Molecular Phylogenetics and Evolution 58 (2011) 456–468

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny of tribe Rhipsalideae (Cactaceae) and taxonomic implications for *Schlumbergera* and *Hatiora*

Alice Calvente [a,*], Daniela C. Zappi [b], Félix Forest [c], Lúcia G. Lohmann [a]

[a] Laboratório de Sistemática Vegetal, Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-090 São Paulo, SP, Brazil
[b] Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK
[c] Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK

ARTICLE INFO

Article history:
Received 15 July 2010
Revised 20 December 2010
Accepted 6 January 2011
Available online 12 January 2011

Keywords:
Atlantic forest
Cactoideae
Epiphytic cacti
Lepismium
Rhipsalis
Character evolution

ABSTRACT

Tribe Rhipsalideae is composed of unusual epiphytic or lithophytic cacti that inhabit humid tropical and subtropical forests. Members of this tribe present a reduced vegetative body, a specialized adventitious root system, usually spineless areoles and flowers and fruits reduced in size. Despite the debate surrounding the classification of Rhipsalideae, no studies have ever attempted to reconstruct phylogenetic relationships among its members or to test the monophyly of its genera using DNA sequence data; all classifications formerly proposed for this tribe have only employed morphological data. In this study, we reconstruct the phylogeny of Rhipsalideae using plastid (*trn*Q-*rps*16, *rpl*32-*trn*L, *psb*A-*trn*H) and nuclear (ITS) markers to evaluate the classifications previously proposed for the group. We also examine morphological features traditionally used to delimit genera within Rhipsalideae in light of the resulting phylogenetic trees. In total new sequences for 35 species of Rhipsalideae were produced (out of 55; 63%). The molecular phylogeny obtained comprises four main clades supporting the recognition of genera *Lepismium*, *Rhipsalis*, *Hatiora* and *Schlumbergera*. The evidence gathered indicate that a broader genus *Schlumbergera*, including *Hatiora* subg. *Rhipsalidopsis*, should be recognized. Consistent morphological characters rather than homoplastic features are used in order to establish a more coherent and practical classification for the group. Nomenclatural changes and a key for the identification of the genera currently included in Rhipsalideae are provided.

1. Introduction

Cactaceae comprises 127 genera and 1438 species divided in four subfamilies: Cactoideae, Pereskiodeae, Maihuenioideae and Opuntioideae (Hunt et al., 2006). Cacti are distributed exclusively in the New World except for *Rhipsalis baccifera*, which also occurs in Africa and Asia. The taxonomy of cacti has been traditionally based on comparative observation of morphological and biogeographic data (Metzing and Kiesling, 2008). More recently, molecular phylogenetic evidence has also been incorporated into systematic studies of this group leading to more comprehensive classifications and an improved understanding of cactus evolution. Initial molecular phylogenetic studies in Cactaceae allowed the assessment of the monophyletism of subfamily Cactoideae and higher-level phylogenetic relationships within this subfamily using the plastid DNA markers *rpo*C1, *trn*T-*trn*L and *rpl*16 (Applequist and Wallace, 2002; Butterworth et al., 2002; Wallace and Cota, 1996). Subsequently, phylogenetic relationships within Cactaceae as a whole were reconstructed using *trn*K/*mat*K and *trn*L-F sequences (Nyffeler, 2002). This study supported the monophyletism of the Cactaceae and of subfamilies Opuntioideae and Cactoideae however, it indicated that subfamily Pereskiodeae is paraphyletic. Relationships within Pereskioideae were investigated further with more extensive sampling in two studies; one based on two plastid markers (*psb*A-*trn*H, *rpl*16; Butterworth and Wallace, 2005), and the other using a combined analysis of plastid (*psb*A-*trn*H, *trn*K-*mat*K, *rbc*L), mitochondrial (*cox*3) and nuclear markers (*phy*C; Edwards et al., 2005). The molecular phylogeny of Opuntioideae was also investigated further through a more extensive sampling and two molecular markers (*trn*L-F and ITS; Griffith and Porter, 2009).

Cactoideae is the most diverse subfamily of cacti and constitutes a monophyletic group well characterized by a complete reduction of leaves (Nyffeler, 2002). The current knowledge of higher-level phylogenetic relationships in Cactoideae is mostly inferred from the phylogeny produced for Cactaceae as a whole (Nyffeler, 2002). Despite a few molecular phylogenetic studies that have been conducted for some genera of Cactoideae (*Lophocereus*, Hartmann et al., 2002; *Pachycereus*, Arias et al., 2003; *Mammillaria*,

* Corresponding author. Present address: Departamento de Botânica, Ecologia e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Campus Lagoa Nova, 59078-900, Natal, RN, Brazil. Fax: +55 21 2551 6133.
E-mail address: alicecalvente@yahoo.com.br (A. Calvente).

1055-7903 © 2011 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.ympev.2011.01.001

Butterworth and Wallace, 2004; *Peniocereus*, Arias et al., 2005; *Rebutia*, Ritz et al., 2007), tribal and generic relationships within this subfamily remain poorly understood and further studies are needed to clarify relationships in the group.

Rhipsalideae is one of nine tribes of Cactoideae (Anderson, 2001) and is composed of unusual cacti. Most cacti are terrestrial and inhabit arid places presenting many xerophytic features, in particular the modification of leaves into spines, succulence associated with water storage and stems with well-developed photosynthetic tissue. Other common features of cacti are the showy and bright colored flowers with many perianth segments and the large, juicy and colorful fruits (Gibson and Nobel, 1986). The species of Rhipsalideae, however, are epiphytic or lithophytic and inhabit tropical and subtropical forests. Members of this tribe present a reduced vegetative body, a specialized adventitious root system, areoles usually lack spines and flowers and fruits are reduced in size (Fig. 1). The center of diversity of Rhipsalideae is in the Brazilian Atlantic Forest, where species occur from coastal habitats to almost 3000 m in altitude (Barthlott, 1983; Barthlott and Taylor, 1995; Hunt et al., 2006). The group represents an important component of the Atlantic Forest biome, a highly deforested system with several threatened cacti taxa (Calvente et al., 2005; Taylor, 1997); most species of Rhipsalideae are rare and often endemic or with restricted distribution.

Within Cactoideae, tribal and generic classifications have changed extensively throughout the years, which also impacted on Rhipsalideae (Anderson, 2001; Hunt et al., 2006). Because tribes Rhipsalideae and Hylocereeae include all obligatory epiphytic cacti, members of both tribes were considered to be closely related in several classifications provided for the family. This caused uncertainty in the generic classification as several genera of the Rhipsalideae have been combined with genera of Hylocereeae (i.e., *Pseudorhipsalis*, *Disocatus* and *Epiphyllum*) in earlier classifications. Furthermore, generic and infrageneric classifications of Rhipsalideae have been controversial especially regarding the position of *Erythrorhipsalis* (as a genus or as subgenus of *Rhipsalis*), *Rhipsalidopsis* (as a genus or as subgenus *Hatiora*), *Pfeiffera* and *Acanthorhipsalis* (as genera or as subgenera of *Lepismium*). For example, the classifications proposed by Britton and Rose (1923), Buxbaum (1970), Barthlott (1987), Barthlott and Taylor (1995), Doweld (2001) and Hunt et al. (2006) diverged significantly in generic and infrageneric classifications of Rhipsalideae and Hylocereeae taxa (Table 1).

The molecular phylogeny produced for Cactaceae as a whole contained a few representatives of Rhipsalideae and Hylocereeae, what allowed the indication that both tribes represent distinct lineages (Nyffeler, 2002). This phylogenetic framework also corroborated the generic composition of Rhipsalideae as proposed by Barthlott and Taylor (1995) except for the inclusion of *Pfeiffera* (containing *Acanthorhipsalis*) that emerged as a closer relative of Hylocereeae (Nyffeler, 2000, 2002). As a result, in the most recent classification of Rhipsalideae (Hunt et al., 2006; Table 2) species of former *Lepismium* subg. *Pfeiffera*, *Lepismium* subg. *Acanthorhipsalis*, *Lepismium* subg. *Lymanbensonia* and part of *Lepismium* subg. *Houlletia* are segregated from Rhipsalideae and included in the genus *Pfeiffera*.

Despite the controversial classification of Rhipsalideae, no studies have ever attempted to reconstruct the phylogenetic relationships among members of this group or to test the monophyly of its genera using a cladistic approach and a comprehensive sampling. To date, the only members of Rhipsalideae ever considered in a cladistic context were *Rhipsalis floccosa*, *Hatiora salicornioides*, *Lepismium cruciforme* and *Schlumbergera truncata*, which were sampled as representatives of Rhipsalideae in the overall molecular phylogeny of Cactaceae (Nyffeler, 2002). In addition, all classifications proposed for the tribe have only employed morphological data. In this study, we reconstruct phylogenetic relationships within tribe Rhipsalideae using plastid and nuclear markers and use this phylogenetic framework to evaluate the generic classifications previously proposed for the group. We also examine morphological features traditionally used in previous classifications in the light of the resulting phylogenetic trees.

2. Material and methods

2.1. Taxon sampling

Taxa were selected to represent main lineages within Rhipsalideae according to the most recent classification of the family (Hunt et al., 2006) and also according to material availability. All four genera (*Rhipsalis*, *Hatiora*, *Lepismium* and *Schlumbergera*), all subgeneric divisions and 35 of the 55 currently recognized species of the tribe were sampled (63% of all species currently assigned to the group; Table 2) to serve the main purpose of this work, which was to evaluate the relationships at generic level. Outgroup selection was based on the family-wide phylogenetic tree of Cactaceae proposed by Nyffeler (2002). Five outgroup taxa representing major lineages associated with Rhipsalideae and two species belonging to tribe Hylocereeae were sampled (Table 2). Throughout the text we refer to genera and subgenera following the circumscription adopted in the latest classification of Rhipsalideae of Hunt et al. (2006), unless otherwise mentioned.

