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FILOGENIA MOLECULAR, BIOGEOGRAFIA E ASPECTOS EVOLUTIVOS DE *PILOSOCEREUS* (CACTACEAE)

PÂMELA LAVOR ROLIM

Tese de Doutorado
Natal/RN, março de 2017

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Tese apresentada à coordenação do Programa de Pós-Graduação em Sistemática e Evolução da Universidade Federal do Rio Grande do Norte, como parte dos requisitos para obtenção do título de Doutor em Sistemática e Evolução.

Orientador (a): Profª Dra. Alice Calvente
Co-orientador: Profº Dr. Leonardo M. Versieux.

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"O meu mundo não é como o dos outros, quero demais, exijo demais, há em mim uma sede de infinito; sou antes uma exaltada, com uma alma intensa, violenta, atormentada, uma alma que não se sente bem onde está, que tem saudades...sei lá de quê! "

(Florbela Espanca)

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Filogenia molecular, bioeografia e aspectos evolutivos de *Pilosocereus* (Cactaceae)

Resumo- O gênero *Pilosocereus* pertence à Cactaceae (subfamília Cactoideae) e é um dos maiores e mais bem distribuídos gêneros dentro da tribo Cereeae. Com 42 espécies, divididas nos subgêneros *Pilosocereus* e *Gounellea*, o gênero é distribuído de forma disjunta na região neotropical, ocorrendo nos mais diferentes tipos de hábitats, sempre associados a ambientes xéricos, sendo o leste do Brasil o seu maior centro de diversidade. Em razão de suas características morfológicas, as quais lhe conferem grande adaptabilidade a ambientes xéricos (assim como as demais Cactáceas), as espécies de *Pilosocereus* são frequentemente dominantes e assumem um importante papel ecológico e etnobotânico onde ocorrem. No entanto, poucos tem sido os trabalhos até o momento que tiveram como foco o gênero como um todo. Assim, esta tese teve como objetivo investigar o relacionamento filogenético, a biogeografia e aspectos evolutivos do gênero *Pilosocereus.* No capítulo 1 investigou-se os processos de diversificação de *Pilosocereus* nos hábitats áridos da região Neotropical, a partir do relacionamento filogenético, do tempo de divergência de clados e da reconstrução de áreas ancestrais. Concluiu-se que o gênero não é monofilético, e o principal clado (*Pilosocereus sensu stricto*) diversificou-se muito recentemente (principalmente no Pleistoceno tardio), com origem na Caatinga e eventos de migração para outros ambientes xéricos da América do Norte/Central, Noroeste da América do Sul e Caribe. No capítulo 2, reconstruiu-se o relacionamento filogenético do gênero através de análise bayesiana e máxima parcimônia, com uma ampliação da amostragem de táxons e regiões genômicas em relação a trabalhos previamente publicados. Comprovou-se a não monofilia do grupo, bem como de quatro espécies heterotípicas, o que permitiu propor mudanças taxonômicas, incluindo a elevação de categoria de três espécies; três novos sinônimos; um novo nome no ranking de espécie e um novo gênero, *Xiquexique* (composto pelas espécies formalmente posicionadas em *P. subg. Gounellea*), passando então *Pilosocereus* a uma nova circunscrição, com 42 espécies e quatro subespécies. Por fim, no capítulo 3 apresenta-se o padrão de distribuição, riqueza, endemismo e atual situação de conservação de todas as espécies do grupo, onde foi encontrado que alguns táxons apresentam restrições ao tipo de vegetação, mas a maioria se mostra amplamente distribuída em diferentes gradientes ambientais. A maior riqueza de espécies e diversidade filogenética são encontradas nos estados da Bahia e Minas Gerais, com áreas de endemismo sendo apontadas para o leste do Brasil e México. Assim, esta tese teve uma abordagem multidisciplinar a fim de elucidar diferentes aspectos da biologia deste grande e diverso grupo, que até o momento permaneciam desconhecidos.

Palavras-chave: Biogeografia; Conservação; Distribuição; Endemismo; Evolução; Filogenia; *Pilosocereus*.

Molecular phylogeny, biogeography and evolutionary aspects of *Pilosocereus* (Cactaceae)

Abstract- The genus *Pilosocereus* belongs to Cactaceae (subfamily Cactoideae) and is one of the largest and most widely distributed genera within the tribe Cereeae. With 42 species divided in two subgenera, *Pilosocereus* and *Gounellea* the group is disjunctly distributed in the Neotropics and centered in eastern Brazil, occurring in many different types of habitats associated with xeric environments. Due to its morphological features, providing great adaptability to xeric environments (as all Cacti), *Pilosocereus* species are often dominant and play an important ecological and ethnobotanical role where they occur. However, there were few studies so far that focused on the genera as a whole. Thus, this thesis aimed to investigate the phylogenetic relationship, biogeography and evolutionary aspects of the genus *Pilosocereus*. The first Chapter focused in investigating *Pilosocereus* diversification processes in arid habitats in the Neotropics, using phylogenetic relationships, clades divergence times and reconstruction of ancestral areas. The conclusion was that the genus is not monophyletic and that the main clade (*Pilosocereus sensu stricto*) diversified very recently (mainly in Late Pleistocene), with origin in the Caatinga and posterior migration events to other xeric environments in North/Central America, Northwest of South America and Caribbe. In Chapter 2, the phylogenetic relationships in the genus were reconstructed through Bayesian and Maximum Parsimony analyses, with expanded taxon sampling and genomic regions in comparison with previously published works. Again, the genus was found as non-monophyletic, as well as four heterotypic species, what led to the proposition of taxonomic changes, including the resurrection of three species, three new synonyms, a new name in the species rank and a new name in the generic rank, *Xiquexique* (composed of the species formally positioned in *P. subg. Gounellea*), while *Pilosocereus* was newly circumscrybed with 42 species and four subspecies. Finally, Chapter 3 presents the distribution patterns, richness, endemism and current conservation scenario for all species of the group. Some taxa are restricted to vegetation types, but most of them are widely distributed in different environmental gradients. The greatest species richness and phylogenetic diversity are found in the states of Bahia and Minas Gerais, with areas of endemism in Eastern Brazil and Mexico. Thus, this thesis had a multidisciplinary approach in order to elucidate different aspects of the biology of this large and diverse group, which remained unknown until now.

Keywords: Biogeography; Distribution; Conservation; Endemism; Evolution; Phylogeny; *Pilosocereus*.

SUMÁRIO

1. INTRODUÇÃO GERAL

1.1 A FAMÍLIA CACTACEAE

As Cactáceas constituem um dos grupos de angiospermas mais diferenciados e bem adaptados a aridez no reino vegetal (Nyffeler, 2002). Algumas das características intrínsecas deste grupo são a presença marcante de extrema suculência no corpo vegetal, uma grande diversidade de formas de crescimento e hábitos e a ocorrência conspícua em regiões xéricas do Novo Mundo (Hernández-Hernández *et al.,* 2011). Outras características marcantes da família são os caules fotossintetizantes; a fotossíntese do tipo CAM; o sistema radicular especializado, com alta capacidade de absorção de água (Edwards & Diaz, 2006); as folhas reduzidas ou ausentes; as aréolas (regiões meristemáticas que podem dar origem a diversas estruturas, como ramos, cerdas, flores e frutos) que, em geral, apresentam uma grande quantidade de espinhos e tricomas; e flores com ovário ínfero receptacular imerso em tecido caulinar (Gibson & Nobel, 1986; Nyffeler, 2002) (Fig. 1).

Tomando como base todas essas marcadas diferenciações morfológicas (vegetativas e reprodutivas), muitos sistematas classificaram os cactos em uma ordem própria, Cactales, julgando-os demasiadamente distintos das demais angiospermas (Nyffeler, 2002). Entretanto, com os avanços em estudos multidisciplinares (integrando morfologia, embriologia, química e biologia molecular, por exemplo), características compartilhadas entre os cactos e outros grupos de plantas suculentas (como Basellaceae, Didiereaceae, Halophytaceae e Portulacaceae) foram descobertas (como a presença de ovário muitas vezes unilocular, pólen com superfície espinhosa e pigmentos químicos do tipo betalaína) (APG IV, 2016). Atualmente, todos os cactos estão inseridos na família Cactaceae, a qual é posicionada dentro da ordem Caryophyllales junto com mais 39 famílias de Eudicotiledôneas (APG IV, 2016).

Cactaceae apresenta atualmente 130 gêneros e 1.850 espécies divididas em quatro subfamílias: Cactoideae, Opuntioideae, Pereskioideae e Maihuenioideae (Nyffeler & Eggli, 2010). Cactoideae é a maior das subfamílias, com seis tribos (Cereeae com 41 gêneros; Phyllocacteae com 32; Cacteae com 26; Notocacteae com cinco; Rhipsalideae com quatro e Blossfeldieae que é monogenerica); Opuntioideae é a segunda maior das subfamílias com duas tribos (Opuntieae e Cylindropuntieae, com cinco e nove gêneros, respectivamente); e as outras duas subfamílias Pereskioideae e Maihuenioideae são monogenéricas (Nyffeler & Eggli, 2010).

Figura 1. Diferença de hábitos (a – árboreo; b – arbustivo); formas (a,b – colunar; c – globoso); tamanhos e características típicas em Cactaceae, como presença de caules suculentos fotosintetizantes e areolas (setas em "d") que podem dar origem a novos ramos (e); flores (f) e frutos (g).

A subfamília Cactoideae é considerada monofilética com base em vários caracteres morfológicos e moleculares (Nyffeler, 2002; Nyffeler & Eggli, 2010). Ela inclui o maior número de espécies de Cactaceae dentre as quatro subfamílias, contendo cerca de 1.530 espécies (Nyffeler & Eggli, 2010), as quais perfazem cerca de 83% do total de espécies da família. Devido a toda essa riqueza é que por muito tempo grande parte das discussões taxonômicas, principalmente envolvendo a circunscrição tribal e genérica de Cactaceae, estiveram focadas dentro dessa subfamília (Applequist & Wallace, 2002; Nyffeler & Eggli, 2010).

Dentro de Cactoideae, a tribo Cereeae é a mais representativa no Brasil, apresentando uma grande variedade de formas de crescimento, desde indivíduos solitários de pequeno porte (como as espécies do gênero *Rebutia* K.Schum.) a formas gigantescas arborescentes (como a maioria das espécies de *Browningia* Britton & Rose) (Nyffeler & Eggli, 2010). Cereeae é uma tribo que poderia ser reconhecida como quase que exclusivamente Sul-Americana se não fosse a presença dos gêneros *Harrisia* Britton, *Melocactus* Link & Otto e *Pilosocereus* Byles & Rowley, que são amplamente distribuidos nas Américas (Nyffeler & Eggli, 2010).

1.2 O GÊNERO *PILOSOCEREUS*

Pilosocereus é um dos maiores gêneros dentro da tribo Cereeae (Hunt *et al.,* 2006), sendo atualmente aceitas 42 espécies e 8 subespécies (Hunt *et al.,* 2006; Zappi & Taylor, 2011), subdivididas nos subgêneros *Gounellea* (com 3 espécies e uma subespécie heterotípica) e *Pilosocereus* (com 39 espécies e 7 subespécies) (Hunt *et al.,* 2006).

O gênero é composto por cactos colunares, com uma grande variedade morfológica, principalmente em relação ao tamanho (algumas espécies, como *P. machrisii* podem apresentar tamanho de 30 cm, enquanto que espécies de maior porte, como *P. pachycladus* podem apresentar até 10 m de altura); hábito (podem ter desde arbustivo a arborescente); formas de crescimento (ereto na maioria das espécies ou escandente, como em *P. pentaedrophorus*); ramificação (variando de não ramificado a densamente ramificado acima ou a nível da base); e no número e coloração de espinhos, costelas e cor da epiderme (Zappi, 1994) (Fig. 2).

Figura 2. Diversidade de hábitos (a,i – arbóreo; b-h – arbustivo), tamanhos e ramificação (a,i – densamente ramificado acima da base com tronco bem definido; b,g,h – ramificado acima do nível do solo; d,f – não ramificado; c,e – ramificação candelabriforme) em *Pilosocereus*. (a – *P. pachycladus*; b – *P. pusillibaccatus*; c – *P. gounellei*; d – *P. pentaedrophorus*; e – *P. tuberculatus*; f – *P. flavipulvinatus*; g – *P. oligolepis*; h – *P. piauhyensis*; i – *P. catingicola*) (Barras: a,i = 100 cm; b,d-e,h = 50 cm; c,f-g = 20 cm).

As principais características do gênero são a presença de tricomas nas aréolas jovens (reprodutivas ou não) podendo ser glabrescentes quando mais maduras (com exceção de *P. tuberculatus* que não apresenta tricomas em nenhuma fase da vida); cefálio ausente ou presente apenas um pseudocefálio em algumas espécies (como em *P. densiareolatus* e *P. chrysostele*); flores que variam de tubular a infundibuliforme, geralmente noturnas com características de polinização quiróptera; e fruto deiscente por uma fenda transversal, depresso-globoso, com remanescente floral persistente (ereto ou pendente) e com polpa funicular branca ou colorida (Zappi, 1994) (Fig. 3).

Atualmente existe uma única e completa revisão taxonômica publicada do gênero (Zappi, 1994), na qual as espécies brasileiras foram revisadas. Neste trabalho novas combinações e sinonímias foram feitas e foi proposta a divisão de *Pilosocereus* em dois subgêneros: (1) *P.* subg. *Gounellea*, reconhecido pela ramificação candelabriforme (quando novos ramos crescem primeiro horizontalmente ao solo e depois verticalmente); costelas sinuosas com característicos podarios abaixo das aréolas e remanescente floral ereto a pendente, não imerso no ápice do fruto (formando uma inserção circular); e (2) *P.* subg. *Pilosocereus*, caracterizado pela ramificação ereta (crescimento sempre vertical); costelas retas e remanescente floral pendente, imerso no ápice do fruto (formando uma inserção linear) (Zappi, 1994). Além disso, Zappi (1994) também propõe o agrupamento das espécies de *Pilosocereus* subgênero *Pilosocereus* em cinco grupos informais, com base na morfologia floral, espinação e hábito das espécies (Tabela 1).

Essa subdivisão em grupos informais dentro de *Pilosocereus* subgênero *Pilosocereus* foi seguida em trabalhos importantes que incluíram as espécies do gênero, como "*Cacti of Eastern Brazil*" (Taylor & Zappi, 2004) e "*The new cactus lexicon*" (Hunt *et al.,* 2006), sendo complementada neste último, com a substituição da nomenclatura de Grupo P. ULEI por P. LEUCOCEPHALUS (que passa a incluir todas as espécies não brasileiras juntamente com as espécies brasileiras previamente reunidas nesse grupo). Esses agrupamentos, em alguns casos, reuniram espécies que formam complexos (apresentam grande variedade morfológica, sobreposição de caracteres diagnósticos e, portanto, incerteza taxonômica) dentro do gênero, os quais foram foco de alguns trabalhos voltados ao entendimento dos mesmos (Jesus, 2010; Moraes *et al.,* 2012; Bonatelli *et al.,* 2013, 2014 e 2015; Perez *et al.,* 2016; Menezes *et al.,* 2016).

Figura 3. Principais características no gênero *Pilosocereus*. (a) Botão floral de *P. pachycladus* subsp. *pernambucoensis*; (b) flor de *P. gounellei*; (c) fruto com remanescente floral ereto em *P. tuberculatus*; (d) fruto com remanescente floral pendente em *P. pentaedrophorus*; (e) fruto com deiscência em fenda transversal em *P. gounellei*; e (f) aréolas apicais com tricomas em *P. oligolepis*. (*Pilosocereus* subg. *Pilosocereus*: a, d, f; *Pilosocereus* subg. *Gounellea*: b-c, e).

