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Filogenia molecular, evolução e sistemática de
Rhipsalis (Cactaceae)

Molecular phylogeny, evolution and systematics of
Rhipsalis (Cactaceae)

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Co-orientadora: Daniela Cristina Zappi

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Para o Leo,
por todos os segundos
que passamos juntos
nesta jornada.

"Where shall I begin, please your majesty?" she asked. "Begin at the beginning." The king said very gravely, "and go on till you come to the end: then stop."

Lewis Carroll

(*Alice in the Wonderland*, cap. 12)

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A FAMÍLIA CACTACEAE

Cactaceae compreende 124 gêneros e 1.438 espécies distribuídas no novo mundo (Hunt et al. 2006), exceto por *Rhipsalis baccifera* (Mill.) Stearn, cuja distribuição chega até a África e Ásia. Nas Américas, as espécies ocorrem desde o Canadá até a Patagônia, e entre a ilha de Fernando de Noronha (a leste) até o arquipélago de Galápagos (a oeste). Os principais centros de diversidade e endemismo dessa família estão localizados no México e Sudoeste dos Estados Unidos, a região central da cordilheira dos Andes especialmente o Peru e a Bolívia) e o leste do Brasil (Taylor, 1997; Taylor & Zappi 2004). A distribuição do grupo compreende todos os tipos de habitats, ocorrendo desde formações costeiras até 4.500 m s.n.m. na região central Andina do Peru e no Chile (Barthlott & Hunt, 1993).

Numerosas características morfológicas e moleculares sustentam o posicionamento das Cactaceae na Ordem Caryophyllales. Em particular, a presença de um embrião curvo circundado por perisperma na semente, betalainas formando pigmentos vermelhos e amarelos (exclusivos da ordem), e elementos de tubo crivado com um anel de filamentos proteináceos e um cristalóide central angular (Judd et al., 1999). Além disso, as Caryophyllales são comumente herbáceas e suculentas, possuindo o metabolismo CAM e nectários na base adaxial dos estames (Stevens, 2001-2009).

O monofiletismo das Cactaceae e sua divisão em três subfamílias – Pereskioideae, Opuntioideae e Cactoideae – é fortemente sustentado por características morfológicas e moleculares (Nyffeler, 2002; Wallace & Gibson, 2002). Entre as características morfológicas que sustentam o monofiletismo do grupo estão: (1) os meristemas axilares diferenciados em aréolas produzindo espinhos, cerdas, tricomas, flores, frutos e ramos novos; (2) o meristema apical organizado em quatro zonas distintas; e (3) as flores com ovário ínfero receptacular (Boke, 1941; Gibson & Nobel, 1986). Outras características marcantes são as folhas reduzidas ou ausentes, os espinhos abundantes, o caule verde fotossintetizante, que permanece ativo por muitos anos até a formação da periderme, e a presença do parênquima armazenador de água no córtex e medula (Gibson & Nobel, 1986; Nyffeler, 2002).

As diversas especializações para a economia de água, associadas à alta diversidade de formas e hábitos do grupo, contribuíram para que representantes de Cactaceae consigam sobreviver em uma ampla gama de condições climáticas e ecológicas (Gibson & Nobel, 1986; Taylor, 1997). Muitas vezes, espécies de Cactaceae são pioneiras, dominantes ou co-dominantes nos habitats onde ocorrem, constituindo um elemento de grande importância para a sobrevivência de polinizadores e dispersores nesses ambientes (Taylor & Zappi, 2004).

A família apresenta uma considerável importância econômica, com destaque para seu valor ornamental, já que espécies de Cactaceae são cultivadas, colecionadas e comercializadas em diversas partes do mundo. Além disso, muitas espécies são utilizadas como forragem, na alimentação (caules, folhas e frutos) e medicina, entre outros (Barthlott & Hunt, 1993; Hollis & Scheinvar, 1995; Taylor, 1997; Anderson, 2001; Andrade et al., 2006).

Apesar da extensa literatura proveniente da horticultura de cactos e da existência de muitas sociedades e revistas que servem a este hobby, poucos gêneros foram abordados em tratamentos taxonômicos formais (27-29 gêneros, de um total de 124; Hunt et al., 2006). Além disso, a natureza espinhosa e succulenta dos cactos faz com que seus representantes sejam pouco coletados e mal representados em herbários, demandando um maior investimento em estudos de campo (Hunt, 1989).

No Brasil, as Cactaceae estão representadas por 37 gêneros, representando 30% das espécies da família e as três subfamílias de Cactaceae atualmente reconhecidas: Opuntioideae, Pereskioideae e Cactoideae. As espécies ocorrem em ambientes diversos, como o Cerrado, a Caatinga e a Floresta Atlântica (Taylor & Zappi, 2004). O leste do Brasil, em particular, inclui uma alta diversidade de espécies endêmicas; estima-se que 74% do total das espécies da família ali conhecidas sejam endêmicas da região (Taylor & Zappi, 2004). No entanto, as populações de cactos desta região vêm sofrendo degradação acentuada, especialmente em virtude do desmatamento, desenvolvimento agrícola, urbanização, coleta para exploração comercial e mineração (Taylor, 1997). Esta interferência humana tem provocado a rápida diminuição do habitat de espécies endêmicas de Cactaceae, fazendo com que

os estudos sobre a taxonomia, biologia e evolução do grupo que são críticos para o estabelecimento de estratégias para a conservação das espécies tornem-se urgentes.

As ameaças à sobrevivência de espécies de Cactaceae atinge toda a família principalmente em decorrência do alto grau de endemismo das espécies, da destruição do hábitat das espécies e ao extrativismo de representantes ornamentais. Essas graves ameaças levaram à um controle da comercialização e intercâmbio de representantes de Cactaceae segundo a regulamentação dos apêndices I e II da CITES (do inglês “Convention of International Trade in Endangered Species”). O CITES é um acordo internacional entre governos que foi criado para garantir que o comércio e troca de espécimes silvestres não ameace a sua sobrevivência. Esse acordo foi implementado em 1975 e sua adesão ocorre de forma voluntária pelas nações participantes. O Brasil participa da implementação do CITES desde 04/11/1975 e designou o IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) para a administração do seu sistema de licenciamento no País. Nesse sistema, as espécies que necessitam de proteção são listadas em três apêndices, da seguinte maneira: (1) Apêndice 1: inclui as espécies ameaçadas de extinção para as quais o comércio e troca são permitidos somente em circunstâncias especiais; (2) Apêndice 2: inclui espécies não necessariamente ameaçadas de extinção mas para as quais o comércio e troca devem ser controlados para evitar a utilização incompatível com a sua sobrevivência; e (3) Apêndice 3: contém espécies protegidas em pelo menos um país. Os espécimes de uma espécie listada nos apêndices do CITES podem ser exportados ou importados somente após a obtenção de documentação apropriada (CITES, 2009). O comércio e a troca de espécies de Rhipsalideae, assim como a família Cactaceae como um todo, devem seguir as regras dispostas no apêndice 2.

AS FILOGENIAS DE CACTACEAE

Nas primeiras classificações de Cactaceae que incorporaram o componente filogenético houve o estabelecimento de relações de parentesco entre as linhagens com base na morfologia (Metzing & Kiesling, 2008). Entretanto, nenhuma dessas classificações utilizou a metodologia cladística que só foi incorporada nos estudos com

a família a partir da década de 80, com base em caracteres morfológicos. Porém, esses estudos não incluíram representantes da família como um todo, mas focaram em grupos individuais, em particular: os gêneros *Coryphantha* e *Melocactus* (Zimmerman, 1985; Taylor, 1991) e a tribo Cereeae (Taylor & Zappi, 1989). Além desses, Terrazas & Arias (2003) produziram uma análise cladística para a subfamília Cactoideae com base nos numerosos trabalhos que detalharam a morfologia interna e externa de Cactaceae ao longo dos anos.

Os primeiros trabalhos com Cactaceae que utilizaram caracteres moleculares para o estabelecimento de parentesco entre taxa empregaram sítios de restrição do DNA do cloroplasto para investigar populações, espécies ou gêneros (Wallace, 1995). O uso de seqüências de DNA para a reconstrução filogenética em Cactaceae iniciou com o teste do monofiletismo da subfamília Cactoideae, utilizando dados dos marcadores de cloroplasto *rpoC1*, *trnT-trnL* e *rp/16* (Wallace & Cota, 1996; Butterworth et al., 2002; Applequist & Wallace, 2002). Posteriormente, a filogenia da família como um todo foi reconstruída com base em seqüências combinadas dos marcadores de cloroplasto *trnK/matK* e *trnL-F* (Nyffeler, 2002). Esse estudo reconheceu o monofiletismo das Cactaceae e das subfamílias Opuntioideae e Cactoideae, estabelecendo o parafiletismo da subfamília Pereskioideae. Todavia, o parentesco entre representantes de Pereskioideae foi investigado posteriormente em um estudo que incluiu uma amostragem mais extensa e marcadores de cloroplasto (*psbA-trnH*, *trnK-matK*, *rbcL*), mitocôndria (*cox3*) e núcleo (*phyC*) (Edwards et al., 2005). Este trabalho corroborou o parafiletismo de Pereskioideae e definiu as duas primeiras linhagens que divergiram em Cactaceae: (1) “Rodocactus”, a primeira linhagem a divergir na família; e (2) “Eupereskia”, irmã das demais Cactaceae exceto “Rhodocactus”. Uma filogenia molecular de Pereskioideae foi produzida paralelamente utilizando dois marcadores de cloroplasto (*psbA-trnH* e *rp/16*), na qual a subfamília emergiu como monofilética, no entanto com baixa sustentação estatística (Butterworth & Wallace, 2005).

Mais recentemente, a filogenia de Opuntioideae foi reconstruída com base em uma amostragem extensa da subfamília e marcadores de cloroplasto (*trnL-F*) e um nuclear (ITS) (Griffith & Porter, 2009). Esse estudo identificou duas linhagens principais em Opuntioideae: (1) linhagem incluindo as Opuntioideae de caule cilíndrico, e (2)

linhagem incluindo as Opuntioideae de caule aplanado, demonstrando o valor taxonômico do caráter "forma do caule" para a taxonomia de Opuntioideae.

Outras filogenias moleculares foram produzidas para gêneros específicos de Cactoideae, especificamente: (1) *Lophocereus*, com base nos marcadores *trnL-F*, *trnC-trnD* e *trnS-trnfM* (Hartmann et al., 2002); (2) *Pachycereus*, com base nos marcadores *rpl16*, *trnL-F* e ITS (Arias et al., 2003); (3) *Mammillaria*, com base nos marcadores *rpl16* e *psbA-trnH* (Butterworth & Wallace, 2004); (4) *Peniocereus*, com base nos marcadores *rpl16* e *trnL-F* (Arias et al., 2005); e, (5) *Rebutia*, com base nos marcadores *atpB-rbcL*, *trnL-F* e *trnK-rps16* (Ritz et al., 2007).

As filogenias de Cactaceae produzidas até o momento utilizaram 11 marcadores de cloroplasto (*rpl16*, *rpoC1*, *trnT-trnL*, *trnL-F*, *psbA-trnH*, *trnK-matK*, *rbcL*, *atpB-rbcL*, *trnL-rps16*, *trnC-trnD* e *trnS-trnfM*), dois nucleares (ITS e *phyC*) e um de mitocôndria (*cox3*). Estes estudos estabeleceram o parentesco entre os grandes grupos e linhagens da família, especialmente em Opuntioideae e Pereskioideae, ressaltando a necessidade de um maior número de estudos em Cactoideae. A subfamília Cactoideae inclui a maior diversidade de cactos da família e um grande número de tribos e gêneros que ainda permanecem pouco investigados.

O EPIFITISMO EM CACTACEAE

Plantas epífitas são aquelas que passam a maior parte de sua vida sobre outras plantas (Benzing, 1987). Essas plantas podem ser classificadas de diversas formas, como por exemplo, quanto à extensão da presença no habitat epifítico, podendo ser epífitos facultativos ou obrigatórios; e quanto à dependência da vegetação suporte, podendo ser parasíticas ou somente epifíticas. Além disso, epífitas também podem ser classificadas como holoepífitas, que são as epífitas verdadeiras que nascem, vivem e morrem no ambiente epifítico; ou podem ser hemiepífitas primárias, que nascem epífitas, porém suas raízes atingem o solo ao longo da vida; ou hemiepífitas secundárias, que germinam no solo e depois se tornam dependentes do habitat epifítico à medida que a sua conexão com o solo se perde (Benzing, 1987).

A história de vida dos epífitos é influenciada por muitas forças seletivas, incluindo a distribuição e estabilidade do substrato e microclima, os quais são tão diversos no habitat epifítico quanto às condições encontradas para a vegetação terrestre. A biologia reprodutiva dessas plantas é muito variada, entretanto as populações epifíticas são, em geral, mais fragmentadas, o que poderia indicar que elas estariam sujeitas a uma estruturação genética especial e, provavelmente, mais propensas a especiação, o que necessita ser melhor esclarecido (Benzing, 1987). Dessa forma, estudos sobre a filogenia das plantas epifitas, a sua preferência de habitat, diversidade morfológica e biogeografia são necessários para uma melhor compreensão dos processos envolvidos na evolução dessa forma de vida particular.

Algumas pesquisas recentes tem analisado alguns grupos de plantas epifitas. O epifitismo em orquídeas foi estudado sob diversos aspectos incluindo a diversidade, a anatomia da folha e a morfologia da semente de espécies epifíticas em comparação com espécies terrestres (Yukawa & Stern, 2002; Gravendeel et al., 2004; Tsutsumi et al. 2007). Além das orquídeas, entre os grupos mais estudados estão as espécies epifíticas de samambaias e licófitas, para as quais filogenias e estudos sobre a evolução do epifitismo e numerosas adaptações morfológicas foram produzidos (Wikström et al., 1999; Tsutsumi & Kato, 2006; Dubuisson et al., 2009). Além desses grupos, as melastomataceas epifíticas também foram estudadas do ponto de vista filogenético e anatômico (Clausing & Renner, 2001). E para as bromeliáceas, diversas evoluções independentes para o epifitismo, bem como para metabolismo CAM foram encontradas (Crayn et al., 2004).

A maioria dos cactos apresentam caules suculentos e habitam regiões áridas e semi-áridas. No entanto, cerca de 10% das espécies de toda a família são adaptadas ao epifitismo em regiões mais úmidas. Essas espécies pertencem à subfamília Cactoideae e formam dois grupos naturais, distintos principalmente por sua morfologia reprodutiva. A delimitação de tais grupos é também sustentada por caracteres moleculares (Barthlott 1983, Nyffeler 2002). O primeiro grupo, a tribo Hylocereeae, inclui muitos epífitos facultativos e hemiepífitos secundários distribuídos principalmente nos gêneros *Hylocereus* (A. Berger) Britton & Rose, *Selenicereus* (A. Berger) Britton & Rose e *Epiphyllum* Haw. Essa tribo apresenta centros de diversidade no sul do México

e América Central e está mais relacionada filogeneticamente com a tribo Pachycereeae, que habita, principalmente, a América do Norte (Barthlott 1983, Nyffeler 2002). O segundo grupo corresponde à tribo Rhipsalideae. Esta tribo contém quatro gêneros de plantas holoepífitas: *Hatiora* Britton & Rose, *Rhipsalis* Gaertn., *Schlumbergera* Lem. e *Lepismium* Pfeiff. (Barthlott, 1983). Essa tribo possui centro de diversidade no sudeste do Brasil e está mais relacionada filogeneticamente com outras tribos sul-americanas, tais como Cereeae, Browningieae e Trichocereae (Barthlott 1983; Nyffeler 2002).

Os mecanismos envolvidos na transição dos cactos do habitat terrestre para o epífito são ainda desconhecidos, porém alguns autores tem especulado algumas alterações morfológicas que poderiam estar relacionadas com esse fenômeno. De acordo com Wallace & Gibson (2002) os cactos epífitos provavelmente evoluíram de cactos colunares com costelas e essa transição foi acompanhada pelas seguintes modificações estruturais: (1) desenvolvimento de raízes adventícias; (2) desenvolvimento de caules em forma de folha, aumentando a razão entre superfície e volume; (3) surgimento de caules com costelas mais estreitas e menor capacidade de estocagem de água; (4) perda da estrutura sólida, como as costelas grossas e a formação conspícua de madeira e colênquima, que está associada à manutenção e suporte da posição ereta; (5) redução ou perda de espinhos que podem ter uma ação bloqueadora dos raios solares, impedindo que eles atinjam os tecidos fotossintetizantes. Todas essas características são observadas nos cactos epífitos e a variação apresentada entre as espécies pode auxiliar na compreensão de quais são exatamente os mecanismos envolvidos na evolução do epífitismo em Cactaceae, que é uma família predominantemente terrestre.

O GÊNERO RHIPSALIS

Rhipsalis Gaertn. inclui cerca de 35 espécies e constitui o maior gênero de cactos epífitos (Hunt et al., 2006). A maioria das espécies de *Rhipsalis* são holoepífitas, entretanto algumas espécies também são rupícolas, crescendo em ambientes abertos e ensolarados, com poucas exclusivas desses ambientes. As espécies de *Rhipsalis* são, em maioria, endêmicas do Brasil (81%) e diversas espécies apresentam uma

distribuição restrita dentro do País. Apenas três espécies de *Rhipsalis* possuem ampla distribuição na América tropical: *Rhipsalis micrantha* (Kunth) DC. (Peru até a Costa Rica), *Rhipsalis floccosa* Salm-Dyck ex Pfeiff. (Paraguai até a Venezuela) e *Rhipsalis baccifera* (em toda a América do Sul, México, Caribe e Flórida nos EUA). *Rhipsalis baccifera* é a única espécie a extrapolar a distribuição americana da família, ocorrendo amplamente em áreas tropicais úmidas do continente Africano e parte do continente Asiático (Barthlott, 1983). De acordo com Gentry & Dodson (1987), existem poucos gêneros de plantas epífitas vasculares que são amplamente distribuídos nas regiões tropicais e *Rhipsalis* está entre eles, em razão da ampla ocorrência de *R. baccifera*.

As espécies de *Rhipsalis* apresentam ramos cilíndricos, angulares ou com duas a sete alas. As flores estão entre as menores da família (geralmente menores que 2 cm de compr.) e são actinomorfas, laterais ou terminais, com tépalas livres, algumas vezes pouco numerosas, reduzidas a 5-6, geralmente alvas e hialinas. Os frutos são bagas suculentas, brancas ou coloridas e uma mesma espécie pode apresentar frutos de cores diferentes (Barthlott, 1983; Barthlott & Taylor, 1995).

Poucos estudos recentes abordaram especificamente o gênero *Rhipsalis*, estando os trabalhos de Löfgren (1915, 1917), Barthlott (1983), Lombardi (1991, 1995), e Barthlott & Taylor (1995) entre os mais importantes. Alguns trabalhos que trataram a família como um todo também são relevantes, pois apresentam dados sobre a morfologia, distribuição geográfica e conservação das espécies de *Rhipsalis* no Brasil. Entre esses trabalhos estão: Scheinvar (1985), Scheinvar et al. (1996), Freitas (1990/1992, 1996, 1997), Taylor & Zappi (2004), Calvente et al. (2005), Calvente & Andreatta (2007), Calvente et al. (2008) e Freitas et al. (2009).

Apesar desses esforços, a identificação de táxons de *Rhipsalis* permanece problemática em razão da plasticidade natural de caracteres morfológicos, falta de informação dos padrões de variação em populações naturais, existência de complexos de espécies e espécies crípticas (Calvente et al., 2005). Em adição, as coleções de herbário de Cactaceae são frequentemente incompletas (faltam características reprodutivas ou informação sobre a variação do caule e padrão de ramificação) e difíceis de ser interpretadas já que características importantes são perdidas durante o

processo de herborização. Todos esses fatores levaram a complicações ao longo da história taxonômica de *Rhipsalis* como um todo.

Espécies de *Rhipsalis* estão distribuídas em cinco subgêneros (Barthlott & Taylor, 1995; Hunt et al, 2006): *Rhipsalis* subg. *Calamorrhopsalis* K. Schum., *R.* subg. *Epallagogonium* K. Schum., *R.* subg. *Erythrorhipsalis* A. Berger, *R.* subg. *Phyllarthrorhipsalis* Buxb., e *R.* subg. *Rhipsalis*. Entre os subgêneros, *Phyllarthrorhipsalis* (13 spp.) é o mais diverso. Nesse subgênero ocorrem os maiores problemas de delimitação específica, o que torna necessária a realização de estudos que avaliem a fundo a circunscrição dessas espécies. *Phyllarthrorhipsalis* é formado por táxons com segmento aplanados ou com 3 a 5 alas que estão divididos em dois subgrupos (Barthlott & Taylor, 1995). O primeiro deles, referido como “grupo *Rhipsalis crispata*”, é formado por oito espécies endêmicas do Brasil, de ocorrência predominantemente na Floresta Atlântica, indo desde o Nordeste até o Sul do país. As espécies desse grupo possuem plântulas com segmentos primários aplanados e segmentos maduros com aréolas com crescimento comumente indeterminado, florescendo repetidamente em algumas espécies (Barthlott & Taylor 1995). O segundo subgrupo, referido como “grupo *Rhipsalis micrantha*”, é formado por quatro espécies com distribuição predominantemente andina, ocorrendo predominantemente nas Florestas Nebulares (Bosques Nublados, Yungas) com espécies morfológicamente muito similares às espécies do grupo anterior. No entanto, pouco se conhece sobre as espécies deste subgrupo, as quais necessitam de estudos morfológicos adicionais visando aprimorar a circunscrição das espécies. Por exemplo, as posições infragenéricas nesse subgrupo são ainda incertas e precisam ser resolvidas (Barthlott & Taylor, 1995).

Em adição aos problemas de delimitação, a disjunção interamericana apresentada por *Phyllarthrorhipsalis* é um ponto adicional que precisa ser melhor investigado. Apesar da semelhança morfológica de alguns dos táxons de *Phyllarthrorhipsalis* andinos com os táxons da Floresta Atlântica, o relacionamento entre eles é ainda desconhecido. Recentemente Bauer (2008), transferiu as espécies do “grupo *Rhipsalis micrantha*” para o subgênero *Rhipsalis*, alegando que esses táxons estariam mais proximamente relacionados a *Rhipsalis baccifera* (subgênero *Rhipsalis*),

que também ocorre na região andina. Essa questão permanece ainda controversa e deve ser melhor investigada.

Por fim, diversas espécies de *Phyllarthrorhopsalis* ocorrem como rupícolas em ambientes abertos e ensolarados. Algumas delas, como *R. pachyptera* Pfeiff. e *R. russellii* Britton & Rose podem ocorrer como epífitas ou rupícolas. Entretanto, pouco se sabe sobre a evolução do epifitismo no grupo e um estudo detalhado da evolução desses táxons pode fornecer elementos para a melhor compreensão da evolução do epifitismo como um todo. Todas as questões taxonômicas, morfológicas, evolutivas e biogeográficas que permanecem pouco elucidadas até o momento apenas poderão ser avaliadas à luz de uma filogenia robusta.

A MORFOLOGIA DE CACTACEAE E DOS CACTOS EPÍFITOS

A grande diversidade de hábitos é característica marcante das Cactaceae, as quais incluem desde pequenos arbustos até grandes árvores, assumindo as mais variadas formas. O hábito das Cactaceae é determinado pela velocidade do crescimento longitudinal em relação ao crescimento lateral do caule primário, pela simetria longitudinal, pelo crescimento secundário dos ramos laterais, pelo tipo e ângulo de ramificação e pela simetria radial ou bilateral do caule (Buxbaum, 1951). Tais fatores são controlados principalmente pela hereditariedade mas, em alguns casos sofrem também a influência de variações ambientais, como disponibilidade de luz ou gravitropismo. Espécies de Cactaceae possuem uma alta plasticidade fenotípica o que, muitas vezes, lhes confere uma ampla variação morfológica. Indivíduos de Cactaceae podem estabelecer-se como epífitos, rupícolas, saxícolas ou geófitos, formando moitas cespitosas, decumbentes, escandentes, eretas e prostradas (Barthlott & Hunt, 1993). Nos grupos de cactos epífitos, além do epifitismo é comum também encontrar formas rupícolas. Dentro do habitat epifítico existe uma grande variedade de fatores ambientais interagindo e acredita-se que isso possa levar a uma estratificação do ambiente, pois algumas espécies são exclusivas em áreas superiores do dossel, enquanto outras ocorrem no sub-bosque.

As raízes das Cactáceas são fibrosas ou tuberosas e os sistemas radiculares são diversos (Barthlott & Hunt, 1993). A maioria dos táxons apresenta longas raízes

horizontais superficiais. Em algumas espécies colunares, esse sistema é combinado com uma raiz central que penetra profundamente no solo. Vários cactos globulares exibem um sistema compacto de raízes laterais, curtas e finas, que permitem a planta absorver a água que escorre de seu próprio corpo vegetativo. Outros, especialmente os cactos geófitos, apresentam uma única raiz pivotante, carnosa, freqüentemente muito maior que o próprio caule da planta. Alguns cactos com hábito escandente desenvolvem raízes de armazenamento subterrâneas, que podem ser solitárias ou múltiplas. Os cactos epífitos podem apresentar um sistema de raízes principal, originário do estágio de plântula, mas, freqüentemente, exibem apenas o sistema de raízes adventícias. O sistema radicular das Cactaceae sofre alterações de acordo com a disponibilidade de água do ambiente (Gibson & Nobel, 1986). Durante períodos de seca as raízes superficiais apresentam poucos ramos laterais com função de absorção de água e quase todo o sistema de raízes é coberto pela periderme. Quando há disponibilidade de água, as raízes se alongam rapidamente, às vezes sem muitas divisões celulares, e a maior umidade do solo hidrata as raízes antigas aumentando o seu poder de condução. Quando a umidade é reduzida, as novas raízes degeneram e caem e a condutância das raízes antigas diminui, fazendo que a planta retorne ao sistema apropriado para as condições de seca.

O caule das Cactaceae pode formar dois tipos de ramos vegetativos: ramos longos e fotossintetizantes e ramos curtos (aréolas). Os ramos longos são freqüentemente segmentados e possuem uma grande variedade de formas: colunares, cilíndricos, globulares, tuberculados, alados, aplanados ou com costelas (Barthlott & Hunt, 1993). Nos cactos epífitos, é comum observar a formação de alas ou ângulos que podem se assemelhar a folhas. Além das formas aladas, são freqüentes as formas cilíndricas extremamente reduzidas. O tipo de ramificação e disposição dos segmentos do caule dos epífitos, principalmente da tribo Rhipsalideae, tem sido utilizado como um importante caráter diagnóstico para gêneros e espécies. Essa grande variedade de conformações dos segmentos proporciona uma formato variado ao hábito que é característico de cada táxon.

A aréola é a característica vegetativa mais marcante dos cactos, e pode ocorrer ao redor dos ramos, no topo de um tubérculo ou nas margens das costelas ou alas.

São formadas por gemas axilares ativas que podem produzir um aglomerado de espinhos e tricomas, sendo também de onde surgem as flores, frutos, folhas ou ramos. Aréolas que produzem elementos reprodutivos freqüentemente possuem espinhos finos ou uma rica produção de tricomas (Gibson & Nobel, 1986). Nos cactos epífitos, é rara a presença de espinhos e a maioria dos táxons apresenta aréolas glabras ou com pequenas escamas e pêlos escassos. Dessa forma, os táxons que apresentam aréolas pilosas ou com espinhos são facilmente identificados, pois se destacam dos demais. A modificação das aréolas após a floração é bem discreta nesse grupo, podendo apresentar maior pilosidade e maior número de escamas, uma característica importante para a identificação de algumas espécies. As aréolas podem estar posicionadas no mesmo nível das demais células da epiderme (emersas) ou podem ser imersas no caule.

A filotaxia de Cactaceae é alternada helicoidal, pois o ápice produz um primórdio foliar de cada vez, disposto em uma curva helicoidal ascendente (Gibson & Nobel, 1986). O primórdio foliar, em geral, não desenvolve a lâmina e o pecíolo, mas sim uma ampliação da base foliar, denominada podário. Quando bem desenvolvidos, os podários dão origem a tubérculos que, unidos em séries verticais, podem formar costelas. Um cacto pode produzir 5, 8, 13, 21, 34 ou 55 costelas (Série de Fibonacci) sendo que, o número de costelas pode variar em uma mesma planta. Nos cactos epífitos podem ocorrer podários, ângulos e alas, os quais configuram características taxonômicas de gêneros, subgêneros e espécies.

Folhas constituem um caráter plesiomórfico nas Cactaceae (Gibson & Nobel, 1986). Nos cactos epífitos, essas estruturas podem estar ausentes ou são vestigiais e rudimentares, sob a forma de escamas que podem aparecer nas aréolas somente durante o período reprodutivo.

As flores dos cactos são geralmente zoófilas, quase sempre bissexuais, sésseis, actinomorfas; conspícuas, solitárias, raramente aglomeradas e dispostas em inflorescências terminais, paniculadas ou cimosas (Barthlott & Hunt 1993). Nos cactos epífitos as flores são variadas, podendo ser grandes, vistosas e coloridas (tribo Hylocereeae) ou reduzidas (atingindo de diâmetro mínimo de 6 mm), em tons freqüentemente claros e hialinos (Rhipsalideae). As flores podem ocorrer nas aréolas

laterais ou subapicais, como na maioria dos táxons ou a floração pode ser terminal no ápice dos caules como, ocorre nas espécies dos gêneros epifíticos *Hatiora* e *Schlumbergera* e em *Rhipsalis* subg. *Erythrorhipsalis*.

O gineceu é composto por ovário ínfero, unilocular, com 3 a 20 carpelos, e geralmente muitos óvulos campilótopos com funículos simples ou ramificados; estilete longo comumente papilado, e estigma 3-20 lobado (Buxbaum 1953). O ovário ínfero receptacular, com os estames e o perianto surgindo no terço terminal do pericarpelo representa a característica mais marcante da flor dos cactos (Gibson & Nobel, 1986). O ovário ínfero receptacular se origina da imersão do ovário dentro do tecido caulinar, contrário à maioria das plantas de ovário ínfero, nas quais a base do receptáculo, sépalas, pétalas e estames formam um hipanto fundido ao ovário. Nos cactos, as aréolas cobrem a parte externa do ovário, o pericarpelo (i.e. o termo pericarpelo se refere ao tecido que envolve o ovário; Buxbaum, 1953). O pericarpelo pode fundir-se à parte superior do hipanto, formando um tubo floral mais ou menos extenso, glabro ou revestido por escamas bractiformes e aréolas. Entre os cactos epífitos da tribo Hyloceeae, é comum as espécies apresentarem indumento e brácteas no pericarpelo e um tubo floral longo. Na tribo Rhipsalideae o pericarpelo pode ser glabro, com tubo floral pouco desenvolvido e com o número de carpelos reduzido, assim como os lobos do estigma.

O androceu é composto por estames numerosos e dispostos em uma série igualmente distribuída pelo terço inferior interno do tubo ou em séries separadas. As anteras são bitecas, tetraesporângiadas, com deiscência rimosa completa. Em Rhipsalideae os estames são em menor número, geralmente livres e inseridos em uma só série na base no ovário. Entretanto, em *Schlumbergera*, os estames assumem alturas diferentes, o que em conjunto com a organização das tépalas confere zigomorfia à flor da maioria das espécies.

As escamas do pericarpelo são geralmente maiores e mais tepalóides em direção ao ápice, numa transição gradual com as séries externas do perianto. As tépalas são numerosas e inseridas de forma espiralada. Há uma mudança gradual de forma, textura e coloração desde as tépalas mais externas até as mais internas, impedindo a diferenciação entre cálice e corola, por função e posicionamento. A dificuldade da

aplicação das terminologias tradicionais para as peças do perianto, seja pela origem incerta (brácteas x estames), seja pela diferenciação gradual ou não-diferenciação é comum entre famílias da ordem Caryophyllales (Brockington et al., 2009). Dessa forma, a utilização dos termos tépalas petalóides e tépalas sepalóides são mais adequados, uma vez que remetem à uma função homóloga às peças do perianto das demais eudicotiledôneas, sem necessariamente implicar em uma homologia de posição (Brockington et al., 2009).

O néctar é secretado por um disco, ou por uma câmara nectarífera ao longo da metade inferior do tubo, naqueles que apresentam tubo floral (Barthlott & Hunt 1993). Nas espécies com tubo floral bem desenvolvido, o terço inferior dos filetes é mais espesso e apresenta-se curvado em direção ao estilete impedindo que o néctar esorra das flores, que geralmente estão perpendiculares, inclinadas ou mesmo pêndulas no ramo. As espécies de Hylocereeae apresentam câmara nectarífera, enquanto que em Rhipsalideae, com exceção de *Schlumbergera*, a secreção apresenta-se sobre um disco nectarífero.