2.2. DNA sequencing

Total genomic DNA was extracted from silica-dried stems using the CTAB protocol of Doyle and Doyle (1987). A pilot study including 13 markers (plastid *trn*Q-*rps*16, *rpl*32-*trn*L, *psb*A-*trn*H, *acc*D, *trn*K-*mat*K, *trn*L-F, *rpo*B, *rpo*C1, *trn*C-*pet*N; nuclear ITS, *Phy*C, MS; and mitochondrial *Cox*3) was conducted to evaluate the suitability of various markers for the present study. The markers *trn*Q-*rps*16 (Shaw et al., 2007), *rpl*32-*trn*L (*rpl*32 and *trn*L (UAG), Shaw et al., 2007; *rpl*32Cact, 5'- GTT ATC TTA GGT TTC AAC AAA CC, this study), *psb*A-*trn*H (*psb*A, Sang et al., 1997; *trn*H2, Tate and Simpson, 2003) and ITS (17SE and 26SE, Sun et al., 1994) presented the most appropriate levels of variation for the reconstruction of phylogenetic relationships within Rhipsalideae and were selected for the present study. Amplification conditions for *trn*Q-*rps*16 and *rpl*32-*trn*L followed Shaw et al. (2007). The plastid spacer *psb*A-*trn*H and the nuclear internal transcribed spacer region (ITS) were amplified in 20 μl reactions containing: 2 μl of 5× GoTaq Buffer (Promega, Southampton), 2 μl of bovine serum albumin (0.4%; BSA), 1 μl of 25 mM $MgCl_2$, 1 μl of each primer (10 mM), 0.4 μl of GoTaq (Promega, Southampton), 0.4 μl of 10 mM dNTPs, 0.8 μl of dimethyl sulfoxide (4%; DMSO), 0.8 μl of genomic DNA and 11.6 μl of water. PCR reaction conditions for the amplification of *psb*A-*trn*H followed Edwards et al. (2005). PCR reaction conditions for the amplification of ITS were as follows: 94 °C for 2 min followed by 28 to 35 cycles of 94 °C for 1 min, 52 to 55 °C for 1 min, 72 °C for 3 min and a final extension of 72 °C for 7 min. Amplification products were purified using either the NucleoSpin Extract II Kit (Macherey–Nagel, Düren) or the QIAquick PCR purification Kit (Qiagen, Crawley), following the manufacturers' protocol. Automated sequencing was performed using the BigDye Terminator Cycle Sequencing Standard Version 3.1 Kit (Applied Biosystems, Warrington) and run on an ABI 3730 DNA Analyzer or sent to Macrogen Inc. (Korea). GenBank accession numbers for the sequences produced as part of this study are provided in Appendix A.

2.3. Sequence analyses

Sequences were assembled in Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually in MacClade v. 4.08

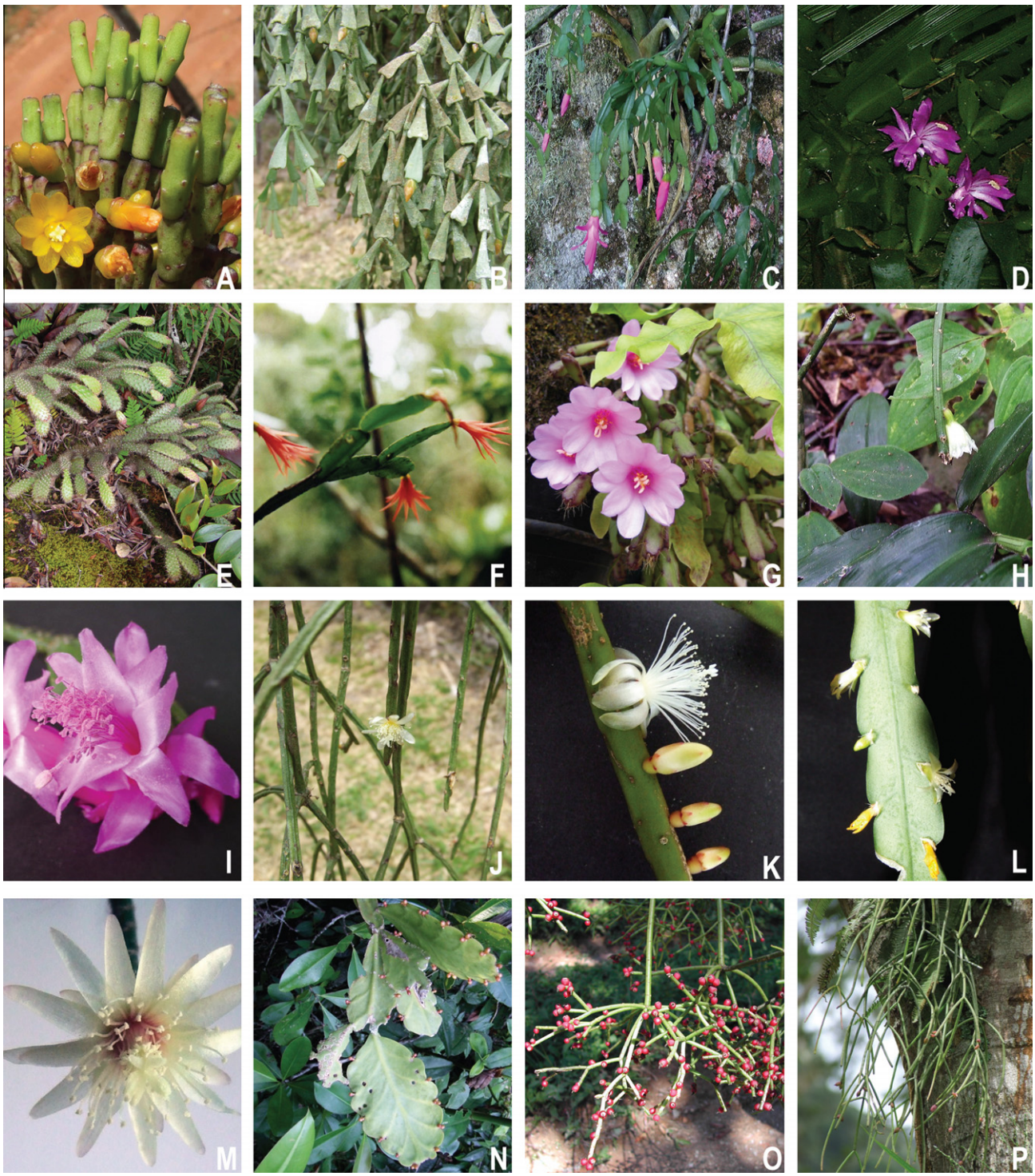

Fig. 1. Morphological diversity in Rhipsalideae. A. *Hatiora cylindrica*. B. *H. epiphylloides*. C. *Schlumbergera russelliana*. D. *S. orssichiana*. E. *S. opuntioides*. F. *H. gaertneri*. G. *H. rosea*. H. *Lepismium lumbricoides*. I. *S. opuntioides*. J. *Rhipsalis floccosa*. K. *R. grandiflora*. L. *L. cruciforme*. M. *R. pilocarpa*. N. *R. pachyptera*. O. *R. teres*. P. *R. baccifera* (photo credits: A. Calvente – A, C, H, L, M, N, Q; L. Versieux – B, J, O, P; H. Freitas – D; N. Mota – E; M. Khaeler – F, G; S. Martins – I).

(Maddison and Maddison, 2005). Indels were coded separately using the simple indel coding method (Simmons and Ochoterena, 2000). Regions with ambiguous alignments were excluded. Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP* version 4.0b10 (Swofford, 2002). MP and ML heuristic searches used 1000 replicates of random-taxon addition (retaining 20 trees at each replicate), tree bisection reconnection (TBR) branch swapping, and equal weighting of all characters. For ML searches, the best-fit model of nucleotide substitution and model parameters were determined for a combined plastid data set (*trn*Q-*rps*16, *rpl*32-*trn*L, *psb*A-*trn*H) and for ITS using ModelTest 3.04 (Posada and Crandall, 1998); HKY85 and HKY85 + G + I were respectively identified as the most appropriate models of evolution for these partitions. Support was assessed with non-parametric bootstrap analyses for MP and ML using random-taxon addition, and TBR branch swapping. MP bootstrap analyses

Table 1
Classifications proposed for taxa of Rhipsalideae illustrating divergent generic and infrageneric circumscriptions and earlier associations with Hylocereeae.

Author	Classification proposed
Britton and Rose (1923)	Positioned all epiphytic cacti in tribe Cereeae together with most part of the globular and columnar cacti. Subtribe Rhipsalidanae included genera *Erythrorhipsalis*, *Rhipsalidopsis*, *Pfeiffera*, *Acanthorhipsalis*, *Pseudorhipsalis*, *Lepismium*, *Hatiora* and *Rhipsalis*, while subtribe Epiphyllanae included genera *Zygocactus* (= *Schlumbergera sensu* Hunt et al., 2006), *Epiphyllanthus* (= *Schlumbergera sensu* Hunt et al., 2006), *Schlumbergera*, *Epiphyllum*, *Disocactus*, *Chiapasia*, *Eccremocactus*, *Nopalxochia* and *Wittia*
Buxbaum (1970)	Positioned Rhipsalinae in the tribe Hylocereeae together with subtribes Nyctocereinae, Hylocereinae, Epiphyllinae and Disocactinae. Divided the subtribe Rhipsalinae in the lineae Pfeifferae (genera *Pfeiffera* and *Acanthorhipsalis*), Schlumbergerae (genera *Erythrorhipsalis*, *Hatiora*, *Rhipsalidopsis*, *Schlumbergera* and *Zygocactus*) and Rhipsales (*Rhipsalis* and *Lepismium*)
Barthlott (1987) and Barthlott and Taylor (1995)	Considered Rhipsalideae apart from Hylocereeae and recognized four genera within Rhipsalideae: *Rhipsalis* (subgenera *Rhipsalis*, *Erythrorhipsalis*, *Calamorhipsalis*, *Epallagogonium* and *Phyllarthrorhipsalis*), *Hatiora* (subgenera *Hatiora* and *Rhipsalidopsis*), *Schlumbergera* and *Lepismium* (subgenera *Lepismium*, *Pfeiffera*, *Ophiorhipsalis*, *Acanthorhipsalis*, *Lymanbensonia* and *Houlletia*)
Doweld (2001)	Recognized two subtribes within Rhipsalideae: (1) Rhipsalidinae composed of *Nothorhipsalis* Doweld (= *Lepismium* subg. *Houlletia sensu* Hunt et al., 2006), *Lepismium*, *Erythrorhipsalis*, *Rhipsalis* (subgenera *Calamorhipsalis*, *Phyllarthrorhipsalis*, *Cereorhipsalis* Doweld and *Rhipsalis*) and *Hatiora* (2) Rhipsalidopsidinae Doweld composed of *Epiphyllanthus* (*S. opuntioides* and *S. microsphaerica*), *Rhipsalidopsis* (*S. rosea*), *Epiphyllopsis* (*S. gaertneri*) and *Rhipsaphyllopsis* (*H. x graeseri*, hybrid between *S. rosea* and *S. gaertneri*). Furthermore, transferred some species previously circumscribed in Rhipsalideae by Barthlott and Taylor (1995) to Hylocereeae, placing them in three subtribes: (1) Pfeifferinae including genera *Pfeiffera*, *Acanthorhipsalis* and *Lymanbensonia*; (2) Schlumbergerinae including genera *Schlumbergera* and *Pseudozygocactus* and nothogenera *Schlumbergeranthus*, *Schlumberphyllum*, *Schlumbergopsis*, *Schlumisocactus*; and (3) Hylorhipsalidinae Doweld including genera *Ophiorhipsalis* and *Hylorhipsalis* Doweld (containing species of *Rhipsalis* subg. *Epallagogonium*)
Hunt et al. (2006)	Used the same classification of Barthlott and Taylor (1995), but excluded species of *Lepismium* subg. *Pfeiffera*, *Lepismium* subg. *Acanthorhipsalis*, *Lepismium* subg. *Lymanbensonia* and part of *Lepismium* subg. *Houlletia* from Rhipsalideae; these taxa were included in *Pfeiffera*

Table 2
Sampling of Rhipsalideae and outgroup taxa used in the present study (following Hunt et al., 2006); type species are highlighted in bold.

