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MOLECULAR PHYLOGENY, EVOLUTION, AND BIOGEOGRAPHY OF SOUTH AMERICAN EPIPHYTIC CACTI

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Epiphytes represent an important element of the tropical flora and are widely distributed across vascular plants. Despite this diversity, however, little is yet known of the evolutionary history, habitat preference, morphological diversity, and biogeographical patterns of epiphytes as a whole. Approximately 10% of cacti are epiphytes inhabiting humid regions, and *Rhipsalis* represents the largest genus of these. Here we reconstruct relationships among species of the genus *Rhipsalis* on the basis of plastid and nuclear DNA markers (*trn*Q-*rps*16, *rpl*32-*trn*L, *psb*A-*trn*H, internal transcribed spacers, and malate synthase) and use them as a basis to study the evolution of habit, key morphological features, and the biogeographical history of the genus. *Rhipsalis* is highly supported as monophyletic, presenting three main lineages. Two lineages are marked by unique floral morphologies and one presents an exclusive stem-shape morphology. In spite of this, neither of these features seems to have been associated with small-scale habit transitions or large-scale transitions through different biogeographical regions. Several lineages of the genus seem to have originated in coastal Brazil and subsequently occupied other tropical forests in South America, North America, Africa, and Asia. These events occurred in relatively recent times, with most of them taking place on terminal branches, thus suggesting recent associations between South American epiphytic flora.

Keywords: Andes, Atlantic forest, Brazil, Cactaceae, *Rhipsalis*.

Online enhancements: appendixes.

Introduction

Epiphytes represent an important element of the tropical flora, being particularly fragile and dependent on the overall maintenance of forests where they occur (Gentry and Dodson 1987). Epiphytism is widely distributed across vascular plants, and 44% of all vascular plant orders contain at least one epiphytic species (Benzing 1987). Research published to date has addressed the evolution of the epiphytic habit in Orchidaceae (Gravendeel et al. 2004; Tsutsumi et al. 2007; Yukawa and Stern 2002), ferns, and lycophytes (Wikström et al. 1999; Tsutsumi and Kato 2006; Dubuisson et al. 2009), Melastomataceae (Clausing and Renner 2001), and Bromeliaceae (Crayn et al. 2004). However, little is still known regarding the potential drivers that triggered the diversification of epiphytes as a whole. Studies combining phylogenetic data, habitat preference, morphological diversity, and biogeography of epiphytes are needed in order to better understand the processes involved in the evolution of these plants.

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Approximately 10% of all cacti are epiphytes that inhabit humid regions (Barthlott 1983). All obligate epiphytic cacti belong to the subfamily Cactoideae and are mainly assigned to two distinct tribes, Hylocereeae and Rhipsalideae (Nyffeler 2002; Hunt et al. 2006). Hylocereeae includes many facultative epiphytes or secondary hemiepiphytes, comprising *Hylocereus* (A. Berger) Britton & Rose, *Epiphyllum* Haw., *Pseudorhipsalis* Britton & Rose, *Disocactus* Lindl., *Selenicereus* (A. Berger) Britton & Rose, and *Weberocereus* Britton & Rose (Wallace and Gibson 2002; Bauer 2003). Rhipsalideae contains four genera, mainly of holoepiphytes: *Hatiora* Britton & Rose, *Rhipsalis* Gaertn., *Lepismium* Pfeiff., and *Schlumbergera* Lem. (Barthlott 1983). A recent phylogenetic analysis of Rhipsalideae based on plastid DNA regions (*psb*A-*trn*H, *trn*Q-*rps*16, *rpl*32-*trn*L) and nuclear internal transcribed spacers (ITSs) supported the monophyly of Rhipsalideae and the major lineages within the tribe (Calvente 2010; Calvente et al. 2011). In particular, it showed that the genera *Rhipsalis* and *Lepismium* are monophyletic, while *Hatiora* and *Schlumbergera* are paraphyletic. *Rhipsalis* represents the largest genus of epiphytic cacti, with five subgenera (*Calamorhipsalis*, *Epallagogonium*, *Erythrorhipsalis*, *Phyllarthrorhipsalis*, and *Rhipsalis*) and 35 species. This genus also includes epilithic species, which grow on open habitats and rocky outcrops (fig. 1; Hunt et al. 2006).

Several hypotheses have been put forward to explain the evolution of epiphytic cacti. These cacti are thought to have

Fig. 1 Morphological and habit diversity in *Rhipsalis*. *A*, *R. russellii*. *B*, *R. triangularis*. *C*, *R. cereoides*. *D*, *R. olivifera*. *E*, *F*, *R. micrantha*. *G*, *R. cuneata*. *H*, *R. pachyptera*. *I*, *R. pulchra*. *J*, *R. clavata*. *K*, *R. lindbergiana*. *L*, *R. grandiflora*. *M*, *R. floccosa*. *N*, *R. paradoxa*. *O*, *R. puniceodiscus* *P*, *R. neves-armondii*.

evolved from ribbed terrestrial columnar cacti, with the transition being accompanied by several structural modifications such as (1) development of adventitious roots so that stems can remain attached to the host plant; (2) development of leaflike stems, which thus increases stem surface-to-volume ratio (Mauseth 2000); (3) stems with narrower ribs and pith, which decreases the ability for water storage; (4) loss of structures associated with maintenance of an upright position (e.g., thick ribs, conspicuous wood formation, and collenchyma); and (5) reduction or loss of spination, which prevents sunlight from reaching photosynthetic tissues (Wallace and Gibson 2002). All of these modifications are observed in

different degrees in *Rhipsalis*, making this genus particularly interesting for the study of the evolution of epiphytic and epilithic habits and the associated morphological features.

Biogeographical patterns within *Rhipsalis* are also particularly interesting, as the genus comprises taxa with inter- and intracontinental disjunct distributions. The species of Cactaceae (ca. 1800; Nyffeler and Eggli 2010) are endemic to the New World, with the exception of *Rhipsalis baccifera*, which spontaneously occurs in Africa and Asia. Some authors believed that this disjunct distribution resulted from vicariance acting on an ancient Gondwanan distribution (Backeberg 1942; Croizat 1952). However, molecular phylogenetic data indicates that cacti originated during the mid-Tertiary, after the Gondwana split, which allowed for a rapid diversification in the newly developing American deserts (Hershkovitz and Zimmer 1997). In the Americas, cacti are distributed across a wide variety of habitats, from the American coast to the Andes and from deserts to humid evergreen forests. The main centers of diversity and endemism are located in Mexico, the United States, and Brazil (Taylor 1997; Hernández et al. 2001). Among the epiphytic cacti, the tribe Hylocereeae is centered in southern Mexico and Central America and Rhipsalideae is centered in southeastern Brazil. The majority (81%) of species assigned to *Rhipsalis* are endemic to Brazil, with several narrowly endemic taxa. Rhipsalis is one of a few genera of vascular plants that are widely distributed across the tropical regions in which epiphytism is extensively prevalent (Gentry and Dodson 1987). Apart from the disjunct distribution of *R. baccifera* and two other species with wide distribution in South America (*Rhipsalis cereuscula* and *Rhipsalis floccosa*), *Rhipsalis* also presents an intracontinental disjunction between the Brazilian Atlantic forest and the Andean forests. Our molecular phylogenetic study of *Rhipsalis* may shed light on the biogeographical patterns observed in this group today.

Here we reconstruct relationships among species of *Rhipsalis*, using plastid and nuclear DNA markers. The resulting phylogenetic tree is used as basis for the study of the evolution of key morphological features, habit, and biogeographical history, and a brief evaluation of the monophyly of subgenera circumscribed within *Rhipsalis*.