O fruto das cactáceas é do tipo bacóide, suculento ou raramente seco, glabro, com escamas, cerdas e espinhos, indeiscente, ou variadamente deiscente (Barroso, 1999; Barthlott & Hunt 1993). Geralmente é conspicuo, globoso a cilíndrico com sementes embebidas em uma polpa carnosa de origem funicular. As sementes são de três a numerosas. A morfologia da semente, em particular a configuração do hilo, micrópila e da microescultura da testa são altamente variáveis e importantes taxonomicamente (Barthlott & Hunt, 1993). Em Cactoideae, a testa é geralmente negra ou marrom escura e pode ser rugosa ou ruminada. A semente madura contém um embrião curvo cercado por tecido nutritivo formado por perisperma (Gibson & Nobel, 1986). O fruto dos cactos epífitos é carnoso e o indumento dessas espécies segue o padrão encontrado no pericarpelo, sendo que a maioria das espécies de Rhipsalideae apresenta frutos glabros. Uma característica marcante dos frutos de Rhipsalideae é a polpa mucilaginoso pegajosa, translúcida, com papel importante na dispersão das sementes no ambiente epífítico.

OBJETIVOS

- (1) Reconstruir a filogenia da tribo Rhipsalideae com base em caracteres moleculares visando o teste do monofiletismo dos gêneros da tribo;
- (2) Reconstruir a filogenia do gênero *Rhipsalis* com base em caracteres moleculares visando o teste do monofiletismo dos seus subgêneros, o estudo da evolução de caracteres morfológicos e da ocupação dos habitats epífítico e rupícola e o estudo da biogeografia;
- (3) Elaborar uma nova classificação para os subgêneros de *Rhipsalis* com base em dados morfológicos e moleculares;
- (4) Revisar o "clado caule-alado" (= *Rhipsalis* subg. *Phyllarthrorhipsalis*) objetivando uma melhor delimitação e identificação dos táxons específicos e infraespecíficos.

JUSTIFICATIVA

Este estudo visa aprofundar o conhecimento da tribo Rhipsalideae e do gênero *Rhipsalis*, grupos de plantas epífitas extremamente abundantes e diversos na região Neotropical. Epífitas representam um importante elemento da flora tropical, constituindo uma alta porcentagem da diversidade vegetal das florestas úmidas. Plantas epífitas são particularmente diversas e abundantes na região neotropical, onde encontramos um número de espécies seis vezes maior do que na Australásia, e o dobro das espécies encontradas na África. É interessante, no entanto, que o número de gêneros e famílias de espécies epífitas é similar nos três continentes, indicando que a especiação de epífitas foi dramaticamente maior nos neotrópicos (Benzing, 1987; Gentry & Dodson, 1987). Apesar da grande importância das plantas epífitas para as florestas tropicais, pouco ainda se sabe sobre a taxonomia, biologia, evolução e biogeografias deste grupo de plantas.

A área de estudo do presente trabalho compreende a Floresta Atlântica, uma área biologicamente muito importante, mas extremamente ameaçada (Fonseca et al. 2004). A grave situação de conservação em que se encontra esse bioma em

decorrência do desmatamento e da urbanização acelerada está ameaçando a diversidade genética de vários grupos de organismos e, até mesmo a sobrevivência das espécies que são o foco do presente estudo. A elevada diversidade e taxa de endemismo de representantes da tribo Rhipsalideae na Mata Atlântica tornam este grupo particularmente interessante para um melhor entendimento dos processos geradores da alta diversidade de espécies encontradas atualmente na Mata Atlântica. Assim, o presente estudo visa contribuir não somente para uma melhor compreensão da taxonomia, morfologia e evolução da tribo Rhipsalideae especificamente, mas também gerar dados importantes para um melhor entendimento dos processos que resultam na alta diversidade encontrada atualmente na Floresta Atlântica e assim, auxiliar na conservação e manejo deste ecossistema tão ameaçado.

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CAPÍTULO 1

MOLECULAR PHYLOGENETICS OF RHIPSALIDEAE AND TAXONOMIC IMPLICATIONS FOR

HATIORA AND *SCHLUMBERGERA*

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

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ABSTRACT

The epiphytic cacti of the tribe Rhipsalideae include some of the smallest and least colorful members of the family. They present a reduced vegetative body and specialized adventitious root systems that may have contributed for the occupation of lithophytic and epiphytic habitats. Despite the controversial classification of the Rhipsalideae, all classifications ever proposed for the tribe have only considered morphological features, with no studies ever attempting to reconstruct the phylogenetic relationships among its members or to test the monophyly of its genera. In this study, we reconstruct the phylogeny of the tribe Rhipsalideae using plastid and nuclear markers and evaluate the classification systems previously proposed for the group. Furthermore, species distributions and morphological features traditionally used in previous classifications are examined in light of the resulting phylogeny. The molecular phylogeny of Rhipsalideae associated with the analysis of morphological features of the group indicates that a broader genus *Schlumbergera* should be recognized, including *Hatiora* subg. *Rhipsalidopsis*. Morphological synapomorphies rather than homoplastic characters are here used in order to establish a more stable and practical classification for the group. Nomenclatural changes and a key for the identification of the genera currently included in Rhipsalideae are provided.

Keywords: Atlantic Forest, Cactoideae, epiphytic cacti, *Lepismium*, *Rhipsalis*, systematics.

1.1. INTRODUCTION

Cacti are well known for their xerophytic features, in particular the modification of leaves into spines, succulence associated with water storage and stems with well-developed photosynthetic tissue. In general, cacti present showy and bright colored flowers with many perianth segments, as well as large, juicy and colorful fruits. The epiphytic cacti of the tribe Rhipsalideae, however, include some of the smallest and least colorful flowers and fruits of the family and areoles usually lack spines. In addition, members of Rhipsalideae present a reduced vegetative body and specialized adventitious root systems that may have contributed for the occupation of lithophytic and epiphytic habitats (Fig. 1).

The tribe Rhipsalideae is centered in the Brazilian Atlantic Forest, where species occur from coastal habitats to almost 3000m of altitude in the Pico da Bandeira. The group represents an important component of the Atlantic Forest, a highly deforested system with several threatened cacti taxa (Taylor, 1997; Calvente & al., 2005). Most species of Rhipsalideae are rare and often endemic or with restricted distribution, however *Rhipsalis baccifera* is widespread, reaching Africa and Asia and representing the only cactus species that occurs naturally outside the Americas.

The Rhipsalideae belongs to the Cactoideae, the most diverse subfamily of cacti (Hunt & al., 2006). This subfamily represents the last diverging lineage in the Cactaceae and constitutes a monophyletic group well characterized by a complete reduction of its leaves (Nyffeler, 2002). The remaining subfamilies of cacti, Pereskioideae and Opuntioideae also represent distinct lineages characterized by unique morphological and molecular features. The Pereskioideae is paraphyletic and includes the earliest diverging lineages in the Cactaceae (Edwards & al., 2005): “Rhodocactus” (the first diverging lineage) and “Eupereskia” (sister to the remainder of the family except “Rhodocactus”). Phylogenetic relationships within Opuntioideae are still poorly understood. However, representatives of Opuntioideae were included in a family level phylogeny of the Cactaceae (Nyffeler, 2002) and were shown to represent a monophyletic group, sister to the Cactoideae.

Within Cactoideae, tribal and generic classifications have suffered major changes throughout the years which have also impacted the Rhipsalideae. Because Rhipsalideae and Hylocereeae include all obligatory epiphytic cacti, members of both tribes were thought to be closely related. However, a family-wide molecular phylogeny of the Cactaceae (Nyffeler, 2002) made it clear that the epiphytic habit has evolved at least twice in the family and that the Rhipsalideae and the Hylocereeae are distantly related.

The last comprehensive classification of the Rhipsalideae (Barthlott & Taylor, 1995) recognized four genera — *Rhipsalis*, *Hatiora*, *Lepismium* and *Schlumbergera* — characterized by distinct morphologies and geography. Some species of *Lepismium* were, however, subsequently transferred to *Pfeiffera* and placed within Hylocereeae (Nyffeler, 2000). A year later, drastic changes in the classification of Rhipsalideae and Hylocereeae were proposed, including the description of several new genera and subtribes to accommodate novel groups that were created based in flower anatomy and seed morphology (Doweld, 2001). This classification was, however, rejected by Hunt & al. (2006), who again recognized four genera with slightly different species compositions than those recognized by Barthlott & Taylor (1995): a large *Rhipsalis* (36 species), *Hatiora* (6 species), *Lepismium* (6 species) and *Schlumbergera* (6 species).

Despite the controversial classification of the Rhipsalideae, all classifications proposed for the tribe have only considered morphological features, with no studies ever attempting to reconstruct the phylogenetic relationships among its members or to test the monophyly of its genera. To date, the only members of Rhipsalideae ever considered in a phylogenetic context were *Rhipsalis floccosa*, *Hatiora salicornioides*, *Lepismium cruciforme* and *Schlumbergera truncata*, which were included as representatives of Rhipsalideae in the overall phylogeny of Cactaceae (Nyffeler, 2002). In this study, we reconstruct the phylogeny of the tribe Rhipsalideae using plastid and nuclear markers and re-evaluate the classification systems previously proposed for the group. We also examine species geographical distributions and morphological features traditionally used in previous classifications in the light of the resulting phylogeny.

1.2. MATERIALS AND METHODS

Taxon sampling. — Taxa were selected according to the most recent classification of the family (Hunt & al., 2006). All four genera (*Rhipsalis*, *Hatiora*, *Lepisimium* and *Schlumbergera*), all subgeneric divisions and 35 of the 54 currently recognized species of the tribe were sampled, representing 65% of all species currently assigned to the group (Table 1). Outgroup selection was based on the phylogeny of the Cactaceae proposed by Nyffeler (2002). Five outgroup species representing major lineages associated with Rhipsalideae and two species belonging to the tribe Hylocereeae were sampled (Table 1).

DNA extraction, PCR amplification and sequencing. — Total genomic DNA was extracted from silica-gel dried stems using the CTAB protocol (Doyle & Doyle, 1987). A pilot study including 13 markers (*trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*, ITS, *Cox3*, *PhyC*, *MLS*, *trnK-matK*, *trnL-F*, *rpoB*, *rpoC1*, *trnC-petN*, and *accD*) was conducted to evaluate the suitability of various markers for the present study. The markers *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH* and ITS 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 *trnQ-rps16* and *rpl32-trnL* followed Shaw & al. (2007). The plastid spacer *psbA-trnH* and the nuclear internal transcribed spacer region (ITS) were amplified in 20 µl reactions containing: 2 µl of 5x Go taq Promega Buffer, 2 µl of bovine serum albumine (BSA), 1 µl of 25mM MgCl₂, 1 µl of each primer (10 mM), 0.4 µl of Promega Go Taq, 0.4 µl of 10mM dNTPs, 0.8 µl of dimethyl sulfoxide (DMSO), 0.8 µl of genomic DNA and 11.6 µl of water. PCR reaction conditions for the amplification of *psbA-trnH* followed Edwards & 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. All primers used for the amplifications and sequencing are listed in Table 2. Amplification products were purified using either the NucleoSpin Extract II Kit (Macherey-Nagel) or the QIAquick PCR purification Kit (Qiagen), following the manufacturer's protocol. Automated sequencing was performed using the BigDye Terminator Cycle Sequencing Standart Version 3.1 Kit (Applied Biosystems) and run on an ABI 3730 DNA Analyzer sequencer at Jodrell Laboratory or sent to MacroGen Inc.

(Korea). Sequences are available in GenBank (accessions numbers provided in the Appendix).

Table 1. Sampling of Rhipsalideae and outgroups used in this study following Hunt et al. (2006); type species are highlighted in bold.

Genera	Subgenera	Species	Voucher
<i>Rhipsalis</i> 21 out of 37 spp. 57%	<i>Phyllarthrorhipsalis</i> 7 out of 13 spp.	<i>R. cereoides</i> (Backeb. & Voll) Backeb.	Barros 2302 (RB)
		<i>R. crispata</i> (Haw.) Pfeiff.	A. Calvente 215 (SPF)
		<i>R. elliptica</i> G.A. Lindberg ex K. Schum.	A. Calvente 214 (SPF)
		<i>R. micrantha</i> (Kunth) DC	A. Calvente 396 (SPF)
		<i>R. olivifera</i> N.P. Taylor & Zappi	A. Calvente 226 (SPF)
		<i>R. pachyptera</i> Pfeiff.	A. Calvente 211 (SPF)
		<i>R. russellii</i> Britton & Rose	A. Calvente 313 (SPF)
	<i>Calamorhipsalis</i> 2 out of 3 spp.	<i>R. neves-armondii</i> K. Schum.	L. Versieux 196 (SPF)
		<i>R. puniceodiscus</i> G.A. Lindberg	A. Calvente 177 (SPF)
	<i>Epallagogonium</i> 4 out of 7 spp.	<i>R. dissimilis</i> (G.A. Lindberg) K. Schum.	A. Calvente 401 (SPF)
		<i>R. floccosa</i> Salm-Dyck ex Pfeiff.	A. Calvente 276 (SPF)
		<i>R. paradoxa</i> (Salm-Dyck ex Pfeiff.) Salm-Dyck	A. Calvente 145 (SPF)
		<i>R. trigona</i> Pfeiff.	A. Calvente 404 (SPF)
	<i>Rhipsalis</i> 4 out of 6 spp.	<i>R. baccifera</i> (J.S. Muell.) Stearn	A. Calvente 379 (SPF)
		<i>R. lindbergiana</i> K. Schum.	A. Calvente 161 (SPF)
		<i>R. mesembryanthemoides</i> Haw.	F. Freitas s/n (RB)
		<i>R. teres</i> (Vell.) Steud.	A. Calvente 255 (SPF)
<i>Hatiora</i> 6 out of 6 spp. 100%	<i>Erythrorhipsalis</i> 4 out of 9 spp.	<i>R. clavata</i> F.A.C. Weber	A. Calvente 240 (SPF)
		<i>R. pulchra</i> Loefgr.	A. Calvente 232 (SPF)
		<i>R. cereuscula</i> Haw.	Kew living collection (1991-1439)
	<i>Hatiora</i>	<i>R. pilocarpa</i> Loefgr.	A. Calvente 357 (SPF)
		<i>H. salicornioides</i> (Haw.) Britton & Rose	A. Calvente 239 (SPF)
		<i>H. cylindrica</i> Britton & Rose	A. Calvente 278 (SPF)
		<i>H. herminiae</i> (Porto & A. Cast.) Backeb. ex Barthlott	Martins s/n (SPF)
		<i>H. gaertneri</i> (Regel) Barthlott	Kew living collection (1985-3156)
		<i>H. rosea</i> (Lagerh.) Barthlott	M. Kaehler s/n (SPF)
		<i>H. epiphyllloides</i> (Porto & Werderm.) Buxb.	A. Calvente 363 (SPF)
<i>Lepismium</i> 4 out of 6 spp. 66%	<i>Lepismium</i> 1 out of 2 spp.	<i>L. cruciforme</i> (Vell.) Miq.	A. Calvente 26 (RUSU)
	<i>Ophiorhipsalis</i> 1 out of 1 spp.	<i>L. lumbricoides</i> (Lem.) Barthlott	A. Calvente 260 (SPF)
	<i>Houlletia</i> 2 out of 3 spp.	<i>L. houlletianum</i> (Lem.) Barthlott	A. Calvente 242
		<i>L. warmingianum</i> (K.Schum.) Barthlott	A. Calvente 259 (SPF)
<i>Schlumbergera</i> 4 out of 6 spp. 66%		<i>S. truncata</i> (Haw.) Moran	A. Calvente (SPF)
		<i>S. russeliana</i> (Hook.) Britton & Rose	A. Calvente 233 (SPF)
		<i>S. opuntoides</i> (Loefgr. & Dúsen) D.R. Hunt	N.F. O. Mota 1047 (BHCB)
		<i>S. orssichiana</i> Barthlott & McMillan	H. Freitas 28 (SPF)
Rhipsalideae total: 35 out of 54 spp (65%)			
Outgroup species: <i>Pereskia bahiensis</i> Gürke, <i>Calymmanthium substerile</i> F. Ritter, <i>Praecereus saxicola</i> (Morong) N.P. Taylor, <i>Pfeiffera ianthothele</i> F.A.C. Weber (Kew living collection), and <i>Epiphyllum phyllanthus</i> (L.) Haw. (A. Calvente - SPF)			

Sequence analyses. — Sequences were assembled in Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually in MacClade v. 4.08 (Maddison & Maddison, 2005). Gaps were coded separately and excluded from the analyses. Regions with ambiguous alignments were also excluded. Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP*, version 4.0b10 (Swofford, 2002). MP and ML heuristic searches used 1,000 replicates of random taxon stepwise-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 (cpDNA; *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*) and for ITS using ModelTest 3.04 (Posada & Crandall, 1998); HKY85 and HKY85+G+I were respectively identified as the most appropriate models of evolution. Support was assessed with non-parametric bootstrap analyses for MP and ML using random-taxon addition, and TBR branch swapping. MP bootstrap analyses were carried out with 1000 replicates, and ML bootstrap analyses with 100 replicates. Clades with bootstrap percentages of 50–74% were described as weakly supported, 75–89% moderately supported and 90–100% strongly supported. An additional constraint analysis to test the hypothesis of a monophyletic *Hattoria* was performed using the MP heuristic search protocol described above and keeping trees only compatible with the constraint-tree.

Bayesian analyses were performed with MrBayes 3.1.1 (Ronquist & Huelsenbeck, 2003). Searches were conducted using two independent runs, each run 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 substitutions were estimated separately for each partition. The F81+G, HKY+G, F81+I+G, GTR+I+G were selected for *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL* and ITS respectively. Posterior probabilities were used to evaluate support for all nodes (Ronquist & Huelsenbeck, 2003); clades with posterior probabilities above 0.95 were considered strongly supported.

Congruence testing and tree statistics. — Incongruence between data sets was evaluated using the Incongruence Length Difference test (ILD; Farris & 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 1,000 homogeneity replicates, saving a maximum of 1,000 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.

Table 2. Primers used in the present study.

Marker	Primers	Source
ITS	17SE - ACG AAT TCA TGG TCC GGT GAA GTG TTC G	Sun & al., 1994
	26SE - TAG AAT TCC CCG GTT CGC TCG CCG TTA C	
<i>psbA-tmH</i>	<i>psbA</i> - GTTATGCATGAACGTAATGCTC	Sang & al., 1997
	<i>tmH2</i> - CGCGCATGGTGGATTACAAATC	
<i>rpl32-tmL</i>	<i>rpl32Cact</i> - GTT ATC TTA GGT TTC AAC AAA CC	this study
	<i>rpl32</i> - CAG TTC CAA AA A AAC GTA CTT C	
	<i>tmL(UAG)</i> - CTG CTT CCT AAG AGC AGC GT	
<i>tmQ-rps16</i>	<i>tmQ(UUG)</i> - GCG TGG CCA AGY GGT AAG GC	Shaw & al. 2007
	<i>rps16x1</i> - GTT GCT TTY TAC CAC ATC GTT T	

Morphological characters and geographical distribution. —

Morphological data were obtained through the analysis of plant material coupled with information provided in descriptions and monographs of Cactaceae and Rhipsalideae (Britton & Rose, 1923; Barthlott & Taylor, 1995; Calvente & al., 2007; Zappi et al., 2007). Morphological characters chosen for the ancestral state reconstructions were those used in previous classifications to delimit groups. Potential synapomorphies were also evaluated through ancestral state reconstructions. All characters were discrete and binary. The ancestral state reconstructions were performed in MacClade 4.08 (Maddison & Maddison, 2005), considering unambiguous events exclusively. The Bayesian combined cpDNA tree was used for character mapping. The ancestral condition of six morphological characters were reconstructed. These characters were: stem segments growth and branching pattern, coded as: (0) indetermined and laterally branched, and (1) determined and apically branched; two-winged determined stems coded as: (0) present, and (1) absent; flower symmetry coded as: (0) actinomorphic, and (1) zygomorphic; perianth segment disposition coded as: (0) internal perianth segments longer than the medium perianth segments, and (1) internal perianth

segments shorter than the median perianth segments; flower tube coded as (0) conspicuous and exceeding the pericarpel, and (1) inconspicuous and not exceeding the pericarpel; and bright colored flowers coded as (0) present, (1) absent.

Information regarding the geographical distribution of species was obtained from the literature and from an extensive herbarium survey. The geographic area of occurrence of each clade was determined and graphically mapped onto the cladograms. Geographical regions were based on the occurrence of endemism and discontinuities in species distributions.

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. uninf.)	Retention Index
			(no.)	% of total size	% excl gaps				
ITS	724	558	44	6	7.9	179	3414	0.43	0.75
<i>psbA-tmH</i>	423	303	58	13.7	19.1	166	136292	0.68	0.90
<i>rpl32-tmL</i>	1493	1194	119	7.9	10	412	9543	0.65	0.82
<i>trnQ-rps16</i>	614	296	46	7.5	15.5	158	83023	0.78	0.86
Combined (plastid)	2530	1793	223	8.8	12.4	747	276	0.66	0.82

1.3. RESULTS

Phylogenetic analyses of separate cpDNA partitions. — Sequences for all ingroup and outgroup species were generated for all three cpDNA markers, except for *Praecereus saxicola* and *Calymmanthium substerile*, to which we were unable to obtain *rpl32-trnL* sequences. The three data sets presented different levels of variation and contained varied amounts of indels (Table 3).

The *trnQ-rps16* data set included a large gap (~250bp) between all species of *Rhipsalis* and the remaining genera, leaving less than 300bp of aligned sequence length. The MP analysis of the *trnQ-rps16* 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 parsimony informative. The *psbA-trnH* data set included a gap of ~100bp leading to an aligned sequence matrix with 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 parsimony informative. The *rpl32-trnL* data set contained several small gaps (up to 20 bp in length) and an aligned matrix with 1,194 characters. The MP analysis for this marker resulted in 9,543 trees of 412 steps with a CI of 0.65 and a RI of 0.82 (Table 3); 10% of the sites included in the analyses were informative.

Phylogenetic analysis of the combined cpDNA data set. — The ILD test demonstrated that the *psbA-trnH*, *trnQ-rps16* and *rpl32-trnL* data sets did not show significant incongruence ($P=0.3$). Hence, these markers were analyzed in combination. The combined MP analysis of the cpDNA data resulted in 276 equally parsimonious trees of 747 steps (CI=0.66; RI=0.82). The strict consensus of all 276 trees led to a well-resolved topology for Rhipsalideae as a whole (Fig. 2). The constraint analysis forcing all species of *Hatiora* to be monophyletic (sensu Hunt & al., 2006) resulted in two trees of 954 steps. The ML analysis of the combined cpDNA data set led to a single tree with $-\ln L = 6738.16029938$ (Fig. 3). The MP, ML and Bayesian analyses led to similar topologies. Bootstrap values and posterior probabilities (PP) were strong overall, with few moderately or weakly supported clades (Fig. 4).

Phylogenetic analysis of ITS. — ITS sequences were generated for all ingroup and outgroup taxa, except for *Epiphyllum phyllanthus*. The MP search resulted in 3,414 trees of 179 steps (CI=0.43; RI=0.75). The aligned matrix resulted in 558 characters of which 7.9% were parsimony informative (Table 3). The ML search led to a single tree with $-\ln L = 1807.26573$ (Fig. 3). The topologies obtained through the MP, ML and Bayesian analyses were congruent 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 of the individual plastid and with the cpDNA combined data set. Furthermore, several contradictory relationships were found between the plastid and ITS topologies (see taxa in bold in Fig. 2). Hence, the ITS data set was not analyzed in combination with the cpDNA data sets.

Ancestral character state reconstructions. — The ancestral condition of six morphological characters was reconstructed. These characters were: stem segments growth and branching pattern, two-winged determined stems, flower symmetry, perianth segment disposition; flower tube, and bright colored flowers (Fig. 5). Indetermined and laterally branched stems were found to represent the ancestral condition in the group, which was followed by multiple evolutions of determined and apically branched stems. Absence of the two-winged, determined stems is the ancestral condition in the group, with at least two shifts to two-winged determined stems. As far as flower symmetry is concerned, actinomorphic flowers were found to represent the ancestral condition in the group, which was followed by at least two shifts to zygomorphic corollas, with both shifts occurring within *Schlumbergera* (Fig. 5). Longer internal perianth segments were found to represent the ancestral condition in the group, with shorter internal perianth segments evolving a single time within *Hatiora s.str.* (Fig. 4, 5). The presence of a conspicuous well-developed flower tube exceeding the pericarpel was found to represent the ancestral condition for the group; this condition was lost at least four times during the evolution of Rhipsalideae. The ancestral condition for the character “bright colored flowers” was ambiguous, with bright colored flowers being lost in *Rhipsalis* and *Lepismium* (Fig. 5).

1.4. DISCUSSION

In this study, we used three cpDNA markers (*psbA-trnH*, *trnQ-rps16* and *rpl32-trnL*) and a nuclear data set (ITS) to investigate phylogenetic relationships within the tribe Rhipsalideae. The three cpDNA markers produced congruent topologies while ITS topology suggested a slightly different scenario than the one recovered with the plastid data. In the following sections, we discuss the results from the phylogenetic analyses, differences between the ITS and plastid topologies, and the implications of the results for the systematics of Rhipsalideae.

cpDNA analyses. — The MP semi-strict consensus tree of the *trnQ-rps16* 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, while better resolution was encountered for the other three genera. The MP semi-strict consensus tree of the *psbA-trnH* show a monophyletic *Rhipsalis* sister to an unresolved clade composed of species of *Lepismium*, *Hatiora* and *Schlumbergera* (not shown). Overall, the *psbA-trnH* provided better resolution at lower levels, with several small clades within *Rhipsalis* being also found in the combined analysis. On the other hand, lower resolution was found at the generic level. The marker *psbA-trnH* presented the highest percentage of informative sites of all four markers examined in the present study yet it led to the highest number of most parsimonious trees (Table 3). The MP strict consensus tree resulting from the analysis of the *rpl32-trnL* led to a better resolved tree at all levels within Rhipsalideae. The topology obtained with *rpl32-trnL* (not shown) was congruent with the topology obtained with the other two plastid markers and similar to the topology presented in the combined plastid tree. Overall, *rpl32-trnL* presented the lowest percentage of informative sites and the lowest number of most parsimonious trees (Table 3).

In the plastid combined analysis, the 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 larger clade strongly supported including all species of *Rhipsalis* (Fig. 4). Within the first clade, *Lepismium* is strongly supported as monophyletic, while *Schlumbergera* and

Hatoria (as traditionally recognized) are paraphyletic (Fig. 4, Table 1). Clades within *Lepismium* are poorly supported and the subgenus *Houlletia* appears to be paraphyletic; however, this relationship is only weakly to moderately supported (Fig. 4, Table 1). Further sampling in *Lepismium* is needed in order to clarify relationships within the group. Three species of *Hatoria* form a strongly supported *Hatoria* s.str. clade corresponding to *Hatoria* subgenus *Hatoria* (Fig. 4, Table 1). *Schlumbergera* s.l. includes species of *Schlumbergera* and *Hatoria* subgenus *Rhipsalidopsis* (Fig. 4, Table 1). *Hatoria* subgenus *Rhipsalidopsis* is paraphyletic, with *H. rosea* and *H. gaertneri* belonging to a strongly supported clade (Figs. 4, Table 1).

The second clade including all species of *Rhipsalis* recovered four well-supported clades (Fig. 4). The "floccosa group" contains some species of *Rhipsalis* subg. *Epallagogonium*, however *R. paradoxa* (the type species of this subgenus) appears as sister to "core *Rhipsalis*" (Fig. 4, Table 1). *Rhipsalis* subg. *Calamorhipsalis* and *Rhipsalis* subgenus *Erythrorhipsalis* are both monophyletic (Fig. 4, Table 1). The fourth clade corresponds to the "core *Rhipsalis*" and holds species of *Rhipsalis* subg. *Rhipsalis* and *R.* subg. *Phyllarthrorhipsalis* (Figs. 4, Table 1).

Comparisons between the combined cpDNA data set and ITS. —

Overall, the ITS data set presented a weaker signal and higher homoplasy levels than the cpDNA data sets (Table 3). The ITS topology was also less resolved and presented lower support overall compared to the cpDNA results (Fig. 2). Visual inspection revealed that major relationships recovered by the cpDNA data set were congruent with those based on ITS. However, the placement of four species of *Rhipsalis* differed between the combined cpDNA and the ITS topology. In particular, *R. clavata* and *R. pilocarpa* are within *Rhipsalis* subg. *Erythrorhipsalis* in the plastid trees but outside this subgenus in the ITS tree (Fig. 2, Table 1). The other two species, *R. neves-armondii* and *R. puniceodiscus*, appear as sister to the "floccosa group" in the ITS tree (making *Rhipsalis* subgenus *Calamorhipsalis* paraphyletic) but form a monophyletic group in the tree based on the combined cpDNA. Lastly, *R. mesembryanthemoides* appears as sister to the core *Rhipsalis* in the ITS topology but is sister to *R. teres* and *R. baccifera* in the combined cpDNA topology (Fig. 2).

Even though we did not encounter any evidence of ITS paralogues (either through multiple bands or explicit ambiguity in the chromatograms), it is possible that divergent ITS paralogues may have been amplified in this study, including pseudogenes and recombinants as previously found for other cacti and other plant groups (e.g. Buckler IV & al., 1997; Harpke & Peterson, 2006). Divergent ITS paralogues could explain the conflict between the ITS data set and the combined cpDNA topology. Another possible explanation is hybridization and introgression, what may have led to the divergent phylogenetic evidence gathered from the plastid and nuclear markers. However, the ITS phylogeny divergence was not particularly problematic for this study because we emphasize the evaluation and the positioning of major clades resulting in genera circumscription what was not affected by this conflict even when both data sets were combined (data not shown). Strongly supported incongruence only affected four species of *Rhipsalis* whose placement diverged between the plastid and ITS trees (see above). Given this and the higher resolution obtained by the analyses of the cpDNA data set, we choose to consider the combined cpDNA topology as representing the best estimate of the phylogeny of the tribe Rhipsalideae. This topology was here used for a further interpretation on the evolution of selected morphological characters in the group and for a re-evaluation of the current classification system of Rhipsalideae.

Systematics of Rhipsalideae. — The combined cpDNA data set corroborated the monophyly of the tribe Rhipsalideae and reconstructed major clades within the tribe. The relationships reconstructed are generally in agreement with the latest generic classifications proposed for Rhipsalideae (Barthlott & Taylor, 1995; Hunt & al., 2006). The only generic disagreement is associated with *Hatiora* and *Schlumbergera*, which are both paraphyletic. The current delimitation of both genera goes back to Barthlott (1987), who separated *Schlumbergera* from *Hatiora* on the basis of the zygomorphic flowers of *Schlumbergera*. This character was here shown to be homoplastic, with parallel evolution of zygomorphic flowers in *S. opuntioides* and *S. orssichiana/S. truncata* (Fig. 5). Previous classifications had already linked *S. russelliana* to *H. gaertneri* on the basis of their actinomorphic flowers (Britton & Rose, 1923). In fact, *H. gaertneri* was first described as an infraspecific taxa of *S. 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 determined stem segments (or angular stem segments in *S. microsphaerica*) in opposition to *Hatiora* subg. *Hatiora* (*Hatiora s.str.*), which presents cylindrical stem segments (Fig. 5, Table 4). A large number of perianth segments with acute apex also help to distinguish *Schlumbergera s.l.* from *Hatiora s.str.* Flowers of *Hatiora s.str.* present fewer perianth segments with obtuse apices, inner perianth segments shorter than medium perianth segments, and erect stems and flowers. *Schlumbergera s.l.*, on the other hand, presents many perianth segments with acute or apiculate apices, inner perianth segments longer than medium perianth segments, and generally pendulous stems and flowers (Fig 1, Table 4).

Table 4 Major clades here recognized and potential morphological synapomorphies.

Clade	Potential morphological synapomorphy
<i>Schlumbergera s.l.</i>	Stems generally pendulous, 2-winged or irregularly angular, short (< 5 cm), determined, apically branched; flowers terminal, pendulous, zygomorphic or actinomorphic, showy and strongly colored (rose, golden yellow, pink, red) or opaque white (in cultivars); perianth segments apiculate, acute; internal perianth segments longer than median perianth segments; flower tube conspicuous exceeding the pericarpel to absent or reduced, not markedly exceeding the pericarpel.
<i>Hatiora s.str.</i>	Stems cylindrical or bottle-shaped, short, determined, apically branched; flowers terminal, erect or pendulous, actinomorphic, strong colored (rose or golden yellow); internal perianth segments shorter than median perianth segments; flower tube absent or reduced, not markedly exceeding the pericarpel. Perianth segments rounded to obtuse at the tips; stems and flowers erect
<i>Lepismium</i>	Stems creeping or pendulous, cylindrical or 2-3-winged, long, laterally branched, indetermined,; flowers lateral, generally pendulous, actinomorphic, translucent white, pinkish or yellowish; internal perianth segments longer than median perianth segments; flower tube conspicuous, exceeding the pericarpel.
<i>Rhipsalis</i>	Stems erect or pendulous, 2-winged, cylindrical or ribed, indetermined or determined, > 5 cm, laterally or apically branched; flowers lateral or terminal, patent to the stem or pendulous, actinomorphic, translucent white, pinkish or yellowish; internal perianth segments longer than median perianth segments; flower tube absent or never exceeding the pericarpel.

