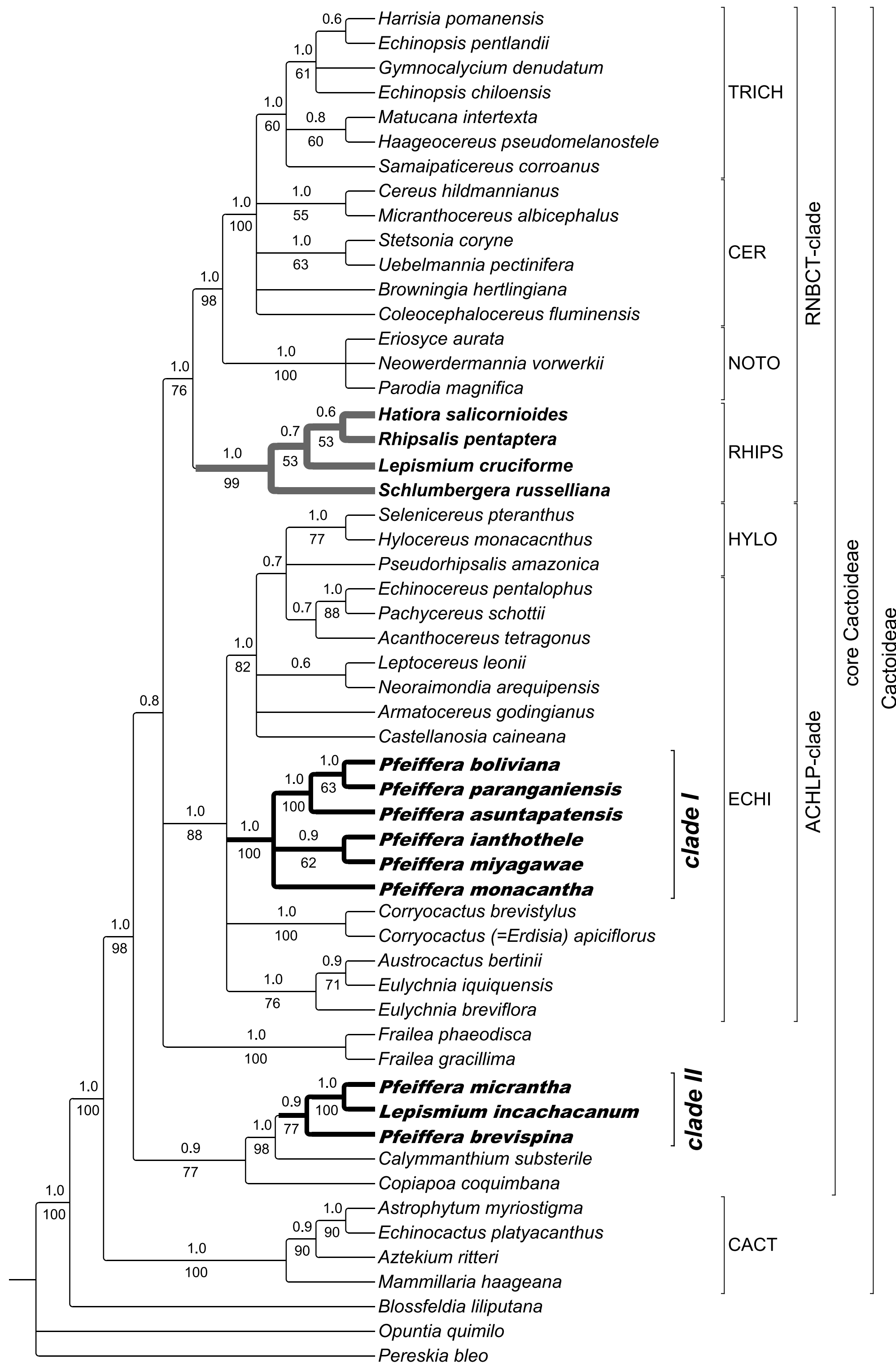

Figure 2.1 Overview tree of the Cactoideae based on *trnK*/*matK* with coded indels. Tree topology as inferred from Bayesian Inference (50-majority-rule consensus tree). Numbers above branches are Bayesian posterior probabilities, numbers below braches are jackknife support values from 10.000 replicates. Tree annotation above tribal level follows Nyffeler (2002), tribal classification follows the New Cactus Lexicon; 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: Echinocereeae, HYLO: Hylocereeae, RHIPS: Rhipsalideae, NOTO: core Notocacteae, CER: Cereeae, TRICH: Trichocereeae.

Table 2.3 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 x in *L. brevispina*, 6 x in *L. incachacana* and *L. micrantha* (missing data for *Calymmanthium*)
	45	3081-3143	Gap in *Lymanbensonia* (missing data for *Calymmanthium*)
	65	3984	Gap in *L. 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 *L. incachacana* and *L. micrantha*
psbA-trnH	130	6166-6204	Gap in *Pfeiffera ianthothele*
	133	6201	Gap in *L. 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 *L. incachacana* and *L. micrantha* (missing data for *L. brevispina*)

2.3.4.1 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. 2.2 and Table 2.4. The parsimony trees inferred from single markers were slightly incongruent and not fully resolved.

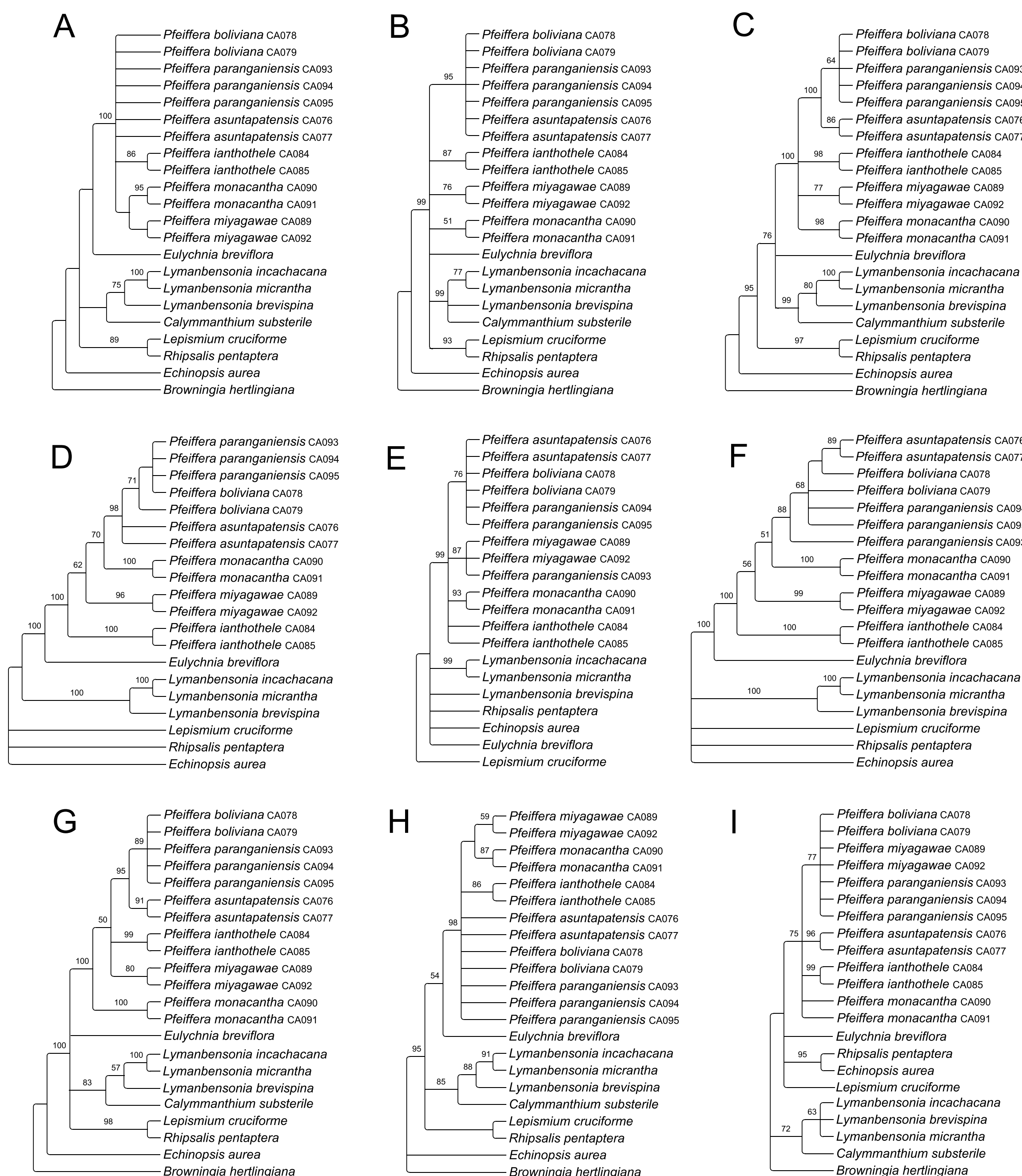

Figure 2.2 Trees inferred from single markers. A: *matK*, B: *trnK* intron, C: *trnK/matK*, D: *trnS-G* spacer, E: *trnG* intron, F: *trnS-G*, G: *rpl16* intron, H: *psbA-trnH*, I: *trnQ-rps16*. All trees are strict consensus trees found by the parsimony ratchet. Numbers above branches are jackknife support values from 10000 replicates.

Table 2.4 Comparison of trees from parsimony analysis of single markers.

	matK	***trnK* intron**	***trnK/matK***	***trnS-G* 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

2.3.4.2 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 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. Figure 2.3 shows the Bayesian topology with additional JK support values.

Two main 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, (100% JK, 1.00 PP) comprising *P. boliviana, P. asuntapatensis* and *P. paranganiensis*. The specimens 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* specimens formed a clade (51 % JK/98 PP) whereas *P. boliviana* was still not found as monophyletic.

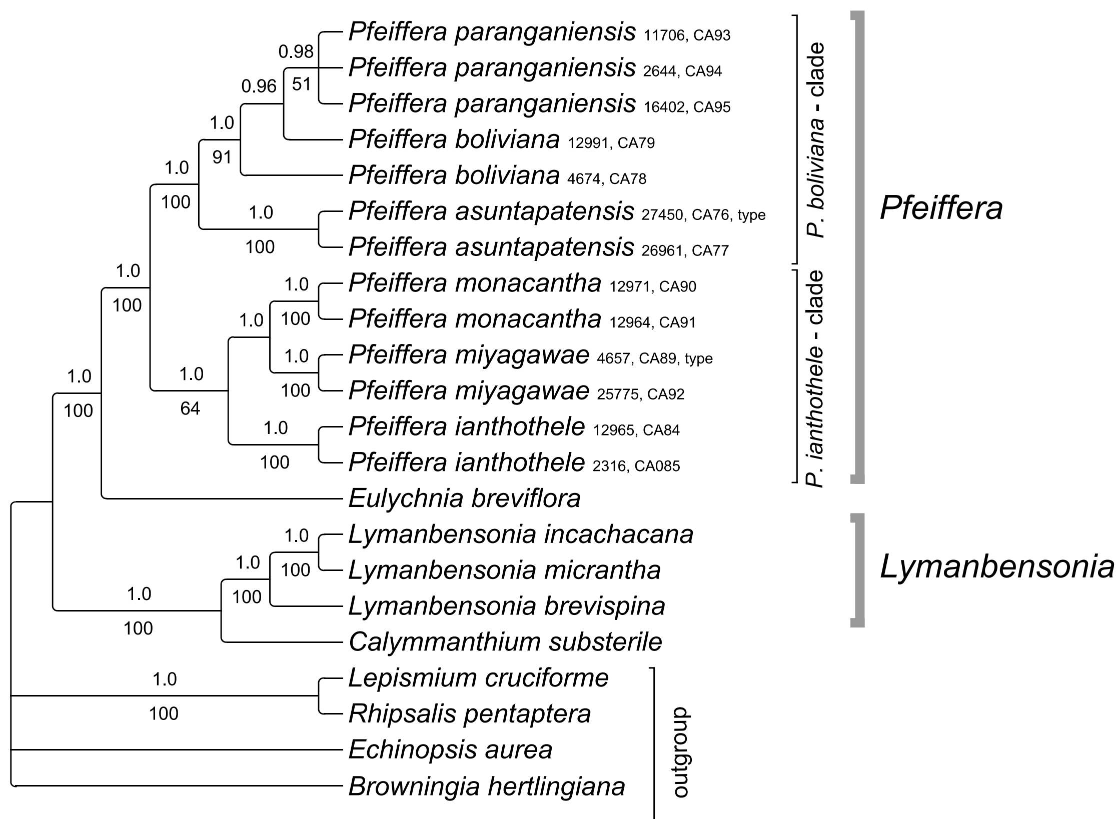

Figure 2.3 Majority-rule consensus tree based on combined chloroplast dataset (*trnK/matK*, *trnS-G*, *rpl16*, *psbA-trnH*, *trnQ-rps16*) and coded indels showing relationships in *Pfeiffera.* Numbers above branches are Bayesian posterior probabilities, jackknife support values from 10.000 replicates are given below the branches. For each *Pfeiffera* sampled, the accession from the Bonn Botanical Garden and the CA-isolate number are given next to the name.

2.4 DISCUSSION

2.4.1 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. 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 2.4). 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 *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 *trnK* CDS was found to be homoplastic. An inferred secondary structure shows the inversion to affect only the terminal loop of a hairpin. 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 *trnK* CDS reveals that only one amino acid is changed due to the inversion. Since *trnK* 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.

2.4.2 Crcumscription 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*.

2.4.3 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 Hylocereeae.

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 suggested *Erdisia* Britton & Rose. The terrestrial genera *Corryocactus* and *Erdisia* (currently included in *Corryocactus* as the *C. squarrosus* - group), a group of shrubby slender-stemmed cacti from Peru, Bolivia and Chile have constantly been proposed as the nearest relatives of *Pfeiffera* subsequently, 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 could be 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 he 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 F. 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 and 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 *Pfeiffera* and *Acanthorhipsalis* to 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.

2.4.4 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 *rbc*L 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 (reported in Cactaceae Cons. Init. 5, 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 a new name Lymanbensonieae, since *Lymanbensonia* is the larger genus; the tribal name is formally proposed below. Although merging of both genera under the 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.

2.4.5 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 data indicate these groups as polyphyletic: the *Lymanbensonia*-group has to be excluded and expanded, while the *Pfeiffera*-group has additionally to include *P. monacantha* and the *Acanthorhipsalis*-group is highly polyphyletic; a part of it belongs in *Lymanbensonia*.

Our data find two clades within *Pfeiffera*. One, informally termed *P. boliviana*-clade includes *P. asuntapatensis*, *P. boliviana* and *P. paranganiensis* (100 % JK, 1.0 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. *Pfeiffera 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* specimens sampled are resolved as distinct, indicating that this species might not be monophyletic. It is variable, especially in flower shape and colour (Fig. 2.4 E), showing the highest colour variation within *Pfeiffera*. The need for further population-level and taxonomic studies has been pointed out by Ibisch & al. (2000).

The second *Pfeiffera* lineage is termed *P. ianthothele*-clade and comprises to 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. *Pfeiffera 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. *Pfeiffera 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).

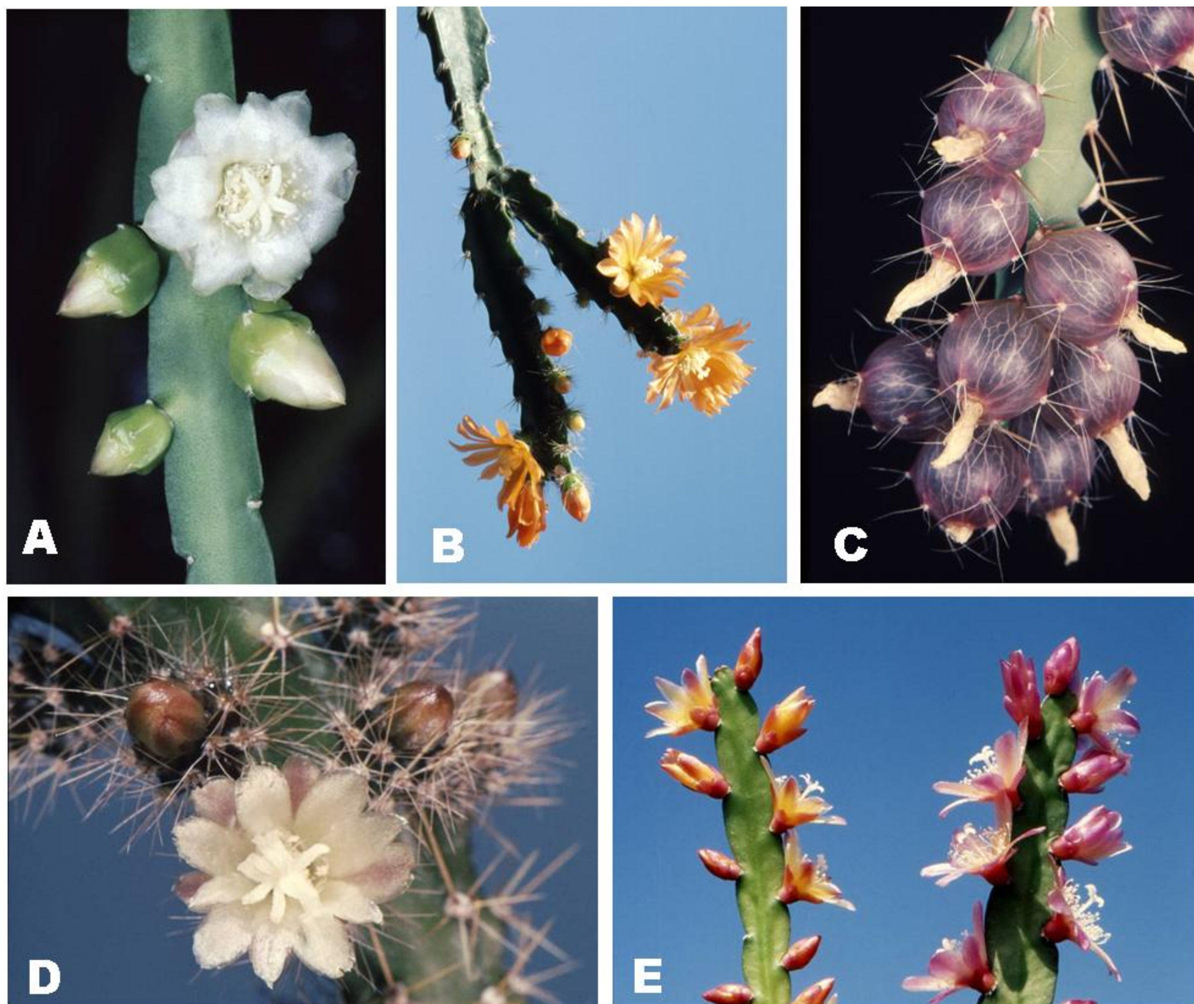

Figure 2.4 *Pfeiffera*– A: *Pfeiffera paranganiensis* (*Ritter 343*, cult. ZSS); B: *Pfeiffera miyagawae* (type collection *Miyagawa s.n.,* 1974, iso HEID 32857, cult. BG Bonn 4657); C – D: *P. ianthothele*, cult. BG Bonn 2316; C: fruits, D: flowering stems; E: flower colour variation in *P. boliviana,* left: BG Bonn 4675 *(Kimnach 2546)*, right BG Bonn 4674 without locality data). Photos: W. Barthlott.

2.4.6 Relationships within *Lymanbensonia*

Our data find *L. brevispina* as sister to *L. micrantha* and *L. incachacana* (97% JK, 1.00 PP, Fig. 2.1; 100% JK, 1.00 PP, Fig. 2.3). 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. It was placed next to *Lepismium monacanthum* (=*Pfeiffera monacantha*) by Barthlott & Taylor (1995) while Kimnach (1984) suggested a close relationship to *Rhipsalis crenata* (= *Pfeiffera crenata*). The position of *Lepismium incachacanum* (= *Lymanbensonia incachacana*) 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. 2.5 D). 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). The intensely red or magenta coloured flowers and also the scarcely expanded perianth of *L. incachacana* make it fit well into *Lymanbensonia*. It is resolved next to *L. micrantha*, an easily recognisable species with magenta flowers and a well developed receptacle-tube (Fig. 2.5 E-F). Barthlott & Taylor (1995) placed *L. micrantha* within *Lepismium* 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 Botanic Gardens (Bolivia, near Corvico, *Kirschnek s.n.*, 1981, BONN, in spirit, Fig. 2.5 A-C) was probably the only cultivated specimen worldwide. Unfortunately the plant did not survive and we could not sample it here. But the studies of the plant's morphology, especially the floral morphology convincingly supports placement within *Lymanbensonia*.

2.4.7 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 recognized 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 recognized *Acanthorhipsalis* as a genus with *A. monacantha*, *A. crenata* and *A. micrantha* (which they chose as type species, erroneously overlooking Schumann's type, *A. monacantha*). The main characters separating *Acanthorhipsalis* from *Rhipsalis* were the spiny areoles and

receptacle tube. Kimnach (1983) argued that *Acanthorhipsalis* should not be recognized 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 and Barthlott (1987), who 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.

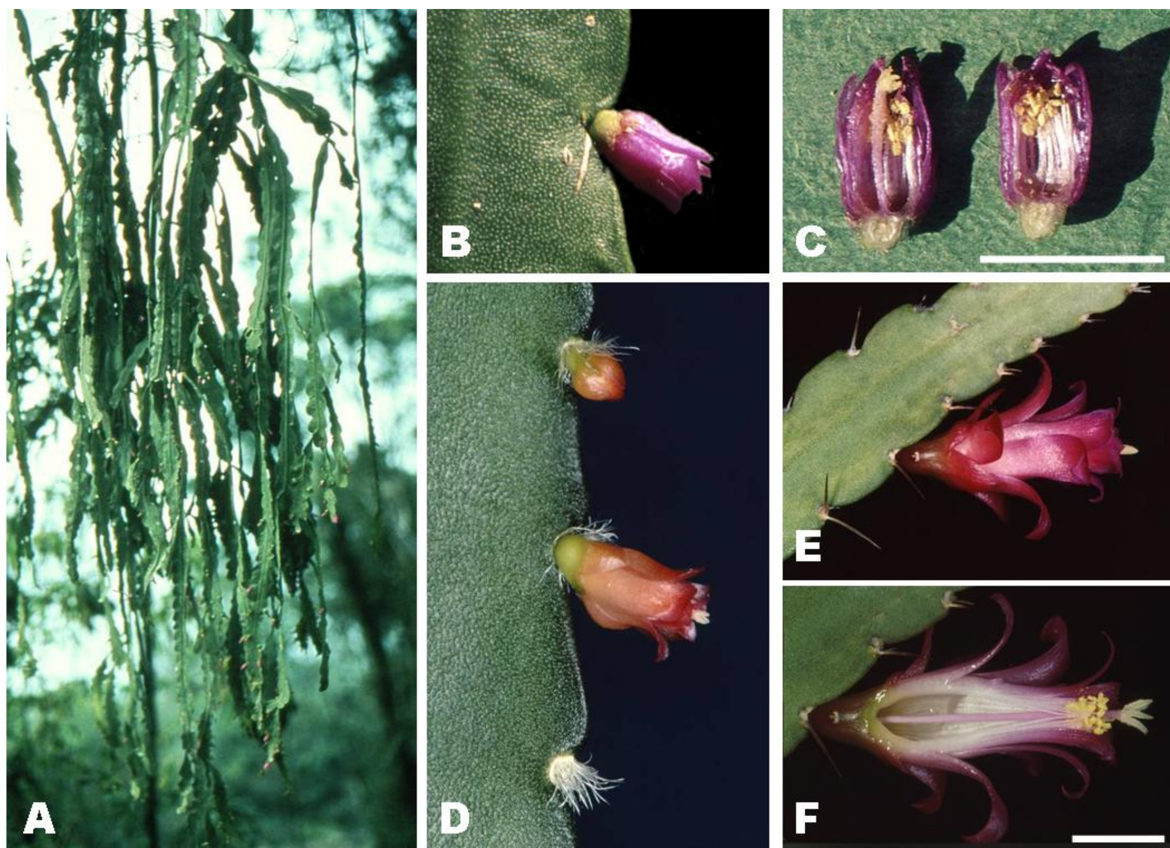

Figure 2.5 *Lymanbensonia* – A-C: *L. crenata* (*Kirschnek s.n.* 1981, voucher BONN) A: plant in habitat, B: flower, C: flower section; D: *L. incachacana* (*Miyagawa 2*, cult. BG Bonn 2639, without locality data, voucher BONN); E-F: *L. micrantha* (*Vargas s.n.*, voucher HNT, cult. BG Bonn 13602, ex UCBG 59.1196, ISI 1164.), E: flower, F: flower section showing the well developed receptacle-tube. Scale bars: 1 cm. Photos A: E. Kirschnek, B-C: R. Bauer, D-F: W. Barthlott.

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; *Pfeiffera* had been accepted as monotypic until the inclusion of *P. miyagawae*. The "cereoid" habit of *P. ianthothele* is now shown to be not so unique as had been thought; it is shared by *P. miyagawae* and most likely represents 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 stems 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 recognized 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 2.5. The main differences are the habit and flower shape as well as the fruits. A further character, already pointed out by Berger (1926), is 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 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), 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 support 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 macromorphological characters are regarded. The micromorphology of seeds and pollen as well as anatomical characters might provide further informative characters.

2.4.8 Biogeographical patterns

Pfeiffera, *Lymanbensonia* and *Lepismium* have separate distribution areas. *Pfeiffera* is distributed from 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 is found sympatrically with *L. brevispina* (Kimnach 1984). *Lepismium*, together with other Rhipsalideae has its distribution centre in South-eastern 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 South-eastern Brazil with the other Rhipsalideae and reached the Andes later, whereas *Pfeiffera* and *Lymanbensonia* evolved in the Andes of Bolivia or Peru.

Table 2.5 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	farrowly campanulate, tepals not entirely expanded, spreading at the apex	flowers somewhat erumpent and pendent, campanulate, tepals expanded to ca. 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	south-eastern Brazil to northern Argentina and southern Bolivia

2.5 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, rps*3-*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 data sets of several markers selected for their high phylogenetic structure are needed as emphasised by (Borsch & Quandt 2009, 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 the Rhipsalideae are still not known with confidence and remain among of 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.

2.6 TAXONOMIC CONCLUSIONS

New circumscriptions of *Pfeiffera* (Echinocereeae) and *Lymanbensonia* (Lymanbensonieae), with a key to their species

Echinocereeae (Britton & Rose) F.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 infraspec.), *Escontria* Rose (1 sp.), *Eulychnia* Phil. (4 spp. + 1 infraspec.), *Jasminocereus* Britton & Rose (1 sp.), *Leptocereus* Britton & Rose (11 sp.), *Myrtillocactus* Console (4 spp.), *Neobuxbaumia* Backeb. (8 spp.), *Neo-raimondia* 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 infraspec.), *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, South-western USA, Brazil, Peru, Bolivia, Chile, Western and Southern Argentina.

***Pfeiffera* Salm-Dyck**

in Cact. Hort. Dyck. ed. I. 40.: 1845. Type species: *P. cereiformis* Salm-Dyck in Cact. Hort. Dyck. ed. I.: 40. 1845. ≡ *Cereus ianthothele* Monv. in Monv. Hort. Universel 1: 218. 1839, (as "*Cereus ianthothelus*") ≡ *Pfeiffera ianthothele* (Monv.) F.A.C. Weber Dict. Hort. [Bois] 2: 944. 1898.

Generic synonyms: *Acanthorhipsalis* Britton & Rose in Cactaceae (Britton & Rose) 4: 211. 1923. Type species: *A. micrantha* (Vaupel) as incorrectly designated by Britton & Rose in Cactaceae (Britton & Rose) 4: 212. 1923. *Rhipsalis* subg. *Acanthorhipsalis* K.Schum. in Gesamtbeschr. Kakt.: 615. 1898.

Type species: *R. monacantha* Griseb. Abh. Königl. Ges. Wiss. Göttingen 24: 140. 1879.

Accepted species: 6 (+2 infraspec.)

Note. — The name *Acanthorhipsalis* can no longer be maintained for a potential subgenus because its type species *P. 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, ca. 4 cm in diameter, intensely orange, shimmering, pericarpel with prominent, long, dark spines ....4. *P. miyagawae*

– Flowers smaller, ca. 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, Ibisch & Barthlott) Ralf Bauer in Cactaceae Syst. Init. 20: 6. 2005. ≡ *Lepismium asuntapatense* M.Kessler, Ibisch & Barthlott 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. Holotype (syntypes): Bolivia, La Paz, 1890 *Bang 601* (US, lectotype K, designated in Barthlott & Taylor in Bradleya 13:46. 1995.), *Rusby 2048* (US, NY, lectoparatype).

3. *Pfeiffera ianthothele* (Monv.) F.A.C. Weber in Dict. Hort. [Bois] 2: 944. 1898 ≡ *Cereus ianthothele* Monv. in Monv. Hort. Universel 1: 218. 1839. Holotype: 'Montevideo' cult. *Hort. Monville*, not known to have been preserved. Neotype designated by Barthlott & Taylor in Bradleya 13: 45. 1995: Argentina, Salta, 15. Jan. 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. Oct. 1974, *Miyagawa s.n.* (HEID 32854). Isotypes: BONN, ZSS, HNT. 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. Oct. 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 in Bauer (2005)].

5. *Pfeiffera monacantha* (Griseb.) P.V.Heath in Calyx 4(4): 158. 1994. ≡ *Rhipsalis monacantha* Griseb. Abh. Königl. Ges. Wiss. Göttingen 24: 140. 1879. Holotype: Argentina, Salta, San Andrés (west of San Ramón de la Nueva) Orán, 25. Sep. 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*** (Doweld) Ralf Bauer in Cactaceae Syst. Init. 19: 8. 2005.

≡ *Acanthorhipsalis monacantha* subsp. *kimnachii* Doweld in Sukkulenty 4(1-2): 41. 2001 publ. 2002. Replaced synonym: *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 ≡ *Acanthorhipsalis paranganiensis* Cárdenas in Cactus (Paris) no. 34: 126. 1952. Holotype: Bolivia, Cochabamba, Ayapaya, Parangani, Oct. 1947, Cárdenas 4856 (LIL 531577), isotype US.

Lymanbensonieae N. Korotkova & Barthlott in Willdenowia 40:166. 2010.
[– Calymmanthieae Lakomski in Swiat Kakt. 38 (1 – 2): 66. 2003, nom. inval., without Latin diagnosis (ICBN Art. 36.1)].

Type: *Lymanbensonia* Kimnach.

Diagnosis. — 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(3): 101 1984.

Type species: *Cereus micranthus* Vaupel, Bot. Jahrb. Syst. 50: Beibl. 111: 19. 1913.

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

Accepted species: 4

Note. — In his revision of *Acanthorhipsalis* 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 epiphytic, epiphytic habit obligatory or facultative. Plants usually erect at first, then spreading, pendent. *Adventitious roots* lacking. *Branching* mesotonic, *Stems* of indeterminate growth, old stem-segments not deciduous, stems flattened, angled at first in *L. micrantha*. *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

with 1–10 yellowish whitish or grey spines 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 to 3 cm long, narrowly tubular bell-shaped, tepals not fully expanding, spreading at apices perianth intensely coloured (red, pink, orange, magenta) or white in *L. brevispina*. *Hypanthium* (receptacle-tube) not conspicuously developed, except in *L. micrantha*. *Stamens* ca. 20–50, filaments and anthers white or whitish/ cream, *Fruits* globose or subglobose, coloured (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 coloured (orange, pink, 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 in Willdenowia 40:166. 2010. Basionym ≡ *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 *Acantho-rhipsalis brevispina* was validated by Doweld, but, as currently known, published later than Hunt's name (Hunt 2003: 3; Eggli & Zappi 2003: 10), thus rendering Doweld's name illegitimate. As the name *Pfeiffera 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(1-2): 34, 2001 publ. 2002. ≡ *Hariota crenata* Britton in Bull. Torrey Bot. Club xviii. 35. 1891. Holotype: Bolivia, La Paz, Yungas, 1885, *Rusby 2047* (US).

3. *Lymanbensonia incachacana* (Cárdenas) Barthlott & N. Korotkova in Willdenowia 40:167. 2010. ≡ *Rhipsalis incachacana* Cárdenas in Cactus (Paris) No. 34, 125. 1952. Holotype: Bolivia, Cochabamba, Incachaca, *Cárdenas 4855*, June 1950 (LIL 511565).

4. *Lymanbensonia micrantha* (Vaupel) Kimnach in Cact. Succ. J. (Los Angeles) 56(3): 101. 1984. ≡ *Cereus micranthus* Vaupel in Bot. Jahrb. Syst. 50: Beibl. 111: 19. 1913. Holotype: Peru, Puno, near Sandía, 31. July 1902, *Weberbauer 1353* (B, destroyed), isotype: US.

Chapter 3

How much does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalideae (Cactaceae)

Summary

The taxonomic units and species limits in the Cactaceae have been difficult to define and molecular phylogenetic studies so far yielded largely unresolved trees, so relationships within Cactaceae remain insufficiently understood. This study focuses on the predominantly epiphytic tribe Rhipsalideae and evaluates the utility of a spectrum of rapidly evolving and non-coding plastid genomic regions. The study including 51 of the 52 accepted species, and 11 of 13 of the infraspecific taxa. Six plastid regions were sequenced, comprising two group II introns (*trnK*, *rpl16*), three intergenic spacers (rps3-*rpl16*, *psbA-trnH*, and *trnQ-rps16*) and *matK*, totalling c. 4200 nucleotides per sample. These regions were evaluated for their phylogenetic signal and for their species discrimination power for DNA based species recognition based on beforehand defined operational taxonomic units (OTUs). A well resolved and supported species-level tree could be inferred. The Rhipsalideae were found to be monophyletic and to contain five major clades that correspond to the genera *Rhipsalis*, *Lepismium*, *Schlumbergera*, *Hatiora*, and *Rhipsalidopsis*. The species-level tree was well resolved and supported and the *rpl16* and *trnK* introns yielded the best phylogenetic signal and the best OTU identification potential while *matK*, *psbA-trnH* and *trnQ-rps16* were less effective in both ways. The highest OTU identifications rate of 97% was found using c. 2500 nt. The phylogenetic performance of the markers was not determined by the level of sequence variability and the species discrimination power did not necessarily correlate with the phylogenetic utility of the markers.

3.1 INTRODUCTION

Cactaceae are one of the major floristic components of the New World's arid as well as seasonally moist tropical regions and at the same time one of the most popular plant families in horticulture. While there is little doubt that Cactaceae are a natural group considering morphological and molecular synapomorphies (Barthlott & Hunt 1993, Nyffeler 2002, Wallace & Gibson 2002), the recognition of tribes, genera and species within the family has always been difficult. Many cacti look similar due to convergent evolution, which is frequent in the family – large columnar forms, small globular cacti and epiphytes with flattened, leaf-like stems are suspected to have evolved each several times (Barthlott & Hunt 1993, Wallace & Gibson 2002).

Until now, relationships within the Cactaceae are insufficiently understood and fairly few molecular phylogenetic studies have been conducted, contrary to other popular plant families such as orchids or bromeliads. So far, only major clades of Cactaceae have been identified but their interrelationships remained largely unresolved (Nyffeler 2002, Wallace & Gibson 2002, Hernández-Hernández & al. 2011; Bárcenas & al. 2011). But many tribes and genera were shown to be either poly- or paraphyletic, indicating that they had either been based on plesiomorphic or convergent morphological characters (Applequist & Wallace 2002, Butterworth & Wallace 2004, Arias & al. 2005, Edwards & al. 2005, Ritz & al. 2007, Korotkova & al. 2010). Besides, species-level trees for Cactaceae hitherto remained largely unresolved or weakly supported statistically due to low sequence divergences or insufficient data and sampling. Strongly increased taxon sampling (666 taxa) did not improve on this (Bárcenas et al., 2011). Attempting to resolve a Cactaceae tree, especially at the species level, seems therefore challenging and a combined analysis of genomic regions selected for their high phylogenetic utility and putative performance at species-level was therefore tempting. A recent comparison of the mutational dynamics of non-coding chloroplast regions (introns and spacers) indicated differences in phylogenetic structure even among highly variable non-coding DNA (Borsch & Quandt, 2009). At lower distance levels, i.e. between genera and species, the addition of more chloroplast intron and spacer sequences into combined matrices has generally resulted in increased resolution and support for the inferred trees (e.g. Barfuss & al. 2005, Löhne & al. 2007, Tesfaye & al. 2007). However, phylogenetic structure per informative site has not been compared in detail and the combined dataset of six markers in this survey provides a good case for study.

This study focuses on the tribe Rhipsalideae DC., which is one major group of in total four lineages of epiphytic cacti (Korotkova & al. 2010). The Rhipsalideae occur mainly in South American tropical and subtropical rainforests, with a center of diversity in the Mata Atlântica. A few species are also found in the Northern and

Central Andes. All Rhipsalideae are predominantly epiphytic and/or epilithic and only rarely terrestrial; exhibiting mostly a pendent or semi-erect, shrubby habit with terete, angular or flattened and sometimes almost leaf-like stems. Flower morphology ranges from medium-sized colored bird-pollinated flowers in *Schlumbergera* Lem. to small insect pollinated white flowers in *Rhipsalis* Gaertn. and *Lepismium* Pfeiff. *Rhipsalis* is the largest and most widely distributed genus of epiphytic cacti and *Rhipsalis baccifera* (Mill.) Stearn is the most widespread of all Cactaceae species. Besides it is the only cactus with a natural distribution area extending beyond the Americas into tropical Africa, Madagascar and Sri Lanka (Barthlott 1983).

Rhipsalideae is the oldest name for any epiphytic Cactaceae group at higher rank, and was established by A. P. de Candolle (1828). The tribe in its initial circumscription contained only *Rhipsalis*; other genera were yet to be described. Following the addition of more and more species and genera, generic limits became controversial. Establishing sound generic concepts was difficult due to intergrading vegetative characters, phenotypic plasticity and the largely uniform flower morphology. The two main kinds of treatments were either combining most of the small flowered taxa in an expanded genus *Rhipsalis* (Schumann 1899, Vaupel 1925-1926, Hunt 1967) while recognizing the larger-flowered taxa as generically distinct, or to accept several small genera (e.g. Britton & Rose 1923, Buxbaum 1962). The total number of genera recognized in the past has consequently varied from two (Vaupel, 1925-1926, Hunt 1967) to nine (Backeberg 1959, 1966), reflecting differing emphases on similarities or on differentiating characters.

The Rhipsalideae currently comprise four genera *Lepismium*, *Rhipsalis*, *Hatiora* Britton & Rose and *Schlumbergera*, totaling 52 accepted species (Hunt 2006). That treatment is largely based on the nomenclatural proposals of Barthlott (1987a) and the commented checklist of Barthlott & Taylor (1995), but molecular data subsequently revealed *Lepismium* as polyphyletic and a part of it is now excluded from the Rhipsalideae (Nyffeler 2002, Korotkova & al. 2010). Leaving aside the species excluded from *Lepismium*, a clade that could be referred to as “core Rhipsalideae” was resolved with 100% bootstrap support, but this finding was based on sampling only a single species for each genus (Nyffeler, 2002; Hernández-Hernández et al., 2011; Bárcenas et al., 2011).

More detailed hypotheses on Rhipsalideae relationships based on sequence data of *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH* and ITS have been recently published, focussing on *Schlumbergera* and *Hatiora* (Calvente & al. 2011). The Rhipsalideae and the genera besides *Hatiora* were found as monophyletic, but only based on the plastid data. ITS trees depicted a basal polytomy and the relationships between genera and especially between species remained largely resolved or weakly supported.

Unstable generic limits and constant movement of species between Rhipsalideae genera has resulted in instability of names. Species boundaries have also been controversial, and often gradual variation in morphological characters fostered extreme divergence of "lumping" and "splitting" treatments. As a result, there are about 450 names for the currently accepted 52 Rhipsalideae species (listed by Barthlott & Taylor, 1995). To give one example: *Lepismium cruciforme*, the type species of *Lepismium* has been described under more than 30 names (Britton & Rose 1923). Although DNA barcoding has emerged as a new tool to recognize and later identify species (Hebert & al. 2003), no such approach has yet been attempted for the Cactaceae, albeit necessary. Due to the problems described above, Cactaceae taxonomy is still far from reliable. A high proportion of cacti are believed to be threatened with extinction, and most are CITES-listed (Hunt 1999). An accurate understanding of species limits and the availability of reliable identification tools is therefore desirable for Red Listing and conservation planning.

In addition to phylogenetics, we will therefore also examine our data sets with respect to species identification power of different plastid regions. The Rhipsalideae are well-suited for this purpose: they are a comparatively small group and most of the taxa are well known morphologically and thus allow for the clear determination of Operational Taxonomic Units (OTUs). In addition to that, Rhipsalideae are among the best-collected Cactaceae groups and well represented in botanical collections so that enough documented material exists and all but one species were available for inclusion in this study.

Only few DNA barcoding studies in flowering plants so far used a full taxonomic setting of all known species of a group and also multiple individuals to assess intraspecific variation. Examples include *Paeonia* sect. *Moutan* (Paeoniaceae, Zhang & al. 2009), *Crocus* (Iridaceae, Seberg & Petersen, 2009) and *Psiguria* (Cucurbitaceae, Steele & al. 2010). One of the major challenges of such barcoding approaches is to find the most effective markers that allow as many species as possible to be distinguished. This requires a large number of sequence characters in order to accumulate enough variable sites, especially in recently diverged groups with low levels of sequence divergence. Seberg and Petersen (2009) concluded that about 5800 bp would be necessary to identify all *Crocus* species, which corresponds to 8-9 chloroplast regions and Steele & al. (2010) found at least four regions were required for *Psiguria*.

Two chloroplast markers, the *rbcL* gene and the fast evolving *matK* gene, have been recently adopted as plant barcodes by the Consortium for the Barcoding of Life (CBOL Plant Working Group 2009). Both markers had been among the most frequently proposed barcoding regions, among with the *psbA-trnH* spacer (Kress & al. 2005, Cowan & al. 2006, Kress & Erickson, 2007), although various other markers had also been evaluated for barcoding purposes (Taberlet & al. 2007, Fazekas & al. 2008,

Ford & al. 2009). Usually, these were suggested in view of their simple similarity-based discrimination utility (BLAST approach) irrespective of their phylogenetic signal.

For our study of Rhipsalideae, we have selected six structurally different rapidly evolving plastid regions: two group II introns (*trnK*, *rpl16*), three intergenic spacers (*psbA-trnH*, *trnQ-rps16*, and *rps3-rpl16*) and *matK*. All regions were known to be highly variable at low taxonomic levels and/or have been proposed as candidate regions for DNA barcoding. In addition, *trnK/matK*, *rpl16* and *psbA-trnH* have already been successfully applied within Cactaceae, offering possibilities to compare phylogenetic performance or patterns of molecular evolution and combining datasets.

The *trnK/matK* region is one of the best established phylogenetic markers. It provides a high number of informative characters, even at low taxonomic levels, exhibits high phylogenetic structure (Müller & al. 2006, Borsch & Quandt, 2009) and, as stated above, *matK* is among the most promising candidates for a barcode (e.g. Chase & al. 2007, Lahaye & al. 2008). The *psbA-trnH* spacer is among the most variable chloroplast spacers. Although there are some problems limiting its usage, such as frequent indels, microsatellites, inversions and a high degree of homoplasy (Borsch & Quandt, 2009, Devey & al. 2009, Whitlock & al. 2010), *psbA-trnH* may still be a successful barcode marker due to its high intraspecific variability (Cowan & al. 2006, Chase & al. 2007, Kress & Erickson 2007, Seberg & Petersen 2009). The *rpl16* intron is the most variable chloroplast intron (Kelchner 2002) and is one of the most frequently used markers in phylogenetics. It has so far shown high intraspecific variability and yielded good phylogenetic signal between closely related taxa, compared to other chloroplast markers in the same taxon set (Löhne & al. 2007, Tesfaye & al. 2007, Sánchez del-Pino & al. 2009). Although rarely used so far, the *trnQ-rps16* spacer is expected to be informative at low taxonomic levels as well. Evidence for this comes from the high percentage of potentially informative characters (PICs) as found by Shaw & al. (2007) and the results of Calviño & Downie (2007) and Fleischmann & al. (2010).

Phylogeny reconstruction and barcoding are different approaches. Even if the sequence data would not resolve the evolutionary relationships due to lack of information or conflict among informative sites, the same markers may provide enough autapomorphic substitutions to distinguish between species. Nevertheless, it is likely that markers which contain sufficient information to resolve phylogenetic relationships will be valuable DNA barcodes as well. We were therefore interested to examine if there is a correlation between overall variability of a genomic region (useful for barcoding) and phylogenetic structure (required for tree inference). Our approach is twofold: Using the same data set, we first aim at resolving phylogenetic relationships at species level. Secondly, we evaluate which are the best suited markers for DNA-

based species recognition within Rhipsalideae, either alone or in combination. Moreover, we will discuss the impact of molecular characters for delimitations of genera and species within Rhipsalideae also in light of the evolution of morphological characters.

3.2 MATERIAL AND METHODS

3.2.1 Plant material and taxon sampling

The plant material used in this study was largely obtained from the living collections of the Botanical Gardens of the University of Bonn, where the world's probably most comprehensive living collection of the Rhipsalideae has been established over three decades by W. Barthlott. Further material was obtained from the Rhipsalideae collections of the Botanical Garden Berlin-Dahlem and the Royal Botanic Gardens, Kew, as well as from the Sukkulenten-Sammlung Zürich. We have sampled 52 species including all the infraspecific taxa. Taxon sampling followed the most up-to-date reference work for the Cactaceae (Hunt, 2006) where 53 species are accepted in Rhipsalideae. *Rhipsalis goebeliana* Backeb. was sampled additionally. *Lepismium incachacanum* (Cárdenas) Barthlott, classified as Rhipsalideae therein, was not sampled since we recently found it not to belong therein (Korotkova et al., 2010). No material was available of *Rhipsalis ormindoi* N.P. Taylor & Zappi and the recently described *Rhipsalis aurea* M. F. Freitas & J. M. A. Braga (de Fatima Freitas et al., 2009).

Morphologically variable and widely distributed species such as *R. micrantha*, *R. teres* and *R. baccifera* were represented by specimens from different countries or collection sites, thus covering some of their intraspecific variation. In total, our analysis contains 110 ingroup and 5 outgroup taxa. All taxa sampled with their origins and voucher information are listed in Appendix 1.

3.2.2 Isolation of genomic DNA

Isolation of DNA from cacti is troublesome due to the high mucilage content of the tissue. Initial attempts using a commercial DNA extraction Kit (Plant Genomic DNA Mini Kit, Avegene Life Science Corp., Taiwan) yielded poor results because columns were easily clogged, DNA yield was low (c. 5-30 ng/µl) and the DNA was impure (A260/A280 values were usually between 2.5 and 3). For efficient isolation of DNA we removed most of the water-storing tissue as soon as possible after collection and dried the remaining cortex tissue over silica-gel in a drying chamber for one or two days at 50°C. This treatment significantly lessened the amount of mucilage during extraction. The dried plant material was homogenized (Retsch mixer mill MM200,

Haan, Germany), 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 0.2 vol% mercaptoethanol. Further steps followed the procedure described by Borsch & al. (2003). Only two extractions were carried out, since measurements of DNA concentration showed a very low amount of DNA (less that 5 ng/µl) in the third fraction. Concentration and purity of the DNA (A260/A260 as well as A260/A230 ratio) were measured using a spectrophotometer (NanoDrop. peqLab, Erlangen, Germany). This isolation method yielded a high amount (120 to 1000 ng/µl) of clean DNA, with an A260/A280 value between 1.7 and 2.1. Original genomic DNA was stored at -30°C and working dilutions with a standard concentration of 10ng/µl were made for use in PCR.

3.2.3 Amplification and sequencing

Amplification conditions and primers used were the same as described in Chapter 2. All primers used for amplification and sequencing are listed in the Appendix 2. All PCR products were stained with 100x SybrGreen nucleic acid stain and electrophoresed on a 2% agarose gel, excised and purified using the Gel/PCR DNA Fragment Extraction Kit (Avegene Life Science Corp., Taiwan) and sequenced via Macrogen Inc. (Seoul, South Korea). All chloroplast regions were easily amplified and sequencing was also straightforward. All regions were sequenced using the amplification primers, additional internal sequencing primers (see Appendix 2) were used if reads were short. Pherograms were edited and sequences were assembled using PhyDe v. 995 (Müller & al. 2005+, www.phyde.de).