Material and Methods

Taxon Sampling

We sampled 33 of the 37 species currently circumscribed in *Rhipsalis*, including all subgenera and their type species (app. A; app. B in the online edition of the *International Journal of Plant Sciences*); only *Rhipsalis pacheco-leonis* and *Rhipsalis sulcata* (subgenus *Epallagogonium*), *Rhipsalis hoelleri* (subgenus *Calamorhipsalis*), and *Rhipsalis burchellii* (subgenus *Erythrorhipsalis*) were not sampled. Whenever species were morphologically polymorphic, multiple individuals were included in the analysis; this condition was particularly predominant within the subgenus *Phyllarthrorhipsalis*, in which species delimitation is particularly problematic. A total of 71 specimens were sampled (apps. A, B). *Schlumbergera orssichiana* and *Hatiora salicornioides* were defined as an outgroup in all analyses because they were shown to form sister lineages of *Rhipsalis* in a recent phylogeny of tribe Rhipsalideae (Calvente et al. 2011).

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from silica-gel-dried stems using a modified CTAB protocol (Doyle and Doyle 1987). Five molecular markers were selected for this study: the plastid spacers *trn*Q-*rps*16, *rpl*32-*trn*L, and *psb*A-*trn*H; the nuclear ITSs; and the nuclear low-copy malate synthase (MS). Amplification primers used are listed in appendix C in the online edition of the *International Journal of Plant Sciences*. Amplifications of *trn*Q-*rps*16 and *rpl*32-*trn*L followed the procedure outlined by Shaw et al. (2007). Amplifications of *psb*A-*trn*H, ITS, and MS were conducted in 20-μL reactions containing 2 μL of 5X GoTaq buffer, 2 μL of bovine serum albumin (0.4%), 1 μL of 25mM $MgCl_2$, 1 μL of each primer (10 mM), 0.4 μL of GoTaq (Promega, Southampton), 0.4 μL of 10mM dNTPs, 0.8 μL of dimethyl sulfoxide, 0.8 μL of template DNA, and 11.6 μL of water. Polymerase chain reaction (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 28–35 cycles of 94°C for 1 min, 52°–55°C for 1 min, 72°C for 3 min, and a final extension of 72°C for 7 min. MS was initially amplified with the degenerate primers 400f and 943r (Lewis and Doyle 2001), using a touchdown protocol starting at 95°C for 3 min that was followed by 15 cycles of 94°C for 1 min, 52°C (−1°C per cycle) for 2 min, and 72°C for 2 min, followed by 23 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 7 min. Specific primers were designed for *Rhipsalis*, using the sequences obtained with the primers of Lewis and Doyle (2001; see app. C). PCR conditions for amplification with these primers were as follows: 95°C for 3 min, followed by 35 cycles of 94°C for 1 min, 56°C for 2 min, 72°C for 2 min, and a final extension of 72°C for 7 min. Amplification products were purified, using the NucleoSpin Extract II Kit (Macherey-Nagel, Düren) or the QIAquick PCR Purification Kit (QIAGEN, Crawley), following the manufacturers' protocols. Automated sequencing was performed, using the BigDye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster City, CA), and it was run on an ABI 3730 DNA Analyzer at the Jodrell Laboratory or sent to Macrogen (Seoul). GenBank accession numbers are provided in appendix A.

Cloning of the MS amplification products was performed whenever length difference between alleles was detected or when more than 10 base ambiguities were found within a sequence. PCR products of the following species were cloned: *R. baccifera*, *R. cereuscula*, *R. crispata*, *R. mesembryanthemoides*, *R. micrantha*, *R. oblonga*, *R. occidentalis*, *R. teres*, and *R. lindbergiana*. For the cloning procedure, purified PCR products were run on agarose gel and bands were excised and purified, using the QIAquick Gel Extraction Kit (QIAGEN). Ligation and transformation were performed, using the pGEM-T Vector System and JM109 competent cells, following the manufacturer's protocol (Promega, Southampton). Up to 10 colonies per species were selected and used as templates in PCR reactions, with the same primers and con-

ditions as the initial PCR reactions. Likewise, automated sequencing for cloned products followed the same procedure as described above.

Phylogenetic Analyses

Complementary sequences were assembled in Sequencher 3.0 (Gene Codes, Ann Arbor, MI) and aligned manually in MacClade, version 4.08 (Maddison and Maddison 2005). Indels were coded separately. Sequenced regions with ambiguous alignments were excluded. For MS, sequences from clones were examined and compared for each specimen cloned. All nonidentical sequences encountered for every specimen cloned were included as independent terminals in the analyses. Polymorphic characters (found within a single specimen) were excluded from the analyses. All analyses were performed, using the complete matrix, including multiple individuals per species and multiple clones per individual whenever applicable.

Analyses were performed on Bioportal (http://www.bioportal.uio.no) or with a personal computer. Maximum parsimony (MP) analyses were performed in PAUP*, version 4.0b10 (Swofford 2002), using the heuristic search option with 1000 replicates of random stepwise addition (retaining 20 trees at each replicate), tree bisection reconnection branch swapping, and equal weighting of all characters. Maximum likelihood (ML) analyses were run on GARLI-PART, version 0.97 (Zwickl 2006), using three independent searches with five search replicates each. Two partitions were defined, one containing nucleotide data and the other containing gap-coded information. For the nucleotide partition, the best-fit model of nucleotide substitution was determined with ModelTest 3.04 (Posada and Crandall 1998) for two data sets, one composed of plastid (*trn*Q-*rps*16, *rpl*32-*trn*L, *psb*A-*trn*H) and ITS data (plastid/ITS data set) and the other including plastid, ITS, and MS data (plastid/ITS/MS data set). F81 + I + G and K81uf + I + G, respectively, were identified as the most appropriate models of evolution for these data sets. For the gap partition, default settings for the Mk model were employed (Lewis 2001; Zwickl 2010). All three independent ML searches resulted in identical likelihood scores and similar topologies. Bayesian analyses were performed using MrBayes 3.1.1 (Ronquist and Huelsenbeck 2003). Searches were conducted with two independent runs, each with four simultaneous chains. Each Markov chain was initiated with a random tree and run for 10^7 generations and sampled every 100 generations. Likelihood values were monitored graphically to determine stationarity and the appropriate burn-in set of trees. Best-fit models of nucleotide substitutions were estimated separately for each partition (F81 + G for *psb*A-*trn*H, F81 + G for *trn*Q-*rps*16, F81 + I + G for *rpl*32-*trn*L, HKY + I + G for ITS, and HKY + G for MS). Gap-coded information was included in a separate partition for which the standard discrete model was applied (Lewis 2001; Ronquist and Huelsenbeck 2003).

Support was assessed with ML and MP nonparametric bootstrapping and posterior probabilities. Heuristic searches with 1000 MP bootstrap replicates were conducted in PAUP*, using the same parameters as the MP analyses. Two independent searches of 100 ML bootstrap replicates were performed with GARLI-PART, version 0.97 (Zwickl 2006), using the same settings as for the ML analyses. All replicates were used to build a ML bootstrap 50% majority rule consensus tree in PAUP*. Clades with bootstrap support of 50%–74% were considered to be weakly supported, 75%–89% were moderately supported, and 90%–100% were strongly supported. Only clades with posterior probabilities above 0.95 were considered to be strongly supported, as posterior probability and bootstrap measures of support differ (Suzuki et al. 2002; Cummings et al. 2003; Simmons et al. 2004).

Incongruence among data sets was evaluated, using the incongruence length difference (ILD) test (Farris et al. 1994) and the Templeton test (Templeton 1983) as implemented in PAUP*. 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, the MP strict-consensus tree containing branches with bootstrap support above 80% was tested against a rival tree; the reverse approach was also adopted. To avoid "soft incongruence" due to lack of resolution in rival trees, polytomies in rival trees were resolved according to the topology of the test tree.