Genera	Subgenera	Species	Voucher
Rhipsalis 21 out of 37 spp. 57%	*Phyllarthrorhipsalis* 7 out of 13 spp.	*R. cereoides* (Backeb. & Voll) Backeb.	A.A. Barros 2302 (RB)
		R. crispata (Haw.) Pfeiff.	A. Calvente 215 (SPF)
		R. elliptica G.A. Lindberg *ex* K. Schum.	A. Calvente 214 (SPF)
		R. micrantha (Kunth) DC	A. Calvente 396 (SPF)
		R. olivifera N.P. Taylor & Zappi	A. Calvente 226 (SPF)
		R. pachyptera Pfeiff.	A. Calvente 211 (SPF)
		R. russellii Britton & Rose	A. Calvente 313 (SPF)
	Calamorhipsalis 2 out of 3 spp.	*R. neves-armondii* K. Schum.	L. Versieux 196 (SPF)
		R. puniceodiscus G.A. Lindberg	A. Calvente 177 (SPF)
	Epallagogonium 4 out of 7 spp.	*R. dissimilis* (G.A. Lindberg) K. Schum.	A. Calvente 401 (SPF)
		R. floccosa Salm-Dyck *ex* Pfeiff.	A. Calvente 276 (SPF)
		R. paradoxa (Salm-Dyck *ex* Pfeiff.) Salm-Dyck	A. Calvente 145 (SPF)
		R. trigona Pfeiff.	A. Calvente 404 (SPF)
	Rhipsalis 4 out of 6 spp.	*R. baccifera* (J.S. Muell.) Stearn	A. Calvente 379 (SPF)
		R. lindbergiana K. Schum.	A. Calvente 161 (SPF)
		R. mesembryanthemoides Haw.	F. Freitas s/n (RB)
		R. teres (Vell.) Steud.	A. Calvente 255 (SPF)
	Erythrorhipsalis 4 out of 9 spp.	*R. clavata* F.A.C. Weber	A. Calvente 240 (SPF)
		R. pulchra Loefgr.	A. Calvente 232 (SPF)
		R. cereuscula Haw.	Kew living collection (1991–1439)
		R. pilocarpa Loefgr.	A. Calvente 357 (SPF)
Hatiora 6 out of 6 spp. 100%	*Hatiora*	*H. salicornioides* (Haw.) Britton & Rose	A. Calvente 239 (SPF)
		H. cylindrica Britton & Rose	A. Calvente 278 (SPF)
		H. herminiae (Porto & A. Cast.) Backeb. ex Barthlott	S. Martins s/n (SPF)
	Rhipsalidopsis	*H. gaertneri* (Regel) Barthlott	Kew living collection (1985–3156)
		H. rosea (Lagerh.) Barthlott	M. Kaehler s/n (SPF)
		H. epiphylloides (Porto & Werderm.) Buxb.	A. Calvente 363 (SPF)
Lepismium 4 out of 6 spp. 66%	*Lepismium* 1 out of 2 spp.	*L. cruciforme* (Vell.) Miq.	A. Calvente 26 (RUSU)
	Ophiorhipsalis 1 out of 1 spp.	*L. lumbricoides* (Lem.) Barthlott	A. Calvente 260 (SPF)
	Houlletia 2 out of 3 spp.	*L. houlletianum* (Lem.) Barthlott	A. Calvente 242
		L. warmingianum (K. Schum.) Barthlott	A. Calvente 259 (SPF)
Schlumbergera 4 out of 6 spp. 66%		*S. truncata* (Haw.) Moran	A. Calvente (SPF)
		S. russelliana (Hook.) Britton & Rose	A. Calvente 233 (SPF)
		S. opuntioides (Loefgr. & Dúsen) D.R. Hunt	N.F. O. Mota 1047 (BHCB)
		S. orssichiana Barthlott & McMillan	H. Freitas 28 (SPF)

Rhipsalideae total: 35 out of 55 spp. (63%)
Outgroup species: *Pereskia bahiensis* Gürke, *Calymmanthium substerile* F. Ritter, *Praecereus saxicola* (Morong) N.P. Taylor, *Pfeiffera ianthothele* F.A.C. Weber (Kew living collection), and *Epiphyllum phyllanthus* (L.) Haw. (A. Calvente – SPF)

were carried out with 1000 replicates, and ML bootstrap analyses with 100 replicates. Clades with bootstrap percentages of 50–74% are described as weakly supported, 75–89% as moderately supported and 90–100% as strongly supported.

Bayesian analyses were performed with MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). Searches were conducted using two independent runs, each performed with four simultaneous chains. Each Markov chain was initiated with a random tree and

run for 10^7 generations, sampled every 100 generations. Likelihood values were monitored graphically to determine stationarity and the appropriate burn-in. Best-fit models of nucleotide substitution were estimated separately for each partition. The F81 + G, HKY + G, F81 + I + G, GTR + I + G were selected for *psb*A-*trn*H, *trn*Q-*rps*16, *rpl*32-*trn*L and ITS, respectively. Posterior probabilities were used to evaluate support for all nodes (Ronquist and Huelsenbeck, 2003); clades with posterior probabilities above 0.95 are considered strongly supported.

A second Bayesian analysis with a constraint forcing all species currently assigned to *Hatiora* to form a monophyletic group was performed to evaluate the hypothesis of a monophyletic *Hatiora* (*sensu* Hunt et al., 2006) against the relationships recovered in the unconstraint analysis. This analysis was performed with the same conditions described above. The topologies resulting from the unconstraint and constraint analyses (maximum clade credibility trees) were compared using a Shimodaira–Hasegawa test (SH-test; Shimodaira and Hasegawa, 1999) to determine if the constraint tree is statistically worse than the unconstraint tree. The SH-test was performed in PAUP* (Swofford, 2002) using the same parameters applied for the ML analyses (see Section 2.3), 1000 bootstrap replicates and the RELL method.

2.4. Congruence testing and tree statistics

Incongruence between data sets was evaluated using the Incongruence Length Difference test (ILD; Farris et al., 1994), and the Templeton test (Templeton, 1983) as implemented in PAUP* version 4.0b10 (Swofford, 2002). For the ILD test, separate partitions were created for each marker and a heuristic search was performed with 1000 homogeneity replicates, saving a maximum of 1000 trees. For the Templeton test, a matrix and a tree for each data set was tested against a rival tree; the reverse approach was also adopted. The test and rival trees used were MP semi-strict consensus trees.

2.5. Ancestral character state reconstructions

Morphological data were compiled from the examination of plant material combined with information obtained in descriptions and monographs (Barthlott and Taylor, 1995; Britton and Rose, 1923; Calvente and Andreata, 2007; Taylor and Zappi, 2004; Zappi et al., 2007). Morphological characters used in previous classifications to delimit groups were chosen for ancestral state reconstructions and to identify potential synapomorphies for clades. All characters were discrete and binary coded. The ancestral state reconstructions were performed in MacClade 4.08 (Maddison and Maddison, 2005), considering unambiguous events exclusively. The Bayesian combined plastid tree was used for character mapping with outgroup species removed. Six morphological characters were optimized: flower symmetry (0, actinomorphic; 1, zygomorphic), flower tube (0, conspicuous and exceeding the pericarpel; 1, inconspicuous and not exceeding the pericarpel), branching pattern (0, mesotonic; 1, acrotonic), stem growth (0, indeterminate; 1, determinate), stem shape (0, winged or angled; 1, cylindrical), and flower color (0, strong and bright – red, pink, rose, yellow; 1, translucent – white, greenish, yellowish, pinkish). Ancestral state reconstructions were also performed using a Bayesian approach as implemented in the software SIMMAP v. 1.5 (Bollback, 2006). Posterior probabilities for ancestral characters states were calculated using 1000 trees sampled from the trees (excluding burn-in) obtained in the Bayesian analysis (see section 2.3). Prior parameters were calculated using a two-step approach (Bollback, 2009). First, we performed an MCMC analysis in SIMMAP v. 1.5 (Bollback, 2006) to sample overall rate values (gamma and beta priors). Second, best fitting values of gamma and beta parameters were estimated using the posterior distribution of gamma and beta performed in the R Statistical Package (R Foundation for Statistical Computing).

3. Results

3.1. Phylogenetic analyses of separate plastid partitions

Sequences for ingroup and outgroup species were generated for all three plastid markers selected (*trn*Q-*rps*16, *psb*A-*trn*H and *rpl*32-*trn*L) except for *Praecereus saxicola* and *Calymmanthium substerile*, for which we were unable to obtain *rpl*32-*trn*L sequences. The three data sets presented different levels of sequence variation and contained varied amounts of indels (Table 3).

The *trn*Q-*rps*16 data set included a large gap (~250 bp) between all species of *Rhipsalis* and remaining genera, leaving less than 300 bp of aligned sequence for all taxa of *Rhipsalis*. The MP analysis of the *trn*Q-*rps*16 data set resulted in 83,023 equally parsimonious trees of 158 steps, with a CI of 0.78 and a RI of 0.86 (Table 3); 15.5% of all characters were potentially parsimony informative. The *psb*A-*trn*H data set included a gap of ~100 bp in species of *Rhipsalis* leading to an aligned sequence matrix of 303 characters. The MP analysis for this marker resulted in 136,292 most parsimonious trees of 166 steps with a CI of 0.68 and a RI of 0.90 (Table 3). For this marker, 19.1% of the sites included in the analyses were potentially parsimony informative. The *rpl*32-*trn*L data set contained several small gaps (up to 20 bp in length) resulting in an aligned matrix of 1194 characters, of which 10% of all sites were potentially parsimony informative. The MP analysis for this marker resulted in 9543 trees of 412 steps with a CI of 0.65 and a RI of 0.82 (Table 3).

The MP semi-strict consensus tree of the *trn*Q-*rps*16 reconstructed two main clades: one including all species of *Rhipsalis* and the other comprising the remaining genera of the tribe, *Schlumbergera*, *Hatiora* and *Lepismium* (not shown). Within *Rhipsalis* a large polytomy was obtained however, better resolution was encountered for the other three genera. The MP semi-strict consensus tree of the *psb*A-*trn*H showed a monophyletic *Rhipsalis* that is sister to an unresolved clade composed of species of *Lepismium, Hatiora* and *Schlumbergera* (not shown). The *psb*A-*trn*H presented the highest percentage of informative sites of all four markers examined yet it led to the highest number of most parsimonious trees (Table 3). Overall, the topology obtained with *psb*A-*trn*H provided better resolution at lower levels, with several small clades within *Rhipsalis*. On the other hand, lower resolution was found at the generic level. The MP semi-strict consensus tree resulting from the analysis of the *rpl*32-*trn*L led to a better resolved tree at all levels within Rhipsalideae. The topology obtained with *rpl*32-*trn*L (not shown) was similar to the topology presented in the combined plastid tree (see Section 3.2). Overall, *rpl*32-*trn*L presented the lowest percentage of informative sites and the lowest number of most parsimonious trees (Table 3).