3.2.4 Sequence alignment, coding of length mutational events

Sequences were aligned manually using PhyDe v. 0995 (Müller & al. 2005+). Rules for the alignment of length variable DNA followed Kelchner (2000) and Löhne & Borsch (2005). All sequences could be aligned unambiguously and only homonucleotide stretches and one $(AT)_n$ microsatellite had to be excluded from the matrices (Appendix 3). Indels were coded according to the Simple Indel Coding method using the Indel Coder option of SeqState v. 1.40 (Müller, 2005b). A list of hypothesized microstructural mutations was compiled (Appendix 4) to allow later testing of homology hypotheses (see Borsch & al. 2007, Morrison 2009, Ochoterena 2009). Inversions were placed separately during alignment and reverse-complemented prior to phylogenetic analyzes. Secondary structures of sequence parts with inversions were calculated using RNA structure 5.0 (Mathews & al. 1996+) to check whether these inversions were associated with hairpins. The inversions were coded manually (assumed plesiomorphic state: 0, inverted state: 1) and traced on the phylogenetic trees using the “Trace Character history” option of Mesquite v. 2.72 (Maddison & Maddison, 2009).

3.2.5 Phylogenetic analyses

Most parsimonious tree search was carried out using the ratchet as implemented in PRAP (Müller 2004) with the combined dataset and each marker individually. The analysis with the combined dataset was performed including all accessions and also with a reduced dataset with only one accession per OTU. Ratchet settings were 200 iterations with 25% of the positions randomly upweighted (weight = 2) during each replicate and 10 random addition cycles. Tree lengths and homoplasy indices (CI, RI, and RC) were calculated in PAUP* v. 4.0b10 (Swofford 1998). Support for the nodes found by the parsimony ratchet was calculated by jackknifing (JK) with 10.000 replicates, TBR branch swapping, 36.788% of characters being deleted in each replicate and one tree held during each replicate. These settings are based on optimal jackknife parameters described by Müller (2005a).

Bayesian Inference (BI) was performed with the combined dataset using MrBayes 3.1 (Huelsenbeck & Ronquist, 2001) with GTR+Γ+I as the best-fitting substitution model as evaluated with jModeltest (Guindon & Gascuel, 2003, Posada, 2008) using the Akaike Information Criterion (AIC). Analyses were performed based on substitutions only and in combination with coded indels, then applying the restriction site (binary) model for the indels partition. Four simultaneous runs of Metropolis-coupled Markov Chain Monte Carlo analyzes, 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 500000, the remaining trees were summarized in a majority rule consensus tree. All trees were imported into the tree editor TreeGraph2 (Stöver & Müller, 2010) for annotation and layout.

3.2.6 Comparison of marker performance / phylogenetic structure *R*

Phylogenetic structure *R* sensu Müller & al. (2006) was estimated with help of a Perl script as described therein, modified to better account for severely staggered alignments (Krug & al. in prep.). The data partitions were defined as: *trnK* intron, partial *matK* – c. 950 nt, as they would be amplified by the primers designed for Caryophyllales by (Cuénoud & al. 2002) and proposed by (Lahaye & al. 2008) for the amplification of *matK* for barcoding purposes, the entire *matK* CDS, the *rpl16* intron, *psbA-trnH* and *trnQ-rps16*. All partitions were compared with each other and analyzes were run with all characters included and only with the informative characters.

3.2.7 Definition of operational taxonomic units (OTUs)

A concept using OTUs instead of species names was employed as a basis for any calculations of intraspecific variability or species identification potential of markers. This was done because species limits within the Rhipsalideae have often changed. There are several taxa that have been described as species and later have been downgraded to subspecies or forms or vice versa. Hence, we did not assume that all currently accepted species names reflected "good" species; there might be subspecies that probably merit specific status and vice versa. Then, the phylogenetic hypothesis provided a reliable estimation on OTU delimitation. A list of the defined OTUs is given in Appendix 5. All the OTUs are morphologically recognisable and do correspond to species or subspecies or forms, no OTU was defined just based on sequences.

3.2.8 Testing of OTU identification success

The OTU identification success rate for each data partition/marker and any combination of these was computed via a Perl script written by K. Müller (University of Münster) that comprised the following computational steps: First, the individual accessions were assigned to OTUs and this information was read from an OTU definition file. Second, all possible combinations of the data partitions were constructed by reading Nexus files and concatenating sequences accordingly. In doing so, the average number of nucleotides sequenced for each set was computed as a coarse proxy for sequencing effort. The data partitions for testing of OTU identification were defined as above for comparisons of phylogenetic structure, with the only exception that "partial *matK*" was not included in the successive marker combination analysis as it requires non-overlapping data partitions. All matrices were the same as used for the phylogenetic analyzes, i.e. with mutational hotspots excluded and inversions reverse-complemented.

An OTU was considered identifiable if none of the sequences of a given OTU was identical to any of the sequences of another OTU. OTU monophyly was therefore not a requirement for identifiability. In testing equality of two sequences, alignment positions with '?' or ambiguity codes in any of the two sequences were ignored. Uppercase and lowercase letters (the latter reflecting manually edited bases deviating from automated base calls) were treated equally. If one sequence had a gap character at a given position while the other had not, the sequences were treated as different. The percentage of OTUs uniquely identified this way was computed, and this was repeated for all possible combinations of markers.

3.3 RESULTS

3.3.1 Sequence characteristics

The final combined matrix comprised 5201 aligned characters, with an average length of 4287 nt per taxon. In total, 15 sequence parts of uncertain homology (mutational hotspots) had to be excluded (Appendix 3). After their exclusion, 4887 aligned characters remained within the matrix with on average 4195 nt per taxon. The full characteristics of the individual regions for the dataset including and excluding hotspots are given in Table 3.1. The *psbA-trnH* spacer provided the highest percentage of variable and informative characters, followed by the *rps3-rpl16* spacer and the *rpl16* intron while the *trnK* intron and the *matK* gene were least variable. Alignment was straightforward for *matK*, the *trnK* intron and *rpl16* where mutational hotspots were restricted to poly-A or poly-T stretches but more troublesome for *psbA-trnH* and especially for *trnQ-rps16* where homology of numerous overlapping indels had to be assessed carefully and inversions required further attention.

3.3.2 Microstructural mutations

The individual sequence parts marked as mutational hotspots were between 1-3 and 32 nt in length (Table 3.1), the largest hotspots occurred in the *rpl*16 intron. All hotspots taken together comprised only a small portion of combined dataset, on average 59 nt in length ranging from 42-89 nt. All hotspots were mononucleotide stretches (poly-A or poly-T) or in one case a dimeric $(AT)_n$ simple sequence repeat in the *rpl16* intron, there were no unalignable sequence parts. Six inversions were observed in all regions except *rps3-rpl16* and the *rpl16* intron (Table 3.1). All inversions were associated with hairpins and affected the nucleotides forming the terminal loops or stem-loops.

The *trnK*/*matK* region showed few indels apart from length variable homo-nucleotide strands. All indels within the *matK* CDS had a length of multiples of three so the codon structure of the gene is maintained. Highest length variability was ob-served in the *rpl16* intron where six gaps spanned more that 100 nt, the largest being 410 nt. Gaps larger than 100 nt occurred in *psbA-trnH* and *trnQ-rps16* in *Rhipsalis* and *Lepismium*.

Table 3.1 Sequence statistics of individual regions in the combined dataset

	trnK intron	*matK*	*psbA-trnH*	*rps3-rpl16*	*rpl16* intron	*trnQ-rps16*	combined
Dataset including hotspots							
Position in the alignment	1-728, 2272-2579	729-2271	2580-3088	3089-3246	3256-4535	4536-5201	1-5201
Aligned length	1036	1543	509	158	1280	666	5201
Length range	831-974	1521-1536	129-371	136-152	811-1152	149-559	3787-4645
Mean length (SD)	917 (28)	1529 (2)	302 (53)	145 (2)	1068 (62)	315 (104)	4287 (161)
Mutational hotspots	2	0	3	1	5	4	15
Length range of all hotspots	1-5	0	5-32	4-6	3-26	1-25	42-89
Mean length of all hotspots	2 (1)	0	17 (6)	5 (1)	15 (3)	12 (3)	59 (8)
% GC	33,608	32,7	25,5	27,7	28,6	25	30,583
Inversions	1	1	1	0	0	3	6
Dataset excluding hotspots							
Position in the alignment	1-695, 2251-2470	696-2234	2471-2915	2916-3067	3068-4297	4298-4887	1-4887
Aligned length	915	1539	445	152	1230	590	4887
Length range	812-889	1518-1536	118-346	130-146	802-1127	134-534	3721-4561
Mean length (SD)	875 (13)	1529 (2)	284 (50)	140 (2)	1049 (62)	299 (103)	4195 (155)
% variable characters	11,3	10,1	30,5	32,2	22	25	17,7
% informative characters	7,3	6,4	16,6	21	16	12,8	11,2
Number of coded indels	17	5	43	7	63	30	165

Table 3.2 Comparisons of phylogenetic structure R in different data partitions. S.E.:standard error.

			informative characters					**all characters**				
Comparison			***R***	**S.E.**	**95% confidence interval**		**better performance**	***R***	**S.E.**	**95% confidence interval**		**better performance**
trnK intron	-	*matK* partial	0.0609	0.0016	0.0577	0.0641	*trnK* intron	0.0463	0.0014	0.0436	0.0490	*trnK* intron
trnK intron	-	*matK*	0.1254	0.0030	0.1195	0.1312	*trnK* intron	0.1120	0.0022	0.1077	0.1164	*trnK* intron
trnK intron	-	*psbA-trnH*	0.0057	0.0051	0.0157	0.0044	insignificant	0.0361	0.0043	0.0444	0.0277	*trnK* intron
trnK intron	-	*rpl16* intron	-0.1550	0.0019	-0.1587	-0.1514	*rpl16* intron	-0.1301	0.0022	-0.1343	-0.1258	*rpl16* intron
trnK intron	-	*trnQ-rps16*	0.0080	0.0048	0.0174	0.0013	insignificant	0.0822	0.0033	0.0887	0.0756	*trnK* intron
matK partial	-	*matK*	0.0272	0.0024	0.0225	0.0319	*matK* partial	0.0274	0.0023	0.0229	0.0320	*mat*K partial
matK partial	-	*psbA-trnH*	0.0418	0.0052	0.0316	0.0519	*psbA-trnH*	0.0365	0.0043	0.0281	0.0448	*psbA-trnH*
matK partial	-	*rpl16* intron	-0.2481	0.0018	-0.2517	-0.2446	*rpl16* intron	-0.2074	0.0023	-0.2118	-0.2029	*rpl16* intron
matK partial	-	*trnQ-rps16*	0.0399	0.0052	0.0296	0.0501	*trnQ-rps16*	-0.0099	0.0034	-0.0166	-0.0032	*matK* partial
matK	-	*psbA-trnH*	-0.0716	0.0050	-0.0619	-0.0813	*psbA-trnH*	-0.0547	0.0039	-0.0471	-0.0623	*psb*A-*trn*H
matK	-	*rpl16* intron	-0.2783	0.0021	-0.2742	-0.2824	*rpl16* intron	-0.2500	0.0016	-0.2469	-0.2532	*rpl16* intron
matK	-	*trnQ-rps16*	-0.0630	0.0035	-0.0561	-0.0699	*trnQ-rps16*	-0.0291	0.0028	-0.0237	-0.0346	insignificant
psbA-trnH	-	*rpl16* intron	-0.0163	0.0037	-0.0235	-0.0092	*rpl16* intron	-0.0901	0.0038	-0.0976	-0.0827	*rpl16* intron
psbA-trnH	-	*trnQ-rps16*	0.0090	0.0015	0.0060	0.0120	*psbA-trnH*	0.0390	0.0022	0.0347	0.0433	*psbA-trnH*
rpl16	-	*trnQ-rps16*	0.0434	0.0039	0.0510	0.0357	*rpl16* intron	0.1626	0.0039	0.1703	0.1549	*rpl16* intron

3.3.3 Trees from the single loci

The single data partitions do not resolve the tree of the Rhipsalideae (Appendices 6 and 7). The *trnK* intron was least homoplastic (HI 0.158) while the *rpl16* intron showed the highest degree of homoplasy in the dataset (HI 0.307). Best resolution from a single partition is obtained from *rpl16*, albeit with lower support compared to the combined dataset. Besides *rpl16* is the only marker to find all major Rhipsalideae clades with high support. Trees from *psbA-trnH* and *trnQ-rps16* result in a large and weakly supported polytomies, with few terminal clades found. Resolution and support from *psbA-trnH* is weakest, none of the major nodes is found and even the Rhipsalideae are not found as monophyletic, and similar results are obtained from *trnQ-rps16* that finds only two clades with support.

3.3.4 Trees from the combined plastid data set

Of 4887 total characters in the combined matrix, 546 were parsimony-informative. The addition of indels provided 113 additional informative characters (of total 165 coded indels). The parsimony analysis including indels resulted in a strict consensus of 144 trees of 1669 steps (CI: 0.712, RI: 0.905, RC: 0.644, HI 0.288), not shown. Figure 3.1 shows the majority-rule consensus tree derived from Bayesian Inference as phylogram. The parsimony tree resulting from the reduced dataset is shown in Figure 3.2.

The Rhipsalideae tree was well resolved and supported in both parsimony and Bayesian analyses and species-level resolution could be obtained with high confidence. Rhipsalideae were maximally supported as monophyletic and comprised five well supported clades, which largely agree with the Rhipsalideae genera as currently understood. *Rhipsalis* and *Lepismium* are confirmed as monophyletic while the two *Hatiora* subgenera *Hatiora* and *Rhipsalidopsis* p.p. are found as two separate clades and *H. epiphylloides* is within *Schlumbergera*. The topologies from both analyzes differ in the position of the genera: the MP topology finds *Schlumbergera* as sister to the rest of the Rhipsalideae, *Hatiora* subg. *Hatiora* to branch off next, followed by *Hatiora* subg. *Rhipsalidopsis*, and *Lepismium* as sister to *Rhipsalis*, but none of these backbone nodes gets support. The Bayesian analysis finds a weakly supported clade of *Lepismium* and *Hatiora* subg. *Rhipsalidopsis* (0.6 PP) while the positions of the other genera are unresolved. Within the individual genera, the trees from both analyses were almost identical, but the Bayesian analysis provided generally better resolution and higher support values.

3.3.5 Phylogenetic structure R

The results are shown in Table 3.2. The *rpl16* intron (along with the *rps3-rpl16* spacer) showed highest phylogenetic structure *R* compared to all other markers in this dataset, regardless whether all or only the informative characters were included. The *trnK* intron had the second-best phylogenetic structure but performed equally well as *psbA-trnH* when only informative characters were considered. The performances of the other markers differed in the analyses, especially *trnQ-rps16* was found to perform better based on the informative characters only. The *matK* gene, either entire or partial, exhibited lower *R* then the two introns in the dataset. When compared directly with each other, partial *matK* showed higher *R* than the complete gene, the entire *matK* showed lowest R in both comparisons.

3.3.6 Success of OTU identification

The comparison OTU identifications success of each marker is shown in the Appendix 5. The percentage of identified OTUs for each marker combination and in relation to the number of nucleotides sequenced is shown in Fig. 3.3 and Fig. 3.4. The number of successfully identified OTUs increased with more nucleotides and the value of 90% identified OTUs is already reached with slightly more than 1600 nt (*psbA-trnH* + *rpl16* intron + *trnQ-rps16*). The maximal value of 97% successfully identified OTUs is first reached by 2500 nt (*psbA-trnH* + *rpl16* intron + *trnK* intron + *trnQ-rps16*) and even the combination of all markers and 4207 nt does not find more. Hence, of the 61 defined OTUs, 59 could be successfully identified. The only OTUs that could not be found by any marker or combinations were *Rhipsalis sulcata* and *Rhipsalis teres* - in each case the *R. sulcata* sequence was identical with one of the *R. teres* accessions.

Figure 3. 1 Phylogram from Bayesian Inference based on the combined dataset and coded indels for A) *Schlumbergera*, *Lepismium*, *Hatiora* ad *Rhipsalidopsis* and B) *Rhipsalis*. Posterior Probabilities are shown above, JK support values from 10000 replicated below the branches. OTUs with multiple accessions are annotated with square brackets, the Bonn Botanic Garden accession numbers for each sample, the CA-isolate numbers and the countries of origin are given next to the names. Species classification follows Hunt (2006).

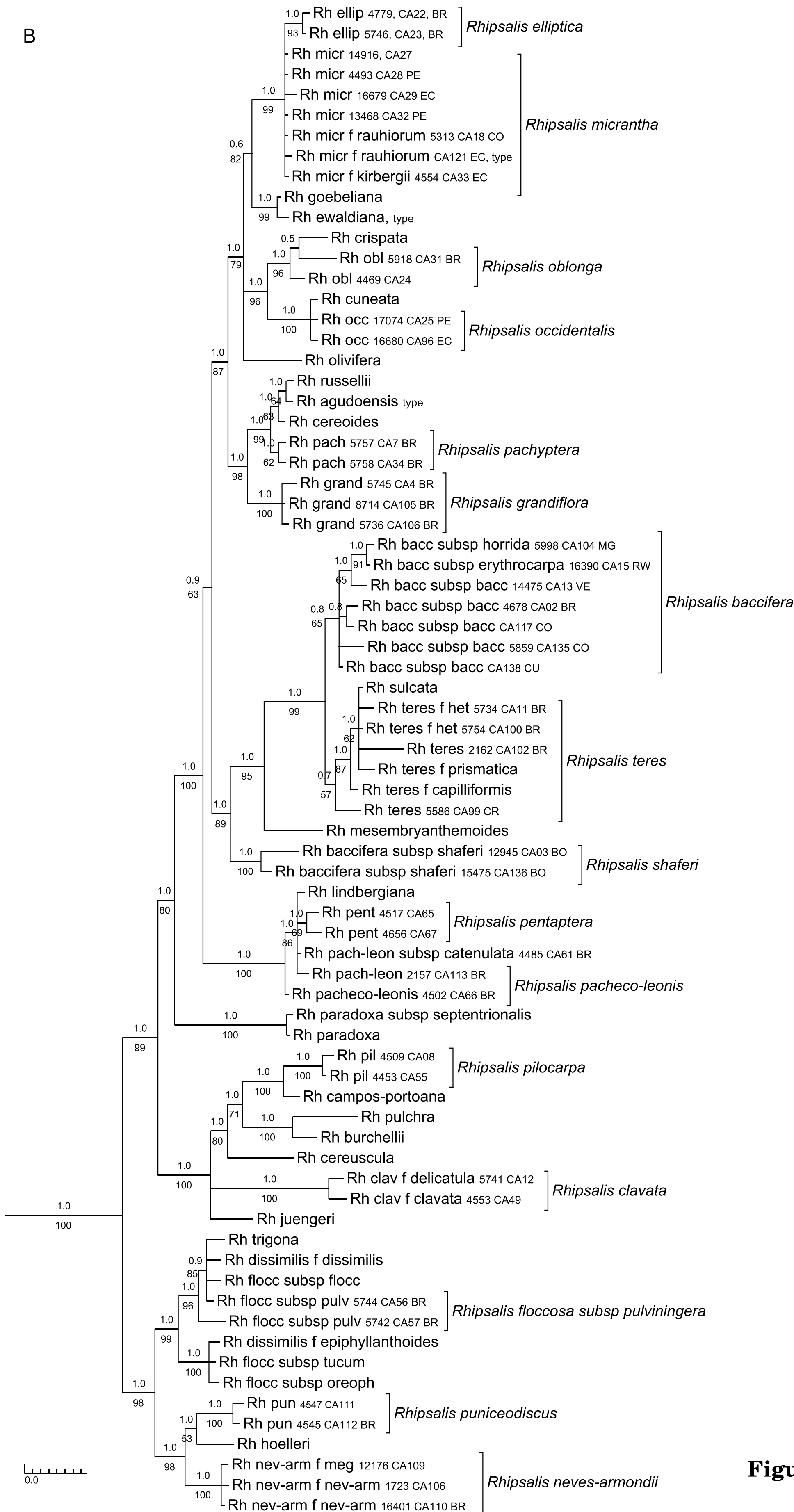

Figure 3.1, continued

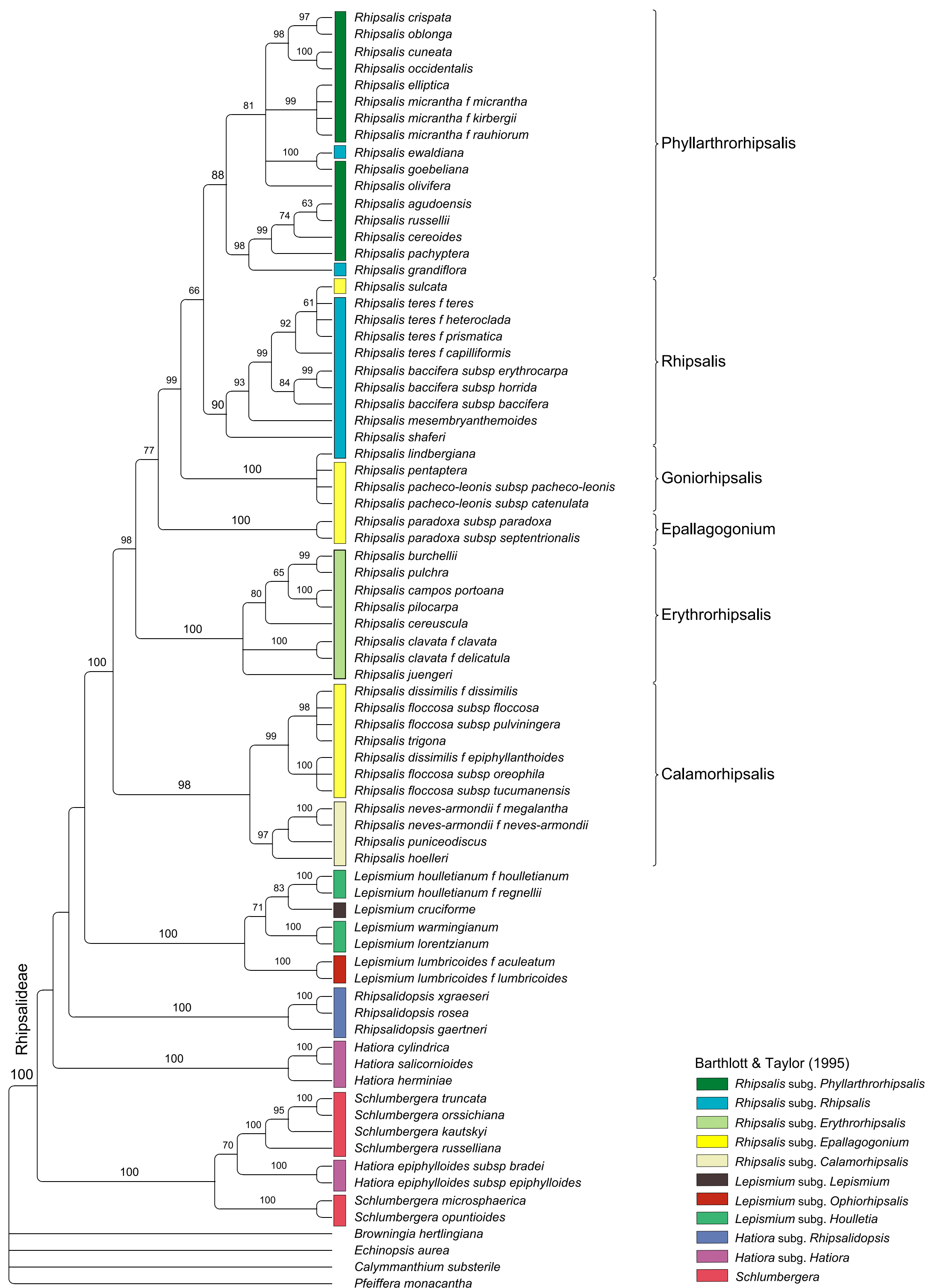

Figure 3.2 Strict consensus of 11 trees (1556 steps, CI: 0.726, RI: 0.856, RC: 0.621) found by the parsimony ratchet based on the combined dataset and coded indels, annotated with the subgeneric classification of Barthlott & Taylor (1995) and new subgenera as they are proposed here. Jackknife support values from 10.000 replicates are shown above the branches.

3.4 DISCUSSION

3.4.1 Major relationships within Rhipsalideae

The dense taxon sampling in our study for many characters unraveled the five major lineages of Rhipsalideae with much improved confidence over previous studies but still could not clarify the relationships between them.

The maximum parsimony consensus tree based on plastid data of Calvente et al. (2011) is just inconsistent with weak support, whereas a Bayesian posterior probability of 0.91 alone supports a *Hatiora-Lepismium-Schlumbergera*-clade. However, a clade hypothesis based on a posterior probability alone, not even reaching 0.95, should be valued with caution (Simmons et al. 2004, Suzuki et al. 2002).

There are also only three earlier hypotheses on relationships within the tribe. Berger (1926), Buxbaum (1967) and Barthlott (1987b) had developed their phylogenetic schemes based on an evaluation of characters and an assumed "direction" of evolution. Berger further discussed hypothetical ancestral character states. But most of these earlier assumptions can not be confirmed in view of our data.

The basal grade of *Schlumbergera*, *Hatiora* and *Rhipsalidopsis* is unsupported, but all genera share colored flowers and strictly determinate stem-segments, with new segments arising from composite apical areoles in a somewhat oblique position to the preceding one. This indicates that these genera might possess the plesiomorphic states for these characters. In light of the evolution of many other vegetative and floral characters (Chapter 3, this study), the parsimony topology, although the backbone is unsupported, may well reflect the organismic phylogeny.

Schlumbergera is found as sister to the rest of the Rhipsalideae. A common earlier view was to regard the morphology of *Schlumbergera* (or *Zygocactus*) as most "derived", because of the zygomorphic flowers (e.g. Barthlott 1987b). Berger (1926) and Buxbaum (1967) further supposed *Schlumbergera* (and *Zygocactus*) to have evolved from flat-stemmed taxa with colored actinomorphic flowers as they are found in *Rhipsalidopsis*. Thus, they assumed close relationships of the two genera but our data do not provide evidence for such a relationship.

Our data reveal *Hatiora* sensu Barthlott and Taylor (1995) as polyphyletic, as also found by Calvente et al. (2011). Barthlott (1987b) classified *Hatiora* and *Rhipsalidopsis* both as subgenera of *Hatiora*, while all preceding authors regarded them as distinct from each other. Our data reveal *Hatiora* in this expanded circumscription as polyphyletic and find both subgenera as subsequently branching lineages. Alternatively, *Hatiora* s.str has been regarded as close to *Rhipsalis* and has even been included in it by Vaupel (1925-1926) and Hunt (1967), mainly because both

genera produce small flowers. Still, our study does not find any evidence of a close relationship of *Hatiora* and *Rhipsalis*.

Lepismium and *Rhipsalis* have been regarded as closely related by Berger (1926) and Buxbaum (1967), although in a different circumscription with only *L. cruciforme*, while Barthlott (1987a) assumed *Lepismium* including *Pfeiffera*, *Acanthorhipsalis* and *Lymanbensonia* to be sister to the other genera and the most "ancestral" group of the Rhipsalideae. *Rhipsalis* and *Lepismium* are morphologically similar in having small white flowers and terete or flattened stems, but there are no apparent morphological synapomorphies of the two genera. *Lepismium* as a whole, or parts of it had sometimes been merged in *Rhipsalis*. Our results provide evidence that both may indeed be sister groups, although the relevant node remains unsupported.

3.4.2 Relationships within main Rhipsalideae lineages, circumscription of genera and subgeneric classification

The relationships within the genera of Rhipsalideae could be resolved with high confidence and our results largely confirm the circumscriptions of genera and subgenera as currently understood. Unless stated otherwise, the relationships depicted in our study will be discussed in comparison with the treatments of Barthlott and Taylor (1995) and Hunt (2006). Figure 3.2 shows the earlier classification in comparison with the revised classification as proposed and discussed here.

3.4.2.1 *Schlumbergera*

A clade consisting of the six recognised *Schlumbergera* species is supported with 100% JK, 1.00 PP, but it additionally includes *Hatiora epiphylloides*. The *Schlumbergera* clade as depicted by our data consists of three sublineages: *S. opuntioides* and *S. microsphaerica* are sister to the rest of the genus, a position which is also supported by their morphology. They differ in having cylindrical or compressed stem-segments bearing areoles all over the surface of the stems. These two species were originally treated as a separate genus *Epiphyllanthus* Berger but later interpreted as neotenic forms of *Schlumbergera* (Barthlott & Rauh, 1975).

Hatiora epiphylloides was originally described as *Rhipsalis epiphylloides* Porto & Werderm. Backeberg (1938) established a monotypic genus *Pseudozygocactus* Backeb. for it, which was included in *Hatiora* by Buxbaum (1970b). The current view of this species being part of *Hatiora* subg. *Rhipsalidopsis* was proposed by Barthlott (1987a). The placement of *Hatiora epiphylloides* within *Schlumbergera* s. str. is unexpected but but was also found by Calvente et al. (2011) and is supported by the plant's stem morphology. The plants are usually smaller in size, but large specimens have been observed in the collection of Countess B. Orssich (W. Barthlott, pers. obs.). The flowers have the structure of a *Hatiora* flower and yellow color, which is typical for *Hatiora* but does not occur in any other *Schlumbergera* species. Actually, the flowers of *Hatiora*

epiphylloides generally lack all flower synapomorphies of *Schlumbergera*, such as stamens inserted in two series, a perianth tube and a nectar chamber. The species therefore seems a morphological intermediate. Possible explanations are either morphological homoplasy or convergence or ancient hybridization between a *Hatiora* s. str. and a *Schlumbergera*. This hypothesis still needs confirmation from sequences of nuclear markers, in view of the maternal inheritance of the plastid genome. *Schlumbergera* is known to hybridize freely, the commonly cultivated Christmas Cactus (*Schlumbergera* × *buckley*) is a hybrid between *S. truncata* and *S. russelliana* and a hybrid between *S. truncata* and *S. opuntioides* (*Schlumbergera* × *exotica*) is also known (Barthlott & Rauh, 1975). Hybridization may therefore also have played a role during speciation in *Schlumbergera*. Calvente et al. (2011) did sequence the nuclear ITS region but the ITS tree is basically a large polytomy and their data neither confirm nor reject the possibility of hybridization within *Schlumbergera*, so other nuclear loci would be needed.

The clade consisting of *S. russelliana* (the type species of *Schlumbergera*), *S. kautskyi*, *S. orssichiana* and *S. truncata* is well supported (100% JK, 1.00 PP) and can be regarded as *Schlumbergera* in the strict sense. *Schlumbergera kautskyi*, which had originally been described as a variety of *S. truncata* and later raised to species rank, is resolved as distinct and confirmed as a “good” species. *Schlumbergera truncata* and *S. orssichiana* are supported as closely related, cannot be separated by the phylogenetic analyzes, but are still found as distinct OTUs. *Schlumbergera orssichiana* differs considerably from *S. truncata* by shape and size of its stem-segments, flower morphology and an unusual flowering behaviour, including flowering in summer (Barthlott & McMillan, 1978).

Although *Schlumbergera* consists only of six species and is morphologically well defined, it has had a complex taxonomic history. Some species had been separated as distinct genera (Hunt 1969, McMillan & Horobin, 1995). Our study supports an expanded *Schlumbergera* to include *Hatiora epiphylloides*, as it was also suggested by Calvente et al. (2011). But including *H. epiphylloides* also poses some problems. *Schlumbergera* is one of the morphologically best defined Rhipsalideae genera, maybe even one of the best defined Cactaceae genera. The features characteristic for it are predominantly zygomorphic flowers with a nectar chamber, a perianth tube, erect, connivent stigmas and stamens inserted in two series. None of these are found in *H. epiphylloides*. Including it in *Schlumbergera* would make the genus morphologically heterogeneous. It remains to be tested if nuclear genes result in a deviating phylogeny and if *H. epiphylloides* perhaps a striking case of reticulate speciation.

3.4.2.2 *Hatiora*

Our data reveal *Hatiora* as polyphyletic. The generic name should only be applied to subgenus *Hatiora* which includes taxa with cylindrical stems, a terete pericarpel and small yellow-orange or magenta flowers. The corresponding clade of *H. salicornioides* (type species), *H. cylindrica* and *H. herminiae* is highly supported (100% JK/1 PP) and the morphologically different magenta-flowered *H. herminiae* is resolved as sister to the other two species. *Hatiora cylindrica* falls into a clade of *H. salicornioides* specimens. The main characteristics of *H. cylindrica* are cylindrical stem-segments, a fully expanded perianth and deep red fruits, while *H. salicornioides* has bottle-shaped stem-segments, flowers which do not open widely and white fruits. Our data indicate that *H. cylindrica* might either not be a "good" species but a form or variety of *H. salicornioides*. But it is also possible that what is known as *H. salicornioides* is more than one species. This is even likely because very distinct races and ecotypes exist in the wild (N. Taylor, pers. obs). Some *H. salicornioides* forms have been described as separate taxa, but species-limits are hard to define because of intergrading characters and further differences possibly attributable to cultivated plants, so the additional species names are currently treated as synonyms.

3.4.2.3 *Rhipsalidopsis*

The clade consisting of *Rhipsalidopsis* (= *Hatiora*) *rosea* and *R.* (= *Hatiora*) *gaertneri* together with their hybrid *R.* × *graeseri* is supported with 100% JK, 1 PP. *Rhipsalidopsis* was originally established as a genus by Britton & Rose (1923) for *R. rosea*, which they had separated from *Rhipsalis*. *Rhipsalidopsis gaertneri* was at first placed in Schlumbergera but later Moran (1953) combined into *Rhipsalidopsis*. Barthlott (1987a) had merged *Rhipsalidopsis* in *Hatiora*, but as stated above, this expanded *Hatiora* is polyphyletic. Contrary to the proposal of Calvente et al. (2011), we do not suggest a merger of *Rhipsalidopsis* with *Schlumbergera*. First, our data do not find a close relationship of these two. And second, it was already pointed out by several authors that *Rhipsalidopsis* and *Schlumbergera* only share vegetative characters but differ considerably in floral characters (e.g. Moran 1953). None of the characters unique for *Schlumbergera* is found in *Rhipsalidopsis*. The best taxonomic and nomeclatural conclusion from our results is recognizing *Rhipsalidopsis* again as a separate genus. It is characterized by flattened stem-segments, an angled pericarpel and large actinomorphic, campanulate pink or red flowers.

3.4.2.4 *Lepismium*

The genus is supported as monophyletic with 100% JK, 1.00 PP. Several considerably different generic concepts have been suggested for *Lepismium* (Table 1). It was either included into *Rhipsalis* (Schumann 1899, Vaupel 1925-1926) or recognized as monotypic for *L. cruciforme* (e.g. Britton and Rose, 1923). Backeberg

(1959) proposed a very different generic concept based mainly on the sunken pericarpel, thus including many species of *Rhipsalis*, recognizing in total 17 species. Barthlott (1987a) established an altered *Lepismium* with 14 species and included the former *Rhipsalis* subgenera *Ophiorhipsalis* and *Houlletia* as well as *Acanthorhipsalis*, *Lymanbensonia* and *Pfeiffera*, based on the mesotonic branching as the main diagnostic character. *Lepismium* in this circumscription was found as polyphyletic and distant from the Rhipsalideae (Nyffeler 2002), and part of it is now treated as *Pfeiffera* Salm-Dyck and *Lymanbensonia* Kimnach (Korotkova & al. 2010). In our new circumscription, *Lepismium* contains 5 species and is characterized by mesotonic branching, indeterminate stem-segments, small, usually white flowers positioned laterally, angled pericarpels and naked fruits.

3.4.2.5 *Rhipsalis*

Rhipsalis is found as monophyletic and contains six lineages basically corresponding to the subgenera sensu Barthlott & Taylor (1995). *Erythrorhipsalis* is the only subgenus entirely confirmed as monophyletic by our data while subg. *Epallagogonium* is highly polyphyletic; its species being found in three different lineages. There are four species that do not “fit” in morphologically otherwise well defined clades but are rather morphological intermediates between the clade they are part of and another, more distant clade. These species are *Rhipsalis pulchra*, *R. grandiflora*, *R. ewaldiana* and *R. sulcata*. Their morphology might either be plesio-morphic, result from homoplasy or convergences, or to be the result of ancient hybridization events. However, no verifiable hybrids between *Rhipsalis* are currently known, and this hypothesis will have to be investigated using nuclear markers if firm evidence for hybridization in *Rhipsalis* is to be obtained.

Subg. *Calamorhipsalis* K.Schum. (incl. subg. *Epallagogonium* K.Schum. p.p.)

Subgenus *Calamorhipsalis* as defined by Barthlott & Taylor (1995) with *R. hoelleri*, *R. neves-armondii* and *R. puniceodiscus* is supported as monophyletic by 98% JK / 1.00 PP. *Rhipsalis neves-armondii*, which has strictly determinate stem-segments is sister to the pair of *R. hoelleri* and *R. puniceodiscus*. Both these species are similar, having indeterminate growth, but *R. hoelleri* differs in having red flowers.

Rhipsalis floccosa, R. trigona and *R. dissimilis* form a well supported clade (100% JK / 1.00 PP) which is sister to *Calamorhipsalis*. These three species were referred to as the *Rhipsalis floccosa* group within subgenus *Epallagogonium* by Barthlott & Taylor (1995) and are characterized by stem-segments of determinate growth and strictly acrotonic branching, often woolly (floccose) areoles post-anthesis and repeatedly flowering areoles. They furthermore exhibit stem-dimorphism with juvenile segments bearing spines, especially in *R. dissimilis*, and the seedlings of *R. floccosa* show developmental phases which pass from ribbed, spiny and cereoid

through triangular spineless stem-segments before the adult cylindrical segments appear, thereby resembling first *R. dissimilis* then *R. trigona* in its ontogenetic stages (N. Taylor, pers. comm.).

The subspecies of *R. dissimilis* and *R. floccosa* sampled do not form separate clades, but are intermixed. *Rhipsalis dissimilis* f. *epiphyllanthoides* was originally described as *Lepismium epiphyllanthiodes* Backeb., then later regarded as just a form of *Rhipsalis dissimilis* (Barthlott & Taylor 1995). This form has a small distribution area and is clearly recognizable whereas forma *dissimilis* is more widespread and varies considerably, depending on its habitat. The two taxa prove to be very distinct in our study, forma *dissimilis* is part of a clade formed by *R. floccosa* subsp. *pulviningera*, subsp. *floccosa*, subsp. *hohenauensis* and *R. trigona* while forma *epiphyllanthoides* is close to *R. floccosa* subsp. *oreophila* and subsp. *tucumanensis*. *Rhipsalis floccosa* is widespread and has the second largest distribution area of all *Rhipsalis* after *R. baccifera*. It is a variable species with five morphologically different and geographically separated subspecies currently recognized (Hunt, 2006), most of them originally described as distinct species. Our data find *R. floccosa* as not monophyletic, but apparently forming a complex of closely related morphologically similar species, unless the complex as a whole is not considered as a single species. This alliance also included *R. trigona* which can not be separated from *R. floccosa* by DNA sequences although the adult plants are morphologically different.

Our data reliably support an expanded subg. *Calamorhipsalis*, including the *R. floccosa* group. This circumscription partly corresponds to the original proposal of Schumann (1899), the group "Floccosae" of Vaupel (1925) and almost meets the one proposed by Backeberg (1959), as a subgenus of *Lepismium*. The subgenus as newly defined is characterized by mainly terete stems (trigonous in *R. trigona*), a sunken pericarpel, erumpent flower-buds and areoles that are often densely woolly post-anthesis.

Subg. *Erythrorhipsalis* Berger

This subgenus was originally monotypic and based on *R. pilocarpa*, then treated as a subgenus of *Rhipsalis* (Barthlott 1987a), including more species and in a circumscription which is entirely confirmed by our data). *Erythrorhipsalis* is well defined by a characteristic habit with indeterminate basal extension shoots and subsequent stem-segments decreasing in size toward the branch apex, pendent, slender terete stems, campanulate flowers borne apically on the terminal or penultimate segments (subapically in *R. pulchra*) and directed downwards. Although relationships between its species could not be fully resolved, all species are found as distinct. *Rhipsalis ormindoi*, which is currently also included in *Erythrorhipsalis*, and also has the typical morphology of this subgenus, could not be sampled here.

Subg. *Epallagogonium* K.Schum.

This subgenus was originally established for *R. paradoxa* then later expanded to include further species with angular stems and sunken pericarpels. *Rhipsalis paradoxa* appears isolated within *Rhipsalis*. It is characterized by stem-segments of determinate growth with three to four discontinuous ribs/angles (i.e. each rib that is actually a podarium is shifted by c. 90° from the preceding one). But excepting its indeterminate stem-segments, *R. paradoxa* is very similar to *R. pacheco-leonis* which is almost like *R. paradoxa* in miniature, so *R. paradoxa* is morphologically not very distinct. Still, since our data show the subgenus *Epallagogonium* as polyphyletic, we suggest it should be circumscribed in the sense of Schumann to include only *R. paradoxa*.

Subg. *Goniorhipsalis* K.Schum. (incl. subg. *Epallagogonium* p.p., subg. *Rhipsalis* p.p.)

Rhipsalis lindbergiana, *R. pentaptera* and *R. pacheco-leonis* form a well supported clade (100% JK /1.00 PP). The two latter species were part of the *R. pentaptera* group of subg. *Epallagogonium* and are characterised by angular stems. Rather unexpectedly, *Rhipsalis lindbergiana* is also part of this grouping. It was believed to be closely related to *R. baccifera* and *R. teres* (and is even occasionally mixed up with these). But a closer examination of the plant's morphology shows *R. lindbergiana* is indeed similar to *R. pentaptera* and *R. pacheco-leonis* and differs mainly by having terete stems.

We assign *R. lindbergiana*, *R. pacheco-leonis* and *R. pentaptera* to an additional subgenus *Goniorhipsalis*, which had not been recognized by Barthlott & Taylor (1995). This subgenus as originally described by Schumann (1899) included *R. pentaptera* along with *R. micrantha* and *R. trigona*, with no type species indicated; *R. pentaptera* was later chosen as the type by Buxbaum (1970a). We therefore decided to resurrect Schumann's infrageneric name for our newly found clade of *R. pentaptera, R. pacheco-leonis* and *R. lindbergiana*. In this new circumscription, the subgenus is characterised by alternating podaria, reduced flowers borne perpendicular to the stem and well-developed scale-leaves. However, the differences to *R. paradoxa* are only ones of relative size of parts and there does not seem to be a single morphological character that absolutely distinguishes *R. paradoxa* from subg. *Goniorhipsalis*.

Subg. *Phyllarthrorhipsalis* Buxb. (including subg. *Rhipsalis* p.p.)

Subgenus *Phyllarthrorhipsalis* is supported as monophyletic (87% JK / 1.00 PP) but has to be expanded to include *Rhipsalis grandiflora* and *R. ewaldiana*. The entire subgenus *Phyllarthrorhipsalis* except for *R. grandiflora* and *R. ewaldiana* can be characterised by strictly determinate stem-segments and either angled or flattened stems (*R. ewaldiana* has additional indeterminate basal extension shoots). The angled

stems are not restricted to any of its subclades and besides also occur in subgenera *Calamorhipsalis*, *Epallagogonium* and *Goniorhipsalis* (as newly circumscribed above), indicating that this feature is highly homoplastic within *Rhipsalis*. On the contrary, flattened stems are restricted to subg. *Phyllarthrorhipsalis* although they occur in several subclades within it, indicating that shifts to flattened stems are quite easy and happened several times. Besides, *Phyllarthrorhipsalis* differs in its seedling morphology: its species have flattened first stems as seedlings, whereas other Rhipsalis taxa observed have initially terete-ribbed seedlings even if subsequent ones are angled (Taylor & Zappi 2004).

The placement of *R. grandiflora* as sister to the rest of the *R. pachyptera*-alliance is rather unexpected. It had originally been placed in subg. *Rhipsalis* based on similarities in stem morphology and flower bud development and is morphologically different from the other *Phyllarthrorhipsalis* in having terete stems, which do not occur in other taxa of this clade. Since all three specimens of *R. grandiflora* sampled occur in this position, the placement is unlikely the results of any artefacts. The numerous stamens of *R. grandiflora* and its ability to produce several flowers per areole tentatively indicate the relationship to *Phyllarthrorhipsalis* and the deviant morphology could also been explained by *R. grandiflora* being a hybrid.

A clade of *R. pachyptera, R. russellii, R. cereoides* and *R. agudoensis* is found with maximal support. All four are morphologically similar and *R. agudoensis* has even been misinterpreted as an unusual form of *R. pachyptera* prior to its description. All four species grow semi-erect and have 3-5 ribbed, sometimes also flattened stem segments (*R. pachyptera, R. russellii*), often produce several flowers per single areole and have fruits that change their colour from white to pink. They are also found growing predominantly as lithophytic, not epiphytic.

A clade of *R. oblonga, R. crispata*, *R. cuneata* and *R. occidentalis*, is supported by 97% JK and 1 PP and contains morphologically similar species with thin, flattened and leaf-like stems. This grouping contains geographically distinct species. While *R. oblonga* and *R. crispata* are native to Brazil, *R. occidentalis* and *R. cuneata* occur in the Andes, mainly in Bolivia and Ecuador.

Rhipsalis micrantha and *R. elliptica* appear in a polytomy, although the two samples of *R. elliptica* are resolved in a distinct lineage. *Rhipsalis elliptica* is a flat-stemmed species native to SE Brazil while *R. micrantha* is a widespread and morphologically very variable species that occurs in the Andes of Ecuador and Peru and extends into Central America. Its stem morphology ranges from narrow flattened or angular stems in the typical forma *micrantha* and especially in forma *kirbergii* to the more broadly flattened stems of forma *rauhiorum*. These three forms had originally been described as distinct species closely related to *R. micrantha* (Barthlott 1974) but were later interpreted as variations in different habitats. The question therefore is

whether *R. micrantha* represents a species complex, or if there is a case of incomplete lineage sorting with *R. elliptica* in fact being derived from ancestral populations of *R. micrantha* or vice versa. More sequence data and a population-level sampling are needed to get further insights.

The placement of *R. ewaldiana* within subgenus *Phyllarthrorhipsalis* as sister to *R. goebeliana* is unexpected. *Rhipsalis ewaldiana* has been regarded as closely related to *R. mesembryanthemoides* since both species share dimorphic stem segments with long and short shoots and exhibit partly mesotonic branching. Nevertheless, *R. ewaldiana* still shares angled stems with other members of subgenus *Phyllarthrorhipsalis*.

Subgenus *Rhipsalis*

As inferred here, subg. *Rhipsalis* is not monophyletic as circumscribed by Barthlott & Taylor (1995). One erstwhile subspecies of *R. baccifera* merit species rank, *R. sulcata* is additionally included and *R. lindbergiana*, *R. grandiflora* and *R. ewaldiana* have to be excluded (see discussion above).

Rhipsalis baccifera and *R. teres* are the “typical” *Rhipsalis* with strictly acrotonic branching, terete stems, and a characteristic habit with indeterminate basal extension shoots (as in subg. *Erythrorhipsalis*) and small whitish flowers with few perianth segments. Both species are widespread, highly variable in morphology and numerous additional names at species and subspecies level have been proposed but are now regarded as synonyms. The *R. teres* specimens sampled form a clade supported by 60 % JK and 0.95 PP that also includes *R. sulcata*. The latter can not be recognized as distinct from *R. teres* based on plastid sequences. It is a poorly known species which had been placed within subg. *Epallagogonium* and regarded as closely related to *R. pentaptera*. Although the placement found by our data is unexpected, the plant's morphology does support it. *Rhipsalis sulcata* has stem-segments with strictly acrotonic branching and shares the habit of *R. teres* and *R. baccifera* with indeterminate basal extension shoots. The main differences are the slightly angled stems, which are, however, also sometimes developed in *R. teres* f. *prismatica*. A specimen from Costa Rica (*C. Horich 4/88*, vouchered at BONN) is resolved as sister to the rest of the *R. teres*-clade. It has to be investigated whether this taxon deserves at least subspecies rank and whether it may represent an alien introduction to the Costa Rican flora (the nearest naturally-occurring populations of *R. teres* are some 5,000 km distant in SE Brazil).

Rhipsalis baccifera is the most widespread of all Cactaceae species and has been described under numerous synonyms. Currently six subspecies are recognized (Barthlott & Taylor 1995). The *Rhipsalis baccifera* specimens sampled, excluding

subsp. *shaferi*, form a moderately supported clade (0.82 PP), only found by BI including indels.

Rhipsalis baccifera subsp. *shaferi* is resolved as sister to the rest of subg. *Rhipsalis*, indicating it should be treated as a distinct species. It was indeed originally described as *R. shaferi* and is geographically distinct from subsp. *baccifera*, ranging through Paraguay, southern Bolivia and northern Argentina to São Paulo state, SE Brazil, and is replaced in northern Bolivia by *R. baccifera* and in Brazil by *R. teres*. It also differs morphologically from the rest of the subg. *Rhipsalis* by having indeterminate stem segments.

Rhipsalis baccifera and *R. teres* may not be exclusive lineages, respectively, and what is known under these names is a complex of very similar taxa. More sequence data and a manifestly larger taxon sampling as well as population-level studies are needed to reliably infer species limits.