Tabela 1. Agrupamentos informais propostos por Zappi (1994) dentro do gênero *Pilosocereus* subgênero *Pilosocereus*.

Grupo	Características	Espécies
P. ARRABIDAE	Espécies arbóreas, com flores amplas e grandes frutos; pequeno número de costelas (3-6); ocorrem em vegetação florestal ou dunas de areia; amplamente distribuídas	*P. arrabidae* (Lem.) Byles & G. D. Rowley *P. catingicola* (Gürke) Byles & G. D. Rowley *P. catingicola* subsp. salvadorensis (Werderm.) Zappi
P. PENTAEDROPHORUS	Espécies arbóreas ou semi-escandentes, botão floral obtuso e estreito e flores curvadas; algumas apresentam de 3-4 costelas quando plântulas; ocorrem em vegetação florestal	*P. albisummus* P. J. Braun & Esteves *P. brasiliensis* (Britton & Rose) Backeb. *P. brasiliensis* subsp. *ruschianus* (Buining & Brederoo) Zappi *P. flavipulvinatus* (Buining & Brederoo) F. Ritter *P. flexibilispinus* P. J. Braun & Esteves *P. floccosus* (Backeb. & Voll) Byles & G. D. Rowley *P. floccosus* subsp. *quadricostatus* (F. Ritter) Zappi *P. glaucochrous* (Werderm.) Blyles & G. D. Rowley *P. oligolepis* (Vaupel) Byles & G. D. Rowley *P. pentaedrophorus* (Labour.) Byles & G. D. Rowley *P. pentaedrophorus* subsp. *robustus* Zappi
P. ULEI	Espécies com grande quantidade de cera na epiderme; pequeno número de costelas (ca. 6); cilindro vascular lignificado; ocorrem geralmente em habitats abertos, em geral associadas a formações rochosas na Caatinga e campos rupestres	*P. fulvilanatus* (Buining & Brederoo) F. Ritter *P. fulvilanatus* subsp. *rosae* (P. J. Braun) Zappi *P. magnificus* (Buining & Brederoo) F. Ritter *P. pachycladus* F. Ritter *P. pachycladus* subsp. *pernambucoensis* (F. Ritter) Zappi *P. ulei* (K. Schum.) Byles & G. D. Rowley
P. AURISETUS	Espécies arbustivas; com ramificação apenas a nível do solo; aréolas reprodutivas diferenciadas; cerca de 9 ou mais costelas; flores rosas ou vermelhas e algumas vezes fruto com polpa branca; distribuição restrita ou disjunta no Cerrado e campos rupestres	*P. aureispinus* (Buining & Brederoo) F. Ritter *P. aurisetus* (Werderm.) Byles & G. D. Rowley *P. aurisetus* subsp. *aurilanatus* (F. Ritter) Zappi *P. machrisii* (E. Y. Dawson) Backeb. *P. vilaboensis* (Diers & Esteves) P. J. Braun
P. PIAUHYENSIS	Espécies com grande número de costelas; espinhos de coloração amarelada; algumas espécies formam um pseudo-cefálio; encontradas em afloramentos rochosos na Caatinga e formações calcárias em Goiás	*P. chrysostele* (Vaupel) Byles & G. D. Rowley *P. densiareolatus* F. Ritter *P. diersianus* (Esteves) P. J. Braun *P. multicostatus* F. Ritter *P. piauhyensis* (Gürke) Byles & G. D. Rowley.

No entanto, a grande maioria dos trabalhos envolvendo *Pilosocereus* são provenientes de pesquisas com espécies isoladas e tiveram como foco o estudo descritivo da sua morfologia (Godofredo, 2009; Menezes & Loiola, 2015), biologia reprodutiva (Locatelli *et al.*, 1997; Rivera-Marchand & Ackerman, 2006; Lucena, 2007; Rocha *et al.*, 2007a,b; Meiado *et al.*, 2008; Munguia-Rosas *et al.*, 2009; Abud *et al.*, 2010; Martins *et al.*, 2012) ou genética de populações (Nassar et al., 2003; Moraes *et al.*, 2005; Figueredo *et al.*, 2010; Kattab *et al.*, 2014; Monteiro *et al.*, 2015).

1.3 ESTUDOS MOLECULARES

O histórico de estudos de sistemática molecular dentro da família Cactaceae inicia à partir da década de 90, quando diversos estudos com diferentes técnicas e regiões moleculares passaram a ser empregados e publicados nos grandes grupos dentro da família (como as subfamílias e tribos) (por exemplo: Wallace & Cota, 1996; Nyffeler, 2002; Wallace & Dickie, 2002; Applequist & Wallace, 2002; Crozier, 2005; Edwards *et al*., 2005; Butterworth & Wallace, 2005; Butterworth, 2006; Griffith & Porter, 2009; Ocampo & Columbus, 2010; Arakaki *et al*., 2011; Barcenas *et al.*, 2011; Calvente *et al*., 2011a; Hernández-Hernández *et al*., 2011; Korotkova *et al*., 2011; Majure *et al*., 2012; Ritz et al., 2012; Hernández-Hernández *et al*., 2014).

Entretanto, devido à grande diversidade e riqueza dentro de grandes grupos de Cactaceae, alguns grupos ainda apresentam relacionamentos mal compreendidos, o que tem gerado dificuldades de desenvolvimento de trabalhos mais aplicados, como de biogeografia e evolução. Um exemplo é a tribo Cereeae, que em grandes filogenias da família, como a de Nyffeler (2002), aparece como grupo irmão de outras duas tribos (Browningieae e Trichocereeae) formando o que seria o clado BCT, agrupando grande parte dos cactos colunares e globosos sul-americanos (cerca de 30 gêneros e 400 espécies) (Nyffeler, 2002). No entanto, em trabalhos posteriores (p.e. Applequist & Wallace, 2002) a monofilia deste grande clado é questionada, e Nyffeler e Eggli (2010) propõem então a mudança de circunscrição, onde todo o clado BCT seria agora delimitado como a tribo Cereeae, e esta seria subdividida nas subtribos Rebutiinae, Cereinae e Trichocereinae.

Isto demonstra o grande desafio do estudo de grupos dentro da família Cactaceae pois, mesmo com estudos mais globais, muitos relacionamentos principalmente em níveis infra e supragenéricos permanecem ainda não resolvidos. Filogenias muito amplas e com grande densidade de amostragem, tem revelado nós mais inclusivos e terminais relativamente mal suportados e controversos, apesar de apresentarem relacionamentos consistentes em níveis intermediários (Barcenas *et al*., 2011). Estudos que visam elucidar o relacionamento a nível genérico e específico dentro de Cactaceae ainda requerem uma grande demanda de pesquisa, pois muitos destes permanecem sem ter sido estudados com ferramentas moleculares ou ainda necessitam de estudos mais aprofundados (Barcenas *et al*., 2011). Muitos destes gêneros abrigam um grande número de espécies com grande similaridade morfológica que pode ser resultado de evolução convergente (processo considerado comum na família) (Hernández-Hernández *et al*., 2011).

Nesse aspecto, estudos filogenéticos dos gêneros *Lophocereus* Britton & Rose (Hartmann *et al*., 2002), *Mammillaria* Haw. (Butterworth & Wallace, 2004), *Pereskia* Mill. (Edwards *et al*., 2005; Butterworth & Edwards, 2008), *Peniocereus* (A. Berger) Britton & Rose (Arias *et al*., 2005), *Rebutia, Sulcorebutia* Backeb., *Weingartia* Werderm. (Ritz *et al*., 2007), *Pfeiffera* Salm-Dyck (Korotkova *et al*., 2010), *Rhipsalis* Gaertn. (Calvente *et al*., 2011b), *Gymnocalycium* Pfeiff. ex Mittler (Demaio *et al*., 2011), *Trichocereus* (A.Berger) Riccob. (Albesiano & Terrazas, 2012), *Echinopsis* Zucc. (Schlumpberger & Renner, 2012) e *Harrisia* (Frank *et al*., 2013) foram desenvolvidos nos últimos anos a fim de auxiliar no melhor entendimento dos relacionamentos dentro da família. No entanto, destes grupos, apenas *Harrisia* e *Rhipsalis* se mostraram monofiléticos na circunscrição em que foram testados, enquanto que os demais gêneros são para- ou polifiléticos e necessitam de estudos adicionais. Talvez por isso, Hunt *et al*. (2006) mencionam que a "grande batalha" de delimitação em Cactaceae ocorre a nível genérico, já que é neste ranking que ocorrem as maiores incertezas e instabilidades taxonômicas na família.

Diante deste tipo de problemática, nos grandes gêneros onde o número de espécies é considerável, estudos em nível infraespecífico podem ser de grande ajuda como fonte de informação para se entender alguns aspectos do grupo como um todo. Um exemplo disso são estudos voltados aos complexos de espécies dentro de *Pilosocereus*, como *P. machrisii* (Moraes *et al*., 2005; Perez *et al*., 2011) e o grupo P. AURISETUS (Jesus, 2010; Moraes *et al*., 2012; Bonatelli *et al*., 2013, 2014 e 2015; Perez *et al*., 2016) que ajudaram a entender que as espécies, apesar de possuírem populações pequenas e isoladas, apresentam uma alta variabilidade genética. (Bonatelli *et al*. (2014) chegaram à conclusão de que uma possível explicação da existência desses complexos, seria uma história filogeográfica complexa, marcada por um conjunto de diferentes fatores como, fragmentação de distribuição; isolamento reprodutivo (que levaria a diferenciação alopátrica) e possível contato secundário entre linhagens divergentes dentro do complexo (afetadas por questões ambientais, como os ciclos climáticos do Pleistoceno).

Em um estudo sobre o grupo P. ARRABIDAE, Menezes *et al*. (2016) concluiram que o grupo não forma um complexo e não pode ser considerado um clado real, já que não é monofilético. Além disso, estes autores também tentaram inferir os relacionamentos de todo o gênero (com adição de amostragem incompleta em outros taxa), mas seus resultados não foram satisfatórios e conclusivos.

Em termos de estudos tratando uma única espécie, os mesmos são ainda escassos no gênero, mas podem ser destacados os das espécies não brasileiras *P. lanuginosus* (Nassar *et al*., 2003) e *P. tillianus* (= *P. lanuginosus sensu* Hunt *et al.* (2006); Figueredo *et al*., 2010), onde foram encontrados uma alta diversidade genética e o destaque da importância da fauna como dispersores na demanda de fluxo gênico entre as populações destas espécies. Um outro estudo produzido na espécie brasileira *P. gounellei* (Monteiro *et al*., 2015) encontrou baixos índices de variabilidade genética e heterozigose, explicados pela influência da ação antrópica, que atualmente reduz o habitat de populações naturais e utiliza a espécie como complemento para alimentação humana e animal, cultivando-a através da propagação clonal. Dessa forma, estes estudos apresentam indícios de algumas das dificuldades encontradas em se estudar os relacionamentos entre as diferentes espécies de *Pilosocereus*, onde aspectos da história natural e evolutiva do gênero como um todo eram até o momento desconhecidos.

1.4 DISTRIBUIÇÃO E BIOGEOGRAFIA

Na região neotropical a família Cactaceae é a segunda maior, entre os grupos de plantas que são endêmicos ou quase restritos a esta região, perdendo apenas para a família Bromeliaceae (Taylor & Zappi, 2004). A família é considerada endêmica do Novo Mundo (com exceção de *Rhipsalis baccifera* (Mill.) Stearn, cuja distribuição chega até a África e Ásia), e ocorre amplamente distribuída, desde o Canadá até a Patagônia, em diversos tipos de habitats (Barthlott & Hunt, 1993). Estudos tem demonstrado que a origem de Cactaceae foi na América do Sul (na região central andina, a norte do Chile e noroeste da Argentina, Bolívia e Peru) à 35 milhões de anos (Ma) durante o Eoceno e que os eventos de diversificação dos grandes clados dentro da família são mais recentes (datam do Mioceno tardio, a cerca de 10-5 Ma) (Arakaki *et al*., 2011), onde várias linhagens passaram por processos independentes de expansão e dispersão para outras regiões das Américas (Hernández-Hernández *et al*., 2014).

Desta forma, Cactaceae possui três grandes centros de diversidade: o primeiro e mais significativo é no México e a sudoeste dos EUA; o segundo ocorre nos Andes, onde o Peru e a Bolívia são regiões especialmente ricas; e o terceiro é o leste do Brasil (que abrange a região nordeste e grande parte do sudeste brasileiro, excluindo o sul do Rio de Janeiro e o estado de São Paulo) (Taylor & Zappi, 2004; Zappi *et al*., 2011). Todos estes grandes centros de distribuição e diversidade da família estão associados a ambientes xéricos nas Américas.

Pilosocereus é um dos gêneros de Cactaceae com maior amplitude de distribuição geográfica, ocorrendo nos EUA (Florida), México, Ilhas do Caribe, Venezuela, Suriname, Guiana, Peru, Equador, Brasil e Paraguai (Taylor & Zappi, 2004). Suas espécies ocorrem predominantemente em ambientes xéricos, como os biomas Caatinga e Cerrado, no Brasil; em savanas e florestas tropicais sazonalmente secas (*sensu* Pennington *et al*., 2000) da América Central e do Sul; e em regiões desérticas da América do Norte e Central. Porém, também habitam regiões de formações florestais úmidas, como a Floresta Atlântica, no Brasil, e as Florestas Amazônica, Montana e de Pinheiros, na America Central e do Sul, mas, neste caso, sempre associadas a afloramentos rochosos ou cordões arenosos (Zappi, 1994; Taylor & Zappi, 2004). O principal centro diversidade e distribuição do gênero é no leste do Brasil, onde um grande número de espécies são encontradas (Zappi, 1994).

1.5 CONSERVAÇÃO DE *PILOSOCEREUS*

Cactaceae é uma das famílias de angiospermas que mais sofrem ameaça de extinção na atualidade. Isso se deve a uma gama de fatores, como por exemplo, restrição de distribuição (o que torna a perda de hábitat preocupante) e vulnerabilidade a perturbações antrópicas (por terem uma baixa taxa de crescimento individual e ciclo de vida longo). Além disso, as espécies são afetadas por coleta e comercio ilegais (que reduzem drasticamente suas populações) e por serem altamente adaptadas às regiões áridas, zonas em sua maioria ocupadas por comunidades rurais, nas quais o uso da terra é um dos únicos meios de sobrevivência (Godinez-Alvarez *et al*., 2003).

O gênero *Pilosocereus* não é uma exceção dentro da família. Sua ampla distribuição em diversos hábitats não o torna menos susceptível a ameaças, já que alguns desses ambientes estão em áreas consideradas hotspots nas Américas (como as ilhas do Caribe; Floresta de Pinheiro-Carvalho de Madrean; a região Mesoaméricana; os Andes Tropical; a Mata Atlântica e Cerrado brasileiros) e que são ameaçados ou vulneráveis à ação humana (Conservation International, 2016). Além disso, muitas das espécies do gênero estão classificadas atualmente dentro das principais categorias de ameaça de Lista Vermelha de espécies ameaçadas de extinção da IUCN (um total de 20 taxa) (IUCN, 2016). Muitas dessas espécies que estão classificadas nas categorias de "Criticamente ameaçada" (como *P. azulensis, P. diersianus, P. frewenii* e *P. fulvilanatus* subsp. *rosae*); "Ameaçada" (como *P. aurisetus* subsp. *aurilanatus, P. magnificus* e *P. multicostatus*)*;* e "Vulnerável" (como *P. aureipinus* e *P. parvus*), possuem uma distribuição muito restrita, chegando a serem consideradas micro endêmicas, e ocorrem em localidades que não são oficialmente protegidas (IUCN, 2016).