3.2. Phylogenetic analysis of the combined plastid data set

The ILD test demonstrated that the *psb*A-*trn*H, *trn*Q-*rps*16 and *rpl*32-*trn*L partitions were not significantly incongruent ($P = 0.3$), thus they were analyzed in combination. This analysis resulted in 276 equally parsimonious trees of 747 steps (CI = 0.66; RI = 0.82). The strict consensus tree obtained is well resolved for Rhipsalideae as a whole (Fig. 2). The MP (Fig. 2), ML (Fig. 3) and Bayesian analyses (Fig. 4) produced similar topologies. Bootstrap values and posterior probabilities (PP) were strong overall, with few moderately or weakly supported clades (Figs. 2–4). The SH-test shows that the constraint tree in which genus *Hatiora* is

Table 3
Characterization of DNA sequences and parsimony analyses conducted for each molecular marker used in this study.

Marker	Total size (bp)	Size excl. gaps	Informative sites			Best tree length	No. of most parsimonious trees	Consistency index (excl. uninformative characters)	Retention index
			(No.)	% of total size	% excl. gaps				
ITS	724	558	44	6	7.9	179	3414	0.43	0.75
*psb*A-*trn*H	423	303	58	13.7	19.1	166	136,292	0.68	0.90
*rpl32-trn*L	1493	1194	119	7.9	10	412	9543	0.65	0.82
*trn*Q-*rps16*	614	296	46	7.5	15.5	158	83,023	0.78	0.86
Combined (plastid)	2530	1793	223	8.8	12.4	747	276	0.66	0.82

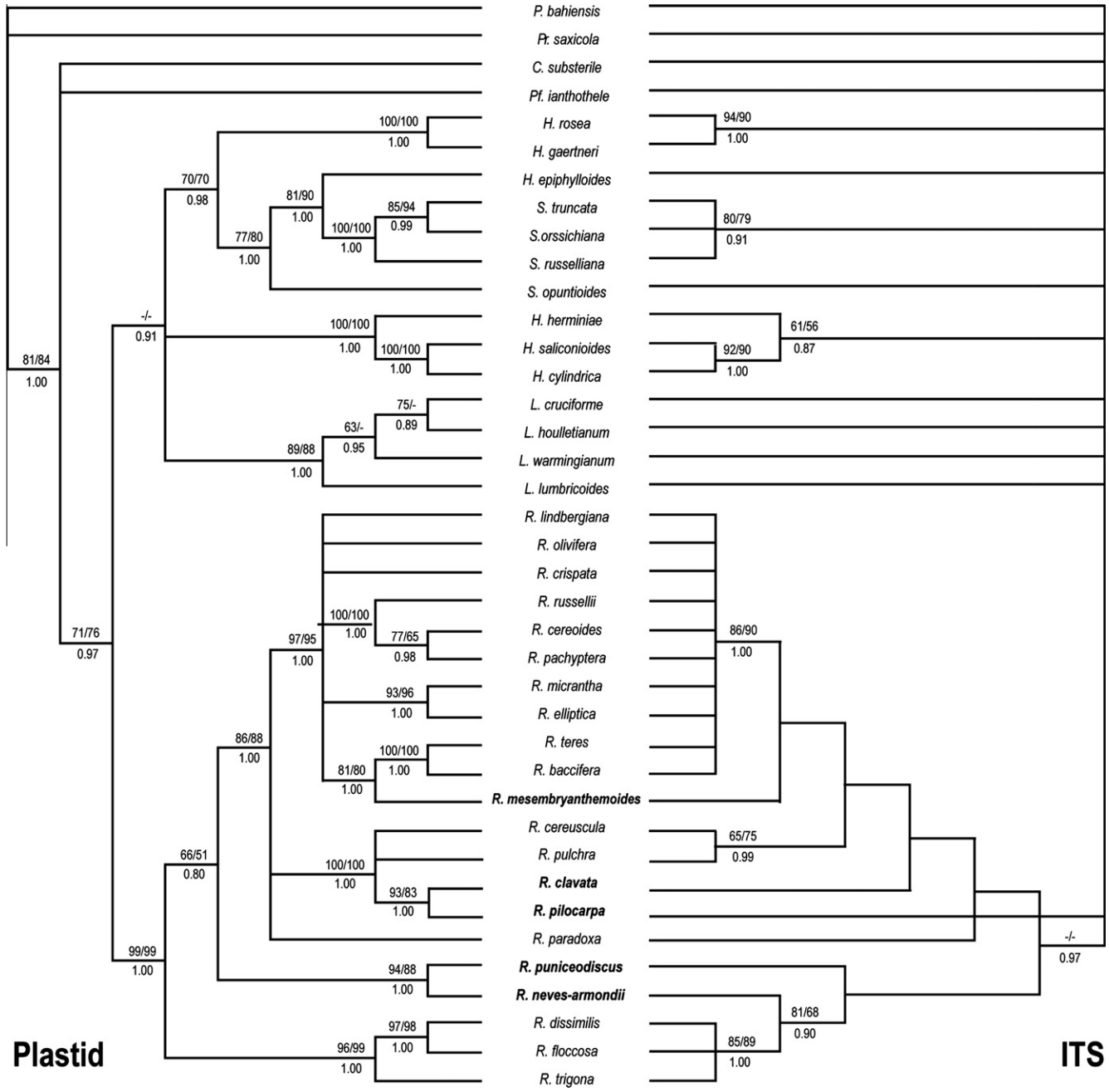

Fig. 2. Comparison between plastid and ITS strict consensus topologies resulting from the maximum parsimony analyses. Maximum parsimony and maximum likelihood bootstrap values are shown above branches and posterior probabilities values from the Bayesian analyses are shown below branches. Species with a controversial position are marked in bold.

monophyletic is significantly different from the unconstraint analysis in which species of the genus form three different lineages (unconstraint-lnL = 6818.60444, constraint-lnL = 6852.43251, difference = 33.82807, $P = 0.012$).

In the plastid combined analysis, tribe Rhipsalideae emerged as monophyletic with moderate support (Fig. 4). Two main clades are reconstructed within the tribe: (1) a smaller, weakly supported clade including *Schlumbergera*, *Hatiora* and *Lepismium* and (2) a lar-

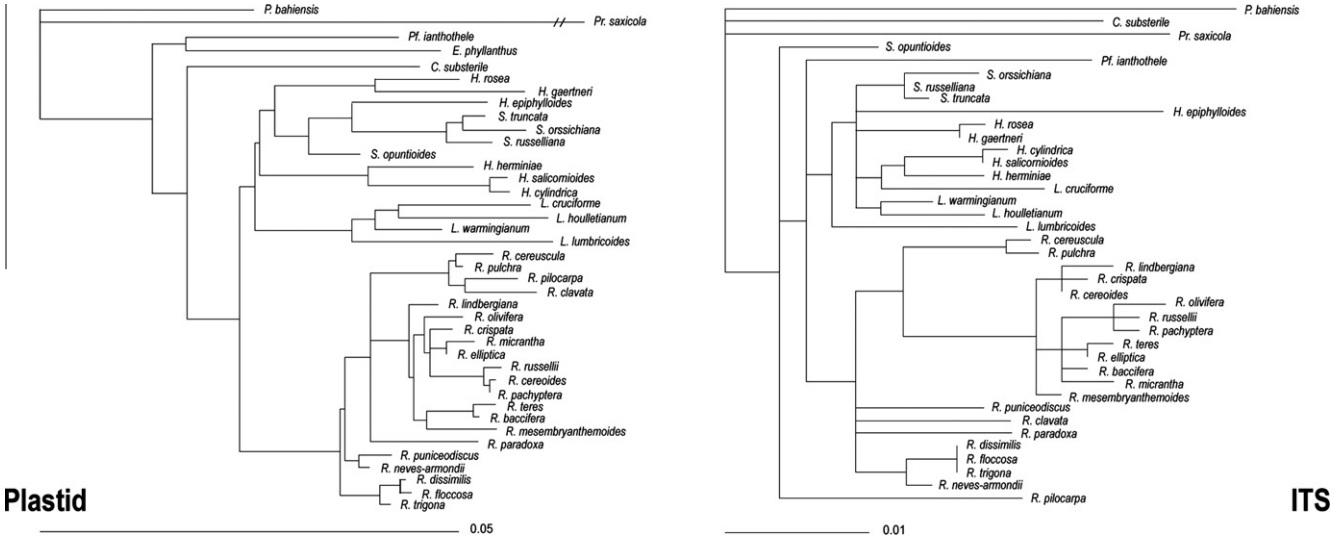

Fig. 3. Maximum likelihood phylograms resulting from the analysis of plastid and ITS data (plastid, −lnL = 6738.16029938; ITS, −lnL = 1807.26573). The terminal branch of Pr. saxicola in the tree based on plastid data has been reduced to half its size for practical reasons (indicated with "//").

ger clade strongly supported including all species of *Rhipsalis* (Fig. 4). Within the first clade, *Lepismium* is strongly supported as monophyletic, while *Schlumbergera* and *Hatiora* are paraphyletic (Fig. 4; Table 2). Clades within *Lepismium* are poorly supported and subgenus *Houlletia* appears to be paraphyletic; however, this relationship is only weakly to moderately supported (Fig. 4; Table 2). Three species of *Hatiora* form a strongly supported *Hatiora s.str.* clade corresponding to *Hatiora* subg. *Hatiora* (Fig. 4; Table 2). *Schlumbergera s.l.* includes species of *Schlumbergera* and *Hatiora* subg. *Rhipsalidopsis* (Fig. 4; Table 2). *Hatiora* subg. *Rhipsalidopsis* is paraphyletic, with *H. rosea* and *H. gaertneri* belonging to a strongly supported clade, and *Hatiora epiphylloides* appearing as sister to *Schlumbergera* (Fig. 4; Table 2).

The second clade including all species of *Rhipsalis* recovered four well-supported clades (Fig. 4). The "*floccosa* group" contains some species of subgenus *Epallagogonium*, however *R. paradoxa* (the type species of this subgenus) is part of an unresolved clade together with "core *Rhipsalis*" (Fig. 4; Table 2) and subgenus *Erythrorhipsalis*. Subgenera *Calamorhipsalis* and *Erythrorhipsalis* are both monophyletic (Fig. 4; Table 2). The fourth clade corresponds to the "core *Rhipsalis*" and holds species of subgenera *Rhipsalis* and *Phyllarthrorhipsalis* (Fig. 4; Table 2).