Habit and Morphological Evolution

Three morphological features of putative importance in the evolution of *Rhipsalis*—flower type, pericarpel position, and stem shape—were selected for ancestral-state reconstructions. Characters were coded as discrete binary (flower type and pericarpel position) or discrete multistate (stem). Flower type was coded as (0) patent (flowers lateral and perpendicular to the stem, frequently with patent or reflexed tepals) or (1) pendent (flowers pendent in relation to the stem, frequently terminal, and with pendent tepals). Pericarpel position was coded as (0) emersed (not inserted in stem) or (1) immersed (inserted in the stem). Stem shape was coded as (0) cylindrical, (1) angular or with narrow wings (<1 cm), or (2) with expanded wings (>1 cm). Habit condition was also reconstructed as one multistate discrete character, as follows: (0) epiphytic, (1) rupicolous, or (2) epiphytic and rupicolous (coded as a separate state to prevent ambiguous coding in ancestral-state reconstruction analyses).

Ancestral-state reconstructions were performed in MacClade 4.08 (Maddison and Maddison 2005), using unordered parsimony and allowing ambiguous reconstructions. Reconstructions were conducted on a simplified version of the Bayesian plastid/ITS/MS combined tree in which selected individuals were trimmed so that a single accession represented each monophyletic species (fig. 2). In the case of nonmonophyletic taxa, all terminals were kept in the simplified tree. Selection and analysis of the reconstructed features were based on specimen observation during extensive herbarium studies and fieldwork, which allowed collection of data from the whole distribution range of the genus.

Ancestral-state reconstructions were also performed, using a Bayesian approach as implemented in the software SIMMAP, version 1.5 (Bollback 2006). Posterior probabilities for ancestral character states were calculated, using 1000 trees sampled from the trees (excluding burn-in) obtained from the Bayesian analysis of the plastid/ITS/MS data set. Prior parameters were calculated, using a two-step approach (Boll-

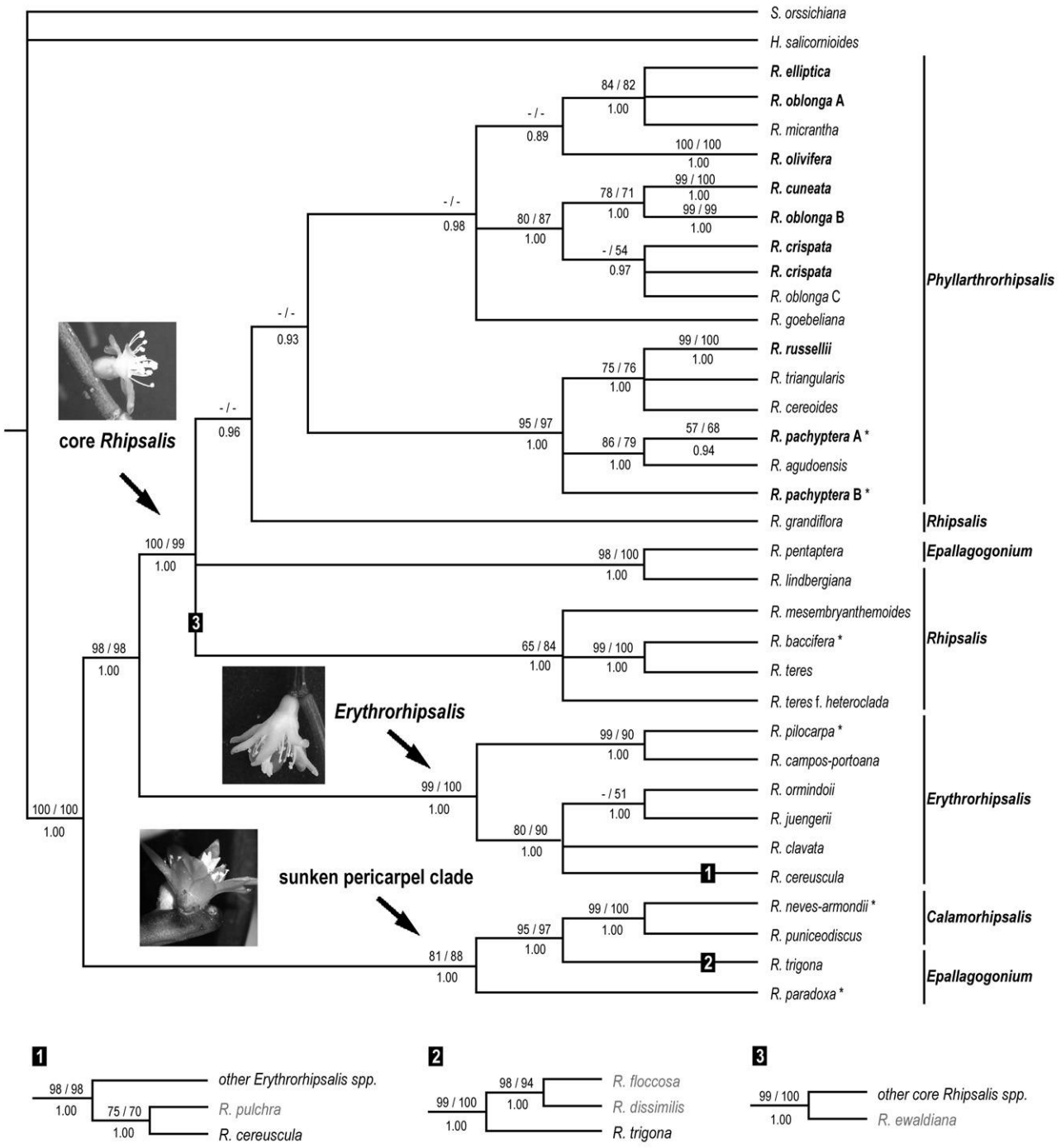

Fig. 2 Phylogenetic tree of *Rhipsalis* derived from the Bayesian analysis of the combined plastid/internal transcribed spacer (ITS)/malate synthase (MS) data set. Maximum parsimony and maximum likelihood bootstrap values are indicated above branches, and posterior probabilities are indicated below branches. Species for which multiple specimens were sampled are highlighted in bold; monophyletic species are represented by a single terminal (others were removed); nonmonophyletic species are indicated by the letter *A*, *B*, or *C* following the species name. Traditionally recognized subgenera are indicated on the right, with type species for each indicated by an asterisk. Clades 1, 2, and 3 were recovered from the analysis of the combined plastid/ITS data set and reconstructs the position of species (in gray) not included in the plastid/ITS/MS data set.

back 2009). First, we performed a Markov chain Monte Carlo analysis in SIMMAP to sample overall rate values (gamma and beta priors). Second, best-fitting values of gamma and beta parameters were estimated, using the posterior distributions of gamma and beta as performed in the R Statistical Package (R Foundation for Statistical Computing; R Development Core Team 2010). For multistate characters, the beta parameter was estimated with an empirical prior.

Biogeography

The geographical distribution of species was determined, using information compiled form herbarium specimens and from Barthlott and Taylor (1995). Five main biogeographical areas were assigned to species as follows: (0) southern Brazil, (1) coastal Brazil (southeastern and northeastern Brazil), (2) southern South America, (3) Andes and Central America, and (4) Central and North America, Africa, and Asia. Ancestral biogeographical areas were reconstructed in MacClade 4.08 (Maddison and Maddison 2005), using the simplified plastid/ITS/MS combined Bayesian topology and the same procedures described above for morphological characters.

Ancestral distributions were also reconstructed, using the statistical dispersal-vicariance analysis method (S-DIVA; Ronquist 1996; Nylander et al. 2008; Harris and Xiang 2009; Yu et al. 2010) implemented in RASP (Yu et al. 2011). Posterior probabilities for ancestral distribution were calculated, using 1000 trees sampled from the trees (excluding burn-in) obtained in the plastid/ITS/MS combined Bayesian analysis and the final Bayesian 50% major rule consensus tree topology. Polytomies in the Bayesian consensus tree (which are not accepted by RASP) were resolved following two procedures: (1) taxa in polytomies with the same distribution were combined into the same clade and (2) species with unknown distribution (*R. goebeliana*) were excluded.