In conclusion, the phylogeny obtained in the present study provided important evidence for a re-definition of generic limits in Rhipsalideae, especially in *Hatiora* and *Schlumbergera*. In this new generic delimitation, the species once included in *Rhipsalidopsis* are transferred to *Schlumbergera* (*Schlumbergera s.l.*) while *Hatiora* is reduced to a genus with only three species (*Hatiora s.str.*) so that monophyletic *Hatiora* and *Schlumbergera* can be recognized. Even though the bootstrap support for *Schlumbergera s.l.* is moderate (70%), good morphological synapomorphies

corroborate the new circumscription of *Schlumbergera*, facilitating the taxonomy and the identification of Rhipsalideae genera as a whole (Table 4). Furthermore, the constraint analysis forcing *Hatiora* (*sensu* Hunt & al., 2006) as monophyletic presented a considerable increase in the number of steps of the most parsimonious trees (954 versus 747 steps) further corroborating the hypothesis that *Hatiora* is unlikely monophyletic under its current circumscription.

Morphological evolution in Rhipsalideae. — In order to further evaluate the utility of the morphological characters traditionally used in the taxonomy of Rhipsalideae, selected characters were mapped onto the combined cpDNA phylogeny of Rhipsalideae. The evolutionary patterns encountered showed that morphological characters traditionally used in the classification of Rhipsalideae are highly homoplastic within the group (Fig. 5). For example, in addition to flower zygomorphy previously discussed, the presence of short and determined stem segments was also shown to be highly homoplastic as this character is present in species of *Hatiora*, *Schlumbergera*, and species of *Rhipsalis* subg. *Erythrorhipsalis* (e.g. *R. clavata*, *R. cereuscula*). Similarly, the long and indetermined stem segments, traditionally used to recognize *Lepismium*, also occurs in *Rhipsalis* (e.g. *R. pulchra*, *R. lindbergiana*, *R. puniceodiscus*).

On the other hand, other morphological features emerged as potential synapomorphies of the various generic clades. Specifically, after the exclusion of *Hatiora* subg. *Rhipsalidopsis*, perianth segments disposition emerged as a synapomorphy of *Hatiora*. The presence of two-winged determined stems also emerged as a diagnostic character of *Schlumbergera* and *Rhipsalis* subg. *Phyllarthrorhipsalis*. Although appearing as homoplastic between these two lineages the two-winged determined stems can be confidently used because the two-winged stem segments of *Schlumbergera* s.l. are always smaller than 5 cm, while those found in *Rhipsalis* subg. *Phyllarthrorhipsalis* are larger than 5 cm (Fig.1, Table 4). The presence of a conspicuous flower-tube is not exclusive of *Lepismium*, even though this character still represents a good way of distinguishing *Rhipsalis* and *Lepismium*, which are very similar with respect to all other morphological characters examined.

This study also illustrated the difficulties of finding morphological synapomorphies within *Rhipsalis*. Most characters examined are homoplasious, with all potential

synapomorphies appearing in overlapping combinations amongst *Rhipsalis* species and other genera of Rhipsalideae. This finding suggests the evolution and radiation of *Rhipsalis* appears to be associated with the loss of typical cactus morphological traits instead of the gain of morphological innovations. Such morphological simplification might be associated with the occupation of epiphytic mesic habitats within forests concomitantly with the loss of characters associated with desertic and terrestrial environments inhabited by most other cacti.

Biogeography of Rhipsalideae. — Mapping geographical regions onto the cpDNA phylogeny of Rhipsalideae allowed an examination of specific hypotheses regarding the geographical occupation of various biomes by members of Rhipsalideae. The lack of resolution in the early history of the tribe makes it difficult, however, to establish the ancestral distribution of Rhipsalideae, leaving it ambiguous whether the ancestor of all Rhipsalideae was restricted to the Atlantic forest or more broadly distributed throughout South America (Figs. 6 and 7).

The molecular evidence presented here indicates that the ancestor of *Rhipsalis* was restricted to the forests of Eastern Brazil, where the Atlantic Forest is currently present. Subsequently, other events seem to have occurred to explain the present-day distribution of *Rhipsalis* species, among them, a long-dispersal event to the Andean region (ancestor of *R. micrantha*), two expansions to the southern American continent (ancestors of *R. floccosa* and *R. cereuscula*) and one major expansion of *R. baccifera* to the North and South American, African and Asian continents. The hypothesis that the current distribution of *R. baccifera* represents the outcome of long dispersal rather than representing a vicariant relict of a broader distribution of the family in the past is the most likely scenario (Anderson, 2001). However, a more detailed biogeographic analysis, involving more extensive sampling within *Rhipsalis* is needed in order to further explain the individual distribution transitions in the genus (Calvente & al., in prep.).

The wide distribution of *R. baccifera* is intriguing when compared to the remarkable endemism that is so predominant in Rhipsalideae. Furthermore, *R. baccifera* has a macro-morphological structure that is extremely reduced and less specialized when compared to other Rhipsalideae (Fig. 1). On the other hand, the highest specialization in morphological features in the tribe is found in *Schlumbergera*, which

includes exclusively micro-endemic and rare species. In a broad spectrum this situation suggests that the specialized traits found in *Schlumbergera* can be associated with specific ecological and habitat conditions therefore acting as a limiting factor in the ability to expand the amplitude of distribution. The opposite can also be true and the lack of specialization and reduction of the overall morphology could be associated with occurrence in many different habitats that the wide distributed *R. baccifera*, *R. floccosa* and *R. cereuscula* are able to grow. However, other species of *Rhipsalis* also with unspecialized morphological features present restricted distribution patterns (e.g., *R. cereoides* and *R. mesembryanthemoides*), suggesting that other factors are also likely shaping niche size in Rhipsalideae.

Taxonomic changes within *Hatiora* and *Schlumbergera*. — The molecular phylogeny of Rhipsalideae associated with the analysis of morphological features of the group suggested that a broader *Schlumbergera* should be recognized, including *Hatiora* subg. *Rhipsalidopsis*. Morphological synapomorphies rather than homoplastic characters are here used to establish a more practical, and hopefully more stable, classification for the group (Table 4, Fig. 5). 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

= *Hattoria* subgen. *Rhipsalidopsis* (Britton & Rose) Barthlott, *syn nov.*

Type: *S. epiphyllodes* Lem. *nom. ileg.* (= *S. russelliana* (Hook.) Britton & Rose)

2.1 – *Schlumbergera gaertneri* (Regel) Britton & Rose

≡ *Hattoria gaertneri* (Regel) Barthlott, *syn nov.*

2.2 - *Schlumbergera kautskyi* (Horobin & McMillan) N.P. Taylor

2.3 - *Schlumbergera lutea* Calvente & Zappi, *nom. nov.*

≡ *Rhipsalis epiphyllodes* Porto & Werderm., Jahrb. Deutsch. Kakteen-Ges. 1(7): 47. 1935. *Hattoria epiphyllodes* (Porto & Werderm.) Buxb., *syn nov.* *Hattoria epiphyllodes* (Porto & Werderm.) Buxb. subsp. *epiphyllodes*, *syn nov.*

2.3.2 - *Schlumbergera lutea* subsp. *bradei* (Porto & A. Cast.) Calvente & Zappi, *comb. nov.*

≡ *Hattoria epiphyllodes* var. *bradei* Porto & A. Cast., Rodriguésia 5(14): 354. 1941. *Hattoria epiphyllodes* subsp. *bradei* (Porto & A. Cast.) Barthlott & N.P. Taylor, *syn. nov.*

2.4 - *Schlumbergera microsphaerica* (K. Schum.) Hoevel

2.5 - *Schlumbergera opuntoides* (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. *Hattoria rosea* (Lagerh.) Barthlott, *syn. nov.*

2.8 - *Schlumbergera russelliana* (Hook.) Britton & Rose

2.9 - *Schlumbergera truncata* (Haw.) Moran

Key to genera of Rhipsalideae

1. Stem segments determined, apically branched and short (< 5 cm). Flowers actinomorphic or zygomorphic, strong colored (or opaque white in cultivars) 2
- 1'. Stem segments determined or indetermined, apically or laterally branched and long segments (> 5 cm) always present. Flowers actinomorphic, translucent 3
2. Stem segments cylindrical or bottle shaped. Flowers actinomorphic, with internal perianth segments shorter than median perianth segments *Hatiora*
- 2'. Stem segments 2-winged or angular. Flowers actinomorphic or zygomorphic, with internal perianth segments longer than median perianth segments *Schlumbergera*
3. Stem segments determined or indetermined, apically or laterally branched. Flower tube absent or never exceeding the pericarpel *Rhipsalis*
- 3'. Stem segments always indetermined and laterally branched, Flower tube conspicuous, exceeding the pericarpel *Lepismium*

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Figure 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)

Figure 2. Comparison between plastid and ITS maximum parsimony strict consensus topologies. Maximum parsimony and maximum likelihood bootstrap values are shown above branches and posterior probabilities values below branches. Species with controversial positioning are marked in bold.

Figure 3. Maximum likelihood phylograms based on plastid and ITS data. Branches indicated with "/" were reduced half size (50%).

Figure 4. Bayesian inference tree based on the combined analysis of plastid markers *psbA-trnH*, *trnQ-rps16* and *rpl32-trnL*. Maximum parsimony and maximum likelihood bootstrap values are shown above branches and posterior probabilities values below branches. Biogeographical distributions are indicated on the right.

Figure 5. Reconstruction of ancestral states of morphological traits using unordered parsimony. A. Flower symmetry (white=actinomorphic, black=zygomorphic). B. Stem segments growth and branching pattern (white=indetermined laterally branched, black=determined apically branched). C. Perianth segment disposition (white=internal perianth segments longer, black=internal perianth segments shorter). D. Two-winged determined stems (white=absent, black=present). E. Flower tube (white= conspicuous and exceeding the pericarpel, black=inconspicuous and not exceeding the pericarpel). F. Bright colored flowers (white=present, black=absent).

Appendix. GeneBank accession numbers for each species used in this study. (note to reviewers: numbers will be provided upon acceptance of the ms.)

Species; accession numbers (*psbA-trnH*; *trnQ-rps16*; *rpl32-trnL*; ITS).

R. cereoides; XXXXXXXX. *R. crispata*; XXXXXXXX. *R. elliptica*; XXXXXXXX. *R. micrantha*; XXXXXXXX. *R. olivifera*; XXXXXXXX. *R. pachyptera*; XXXXXXXX. *R. russellii*; XXXXXXXX. *R. neves-armondii*; XXXXXXXX. *R. puniceodiscus*; XXXXXXXX. *R. dissimilis*; XXXXXXXX. *R. floccosa*; XXXXXXXX. *R. paradoxa*; XXXXXXXX. *R. trigona*; XXXXXXXX. *R. baccifera*; XXXXXXXX. *R. lindbergiana*; XXXXXXXX. *R. mesembryanthemoides*; XXXXXXXX. *R. teres*; XXXXXXXX. *R. clavata*; XXXXXXXX. *R. pulchra*; XXXXXXXX. *R. cereuscula*; XXXXXXXX. *R. pilocarpa*; XXXXXXXX. *H. salicornioides*; XXXXXXXX. *H. cylindrical*; XXXXXXXX. *H. herminiae*; XXXXXXXX. *H. gaertneri*; XXXXXXXX. *H. rosea*; XXXXXXXX. *H. epiphylloides*; XXXXXXXX. *L. cruciforme*; XXXXXXXX. *L. lumbricoides*; XXXXXXXX. *L. houletianum*; XXXXXXXX. *L. warmingianum*; XXXXXXXX. *S. truncata*; XXXXXXXX. *S. russeliana*; XXXXXXXX. *S. opuntioides*; XXXXXXXX. *S. orssichiana*; XXXXXXXX. *Pereskia bahiensis*; XXXXXXXX. *Calymmanthium substerile*; XXXXXXXX. *Praecereus saxicola*; XXXXXXXX. *Pfeiffera ianthothele*; XXXXXXXX. *Epiphyllum phyllanthus*; XXXXXXXX.

CAPÍTULO 2

MOLECULAR PHYLOGENY, EVOLUTION AND BIOGEOGRAPHY OF SOUTH AMERICAN

EPIPHYTIC CACTI

Calvente et al., Phylogeny of *Rhipsalis*

**Molecular Phylogeny, Evolution and Biogeography of South American
Epiphytic Cacti¹**

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ABSTRACT

Epiphytes represent an important element of the tropical flora and are widely distributed across vascular plants. Despite that, little is still known about the evolutionary history, habitat preference, morphological diversity and biogeography of epiphytes as a whole. Approximately 10% of cacti are epiphytes inhabiting humid regions, with *Rhipsalis* representing the largest genus of epiphytic cacti. Here we reconstruct the molecular phylogeny of *Rhipsalis* using plastid and nuclear DNA markers, using it as the basis to study the evolution of habit and key morphological features, and to study the biogeographical history of the genus. Several lineages of *Rhipsalis* seem to have originated in the Atlantic Forest and subsequently occupied other tropical forests in South America, North America, Africa and Asia. These transitions occurred at different times, with some taking place long ago, and others being more recent, suggesting both ancient and recent associations within the Southern American epiphytic flora.

Key words: Andes, Atlantic Forest, Brazil, Cactaceae, *Rhipsalis*

2.1. INTRODUCTION

Epiphytes represent an important element of the tropical flora, being particularly fragile and dependent on the overall maintenance of tropical forests (Gentry and Dodson, 1987). The life history of epiphytes has been influenced by many selective forces including the distribution and stability of various substrates, rooting system and microclimate (Benzing, 1987). Epiphytism is widely distributed across vascular plants, with 44% of all vascular plant orders containing at least one epiphytic species (Benzing, 1987). Research on epiphytes has addressed the evolution into epiphytic habit in Orchidaceae (Gravendeel et al., 2004; Tsutsumi et al. 2007; Yukawa and Stern, 2009), 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). Despite that, little is still known about the drivers of diversification of epiphytes as a whole. Studies on the phylogeny, habitat preference, morphological diversity and biogeography of epiphytes are needed in order to better understand the processes involved in the evolution of these plants.

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 distributed through two distinct tribes: Hylocereae and Rhipsalideae (Nyffeler, 2002). Hylocereae are centered in Southern Mexico and Central America, and includes many facultative epiphytes or secondary hemiepiphytes distributed across genera *Hylocereus*, *Epiphyllum*, *Pseudorhipsalis*, *Disocactus*, *Selenicereus* and *Weberocereus* (Bauer, 2003; Wallace and Gibson, 2002). Rhipsalideae are centered in southeastern Brazil and contain four genera of mainly holoepiphytes: *Hatiora*, *Rhipsalis*, *Lepismium* and *Schlumbergera* (Barthlott, 1983). A recent phylogeny of Rhipsalideae based on *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL* and ITS tested the monophyly of Rhipsalideae and reconstructed major relationships within the tribe (Calvente et al, submitted). In particular, this study reconstructed a monophyletic *Rhipsalis*, a monophyletic *Lepismium*, and paraphyletic *Hatiora* and *Schlumbergera*; taxonomic changes were proposed to ensure that only monophyletic genera are included in this tribe. *Rhipsalis*, with five subgenera (*Calamorhipsalis*, *Epallagogonium*, *Erythrorhipsalis*, *Phyllarthrorhipsalis*, and *Rhipsalis*) and 35 species of epiphytic and rupicolous plants

that grow on open habitats and rocky outcrops, represents the largest genus of epiphytic cacti (Hunt et al., 2006).

Several processes have been used to explain the evolution of epiphytic Cacti. They are thought to have evolved from ribbed terrestrial columnar cacti, with this transition being accompanied by several structural modifications (Wallace and Gibson, 2002) such as: (1) development of adventitious roots so that stems can remain attached to the host plant; (2) development of “leaf-like” stems, increasing stem surface to volume ratio; (3) stems with narrower ribs and pith, decreasing the ability for water storage; (4) loss of structures associated with maintenance of upright position (e.g., thick ribs, conspicuous wood formation and collenchyma); and, (5) reduction or loss of spination, which blocks sunlight from reaching photosynthetic tissues. All of these modifications are observed in *Rhipsalis*, making this genus particularly interesting for the study of the evolution into epiphytic habit and associated morphological features. In particular, the stem of species of *Rhipsalis* varies in shape from cylindrical to alate, with considerable variation of the ratio between surface and volume (Fig. 1). The correlation of this particular character with the evolution of epiphytism versus the occupation of rupicolous habit could provide interesting evidence for the understanding of epiphytism in cacti.

Cactaceae is exclusive to the New World with the exception of *R. baccifera*, which spontaneously occurs in Africa and Asia. Some authors believed that this disjunct distribution resulted from vicariance acting upon an old Gondwanan distribution (Backeberg, 1942; Croizat, 1952). However, molecular phylogenetic data indicates that cacti originated during the mid-tertiary, after the Gondwana split, allowing a rapid diversification in the newly developing American deserts (HersHKovitz and Zimmer, 1997). In the Americas, cacti are distributed within a wide variety of habitats with species occurring from the American coast to the Andes and from deserts to evergreen humid forests. The main centers of diversity and endemism are located in Mexico, USA and Brazil (Taylor, 1997). Among epiphytic cacti, tribe Hylocereeae is centered in Southern Mexico and Central America and Rhipsalideae is centered in southeastern Brazil. The majority of species belonging to *Rhipsalis* are endemic to Brazil (81%), with several narrowly endemic species. According to Gentry & Dodson (1987) there are few

genera of vascular plants widely distributed across the tropical regions in which epiphytism is widely prevalent and *Rhipsalis* is among them. Apart from the disjunct distribution of *R. baccifera* and two species with wider distribution in south america (*R. cereuscula* and *R. floccosa*), *Rhipsalis* presents also an intra-continental disjunction between the Brazilian Atlantic Forest and the Andean mountainous forests, with two additional species presenting wider continuous distribution throughout South America. Andean vegetation is also rich in epiphytic species, found usually on humid eastern Andean slopes that present a steep topography that results in high rainfall. In these evergreen forests, epiphytes may contribute up to 35% of the total species diversity (Ibisch et al., 1996).

Robust phylogenetic trees would provide the evolutionary frameworks necessary for the study of the interesting biogeographical patterns found in the epiphytic flora of South American tropical forests. Here, we reconstruct the molecular phylogeny of *Rhipsalis* using plastid and nuclear DNA markers and evaluate the monophyly of subgenera formerly circumscribed within *Rhipsalis*. This phylogenetic tree also serves as basis for the study of the evolution of key morphological features, and to reconstruct the ancestral habit condition and biogeographical history of *Rhipsalis*.

2.2. MATERIALS AND METHODS

Taxon sampling— We sampled 34 of the 37 species currently circumscribed in *Rhipsalis*, including samples from all subgenera and respective type species (Appendices 1, 2); only *R. pacheco-leonis* (subgenus *Epallagogonium*), *R. sulcata* (subgenus *Epallagogonium*), *R. hoelleri* (subgenus *Calamorrhypsalis*) and *R. burchellii* (subgenus *Erythrorhipsalis*) were not sampled. Whenever species were polymorphic, multiple individuals were included in the analysis; this condition was particularly predominant within subgenus *Phyllarthrorhipsalis* in which species delimitation is particularly problematic. A total of 72 specimens were sampled. *Schlumbergera orssichiana* and *Hatiora salicornioides*, representing sister lineages of *Rhipsalis* based on a recent phylogeny of the tribe Rhipsalideae (Calvente et al., submitted), were defined as outgroup in all analyses.

DNA Extraction, Amplification and Sequencing— Genomic DNA was extracted from silica-gel dried stems using a CTAB extraction protocol (Doyle and Doyle, 1987). Five molecular markers were selected for the present study: the plastid spacers *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*, the nuclear internal transcribed spacer (ITS) and the nuclear gene malate synthase (MS). Amplification primers used are listed in Appendix 3. Amplifications of *trnQ-rps16* and *rpl32-trnL* followed the procedure outlined in Shaw et al. (2007). Amplifications of *psbA-trnH*, ITS and MS were conducted in 20 µl reactions containing: 2 µl of 5X Go taq Promega buffer, 2 µl of bovine serum albumine (BSA), 1 µl of 25mM MgCl₂, 1 µl of each primer (10 mM), 0.4 µl of Promega Go Taq, 0.4 µl of 10mM dNTPs, 0.8 µl of dimethyl sulfoxide (DMSO), 0.8 µl of genomic DNA and 11.6 µl of water. PCR reaction conditions for the amplification of *psbA-trnH* 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. 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, followed by 15 cycles of 94°C for 1 min, 52°C (-1°C per cycle) for 2 min, 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. More specific primers were designed for *Rhipsalis* using the sequences obtained with the primers of

Lewis and Doyle (2001). PCR conditions for the amplification using the new 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, Germany) or the QIAquick PCR Purification Kit (QIAGEN), following the manufacturer's protocol. Automated sequencing was performed using the BigDye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster City, California, USA), and run on an ABI 3730 DNA Analyzer at Jodrell Laboratory or sent to Macrogen Inc. (Korea). GenBank accessions numbers are provided in Appendix 1.

Cloning of MS amplification products was performed whenever difference between the length of the alleles was detected, or when more than 10 base ambiguities were found within a sequence. Cloned species were: *R. baccifera*, *R. cereuscula*, *R. crispata*, *R. mesembryanthemoides*, *R. micrantha*, *R. oblonga*, *R. occidentalis*, *R. teres* and *R. lindbergiana*. For cloning, purified PCR products were run on agarose gel and bands 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 manufacture's protocol (Promega, Madison, Wisconsin, USA). Up to 10 colonies per species were selected and use as template in PCR reactions using the same primers and conditions as outlined above. Cycle sequencing also followed the same procedure as described above.

Phylogenetic Analyses—Complementary sequences were assembled in Sequencher 3.0 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually in MacClade v. 4.08 (Maddison & Maddison, 2005). Gaps were coded separately. Sequence regions with ambiguous alignments were excluded. For MS, sequences from clones were examined for each cloned individual and compared; all different divergent sequences encountered among the sample sequenced for a given individual were included as independent terminals in the matrix. Polymorphic characters (found within a single individual) 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.

Maximum parsimony (MP) and maximum likelihood (ML) analyses were performed in PAUP*, version 4.0b10 (Swofford, 2002) using heuristic searches set to run 1,000 replicates of random stepwise-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 was determined using ModelTest 3.04 (Posada & Crandall, 1998) for a “small combined data set,” composed of the plastid (*trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*) and ITS data, as well as for a “large combined data set,” which included species for which data were available for all markers (*trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*, ITS and MS); F81+I+G and K81uf+I+G were respectively identified as the most appropriate models of evolution for each of these data sets. Support was assessed with non-parametric bootstrapping; heuristic searches with 1000 replicates for MP and 100 replicates for ML were conducted using the same parameters as described above. Clades with bootstrap support of 50–74% were considered weakly supported, 75–89% moderately supported and 90–100% strongly supported.

Bayesian analyses were performed with the “large combined data set” only using MrBayes 3.1.1 (Ronquist & Huelsenbeck, 2003). Searches were conducted using two independent runs, each run 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, F81+G, F81+I+G, HKY+I+G, and HKY+G were selected for *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, ITS and MS, respectively. Posterior probabilities were used to evaluate support for all nodes (Ronquist & Huelsenbeck, 2003); clades with posterior probabilities above 0.95 were considered strongly supported.

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 1,000 homogeneity replicates, saving a maximum of 1,000 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— Two morphological features putatively involved in the evolution of main clades within *Rhipsalis* were selected for ancestral state reconstructions: flower type and stem shape. Characters were coded as multi-state and discrete. Three independent characters were combined to represent the flower types found in the main lineages of *Rhipsalis*: flower position (terminal or lateral to the stem), pericarpel immersion (immersed or not immersed in stem), and flower shape (rotate or campanulate). Given that, three flower type states were coded as follows: (0) flowers rotate, lateral, with pericarpel not immersed in stems, (1) flowers rotate, lateral, with pericarpel immersed in stems; and, (2) flowers campanulate, and/or terminal, with pericarpel not immersed in stems. Stem shape was coded as: (0) cylindrical, (1) angular or with narrow wings (< 1cm), and (2) with expanded wings (> 1cm). Habit condition was also reconstructed as one multi-state discrete character coded as follows: (0) epiphytic, (1) rupicolous, growing in shaded habitat, (2) rupicolous, growing in open and sunny habitat.

Ancestral state reconstructions were performed in MacClade 4.08 (Maddison & Maddison, 2005), using unordered parsimony and allowing ambiguous reconstructions. Reconstructions were conducted on a simplified version of the Bayesian cpDNA + 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 non-monophyletic taxa, all terminals were kept in the simplified tree. Selection and analysis of the reconstructed features were based on specimen observation during extensive herbarium and field work, which allowed data collection from the whole distribution range of the genus.

Biogeography— The geographical distribution of species was determined using information compiled from herbarium specimens and from Barthlott and Taylor (1995). Four 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 & Central America, (4) Central & North America, Africa and Asia. Ancestral biogeographical areas were reconstructed in MacClade 4.08 (Maddison & Maddison, 2005), using the simplified combined Bayesian topology and the same procedures described above for morphological characters.

Ancestral distributions were also reconstructed using the dispersal-vicariance analysis implemented in DIVA 1.1 (Ronquist, 1996). A matrix containing information on the (1) presence and (0) absence of species in the five biogeographical areas defined above was prepared in MacClade 4.08 (Maddison & Maddison, 2005). The same Bayesian simplified topology was used as topological backbone for the DIVA analysis, except that polytomies were resolved following two procedures: (1) taxa in polytomies with the same distribution were combined into a single terminal; (2) species with unknown distributions were excluded. The analysis was set to run using default optimizations (maxareas=5, bound=250, hold=1000).

2.3. RESULTS

Phylogenetic Analyses— The ILD test did not indicate significant incongruence between the cpDNA individual data sets ($P=0.83$), but indicated significant incongruence between the combined cpDNA data set and ITS ($P=0.001$), and between the “small combined” and MS data sets ($P=0.001$). The Templeton test did not indicate significant incongruence between the cpDNA data set and ITS (rival tree ITS, $P=0.25$; rival tree cpDNA, $P=1.0$), but indicated incongruence between the “small combined” and MS data sets (rival tree “small combined”, $P=0.0001$; rival tree MS, $P=0.1185$). The non-significant results encountered in the comparison between the cpDNA data set and ITS by the Templeton test suggest that the incongruence detected by the ILD likely represents “soft incongruence” due to lack of resolution in the individual data sets compared (Soltis et al. 1998). Hence the cpDNA and ITS data sets were analyzed in combination; this data set is here denominated “small combined data set”. Visual comparison of the “small combined data set” and the MS topology indicated that the varying position of *R. ormindoi* (sister to *R. juengeri* in the MS topology and sister to *R. clavata* in the “small combined” tree) was likely responsible for the incongruence detected. We reduced these clades to polytomies in both topologies and conducted the Templeton test again, which then lead to a non-significant result (rival tree “small combined”, $P=0.125$; rival tree MS, $P=0.5$). The exclusion of *R. ormindoi* in either tree or in both trees also resulted in a non-significant result. We then combined the cpDNA, ITS and MS data sets (here denominated “large combined”) but collapsed the clade formed by *R. ormindoi* and *R. juengeri* in the Bayesian consensus tree presented (Fig. 2), representing *R. ormindoi*, *R. clavata* and *R. juengeri* as a polytomy.

Sequences for 72 ingroup and outgroup terminals were generated for each of the cpDNA markers (*psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*) and ITS. For MS, the final data set included 67 terminals, as we were unable to obtain sequences for *R. pulchra*, *R. floccosa*, *R. dissimilis* and *R. ewaldiana*. The sizes of individual matrices and variation obtained in each data set are presented in Table 1.

The *psbA-trnH* data set included 295 bp of which 10.8% were informative; the MP analysis of this data set led to 15906 most parsimonious trees of length 78 (CI=0.78, RI=0.93). The *trnQ-rps16* marker included 220 bp of which 4.6% were informative; the

MP analysis of this data set led to 5463 most parsimonious trees of length 28 (CI=0.96, RI=0.94). The *rpl32-trnL* data set included 1165 bp of which 6.0% were informative; the MP analysis of this data set led to 273 most parsimonious trees of length 186 (CI=0.71, RI=0.93).

The ITS data set included 652 bp of which 4.6% were informative. The MP analysis of the ITS data set led to 9885 most parsimonious trees of length 89 (CI=0.52, RI=0.86). The combined cpDNA+ITS data set (“small combined”) included 2331 bp of which 7% were parsimony informative. The MP analysis of this data set led to 17905 most parsimonious trees of length 413 (CI=0.59, RI=0.87) and the ML search resulted in two best trees of -lnL 6112.84676223.

The MS data set contained 67 terminals and two terminals for each cloned individual; never more than two different sequences were found in individual selected for cloning. Nine species presented sequence ambiguities that suggested allelic polymorphism and were cloned for verification. In the case of *R. micrantha* and *R. crispata*, a high number of polymorphic sites were detected (up to 28). In the case 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. Although several specimens and infraspecific taxa of *Rhipsalis micrantha* were sequenced for cpDNA markers and ITS (Appendix 1), a single cloned specimen of *R. micrantha* was included in the MS data set given the high similarity between the MS sequences obtained for this species. The final MS matrix contained 1258 bp of which 7.4% were potentially parsimony informative. The parsimony analysis of the MS data set produced 15300 most parsimonious trees of length 315 (CI=0.81, RI=0.90).

The combined matrix containing all five markers (*psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, ITS, and MS), here denominated “large combined,” included 3536 bp of which 6.6% were parsimony informative. The MP analysis of this data set resulted in 12397 most parsimonious trees of length 730 (CI=0.65, RI=0.86). The ML search led to a single tree of -lnL 10186.74985. Results derived from the Bayesian analyses were congruent with those from the MP and ML analyses. The Bayesian analysis of the “large combined” matrix recovered a monophyletic *Rhipsalis* containing three highly supported clades (Fig. 2). The first clade here named “sunken pericarpel clade” includes species of

the subgenera *Calamorhipsalis* and most species from *Epallagonium*, 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 *Epallagonium*. Only two clades of the “core *Rhipsalis*” lineage are supported by high posterior probabilities and bootstrap values: (1) *R. pentaptera* + *R. sp.* + *R. lindbergiana* and, (2) ((*R. russellii* + *R. triangularis* + *R. cereoides*) (*R. pachyptera* A + *R. agudoensis*) *R. pachyptera*). All species sampled were monophyletic or unresolved within a larger polytomy, except for *R. oblonga* and *R. pachyptera* (Fig. 2).

Table 1. Characteristics of each partitions and information derived from the maximum parsimony analyses of the individual and combined data sets (CI, consistency index, calculated excluding uninformative characters; RI, retention index).

	<i>psbA-trnH</i>	<i>trnQ-rps16</i>	<i>rpl32-trnL</i>	ITS	MLS	"small combined	"large combined
No. of terminals	72	72	72	72	67	72	66
Aligned matrix (bp)	295	220	1165	651	1258	2331	3526
Informative sites	32 / 10.8%	10 / 4.6%	70 / 6.0%	30 / 4.6%	93 / 7.4%	162 / 7.0%	232 / 6.6%
No. trees retained	15906	5463	273	9885	15300	17905	12397
Length of best trees	78	28	186	89	315	413	730
CI	0.78	0.96	0.71	0.52	0.81	0.59	0.65
RI	0.93	0.94	0.93	0.86	0.90	0.87	0.86

The relationships recovered from the analyses of the “small combined” data set are similar overall with the results from the analysis of the “large combined” data set. This data set included four species that were not sampled for MS (*R. pulchra*, *R. floccosa*, *R. dissimilis* and *R. ewaldiana*). The position of these species does not create conflict with the overall topology obtained from the analysis of the “large combined” data set (Fig. 2). The analysis of the “small combined” data set shows that *R. pulchra* is sister to *R. cereuscula*; the clade (*R. floccosa* + *R. dissimilis*) is sister to *R. trigona*; and that *R. ewaldiana* is sister to the “core *Rhipsalis*” clade (not shown).