3.3. Phylogenetic analysis of ITS

ITS sequences were generated for all ingroup and outgroup taxa, except for *Epiphyllum phyllanthus*. The MP search resulted in 3414 trees of 179 steps (CI = 0.43; RI = 0.75). The aligned matrix comprises 558 characters of which 7.9% are potentially parsimony informative (Table 3). The topologies obtained through the MP (Fig. 2), ML (Fig. 3) and Bayesian (not shown) analyses were similar with respect to all strongly supported clades. The ILD ($P = 0.001$) and Templeton tests (rival tree ITS, $P < 0.0001$; rival tree plastid, $P = 0.34$) suggested that the ITS data set is incongruent with each individual plastid partition as well as with the plastid combined data set. Furthermore, several well-supported contradictory relationships were found between the plastid and ITS topologies (Fig. 2). Hence, the ITS data set was not analyzed in combination with the plastid data set.

3.4. Ancestral character state reconstructions

We reconstructed the ancestral character states of six morphological characters. These characters were: flower symmetry, flower tube, branching pattern, stem growth, stem shape, and flower color (Figs. 5 and 6). Results of this analysis indicate that actinomorphic flowers represent the ancestral condition in the group with at least two shifts to zygomorphic flowers, with both shifts occurring within *Schlumbergera* according to parsimony reconstruction (Fig. 5). The Bayesian reconstruction suggests a different scenario with a marginally greater probability that the common ancestral state of *S. opuntioides*, *S. russeliana*, *S. orssichiana*, *S. truncata* and *H. epiphylloides* is zygomorphic flowers. In this case, two reversions to actinomorphic flowers in *H. epiphylloides* and *S. russeliana* are needed to explain the patterns encountered.

The inconspicuous flower tube not exceeding the pericarpel represents the ancestral condition for Rhipsalideae in both parsimony and Bayesian reconstruction. One shift to conspicuous flower tubes exceeding the pericarpel occurred in *Lepismium*, supported both by the parsimony and Bayesian reconstructions (Fig. 5). The Bayesian reconstruction indicates a greater probability that another shift to conspicuous flower tubes occurred in the common ancestor of *Schlumbergera s.l.* followed by reversions to inconspicuous flower tubes in *H. epiphylloides* and in the ancestor of *H. rosea* and *H. gaertneri*. The parsimony reconstruction is equivocal for a few branches, but could also support a scenario identical to the one shown by the Bayesian optimization. It also indicates another possible scenario with two shifts to conspicuous flower tubes occurring in *Schlumbergera* (Fig. 5).

An acrotonic branching pattern is the ancestral condition for Rhipsalideae. One shift to mesotonic branching pattern occurred in *Lepismium* and three others occurred in three *Rhipsalis* species based on both parsimony and Bayesian approaches (Fig. 5).

Parsimony reconstruction of stem growth in Rhipsalideae resulted in several branches with equivocal reconstructions, including the root. However, Bayesian reconstruction indicates that determinate stems are the ancestral condition for the group. Shifts to indeterminate stems occurred in *Lepismium* and *Rhipsalis*. In the latter, the first diverging lineages in the genus are assigned determinate stem growth followed by one shift to indeterminate stem

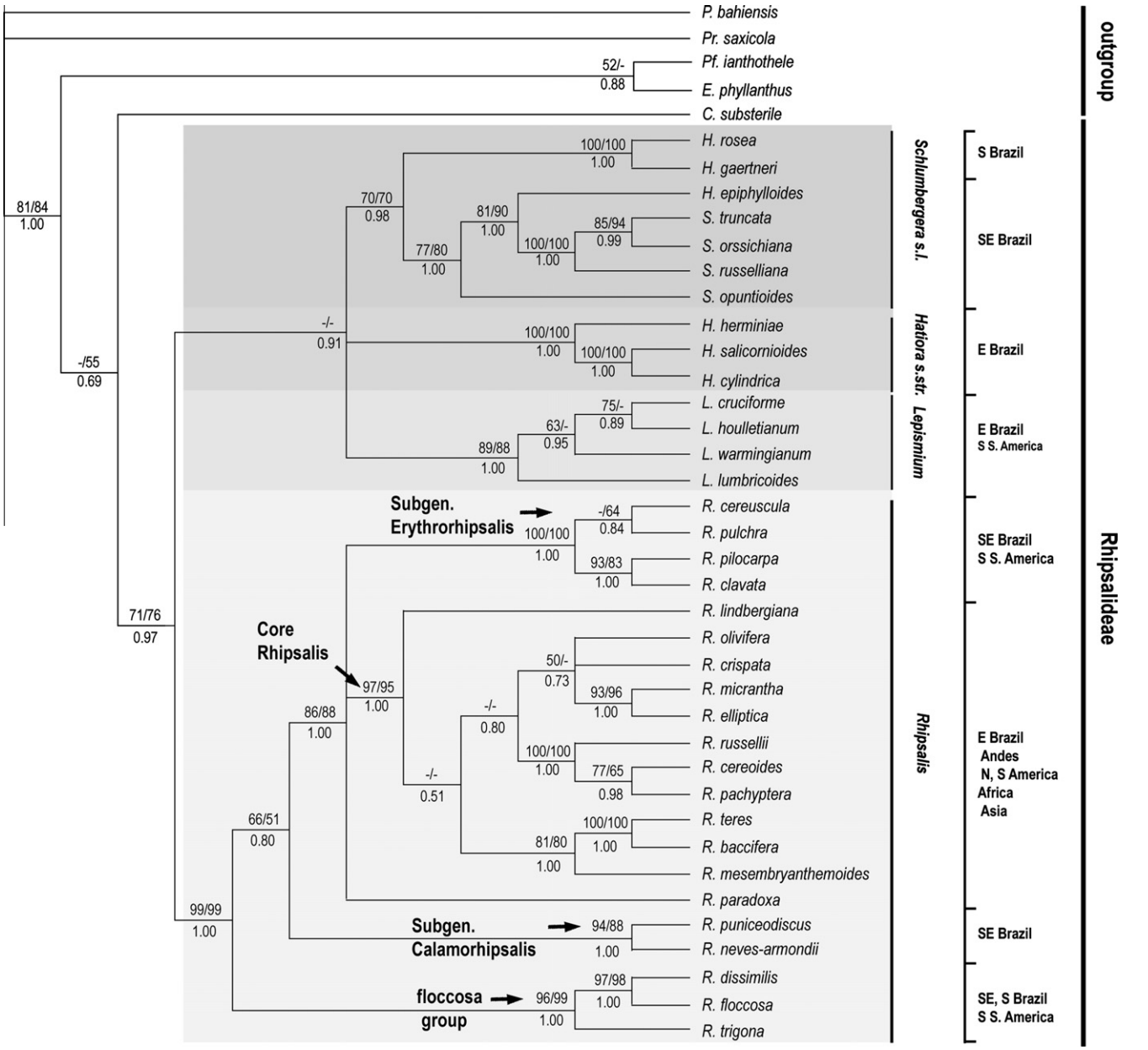

Fig. 4. Bayesian inference tree based on the combined analysis of plastid markers *psb*A-*trn*H, *trn*Q-*rps*16 and *rpl*32-*trn*L. Maximum parsimony and maximum likelihood bootstrap values are shown above branches and posterior probabilities values below branches. Biogeographic distributions are indicated on the right, based on information obtained from the literature and an extensive survey of herbarium specimens.

growth and at least two reversals to the determinate state (Fig. 6). However, due to the uncertainty in the optimization within *Rhipsalis*, other scenarios are also probable.

Parsimony reconstruction indicates that cylindrical stems are the ancestral condition for Rhipsalideae and that at least five shifts to winged or angled stems occurred in the group. Bayesian reconstruction indicates a different scenario with winged or angled stems as the ancestral condition for Rhipsalideae. Three subsequent shifts to cylindrical stems occurred in *Hatiora*, *Lepismium lumbricoides* and *Rhipsalis*, followed by three reversals to winged or angled stems within *Rhipsalis* (Fig. 6).

Parsimony reconstruction indicates that translucent flower colors (white, greenish, yellowish. pinkish) are the ancestral condition for Rhipsalideae and that at least one shift to strong and bright colors (red, pink, rose, yellow) occurred in *Hatiora* and *Schlumbergera* depending on the relationship of these groups (a polytomy in this study; Fig. 6). On the contrary, the Bayesian reconstruction indicates that there is a slightly higher probability that strong and bright flower colors are the ancestral condition for Rhipsalideae and that two independent shifts occurred to translucent flower colors in *Lepismium* and *Rhipsalis* (Fig. 6).

4. Discussion

4.1. Comparison between ITS and the combined plastid data sets

Overall, the ITS data set presented a weaker signal and higher homoplasy levels than the plastid data sets (Table 3). The ITS topology was also less resolved and presented lower support overall when compared to the plastid results (Fig. 2). Visual inspection revealed that major relationships recovered by the plastid data set