The Old World *Rhipsalis*

The occurrence of *Rhipsalis baccifera* in Africa, Madagascar and Sri Lanka has puzzled taxonomists and biogeographers for more than 100 years. These plants have been considered to be Gondwanan relicts (Croizat 1952) or in the other extreme as recently introduced by man (Buxbaum 1970a). The most commonly accepted hypothesis, however, was dispersal to Africa by migratory birds, early in the evolutionary history of *Rhipsalis baccifera* (Backeberg 1942).

There are more examples of taxa of an exclusively New World family occurring in tropical Africa. One species of Bromeliaceae, *Pitcairnia feliciana* has a small distribution area in West Africa (Porembski & Barthlott 1999). Its dispersal from South America to Africa has recently been estimated to have happened around 10 Mya (Givnish & al. 2007). A similar figure of c. 6 Mya has been estimated for the dispersal of *Maschalocephalus dinklagei* (Rapateaceae) to Africa (Givnish & al. 2004). Recently, an age of 19.1 – 3.1 Mya has been inferred for the Cactaceae (Ocampo & Columbus 2010). Although no timeframe for the dispersal of *Rhipsalis baccifera* to Africa was inferred, this age estimate is comparable to the figures as quoted above.

The African *Rhipsalis baccifera* populations differ from their New World relatives in gross morphology, ploidy level, anatomical characters and pollen morphology (Barthlott 1983). The two African specimens sampled here (subsp. *erythrocarpa* from East Africa and subsp. *horrida* from Madagascar) are depicted as sisters with high confidence (91%JK, 1.00 PP) within the grouping of South American *Rhipsalis baccifera* specimens. The divergent sequences of the two specimens sampled here provide another evidence for a long independent evolution of these populations thus arguing against a recent introduction to Africa by man.

3.4.3 The potential of markers for OTU identification within Rhipsalideae

The underlying principle of DNA barcoding is that *a priori* defined species are recognizable by specific DNA sequences (e.g. Hebert & al. 2003). However, there is yet no standardized approach how to distinguish species by DNA sequences and how many sequence characters of a given set of markers will be needed for unambiguous recognition. The accuracy, i.e. the ability of a barcode to identify a species correctly will be highest if it does not only distinguish randomly chosen species or species that occur in single geographical settings (e.g. plots) but provides enough variation to separate closely related species. Nevertheless, it does not seem appropriate to use a generally applicable threshold value for distinguishing sister species due to varying degrees of sequence divergence resulting from rate heterogeneity of markers and lineages. Meyer & Paulay (2005) further argued that thresholds would result either in false positives or false negatives, as there is no discontinuity between intraspecific and interspecific sequence divergence. Therefore, some authors use an approach in which infraspecific p-distances must be smaller compared to interspecific ones (Lahaye & al. 2008, CBOL Plant Working Group 2009). Others simply regard a taxon as unique if it does not share its sequence with any other taxon in the sampling, e.g. Seberg & Petersen (2009). On the other hand, DNA sequences are also useful to evaluate if morphologically similar individuals belong to a species, thereby evaluating alpha-taxonomy or searching for cryptic or otherwise unrecognized species. Likelihood methods were developed recently that determine the point of transition between population level evolutionary processes and stochastic lineage growth (Pons & al. 2006, Fontaneto & al. 2007, Monaghan & al. 2009). Such methods were also applied in angiosperms to test monophyly of species (Lahaye & al. 2008). On the other hand, extant patterns of angiosperm species diversity, including those of cacti, may involve considerable incomplete lineage sorting (e.g. Jakob & Blattner 2006) or reticulate evolution (e.g., Sang & al. 1997). Complex, multi-faceted approaches are therefore needed to assess and later identify Cactaceae species using molecular markers.

In our study, we focus on the molecular evaluation of OTUs that were *a priori* defined using morphology (Appendix 5). Being well studied and completely available in cultivation, we assume that carefully defined OTUs of Rhipsalideae will already closely match species in most cases. As one of the facets of the above described approach we analyze the species (= OTU) identification potential of a wide spectrum of plastid markers. So far, comprehensive comparative sequence data sets for taxonomically fully sampled lineages of plants are hardly available. In addition, we will discuss situations where OTUs appear not be monophyletic to guide future research on species limits using nuclear sequences and population level sampling.

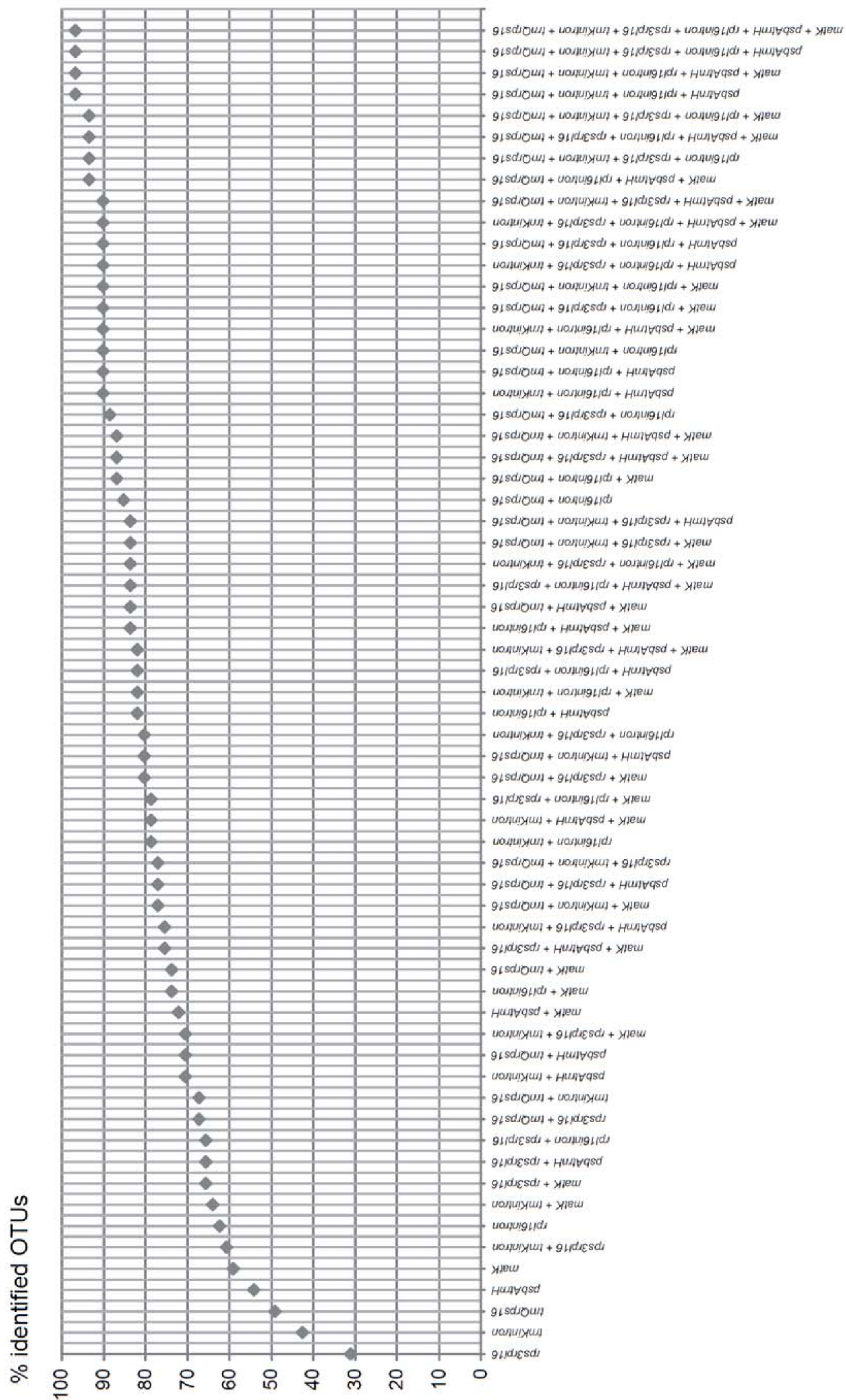

Figure 3.3 Results from OTU-identification test: percentage of identified OTUs from single markers and all possible combinations.

Using our approach, maximally 59 out of 61 (97%) of all OTUs could be successfully identified (Figs. 3.3 and 3.4) using a maximum number of sequence characters. The main trend was that all morphologically well recognizable OTUs had distinct sequences as well and appeared as monophyletic in the phylogenetic tree. In contrast, those species which can not be easily separated by morphological features or are morphologically variable, were either not easily resolved by our sequence data or intraspecific sequence variation was observed (1-3 mutations within OTUs). The lineages of *Hatiora salicornioides* and of *Rhipsalis baccifera*, *R. floccosa, R. teres* and probably *R. micrantha* (individuals of *Rh. micrantha* lack resolution to *Rh. elliptica*, although the latter share potential synapomoprhies) are paraphyletic to other morphologically recognizable taxa (*Hatiora cylindrica*, the African subspecies of *R. baccifera*, *R. dissimilis* & *R. trigona*, *R. sulcata*).

This identification success is higher than observed in other barcoding studies that used a taxonomic setting. Hollingsworth & al. (2009) used seven loci (*rpoC1*, *rpoB*, *rbcL*, *matK*, *psbA-trnH*, *atpF-atpH*, *psbK-psbI*) but could identify only 69% species of *Inga* (Fabaceae), and 32% of *Araucaria*. Seberg and Petersen (2009) found that even six regions (*ndhF*, *matK*, *psbA-trnH*, *rps8-pl36*, *accD*, *rpoC1*, c. 4500 nt per sample) were not sufficient for discriminating more than 92% of *Crocus* species. In contrast, already c. 2500 nt of four highly performing regions used here (*rpl16* intron, *trnK* intron, *psbA-trnH*, *trnQ-rps16*) were sufficient to identify 97% of the OTUs.

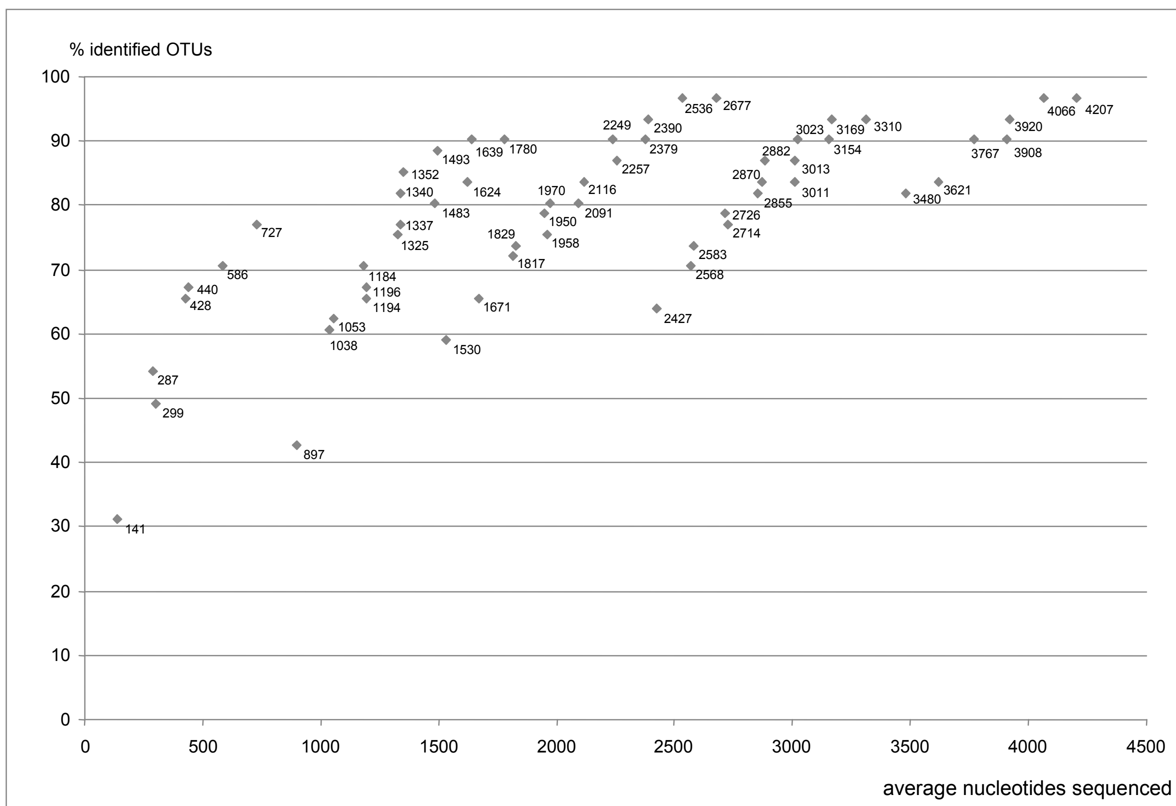

Figure 3.4 Percentage of identified OTUs in relation to the number of sequenced nucleotides.

Among all possible marker combinations this one was the most successful with the least number of sequenced nucleotides; the combination of all markers (4207 nt) yielded the same identification success. Other marker combinations with a comparable number of nucleotides, however, often resulted in lower identification success (Fig. 3.5) – for example, the combination of *matK*, *trnK* intron and *psbA-trnH* (2714 nt) which found only 48 (79%) OTUs.

The *rpl16* intron was the best single-locus barcode, identifying (38 OTUs, 62%, Appendix 3), followed by *matK* (36 OTUs, 59%, Appendix 3). The *rpl16* intron has not yet been suggested as a barcode but is frequently used in phylogenetic studies at low taxonomic levels. Our results provide evidence that it is not only a powerful phylogenetic marker but should seriously be considered also an effective barcode. The *matK* gene or a part of it has been repeatedly suggested as plant barcode and its good performance has been corroborated by recent studies (Chase & al. 2007, Little & Stevenson, 2007, Lahaye & al. 2008, Ford & al. 2009). Remarkably, *matK* alone found even more OTUs than the *trnK* intron (Appendix 5), although the intron is more variable (Table 2). When looking at single data partitions, each *matK* and *trnK* also identified some OTUs uniquely. We have additionally compared the identification success of the entire *matK* CDS with a part of the gene. This corresponded to the c. 950 bp fragment (partial *matK*) proposed by Lahaye & al. (2008) as a universal plant barcode. However, partial *matK* finds only 46% while the entire gene finds 59% of the OTUs, and is therefore more successful.

PsbA-trnH has been regarded as one of the most promising angiosperm barcodes (Kress & al. 2005, Cowan & al. 2006, Chase & al. 2007, Kress and Erickson, 2007). In our study it identified only 54% of the OTUs (Fig. 3.4, Appendix 3). The *trnQ-rps16* spacer has recently been demonstrated as a good barcode for *Paeonia* (Zhang & al. 2009) or *Psiguria* (Steele & al. 2010) but was among the less effective barcode regions with a performance comparable to that of *psbA-trnH*.

3.4.4 Phylogenetic utility of the regions used

None of the single partitions yielded fully or even nearly fully resolved trees. Only the combined dataset of *trnK/matK*, *rps3-rpl16*, *rpl16* intron, *psbA-trnH* and *trnQ-rps16* provided sufficient resolution and good support. The combined dataset provided not only high resolution, even at species level, but also yielded a highly supported tree with 46 of the 86 nodes gaining JK values higher than 95% in parsimony analyzes. Posterior probabilities from Bayesian analyses were higher; out of 89 supported nodes, 75 have a PP>0.95 and 63 nodes are maximally supported.

The two best-performing markers in our dataset were the group II introns in *rpl16* and *trnK*, and this is another peace of evidence for the high phylogenetic performance of GII introns – regardless of taxonomic level – as pointed out by (Borsch &

Quandt, 2009). Chloroplast spacers were found least informative, in congruency with the results of Löhne & al. (2007) who also found introns to perform better than spacers.

Most of the nodes found by an analysis of the combined dataset were also resolved by *rpl16*, albeit with lower support (Appendix 6). The *rpl16* intron also had the highest phylogenetic structure (Table 3.2). Resolution and support from *trnK/ matK* was comparable to *rpl16*, although fewer backbone nodes were found and support was lower. The high phylogenetic structure *R* in *rpl16* and *trnK/matK* as compared to other chloroplast genomic regions has also been observed by Löhne & al. (2007) in Nymphaeales. However, the *trnK* intron and *matK* gene differ in their phylogenetic structure and partial *matK* showed higher phylogenetic structure *R* that the entire gene when compared directly to each other (Table 3.2). The tree based on the entire gene was better resolved and supported compared to the one from partial *matK* (Appendix 6 and 7). This could be explained by the different degree of conservation: the 3' part of the generally fast evolving gene is fairly conserved while the 5' region is less conserved (Hilu & Liang, 1997) and therefore different parts of the gene may yield different levels of phylogenetic signal or signal directed towards other parts of the tree.

The *psbA-trnH* spacer was the most variable region in our study (Table 3.1). It showed higher phylogenetic structure *R* compared to *matK* and *trnQ-rps16* but the parsimony tree derived from *matK* was much better resolved and supported than from *psbA-trnH* that is very short in Cactaceae (Table 3.1).

When analyzed separately, *psbA-trnH* just yielded a large unsupported polytomy. The inferiority of the phylogenetic performance of *psbA-trnH* compared to other markers (e.g. *matK*, *trnL-F*, ITS) has previously been noted (Sang & al. 1997, Kim & al. 1999) and corresponds to our result here and our recent experience in a study of the genus *Pfeiffera* (Korotkova & al. 2010).

The *trnQ-rps16* spacer has hitherto been hardly applied in phylogenetics but was proposed as promising for low taxonomic level studies by Shaw & al. (2007) based on a high percentage of potentially informative characters (PICs). It was successfully applied for *Genlisea* (Lentibulariaceae) (Fleischmann & al. 2010) and Apiaceae subfamily Saniculoideae (Calviño & Downie, 2007), where it indeed provided a high number of informative characters, but trees based on single markers were not discussed therein. Compared to these lineages, *trnQ-rps16* is much shorter in Rhipsalideae (mean length 300 nt vs. 576 and 1370 nt), thus the amount of potentially informative characters is limited.

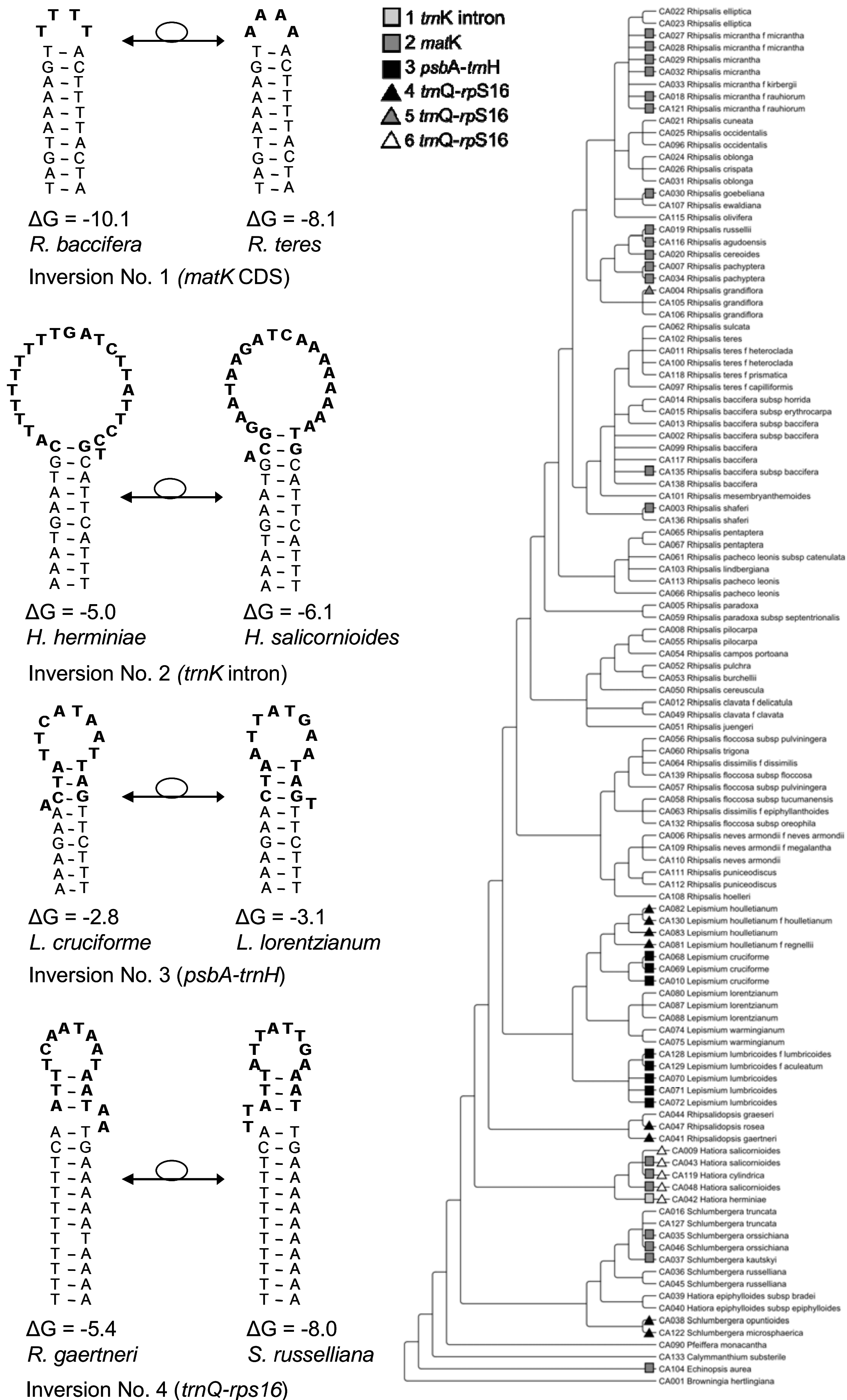

Figure 3. 5 Inversions found a) in the *trnK* intron, b) in the *matK* CDS, c) in *psbA-trnH* and d) in *trnQ-rps16* plotted on the parsimony consensus tree of the Rhipsalideae. Inverted states shown on the right, assumed plesiomorphic states on the left.

We observed inversions in all markers used with the exception of *rps3-rpl16* and the *rpl16* intron. The reconstruction of the original and inverted states of these inversions on the parsimony tree showed most of them to be homoplastic (Fig. 3.5). All affect the terminal loops of hairpins. This is the most common pattern for small inversions in the plastid genome (Kelchner and Wendel, 1996; Kelchner and Clark, 1997; Borsch and Quandt, 2009). Such hairpin-associated inversions have already been shown to switch easily, even at population level (Quandt et al., 2003; Quandt and Stech, 2004). Inversions are known to be a problem in phylogenetic analyses. They will influence phylogenetic signal if overlooked in the alignment (Quandt et al., 2003). Perhaps the most severe potential problem, at least in Cactaceae, is the inversion in the *matK* CDS. It was also observed in other Cactaceae genera (Korotkova et al., 2010) and is highly homoplastic and the two states switch within OTUs. The *matK* region has to be checked carefully despite of its coding nature since other variable inversions were found for example in Amaranthaceae - Gomphrenoideae (Borsch et al. 2011).

Phylogenetic studies in Cactaceae all have shown a comparatively low sequence variation of the markers used and most authors combined at least two regions. The *rpl16* intron as a sole marker for the Cacteae resulted in a largely unsupported tree (Butterworth & al. 2002). A combination of the *rpl16* intron and *psbA-trnH* for *Mammillaria* still did not provide much better resolution (Butterworth and Wallace, 2004). Improved resolution for closely related species was obtained from the *rpl16* intron and *trnL-F* within *Peniocereus* (Arias & al. 2005). Within *Pereskia*, only a combination of five regions (*psbA-trnH*, *trnK/matK*, *rbcL*, *phyC* and *cox3)* could clarify the relationships (Edwards & al. 2005). A combination of three chloroplast spacers (*atpB-rbcL*, *trnL-F* and *trnK-rps16)* for *Rebutia* and allied genera could identify clades within the genera but did not produce full resolution at species level (Ritz & al. 2007). In our recent study of *Pfeiffera*, only a combination of *trnK/matK*, *trnS-G, rps3-rpl16,* the *rpl16* intron, *trnQ-rps16* and *psbA-trnH* provided full resolution between all species (Korotkova & al. 2010). A comparison of our results and former studies within Cactaceae leads to the conclusion that *trnK/matK* and *rpl16* are among the best performing regions within Cactaceae and should be considered as routine markers in future studies, whereas *psbA-trnH* and *trnQ-rps16* cannot be recommended.

The usage of *psbA-trnH* and *trnQ-rps16* also has practical limitations: relative to their shortness, both required high sequencing efforts. Obtaining the whole sequence of the spacers with one primer was possible only in an estimated 30% of the taxa; usually two primer reads were necessary because of large homonucleotide stretches. The occurrence of such homonucleotides is also a putative problem for barcoding, as pointed out by Devey & al. (2009).

3.4.5 Comparison of phylogenetic utility and species identification potential of the markers used

Similar to phylogenetic utility, identification utility depends on the mutational dynamics of the genomic region at hand and the amount of mutations per sequenced nucleotide. A major difference to phylogenetic utility is that patterns of homoplasy matter a lot when tree reconstruction is the goal. Our data suggest that introns and spacers outperform coding genes in phylogenetic utility, but not in identification utility. The ranking of markers according to their phylogenetic structure (based on identical numbers of characters) is *rpl16* intron > *trnK* intron > *psbA-trnH* > *trnQ-rps16* > partial *matK* > complete *matK*. The best-performing regions are not necessarily those that provide the largest percentage of variable characters; the percentage of variable and informative characters is in fact low in *trnK*/*matK* and that of *rpl16* is comparable to *psbA-trnH* and *trnQ-rps16* (Table 3.1). Regarding species identification potential, the ranking would look different: *rpl16* intron > *matK* > *psbA-trnH* > *trnQ-rps16* > *trnK* intron > partial *matK*. It is interesting that, apart from the different ranking, the best performing phylogenetic marker in our study is also the most successful single-locus species identifier. But apart from this, it seems that levels of variability do not necessarily correlate with phylogenetic signal, since the most variable regions do not provide the highest phylogenetic structure.

3.4.6 An improved classification system for Rhipsalideae

Our study has provided a robust framework for a phylogeny-based classification of the Rhipsalideae. Several taxonomic and nomenclatural changes are proposed, as summarized in the following.

Since *Hatiora* was found as polyphyletic, the name should only be applied to the former *Hatiora* subgenus *Hatiora*. Subg. *Rhipsalidopsis* should be recognized again at the genus level, following the "classical" circumscription that includes only *R. rosea* and *R. gaertneri*. Furthermore, *Hatiora epiphylloides* needs to be included into *Schlumbergera* (necessary new names and combinations are provided below). Within *Lepismium*, an altered circumscription results from the exclusion of *L. incachacanum*, which is now part of *Lymanbensonia*. Subgeneric limits within *Lepismium* also need to be re-defined. Our data support to recognize subgenus *Ophiorhipsalis* with its only species *L. lumbricoides*, but neither confirm subg. *Houlletia* nor subg. *Lepismium* as natural groups. We therefore propose uniting *L. cruciforme*, *L. houlletianum*, *L. warmingianum* and *L. lorentzianum* into subgenus *Lepismium* and keeping subg. *Ophiorhipsalis* with *L. lumbricoides*. Within *Rhipsalis*, slightly altered subgeneric circumscriptions are proposed for all subgenera but *Erythrorhipsalis* (see discussion above). Most changes should be made for subgenus *Epallagogonium*, as its species are found in three *Rhipsalis* clades. It should to be split and only circumscribed to contain the type species *R. paradoxa* while the rest is transferred an expanded subg.

Calamorhipsalis, subg. *Rhipsalis* and the resurrected subgenus *Goniorhipsalis*. Subgenus *Rhipsalis* is reduced and two species are transferred to subg. *Phyllarthrorhipsalis*. This revised classification is also shown in Fig. 3.2.

Rhipsalis baccifera subsp. *shaferi* merits species rank. Its old name *Rhipsalis shaferi* Britton & Rose can easily be reinstated. A complicated case is the *R. floccosa* / *R. dissimilis* alliance where the gross-morphology does not correspond with the molecular phylogeny. A possibility derived from the phylogenetic hypothesis and the OTU recognition analyses would be species ranks for all *R. floccosa* subspecies and the two *R. dissimilis* forms, most of the names even already exist. Alternatively all the subspecies/forms could be merged into a much expanded *R. floccosa*. This would be in line with their ontogenetic stages that resemble each other. But this would likely make taxa of this complex hard to identify because many intergrade in their morphological characters. For the time being we do not propose any nomenclatural changes for this complex. We feel that more detailed studies of this species complex would be needed, sampling more populations or studying the ontogeny in more detail. Altering the formal taxonomy too early might result in taxa that can not be identified easily except with sequence data. There is not even any clear geographical pattern to be observed within the complex and it is not known whether the taxa of this complex interbreed or not.

Chapter 4

Morphology and character evolution of the Rhipsalideae

Summary

Only few character surveys exist for the Rhipsalideae, apart from compilations of characters in taxonomic treatments. A reconstruction of character evolution in a phylogenetic context is also lacking. Especially hypotheses on characters associated with the epiphytic life-form and the floral traits are missing. Synapomorphies for clades that are formally described as genera or subgenera also still need to be found. The well resolved phylogenetic hypothesis for the Rhipsalideae now enables a detailed study of character evolution. A matrix of 36 characters was compiled and the evolution of these characters was reconstructed on the phylogenetic tree using a Bayesian approach and ACCTRAN and DELTRAN optimization schemes. Epiphytism is reconstructed as crown group synapomorphy of the Rhipsalideae and epilithic and terrestrial growth are found to be reversals or further shifts. The Rhipsalideae are supported by several synapomorphies some of which are adaptations to the epiphytic life-form, such as the thin terete stems and the shrubby, pendent habit. The ancestral flowers of the tribe were reconstructed as actinomorphic, small, with free perianth segments, and not intensely coloured. Innovations in floral characters are zygomorphy, adaptations to bird-pollination, decrease in flower size, reflexion of the perianth and prominent stamen exposure. The degree of homoplasy is high, especially concerning vegetative characters. Reversals are also common. Many characters used to define genera and subgenera in the past are homoplastic. But several characters are homogenous within the respective clades and therefore can be used as diagnostic. So as a result, all the highly supported clades found by the molecular phylogenetic analyses can be defined morphologically.

4.1 INTRODUCTION

According to the current knowledge, epiphytism has evolved four times in the Cactaceae (Chapter 1 this study, Hernández-Hernández & al. 2011, Wallace & Gibson 2002). The colonization of the tree canopy as new habitats went along with ample changes in morphology and there are several characters shared by all the epiphytic groups. These morphological shifts include the formation of flattened or terete stems in contrast to multi-ribbed stems in the terrestrial cacti. The stems of the epiphytes are much thinner; spines are mostly absent, inconspicuously developed or bristle-like. Adventitious roots occur in many Cactaceae genera but are especially frequent in creeping terrestrial species and in the epiphytes; some epiphytes can exhibit an exclusively adventitious root system. All epiphytic groups vary in flower morphology: small whitish flowers are found in all groups and at the same time also intensely coloured flowers (red, yellow, pink, magenta), likely bird pollinated. The flowers of some of the epiphytic genera are smaller compared to many terrestrial cacti. Those of *Rhipsalis* and *Pseudorhipsalis* are even among the smallest in the whole family.

The Rhipsalideae are one of the two largest epiphytic tribes. All molecular phylogenetic studies (Bárcenas & al. 2011, Nyffeler 2002, Hernández-Hernández & al. 2011) resolve the Rhipsalideae as the sister group of a diverse and speciose clade of South American columnar cacti, the tribes Trichocereeae, Browningieae and Cereeae (BCT clade). While the earlier studies yielded only moderate support for the node of the Rhipsalideae+BCT clade (72% BS support in Nyffeler's study, 61 ML BS support in the Hernández-Hernández & al. study), the most recent phylogenetic study based on *trnK/matK* provides 0.99 PP for this node (Bárcenas & al. 2011). It appears that the Rhipsalideae are phylogenetically isolated within the Cactaceae, and they are also morphologically very different from their sister group.

4.1.1 Characters applied as diagnostic for taxonomic groups in the Rhipsalideae

One of the main characters used to define genera and subgenera was stem morphology – whether the stems are flattened or terete or ribbed was considered significant by all authors. The presence of spines was also considered significant. Floral characters played a key role, mainly the floral symmetry (actinomorphic vs. zygomorphic), the position of the flowers (lateral vs. apical), and the size and coloration of the flowers were considered significant. The taxa with small whitish or white flowers (*Rhipsalis*, *Lepismium*) were usually separated from those with larger and coloured flowers (*Schlumbergera*, *Rhipsalidopsis*). The presence or absence of a floral tube was sometimes used as a diagnostic character. Another character often considered significant is linked to the development of the areoles. In some Cactaceae species, most commonly in *Rhipsalis*, the areoles are sunken into the stem tissue. The flowers

develop within the stem and burst through the stem epidermis where the areole would normally be. The ovary is sunken into the stem tissue so that both form a unit termed the pericarpel. The actual ovary is not visible. This sunken pericarpel is easily observable and was therefore also used as a diagnostic character.

The interpretation of the morphology of the Rhipsalideae in the past was usually linked with taxonomic treatments of the group. Although the authors were probably implying that the characters of a given group were of common origin, hardly any clear statements about assumed character evolution were made.

The first comprehensive Cactaceae monograph was provided by Schumann (1899). The Rhipsalideae subgenera were also first established therein. The characters he considered significant were the sunken vs. superficial pericarpel, the stem, ribbed or flattened and also the presence or absence of spines.

Britton & Rose (1923) also emphasized stem morphology, the flower position (lateral or terminal) and the presence or absence of spines. They also considered the flower shape and size significant: their Rhipsalideae (or rather Rhipsalidinae) contained only the small flowered epiphytic species. The species with large, coloured flowers and flattened stems (*Schlumbergera*, *Rhipsalidopsis*) were part of a separate subtribe, the Epiphyllanae.

The first hypotheses on common ancestry of characters were provided by Berger (1926). He attempted to define groupings within the Rhipsalideae based on assumptions of common origins of characters. Berger regarded the stem morphology, the position of the flowers and the floral symmetry as the most important characters. He assumed the putative ancestor of the Rhipsalideae had thin, terete stems that were retained in some *Rhipsalis*, in *Hatiora* and in *Erythrorhipsalis*. In contrast, he assumed flattened stems to have evolved twice in *Rhipsalis* and *Rhipsalidopsis* + *Zygocactus* / *Schlumbergera* and *Lepismium* + *Pseudorhipsalis* + *Acanthorhipsalis*. Remarkably, Berger did not mention taxa with angled stems, although most of them had already been described. Berger for the first time examined the funiculi and pointed out their potential diagnostic value. He noted that these of *Pfeiffera* were long-stalked and branched, while those of the Rhipsalideae are short-stalked and unbranched and this could be a character separating *Pfeiffera* from the Rhipsalideae (see Chapter 1).

Some further assumptions on character evolution are found in Backeberg's works, even though his approach was generally phenetic. Backeberg (1959) also emphasized the position and morphology of flowers. He considered the "Rhipsalides" with their small flowers with a reduced hypanthium and short funiculi to be the most "ancient" group. The tendency to smaller flowers, a reduced hypanthium and simple funiculi he recognised in the majority of the Rhipsalidineae. In contrast, the comparatively large zygomorphic flowers with a perianth tube (e.g. *Schlumbergera*, *Zygocactus*) were considered as more derived (Backeberg 1959).

Although all authors used basically the same characters to define genera and subgenera, they often came to different conclusions or points of view as to which the most significant characters were. The sunken pericarpel was used by Schumann (1899) and later by Barthlott & Taylor (1995) to define *Rhipsalis* subgenera *Calamorhipsalis* and *Epallagogonium* (that included Schumann's *Calamorhipsalis*). Backeberg (1959) based *Lepismium* on the sunken pericarpel while Barthlott (1987) based *Lepismium* on the mesotonic branching. The prominent bristle-spines of *Rhipsalis pilocarpa* were interpreted as a character that separates this species from other *Rhipsalis* and it was placed in a monotypic genus *Erythrorhipsalis* by Berger and later combined into *Rhipsalis* subgenus *Erythrorhipsalis* that was based on apical flowers (Barthlott 1987). *Schlumbergera* had been based on the actinomorphic flowers while *Zygocactus* was based on zygomorphic flowers. *Epiphyllanthus* was treated as a separate genus because of the well developed spines. The fact that it has the same flowers as *Zygocactus* was not considered (McMillan & Horobin 1995). Hunt (1968) pointed out that all share stamens arranged in two series and erect, connivent stigmas, which in combination with the zygomorphic flowers became the new diagnostic characters of an expanded *Schlumbergera*.

A reconstruction of character evolution in a phylogenetic context is still lacking. Thus synapomorphies for clades that are also formally described as genera or subgenera still need to be found.

4.1.2 State of knowledge on morphological characters and earlier character surveys

Apart from compilations of characters in taxonomic treatments of the Rhipsalideae and the genera, few character surveys exist. The gross morphology, including the vegetative characters, the flower and fruit characters are well covered in many of the taxonomic treatments. Among the most detailed literature sources are the studies of Buxbaum. He undertook a detailed examination of some areole characters, especially of the composite apical areoles (Buxbaum 1942). He also provided many very detailed listings and drawings of vegetative, floral, fruit and seed characters at the generic level, including hypotheses on the homology of these characters in the Rhipsalideae genera studied (e.g. Buxbaum 1970a, b, c). Further characters were discussed in the "Morphologie der Kakteen" (Buxbaum 1957-1960). A survey of seed characters is available for the Cactoideae (Barthlott & Hunt 2000) but therein, seeds of only 9 Rhipsalideae species were analysed.

Pollen characters of the Rhipsalideae were first studied by Leuenberger (1976) in the context of a survey of pollen morphology of the Cactaceae. Barthlott & Rauh (1977) have studied the pollen of *Schlumbergera* and pollen morphology of all Rhipsalideae was analyzed in a diploma thesis (Binski 2002, unpublished), carried out at the Nees Institute in the working group of T. Borsch.

There are several studies of Rhipsalideae stem anatomy, starting with a first survey of Vöchting (1873). However, all these studies included only very few species. Dettke & Milaneze-Gutierre (2008) characterised the stems of seven Cactaceae epiphytes: *Epiphyllum phyllanthus*, 3 *Lepismium* species, 3 *Rhipsalis* species, and *Hatiora salicornioides*. The authors suggested that the anatomical characters had taxonomic value and would be useful for separating species. Calvente & al. (2008) also provided a survey of anatomical, especially epidermis characters. They examined six *Rhipsalis* species aiming at the evaluation of the taxonomic relevance of these characters and concluded epidermis characters were useful to differentiate *Rhipsalis* species.

A very detailed anatomical and crystallographic study of the Rhipsalideae has been made as part of a dissertation (Hartl 2000) carried out at the Nees Institute in the working group of W. Barthlott. The results are largely unpublished besides the survey of the generation of calcium oxalate crystals in all Rhipsalideae species (Hartl & al. 2003). They found a unique crystal type in *Rhipsalis*, which forms exclusively monoclinic calcium monohydrate crystals and besides found the crystal types useful for differentiation of genera.

The first molecular phylogenetic analysis of the Rhipsalideae, focussing on *Hatiora* and *Schlumbergera* was published recently (Calvente & al. 2011). This study also included ancestral state reconstructions for six vegetative and floral characters which all had been or still are used in classification systems. Ancestral states were reconstructed for the flower symmetry, the presence or absence of a flower tube, the branching pattern, the stem growth (indeterminate or determinate), the stem shape and the flower colour.

The study of Calvente & al. (2011) which appeared during the final phase of this dissertation yielded some first insights into the character evolution within the Rhipsalideae. Only six characters were included, so there are still numerous characters to be analysed. Also, some of the relevant nodes, including most of the nodes in *Rhipsalis* were unresolved. The well resolved phylogenetic tree based on a complete taxon sampling presented in Chapter 3 now provides the framework for the detailed study of character evolution in the Rhipsalideae. The aims of the survey presented in this chapter were first, to compile a detailed dataset of morphological characters for the Rhipsalideae and to infer whether the clades (genera, subgenera) found by the molecular phylogenetic analyses can also be characterised morphologically and which characters are synapomorphic for these clades. The second aim was to reconstruct the evolution of these characters, with emphasis on the characters associated with the epiphytic life-form and the floral traits.

4.2 MATERIAL AND METHODS

4.2.1 Taxon sampling

Morphological characters were scored for all Rhipsalideae species that were also represented in the molecular analysis, thus covering all but one species of the tribe. The phylogenetic hypothesis was used as a guideline to which infraspecific taxa should also be included in the morphological matrix. Some taxa currently ranked as forms or subspecies were found as distinct by the molecular analyses (e.g. the forms of *R. dissimilis* or *Rhipsalis baccifera* subsp. *shaferi*). These taxa were also included in the morphological matrix. Other infraspecific taxa were included because they differ in morphological character states, e.g. the forms of *Rhipsalis micrantha*. *Browningia hertlingiana*, *Calymmanthium substerile*, and *Echinopsis aurea* were included as outgroup taxa.

4.2.2 Morphological data

A matrix comprising 36 characters listed in detail below was compiled. The complete matrix is shown in the Appendix 8. The morphological data were obtained from own observations of the living plants in the Botanical Gardens Bonn, from literature data and from the original diagnoses. The main literature sources were McMillan & Horobin (1995) and Barthlott & Rauh (1975) for *Schlumbergera*, the studies of Buxbaum (1942, 1970a, b, c), the Rhipsalideae checklist of Barthlott & Taylor (1995) and the treatment of the eastern Brazilian cacti of Taylor & Zappi (2004). The terminology for characters and their states was adopted from the last two sources. Data on ploidy levels were available from the chromosome counts of Barthlott (1976). Some pollen characters were scored from Binski (2002). A detailed survey on the Rhipsalideae pollen will be the task of future studies.

4.2.3 Analysis of character evolution

Characters were coded as categorical data with multiple states, and with polymorphisms, if polymorphisms have been observed. The Bayesian majority-rule consensus tree was considered as the best approximation of the organismal phylogeny for the character reconstructions. Character state transformations were mapped using WinClada v. 0.9.9 (Nixon 2002), examining unambiguous transformations as well as accelerated (ACCTRAN) and delayed (DELTRAN) optimization schemes. Homoplasy was mapped by character states, i.e. only discontinuous states were mapped as homoplastic. Under parsimony, ancestral states were reconstructed using the parsimony model with unordered states and the "trace character history" option of Mesquite v. 2.74 (Maddison & Maddison 2010). Posterior probabilities for ancestral states were reconstructed using BayesTraits (Pagel & al. 2004). As polymorphisms are

not allowed for calculations in BayesTraits, a modified and reduced version of the matrix with 20 characters was constructed (Appendix 8). Here, either only the predominant character states were scored or the coding was modified so that each of the states was coded separately. The modifications are described below in more detail.

The trees used for the Bayesian character state reconstruction were obtained using BEAST 1.0 (Drummond & Rambaut 2003). The trees obtained before with MrBayes contained polytomies which produced error messages in BayesTraits so that using these trees for the character reconstruction was not possible. A sample of 500 trees from the BEAST run was extracted using a perl script (K. Müller, unpubl.) which generates a BayesTraits input file from BEAST or MrBayes output files. The nodes for which ancestral states were wanted, were added to the ancestral state reconstruction using the "AddNode" command. The chain was run for 5050000 generations and rate coefficients and ancestral states were sampled every 100 generations. The mean values of all the posterior probabilities found were afterwards calculated with Excel and illustrated as pie chart diagrams using TreeGraph 2 (Stöver & Müller 2010).

4.2.4 Modifications of the matrix for BayesTraits analyses

The matrix was reduced to the most significant vegetative and floral characters and included 20 characters (Appendix 8). Some of the characters were modified to remove polymorphisms, which are not allowed for BayesTraits analyses. These modifications are described in the following; all the other characters in the matrix were the same as for the ancestral states reconstruction under parsimony.

The life forms were scored as separate characters and the different states were coded as follows: 1) Epiphytic growth: (0): not epiphytic, (1): epiphytic; 2) Epilithic growth: (0): not epilithic, (1): epilithic; 3) Terrestrial growth: (0) not terrestrial, (1): terrestrial. Only the predominant states of the habits were scored. The flower colours were reduced just to two states: (0): flower white or whitish, not conspicuously coloured, (1): flowers intensely coloured (bright yellow, orange, red, magenta).

The following characters were removed from the matrix: Adventitious roots, stem diameter, hair, flower buds position, flower size, stamen colour, anther colour, pollen colour, stamen insertion, style colour, stigma shape, pericarpel, fruit colour, and chromosome numbers.

4.3 LIST OF MORPHOLOGICAL CHARACTERS AND THEIR STATES

Life-form and main vegetative characters

Growth form

(0): tree-like, with a conspicuous woody trunk, (1): large columnar, (2): medium-sized to small columnar, (3): shrubby, (4): globular / barrel

Life-form

(0): terrestrial, (1): epiphytic, (2): epilithic

Habit

(0): erect, (1): sub-erect or semi-erect, (2): pendent, (3): creeping, (4): spreading, (5): arching
The states apply to adult plants; many species are erect in their juvenile stage, then pendent. In these cases, only the state "pendent" was scored. Furthermore, only the predominant states were scored as transitions between the states are often observed.

Branching pattern

(0): mesotonic, (1): acrotonic (incl. subacrotonic), (2): basitonic

Adventitious roots

(0): absent/rarely developed, (1): present

Stem-segments growth habit

(0): indeterminate, (1): (strictly) determinate, i.e. after the primary stem segment reaches a certain, probably predetermined size/length, the growth stops and a new segment or a new order of segments begins to develop. (2): "Firework habit": a special pattern of indeterminate basal extension shoots, and other segments decreasing in size towards the distal part of the plant, e.g. in subg. *Rhipsalis*, (3): "mixed": primary axes indeterminate, lateral axes determinate (e.g. *Rhipsalis mesembryanthemoides*).

Shedding of old segments

(0): old segments not deciduous, (1): old segments deciduous = shed by well developed abscission zones at the joints

Stem form

(0): ribbed (angled); with 3-5 ribs, (1): flattened (only 2 ribs), (2): terete, (3): 5-more ribs, (4): cladodes, i.e. flattened stems but resulting not from reduction of ribs but resembling the stem segments of *Opuntia* (only *Schlumbergera opuntioides*). *Opuntia* is the only Cactaceae genus besides the epiphytes with flattened stems joints, but they are of different origin compared to the flattened stems of the epiphytes. The cladodes of *Opuntia* result from flattened cylindrical stems, while the flattened portion of a flattened stem of an epiphyte is produced in the same way as a rib (Gibson & Nobel 1986). Therefore, this character is scored separately, not homologous to state 1.

Podaria

(0): absent, (1): present

The podarium is a structure unique to the Cactaceae. It is a product of the fusion of the leaf base and the stem. The result is either a tubercle or, if all podaria are arranged longitudinally, the cactus ribs (Buxbaum 1937).

Stem diameter

The stem diameter is used here as an approximation of the degree of succulence. There are several ways for its measurement, as demonstrated for example in a recent study of *Crassula* (Jones & al. 2011). The degree of succulence is commonly defined as the "water content per unit area of surface" (Delf 1912). This first attempt measured succulence as the amount of water in grams per square decimetre (dm^2) of a leaf. Alternatively succulence can be measured in grams of water per gram of plant tissue (von Willert & al. 1992). Categories for thickness of stems were defined based on the average diameters of terete or angled stems and the size and thickness of the flattened segments.

(0): lowest: filiform terete stems ≤ 0,5 cm, or very thin cladodes, (1): low: filiform terete stems 0,6 – 1 cm, or thin flattened stems, (2): medium: thick angled or terete stems 1-5 cm or thick flattened stems, (3): succulent: thick angled stems +5 cm

Areoles, spines and hair

Position and development of the areoles

(0): all areoles superficial, never sunken, growing throughout the life-cycle of the plant, (1): all areoles sunken, also the apical areole, (2): areoles sunken, except the apical areole, (3): areoles depressed (deepened), covered by a podarium/leaf primordium/primordial scale

Those areoles that are truly sunken into the cortical tissue are regarded and termed as sunken. The flower buds and new stem segments developing at those areoles burst through the stem-epidermis where the areole would normally be (termed erumpent). Areoles of *Lepismium*, however, appear sunken but develop in a different way

(Buxbaum 1970). They are almost superficial at the beginning of their development and deepened later and therefore are not treated as homologous to the sunken areoles of *Rhipsalis* but instead termed "depressed".

Apical composite areoles

(0): absent, (1): present (but sometimes hidden)

Spines

(0): absent or inconspicuous (1): present, well developed, stiff, (2): present, bristly
In their juvenile stages, many Rhipsalideae bear spines which are reduced later. Here, only the presence or absence and appearance of spines on adult stems were considered. Sometimes there are also spines on some basal extension shoots of mature plants that are otherwise spineless. In this case spines were scored as absent.

Trichomes

(0): absent or not significantly developed, (1): dense wool

Flower characters

Position of the flowers

(0): lateral to apical, (1): only lateral, (2): only apical, at composite apical areoles ("terminal"). Although the flowers on apical composite areoles appear to be terminal, and are sometimes termed as such, they do not terminate the stem – stem growth continues from the composite areole. Therefore the term "apical" is preferred.