Habit and Morphological Evolution— Ancestral reconstructions of the habit condition and of two selected morphological traits (flower type and stem shape) are presented in Fig. 3. The ancestral condition of flower type is ambiguous. We observe one evolution to “flowers campanulate, and/or terminal, with pericarpel not immersed in stems” in *Erythrorhipsalis* and one evolution of “flowers rotate, lateral, with pericarpel immersed in stems” within the “sunken pericarpel clade” (Figs 2, 3). The character state “flowers rotate, lateral, with pericarpel not immersed in stems” was present in the ancestor of three lineages of the “core *Rhipsalis*” clade. However, it remains uncertain whether this character evolved once or multiple times within the “core *Rhipsalis*” clade (Fig. 3). The polytomy found at the crown node of the core *Rhipsalis* clade is the cause of the uncertainty in the optimization of flower types (Fig 3), but it is relatively safe to assume that the ancestral state for this character in this group is flowers rotate, lateral, with pericarpel immersed in stems. If not, it certainly appeared early in the evolutionary history of this group.

As far as stem shape is concerned, the character states “cylindrical” and “angular or with narrow wings (< 1cm)” seem to have evolved multiple times, and “stems with expanded wings (> 1cm)” a single time, within *Phyllarthrorhipsalis*, with a shift to angular or with narrow wings in *R. cereoides* (Fig. 3). The ancestral condition of this character remains ambiguous in *Rhipsalis*.

Ancestral state reconstructions indicated that the epiphytic condition is ancestral, being followed by at least five independent shifts to rupicolous in open and sunny habitats (Fig. 3). A transition to rupicolous habit, with individuals growing in shaded habitats is observed in *R. crispata*.

Biogeography— Reconstructions of ancestral areas in *Rhipsalis* suggested that the ancestor of all *Rhipsalis* occurred in Costal Brazil, being followed by at least six independent range expansions into southern Brazil (*R. elliptica*, *R. pachyptera*/*R. agudoensis*, *R. teres*, *R. pilocarpa*, *R. campos-portoana* and *R. cereuscula*) and one independent transitions to southern South America (*R. cereuscula*). Two independent transitions to the “Andes & Central America” (*R. micrantha* and *R. cuneata*) and one transition to “Central & North America, Africa and Asia” (*R. baccifera*) are also observed.

Examination of the ancestral distribution using dispersal-vicariance analysis detected an optimal reconstruction requiring 18 dispersal events. Coastal Brazil is reconstructed as the ancestral area of *Rhipsalis*, of the “sunken pericarpel clade,” of *Erythrorhipsalis*, and of the “core *Rhipsalis*” clade (Fig. 3). Most dispersal events were found in terminal branches, except for four dispersal events in more internal branches (Fig. 3), in particular: (1) one dispersal to Southern Brazil by the ancestor of *R. russellii* to *R. pachyptera* or by the ancestor of *R. agudoensis* and *R. pachyptera*; (2) one dispersal to the “Andes & Central America” by the ancestor of *R. cuneata* and *R. oblonga*; (3) one dispersal to the “Andes & Central America” by the ancestor of *R. micrantha* and *R. elliptica*; and, (4) one dispersal to southern Brazil either by the ancestor of *R. elliptica* and *R. micrantha* or by *R. elliptica*.

2.4. DISCUSSION

Phylogenetic analyses— Out of all molecular markers analyzed, *psbA-trnH* presented the greatest percentage of informative sites (Table 1), while the MS and *rpl32-trnL* markers 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 cpDNA, ITS and MS data sets were generally congruent, except for the position of *R. ormindoi*, which appeared as sister to *R. juengeri* in the analysis of the MS (bs=99), *rpl32-trnL* (bs=64), and *trnQ-rps16* (bs=63), but as sister to *R. clavata* in the analysis of ITS (bs=86), and “small combined” data set (bs = 96). Furthermore, in the *psbA-trnH* tree, *R. ormindoi* appeared in a polytomy within the *Erythrorhipsalis* clade together with *R. clavata* while *R. juengeri* appeared as closely related to the remaining *Erythrorhipsalis* species (low support; data not shown). This data suggests a hybrid origin of *R. ormindoi*, which is also supported by morphological evidence. In particular, the vegetative morphology of *R. ormindoi* is intermediate between *R. clavata* and *R. jungeri* as the branching pattern of stems resembles *R. jungeri*, while the final stem segments are clavate like those of *R. clavata*.

Rhipsalis is highly supported as monophyletic but not all subgenera within *Rhipsalis* are demonstrated to be monophyletic in this analysis (Fig. 2). Specifically, subgenera *Epallagogonium* and *Rhipsalis* are polyphyletic. Furthermore, three main lineages are recognized within *Rhipsalis*: “sunken pericarpel clade”, *Erythrorhipsalis* and “core *Rhipsalis*”. 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., submitted). However, Calvente et al. (submitted) found that subgenus *Calamorhipsalis* and *R. paradoxa* formed independent lineages that were sister to the “core *Rhipsalis*” and not to the remaining taxa of the “sunken pericarpel clade” (supported by moderate and low bootstrap values only). The higher number of markers and more comprehensive sampling within *Rhipsalis* in the present 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 rainforest epiphytes indicates that bees are the most common pollinators of epiphytes (van Dulmen, 2001). Even though pollination studies have never been conducted with *Rhipsalis* specifically, the flower morphology of representatives of this genus has led researchers to assume bee pollination for most species. The ancestral reconstruction of flower type indicated that the campanulate and/or terminal flowers with pericarpel not immersed in stems are exclusive to subgenus *Erythrorhipsalis* (Fig. 3). These flowers are pendent, generally white and delicate, often with colored filaments (Figs 1, 2), representing an innovation that is likely associated with a specific group of pollinators.

The lateral rotate flowers with immersed pericarpel appear in the “sunken pericarpel clade”. 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.

Rotate lateral flowers with pericarpel not immersed is characteristic of the “core *Rhipsalis*” clade (Fig. 3), although rotate lateral flowers also appear in the “sunken pericarpel clade.” It would be interesting to investigate the ontogenic history of this character in both lineages to see whether any developmental differences are encountered. Flowers from the “core *Rhipsalis*” clade are generally white, smaller and reduced, suggesting that the diversification of this group is associated with morphological simplification and reduction of flower parts rather than the origin of floral innovations (Calvente et al., submitted). It is still unclear whether the reduction of flower parts and the evolution to rotate lateral flowers with non-immersed pericarpels within the “core *Rhipsalis*” clade are related to ecological pressures by pollinators or from historical factors.

Ancestral state reconstructions of stem shape in the genus revealed that this character is constant in *Phyllarthrorhipsalis* but homoplasious in all other lineages of *Rhipsalis*. All species of *Phyllarthrorhipsalis* present stems with expanded wings, with only a few exceptions (*R. cereoides* and forms of *R. micrantha*).

The evolution of stem shape has been investigated for decades and is thought to be highly related to environmental pressures and habitat conditions. Specifically in

Cactaceae, studies have found a correlation between stem shape, leaf morphology and habitat (Griffith, 2009; Wallace and Gibson, 2002). In subfamily Opuntioideae, flat stems and leaves are only found in areas in which aridity is not an absolute limiting factor; it is possible that the increased surface area of the flattened stems and reversal for the production of leaves might represent adaptations to overcome limited photosynthetic capacity (Griffith, 2009). Epiphytes need to be highly adaptive to challenging environments, with varying supplies of water, nutrients and light. In those environments, survival is dependent on efficient storage capacity, economical use of water and rapid ability to rebound from drought-imposed stress (Benzing, 1987). Even in the humid tropics, sporadic or seasonal periods of water shortage occur, representing one of main abiotic stress factor to which epiphytes are exposed to (Zotz and Thomas, 1999). We hence hypothesized that stem shape is critical for the evolution of successful epiphytic cacti, and expect this character to be correlated with the occupation of different habitats. In particular, stems with expanded wings 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 reduced surface area, reducing water loss through transpiration, and thus being more effective in dry areas. Unfortunately, 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. However, the sister taxa to *Rhipsalis* (i.e., *Hatiora* and *Schlumbergera*; Calvente et al., Submitted) were already epiphytic, making it unclear at what point in the evolution of Rhipsalideae the evolution of epiphytic habit took place. 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, followed by multiple independent transitions to the rupicolous habit. Rupicolous species inhabit gneiss-granitic mountains (inselbergs) that occur within the Atlantic Forest. The vegetation of inselbergs differs markedly from that of their surroundings due to edaphic and microclimate conditions (Porembski et al., 1998). Species of *Rhipsalis* that occur within these open and sunny habitats generally grow exclusively in these environments. However, some species (e.g., *R. pachyptera*, *R. russellii*, and *R. floccosa*) are

rupicolous-facultative in inselbergs, being also found as epiphytes. These species normally occur in more sunny epiphytic environments (e.g., tree canopy), indicating that the environmental conditions offered by this microhabitat may be similar to the conditions found in rupicolous habitats. Furthermore, *R. micrantha* also inhabits Andean epiphytic and rupicolous habitats, while *R. baccifera* also occupies the same niche in Africa, illustrating a connection between epiphytic and rupicolous habitats in other tropical forest formations.

Although flower type and stem shape represent the most variable macro-morphological features in *Rhipsalis*, the evolution of neither character seem to be correlated with habit evolution in *Rhipsalis*. Other studies found correlations between leaf anatomical characters and the occupation of epiphytic and rupicolous habitats in *Cymbidium* (Yukawa and Stern, 2002), as well as correlations between embryo size and habitat in Orchidaceae (Tsutsumi et al., 2007). In Cactaceae, specifically, an variation between epidermal cells, stomatal position and sclerenchyma distribution were also documented (Calvente et al., 2008), indicating that further research on the stem anatomy and seed morphology of *Rhipsalis* might bring interesting conclusions on the evolution of habit in the genus.

Biogeography— Epiphytism evolved prior to the split between South America and Africa 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, ca. 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 the biogeographical areas of *Rhipsalis* indicated that the genus originated in Coastal Brazil and subsequently diversified and expanded into other biogeographical areas. This finding and the results from the DIVA analysis corroborate 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 (ancestors of *R. cuneata* and *R. micrantha*). Alternatively, it is also possible that two distribution expansions may have occurred in the ancestors of *R. cuneata* and *R. micrantha*, leading to a wider

distribution of these taxa, subsequently divided by vicariance events. Because epiphytic diversity is correlated with wet forests (Gentry and Dodson, 1987), this scenario would imply that the ancestors of both clades would have had to find a path through wet tropical forests in order to expand their distributions to the west. A link between the Brazilian Paramos (*campos de altitude*) and Andean highland vegetation has been documented (Safford, 2007), suggesting a potential migration route through northern Argentina/Paraguayan lowlands, the highlands of Uruguay and southern Brazil. Although *R. elliptica*, *R. oblonga* and *R. crispata* do not occur in the Brazilian Paramos, these taxa inhabit the marginal highland forests that surround this vegetation. Therefore, it is possible that the ancestors of these taxa may have been distributed along this route as well. The fact that *R. crispata* can occur further 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* (and perhaps even the ancestors of *R. micrantha*, *R. elliptica* and *R. oblonga* A) may have been tolerant to drier climates, having reached the Bolivian rising Andean vegetation by transposing the Brazilian quaternary dryer forests expansion and Chaco region (Pennington et al., 2000).

DIVA analysis 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 also frequent in these areas, commonly reaching 2000 m above sea level. Therefore, expansion to Southern Brazil would be a natural phenomenon following similar climatic and environmental conditions. This expansion is likely recent as the transitions are hypothesized to have occurred in terminal branches and to not involve closely related taxa.

Conclusions — A robust phylogeny of *Rhipsalis* provided the basis for a study of the evolution of key morphological features and for biogeographical inferences in the genus. Overall, several species of *Rhipsalis* seem to have expanded their geographic range from the Atlantic Forest into other tropical forests in South America (*R. floccosa*, *R. cereuscula*, *R. cuneata* and *R. micrantha*), North America, Africa and Asia (*R. baccifera*), either by dispersal or vicariance. These transitions occurred at different times, with some of the transitions having happened long ago, and others being recent, suggesting ancient and recent associations between the Southern American epiphytic

flora. However, neither flower type, nor stem shape seems to have been associated with small scale habitat transitions or large scale transitions through different biogeographical regions. Given that flower type and stem shape represent the main morphological features involved in the diversification of *Rhipsalis*, it is possible that historical processes and other physiological or micro-morphological traits may have played key roles in the diversification of these South American epiphytic cacti.

2.5. LITERATURE CITED

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Figure 1. Morphological and habitat diversity of *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. pilocarpa*. K. *R. baccifera*. L. *R. grandiflora*. M. *R. floccosa*. N. *R. paradoxa*. O, P. *R. puniceodiscus*.

Figure 2. Phylogenetic tree of *Rhipsalis* derived from the Bayesian analysis of the “large combined” data set (cpDNA *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, and nuclear ITS and MS). Maximum parsimony bootstrap and maximum likelihood bootstrap 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 (additional specimens were trimmed); non-monophyletic species are indicated with letters A, B or C following species names. Subgenera are indicated on the right, with type species for each indicated by an asterisk (*). Clades 1, 2, 3 were recovered from the analysis of the “small combined” data set (ITS + cpDNA) and reconstructs the position of species (in grey) not included in the “large combined” data set.

Figure 3. Ancestral state reconstructions of selected morphological traits (flower, stem), habit and biogeographical areas of *Rhipsalis*. Ancestral areas obtained with dispersal-vicariance analysis (DIVA) are indicated by letters A, B, C, D, E on the branches.

Online Appendix 1. Sampling, vouchers and GenBank accession numbers.

Species; vouchers - accession numbers *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, ITS, MS.

R. agudoensis; Hoerst-Uebelman 821, living collection (Uhlig-Kakteen) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. cereoides***; Barros 2302, RJ, Brazil, (RB) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. crispata***; Calvente 368, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 215, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 366, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 365, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. cuneata***; Aguillar s.n. (Cult. Bauer 105) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Glatz s.n. (Cult. Bauer 270) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Ruiz s.n. (Cult. Bauer 343) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Krahn s.n. (Cult. Bauer 346) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Ibisch 93766A (Cult. Bauer 776) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. elliptica***; Calvente 214, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 96, RJ, Brazil (RUSU) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 350 SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 337 SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 369 SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 194, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. goebeliana***; living collection (Kew 2000-1071) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. micrantha***; Versieux 442, Puntarenas, Costa Rica (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 360, El Rodeo, Costa Rica (INBIO) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 361, El Rodeo, Costa Rica (INBIO) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 388, El Oro, Ecuador (QCNE) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 386, Loja, Ecuador (QCNE) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 383, Loja, Ecuador (QCNE) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 392, Cañar, Ecuador (QCNE) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 396, Cajamarca, Peru (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 394, Cajamarca, Peru (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. oblonga***; Calvente 407, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 342, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 202, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 196, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 218, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 245, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. occidentalis***; Calvente 381, Loja, Ecuador (QCNE) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; living collection (Kew 1990-1883) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. olivifera***; Calvente 221, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 151, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 226, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. pachyptera***; Calvente 250 RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 272, ES, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 211, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 354, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 277, ES, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. russellii***; Calvente 309, BA, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 326, ES, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 313, BA, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Zappi 195, MG, Brazil (K) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. triangularis***; Calvente 88, RJ, Brazil (RUSU) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. (53) ***R. sp.***; Calvente 284, ES, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. neves-armondii***; Versieux 196, ES, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. puniceodiscus***; Calvente 177, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. (3) ***R. dissimilis***; Calvente 401, PR, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. floccosa***; Calvente 276, ES, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. paradoxa***; Calvente 145, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. pentaptera***; Calvente 100, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. trigona***; Calvente 404, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. baccifera***; Calvente 379, Loja, Ecuador (QCNE) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. ewaldiana***; living collection (Kew 1996-758) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. grandiflora***; living collection (Kew 1996-540) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. lindbergiana***; Calvente 161, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. mesembryanthemoides***; Freitas s/n, RJ, Brazil (RB) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. teres***; Calvente 86, RJ, Brazil (RUSU) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX; Calvente 255, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. (12) ***R. campos-portoana***; living collection (Kew 1996-2332) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. clavata***; Calvente 240, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. juengeri***; Calvente 266, MG, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. ormindoi***; Calvente 154, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. pulchra***; Calvente 232, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. cereuscula***; living collection (Kew 1991-1439) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***R. pilocarpa***; Calvente 357, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. (7) ***H. salicornioides***; Calvente 239, RJ, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. ***S. orssichiana***; Freitas 28, SP, Brazil (SPF) – XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX, XXXXXXXX. (2)

Online Appendix 2. Circumscription of *Rhipsalis* subgenera (Hunt et al., 2006, with minor modifications), and geographic distribution of species sampled. Asterisks indicate species not sampled in this study. Type species are marked in bold, Brazilian states are given with acronym abbreviations. Endemic Brazilian species are indicated by an asterisk following the distribution. **(online only)**

Subgenera	Species	Geographic distribution
		Coding used (actual distribution)
<i>Phyllarthrorhipsalis</i>	<i>R. agudoensis</i> N.P. Taylor	S Brazil (RS)*
	<i>R. cereoides</i> (Backeb. & Voll) Backeb.	Coastal Brazil (RJ)*
	<i>R. crispata</i> (Haw.) Pfeiff.	Coastal Brazil (RJ, SP)*
	<i>R. cuneata</i> Britton & Rose	Andes (Ecuador, Peru, Colombia, Bolivia)
	<i>R. elliptica</i> G.A. Lindberg ex K. Schum.	Coastal, S Brazil (widespread)*
	<i>R. goebeliana</i> Backeb.	unknown
	<i>R. micrantha</i> (Kunth) DC	Central America, Andes (Costa Rica, Ecuador, Peru, Colombia)
	<i>R. oblonga</i> Loefgr.	Coastal Brazil (BA, RJ, SP)*
	<i>R. occidentalis</i> Barthlott	considered = <i>R. cuneata</i>
	<i>R. olivifera</i> N.P. Taylor & Zappi	Coastal Brazil (RJ)*
	<i>R. pachyptera</i> Pfeiff.	Coastal, S Brazil (widespread)*
	<i>R. russellii</i> Britton & Rose	Coastal Brazil (BA, MG)*
	<i>R. triangularis</i> Werderm.	Coastal Brazil (RJ)*
<i>Calamorphipsalis</i>	<i>R. hoelleri</i> Barthlott & N.P. Taylor (*)	Coastal Brazil (ES)*
	<i>R. neves-armondii</i> K. Schum.	Coastal Brazil (ES, RJ)*
	<i>R. puniceodiscus</i> G.A. Lindberg	Coastal Brazil (RJ, SP, PR, SC)*
<i>Epallagonium</i>	<i>R. dissimilis</i> (G.A. Lindberg) K. Schum.	Coastal, S Brazil (SP, PR)*
	<i>R. floccosa</i> Salm-Dyck ex Pfeiff.	S. South America / Coastal Brazil / S Brazil (widespread)
	<i>R. pacheco-leonis</i> Loefgr. (*)	Coastal Brazil (RJ)*
	<i>R. paradoxa</i> (Salm-Dyck ex Pfeiff.) Salm-Dyck	Coastal, S Brazil (widespread)*
	<i>R. pentaptera</i> A. Dletrich.	Coastal Brazil (RJ)*
	<i>R. sulcata</i> F.A.C. Weber (*)	Unknown (Brazil, unknown)*
	<i>R. trigona</i> Pfeiff.	Coastal, S Brazil (SP, PR, SC)*
<i>Erythrorhipsalis</i>	<i>R. burchellii</i> Britton & Rose (*)	Coastal Brazil (RJ, SP)*
	<i>R. campos-portoana</i> Loefgr.	Coastal, S Brazil (MG, RJ, SP, PR, SC)*
	<i>R. clavata</i> F.A.C. Weber	Coastal Brazil (RJ, SP)*
	<i>R. juengeri</i> Barthlott & N.P. Taylor	Coastal Brazil (MG, RJ)*
	<i>R. ormindoi</i> N.P. Taylor & Zappi	Coastal Brazil (RJ)*
	<i>R. pulchra</i> Loefgr.	Coastal Brazil (MG, RJ, SP)*
	<i>R. cereuscula</i> Haw.	Coastal Brazil / S South America (widespread)
	<i>R. pilocarpa</i> Loefgr.	Coastal Brazil, S. Brazil (RJ, SP, PR)*
<i>Rhipsalis</i>	<i>R. baccifera</i> (J.S. Muell.) Stearn	All areas (widespread)
	<i>R. ewaldiana</i> Barthlott & N.P. Taylor	Unknown (Brazil, unknown)*
	<i>R. grandiflora</i> Haw.	Coastal Brazil (RJ, SP)*
	<i>R. lindbergiana</i> K. Schum.	Coastal Brazil (widespread)*
	<i>R. mesembryanthemoides</i> Haw.	Coastal Brazil (RJ)*
	<i>R. teres</i> (Vell.) Steud.	Coastal, S Brazil (widespread)*

Online Appendix 3. Primers used in this study.

Marker	Primers	Source
ITS	17SE - ACG AAT TCA TGG TCC GGT GAA GTG TTC G	Sun et al., 1994
	26SE - TAG AAT TCC CCG GTT CGC TCG CCG TTA C	
MS	400f - GGA AGA TGR TCA TCA AYG CNC TYA AYT C	Lewis & Doyle, 2001
	943r - GTC TTN ACR TAG CTG AAD ATR TAR TCC C	
	Rh.F - ATG CRT GTA AAT TAA TGT GGT CTC ACT C	This study
	Rh.R_int - CCG GAG ATC TAT AGC CTA ATT TGA GTG TC	
	Rh.F_int - CTA AGA TGG ARC ACT CCA GGC AAG TTG	
	Rh. R - AAT ACT GCA GGA AGT GTC TCT ATC AGC AC	
<i>psbA-tmH</i>	<i>psbA</i> - GTTATGCATGAACGTAATGCTC	Sang et al., 1997
	<i>tmH2</i> - CGCGCATGGTGGATTCACAAATC	Tate et al., 2003
<i>rpl32-tmL</i>	<i>rpl32Cact</i> - GTT ATC TTA GGT TTC AAC AAA CC	This study
	<i>rpl32</i> - CAG TTC CAA AA A AAC GTA CTT C	Shaw et al. 2007
	<i>tmL(UAG)</i> - CTG CTT CCT AAG AGC AGC GT	
<i>tmQ-rps16</i>	<i>tmQ(UUG)</i> - GCG TGG CCA AGY GGT AAG GC	
	<i>rps16x1</i> - GTT GCT TTY TAC CAC ATC GTT T	

CAPÍTULO 3

A NEW SUBGENERIC CLASSIFICATION OF *RHIIPSALIS* (CACTOIDEAE, CACTACEAE)

CALVENTE ET AL., SYSTEMATICS OF *RHIPSALIS*

A new subgeneric classification of *Rhipsalis* (Cactoideae, Cactaceae)

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ABSTRACT

Most Cactaceae have succulent stems and inhabit dry or arid areas, although some are epiphytes that occur in humid regions. *Rhipsalis* is the largest epiphytic genus of cacti. Despite their ecological importance, species of *Rhipsalis* are notoriously difficult to identify, and the subgeneric classification of the genus has remained controversial. Between 1837 and 1995, eight different subgeneric classifications have been proposed for *Rhipsalis*. The most comprehensive taxonomic treatment of the genus recognized five subgenera, *Phyllarthrorhipsalis*, *Rhipsalis*, *Epallagonium*, *Calamorhipsalis*, and *Erythrorhipsalis*, mainly characterized by stem morphology. Here, we use new morphological data and molecular phylogenetic information as basis to re-evaluate the former subgeneric classifications proposed for the genus. We recognize three monophyletic subgenera, *Rhipsalis*, *Calamorhipsalis* and *Erythrorhipsalis*, that are mainly characterized by floral traits and molecular data. The changes proposed mainly include expanding the circumscription of *Rhipsalis* by the inclusion of species previously included in *Phyllarthrorhipsalis*, *Epallagonium* and *Calamorhipsalis* and recognizing a broader *Calamorhipsalis* (also includes species from subg. *Epallagonium*). The circumscription of *Erythrorhipsalis* remains unchanged. For each subgenus we present a list of synonyms, description and a list of species included. Furthermore, a key for the identification of subgenera is also provided.

Keywords — Atlantic Forest, epiphytic cacti, Rhipsalideae.

3.1. INTRODUCTION

Cactaceae includes 124 genera and 1.438 species distributed throughout the Neotropics, except for *Rhipsalis baccifera* (J.S.Muel.) Stearn, that also occurs in Africa and Asia (Hunt et al. 2006). The family has been traditionally divided in three subfamilies, Pereskioideae, Opuntioideae and Cactoideae (Nyffeler 2002), of which Cactoideae is the most diverse, including the widest morphological variation and the highest number of taxa (Barthlott and Hunt 1993).

Most Cactoideae have succulent stems and inhabit dry or arid areas, although some are epiphytes that occur in humid regions. The tribes Hylocereae and Rhipsalideae include most epiphytic species (Hunt et al. 2006). Rhipsalideae, in particular, is composed of four genera, *Hattoria* Britton & Rose, *Lepismium* Pfeiff., *Rhipsalis* Gaertn., and *Schlumbergera* Lem., of which *Rhipsalis* is the largest. A recent molecular phylogeny of the Rhipsalideae supported the monophyly of *Rhipsalis* and *Lepismium* but indicated that *Hattoria* and *Schlumbergera* are paraphyletic as previously circumscribed (Calvente et al. submitted). The majority of species belonging to this tribe are endemic to Brazil, with several species presenting very restricted distribution ranges and being threatened due to habitat reduction.

The most recent and comprehensive taxonomic treatment of *Rhipsalis* included 33 species distributed in five subgenera: *Calamorhipsalis* K.Schum., *Epallagonium* K.Schum., *Erythrorhipsalis* A.Berger, *Phyllarthrorhipsalis* Buxb., and *Rhipsalis* (Barthlott and Taylor, 1995). The subgeneric classification of *Rhipsalis* proposed by Barthlott and Taylor (1995) was also adopted in the most recent account of the Cactaceae, which recognized 35 species in *Rhipsalis* (Hunt et al. 2006). However, a recent molecular phylogeny of *Rhipsalis* (Calvente et al. in prep.) indicated that three subgenera are polyphyletic as previously circumscribed (*Rhipsalis*, *Calamorhipsalis*, and *Epallagonium*). Here, we propose a new subgeneric classification for *Rhipsalis* based on morphological characters and molecular phylogenetic data (Calvente et al. in prep). Only monophyletic subgenera, diagnosed by morphological synapomorphies are recognized. The proposed changes mainly include expanding the circumscription of *R.* subg. *Calamorhipsalis* by the inclusion of some species previously placed within *R.* subg. *Epallagonium*, and widening the circumscription of *R.* subg. *Rhipsalis* by the inclusion of

species previously comprised in *R.* subg. *Phyllarthrorhipsalis*, *R.* subg. *Epallagogonium* and *R.* subg. *Calamorhipsalis*. The circumscription of *R.* subg. *Erythorhipsalis* remains unchanged.

3.2. TAXONOMIC HISTORY

The first species of *Rhipsalis* was described as *Cassytha baccifera* J.S. Muell. However, *C. baccifera* was an illegitimate name at generic level due to the previous publication of *Cassytha* L., within the Lauraceae (Linneus, 1753). Gaertner (1788) subsequently described *Rhipsalis* and transferred *Cassytha baccifera* J.S. Muell. into *Rhipsalis* as *R. cassutha* (J.S. Muell.) Gaertner (often corrected to *R. cassytha*); he also recognized *R. cassutha* as the type of *Rhipsalis*. Even though Gaertner (1788) used the same type-material of *Cassytha baccifera*, he adopted a different specific epithet, making *R. cassutha* (J.S. Muell.) illegitimate. Stearn (1839) noticed this equivocal combination and published *Rhipsalis baccifera* (J.S. Muell.) Stearn (the correct type of *Rhipsalis*). It is also important to note that the genus *Hariota* was described by Adanson (1763) prior to the publication of *Cassytha baccifera* J.S. Muell. However, the problematic application of Adansonian uninomials (see Parkinson 1987, 1988) led to the conservation of *Rhipsalis* Gaertn. against *Hariota* Adans.

De Candolle (1828) represents the first comprehensive treatment of *Rhipsalis*, where 7 species are recognized without infrageneric classification. *Rhipsalis* was subsequently subdivided into four series (*Alatae*, *Angulosae*, *Teretes* and *Articuliferae*), characterized by the shape of the stems (Pfeiffer 1837). A modified version of this subgeneric classification was adopted by Salm-Dyck (1850), who also recognized *Sarmentosae* in addition to *Alatae*, *Angulosae*, *Teretes* and *Articuliferae*. Schumann (1890) maintained the same subgeneric division of *Rhipsalis* in series, but divided *Alatae* into *Perpetuae* and *Terminatae*, and did not recognize *Articuliferae*.

Schumann (1899) was the first author to divide the genus into subgenera and to use flower morphology as another key diagnostic feature of infrageneric taxa of *Rhipsalis*. He described eight subgenera (*Eurhipsalis*, *Goniorhipsalis*, *Ophiorhipsalis*, *Phyllorhipsalis*, *Acanthorhipsalis*, *Calamorhipsalis*, *Epallagogonium*, and *Lepismium*) and placed all species with immersed pericarpels within *Calamorhipsalis*, *Epallagogonium* and *Lepismium*. Löfgren (1915, 1917) subsequently accepted the eight subgenera proposed by Schumann (1899), but widened the circumscription of *Rhipsalis* to include two additional subgenera (*Pfeiffera* and *Hariota*), both of which included species currently placed within *Lepismium* and *Hatoria*. Britton and Rose (1923) did not follow

the classifications proposed by Schumann (1899) or Löfgren (1915, 1917) and divided *Rhipsalis* into 16 new series.

More recently, Buxbaum (1970) recognized the subtribe Rhipsalinae with three “linae” (Pfeifferae, Schlumbergereae and Rhipsales) and divided *Rhipsalis* into four subgenera *Goniorhipsalis*, *Phyllorhipsalis* (= Perpetuae, current *Lepismium* winged spp.), *Phyllarthrorhipsalis* (= Terminatae, current *Rhipsalis* winged spp.) and *Rhipsalis*. In addition, Buxbaum (1970) subdivided the subgenus *Rhipsalis* in four series: *Ophiorhipsalis*, *Mesembryanthoides*, *Cereusculii* and *Rhipsalis*. Most species currently recognized as *Rhipsalis* were included in *Erythrorhipsalis* (Schlumbergereae) and in *Rhipsalis* (Rhipsales) itself under Buxbaum’s classification.

The classifications of Barthlott (1987) and Barthlott & Taylor (1995) transferred all species of *Rhipsalis* with basal and lateral branching and indeterminate growth to *Lepismium*, recognizing *Rhipsalis* in a narrower sense. This classification also recognized five subgenera of *Rhipsalis* (*Rhipsalis*, *Calamorhipsalis*, *Epallagonium*, *Phyllarthrorhipsalis* and *Erythrorhipsalis*), mainly characterized by stem shape, and a few flower characters. The latest classification of the genus (Hunt et al. 2006) recognized the same five subgenera proposed by Barthlott (1987) and Barthlott & Taylor (1995), and a tribe Rhipsalideae that included four genera (*Hatiora*, *Lepismium*, *Rhipsalis*, and *Schlumbergera*), however several species previously circumscribed in *Lepismium* were transferred to Pfeiffera (tribe Hylocereeae).

Table 1. History of the infrageneric classifications of *Rhipsalis*.

Author	Notes:
De Candolle 1828	Listed <i>Rhipsalis</i> spp. without subdivisions
Pfeiffer 1837	Divided <i>Rhipsalis</i> in 4 series based on stem shape (including species currently placed in <i>Lepismium</i>): Alatae, Angulosae, Teretes and Articuliferae
Salm-Dyck 1850	Divided <i>Rhipsalis</i> in 5 series: Alatae, Angulosae, Teretes, Sarmentosae and Articuliferae
Schumann 1890	Divided <i>Rhipsalis</i> in 4 series based on stem shape (including species currently placed in <i>Lepismium</i>): Teretes, Angulatae, Alatae (divided in Perpetuae and Terminatae) and Sarmentosae.
Schumann 1899	Divided <i>Rhipsalis</i> (including <i>Lepismium</i> spp) in eight subgenera based on stem and flower morphology: <i>Eurhipsalis</i> , <i>Goniorhipsalis</i> , <i>Ophiorhipsalis</i> , <i>Phyllorhipsalis</i> , <i>Acanthorhipsalis</i> , <i>Calamorhipsalis</i> , <i>Epallagogonium</i> , <i>Lepismium</i>
Löfgren 1915, 1917	Described several Brazilian new species and a taxonomic treatment adopting the subgenera proposed by Schumann (1899) and two new: <i>Pfeiffera</i> and <i>Hariota</i> (including <i>Lepismium</i> and <i>Hattoria</i> species).
Britton & Rose 1923	Divided <i>Rhipsalis</i> in 16 series (small groups of morphological similar species)
Buxbaum 1970	Recognized the tribe Hylocereae, subtribe Rhipsalinae with 3 "linae": Pfeifferae, Schlumbergereae and Rhipsales. Current <i>Rhipsalis</i> species are positioned in genera <i>Erythrorhipsalis</i> (Schlumbergereae) and <i>Rhipsalis</i> (Rhipsales). <i>Rhipsalis</i> is divided in four subgenera: <i>Goniorhipsalis</i> , <i>Phyllorhipsalis</i> (= Perpetuae, current <i>Lepismium</i> winged spp.), <i>Phyllarthrorhipsalis</i> (=Terminatae, current <i>Rhipsalis</i> winged spp.) and <i>Rhipsalis</i> . He further subdivided the subgenus <i>Rhipsalis</i> in four series: <i>Ophiorhipsalis</i> , <i>Mesembryanthoides</i> , <i>Cereusculii</i> and <i>Rhipsalis</i> .
Barthlott 1987; Barthlott & Taylor 1995	Transferred species from <i>Rhipsalis</i> to <i>Lepismium</i> . Divided the remaining species of <i>Rhipsalis</i> in five subgenera: <i>Rhipsalis</i> , <i>Calamorhipsalis</i> , <i>Epallagogonium</i> , <i>Phyllarthrorhipsalis</i> and <i>Erythrorhipsalis</i> .
Hunt 2006	Followed the subgeneric classification of Barthlott & Taylor (1995) and recognized five subgenera: <i>Rhipsalis</i> , <i>Calamorhipsalis</i> , <i>Epallagogonium</i> , <i>Phyllarthrorhipsalis</i> and <i>Erythrorhipsalis</i> .