Results

Phylogenetic Analyses

Sequences for 71 ingroup and outgroup terminals (35 species) were generated for each of the plastid markers (*psb*A-*trn*H, *trn*Q-*rps*16, and *rpl*32-*trn*L) and ITS. We were unable to obtain MS sequences for *R. pulchra*, *R. floccosa*, *R. dissimilis*, and *R. ewaldiana*. The MS data set and the final combined plastid/ITS/MS data set included 65 terminals (31 species). The sizes of individual matrixes and the variation obtained in each data set are presented in table 1.

The *psb*A-*trn*H data set included 295 bp, of which 10.8% were potentially parsimony informative; the MP analysis of this data set led to 15,442 most parsimonious trees of length 78 (consistency index [CI], 0.78; retention index [RI], 0.93). The *trn*Q-*rps*16 data set included 220 bp, of which 3.6% were potentially parsimony informative; the MP analysis of this data set led to 5381 most parsimonious trees of length 27 (CI, 0.90; RI, 0.96). The *rpl*32-*trn*L data set included 1165 bp, of which 6.0% were potentially parsimony informative; the MP analysis of this data set led to 249 most parsimonious trees of length 186 (CI, 0.70; RI, 0.93). The ITS data set included 651 bp, of which 4.6% were potentially parsimony informative. The MP analysis of the ITS data set led to 7696 most parsimonious trees of length 87 (CI, 0.52; RI, 0.86).

In the MS data set, nine species presented sequence ambiguities that suggested allelic polymorphism and thus were cloned for verification. In the cases of *R. micrantha* and *R. crispata*, a high number of polymorphic sites were detected (up to 28). In the cases of *R. baccifera*, *R. cereuscula*, *R. mesembryanthemoides*, *R. oblonga*, *R. occidentalis*, *R. teres*, and *R. lindbergiana*, differences in sequence size (indels of 1–8 bp) were detected. No more than two divergent sequences were ever found for the same specimen. Two terminals for each cloned plant specimen, corresponding to the two divergent sequences found for each, were included in the analyses. Although several specimens and the infraspecific taxa of *Rhipsalis micrantha* were sequenced for plastid markers and ITSs (app. A), a single cloned specimen of *R. micrantha* was included in the MS data set given that MS sequences obtained for different specimen of this species were all identical. The final MS matrix contained 1195 bp, of which 7.3% were potentially parsimony informative. The parsimony analysis of the MS data set produced 15,180 most parsimonious trees of length 308 (CI, 0.80; RI, 0.90).

Two total-evidence phylogenetic analyses were conducted. The first was based on plastid (*psb*A-*trn*H, *trn*Q-*rps*16, and *rpl*32-*trn*L) and ITS sequence data for 71 terminals (the plastid/ITS data set), and the second was based on the plastid and ITS (as above) sequences as well as MS sequences (the plastid/ITS/MS data set) for 65 terminals. Both data sets were tested for topological incongruence. The ILD test did not indicate significant incongruence among each individual plastid data set ($P = 0.733$), whereas the plastid and ITS data sets were shown to be significantly incongruent ($P = 0.001$). The Templeton test was used to further examine incongruence between plastid and ITS topologies. Tree score differences were nonsignificant when the ITS topology was tested as the rival topology (test tree score: 334, rival tree score: 340; $P = 0.0703$). However, the plastid topology as rival was shown to have the best fit to the ITS data set (test tree score: 211, rival tree score: 139; $P = 0.0023$), as the ITS consensus topology provided very low resolution. Visual inspection did not indicate sup-

Table 1

Characteristics of Each Partition and Information Derived from the Maximum Parsimony Analyses of the Individual and Combined Data Sets

	*psb*A-*trn*H	*trn*Q-*rps*16	*rpl*32-*trn*L	ITS	MS	Plastid/ITS	Plastid/ITS/MS
No. terminals	71	71	71	71	65	71	65
Aligned matrix, bp	295	220	1165	651	1195	2331	3526
Potentially informative sites, no. (%)	32 (10.8)	8 (3.6)	70 (6.0)	30 (4.6)	87 (7.3)	140 (6.0)	227 (6.4)
No. trees retained	15,442	5381	249	7696	15,180	12,121	3450
Length of best trees, steps	78	27	186	87	308	410	719
CI	.78	.90	.70	.52	.80	.59	.65
RI	.93	.96	.93	.86	.90	.87	.86

Note. CI, consistency index, calculated excluding uninformative characters; ITS, internal transcribed spacer; MS, malate synthase; RI, retention index.

ported topological incongruence between the ITS and plastid topologies; it did confirm, however, the major difference in the amount of resolution provided by each data set. Furthermore, preliminary phylogenetic analyses of the plastid and plastid/ITS data sets resulted in the same topological structure, but the plastid/ITS data set offered increased support for several branches (data not shown). These results suggest that the incongruence detected by the ILD between the plastid and the ITS partitions likely represents "soft incongruence" due to lack of resolution in one individual partition.

The ILD test also indicated significant incongruence between the *psb*A-*trn*H, *trn*Q-*rps*16, *rpl*32-*trn*L, ITS, and MS data sets ($P = 0.001$). The Templeton test was also used to further examine the incongruence between the plastid and the MS data sets and between the ITS and the MS data sets. Testing between the MS and the plastid data sets indicated a nonsignificant result (test tree MS score: 335, rival tree plastid score: 344; $P = 0.0639$; test tree plastid score: 322, rival tree MS score: 324; $P = 1$). Testing between the ITS and the MS data sets, using the Templeton test, showed results that were similar to those of the comparison of the ITS and the plastid data sets. Tree score differences were nonsignificant when the ITS topology was tested as the rival topology (test tree score: 335, rival tree score: 338; $P = 0.2188$). However, the MS topology as rival was significantly the best fit to the ITS data set (test tree score: 208, rival tree score: 167; $P = 0.0213$), as the ITS consensus topology provided very low resolution. A visual comparison of all of the topologies obtained did not indicate any major and supported topological incongruence between them. However, it did indicate differences in the amount of resolution provided by each data set, causing the "soft incongruence" detected by the ILD test.

The plastid/ITS data set included 2331 bp, of which 6% were potentially parsimony informative. The MP analysis of this data set produced 12,121 most parsimonious trees of length 410 (CI, 0.59; RI, 0.87), and the ML search resulted in one topology of $-\ln L 6072.6036$. The plastid/ITS/MS data set included 3526 bp, of which 6.4% were potentially parsimony informative. The MP analysis of this data set resulted in 3450 most parsimonious trees of length 719 (CI, 0.65; RI, 0.86). The ML search led to two topologies of $-\ln L 10,074.0531$. Results derived from the Bayesian analyses were congruent with those from the MP and ML analyses, for both data sets.

The Bayesian analysis of the plastid/ITS/MS data set recovered a monophyletic *Rhipsalis* containing three highly supported clades (fig. 2). The first clade, here named the "sunken pericarpel clade," includes species of the subgenera *Calamorhipsalis* and most species from *Epallagogonium*, while the second clade comprises all species of the subgenus *Erythrorhipsalis*. The third clade, here named "core *Rhipsalis*," includes all species of the subgenera *Phyllarthrorhipsalis* and *Rhipsalis* and one species belonging to *Epallagogonium*. Only two clades of the core *Rhipsalis* lineage are supported by high posterior probabilities and bootstrap values, (1) (*R. pentaptera* + *R. lindbergiana*) and, (2) [(*R. russellii* + *R. triangularis* + *R. cereoides*)(*R. pachyptera* A + *R. agudoensis*) *R. pachyptera*)]. All species with multiple specimens that were included in the analysis were monophyletic or unresolved within a larger polytomy, except for *R. oblonga* and *R. pachyptera* (fig. 2).