Orientation of the flowers (relative to the surface of the ground)

(0): random, not conspicuously oriented, (1): pendent or directed downwards (e.g. *Rhipsalis* subg. *Erythrorhipsalis*)

Position of flower buds

(0): oblique, (1): perpendicular, (2): aligned with stem-axis (e.g. *Rhipsalis* subg. *Erythrorhipsalis*, *Schlumbergera*)

Number of flowers at a solitary lateral areole contemporaneously

(0): one, (1): two or more flowers. This character only applies to the production of several flowers at a solitary lateral areole. Composite areoles often produce more than one flower.

Repeated flowering at one areole

(0): areoles flower only once, (1): areoles flower repeatedly
This character does not apply to repeated flowering at a collective areole but only at a single lateral areole.

Floral symmetry

(0): actinomorphic, (1): zygomorphic

Perianth segments fusion

(0): free, (1): fused, forming a tube

Perianth segments curvature

(0): not reflexed, i.e. partially expanded to patent, (1): reflexed

Flower size (diameter or length if the flower is tubular)

(0): very small (smaller than 1 cm), (1): small (1-3 cm), (2): medium-sized (4 – 6 cm), (3): large (+7 cm)

Flower colour

(0): white / whitish, (1): yellowish, (2): bright yellow, (3): pink / magenta, (4): red, (5): orange, (6): pale pink

Androecium and gynocecium

Nectaries

(0): unspecific, (1): disc, (2): nectar chamber

Stamen / filament colour

(0): white / whitish or cream = not conspicuously coloured, (1): coloured

Stamens insertion

(0): stamens inserted in one series, (1): stamens inserted in two series

Style colour

(0): white/whitish or cream = not conspicuously coloured, (1): coloured

Stigma shape

(0): stigma lobes spreading, (1): stigma lobes erect, connivent

Pericarpels and fruits

Pericarpels

(0): smooth, not angled, (1): angled, (2): ridged, (3): slightly or inconspicuously angled

Fruit shape

(0): longer than broad, (1): globose, (2): subglobose

Fruit colour

(0): white / whitish, (1): red, (2): pink, (3): yellow, (4): greenish, (5): dark red to almost black, (6): orange
In some species, fruits are white at first then changing their colour to pink so that both colours can be observed at the same time. In this case, the colour of the ripe fruits was coded. Some other species, e.g. *R. puniceodiscus* have forms with differing fruit colours and consequently both states were coded in such cases. Those white fruits that have a reddish ring around the perianth scar were scored only as whitish.

Pollen characters

Pollen colour

(0): white / whitish or cream = not conspicuously coloured, (1): coloured (mostly yellow or red)

Pollen size (average diameter)

(0): small (< 40 µm), (1): medium-size (41–50 µm), (2): large (51–100 µm)

Aperture numbers

(3): 3 apertures, (6): 6 apertures, (9): 9 apertures, (1): 12 apertures
Rhipsalideae pollen is uniformly colpate. Aperture number variation within species or sometimes individuals is common in the Rhipsalideae. Therefore all the observed states within a species were coded. It is not always possible to determine the number of apertures from SEM images, and especially 6-colpate and 9-colpate pollen cannot always be distinguished. In such cases, it was decided to score 6 colpi since Leuenberger (1976) reports 9-colpate pollen to be rare within Cactaceae.

Chromosome number

(2): diploid 2n=2x=22, (4): tetraploid 4n=4x=44, (6): hexaploid 6n=6x=66, (8): octoploid 8n=8x=88.
All chromosome count for the Cactaceae so far yield a basic chromosome number of 11 and multiples of 11 in the polyploidy taxa (Arakaki & al. 2007, Cota-Sanchez & Wallace 1995, Das & al. 1999, Negron-Ortiz 2007, Pinkava & McLeod 1971, Pinkava & al. 1998, Ross 1981). So far no dysploid changes were observed.

4.4 RESULTS AND DISCUSSION

CHARACTER EVOLUTION IN THE RHIPSALIDEAE

4.4.1 Synapomorphies of the Rhipsalideae

Considering unambiguous character changes only, the epiphytic life-form, the pendent habit, the thin stems, the absence of spines and trichomes are found as synapomorphic for the Rhipsalideae. The ACCTRAN optimization finds additionally the shrubby habit, the acrotonic branching, the terete stems, the small flowers and small pollen (< 40 µm diameter). The DELTRAN optimization finds the same characters except the acrotonic branching and the small pollen, but suggests the determinate stem-segments and the 6-colpate pollen as further potential synapomorphies.

4.4.2 Apomorphic versus highly homoplastic characters

The trees summarizing character states transformations are shown in Figs. 4.1 – 4.3 and the results of the Bayesian ancestral state reconstructions are shown in Figs 4.4 – 4.9. There is a strikingly high degree of homoplasy and also numerous reversals in character states. There are only 3 unambiguous apomorphic state transformations that characterise larger clades (Fig. 4.1). The non-"deciduous" stem-segments are observed only in *Lepismium* and are consequently found as a synapomorphy of this genus by all optimization methods (Fig. 4.1-4.3). The fruit colour changed to white or whitish in *Rhipsalis* (but other fruit colours are also found within *Rhipsalis*. More than one flower at a lateral areole is synapomorphic for *Rhipsalis* subg. *Phyllarthrorhipsalis*.

Apart from these characters which changed only once, there are several characters which characterise a given clade but are also convergently found in one or two species outside it (compare also Table 4.1). The branching pattern was considered an informative character in the Rhipsalideae already by Barthlott (1987), especially to separate the mesotonically-branched *Lepismium* from *Rhipsalis.* Acrotonic branching is reconstructed as plesiomorphic within the Rhipsalideae (PP 0.78). Only those *Rhipsalis* that no longer develop the apical composite areoles (e.g. *R. puniceodiscus*, *R. hoelleri*) exhibit subacrotonic branching. Within the Rhipsalideae, mesotonic branching is characteristic for *Lepismium* and is otherwise found only in *Rhipsalis mesembryanthemoides* and *R. ewaldiana* (but they are not sister species). The exceptional mesotonic branching in *Lepismium* results from the loss of the apical composite areoles. This is another feature characteristic for *Lepismium*, but also found in four *Rhipsalis* species.

The flower morphology of *Schlumbergera* is exceptional within the Rhipsalideae. The flowers have two series of perianth segments with the inner segments fused and forming a perianth tube, synapomorphic for the genus. Tubular flowers are common in the Cactaceae, but the tube is commonly formed by the pericarpel, not by the perianth. Apart from *Schlumbergera*, a perianth tube is found only in *Disocactus* and *Pseudorhipsalis* (Hylocereeae), notably also epiphytes. *Schlumbergera* has predominantly zygomorphic flowers (except *S. russelliana*); the rest of the tribe has exclusively actinomorphic flowers. The stigma lobes are erect and connivent, this is also exceptional. All these characters can be regarded as synapomorphies of *Schlumbergera* (e.g. Hunt, 1969). However, *Hatiora epiphylloides* that falls in *Schlumbergera* based on the sequence data, lacks all these synapomorphies (discussed in more detail below), thus causing difficulties for the character reconstruction because reversals for almost all the character states have to be assumed in this taxon.

The fruits of *Schlumbergera*, *Hatiora* and *Rhipsalidopsis* are mostly longer than broad and obconic in shape; pericarpels are mostly angled. In contrast, the fruits of *Rhipsalis* are predominantly globose (spherical) or subglobose, barrel-shaped. Especially subgenus *Rhipsalis* is characterised by such fruits. Pericarpels are never angled in *Rhipsalis* and in *Hatiora*. Coloured fruits, usually pink, are found in many *Rhipsalis* species and are especially characteristic for the *R. cereoides*-clade and some *Erythrorhipsalis*. *Lepismium* is exceptional by having very dark red, almost black fruits.

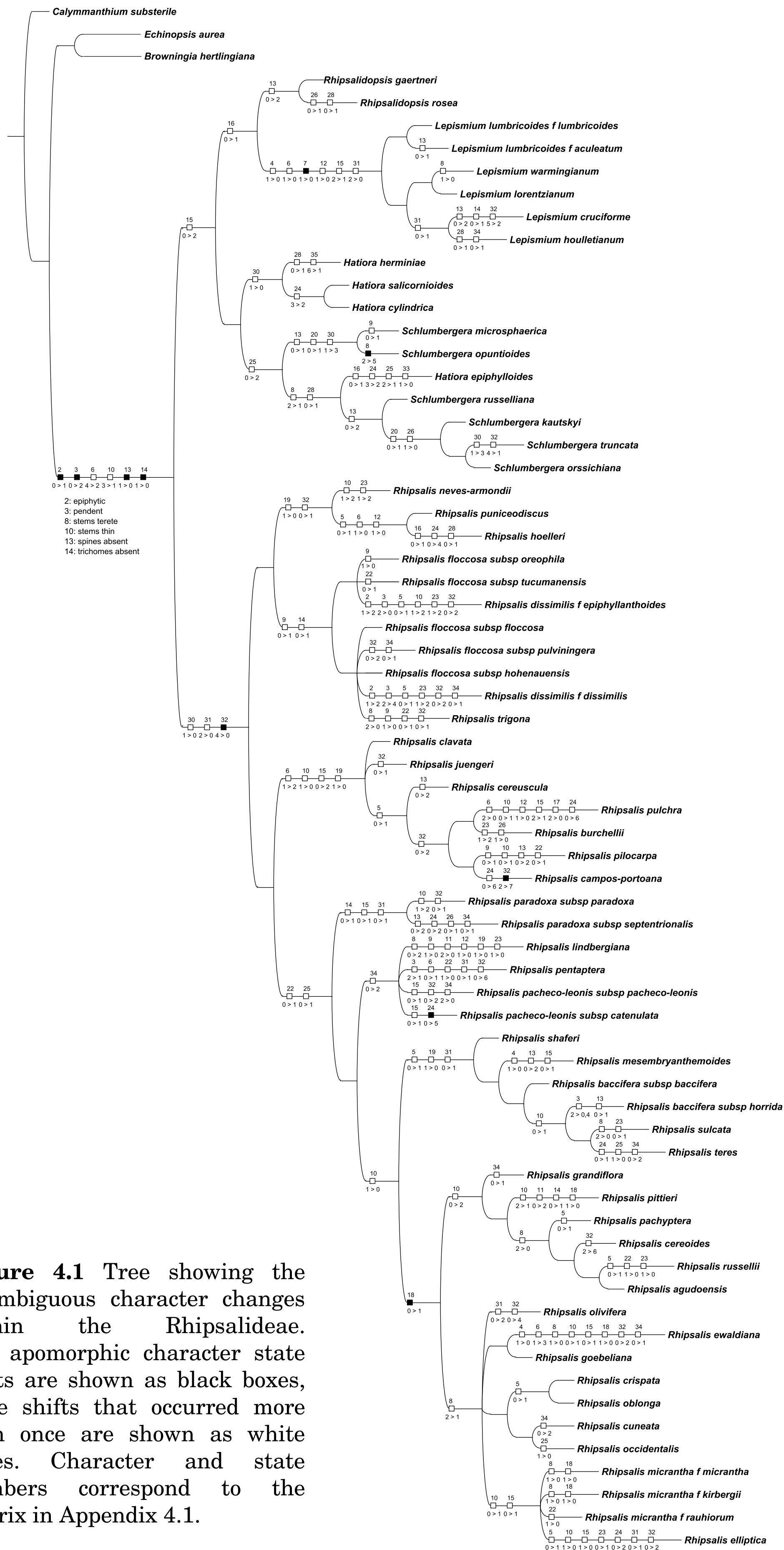

Figure 4.1 Tree showing the unambiguous character changes within the Rhipsalideae. The apomorphic character state shifts are shown as black boxes, state shifts that occurred more than once are shown as white boxes. Character and state numbers correspond to the matrix in Appendix 4.1.

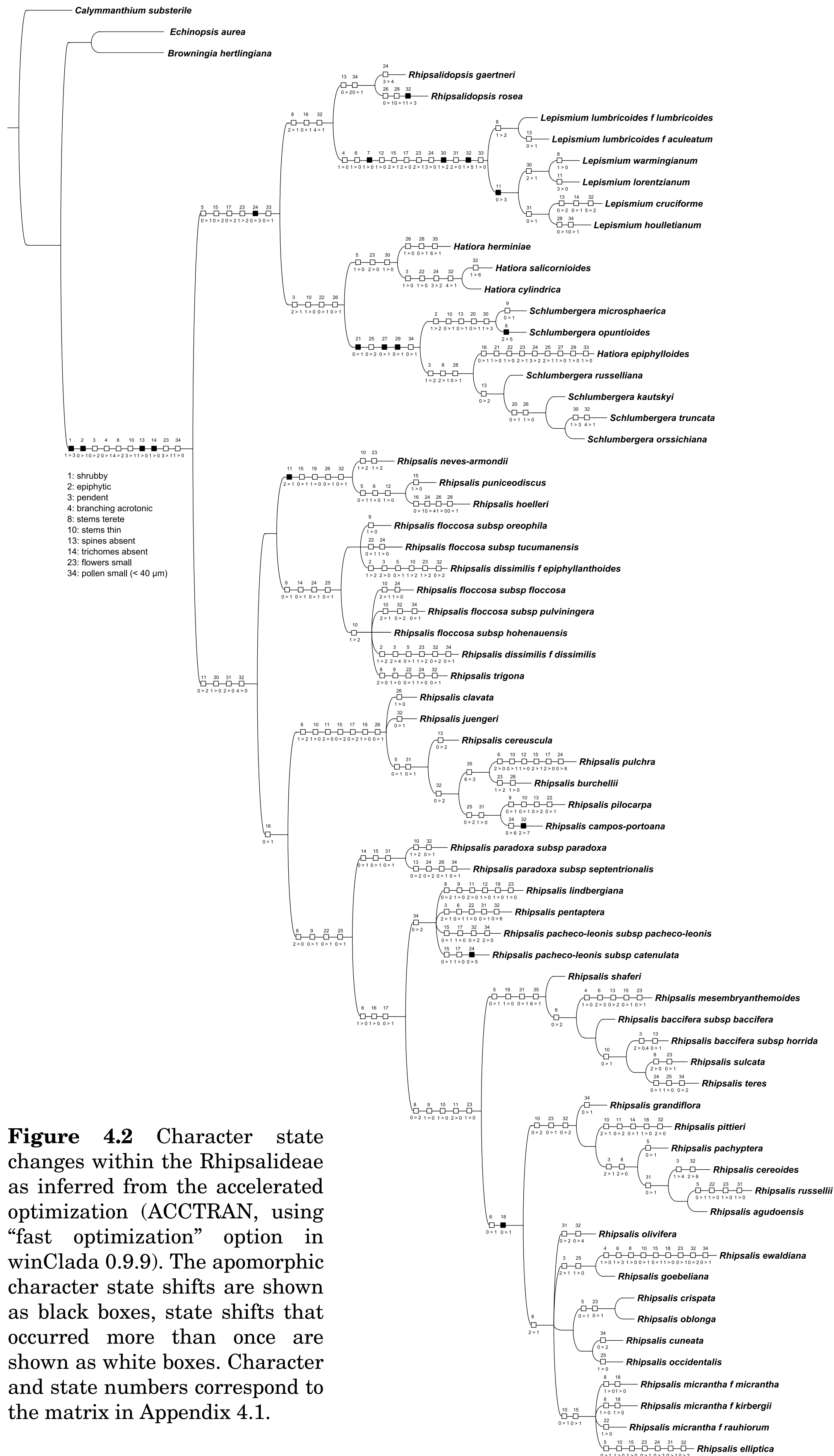

Figure 4.2 Character state changes within the Rhipsalideae as inferred from the accelerated optimization (ACCTRAN, using "fast optimization" option in winClada 0.9.9). The apomorphic character state shifts are shown as black boxes, state shifts that occurred more than once are shown as white boxes. Character and state numbers correspond to the matrix in Appendix 4.1.

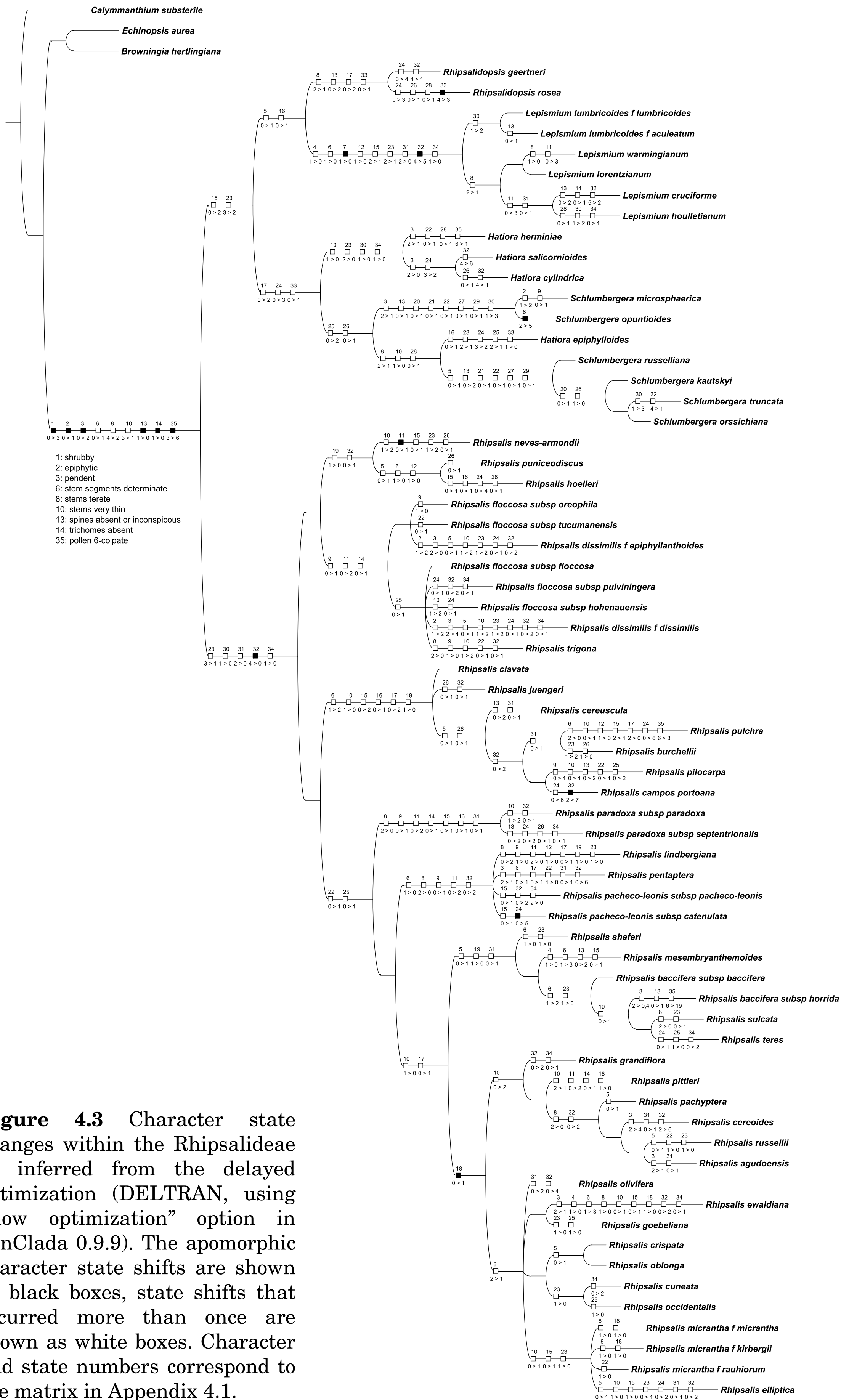

Figure 4.3 Character state changes within the Rhipsalideae as inferred from the delayed optimization (DELTRAN, using "slow optimization" option in winClada 0.9.9). The apomorphic character state shifts are shown as black boxes, state shifts that occurred more than once are shown as white boxes. Character and state numbers correspond to the matrix in Appendix 4.1.

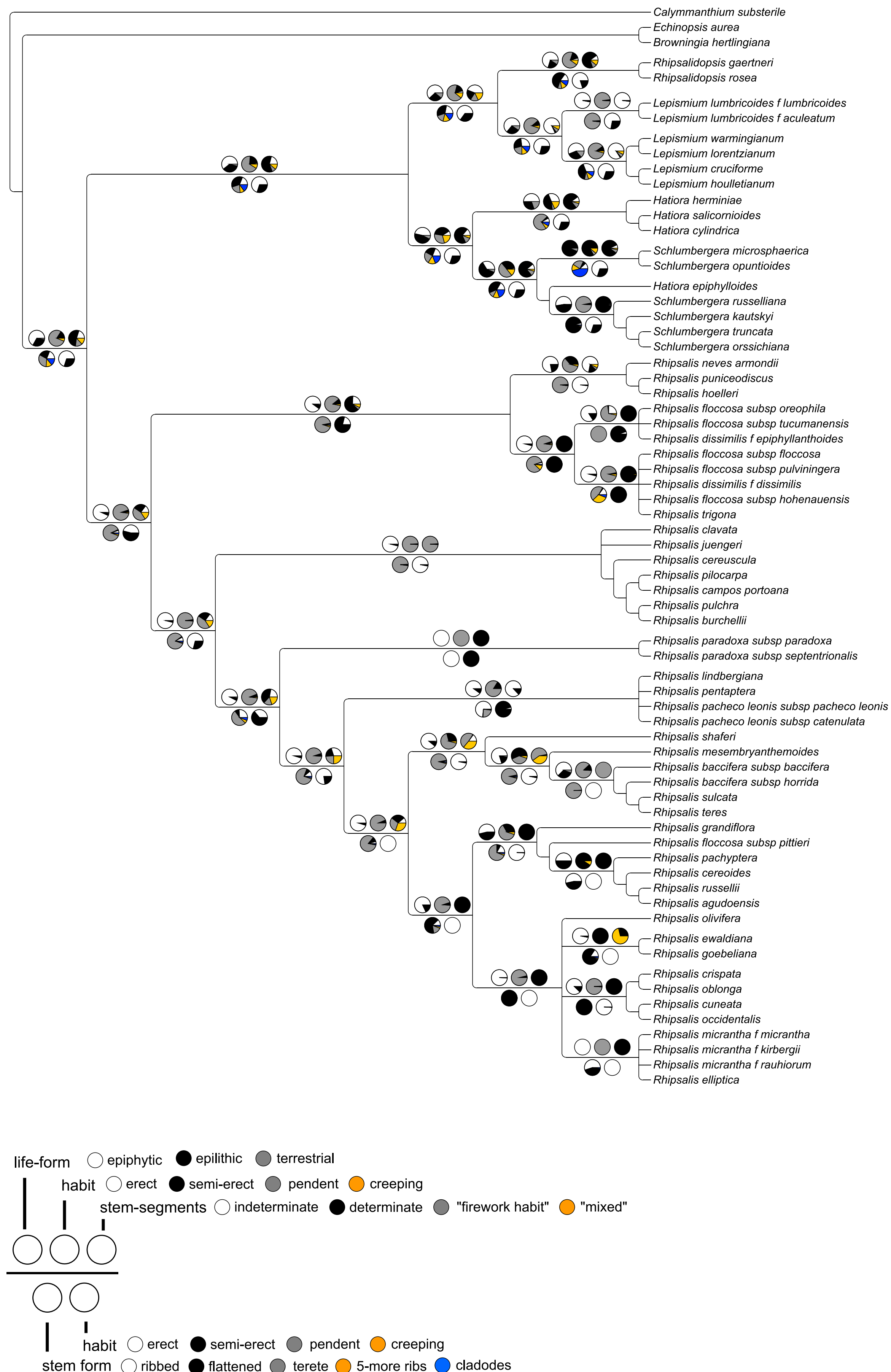

Figure 4.4 BayesTraits ancestral states reconstruction for the life-forms and the main vegetative characters. Posterior Probabilities are shown as pie chart sectors.

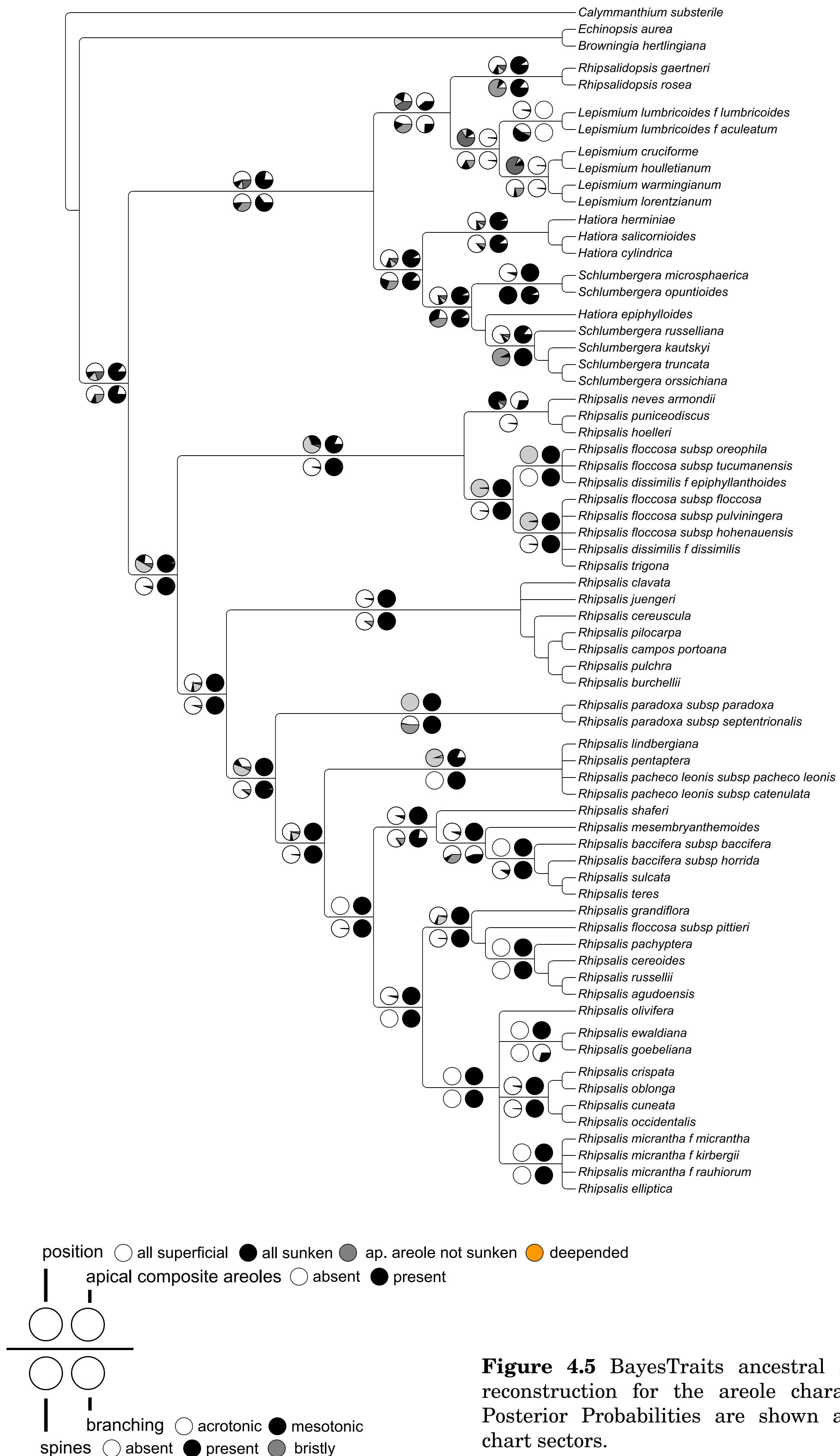

Figure 4.5 BayesTraits ancestral states reconstruction for the areole characters. Posterior Probabilities are shown as pie chart sectors.

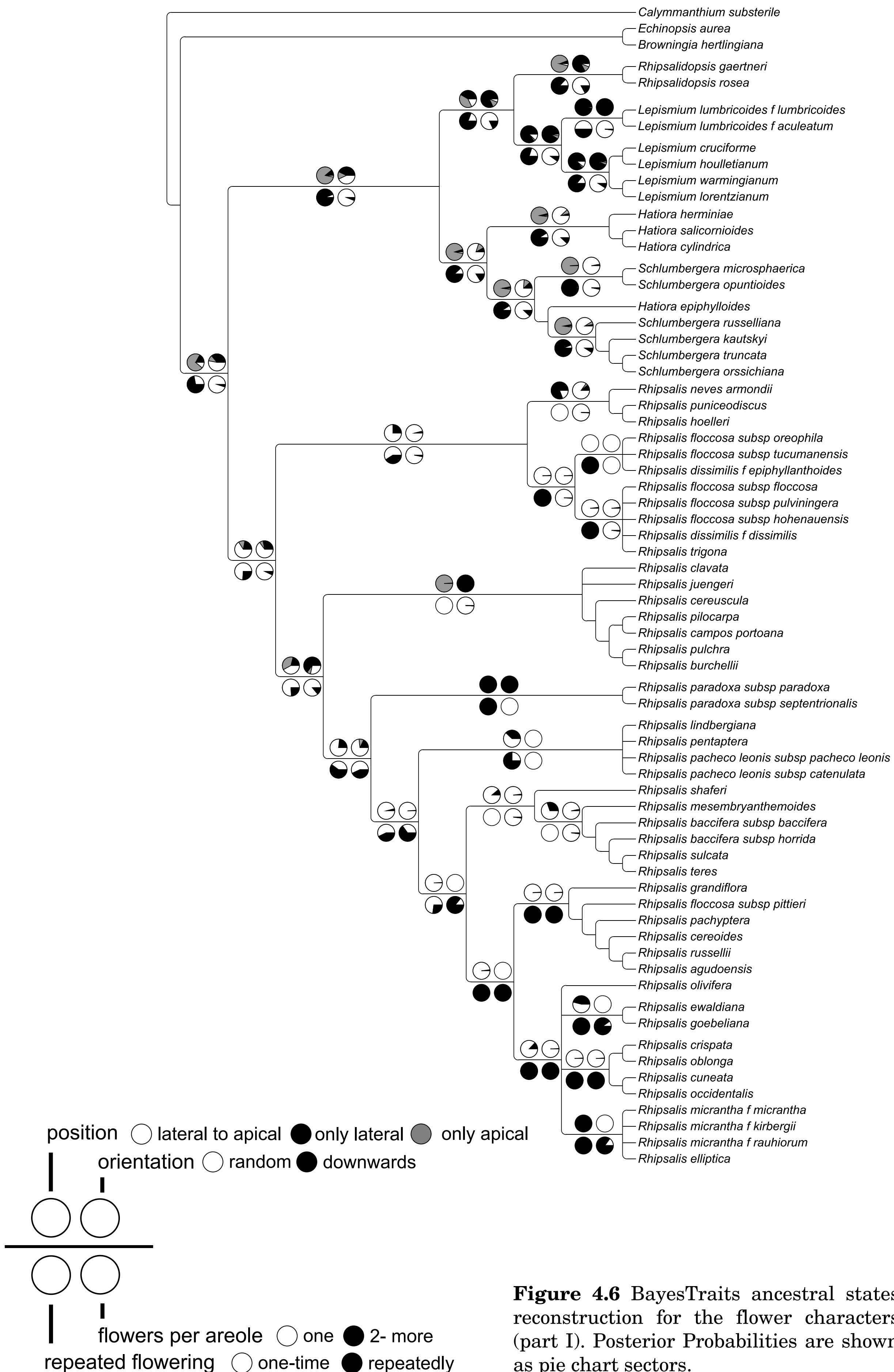

Figure 4.6 BayesTraits ancestral states reconstruction for the flower characters (part I). Posterior Probabilities are shown as pie chart sectors.

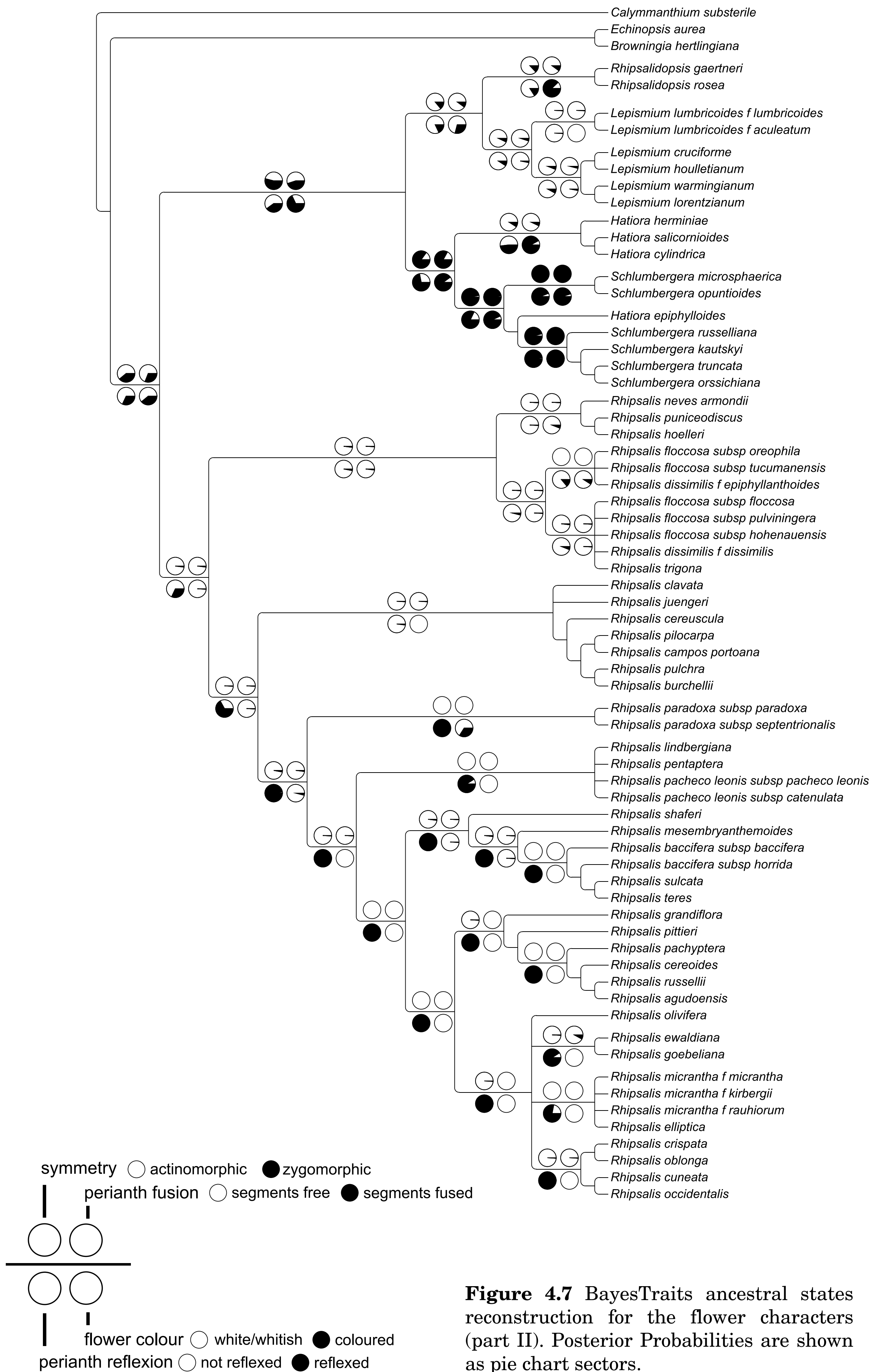

Figure 4.7 BayesTraits ancestral states reconstruction for the flower characters (part II). Posterior Probabilities are shown as pie chart sectors.

Besides the characters discussed above, most characters, including also most of those used to define genera and subgenera, evolved at least twice and should therefore be considered highly homoplastic.

Determinate stem-segments are reconstructed as the predominant plesiomorphic state in the Rhipsalideae (PP 0.53). Shifts to indeterminate stem-segments happened 4 times: in *Lepismium*, in *Rhipsalis* subg. *Goniorhipsalis*, in the pair of *R. hoelleri* and *R. puniceodiscus* and also in *R. pulchra* (Fig. 4.3, DELTRAN optimization). The independent shifts to indeterminate segments are probably connected with the loss of composite apical areoles because both states occur predominantly together, subg. *Goniorhipsalis* being the only exception. The apical composite areoles were lost four times: in *Rhipsalis puniceodiscus* and *R. hoelleri*, in *R. lindbergiana*, in *R. puchra* (this one can produce apical composite areoles on rare occasions), and in *Lepismium*.

The "firework habit", a specialized pattern of indeterminate basal extension shoots and segments decreasing in size towards the distal part of the plant, evolved independently in *Rhipsalis* subg. *Rhipsalis* (except *R. shaferi*) and in subg. *Erythro-rhipsalis*.

All the stem forms likely evolved several times independently. Therefore, the same character state in different genera is often not homologous; for example the flattened stems of *Schlumbergera* and *Rhipsalidopsis* or of *Schlumbergera* and *Rhipsalis*. The highest posterior probability for any of the states plesiomorphic for the Rhipsalideae is 0.36 for terete stems (Fig. 4.4), and they are also found as synapomorphic for the tribe (Figs. 4.1-4.3). Terete stems also found as plesiomorphic in *Rhipsalis* (PP 0.89) and they predominate in the subgenera *Calamorhipsalis*, *Erythrorhipsalis* and *Rhipsalis*. Flattened stems, in contrast, evolved in *Rhipsalidopsis*, in part of *Lepismium*, *Schlumbergera,* except *S. opuntioides* and *S. microsphaerica,* and in *Rhipsalis* subg. *Phyllarthrorhipsalis*.

While areoles are superficial in most Cactaceae and also in most Rhipsalideae, areoles that are sunken into the stem tissue are characteristic for *Rhipsalis*, although they are found in only some of its species (Fig. 4.2, ACCTRAN optimization). They seem to have evolved three times: in the *Rhipsalis floccosa*-group, *R. paradoxa,* and *Rhipsalis* subg. *Goniorhipsalis* (Fig. 4.3, DELTRAN optimization). The deepened/ depressed areoles evolved only in *Lepismium*, most likely in the subgenus *Lepismium* (Fig. 4.2, ACCTRAN, and PP 0.72), then lost in *L. lorentzianum*. The different positions and development of areoles, leading to sunken pericarpels has been regarded as informative in the past: Backeberg (1959) based his *Lepismium* almost solely on this character while Barthlott & Taylor (1995) regarded it significant for *Rhipsalis* subg. *Epallagogonium*. As noted above, the sunken pericarpels of *Lepismium* and *Rhipsalis* are not homologous because they develop in a different way (Buxbaum 1970) and the deepened areoles of *Lepismium* are unique and characteristic for part of it. The sunken

areoles in *Rhipsalis* could either be considered homoplastic or as a synapomorphy of the genus, as suggested by the ACCTRAN optimization.

While trichomes are either absent or inconspicuous in all the other Rhipsalideae, densely woolly areoles evolved independently the *R. floccosa* group and *R. paradoxa* (Figs. 4.1-4.3).

The flower position is one of the characters often considered significant for the delimitation of genera. Indeed, especially the apical flowers define some clades. The clade of *Schlumbergera*, *Hatiora* and *Rhipsalidopsis* is characterised by flowers only at apical composite apical areoles (Fig. 4.1-4.3, PP 0.86). Within *Rhipsalis*, apical flowers are observed only in subg. *Erythrorhipsalis* (except *R. pulchra*) and are likely the results of a shift from lateral or lateral to apical flowers, which are found as plesiomorphic for *Rhipsalis* (PP 0.66 for lateral to apical, Fig. 4.6). This result confirms one of Barthlott's earlier assumption (1987). He was the first to define *Rhipsalis* subg. *Erythrorhipsalis* by the apical, often campanulate flowers and they are not synapomorphic but still characteristic for this subgenus. In contrast, lateral or lateral to apical flowers occur in all other *Rhipsalis* subgenera. Different kinds of flower orientation characterise some *Rhipsalis* clades but evolved independently in each of them. Flowers oriented downwards occur in *Rhipsalidopsis* and *Lepismium*, *Rhipsalis* subg. *Erythrorhipsalis*, *R. paradoxa*, *R. hoelleri* and *R. puniceodiscus*. The flower orientation is not necessarily linked with the flower position - apical flowers are not necessarily directed downwards. Oblique flower buds are characteristic for *Lepismium.* This is due to the fact that the depressed areoles are themselves aligned obliquely to the stem axis. Oblique flower buds are also found in *Rhipsalis* subg. *Calamorhipsalis* p.p. (not conspicuous in the *R. floccosa*-group), also for *Rhipsalis paradoxa* and *R. pacheco-leonis* and *R. pulchra*, but the oblique or perpendicular flower buds are not linked with flower position or orientation. In contrast, all species with exclusively apical flowers also have flower-buds aligned with the stem axis. Areoles that grow throughout the plant's life cycles and flower repeatedly are found throughout the Rhipsalideae and are likely plesiomorphic (PP 0.72). In contrast, one-time flowering is only found in the clade of *R. puniceodiscus*, *R. hoelleri* and *R. neves-armondii*, also in *Rhipsalis* subg. *Erythrorhipsalis*, subg. *Rhipsalis* and in *R. lindbergiana*. The ability for repeated flowering at one areole seems to be lost independently in these species.

The largest pollen grains occur in *Schlumbergera* and *Rhipsalidopsis* and the reduction of the pollen grain size is a trend throughout the Rhipsalideae. But at the same time, there seem to be also several independent secondary increases in pollen size. There are different scenarios for the evolution of the pollen size. The ACCTRAN optimization suggests small pollen in all Rhipsalideae and then independent increases in *Rhipsalidopsis* and *Schlumbergera*, also in *Rhipsalis* subg. *Goniorhipsalis*. The DELTRAN optimization finds three shifts from medium-sized to small pollen in *Lepismium*, in *Hatiora* and in *Rhipsalis*, with secondary increases in *Rhipsalis* subg.

Goniorhipsalis. A varying number of apertures is found in all Rhipsalideae genera except *Schlumbergera*, which is uniformly 6-colpate. Aperture number therefore is not informative within Rhipsalideae. Pollen with 3 colpi is still found within the first branching *Rhipsalis* clades (subg. *Calamorhipsalis* and *Erythrorhipsalis*) while the rest has higher aperture numbers, most commonly 6, which is found as a synapomorphy of the Rhipsalideae by the DELTRAN optimization. A further increase in aperture number is characteristic for subg. *Rhipsalis* that has more or less uniformly 6 and 12 colpi, and usually both states are observed within a taxon; and within subg. *Phyllarthrorhipsalis* some species also have 12-colpate pollen. There are no reversals from 6- or 12-colpate to 3-colpate pollen throughout the Rhipsalideae. The general pattern seems to be what is termed successiformy i.e. the increase of aperture numbers by doubling. This appears to happen frequently and independently.

4.4.3 Evolution of characters associated with the epiphytic life-form

It is difficult to formulate hypotheses on the evolution of epiphytism in the Rhipsalideae from a comparison with their closest relatives. The sister group of the Rhipsalideae, the BCT-clade is morphologically very different. Notably, there seems to be a slight but recurrent tendency for epiphytism in the BCT-clade: two *Cleistocactus*, one *Samaipaticereus* and two *Echinopsis* species are commonly found as epiphytes in Bolivia (Ibisch & al. 2000); *Echinopsis arboricola* is even an obligate epiphyte (Kimnach 1990). Using a Bayesian approach for ancestral states reconstruction, and sampling genera from all major Cactaceae clades, Hernández-Hernández & al. (2011) reconstructed the common ancestor of the Rhipsalideae and the BCT clade as an erect and ribbed, less probably barrel-like cactus (PP ribbed 0.99, erect 0.71, barrel-like 0.56). They suggest that the steps during the evolution of epiphytism therefore would have involved first a shift to shrubby habit in the ancestor of the Rhipsalideae and in the next step, the evolution of the pendent habit (PP shrubby 0.47, epiphytic 0.99, non-erect 0.99). The results of this study confirm this; the shrubby and pendent habit is reconstructed as synapomorphic for the Rhipsalideae, with even higher Posterior Probabilities (PP shrubby 0.99, pendent 0.78). It is therefore likely that the ancestor on the Rhipsalideae was a terrestrial plant that had the ability to grow epiphytic and finally shifted to fully epiphytic. Within the Rhipsalideae, most of the species are obligate epiphytes, epilithic growing species and also terrestrials are observed, but no hemiepiphytic species. The epilithic growth predominates in some clades or taxa: in *Schlumbergera*, *Rhipsalis teres* and especially in the *R. cereoides*-group but there are only two obligate lithophytes which are the two forms of *R. dissimilis*. All other species growing as lithophytes are also found as epiphytes.

It appears there was no “transition” from terrestrials to lithophytes to epiphytes but rather a direct shift from terrestrials to epiphytes. However, the possibility of extinction at the branch leading to the Rhipsalideae must also be considered.

Epiphytism is reconstructed as a crown group synapomorphy in the Rhipsalideae (PP 1.0), Figs. 4.2-4.4. The epilithic and terrestrial growth in the Rhipsalideae appear to be reversals or further shifts. There are different possible scenarios for the epilithic growth. It may represent an ancestral condition within the tribe (PP 0.44) which has been retained and became predominant in some clades. Within *Rhipsalis*, the probability for epilithic growth found for the backbone nodes is small and increases only in the ancestor of the *R. cereoides*-clade and the *R. teres* - *R. baccifera* clade. It seems therefore, that these groups have independently shifted from epiphytic to predominantly or facultative epilithic habit. The terrestrial growth in *Hatiora* is also found as a reversal (Fig. 4.4), not as an ancestral condition. The pendent habit appears connected with the epiphytic life-form. The semi-erect or spreading habit appears derived and often connected to epilithic life-form.

Adventitious roots are commonly found in epiphytic cacti and are believed to be connected with the epiphytic life-form to allow the plants to attach themselves to the tree bark or rock and to absorb water and minerals (Gibson & Nobel, 2002). It is therefore not surprising that adventitious roots are also frequent in the Rhipsalideae. They are developed in *Schlumbergera*, *Lepismium*, *Rhipsalis* subg. *Erythrorhipsalis* and subg. *Rhipsalis* and in 9 other *Rhipsalis* species.

The epiphytes often have a different stem morphology compared to terrestrial cacti. Especially the formation of flattened stems is characteristic and found in all the epiphytic genera. In Rhipsalideae, thin terete stems are also very common and they are found as one synapomorphy of the tribe (Figs 4.1-4.4). Reconstructing the evolution of the different stem-forms is not straightforward. There are numerous shifts between the different stem forms, involving convergent evolution and reversals. The two principal states – the flattened and terete stems evolved both from multi-ribbed stems but it seems that multi-ribbed stems can be either transformed in terete or flattened stems. The flattened stems are derived from ribbed stems as result of the formation of only two ribs (Gibson & Nobel 1986, Wallace & Gibson 2002). They occur consequently only in those clades where also ribbed stems are found and evolved independently in *Schlumbergera* and in *Rhipsalis* subg. *Phyllarthrorhipsalis*. It appears that there are several reversals from flattened to 3-ribbed stems in subg. *Phyllarthrorhipsalis*. These shifts therefore must be rather easy, and some species (e.g. *Rhipsalis pachyptera*) produce segments with 3-4 ribs before producing a flattened segment, often also both types of segments are observed on one plant.

The thin terete stems which are so typical for the Rhipsalideae could have evolved from cylindrical stems with prominent podaria, as they can be observed for example in *Schlumbergera microsphaerica*. The podaria were subsequently reduced until they were finally not visible any more, resulting in perfectly terete stems. Such a case can be observed in the *R. floccosa*-group: most of the taxa do have podaria but they are less prominent in some of the species (e.g. in *R. floccosa* subsp. *oreophila*). The

sister group, the *R. puniceodiscus*-clade have perfectly cylindrical stems, indicating a reduction of the podaria. The Bayesian reconstruction find a PP of 0.7 for the absence of podaria at the node leading to the Rhipsalideae (Fig. 4.4) but the presence or absence of podaria in *Rhipsalis* appear equally likely (PPs 0.46 absent, 0.54 present). Low or moderate posterior probabilities to form podaria are also found for most of the *Rhipsalis* clades (Fig. 4.4.), especially for those clades with terete stems, e.g. *Rhipsalis* subg. *Erythrorhipsalis* and *Phyllarthrorhipsalis*.

The stem diameter was used here as an approximate indicator for the degree of succulence, assuming that thin stems do not store large amounts of water (Gibson & Nobel 1986). Even if this is only an approximation, there seems to be a tendency for the reduction of succulence in the whole tribe. All optimization schemes find the thin stems with an assumed low degree of succulence as one of the synapomorphies of the Rhipsalideae (Figs. 4.1-4.3) and shifts to the smallest stem diameters in *Hatiora*, *Schlumbergera* and in the *Rhipsalis* subgenera *Phyllarthrorhipsalis*, *Erythrorhipsalis* and *Rhipsalis*. The more succulent stems in the *Rhipsalis cereoides*-clade would then result from an increase. This increase in succulence is possibly connected with the predominant epilithic habit of this clade; a higher degree of succulence is also found in the likewise epilithic *Rhipsalis dissimilis* forms.