3.3. A NEW SUBGENERIC CLASSIFICATION OF RHIPSALIS

The molecular phylogeny of Rhipsalideae (Calvente et al. submitted) supports the segregation of *Lepismium* from *Rhipsalis* and a “narrower” circumscription of *Rhipsalis* as proposed by Barthlott (1987), Barthlott & Taylor (1995) and Hunt et al. (2006). However, recent molecular phylogenetic data (Calvente et al. in prep.) do not support an infrageneric division of *Rhipsalis* based on stem shape. Instead, it suggests that a subdivision of the genus in three main groups characterized by flower traits would be more appropriate. More specifically, it suggests that the three main clades reconstructed in the molecular phylogeny (Fig. 1) should be recognized, as three subgenera, as follows: (1) *Rhipsalis* s.l. (“core *Rhipsalis*”); (2) *Erythrorhipsalis*; and (3) *Calamorhipsalis* s.l. (=“sunken pericarpel” clade).

The “core *Rhipsalis*” clade (pp = 1.00, pb = 100, pl = 99) includes all species previously included in *Rhipsalis* subg. *Phyllarthrorhipsalis* and *Rhipsalis* subg. *Rhipsalis*, plus *R. pentaptera* (subg. *Epallagogonium*). While *Rhipsalis* subg. *Phyllarthrorhipsalis* is monophyletic (pp = 0.94), *Rhipsalis* subg. *Rhipsalis* is polyphyletic. All species from the “core *Rhipsalis*” clade are here included in a broader subg. *Rhipsalis*.

Erythrorhipsalis (pp = 1.00, pb = 100, pl = 100) presents campanulate and/or terminal flowers and is the only subgenus whose original circumscription is corroborated by the molecular phylogenetic data (Barthlott 1987, Barthlott and Taylor, 1995; Hunt et al. 2006). The four remaining subgenera (*Rhipsalis*, *Calamorhipsalis*, *Epallagogonium*, and *Phyllarthrorhipsalis*) are either paraphyletic, polyphyletic, or not strongly supported.

The “sunken pericarpel” clade (pp = 1.00, pb = 84, pl = 85) includes all species previously placed within subgenera *Calamorhipsalis* and *Epallagogonium*, except for *R. pentaptera*, a species previously included within subg. *Epallagogonium* and which emerges within the “core *Rhipsalis*” clade. However *R. pentaptera* presents rotate flowers with the pericarpel not immersed in the stem, matching perfectly the flower morphology found in the remaining species of the “core *Rhipsalis*” clade. All species belonging to the “sunken pericarpel” clade, on the other hand, are characterized by the

rotate flowers and a pericarpel deeply immersed in the stem. All species from the “sunken pericarpel” clade are here included in a broader subg. *Calamorhipsalis*.

Below we propose a new subgeneric classification of *Rhipsalis* that only recognizes monophyletic subgenera characterized by morphological synapomorphies. The changes here proposed mainly include expanding the circumscription of *Rhipsalis* by the inclusion of species previously included in *Phyllarthrorhipsalis*, *Epallagonium* and *Calamorhipsalis* and recognizing a broader *Calamorhipsalis* (also includes species from subg. *Epallagonium*). The circumscription of *Erythrorhipsalis* remains identical.

RHIPSALIS Gaertn., Fruct. Sem. pl. 1: 137. 1788, *nom. cons.* *Hariota* Adans., Fam. Pl. 2: 243. 1763. *Cassytha* J.S.Muell., Gard. Dict., ed. 8. 1768, non L., 1753.—TYPE: *Rhipsalis cassutha* Gaertner (= *Rhipsalis baccifera* (J.S.Muell.) Stearn).

1. RHIPSALIS subg. RHIPSALIS

Rhipsalis subg. *Phyllarthrorhipsalis*, Buxb. in Krainz, Kakteen 44-45: 1970, *syn. nov.*—TYPE: *Rhipsalis pachyptera* Pfeiff.

Rhipsalis subg. *Goniorhipsalis* K.Schum., Gesamtbeschr. Kakt.: 615. 1898, *syn. nov.*—TYPE: *Rhipsalis pentaptera* A.Dietr.

Branching apical, subapical or lateral. Stem segments cylindrical or 2-6 winged. Flowers rotaceous, lateral or sublateral, rarely also apical in stem segments; pericarpel not immersed in the areole.

Species Included: *R. baccifera* (Mill.) Stearn, *R. cereoides* (Backeb. & Voll) Backeb., *R. crispata* (Haw.) Pfeiff., *R. crispimarginata* Loefgr., *R. cuneata* Britton & Rose, *R. elliptica* G.Lindb. ex K.Schum., *R. ewaldiana* Barthlott & N.P.Taylor, *R. goebeliana* Backeb., *R. grandiflora* Haw., *R. lindbergiana* K.Schum., *R. mesembryanthemoides* Haw., *R. micrantha* (Kunth) DC., *R. oblonga* Loefgr., *R. olivifera* N.P.Taylor & Zappi, *R. pachyptera* Pfeiff., *R. pentaptera* A. Dietr., *R. russellii* Britton & Rose, *R. sulcata* F.A.C.Weber, *R. teres* (Vell.) Steud., *R. triangularis* Werderm.

2. RHIPSALIS subg. ERYTHORHIPSALIS A.Berger, Monatsschr. Kakteenk. 30: 4. 1920.—TYPE: *Rhipsalis pilocarpa* Loefgr.

Stem segments cylindrical to clavate. Flowers campanulate, apical or lateral in stem segments or rotaceous and strictly apical in stem segments; pericarpel not immersed in the areole.

Species Included: *R. aurea* M.F.Freitas & J.M.A.Braga, *R. burchellii* Britton & Rose, *R. campos-portoana* Loefgr., *R. clavata* F.A.C.Weber, *R. juengeri* Barthlott & N.P.Taylor, *R. ormindoi* N.P.Taylor & Zappi, *R. pulchra* Loefgr., *R. cereuscula* Haw., *R. pilocarpa* Loefgr.

3. RHIPSALIS subg. CALAMORHIPSALIS K.Schum., Gesamtbeschr. Kakt.: 615. 1898.—TYPE: *Rhipsalis neves-armondii* K.Schum.

Rhipsalis subg. *Epallagonium* K.Schum., Gesamtbeschr. Kakt.: 615. 1898, *syn. nov.* —TYPE: *Rhipsalis paradoxa* (Salm-Dyck ex Pfeiff.) Salm-Dyck.

Branching apical, subapical or lateral. Stem segments cylindrical, with well developed podaria, angled or with narrow wings, not continuous in stem segments. Flowers rotaceous, lateral or sublateral in stem segments; pericarpel conspicuously immersed in the areole.

Species Included: *R. dissimilis* (G.Lindb.) K.Schum., *R. floccosa* Salm-Dyck ex Pfeiff., *R. hoelleri* Barthlott & N.P.Taylor, *R. neves-armondii* K.Schum., *R. pacheco-leonis* Loefgr., *R. paradoxa* (Salm-Dyck ex Pfeiff.) Salm-Dyck, *R. puniceodiscus* G.Lindb., *R. trigona* Pfeiff.

3.4. IDENTIFICATION KEY TO SUBGENERA OF *RHIPSALIS*

- 1a. Pericarpel immersed in the areole *Rhipsalis* subg. *Calamorhipsalis*
- 1b. Pericarpel not immersed in the areole 2
- 2a. Stems cylindrical or clavate; flowers campanulate and/or exclusively apical in stem segments *Rhipsalis* subg. *Erythrorhipsalis*
- 2b. Stems cylindrical or winged; flowers rotate and lateral in stem segments (rarely apical) *Rhipsalis* subg. *Rhipsalis*

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Figure 1. Majority rule consensus tree of *Rhipsalis* derived from the Bayesian analysis of cpDNA *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, ITS and MS. Maximum parsimony bootstrap and maximum likelihood bootstrap 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 (additional specimens were trimmed); non-monophyletic species are indicated with letters A, B or C following species names. *Rhipsalis* subgenera are indicated in the right, with subgenera type species indicated by an asterisk following species names. Clades 1, 2, 3 were recovered from the analysis of ITS + cpDNA data set and reconstructs the position of species (in grey) not included in the cpDNA, ITS and MS data set. Extracted from Calvent et al (in prep).

CAPÍTULO 4

TAXONOMIC REVISION OF THE "WINGED-STEM CLADE" (RHIPSALIS, CACTACEAE)

CALVENTE ET AL., REVISION OF "WINGED-STEM CLADE"

Taxonomic revision of the "winged-stem clade" (*Rhipsalis*,
Cactaceae)

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ABSTRACT

Rhipsalis is the largest genus of epiphytic cacti. It currently includes 37 species distributed among three subgenera: *Rhipsalis* (20 spp.), *Erythrorhipsalis* (9 spp.), and *Calamorhipsalis* (8 spp.). The identification of taxa within *Rhipsalis* has always been problematic due to the natural plasticity in morphological traits, lack of information on the patterns of morphological variation in natural populations, existence of species complexes, and cryptic species. The “winged-stem clade” represents a monophyletic and morphologically homogenous group within *Rhipsalis* subg. *Rhipsalis*. The high level of morphological similarity among species from this clade has led to several taxonomic problems within this lineage and a particularly complicated circumscription of taxa. This taxonomic revision of taxa belonging to the “winged-stem clade” uses novel molecular phylogenetic data, field observations (including an extensive analyses of morphological traits in natural populations and type localities), and a careful examination of herbarium collections (including type specimens), and literature. Only monophyletic species are recognized and new circumscriptions are proposed for several taxa. For each taxa we present a complete description, a taxonomic commentary, information on the distribution and illustrations. Furthermore, a key for the identification of all species within *Rhipsalis* subg. *Rhipsalis* is presented.

Keywords — Rhipsalideae, epiphytic cacti, Atlantic Forest.

4.1. INTRODUCTION

Rhipsalis is the largest genus of epiphytic cacti. It currently includes 37 species distributed among three subgenera: *Rhipsalis* (20 spp.), *Erythrorhipsalis* (9 spp.), and *Calamorhipsalis* (8 spp.) (Calvente et al. in prep). Only one species of *Rhipsalis* occurs outside the (R. *baccifera* in Africa and Asia), while the majority of species are restricted to the Atlantic forest of Eastern Brazil, where many are endangered due to habitat loss (Taylor 1997).

Studies on *Rhipsalis* have focused on the taxonomy (Löfgren 1915, 1917; Barthlott 1987; Lombardi 1991, 1995; Barthlott and Taylor 1995), molecular phylogeny and evolution of the genus and closest relatives (Calvente et al. in prep; Calvente et al. submitted). Despite that, the identification of taxa within *Rhipsalis* remains problematic due to the natural plasticity in morphological traits, lack of information on the patterns of morphological variation in natural populations, existence of species complexes, and cryptic species (Calvente et al. 2005). Furthermore, herbarium collections of Cactaceae are often incomplete (lacking reproductive features or information on stem variation and branching patterns) and often difficult to be interpreted, as many important features are lost during the herborization process. All of these factors have led to the complicated taxonomic history of *Rhipsalis* as a whole.

The “winged-stem clade” is composed of species circumscribed in *Rhipsalis* subg. *Rhipsalis*. Species from this clade were traditionally included in *Rhipsalis* subg. *Phyllarthrorhipsalis*. However, the recognition of *Rhipsalis* subg. *Phyllarthrorhipsalis* would lead to a paraphyletic *Rhipsalis* subg. *Rhipsalis*. Hence, a broader *Rhipsalis* subg. *Rhipsalis* is now recognized, including all species previously included in *Rhipsalis* subg. *Phyllarthrorhipsalis* (Calvente et al. in prep). Species from the “winged-stem clade” are morphologically uniform and include two species complexes: (1) the *R. micrantha* complex and (2) the *R. crispata* complex (Barthlott and Taylor 1995). The high level of morphological similarity among species from this clade has led to several taxonomic problems within this lineage and a particularly complicated circumscription of taxa. A

recent molecular phylogeny of *Rhipsalis* (Calvente et al. in prep), including extensive sampling of specimens of the "winged-stem clade" provided critical information for the delimitation of taxa. This phylogeny indicated that two species (*R. oblonga* and *R. pachyptera*) were paraphyletic (Fig 1) and needed to be re-circumscribed.

This taxonomic revision of taxa belonging to the "winged-stem clade" incorporates novel molecular phylogenetic data with field observations (including an extensive analyses of morphological traits in natural populations and type localities) and careful examination of herbarium collections (including type specimens) and literature. Only monophyletic species are recognized and new circumscriptions are proposed for several taxa. For each taxa we present a complete description, a taxonomic commentary, information on the distribution and illustrations. Furthermore, a key for the identification of all species within *Rhipsalis* subg. *Rhipsalis* is presented.

4.2. MATERIALS AND METHODS

The taxonomic treatment, identification keys and geographical distribution of taxa are based on extensive field research conducted throughout the Neotropics, and on a detailed examination of the literature and collections from the following herbaria: BHCB, C, CR, GH, HB, HBR, HEID, HNT, HRCB, HUEFS, INB, JBSD, LOJA, LPB, K, MBM, MBML, MO, NY, P, QCA, QCNE, RB, S, SP, SPF, US, USM. A complete list of the examined specimens is provided in Appendix 1.

Molecular phylogenetic data (Calvente et al. in prep) were used as basis to test the monophyly of taxa. Only monophyletic taxa, diagnosable by distinct morphological characters were recognized. Whenever a species presented morphologically and geographically distinct populations, but with somewhat overlapping diagnostic features among populations, “subspecies” were recognized.

4.3. IDENTIFICATION KEY TO SPECIES OF *RHIPSALIS* SUBG.

RHIPSALIS

- 1a. Primary stem segments winged or angular, sometimes with cylindrical base, but never entirely cylindric 2
- 2a. Stem segments not conspicuously winged, angular, often with well-developed podaria 3
- 3a. Stem segments determined, all with similar size, branching apical *R. sulcata*
- 3b. Primary stem segments indetermined, long, secondary stem segments shorter *R. ewaldiana*
- 2b. Stem segments conspicuously winged, never with well-developed podaria 4
- 4a. Stem segments (4-)5(-6)-winged (never 2 or 3-winged) *R. pentaptera*
- 4b. Stem segments 2-6-winged, but 2 or 3-winged ones always present ... 5
- 5a. Sterile mature areoles < 1 mm diam 6
- 6a. Longest petaloid tepal < 4.5 mm long *R. cuneata*
- 6b. Longest petaloid tepal > 5 mm long 7
- 7a. Wings of secondary stem segments < 1 cm wide *R. micrantha*
- 7b. Wings of secondary stem segments > 1.5 cm wide 8
- 8a. Sterile mature areoles glabrous; longest petaloid tepal > 7 mm long; stigma lobes spreading and ligulate *R. elliptica*
- 8b. Sterile mature areoles with vestigial scales; longest petaloid tepal 6.5 mm; stigma lobes erect and sagitate *R. crispimarginata*
- 5b. Sterile mature areoles > 1 mm diam 9

-
- 9a. Wings of stem segments < 1 cm wide 10
- 10a. Flowers > 15 mm diam; longest petaloid tepal > 8 mm long (Brazil)
..... *R. cereoides*
- 10b. Flowers < 12 mm diam; longest petaloid tepal < 6.5 mm long
(Andes and Central america) *R. micrantha*
- 9b. Wings of stem segments > 1 cm wide 11
- 11a. Flowers < 7 mm diam; fruits deep magenta *R. russellii*
- 11b. Flowers > 7.1 mm diam; fruits white or pinkish 12
- 12a. First areoles of each segment 6-13 cm away from the segment
base; pericarpel with 1-4 bracts, petaloid tepals pinkish *R. olivifera*
- 12b. First areoles of each segment < 5.6 cm away from segment
base; pericarpel glabrous, petaloid tepals white, yellowish or reddish
..... 13
- 13a. All stem segments with margin projections 2-4 mm 14
- 14a. Stem segments 0.5-0.8 mm diam, margin serrate, areoles
between margin projections (Brazil) *R. oblonga*
- 14b. Stem segments 1-2 mm diam, margin serrate, slightly lobed
or dentate, areoles between or on margin projections (cult.)
..... *R. goebeliana*
- 13b. Stem segments with margin projections > 5 mm present 15
- 15a. Style 4-5 mm long; stigma lobes ligulate and spreading
..... *R. crispata*
- 15b. Style 6-8.5 mm long; stigma lobes sagitate and erect, rarely
spreading 16
- 16a. Stem segments 2-3-winged, wings (1.6-)2-7 cm wide
..... *R. pachyptera*

- 16b. Stem segments 3-5-winged, (0.5-)1-2(-3) cm wide
 R. triangularis
- 1b. Primary stem segments completely cylindrical 17
- 17a. Stem segments monomorphic, all with similar size 18
- 18a. Stem segments determined, < 15 cm, branching apical
 R. grandiflora
- 18b. Stem segments indetermined, > 16 cm branching subapical, lateral,
 rare apical *R. lindbergiana*
- 17b. Stem segments dimorphic, primary longer and secondary shorter 19
- 19a. Stems branching lateral or sub-apical, secondary stem segments < 15
 mm long, and conspicuously thicker than primary segments
 R. mesembryanthemoides
- 19b. Stems branching apical or sub-apical, secondary segments > 20 mm
 long), similar in thickness to primary segments 20
- 20a. Pericarpel as long as or longer than perianth; fruits elongated
 R. baccifera
- 20b. Pericarpel shorter than perianth; fruits globose *R. teres*

4.4. TAXONOMIC TREATMENT

All taxa from the “winged-stem clade” share stem segments with definite growth; pericarpel not immersed on stems, lateral, usually rotaceous, with deltoid external tepals and longer internal tepals; floral tube inconspicuous; style whitish and papillate; stamens free with base and filament of the same color; parietal placentation with ovules distributed in incomplete septa or defined longitudinal rows; nectary ring involving the base of the style.

1. RHIPSALIS CEREOIDES (Backeb. & Voll) A.Cast., Anais Reuniao Sul-Amer. Bot. 3: 12. 1940. *Lepismium cereoides* Backeb. & Voll in Backeb. & Knuth, Kaktus-ABC: 411. 1935. *Rhipsalis cereoides* Backeb. & Voll in Backeb. & Knuth, Kaktus-ABC: 411. 1935, *pro syn.*—TYPE: BRAZIL. Rio de Janeiro: Maricá, Itaipuaçu, 1936, *Voll s.n.* (neotype in Barthlott and Taylor 1995: RB10258!).

Lithophyte in open habitat, rarely epiphyte, 30-100 cm long, branching apical. Stem segments triangular to quadrangular in longitudinal section, rarely pentagonal, 2-5 mm diam, olive green with reddish margin, succulent, stiff, monomorphic, 6-12 cm long, base attenuate, apex truncate, wings 3-4(-5), 0.7-1 cm wide, margin entire to slightly crenate, straight, with 0-1(-3) mm projections, midrib ca. 6 mm diam, with shape not evidently marked (hidden by stem succulence). Areoles between margin projections, 1-1.5 cm apart, first of segment 1-1.5 cm distant from segment base; when sterile 2 mm diam, pilose sometimes with vestigial scales; when fertile 2.5-3 mm diam, pilose with numerous acicular scales, some spine-like, 1-3 flowers/fruits. Flowers ca. 15 mm diam; pericarpel 3.5-4 X 3.5 mm, obovoid, greenish, sometimes reddish, glabrous or with sepaloid bract near apex; with 1-4 sepaloid tepals, 1.5-3.5 mm long and 8-10 petaloid tepals, 4.5-9 X 2.5-4 mm, oblong to elliptic, patent, whitish, apex rounded, acute or mucronate, cucullate, margin straight or curved inwards. Style ca. 5 mm long; stigma with 4-6 lobes, 1.5-2.5 mm long, ligulate, curved, sub-reflexed. Ovules in 3-4 rows, funicle short (< 0.5 mm long).

Stamens ca. 50, 2-5.5 mm long, internal shorter, spreading, white. Nectary ca. 0.6 mm long. Fruit 5.5 X 5 mm, globoid, pinkish or whitish, glabrous. Figure 2: B, F.

Notes: *Rhipsalis cereoides* is a well-circumscribed species, easily identified by the stem segments with narrow succulent wings and almost straight margin. The only other species that shares the same habitat type (open and sunny coastal inselbergs of Rio de Janeiro and Niterói) is *Rhipsalis triangularis*. However, *R. triangularis* occurs further south in Rio de Janeiro.

Habitat and distribution: Occurs in open and sunny inselbergs in Rio de Janeiro and Niterói (Rio de Janeiro, Brazil). Figure 3.

2. RHIPSALIS CRISPATA (Haw.) Pfeiff., Enum. Diagn. Cact.: 130. 1837. *Epiphyllum crispatum* Haw., Philos. Mag. J. 7: 111. 1830. *Cereus crispatus* Pfeiff., Enum. Diagn. Cact.: 130. 1837, *pro syn.* *Rhipsalis crispa* F.Först., Handb. Cacteenk.: 450. 1846, *pro syn.* *Rhipsalis rhombea* var. *crispata* K.Schum., Gesamtbeschr. Kakt.: 638. 1898.—TYPE: BRAZIL. São Paulo: Rio Claro, Fazenda São José, "à beira da Lagoa", A. Cardoso in *Zappi* 249 (neotype, here designated: HRCB!).

? *Rhipsalis crispata* β *latior* Salm-Dyck ex Pfeiff., Enum. Diagn. Cact.: 130. 1837.

Epiphyte in shaded or open habitat or lithophyte in shaded habitat, 1-2 m long, branching apical or sub-apical, rare lateral. Stem segments flattened in longitudinal section, 0.8-2 mm diam, olive green sometimes with pinkish margin, slightly succulent but midrib stiff, dimorphic or monomorphic, midrib 2.5-5 mm diam, cylindric; primary stem segments up to 50 cm long, 2-winged, cylindric at base, wings 1.5 cm wide; secondary stem segments (10-)11.5-20(-30) cm long, base acute, attenuate or wide attenuate, apex truncate; wings 2, (1.5-)2-5.5 cm wide, margin strong crenate to lobed, undulate or strong undulate, with 4-10(-17) mm projections. Areoles between margin projections, 1.3-5 cm apart, first of

segment 0.5-4.5 cm distant from segment base; when sterile 1.3-2.5 mm diam, glabrous or with 1-4 acicular scales; when fertile 2-5 mm diam, with 3 pink acicular scales or scarce hairs at margin, 1-7 flowers/fruits. Flowers 15-17.5 mm diam; pericarpel 3.2-3.5 X 3-4 mm, cylindric to obovoid, yellowish green with dark reddish apex, glabrous; with 2-4 sepaloid tepals, 0.5-2 mm long and 6-7 petaloid tepals, 3-9 X 3-5 mm, wide elliptic or elliptic, sub-erect to reflexed, yellowish with dark redish apex, apex rounded, curved inwards to cucullate, margin straight or cuved inwards. Style 4-5 mm long; stigma with 3-7 lobes, 2.2-2.3 mm long, ligulate, spreading. Ovules in 4 incomplete septa, funicle short (< 0.5 mm long). Stamens 70-80, 4-7 mm long, internal shorter, internal erect and external facing inwards, white. Nectary ca. 0.8 mm long. Fruit 5-7 X 3.5-5.5 mm, elongate globoid to sub-cylindric, whitish green, sometimes with pinkish apex, glabrous. Figure 4: A, G.

Notes: This species was originally described from cultivated material of unknown provenance. Barthlott and Taylor (1995) selected the material that was cultivated in Europe and kept under this name at the time of the publication of the neotype for this species. However, the specimen selected by Barthlott and Taylor (1995) was not found (A. Cardoso in *Zappi 249*, Kew spirit collection), and extant material of the collection A. Cardoso in *Zappi 249* (HRCB) was here selected as a new neotype of *R. crispata*. An ex-neotype is also preserved at Kew's living collection. Because *Rhipsalis crispata* remained without a clear type between 1830 (original description of the species) and 1995 (neotypification by Barthlott and Taylor), the circumscription of thie species remained problematic for over 150 years. More specifically, several authors misidentified specimens of *R. pachyptera*, *R. elliptica*, *R. oblonga*, *R. russellii* and *R. olivifera* as *R. crispata*. This confusion is well justified given the morphological similarity between *Rhipsalis crispata* and those species, particularly in terms of the white fruits and stem segments with crenate to lobed margins, sometimes undulate. Nevertheless, *R. crispata* can be distiguated from these taxa by its deeply pronounced stem margin projections, large areoles, conspicuously apparent mirib and stem venation.

Habitat and distribution: Occurs mainly in Rio de Janeiro and São Paulo in the Atlantic Forest *sensu lato*, also occurring in gallery forests and semideciduous Forests towards the interior of the state of São Paulo. It also occurs further North in Bahia (Brazil). Figure 5.

3. RHIPSALIS CRISPIMARGINATA Loefgr., Arch. Jard. Bot. Rio de Janeiro 2: 37. 1917.—TYPE: BRAZIL. Rio de Janeiro: Ilha Grande, 1915, *Rose 20401* (lectotype in Britton and Rose 1917: US!).

Epiphyte in shaded habitat strictly in forest understory, 1.5-1.8 m long, branching apical or sub-apical. Stem segments flattened in longitudinal section, 0.1-0.5 mm diam, medium green, delicate but flexible, monomorphic, 9-20 cm long, base cylindric to attenuate, apex truncate, attenuate or rounded, wings 2, 1-3 mm, margin serrate to slightly lobed or crenate, undulate, with 3-7 mm projections, midrib 1.5-2.5 mm diam, cylindric to flattened. Areoles between margin projections, 2-3.3 cm apart, first of segment (2.5-)3-7 cm distant from segment base; when sterile 1 mm diam, with vestigial scales; when fertile 1.5 mm diam, with 1-3 acicular scales, 1-2 flowers/fruits. Flowers 12-16 mm diam; pericarpel 3.2-5.7 X 2.3-3.2 mm, cylindric, greenish white, with 1 sepaloïd bract near apex; with 3 sepaloïd tepals, 0.7-2 mm long and 6-7 petaloïd tepals, 3.5-6.3-3.5 mm, oblong to elliptic, patent to reflexed, greenish or whitish, apex rounded, slightly cucullate to cucullate, margin straight or cued inwards. Style ca. 4.5 mm long; stigma with 5 lobes, ca. 1.5 mm long, sagitate, erect. Ovules in 5 incomplete septa, funicle short (< 0.5 mm long). Stamens ca. 70, 3.5-6 mm long, internal shorter, internal facing outwards and external facing inwards, white. Nectary 0.7 mm long. Fruit 7-8 X 6-7 mm, ovoid to globoid, pinkish translucent, glabrous. Figure 6: A, G.

Notes: The type material originally proposed for *R. crispimarginata* by Loefgren (1917) was a material collected at Ilha Grande (Rio de Janeiro) by Loefgren, Rose, and Campos Porto (s.n.). Subsequently, Britton and Rose (1923) cited the collection Rose 20401 from Ilha Grande as the type of *R.*

crispimarginata. The collection Rose 20401 had duplicates deposited at both US and NY. However, the specimen (Rose 20401) at NY was preserved after cultivation and is excluded from the type material as it is reduced and seems to be juvenile not allowing the correct identification. Furthermore, the illustration for *R. crispimarginata* presented in Britton and Rose (1923) represents a stouter and apparently stiffer plant that actually resembles *R. crispata*. Additional specimens at NY labeled as "Shafer by Löfgren" (material in cultivation sent by Löfgren to Shafer) are also distinct from the type specimen.

Barthlott and Taylor (1995) considered *R. crispimarginata* as a synonym of *R. oblonga*. However, *R. crispimarginata* differs from *R. oblonga* by the larger and wider stem segments with smaller mature areoles and translucent rose fruits, in contrast to the greenish translucent fruits of *R. oblonga*. Thinner forms of *R. crispimarginata* can, however, be confused with *R. oblonga* in the herbarium.

The molecular phylogeny of Calvente et al. (in prep.; Fig. 1) indicated that *R. crispimarginata* is sister to *R. cuneata*, both of which share thin and delicate (but flexible) stem segments with narrow-attenuate to almost cylindric base.

Habitat and distribution: Occurs in Rio de Janeiro and Northern São Paulo in coastal Atlantic Forest (Brazil). Figure 3.

4. RHIPSALIS CUNEATA Britton & Rose, Cactaceae 4: 146. 1923. —TYPE: BOLIVIA. Above San Juan, alt. 5,500 ft., april 2, 1902, *Williams 2458* (holotype: NY452436!; isotype: NY452435!).

Rhipsalis occidentalis Barthlott & Rauh, Kakteen Sukk. 38: 17. 1987. —TYPE: PERU. San Martin: Rioja, 800m, 1973, *Rauh 35392* (lectotype, here designated: HNT!).

Notes: This species was originally described from a collection from Bolivia (Williams 2458). However, the whole range of geographic distribution and morphological variation in natural populations of this species was unknown at the time of its publication. For many years nobody was able to re-locate

populations of *R. cuneata* growing next to the type locality in Bolivia, leading to taxonomic confusion. In particular, it was unclear whether specimens with more delicate and narrower stem segments with margins presenting narrower projections collected in Bolivia, Peru and Ecuador belonged to *R. cuneata* or not. Barthlott and Rauh (1987) preferred to recognize new collections from Peru and Ecuador of individuals that resembled *R. cuneata* as a new species (*R. occidentalis*), while Barthlott and Taylor (1995) named the thinner forms of *R. cuneata* from Bolivia as *R. goebeliana*. More recently, however, Bauer (2008) made new collections of specimens thought to represent *R. cuneata*, and *R. occidentalis* in Ecuador, Peru and Bolivia and cultivated those specimens under standardized conditions. Bauer (2008) observed that these specimens were very similar morphologically when cultivated under standardized conditions. Even the stem segments of *R. cuneata* from the type collection (larger and stouter, with margins with deeper projections) changed in cultivation demonstrating that the variation in stem morphology is likely phenotypic plasticity, resulting from varying environmental conditions and suggesting that all taxa listed above should actually be recognized as a single species. Only a narrow form of *R. cuneata*, from the Bolivian Chaparre seemed to maintain its morphological variation in cultivation, hence deserving to be treated as a subspecies (*R. cuneata* subsp. *australis*). *Rhipsalis goebeliana* was excluded from this context, as it was originally described from cultivated material with unknown provenance (see notes under this species).

4.1. RHIPSALIS CUNEATA subsp. CUNEATA

Epiphyte in shaded habitat, 1 m long, branching apical or sub-apical. Stem segments flattened to triangular in longitudinal section, 0.5-1.5 mm diam, dark green, delicate, fragile, dimorphic, midrib 2-3 mm diam, cylindric; primary stem segments 13-14 cm long; wings 2-3, with cylindric base, 0.4-0.7 cm wide; secondary stem segments 5.5-17 cm long, base attenuate, apex truncate, wings 2(-3), 1.2-2.5 cm wide, margin serrate, plane, rare slightly undulate, with 2-5 mm projections. Areoles between margin projections, 1.7-3.8 cm apart, first

of segment 4-8(-10) distant from segment base; when sterile 1 mm diam, with 2 acicular reddish scales; when fertile 1.5 mm diam, with 1-3 acicular scales, 1(-2) flowers/fruits. Flowers ca. 8 mm diam; pericarpel 2-4 X 2-3 mm, cylindric, greenish, glabrous or with sepaloid bract; with 1-3 sepaloid tepals, 0.3-1.5 mm long and 6 petaloid tepals, 3-4.2 X 2-2.3 mm, wide elliptic, patent to sub-erect, whitish, apex rounded, slightly cucullate, margin straight. Style 3-4 mm long; stigma with 3-5 lobes, ca. 1 mm long, ligulate, spreading. Ovules in 3 rows, funicle short (< 0.5 mm long). Stamens ca. 40, 1.5-2.5 mm long, internal shorter, internal erect and external facing inwards, white. Nectary ca. 0.4 mm long. Fruit 7-9 X 7-8 mm, globose, whitish, glabrous. Figure 7: A, H.