Outro fator a ser destacado são as espécies que ainda estão listadas na categoria de "Dados deficientes", como *P. albisummus, P. mollispinus, P. oligolepis e P. splendidus* ou foram retirados da lista devido à falta de avaliação (como *P. chrysostele* subsp. *cearensis*), sendo que todas elas também possuem uma distribuição muito restrita e não ocorrem em unidades de conservação (IUCN, 2016). Muitas espécies são classificadas como dados deficientes devido principalmente a fatores como ausência de informações precisas sobre a localidade tipo e consequente falta de registros recentes que potencialmente venham a contribuir com informações sobre seu tamanho populacional, amplitude de distribuição e atuais ameaças (Zappi & Taylor, 2013). O simples aumento no esforço de coleta, nesses casos, pode auxiliar no incremento dessas informações, caso de *P. oligolepis,* em que a realização de coletas recentes no estado de Roraima (Norte do Brasil) no ano de 2014 resultou na descoberta de novas populações desta espécie até então com dados conhecidos apenas para 1927 (Lavor *et al.*, 2016).

Assim, o primeiro passo na tomada de medidas de conservação para muitas Cactáceaes, incluindo *Pilosocereus,* é o aumento de esforço de coleta e trabalhos de campo. Essas medidas tem que visar reunir ou aumentar o conhecimento básico de taxa pouco estudados e que estejam em áreas consideradas de risco pela ação antrópica, e que são pouco contempladas pelas politicas de conservação, como as regiões aridas e semi-áridas nos Neotrópicos, que muitos acreditam serem menos "ricas" que as grandes florestas.

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2. OBJETIVOS

2.1 OBJETIVOS GERAIS

Investigar o relacionamento filogenético, a biogeografia e aspectos evolutivos do gênero *Pilosocereus.*

2.2 OBJETIVOS ESPECÍFICOS

1. Reconstruir a história da diversificação de *Pilosocereus* nos neotrópicos com base no relacionamento filogenético, datação molecular e diferentes análises biogeográficas;

2. Apresentar uma hipótese filogenética para o gênero com base em caracteres moleculares e avaliar a sistemática do grupo frente à essas evidências;

3. Descrever os padrões de distribuição geográfica, riqueza, endemismo e diversidade filogenética do gênero e discutir as implicações destes fatores na conservação do gênero.

3. CAPITULOS

CAPITULO 1

THE ROLE OF PLEISTOCENE CLIMATIC FLUCTUATIONS IN THE GEOGRAPHIC DISTRIBUTION AND DIVERSIFICATION OF THE WIDESPREAD NEOTROPICAL GENUS *PILOSOCEREUS* (CACTACEAE)

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Original Research Article

Title: The role of Pleistocene climatic fluctuations in the geographic distribution and diversification of the widespread Neotropical genus *Pilosocereus* (Cactaceae)

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Short title: Spatiotemporal evolution of cacti genus *Pilosocereus*

ABSTRACT

Aim Diversification mechanisms underlying the extraordinary plant diversity levels of the Neotropics have long attracted attention. However, focus has been mostly on the humid tropical forests and less so in other biomes. Here, we used an endemic genus of cacti, *Pilosocereus*, to investigate diversification processes in Neotropical arid environments. Specifically, we wanted to assess the impact of Pleistocene climatic fluctuations on the diversification of this type of flora.

Location The Neotropical region, from Mexico to Southwest Brazil.

Methods Based on plastid and nuclear sequences, we estimated phylogenetic relationships and divergence times in *Pilosocereus* using Bayesian relaxed molecular clocks. Ancestral ranges and the main history of migration events were estimated using likelihood-based and Bayesian methods. We also explored potential shifts in diversification rates over time and across clades.

Results *Pilosocereus* was recovered as paraphyletic, with representatives of other Cereinae nested within. Diversification within the *Pilosocereus sensu stricto* clade was dated very recent, with most lineages diversifying in the Late Pleistocene. Biogeographic analyses inferred an origin in the Brazilian Caatinga region, with migration events into other xeric habitats (savannas, rock outcrops in moist forests) of North/Central America, northwestern South America, and the Caribbean, driving diversification.

Main conclusions Patterns of diversification and biogeographic evolution in *Pilosocereus* were associated to the rapid climatic oscillations of the Pleistocene. Biotic connections among dry and open formations during the arid glacial stages, and expansion of tropical forests during the humid interglacial stages, would explain the widespread but disjunct distribution observed today in this genus and other cacti taxa.

Key-words Biogeography, Cacti, Caatinga, diversification, Neotropical region, Northeastern Brazil, Pleistocene climatic changes.

INTRODUCTION

The Neotropics are among the regions with the greatest floristic diversity in the world (Gentry, 1982; Prance, 1994; Kier *et al.*, 2005). Moist (e.g. tropical rainforest, tropical wetlands, and lowland rainforests) to dry plant formations (e.g. seasonally dry tropical forests (SDTFs), savannas and rocky fields, high-elevation Andean grasslands and deserts) arise under a complex physiography which embraces many different topographic, climatic and habitat conditions (Burnham & Graham, 1999; Hughes *et al.*, 2013). The unique features of this region have inspired numerous studies that - through multidisciplinary approaches integrating systematic, geological, paleobiological, biogeographical, and ecological data - seek to understand the biotic and abiotic factors driving diversity patterns in the Neotropical flora (Gentry, 1982; Burnham & Graham, 1999; Pennington *et al.*, 2000; 2004; Jaramillo *et al.*, 2006; Antonelli & Sanmartín, 2011a,b; Rull, 2011; Hughes *et al.*, 2013; Leite *et al.*, 2016).

The STDFs, savannas and tropical rainforests are vegetation types or biomes commonly found in the Neotropical region (Gentry, 1982; Pennington *et al.*, 2000; Werneck, 2011; Carnaval & Moritz, 2008). The STDFs are dry low forests with thorny and deciduous elements (e.g. Fabaceae, Bignoniaceae, Bromeliaceae, and Cactaceae) that can withstand long periods of draught (Pennington *et al.*, 2000). They occur in Northeastern Brazil (Caatinga), Southern Brazil, Eastern Paraguay and Northeastern Argentina (Misiones); Southeastern Bolivia and Northwestern Argentina (Piedmont) (Pennington *et al*. 2000; Prado & Gibbs, 1993; Oakley & Prado, 2011; Banda *et al.*, 2016), and in the Colombian and Venezuelan Caribbean coasts. Savannas are open formations over oligotrophic or rocky soils with low evergreen sclerophyllous and grassy components, where seasonal fires are common (Pennington *et al.*, 2000; Werneck, 2011). Main Neotropical savannas occur in central Brazil (Cerrado);

Venezuela (Llanos); Northern Brazil and Guyana (Pennington *et al*., 2000). Tropical rainforests are dense moist forests with a dominant angiosperm component (e.g. Leguminosae, Moraceae and Annonaceae) that flourish under high annual precipitation, low seasonality and high mean annual temperatures (Burnham & Johnson, 2004; Jaramillo & Cárdenas, 2013). In the Neotropics, they extend from Northern Brazil to Peru, Ecuador, Colombia, Venezuela and Guyana (Amazon) and along the Eastern Brazilian Atlantic coast (Atlantic Forest) (Olson & Dinerstein, 2002). Patches of different sizes of alternating plant formation types (moist forests, STDFs, and savannas) throughout the Neotropical region create a mosaic-like vegetation landscape (see fig. 1 in Olson *et al*., 2001 and Antonelli & Sanmartin, 2011a).

This landscape heterogeneity offers fine-scale partitioning of environmental resources and a diverse array of niches that may have favored rapid diversification in Neotropical lineages through colonization of novel niches and ecological release (Wiens *et al*., 2010). Bignoniaceae (Lohmann *et al*., 2013), Caricaceae (Carvalho & Renner, 2012) and Rubiaceae (Motley *et al*., 2005) are examples of lineages widely distributed in the Americas where diversification has been linked to different vegetation types. In contrast, for lineages that tend to occur in rather similar habitat conditions (i.e., habitat conservatism), landscape heterogeneity does not seem to be a key element associated with diversification. Habitat conservatism often leads to a disjunct distribution pattern across unconnected patches of similar vegetation types. This can be observed, for example, in Leguminosae, with several clades restricted to different nuclei of STDFs (Pennington et al., 2000), or in Cactaceae lineages such as *Rhipsalis*, which are centered in two regions of Neotropical moist forests: the Atlantic Forest (Brazil) and the *yungas* (Andean slopes) (Calvente *et al*., 2011). In these cases, diversification seems to be more closely associated to dispersal across barriers and allopatric speciation (Antonelli &

Sanmartín, 2011b; Hughes *et al*., 2013) than to local adaptation to different ecological conditions. On the other hand, whether geographic or ecological, speciation in Neotropical lineages seems to have been similarly driven by global changes in the landscape through time, including mountain building and retraction and expansion events of several vegetation types during the climatic oscillations of the late Cenozoic (Antonelli & Sanmartín, 2011a).

Geological and palynological evidences suggest that the rapid and intense climatic fluctuations of the Pleistocene – the last 2.4 million years (Ma) – led to profound changes in the flora of the Neotropical region (Van der Hammen, 1991). Periods of dry and cold conditions (glacial) were interspersed with interglacial stages, dominated by warm and humid conditions (Hewitt, 2000; Haffer & Prance, 2002). This cyclical process probably played a pivotal role in the shaping of patterns of distribution and species richness in major Neotropical biomes, such as the SDTFs, savannas and rainforests (Carnaval & Moritz, 2008; Carnaval *et al*., 2009; Pennington *et al*., 2000, 2004, 2009; Werneck, 2011; Werneck *et al*., 2011, 2012; but see Hoorn *et al*., 2010 for a different view). During glacial periods, moist forests contracted and dry and open vegetation such as SDTFs and savannas expanded, and the opposite occurred during the interglacial periods (Prado & Gibbs, 1993; Burnham & Graham, 1999; Pennington *et al*., 2000; Werneck, 2011).

The disjunct distribution pattern observed today in some STDF and savanna lineages, with three main centers located in the Caatinga, Misiones and Piedmont regions, might be a result of vicariance (an ancient widespread distribution of these vegetation types fragmented by climate change) or might have originated from independent dispersal events during Pleistocene climatic fluctuations (Pennington *et al*., 2000; Werneck, 2011; Banda et al., 2016). Based on palynological, geomorphological and biological evidence from several STDF woody lineages, Pennington *et al*. (2000) suggested that during the dry and cold conditions of Pleistocene glacial periods, dry South American plant formations expanded far beyond the current borders of the three nuclei, penetrating the Amazonian plain and reaching Central America. Corroborating this hypothesis, however, would require in-depth studies on plant taxa with: (1) a widespread distribution across the Neotropics but restricted to STDFs patches (i.e., habitat conservatism); (2) a recent origin and diversification in the Pleistocene; and (3) sister species occupying different STDFs' nuclei (Pennington *et al*., 2009). If the focus is expanded to lineages occurring in other Neotropical plant formations, such as savannas and rainforests, such studies could provide general insights onto the genesis of the main Neotropical vegetation types, and the link between their present disjunct, mosaic-like distribution and Pleistocene climatic fluctuations.

The angiosperm family Cactaceae fits these three criteria, since the group is widely diversified in the Neotropics though mostly restricted to xeric habitats, and its origin is relatively recent compared to other families, with diversification events ranging from the Miocene to the Pleistocene (Hunt *et al*., 2006; Arakaki *et al*., 2011; Hernández-Hernández *et al*. 2014; Hernández-Ledesma *et al*., 2015). Within the family, the genus *Pilosocereus* Byles & Rowley offers a model group for biogeographical studies (Nyffeler & Eggli, 2010). It is a conspicuous element of the flora of dry zones, with species distributed mostly in xeric habitats of the SDTFs, savannas and deserts (Zappi, 1994), though a few species occur also in the moist forests of Central and South America (Amazon and Atlantic Forest), always associated with rocky outcrops. The genus shows a disjunct distribution with two core areas: (1) Eastern and Central Brazil (harboring the highest species diversity), and (2) Northern South America, Central America and North America (Taylor & Zappi, 2004). Previous studies, focusing on less inclusive species complexes within *Pilosocereus*, revealed a role for Pleistocene climatic fluctuations in species diversification, through reproductive isolation during interglacial periods and subsequent gene flow by secondary contact during glacial periods (Bonatelli *et al*., 2014).

In this study, we use *Pilosocereus* as a model group to investigate the diversification of plant lineages associated to Neotropical xeric environments. We reconstruct divergence times and historical distribution patterns and discuss the processes that led to the genus' present disjunct distribution. Specifically, we aim to answer the following questions: (1) Did Pleistocene climatic fluctuations have a major role in the diversification of the xerophitic Neotropical flora, as suggested for other vegetation types? (2) Was diversification in this vegetation type mainly driven by dispersal and colonization events of other xeric habitats (habitat conservatism), or geology and landscape heterogeneity played a role, as well?

MATERIAL AND METHODS

Study group

Cactaceae includes 130 genera and 1,850 species divided into four subfamilies (Cactoideae, Opuntioideae, Pereskioideae and Maihuenioideae) centered in the Neotropical region (Nyffeler & Eggli, 2010). *Rhipsalis baccifera* (Mill.) Stearn is the only species to exceed the Neotropical distribution, reaching Africa and Asia. *Pilosocereus* belongs to Cactoideae, which is the richest and more diverse subfamily and includes six tribes: Blossfeldieae, Cacteae, Phyllocacteae, Rhipsalideae, Notocacteae and Cereeae (Nyffeler & Eggli, 2010). Cereeae encompasses the greater number of genera (41) and a great variety of growth forms, of which columnar and globular taxa are centered in South America (Brazil) (Nyffeler & Eggli, 2010). The tribe includes a few taxon-rich genera, such as *Pilosocereus*, and phylogenetic relationships at the suprageneric and infrageneric levels are still uncertain. *Harrisia* Britton, *Melocactus* Link & Otto and *Pilosocereus* are the only genera in Cereeae to exceed the South American distribution, reaching Central and North America (Hunt *et al*., 2006).

Genus *Pilosocereus* has one of the widest distributional ranges within Cactaceae. It is disjunctly distributed in the New World, occurring in USA (Florida), Mexico, the Caribbean islands, Venezuela, Suriname, Guyana, Peru, Ecuador, Brazil and Paraguay, predominantly in xeric habitats, such as SDTFs, savannas and deserts of North, Central and South America (including the Caatinga and Cerrado biomes in Brazil) (Taylor & Zappi, 2004). Species of *Pilosocereus* are also present in moist forest habitats (the Atlantic, Amazonian, Pine and Mountain Forests of Central and South America), though in this case they appear associated with rocky fields or rock outcrops (Zappi, 1994; Taylor & Zappi, 2004). The genus comprises 42 species (Hunt *et al*., 2006; Zappi & Taylor, 2011), and traditionally subdivided into two subgenera: *Gounellea* (with 3 species: *P. gounellei*, *P. tuberculatus* and *P. frewenii*) and *Pilosocereus* (with 39 species) (Hunt *et al*., 2006), and shows its highest diversity in eastern Brazil (Zappi, 1994).

Previous phylogenetic work has been limited to a few *Pilosocereus* species included in family-level phylogenetic studies (e.g. Terrazas & Arias, 2002; Crozier, 2005; Bárcenas *et al.*, 2011; Hernández-Hernández *et al*., 2011, 2014) and, more recently, a phylogenetic study centered on the genus (Calvente *et al*., 2016), which recovered a paraphyletic *Pilosocereus*. Other studies have focused on phylogenetic, phylogeographic, or population genetic aspects of less inclusive species complexes (Moraes *et al.*, 2012; Bonatelli *et al.*, 2013, 2014, 2015; Menezes *et al.*, 2016; Perez *et al.*, 2016).