Spines are either absent or reduced to bristles in the majority of the Rhipsalideae and are found as one of the synapomorphies (Figs. 4.1-4.3). Prominent spines are only developed in *Schlumbergera opuntioides* and *S. microsphaerica*, in *Lepismium lumbricoides* forma *aculeatum* and in *Rhipsalis baccifera* subsp. *horrida*. The probability for spines is low (PP 0.23) in the node leading to the Rhipsalideae. This would mean that the prominent spines result from reversals. Barthlott (1983) and Barthlott & Rauh (1975) considered spines on the adult stems of the Rhipsalideae neotenic. Most of the species do have spines in their juvenile stage and often also on primary stem-segments but later reduce the spines. Neoteny is an evolutionary mechanism that allows retaining juvenile traits in the adult stage and thus to re-gain traits that have been reduced or lost. Neoteny is especially common and well known in animals but probably also relevant for angiosperms (Takhtajan 1972).

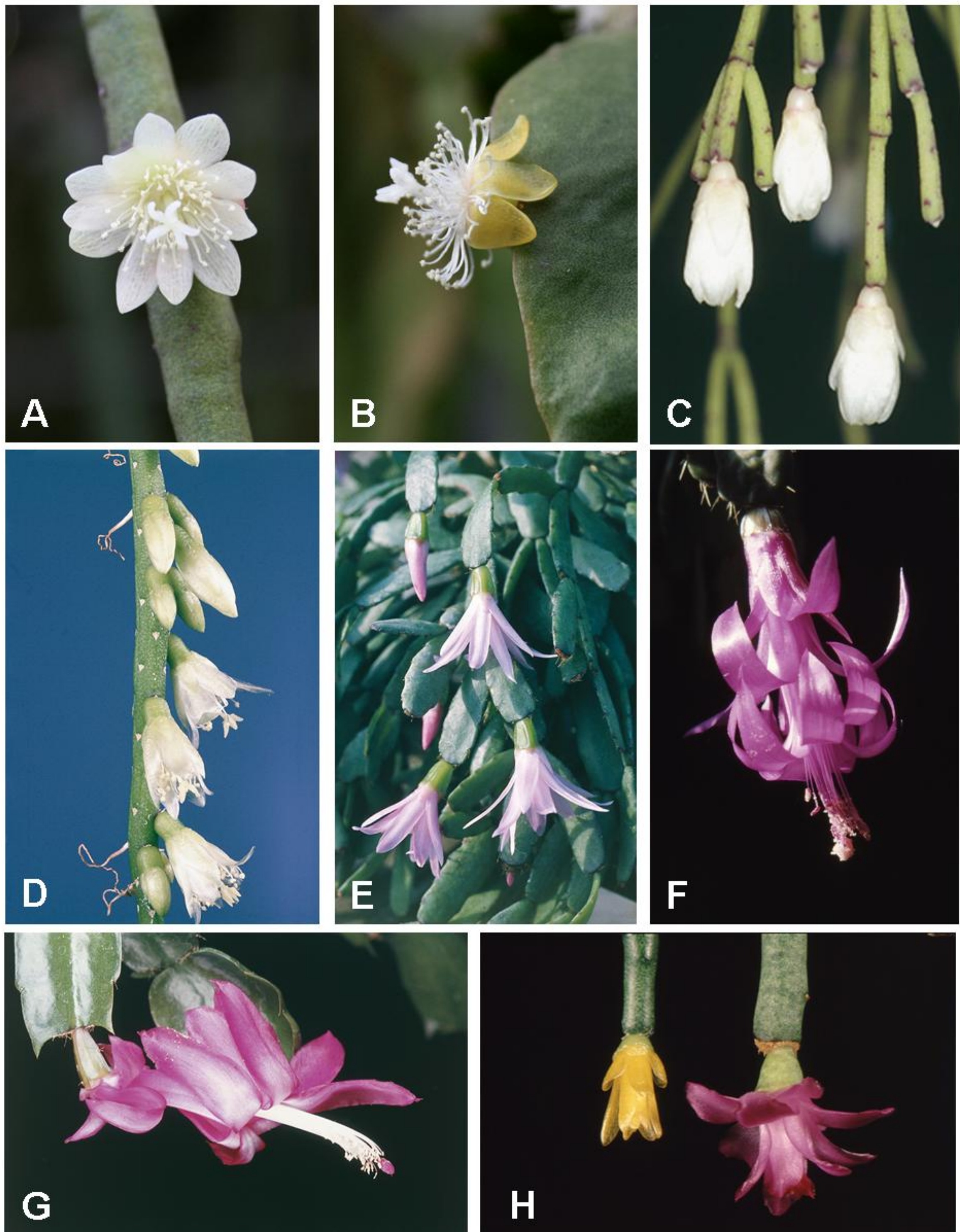

Figure 4.8 Flowers of the Rhipsalideae. A-C: *Rhipsalis*. A: *R. floccosa*: actinomorphic, otherwise unspecialised flowers characteristic for subg. *Calamorhipsalis*, B: *R. elliptica* showing the “stamen brush” syndrome with reflexed perianth segments, and exserted stamens as the main visible attractant. This flower type is typical for *Rhipsalis*. C: *R. clavata*: apical, campanulate flowers directed downwards, as characteristic for subg. *Erythrorhipsalis*. D: *Lepismium lumbricoides* has the same floral syndrome as *Rhipsalis* subg. *Erythrorhipsalis* but the flowers are lateral, not apical. E: *Rhipsalidopsis rosea*: intensely coloured, apical, campanulate flowers. F-G: *Schlumbergera*. F: *S. russelliana*: apical actinomorphic flowers with perianth segments fused and reflexed, G: *Schlumbergera truncata*: zygomorphic flowers with perianth segments fused and reflexed. H: *Hatiora salicornioides* (left) and *H. herminiae* (right): apical, campanulate coloured flowers.Photos C-H: W. Barthlott.

4.4.4 Evolution of floral traits and assumed pollination syndromes

A first attempt to reconstruct the flower morphology of the Rhipsalideae in a phylogenetic context was recently done by Calvente & al. (2011). They analysed three flower characters: the floral symmetry (actinomorphic vs. zygomorphic), the flower tube (conspicuous vs. inconspicuous) and the flower colour (strong vs. translucent) but functional aspects of the flowers and possible pollination syndromes were not discussed.

The ancestral state reconstruction of floral traits in this study indicates that the ancestral flowers of the tribe were actinomorphic (PP 0.6), small (1-3 cm in diameter), with free perianth segments (PP 0.7), and not intensely coloured (PP 0.6). *Schlumbergera* has many floral innovations, which are the fusion of the perianth segments and thus the formation of a floral tube (PP 0.98), a nectar chamber and zygomorphic flowers. All character optimization schemes suggest that the zygomorphic flower evolved even twice within *Schlumbergera*, in *S. opuntioides* and *S. microsphaerica* and independently in the *S. truncata*-clade. The Bayesian approach, however, suggests a common origin of zygomorphic flowers already at the node leading to *Schlumbergera* (PP 0.97). The actinomorphic flowers of *S. russelliana* would then be the result of a reversal. The same scenario was also found by Calvente et al. (2001).

Hatiora epiphylloides that is resolved as belonging in *Schlumbergera* however has actinomorphic, campanulate flowers and lacks the perianth tube as well as all the other floral synapomorphies of *Schlumbergera*, such as stamens in two series and connivent stigma lobes. It has the vegetative morphology of *Schlumbergera* but flowers of *Hatiora* or *Rhipsalidopsis.* The different flower morphology of *Hatiora epiphylloides* is thus not straightforward to interpret and many reversals have to be assumed. But as already discussed in Chapter 3, *H. epiphylloides* could be a hybrid of a true *Hatiora* and a *Schlumbergera* and this might be an explanation of its intermediate morphology.

The reduction of flower size seems to be a tendency throughout the Rhipsalideae. *Schlumbergera* and *Rhipsalidopsis* have the largest flowers within the tribe while flower size is highly reduced in *Hatiora*. Within *Rhipsalis*, small flowers predominate and flower size is even further reduced to a diameter less than 1 cm in subg. *Rhipsalis* and in some species of subg. *Phyllarthrorhipsalis*. The character reconstruction suggests small to medium-sized flowers as plesiomorphic, medium-sized to small flowers in the ancestor of the SHLR-clade and a reduction of flower size independently in *Lepismium,* in *Hatiora* and in *Rhipsalis*.

Flowers with reflexed perianth segments evolved independently in *Schlumbergera*, in *Hatiora herminiae*, and in *Rhipsalis*. In *Rhipsalis*, this character is synapomorphic for the clade formed by the subgenera *Epallagogonium*, *Goniorhipsalis*, *Rhipsalis* and *Phyllarthrorhipsalis*. It is also found convergently in *Rhipsalis pilocarpa*, *R. trigona* and *R. floccosa* subsp. *tucumanensis*. A nectar disc is also

developed. The reflexed perianth in *Rhipsalis* causes prominent exposure of the stamens leading to a unique flower type termed "stamen-brush" flowers in the following. (Fig. 4.8 B).

Coloured flowers are found with high probability as absent in *Rhipsalis* (PP 0.99). One of the exceptions is *R. hoelleri,* which has intensely red coloured flowers, indicating it attracts different pollinators than the rest of *Rhipsalis*. Some *Rhipsalis* have yellowish or yellow flowers (e.g. *R. elliptica* Fig. 4.8 B). The presence of coloured flowers appears to be the result of reversals, so it seems shifts from coloured to non-coloured flowers or vice versa are rather easy.

The pollination of epiphytic cacti is difficult to study in the field and consequently, there is only very limited information on their pollination biology from field observations. In general, very few cactus genera have been studied in the field for their pollination biology (Pimienta-Barrios & del Castillo 2002). Nevertheless, Cactaceae flowers can be classified in different pollination syndromes, based on flower shape and colour. The major pollinator groups are bats, moths, birds and bees (Pimienta-Barrios & del Castillo 2002, Porsch 1938, 1939). For the Rhipsalideae, it is possible to classify the flowers in two main groups: as bird- or insect pollinated. The character reconstruction allows four main flower "types" found in the Rhipsalideae, based on combinations of different characters. The most distinct flowers are the comparatively large, tubular, flowers of *Schlumbergera* which have an intensely magenta coloured perianth and also coloured styles, filaments and pollen. They can be considered bird-pollinated and hummingbirds have indeed been reported visiting *Schlumbergera* flowers (McMillan & Horobin 1995). Rose & Barthlott (1994) also suggest that red coloured pollen is also part of the bird-pollination syndrome, as a mimetic adaptation. The pollen colour is similar to the colour of the bird's beak and is thus less irritation for the bird than a contrasting pollen colour would be. *Rhipsalidopsis* has actinomorphic, campanulate flowers, also intensely coloured and probably pollinated by birds as well. The flowers of *Hatiora* are more difficult to classify, they are actinomorphic, small, either intensely yellow or magenta and may be visited by birds or/and by insects. Within *Rhipsalis*, there are three different flower "types" which possibly attract different pollinators. Most common are the white or whitish stamen-brush flowers with a reflexed perianth (Fig. 4.8 B). Other *Rhipsalis* have no reflexed perianth and the flowers are either funnel-shaped or more or less campanulate but with no apparent species characteristics and usually not coloured (Fig. 4.8 A). Finally, *Lepismium* and *Rhipsalis* subg. *Erythrorhipsalis* share the campanulate, pendent flowers directed downwards with a white perianth (pink only in some forms of *L. cruciforme*). It is therefore likely that *Rhipsalis* subg. *Erythrorhipsalis* attracts other pollinators compared to the rest of *Rhipsalis* but the same pollinators as *Lepismium*.

4.4.5 Morphological intermediates in the Rhipsalideae

There are five *Rhipsalis* species which seem misplaced because they do not "fit" in morphologically well defined clades. In fact, their morphology appears intermediate between the clade they are part of and another, more distant clade. *Rhipsalis pulchra* lacks the apical flowers characteristic for subg. *Erythrorhipsalis* and resembles *R. puniceodiscus*. *Rhipsalis grandiflora* and *R. pittieri* have neither angled nor flattened stems characteristic for subg. *Phyllarthrorhipsalis* but instead have terete stems. On the other hand, *R. grandiflora* shares most characters with *Phyllarthrorhipsalis*, such as multiple flowering at one areole and repeated flowering. The placement of *R. pittieri* in *Phyllarthrorhipsalis* is more difficult to explain, as it shares hardly any characters with the rest of this subgenus but is instead very similar to the other *R. floccosa*-clade and has been included in it, as a subspecies of *R. floccosa,* which it very closely resembles. *Rhipsalis ewaldiana* has a characteristic vegetative habit with long shoots and short shoots and mesotonic branching, found besides only in *R. mesembryanthemoides*. Based on this, these two species have been regarded as sister species. But Barthlott & Taylor (1995) in their first description of *R. ewaldiana* also stated that it might even be a hybrid of *R. mesembryanthemoides* and a species with winged or angled stems. But *R. ewaldiana* fits well into subgenus *Phyllarthrorhipsalis* by having angled stems. Considering that the sister relationship of *R. ewaldiana* and *R. goebeliana* is supported with 100%, *R. goebeliana* might have been the second hybrid parent with flattened stems. Finally, *Rhipsalis sulcata* falls in the *R. teres* alliance and cannot even be separated from the *R. teres* accessions sampled. This species has angular stems and Barthlott & Taylor (1995) therefore considered it related to the other species with angular stems, e.g. *R. paradoxa* or *R. pentaptera*. But at the same time, *Rhipsalis sulcata* has the habit of *R. teres* and *R. baccifera*: pendent stems, indeterminate basal extension shoots and strict acrotonic branching. Even if the angled stems appear exceptional in subg. *Rhipsalis*, they are sometimes developed also in *R. teres* (f. *prismatica*).

One possible explanation for the placement of these taxa is that they are hybrids and found next or close to their hybrid mother-parent. Hybrids are common in Cactaceae and known from *Schlumbergera* and *Rhipsalidopsis*. No definite hybrids in *Rhipsalis* are known from cultivation so far; although Taylor (1999) reports a plant from cultivation which appears to be an intermediate between *Rhipsalis puniceodiscus* and *R. neves-armondii* and he assumes to be of hybrid origin. Therefore, these morphological intermediates can be a first hint towards hybridization in *Rhipsalis,* but this has to be confirmed using nuclear markers.

4.4.6 Morphological characterization of the clades inferred by sequence data corresponding to genera

The aim of a classification based on a phylogenetic hypothesis is to classify (=name) monophyletic entities. The formally recognized taxa such as genera or subgenera should desirably be also recognizable by their morphology. However, Cactaceae pose problems for finding morphological characters defining taxonomic entities, as already discussed in the Chapters 1 and 2. The result from the character reconstruction here is that most vegetative characters are homoplastic, including many of the characters used to define genera in the past. Emphasis of single characters may therefore be misleading when they are used to define taxonomic units. Therefore, not all characters are equally useful for delimitation of all the genera and subgenera. While stem morphology defines some clades, flower morphology defines others and mostly only combinations of characters allow an unambiguous diagnosis. But although most of the characters appear more than once, they are homogenous within the respective clades and therefore can be used as diagnostic characters. So as a result, all the highly supported clades found by the molecular phylogenetic analyses can be defined morphologically. The most relevant characters are the branching pattern, the determinate growth of stem-segments, the shedding of old segments, the stem form, the position of the areoles (superficial compared to sunken areoles), woolly areoles post-anthesis, the flower position, flower orientation, flower number per lateral areole. The diagnostic characters for the genera of Rhipsalideae as found by the character optimization schemes are summarised in Tables 4.1 and the characters defining the subgenera of *Rhipsalis* are listed in Table 4.2.

Table 4.1 Morphological characteristic for the Rhipsalideae genera. Apomorphic characters are highlighted in bold.

	Rhipsalis	*Lepismium*	*Schlumbergera*	*Hatiora*	*Rhipsalidopsis*
Branching	strictly acrotonic or subacrotonic	**mesotonic**	strictly acrotonic	strictly acrotonic	strictly acrotonic
Stem segments	commonly determinate, in some subgenera indeterminate	**indeterminate**	Determinate growth	Determinate growth	Determinate growth
Stem form	terete, flattened or 3-ribbed, sometimes with prominent podaria	3-ribbed or flattened	flattened	terete, cylindrical	flattened
Old segments	deciduous	**not deciduous**	deciduous	deciduous	deciduous
Apical composite areoles	present (rarely absent)	**absent**	present	present	present
Flower position	predominantly lateral or lateral to apical or only apical at composite areoles	**Only lateral**	apical (at composite areoles)	apical (at composite areoles)	apical (at composite areoles)
Flower morphology	actinomorphic, perianth often reflexed, **stamen-brush**, OR campanulate, pendent, oriented downwards, stamen-brush not developed	actinomorphic, campanulate, pendent, oriented downwards	**zygomorphic**, rarely actinomorphic with a well developed **perianth tube**	actinomorphic, campanulate	actinomorphic, campanulate
Flower size	small to very small	small	medium-sized	small/ very small	medium-sized
Flower colour	mostly white/ whitish, or pale yellow. Rarely intense yellow or pink	white/whitish, pink only in *L. cruciforme*	pink/magenta	yellow or pink	red or pink
Pericarpel	smooth, **never angled**, terete and naked (bristly in *R. pilocarpa*)	angled or **ridged**	angled (sometimes only slightly angled)	smooth, not angled	angled
Stamen insertion	one series	one series	**two series**	one series	one series
Fruits	globose or subglobose (barrel-shaped)	globose	elongate	elongate	elongate
Fruit colour	**white** or coloured	coloured, mostly **dark red to almost black**	greenish	greenish or pink	red or yellow

Table 4.2 Diagnostic features of the *Rhipsalis* subgenera. Apomorphic characters are highlighted in bold

	Calamorhipsalis	***Erythrorhipsalis***	***Goniorhipsalis***	***Epallagogonium***	***Rhipsalis***	***Phyllarthrorhipsalis***
determination of stem segments	predominantly determinate	firework habit	predominantly indeterminate	determinate	firework habit	determinate
stem form	terete + offset podaria	terete	offset podaria	offset podaria	terete	flattened angled
areoles development	sunken, sometimes **also the apical areoles**	not sunken	sunken, except apical areole (not in *R. lindbergiana*)	sunken, except apical ar	not sunken	not sunken
woolly areoles post-anthesis	developed	absent	absent	developed	absent	absent
flower buds position	oblique, perpendicular	aligned with stem axis	oblique	oblique, perpendicular	perpendicular	perpendicular
flower position	lateral to apical	only apical	lateral to apical	only lateral	lateral to apical	lateral to apical
flower orientation	random	downwards	random	downwards	random	random
flowers per lateral areole	one	one	one	one	one	**several (rarely one)**
flower morphology	actinomorphic, perianth not reflexed	actinomorphic, campanulate, pendent	perianth reflexed, stamen brush developed	perianth reflexed, stamen brush developed	perianth reflexed, stamen brush developed	perianth reflexed, stamen brush developed

Chapter 5

Towards understanding the historical phylogeography of *Rhipsalis baccifera*, the most widespread cactus

Summary

Rhipsalis baccifera is the most widespread cactus and the only cactus that is native to tropical Africa. The distribution patterns of *Rhipsalis baccifera* are addressed in this chapter using tree building methods and haplotype network algorithms. The taxon sampling included 42 *Rhipsalis baccifera* specimens covering most of the area. A haplotype network based on the *rps3-rpl16* spacer and the *rpl16* intron was constructed using the statistical parsimony as implemented in TCS, and Maximum Likelihood (ML) methods. The TCS algorithm found 10 haplotypes whereas a network derived from ML analysis found 17 haplotypes. Two main groups of plastid haplotypes were found using both methods: a northern South American haplotype that included specimens from the Caribbean and Mesoamerica and a haplotype shared by the African specimens. Besides, unique haplotypes were found in several South American and African specimens. These results suggest a single dispersal of *Rhipsalis baccifera* to Africa and reveal high genetic diversity within its populations.

5.1 INTRODUCTION

Most Cactaceae have distribution areas of about 10.000 km^2 but *Rhipsalis baccifera* occupies an area which is estimated to be 2000 times larger (Barthlott et al., unpublished data). Its range, as illustrated in Fig. 5.1, covers large parts of northern tropical South America to the Caribbean and Mexico. *Rhipsalis baccifera* is thus the most widespread cactus and besides, it is the only cactus with a natural occurrence in the old World where it ranges through large parts of tropical Africa to Madagascar and to Sri Lanka (Barthlott, 1983).

There are currently 5 accepted subspecies of *Rhipsalis baccifera* (Barthlott & Taylor 1995, Hunt 2006) which also have distinct geographical distributions – their areas do not overlap. The subsp. *baccifera* is found throughout northern South America, in the Caribbean, in southern Florida and in Mexico. The subsp. *hileiabaiana* is endemic to the state Bahia in south eastern Brazil, in the state of Bahia. The erstwhile subsp. *shaferi* (= *Rhipsalis shaferi*, see Chaper 2) replaces subsp. *baccifera* in Paraguay, Bolivia and northern Argentina.

In Africa, subsp. *mauritiana* is found throughout tropical Africa, subsp. *erythrocarpa* occurs in the mountains of tropical east Africa and subsp. *horrida* is endemic on Madagascar.

The occurrence of *Rhipsalis baccifera* in the Old World has long been known and has puzzled taxonomists and biogeographers for more than 100 years. The fist formally proposed name (*Rhipsalis aethiopica* Welw.) was published in 1859 but the plants were known at least 50 years before (references in Barthlott 1973). Most authors considered them to be identical with or closely related to the South American *Rhipsalis baccifera*. However, the exact origin of the African populations is unknown. The only suggestion is that of Backeberg (1942) who assumed dispersal to Africa from north-eastern South America.

The most commonly accepted hypothesis how *Rhipsalis baccifera* may have reached Africa was and still is dispersal by migratory birds (Backeberg 1942, Barthlott, 1983). But there are no migratory birds known that cross the Atlantic Ocean and may have brought *Rhipsalis baccifera* seeds to Africa. Consequently, these plants have also been considered to be Gondwana relicts (e.g. Croizat, 1952) or in the other extreme as introduced by man in the last 200 years (e.g. Buxbaum, 1970a). But these two theories were purely speculative, with no supporting data.

The first detailed study of the palaeotropic *Rhipsalis baccifera* was conducted by Barthlott (1973, 1984). He examined the morphology, pollen and ploidy level of the palaotropic *Rhipsalis*. He found the African populations to show more variability and also unique characters, not found in their South American relatives. All the African

populations studied so far are polyploid (4n=44, 6n=66 and 8n=88). The subsp. *erythrocarpa* has red fruits, otherwise not found in subgenus *Rhipsalis*. The populations from Madagascar are often terrestrial and spinyand their pollen has a unique reticulate tectum (Barthlott 1973, 1976, 1983).

These results argued against a recent introduction of *Rhipsalis baccifera* into Africa but rather suggested a long independent evolution of these populations. The theory that *Rhipsalis* was a Gondwana relict was also rejected because it appeared too derived to be an old Gondwana taxon (Barthlott 1983).

The population of *Rhipsalis baccifera* have not yet been analysed using molecular data. So far, sequences of plastid markers have already revealed some genetic diversity within the specimens sampled (Chapter 2, this study) and therefore a more detailed study seemed promising and was conducted here. The main questions on the biogeography of *Rhipsalis baccifera* are: How different are the African populations in comparison to their South American relatives? A high genetic distance should support the hypothesis of a long independent evolution thus arguing against a recent introduction. The next immediate question is therefore: When was the dispersal to Africa? Was there a single dispersal event or were there even independent dispersals? From where in South America or Mesoamerica did the dispersal take place? Do the morphologically different Malagasy populations result from further independent evolution on Madagascar?

The analyses presented in this chapter are a first step towards understanding the evolutionary history and distribution patterns of *Rhipsalis baccifera*. Traditional tree-building methods and haplotype network construction algorithms are applied. Both rely on sequence data from the *rps3-rpl16* spacer and the *rpl16* intron. This region is very variable, as found in the datasets of Chapters 1 and 2. It also shows variation at population level and was therefore chosen for the analyses here.

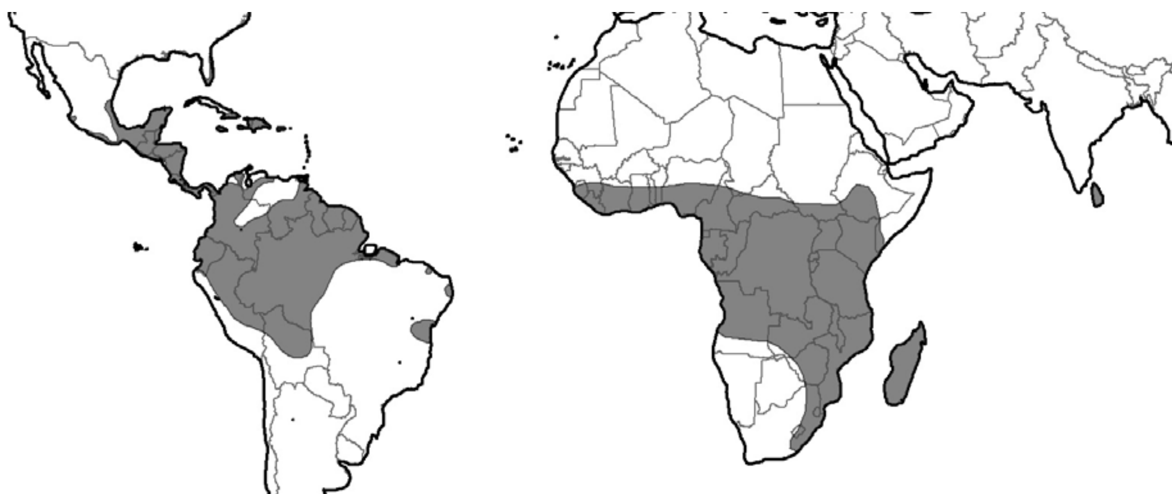

Figure 5.1 Estimated distribution area of *Rhipsalis baccifera*. Distribution data from Barthlott 1983, Taylor & Zappi 2004,

5.2 MATERIAL AND METHODS

5.2.1 Plant material and taxon sampling

The plant material was obtained from the living collections of the Botanic Gardens Bonn and the Botanical Garden Berlin-Dahlem. The sampling strategy was to include as many accessions from different origins as possible thus trying to cover most of the area. Totaling 42 accessions of the subspecies *baccifera*, *mauritiana*, *erythrocarpa* and *horrida* were sampled, with 20 specimens from South America, and 22 specimens from the Old World distribution area. The complete source information is provided in the Appendix 1.

5.2.2 Isolation of genomic DNA, amplification and sequencing

The plant material freshly collected then cut in small pieces and dried on silica-gel in a drying chamber at 35°C for app. 24 hrs. Genomic DNA was then isolated using a CTAB method as described in Chapter 1. DNA concentration and purity (A260/A280 ratio) were measured using a NanoDrop ND-1000 (peqLab, Erlangen, Germany). A working dilution of 10ng/µl was made to be used for PCR. The *rpl16* intron and the *rps3-rpl16* spacer were co-amplified as described in Chapters 1 and 2, the primer sequences are listed in the Appendix 2. All PCR products were stained with 100x 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). Manual editing of pherograms, assembly of sequences and manual sequence alignment was done using PhyDE v.0995 (Müller & al. 2005+).

5.2.3 Phylogenetic analyses and haplotype network construction

All the sequences of *Rhipsalis baccifera* accessions sampled were added to the *rpl16* dataset of Chapter 2 and analysed using Bayesian Inference as described therein. The analysis was run for 5000000 generations. The first 2000 trees were discarded and the remaining trees were summarised into a majority-rule-consensus tree.

A haplotype network was constructed using TCS (Clement & al. 2000) which implements the Statistical parsimony (Templeton & al. 1992). Standard phylogenetic reconstruction methods were additionally applied as they have recently been shown to perform well for haplotypic data (Salzburger & al. 2011). Trees were build using Bayesian Inference with MrBayes 3.1 (Huelsenbeck & Ronquist 2001), also using the heuristic search in PAUP* (Swofford 1998) under Maximum Parsimony (MP) and Maximum Likelihood (ML) and using Neighbour-Joining (NJ). The ML search was based on the best-fitting nucleotide substitution model (F81) as evaluated with jModeltest (Posada 2008) and the AIC information criterion. The resulting trees were imported into Haplotype Viewer (G. Ewing, available at www.cibiv.at/~greg/

haploviewer). This software converts trees build from traditional phylogenetic methods to haplotype genealogies.

5.3 RESULTS

5.3.1 Sequence characteristics, phylogenetic analyses and haplotype network construction

The amplified fragment of the *rps3-rpl16* spacer and the *rpl16* intron was 1183-1269 nt in length with a mean length of 1200 nt. and 1,2 % variable characters. Most variability was found in length variable mononucleotide stretches within the *rpl16* intron. Larger indels were also found, a 19 nt gap occurs in four of the sampled accessions.

The relationships found within subg. *Rhipsalis*, including all the 42 *Rhipsalis baccifera* accessions sampled, are shown in Fig. 5.2. The TCS haplotype network of *Rhipsalis baccifera* is shown in Fig. 5.3 and the results from the haplotype network derived from the ML analysis is shown in Fig. 5.4. These different methods find a different number of haplotypes: The TCS algorithm found 9 haplotypes whereas the network derived from ML analysis found 16 haplotypes. There are two haplotypes characterising the majority of the samples. The first haplotype is found in specimens from northern South America (Brazil, Colombia, and Venezuela) and from Mesoamerica (Costa Rica, Guatemala) and the Caribbean (Cuba and Jamaica).

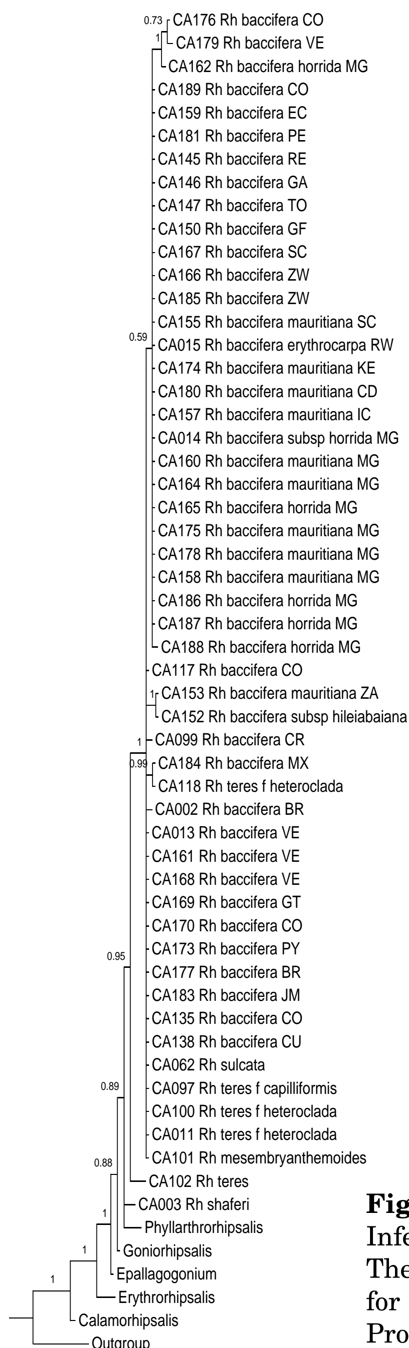

Figure 5.2 50% majority-rule consensus tree from Bayesian Inference including all the *Rhipsalis baccifera* accessions sampled. The other *Rhipsalis* subgenera have been reduced to single branches for better readability. Numbers above branches are Posterior Probabilities.

The second haplotype comprises all the specimens sampled from tropical Africa, Madagascar, the Seychelles and Réunion. It is also found in 3 samples from Northern South America (northern Colombia, Ecuador, Peru, French Guiana).

Besides, TCS and ML find 7 and 15 unique haplotypes, respectively. These are derived from either the northern South American or the African haplotype and found in only 1 or 2 specimens.

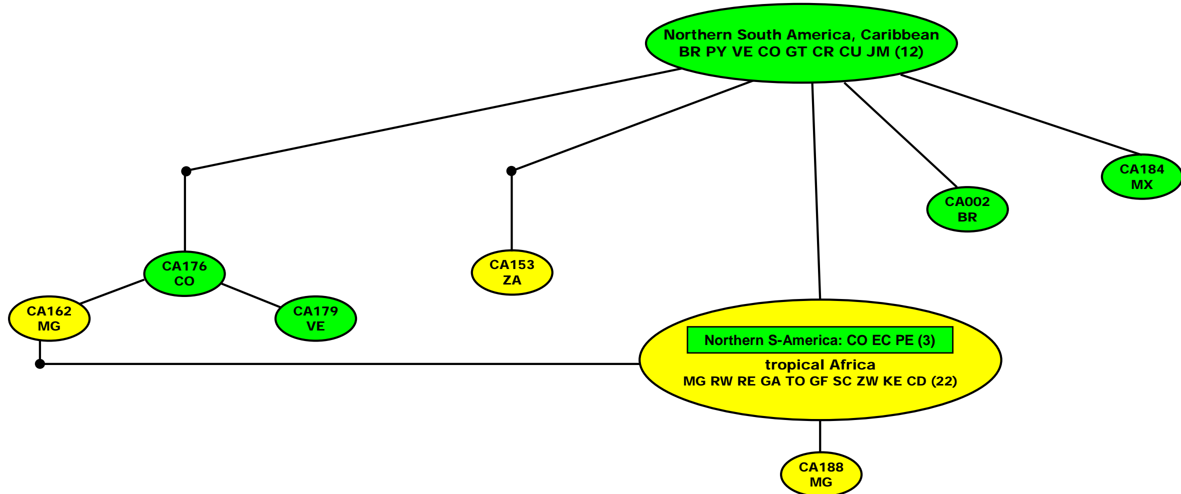

Figure 5. 3 TCS Haplotype network of *Rhipsalis baccifera* based on sequence data of *rps3-rpl16* and the *rpl16* intron.

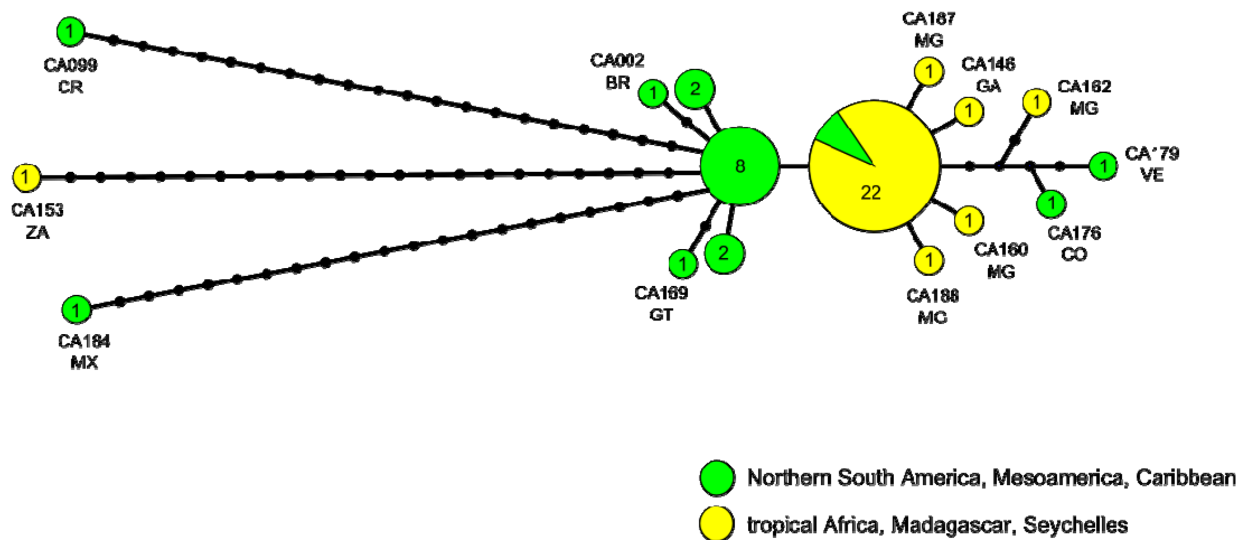

Figure 5.4 Haplotype network generated from a Maximum Likelihood analysis of *Rhipsalis baccifera* based on sequence data of *rps3-rpl16* and the *rpl16* intron.

5.4 DISCUSSION

5.4.1 First insight into the biogeography of *Rhipsalis baccifera*

As outlined in the introduction, the commonly accepted hypothesis for the origin of the African *Rhipsalis baccifera* is that they are derived from South American populations. This is supported by all the data on these populations available so far (Barthlott 1983).

The haplotype network analysis here finds one haplotype characterising all the African populations and derived from the northern South American haplotype. This supports the assumption of a single dispersal to Africa from South America. There are also unique haplotypes found in specimens from Africa which are secondly derived from the African haplotype. Of the 10 specimens sampled from Madagascar, 2 unique haplotypes are found by the TCS algorithm and 4 by the ML analysis. The ML analysis additionally finds a unique haplotype in the specimen from Gabun (CA146). These unique haplotypes suggest a long independent evolution and argue against a recent introduction of *Rhipsalis baccifera* to Africa by man. These results suggest further diversification and independent evolution of the African and especially Malagasy populations. This is in line with the hypothesis of Barthlott (1984) who assumed Madagascar to be an evolutionary centre of *Rhipsalis baccifera*.

The haplotypes found also reveal genetic diversity within the South American and Mesoamerican populations. The specimens from Mexico, Costa Rica, Guatemala and from Brazil have each unique haplotypes. This suggests several dispersals from northern South America further southwards and northwards.

There are some haplotypes that differ from the two frequent haplotypes, especially in South America. The intermediate haplotypes are missing (marked by black dots in the network). Several mutational steps have to be assumed in order to explain these haplotypes. The missing intermediate haplotypes could either be explained by incomplete taxon sampling or by the loss of these haplotypes.

Most difficult to explain is the fact that some South American populations have the African haplotype (Figs. 5.3 and 5.4). The TCS network finds reticulations involving the African haplotype and the samples CA179 and CA176. The ML analysis also haplotypes in specimens from Colombia (CA176) and Venezuela (CA179) to be derived from the African haplotype, with missing haplotypes in between (Fig. 5.3).

There is also a unique haplotype in the South African specimen (CA153). It is derived from the northern South American, not from the African haplotype.

A possible explanation for this would be multiple dispersals, including dispersal from Africa back to South America and maybe even a second dispersal to Africa. But

multiple dispersals of *Rhipsalis baccifera* have not yet been assumed and therefore need further investigation with a more thorough taxon sampling.

5.5 CONCLUSIONS AND OUTLOOK

The study presented in this chapter is just a first step towards a more detailed study of *Rhipsalis baccifera*. Unfortunately, the data do not provide any structure within the northern South American and African specimens, respectively. Therefore no conclusions about ancestral distribution and possible migration routes are possible at this point. Future work will be based on a larger taxon sampling, including desirably more specimens from individual populations. Also more markers, plastid and nuclear, will be added for the construction of the haplotype network and microsatellite markers will be used as well. Chromosome numbers for all the samples would need to be collected provide insights whether there was one polyploidization event connected to the dispersal to Africa and whether or if there are polyploids, maybe independently, already in South America or Mesoamerica. The timeframe for the dispersal to Africa also still has to be inferred using molecular clock dating.

Chapter 6

Development of microsatellite loci for *Rhipsalis baccifera* using 454 sequencing

6.1 INTRODUCTION

Rhipsalis baccifera is the most widespread Cactaceae species and the only cactus that ranges through a large part of tropical Africa. However, its biogeographic patters have not been studied in detail and especially the origin of the African populations is an open question. At the same time, it seems that the morphological differentiation in *Rhipsalis baccifera* is connected with its distribution patterns. Some populations in northern South America and especially the African populations differ morphologically. But it is hardly possible to assess this variation with "classical" methods studying the morphology.

It is well possible that there are still unrecognised cryptic species under the name *Rhipsalis baccifera*. A detailed study of the biogeography of *Rhipsalis baccifera* should therefore provide insights into its evolutionary history and possibly also into the mode of speciation. A better understanding on the genetic variation within the populations could also be of value for conservation assessments if genetically unique populations will be found.

So far, sequences of plastid markers have already revealed some genetic diversity within the *Rhipsalis baccifera* specimens sampled (Chapter 2 and 5, this study). But the plastid markers do not provide enough resolution between the individual populations and therefore more variable markers are needed. Microsatellite loci appear especially promising for this purpose. Microsatellites are highly variable DNA stretches with tandem repeats of few nucleotides, most commonly one, two, three and four nucleotides. Microsatellites offer some advantages in comparison to other population-level markers such as AFLPs (Vos & al. 1995). Using microsatellites, partial datasets can be generated that can be later expanded once suitable primers are designed. One of the disadvantages of using microsatellite loci was in the past that their development was laborious and expensive and required extensive cloning. The cloning steps unnecessary when next generation sequencing methods are applied. Using the next generation 454 sequencing ten thousands of reads can be generated with just one run. The reads obtained can then be screened for repeat-containing motifs and several algorithms and software is available for that purpose. The

development of microsatellite loci using 454 sequencing was initially tested by Santana et al. 2009. Since then, an increasing number of studies develop microsatellite markers using 454 sequencing (Abdelkrim & al. 2009, Allentoft & al. 2009, Castoe & al. 2010, Csencsics & al. 2010, Lee & al. 2009, Tangphatsornruang & al. 2009)

In this chapter, microsatellite markers for *Rhipsalis baccifera* have been developed using 454 sequencing and based on a genomic library enriched for repeat motifs. The first followed the AFLP protocol of Vos & al. (1995) and subsequent steps largely followed the protocol for isolation of microsatellite loci provided by Glenn & Shable (2005). The actual testing of primers and the application of the selected loci are beyond the scope of this study and will be the object of further work.

6.2 MATERIAL AND METHODS

6.2.1 Plant material and taxon sampling

The *Rhipsalis baccifera* accession no. 166048323 (*Leuenberger 3088*, Brazil, Bahía, Camarca, isolate number CA148, vouchered at B) was chosen from the Cactaceae living collection of the Botanical Garden Berlin-Dahlem.

6.2.2 Chromosome count

The number of chromosomes was determined since the plant had to be desirably diploid for the following genomic library construction and polyploidy is occasionally observed in *Rhipsalis baccifera* (Barthlott 1976), although so far only in specimens from the Caribbean and Florida and the African populations. Growing root tips of the aerial roots were collected at c. 8:40 h in the morning and pre-treated in 0,002 M solution of 8-hydroxychenoline for c. 4 h in a refrigerator at 5-8°C. They were then fixed with a mixture of 3:1 ethanol 96%-acetic acid for app. 24 h in a refrigerator at 5-8°C. The root tips were then hydrolyzed in 1 N HCL for 10 min. at 60°C then transferred into dest. water. A piece of c. 2 mm of the root tip was carefully squashed with a needle, then stained with aceto-orcein and carefully squashed under a cover glass. The root tips were examined using a light microscope (Zeiss standard 14) and documented using a digital camera (Zeiss AxioCam MRc).

6.2.3 Isolation of genomic DNA

The plant material freshly collected then cut in small pieces and dried on silica-gel in a drying chamber at 35°C for app. 24 hrs. Genomic DNA was then isolated using a CTAB method as described in Chapter 2. DNA concentration and purity (A260/A280 ratio) were measured using a NanoDrop ND-1000 (peqLab, Erlangen, Germany).

6.2.4 Genomic library construction

6.2.4.1 Restriction digest

The genomic DNA was digested using the restriction enzymes EcoRI and MseI (New England Biolabs) using a reaction mixture of 2.50 µL NEB 10x Ligase Buffer (pre-heated to 50°C to get all components in solution), 0,25 µL 100x Bovine Serum Albumine (New England Biolabs, supplied with the enzymes), 0.25µL 5M NaCl (50 mM final), 1 µL EcoRI, 1 µL MseI, 20 µL genomic DNA (@concentration 120 ng/µL). The restriction digest set-up was incubated in a thermal cycler at 37°C for 2 hours. The success of the digestion was verified by running 4 µl of the digested DNA on a 1% agarose gel (30 min, 100 V). To ensure that the DNA fragments to be used for the following steps were between 400-800 nt in length, the whole volume of the digested DNA from the previous step was run on a 2% agarose gel for c. 1 h. DNA of the desired size was excised from the gel and purified using the QIAquick Gel Extraction Kit (Quiagen) according to the manufacturer's instructions.

6.2.4.2 Ligation of EcoRI and MseI adapters to the restriction fragments

To form double stranded adapters, equal volumes of equal molar amounts of EcoRI and MseI adapters were mixed (6,5 µL EcoRI-linker, 6,5 µl MseI-linker, (10 µm each). The mixture was heated to 95°C and cooled down in a water bath to room temperature and then incubated at 16°C overnight.

Adaptors used:

EcoRI adaptor:	MseI adaptor:
5'-CTCGTAGACTGCGTACC	5'-GACGATGAGTCCTGAG
CATCTGACGCATGGTTAA-5'	TACTCAGGACTCAT-5'

For the adapter ligation, 6,5 µl of EcoRI and 6,5 µl of MseI adapters were mixed with 4 µl of 10x T4 DNA ligase buffer and 3 µl T4 DNA ligase (New Englad Biolabs, Cat. No. Mo202S) (400 U/µl). This mixture was added to the DNA from the previous step. To test the success of the ligation, a test PCR was run. A 50µl reaction containing 4 µl of the linker ligated DNA, 5 µl peqlab Taq polymerase buffer, 10 µl peqlab PCR enhancer solution, 5 µl BSA @ 350 µg/ml, 2, 6 µl EcoRI primer EcoRI primer: 5'-CTCGTAGACTGCGTACCAATTC, 2,6 µl MseI primer 5'-GACGATGAGTCCTGAGTAA, 3 µl dNTPs, 0,4 µl peqlab Taq DNA polymerase and 17,4 µl ultrapure H_2O. The amplification conditions were: 95°C for 2 min. followed by 20 cycles of 95°C for 20 sec., 60°C for 20 sec., 72°C for 1.5 min., then the reaction was held at 15°C. Success of the PCR was checked by running 4µl of the product on a 1.5% agarose gel.

6.2.4.3 Enrichment of the genomic library for repeat motifs

The PCR products from the previous step were used. Two enrichments of repeat-containing sequence motifs were carried out using two different 3-biotinylated oligo-mixes.

Oligo mix 1: $(AG)_{12}$, $(TG)_{12}$, $(AAG)_{8}$, $(AAT)_{12}$, $(ACT)_{12}$

Oligo mix 2: $(AAAC)_{6}$, $(AAAG)_{6}$, $(AATC)_{6}$, $(AATG)_{6}$, $(ACAG)_{6}$, $(ACCT)_{6}$, $(ACTC)_{6}$, $(ACTG)_{6}$

The linker ligated DNA was hybridized with the two oligo mixes following the protocol of Glenn & Shable (2005). The hybridized DNA was afterwards added to 150 µl of Dynabeads washed as described by (Glenn & Schable 2005). The following steps also followed the procedure described therein.

The enriched DNA was recovered using a PCR with 2 µl of the DNA and the same reaction set-up as described above for the testing of adaptor ligation success. The cycling conditions were 95°C for 2 min. followed by 25 cycles of 95°C for 20 sec., 60°C for 20 sec., 72°C for 1.5 min.; then 72°C for 10 min.; then hold at 15°C. Success of the PCR was checked by running 4µl of the product on a 1.5% agarose gel. The product from this PCR was used for the second enrichment.

6.2.5 454 sequencing

The genomic library was first purified using the Amplicon Library Preparation Protocol (Roche) according to the manufacturer's instructions. Then the library was sequenced of a GS FLX System using the LibL Kits and the GS FLX Titanium Sequencing Kit XLR70 (both Roche) according to the manufacturer's instructions.

6.2.6 Screening for repetitive motifs and primer design

The search for repetitive sequence motifs was done using QDD (Meglécz & al. 2010). This software uses a series of perl scripts in combination with BLAST (ftp://ftp.ncbi.nih.gov/blast/executables/), Clustal X (Larkin & al. 2007) and primer3 (Rozen & Skaletsky 2000). In the first step, contigs from reads containing the same sequence motifs containing repetitive motifs are assembled. In the following step primers are automatically designed for the loci selected by the software.

6.3 RESULTS

6.3.1 Chromosome counts

The selected plant is diploid and has a chromosome number of 2n=22.

6.3.2 454 sequencing and microsatellite loci found

The 454 run produced 86125 reads with a length between 130 and 400 bp. The screening for repetitive elements using QDD produced 716 loci found for which primers could be designed of which 103 were marked as best by the software. The output containing the repeat motif, their length and the designed primers and their properties is shown in Table 6.1. Dinucleotide repeats are most common, tri and –tetranucleotide repeats were also found and the largest repeat motif comprises 6 nucleotides. Most microsatellites are between 10 and 24 nt in length, few are between 30-36 nt, only three longer microsatellites (of 42 nt) were detected.