The relationships recovered from the analyses of the plastid/ITS data set are similar to the results obtained from the analysis of the plastid/ITS/MS data set. Although the plastid/ITS data set included four species that were not sampled for MS (*R. pulchra*, *R. floccosa*, *R. dissimilis*, and *R. ewaldiana*), the positions of these species do not create conflict with the overall topology obtained with the plastid/ITS/MS data set (fig. 2). The analysis of the plastid/ITS data set shows that *R. pulchra* is sister to *R. cereuscula*, the clade (*R. floccosa* + *R. dissimilis*) is sister to *R. trigona*, and *R. ewaldiana* is sister to the core *Rhipsalis* clade (fig. 2).

Habit and Morphological Evolution

Ancestral reconstructions of the habit condition and three selected morphological traits (flower type, pericarpel position, and stem shape) are presented in figure 3. The ancestral flower-type condition is patent flowers, with a single evolution of pendent flowers in *Erythrorhipsalis* on the basis of both parsimony and Bayesian reconstructions (figs. 2, 3). The ancestral condition of pericarpel position for *Rhipsalis* is uncertain, according to parsimony reconstruction. The Bayesian reconstruction indicates a slightly higher probability for an emersed pericarpel as the ancestral condition for the group (fig. 3). However, the ancestral condition for the first two lineages within *Rhipsalis* is evident for both parsimony and Bayesian reconstructions. An immersed pericarpel is the ancestral condition for the sunken pericarpel clade, and an emersed pericarpel is the ancestral condition for the core Rhipsalis + *Erythrorhipsalis* clade (figs. 2, 3). Cylindrical stems are the ancestral condition for *Rhipsalis* on the basis of the Bayesian reconstruction, while the parsimony reconstruction is ambiguous, with both cylindrical stems and narrow wing states being probable. In both the parsimony and the Bayesian reconstructions, the character state of being angular or with narrow wings (<1 cm) seems to have evolved multiple times, while stems with expanded wings (>1 cm) evolved a single time within *Phyllarthrorhipsalis*, with shifts to the state of being angular or with narrow wings in *R. cereoides* and *R. micrantha* (fig. 3).

Our optimizations indicate that the epiphytic condition is ancestral within *Rhipsalis*, with at least five independent shifts to the rupicolous and epiphytic conditions (fig. 3). It is uncertain in both reconstruction methods whether one or two independent transitions to an exclusive rupicolous habit occurred in *R. triangularis* and *R. cereoides* or whether one transition to this condition occurred within the clade (*R. russellii* + *R. triangularis* + *R. cereoides*), followed by a subsequent reversal to the rupicolous and epiphytic conditions in *R. russellii*.

Biogeography

Reconstructions of ancestral areas that were made using MacClade indicated that the ancestor of *Rhipsalis* occurred in coastal Brazil, and this was followed by multiple independent range expansions into southern Brazil (*R. elliptica*, *R. pachyptera*, *R. agudoensis*, *R. teres*, *R. pilocarpa*, *R. camposportoana*, *R. cereuscula*, *R. puniceodiscus*, *R. trigona*, and *R. paradoxa*) and two expansions to southern South America (*R. cereuscula*, *R. baccifera*; fig. 3). We also observed two in-

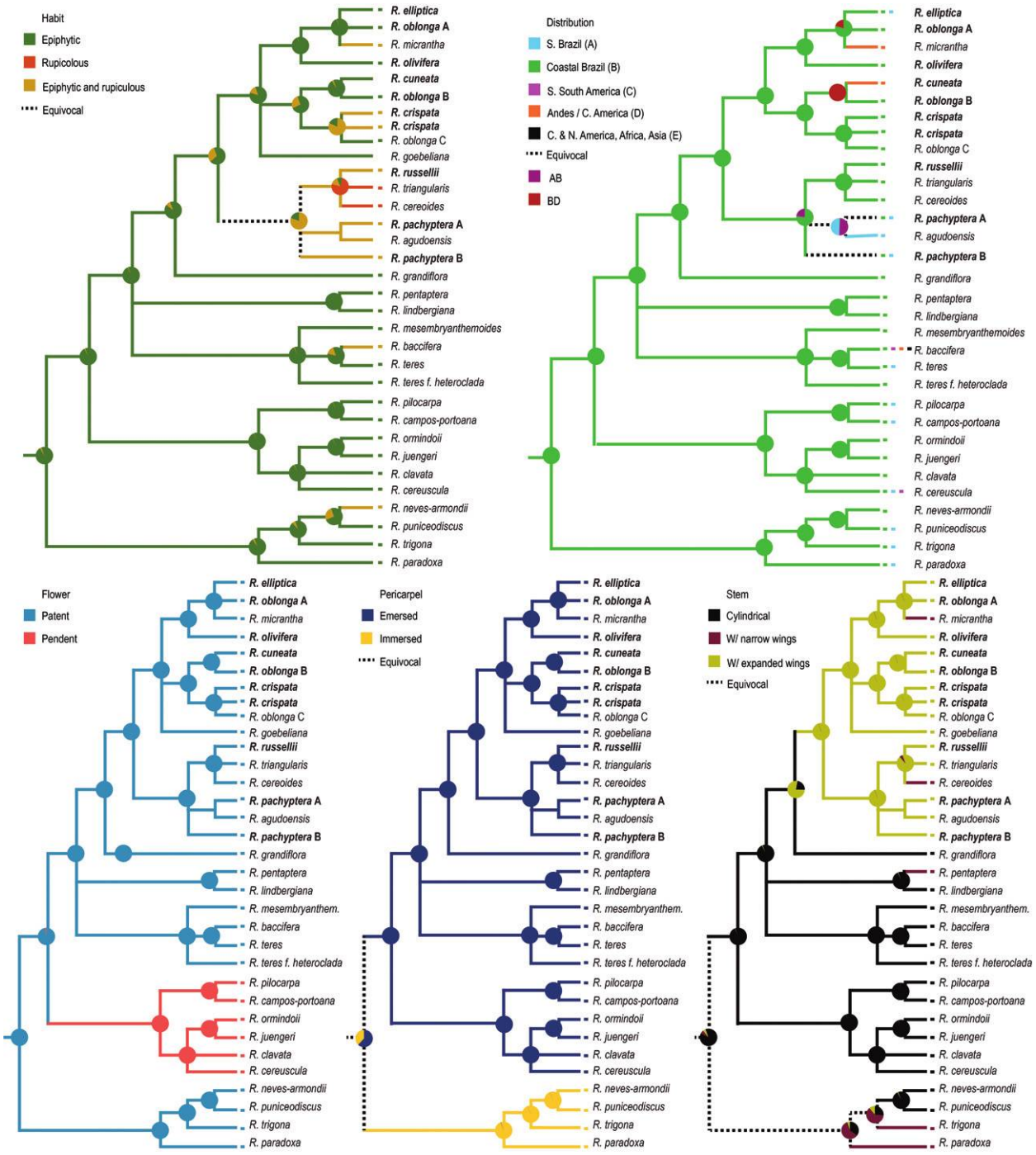

Fig. 3 Ancestral-state reconstructions of selected morphological traits (flower type, pericarpel position, and stem shape), habit, and biogeographical areas of *Rhipsalis*. Branches are colored according to the parsimony reconstruction, and the pie charts on the nodes show the results of the Bayesian reconstruction. Colored dots next to species names indicate the state(s) assigned to this species (missing data are indicated by the absence of a dot). Results of the ancestral areas that were reconstructed using the statistical dispersal-vicariance analysis (S-DIVA; see text) are indicated by pie charts. *Rhipsalis goebeliana* was excluded from the biogeographical reconstruction because its natural distribution is unknown. *Rhipsalis mesembryanthemoides* is abbreviated as *R. mesembryanthem.* on the flower, pericarpel, and stem optimizations.

dependent transitions and one expansion to the Andes and Central America (*R. micrantha*, *R. cuneata* and *R. baccifera*) and one expansion to the region including Central and North America, Africa, and Asia (*R. baccifera*).