Taxon sampling and occurrence data

In this work, we added 145 new sequences to the data set used by Calvente *et al*. (2016), totalizing 276 sequences for 48 species from six DNA regions in this study (Appendix S1). In total, we used 38 ingroup and ten outgroup taxa from two distinct subfamilies, to sample relevant nodes from the phylogeny of the family and provide points for calibration: *Pereskia grandifolia*, *Copiapoa cinerea*, *Rhipsalis baccifera*, *Browningia microsperma*, *Cleistocactus* sp., *Oreocereus hempelianus*, *Arrojadoa rhodantha*, *Cereus jamacaru*, *Melocactus zehntneri* and *Stephanocereus leucostele* (Appendix S1, table S1).

Information on the geographic distribution for *Pilosocereus* species was compiled from occurrence data from online databases such as the SpeciesLinks (CRIA, http://splink.cria.org.br/), Global Biodiversity Information Facility (GBIF, http://www.gbif.org/), and the Virtual Herbarium REFLORA (Flora do Brasil 2020, http://floradobrasil.jbrj.gov.br/), and further complemented with literature records (Zappi, 1994; Taylor & Zappi, 2004; Hunt *et al*., 2006). Application of names and synonyms in this work followed Hunt *et al*. (2006). We excluded records unidentified to species level, with imprecise locality data, or duplicated. The edited occurrence data for the species obtained from online databases was then visualized in Quantum Gis v2.14.0 (QGis, 2011) and compared to literature records (Zappi, 1994; Taylor & Zappi, 2004; Hunt *et al*., 2006) to search for outliers or unreliable records. These records were then individually examined for consistency, with preference given to those identified by group specialists. Distribution plots of all species (total of 2624 records) (Fig. 1a) and for sampled species in our study (1312 records) (Fig. 1b) were compared in QGis.

DNA extraction, amplification, sequencing and alignment

To obtain sequence data, genomic DNA was either extracted from silica dried stems or roots or from herbarium specimens using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) or the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) in accordance to the manufacturers protocols. Five molecular markers – four non-coding intergenic spacers of chloroplast DNA (cpDNA): *trn*S-*trn*G, *psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E; one low-copy nuclear gene, phytochrome C (PHYC) (followed protocols described in Calvente *et al*. (2016)] and cpDNA *ycf*1 gene was used in this study. Primers and amplification reaction conditions is presented in Appendix S1 (Appendix S1, table S2). Amplification products were purified using the NucleoSpin Gel or PCR clean-up Kit (Macherey-Nagel, Düren, Germany) and the QIAquick PCR Purification Kit (QIAGEN, Crawley, UK), following the manufacturer's protocol. Automated sequencing was performed by Macrogen Inc. Korea and Netherlands. We were able to amplify *trn*S-*trn*G for 18 taxa, *psb*D-*trn*T for 23 taxa, *trn*L-*trn*T for 19 taxa, *pet*L-*psb*E for 23 taxa, PHYC for 19 taxa, and *ycf*1 for 43 taxa (which were added to Calvente *et al*. (2016)'s previous dataset). Missing data (some regions could not be sequenced for few species) were coded as (-) (Appendix S1, table S1).

Complementary sequences were assembled in Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually using Mesquite v. 3.04 (Maddison & Maddison, 2015). Indels were coded using the simple indel-coding method (Simmons & Ochoterena, 2000) and included in phylogenetic analyses as presence/absence data. *Pereskia grandifolia* was used as outgroup to root the trees in all analyses, based on previous knowledge of phylogenetic relationships in Cactaceae and its position within the basalmost cacti subfamily Pereskioideae (Arakaki *et al*., 2011; Hernández-Hernández *et al*., 2014).

Phylogenetic reconstruction

We infer phylogenetic relationships using Bayesian inference implemented in MrBayes 3.2.2 (Ronquist *et al*., 2012), hosted on the CIPRES Science Gateway (Miller *et al*., 2010). Choice of substitution models was based on the Akaike Information Criterion implemented in MrModelTest 2.2 (Nylander, 2004) and run in PAUP* v4.0a147 (Swofford, 2002). Bayesian analyses were performed on individual genes, using the selected molecular substitution models *psbD-trnT, petL- psbE, ycf1, trnS-trnG, trnL-trnT* and PHYC were GTR, GTR+I, GTR+G, GTR+I+G, F81+I and HKY+G, respectively (data not shown). After rejecting significant phylogenetic incongruence (clades supported with > 90% pp were shared by all individual gene trees), we built a concatenate matrix with two partitions, plastid markers/PHYC, using GTR+I+G and HKY+G as the best-fit models, respectively, and with the overall substitution rate unlinked between partitions. Two independent analyses of four chains each were run in MrBayes for 10 million generations, sampling every 1000th generation. Convergence between runs was assessed by monitoring the standard deviation of split frequencies (< 0.01), and using the Potential Scale Reduction Factor (PSRF). After discarding the first 25% samples as burnin, the remaining trees were pooled to construct a 50% majority rule consensus tree. Clade posterior probability (PP) values were considered as "weak support" if PP < 0.8; moderate support if 0.8 < PP > 0.95, and strong support if PP > 0.95.

Molecular Dating and diversification analyses

Lineage divergence times within *Pilosocereus* and between the genus and outgroups were estimated with Bayesian relaxed-clock models implemented in BEAST v1.8.3 (Drummond *et al*., 2012a) hosted on CIPRES. We used the partitioned plastid/nuclear concatenate matrix, with the models used above. The Birth-Death model and the uncorrelated lognormal distribution were used as the tree and clock-model priors, respectively. Two MCMC chains were run for 10 x10^7 generations, sampling every 1000th generation. Convergence and EES values (> 200) for all parameters and runs were monitored in Tracer v.1.6 (Rambaut *et al*., 2013). A maximum clade credibility (MCC) tree (burnin of 10%) with posterior probability limit set to 0.5 was constructed in TreeAnnotator v.1.8.2 (Drummond & Rambaut, 2016).

There are not known fossils of Cactaceae. To obtain absolute divergence time estimates for *Pilosocereus*, we used secondary calibration points obtained from two more inclusive, higher-level studies on succulent plants that included Cactaceae taxa (Arakaki *et al*., 2011; Hernández-Hernández *et al*., 2014). Because age estimates for key calibration points differed between these two studies, we performed three different analyses to compare the influence of calibration constraints on our diversification age estimates; a normal distribution prior was used for all calibration points in these three analyses: a) Analysis “AE 1" included two calibration points based on Arakaki *et al*. (2011): the crown age of Cactaceae (mean = 28.6 Million year (Ma), standard deviation (SD) = 1.9, 95% high posterior density (HPD) credibility interval = 25.47-31.73 Ma) (the root node in our dataset), and the crown age of subfamily Cactoideae (mean = 21.8 Ma, SD = 1.7, HPD = 19-24.6 Ma) (the node splitting *Pereskia grandifolia* from all other taxa); b) Analysis "AE 2" included the same calibration points (the root node, Cactaceae crown age (mean = 26.88 Ma, SD = 6.2, HPD = 16.68-37.08 Ma) and the Cactoideae crown node (mean = 17.15 Ma, SD = 3, HPD = 12.22-22.08 Ma), based on Hernández-Hernández *et al*. (2014); c) Analysis "AE 3" included the same calibration point plus a third calibration from Hernández-Hernández *et al*. (2014): the crown age estimate of the Cereeae node (mean = 5.28 Ma, SD = 1.3, HPD = 3.14-7.41 Ma), which corresponds in our dataset to the node splitting *Pereskia grandifolia*, *Copiapoa cinerea* and *Rhipsalis baccifera* from all other taxa.

Since a previous study (Calvente *et al*., 2016), and our own results (below), supported a non-monophyletic *Pilosocereus*, we used the clade containing the majority of species of the genus (*Pilosocereus sensu stricto* (*s.s*) (Calvente *et al*., 2016) for diversification analyses. The dated MCC tree obtained from Analysis AE2 was used for these analyses because it provided the narrowest 95% HPD confidence intervals for most nodes, while giving mean age estimates very similar (within the 95% HPD interval) to the other analyses (AE1 and AE3, see Results). A lineage-through-time (LTT) plot was constructed for *Pilosocereus s.s.* with the R package ape (Paradis, 2012). Whole-tree episodic birth-death models implemented in the R package TreePar (Stadler, 2015) were used to compare a birth-death model implementing a time-homogeneous diversification process with constant rates of diversification (*r* = speciation minus extinction) and turnover (epsilon = extinction/speciation), *versus* a time-heterogeneous diversification model in which these two parameters can vary at discrete points in time (Stadler, 2011). We estimated the magnitude and times of potential rate shifts assuming that all lineages are sampled at shift times (option ME = FALSE in TreePar), except at present, when we accounted for incomplete taxon sampling in the reconstructed phylogeny using a sampling fraction (rho=0.92). Different models with an increasing number of rate shifts were explored using likelihood ratio tests.

Ancestral area reconstruction

To investigate the history of the distribution and spatiotemporal patterns of *Pilosocereus* in different Neotropical areas, we performed two different ancestral area reconstruction analyses based on Maximum Likelihood (ML) and Bayesian inference methods. Analyses were ran exclusively on the *Pilosocereus s.s.*, clade to avoid the influence of outgroups. For the likelihood-based Dispersal-Extinction-Cladogenesis (DEC) model (Ree *et al*., 2005), three areas were delimited based in vegetation types (modified from Olson *et al*., 2001) in order to understand the dynamics of occupation in xeric habitats:

(A) dry formations; (B) savanna, and (C) moist forest. This model allows lineages to occur more than one character state (i.e., taxa distributed in more than one vegetation type). We ran DEC as implemented in the python software LAGRANGE 2.0 (Ree & Smith, 2008) and estimated global rates of dispersal (range expansion) and extinction (range contraction) and node-by-node range inheritance scenarios (describing the division of ancestral ranges among descendant nodes) based on the MCC tree from the BEAST AE2 analysis.

Additionally, we used the discrete phylogeographic approach (DPA) of Lemey *et al*. (2009) implemented in BEAST v1.8.3 (Drummond *et al*., 2012a) to estimate rates of migration among areas and ancestral ranges by MCMC Bayesian inference. DPA uses a continuous-time Markov chain process analogous to models of nucleotide substitution in which a lineage can occur only in a single state at any point in time (i.e., no widespread taxa). Unlike in DEC, phylogenetic relationships, divergence times, and the history of migration events are simultaneously estimated using the molecular sequences and the geographic location of species (Ronquist & Sanmatín, 2011). For this analysis, seven discrete single areas were delimited based on distribution patterns and richness of the genus, the geological history of the region, as well areas of endemism used in previous analyses in an effort to maximize comparisons with biogeographic studies in other floras (Gentry, 1992; Zappi, 1994; Antonelli *et al*., 2009; Antonelli & Sanmartin, 2011b; Hernández-Hernández *et al*., 2014). Areas included: (A) Caatinga; (B) Cerrado; (C) Atlantic Forest; (D) Guiana Shield; (E) North-western South America; (F) Central America and Mexico and (G) Caribbean. Since DPA accepts only single-area coding, for species such as *P. pentaedrophorus* and *P. catingicola*, have more distribution to the Caatinga but extending its range into the adjacent Atlantic Forest, the present range was coded as only Caatinga (A); the same procedure was adopted for *P. flavipulvinatus* and

P. pachyclaudus, coded as Caatinga; *P. floccosus, P. aurisetus* and *P. densiareolatus* (Cerrado), even though these species extend their distribution to adjacent biomes.

The BEAST DPA analysis was ran on the CIPRES Science Gateway (Miller *et al*., 2010) with a root node calibration distribution obtained from the AE2 analysis of the full dataset, i.e., we set a normal prior with mean=2.71 Ma, and SD=0.5 (95%HPD = 1.19–4.02 Ma) for the crown node of *Pilosocereus* s.s. Because introducing a new (geographic) character might change phylogenetic relationships for some clades (i.e., especially those with low support), we fix the topology of the tree to follow the one obtained from the more inclusive BEAST dating analysis (Analysis AE2); this way we ensured that DEC and DPA results were comparable. Besides, migration rates between areas (reversible rates) were modelled using: a) default gamma prior distributions (mean 1, stdev 0), or b) geographic-informed priors, defined as normalized inverse distances from area centroid geographic coordinates (Lemey *et al*. 2009). An exponential prior was used for the geographic rate scalar. A single MCMC chain was run for 50 million generations, sampling every 1000th steps, and 50% MCC tree built in TreeAnnotator as described above with burnin 10%.

RESULTS

Phylogenetic relationship of *Pilosocereus*

The MCC tree obtained from the BEAST AE2 analyses (Fig. 2) and the majority-rule consensus tree produced by MrBayes (Appendix S2, Fig. S1) were largely congruent, with all major clades receiving strong support (PP > 0.95). Both analyses recovered *Pilosocereus* as paraphyletic, with one clade comprising three outgroup genera within subtribe Cereinae (*Arrojadoa rhodantha, Cereus jamacaru* and *Melocanthus zehntneri*) nested inside the genus (BEAST AE2: node 11, PP=0.98; majority-rule consensus tree: node 10, PP=0.99). Diverging basally to this clade are a clade formed by sister-species

Pilosocereus gounellei and *P. tuberculatus*, and species *P. bohlei*. The remaining *Pilosocereus* species form a strongly supported clade, *Pilosocereus s.s.* (PP=1). Within this clade, *P. aureispinus* is placed as the sister-group of two clades grouping the majority of species in the genus: Clade A - (node 16 and 15, PP=1) comprises a clade of Brazilian species (clade AI) *P. glaucochorus*, *P. pentaedrophorus* and *P. piauhyensis* (node 17 and 16, PP=1) and a second clade comprising all non-Brazilian species (clade AII, node 19 and 18, PP=1); Clade B - clusters all other Brazilian species with strong support (node 27 and 25, PP=1), and includes three subclades (BI-III), with poor resolution for basal relationships (low to moderate PP values); support is higher in the BEAST MCC tree than in the MrBayes tree (Fig. 2 and Appendix S2, Fig. S1).

Dating and diversification analyses

The three calibration settings gave slightly different divergence times for major clades but with overlapping confidence intervals. All of them support a recent origin for genus *Pilosocereus*, with the age of divergence events among major clades ranging between Late Miocene (AE1 analysis) and Early to Late Pliocene (AE2 and AE3 analyses, respectively) (Appendix S2, Table S1). In the following sections, we discuss results from Analysis AE2, which used less restrictive calibration points and generated narrower 95% HPD credibility intervals) (Fig. 2). Analysis AE2 supported a first divergence event within *Pilosocereus* (node 8) in the Early Pliocene (4.99 Ma, HPD=2.35-8.24 Ma, PP=1), while initial divergence within *Pilosocereus s.s.* (node 14) occurred around the Pliocene-Pleistocene boundary (2.70 Ma, HPD=1.19-4.74 Ma, PP=1). The split between clades A and B (node 16) is dated in the Late Pleistocene (1.73 Ma, HPD=0.68-3.16 Ma, PP=1), whereas splits within clade B (node 27) range Late Pleistocene (1.42 Ma) and Holocene (0.02 Ma).

In the results from the diversification analyses, the LTT plot shows an increase in the number of lineages at 1.5 Ma (Appendix S2, Fig. S2) and Treepar supported a model with no significant shifts in the rate of diversification over time (*p* = 0.92).