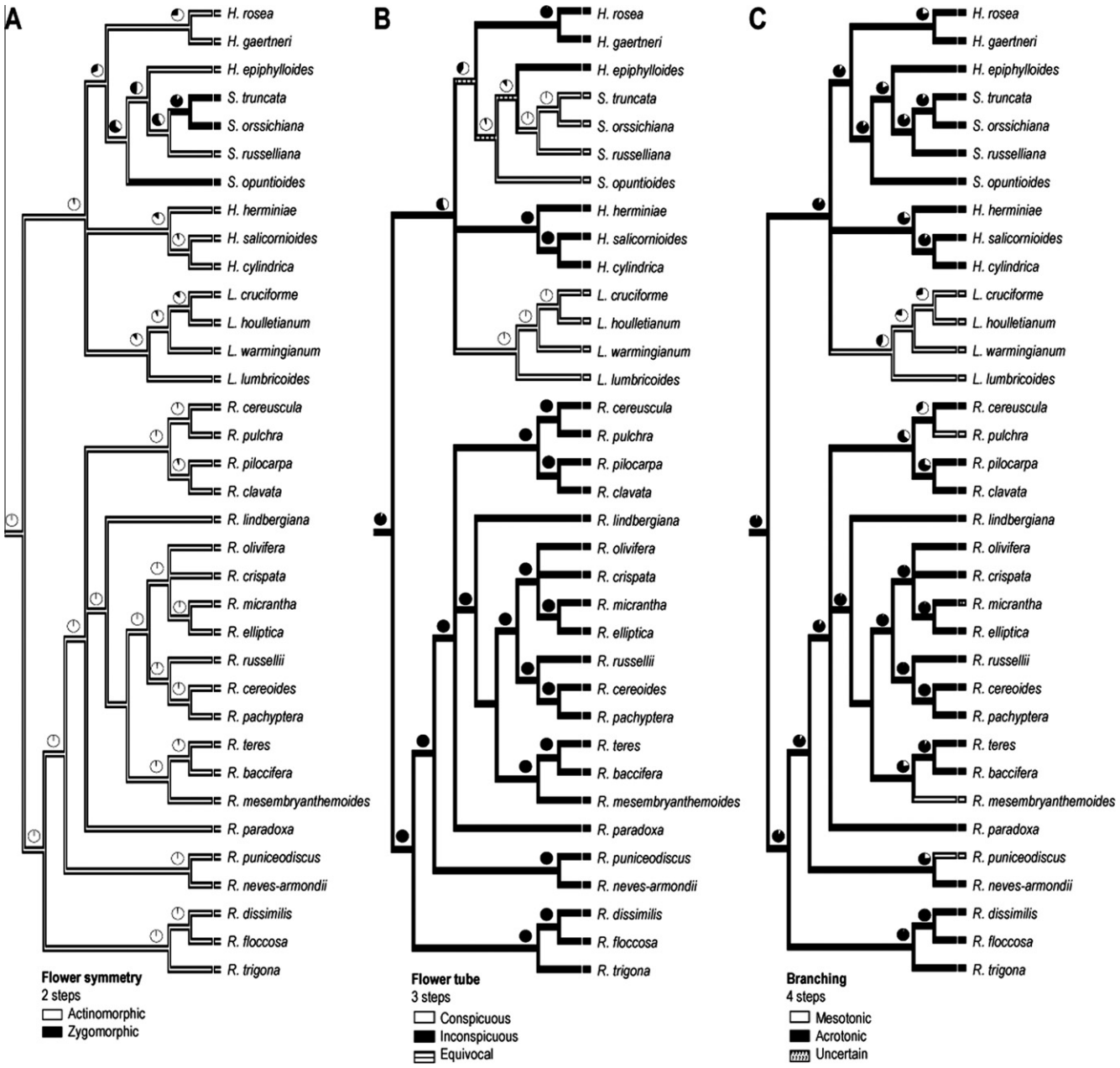

Fig. 5. Reconstruction of ancestral states of morphological traits using parsimony and Bayesian approaches. Posterior probabilities for each character state are indicated as pie charts.

were congruent with those based on ITS. However, the placement of five species of *Rhipsalis* differed between the combined plastid and the ITS topology. In particular, *Rhipsalis clavata* and *Rhipsalis pilocarpa* are within *Rhipsalis* subg. *Erythrorhipsalis* in the plastid trees but outside this subgenus in the ITS tree (Fig. 2; Table 2). Other two species, *Rhipsalis neves-armondii* and *Rhipsalis puniceodiscus*, appear as sister to the "*floccosa* group" in the ITS tree (making *Rhipsalis* subg. *Calamorhipsalis* paraphyletic) but form a monophyletic group in the tree based on the combined plastid data set. Lastly, *Rhipsalis mesembryanthemoides* appears as sister to the core *Rhipsalis* clade in the ITS topology but is sister to *Rhipsalis teres* and *Rhipsalis baccifera* in the combined plastid topology (Fig. 2).

Divergent ITS paralogues could explain the conflict between the ITS data set and the combined plastid topology. We did not encounter any evidence of ITS paralogues either through multiple bands or explicit ambiguity in the chromatograms. Even though, it is possible that divergent ITS paralogues may have been amplified in this study, including pseudogenes and recombinants as previously found for other cacti as well as other plant groups (e.g., Buckler IV et al., 1997; Harpke and Peterson, 2006). Although Harpke and Peterson (2006) had strong evidence of the presence of pseudogenes for *Mammillaria* species it is unclear whether ITS paralogy issues are common to all cacti or only occur in this genus. Other phylogenetic studies with different cacti using ITS did not encounter evidence of paralogy. Arias et al. (2003) did not mention evidence of paralogues for *Pachycereus* but reported ITS topology congruent with plastid topology in spite of finding higher homoplasy in ITS data. Griffith and Porter (2009) also found ITS congruent with plastid topology and did not report observation of paralogues. Future studies employing a broader sampling may shed light on the evolution of ITS in Cactaceae as a whole.

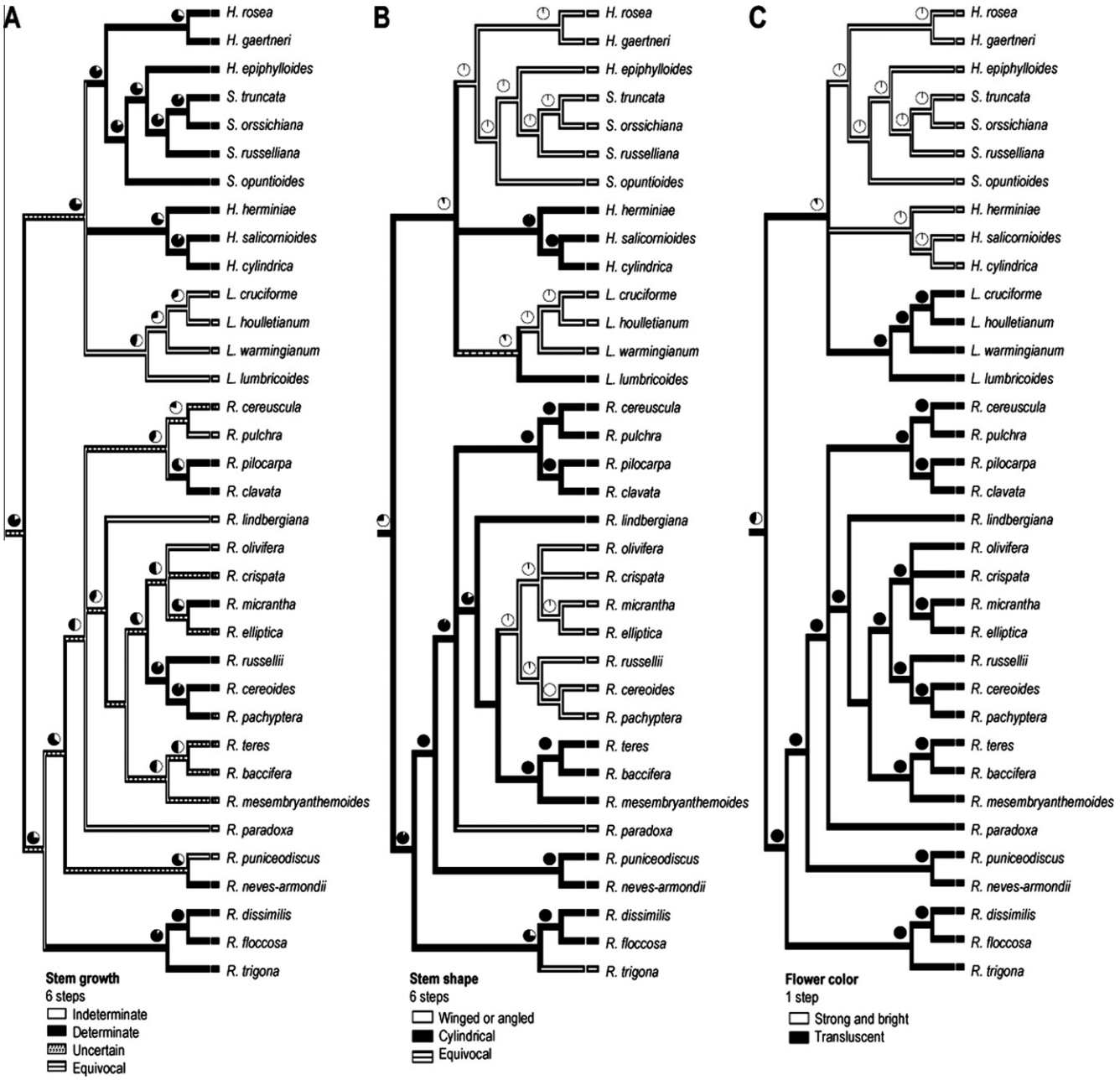

Fig. 6. Reconstruction of ancestral states of morphological traits using parsimony and Bayesian approaches. Posterior probabilities for each character state are indicated as pie charts.

Another possible explanation is hybridization and introgression, which may have led to the divergent phylogenetic evidence gathered from the plastid and nuclear markers. Several interspecific and intergeneric hybrid species have been produced in cultivation using *Hatiora* and *Schlumbergera* but apparently these do not hybridize naturally due to ecological isolation (Boyle, 2007). Hybrids involving *Rhipsalis* species have not been yet documented. Hypotheses have been drawn as whether *R. puniceodiscus* and *R. neves-armondii* hybridize in cultivation (specimen of these species included in the present work were collected in the wild), although no formal studies have been conducted to confirm or refute this matter (Taylor, 1999). In general, this work has not provided consistent evidence of hybridization and introgression events in Rhipsalideae as the ITS did not provide strong support for the conflicting relationships. Furthermore, no additional insights are drawn from the ITS conflicting topology as the plastid topology is more congruent with morphological patterns encountered in the tribe. Nevertheless, this must be further evaluated using a broader sampling within *Rhipsalis* and additional genomic regions.

The incongruence between the nuclear and plastid partitions is not particularly problematic for this study, as it focuses on (1) the evaluation of major groups, (2) the relationships between these groups, and (3) the resulting generic circumscription. Combining the ITS and plastid data sets did not change the outcomes with regard to the relationships of major clades and genera (data not shown). Incongruence only affected five species of *Rhipsalis* whose placement diverged between the plastid and ITS trees (see Section 4.1). Given this, the higher resolution and support obtained by the analyses of the plastid data set, we choose to consider the combined plastid topology as representing the best estimate of genera relationships in tribe Rhipsalideae. This topology was used to assess the evolution of selected morphological characters and

Table 4
Major clades recognized in this study and their morphological characterization.

Clade	Morphological characterization
Schlumbergera s.l.	Branching acrotonic, stems generally pendulous, 2-winged or irregularly angular, short (<7 cm), determinate; flowers terminal, pendulous, zygomorphic or actinomorphic, showy and strongly colored (rose, golden yellow, pink, red) or opaque white (in cultivars); perianth segments apiculate, acute; flower tube conspicuous exceeding the pericarpel to absent or reduced, not markedly exceeding the pericarpel
Hatiora s.str.	Branching acrotonic, stems cylindrical or bottle-shaped, short, determinate; flowers terminal, erect or pendulous, actinomorphic, strong colored (rose or golden yellow); flower tube absent or reduced, not markedly exceeding the pericarpel. Perianth segments rounded to obtuse at the tips; stems and flowers erect
Lepismium	Branching mesotonic, stems creeping or pendulous, cylindrical or 2–3-winged, long, indeterminate; flowers lateral, generally pendulous, actinomorphic, translucent white, pinkish or yellowish; flower tube conspicuous, exceeding the pericarpel
Rhipsalis	Branching acrotonic or mesotonic, stems erect or pendulous, 2-winged, cylindrical or ribbed, indeterminate or determinate, >7 cm; flowers lateral or terminal, patent to the stem or pendulous, actinomorphic, translucent white, pinkish or yellowish; flower tube absent or never exceeding the pericarpel

will serve as basis for a re-evaluation of the current classification system for Rhipsalideae.