Examination of the ancestral distribution with S-DIVA resulted in an optimal reconstruction requiring dispersal, expansion, and vicariance events. Most events were assigned to terminal branches, but a few events in more internal

branches are also inferred. Coastal Brazil is reconstructed as the ancestral area of *Rhipsalis*, the sunken pericarpel clade, *Erythrorhipsalis*, and the core *Rhipsalis* clade (fig. 3). Several range expansions to southern Brazil are indicated in terminal branches. The ancestor of *R. pachyptera* and *R. agudoensis* occurred either in southern Brazil and coastal Brazil or only in southern Brazil, having dispersed to this area from coastal Brazil. One expansion from coastal Brazil to the Andes and Central America is observed in the ancestor of *R. cuneata* and *R. oblonga* B, and this was followed by a vicariance event that separated these two species into each of these areas. One dispersal to the Andes and Central America is observed in *R. micrantha*, range expansions to southern South America are observed in *R. cereuscula* and *R. baccifera*, and one expansion to the region including Central and North America, Africa, and Asia is observed in *R. baccifera*.

Discussion

Phylogenetic Analyses

Of all of the molecular markers analyzed, *psb*A-*trn*H presented the greatest percentage of potentially informative sites (table 1), while MS and *rpl*32-*trn*L presented the greatest absolute number of informative sites. ITS, on the other hand, presented the highest amount of homoplasy (CI, 0.52; RI, 0.86), resulting in a poorly resolved topology (tree not shown). The individual topologies resulting from the plastid, ITS, and MS data sets were generally congruent.

Rhipsalis is highly supported as monophyletic, but not all subgenera within *Rhipsalis* are monophyletic; subgenera *Epallagogonium* and *Rhipsalis* are paraphyletic (fig. 2). Furthermore, three main lineages are recognized within *Rhipsalis*: the sunken pericarpel clade, *Erythrorhipsalis*, and the core *Rhipsalis* clade (fig. 2). These results are congruent with a previous molecular phylogeny of Rhipsalideae that recovered a monophyletic *Rhipsalis* containing two main clades, core *Rhipsalis* and *Erythrorhipsalis* (Calvente et al. 2011). This previous work, however, did not recover a monophyletic sunken pericarpel clade; instead, the subgenus *Calamorhipsalis* and *R. paradoxa* formed independent lineages that were sister to the core *Rhipsalis* clade and not to the remaining taxa of the sunken pericarpel clade (supported by only moderate and low bootstrap values in that work). The higher number of markers and more comprehensive sampling within *Rhipsalis* in this study provided additional characters that led to the recovery of a monophyletic sunken pericarpel clade with strong support.

Morphological Evolution

Research on the reproductive biology of rain forest epiphytes indicates that bees are these plants' most common pollinators (van Dulmen 2001). In cacti, morphological specialization of flowers has been driven by the action of multiple pollinators, mainly bees, moths, hummingbirds, and bats (Pimienta-Barrios and Castillo 2002). Even though pollination studies have never been conducted in *Rhipsalis* specifically, the general flower morphology (actinomorphic nontubular flowers with mostly diurnal anthesis) of representatives of this genus has led researchers to assume that bees were the pollinators of most species in the group. However, results from this study show the existence of three distinct flower morphologies, each corresponding to a single lineage of *Rhipsalis*, which indicates that flower evolution may have played a significant role in *Rhipsalis* diversification. Since differences in flower morphology as size, structure, and color are common morphological adaptations of plants to pollination (Fleming et al. 2001; Fenster et al. 2004; Glover 2007), the observed changes in flower morphology may reflect the existence of distinct groups of pollinators (e.g., different bee groups, butterflies, Diptera, and beetles) and reproduction strategies in the genus.

The ancestral reconstruction of flower type indicated that pendent flowers are exclusive to a single clade, the subgenus *Erythrorhipsalis* (fig. 3). The pendent position of flowers may be a strongly selective trait, as many flower visitors lack the ability to encounter floral resources with this particular flower disposition (Pellmyr 2002; Castellanos et al. 2004). In addition to being pendent, these flowers are generally white and delicate, often with colored filaments (figs. 1, 2), representing a distinct morphological flower type in the group.

The immersed pericarpel is exclusive of the sunken pericarpel clade and represents another flower morphology type within the genus. The immersed pericarpel is a potential adaptation for fruit protection during development (given that the ovary is protected by being immersed in the stem). It is also possible that this trait might provide protection for the flower meristems and areoles (sunken areoles) from desiccation. Marked habitat differences among the sunken pericarpel clade species and other *Rhipsalis* lineage species are not evident, so it is not clear whether this feature is correlated to the environment or to a particular pollinator group or reproductive strategy.

Flowers in the core *Rhipsalis* clade do not present particular morphological features that are as distinctive as those that are characteristic of the other main lineages in *Rhipsalis*. In this group, flowers are patent, as in the sunken pericarpel clade, but with emersed pericarpels, as in subgenus *Erythrorhipsalis*, thus potentially representing in general a plesiomorphic flower type. Flowers in the core *Rhipsalis* clade are generally white, smaller, and more reduced in the number of parts when compared with the flowers of the other two lineages of *Rhipsalis*. Therefore, the unique flower morphology type of this group may be associated with the reduction of flower per se, which also may be related to specific pollinators, reproductive strategies, or other ecological pressures.

Ancestral-state reconstructions of stem shape in the genus revealed that a cylindrical stem is likely to be the ancestral state for the genus, with a shift to stems with expanded wings in subgenus *Phyllarthrorhipsalis*. The presence of stems with narrow wings has occurred independently at least four times throughout the genus.

The evolution of stem shape is probably related to environmental pressures and habitat conditions. Studies in Cactaceae have found a correlation between stem shape, leaf morphology, and habitat (Wallace and Gibson 2002; Griffith 2009). In the subfamily Opuntioideae, flat stems and leaves are found only in areas in which aridity is not an absolute limiting factor; it is possible that the increased surface area of the

flattened stems and the reversal for the production of leaves might represent adaptations to overcome limited photosynthetic capacity (Griffith 2009). Epiphytes must be highly adaptive to challenging environments with varying supplies of water, nutrients, and light. In those environments, survival is dependent on efficient storage capacity, the economical use of water, and a rapid ability to recover from drought-imposed stress (Benzing 1987). Even in the humid tropics, sporadic or seasonal periods of water shortage do occur. This represents one of the main abiotic stress factors to which epiphytes are exposed (Zotz and Thomas 1999). We therefore hypothesized that stem shape is critical for the evolution of successful epiphytic cacti, and we expect this character to be correlated with the occupation of different habitats. Stems with expanded wings, in particular, can represent an adaptation to shaded environments by increasing light capture. However, it is also possible that wing expansion might increase water storage in xeric environments. Cylindrical stems, on the other hand, are more compact and present a reduced surface area, which reduces water loss through transpiration and is thus more effective in dry environments. However, such correlation was not found in *Rhipsalis*, as both cylindrical and expanded, winged stems were found in epiphytic and rupicolous species.