Ancestral area reconstruction

Patterns of spatiotemporal evolution in DEC and DPA were largely congruent, showing an origin of *Pilosocereus s.s.* in dry formations in Brazil (Caatinga), at the end of the Late Pliocene (Piacensian) (node 1, 2.70 Ma, Fig. 3) in DEC, and slightly later, in Early Pleistocene (Gelasian) (node 1, 2.33 Ma, Fig. 4) in DPA. The DPA analyses with and without migration rates constrained by geographic distance gave very similar reconstructions, with the same migration events (Fig. 4 and Appendix S2, Fig.S3); model likelihood was only slightly higher for the unconstrained model over the distance model (-8179,496, 8179,711). Both DEC (Fig. 3) and the unconstrained DPA analysis (Fig. 4) reconstructs the dry Caatinga biome was also the ancestral area of the basal Brazilian clade AI (node 4, 0.38- 0.32 Ma, PP=1), with a recent migration to the adjacent moist forest biome in *P. pentaedrophorus* in DEC.

Migration out of the Caatinga to other dry formations in Central America took place in the Late Pleistocene (node 3, 1.72-1.44 Ma, PP=1; Figs. 3-4). Subsequent speciation within clade AII (node 6) (*P. royenii*-*P. chrysacanthus*) involved several migration events from dry to moist forest and savanna formations in the northwestern South America (*P. lanuginosus*) and Caribe (*P. polygonus*) (Figs. 3-4). Species such as *P. leucochephalus* and *P. royenii* also expanded their distribution from dry formations to other formations in Mexico/Central America and Caribe (to the last) in more recent times. Clade B - comprising the remaining Brazilian species (node 14) - is inferred to have undergone an early vicariance (biome/range division) event between the savanna (Cerrado) and the moist forest formations (Atlantic Forest) in the Late Pleistocene

(1.54-1.30 Ma), preceded by a migration event from the dry formation Caatinga during the Early Pleistocene (node 2, between 2.28/1.93 Ma, Figs. 3-4).

Clade BI is reconstructed as having originated within the Atlantic moist forest biome, with subsequent dispersal back to the dry formation Caatinga in *P. catingicola*. Ancestral area reconstruction for Clade BII implies several in-situ diversification events within the savanna Cerrado but also migration to the adjacent moist forest biome (Atlantic forest), with subsequent vicariance giving rise to the clade *P. brasiliensis-P. multicostatus-P. magnificus*. Allopatric/ecological speciation between different geographic regions/biomes is also responsible for the divergence between *P. azulensis* (Atlantic Forest) and *P. floccosus* (Cerrado) in relatively recent times (Late Pleistocene, 0.42-0.32 Ma) (Figs. 3,4). Clade BII *P. oligolepis-P. chrysostele-P. flavipulvinatus* is inferred in DEC to have originally occupied the savanna regions of northern South America (Cerrado and Guiana Shield), with subsequent dispersal to the Caatinga dry biome in *P. chrysostele*, and expansion to the Caatinga in *P. flavipulvinatus*, but DPA infers instead an origin in the Caatinga with subsequent migration to the Guiana Shield in *P. oligolepis*. Clade BIII *P. fulvilanatus-P. densiareolatus* is inferred to have origined in the savanna Cerrado with expansion to the Atlantic Forest in *P. aurisetus* in DEC. Clade *P. splendidus-P. pachyclaudus* is inferred to have originated in the Caatinga in DPA (Fig. 4) or Caatinga-Cerrado in DEC (Fig. 3), with subsequent migration events back to the Cerrado in *P. albisummus* and wide expansion to other formations in *P. pachycladus* in DEC.

DISCUSSION

STDF habitat conservatism and the expansion of xeric environments

Cactaceae originated in South America (Andean region) at the end of the Eocene (35 Ma), coincident with a global drop in temperatures and CO2 levels (Arakaki *et al.*, 2011). Diversification among major clades took place more recently in the Late Miocene (10-5 Ma), following a global cooling trend and the expansion of arid habitats in the New World after the initial closing of the Panama Isthmus (Arakaki *et al*., 2011; Hernandez-Hernandez *et al*., 2014; Montes *et al*., 2015). Cacti are well known by their various adaptations to arid environments, including photosynthetic stems, leaves modified into spines, and extreme succulence (Arakaki *et al*., 2011).

In angiosperms, the evolution of new morphological and physiological traits lead to selective advantages in reproductive, ecological, and dispersal capacities (Dodd *et al*., 1999), driving events of rapid diversification within some families such as Aizoaceae (Klak *et al*., 2004), Bromeliaceae (Krapp *et al*., 2014), Costaceae (Kay *et al*., 2005) and Leguminosae (Drummond *et al*., 2012b). In Cactaceae, major radiation events have been linked to the global expansion of arid and semi-arid habitats in the New World during the Late Miocene, but also with the appearance of morphological innovations, such as novel growth forms and pollination syndromes (Hernández-Hernández *et al*., 2014). These environmental and morphological changes would have favored the colonization of new habitats, triggering a process of rapid diversification within the family (Hernández-Hernández *et al*., 2014).

By contrast, morphological characters are fairly homogeneous in *Pilosocereus* (e.g., floral morphology), and variation seems to be uncoupled from any phylogenetic signal (Calvente *et al*., 2016). We also did not find evidence of accelerated speciation rates within *Pilosocereus s.s.* Instead, patterns of species richness seem to be explained by a gradual diversification process driven by allopatric speciation and the colonization of new (xeric) habitats in neighboring geographic regions.

According to the DEC and DPA reconstructions, *Pilosocereus s.s*. originated and first diversified within the Caatinga region, a SDTF-type dry formation in northeastern

Brazil (Pennington *et al*., 2000), during the Late Pliocene and Pleistocene. Pennington *et al*. (2009) argued that SDTF formations are old (dated as Middle Eocene in North America), and that their mostly endemic taxa have evolved by in-situ diversification, with few migration events to other habitat types. This has been considered as strong evidence of niche conservatism (habitat) in STDF taxa (Pennington *et al*. 2000). Among the SDTF nuclei, the Brazilian Caatinga is the widest, richest, and exhibits the highest endemism levels (Cardoso & Queiroz, 2011). Werneck *et al*., (2011) highlighted the historical stability of this biome, which might have acted as species “refugia” favoring the establishment and diversification of arid-adapted taxa during Pleistocene climatic cycles. Genetic isolation and genetic drift would be the main mechanisms generating new Caatinga species (Latimer *et al*., 2005). The extensive Caatinga (that currently occupies 850.000 km^2) might also have acted as a "corridor" of dispersion, facilitating biotic interchange between southern and southwest South America with xeric vegetation nuclei in Central America and North America. For example, cacti genera *Opuntia* (Majure *et al*., 2012) and *Harrisia* (Franck *et al*., 2013) apparently originated in southern/ southwestern South America and used the Caatinga biome as a dispersal path for Pleistocene migration into Northern South America, Central America, Mexico, and the Caribbean. A similar pattern is found here in *Pilosocereus s.s*., which shows several migration events from the Brazilian Caatinga to other dry formations in Central America in the Pleistocene, such as tropical and subtropical Dry Broadleaf and Coniferous Forests, deserts and xeric shrublands (Olson *et al*., 2001). The first of these dispersal events took place at the end of the Pleistocene (between 2.28 and 1.72 Ma) following the final closing of the Panama Isthmus (and the Great American Biotic Interchange, Simpson (1950)]. There is evidence that Central and North America offered favorable conditions to xerophytes and that cacti were already established in this region before and during this time (Hernández-Hernández *et al*., 2014; Vázquez-Lobo *et al*., 2015).

One possible hypothesis for this migration event is long-distance dispersal through biotic vectors. Chiropterochory (i.e., seed dispersal by bats) is common in *Pilosocereus* (Zappi, 1994). Also, fossil bat species, such as *Furipterus horrens* (Furipteridae), *Chrotopterus auritus* (Phyllostomidae), and *Mormoops* cf. *megalophylla*, *Pteronotus gymnonotus* and *Pteronotus parnellii* (Mormoopidae) - dated between the Late Miocene and the Holocene - have been found disjunctly distributed in Eastern Brazil (mainly in rocky caves inside the Caatinga), Central America and Mexico (Salles *et al*., 2014). Long-distance bat dispersal could also explain the more recent colonization of the Caribbean islands (Figs. 3, 4). After dispersal, establishment and speciation could have been favored by the mosaic-like structure of the landscape, with highly distinct vegetation types (Olson *et al*., 2001) and numerous rocky formations in the mountain range systems of Mexico-Central America (Sierra Madre), the Caribe, or northwestern South America (Andes) (Pindell & Kennan, 2009). *Pilosocereus* species are seemingly well adapted, if not restricted, to rocky substrates in these regions (Zappi, 1994; Taylor & Zappi, 2004; Hunt *et al*. 2006).

An alternative hypothesis is short-range dispersal during the arid glacial stages of the Pleistocene, when dry vegetation expanded in northwestern South America and Central America (Pennington *et al*., 2000, 2004; Werneck, 2011; Bonatelli *et al*., 2014). Retraction of tropical moist forests would have facilitated connections through dispersal corridors between the South American STDFs and savannas, i.e., Caatinga, Cerrado, and savanna formations in northwestern Amazonia (Werneck *et al*., 2011, 2012; Bonatelli *et al*., 2014). One example of this migration would be *P. oligolepis* (Figs. 3, 4), currently endemic to savanna formations in the Guiana Shield region (Lavor *et al*., 2016).

In sum, our study provides support to the hypothesis of strong habitat conservatism in STDF's taxa and the existence of historic floristic connections among STDFs nuclei during Pleistocene climatic fluctuations (Pennington *et al*., 2009; Werneck et al, 2011). Divergence time and ancestral range estimates in *Pilosocereus* s.s indicate that diversification within the genus was mainly driven by migration events from an ancestral Caatinga origin to other xeric habitats in neighboring regions (STDFs, savannas, and rocky formations in moist forests). The expansion and retraction of dry vegetation during Pleistocene climatic fluctuations could have favored the appearance of novel ecological niches and provided establishment opportunities for organisms with high adaptive potential to extreme habitats such as Cactaceae. Cycles of range expansion (arid glacial stages) and range contraction (humid interglacial stages), allied with medium to long distance dispersal events, are likely responsible for the widespread but disjunct geographic distribution observed today in *Pilosocereus* (Fig. 1) and other cacti (such as *Opuntia* (Majure *et al*., 2012) and *Harrisia* (Franck *et al*., 2013) and STDF taxa (see Banda *et al*. 2016).

Effect of Pleistocene climatic fluctuations on savanna and moist forest lineages

The Pleistocene retraction/expansion scenario described above might also explain the occurrence of *Pilosocereus* species in savanna formations of Central Brazil (Cerrado). Cacti are sensitive to fire and therefore are relatively rare in the Cerrado, where natural fires occur seasonally (Taylor & Zappi, 2004). *Pilosocereus* species are an exception because their occurrence is restricted to rock outcrops (very abundant in the Brazilian Central plateau), where fires cannot penetrate (Taylor & Zappi, 2004). During Pleistocene glacial stages, expansion of the Caatinga vegetation into Cerrado rock outcrops could have favored the entrance of *Pilosocereus* into this biome and its establishment in historical stable zones. These populations would have become isolated with the expansion of moist forest vegetation during the humid interglacial stages, followed by range expansion to other outcrops when conditions became favorable during dry glacial periods.

This cyclical process may have affected species divergence and promoted fuzzy species boundaries. For example, the *P. aurisetus*-species complex (comprising *P. aurisetus*, *P. aureispinus*, *P. bohlei*, *P. jauruensis*, *P. machrisii*, *P. parvus*, *P. pusillibaccatus* and *P. vilaboensis*, Zappi (1994); Hunt *et al*., 2006) exhibits great morphological similarity (Zappi, 1994) and occurs in isolated rock outcrops in the Cerrado. Bonatelli *et al*. (2014) showed that of estimation of divergence times and phylogeographical analyzes for this group suggest that Pleistocene microrefugia during interglacial stages (retraction of dry and open vegetation) caused reproductive isolation and subsequent secondary contact driven by dispersal between outcrops helped reestablish gene flow during glacial stages (expansion of dry and open vegetation). Werneck *et al*. (2012) hypothesized two potential climatic stability areas in Cerrado: (1) one large area in Central Cerrado and a few smaller areas next to the western and eastern boundaries of the biome, in the Beni and Espinhaço range; or (2) a long and narrow area extending north to south of the biome, including the Brazilian states of Goiás, Tocantins and Bahia. In both hypotheses, the putative stability areas coincide with present day occurrence sites of the *P. aurisetus*-species complex.

A mechanism of expansion/retraction of dry and open formations can also explain the isolated occurrence of *P. oligolepis* in rock outcrops inside savanna and moist forest areas of northern Brazil and Guyana (Lavor *et al*., 2016). For Taylor and Zappi (2004) this species would be sister to *P. chrysostele* and *P. flavipulvinatus* (both distributed principally in Caatinga) due morphologically similarity and would demonstrate the existence of the historical dispersal corridor in the northern Atlantic Brazilian coast, connecting the Cerrado, Caatinga, and the Guiana Shield savannas. Similar case is pointed to *Cereus hexagonus* Mill. (found in Northern Brazil and Guyana) and *Cereus jamacaru* (found widespread in Northeastern Brazil) (Taylor & Zappi, 2004). This coastal corridor - mentioned in other works (Silva & Bates, 2002), was later corroborated by Werneck *et al*. (2012), who showed a relatively climatic stable connection between Cerrado and the Guyana savannas during the Pleistocene. Our phylogeny corroborates the close relationship, proposed by Taylor and Zappi (2004) of these three *Pilosocereus* species (Appendix S2, Fig. S1). Ancestral range reconstructions (Figs. 3, 4) support also historical migration from the dry formation Caatinga to savanna biomes during the Pleistocene in the latter species.

The presence of *Pilosocereus* species, such as *P. ulei*, *P. brasiliensis*, or *P. leucocephalus*, in moist forest formations of Brazil and Central America can be similarly explained by expansion/retraction of dry vegetation during Pleistocene climatic cycles. Expansion of dry and open formation would have allowed *Pilosocereus* species to reach habitats with xeric conditions, such as rock outcrops, inside these moist forest domains. This can be observed in the Central-North American clade AII, where each lineage is reconstructed as having originated in dry vegetation areas, with only a few of them having more recently expanded their range to rock outcrop areas inside moist forests (e.g., *P. leucocephalus*). For Atlantic Forest lineages (clade BI and the *P. azulensi*-*P. magnificus* clade in BII), expansion of the Caatinga and Cerrado vegetation during the Late Pleistocene – concomitant with Atlantic Forest retraction – likely facilitated colonization and persistence in local habitats under xeric conditions.

The Atlantic Forest is a moist forest formation with some degree of heterogeneity. It includes distinct interconnected vegetation types, each of them with a particular set of environmental conditions, including evergreen and semi-evergreen forests, riverine forests, gneiss-granite rock outcrops formations (inselbergs), and coastal vegetation on sandy soil (restinga) (Taylor & Zappi, 2004). Several transitions within clade B from the Caatinga and Cerrado into the Atlantic Forest are dated in the Early/Late Pleistocene (Figs. 3, 4), when changes in vegetation spatial distribution allowed these lineages to establish in rocky or sandy substrate habitats with xeric environmental conditions akin to the ancestral ones. When moist forest expanded, these populations became isolated, and are today restricted to “island” climatic refugia inside the Atlantic Forest (e.g., *P. azulensis*, *P. brasiliensis*).