6.4 DISCUSSION

The approach using the enriched genomic library and 454 sequencing was very effective successful. The genomic library construction took about one week but can be even done faster. The whole procedure from isolation of genomic DNA to the 454 sequencing can be done less that two weeks each steps are done immediately after the previous step. The 454 reads are rather short, the run here produced reads with often only 100-130 bp. But usually read lengths of c. 400 nt are expected. During the genomic library construction, attention was paid to obtain restriction fragments of about 400 nt in leght to ensure reads which are long enough for primer binding sites. Nevertheless, the reads were still suitable for primer design. A high number of reads containing microsatellites was found. This is a very good prerequisite for selecting the loci for initial testing. The loci to be testes will include those with di, tri, tetra and hexa-nucleotide repeats, preferably with longer repeat stretches. Those fragment which have primer combinations with a similar Tm will be preferred so that the same annealing temperature can be used for all primer combinations. Following the approach of Csencsics et al. (2010), the following criteria will be applied: amplification products larger than 100 bp, primer melting temperature of 60.0 °C, primer GC content of 50% and low levels of self- or paircomplementarity of the primers. Successfully amplified loci will then be amplified for c. 20 samples to test whether they are polymorphic and heterozygous.

Table 6.1 Primers for the microsatellite loci found.

No.	motif	Length		Primer sequence	Tm	% GC	PCR product size
1	GT	10	F	TATGTTGAGCAGGGTAGGGG	59,9	55	132
			R	CTGAGGACCCCATAGTCGAA	60,1	55	
2	CT	20	F	AATCACCTTTCCACTGGTCG	60	50	129
			R	CTGTTGTTGCTGTGGTGCTT	59,9	50	
3	CA	18	F	CGTACCAATTCCCTAAGCCA	60	50	129
			R	CCTGAGTAATTGCTGGGTGA	58,7	50	
4	CT	12	F	TGCGTCTCGTAGACTGCGTA	60,8	55	93
			R	TGACACTCTCGTCTTGTATTTCC	58,4	43	
5	CT	20	F	TGCGTCTCGTAGACTGCGTA	60,8	55	111
			R	CTAGAGGACTGCGTCTTTGTCT	57,9	50	
6	TC	18	F	GAGTGCGCTCGTAGACTGC	59,9	63	95
			R	ATCCACAACGCCGTCCAT	62,4	55,5	
7	GA	10	F	AGGTTCGAATTGATGAACGAA	59,6	38	187
			R	CAACGGTTGTTTGTCGAGG	60,1	52,6	
8	AC	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	94
			R	CAGCATTGAGCAGCAGATGT	60,2	50	
9	AG	26	F	TGCGTCTCGTAGACTGCGTA	60,8	55	96
			R	GAGGGCGACCCTACCCTAC	60,9	68	
10	CT	22	F	TGTATGGAGCCGTGGTGTAA	60	50	112
			R	GGTTTCACAAAACCCTAGCTG	58,8	47,6	
11	TC	26	F	AAACGACGTTCCTTGTTTCG	60,1	45	149
			R	TTCTCTGGACAGCGGAGG	60,1	61,1	
12	TCT	18	F	TGCGTCTCGTAGACTGCGTA	60,8	55	155
			R	TGCAAAATTGATTGGATGGA	59,9	35	
13	CA	20	F	TGCGTCTCGTAGACTGCGTA	60,8	55	97
			R	CGATGAGTCCTGAGTAAGTCATTT	58,9	41,7	
14	CA	16	F	CTTGCGGTCTTGACACTTGA	60	50	277
			R	CGATGAGTCCTGAGTAAATGGTT	59,5	43,5	
15	GA	20	F	TGCGTCTCGTAGACTGCGTA	60,8	55	272
			R	TTCAGTTGGGAATGTTGGAAG	60	42,9	
16	AG	18	F	TTCGTCGGTGGTAGGAACTC	60,1	55	178
			R	TCAACGGTCGGTCACTATCA	60,1	50	
17	AC	34	F	TGCGTCTCGTAGACTGCGTA	60,8	55	101
			R	GCCAGTTGGCTCAGAGGTAG	60	60	
18	AG	18	F	CATCTGCAAAACCCCATTTT	59,8	40	97
			R	CCAAGCAAACCCAAACACTT	60	45	
19	CT	12	F	TGCGTCTCGTAGACTGCGTA	60,8	55	90
			R	AGTCCTGAGTAAATCGAGAACCC	60	47,8	
20	AC	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	96
			R	TGAGTCCTGAGTAACTTGGCG	60,4	52,4	
21	TC	10	F	ACTGCGTACCAATTCCCAAG	60	50	170
			R	CAGAATCGAAGTAGAGGGAGGA	59,8	50	
22	CT	26	F	TGCGTCTCGTAGACTGCGTA	60,8	55	96
			R	AACAAAGGAAACAAGAGACAACA	58	34,8	
23	TC	20	F	GACCGCCGTTCATCTTAGAA	60,2	50	111
			R	CACTGCAGACGCAGAGGTAG	59,8	60	
24	ACA	18	F	GGAATTGGGTCTTGGATCTG	59,3	50	92
			R	CCTACATCTGGATCTCCCCA	59,9	55	
25	ACAA	24	F	TGCGTCTCGTAGACTGCGTA	60,8	55	168
			R	CTCCCTCTGCATCCATCAGT	60,2	55	

Table 6.2, continued

No.	motif	Length		Primer sequence	Tm	% GC	PCR product size
26	ACA	24	F	ACCAATTCAAAATGAGGCCA	60,3	40	114
			R	GCCTACATCTGGATCTCCCA	60	55	
27	TC	30	F	TGCGTCTCGTAGACTGCGTA	60,8	55	91
			R	GATGAGTCCTGAGTAAGGCAGC	60,4	54,5	
28	AG	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	154
			R	CAAGCCTGCGAATTTCAAGT	60,4	45	
29	CT	14	F	TGCGTACCAATTCCTCACTG	59,7	50	92
			R	ATCAGGAGCAAGAGCGAGAG	59,9	55	
30	TC	10	F	TTGTTCCTTGCACTGTGAGC	60	50	220
			R	CCAAATCATACCTCCCCAGA	59,7	50	
31	AG	10	F	TGCGTACCAATTCGGTTGTA	60	45	126
			R	CGATGAGTCCTGAGTAACAGC	57,6	52,4	
32	GA	10	F	AGTGCCCAAATTACGATTGG	59,8	45	141
			R	AAACCTCCCTTGTTCATCCC	60,2	50	
33	AG	10	F	TGATGATCTGGTGGGAATGA	59,9	45	223
			R	CCTGAGTAAACCCTCCCACA	60	55	
34	CT	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	101
			R	AAGGAGTTGAATCACGCTCG	60,4	50	
35	TC	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	107
			R	ATTGCCCCACGAAAATAACA	60,2	40	
36	TG	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	228
			R	TCCAACACGGAAAATACCAA	58,9	40	
37	GA	24	F	CTGCGTACCAATTCAAAGCA	59,9	45	106
			R	CAATCTTCGCTTCTTCCTGG	59,9	50	
38	GAA	16	F	TACCAAATCATTGCAGCCAG	59,7	45	166
			R	TTCTTCTCTTTCTTCCCTTCTCC	59,5	43,5	
39	GA	18	F	GAAAATGAGCGCTGCAAGAT	60,5	45	103
			R	AACTTGATCAACATACGCAACA	58,2	36,4	
40	GTG	12	F	ATGTGGTGGTTGTGGTTGTG	60,2	50	154
			R	GCACGCATTCCATGAAACTA	59,7	45	
41	CA	15	F	TGCGTCTCGTAGACTGCGTA	60,8	55	108
			R	CGATGAGTCCTGAGTAAGTCATTT	58,9	41,7	
42	GA	28	F	TGCGTCTCGTAGACTGCGTA	60,8	55	118
			R	GCATCTAAGGGACACCTCCA	60,1	55	
43	CA	10	F	GCTTATGTTGCAGCTCATGG	59,4	50	127
			R	ATCATGGGTGTTGCATCTCA	59,9	45	
44	AG	12	F	TGAAGATGATGACACTTTGCTTT	58,9	34,8	90
			R	TTGTGTCTTCTGCTACTACTGCTACA	59,7	42,3	
45	GA	10	F	AATTCTGGCTGTGGAGGAGA	59,8	50	105
			R	ACTCTCATCATTTCCCAGGC	59,1	50	
46	AG	14	F	GATGACTCATTTGGGTTGGG	60,2	50	100
			R	ACTGCAATGGTGAGGTCTGA	59,3	50	
47	CA	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	111
			R	AGGAACCCTAGATGCAAGGC	60,6	55	
48	TC	18	F	TGCGTCTCGTAGACTGCGTA	60,8	55	114
			R	TTAGAGCATCGCCCTACGTC	60,4	55	
49	CT	18	F	TACTGCCCTTTGTTCAGCCT	59,9	50	127
			R	AGCCACAGGAGAGAAGAGAAGA	59,8	50	
50	ACGT	10	F	AACCAAAACGGAAGGGTACG	61,1	50	117
			R	CCCTAACCGTTTCGTTTCCTA	60,3	47,6	

Table 6.3, continued

No.	motif	Length		Primer sequence	Tm	% GC	PCR product size
51	CGTA	24	F	GGAAACGAAACGGTTAGGGT	60,2	50	132
			R	TCGTACGTTAGGTTCGTTTCG	60,2	47,6	
52	AG	20	F	TGCGTCTCGTAGACTGCGTA	60,8	55	91
			R	CTGCTCTTCCAGTCTCTGCC	60,3	60	
53	AG	10	F	GGCAGAGACTGGAAGAGCAG	60,3	60	91
			R	AAACATTAGGGTTCCATTCTCG	59,4	40,9	
54	GA	10	F	CCAATTCTAGACGAACCGGA	60,1	50	99
			R	AAACACTCCATTCTCACCAATC	58	40,9	
55	AG	10	F	GACTGCGTACCAATTCTGGC	60,7	55	91
			R	TCCTGAGTAAAGCACAGGCA	59,6	50	
56	CA	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	91
			R	CGATGAGTCCTGAGTAAGATGTG	58,9	47,8	
57	CT	24	F	TAAGTTGAACAGGGCAACCC	60	50	320
			R	CTAGACAGAGCCAGCAGCG	60	63,2	
58	CT	24	F	TTCCCTAAACTACCCCCACC	60	55	93
			R	CGATGAGTCCTGAGTAACGGA	60,3	52,4	
59	TC	14	F	TGCGTCTCGTAGACTGCGTA	60,8	55	90
			R	TTTATGATCAGTGACAGAAGGACA	58,8	37,5	
60	TG	10	F	GTACCAATTCACCCAAACCG	60,1	50	191
			R	TGGGTCATGTTTCGAGTCAA	60,1	45	
61	AG	26	F	TGCGTACCAATTCGTTGAAA	60,1	40	102
			R	TACCCTTTCCTACGCCTCCT	60,1	55	
62	CA	20	F	TGCGTCTCGTAGACTGCGTA	60,8	55	97
			R	AAACTACTCGGTGTCGGAAATC	59,5	45,5	
63	GA	20	F	TGCGTCTCGTAGACTGCGTA	60,8	55	125
			R	ATACTCGACTCCTCGCTCCA	60	55	
64	AAC	30	F	CGACTCAAGCCAAGATGTCA	60	50	117
			R	TCTTGTTCGGGAGCTGATTT	59,8	45	
65	CA	15	F	GCTCGTAGACTGCGTACCAA	59,1	55	90
			R	CTAATAGGTCTCCCCACCCC	59,6	60	
66	CT	30	F	TGCGTCTCGTAGACTGCGTA	60,8	55	110
			R	TCAAACCAGCGACTCATCAA	60,4	45	
67	GAA	16	F	CCGTGACCCTAATGCTGATT	60	50	122
			R	ATCTCAAACCTCCCTCCCTC	59,5	55	
68	CT	15	F	CAAGATGATGAAGGCAAGCA	59,9	45	223
			R	TCCTACAGCCTAGATGGACAGA	59	50	
69	TC	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	106
			R	CAATAGGGGACACCATCAGG	60,2	55	
70	GA	22	F	AAGCCTTTTGTGTTGAGGGA	59,7	45	153
			R	TGAGTAACTGTGGCATCCGA	60,3	50	
71	TC	10	F	GACTGCGTACCAATTCCTCC	59,6	55	104
			R	GACAGACACGAATGGCAATG	60,1	50	
72	AG	24	F	TGTGGACGTTGGAATCTGTG	60,6	50	92
			R	TCATTACCCCTGATTTTGTTCA	59,3	36,4	
73	GA	16	F	TGCGTCTCGTAGACTGCGTA	60,8	55	107
			R	TTTCGACCTTAACCGTTTCAC	59,1	42,9	
74	TTC	10	F	TGTTGTATGACGCCCATTGT	59,8	45	90
			R	ATTCACCACAACCACAGCAA	60	45	
75	GA	15	F	GCGGAATCGAAGTTTCAGAG	60	50	114
			R	GAAGCACTGAAAGACGCACA	60,2	50	

Table 6.4, continued

No.	motif	Length		Primer sequence	Tm	% GC	PCR product size
76	TC	22	F	TGCGTCTCGTAGACTGCGTA	60,8	55	153
			R	CTGCAGTATGGGAGAGGAGG	59,8	60	
77	AG	24	F	CTCCGAAGCTTTCAGCAAAC	60,1	50	114
			R	GAAGGCTACTGCTTCAAACCAT	59,8	45,5	
78	CT	10	F	TCGATTGAAATCAGACACGC	59,8	45	93
			R	GGCATAACTCCCATCAGTCC	59,4	55	
79	AG	18	F	TGCGTCTCGTAGACTGCGTA	60,8	55	90
			R	TGAGTCCTGAGTAACCTAATCACC	58,7	45,8	
80	TCT	18	F	CTCTCATTCCTCCCATTCCA	60	50	123
			R	AGTCCTGAGTAAAGCAGCCG	59,6	55	
81	CT	15	F	GTTTGGTGGCCTGAATATGG	60,2	50	108
			R	AGTCAGGTCAAGAGGAGCCA	60	55	
82	AG	18	F	CCCATGGAATGTTGTGTCAA	60,2	45	124
			R	TGGTAACGTGGTGATGCACT	60	50	
83	AGAC	24	F	TGCGTCTCGTAGACTGCGTA	60,8	55	174
			R	GGTTGATGCTTATCCTCCCC	60,7	55	
84	GA	20	F	AGACTGCGTACCAATTCCGT	59,6	50	97
			R	GTGTACTCGTCCGCTCACAA	59,9	55	
85	AG	10	F	TCAACGAAAGGGGAAAGAGA	59,8	45	149
			R	TCGTCATCGTTCACGCTAAG	60	50	
86	CT	12	F	TGCGTCTCGTAGACTGCGTA	60,8	55	99
			R	CGATGAGTCCTGAGTAATAAAGG	57,1	43,5	
87	CT	26	F	TGCGTCTCGTAGACTGCGTA	60,8	55	94
			R	CGATGAGTCCTGAGTAATGTTCC	60	47,8	
88	TG	20	F	CGTAGACTGCGTACCAATTCC	59,6	52,3	99
			R	CCTTAGATTCCACGTGACCAA	60	47,6	
89	TC	10	F	TGCGTCTCGTAGACTGCGTA	60,8	55	98
			R	ACGATGAGTCCTGAGTAACGA	57,4	47,6	
90	TC	22	F	CTATGCTGCTTCGGCTATGG	60,9	55	93
			R	CGTTGTTGAGGTTGAGAGCA	60	50	
91	CTTCTA	12	F	AACTTGATGCCCGTTTCATC	59,9	45	167
			R	TGCCTGAAATCATCAGCATC	59,8	45	
92	GA	36	F	TGCCGATTGAATTAGGAACC	59,9	45	105
			R	TGAGCTGCTGTGCTGATCTC	60,5	55	
93	TG	16	F	TGCGTCTCGTAGACTGCGTA	60,8	55	148
			R	GAGCCTTGCGTGTTACATCA	59,9	50	
94	CA	26	F	TGCGTCTCGTAGACTGCGTA	60,8	55	106
			R	CGATGAGTCCTGAGTAATATTATGTGT	59	37	
95	CTT	20	F	TCCATTGTTTATCCTTAGGCG	59,1	42,8	260
			R	AAAAGAATGGAAGGGTCGGT	59,8	45	
96	AT	33	F	TCCATGCTAGGTGGAAAACC	59,9	50	211
			R	GTTTGAACGGGATGGTATGG	60,1	50	
97	TC	10	F	CGTCTCGTAGACTGCGTACC	58,6	60	91
			R	AAATGCGCAGTAAGGGAGAA	59,8	45	
98	AC	12	F	GCGTACCAATTCCTCCTCAA	60,1	50	170
			R	GTGTGAAGGCACTCCTGGAT	60,1	55	
99	GT	24	F	GGAACAGGGAGCTAGGGAGT	59,7	60	149
			R	GGTACGTGATAGAGGAGGAAGG	59,1	54,5	
100	CT	34	F	AGTGCGTCTCGTAGACTGCGTA	62,4	54,5	90
			R	CTCCGACCCGAAGCAGAGTA	62,8	60	

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Summary

Korotkova, Nadja. 2011. *Phylogeny and evolution of the epiphytic Rhipsalideae (Cactaceae)*. PhD thesis, Mathematisch-Naturwissenschaftliche Fakultät, Rheinische Friedlich-Wilhelms-Universität Bonn, Germany.

Cactaceae are one of the major floristic components of the New World's arid as well as seasonally moist tropical regions and at the same time one of the most popular plant families in horticulture. The taxonomic units (tribes, genera) and species limits in the Cactaceae have been difficult to define due to intergrading vegetative characters, phenotypic plasticity and the largely uniform flower morphology. Molecular phylogenetic studies so far yielded largely unresolved or poorly supported trees so relationships within Cactaceae remained insufficiently understood. Besides, Cactaceae taxonomy is still often unreliable. But a high proportion of cacti is CITES-listed and accurate species delimitation and identification are therefore desirable for conservation Red List assessments.

This study focuses on the Rhipsalideae, a predominantly epiphytic tribe of Cactaceae from the tropical rainforests of South and Central America. The Rhipsalideae have hitherto not been subject of a detailed phylogenetic study so far but are well-suited for this purpose: they are a comparatively small group and are well known morphologically. All but one species were available for this study so it is one of the most comprehensive species-level studies carried out within the Cactaceae so far.

The major aims of this study were to resolve species-level relationships in the Rhipsalideae, also to get better insights into species limits and to find morphological characters synapomorphic or at least characteristic for the genera and subgenera.

In order to resolve relationships between so closely related species, rapidly evolving plastid markers with high phylogenetic structure were selected. The phylogenetic relationships were analysed using sequence data from intergenic spacers (*psbA*-*trnH*, *rps3*-*rpl16*, *trnS*-*trnG*, *trnQ*-*rps16*), group II introns (*trnK*, *rpl16*, *trnG*) and the coding region *matK*. Trees were inferred with Maximum Parsimony and Bayesian Inference. Haplotype network construction was carried out for examining patterns within *Rhipsalis baccifera* and allies.

First, the position and circumscription of the genus *Pfeiffera* was addressed. It had formerly been included in the Rhipsalideae but earlier studies showed it to be distantly related. A dataset of seven regions was generated with c. 7000 nucleotides sequenced per sample. All but one *Pfeiffera* species with multiple accessions were sampled. Detailed phylogenetic analyses of this study revealed *Pfeiffera* polyphyletic, comprising two unrelated clades, both well resolved and highly supported. One clade includes the type species, *P. ianthothele*; the second contains two *Pfeiffera* and one

erstwhile *Lepismium* species. These results and a re-evaluation of the morphological characters justify a generic status for this newly found clade. It includes the type species of the earlier-proposed monotypic genus *Lymanbensonia* and, therefore, its reinstatement is proposed in an amplified circumscription. A further taxonomic and nomenclatural consequence is the establishment of a separate tribe Lymanbensonieae, formally proposed here, to contain the genera *Lymanbensonia* and *Calymmanthium*. The results further underscore that epiphytism evolved more frequently in Cactaceae than hitherto assumed.

To resolve phylogenetic relationships in the Rhipsalideae, a dataset of six regions was generated with c. 4200 nucleotides sequenced per sample for 120 accessions. The regions used were evaluated for their phylogenetic performance and species discrimination power for DNA based species recognition (DNA barcoding) based on beforehand defined operational taxonomic units (OTUs).

The Rhipsalideae were found as monophyletic and contain five major clades that correspond to the genera *Rhipsalis*, *Lepismium*, *Schlumbergera*, *Hatiora* subg. *Hatiora* and *Hatiora* subg. *Rhipsalidopsis*. The relationships between the major clades corresponding to genera could not be clarified. But the species-level relationships were well resolved and supported. Based on the results, a reinstatement of *Rhipsalidopsis* at generic level and a revised subgeneric classification for *Rhipsalis* are proposed.

Already c. 2500 nt of four regions (*rpl16* intron, *trnK* intron, *psbA-trnH*, *trnQ-rps16*) were sufficient to identify 97% of the OTUs in the Rhipsalideae. Among all possible marker combinations this one was the most successful with the least number of sequenced nucleotides. The combination of all markers (4207 nt) yielded the same number of identified OTUs. The *rpl16* intron was the best single-locus barcode, finding 60% of the OTUs.

The two markers providing the best phylogenetic signal for the Rhipsalideae were the group II introns in *rpl16* and *trnK*. The phylogenetic performance of the markers was found to be not determined by the level of sequence variability. Comparisons of the OTU identification potential of the markers with their phylogenetic performance revealed that these two qualities are not necessarily correlated.

The reliable phylogenetic hypothesis for the Rhipsalideae provided a framework for a detailed study of character evolution. A matrix of 36 characters was compiled and ancestral states were reconstructed using a Bayesian approach. A focus was put on the characters associated with the epiphytic life form and the floral traits. The degree of homoplasy was found to be high but many characters were homogenous within the clades and all the highly supported clades (genera, subgenera) found by the molecular phylogenetic analyses could also be defined morphologically.

Rhipsalis baccifera is the most widespread cactus and the only cactus native to Africa. To get more insights into the relationships between the South American and

the African populations, the distribution patterns of *Rhipsalis baccifera* were analysed. Tree building methods and haplotype network algorithms were applied to sequences of the *rps3-rpl16* spacer and the *rpl16* intron. Two main groups of plastid haplotypes were found: a northern South American / Caribbean / Central American haplotype and an African haplotype. These results suggest a single dispersal of *Rhipsalis baccifera* to Africa and reveal high genetic diversity within its populations on both continents. To obtain further resolution among the populations, microsatellite markers for *Rhipsalis baccifera* have been developed using 454 sequencing.

The analyses resulted in almost completely resolved and well supported species level trees which were hitherto hardly achieved in the Cactaceae. This study could therefore serve as a case study for resolution of species-level relationships between closely related and recently diverged species, in other Cactaceae groups or in other plant families that pose similar problems. The results also lead to the conclusion that morphology-based taxonomic units can be misleading to guide taxon sampling and the best solution is to sample the study group as completely as possible for a reliable phylogeny inference. This study is also the first DNA barcoding study for the Cactaceae. The identification success here is higher than observed in other studies that also used a taxonomic setting and can serve as an example for future studies. The results furthermore emphasize that the outcome of a phylogenetic study and a barcoding study will largely depend on the markers chosen.

So far, plastid markers have provided a solid phylogenetic hypothesis for the Rhipsalideae which is also in line with morphological characters. But hybridization is common in Cactaceae (although supposed to be rare in the Rhipsalideae). Future work should aim at including nuclear markers which are so far hardly applied in Cactaceae.

Appendices

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 are 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* and the isolated / unplaced genera.

Outgroups (Cactaceae dataset)

Opuntia quimilo K.Schum., Argentina, *Leuenberger 3558* (B 159-94-86-10), *trnK/matK* AY015279 (Nyffeler 2002).

Pereskia bleo (Kunth) D.C., BGBM 277-01-88-80; *Schwerdtfeger 12678* (B-*G*artenherbar), *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).

Cereeae Salm-Dyck

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, *psbA-trnH* FN995427, *trnQ-rps16* FN677806.

Cereus hildmannianus Pfeiff., Brazil, *Eggli et 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 et 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).

Lymanbensonieae N. Korotkova & Barthlott

Calymmanthium substerile F.Ritter, ZSS 893442 hort. ZSS, without locality data *presumably F. Ritter collection from ca. 1960* (no voucher), CA133, *trnS-G* --, *trnK/matK* AY015291 (Nyffeler 2002), *rpl16* FN673676 (Korotkova et al. 2010), *psbA-trnH* FN669004 (Korotkova et al. 2010), *trnQ-rps16* FN677924 (Korotkova et al. 2010).

Lymanbensonia brevispina (Barthlott) Barthlott & N. Korotkova, Peru, Prov. Amazonas, east of Balsas *Charles GC1065.02* (photo voucher), CA131, *trnS-G* FR716737, *trnK/matK* FR716759, *rpl16* FR716770, *psbA-trnH* FR716780, *trnQ-rps16* FR716790.

Lymanbensonia incachacana (Cárdenas) Barthlott & N. Korotkova, BG Bonn 2639 Bolivia, Prov. Sud-Yungas *Miyagawa 2* (BONN, photos), CA086, *trnS-G* FR716738, *trnK/matK* FN669728, *rpl16* FN673634, *psbA-trnH* FN669038, *trnQ-rps16* FN677881.

Lymanbensonia 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, *trnK/matK* FN669722, *rpl16* FN673628, *psbA-trnH* FN669039, *trnQ-rps16* FN677877.

Echinocereeae (Britton & Rose) F.Buxb.

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, *trnK/matK* FN669772, *rpl16* FN673680, *psbA-trnH* FN669003, *trnQ-rps16* FN677928.

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, Ibisch & Barthlott) Ralf Bauer, BG Bonn 27450 Bolivia, La Paz *Kessler 9800* (holo LPB, iso GOET, K), CA076, *trnS-G* FR716742, *trnK/matK* FR716760, *rpl16* FR716771, *psbA-trnH* FR716781, *trnQ-rps16* FR716791; BG Bonn 26961 Bolivia, La Paz, *Krahn 970* (B), CA077, *trnS-G* FR716741, *trnK/matK* FR716761, *rpl16* FR716772, *psbA-trnH* FR716782, *trnQ-rps16* FR716792.

Pfeiffera boliviana (Britton) D.R. Hunt, BG Bonn 4674 without locality data (B), CA078, *trnS-G* FR716743, *trnK/matK* FR716762, *rpl16* FR716773, *psbA-trnH* FR716783, *trnQ-rps16* FR716793; BG Bonn 12991 Bolivia, Santa Cruz *Ibisch 93.438* (B), CA079, *trnS-G* FR716744, *trnK/matK* FR716763, *rpl16* FR716774, *psbA-trnH* FR716784, *trnQ-rps16* FR716794.

Pfeiffera ianthothele (Monv.) F. A. C. Weber, BG Bonn 12965 Bolivia, Santa Cruz *C. & P. Ibisch 93.884* (LPB, FR), CA084, *trnS-G* FR716748, *trnK/matK* FR716764, *rpl16* FR716775, *psbA-trnH* FR716785, *trnQ-rps16* FR716795; BG Bonn 2316 without locality data (B), CA085, *trnS-G* FR716749, *trnK/matK* FR716765, *rpl16* FR716776, *psbA-trnH* FR716786, *trnQ-rps16* FR716796.

Pfeiffera 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, *trnK/matK* FN669731, *rpl16* FN673637(Korotkova et al. 2010), *psbA-trnH* FN995429, *trnQ-rps16* FN677885; BG Bonn 25775 Bolivia, La Paz, prov. Sud Yungas *Krahn 1044* (B, BONN), CA092, *trnS-G* FR716751,

trnK/matK FN669734, *rpl16* FN673640(Korotkova et al. 2010), *psbA-trnH* FN995432, *trnQ-rps16* FN677888.

Pfeiffera monacantha (Griseb.) P.V.Heath, BG Bonn 12971 Bolivia, Dept. Tarija, *C. & P. Ibisch 93.1228* (FR), CA090, *trnS-G* FR716752, *trnK/matK* FN669732, *rpl16* FN673638, *psbA-trnH* FN995430, *trnQ-rps16* FN677886; BG Bonn 12964 Bolivia, Santa Cruz *C.& P.Ibisch 93.874* (BOLV, LPB, FR), CA091, *trnS-G* FR716753, *trnK/matK* FN669733, *rpl16* FN673639, *psbA-trnH* FN995431, *trnQ-rps16* FN677887.

Pfeiffera paranganiensis (Cárdenas) P.V.Heath, BG Bonn 11706 Bolivia, between Morochata and Parangani *Augustin s.n.* (B), CA093, *trnS-G* FR716754, *trnK/matK* FR716767, *rpl16* FR716777, *psbA-trnH* FR716787, *trnQ-rps16* FR716797; BG Bonn 2644 Bolivia, La Paz, Lambate Miyagawa 7 (B), CA094, *trnS-G* FR716755, *trnK/matK* FR716768, *rpl16* FR716778, *psbA-trnH* FR716788, *trnQ-rps16* FR716798; BG Bonn 16402 ex HBG 15931, UCBG 56.1257, ISI 1102 Bolivia, La Paz, Prov. Inquisivi Cárdenas s.n. (HNT), CA095, *trnS-G* FR716756, *trnK/matK* FR716769, *rpl16* FR716779, *psbA-trnH* FR716789, *trnQ-rps16* FR716799.

Hylocereeae (Britton & Rose) F.Buxb.

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 F.Buxb.

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).

Trichocereeae F.Buxb.

Echinopsis aurea Britton & Rose, BG Bonn 24068 without locality data (no voucher), CA104, *trnS-G* FR716745, *trnK/matK* FN669743, *rpl16* FN673649, *psbA-trnH* FN669005, *trnQ-rps16* FN995670.

E. chiloensis (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, *rpl16* FN673557, *psbA-trnH* FN669002, *trnQ-rps16* FN677918.

Frailea gracillima (Lem.) Britton & Rose, Brazil, *Hofacker 382* (ZSS 19927), *trnK/matK* AY015285 (Nyffeler 2002).

F. phaeodisca (Speg.) Speg., Brazil, *Hofacker 25* (ZSS 893932), *trnK/matK* AY015286 (Nyffeler 2002).

Rhipsalideae DC.

Hatiora cylindrica Britton & Rose, BG Bonn 30881 ex RGB Kew 1991-1436, Brazil, Minas Gerais, Camanducaia, *Catarino s.n.* (B), CA119, *trnK/matK* FN669758, *rpl16* FN673664, *psbA-trnH* FN669031, *trnQ-rps16* FN677911.

Hatiora epiphylloides* subsp. *bradei, BG Bonn 13647, Brazil, *W. Rauh 64291* (BONN, in spirit, photos), CA039, *trnK/matK* FN669689, *rpl16* FN673594, *psbA-trnH* FN669028, *trnQ-rps16* FN677844.

Hatiora epiphylloides* subsp. *epiphylloides, BG Bonn 11649, Brazil, *B. Orssich 2.1990* (BONN in spirit, photos), CA040, *trnK/matK* FN669690, *rpl16* FN673595, *psbA-trnH* FN669029, *trnQ-rps16* FN677845.

Hatiora herminiae (Porto & A.Cast.) Backeb. ex Barthlott, BG Berlin-Dahlem BR-0-B-1802001, Brazil, Sâo Paulo, Campos do Jordao, *Friedrich, 5.1975* (B Gartenherbar 42725), CA042, *trnK/matK* FN669692, *rpl16* FN673597, *psbA-trnH* FN669034, *trnQ-rps16* FN677847.

Hatiora salicornioides (Haw.) Britton & Rose, BG Bonn 4667, Brazil, Espírito Santo, Vila Velha, *K. Friedrich K 0277, 4.1980* (B), CA009, *trnK/matK* FR852589, *rpl16* FN673564, *psbA-trnH* FN669032, *trnQ-rps16* FN677814; BG Bonn 4637, without locality data, (B), CA048, *trnK/matK* FN669698 (Korotkova et al., 2010), *rpl16* FN673603 (Korotkova et al., 2010), *psbA-trnH* FN669030 (Korotkova et al., 2010), *trnQ-rps16* FN677853 (Korotkova et al., 2010); BG Bonn 1717, without locality data, (B), CA043, *trnK/matK* FN669693, *rpl16* FN673598, *psbA-trnH* FN669033, *trnQ-rps16* FN677848.

Lepismium cruciforme (Vell.) Miq., BG Bonn 5760, Brazil, Párana, descent Itatiaia to Ponta Crossa, *W. Barthlott 90-27* (B), CA010, *trnK/matK* FN669662 (Korotkova et al., 2010), *rpl16* FN673565 (Korotkova et al., 2010), *psbA-trnH* FN669012 (Korotkova et al., 2010), *trnQ-rps16* FN677815 (Korotkova et al., 2010); BG Bonn 14531, without locality data, (B), CA068, *trnK/matK* FN669717, *rpl16* FN673623, *psbA-trnH* FN669014, *trnQ-rps16* FN677872; BG Bonn 2239, Brazil, *W. Rauh 70774* (B), CA069, *trnK/matK* FN669718, *rpl16* FN673624, *psbA-trnH* FN669013, *trnQ-rps16* FN677873.

Lepismium houlletianum (Lem.) Barthlott, BG Bonn 2176, Brazil, Rio Grande do Sul, between Candelaria und Agudo, *Horst & Uebelmann HU1003* (B), CA082, *trnK/matK* FN669726, *rpl16* FN673632, *psbA-trnH* FN669006, *trnQ-rps16* FN677880; BG Bonn 4557, Brazil, *Friedrich s.n.* (B), CA083, *trnK/matK* FN669727, *rpl16* FN673633, *psbA-trnH* FN669007, *trnQ-rps16* FN677882; BG Bonn 26962, without locality data, (B), CA130, *trnK/matK* FN669768, *rpl16* FN673674, *psbA-trnH* FN669157, *trnQ-rps16* FN677922.

Lepismium houlletianum* f. *regnellii (G.Lindb.) Barthlott & N.P.Taylor, BG Bonn 5748, Brazil, Rio Grande do Sul, above Torres, *W. Barthlott 90-61* (B), CA081, *trnK/matK* FN669725, *rpl16* FN673631, *psbA-trnH* FN669008, *trnQ-rps16* FN677879.

Lepismium lorentzianum (Griseb.) Barthlott, BG Bonn 21783, Argentina, Jujuy, road from Sta. Clara to El Fuerte, *B. O. Schlumpberger BOS 157* (B), CA080, *trnK/matK* FN995676, *rpl16* FN995673, *psbA-trnH* FN669016, *trnQ-rps16* FN995669; BG Bonn 12972, Bolivia, Dep.Tarija, Prov. Arce, *C. & P. Ibisch 93.1230* (BOLV, LPB, FR), CA087, *trnK/matK* FN669729, *rpl16* FN673635, *psbA-trnH* FN669017, *trnQ-rps16* FN677883; BG Bonn 12976, Bolivia, Dep.Tarija, Prov. O'Connor, *C. & P. Ibisch 93.1261* (FR), CA088, *trnK/matK* FN669730, *rpl16* FN673636, *psbA-trnH* FN669015, *trnQ-rps16* FN677884.

Lepismium lumbricoides (Lem.) Barthlott, BG Bonn 12181, Brazil, Paraná, betw. Palmeira und Matra, *Horst & Uebelmann HU985* (B), CA070, *trnK/matK* FN669719, *rpl16* FN673625, *psbA-trnH* FN669011, *trnQ-rps16* FN677874; BG Bonn 5755, Brazil, Rio Grande du Sul, near Arroio da Sêca, *W. Barthlott 90-52* (B), CA071, *trnK/matK* FN669720, *rpl16* FN673626, *psbA-trnH* FN669009, *trnQ-rps16* FN677875; BG Bonn 12977, Bolivia, Dep. Tarija, Prov. O'Connor, *C. & P. Ibisch 93.1274* (B), CA072, *trnK/matK* FN669721, *rpl16* FN673627, *psbA-trnH* FN669010, *trnQ-rps16* FN677876; BG Bonn 8571, Brazil, Rio Grande do Sul, São Francisco de Assis - Santa Maria, *Horst & Uebelmann HU1100* (B), CA128, *trnK/matK* FN669766, *rpl16* FN673672, *psbA-trnH* FN669160, *trnQ-rps16* FN677920.

Lepismium lumbricoides* f. *aculeatum (F.A.C. Weber) Barthlott & N.P.Taylor, BG Bonn 14123, Brazil,(B), CA129, *trnK/matK* FN669767, *rpl16* FN673673, *psbA-trnH* FN669156, *trnQ-rps16* FN677921.

Lepismium warmingianum (K.Schum.) Barthlott, BG Berlin-Dahlem BR-0-B-0611105, Brazil, Rio Grande do Sul, near Arroio da Sêca, *W. Barthlott 90-51* (B), CA074, *trnK/matK* FN669723, *rpl16* FN673629, *psbA-trnH* FN669026, *trnQ-rps16* FN677878; BG Bonn 4837, Brazil, Rio Grande do Sul, Morro Santana rainforest, *S. Porembski 12.1990* (B), CA075, *trnK/matK* FN669724, *rpl16* FN673630, *psbA-trnH* FN669027, *trnQ-rps16* FN677879.

Rhipsalidopsis gaertneri (Regel) Linding, BG Bonn 16396, Brazil, Rio Grande do Sul, Pró Mata reserve, *B.O. Schlumpberger, 1996 s.n.* (B), CA041, *trnK/matK* FN669691, *rpl16* FN673596, *psbA-trnH* FN669036, *trnQ-rps16* FN677846.

Rhipsalidopsis rosea (Lagerh.) Britton & Rose, BG Bonn 2172, without locality data, (B), CA047, *trnK/matK* FN669697, *rpl16* FN673602, *psbA-trnH* FN669037, *trnQ-rps16* FN677852.

Rhipsalidopsis xgraeseri (Werderm.) Moran, BG Bonn 5579, cultivated hybrid, (B), CA044, *trnK/matK* FN669694, *rpl16* FN673599, *psbA-trnH* FN669035, *trnQ-rps16* FN677849.

Rhipsalis agudoensis N.P. Taylor, BG Bonn 26964, Brazil, Rio Grande do Sul, Morro de Agudo near Agudo, type collection *Horst & Uebelmann HU821* (holotype K ID K000372524), CA116, *trnK/matK* FN669755, *rpl16* FN673661, *psbA-trnH* FN669083, *trnQ-rps16* FN677908.

Rhipsalis baccifera* subsp. *baccifera (J.S.Muell.) Stearn, Colombia, Huila, betw. Gigante and Río Loro, *R. Bauer 23* (B), CA117, *trnK/matK* FN669756, *rpl16* FN673662, *psbA-trnH* FN669073, *trnQ-rps16* FN677909; Cuba, Prov. Cienfuegos, Mun. Cumanayagua, *W. Greuter, T. Borsch, R. Rankin, J. León, D. Suárez 26982* (B, HAJB), CA138, *trnK/matK* FN995679, *rpl16* FN995674, *psbA-trnH* FN995435, *trnQ-rps16* FN995671; BG Bonn 14745, Venezuela, Sucre, Paria, *R. Bauer s.n.*, 1997 (B), CA013, *trnK/matK* FN669665, *rpl16* FN673568, *psbA-trnH* FN669069, *trnQ-rps16* FN677818; BG Bonn 14254 ex. Marie Selby Garden 79-0932, USA, Florida, Everglades National Park, near Flamingo, *Dodson s.n.* (B), CA125, *trnK/matK* FN669764, *rpl16* FN673670, *psbA-trnH* FN669154, *trnQ-rps16* FN677917; BG Bonn 5859, Colombia, north of La Paila, *M. Koenen & S. Porembski 44* (B), CA135, *trnK/matK* FN669771, *rpl16* FN673678, *psbA-trnH* FN669161, *trnQ-rps16* FN677926; BG Bonn 4678, Brazil, Pernambuco, Recife, *P. Braun s.n.*, 1988 (B), CA002, *trnK/matK* FN669655, *rpl16* FN673556, *psbA-trnH* FN669070, *trnQ-rps16* FN677807, BG B 241060640, Réunion, Ravine à Malheur, (no voucher yet), CA145, *rpl16* not yet submitted; BG B 177181030, Gabun, Ivindo, observation platform, *Scharf s.n.* (no voucher yet), CA146, *rpl16* not yet submitted; BG B 11337820, Togo, (B Gartenherbar 23131), CA147, *rpl16* not yet submitted; BG B 201018724, French Guyana, Saül, 206m, *Freiberg 309* (B Gartenherbar 28222), CA150, *rpl16* not yet submitted; BG Bonn 4435, Ecuador, close to Locha c. 400 m, *J. Wacker* (no voucher yet), CA159, *rpl16* not yet submitted; BG Bonn 14744, Venezuela, Monagas, Caripe, *R. Bauer 4* (no voucher yet), CA161, *rpl16* not yet submitted; BG Bonn 9806, Venezuela, between Altagiacia de Orituco and Caicara, 120 m, *N. Biedinger & M. Koenen 49* (no voucher yet), CA163, *rpl16* not yet submitted; BG Bonn 13751, Zimbabwe, Mtarazi Falls National Park, *R. Seine 1384* (no voucher yet), CA166, *rpl16* not yet submitted; BG Bonn 4676, bRAZIL, Pernambuco, Primavera close to Recife, *P. Braun* (no voucher yet), CA177, *rpl16* not yet submitted; BG Bonn 14135, Venezuela, Orinoko delta, Delta Amacuro, Deltaarm close to Campo Simuina, *R. Bauer 1* (no voucher yet), CA168, *rpl16* not yet submitted; BG Bonn 24266, Guatemala, Peten, El Ceibal, *R. Bauer 10* (no voucher yet), CA169, *rpl16* not yet submitted; BG Bonn 2890, Colombia, 1100 m, *W. Rauh* (no voucher yet), CA176, *rpl16* not yet submitted; BG Bonn 9808, Venezuela, Ocumare de la Costa, near National park Rancho Grande, 0-100 m, *N. Biedinger & M.Koenen 54* (no voucher yet), CA179, *rpl16* not yet submitted; BG Bonn 13474, Peru, Cuzco - Pisac, (no voucher yet), CA181, *rpl16* not yet submitted; BG Bonn 15469, Jamaica, (no voucher yet), CA183, *rpl16* not yet submitted; BG Bonn 15948, Mexico, Tamaulipas, surroundings of Ciudad Mante, *D. Waldeis* (no voucher yet), CA184, *rpl16* not yet submitted; BG Bonn 16374, Colombia, Antiquia, road from Manizales to Medellin, c. 55 km before Medellin, *P. Braun 27* (no voucher yet), CA189, *rpl16* not yet submitted.

Rhipsalis baccifera **subsp. *erythrocarpa*** (Schumann) Barthlott, BG Bonn 16390, Rwanda, Nyagatare, Umutara, *E. Fischer 8051* (B), CA015, *trnK/matK* FN669667, *rpl16* FN673570, *psbA-trnH* FN669084, *trnQ-rps16* FN677820.

Rhipsalis baccifera subsp. hileiabaiana N.P.Taylor, RBG Kew 1966-48932, Brazil, Bahia, Floresta Azul, *Martins in Brieger 43*, coll. before 1966 (K, in spirit), CA152, *trnK/matK* FR852591, *rpl16* FR853121, *psbA-trnH* FR853114, *trnQ-rps16* FR853126**.**

Rhipsalis baccifera **subsp. *horrida*** (Baker) Barthlott, BG Bonn 5998, Madagascar, Fort Dauphin, *W. Rauh 68614* (B), CA014, *trnK/matK* FN669666, *rpl16* FN673569, *psbA-trnH* FN669082, *trnQ-rps16* FN677819; BG Bonn 1704, Madagascar, Fort. Dauphin, *W. Rauh 7106* (no voucher yet), CA162, *rpl16* not yet submitted; BG Bonn 4922, Madagascar, north of Taolanaro, *H. Löschper* (no voucher yet), CA165, *rpl16* not yet submitted; BG Bonn 8550, Madagascar, Fort Dauphin, on granite outcrop, *H. Löschper 53* (no voucher yet), CA186, *rpl16* not yet submitted; BG Bonn 4531, Madagascar, Fort Dauphin, *W. Rauh 68614* (no voucher yet), CA187, *rpl16* not yet submitted; BG Bonn 5648, Madagaskar, Andranokoditra, *J. Bogner 2082* (no voucher yet), CA188, *rpl16* not yet submitted.

Rhipsalis baccifera **subsp. *mauritiana*** (DC.) Barthlott, BG Bonn 6983, Ivory Coast, Taï National Park, *S. Porembski, 5.5.1990* (B), FR853118 *trnK/matK* FR852593, *rpl16* FR853124, *psbA-trnH* -, *trnQ-rps16* FR853125; BG Bonn 1684, S-Africa, Transvaal, (no voucher yet), CA153, *rpl16* not yet submitted; BG Bonn 13674, Seychelles, Mahé, following the "La Misere route", granite outcrop, 110m, *S.Porembski & N.Biedinger* 2155 (no voucher yet), CA155, *rpl16* not yet submitted; Ivory Coast, Tai National park*, S. Porembski, 5.5.1990* (no voucher yet), CA157, *rpl16* not yet submitted; BG Bonn 4424, Madagascar, Perinet, mountain rain forest, 1200 m, *W. Rauh 7165* (no voucher yet), CA158, *rpl16* not yet submitted; BG Bonn 4432, Madagascar, Ocacombe, *W. Rauh 67120* (no voucher yet), CA160, *rpl16* not yet submitted; BG Bonn 5817, Madagascar, Andasibe, *H. Löschper s.n.* (no voucher yet), CA164, *rpl16* not yet submitted; Seychelles, Mahé, near Victoria, on rocks, ca. 50m, *W. Krahn 1257* (no voucher yet), CA167, *rpl16* not yet submitted; BG Bonn 4429, Kenya, Ngangoa forest, Taita-Hills, 1800 m, (no voucher yet), CA174, *rpl16* not yet submitted; BG Bonn 4425, Madagascar, Fort Dauphin, W. Rauh s.n. (no voucher yet), CA175, *rpl16* not yet submitted; BG Bonn 1698, Madagascar, Pic St.Louis, Fort Dauphin, gneiss, *W. Rauh 7555* (no voucher yet), CA178, *rpl16* not yet submitted; BG Bonn11831, Democratic Republic Congo (ex Zaire), *E. Fischer 1085* (no voucher yet), CA180, *rpl16* not yet submitted; BG Bonn 4437, Zimbabwe, Road from Masvingo to Great Zimbabwe, 6 km from turnoff towards Mission Morgenstern, *D. Supthut 88311* (no voucher yet), CA185, *rpl16* not yet submitted.

Rhipsalis burchellii Britton & Rose, BG Berlin-Dahlem BR-0-B-0610905, Brazil, Paraná, descent Itaitaia to Ponta Crossa, *W. Barthlott 90-28* (B), CA053, *trnK/matK* FN669702, *rpl16* FN673608, *psbA-trnH* FN669066, *trnQ-rps16* FN677858.

Rhipsalis campos-portoana Loefgr., BG Bonn 5738, Brazil, Paraná, descent Itaitaia to Ponta Crossa, *W. Barthlott 90-33* (B), CA054, *trnK/matK* FN669703, *rpl16* FN673609, *psbA-trnH* FN669065, *trnQ-rps16* FN677859.

Rhipsalis cereoides Backeb. & Voll, BG Bonn 4462, Brazil, Espírito Santo, Domingos Martins, *W. Rauh & R. Kautsky 67557* (K), CA020, *trnK/matK* FN669671, *rpl16* FN673575, *psbA-trnH* FN669054, *trnQ-rps16* FN677825.

Rhipsalis cereuscula Haw., BG Bonn 12179, Bolivia, La Paz, Prov. Nor Yungas, close to Chulumani, *M. Miyagawa, 9.4.1987* (B), CA050, *trnK/matK* FN669700, *rpl16* FN673605, *psbA-trnH* FN669064, *trnQ-rps16* FN677855.

Rhipsalis clavata **f. *clavata*** F.A.C. Weber, BG Bonn 4553, without locality data, *Marnier-Lapostolle 1974 s.n.* (B), CA049, *trnK/matK* FN669699, *rpl16* FN673604, *psbA-trnH* FN669061, *trnQ-rps16* FN677854.

Rhipsalis clavata **f. *delicatula*** (Loefgr.) Barthlott & N.P.Taylor, BG Bonn 5741, Brazil, São Paulo, close to Ubatuba, *W. Barthlott 90-18, 3.1990* (B), CA012, *trnK/matK* FN669664, *rpl16* FN673567, *psbA-trnH* FN669062, *trnQ-rps16* FN677817.

Rhipsalis crispata Pfeiff., BG Bonn 4472, without locality data, (B), CA026, *trnK/matK* FN669677, *rpl16* FN673581, *psbA-trnH* FN669094, *trnQ-rps16* FN677831.