Notes: *Rhipsalis cuneata* subsp. *cuneata* can be distinguished from *R. cuneata* subsp. *australis* by the wider stem segments and deep projections in the margin.

Habitat and distribution: Occurs in the eastern lower Andean slopes of Ecuador and Peru, reaching marginal Amazonian formations on lower elevations (200-1500 m). In Bolivia occurs in the "Yungas" of La Paz, Cochabamba and Santa Cruz. An old collection from Suriname was also analyzed. Although we did not examine specimens from other countries, it is possible that this species might also occur in Colombia and other countries of northern South America. Figure 8.

- 4.2. RHIPSALIS CUNEATA subsp. AUSTRALIS Ralf Bauer, EPIG 62: 26. 2008. —
TYPE: BOLIVIA. Cochabamba: Prov. Chaparre, Km 130 Cochabamba-Vila Tunari, 1977, *Aguilar s.n.* (holotype: ZSS28443; isotype: HNT!).

Epiphyte in shaded habitat, 1-1.5 m long, branching apical. Stem segments flattened in longitudinal section, 0.5-1.5 mm diam, dark green, delicate, fragile, monomorphic, 7-12 cm long, base attenuate, apex truncate, wings 2, (0.2-)0.45-1(-1.2) cm wide, margin serrate, plane, rare slightly undulate, with 2-3 mm projections, midrib 2-3 mm diam, cylindric. Areoles between margin projections, 1.5-3.5 cm apart, first of segment 4-8(-10) distant from segment base; when sterile 1 mm diam, with 2 acicular reddish scales; when fertile 1.5 mm diam,

with 1-3 acicular scales, 1(-2) flowers/fruits. Flowers ca. 8 mm diam; pericarpel 2-4 X 2-3 mm, cylindric, greenish, glabrous or with sepaloïd bract; with 1-3 sepaloïd tepals, 0.3-1.5 mm long and 6 petaloïd tepals, 3-4.2 X 2-2.3 mm, wide elliptic, patent to sub-erect, whitish, apex rounded, slightly cucullate, margin straight. Style 3-4 mm long; stigma with 3-5 lobes, ca. 1 mm long, ligulate, spreading. Ovules in 3 rows, funicle short (< 0.5 mm long). Stamens ca. 40, 1.5-2.5 mm long, internal shorter, internal erect and external facing inwards, white. Nectary ca. 0.4 mm long. Fruit 7-9 X 7-8 mm, globose, whitish, glabrous. Figure 7: G.

Notes: *Rhipsalis cuneata* subsp. *australis* can be distinguished from *R. cuneata* subsp. *cuneata* by the narrow stem segments with narrow projections in the margin.

Habitat and distribution: Occurs in the Chaparre region from 200-700 m (Cochabamba and Santa Cruz, Bolivia). Figure 8.

5. RHIPSALIS ELLIPTICA G.Lindb. ex K.Schum. in Martius, Fl. Bras. 4(2): 293. 1890. —TYPE: BRAZIL. São Paulo: Santos, "prope Sororocaba in adscensu montis Espigão do Curupira ad arbores silvae primaevae", Mosén 3630 (lectotype in Barthlott and Taylor 1995: SI).

? *Rhipsalis chloroptera* F.A.C.Weber in Bois, Dict. Hort. 2: 1045. 1898.—TYPE: BRAZIL. São Paulo: Santos, before 1898, Weber s.n. (not found).

Rhipsalis elliptica var. *helicoidea* Loefgr., Arch. Jard. Bot. Rio de Janeiro 2: 44. 1917.—TYPE: Loefgren, Arch. Jard. Bot. Rio de Janeiro 2: Tab. XVI. 1917 (lectotype, here designated).

Notes: The original description of *R. elliptica* in the Flora Brasiliensis by Schumann (1890) included a typo in the locality of the collection Mosén 3630 (cited), there designated as one of the syntypes of *R. elliptica*. The collection Mósen 3630 was erroneously published as "Sorocaba" (São Paulo, Brazil), instead of "Sororocaba" (Santos, Brazil) as written in the specimen label.

When *R. chloroptera* was described, a collection from Santos (Weber s.n.) was selected as the type. Even though this collection could not be located, a detailed analysis of the original description of *R. chloroptera* allowed us to determine that *R. chloroptera* is very likely a synonym of *R. elliptica*. The fact that the type locality of *R. chloroptera* (Santos, Brazil) corresponds to the same type locality of *R. elliptica* supports our hypothesis.

R. elliptica var. *helicoidea* was originally described by Loefgren (1917) as being characterized by an helicoidal disposition of the branches. However, this morphological trait is quite variable and also commonly found in *Rhipsalis elliptica*. As a matter of fact, a single specimen of *Rhipsalis elliptica* can include branches with helicoidal and plane dispositions, making the recognition of both taxa inappropriate.

The original circumscription of *R. elliptica* was based on only a few specimens, all of which presented deep magenta fruits. A careful examination of a higher number of specimens of *R. elliptica* indicated that *R. elliptica* is much more widespread and morphologically variable than originally thought. In particular, the deep magenta fruits can be white when immature. Furthermore, several specimens that perfectly match the description of *R. elliptica* in terms of vegetative and floral traits, presented white fruits, indicating that fruit color is variable within this species. Variation in fruit color is not uncommon in *Rhipsalis*. For example, *R. teres* and *R. lindbergiana* present specimens with either whitish, pinkish or magenta. Hence, we do not consider fruit color as being sufficient to diagnose species in *Rhipsalis* and adopt a broader circumscription of *R. elliptica*. The new circumscription of *R. elliptica* also includes specimens with white fruits, instead of treating those specimens as a separate taxon (*R. oblonga*) like previous treatments (Barthlott & Taylor 1995; Hunt et al 2006; see notes under *R. oblonga* for further information). Furthermore, the white fruited individuals differ from *R. oblonga* by the larger stem segments with crenate margins and small areoles, which are diagnostic characters of *R. elliptica*. Two subspecies are recognized here.

5.1. RHIPSALIS ELLIPTICA subsp. ELLIPTICA

Epiphyte in shaded habitat, 0.4-1.5 m long, branching apical or sub-apical. Stem segments flattened in longitudinal section, 0.3-2 mm diam, medium green, dark green or reddish, slightly succulent to succulent, dimorphic or monomorphic, midrib 2-3.5 mm diam (up to 6mm diam in primary segments), cylindric; primary stem segments 12.5-24 cm long; wings 2-3, 0.5-2 cm wide; secondary stem segments 7-16 cm long, base wide attenuate to attenuate, apex attenuate, wide attenuate, rounded or rarely truncate; wings 2, 0.5-4.5 cm wide, margin crenate or slightly crenate, plane to undulate, with 1-6 mm projections. Areoles between margin projections, 0.8-3 cm apart, first of segment 1-5 cm distant from segment base; when sterile 1 mm diam or less, glabrous; when fertile 1.2-2.5 mm diam, glabrous or with 1 acicular scale, rarely with scarce hairs at margin, 1-2 flowers/fruits. Flowers 11-14 mm diam; pericarpel 3-5 X 2.5-4.5 mm, cylindric, greenish or pinkish, glabrous; with 2-4 sepaloid tepals, 0.4-2 mm long and 5-7 petaloid tepals, 3-8 X 2-5 mm, wide elliptic, elliptic or oblong, patent to reflexed, yellowish or greenish, sometimes external with reddish apex, apex rounded, straight to slightly cucullate, margin straight or curved inwards. Style 4.5-5 mm long; stigma with 3-5 lobes, 1.5-2.7 mm long, ligulate, spreading. Ovules in 3-4 incomplete septa, funicle short to long (0.5-1 mm long). Stamens 60-100, 2-7 mm long, internal shorter, internal erect and external facing inwards, whitish. Nectary 0.5-0.8 mm long. Fruit 5.5-7.5 X 5-6 mm, globoid to elongate, deep magenta to white, glabrous. Figures 4: B, C, H; 6: B, C.

Notes: *Rhipsalis elliptica* subsp. *elliptica* is distinct from *R. elliptica* subsp. *microflora* by the larger flowers with longer petaloid tepals and stamens. All specimens with deep magenta fruits are circumscribed under this subspecies

Habitat and distribution: Occurs widespread in southern and southeastern portions of the Atlantic Forest (Brazil). Figure 5.

5.2. **Rhipsalis elliptica subsp. microflora** Calvente, *subsp. nov.* —

TYPE: BRAZIL. Minas Gerais: Santa Maria do Salto, jan 2007, *Calvente* 320 (holotype: SPF; isotypes: K, RB, MO, NY, HUEFS).

Subspecies nova, Rhipsalis elliptica G.Lindb. ex K.Schum. *affinis sed floribus minoribus, tepalis brevioribus, staminibus minoribus et quad numerum inferioribus, pericarpelis albis differt. A Rhipsalis oblonga* Loefgr. *similis, sed alis latioribus et areolis minoribus, glabris differt.*

Epiphyte, up to 2m long, branching apical, rare lateral. Stem segments flattened in longitudinal section, 0.3-1 mm diam, medium green with reddish margin to reddish, slightly succulent, fragile, dimorphic, midrib 1-2 mm diam, cylindric to flattened; primary stem segments ca. 30 cm long; wings 2, with cylindric base, up to 0.5 cm wide; secondary stem segments (6-)8-16 cm long, base attenuate, apex attenuate, truncate ou rounded; wings 2(-3), 1.5-3.5 cm wide, margin crenate to slightly lobed, curved to slightly undulate, with 3-6 mm projections. Areoles between margin projections, 1-3 cm apart, first of segment 1.5-5 cm distant from segment base; when sterile 0.7-1 mm diam, glabrous or with 1 acicular scale; when fertile 1-1.3 mm diam, glabrous, with 1 flower/fruit. Flowers ca. 8 mm diam; pericarpel 3.5 X 2.5 mm, cylindric, whitish, glabrous; with 1-2 sepaloid tepals, 0.6-1.3 mm long and ca. 5 petaloid tepals, 1.7-7.2 X 2-3 mm, oblong to elliptic, patent to reflexed, yellowish, apex rounded, straight to slightly cucullate, margin straight. Style ca. 3.5 mm long; stigma with 4-5 lobes, 1.5 mm long, ligulate, erect to spreading. Ovules in 4 incomplete septa, funicle short (< 0.5 mm long). Stamens ca. 40, 1.7-3.5 mm long, internal shorter, internal erect and external facing inwards, whitish. Nectary ca. 0.6 mm long. Fruit 6-7.4 X 5-6 mm, globoid, white, glabrous. Figure 6: E, I.

Notes: This new subspecies was included by Taylor & Zappi (2004) in the circumscription of *R. oblonga* due to its white fruits. However, *R. elliptica* subsp. *microflora* and *R. elliptica* subsp. *elliptica* are distinct of *R. oblonga* for their larger stem segments with crenate margin and smaller areoles. *R. elliptica* subsp. *microflora* differs from *R. elliptica* subsp. *elliptica* by its smaller flowers

and petaloid tepals, fewer and shorter stamens, with a whitish pericarpel, and white fruits.

Habitat and distribution: Occurs in southern Bahia, Espírito Santo and northeastern Minas Gerais in the Atlantic Forest (Brazil). Figure 5.

6. *RHIPSALIS GOEBELIANA* Backeb., Descr. Cact. Nov. 1: 10. 1957. —TYPE: Backeberg, Cactaceae 2: 676, Abb. 633. 1959 (neotype in Barthlott and Taylor 1995); ex. hort. Botanischer Garten Bonn no. 4467 (epitype in Bauer 2008: ZSS28438).

Habitat unknown, ca. 30 cm long (cultivated), branching apical or sub-apical, rare lateral. Stem segments flattened to triolate in longitudinal section, 1-2 mm diam, pale olive green, succulent, monomorphic, 10.4-13.9 cm long, base attenuate, apex truncate or rounded; wings 2(-3), 0.7-1.5 cm wide, margin serrate, slightly lobed or dentate, plane, with 3-4 mm projections, midrib 1.5-3.4 mm diam, cylindric. Areoles between or on margin projections, 1-2.5 cm apart, first of segment 0.5-4 cm from segment base; when sterile 1.5-2.5 mm diam, glabrous; when fertile 1.5-2.5 mm diam, with scarce hairs at margin, 1-2 acicular scales, 1 flower/fruit. Flowers ca. 8.5 mm diam; pericarpel 3 X 2.3 mm, cylindric, greenish, glabrous; with 2-3 sepaloid tepals, 0.4-1.5 mm long and 7 petaloid tepals, 2.5-6 X 2-2.5 mm, wide elliptic or elliptic, patent to sub-erect, pinkish green, apex rounded, slightly cucullate, margin straight. Style ca. 3 mm long; stigma with 4 lobes, 1.5 mm long, ligulate, spreading. Ovules in 3 incomplete septa, funicle short (< 0.5 mm long). Stamens ca. 35, 3.5-5 mm long, internal shorter, facing inwards, white. Nectary ca. 0.4 mm long. Fruit 4.5-5 X 4.7-5 mm, globoid, greenish white, glabrous. Figure 6: D, H.

Notes: Backeberg (1959) described *R. goebeliana* based on a cultivated specimen of unknown provenance. *R. goebeliana* resembles forms of *R. cuneata* and *R. oblonga* however, the pale olive-green stems with serrate, slightly lobed or dentate margins are sufficient to recognize it as a separate

taxon. Furthermore, the recent molecular phylogeny of *Rhipsalis* (Calvente et al. in prep) indicates that this species is not linked to *R. cuneata* nor to *R. oblonga* (Fig 1). Therefore, to avoid confusion with Brazilian and Andean species the circumscription here applied only includes the cultivated specimens.

Habitat and distribution: This species is only known from cultivated material.

7. RHIPSALIS MICRANTHA (Kunth) DC., Prodr. 3: 476. 1828. *Cactus micranthus* Kunth in Humb., Bonpl. & Kunth, Nov. Gen. Sp. 4: 65. 1823. —TYPE: PERU. Olleros: "Crescit in arboribus", 1330m, *Humboldt & Bonpland s.n.* (holotype: P!; isotype: P!).

Rhipsalis werkleii Berger, Monatsschr. Kakteenk 16: 64. 1906.— TYPE: The Alwin Berger Succulent Hebarium, *Alwin Berger s.n.* (lectotype, here designated: NY386146!)

Rhipsalis roseana Berger, Z. Sukkulentenk. 2: 22. 1923.—TYPE: The Alwin Berger Succulent Hebarium, *Alwin Berger s.n.* (lectotype, here designated: NY386145!).

Notes: *Rhipsalis micrantha* is very diverse morphologically. Its morphological diversity is linked with geography, altitude ranges and with the spatial distribution of its populations, perhaps as a result from the Andean discontinuous mosaic of environmental conditions and isolated microhabitats. The four geographically isolated and morphological distinct populations of *R. micrantha* are here recognized as four subspecies: *R. micrantha* subsp. *micrantha*, *R. micrantha* subsp. *rauhiorum*, *R. micrantha* subsp. *tonduzii*, and *R. micrantha* subsp. *monticola*. The fact that no molecular variation was found among subspecies (Calvente et al. in prep.), suggests a rapid diversification. Apart from differences in geographical distribution, variation in stem morphology is also important to diagnose subspecies. Despite that, all subspecies of *R.*

micrantha are difficult to be separated in the herbarium because the diagnostic features are often lost during the herborization process.

7.1. RHIPSALIS MICRANTHA subsp. MICRANTHA

Lithophyte or epiphyte in open habitat, 0.6 m long, branching apical or rare sub-apical. Stem segments flattened to triangular in longitudinal section, 2-3 mm diam, olive green, sometimes with reddish margin, succulent, dimorphic, midrib 3-4 mm diam, cylindric; primary stem segments 20-25 cm long; wings 3-4, 0.4-0.5 cm wide; secondary stem segments (6.5-)-8-12(-15) cm long, base attenuate, apex truncate; wings 2-3, 0.4-0.5(-0.7) cm wide, margin slightly serrate, plane, with 1-2 mm projections. Areoles between margin projections, 0.9-4.5 cm apart, first of segment 1(-2) cm distant from segment base; when sterile 1.5-2 mm diam, with vestigial hairs; when fertile 2 mm diam, with 1-2 acicular scales and scarce hairs at margin, 1(-3) flowers/fruits. Flowers ca. 10.5 mm diam; pericarpel ca. 4 X 3 mm, cylindric, greenish, glabrous; with 1-2 sepaloid tepals, 0.5-1 mm long and 7 petaloid tepals, (2-)5-6 X 2-3 mm, oblong to elliptic, patent or sub-erect, whitish, apex rounded, slightly cucullate, margin straight. Style 4.5 mm long; stigma with 5 lobes, 1.5 mm long, ligulate, curved, sub-reflexed. Ovules in 5 rows, funicle short (< 0.5 mm long). Stamens ca. 35, 2-4.5 mm long, internal shorter, spreading, whitish. Nectary ca. 0.5 mm long. Fruit 5.5-6.5 X 5-5.5 mm, obovate, elongate, white, glabrous. Figure 7: D, I.

Notes: *R. micrantha* subsp. *micrantha* is characterised by the succulent, mostly 2-winged stem segments, as well as narrow wings and margins with narrower projections than *R. micrantha* subsp. *rauhiorum*.

Habitat and distribution: Occurs in the highland Andean forests of Ecuador and Peru, from 1000-1800 m. Figures 8, 9.

7.2. ***Rhipsalis micrantha* subsp. *monticola*** (Barthlott) Calvente, *stat. nov.* *Rhipsalis kirbergii* var *monticola* Barthlott, Trop. Subtrop. Pflanzenwelt 10: 15. 1974.—TYPE: Barthlott, Trop. Subtrop. Pflanzenwelt 10: Abb. 8. 1974 (lectotype, here designated); ECUADOR.

Loja: Catamayo, estrada Catamayo-Catacocha, Jan 2008, *Calvente* 383 (epitype, here designated: QCNE!; isoeotype: SPF, QCA, LOJA, K, RB, NY).

Epiphyte, terrestrial or lithophyte in open and sunny habitat, 0.7 m long, branching apical, sub-apical, rare lateral. Stem segments flattened, cylindric to quadrangular in longitudinal section, ca. 3 mm diam, olive green sometimes with pinkish margin, succulent to stiff and woody at more basal segments, dimorphic, midrib 3-5 mm diam, with shape not evidently marked (hidden by stem succulence); primary stem segments 15-25 cm long, cylindric to 4-winged, wings 0-0.3 cm wide; secondary stem segments 6-20 cm long, base and apex truncate; wings 3-4(-5), 0.3-0.5(-0.6) cm wide, margin crenate, plane, with 1-3 mm projections. Areoles between margin projections, 2.5-5.3 cm apart, first of segment 1-2 cm distant from segment base; when sterile 2-3 mm diam, glabrous or with 1 acicular scale; when fertile 3 mm diam, glabrous, with 1(-2) flowers/fruits. Flowers unknown. Fruit 7 X 5-6 mm, globoid, elongate, whitish, glabrous. Figure 7: C.

Notes: Barthlott linked *R. micrantha* subsp. *monticola* to *R. kirbergii* (= *R. micrantha* subsp. *tonduzii*) because of the shared multi-winged stems. However, *R. micrantha* subsp. *monticola* is endemic to Loja (southern Ecuador) and well characterized by the succulent, curved, stout and 4-5-winged stems. *R. micrantha* subsp. *monticola* is further characterized by the basal stem segments with areoles including up to 10 bristle-like, long acicular scales (ca. 3 mm long), and greenish to reddish immature fruits.

Habitat and distribution: Occurs in Loja region (Ecuador) in mostly dry (with daily fluctuations of fog), open formations of higher altitude, ranging from 1300-2300 m. Figures 8, 9.

- 7.3. ***Rhipsalis micrantha* subsp. *rauhiorum*** (Barthlott) Calvente, *stat. nov.* *Rhipsalis rauhiorum* Barthlott, Trop. Subtrop. Pflanzenwelt 10: 15. 1974. *Rhipsalis micrantha* f. *rauhiorum* (Barthlott) Süplie, Rhipsalidinae: 101. 1994. *Rhipsalis micrantha* f. *rauhiorum* (Barthlott)

Barthlott & N.P.Taylor, *Bradleya* 13: 62. 1995.—TYPE: ECUADOR. "tal des Rio Catamayo", 1300 m, Sept. 1973, *Rauh* 35278 (holotype: HEID702584!; isotype: HEID702585!, HNT!).

Terrestrial or rare epiphyte in sunny habitat, 0.8 m long, branching apical or sub-apical. Stem segments flattened to triangular, rarely quadrangular in longitudinal section, 1.5-3 mm diam, olive green, succulent, monomorphic, 5-14 cm long, base attenuate, apex truncate; wings 2-3(-4), sometimes discontinuous, 0.4-1 cm wide, margin crenate, plane, with 1-4 mm projections, midrib 3-4 mm diam, cylindric. Areoles between margin projections, 1-3.5 cm apart, first of segment 1-5 distant distant from segment base; when sterile 2 mm diam, with vestigial scales; when fertile 1.5 mm diam, with 1-2 acicular scales and scarce hairs at margin, 1(-2) flowers/fruits. Flowers ca. 9.5 mm diam; pericarpel 3-3.5 X 2.5-3 mm, cylindric, greenish, glabrous or with sepaloid bract; with 1-3 sepaloid tepals, 0.3-1.2 mm long and 7 petaloid tepals, 4-5 X 2.3-2.7 mm, oblong to elliptic, patent or sub-erect, greenish yellow, apex rounded, slightly cucullate to straight, margin straight. Style 4 mm long; stigma with 4-5 lobes, 1.5 mm long, ligulate, curved, sub-reflexed. Ovules in 4 rows, funicle short (< 0.5 mm long). Stamens ca. 40, 1.5-3.5 mm long, internal shorter, internal facing outwards and external facing inwards, whitish. Nectary ca. 0.5 mm. Fruit 7 X 5-6 mm, globoid, whitish, sometimes with reddish apex, glabrous. Figure 7: F, K.

Notes: *Rhipsalis micrantha* subsp. *rauhiorum* is distinct from the other four subspecies of *Rhipsalis micrantha* by the wider wings with margins with deeper projections.

Habitat and distribution: This species is restricted to the Catamayo River Valley, in Loja (Ecuador), occurring in mostly arid formations, ranging from 1600-1700 m. Figures 8, 9.

7.4. *Rhipsalis micrantha* subsp. *tonduzii* (F.A.C.Weber) Calvente, *comb. nov., stat. nov.* *Rhipsalis tonduzii* F.A.C.Weber in Bois, *Dict. Hort.* 2: 1046. 1898.—TYPE: COSTA RICA. "prés San Marcos, pont du

rio Tarrazu", 1306 m, Apr. 1897, C. Wercklé 2312 (neotype, here designated: P!).

Rhipsalis kirbergii Barthlott, Trop. Subtrop. Pflanzenwelt 10: 11. 1974.

Rhipsalis micrantha f. *kirbergii* (Barthlott) Süpple, Rhipsalidinae: 100. 1994.

Rhipsalis micrantha f. *kirbergii* (Barthlott) Barthlott & N.P.Taylor, Bradleya 13: 62. 1995.—TYPE: ECUADOR. Manabi: chone, "10km nördl.", 200 m, *Rauh* 34364 (holotype: HEID702582!; isotype: HEID702580!, HEID702581!).

Epiphyte, in open or shaded habitat, 1-2 m long, branching apical or sub-apical, rare lateral. Stem segments flattened, triangular, quadrangular or hexagonal in longitudinal section, ca. 1-2 mm diam, olive, medium or dark green, succulent but stiff and sometimes woody, monomorphic or dimorphic, (5-)11-20(-30) cm long, base attenuate or truncate, apex truncate; wings 2-6, 0.1-0.4(-0.5) cm wide, margin slightly serrate to entire, plane, with 0-1.5(-2) mm projections, midrib 1.5-2(-5) mm diam, cylindric. Areoles between margin projections, 1-4.5 cm apart, first of segment 0.5-3.5 cm distant from segment base; when sterile 1-3 mm diam, glabrous or with 1-4 acicular scales; when fertile 1.5-3 mm diam, glabrous or pilose, with 1(-2) flowers/fruits. Flowers 6-8 mm diam; pericarpel ca. 3.4-4 X 3-3.7 mm, cylindric, greenish, glabrous or with sepaloid bract; with 1-4 sepaloid tepals, 0.5-1.5 mm long and 5-8 petaloid tepals, (2-)5-6.5 X 1.5-1.8 mm, oblong to elliptic, patent or sub-erect, whitish, apex rounded, slightly cucullate, margin straight. Style ca. 4 mm long; stigma with 3-4 lobes, 1.5 mm long, ligulate, curved, sub-reflexed. Ovules in 3 rows, funicle short (< 0.5 mm long). Stamens ca. 40, 2-3.7 mm long, internal shorter, facing inwards, pinkish. Nectary ca. 0.7 mm long. Fruit 6-8 X 4-6 mm, globoid, elongate, whitish, glabrous. Figure 7: B, E, J.

Notes: Some forms of *R. micrantha* subsp. *tonduzii* from Ecuador have 2-winged and narrow secondary stem segments that can present dark-brown and floccose deposition in the stems (wax?) after drying. It can be separated from *R. micrantha* subsp. *micrantha* and *R. micrantha* subsp. *rauhiorum* by the narrower and stiffer wings and from *R. micrantha* subsp. *monticola* by the 2-

winged secondary segments. When the 2-winged secondary segments are lacking, this species can be distinguished from *R. micrantha* subsp. *monticola* by the stem segments that are straight and less succulent, with almost entire margins.

Habitat and distribution: It occurs in Ecuador and Costa Rica, usually in coastal and lowland forests, but also in higher altitudes, ranging from 50-2250 m. Figures 8, 9.

8. RHIPSALIS OBLONGA Loefgr., Arch. Jard. Bot. Rio de Janeiro 2: 36. 1917.—
TYPE: Loefgren, Arch. Jard. Bot. Rio de Janeiro 2: Tab. VIII. 1918.
(lectotype in Barthlott and Taylor 1995).

Epiphyte in shaded habitat, 2.50 m long, branching apical or lateral. Stem segments flattened to rarely triangular in longitudinal section, 0.5-0.8 mm diam, olive-green or light green, slightly succulent, monomorphic, 8-15(-16.2) cm long, base attenuate, apex truncate, wings 2(-3), sometimes discontinuous (fin-like), 0.5-1.6(-2) cm wide, margin serrate, strong undulate to plane, with 2-4 mm projections, midrib 1.5-2.5 mm diam, cylindric. Areoles between margin projections, 1.4-3.3 cm apart, first of segment 3-5.5 cm distant from segment base; when sterile 1-2.5 mm diam, with vestigial hairs and scales; when fertile 1-2.5 mm diam, with 1 acicular scale, scarce marginal hairs, 1 flower/fruit. Flowers not observed. Fruit 5-8 X 5-7 mm, globose, greenish translucent (sometimes with pink ring at apex when immature), glabrous. Figure 6: F.

Notes: *Rhipsalis oblonga* can be easily identified by the olive or light green, small and narrow stem segments, with serrate margins, and the large areoles that may remain active for several bloomings. We here adopt a narrower circumscription of *R. oblonga*, following the original circumscription of Löfgren. Other taxonomic treatments (Barthlott and Taylor 1995; Taylor & Zappi 2004, Hunt et al. 2006) have adopted a broader circumscription for *R. oblonga* and have also included *Rhipsalis elliptica*-like specimens with white fruits under *R.*

oblonga. However, several morphological traits (as the areoles, flowers and stem morphology) indicate that those individuals are better placed under *R. elliptica* and *R. crispimarginata*. Furthermore, the inclusion of all white fruited *Rhipsalis elliptica*-like specimens under *R. oblonga* would lead to the recognition of a polyphyletic *R. oblonga* (Calvente et al. in prep; Fig. 1). Therefore, some specimens previously included in *R. oblonga* are now placed in *R. crispimarginata*, *R. elliptica* subsp. *elliptica* and *R. elliptica* subsp. *microflora*. Even though flowers of *R. oblonga* were not observed in the field or in the herbarium, these flowers were previously described as having 3-4 sepaloid tepals and 5 petaloid tepals, up to 8 mm, reflexed, yellowish with reddish apex, numerous whitish stamens and style with 4 lobes (Löefgren 1917).

Habitat and distribution: Occurs in Rio de Janeiro and northern São Paulo in coastal or montane Atlantic Forest. Figure 3.

9. RHIPSALIS OLIVIFERA N.P.Taylor & Zappi, Cactaceae Syst. Init. 3: 8. 1997.—
TYPE: BRAZIL. Rio de Janeiro: Teresópolis, Parque Nacional da Serra dos Órgãos, "caminho para o Campo das Antas", 1600-1800m, Feb. 1983, *Martinelli 9038* (holotypus: RB!; isotypus: K!).

Epiphyte, 1.5-2 m long, branching apical. Stem segments flattened to rarely triangular in longitudinal section, 0.5-2 mm diam, medium green, slightly succulent but flexible, monomorphic, 12.5-27 cm long, base attenuate to almost cylindric, apex truncate, wings 2, (0.7-)1.3-3.6 cm wide, margin crenate to lobed, plane to undulate, with 3-6 mm projections, midrib 1.7-4.5 mm diam, cylindrical. Areoles between margin projections, 2-5.5 apart, first of segment 6-13 cm distant from segment base; when sterile 2-4 mm diam, glabrous or with scarce vestigial hairs; when fertile 2-5 mm diam, with 0-3 acicular scales, scarce hair at margin, 1-5 flowers/fruits. Flowers 13-16 mm diam; pericarpel 2.4-4 X 3.3-4.5 mm, turbinate, greenish, white with pink apex, with 1-4 sepaloid bracts; with 2-3 sepaloid tepals, 1.2-2.5 mm long and 7-8 petaloid tepals, 3.5-11 X 2.5-4.4 mm, oblong to elliptic, patent to reflexed, yellowish, external pinkish

or with pinkish apex, apex attenuate to acute, rounded or mucronate, curved inwards to cucullate, margin straight. Style 6-7 mm long; stigma with 3-4 lobes, 1-1.5 mm long, sagitate, erect to spreading. Ovules in 3-4 incomplete septa, funicle short (< 0.2 mm long). Stamens 80-130, 3-7 mm long, internal shorter, facing inwards, white. Nectary 0.3-1 mm long. Fruit 5-7 X 5.5-6.6 mm, globoid, whitish at base, tinged dark red at apex with 1 deltoid scale (1x1.6 mm). Figure 4: D, I.

Notes: *R. olivifera* is easily identified by the pale-pinkish flowers, and large stem segments, with margins presenting lobed projections. The young areoles can present several long acicular scales (up to 3 mm).

Habitat and distribution: Occurs in montane Atlantic Forest in the “Serra dos Órgãos” and “Serra da Mantiqueira” (Brazil). Figure 10.

10. *Rhipsalis pachyptera* Pfeiff., Enum. Diagn. Cact.: 132. 1837.—TYPE: Curtis's Bot. Mag. 55: tab 2820. 1828 (lectotype in Barthlott and Taylor, 1995).

Rhipsalis dusenii Hjelmq., Bot. Not. 4: 349. 1941.—TYPE: BRAZIL. Paraná: Jacarehy, "in silvula ad trunc. arb.", May 1915, *Dusén 17074*, (holotype: S5470!; isotype: S04-768!, GH!).

? *Rhipsalis pachyptera* var. *crassior* Salm-Dyck ex Pfeiff., Hort. Dyck.: 59. 1849, *nom. nud.*

? *Rhipsalis pachyptera* f. *rubra* Süplie, Epi-flora 1: 16-17, 2005.

Rhipsalis agudoensis N.P.Taylor, Cactaceae Syst. Init. 16: 12. 2003, *syn. nov.* — TYPE: BRAZIL. Rio Grande do Sul: Agudo, *Horst-Uebelmann 821*, before 1989, cult. Hort. Uhlig-Kakteen, Germany (holotype: K!).