Brazilian *Pilosocereus* species occurring outside the Caatinga (i.e., in the Cerrado and Atlantic Forest biomes), are frequently narrowly distributed and micro-endemic. These species are currently restricted to specific substrates and limited in their ability to disperse by the surrounding habitat conditions. Therefore, under a scenario of niche conservatism, historical migration into these regions must have been facilitated by changes in the landscape during the Pleistocene, which is also supported by our divergence time and ancestral area estimates for these lineages. Further studies on other cacti and STDF taxa could help corroborate our hypothesis on the pivotal role of Pleistocene climatic cycles on the evolution of Neotropical lineages in xeric habitats.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary tables

Appendix S2. Supplementary results

BIOSKETCH

Pamela Lavor is a PhD student at the Universidade Federal do Rio Grande do Norte, working on the systematics and evolution of cacti. Her interests lie in Neotropical biogeography, with emphasis in the assembly of arid environment floras. The other authors are specialists on Cactaceae systematics and analytical biogeography.

Author Contributions: PL, AC and IS designed the study; PL performed molecular sequencing and ran the analyses with help from AC and IS; PL, AC and IS wrote the manuscript with contributions from LMV.

TABLES

Table 1. Relative likelihoods (> 0.1) for alternative nodal range inheritance scenarios obtained by Dispersal–Extinction–Cladogenesis (DEC), and marginal probabilities for ancestral ranges obtained by MCMC Bayesian Discrete Phylogeographic Approach (DPA) implemented in BEAST. Analyses were run on the BEAST A2 MCC tree (DEC) or the *Pilosocereus sensu stricto* data set with A2 settings (BEAST DPA). Region codes and node numbers follow figures 3 and 4.

	Ancestral range reconstructions	
Nodes	*DEC	DPA
1	[A|A] 0.50; [A|AC] 0.14; [A|AB] 0.11	A-0.99
2	[A|A] 0.30; [A|BC] 0.11; [A|C] 0.11	A-0.99
3	[A|A] 0.64	A-0.99
4	[A|A] 0.59; [A|AC] 0.35	A-1.00
5	[A|AC] 0.71; [A|A] 0.27	A-1.00
6	[A|A] 0.41; [ABC|A] 0.17; [AC|A] 0.12; [AB|A] 0.10	F-1.00
7	[A|A] 0.95	F-1.00
8	[A|A] 0.91	F-1.00
9	[A|A] 0.93	F-1.00
10	[A|A] 1.00	F-1.00
11	[A|A] 0.66	F-1.00
12	[A|A] 0.66; [AC|A] 0.11; [AB|A] 0.11	F-1.00
13	[A|A] 1.00	F-1.00
14	[C|B] 0.21; [AC|B] 0.14; [C|BC] 0.12; [A|B] 0.11	B-0.73; A-0.19; C-0.08
15	[C|C] 0.55; [C|AC] 0.41	C-0.98
16	[C|AC] 0.48; [C|C] 0.47	C-0.98
17	[B|B] 0.43; [BC|B] 0.21; [ABC|B] 0.12	B-0.98
18	[B|B] 0.35; [B|BC] 0.22; [B|ABC] 0.14	B-0.98
19	[B|B] 0.74; [B|BC] 0.13; [B|C] 0.12	B-0.99
20	[C|BC] 0.77; [C|B] 0.12; [C|C] 0.10	B-0.99
21	[C|B] 0.60; [C|AB] 0.33	B-0.63; C-0.25; A-0.12
22	[C|C] 1.00	C-1.00
23	[C|C] 1.00	C-1.00
24	[B|B] 0.44; [B|AB] 0.34; [B|A] 0.21	A-0.54; B-0.32; C-0.13
25	[A|AB] 0.75; [A|A] 0.17	A-1.00
26	[B|B] 0.87	B-1.00
27	[B|B] 0.84; [B|BC] 0.12	B-1.00
28	[B|B] 0.83	B-1.00
29	[B|B] 1.00	B-1.00
30	[B|B] 0.50; [B|AB] 0.17; [B|ABC] 0.10	B-1.00
31	[B|B] 0.93	B-1.00
32	[B|B] 0.86; [B|AB] 0.12	B-1.00
33	[A|ABC] 0.41; [A|AB] 0.40	A-0.54; B-0.46
34	[B|ABC] 0.87	A-0.54; B-0.46

* Split format: [left|right], where 'left' and 'right' are the ranges inherited by each descendant branch (on the tree, 'left' is the upper branch and 'right' the lower branch).

FIGURE CAPTIONS

Figure 1. Geographic distribution plots for all species of *Pilosocereus* (a), and for those species sampled in our ancestral range reconstructions analyses (b).

Figure 2. Maximum-clade-credibility (MCC) tree with 95% HPD confidence intervals for phylogenetic relationships and lineage divergence times obtained in BEAST for *Pilosocereus* (Analysis AE2). (a) Outgroup taxa and species of *Pilosocereus* subgenus *Gounellea* and *P. bohlei*; (b) *Pilosocereus sensu stricto* data set. Numbers above branches indicate mean ages and below branches correspond to posterior probability values. Clades with numbers in front of the nodes (in grey) are referenced in Appendix S5.

Figure 3. Ancestral biome reconstruction analysis using the Dispersal–Extinction–Cladogenesis (DEC) model implemented in Lagrange. The tree is the BEAST MCC tree (AE2) based on the *Pilosocereus sensu stricto* clade. Numbers above branches indicate mean ages; those below branches correspond to posterior probability values; numbers in front of each cladogenetic event (in grey) refer to nodes numbered in Table 1. (a) Discrete biome states used in the analysis. (b) Range inheritance scenarios receiving the highest relative likelihood in the DEC analysis, indicating the ranges inherited by each descendant branch; color codes as in (a). Empty squares to the right contain alternative, less probable range inheritance scenarios, where size is proportional to the relative likelihood of each scenario (those < 0.1 are not represented). Coloured circles close to taxon names indicate the present distributions.

Figure 4. Ancestral range reconstruction analysis using the MCMC Bayesian discrete phylogeographic approach (DPA) in BEAST applied to the *Pilosocereus sensu stricto* data set (A2 settings). Numbers above branches indicate mean ages and numbers in front of each cladogenetic event (in grey) refer to nodes numbered in Table 1. (a) Discrete geographic areas used in the analysis. (b) Chronogram showing results from the DPA analysis; coloured branches represent for each lineage the ancestral range receiving the highest marginal posterior probability. Pie charts at nodes represent uncertainty in the estimation, with black colour representing ancestral areas receiving < 0.1 pp values.

FIGURES

Figure 1

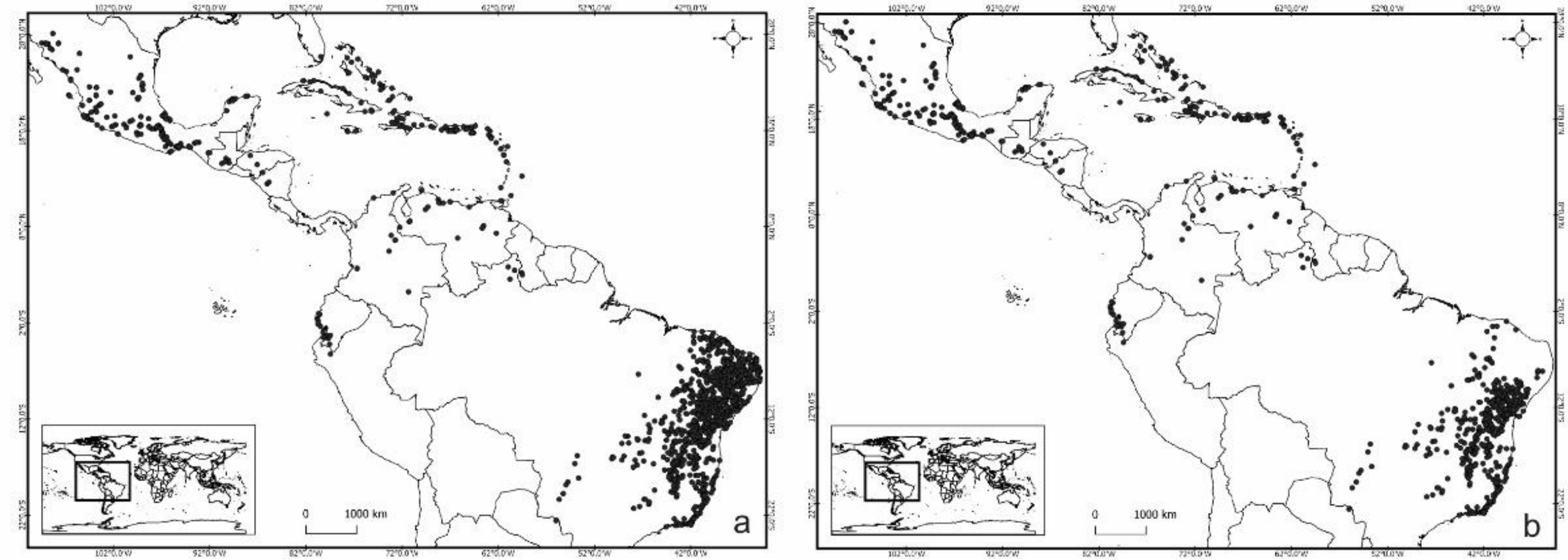


Figure 2

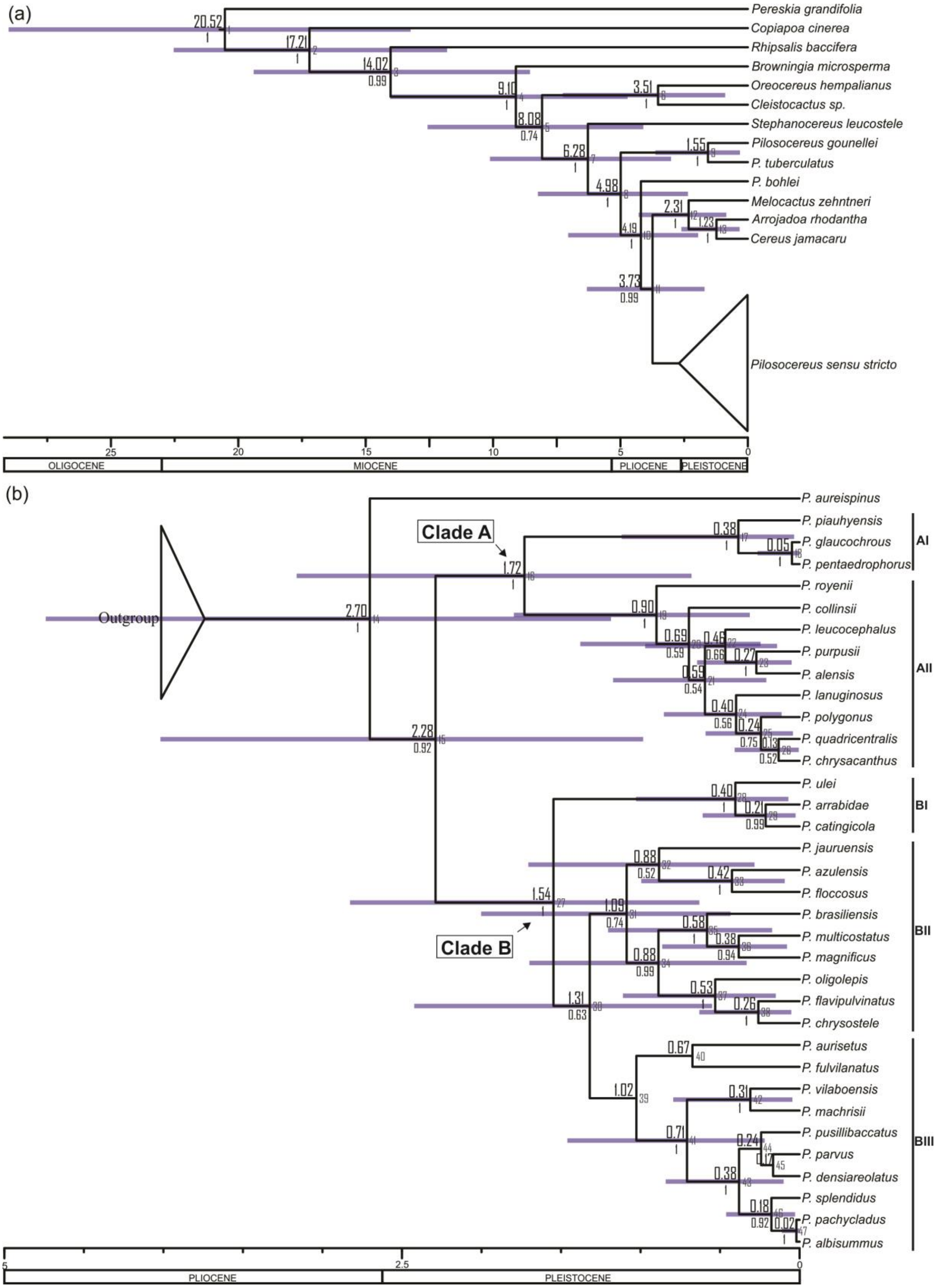

(a)
Pereskia grandifolia
Copiapoa cinerea
Rhipsalis baccifera
Browningia microsperma
Oreocereus hempalianus
Cleistocactus sp.
Stephanocereus leucostele
Pilosocereus gounellei
P. tuberculatus
P. bohlei
Melocactus zehntneri
Arrojadoa rhodantha
Cereus jamacaru
Pilosocereus sensu stricto
20.52
17.21
14.02
9.10
8.08
3.51
6.28
1.55
4.98
2.31
1.23
4.19
3.73
25
20
15
10
5
0
OLIGOCENE
MIOCENE
PLIOCENE
PLEISTOCENE
(b)
Outgroup
Clade A
Clade B
P. aureispinus
P. piauhyensis
P. glaucochrous
P. pentaedrophorus
P. royenii
P. collinsii
P. leucocephalus
P. purpusii
P. alensis
P. lanuginosus
P. polygonus
P. quadricentralis
P. chrysacanthus
P. ulei
P. arrabidae
P. catingicola
P. jauruensis
P. azulensis
P. floccosus
P. brasiliensis
P. multicostatus
P. magnificus
P. oligolepis
P. flavipulvinatus
P. chrysostele
P. aurisetus
P. fulvilanatus
P. vilaboensis
P. machrisii
P. pusillibaccatus
P. parvus
P. densiareolatus
P. splendidus
P. pachycladus
P. albisummus
AI
AII
BI
BII
BIII
2.70
2.28
1.72
0.38
0.05
0.90
0.69
0.46
0.27
0.59
0.40
0.24
0.13
1.54
0.40
0.21
1.09
0.88
0.42
0.58
0.38
0.88
0.53
0.26
1.31
1.02
0.67
0.31
0.71
0.24
0.17
0.38
0.18
0.02
5
2.5
0
PLIOCENE
PLEISTOCENE