4.2. Systematics of Rhipsalideae

The combined plastid data set corroborated the monophyly of Rhipsalideae and reconstructed major clades within the tribe (Fig. 4). Relationships reconstructed are generally in agreement with the latest generic classification proposed for the tribe (Hunt et al., 2006). The only discrepancy is associated with *Hatiora* and *Schlumbergera*, which were both shown to be paraphyletic. For this reason and in order to recognize exclusively monophyletic genera we propose a re-definition of generic limits in Rhipsalideae involving *Hatiora* and *Schlumbergera*. In this new generic delimitation, species once included in *Hatiora* subg. *Rhipsalidopsis* are transferred to *Schlumbergera* (*Schlumbergera s.l.*) while *Hatiora* is reduced to a genus with only three species (*Hatiora s.str.*).

The current delimitation of these genera goes back to Barthlott (1987), who separated *Schlumbergera* from *Hatiora* on the basis of the zygomorphic flowers found in the former. This character was here shown to be homoplastic, with parallel evolution of zygomorphic flowers in *S. opuntioides* and the pair *S. orssichiana/S. truncata* (Fig. 5). Previous classifications had already linked *S. russeliana* to *H. gaertneri* on the basis of their actinomorphic flowers (Britton and Rose, 1923). In fact, *H. gaertneri* was first described as infraspecific taxa of *Schlumbergera russelliana* and hence, positioned within *Schlumbergera*. All species of *Hatiora* subg. *Rhipsalidopsis* and *Schlumbergera* (*Schlumbergera s.l.*) are very similar vegetatively and characterized by two-winged, short determinate stem segments (or angular stem segments in *S. microsphaerica*), while species in *Hatiora* subg. *Hatiora* (*Hatiora s.str.)* have cylindrical stem segments (Fig. 6; Table 4). A large number of perianth segments with acute or apiculate apices and generally pendulous stems and flowers also help distinguishing *Schlumbergera s.l.* from *Hatiora s.str*. Flowers of *Hatiora s.str.* present fewer perianth segments with obtuse apices and erect stems and flowers (Fig. 1, Table 4).

Even though the bootstrap support for *Schlumbergera s.l.* is weak (70%), this consistent morphological information corroborates the new circumscription of *Schlumbergera* and facilitates the taxonomy and identification of genera within Rhipsalideae as a whole (Table 4). Furthermore, the constraint topology forcing a monophyletic *Hatiora* was shown to be significantly different from the topology found in the unconstraint Bayesian analysis (SH-test; see section 3.2), thus adding to the evidence presenting *Hatiora* as paraphyletic under its current circumscription.

4.3. Morphological evolution in Rhipsalideae

In order to further evaluate the suitability of the morphological characters traditionally used in the taxonomy of Rhipsalideae, selected morphological characters were mapped onto the combined plastid phylogeny of Rhipsalideae. The evolutionary patterns encountered illustrate that some morphological characters traditionally used in the classification of Rhipsalideae are homoplastic and are not adequate for the delimitation of generic boundaries (Figs. 5 and 6). For example, flower zygomorphy that has been previously used to separate *Schlumbergera* from *Hatiora* is homoplastic and appears to have evolved independently at least twice (see Section 4.2). Conspicuous flower tubes were also previously used to separate *Hatiora* from *Schlumbergera*, but the phylogenetic relationships depicted here indicated that this character is homoplastic for *Schlumbergera s.l.* as *H. epiphylloides*, *H. rosea* and *H. gaertneri* present inconspicuous flower tubes (Fig. 5). Conspicuous flower tubes also occur in *Lepismium*, however in this group this character state presents a different appearance and size, indicating the possibility that these morphologies may have been derived from different evolutionary pathways.

On the other hand, other morphological features corroborate clades representing genera and are good alternative for taxonomic use at this level. For example, branching pattern has been mainly used to distinguish *Rhipsalis* from *Lepismium* and *Hatiora*. Even though branching pattern is homoplastic within *Rhipsalis* (hence inadequate to distinguish *Rhipsalis* from the other genera of Rhipsalideae), it is reliable for the separation of *Lepismium* from *Hatiora* and *Schlumbergera* as the branching is strictly acrotonic in *Hatiora* and *Schlumbergera* and strictly mesotonic in *Lepismium*. Similarly, stem growth is highly variable within *Rhipsalis* and is only adequate to separate *Lepismium* from *Hatiora* and *Schlumbergera* (Table 4). Stem shape is a good character to separate *Schlumbergera s.l.* from *Hatiora s.str.*, although it is variable among species of *Lepismium* and *Rhipsalis*. Flower color is also consistent at generic level in the group and is a good character for the separation of *Lepismium* and *Rhipsalis* from *Hatiora* and *Schlumbergera*.

This study also illustrated the difficulties of finding morphological synapomorphies within *Rhipsalis*. Most characters examined are homoplasious, with all characters analyzed appearing in overlapping combinations amongst *Rhipsalis* species and other genera of Rhipsalideae. This finding suggests that morphological evolution of *Rhipsalis* was complex. The principal macromorphological features varying among Rhipsalideae genera are the main focus here, but it would be interesting to examine micro-morphological, ecological and physiologic data in future investigations.

5. Taxonomic changes within *Hatiora* and *Schlumbergera*

The molecular phylogenetic analyses of Rhipsalideae associated with the study of morphological features in the group indicate that a broader *Schlumbergera* (including *Hatiora* subg. *Rhipsalidopsis*) should be recognized. Consistent morphological

characters rather than homoplastic characters are here used to establish a more practical, and hopefully more stable, classification for the group (Figs. 5 and 6; Table 4). The taxonomic and nomenclatural changes proposed for *Hatiora* and *Schlumbergera* are outlined below, as well as a complete list of the species currently recognized in those two genera. Synonyms, infra-specific taxa are only listed when associated with new combinations. A taxonomic key for the identification of genera of Rhipsalideae is also provided.

1 –***Hatiora*** Britton & Rose, Stand. Cycl. Hort. 3: 1432. 1915.
Type: *H. salicornioides* (Haw.) Britton & Rose
1.1 *Hatiora cylindrica* Britton & Rose
1.2 *Hatiora herminiae* (Porto & A. Cast.) Backeb. ex Barthlott
1.3 *Hatiora salicornioides* (Haw.) Britton & Rose

2 –***Schlumbergera*** Lem., Rev. Hort. 4(7): 253. 1858.
= *Zygocactus* K. Schum.
= *Epiphyllanthus* A. Berger
= *Rhipsalidopsis* Britton & Rose
= *Hatiora* subg. *Rhipsalidopsis* (Britton & Rose) Barthlott, *syn nov.*
Type: *S. epiphylloides* Lem. *nom. ileg.* (= *S. russelliana* (Hook.) Britton & Rose)

2.1 *Schlumbergera gaertneri* (Regel) Britton & Rose
≡ *H. gaertneri* (Regel) Barthlott, syn nov.
2.2 *Schlumbergera kautskyi* (Horobin & McMillan) N.P. Taylor
2.3 Schlumbergera lutea Calvente & Zappi, nom. nov.
≡ *Rhipsalis epiphylloides* Porto & Werderm., Jahrb. Deutsch. Kakteen-Ges. 1(7): 47. 1935. *H. epiphylloides* (Porto & Werderm.) Buxb., *syn nov. H. epiphylloides* (Porto & Werderm.) Buxb. subsp. *epiphylloides*, *syn nov.*
2.3.2 *Schlumbergera lutea* subsp. *bradei* (Porto & A. Cast.) Calvente & Zappi, *comb. nov.*

≡ *Hariota epiphylloides* var. *bradei* Porto & A. Cast., Rodriguésia 5(14): 354. 1941. *Hatiora epiphylloides* subsp. *bradei* (Porto & A. Cast.) Barthlott & N.P. Taylor, *syn. nov.*
2.4 *Schlumbergera microsphaerica* (K. Schum.) Hoevel
2.5 *Schlumbergera opuntioides* (Loefgr. & Dúsen) D.R. Hunt
2.6 *Schlumbergera orssichiana* Barthlott & McMillan
2.7 *Schlumbergera rosea* (Lagerh.) Calvente & Zappi, *comb nov.*
≡ *Rhipsalis rosea* Lagerh., Svensk Bot. Tidskr. 6: 717. 1912. *Hatiora rosea* (Lagerh.) Barthlott, *syn. nov.*
2.8 *Schlumbergera russelliana* (Hook.) Britton & Rose
2.9 *Schlumbergera truncata* (Haw.) Moran

5.1. Key to genera of Rhipsalideae

1. Branching acrotonic, stem segments determinate and short (<7 cm). Flowers actinomorphic or zygomorphic, strong and bright colored (or opaque white in cultivars) .....2.
1'. Branching acrotonic or mesotonic, stem segments determinate or indeterminate, long segments (>7 cm) always present. Flowers actinomorphic, translucent .....3.
2. Stem segments cylindrical or bottle-shaped. Flowers actinomorphic ..... *Hatiora.*
2'. Stem segments 2-winged or angular. Flowers actinomorphic or zygomorphic . . .. *Schlumbergera.*
3. Branching acrotonic or mesotonic, stem segments determinate or indeterminate. Flower tube absent or never exceeding the pericarpel ..... *Rhipsalis.*
3'. Branching strictly mesotonic, stem segments always indeterminate. Flower tube conspicuous, exceeding the pericarpel ..... *Lepismium.*

Acknowledgments

This project represents part of the Ph.D. thesis of A.C. Authors thank FAPESP, IAPT, Universidade de São Paulo and Royal Botanic Gardens, Kew for financial support; the Royal Botanic Gardens, Kew for providing materials from its living collections; Marcelo Sellaro and the staff of the living collections at Kew for assistance; IBAMA and IF-SP for collection permits; Thelma Barbará and Christian Lexer for assistance to A.C. while visiting Kew; Edith Kapinos, Dion Devey, Laura Kelly and the Jodrell Laboratory staff for assistance during lab work; Leonardo Versieux, Pedro Viana, Nara Mota, Miriam Khaeler, Suzana Martins and Herbert Freitas for assistance during field work and/or for providing silica-dried materials; Leonardo Versieux and members of Lúcia Lohmann's Lab Group for comments on an earlier version of this manuscript.