Epiphyte or lithophyte in mostly open habitat, 0.7-1.5 m long, branching apical, rare lateral. Stem segments flattened to triangular in longitudinal section, 1-3.6 mm diam, medium green, succulent, stiff, dimorphic, midrib 3-5(-7) mm

diam, cylindric; primary stem segments 8-40 cm long, wings 2-3, with cylindric base, 0.7-3 cm wide; secondary stem segments (8-)11-25 cm long, base acute, attenuate or truncate, apex truncate or rounded, wings 2-3, (1.6-)2-7 cm wide, margin crenate, rare serrate, plane to slightly undulate, with 6-16 mm projections. Areoles between margin projections, 1-5 cm apart, first of segment 1-6 cm distant from segment base; when sterile 2-3 mm diam, pilose, sometimes with vestigial scales; when fertile 2.8-4 mm diam, dense pilose, with 1-numerous acicular scales, 1-9 flower/fruits. Flowers 13-22 mm diam; pericarpel 3-4 X 3-5 mm, turbinate, rare cylindric, greenish, glabrous; with 1-4 sepaloid tepals, 0.6-2.5 mm long and 6-9 petaloid tepals, 2.5-11 X 2.5-5.5 mm, wide elliptic or elliptic, patent to sub-erect, whitish or yellowish, external with reddish apex, apex rounded, curved inwards to cucullate, margin curved inwards. Style 6-7 mm long; stigma with 3-6 lobes, 1.7-3.5 mm long, sagitate, erect to spreading. Ovules in 3-6 incomplete septa, funicle short (<0.1 mm). Stamens 60-70, 4-8 mm long, median or internal shorter, internal facing outwards and external facing inwards, whitish. Nectary 1-1.3 mm long. Fruit 4.5-5 X 5-5.5 mm, globoid, white (pinkish or reddish when immature), glabrous. Figures 2: A, C, G; 4: E, J.

Notes: *R. pachyptera* is well characterized by the stout stem segments, mostly with truncate apices, marked secondary venation and large pilose areoles. However, flowers present variable sizes, and the fruits can also present varying colors, ranging from pinkish to white during development. Given the high variation in fruit color observed in *R. pachyptera*, we here treat *R. agudoensis* as a synonym given that these species used to only be separated by the pinkish fruits of *R. agudoensis*. Furthermore, the recognition of *R. agudoensis* as a separate species would result in a paraphyletic *R. pachyptera* (fig 1). *R. pachyptera* is often confused with *R. russelli* when flowers and fruits are lacking however, these species can be distinguished by the larger flowers and usually whitish fruits of *R. pachyptera*, in contrast to the small flowers and magenta fruits of *R. russelli*.

Habitat and distribution: Widespread through southern and southeastern Atlantic Forest (Brazil). The occurrence at Agudo, further inland in Rio Grande do Sul is only known from the registered provenance of the type material of *R. agudoensis*, which was described after almost 15 years of cultivation in Europe. Figure 10.

11. RHIPSALIS RUSSELLII Britton & Rose, Cactaceae 4: 242. 1923. —TYPE: BRAZIL. Bahia: near Toca da Onça (Jaguaquara), Rose 20106 (holotype: NY!; isotype: US!).

Epiphyte or lithophyte in open or shaded habitat, 0.4-1.5 m long, branching apical. Stem segments flattened to triangular in longitudinal section (1-)2-5.5 diam, olive or pale green with reddish margin, succulent, stiff sometimes flexible, monomorphic 10-22 cm long, base attenuate, apex truncate, wings 2-3, 1-4 cm wide, margin crenate to lobed, plane to slightly undulate, with (3-)5-10 mm projections, midrib 3.5-7.5 diam, cylindric. Areoles between margin projections, 1.5-2.5 cm apart, first of segment 1-3(-8) cm distant from segment base; when sterile 2-3 mm diam, pilose, with vestigial scales; when fertile 2.5-3 mm diam, pilose, with numerous acicular scales, 2-10 flowers/fruits. Flowers 5-6 mm diam; pericarpel 3 X 2.5-3 mm, globoid, green, glabrous; with 3 sepaloid tepals, 0.6-1 mm long and 5 petaloid tepals, 2-3 X 2.1-2.5 mm, wide elliptic, patent to sub-erect, whitish, apex rounded, curved inwards, margin curved inwards. Style 2.3-2.5 mm long; stigma with 3-5 lobes, 0.8-1 mm long, ligulate, spreading. Ovules in 5 incomplete septa, funicle short (< 0.5 mm long). Stamens 9-12, 1.2-2.2 mm long, side by side, spreading, whitish. Nectary ca. 0.4 mm long. Fruit 6-10 X 6-9 mm, globoid, deep magenta, glabrous. Figure 2: D, H.

Notes: *R. russellii* can be easily identified by the small flowers (ca. 5-6 mm diam.), with few stamens, the deep magenta fruits numerous per areole, and the large and pilose areoles with numerous scales.

Habitat and distribution: Occurs in Minas Gerais, Espírito Santo and Bahia (Brazil), usually in dryer Atlantic forest (*sensu lato*). It can also occur as lithophyte in Bahia (Chapada Diamantina, Brazil). Figure 5.

12. *RHIPSALIS TRIANGULARIS* Werderm., Repert. Spec. Nov. Regni Veg. 42: 3. 1937.—TYPE: BRAZIL. Rio de Janeiro: Rio de Janeiro, Parque Natural Municipal da Prainha, 2003, *Calvente* 37 (neotype, here designated: RUSU; isoneotype: R, RB).

Lithophyte in open habitat, 0.5-1 m long, branching apical. Stem segments, triangular to tetragular, rarely pentangular in longitudinal section, 1-3(-4) mm diam, olive green, succulent, stiff, monomorphic, 6-24 cm long, base attenuate, apex truncate, wings 3-4(-5), (0.5-)1-2(-3) cm wide, margin serrate to crenate, plane, with 4-7 mm projections, midrib 5-7 mm diam, cylindric. Areoles between margin projections, 1.5-2.5 cm apart, first of segment 0.5-2 cm distant from segment base; when sterile 2-5 mm diam, pilose, with vestigial scales; when fertile ca 3 mm diam, pilose at margin, with numerous acicular scales, 1-3 flowers/fruits. Flowers 15-25 mm diam; pericarpel 5 X 6 mm, obovoid, pinkish green to magenta, glabrous; with 3-4 sepaloïd tepals, 1-2.5 mm long and 10-12 petaloïd tepals, 4.5-10 X 3-5.6 mm, wide elliptic or elliptic, patent to sub-erect, white, apex rounded, slightly to strongly cucullate, margin curved inwards. Style 7-8.5 mm long; stigma with 5-7 lobes, 2.5-3 mm long, sagitate, erect. Ovules in 5 rows, funicle short (< 0.5 mm long). Stamens 70-160, 5-7 mm long, median shorter, internal facing outwards and external facing inwards, white. Nectary ca. 1 mm long. Fruit 6-7 X 7-8 mm, globoid, magenta to pinkish, glabrous. Figure 2: E, I.

Notes: *R. triangularis* is well characterized by the large flowers, long style and stem segments with deeply serrate margins. This taxon was described from a cultivated specimen that was sent from the Botanical Garden of Rio de Janeiro to the Dahlem Botanical Garden. However, this specimen was lost requiring the establishment of a neotype. The lack of a type specimen

contributed for the uncertainty on the correct application of this name. However, the recent discovery of new populations of *R. triangularis* in Rio de Janeiro (Calvente and Andreato 2007), allowed for a more careful examination of *R. triangularis* in the field and a better circumscription of this taxon. This species is closely related to another species from the inselbergs of Rio de Janeiro (*R. cereoides*); however, these taxa have non-overlapping distributions.

Habitat and distribution: Occurs in southern coastal inselbergs of Rio de Janeiro (Rio de Janeiro, Brazil). It is currently only known from the Natural Municipal Park of Prainha (Rio de Janeiro, Brazil). Figure 10.

EXCLUDED NAMES:

1. *Rhipsalis rhombea* (Salm-Dyck) Pfeiff., Enum. Diagn. Cact.: 130. 1837.
Cereus rhombeus Salm-Dyck, Hort. Dyck.: 341. 1834.

Notes: *Rhipsalis rhombea* was described from a specimen cultivated in Germany and with unknown provenance. The name *R. rhombea* has been applied in multiple taxonomic senses during the past 90 years (e.g., Löfgren 1915; Britton and Rose 1923) leading to a lot of taxonomic confusion. Unfortunately, however, the original description is also very broad, preventing a correct tipification of this taxon.

2. *Rhipsalis robusta* Lem., Rev. Hort. 4: 520. 1860, *nom. nud.*

Notes: *R. robusta* was described from a specimen cultivated in France. No formal description or diagnosis was presented, preventing a correct application of this name.

3. *Rhipsalis platycarpa* Pfeiff., Enum. Diagn. Cact.: 131. 1837. *Epiphyllum platycarpum* Zucc. ex. Pfeiff., Enum. Diagn. Cact.: 131. 1837, *pro syn.*
Cereus platycarpus Zucc. Plantarum novarum vel minus cognitarum quae in Horto Botanico Herbarioque regio monacensi servantur 3: 736. 1838.—

TYPE: Pfeiff. & Otto, Abbild. Besch. Cact. Taf 17, Fig 2. 1838 (neotype, here designated).

Notes: Britton and Rose (1923) present the illustration of Pfeiffer and Otto (1838) as reference to this name. This illustration is here selected as the neotype of *R. platycarpa*. However, a careful analysis of the neotype and of the original description of this taxon (Zuccarini 1836; Pfeiffer 1837) indicates that *R. platycarpa* presents flowers with a sub-tetragonal ovary and fruit. These traits are not present in any other species of *Rhipsalis*, suggesting that this species likely represents another genus of Cactaceae or a specimen with anomalous flowers.

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Figure 1. Phylogenetic tree of "core *Rhipsalis*" derived from the Bayesian analysis of the cpDNA *psbA-trnH*, *trnQ-rps16*, *rpl32-trnL*, and nuclear ITS and MS data set. Maximum parsimony bootstrap and maximum likelihood bootstrap values are indicated above branches and posterior probabilities are indicated below branches.

Figure 2. A-E. Habit showing stem segment and areole morphology of species of the "winged-stem clade" (*Rhipsalis*). F-I. Longitudinal section of flowers. A, C, G. *R. pachyptera*. B, F. *R. cereoides*. D, H. *R. russellii*. E, I. *R. triangularis*.

Figure 3. Distribution of species of the "winged-stem clade" (*Rhipsalis*) restricted to Rio de Janeiro and São Paulo, Brazil.

Figure 4. A-F. Habit showing stem segment and areole morphology of species of the "winged-stem clade" (*Rhipsalis*). G-J. Longitudinal section of flowers. A. G. *R. crispata*. B, C, H. *R. elliptica* subsp. *elliptica*. D, I. *R. olivifera*. E, F, J. *R. pachyptera*.

Figure 5. Distribution of species of the "winged-stem clade" (*Rhipsalis*) restricted to Brazil.

Figure 6. A-F. Habit showing stem segment and areole morphology of species of the "winged-stem clade" (*Rhipsalis*). G-I. Longitudinal section of flowers. A, G. *R. crispimarginata*. B, C. *R. elliptica* subsp. *elliptica*. D, H. *R. goebeliana*. E, I. *R. elliptica* subsp. *microflora*. F. *R. obonga*.

Figure 7. A-G. Habit showing stem segment and areole morphology of species of the "winged-stem clade" (*Rhipsalis*). H-K. Longitudinal section of flowers. A, H. *R. cuneata* subsp. *cuneata*. B, E, J. *R. micrantha* subsp. *tonduzii*. C. *R. micrantha* subsp. *monticola*. D, I. *R. micrantha* subsp. *micrantha*. F, K. *R. micrantha* subsp. *rauhiorum*. G. *R. cuneata* subsp. *australis*.

Figure 8. Distribution of species of the "winged-stem clade" (*Rhipsalis*) in South and Central America (except Brazil).

Figure 9. Distribution of species of the "winged-stem clade" (*Rhipsalis*) in Peru and Ecuador.

Figure 10. Distribution of species of the "winged-stem clade" (*Rhipsalis*) restricted to Brazil.

Appendix 1. Examined specimens.

R. CEREOIDES. Brazil. Rio de Janeiro: Itaipuaçu, Apr 1936, *Voll s.n.* (RB 10258, neotype). Niterói, Morro do Costão de Itacoatiara, 2 May 1980, *Moutinho 109* (HB); Pedra de Itacoatiara, 500 m, 12 Sep 1982, *Farney 92* (RB); Morro Alto Moirão, 300-325 m, 12 Feb 1985, *Plowman 13935* (RB); entre Mun. Niterói e Maricá, entre praias de Itaipuaçu e Itacoatiara, Alto Moirão, 12 Apr 1989, *Andreato 886* (RB); Charitas, 2 m, 28 Mar 1995, *Scheinvar 6295* (RB); Pedra de Itacoatiara, 16 May 1997, *Lúcio 9* (RB). Rio de Janeiro, Morro da Urca, atrás da estação do teleférico, 10 m, 15 May 1995, *Scheinvar 6226* (RB).

R. CRISPATA. **Brazil.** *Rose 20708* (NY). **Bahia:** Amélia Rodrigues, Margem da BR-324, Mar 1998, *Machado 3* (HUEFS). **Rio de Janeiro:** Araruama, APA - Massambaba, RJ-132, próx. à lagoa de Araruama, 16 Aug 1992, *Freitas 239* (RB). Arraial do Cabo, Arredores da cidade, 17 May 1990, *Zappi 232* (HRCB, K); APA - Massambaba, Reserva Biológica da Massambaba, 27 Aug 1991, *Freitas 225* (RB); ibidem, brejo do Espinho, 16 Aug 1992, *Freitas 240* (RB). Guapimirim, Estrada Rio-Teresópolis, 300 m, 19 Jul 2006, *Calvente 219* (SPF). Rio das Ostras, Restinga de Balneário das Garças, 14 Dec 1999, *Braga 704* (RB). Restinga de Cabo Frio, 14 Oct 1968, *Sucre 3955* (MO, RB). Saquarema, Restinga de Ipitangas, 30 Mar 1989, *Freitas 56* (RB). Organ Mountains, 1915, *Rose 21159* (NY). **São Paulo:** Ilhabela, caminho da praia de Jabaquara, 0-50 m, 14 Aug 2007, *Zappi 839* (SPF); estrada para Jabaquara, 31 m, 1 Dec 2007, *Calvente 366, 368* (SPF). **Cultivated (Ex hortus).** 2 Mar 1967, s. col. (*cult.*) *E5160-0008* (C).

R. CRISPIMARGINATA. **Brazil.** **Rio de Janeiro:** Angra dos Reis, Ilha Grande, 50-100 m, 16 Apr 1992, *Zappi 274* (SPF). Mangaratiba, Reserva Rio das Pedras, 30-650 m, 14 Apr 2006, *Calvente 196, 202* (SPF). Parati, Apa-Cairuçu, trilha para o morro do Cuscuzzeiro, 10 Aug 1994, *Duarte 93* (RB). Petrópolis, Serra da Estrela, Meio da Serra, leito da antiga estrada de ferro, 400 m, 30 Mar 1977, *Martinelli 1575* (RB). Rio das Ostras, Reserva Biológica da União, 19 Jun 1997, *Oliveira 4* (BHCB). Saquarema, APA-Massambaba, Reserva Ecológica de Jacarepiá, 11-12 Dec 1990, *Freitas 206, 210* (RB); ibidem, 28 Aug 1991, *Freitas 229* (RB). **São Paulo:** Ilhabela, estrada para a praia de Castellanos, 13 Jun 1991, *Taylor 1645, 1645-A* (HRCB, K). Ubatuba, Picinguaba, trilha da casa da farinha, Sep 2001, *Udulutsch 454* (HRCB).

R. CUNEATA SUBSP. CUNEATA. **Bolivia.** **Cochabamba:** Carrasco, Confluente del Río Leche con el Río Isarsama, 220 m, 5 May 1979, *Beck 1629* (MO); Parque Nacional Carrasco, al S del acampamento petrolero Ichoa, 700 m, 20 Sep 1997, *Acebey 708* (LPB). Chaparre, Camino Cochabamba-Vila Tunari, km 106, 1450 m, 19 Sep 1993, *Ibisch 930766-A* (LPB); Territorio Indigena Parque Nacional Isiboro-Secure, Cordillera de Mosetenez, laguna Carachupa, 1300 m, 29 Aug 2003, *Kessler 13024* (LPB). José Carrasco Torrico, 147 km antigua carretera Cochabamba-Vila Tunari, 1500 m, 25 Aug 1996, *Kessler 7754* (K, LPB); ibidem, 1100 m, 28 Aug 1996, *Kessler 7955* (K, LPB). **La Paz:** Chulumani, 107 km, Sud Yungas, 900 m, 6 Aug 1983, *Beck 8528* (LPB). **Santa**

Cruz: Caballero, Parque Nacional Amboro, 1420 m, 31 Mar 1996, *Jardim 2575* (LPB, NY). Ichilo, 5 km SW of E. Cóndor, Línea rola, delimiting the boundary of the Paque Nacional Amboró, 500 m, 19 Nov 2000, *Nee 51484* (MO, NY). **Ecuador. Morona-Santiago:** Bomobolza, Río Cuyes, 800 m, 1 Nov 1986, *Cerón 420* (QCNE). Limon Indanza, Region de la Cordillera del Cóndor, 1350-1800 m, 12 Dec 2002, *Katan 102* (QCNE). **Pastaza:** Pastaza, Sector Huamboya a 8 km a la izquierda, 650 m, 6 Nov 1991, *Tipaz 403* (QCNE). **Zamora-Chinchipe:** Bombuscaro, parcela de Tesis, 17 Sep 1988, *Sánchez 53* (LOJA, QCNE); Bombuscaro, junto al camino, 950 m, 9 Mar 1998, *Lozano 983* (LOJA); From parking area to the guard information centre, 1000 m, 11 Jan 2001, *Rosales 7651* (LOJA). Zamora, Estrada Zamora-Sabanilla, 1336 m, 8 Jan 2008, *Calvente 381* (SPF). Zamora-Chinchipe, 10 km West of Zamora on road along left shore of Río Jamboé, 1100 m, 12 Apr 1985, *Harling 24020* (QCA). Road Zamora-Loja Km 11, 1300 m, 5 Dec 1988, *Madsen 75835* (K, LOJA, NY, QCA, QCNE). Zamora-Chinchipe, limit of Parque Nacional Podocarpus, Quebreda de León, affluent of Río Bombuscaro, S of Zamora, 1100 m, 16 Apr 1989, *Madsen 85988* (LOJA, QCA, QCNE). **Peru. Madre de Dios:** Manu, Atalaya, Hacienda Amazonia, 2-3 km west of village, across Río Alto Madre, 600-900 m, 7 Dec 1983, *Foster 7249* (USM). **San Martin:** entre Moyobamba y Rioja, 800-900 m, 30 Sep 1973, *Ferreira 18250-A* (USM). **Suriname.** 1951, *Engelman 1478* (MO). **Brokopondo:** Afobaka, Forest along Sara Creek, Feb 1965, *Douselsar 2087* (K). **Cultivated (Ex hortus):** origin: East Equador, near Sucua, 950 m, Sep 1975, *Rauh 34950* (paratype: HNT). Origin: Peru, between Yanamayo and Huayruruni, 28 Sep 1982, *Baker 4382* (HNT). Origin: San Martin, Rioja, 800 m, Sep 1975, *Rauh 35392* (HNT). Origin: Bolivia, *Johnson s.n.* (*Berkeley 62400-1*) (MO, P).

R. CUNEATA SUBSP. AUSTRALIS. **Bolivia. Cochabamba:** Carrasco, Canton Chuquioma, campamento Isarsame de La UMES, al Río del mismo nombre (afluente río Ichilo), 200-300 m, 3 May 1979, *Beck 1559* (LPB, MO); Estación Proyecto Valle del Sacta, 235 m, 25 Apr 1989, *Smith 12993* (LPB, MO). Chaparre, 159 km antigua carretera Cochabamba-Vila Tunari, 700 m, 7 Sep 1996, *Kessler 8223* (K, LPB). José Carrasco Torrico, Vale del Sacta, 220 m, 1 Oct 1996, *Kessler 8734* (K, LPB); ibidem, 220 m, 8 Oct 1996, *Kessler 8977* (K, LPB); ibidem, 9 Oct 1996, *Kessler 8982* (LPB); ibidem, Parque Nacional Carrasco, al S del acampamento petrolero Ichoa, 300 m, 11 Sep 1997, *Acebey 422* (LPB); ibidem, 650 m, 14 Sep 1997, *Acebey 581* (LPB). **Cultivated (Ex hortus):** origin: Bolivia, Cochabamba, Chaparre, Km 130 along Vila Tunari, between Río Cristal Mayu at Chokolatal, 1977, *Aguilar s.n.* (HNT 8404).

R. ELLIPTICA SUBSP. ELLIPTICA. **Brazil.** 1883-84, *Glaziou 14859*, (syntype: C, K, P); 13 Apr 1989, *Sucre 10957* (RB). **Espírito Santo:** Cachoeiro do Itapemirim, Vargem Alta, May 1949, *Brade 19993* (RB). Castelo, Forno Grande, 12 Oct 2000, *Kollmann 3140* (MBML, SPF). **Minas Gerais:** Felício dos Santos, APA Felício, Mata de Isidoro e arredores, 1320 m, 29 Aug 2008, *Viana 3692* (SPF). Santana do Riacho, Alto do Palácio, Parque Nacional da Serra do Cipó, 4 Mar 2006, *Lemos 3* (SPF). **Paraná:** Antonina, Río Pequeno, 12 Jan 1974,

Hatschbach 33662 (MBM); Rio Xaxim, 22 Sep 1982, *Hatschbach* 45418 (MBM). Bocaiúva do Sul, Serra São Miguel, 8 Jun 1988, *Hatschbach* 52139 (C, HUEFS, MBM, MO). Campina Grande do Sul, Contrafortes da Serra Capivari Grande, 1 Apr 1962, *Hatschbach* 9105 (HB, MBM); Ribeirão do Cedro, 18 Feb 1962, *Hatschbach* 9056 (MBM); Contafortes, Serra de Capivari Grande, 1 Apr 1962, *Hatschbach* 9156 (MBM); Rio Capivari, 6 May 1986, *Kummrow* 2749 (C, MBM). Guaraqueçaba, Tagaçaba, 8 May 1985, *Hatschbach* 49349 (MBM, MO). Paranaguá, Ilha do Mel, Praia Grande, 10 May 1986, *Britez* 732 (MBM); ibidem, Morro do Meio, 6 Mar 1987, *Britez* 1381 (MBM). **Rio de Janeiro:** Angra dos Reis, Ilha Grande, 22 Jul 1915, *Rose* 20346 (NY); Estrada Praia Grande, 10 m, 29 Sep 1973, *Martinelli* 63 (RB); Angra dos Reis, 40 m, 26 Aug 1974, *Martinelli* 481, 482 (RB); Ilha Grande, na estrada entre a Praia do Abraão e a Colônia Penal, lado Nordeste da ilha, 28 Jun 1978, *Carauta* 2906 (RB); Ilha Grande, sobre rochas e na base de uma árvore, 300 m, 14 Apr 1992, *Zappi* 267 (SPF); Ilha Grande, sul da Ilha, 400 m, 14 Apr 1992, *Zappi* 269 (SPF); Ilha Grande, pequena cachoeira entre Abraão e Praia do Iguaçu, 50-100 m, 14 Apr 1992, *Zappi* 276 (SPF); Ilha Grande, trilha Parnaioca-Bosque das Pedras, 2 Mar 2002, *Gomes* 29, 29a (HB). Itatiaia, PNI-EFE., 13 Feb 1980, *Barreto* 121 (RB). Nova Friburgo, Macaé de Cima, estrada para o Sítio Rio das Flores, 1011 m, 21 Nov 2005, *Calvente* 146 (SPF). Nova Iguaçu, Morro do Beco, próximo ao riacho, 17 Nov 1994, *Silva-Neto* 443 (RB). Parati, Faz. São Roque, caminho para Cunha passando o rio São Roque, Serra de Paraty, 30 Nov 1988, *Marquete* 185 (RB); Praia de Jabaquara, 25 Sep 1989, *Freitas* 83 (RB); Paratimirim, 26 Sep 1989, *Freitas* 96, 97, 101 (RB); Ponta da Trindade, 27 Sep 1989, *Freitas* 128 (RB); Fazenda Taquari, 28 Sep 1989, *Freitas* 138 (RB); Fazenda São Gonçalo, Barra do Girimim, Praia do Iririguaçu, 28 Sep 1989, *Freitas* 155 (RB); Fazenda Taquari, 28 Sep 1989, *Freitas* 158, 160 (RB); Mata do lado direito da trilha para Praia de Martim de Sá (em direção ao Cairuçu na APA-CAIRUÇÚ), 190 m, 10 Nov 1990, *Marquete* 258 (RB); Laranjeiras, entre o primeiro e o segundo portão, rumo a Praia do Sono, 18 Oct 1990, *Frutuoso* 96 (RB); Laranjeiras, início da estrada para a praia do sono até o portão para a Fazenda Santa Rita, 18 Oct 1990, *Klein* 943 (RB); Loteamento Frade Paraty, Folha: SF.23-ZC, 10 Nov 1991, *Marquete* 498 (RB); Apa-Cairuçu, alagado Olaria, próximo a Flora de Parati, 20 Oct 1993, *Marquete* 1273 (RB); Condom. Laranjeiras, estrada para Praia do Sono, 9 Aug 1994, *Marquete* 1990 (RB); 1º Distrito, Rodovia Rio-Santos (BR 101) lado esquerdo em direção a São Paulo, mata de baixada após o Trevo de Paraty, Apa-Cairuçu, 23 Nov 1994, *Giordano* 1763 (RB); Estrada próxima ao Rio dos Meros, 70 m, 27 Jun 1995, *Campos* 36 (RB); Trindade, Praia Brava, lado direito, 29 Jun 1995, *Marquete* 2191 (RB). Rio de Janeiro, s.d., *Ribas* 384 (HB); Floresta da Tijuca, 10 Jul 1883, *Glaziou* 14859 (C, K, P); Rio de Janeiro, 1915, *Rose* 20845, 20874 (NY); 28 Jul 1958, *Pereira* 4076 (HB, NY, RB); Vertente NW da Serra da Piaba, 40-80 m, 17 Aug 1970, *Sucre* 7052 (RB); Parque Nacional da Tijuca, Serra dos Pretos Fôrros, Represa dos Ciganos, 200-300 m, 30 Sep 1977, *Martinelli* 3123 (RB); Pq. Estadual da Pedra Branca, Floresta do Camorim, lage do Ravi, 28 Aug 1982, *Costa* 211 (RB); Jacarepaguá, Curicica, 4 Aug 1990, *Farney* 2396 (RB); Recreio dos

Bandeirantes, Parque Natural Municipal da Prainha, 3 Jul 2004, *Cardoso* 202 (RB). Rio de Janeiro, pr. Rodeio, 25 Jul 1964, *Pabst* s.n. (HB 32168). **Santa Catarina:** Ibirama, Horto Florestal I.N.P., 450 m, 12 Apr 1956, *Reitz* 3072 (HBR, NY). Itajaí, Luis Alves, Braço Joaquim, 450 m, 22 Mar 1956, *Reitz* 2885 (HBR); Morro da Ressacada, 350 m, 29 Mar 1956, *Reitz* 2927 (HBR, NY). Palhoça, Pilões, 200 m, 6 Apr 1956, *Reitz* 3057 (HBR, NY). Rio do Sul, Serra do Matador, 550 m, 16 Apr 1969, *Reitz* 8754 (HBR). São Francisco do Sul, Garuva, Três Barras, 50 m, 20 Mar 1958, *Castellanos* 6616 (K). *ibidem*, 50 m, 26 Mar 1958, *Reitz* 6616 (HB, HBR, MBM). Vidal Ramos, Sabiá, 750 m, 16 Jun 1957, *Reitz* 4369 (HBR); Sabiá, 600 m, 6 Apr 1958, *Reitz* 6658 (HBR). **São Paulo:** Bananal, Parque Nacional da Serra da Bocaina, próximo ao marco 22, 1100 m, 23 Jun 1978, *Martinelli* 4672 (RB). Entre Mongaguá e Praia Grande, 3 May 1994, *Godoi* 401 (K). Ilhabela, estrada para a praia de Castellanos, 13 Jun 1991, *Taylor* 1644 (HRCB, K); *ibidem*, 225 m, 1 Dec 2007, *Calvente* 369 (SPF). Iporanga, Fazenda Intervalles, Base do Carmo, 24 Apr 1995, *Sugiyama* 1305 (K). Pariquera-Açu, Propriedade de Antonio Povinski, 31 May 1996, *Ivanauskas* 804 (BHCB, HRCB, K). Ribeirão Grande, Fazenda Intermontes, 800-1000 m, 29 May 2006, *Bazarian* 102 (SPF). Santo André, Reserva Florestal de Paranapiacaba, 30 Feb 1982, *Maruffa* 52 (MBM); Reserva Biológica do Alto da Serra, 14 Oct 1992, *Almeida* s.n. (K 9520, MBM); Santo André, Reserva Biológica de Paranapiacaba, 850 m, *Taylor* 1637 (K). Santos, Fortaleza da Praia Grande, 4 Apr 2006, *Calvente* 194 (SPF). São Vicente, Estrada de Ferro Sororocabana, próximo da Estação Acaraú, 224 m, 30 May 2006, *Calvente* 214 (SPF). Sete Barras, Fazenda Intervalles, Saibadela, 6 Jul 1992, *Mello-Silva* 578, 579 (HUEFS, MBM, SPF); Fazenda Intervalles, Saibadela, trilha de baixada, 5 Jul 1995, *Almeida-Scabbia* 1437 (HRCB). Ubatuba, estrada Rio-Santos, 17 Apr 1979, *Ferreira* 664 (RB); Picinguaba, trilha das 3 lagoas, 4 Dec 1988, *Garcia* 274 (HRCB); Picinguaba, trilha do Morro do Corsário, 6 Aug 1988, *Ribeiro* 428 (HRCB, SPF); Picinguaba, estrada da casa da farinha, 8 Oct 1988, *Cunha* 147 (HRCB); *ibidem*, 28 Jul 1990, *Romero* 109 (HRCB); Trilha do Corisco, 9 Nov 1993, *Barros* 29472 (K); Trilha da Fazenda Capricórnio, 28 Aug 1994, *Assis* 375 (HRCB, K); Picinguaba (flowered in cult.), 19 Jul 1996, *Lombardi* 1353 (BHCB); *ibidem*, trilha atrás da sede, 30 m, 4 Aug 2001, *Salino* 7219 (BHCB); *ibidem*, trilha próxima ao estacionamento de visitantes, 14 m, 5 May 2007, *Calvente* 347 (SPF); *ibidem*, trilha da Restinga em direção ao Mangue, 15 m, 5 May 2007, *Calvente* 345 (SPF); *ibidem*, 15 m, 8 Jun 2007, *Calvente* 348, 349 (SPF); *ibidem*, 9 m, 8 Jun 2007, *Calvente* 337b, 338b (SPF); Picinguaba, caminho para a casa de farinha, 8 Jun 2007, *Calvente* 355 (SPF). S. José dos Campos - Caraguatuba, 750-820 m, 27 May 1970, *Sucre* 6933 (RB). São Paulo-Curitiba Highway, 265 km from SP, 15 Jul 1966, *Hunt* 6316 (K). **Cultivated (Ex hortus):** Origin: Brazil, 1917, *Löfgren* s.n. (NY 45419).

RHIPALIS ELLIPTICA SUBSP. MICROFLORA. **Brazil. Bahia:** Barro Preto, Serra da Pedra Lascada, 2 Nov 2003, *Fiaschi* 1778 (SPF). Camacan, Estrada a Pau Brasil, 19 Jan 1971, *Santos* 1352 (RB). Santa Teresinha, Serra da Jibóia, 23 Nov 2001, *Andrade* 37 (HUEFS). Teixeira de Freitas, Vale do Rio Alcobaça, 12 May 1971, *Soares* 1627 (RB). Una, Estrada Ilhéus-Una, ca. 35-40 km ao sul de

Oliveira, 35-40 m, 2 Dec 1981, *Lewis* 729 (K, NY, P, RB); Km 9 da estrada São José de Burarema-Una, 28 Oct 1983, *Callejas* 1561 (MBML, NY, RB). **Espírito Santo:** Atílio Vivácqua, Moitão do Sul, Serra das Torres, 650 m, 25 Apr 2007, *Fontana* 3185 (MBML, SPF). Santa Teresa, Nova Lombardia, Reserva Biológica Augusto Ruschi, 16 Apr 2002, *Vervloet* 118 (MBML, SPF); 18 Feb 2003, *Vervloet* 1847 (MBML, SPF); Parque do Museu Biológico Melo Leitão, 21 Jan 2005, *Kollmann* 7273 (MBML, SPF). **Minas Gerais:** Santa Maria do Salto, Fazenda Duas Barras, 810 m, 25 Jul 2003, *Lombardi* 5560 (BHCB); ibidem, 776-755 m, 9 Mar 2004, *Lombardi* 5961 (BHCB); ibidem, 763-847 m, 21 Feb 2005, *Stehmann* 4055 (BHCB).