Figure 3

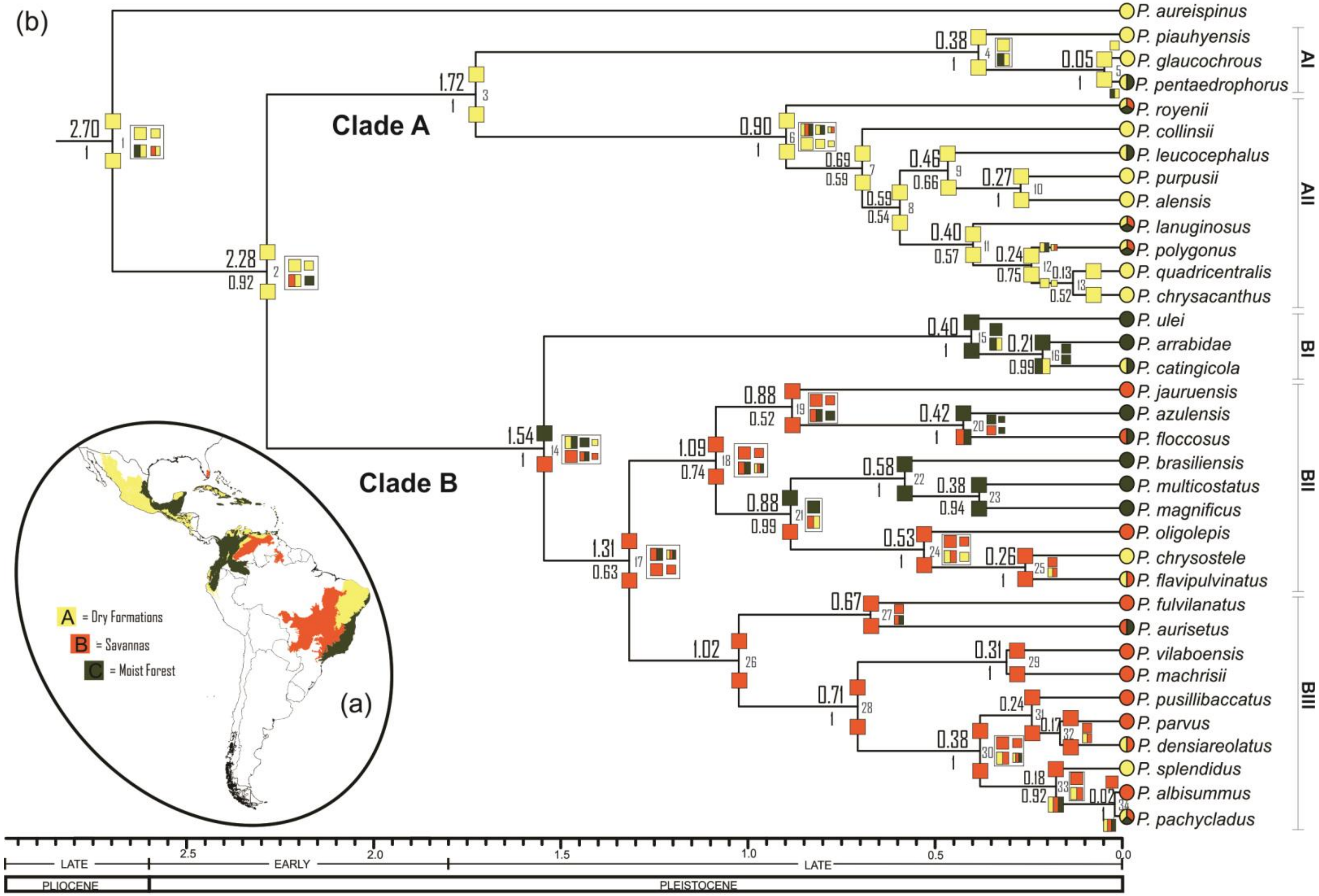
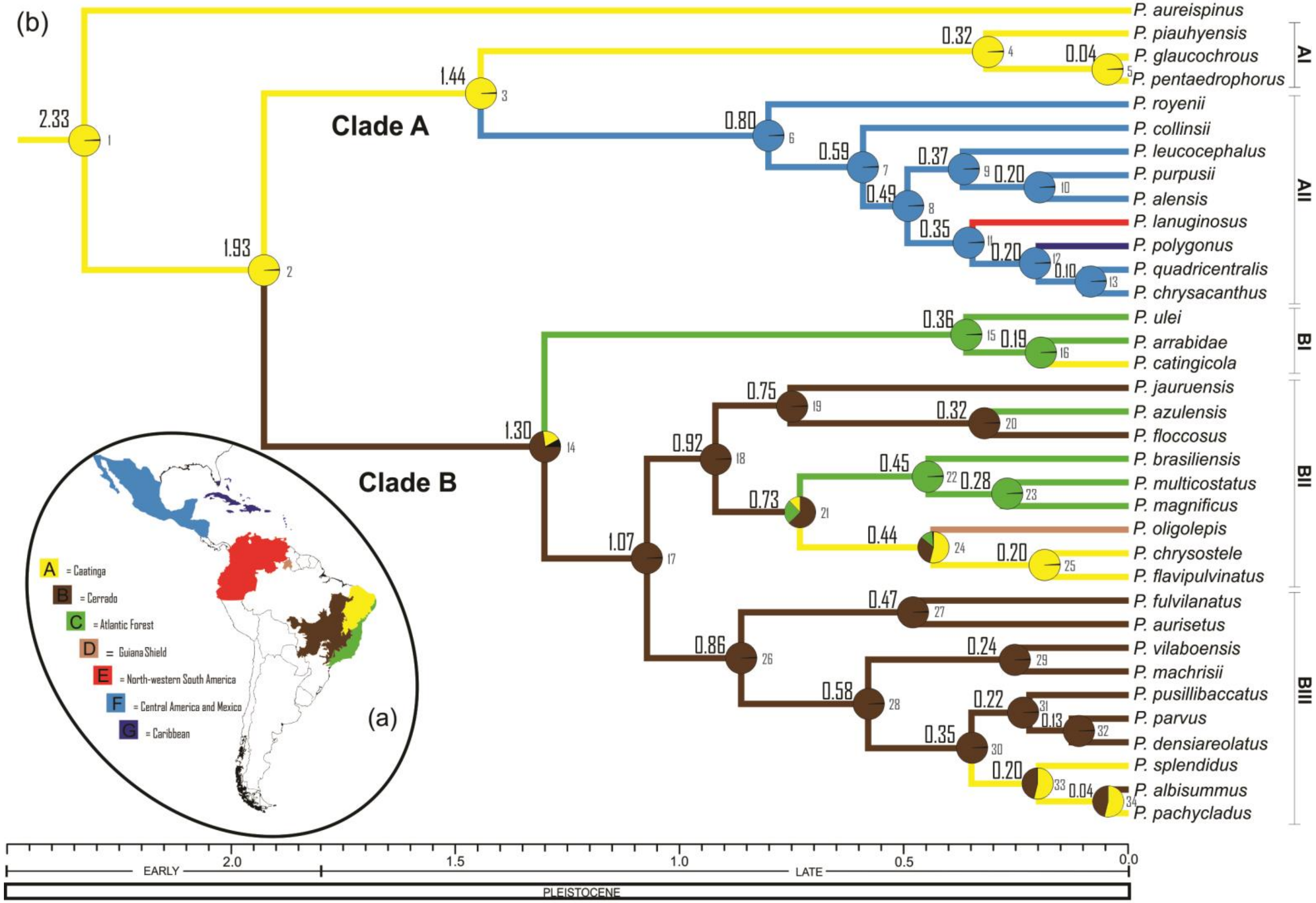

(b)
(a)
P. aureispinus
P. piauhyensis
P. glaucochrous
P. pentaedrophorus
P. royenii
P. collinsii
P. leucocephalus
P. purpusii
P. alensis
P. lanuginosus
P. polygonus
P. quadricentralis
P. chrysacanthus
P. ulei
P. arrabidae
P. catingicola
P. jauruensis
P. azulensis
P. floccosus
P. brasiliensis
P. multicostatus
P. magnificus
P. oligolepis
P. chrysostele
P. flavipulvinatus
P. fulvilanatus
P. aurisetus
P. vilaboensis
P. machrisii
P. pusillibaccatus
P. parvus
P. densiareolatus
P. splendidus
P. albisummus
P. pachycladus
AI
AII
BI
BII
BIII
Clade A
Clade B
2.70
1
2.28
0.92
1.72
1
0.38
1
0.05
1
0.90
1
0.69
0.59
0.59
0.54
0.46
0.66
0.27
1
0.40
0.57
0.24
0.75
0.13
0.52
0.40
1
0.21
0.99
1.54
1
0.88
0.52
0.42
1
1.09
0.74
0.58
1
0.38
0.94
0.88
0.99
0.53
1
0.26
1
1.31
0.63
0.67
1.02
0.31
1
0.71
1
0.24
0.17
0.38
1
0.18
0.92
0.02
1
A = Dry Formations
B = Savannas
C = Moist Forest
2.5
2.0
1.5
1.0
0.5
0.0
LATE
EARLY
LATE
PLIOCENE
PLEISTOCENE

Figure 4


(b)
(a)
Clade A
Clade B
2.33
1
1.93
2
1.44
3
0.32
4
0.04
5
0.80
6
0.59
7
0.49
8
0.37
9
0.20
10
0.35
11
0.20
12
0.10
13
1.30
14
0.36
15
0.19
16
1.07
17
0.92
18
0.75
19
0.32
20
0.73
21
0.45
22
0.28
23
0.44
24
0.20
25
0.86
26
0.47
27
0.58
28
0.24
29
0.35
30
0.22
31
0.13
32
0.20
33
0.04
34
P. aureispinus
P. piauhyensis
P. glaucochrous
P. pentaedrophorus
P. royenii
P. collinsii
P. leucocephalus
P. purpusii
P. alensis
P. lanuginosus
P. polygonus
P. quadricentralis
P. chrysacanthus
P. ulei
P. arrabidae
P. catingicola
P. jauruensis
P. azulensis
P. floccosus
P. brasiliensis
P. multicostatus
P. magnificus
P. oligolepis
P. chrysostele
P. flavipulvinatus
P. fulvilanatus
P. aurisetus
P. vilaboensis
P. machrisii
P. pusillibaccatus
P. parvus
P. densiareolatus
P. splendidus
P. albisummus
P. pachycladus
AI
AII
BI
BII
BIII
A = Caatinga
B = Cerrado
C = Atlantic Forest
D = Guiana Shield
E = North-western South America
F = Central America and Mexico
G = Caribbean
2.0
1.5
1.0
0.5
0.0
EARLY
LATE
PLEISTOCENE

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SUPPORTING INFORMATION

The role of Pleistocene climatic fluctuations in the geographic distribution and diversification of the widespread Neotropical genus *Pilosocereus* (Cactaceae)

Pâmela Lavor, Alice Calvente, Leonardo M. Versieux, Isabel Sanmartín

Appendix S1 Supplementary tables

Appendix S1, Table S1. Species of Cactaceae family used in this study with voucher information and GenBank accession numbers (Newly sequences generated are printed in bold).

Taxon (code)	Collector/Voucher herbarium	Location	*trn*S-*trn*G	*psb*D-*trn*T	*trn*L-*trn*T	*pet*L-*psb*E	PHYC	*ycf*1
Pereskioideae								
Pereskia grandifolia Haw.	Damaso P./UFRN 7796	Rio Grande do Norte, Brazil	-	**KX387707**	**KX387772**	**KX387741**	**XXXXXXXX**	**XXXXXXXX**
Cactoideae								
Copiapoa cinerea (Phil.) Britton & Rose	Rodrigues R. 3120/MA702058/11019	Antofagasta, Chile	-	**KX387702**	**KX387767**	**KX387736**	**XXXXXXXX**	**XXXXXXXX**
Rhipsalideae								
Rhipsalis baccifera (J.S.Mueller) Stearn.	Rodríguez A. 5318/MA733603	Limón, Costa Rica	**KX387797**	**KX387704**	**KX387769**	**KX387738**	**XXXXXXXX**	**XXXXXXXX**
Cereeae								
Rebutiinae								
Browningia microsperma (Werderm. & Backeb.) W.T.Marshall.	Madsen JE. 7311/MA752144	Loja, Ecuador	**KX387798**	**KX387705**	**KX387770**	**KX387739**	**XXXXXXXX**	**XXXXXXXX**
Cereinae								
Arrojadoa rhodantha Britton & Rose	Machado M. 777/HUEFS107367	Bahia, Brazil	KX301205	KX301086	KX301167	KX301129	KX301244	**XXXXXXXX**
Cereus jamacaru DC	Calvente A. 461/UFRN	Bahia, Brazil	KX301200	KX301076	KX301162	KX301119	KX301238	**XXXXXXXX**
Melocactus zehntneri (Britton & Rose) Luetzelb.	Calvente A. 462/UFRN	Rio Grande do Norte, Brazil	KX301198	KX301074	KX301160	KX301117	KX301236	**XXXXXXXX**
Pilosocereus albisummus	Moraes E.M. S141/SORO4530	Minas Gerais, Brazil	KX301216	KX301097	KX301178	KX301140	KX301255	**XXXXXXXX**
P. alensis (F.A.C.Weber) Byles & G.D.Rowley	Sánchez-Mejorada H. 4449/MEXU	Jalisco, Mexico	KX301188	KX301064	KX301150	KX301107	KX301226	**XXXXXXXX**
P. arrabidae (Lem) Byles & G.D.Rowley	Machado MC. S35M1/SORO4488	Bahia, Brazil	**KX387801**	**KX387714**	**KX387774**	**KX387747**	**XXXXXXXX**	**XXXXXXXX**
P. aureispinus (Buining & Brederoo) F.Ritter	Moraes EM. S21/HUFS642	Bahia, Brazil	JN035414/JN035456	KX301080	KX301163	KX301123	KX301240	-
P. aurisetus (Werderm.) Byles & G.D.Rowley	Moraes EM. S11/HUFS646	Minas Gerais, Brazil	JN035403/JN035437	**KX387709**	JN035585	**KX387743**	KC779292.1	**XXXXXXXX**
P. azulensis (F.A.C.Weber ex Rol.-Goss) Byles & G.D.Rowley	Olsthoorn G. 253/SORO 4531	Minas Gerais, Brazil	KX301214	KX301095	KX301176	KX301138	KX301253	**XXXXXXXX**
P. bohlei Hofacker	Moraes EM. S51/CCTS3000	Bahia, Brazil	**KX387802**	**KX387715**	**KX387775**	**KX387748**	**XXXXXXXX**	**XXXXXXXX**
P. brasiliensis (Britton & Rose) Backed	Franco FF. S79E /SORO2654	Espirito Santo, Brazil	KX301223	KX301104	KX301185	KX301147	KX301262	**XXXXXXXX**
P. catingicola (Gürke) Byles & G.D.Rowley	Olsthoorn G. 1026/SORO4532	Bahia, Brazil	KX301217	KX301098	KX301179	KX301141	KX301256	**XXXXXXXX**
P. chrysacanthus (F.A.C.Weber) Byles & G.D.Rowley	Arias S. 858/ MEXU	Oaxaca, Mexico	KX301190	KX301066	KX301152	KX301109	KX301228	**XXXXXXXX**
P. chrysostele (Vaupel) Byles & G.D.Rowley	Lavor P. 36/UFRN17127	Ceará, Brazil	**KX387789**	**KX387695**	**KX387761**	**KX387729**	-	**XXXXXXXX**
P. collinsii (Britton & Rose) Byles & G.D.Rowley	Arias S. 1658/MEXU	Chiapas, Mexico	**KX387784**	**KX387690**	**KX387756**	**KX387724**	-	**XXXXXXXX**
P. densiareolatus F.Ritter	Moraes EM. S43/SORO2650	Minas Gerais, Brazil	KX301208	KX301089	KX301170	KX301132	KX301247	**XXXXXXXX**
P. flavipulvinatus (Buining & Brederoo) F.Ritter	Lavor P. 71/-	Ceará, Brazil	**KX387799**	**KX387706**	**KX387771**	**KX387740**	-	**XXXXXXXX**
P. floccosus (Backeb. & Voll) Byles & G.D.Rowley	Olsthoorn G. 42/SORO4558	Minas Gerais, Brazil	KX301220	KX301101	KX301182	KX301144	KX301259	**XXXXXXXX**
P. fulvilanatus (Buining & Brederoo) F.Ritter	Moraes EM. S42/SORO2655	Minas Gerais, Brazil	KX301207	KX301088	KX301169	KX301131	KX301246	**XXXXXXXX**
P. glaucochrous (Werderm.) Blyles & G.D.Rowley	Machado MC. S35M2/SORO 4536	Bahia, Brazil	KX301202	KX301083	KX301164	KX301126	-	-
P. gounellei (F.A.C.Weber ex K.Schum.) Byles & G.D.Rowley	Lavor P. 08/UFRN16223	Piauí, Brazil	**KX387787**	**KX387693**	**KX387759**	**KX387727**	**XXXXXXXX**	**XXXXXXXX**