Appendix A. GenBank accession numbers for each species used in this study

Species; accession numbers (*psb*A-*trn*H; *trn*Q-*rps*16; *rpl*32-*trn*L; ITS)
R. cereoides; HQ727740; HQ727819; HQ727859; HQ727780. *R. crispata;* HQ727731; HQ727810; HQ727850; HQ727771. *R. elliptica*; HQ727730; HQ727809; HQ727849; HQ727770. *R. micrantha*; HQ727736; HQ727815; HQ727855; HQ727776. *R. olivifera*; HQ727741; HQ727820; HQ727860; HQ727781. *R. pachyptera;* HQ727732; HQ727811; HQ727851; HQ727772. *R. russellii*; HQ727746; HQ727825; HQ727865; HQ727786. *R. neves-armondii*; HQ727737; HQ727816; HQ727856; HQ727777. *R. puniceodiscus*; HQ727749; HQ727828; HQ727868; HQ727789. *R. dissimilis*; HQ727750; HQ727829; HQ727869; HQ727790. *R. floccosa*; HQ727748; HQ727827; HQ727867; HQ727788. *R. paradoxa*; HQ727742; HQ727821; HQ727861; HQ727782. *R. trigona*; HQ727738; HQ727817; HQ727857; HQ727778. *R. baccifera*; HQ727744; HQ727823; HQ727863; HQ727784. *R. lindbergiana*; HQ727755; HQ727834; HQ727874; HQ727795. *R. mesembryanthemoides*; HQ727739; HQ727818; HQ727858; HQ727779. *R. teres*; HQ727754; HQ727833; HQ727873; HQ727794. *R. clavata*; HQ727753; HQ727832; HQ727872; HQ727793. *R. pulchra*; HQ727735; HQ727814; HQ727854; HQ727775. *R. cereuscula*; HQ727765; HQ727844; HQ727882; HQ727805. *R. pilocarpa*; HQ727745; HQ727824; HQ727864; HQ727785. *H. salicornioides*; HQ727743; HQ727822; HQ727862; HQ727783. *H. cylindrica*; HQ727752; HQ727831; HQ727871; HQ727792. *H. herminiae*; HQ727760; HQ727839; HQ727879; HQ727800. *H. gaertneri*; HQ727767; HQ727846; HQ727884; HQ727807. *H. rosea*; HQ727768; HQ727847; HQ727885; HQ727808. *H. epiphylloides*; HQ727751; HQ727830; HQ727870; HQ727791. *L. cruciforme*; HQ727747; HQ727826; HQ727866; HQ727787. *L. lumbricoides*; HQ727758; HQ727837; HQ727877; HQ727798. *L. houlletianum*; HQ727756; HQ727835; HQ727875; HQ727796. *L. warmingianum*; HQ727759; HQ727838; HQ727878; HQ727799. *S. truncata*; HQ727757; HQ727836; HQ727876; HQ727797. *S. russelliana*; HQ727734; HQ727813; HQ727853; HQ727774. *S. opuntioides*; HQ727761; HQ727840; HQ727880; HQ727801. *S. orssichiana*; HQ727733; HQ727812;

(*continued on next page*)

Apendix A (*continued*)

Species; accession numbers (*psb*A-*trn*H; *trn*Q-*rps*16; *rpl*32-*trn*L; ITS)
HQ727852; HQ727773. *Pereskia bahiensis*; HQ727763; HQ727842; HQ727881; HQ727803. *Calymmanthium substerile*; HQ727764; HQ727843; - ; HQ727804. *Praecereus saxicola*; HQ727762; HQ727841; - ; HQ727802. *Pfeiffera ianthothele*; HQ727766; HQ727845; HQ727883; HQ727806. *Epiphyllum phyllanthus*; HQ727769; HQ727848; HQ727886; –.

References

Anderson, E.F., 2001. The Cactus Family. Timber Press, Portland.

Applequist, W.L., Wallace, R.S., 2002. Deletions in the plastid trnT-trnL intergenic spacer define clades within Cactaceae subfamily Cactoideae. Plant Syst. Evol. 231, 153–162.

Arias, S., Terrazas, T., Cameron, K., 2003. Phylogenetic analysis of *Pachycereus* (Cactaceae, Pachycereeae) based on chloroplast and nuclear DNA sequences. Syst. Bot. 28, 547–557.

Arias, S., Terrazas, T., Arreola-Nava, H.J., Vazquez-Sanchez, M., Cameron, K.M., 2005. Phylogenetic relationships in *Peniocereus* (Cactaceae) inferred from plastid DNA sequence data. J. Plant Res. 118, 317–328.

Barthlott, W., 1983. Biogeography and Evolution in Neo and Paleotropical Rhipsalinae (Cactaceae). In: Kubitzki, K. (Ed.), Dispersal and Distribution: An International Symposium, Sonderbande des Naturwissenschaftlichen Vereins, Hamburg, pp. 241–248.

Barthlott, W., 1987. New names in Rhipsalidinae (Cactaceae). Bradleya 5, 97–100.

Barthlott, W., Taylor, N.P., 1995. Notes towards a monograph of Rhipsalideae (Cactaceae). Bradleya 13, 43–79.

Bollback, J.P., 2006. Simmap: stochastic character mapping of discrete traits on phylogenies. BMC Bioinf. 7, 88.

Bollback, J.P., 2009. Simmap 1.5 Documentation Online. <http://www.simmap.com/pgs/docs.html> (accessed 05.10.

Boyle, T., 2007. Cacti: *Schlumbergera truncata*, *S. x buckleyi*, *Hatiora gaertneri*. In: Anderson, N.O. (Ed.), Flower Breeding and Genetics: Issues, Challenges and Opportunities for the 21st Century. Springer, Dordrecht.

Britton, N.L., Rose, J.N., 1923. The Cactaceae: Descriptions and Illustrations of Plants of the Cactus Family, vol. 4. The Carnegie Institution of Washington, Washington, DC, USA.

Buckler IV, E.S., Ippolito, A., Holtsford, T.P., 1997. The evolution of ribosomal DNA divergent paralogues and phylogenetic implications. Genetics 145, 821–832.

Butterworth, C.A., Wallace, R.S., 2004. Phylogenetic studies of *Mammillaria* (Cactaceae) – insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. Am. J. Bot. 91, 1086–1098.

Butterworth, C.A., Wallace, R.S., 2005. Molecular phylogenetics of the leafy cactus genus *Pereskia* (Cactaceae). Syst. Bot. 30, 800–808.

Butterworth, C.A., Cota-Sanchez, J.H., Wallace, R.S., 2002. Molecular systematics of Tribe Cacteae (Cactaceae : Cactoideae): a phylogeny based on *rpl* 16 intron sequence variation. Syst. Bot. 27, 257–270.

Buxbaum, F., 1970. Das phylogenetische system der Cactaceae in die kakteen. In: Krainz, H. (Ed.), Die Kakteen: Eine gesamtdarstellung der eingeführten arten nebst anzucht – und pflegeanweisungen herausgegeben Kosmos-Verlag, Franckh'sche Verlagshandlung, Stuttgart, Germany.

Calvente, A.M., Andreata, R.H.P., 2007. The Cactaceae of the natural municipal park of Prainha, Rio de Janeiro, Brazil: taxonomy and conservation. J. Bot. Res. Inst. Tex. 1, 529–548.

Calvente, A.M., Freitas, M.F., Andreata, R.H.P., 2005. Listagem, distribuição geográfica e conservação das espécies de Cactaceae no Estado do Rio de Janeiro. Rodriguésia 56, 141–162.

Doweld, A.B., 2001. Re-classification of Rhipsalideae, a polyphyletic tribe of the Cactaceae Durande. Succulents 4, 25–45.

Doyle, J.A., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Edwards, E.J., Nyffeler, R., Donoghue, M.J., 2005. Basal cactus phylogeny: implications of *Pereskia* (Cactaceae) paraphyly for the transition to the cactus life form. Am. J. Bot. 92, 1177–1188.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing the significance of incongruence. Cladistics 10, 315–319.

Gibson, A.C., Nobel, P.S., 1986. The Cactus Primer. Harvard University Press, Cambridge, Massachusetts, USA.

Griffith, M.P., Porter, J.M., 2009. Phylogeny of Opuntioideae. Int. J. Plant Sci. 170, 107–116.

Harpke, D., Peterson, A., 2006. Non-concerted ITS evolution in *Mammillaria* (Cactaceae). Mol. Phylogenet. Evol. 41, 579–593.

Hartmann, S., Nason, J.D., Bhattacharya, D., 2002. Phylogenetic origins of *Lophocereus* (Cactaceae) and the senita cactus-senita moth pollination mutualism. Am. J. Bot. 89, 1085–1092.

Hunt, D., Taylor, N., Charles, G., 2006. The New Cactus Lexicon. DH books, Milborne Port, UK.

Maddison, D.R., Maddison, W.P., 2005. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.08. Sinauer Associates, Sunderland, Massachusetts.

Metzing, D., Kiesling, R., 2008. The study of the cactus evolution: the pre-DNA era. Heseltonia 14, 6–25.

Nyffeler, R., 2000. Should *Pfeiffera* be resurrected? Cact. Syst. Initiat. 10, 10–11.

Nyffeler, R., 2002. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trn* K/*mat* K and *trn* L-*trn* F sequences. Am. J. Bot. 89, 312–326.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Ritz, C.M., Martins, L., Mecklenburg, R., Goremykin, V., Hellwig, F.H., 2007. The molecular phylogeny of *Rebutia* (Cactaceae) and its allies demonstrates the influence of paleogeography on the evolution of South American mountain cacti. Am. J. Bot. 94, 1321–1332.

Ronquist, F., Huelsenbeck, 2003. MrBayes3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sang, T., Crawford, D.J., Stuessy, T.F., 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). Am. J. Bot. 84, 1120–1136.

Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am. J. Bot. 94, 275–288.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.

Sun, Y., Skinner, D.Z., Liang, G.H., Hulbert, S.H., 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. Theor. Appl. Genet. 89, 26–32.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts, USA.

Tate, J.A., Simpson, B.B., 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Syst. Bot. 28, 723–737.

Taylor, N.P., 1997. Cactaceae. In: Olfield (Ed.), Cactus and Succulent Plants: Status Survey and Conservation Action Plan. IUCN/SSC. Cactus and Succulent Specialist Group, Gland, Switzerland and Cambridge, pp. 17–20.

Taylor, N., 1999. *Rhipsalis puniceodiscus*. Cactaceae Curti's Bot. Mag. 16, 29–33.

Taylor, N.P., Zappi, D.C., 2004. Cacti of Eastern Brazil. The Royal Botanic Garden, Kew, Richmond, UK.

Templeton, A., 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. Evolution 37, 221–244.

Wallace, R.S., Cota, J.H., 1996. An intron loss in the chloroplast gene *rpoC1* supports a monophyletic origin for the subfamily Cactoideae of the Cactaceae. Curr. Genet. 29, 275–281.

Zappi, D., Aona, L.Y.S., Taylor, N., 2007. Cactaceae. In: Wanderley, M.G.L., Shepherd, G.J., Melhem, T.S., Giulietti, A.M. (Eds.), Flora Fanerogâmica do Estado de São Paulo, vol. 5. Instituto de Botânica, São Paulo, pp. 163–193.