Ancestral-state reconstructions of habit indicate that the ancestor of *Rhipsalis* was epiphytic. Furthermore, the sister taxa to *Rhipsalis* (i.e., *Hatiora* and *Schlumbergera*; Calvente et al. 2011) are also epiphytic, and thus the evolution of the epiphytic habit occurred earlier in the evolutionary history of Rhipsalideae. However, given that most of the diversification of Rhipsalideae occurred in the Atlantic rain forest of Brazil, it is likely that the evolution of epiphytism happened in this region and was followed by multiple independent transitions to the rupicolous habit. Rupicolous species inhabit gneiss-granitic mountains (inselbergs) that occur within the Atlantic rain forest biome. The vegetation of inselbergs differs markedly from that of their surroundings because of edaphic and microclimatic conditions (Porembski et al. 1998). Several species of *Rhipsalis* that occur in these open and sunny habitats (e.g., *R. pachyptera*, *R. russellii*, and *R. floccosa*) are rupicolous-facultative in inselbergs and are also found as epiphytes in the forested habitats. These species normally occur in more sunny epiphytic environments (e.g., tree canopies), indicating that environmental conditions offered by this microhabitat may somehow be similar to the conditions found in rupicolous habitats. Furthermore, *R. micrantha* also inhabits Andean epiphytic and rupicolous habitats, while *R. baccifera* also occupies epiphytic and rupicolous habitats in Africa, illustrating a connection between epiphytic and rupicolous habitats in other tropical forest formations.

Although flower morphology and stem shape represent the most variable macromorphological features in *Rhipsalis*, neither character's evolution seems to be correlated with habit evolution in the genus. Both cylindrical stems and stems with expanded wings apparently offer advantages in both sunny and shaded environments. Nevertheless, a greater number of species with expanded wings occur in sunnier environments (higher in the canopy or in a rupicolous habitat), indicating that the hypothesized increased ability of stems with expanded wings to capture light may not be critical for the group. Instead, the increased ability to store water is probably a stronger advantage for the species in *Rhipsalis* with expanded wings. Micromorphological features are certainly also associated with habitat distribution within the group. For example, species occurring in sunnier environments are stouter and frequently woodier (A. Calvente, personal observation). Other studies found correlations between leaf anatomical characters and the occupation of epiphytic and rupicolous habitats in the orchid genus *Cymbidium* (Yukawa and Stern 2002), as well as correlations between embryo size and habitat in Orchidaceae in general (Tsutsumi et al. 2007). In Cactaceae, variation in epidermal cells, position of stomata, and distribution of sclerenchyma were also documented for some *Rhipsalis* species (Calvente et al. 2008). Further research on stem anatomy and seed morphology of *Rhipsalis* might bring interesting insights on habit and stem evolution in this genus.

Biogeography

Epiphytism evolved before the split between South America and Africa occurred, but the origin of most modern species diversity likely postdates the mid-Cretaceous diversification of flowering plants (Wikström et al. 1999). Subfamily Cactoideae presumably originated in the central Andes, ~30–20 mya, and likely diversified in parallel with the Andean orogeny (25–20 mya; Hershkowitz and Zimmer 1997; Nyffeler 2002; Edwards et al. 2005). Ancestral reconstruction of biogeographical areas in *Rhipsalis* indicated that the genus originated in coastal Brazil and subsequently diversified and expanded into other biogeographical areas. This finding corroborates the hypothesis that *R. baccifera* reached Africa and Asia by long-distance dispersal.

Two other long-distance dispersal events may have occurred from the Atlantic forest to the Andean forests and Central America: the ancestors of *R. cuneata* and *R. micrantha*, according to the MacClade analysis. Alternatively, S-DIVA analysis indicated that the ancestor of *R. cuneata* and *R. oblonga* B had a wider distribution than its descendants (coastal Brazil and the Andes and Central America), potentially indicating a vicariance event. Because epiphytic diversity is correlated with wet forests (Gentry and Dodson 1987), this scenario would imply that the ancestor of this clade would have had to find a path through wet tropical forests in order to expand its distribution to the west.

One possible hypothesis would explain the western expansion of the ancestor of *R. cuneata* and *R. oblonga* B or a migration route for the ancestor of *R. micrantha*. A link between the Brazilian Páramos (*campos de altitude*) and Andean highland vegetation has been documented (Safford 2007), suggesting a potential migration route through northern Argentina or the Paraguayan lowlands, the highlands of Uruguay, and southern Brazil. Although *R. oblonga* and *R. crispata* do not occur in the Brazilian Páramos or in southern Brazil, these taxa inhabit the marginal highland wet forests that surround this vegetation. Therefore, it is possible that the ancestors of these taxa may have been distributed along this route as well.

An alternative hypothesis would be a migration route to the west though dry forests. The fact that *R. crispata* can occur

farther inland in dryer vegetations (marginal cerrado) is consistent with the hypothesis that the ancestors of *R. cuneata*, *R. oblonga* (B and C), and *R. crispata* were tolerant to drier climates, having reached the rising Bolivian Andean vegetation through expansion to the drier Brazilian quaternary forests and the Chaco region (Pennington et al. 2000).

Analyses indicated that expansions to southern Brazil were also frequent in *Rhipsalis* as a whole. Most species of *Rhipsalis* are not restricted to highland vegetation but are frequent in these areas, commonly found in elevations reaching 2000 m above sea level. Therefore, expansion to southern Brazil would be a natural phenomenon, following similar climatic and environmental conditions. Most of these expansions are likely to be recent, as the dispersals occur in terminal branches. However, transitions in more internal branches and involving closely related taxa also occur, possibly indicating a more ancient link between these areas as well.

Conclusions

This robust phylogeny of *Rhipsalis* provides the basis for the study of the evolution of key morphological features and biogeographical patterns within the genus. Overall, several species of *Rhipsalis* seem to have dispersed or expanded their geographic range from coastal Brazil into southern Brazil or other tropical forests in South America, North America, Africa, and Asia. All of these events occurred relatively recently, thereby suggesting recent associations between southern American epiphytic flora. Despite the apparent contribution of flower morphology and stem shape to *Rhipsalis* diversification, neither seems to have been associated with small-scale habit transitions or large-scale transitions through different biogeographical regions. Therefore, it is possible that other physiological or micromorphological traits may have also played key roles in the diversification of these South American epiphytic cacti.

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Appendix A

Sampling, Vouchers, and GenBank Accession Numbers for Specimens Used in the Phylogenetic Analysis of *Rhipsalis*

Species; voucher, accession numbers *psb*A-*trn*H, *trn*Q-*rps*16, *rpl*32-*trn*L, ITS, MS (not cloned), MS clone 1, MS clone 2.