Rhipsalis cuneata Britton & Rose, BG Bonn 12957, Bolivia, Dep. Cochabamba, Prov. Chapare, *C. & P. Ibisch 93.766* (LPB, FR, B), CA021, *trnK/matK* FN669672, *rpl16* FN673576, *psbA-trnH* FN995428, *trnQ-rps16* FN677826.

Rhipsalis dissimilis* f. *dissimilis K.Schum., BG Bonn 4505, without locality data, (B), CA064, *trnK/matK* FN669713, *rpl16* FN673619, *psbA-trnH* FN669048, *trnQ-rps16* FN677868.

Rhipsalis dissimilis* f. *epiphyllanthoides Barthlott & N.P.Taylor, BG Bonn 5743, Brazil, Paraná, close to Villa Velha, *W. Barthlott 90-34* (B), CA063, *trnK/matK* FN669712, *rpl16* FN673618, *psbA-trnH* FN669047, *trnQ-rps16* FN677929.

Rhipsalis elliptica G.Lindb. ex K.Schum., BG Bonn 4679, Brazil, Goias, *P. Braun 879* (B), CA022, *trnK/matK* FN669673, *rpl16* FN673577, *psbA-trnH* FN669087, *trnQ-rps16* FN677827; BG Bonn 5746 ex BG Gent, Brazil, Paraná, descent Itatiaia to Ponta Crossa, *W. Barthlott 90-29*, *3.1990* (B), CA023, *trnK/matK* FN669674, *rpl16* FN673578, *psbA-trnH* FN669089, *trnQ-rps16* FN677828.

Rhipsalis ewaldiana Barthlott & N.P.Taylor, BG Bonn 8780 ex. BG Gent, without locality data, *E. Ewald 31.5.1987* (K, holotype), CA107, *trnK/matK* FN669746, *rpl16* FN673652, *psbA-trnH* FN669093, *trnQ-rps16* FN677899.

Rhipsalis floccosa* subsp. *floccosa Salm-Dyck, RBG Kew, without locality data, *C. Erskine 164* (K, neotype), CA139, *trnK/matK* FN995680, *rpl16* FN995675, *psbA-trnH* FN995436, *trnQ-rps16* FN995672.

Rhipsalis floccosa* subsp. *hohenauensis (F.Ritter) Barthlott & N.P.Taylor, RBG Kew 1991-1448, Paraguay, Reserva de Itabo, *D. Zappi 92* (SPF), CA154, *trnK/matK* FR852592*rpl16* FR853120, *psbA-trnH* FR853115,*trnQ-rps16* FR853129, *rbcL* FR853397.

Rhipsalis floccosa* subsp. *oreophila N.P. Taylor & Zappi, Brazil, Minas Gerais, Monte Azul, *Braun s.n.* (no voucher), CA132, *trnK/matK* FN669769, *rpl16* FN673675, *psbA-trnH* FN669158, *trnQ-rps16* FN677923.

Rhipsalis floccosa* subsp. *pulviningera (G.Lindb.) Barthlott & N.P.Taylor, BG Bonn 5744, Brazil, São Paulo, close to Campos do Jordao, *W. Barthlott 90-2* (B), CA056, *trnK/matK* FN669705, *rpl16* FN673611, *psbA-trnH* FN669050, *trnQ-rps16* FN677861; BG Bonn 5742, Brazil, Paraná, descent Itatiaia to Ponta Crossa, *W. Barthlott 90-32* (B), CA057, *trnK/matK* FN669706, *rpl16* FN673612, *psbA-trnH* FN669051, *trnQ-rps16* FN677862.

Rhipsalis floccosa* subsp. *tucumanensis (F.A.C.Weber) Barthlott & N.P.Taylor, BG Bonn 12956, Bolivia, Dep. Cochabamba, Prov. Chapare, *C. & P. Ibisch. 93.762* (BOLV, LPB, FR, B), CA058, *trnK/matK* FN669707, *rpl16* FN673613, *psbA-trnH* FN669053, *trnQ-rps16* FN677863.

Rhipsalis goebeliana Backeb., BG Bonn 4467, without locality data, (ZSS 28438, B), CA030, *trnK/matK* FN669681, *rpl16* FN673585, *psbA-trnH* FN669092, *trnQ-rps16* FN677835.

Rhipsalis grandiflora Haw., BG Bonn 8714, Brazil, Paraná, Ponta Crossa, *Kirschnek s.n.* (B), CA105, *trnK/matK* FN669744, *rpl16* FN673650, *psbA-trnH* FN669042, *trnQ-rps16* FN677897; BG Bonn 5736, Brazil, Santa Catarina, east of Blumenau, *W. Barthlott 90-38* (B), CA106, *trnK/matK* FN669745, *rpl16* FN673651, *psbA-trnH* FN669041, *trnQ-rps16* FN677898; BG Bonn 5745, Brazil, Paraná, descent Itatiaia to Ponta Crossa, *W. Barthlott 90-25*, 3.1990 (B), CA004, *trnK/matK* FN669657, *rpl16* FN673559, *psbA-trnH* FN669040, *trnQ-rps16* FN677809.

Rhipsalis hoelleri Barthlott & N.P.Taylor, BG Bonn 4841, 12186, Brazil, Espírito Santo, Domingos Martins, *B. Orssich s.n.,1987* (BONN, holotype), CA108, *trnK/matK* FN669747, *rpl16* FN673653, *psbA-trnH* FN669106, *trnQ-rps16* FN677900.

Rhipsalis juengeri Barthlott & N.P.Taylor, BG Bonn 1700, without locality data, (BONN, holotype), CA051, *trnK/matK* FR853119, *rpl16* FN673606, *psbA-trnH* FN669063, *trnQ-rps16* FN677856, *rbcL* FR853330.

Rhipsalis lindbergiana K.Schum., BG Bonn 4670, without locality data, (B), CA103, *trnK/matK* FN669742, *rpl16* FN673648, *psbA-trnH* FN669101, *trnQ-rps16* FN677896.

Rhipsalis mesembryanthemoides Haw., BG Bonn 4482, without locality data, (B), CA101, *trnK/matK* FN669740, *rpl16* FN673646, *psbA-trnH* FN669076, *trnQ-rps16* FN677894.

Rhipsalis micrantha (Kunth) DC., BG Bonn 16679, Ecuador, El Oro, near Machala, *R. Bauer 50* (B), CA029, *trnK/matK* FN669680, *rpl16* FN673584, *psbA-trnH* FN669097, *trnQ-rps16* FN677834; BG Bonn 13468, Peru, *K. Knidze 1648* (B), CA032, *trnK/matK* FN669682, *rpl16* FN673587, *psbA-trnH* FN669098, *trnQ-rps16* FN677837; BG Bonn 14916, locality data given as "Bolivia, Rio Pando" may be incorrect, *K. Knidze 2793* (B), CA027, *trnK/matK* FN669678, *rpl16* FN673582, *psbA-trnH* FN669096, *trnQ-rps16* FN677832; BG Bonn 4493, Peru, Piura, Ayabaca, *W. Rauh & W. Barthlott s.n., 1973* (B), CA028, *trnK/matK* FN669679, *rpl16* FN673583, *psbA-trnH* FN669095, *trnQ-rps16* FN677833.

Rhipsalis micrantha* f. *kirbergii (Barthlott) Barthlott & N.P.Taylor, BG Bonn 4554, Ecuador, Loja, near La Toma, *J. Madsen 61157* (AAU, MO, QCA), CA033, *trnK/matK* FN669683, *rpl16* FN673588, *psbA-trnH* FN669099, *trnQ-rps16* FN677838.

Rhipsalis micrantha* f. *rauhiorum (Barthlott) Barthlott & N.P.Taylor, BG Bonn 5913, Colombia, Tolima, near Ibagué, *M.Koenen & S.Porembski 99* (B), CA018, *trnK/matK* FN669669, *rpl16* FN673573, *psbA-trnH* FN669085, *trnQ-rps16* FN677823; Ecuador, Rio Catamayo valley, *W. Barthlott & W. Rauh 35276* (HEID, holotype), CA121, *trnK/matK* FN669760, *rpl16* FN673666, *psbA-trnH* FN669086, *trnQ-rps16* FN677913.

Rhipsalis neves-armondii K.Schum., BG Bonn 16401 ex I.S.I 1819, Brazil, near Tijuco, (B), CA110, *trnK/matK* FN669749, *rpl16* FN673655, *psbA-trnH* FN669107, *trnQ-rps16* FN677902; BG Bonn 01723, without locality data, (B), CA006, *trnK/matK* FN669659, *rpl16* FN673561, *psbA-trnH* FN669052, *trnQ-rps16* FN677811.

Rhipsalis neves-armondii* f. *megalantha (Loefgr.) Barthlott & N.P.Taylor, BG Bonn 12176, without locality data, (B), CA109, *trnK/matK* FN669748, *rpl16* FN673654, *psbA-trnH* FN669105, *trnQ-rps16* FN677901.

Rhipsalis oblonga Loefgr., BG Bonn 4469, Brazil, Rio de Janeiro, Serra dos Órgãos, *W. Rauh s.n.* (B), CA024, *trnK/matK* FN669675, *rpl16* FN673579, *psbA-trnH* FN669100, *trnQ-rps16* FN677829; BG Bonn 5918, Brazil, São Paulo, Ilha de Sao Sebastiao, *D. Zappi & N. Taylor 1645* (K K000009537), CA031, *trnK/matK* FR853113, *rpl16* FN673586, *psbA-trnH* FN669088, *trnQ-rps16* FN677836, *rbcL* FR853310.

Rhipsalis occidentalis Barthlott & Rauh, BG Bonn 17074, Peru, Rioja, Distr. Yuracyacu, San Martin, Caserio Tambo, *R. Villena Ruiz s.n. / R.Bauer & M. Kimnach 54-1* (USM, ZSS 19799, ZSS 28436), CA025, *trnK/matK* FN669676, *rpl16* FN673580, *psbA-trnH* FN669090, *trnQ-rps16* FN677830; BG Bonn 16680, Ecuador, Sucumbios, near Lago Agrio, *A. Glatz s.n. 5.7.1998* (ZSS 28445), CA096, *trnK/matK* FN669735, *rpl16* FN673641, *psbA-trnH* FN669091, *trnQ-rps16* FN677889.

Rhipsalis olivifera N.P.Taylor & Zappi, BG Bonn 26078, without locality data, (B), CA115, *trnK/matK* FN669754, *rpl16* FN673660, *psbA-trnH* FN669112, *trnQ-rps16* FN677907.

Rhipsalis pacheco-leonis Loefgr., BG Bonn 2157 ex ZSS 861181, Brazil, Rio de Janeiro, Cabo Frio, *P. Frick 13* (B), CA113, *trnK/matK* FN669752, *rpl16* FN673658, *psbA-trnH* FN669110, *trnQ-rps16* FN677905.

Rhipsalis pacheco-leonis* subsp. *catenulata (Kimnach) Barthlott & N.P.Taylor, BG Bonn 4485 ex BG Huntington, Brazil, Rio de Janeiro, Mun. Nova Friburgo, *presumably Fowlie s.n. type collection* (B (also HNT, if holotype), CA061, *trnK/matK* FN669710, *rpl16* FN673616, *psbA-trnH* FN669046, *trnQ-rps16* FN677866; BG Bonn 4502, Brazil, Espírito Santo, Domingos Martins, *W. Rauh & R. Kautskyi 67560* [collection number probably incorrect and W. Rauh 67533/67618 may be the right number] (K, BONN), CA066, *trnK/matK* FN669715, *rpl16* FN673621, *psbA-trnH* FN669045, *trnQ-rps16* FN677870.

Rhipsalis pachyptera Pfeiff., BG Bonn 5758, Brazil, *W. Barthlott 90-44* (BONN, photos), CA034, *trnK/matK* FN669684, *rpl16* FN673589, *psbA-trnH* FN669055, *trnQ-rps16* FN677839; BG Bonn 5757, Brazil, Santa Catarina, east of Blumenau, *W. Barthlott 90-37* (BONN, photos), CA007, *trnK/matK* FN669660, *rpl16* FN673562, *psbA-trnH* FN669057, *trnQ-rps16* FN677812.

Rhipsalis paradoxa Salm-Dyck, BG Bonn 08844, Brazil, Bahia, nortwest of Salvador da Bahia, *W. Barthlott 89-001* (B), CA005, *trnK/matK* FN669658, *rpl16* FN673560, *psbA-trnH* FN669043, *trnQ-rps16* FN677810.

Rhipsalis paradoxa* subsp. *septentrionalis Barthlott & N.P.Taylor, BG Bonn 4489, Brazil, Espírito Santo, Mun. Domingos Martins, *W. Rauh 67565* (K), CA059, *trnK / matK* FN669708, *rpl16* FN673614, *psbA-trnH* FN669049, *trnQ-rps16* FN677864.

Rhipsalis pentaptera A.Dietr., BG Bonn 4517, without locality data,(B), CA065, *trnK/matK* FN669714 (Korotkova et al., 2010), *rpl16* FN673620 (Korotkova et al., 2010), *psbA-trnH* FN669103 (Korotkova et al., 2010), *trnQ-rps16* FN677869 (Korotkova et al., 2010); BG Bonn 4656 ex BG Tübingen, without locality data, (B), CA067, *trnK / matK* FN669716, *rpl16* FN673622, *psbA-trnH* FN669102, *trnQ-rps16* FN677871.

Rhipsalis pilocarpa Loefgr., BG Bonn 4509, without locality data, *W. Rauh & W. Barthlott s.n.* (B), CA008, *trnK / matK* FN669661, *rpl16* FN673563, *psbA-trnH* FN669058, *trnQ-rps16* FN677813; BG Bonn 4453, without locality data, (B), CA055, *trnK/matK* FN669704, *rpl16* FN673610, *psbA-trnH* FN669059, *trnQ-rps16* FN677860.

Rhipsalis pittieri (Britton & Rose) Barthlott & N.P.Taylor, BG Berlin 055220640, Venezuela, Pto. Cabello, (B), CA144, *trnK/matK* FR852590, *rpl16* FR853122, *psbA-trnH* FR853116, *trnQ-rps16* FR853127, *rbcL* FR853395; BG Bonn without acc. no., without locality data, CA156, *trnK/matK* FR852594, *rpl16* FR853123, *psbA-trnH* FR853117, *trnQ-rps16* FR853128, *rbcL* FR853398.

Rhipsalis pulchra Loefgr., BG Bonn 5924, Brazil, Minas Gerais, Parque Estadual Florestal do Ibitipoca, *D. Zappi 260* (K, SPF, CESJ), CA052, *trnK/matK* FN669701, *rpl16* FN673607, *psbA-trnH* FN669060, *trnQ-rps16* FN677857.

Rhipsalis puniceodiscus G.Lindb., BG Bonn 4547, without locality data, *Marnier-Lapostolle, 1974* (B), CA111, *trnK/matK* FN669750, *rpl16* FN673656, *psbA-trnH* FN669109, *trnQ-rps16* FN677903; BG Bonn 4545, Brazil, São Paulo, *Döring 2* (B), CA112, *trnK/matK* FN669751, *rpl16* FN673657, *psbA-trnH* FN669108, *trnQ-rps16* FN677904.

Rhipsalis russellii Britton & Rose, BG Bonn 4474, Brazil, Goiás, *P. Braun s.n.* (B), CA019, *trnK/matK* FN669670, *rpl16* FN673574, *psbA-trnH* FN669104, *trnQ-rps16* FN677824.

Rhipsalis shaferi Britton & Rose, BG Bonn 15475, Bolivia, Santa Cruz, *C. Nowicki 1628* (B), CA136, *trnK/matK* FN995678, *rpl16* FN673679, *psbA-trnH* FN995434, *trnQ-rps16* FN677927; BG Bonn 12945, Bolivia, Santa Cruz, Prov. Florida, 80 km de Santa Cruz, *C. & P. Ibisch 93.327* (FR), CA003, *trnK/matK* FN669656, *rpl16* FN673558, *psbA-trnH* FN669067, *trnQ-rps16* FN677808.

Rhipsalis sulcata F.A.C. Weber, BG Bonn 4490, Brazil, Espírito Santo, Domingos Martins, *W. Rauh & R. Kautskyi 67562* (K), CA062, *trnK/matK* FN669711, *rpl16* FN673617, *psbA-trnH* FN669074, *trnQ-rps16* FN677867.

Rhipsalis teres Steud., BG Bonn 5586, Costa Rica, Llanuras de San Carlos, *C. Horich 4/88* (BONN, in spirit), CA099, *trnK/matK* FN669738, *rpl16* FN673644, *psbA-trnH* FN669079, *trnQ-rps16* FN677892; BG Bonn 2162, Brazil, Rio Grande do Sul, betw. Candelaria and Agudo, *Horst & Uebelmann HU1004*, (B), CA102, *trnK/matK* FN669741, *rpl16* FN673647, *psbA-trnH* FN669077, *trnQ-rps16* FN677895; BG Bonn 2155, Brazil, Rio Grande do Sul, betw. Candelaria and Agudo, *Horst & Uebelmann HU1002* (B), CA134, *trnK/matK* FN669770, *rpl16* FN673677, *psbA-trnH* FN669159, *trnQ-rps16* FN677925.

Rhipsalis teres* f. *capilliformis (F.A.C.Weber) Barthlott & N.P.Taylor, BG Bonn 4455, without locality data, *F. Marnier-Lapostolle, 1974* (B), CA097, *trnK/matK* FN669736, *rpl16* FN673642, *psbA-trnH* FN669071, *trnQ-rps16* FN677890.

Rhipsalis teres* f. *heteroclada (Britton & Rose) Barthlott & N.P.Taylor, BG Bonn 5734, Brazil, Rio de Janeiro, south of Parati, *W. Barthlott 90-16*, 3.1990 (B), CA011, *trnK/matK* FN669663, *rpl16* FN673566, *psbA-trnH* FN669078, *trnQ-rps16* FN677816; BG Bonn 5754, Brazil, Rio Grande do Sul, near Arroio da Sêca, *W. Barthlott 90-54* (B), CA100, *trnK/matK* FN669739, *rpl16* FN673645, *psbA-trnH* FN669075, *trnQ-rps16* FN677893.

Rhipsalis teres* f. *prismatica (Lem.) Barthlott & N.P.Taylor, without locality data, (B), CA118, *trnK/matK* FN669757, *rpl16* FN673663, *psbA-trnH* FN669072, *trnQ-rps16* FN677910.

Rhipsalis trigona Pfeiff., BG Bonn 14128, without locality data, (B), CA060, *trnK/matK* FN669709, *rpl16* FN673615, *psbA-trnH* FN669044, *trnQ-rps16* FN677865.

Schlumbergera kautskyi (Horobin & McMillan) N.P.Taylor, BG Bonn 4595, Brazil, Espírito Santo, Domingos Martins, *W. Rauh & R. Kautsky 67558* (cult. BONN; photos), CA037, *trnK/matK* FN669687, *rpl16* FN673592, *psbA-trnH* FN669022, *trnQ-rps16* FN677842.

Schlumbergera microsphaerica (K.Schum.) Hövel, Brazil, Espírito Santo, Pico da Bandeira, *Thieken s.n.* (no voucher), CA122, *trnK/matK* FN669761, *rpl16* FN673667, *psbA-trnH* FN669020, *trnQ-rps16* FN677914.

Schlumbergera opuntioides (Loefgr. & Dusén) D.R.Hunt , BG Bonn 27452, without locality data, *Thieken s.n.* (BONN, photos), CA038, *trnK/matK* FN669688, *rpl16* FN673593, *psbA-trnH* FN669019, *trnQ-rps16* FN677843.

Schlumbergera orssichiana Barthlott & McMillan, BG Bonn 5584, Brazil, Rio de Janeiro, Serra do Mar, *B. Orssich* (HEID holotype, BONN), CA035, *trnK/matK* FN669685, *rpl16* FN673590, *psbA-trnH* FN669023, trnQ-rpS 16FN677840; BG Bonn 5727, Brazil, Rio de Janeiro, Serra do Mar, *B. Orssich 23* (B), CA046, *trnK/matK* FN669696, *rpl16* FN673601, *psbA-trnH* FN669025, *trnQ-rps16* FN677851.

Schlumbergera russelliana (Hook.) Britton & Rose, BG Bonn 2636, Brazil, Rio de Janeiro, near Teresopolis, *R. Ehlers s.n.* (BONN, photos), CA036, *trnK/matK* FN669686 (Korotkova

et al., 2010), *rpl16* FN673591 (Korotkova et al., 2010), *psbA-trnH* FN669021 (Korotkova et al., 2010), *trnQ-rps16* FN677841 (Korotkova et al., 2010); BG Bonn 4672, without locality data, *B. Orssich* (BONN, photos), CA045, *trnK/matK* FN669695, *rpl16* FN673600, *psbA-trnH* FN669018, *trnQ-rps16* FN677850.

Schlumbergera truncata (Haw.) Moran, BG Bonn 5583, Brazil, Rio de Janeiro, near Teresopolis, *B. Orssich s.n.* (B), CA016, *trnK/matK* -, *rpl16* FN673571, *psbA-trnH* FN669024, *trnQ-rps16* FN677821; BG Bonn 29372, Brazil, Rio de Janeiro, near Teresopolis, *B. Orssich s.n.* (B), CA127, *trnK/matK* FN669765, *rpl16* FN673671, *psbA-trnH* FN995433, *trnQ-rps16* FN677919.

Appendix 2. PCR amplification protocols

DNA working dilution: 10 ng / µl

Primer stock concentration: 100 mmol @l (MWG Biotech, Ebersberg / Germany); primer working concentration: 20mmol

PCR reagents used

Taq Polymerase:

- SAWADY Taq DNA polymerase (Peqlab Biotechnologie GmbH, Erlangen, Germany)
- GoTaq® Flexi DNA polymerase (Promega Corp., Madison, USA, cat. no. M830A)

Taq polymerase buffers and magnesium chloride (all reagents supplied by the manufacturer of the polymerase)

for SAWADY Taq DNA polymerase:

- peqLab Buffer Y: 200 mM Tris-HCl (pH 8.55 at 25 °C), 160 mM $(NH_4)2SO_4$, 0.1% Tween 20 (red cap) and 20 mM $MgCl_2$), increases product yield).
- peqLab Buffer S: (100 mM Tris-HCl (pH 8.8, 500 mM KCl, 0.1 % Tween 20, 15 mM $MgCl_2$), increases polymerase specifity.
- 25mM MgCl2 (cat. no. A351B)

for GoTaq® Flexi DNA polymerase:

- 5X Colorless GoTaq® Flexi Buffer (Promega Corp., Madison, USA, cat. no. M890A)
- 25mM MgCl2 (cat. no. A351H)

dNTPs:

- peqGOLD dNTP-Set 4x25 @mol (Peqlab Biotechnologie GmbH, Erlangen, Germany; cat. no. 20-2010)
- dNTP Set 1 (Carl Roth GmbH, Karlsruhe, Germany; cat. no. K 0.39.1)
- PCR nucleotide mix (Promega Corp., Madison, USA, cat. no. C1141)

PCR additives:

- PVP (Polyvinylpyrrolidone) 10%
- 5 M Betaine

Reaction mixtures

Reaction mixture using SAWADY Taq-DNA-Polymerase, total volume: 50 µl

2 µl	solution of DNA template à 20 ng/µl
5 µl	10x peqLab Taq Buffer S or Y
0 - 3 µl	$MgCl_2$ 25mM
0 - 5 µl	Betaine (5 M)
0 - 2,5 µl	PVP (10%)
2 µl	Forward primer (20pm/µl)
2 µl	Reverse primer (20pm/µl)
10 µl	dNTP (each 1,25mM)
0,3 µl	peqLab Taq polymerase
ad	H_2O p.a. grade

Reaction mixture using GoTaq® Flexi DNA polymerase, total volume: 50 µl

2 µl	solution of DNA template à 20 ng/µl
6 µl	$MgCl_2$ 25mM
10 µl	5x GoTaq® Flexi Buffer
2 µl	Forward primer (20pm/µl)
2 µl	Reverse primer (20pm/µl)
0 - 2,5 µl	Betaine (5 M)
0 - 5 µl	PVP 10%
8 µl	dNTP (each 1,25 mM) *or* 1 µl PCR nucleotide mix
0,25 µl	GoTaq® flexi DNA polymerase @ 5units/µl
ad	H_2O p.a. grade

Primers used and amplification conditions

(Directions: F: forward, R: reverse. Application: A: amplification, S: sequencing).

psbA-trnH **intergenic spacer**

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

PCR conditions

1) initial denaturation: 2 min at 95°C,
2) 5 cycles of 0:30 min at 95°C, 1 min. at 59°C, 1 min at 72°C
3) 30 cycles of 0:30 min at 95°C, 1 min. at 55°C, 1 min at 72°C
4) final extension step of 10 min. at 72°C

trnQ-rps16 **intergenic spacer**

Primers used

primer name	sequence (5'-3')			reference
trnQ2	CCAAGTGGTAAGGCGTCGGG	F	A, S	this study
rps16x1	GTTGCTTTCTACCACATCGTTT	R	A, S	Shaw et al. 2007

PCR conditions:

1) initial denaturation: 2 min at 95°C,
2) 35 cycles of 0:30 min at 95°C, 1 min. at 55°C, 1 min at 72°C
3) final extension step of 10 min. at 72°C.

trnS-trnG **region (*trnS-trnG* intergenic spacer, *trnG* intron)**

Primers used

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

PCR conditions:

1) initial denaturation: 2 min at 95°C,
2) 35 cycles of 0:30 min at 95°C, 1 min. at 58°C, 2 min at 72°C
3) final extension step of 15 min. at 72°C.

rps3-rpl16 **spacer*,* *rpl16* intron**

Primers used

primer name	sequence (5'-3')			Reference
CArps3F	GATTATTGCGCCTATCCG	F	A, S	this study
CArpl16R	CCGATAAGATAATCCCTTCA	R	A, S	this study
CArpl16-400R	GAACTTTGTTCTTGAGCC	R	S	this study
CArpl16-700R	GYTAAAATAAAATTGGAGCCATC	R	S	this study

PCR conditions:

1) initial denaturation: 2 min at 95°C,
2) 35 cycles of 0:30 min at 95°C, 1 min. at 55°C, 1:30 min at 72°C
3) final extension step of 15 min. at 72°C.

***trn*K/*mat*K region**

Primers for amplification (A) and sequencing (S)

primer name	sequence (5'-3')			Reference
trnK-F	GGGTTGCTAACTCAATGGTAGAG	F	A, S	Wicke & Quandt 2009
trnK3914Fdi	GGGGTTGCTAACTCAACGG	F	A, S	Johnson & Soltis 1995
trnK-2R	AACTAGTCGGATGGAGTAG	R	A, S	Johnson & Soltis 1995
ROSmatK-655R	GGATTCGTATTCACATACAT	R	A, S	Worberg 2009
ROSmatK-530F	AGATGCCTCTTCTTTGC	F	A, S	Worberg 2009
ACmatK500F	TTCTTCTTTGCATTTATTACG	F	A, S	Müller 2002
ACmatK650R	GGATTCATATTCACATACATRG	R	S	Müller 2002
ACmatK1300F	ATAAAGTATATACTTCGAC	F	S	Müller & Borsch 2005
trnK-71R	CTAATGGGATGTCCTAATAC	R	S	Nyffeler 2002
CAtrnK-270R	GAGCTTATCTTCGTAATTTG	R	S	Korotkova et al. 2010

PCR conditions:

1) initial denaturation: 1:30 min at 95°C,
2) 35 cycles of 0:30 min at 95°C, 1 min. at 50°C, 1:30 min at 72°C
3) final extension step of 20 min. at 72°C.

Appendix 3. Sequence parts excluded from the phylogenetic analyses

Positions excluded from the Cactaceae dataset (Chapter 2)

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

Positions excluded from the combined *Pfeiffera* dataset (Chapter 2)

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
4475-4496	*trnG* 5'exon	excluded as uninformative
4719-4733	*trnG* intron	poly-T
4816-4824	*trnG* intron	poly-T
5180-5216	*trnG* 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

Positions excluded from the combined Rhipsalideae dataset (Chapter 3)

Position	region	Comment
1-30	*trnK* intron	incomplete beginning
706-708	*trnK* intron	polyT
2364-2366	*trnK* intron	polyA
2535-2579	*trnK* intron	incomplete ending
2712-2731	*psbA-trnH*	polyT
2604-2916	*psbA-trnH*	sequences of *Schlumbergera truncata* and *S. orsicchiana* unreliable due to reading errors after polyT streches
2814-2833	*psbA-trnH*	polyT, polyA
3046-3053	*psbA-trnH*	polyA
3152-3158	*rps3-rpl16*	polyT
3247-3255	*rpl16* exon	uninformative
3300-3302	*rpl16* intron	polyA
3449-3453	*rpl16* intron	polyT
3488-3505	*rpl16* intron	polyA
3527-3544	*rpl16* intron	multiple AT-repeat
4101-4103	*rpl16* intron	polyA
4795-4808	*trnQ-rps16*	polyA
4973-4992	*trnQ-rps16*	polyT (with substitutions)
5047-5064	*trnQ-rps16*	polyA
5085-5088	*trnQ-rps16*	polyA

Appendix 4. List of indels coded from the combined Rhipsalideae dataset

No.	extension	length	Sequence motif
trnK **intron 5' fragment**			
1	129-139	11	Gap in *Echinopsis aurea*
2	140-143	4	Gap in *Calymmanthium substerile*; "CAAA" Simple Sequence Repeat (SSR) in all other taxa
3	160-167	8	"AGAATATC" insertion of unknown origin in *Browningia hertlingiana*
4	211-213	3	"GCC" SSR in *Rhipsalis grandiflora*, *R. pachyptera*, *R. russelli*, *R. cereoides*, *R. agudoensis*; likely synapomorphic for these taxa
5	353-360	8	Gap in *Hatiora herminiae*
6	380-384	5	"CGATT" SSR in *Echinopsis aurea*
7	446-446	1	Inserted "T" in *Rhipsalis baccifera* subsp. *baccifera*, subsp. *horrida* and subsp. *erythrocarpa*
8	505-512	8	"CTTACTTT" SSR in *Schlumbergera truncata*, *S. orssichiana* and *S. kautskyi*; likely synapomorphic for these taxa
9	523-523	1	Inserted "A" in *Pfeiffera monacantha*
10	550-551	2	Gap in *Rhipsalis oblonga* and *R. occidentalis*
11	818-826	9	Gap in *Rhipsalis pentaptera*, *R. pacheco-leonis* and *R. lindbergiana*
matK **CDS**			
12	947-952	6	Gap in *Echinopsis aurea*
13	1057-1059	3	"AAA" insertion in *Lepismium lumbricoides*
14	1260-1265	6	„CGTAAT“ SSR in *Rhipsalidopsis gaertneri*
15	1310-1315	6	Gap in *Schlumbergera truncata* isolate CA127
16	1483-1485	3	Gap in *Pfeiffera monacantha*
trnK **intron 3' fragment**			
17	2281-2286	6	Gap in *Lepismium lorentzianum*
18	2364-2367	4	"TTGA" SSR in *Calymmanthium substerile*
19	2388-2390	3	"AGT" SSR in *Lepismium lorentzianum* and *L. warmingianum*; probably synapomorphic for these two sister species
psbA-trnH **spacer**			
20	2524-2539	16	Gap in *Rhipsalis paradoxa*
21	2561-2575	15	Gap in *Lepismium houlletianum* and *Rhipsalis* subg. *Phyllarthrorhipsalis*
22	2564-2568	5	"AGTTA" insertion of unknown origin in *Browningia hertlingiana* and *Echinopsis aurea*
23	2571-2575	5	"ACTAG" SSR in *Hatiora epiphylloides*, *Rhipsalidopsis gaertneri*, *R. rosea*, *Schlumbergera* and *Hatiora salicornioides* isolate CA009
24	2577-2586	10	Gap in *Calymmanthium substerile*
25	2578-2586	9	"AGTCTTTTT" insertion of unknown origin in *Rhipsalidopsis x graeseri* and *Hatiora herminiae*
26	2588-2596	9	"TTCGTTTAT" SSR in *Rhipsalis* subgenus *Erythrorhipsalis*, likely synapomorphic for this subgenus
27	2603-2684	82	Gap in *Rhipsalis*, likely synapomorphic for the genus
28	2600-2681	91	Gap in *Rhipsalis* subgenus *Goniorhipsalis*, *Echinopsis aurea*, *Calymmanthium substerile*, *Browningia hertlingiana*
29	2603-2618	16	"CTTTTTTTTTTTAGT" insertion in *Lepismium houlletianum*
30	2614-2614	1	1 nt missing in sequence motif of 29 in *Lepismium houlletianum* isolate CA082, CA083 and 130
31	2620-2889	270	Gap in *Lepismium warmingianum*
32	2625-2629	5	Gap in *Hatiora herminiae*, *H. salicornioides* isolate CA009, CA043 and *Rhipsalidopsis x graeseri*
33	2632-2636	5	"TTCAA" SSR in *Lepismium cruciforme* and *L. houlletianum*
34	2642-2647	6	"TTTAA" or "TTTTTT" or similar repeats in *Schlumbergera*, *Lepismium lumbricoides*, *Pfeiffera monacantha*, *Rhipsalidopsis* and *Hatiora*
35	2642-2659	18	"TTAACAGTTAA" SSR in *Lepismium lorentzianum*
36	2642-2905	264	Gap in *Lepismium houlletianum*
37	2647-2659	13	Gap in *Lepismium lumbricoides* isolate CA128 and CA129
38	2648-2659	12	Insertion of "TTAACAG" and repeat of this motif in *Lepismium lorentzianum*
39	2663-2668	6	Gap in *Hatiora salicornioides* isolate CA009, CA043 and *Hatiora herminiae*
40	2663-2672	10	Gap in *Rhipsalidopsis gaertneri*, *R. xgraeseri* and *Hatiora herminiae*
41	2663-2680	18	Gap, homology assessment unclear. Nucleotides present in all taxa except *Schlumbergera*, *Hatiora salicornioides*, *H. cylindrica*, *H. herminiae*, *Rhipsalidopsis gaertneri*, *R. xgraeseri*
42	2664-2664	1	Gap in *Schlumbergera opuntioides* and *S. microsphaerica*

Appendix 4, continued

No.	extension	length	Sequence motif
43	2669-2680	12	“ATTCGTTTATTT” insertion in *H. salicornioides* isolate CA043, CA009 and *Hatiora cylindrica*
44	2682-2837	156	Gap in *Lepismium cruciforme*
45	2685-2690	6	“ATT”$_2$ SSR in *Rhipsalis* subg. *Rhipsalis*
46	2685-2691	7	Gap in *Lepismium lumbricoides*
47	2685-2837	153	Gap in *Lepismium lorentzianum*
48	2688-2690	3	“ATT” SSR in *Rhipsalis baccifera* isolates CA003, CA134, *Rhipsalis teres* isolates CA097, CA118, CA100, CA102, CA011, *Rhipsalis sulcata* and *Rhipsalis mesembryanthemoides*
49	2695-2698	4	“TATA” SSR with one substitution in *Calymmanthium substerile*
50	2695-2837	143	Gap in *Lepismium lumbricoides*
51	2703-2705	3	Gap in *Rhipsalis mesembryanthemoides*
52	2767-2768	2	“TT” SSR in *Rhipsalis shaferi*
53	2776-2781	6	Gap in *Browningia hertlingiana*
54	2802-2811	10	Gap in *Browningia hertlingiana* and *Echinopsis aurea*
55	2847-2861	15	Gap in *Lepismium lumbricoides*
56	2879-2889	11	“TAGGAAAGGGG” insertion in *Lepismium cruciforme*
57	2879-2897	19	Gap in *Calymmanthium substerile*
58	2892-2894	3	“GGA” SSR in *Lepismium lumbricoides*
59	2892-2897	6	Gap in *Lepismium cruciforme* isolate CA010
60	2896-2897	2	“GA” SSR in *Rhipsalis baccifera*, *R. teres* and *R. sulcata*
61	2896-2898	3	Gap in *Rhipsalis shaferi* isolate CA003, *R. grandiflora* CA004 and *R. pachyptera* CA007
62	2900-2903	4	“AAGG” SSR in *Lepismium lorentzianum*, *Hatiora cylindrica* and *H. salicornioides* isolate CA043, CA009
63	2908-2912	5	“AAAGG” SSR in *Rhipsalidopsis gaertneri* and *R. rosea*
***rps3-rpl16* spacer**			
64	2917-2920	4	Gap in Echinopsis aurea
65	2928-2928	1	Inserted "A" in *Lepismium warmingianum*
66	2928-2933	6	Gap resulting from alignment of 64 and 65
67	2929-2933	5	“ACTTG” SSR in the outgroup taxa and *Lepismium lorentzianum* and *L. warmingianum*
68	2976-2976	1	Gap in *Browningia hertlingiana* and *Echinopsis aurea*
69	2980-2990	11	Gap in the outgroup and *Hatiora epiphylloides*
70	2995-2998	4	“TCAA” SSR in *Rhipsalis puniceodiscus*
***rpl16* intron**			
71	3094-3161	68	Gap, in *Rhipsalis* subg. *Erythrorhipsalis*
72	3104-3113	10	“GGCGAAAAAA” SSR in *Rhipsalis elliptica* and *R. micrantha*
73	3104-3154	51	Gap, in *Lepismium warmingianum*
74	3146-3152	7	Gap, in *Echinopsis aurea* and *Browningia hertlingiana*
75	3271-3272	2	“AA” SSR in *Hatiora salicornioides* and *H. herminiae*
76	3276-3296	21	Gap, in *Hatiora herminiae*
77	3297-3314	18	Gap, in *Lepismium lorentzianum*, *L. warmingianum*, *Rhipsalis pilocarpa* and *R. campos-portoana*
78	3302-3314	13	Gap, in *Rhipsalis shaferi* isolate CA003
79	3303-3308	6	“GAAAAA” SSR in *Rhipsalidopsis gaertneri*, *R. dissimilis f. epiphyllanthoides*, *R. floccosa* subsp. *oreophila*
80	3303-3314	12	Gap, resulting from the alignment of 79 and 81
81	3309-3314	6	“TAAAAA” SSR in *Lepismium cruciforme*
82	3323-3323	1	inserted "A" in some *Rhipsalis*, *Hatiora epiphylloides* and *Rhipsalidopsis gaertneri*
83	3323-3327	5	Gap in *Lepismium*
84	3326-3335	10	Gap in *Rhipsalis teres* isolate CA102
85	3330-3335	6	„AAAGGA“ SSR in *Rhipsalidopsis gaertneri*
86	3337-3338	2	“AA” SSR in *Lepismium houlletianum*
87	3414-3421	8	“TCTTTGAA” SSR in *Calymmanthium substerile*
88	3432-3436	5	Gap, occurring in the outgroup taxa, *Rhipsalis* subg. *Goniorhipsalis* and *Rhipsalis neves-armondii*
89	3469-3477	9	Gap in *Lepismium houlletianum*
90	3486-3486	1	Inserted “A” in *Rhipsalis baccifera*, *R. teres* and *R. sulcata*, probably synapomorphic for these taxa, missing in CA102
91	3499-3507	9	Gap in *Lepismium cruciforme*
92	3499-3511	13	Gap in *Echinopsis aurea*
93	3500-3511	12	Gap, *Rhipsalis grandiflora, R. pachyptera, R. russelli, R. cereoides, R. agudoensis*; likely synapomorphic for these taxa
94	3510-3511	2	"AA" SSR in *Rhipsalidopsis gaertneri* and *Calymmanthium substerile*
95	3511-3511	1	Inserted “A” in *Rhipsalidopsis rosea*
96	3532-3534	3	CAA SSR in *Rhipsalis puniceodiscus* isolate CA111
97	3546-3547	2	“GA” insertion in *Rhipsalis pittieri* isolate CA144
98	3649-3673	25	Gap in *Rhipsalis* subgenus *Phyllarthrorhipsalis*

Appendix 4, continued

No.	extension	length	Sequence motif
99	3673-3680	8	Gap in *Rhipsalis cereuscula*
100	3766-3771	6	"AGATAT" SSR in *Echinopsis aurea*
101	3826-3866	41	Gap in *Browningia hertlingiana*
102	3826-4151	326	Gap in *Echinopsis aurea*
103	3869-3869	1	Inserted "T" in *Echinopsis aurea*
104	3904-4210	307	Gap in *Lepismium warmingianum*
105	3907-3907	1	Inserted "A" in *Rhipsalis paradoxa*
106	3907-4144	238	Gap in *Browningia hertlingiana*
107	3922-3926	5	Gap, in *Pfeiffera monacantha*
108	3929-3938	10	"TAAATACAAA" SSR in *Rhipsalis floccosa* isolate CA132 and *R. dissimilis* f. *epiphyllanthoides*
109	3948-3966	19	"TAAATACAAATAGAAAAAT" SSR in *Schlumbergera truncata* and *S. orssichiana*
110	3985-3985	1	Inserted "T" in *Rhipsalis pentaptera*
111	4003-4030	28	Gap in *Pfeiffera monacantha*,
112	4008-4026	19	"GAAAAGAATCTTATGAATA" SSR in *Rhipsalis baccifera* isolate CA002, CA013, CA014, CA015, CA017, CA135, CA138
113	4043-4072	30	Gap in *Schlumbergera opuntioides*
114	4075-4210	136	Gap in *Lepismium houlletianum*
115	4094-4103	10	"TTTTTATTCA" sequence motif in outgroup taxa, *Schlumbergera*, *Hatiora*, *Rhipsalidopsis* and *Lepismium* except *L. cruciforme*
116	4094-4104	11	Gap from alignment of 115 and one missing "T" in *Rhipsalis pittieri* isolate CA156
117	4094-4209	116	Gap, in *Rhipsalis* subgenera *Goniorhipsalis*, and *Rhipsalis* and part of subg. *Phyllarthrorhipsalis*: *R. micrantha, R. ewaldiana, R. cuneata, R. occidentalis, R. goebeliana*
118	4117-4118	2	"AA" insertion in *Calymmanthium substerile*
119	4161-4166	6	Gap in *Lepismium lorentzianum*
120	4161-4170	10	Gap in *Lepismium lumbricoides*
121	4162-4170	9	Gap in *Echinopsis aurea*
122	4165-4170	6	Gap in *Lepismium cruciforme*
123	4170-4170	1	"T" insertion in *Rhipsalis oblonga*, "A" insertion in *R. juengeri*
124	4173-4176	4	Gap in *Rhipsalis hoelleri*
125	4173-4181	9	Gap in *Rhipsalidopsis*
126	4174-4176	3	Gap in *Lepismium lumbricoides*
127	4174-4177	4	Gap in *Rhipsalis juengeri*
128	4181-4181	1	Gap in *Rhipsalis oblonga* isolate CA031
129	4182-4199	18	Gap in *Echinopsis aurea*
130	4183-4186	4	"WAAT" SSR in *Rhipsalis paradoxa*, *R. hoelleri* and *R. clavata*
131	4188-4199	12	Gap in *Rhipsalis clavata*
132	4194-4199	6	"TTCAAT" SSR in *Rhipsalis olivifera*
133	4194-4204	11	Gap in *Rhipsalis cereuscula*
134	4209-4209	1	Gap in *Rhipsalis* subg. *Rhipsalis* and *Phyllarthrorhipsalis*
135	4223-4223	1	„T" insertion in *Rhipsalis* subg. *Goniorhipsalis*
trnQ-rps16 **spacer**			
136	4324-4327	4	"TATA" SSR in *Hatiora herminiae*
137	4358-4359	2	"TT" insertion in *Browningia hertlingiana*
138	4362-4663	302	Gap in *Rhipsalis*, *Schlumbergera opuntioides*, *Lepismium houlletianum*, *L. warmingianum*, *Hatiora salicornioides* isolate CA043, and *Echinopsis aurea*
139	4389-4541	153	Gap in *Lepismium lumbricoides*
140	4433-4437	5	Gap in *Browningia hertlingiana*
141	4433-4438	6	"TTTTTT" in *Rhipsalidopsis gaertneri*
142	4433-4669	237	Gap in *Pfeiffera monacantha*
143	4458-4462	5	"ATAAA" SSR in *Calymmanthium substerile*
144	4467-4474	8	"CAAAAAAG" insertion of unknown origin in *Browningia hertlingiana*
145	4479-4483	5	Gap in *Hatiora herminiae*
146	4566-4567	2	"CC" SSR in *Schlumbergera truncata, S. orssichiana, S. russelliana* isolate CA036, *Schlumbergera kautskyi*, *Hatiora salicornioides* isolate CA048 and *Rhipsalidopsis xgraeseri*
147	4566-4783	218	Gap in *Hatiora epiphylloides*
148	4604-4607	4	"TAGA" SSR in *Lepismium lorentzianum*
149	4624-4731	108	Gap in *Rhipsalidopsis xgraeseri*, *Schlumbergera orssichiana, S. truncata, S. russelliana* isolate CA036 and *S. kautskyi*
150	4631-4631	1	Gap in *Browningia hertlingiana*
151	4631-4733	103	Gap in *Hatiora salicornioides*
152	4673-4733	61	Gap in *Hatiora salicornioides* isolate CA009 and CA043 and *H. cylindrica*
153	4679-4728	50	Gap in *Hatiora herminiae*
154	4684-4693	10	"TTGTTTTAAA" imperfect repeat in *Lepismium cruciforme* and *L. houlletianum*

Appendix 4, continued

No.	extension	length	Sequence motif
155	4705-4706	2	"AC" insertion in *Browningia hertlingiana*
156	4735-4740	6	"TTTCAA" SSR in *Browningia hertlingiana* and "TTTGAA" SSR in *Calymmanthium substerile*
157	4735-4758	24	Gap in *Echinopsis aurea*
158	4743-4758	16	Gap in *Browningia hertlingiana*, *Calymmanthium substerile* and *Pfeiffera monacantha*
159	4744-4747	4	"TATT" SSR in *Rhipsalis oblonga*
160	4753-4758	6	Gap in *Rhipsalis* subg. *Rhipsalis*, subg. *Calamorhipsalis*, *Hatiora salicornioides* isolate CA048, *H. herminiae*, *Rhipsalidopsis graeseri*, *Schlumbergera orssichiana, S. truncata, S. russelliana* isolate CA036, *S. kautskyi*
161	4760-4778	19	Gap in *Rhipsalidopsis rosea*, *Schlumbergera opuntioides* and *S. microsphaerica*
162	4765-4770	6	„CAAAAA" insertion of unknown origin in *Schlumbergera orssichiana, S. truncata, S. russelliana* isolate CA036, *Rhipsalidopsis xgraeseri*, *Hatiora salicornioides* isolate CA048
163	4765-4778	14	Gap resulting from alignment of 162 and 164
164	4771-4778	8	„AATAAAA" insertion in *R. pittieri* CA144*, Rhipsalis trigona, R. dissimilis f. dissimilis, R. floccosa subsp. pulviningera* CA056*, floccosa* CA139*, hohenauensus* CA154
165	4778-4778	1	an additional „A" in sequence motif of 164 in *R. pittieri* CA144
166	4791-4827	37	Gap in *Hatiora salicornioides* isolate CA009

Appendix 5. Success of single partitions and the combined dataset in OTU identification

	trnK intron	*matK*	*matK* partial	*psbA-trnH*	*rps3-rpl16*	*rpl16* intron	*trnQ-rps16*	all markers combined	
aligned length	**913**	**1539**	**950**	**436**	**152**	**1213**	**567**	**4820**	
average no. of nucleotides	**897**	**1530**	**950**	**287**	**141**	**1053**	**299**	**4207**	
Totalling identifiable OTUs	**26 (42.6 %)**	**36 (59 %)**	**28 (45.9 %)**	**33 (54.09 %)**	**19 (31.1 %)**	**38 (62.2 %)**	**30 (49.1 %)**	**59 (96.7 %)**	
OTU									**How often identified?**
Hatiora cylindrica	+	+	+	+	-	+	+	+	7
Hatiora epiphylloides subsp. *bradei*	-	-	-	+	+	+	-	+	4
Hatiora epiphylloides subsp. *epiphylloides*	-	-	-	+	+	+	-	+	4
Hatiora herminiae	+	+	+	+	+	+	+	+	8
Hatiora salicornioides	+	+	+	-	-	+	+	+	8
Lepismium cruciforme	+	+	+	+	+	+	+	+	6
Lepismium houlletianum	-	+	-	+	+	+	+	+	8
Lepismium houlletianum f. *regnellii*	-	+	-	+	+	+	+	+	6
Lepismium lorentzianum	+	+	+	+	+	+	+	+	8
Lepismium lumbricoides	+	+	+	+	+	+	+	+	8
Lepismium warmingianum	+	+	+	+	+	+	+	+	8
Rhipsalidopsis gaertneri	+	+	+	+	-	+	+	+	7
Rhipsalidopsis rosea	+	+	+	+	-	+	+	+	7
Rhipsalis agudoensis	-	-	-	+	-	-	-	+	2
Rhipsalis baccifera	+	+	-	+	-	+	-	+	5
Rhipsalis burchellii	+	+	+	+	-	-	+	+	6
Rhipsalis campos-portoana	-	+	+	+	-	-	+	+	5
Rhipsalis cereoides	+	+	+	-	-	-	-	+	4
Rhipsalis cereuscula	-	+	-	+	+	+	+	+	6
Rhipsalis clavata	+	+	+	+	+	+	+	+	8
Rhipsalis crispata	-	+	+	-	+	+	-	+	5
Rhipsalis cuneata	+	-	-	-	-	-	-	+	2
Rhipsalis dissimilis f. *dissimilis*	-	-	-	-	-	-	+	+	2
Rhipsalis dissimilis f. *epiphyllanthoides*	-	+	+	-	-	+	-	+	4
Rhipsalis elliptica	-	+	+	-	-	+	-	+	4
Rhipsalis ewaldiana	-	-	-	-	-	+	-	+	2
Rhipsalis floccosa subsp. *floccosa*	-	-	-	-	-	+	-	+	2
Rhipsalis floccosa subsp. *oreophila*	-	-	-	+	-	+	-	+	3

	trnK intron	*matK*	*matK* partial	*psbA-trnH*	*rps3-rpl16*	*rpl16* intron	*trnQ-rps16*	all markers combined	
Rhipsalis floccosa subsp. *pulviningera*	-	-	-	-	-	-	-	+	1
Rhipsalis floccosa subsp. *tucumanensis*	-	-	-	-	-	+	-	+	2
Rhipsalis goebeliana	-	-	-	-	-	+	-	+	2
Rhipsalis grandiflora	+	+	+	-	-	+	+	+	6
Rhipsalis hoelleri	-	-	-	-	+	+	-	+	3
Rhipsalis juengeri	+	+	+	+	+	+	+	+	8
Rhipsalis lindbergiana	-	-	-	-	-	-	+	+	2
Rhipsalis mesembryanthemoides	+	+	+	+	-	-	-	+	5
Rhipsalis micrantha	-	+	+	-	-	+	-	+	4
Rhipsalis neves-armondii	-	+	-	+	+	+	-	+	5
Rhipsalis oblonga	-	+	+	-	-	+	-	+	4
Rhipsalis occidentalis	-	-	-	-	-	-	-	+	1
Rhipsalis olivifera	+	+	-	+	-	+	+	+	6
Rhipsalis pacheco-leonis	-	-	-	-	+	-	-	+	2
Rhipsalis pacheco-leonis subsp. *catenulata*	-	-	-	-	-	-	-	+	1
Rhipsalis pachyptera	+	-	-	-	-	-	-	+	2
Rhipsalis paradoxa	-	-	-	-	-	-	+	+	2
Rhipsalis paradoxa subsp. *septentrionalis*	-	-	-	-	-	-	+	+	2
Rhipsalis pentaptera	-	-	-	+	-	+	+	+	4
Rhipsalis pilocarpa	+	+		+	-	-	+	+	5
Rhipsalis pulchra	+	+	-	+	-	-	+	+	5
Rhipsalis puniceodiscus	-	+	+	-	+	+	+	+	6
Rhipsalis russellii	-	-	-	-	-	-	-	+	1
Rhipsalis shaferi	+	+	-	+	-	+	-	+	5
Rhipsalis sulcata	-	-	-	-	-	-	-	-	0
Rhipsalis teres	-	-	-	-	-	-	-	-	0
Rhipsalis trigona	-	+	+	+	-	-	-	+	4
Schlumbergera kautskyi	+	+	+	+	+	+	+	+	8
Schlumbergera microsphaerica	+	+	+	+	-	+	+	+	7
Schlumbergera opuntioides	+	+	+	+	-	+	+	+	7
Schlumbergera orssichiana	-	-	-	+	-	-	-	+	2
Schlumbergera russelliana	+	+	+	-	-	+	-	+	5
Schlumbergera truncata	-	-	-	+	-	-	+	+	3

Appendix 6. Comparison of trees inferred from parsimony analyses of single markers and the complete dataset for the Rhipsalideae (“--“: node not found).