RHIPSALIS MICRANTHA subsp. *MICRANTHA*. **Ecuador. Carchi:** Tulcán, Chical, 1200-1800 m, 7 Dec 2001, *Clark* 6334 (QCNE). **Chimborazo:** Railroad Huigra-Cumandá, Km 5-8 from Huigra, at Río Chanchan, 800-850 m, 13 Nov 1985, *Madsen* 61124 (K, QCA, QCNE). **Loja:** Sozoranga, El Tundo, 1800 m, 22 Nov 1996, *Lozano* 584 (LOJA). Ridge S camp, 2 km w Tambo Negro on Macará-Sozoranga Road, 1000 m, 20 Jan 1991, *Kessler* 2267 (QCA). **Manabí:** San Sebastian-Agua Blanca, 200-400 m 20 Sep 1991, *Cerón* 16625 (QCNE). **Tungurahua:** Baños, Río Negro, 1500 m, 16 Jun 1987, *Cerón* 1580 (K, QCNE). **Peru. Cajamarca:** Contumaza, Platanar - planta electrica, 1400 m, 31 Mar 1994, *Sagástegui* 15204 (QCNE). Estrada Lambayeque-Moyobamba, km 28 após Limón de Porpuia, 1288 m, 17 Jan 2008, *Calvente* 395, 396 (SPF). Santa Cruz, Camino al chorro blanco, 1350 m, 10 Oct 1993, *Leiva* 912 (QCNE). **Lambayeque:** Lambayeque, Hualanga, Penachi, 1400 m, 13 Jan 1996, *Quiroz* 4084 (USM). **Olleros:** *Humboldt* 3494 (P). **Piura:** Ayabaca, on the road to Ayabaca, 13 km above Puente Tandopa, 1600 m, 23 Sep 1964, *Wright* 6669 (USM). **Cultivated (Ex hortus):** Origin: Costa Rica, 21 Mar 1961, *Lankester* s.n. (MO 2287791).

Rhipsalis micrantha subsp. *monticola*. **Ecuador. Gran Chimú:** La Libertad, Platanar, arriba de cascadas, 1300 m, 1 Nov 1995, *Sagástegui* 15816 (QCA). **Loja:** Catamayo, Estrada Catamayo-Catacocha, 2245 m, 9 Jan 2008, *Calvente* 383 (SPF). Zambí, Road La Toma-Loja, Km 12, 2000 m, 2 Sep 1968, *Madsen* 75203 (LOJA, QCA, QCNE); Camino al cerro Trablazo, 1420 m, 21 Jan 1996, *Van den Eynden* 590 (LOJA, QCA). Road La Toma (Catamayo) - Loja, about 5 km from La Toma, 1600-1800 m, 26 Nov 1985, *Madsen* 61157 (K, NY, QCA). Road La Toma-Loja, Km 10-12, 1700-2000 m, 7 Dec 1988, *Madsen* 75908 (LOJA, QCA). Between Cariamanga and Amaluza, 1500 m, 1 Oct 1989, *Madsen* 86220 (QCA). South of Loja, 2000 m, 21 Jul 1989, *Madsen* 86083 (LOJA, QCA, QCNE). Parque Nacional Podocarpus, Reserva El Bosque, above San Pedro de Vilcabamba (5 km east of village), 2100 m, 30 Nov 1994, *Pedersen* 104135 (LOJA, QCNE). Malacato-El Tambo, road near La Merced, 1530 m, 16 May 1995, *Pedersen* 104296 (LOJA). Carretera Loja-Malacatos, cerca de Río Campanas, 1400 m, 13 Nov 1997, *Merino* 5088 (LOJA).

RHIPSALIS MICRANTHA SUBSP. *RAUHIIORUM*. **Ecuador. Loja:** Vale do Rio Catamayo, próximo à estrada Catacocha-San Vicente, 1650 m, 10 Jan 2008, *Calvente* 386 (SPF). Süd-Ecuador, Tal des Río Catamayo, 1300 m, Sep 1973,

Rauh 35278 (HEID). **Cultivated (Ex hortus)**. Origin: S Ecuador, Rio Catamayo Valley, 1330 m, Sep 1975, *Rauh* 35278 (HNT). Jul 1978, *Lorenz* s.n. (HNT 2021). *Rauh* s.n. (HNT 7147).

RHIPSALIS MICRANTHA SUBSP. TONDUZII. **Costa Rica**. 1845, *Orsted* 11154, 11155, s.n. (C). **Alajuela**: San Ramón, 3 km of San Ramón, 1025 m, 22 Jun 1969, *Lenf* 1766 (CR). Santiago de San Ramón, 10 Apr 1933, *Broner* 12183 (CR). **San Jose**: Cantón de Acosta, Z. P. Cerros de Escazú, 1600-1700 m, 14 May 1994, *Morales* 2757 (CR, INB, MO). Cantón de Mora, Zona Protetora El Rodeo, 800 m, 15 Feb 1993, *Hammel* 18841 (CR); Cuenca del Rio Grande de Tárcoles, 800 m, 25 Feb 1993, *Hammel* 18841 (MO); Zona Protetora El Rodeo, 480-550 m, 15 Jul 1997, *Cascante* 1353 (CR). San Jose, Cultivado en el Parque Bolivar, 6 Jun 1935, *Valerio* 1005 (CR); Cultivado en San Jose, Feb 1937, *Valerio* 1072 (CR). San Marcos, Apr 1897, *Wercklé* 2312 (P). **Ecuador**. Route Cariamanga-Macara, 1900 m, 23 Apr 1991, *Huttel* 1997 (QCA). **Azuay**: Road Santa Isabel-Pasaje, below San Francisco, 700 m, 24 Oct 1985, *Madsen* 61079 (K, NY, QCA, QCNE). **Cañar**: Estrada E25, entre San Antonio e Jaime Roldos, 50 m, 10 Jan 2008, *Calvente* 388b, 393 (QCNE, SPF). Estrada E25, entre San Antonio e Jaime Roldos, 51 m, 10 Jan 2008, *Calvente* 392 (QCNE, SPF). **El Oro**: Estrada entre Balsas e entrada para Piñas, 744 m, 10 Jan 2008, *Calvente* 388a (QCNE, SPF). **Esmeraldas**: Cabo San Francisco, Cantón Muisne, 0-200 m, Aug 2004, *Haro-Carrión* 244 (QCA). Quininde, Bilsa Biologica Station, 500 m, 20 Nov 1995, *Clark* 1683 (QCNE). Fundacion Paraiso de Papagayos, 200 m, 10 Oct 1996, *Clark* 3050 (QCNE). Colecciones en el predio Quititos, Eucapacific, 15 Aug 2002, *Pérez* 470 (QCA). **Guayas**: Los Mangas, 8 km west of Manglaralto, 50 m, 27 Jun 1977, *Ittis* E34 (MO, QCA). Santa Elena, Reserva Comunal Loma Alta, 250-500 m, 22 Jan 1997, *Clark* 3858 (QCNE). Rd. Puerto Lopez-Puerto Cayo, 55 km N of Manglaralto, 300 m, 13 Dec 1989, *Hunt* 89143 (K, QCA). Cordillera Chongón-Colonche, comuna Olón, 350-400 m, 8 Jun 1994, *Cornejo* 2865 (QCNE). **Loja**: Desert country between Vilcabamba and Cachiyacu, 1600-2100 m, 6 Oct 1943, *Steyemark* 54389 (K). Road San Pedro de la Bendita-El Clane, c. km 8, Northern extension of the Catamayo Valley, 1900 m, 25 Feb 1989, *Ollgaard* 90730 (LOJA, QCA, QCNE). Loja-Chuquiribamba, km 25, 2250 m, 20 Apr 1994, *Jorgensen* 414 (LOJA, QCA, QCNE). Carretera Playas-Lauro Guerrero, 5 km before Lauro Guerrero, 1580 m, 23 Aug 1994, *Pedersen* 104086 (LOJA, QCA). Hacienda Banderones, pie de montaña, 1000 m, 6 May 1997, *Lozano* 782 (LOJA). **Los Rios**: San Antonio de Columa, Km 39 on the Road Pueblo Viejo-Guaranda, 250 m, 20 Apr 1980, *Holm-Nielsen* 22917 (K, QCA). Near Quevedo, Cantón Vinces, 28 Oct 1934, *Mexia* 6634 (P). Rio Palenque Biological Station, 150-220 m, 18 Mar 1974, *Dodson* 5516 (QCA). Represa Daule-Peripa, 22 Jun 1985, s. col. (*cult.*) s.n. (MO 5683367). **Manabí**: Crucita, Cuenca Rio Ayampe, 500 m, 9 Oct 1994, *Cornejo* 971 (K, QCNE). Jama, Flood Plain of Rio Jama, 20 m, 16 Dec 1998, *Neil* 11600 (QCNE). Montecristi, Cerro Montecristi, eastern slopes above town, 300-400 m, 18 Jul 1986, *Plowman* 14350 (K, NY, QCA); Cerro Montecristi, 300-600 m, 11 Nov 1995, *Nuñez* 362 (QCNE). Puerto Lopez, to the S, near the beach, sandy and stony slopes, 0-10 m, 7 Dec 1985, *Madsen* 61179, 61180 (K). **Pichincha**:

Along N-bank of river 3 km W of Allurquín, 800 m, 20 Oct 1981, *Werling* 519 (QCA); km 9, La Independencia a Puerto Quito, 220 m, 7 Jul 1984, *Dodson* 14715 (QCNE). **Zamora-Chinchipe:** Road Loja-Zamora, ca km 29-31, 2-4 km west of Sabanilla, 1700-1750 m, 1 Jan 1979, *Luteyn* 6636 (QCA). **Cultivated (Ex hortus):** Origin: Costa Rica, 5000-7000 ft, 15 Aug 1960, *Lankester s.n.* (MO 87870). Origin: Ecuador, Los Rios, *Horich s.n.* (*Berkeley* 58758) (P).

RHIPSALIS OBLONGA. **Brazil. Rio de Janeiro:** *Gaudichaud* 914 (P). Parati, Fazenda Taquari, Córrego da Faz. Taquari, caminho beirando o córrego, 200-300 m, 13 Apr 1989, *Jacques* 109 (RB); Fazenda Santa Maria, estrada em direção à Praia do Sono, 27 Sep 1989, *Freitas* 119 (RB). Rio de Janeiro, Horto Florestal, subindo o Rio dos Macacos, córrego afluente esquerdo, 13 Sep 1994, *Marquete* 2007 (RB); Reserva Florestal da Vista Chinesa, perto da FEEMA, 425 m, 2 Jun 1995, *Scheinvar* 6236 (RB); Floresta da Tijuca, Estrada do Redentor, abaixo do Morro Bela Vista, 460 m, 6 Jun 1995, *Scheinvar* 6264 (RB). Teresópolis, Serra dos Orgãos, subsede of Parque Nacional, 600 m, 4 Aug 1966, *Hunt* 6512 (K, NY, RB); Parque Nacional da Serra dos Orgãos, river margin near subsede do Parque, 300-400 m, 21 Oct 1977, *Maas* 3397 (K, RB); 21 Oct 1979, *Pereira* 10827 (HB); ibidem, sub-sede, 800 m, 2 Apr 1989, *Scheinvar* 5571 (RB). **São Paulo:** Ubatuba, Trilha do Corisco, 9 Nov 1993, *Barros* 29471 (K). **Cultivated (Ex hortus).** 25 Jan 1970, s. col. E5160-0010 (C).

RHIPSALIS OLIVIFERA. **Brazil. Glaziou** 14860 (C, K). **Rio de Janeiro:** Magé, 7 Apr 1984, *Guedes* 733 (RB). Nova Friburgo, Macaé de Cima, Reserva Ecológica de Macaé de Cima, caminho para o sítio do David, 22 Jul 1994, *Freitas* 250 (RB); ibidem, Sítio Bacchus, 1473 m, 20 Nov 2005, *Calvente* 144 (SPF); ibidem, Rio das Flores, 1117 m, 21 Nov 2005, *Calvente* 151 (SPF). Organ Mountains, 1915, *Rose* 20818 (NY). Petrópolis, Serra da Estrela, 1883, *Glaziou* 14860 (P); Correa, Faz. Bonfim, 9 May 1989, *Klein* 701 (RB). Resende, Engenheiro Passos, Margem do Rio Sato, 3 Jun 1995, *Parra* 5 (K). Santa Maria Madalena, Santo Antônio do Imbé, estrada para o poço da Mirindiba; Rio Sossêgo, 18 Oct 1994, *Marquete* 2017 (RB). Silva Jardim, Arreia Mochila, caminho para a aldeia velha, 800 m, 18 Nov 2005, *Calvente* 178 (SPF). Teresópolis, Parque Nacional da Serra dos Órgãos, pr. Represa, 6 Aug 1961, *Pabst* 5667-A (HB); ibidem, caminho para Pedra do Sino, 1100-1450 m, 21 Oct 1977, *Martinelli* 3318 (RB). ibidem, 1450 m, 27 Apr 1977, *Martinelli* 1778 (RB); ibidem, caminho para campo das Antas, 1600-1800 m, 1 Feb 1983, *Martinelli* 9038 (K, RB), trilha para a Pedra do Sino, 24 Aug 2003, *Forzza* 2428 (RB); ibidem, 1000-2263 m, 20 Jul 2006, *Calvente* 225, 226, 227, 228, 229 (SPF). **São Paulo:** Bananal, Serra da Bocaina, 28 Sep 1994, *Catharino* 2039 (K, SPF). São José do Barreiro, Parque Nacional da Bocaina, Sítio do Sr. Sebastião Arantes, 18 Jul 1994, *Rossi* 1580 (K).

RHIPSALIS PACHYPTERA. **Brasil. Glaziou** 14862 (K). **Espírito Santo:** Afonso Claudio, Estrada do Garrafão, 3 pontões, Serra Pelada, 23 May 2007, *Kollmann* 9816 (MBML, SPF). Castelo, Parque Estadual do Forno Grande, 30 Oct 2004,

Kollmann 7206 (MBML, SPF); ibidem, 12 Jul 2005, *Kollmann* 7988 (MBML, SPF); Castelo, Mata das Flores, 16 Jul 2005, *Kollmann* 8111 (MBML, SPF); Próximo ao Parque Estadual Forno Grande, 1114-1178 m, 19 Jan 2007, *Calvente* 272, 277 (SPF). Domingos Martins, próximo a cidade de Campinho, 550 m, 27 Aug 1974, *Martinelli* 419 (RB). Santa Maria do Jequitibá, Caramuru, Sítio Jequitibá, *Kollmann* 6236 (MBML, SPF). Santa Teresa, Comunidade Pedra Alegre, Pedra do Cruzeiro, 900 m, 20 Jun 2000, *Kollmann* 3020 (MBML, SPF). **Paraná:** Antonina, Mangue Maior Santo, 20 m, 28 Apr 1983, *Hatschbach* 45275 (MBM); Reserva Biológica de Sapetanduva, 50 m, 8 Apr 1999, *Silva* 2911 (BHCB, C). Guaraguava, Serrinha, 13 Jun 1967, *Hatschbach* 16115 (MBM). Guaratuba, 26 Jul 1974, *Kruger* 13372 (SPF); Rio Quiririm, 1 Apr 1975, *Hatschbach* 31795 (MBM). Jacareí, in silvula ad truncos arborum, 13 Jun 1915, *Dusén* 17074 (NY, P). Jundiá do Sul, Fazenda Monte Verde, 6 Feb 2001, *Carneiro* 1058 (MBM). Morretes, Rio Cruzeiro, 12 Apr 1977, *Hatschbach* 39871 (MBM). Paranaguá, Ilha do Mel, Praia Grande, 5 May 1985, *Souza* 38 (MBM). Praia do Leste, 21 Mar 1973, *Leinig* 541 (HB). **Rio de Janeiro:** Angra dos Reis, Ilha Grande, cachoeira a oeste de Abraão, 50-100 m, 16 Apr 1992, *Zappi* 273 (SPF); trilha Abraão-Lopes Mendes, 41 m, 17 Jul 2006, *Calvente* 216 (SPF). Itatiaia, Parque Nacional do Itatiaia, trilha próxima à Cachoeira Véu da Noiva, 2000 m, 20 Apr 1991, *Zappi* 255 (HRCB). Magé, Serra da Estrela, estrada velha, 4 Apr 1989, *Marquete* 213 (RB). Mangaratiba, Reserva Rio das Pedras, 30-650 m, 14 Apr 2006, *Calvente* 203 (SPF); ibidem, margem do Rio, 30 m, 15 Apr 2006, *Calvente* 313 (SPF); ibidem, topo do Morro do Corisquinho, 450 m, 15 Apr 2006, *Calvente* 211 (SPF). Natividade, 10 km de Raposo na estrada de Natividade, 220 m, 27 Nov 2004, *Aona* 910 (RB); Estrada de dentro da mata entre a RJ-214 e o distrito de S. Gonçalo, 7 Jul 2004, *Pontes* 65 (RB). Nova Iguaçu, Reserva Biológica do Tinguá, 16 Sep 2006, *Calvente* 247, 250 (SPF). Paraty, Fazenda de Laranjeiras, 10 Jan 1974, *Martinelli* 554 (RB); Praia de Jabaquara, 25 Sep 1989, *Freitas* 80 (RB); Estrada para Paratimirim, 26 Sep 1989, *Freitas* 86 (RB); Fazenda Santa Maria, estrada em direção à Praia do Sono, 27 Sep 1989, *Freitas* 117, 118 (RB); Fazenda Taquari, 28 Sep 1989, *Freitas* 137, 157 (RB); Fazenda São Gonçalo, Barra do Girimim, Praia do Iririguaçu, 28 Sep 1989, *Freitas* 153 (RB); Paratimirim, 11 Apr 1989, *Jacques* 89 (RB); Loteamento Frade, 10 Nov 1991, *Marquete* 496 (RB); Subindo pelo lado esquerdo do rio Coriscão, 11 Nov 1991, *Marquete* 504 (RB); 18 km do trevo de Paraty até a entrada de Laranjeiras mais 9 km em direção à Fazenda, 30 Jun 1993, *Marquete* 1100 (RB); Entrada da Praia do Sono, mata ao lado esquerdo, 30 Jun 1993, *Reis* 47 (RB); Laranjeiras, estrada para Praia do Sono, 9 Aug 1994, *Marquete* 1989 (RB). Petropolis, Serra da Estrela, lado direito da estrada em direção a Petrópolis., 25 Oct 1988, *Marquete* 159 (RB); Alto do Imperador, 1883, *Glaziou* 14862 (C, P). Cairú, Jun 1943, *Goes* 633 (RB); Estrada Petrópolis-Rio, próximo à Saída de Petrópolis, 830 m, 29 Dec 2007, *Calvente* 378 (SPF). Rio de Janeiro, Recreio dos Bandeirantes, 12 May 1959, *Duarte* 4807 (RB); Jardim Botânico, 23 Jun 1961, *Pereira* 5731 (HB); Monte Corcovado, 400-500 m, 22 May 1969, *Sucre* 5075 (RB); Barra da Tijuca, 16 Feb 1973, *Sucre* 10026 (RB); P. N. Tijuca, Paineiras, 500 m, 3 Apr 1989,

Scheinvar 5575 (RB); Horto Florestal, caminho para Barris, 22 Jul 1992, *Marquete* 584 (RB); Horto Florestal, afloramento rochoso, 22 Jun 1993, *Marquete* 1038 (RB). São Pedro da Aldeia, Será de Sapiatiba, estrada para as antenas, 18 Jun 2004, *Marquete* 3473 (RB). Saquarema, APA-Massambaba, Reserva Ecológica de Jacarepiá, 12 Dec 1990, *Freitas* 212 (RB); ibidem, 24 Oct 1990, *Freitas* 185 (RB); ibidem, 16 Aug 1992, *Freitas* 238 (RB). Teresópolis, P.N. Serra dos Órgãos, between abrigos 1 e 2, 3 Aug 1966, *Hunt* 6485 (K, NY, RB); P.N. Serra dos Órgãos, sub-sede, 600 m, 4 Aug 1966, *Hunt* 6511 (K, NY, RB); Estrada Teresópolis - Friburgo, 800 m, 4 Apr 1970, *Sucre* 6498 (RB); P.N. Serra dos Órgãos, 10 Aug 1982, *Barros* 1025 (RB). Vassouras, Morro Azul, 9 Jun 1960, *Nagelschmidt* s.n. (HB 18987, MBM). **Santa Catarina:** Blumenau, Bom Retiro, Mata da Cia. Hering 250 m, 3 Jun 1960, *Klein* 2444 (HBR). ibidem, Mata da Cia. Hering, 300 m, 10 Mar 1960, *Klein* 2394 (HBR). Brusque, Azambuja, 35 m, 12 May 1949, *Reitz* 2993 (HBR, NY); Mato de Malucher, 40-50 m, 23 Feb 1952, *Smith* 5812 (RB). Florianópolis, Ilha de Santa Catarina, morro Costa da Lagoa, 19 Apr 1967, *Klein* 7367 (MBM). Governador Colan Ramos, Vargem do Macário, 5 m, 20 Mar 1972, *Brosolin* 524 (MBM). Ibirama, 100 m, 21 Sep 1956, *Reitz* 3757 (HBR). Ilhota, Parque Botânico do Morro do Baú, 620 m, 18 Nov 1980, *Scheinvar* 2973 (HBR). Itajaí, Morro da Fazenda, 70 m, 14 May 1954, *Reitz* 1847 (HBR, NY); Morro da Ressacada, 150 m, 6 May 1955, *Klein* 1351 (HBR); Cunhas, 10 m, 12 May 1955, *Klein* 1372 (HBR, NY). Luis Alves, Braço Joaquim, 450 m, 22 Mar 1965, *Reitz* 2888 (K, NY). Palhoça, Pilões, 50-700 m, 14 Mar 1952, *Smith* 6223 (RB); Pilões, 300 m, 23 Jul 1956, *Reitz* 2818 (HBR, NY). Paulo Lopes, Bom Retiro, 250 m, 27 Jun 1973, *Brosolin* 770 (MBM). **São Paulo:** Cananéia, 24 Feb 1983, *Pirani* 538 (SPF). Cunha, Parque Estadual da Serra do Mar, ao longo do Rio Paraibuna, 31 Mar 1994, *Baitelo* 639 (K). Juréia, 10 Oct 1988, *Simão-Bianchini* 46 (SPF). Pariquera-Açu, Estação experimental do IAC, 28 Apr 1996, *Ivanauskas* 792 (BHCB, HRCB, K). Sete Barras, Parque Estadual Intervales, base Saibadela, May 2002, *Guilherme* 314 (HRCB). Ubatuba, Picinguaba, trilha para o morro da morte e mangue do Rio da Fazenda, 4 Jun 1988, *Ribeiro* 314 (HRCB, SPF); Picinguaba, trilha do Morro do Corsário, 17 Jun 1989, *Ribeiro* 635 (HRCB); Estrada para a Vila de Picinguaba, 12 Jun 1991, *Taylor* 1642 (HRCB, K); Trilha do Camburi, km 01 da Rodovia Rio-Santos, 14 Apr 1994, *Furlan* 1398 (HRCB, K); PESM - Núcleo Picinguaba, trilha próxima ao estacionamento de visitantes, 14 m, 5 May 2007, *Calvente* 339, 346, 346b (SPF); caminho para a casa de farinha, 14 m, 8 Jun 2007, *Calvente* 354 (SPF); trilha da Restinga em direção ao Mangue, 9 m, 8 Jun 2007, *Calvente* 336b (SPF). Rodovia BR-101, 38 Km SW de São Sebastião, 2-3 KM NE de Maresias., 14 Jun 1991, *Taylor* 1649 (HRCB, K). **Cultivated (ex hortus):** Origin: Brasil, São Paulo, Jacareí, 7 May 1954, *Hoene* 15349 (SPF). Origin: Brazil, Rio Grande do Sul, Agudo, 10 Jun 2003, *Horst* 821 (K holotype of *R. agudoensis*). Origin: Brazil, *Berger* s.n. (*Berkeley Hortus* 54088) (P).

RHIPSALIS RUSSELLII. **Brazil. Bahia:** Entre Itabuna e Ubaitaba, Fazenda Almada, 12 Dec 1967, *Castellanos* 26951 (HB). Jaguaquara, 821 m, 6 Feb 2007, *Calvente* 314 (SPF). Lençóis, Morro da Chapadinha, 1050 m, 23 Nov 1994, *Melo* 1283 (K, SPF). Mucugê, 0.5 km da cidade, nas proximidades do

cemitério, 1000 m, 22 Dec 1988, *Harley* 25600 (K, SPF). Santa Teresinha, Serra da Jibóia, 23 Nov 2001, *Andrade* 35 (HUEFS); ibidem, ca. 820 m, 6 Feb 2007, *Calvente* 309 (SPF). **Espírito Santo:** Linhares, Reserva Florestal de Linhares, 20 Sep 1994, *Folli* 2375 (RB). **Minas Gerais:** Alvorada de Minas, Estrada para Serro, 12 Dec 2005, *Calvente* 184 (SPF). Conceição do Mato Dentro, Estrada Conceição do Mato Dentro-Morro do Pilar, 20 Nov 1989, *Zappi* 195 (HRCB, SPF); Parque Natural Municipal do Ribeirão do Campo, 1 Jul 2003, *Mota* 2094 (BHCB); Vila de Tabuleiro, 662 m, 12 Dec 2005, *Calvente* 183 (SPF). Descoberto, Reserva Biológica da Represa do Grama, 26 Jan 2002, *Forzza* 2057 (RB). Poté, Fazenda do Sr. Júlio Tavares, 435 m, 21 Jul 2004, *Lombardi* 6094 (BHCB). Santa Maria do Salto, Fazenda Duas Barras, 810 m, 25 Jul 2003, *Lombardi* 5559 (BHCB, HUEFS, SPF); Próximo à divisa com a Bahia, Fazenda Duas Barras, 850 m, 10 Feb 2007, *Calvente* 326, 327 (SPF). Serro, Milho Verde, 24 Jul 2002, *Lombardi* 1678 (BHCB); ibidem, 2 Sep 2002, *Mota* 1679 (BHCB).

RHIPSALIS TRIANGULARIS. **Brazil. Rio de Janeiro:** Rio de Janeiro, Parque Natural Municipal da Prainha, 6 Jun 1996, *Braga* 3357 (RUSU); 17 Apr 2003, *Calvente* 38 (RUSU); 6 Jun 2003, *Calvente et al.* 42 (RUSU); 12 Dec 2003, *Cardoso* 51 (RB); 15 Mai 2004, *Calvente* 87, 89, 90 (RUSU); 10 Oct 2004, *Calvente* 107a, 112 (RUSU).

RESUMO

Rhipsalis inclui 37 espécies e representa o maior gênero de Cactaceas epífitas. Grande parte das espécies de *Rhipsalis* são endêmicas do Brasil (81%), e apenas três espécies são amplamente distribuídas pela América Tropical: *Rhipsalis micrantha* (Kunth) DC. (Peru, Colômbia, Venezuela e Costa Rica), *Rhipsalis floccosa* Salm-Dyck ex Pfeiff. (Paraguai até a Venezuela) e *Rhipsalis baccifera* (em toda a América do Sul, desde a Argentina e Uruguai até as partes úmidas do México e Caribe, com limite norte no estado da Flórida, EUA). *Rhipsalis baccifera* é a única espécie a extrapolar a distribuição americana de Cactaceae, ocorrendo amplamente em áreas tropicais úmidas do continente Africano e parte do continente Asiático. Apesar de estudos recentes terem abordado a taxonomia do gênero, a identificação de táxons de *Rhipsalis* permanece problemática, principalmente em razão da plasticidade natural de caracteres morfológicos, falta de informação sobre os padrões de variação morfológica em populações naturais, existência de complexos de espécies e espécies crípticas. Além disso, pouco se conhece sobre a biologia e evolução desse grupo. Este trabalho visa aprofundar o conhecimento da tribo Rhipsalideae e do gênero *Rhipsalis*, os quais incluem plantas epífitas extremamente abundantes e diversas na região Neotropical. A área de estudo do presente trabalho compreende a Floresta Atlântica, uma área biologicamente muito importante, mas extremamente ameaçada. A elevada diversidade de espécies e alta taxa de endemismo de representantes da tribo Rhipsalideae na Mata Atlântica tornam este grupo particularmente interessante para um melhor entendimento dos mecanismos associados à diversificação de espécies na Mata Atlântica. Os objetivos do presente estudo são: (1) Reconstruir a filogenia da tribo Rhipsalideae com base em caracteres moleculares visando o teste do monofiletismo dos gêneros da tribo; (2) Reconstruir a filogenia do gênero *Rhipsalis* com base em caracteres moleculares visando o teste do monofiletismo dos seus subgêneros, o estudo da evolução de caracteres morfológicos, o estudo da ocupação dos habitats epifítico e rupícola, e o estudo da biogeografia do grupo; (3) Elaborar uma nova classificação para o grupo com base em dados morfológicos e moleculares; e, (4) Revisar o "clado caule-alado" pertencente ao gênero *Rhipsalis* (= *Rhipsalis* subg. *Phyllarthrorhipsalis*). Estes quatro objetivos correspondem aos objetivos dos quatro capítulos desta tese. No primeiro capítulo, é apresentada a filogenia molecular da tribo Rhipsalideae reconstruída com base nos marcadores moleculares *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH* e ITS. Com base nessa filogenia e num estudo da evolução de caracteres morfológicos, são propostas mudanças taxonômicas para os gêneros *Hatiora* e *Schlumbergera*. No segundo capítulo, é apresentada a filogenia do gênero *Rhipsalis* com base nos marcadores moleculares *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*, ITS e MS. Esta filogenia é então utilizada como base para inferir o padrão de evolução de características morfológicas e do hábito das espécies de *Rhipsalis*, bem como estudar a história biogeográfica do gênero. No terceiro capítulo, uma nova classificação infragenérica para *Rhipsalis* é proposta com base na filogenia do gênero (capítulo 2) e informações morfológicas. Por fim, no quarto capítulo é apresentada a revisão taxonômica do clado "caule-alado", incluindo descrições, informações sobre a distribuição geográfica das espécies, novas circunscrições para diversos táxons e uma chave para a identificação das espécies do subgênero *Rhipsalis*.

ABSTRACT

Rhipsalis includes 37 species and represents the largest genus of epiphytic cacti. Species of *Rhipsalis* are mainly endemic to Brazil (81%), and only three species are widely distributed throughout the Neotropics: *Rhipsalis micrantha* (Peru, Colombia, Venezuela and Costa Rica), *Rhipsalis floccosa* (Paraguay to Venezuela), and *Rhipsalis baccifera* (throughout South America, México, Caribbean region and Florida, USA). *Rhipsalis baccifera* is the only species whose distribution extends beyond the Americas, also occurring in tropical areas of the African continent and part of Asia. Despite recent taxonomic studies in the genus, the identification of taxa within *Rhipsalis* has remained problematic, mainly due to the natural plasticity of morphological characters, lack of information on the patterns of morphological variation in natural populations, existence of species complexes and cryptic species. In addition, little is known about the biology and evolution of this group. This study focuses on the tribe Rhipsalideae and on the genus *Rhipsalis*, very abundant and diverse groups of epiphytes in the Neotropics. The study area comprises the Atlantic Forest, which is an extremely important area biologically but also a highly threatened ecosystem. The high diversity of species and high endemism of Rhipsalideae in the Atlantic Forest makes this group particularly interesting for the study of the processes involved in the diversification of species within Mata Atlântica. The objectives of the present project are to: (1) Reconstruct the molecular phylogeny of the tribe Rhipsalideae and test the monophyly of genera within this tribe; (2) Reconstruct the molecular phylogeny of *Rhipsalis* in order to test the monophyly of its subgenera, study the evolution of morphological features, study the occupation of the rupicolous and epiphytic habitats, as well as the biogeographical history of the group; (3) Propose a new classification for the group based on novel molecular and morphological data; and, (4) Revise the "winged-stem" clade (= *Rhipsalis* subg. *Phyllarthrorhipsalis*). These four objectives correspond to the four chapters of the present thesis. The first chapter includes a molecular phylogeny of Rhipsalideae based on the molecular markers *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH* and ITS. Information derived from this phylogeny and from a study on the evolution of selected morphological characters, is then used as basis to propose taxonomic changes in *Hattiora* and *Schlumbergera*. The second chapter presents a phylogeny of *Rhipsalis* based on the molecular markers *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH*, ITS and MS. This phylogeny is then used as basis to study the evolution of selected morphological traits, study the evolution of the habit, and the biogeographical history of the genus. In the third chapter, a new infrageneric classification for *Rhipsalis* is proposed based on the phylogeny of *Rhipsalis* (chapter 2) and novel morphological information. Lastly, the fourth chapter presents a taxonomic revision of the "winged-stem" clade, including descriptions, information on the geographic distribution of species, novel circumscriptions for several taxa, and an identification key for all species of the subgenus *Rhipsalis*.