R. agudoensis; Hoerst-Uebelman 821, living collection (Uhlig-Kakteen), JF700607, JF700750, JF700655, JF700703, JF700534, -, -. ***R. cereoides***; Barros 2302, RJ, Brazil, (RB), HQ727740, HQ727819, HQ727859, HQ727780, JF700535, -, -. ***R. crispata***; Calvente 368, SP, Brazil (SPF), JF700608, JF700751, JF700656, JF700704, JF700536, -, -; Calvente 215, SP, Brazil (SPF), HQ727731, HQ727810, HQ727850, HQ727771, -, JF700537, JF700538; Calvente 366, SP, Brazil (SPF), JF700609, JF700752, JF700657, JF700705, JF700539, -, -; Calvente 365, SP, Brazil (SPF), JF700610, JF700753, JF700658, JF700706, JF700540, -, -. ***R. cuneata***; Aguillar s.n. (Cult. Bauer 105), JF700611, JF700754, JF700659, JF700707, JF700541, -, -; Glatz s.n. (Cult.Bauer 270), JF700612, JF700755, JF700660, JF700708, JF700542, -, -; Ruiz s.n. (Cult.Bauer 343), JF700613, JF700756, JF700661, JF700709, JF700543, -, -; Krahn s.n. (Cult. Bauer 346), JF700614, JF700757, JF700662, JF700710, JF700544, -, -; Calvente 381, Loja, Ecuador (QCNE), JF700615, JF700758, JF700663, JF700711, -, JF700545, -; living collection (Kew 1990–1883), JF700616, JF700759, JF700664, JF700712, JF700546, -, -; Ibisch 93766A (Cult. Bauer 776), JF700617, JF700760, JF700665, JF700713, JF700547, -, -. ***R. elliptica***; Calvente 214, SP, Brazil (SPF), HQ727730, HQ727809, HQ727849, HQ727770, JF700548, -, -; Calvente 96, RJ, Brazil (RUSU), JF700618, JF700761, JF700666, JF700714, JF700549, -, -; Calvente 350, SP, Brazil (SPF), JF700619, JF700762, JF700667, JF700715, JF700550, -, -; Calvente 337, SP, Brazil (SPF), JF700620, JF700763, JF700668, JF700716, -, -, -; Calvente 369, SP, Brazil (SPF), JF700621, JF700764, JF700669, JF700717, JF700551, -, -; Calvente 194, SP, Brazil (SPF), JF700622, JF700765, JF700670, JF700718, JF700552, -, -. ***R. goebeliana***; living collection (Kew 2000–1071), JF700623, JF700766, JF700671, JF700719, JF700553, -, -. ***R. micrantha***; Versieux 442, Puntarenas, Costa Rica (SPF) , JF700624, JF700767, JF700672, JF700720, -, -, -; Calvente 360, El Rodeo, Costa Rica (INBIO), JF700625, JF700768, JF700673, JF700721, -, JF700554, -; Calvente 361, El Rodeo, Costa Rica (INBIO), JF700626, JF700769, JF700674, JF700722, -, JF700555, JF700556; Calvente 388, El Oro, Ecuador (QCNE) , JF700627, JF700770, JF700675, JF700723, -, -, -; Calvente 386, Loja, Ecuador (QCNE), JF700628, JF700771, JF700676, -, -, -, -; Calvente 383, Loja, Ecuador (QCNE), JF700629, JF700772, JF700677, JF700724, JF700557, -, -; Calvente 392, Cañar, Ecuador (QCNE), JF700630, JF700773, JF700678, JF700725, JF700558, -, -; Calvente 396, Cajamarca, Peru (SPF), HQ727736, HQ727815, HQ727855, HQ727776, JF700559, -, -; Calvente 394, Cajamarca, Peru (SPF), JF700631, JF700774, JF700679, JF700726, -, JF700560, JF700561. ***R. oblonga***; Calvente 407, SP, Brazil (SPF), JF700632, JF700775, JF700680, JF700727, -, JF700562, JF700563; Calvente 342, SP, Brazil (SPF), JF700633, JF700776,

JF700681, JF700728, -, JF700564, JF700565; Calvente 202, RJ, Brazil (SPF), JF700634, JF700777, JF700682, JF700729, JF700566, -, -; Calvente 196, RJ, Brazil (SPF), JF700635, JF700778, JF700683, JF700730, JF700567, -, -; Calvente 218, RJ, Brazil (SPF), JF700636, JF700779, JF700684, JF700731, -, JF700568, JF700569; Calvente 245, RJ, Brazil (SPF), JF700637, JF700780, JF700685, JF700732, -, JF700570, JF700571. ***R. olivifera***; Calvente 221, RJ, Brazil (SPF), JF700638, JF700781, JF700686, JF700733, JF700572, -, -; Calvente 151, RJ, Brazil (SPF) , JF700639, JF700782, JF700687, JF700734, JF700573, -, -; Calvente 226, RJ, Brazil (SPF) , HQ727741, HQ727820, HQ727860, HQ727781, JF700574, -, -. ***R. pachyptera***; Calvente 250 RJ, Brazil (SPF), JF700640, JF700783, JF700688, JF700735, JF700575, -, -; Calvente 272, ES, Brazil (SPF), JF700641, JF700784, JF700689, JF700736, JF700576, -, -; Calvente 211, RJ, Brazil (SPF), HQ727732, HQ727811, HQ727851, HQ727772, JF700577, -, -; Calvente 354, SP, Brazil (SPF), JF700642, JF700785, JF700690, JF700737, JF700578, -, -; Calvente 277, ES, Brazil (SPF), JF700643, JF700786, JF700691, JF700738, -, -, -. ***R. russellii***; Calvente 309, BA, Brazil (SPF), JF700644, JF700787, JF700692, JF700739, JF700579, -, -; Calvente 326, ES, Brazil (SPF), JF700645, JF700788, JF700693, JF700740, JF700580, -, -; Calvente 313, BA, Brazil (SPF), HQ727746, HQ727825, HQ727865, HQ727786, JF700581, -, -; Zappi 195, MG, Brazil (K), JF700646, JF700789, JF700694, JF700741, JF700582, -, -. ***R. triangularis***; Calvente 88, RJ, Brazil (RUSU) , JF700647, JF700790, JF700695, JF700742, JF700583, -, -. ***R. neves-armondii***; Versieux 196, ES, Brazil (SPF), HQ727737, HQ727816, HQ727856, HQ727777, JF700584, -, -. ***R. puniceodiscus***; Calvente 177, RJ, Brazil (SPF), HQ727749, HQ727828, HQ727868, HQ727789, JF700585, -, -. ***R. dissimilis***; Calvente 401, PR, Brazil (SPF), HQ727750, HQ727829, HQ727869, HQ727790, -, -, -. ***R. floccosa***; Calvente 276, ES, Brazil (SPF), HQ727748, HQ727827, HQ727867, HQ727788, -, -, -. ***R. paradoxa***; Calvente 145, RJ, Brazil (SPF), HQ727742, HQ727821, HQ727861, HQ727782, JF700586, -, -. ***R. pentaptera***; Calvente 100, RJ, Brazil (SPF), JF700648, JF700791, JF700696, JF700743, JF700587, -, -. **R. trigona**; Calvente 404, SP, Brazil (SPF), HQ727738, HQ727817, HQ727857, HQ727778, JF700588, -, -. ***R. baccifera***; Calvente 379, Loja, Ecuador (QCNE), HQ727744, HQ727823, HQ727863, HQ727784, -, JF700589, JF700590. ***R. ewaldiana***; living collection (Kew 1996–758), JF700649, JF700792, JF700697, JF700744, -, -, -. ***R. grandiflora***; living collection (Kew 1996–540), JF700650, JF700793, JF700698, JF700745, JF700591, -, -. ***R. lindbergiana***; Calvente 161, RJ, Brazil (SPF), HQ727755, HQ727834, HQ727874, HQ727795, -, JF700592, JF700593. ***R. mesembryanthemoides***; Freitas s/n, RJ, Brazil (RB), clone 1, HQ727739, HQ727818, HQ727858, HQ727779, -, JF700594, JF700595. ***R. teres***; Calvente 86, RJ, Brazil (RUSU), JF700651, JF700794, JF700699, JF700746, -, JF700596, -; Calvente 255, SP, Brazil (SPF), HQ727754, HQ727833, HQ727873, HQ727794, -, JF700597, -. ***R. campos-portoana***; living collection (Kew 1996–2332), JF700652, JF700795, JF700700, JF700747, JF700598, -, -. ***R. clavata***; Calvente 240, RJ, Brazil (SPF), HQ727753, HQ727832, HQ727872, HQ727793, JF700599, -, -. ***R. juengeri***; Calvente 266, MG, Brazil (SPF), JF700653, JF700796, JF700701, JF700748, JF700600, -, -. ***R. ormindoi***; Calvente 154, RJ, Brazil (SPF), JF700654, JF700797, JF700702, JF700749, JF700601, -, -. ***R. pulchra***; Calvente 232, RJ, Brazil (SPF), HQ727735, HQ727814, HQ727854, HQ727775, -, -, -. ***R. cereuscula***; living collection (Kew 1991–1439), HQ727765, HQ727844, HQ727882, HQ727805, , JF700602, JF700603. ***R. pilocarpa***; Calvente 357, SP, Brazil (SPF), HQ727745, HQ727824, HQ727864, HQ727785, JF700604, -, -. ***H. salicornioides***; Calvente 239, RJ, Brazil (SPF), HQ727743, HQ727822, HQ727862, HQ727783, JF700605, -, -. ***S. orssichiana***; Freitas 28, SP, Brazil (SPF), HQ727733, HQ727812, HQ727852, HQ727773, JF700606, -, -.

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