	***trnK* intron**	**partial *matK***	***matK***	***rpl16* intron**	***psb*A-*trnH***	***trnQ-rps16***	***rbcL***	**combined**	**combined + indels**
total characters (excl. hotspots)	928	949	1539	1235	446	590	1340	6244	6412
constant characters	821	857	1383	953	307	443	1283	5308	5310
variable, uninformative	34	32	56	81	64	72	12	336	395
parsimony informative	73	60	100	201	75	75	45	600	707
Number of shortest trees	61	25	437	528	1188	102	119	1028	1109
Tree length	138	127	234	489	231	218	69	1606	1848
CI	0.833	0.787	0.726	0.695	0.740	0.826	0.884	0.682	0.683
RI	0.940	0.935	0.915	0.922	0.912	0.904	0.981	0.895	0.894
RC	0.784	0.736	0.665	0.641	0.675	0.746	0.867	0.611	0.611
HI	0.167	0.213	0.274	0.305	0.260	0.174	0.116	0.318	0.317
Jackknife support for most important nodes									
Rhipsalideae	75	95	--	100	--	--	83	100	100
Rhipsalis	--	70	88	100	96	58	--	100	100
Lepismium	--	61	96	98	--	60	--	100	100
Schlumbergera	91	--	--	95	--	--	--	100	100
Hatiora s. str.	100	53	83	100	--	--	--	100	100
Rhipsalidopsis	95	--	86	100	--	--	--	100	100

Appendix 7. Trees from maximum parsimony analyses of the single markers

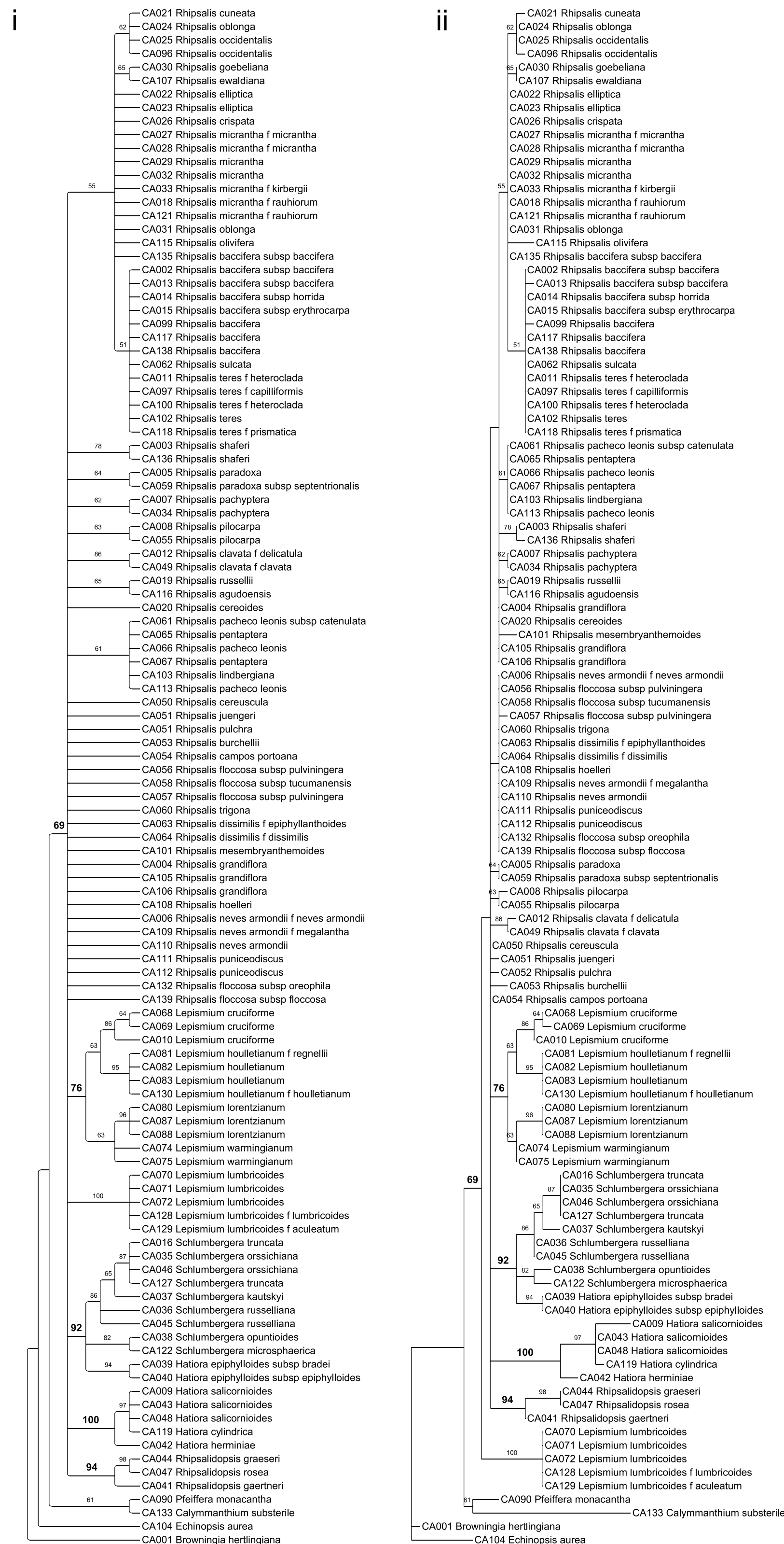

Figure A.1 Strict consensus tree (i) and shortest tree (ii) from the Maximum Parsimony analysis based on the *trnK* intron. Numbers above branches are Jacknife support values from 10.000 replicates.

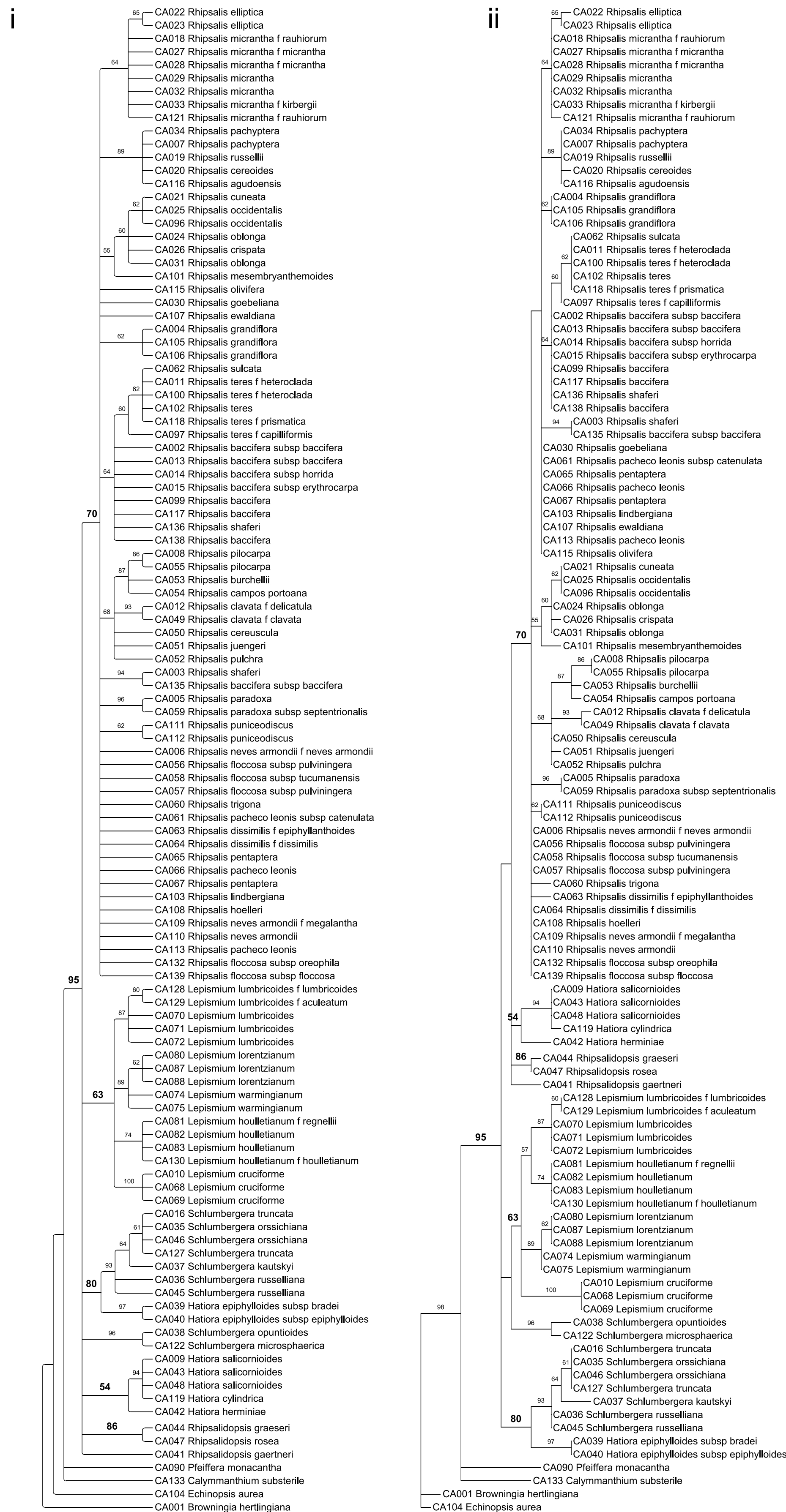

Figure A.1, continued Strict consensus tree (i) and shortest tree (ii) from the Maximum Parsimony analysis based on partial *matK*. Numbers above branches are Jacknife support values from 10.000 replicates.

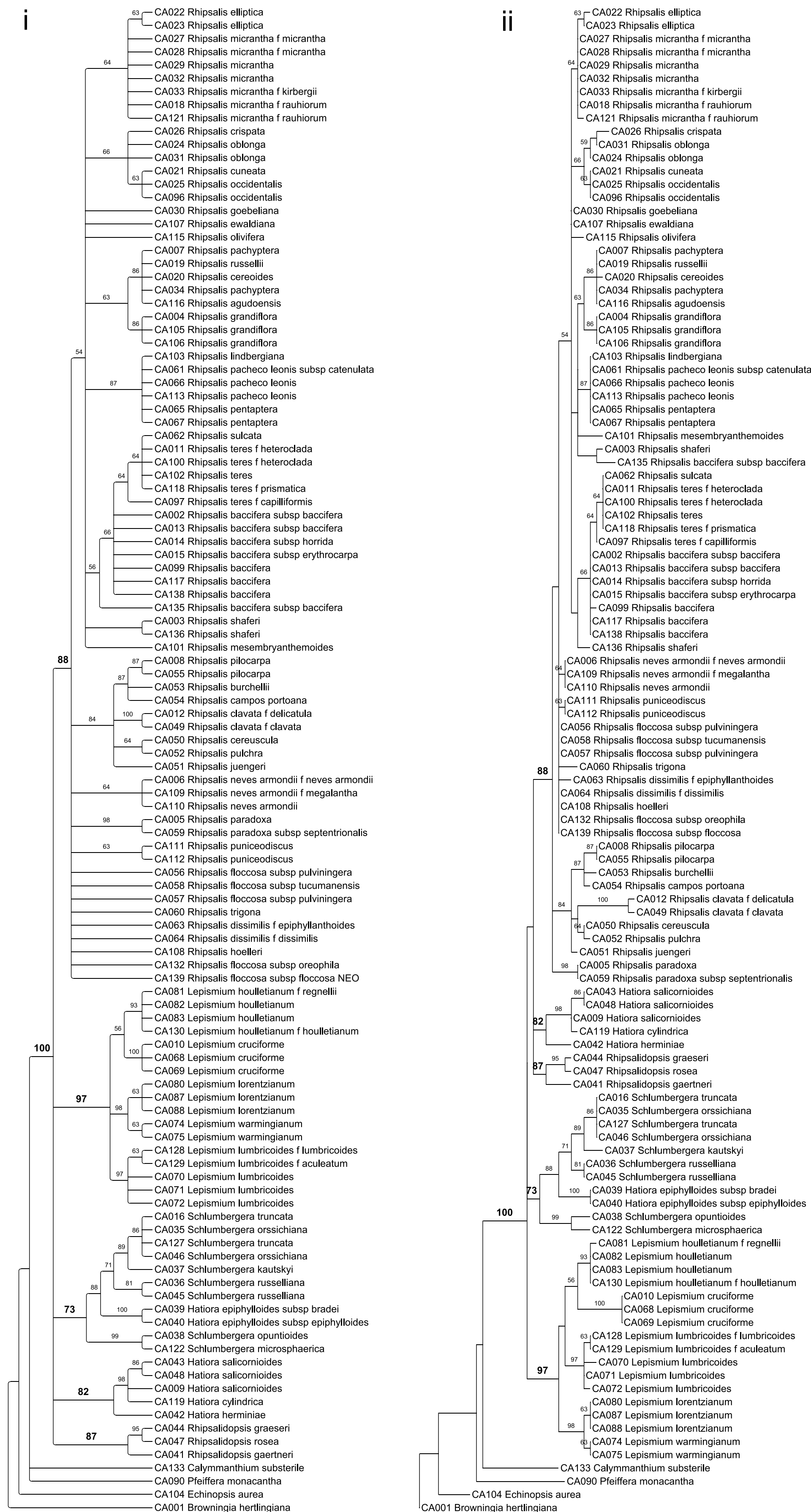

Figure A.1, continued Strict consensus tree (i) and shortest tree (ii) from the Maximum Parsimony analysis based on complete *matK*. Numbers above branches are Jacknife support values from 10.000 replicates.

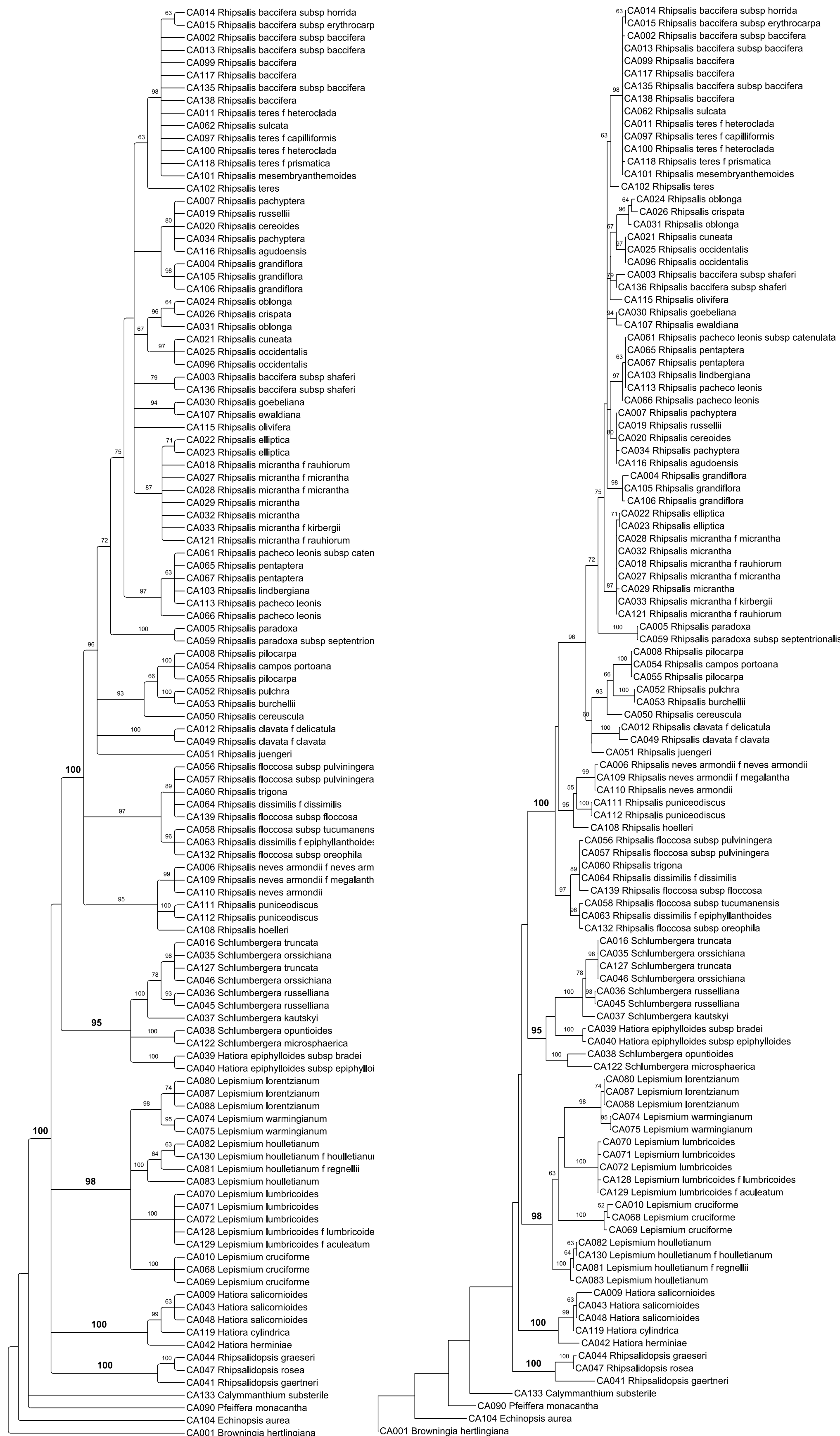

Figure A.1, continued Strict consensus tree (i) and shortest tree (ii) from the Maximum Parsimony analysis based on the *rpl16* intron. Numbers above branches are Jacknife support values from 10.000 replicates.

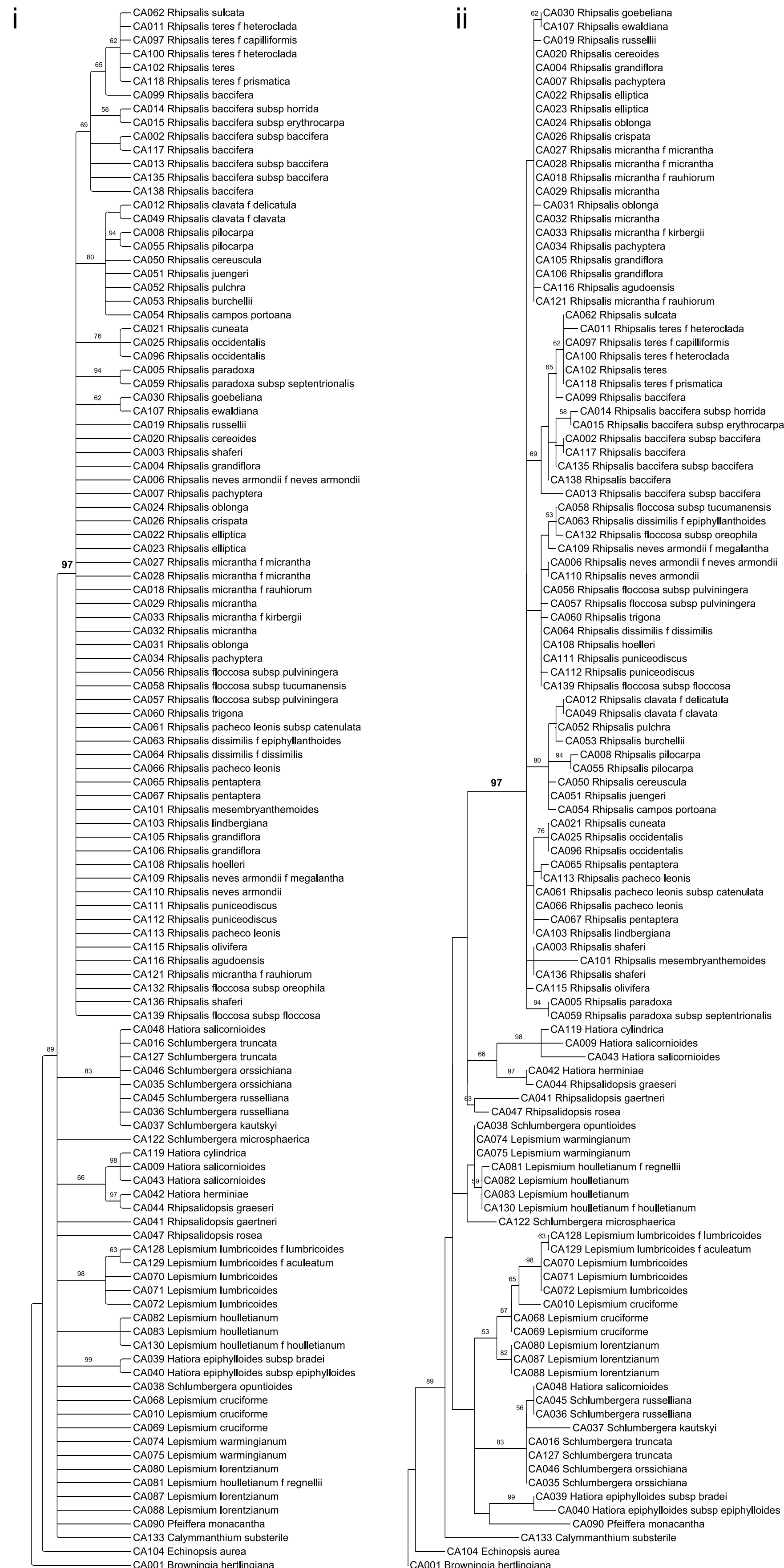

Figure A.1, continued Strict consensus tree (i) and shortest tree (ii) from the Maximum Parsimony analysis based on the *psbA-trnH* spacer . Numbers above branches are Jacknife support values from 10.000 replicates.

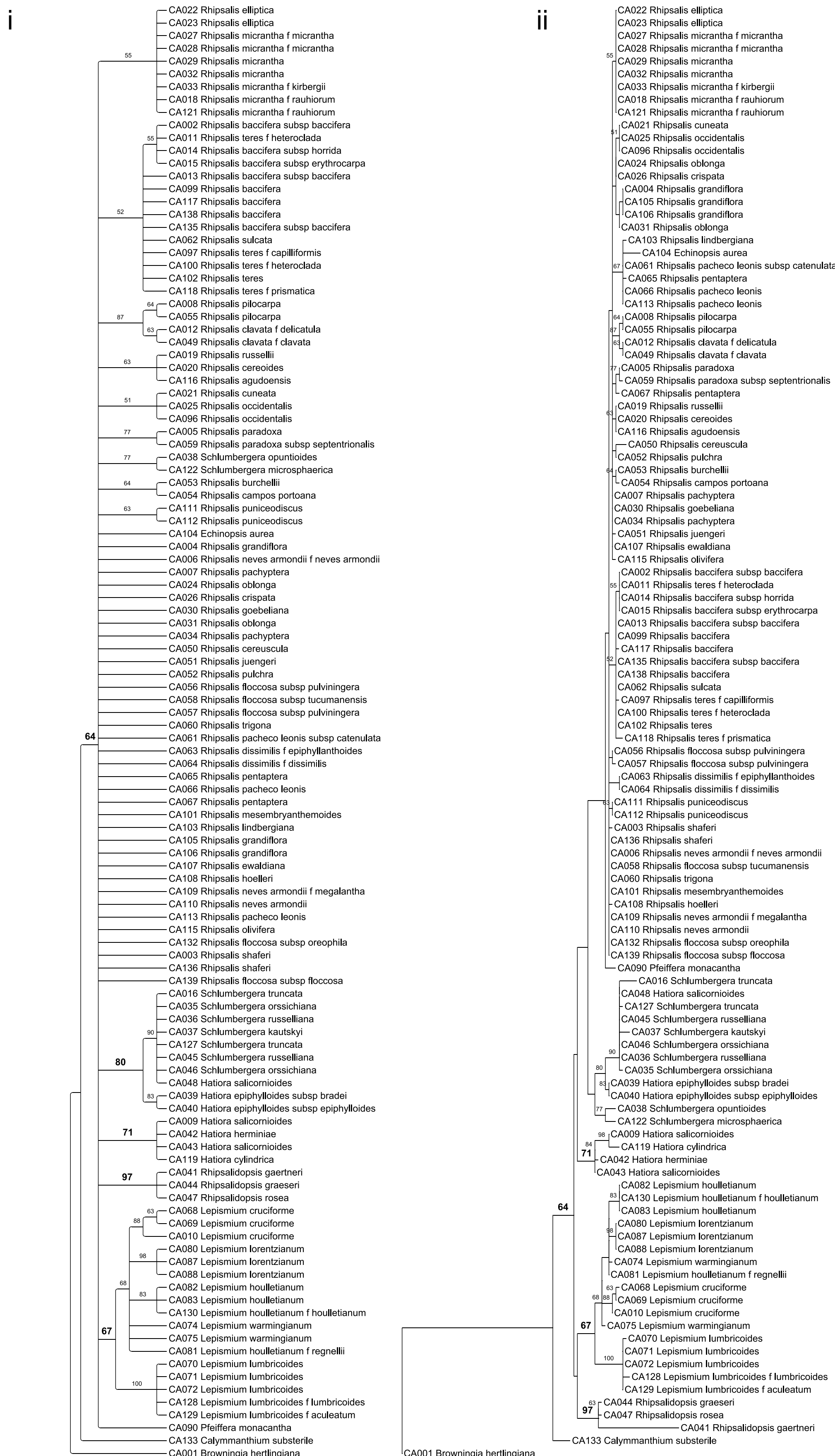

Figure A.1, continued Strict consensus tree (i) and shortest tree (ii) from the Maximum Parsimony analysis based on the *trnQ-rps16* spacer . Numbers above branches are Jacknife support values from 10.000 replicates.

Appendix 4.1 Matrix of morphological characters.

Characters also used for the Bayesian ancestral states reconstruction are highlighted in bold and those modification in character coding are given next to the original coding with the character name in italics.

Character	1	**2**				**3**		**4**	5	**6**	7	**8**		**9**	10	**11**	**12**	13	14	**15**	**16**	17	**18**	**19**	**20**
	Growth form	**Life-form**	***Epiphytic - BayesTraits***	***Epilithic - BayesTraits***	***Terrestrial- BayesTraits***	**Habit**	***Habit - BayesTraits***	**Branching**	Adventitious roots	**Stem segments growth habit**	Old segments	**Stem form**	***Stem form - BayesTraits***	**Podaria**	Stem diameter	**Position / development of the areoles**	**Apical composite areoles**	**Spines**	Trichomes	**Position of the flowers**	**Orientation of the flowers**	Position of flower buds	**Repeated flowering**	**Flowers per areole**	**Floral symmetry**
Lepismium																									
L. cruciforme	3	1, 2	1	1	0	2	2	0	1	0	0	0, 1	0	0	1	3	0	2	1	1	1	0	?	0	0
L. lumbricoides																									
f. *lumbricoides*	3	1	1	0	0	2, 3	2	0	1	0	0	2	2	0	1	0	0	0	0	1	1	0	?	0	0
f. *aculeatum*	3	1	1	0	0	2, 3	2	0	1	0	0	2	2	0	1	0	0	1	?	1	1	0	?	0	0
L. warmingianum	3	1, 2	1	1	0	2	2	0	1	0	0	0	0	0	1	3	0	0	0	1	1	0	1	0	0
L. houlletianum	3	1	1	0	0	2	2	0	?	0	0	1	1	0	1	3	0	0	0	1	1	0	1	0	0
L. lorentzianum	3	1	1	0	1	2	2	0	1	0	0	1	1	0	1	0	0	0	?	1	1	0	?	0	0
Hatiora																									
H. salicornioides	3	0, 1, 2	1	1	0	0	0	1	0	1	1	2	2	0	0	0	1	0	0	2	0	2	1	0	0
H. herminiae	3	1	1	0	0	1	1	1	0	1	1	2	2	0	0	0	1	0	0	2	0	2	1	0	0
H. cylindrica	3	0, 1	1	1	1	0	0	1	0	1	1	2	2	0	0	0	1	0	0	2	0	2	1	0	0
H. epiphylloides	3	1, 2	1	1	0	2	2	1	?	1	1	1	1	0	0	0	1	0	0	2	1	2	1	0	0
Rhipsalidopsis																									
R. gaertneri	3	1	1	0	0	2	2	1	1	1	1	1	1	0	1	0	1	2	0	2	1	2	1	0	0
R. rosea	3	1	1	0	0	2	2	1	1	1	1	1	1	0	1	0	1	2	0	2	1	2	1	0	0
Schlumbergera																									
S. truncata	3	1, 2	1	1	0	2	2	1	1	1	1	1	1	0	0	0	1	2	0	2	0	2	1	0	1
S. orssichiana	3	1	1	0	0	2	2	1	1	1	1	1	1	0	0	0	1	2	0	2	0	2	1	0	1
S. kautskyi	3	1, 2	1	1	0	2	2	1	1	1	1	1	1	0	0	0	1	2	0	2	0	2	1	0	1
S. russelliana	3	1	1	1	0	2	2	1	1	1	1	1	1	0	0	0	1	2	0	2	0	2	1	0	0
S. microsphaerica	3	2	0	1	0	1	1	1	?	1	1	2, 3	2	1	1	0	1	1	0	2	0	2	1	0	1
S. opuntioides	3	12	0	1	0	1	1	1	?	1	1	4	4	0	1	0	?	1	0	2	0	2	1	0	1

Appendix 8, continued

Character	1	**2**				**3**		**4**	5	**6**	7	**8**		**9**	10	**11**	**12**	13	14	**15**	**16**	17	**18**	**19**	**20**
Rhipsalis																									
R. russellii	3	1, 2	1	1	0	1, 2	1	1	1	1	1	0, 1	1	0	2	0	1	0	0	0	0	1	1	1	0
R. agudoensis	3	?	-	0	0	1	1	1	0	1	1	0	0	0	2	0	1	0	0	0	0	1	1	1	0
R. cereoides	3	1, 2	1	1	0	4	3	1	0	1	1	0	0	0	2	0	1	0	0	0	0	1	1	1	0
R. pachyptera	3	1, 2	1	1	0	1, 2	1	1	1	1	1	0, 1	1	0	2	0	1	0	0	0	0	1	1	1	0
R. grandiflora	3	1, 2	1	1	0	2	2	1	0	1	1	2	2	0	2	0	1	0	0	0	0	1	1	1	0
R. pittieri	3	1	1	0	0	2	2	1	0	1	1	2	2	0	1	2	1	0	1	0	0	0, 1	1	0	0
R. ewaldiana	3	?	-	-	0	1	1	0	?	3	1	0	0	0	1	0	1	0	0	1	0	1	1	0	0
R. goebeliana	3	1	1	0	0	1, 2	1	1	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0
R. micrantha																									
f. *micrantha*	3	1	1	0	0	2	2	1	0	1	1	0	0	0	1	0	1	0	0	1	0	1	1	0	0
f. *kirbergii*	3	1	1	0	0	2	2	1	0	1	1	0	0	0	1	0	1	0	0	1	0	1	1	0	0
f. *rauhiorum*	3	1	1	0	0	2	2	1	0	1	1	1	1	0	1	0	1	0	0	1	0	1	1	1	0
R. elliptica	3	1, 2	1	1	0	2	2	1	1	1	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0
R. cuneata	3	1	1	0	0	2	2	1	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0
R. occidentalis	3	1	1	0	0	2	2	1	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0
R. crispata	3	0, 1	1	0	0	2	2	1	1	1	1	1	1	0	0	0	1	0	0	0	0	1	1	1	0
R. oblonga	3	1, 2	1	1	0	2	2	1	1	1	1	1	1	0	0	0	1	0	0	0	0, 1	1	1	1	0
R. olivifera	3	1	1	0	0	2	2	1	0	1	1	1	1	0	0	0	1	0	0	?	?	?	?	?	?
R. sulcata	3	1	1	0	0	2	2	1	1	2	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0
R. teres	3	1, 2	1	1	0	2	2	1	1	2	1	2	2	0	1	0	1	0	0	0	0	1	0	0	0
R. baccifera																									
subsp. *baccifera*	3	1	1	0	0	2	2	1	1	2	1	2	2	0	0	0	1	0	0	0	0	1	0	0	0
subsp. *horrida*	3	0, 1, 2	1	1	1	0, 4	1	0, 1	1	2	1	2	2	0	1	0	1	1	?	0	0	1	0	0	0
R. shaferi	3	1	1	0	0	2	2	1	1	0	1	2	2	0	0	0	1	0	0	0	0	1	0	0	0
R. mesembr.	3	1	1	0	0	2, 4	1	0	1	3	1	2	2	0	0	0	1	2	0	1	0	1	0	0	0
R. lindbergiana	3	1	1	0	0	2	2	1	?	0	1	2	2	0	1	0	0	0	0	0	0	1	0	0	0
R. pentaptera	3	1	1	0	0	1	1	1	?	1	1	3	3	1	1	2	1	0	?	0	0	1	1	0	0
R. pacheco-leonis																									
ssp. *pacheco-leonis*	3	1, 2	1	1	0	2	2	1	?	0	1	3	3	1	1	2	1	0	0	1	0	0	1	0	0
ssp. *catenulata*	3	1	1	0	0	2	2	1	?	0	1	3	3	1	1	2	1	0	0	1	0	0	1	0	0
R. paradoxa																									
ssp. *paradoxa*	3	1	1	0	0	2	2	1	0	1	1	3	3	1	2	2	1	0	1	1	1	0	1	0	0
ssp. *septentrionalis*	3	1	1	0	0	2	2	1	0	1	1	3	3	1	1	2	1	2	1	1	1	0	1	0	0
R. pilocarpa	3	1, 2	1	1	0	2	2	1	1	2	1	2	2	1	1	0	1	2	0	2	1	2	0	0	0
R. campos-portoana	3	1	1	0	0	2	2	1	?	2	1	2	2	0	0	0	1	0	0	2	1	2	0	0	0
R. pulchra	3	1	1	0	0	2	2	1	1	0	1	2	2	0	1	0	0	0	0	1	1	0	0	0	0
R. burchellii	3	1	1	0	0	2	2	1	?	2	1	2	2	0	0	0	1	0	0	2	1	2	0	0	0
R. cereuscula	3	1	1	0	0	2	2	1	1	2	1	2	2	0	0	0	1	2	0	2	1	2	0	0	0
R. clavata	3	1	1	0	0	2	2	1	0	2	1	2	2	0	0	0	1	0	0	2	1	2	0	0	0

Appendix 8, continued

Character	1	**2**				**3**		**4**	5	**6**	7	**8**		**9**	10	**11**	**12**	13	14	**15**	**16**	17	**18**	**19**	**20**
R. juengeri	3	1	1	0	0	2	2	1	0	2	1	2	2	0	0	0	1	0	0	2	1	2	0	0	0
R. floccosa																									
ssp. *floccosa*	3	1	1	0	0	2	2	1	0	1	1	2	2	1	1	2	1	0	1	0	0	0, 1	1	0	0
ssp. *oreophila*	3	1, 2	1	1	0	2	2	1	0	1	1	2	2	0	1	2	1	0	1	0	0	0, 1	1	0	0
ssp. *pulviningera*	3	1, 2	1	1	0	2	2	1	0	1	1	2	2	1	1	2	1	0	1	0	0	0, 1	1	0	0
ssp. *hohenauensis*	3	1	1	0	0	2	2	1	0	1	1	3	3	1	2	2	1	0	1	0	0	0, 1	1	0	0
ssp. *tucumanensis*	3	1	1	0	0	2	2	1	0	1	1	2	2	1	1	2	1	0	1	0	0	0, 1	1	0	0
R. dissimilis																									
f. *dissimilis*	3	2	0	0	0	4	3	1	1	1	1	2	2	1	2	2	1	0	1	0	0	0, 1	1	0	0
f. *epiphyllanthoides*	3	2	0	0	0	0	0	1	1	1	1	2	2	1	2	2	1	0	1	0	0	0, 1	1	0	0
R. trigona	3	1	1	0	0	2	2	1	0	1	1	0	0	0	2	2	1	0	1	0	0	0, 1	1	0	0
R. neves armondii	3	1, 2	1	1	0	2, 4	1	1	0	1	1	2	2	0	2	1	1	0	0	1	0	0	0	0	0
R. puniceodiscus	3	1	1	0	0	2, 3	2	1	1	0	1	2	2	0	1	-	0	0	0	0	0	0	0	0	0
R. hoelleri	3	1	1	0	0	2	2	1	1	0	1	2	2	0	1	-	0	0	0	1	1	0	0	0	0
Outgroup																									
Calymmanthium substerile	0	0	0	0	1	0	0	1	?	?	?	5	5	0	3	0	?	1	1	0	0	1	?	0	0
Browningia hertlingiana	1	0	0	0	1	0	0	0	?	0	?	5	5	0	3	0	?	1	1	0	0	?	?	0	0
Echinopsis aurea	4	0	0	0	1	0	0	-	0	-	-	5	5	0	3	0	?	1	1	0	2	?	?	0	0

Appendix 8, continued

	21	**22**	23	24		25	26	27	28	29	30	31	32	33	34	35	36
	Perianth segments fusion	**Perianth segments reflexion**	Flower size	Flower colour	***Flower colour - BayesTraits***	Nectaries	Filament colour	Stamen insertion	Style colour	Stigma shape	Pericarpel	Fruit shape	Fruit colour	Pollen colour	Pollen size	Aperture number	Chromosome number
Lepismium																	
L. cruciforme	0	0	1	0, 3	0	?	0	0	0	0	?	1	2	0	0	3, 6	2
L. lumbricoides																	
f. *lumbricoides*	0	0	1	0	0	0	0	0	0	0	2	0	5	0	0	1, 6, 9	?
f. *aculeatum*	0	0	1	0	0	0	0	0	0	0	2	0	5	0	0	6	?
L. warmingianum	0	0	1	0	0	0	0	0	0	0	1	0	5	0	0	3, 6	?
L. houlletianum	0	0	1	0	0	0	0	0	1	0	2	1	5	0	1	1, 3, 6	2
L. lorentzianum	0	0	1	0	0	0	0	0	?	0	1	0	5	0	0	3, 6	?
Hatiora																	
H. salicornioides	0	0	0	2	1	0	?	0	0	0	0	2	6	?	0	3, 6	?
H. herminiae	0	1	0	3	1	0	0	0	1	0	0	2	4	1	0	1	?
H. cylindrica	0	0	0	2	1	?	1	0	0	0	?	2	1	1	-	-	?
H. epiphylloides	0	0	1	2	1	1	1	0	1	0	1	2	4	0	1	6	2
Rhipsalidopsis																	
R. gaertneri	0	0	2	4	1	?	0	0	0	0	1	2	1	1	1	16	2
R. rosea	0	0	2	3	1	0	1	0	1	0	1	2	3	1	1	1, 6, 9	2
Schlumbergera																	
S. truncata	1	1	2	3	1	2	0	1	1	1	3	2	1	1	1	6	2
S. orssichiana	1	1	2	3	1	?	0	1	1	1	1	2	4	1	1	6	?
S. kautskyi	1	1	2	3	1	2	0	1	1	1	1	2	4	1	1	6	?
S. russelliana	1	1	2	3	1	2	1	1	1	1	1	2	4	1	1	6	2
S. microsphaerica	1	1	2	3	1	2	?	1	?	1	3	2	4	1	1	6	?
S. opuntioides	1	1	2	3	1	2	1	1	0	1	3	2	4	1	1	6	?

Appendix 4.1, continued

	21	**22**	23	24		25	26	27	28	29	30	31	32	33	34	35	36
Rhipsalis																	
R. russellii	0	0	0	0	0	?	0	0	0	0	0	0	2	0	0	16	?
R. agudoensis	0	1	1	0	0	?	0	0	0	0	0	1	0, 2	0	-	-	?
R. cereoides	0	1	1	0	0	1	0	0	0	0	0	1	6	0	0	1, 6	2
R. pachyptera	0	1	1	0	0	?	0	0	0	0	0	0	2	0	0	1, 6	?
R. grandiflora	0	1	1	0	0	1	0	0	0	0	0	0	2	0	1	1, 6	2
R. pittieri	0	1	1	0	0	1	0	0	0	0	0	0	0	0	-	-	?
R. ewaldiana	0	1	1	0	0	?	0	0	0	0	0	0	2	0	1	1, 6	?
R. goebeliana	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1, 6	2
R. micrantha																	
f. *micrantha*	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1, 6, 9	2
f. *kirbergii*	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	6, 9	2
f. *rauhiorum*	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	6	2
R. elliptica	0	1	1	2	1	1	0	0	0	0	0	1	2	0	0	1, 6	?
R. cuneata	0	1	0	0	0	1	0	0	0	0	0	0	0	0	2	1, 6	?
R. occidentalis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6	?
R. crispata	0	1	1	0	0	?	0	0	0	0	0	0	0	0	0	6	2
R. oblonga	0	1	0	?	0	0	0	0	0	0	0	0	?	0	0	1, 6	0
R. olivifera	-	-	?	?	-	?	0	0	0	0	?	2	4	0	-	-	?
R. sulcata	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1, 6	?
R. teres	0	1	0	1	0	0	0	0	0	0	0	1	0	0	2, 0	1, 6	2
R. baccifera																	
subsp. *baccifera*	0	1	0	0	0	?	0	0	0	0	0	1	0	0	0	1, 6	2, 4
subsp. *horrida*	0	1	0	0	0	?	0	0	0	0	0	1	0	0	0	1, 9	2, 4, 8
R. shaferi	0	1	0	0	0	?	0	0	0	0	0	1	0	0	0	1, 6	2
R. mesembr.	0	1	1	0	0	?	0	0	0	0	0	1	0	0	0	1, 6	2
R. lindbergiana	0	1	0	0	0	?	0	0	0	0	0	0	0, 2	0	2	16	?
R. pentaptera	0	0	1	0	0	1	0	0	0	0	0	1	6	0	2	6	?
R. pacheco-leonis					0												
ssp. *pacheco-leonis*	0	1	1	0		?	0	0	0	0	0	0	2	0	0	6	?
ssp. *catenulata*	0	1	1	5	0	?	0	0	0	0	0	0	0, 1	0	2	1, 6	?
R. paradoxa																	
ssp. *paradoxa*	0	1	1	0	0	1	0	0	0	0	0	1	1	0	0	6	2
ssp. *septentrionalis*	0	1	1	2	1	?	1	0	0	0	0	1	0	0	1	6	2
R. pilocarpa	0	1	1	0	0	2	1	0	0	0	0	0	2	0	0	6	2
R. campos-portoana	0	0	1	6	0	?	1	0	0	0	0	0	7	0	0	3, 6	?
R. pulchra	0	0	1	6	0	?	1	0	0	0	0	1	0, 2	0	0	3	?
R. burchellii	0	0	2	0	0	0	0	0	0	0	0	1	2	0	0	3, 6	?
R. cereuscula	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	3, 6	2
R. clavata	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0, 2	3, 6	2
R. juengeri	0	0	1	0	0	?	1	0	0	0	0	0	1	0	0	3, 6, 9	?

Appendix 4.1, continued

R. floccosa	0																
ssp. *floccosa*	0	0	1	0	0	?	0	0	0	0	0	0	0	0	0	3, 6	?
ssp. *oreophila*	0	0	1	?	0	?	0	0	0	0	0	0	0	0	-	-	?
ssp. *pulviningera*	0	0	1	1	0	1	0	0	0	0	0	0	2	0	1	3, 6	?
ssp. *hohenauensis*	0	0	1	1	0	1	0	0	0	0	0	0	0	0	?	?	?
ssp. *tucumanensis*	0	1	1	0	0	?	0	0	0	0	0	0	0, 1	0	0	3, 6	?
R. dissimilis	0																
f. *dissimilis*	0	0	2	1	1	1	0	0	0	0	0	0	2	0	1	1, 6	?
f. *epiphyllanthoides*	0	0	2	1	1	?	0	0	0	0	0	0	2	0	0	1, 6	2
R. trigona	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1, 6	?
R. neves-armondii	0	0	2	0	0	0	1	0	0	0	0	0	1	0	0	3, 6	?
R. puniceodiscus	0	0	1	0	0	0	1	0	0	0	0	0	1, 3	0	0	3, 6	?
R. hoelleri	0	0	1	4	1	?	0	0	1	0	0	0	1	0	0	3, 6	?
Outgroup																	
Calymmanthium substerile	0	0	3	4	1	2	0	0	0	0	1	2	4	0	?	?	?
Browningia hertlingiana	0	0	3	0	0	?	0	0	0	0	?	?	?	0	?	?	?
Echinopsis aurea	0	0	3	2	1	?	0	0	1	0	?	?	?	1	?	?	?

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig angefertigt und die benutzten Quellen und Hilfsmittel vollständig angegeben habe. Diese Arbeit wurde an keiner anderen Hochschule als Dissertation eingereicht und wurde, abgesehen von den im Folgenden angegebenen Teilpublikationen, noch nicht veröffentlicht.

Kapitel 2 wurde wie folgt veröffentlicht:

Korotkova, N., Zabel, L., Quandt, D. & Barthlott, W. 2010: A phylogenetic analysis of Pfeiffera and the reinstatement of Lymanbensonia as an independently evolved lineage of epiphytic Cactaceae within a new tribe Lymanbensonieae. - Willdenowia 40: 151-172.

Kapitel 3 wurde wie folgt veröffentlicht:

Korotkova, N., Borsch, T., Quandt, D., Taylor, N. P., Müller, K. & Barthlott, W. (im Druck): What does it take to resolve relationships and to identify species with molecular markers? An example from the epiphytic Rhipsalideae (Cactaceae)). – American Journal of Botany 98: 1549-1572.