Table 1 (Continue)

Taxon (code)	**Collector/Voucher herbarium**	**Location**	***trn*S-*trn*G**	***psb*D-*trn*T**	***trn*L-*trn*T**	***pet*L-*psb*E**	**PHYC**	***ycf*1**
P. jauruensis (Buining & Brederoo) P.J.Braun	Moraes EM. S23/HUFS 638	Mato Grosso do Sul, Brazil	KC779348.1	**KX387713**	KC779348.1	**KX387746**	**XXXXXXXX**	**XXXXXXXX**
P. lanuginosus (L.) Byles & G.D.Rowley	Mero 96	?	**KX387791**	**KX387697**	**KX387763**	**KX387731**	**XXXXXXXX**	**XXXXXXXX**
P. leucocephalus (Poselg.) Byles & G.D.Rowley	Arias S. 1654/MEXU	Chiapas/Mexico	KX301193	KX301069	KX301155	KX301112	KX301231	-
P. machrisii (E.Y.Dawson) Backeb.	Moraes EM. S18/HUFS 648	Goiás, Brazil	KC779332.1	**KX387710**	KC621149.1	**KX387744**	**XXXXXXXX**	**XXXXXXXX**
P. magnificus (Buining & Brederoo) F.Ritter	Moraes EM. S37/SORO4550	Minas Gerais, Brazil	**KX387805**	**KX387718**	**KX387777**	**KX387751**	**XXXXXXXX**	**XXXXXXXX**
P. multicostatus F.Ritter	Moraes EM. S41/SORO2649	Minas Gerais, Brazil	**KX387806**	**KX387719**	**KX387778**	**KX387752**	**XXXXXXXX**	**XXXXXXX**
P. oligolepis (Vaupel) Byles & G.D.Rowley	Lavor et al., 60/UFRN 18663	Roraima, Brazil	**KX387792**	**KX387698**	**KX387764**	**KX387732**	**XXXXXXXX**	**XXXXXXXX**
P. pachycladus F.Ritter	Moraes EM. S45/SORO2647	Minas Gerais, Brazil	KX301209	KX301090	KX301171	KX301133	KX301248	**XXXXXXXX**
P. parvus (Diers & Esteves) P.J.Braun	Moraes EM. S47/SORO2648	Goiás, Brazil	**KX387808**	**KX387721**	**KX387780**	**KX387754**	**XXXXXXXX**	**XXXXXXXX**
P. pentaedrophorus (Labour.) Byles & G.D.Rowley	G Olsthoorn 167/SORO4537	Bahia, Brazil	XXXXXXXX	XXXXXXX	XXXXXXXX	XXXXXXX	XXXXXXXX	XXXXXXXX
P. piauhyensis (Gürke) Byles & G.D.Rowley	Lavor P. 14/UFRN16219	Piauí, Brazil	**KX387786**	**KX387692**	**KX387758**	**KX387726**	**XXXXXXXX**	-
P. polygonus (Lam.) Byles & G.D.Rowley	DNA Bank Kew 45353	-	**KX387794**	**KX387700**	-	**KX387734**	**XXXXXXXX**	**XXXXXXXX**
P. purpusii (Britton & Rose) Byles & G.D.Rowley	Blancas Vázquez JJ. 119/MEXU	Nayarit, Mexico	KX301189	KX301065	KX301151	KX301108	KX301227	**XXXXXXXX**
P. pusillibaccatus P.J.Braun & Esteves	Lavor P. 20/UFRN16225	Piauí, Brazil	KX301195	KX301071	KX301157	KX301114	KX301233	**XXXXXXXX**
P. quadricentralis (E.Y.Dawson) Backeb.	Arias S. 2180/MEXU	Oaxaca, Mexico	KX301187	KX301063	KX301149	KX301106	KX301225	**XXXXXXXX**
P. royenii (L.) Byles & G.D.Rowley	S Arias 1098/MEXU	Yucatán, Mexico	KX301192	KX301068	KX301154	KX301111	KX301230	-
P. splendidus F.Ritter	Moraes EM. S139/SORO4539	Bahia, Brazil	KX301213	KX301094	KX301175	KX301137	KX301252	**XXXXXXXX**
P. tuberculatus (Werderm.) Byles & G.D.Rowley	Lavor P. 47/UFRN18650	Pernambuco, Brazil	**KX387790**	**KX387696**	**KX387762**	**KX387730**	**XXXXXXXX**	**XXXXXXXX**
P. ulei (K.Schum.) Byles & G.D.Rowley	Franco FF. S79/SORO4557	Rio de Janeiro, Brazil	KX301221	KX301102	KX301183	KX301145	KX301260	**XXXXXXXX**
P. vilaboensis (Diers & Esteves) P.J.Braun (2)	Moraes EM. S19/CCTS3001	Goiás, Brazil	KC779340.1	KX301079	KC621157.1	KX301122	KC779305.1	**XXXXXXXX**
Stephanocereus leucostele (Guerke) A.	Calvente A. 413/UFRN 13195	Bahia, Brazil	KX301199	KX301075	KX301161	KX301118	KX301237	**XXXXXXXX**
Trichocereinae								
Cleistocactus sp. Lem.	Aedo C. 14512/MA759900	Cochabamba, Bolivia	**KX387795**	**KX387701**	**KX387766**	**KX387735**	**XXXXXXXX**	**XXXXXXXX**
Oreocereus hempelianus (Gürke) D.R.Hunt.	Aedo C. 11369/MA728565	Moquegua, Peru	**KX387796**	**KX387703**	**KX387768**	**KX387737**	**XXXXXXXX**	**XXXXXXXX**

Abbreviations— BHCB: Herbário Universidade Federal de Minas Gerais; CCTS: Herbário da Universidade Federal de São Carlos (Campus Sorocaba), Brazil; CEPEC: Herbário CEPEC; HUEFS: Herbário Universidade Federal de Feira de Santana, Brazil; SORO: Herbário do Centro de Ciências e Tecnologia para Sustentabilidade, Universidade Federal de São Carlos (Campus Sorocaba), Brazil; UFRN: Herbário Universidade Federal do Rio Grande do Norte, Brazil; MA: Vascular Plant Herbarium, Real Jardín Botánico de Madrid, Espanha.

Appendix S1, Table S2. Primers and PCR conditions for the amplification of the different genomic regions used in this study.

Region	Primers	Source	Primer F-R (µL)	PCR buffer (µL)	MgCl2 (µL)	DNTPs (µL)	***Taq 1U*** **(µL)**	Final volume (µL)	PCR conditions
*pet*L-*psb*E	petL: AGTAGAAAACCGAAATAACTAGTT A psbE: TATCGAATACTGGTAATAATATCAGC	Shaw et al., 2007	0.5	5	2.5	0.5	0.2	25	80°C/5 min, followed by 30 cycles of 95°C/1 min, 50ºC/1 min, 65°C/4 min and finishing at 65°C/5 min;
*psb*D-*trn*T^{GGU}	psbD: CTCCGTARCCAGTCATCCATA trnT(GGU)-R: CCCTTTTAACTCAGTGGTAG	Shaw et al., 2007	0.25	5	2.5	0.5	0.2	25	
*trn*L-*trn*T	5’trnLUAAR(TabB): TCTACCGATTTCGCCATATC trnT^{UGU}F (TabA): CATTACAAATGCGATGCTCT	Taberlet et al., 1991	0.25	5	3	0.5	0.2	25	
*trn*S-*trn*G	5’trnG2S: TTTTACCACTAAACTATACCCGC SGFwd2: CACCCATGGTTCCCATTAGA trnS^{GCU}: AGATAGGGATTCGAACCCTCGGT SGRev2: TCCGCTCATTAGCTCTCCTC	Shaw et al., 2005 Bonatelli et al., 2013	0.25	5	2.75	0.6	0.2	25	80°C/5 min, followed by 40 cycles of 95°C/1 min, 62ºC/1 min, 65°C/5 min and finishing at 65°C/5 min;
PHYC	PhyF: AGCTGGGGCTTTCAAATCTT PhyR: TCCTCCACTTGACCACCTCT	Helsen et al., 2009	0.4	5	3	0.5	0.2	25	94°C/5 min, followed by 33 cycles of 94°C/1 min, 55ºC/90 sec, 72°C/2 min and finishing at 72°C/9 min;
*ycf*1	ycf1-4182F*: AAATAYRRATAGAAAATATTTKGATT ycf1-5248R*: GAATTCTYAATTCTCTACGACG	Franck et al., 2012	1.6	4	3	0.5	0.2	19	94°C/3 min, followed by 40 cycles of 94°C/45 sec, 45ºC/45 sec, 72°C/2 min and 30 sec and finishing at 72°C/5 min.

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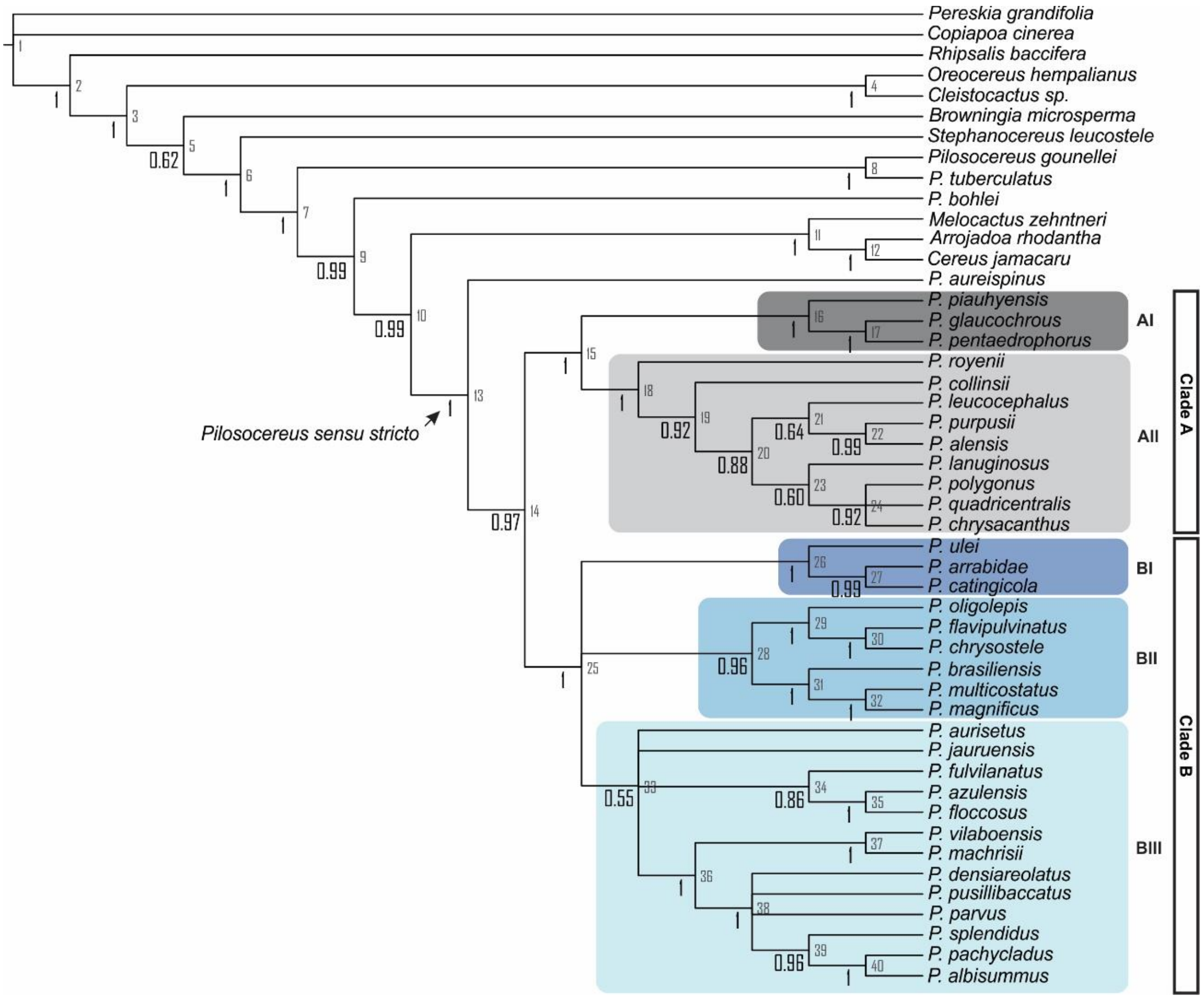
Appendix S2 Supplementary results

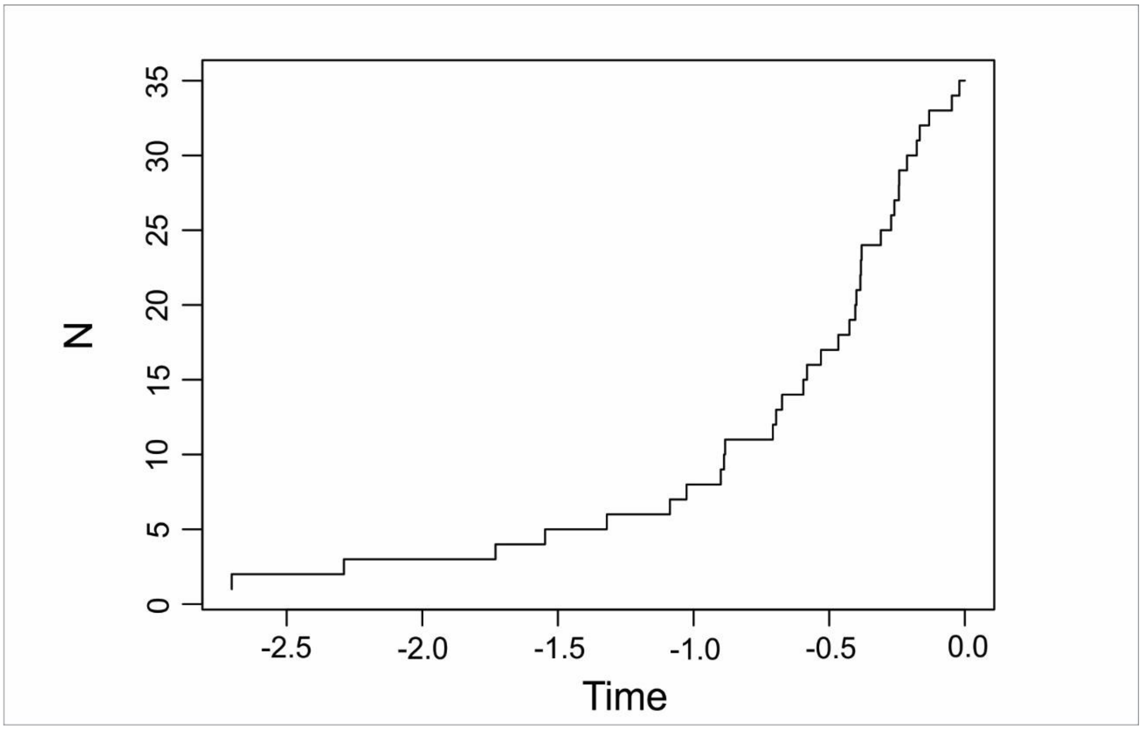
Appendix S2, Figura S1. Majority rule consensus tree derived from Bayesian analyses to of the combined dataset of (*trn*S-*trn*G, *psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E, PHYC and *ycf*1) produced for *Pilosocereus* (ingroup and outgroups). Posterior Probability (PP) values are shown below branches and clades numbered are in front (grey) branches.



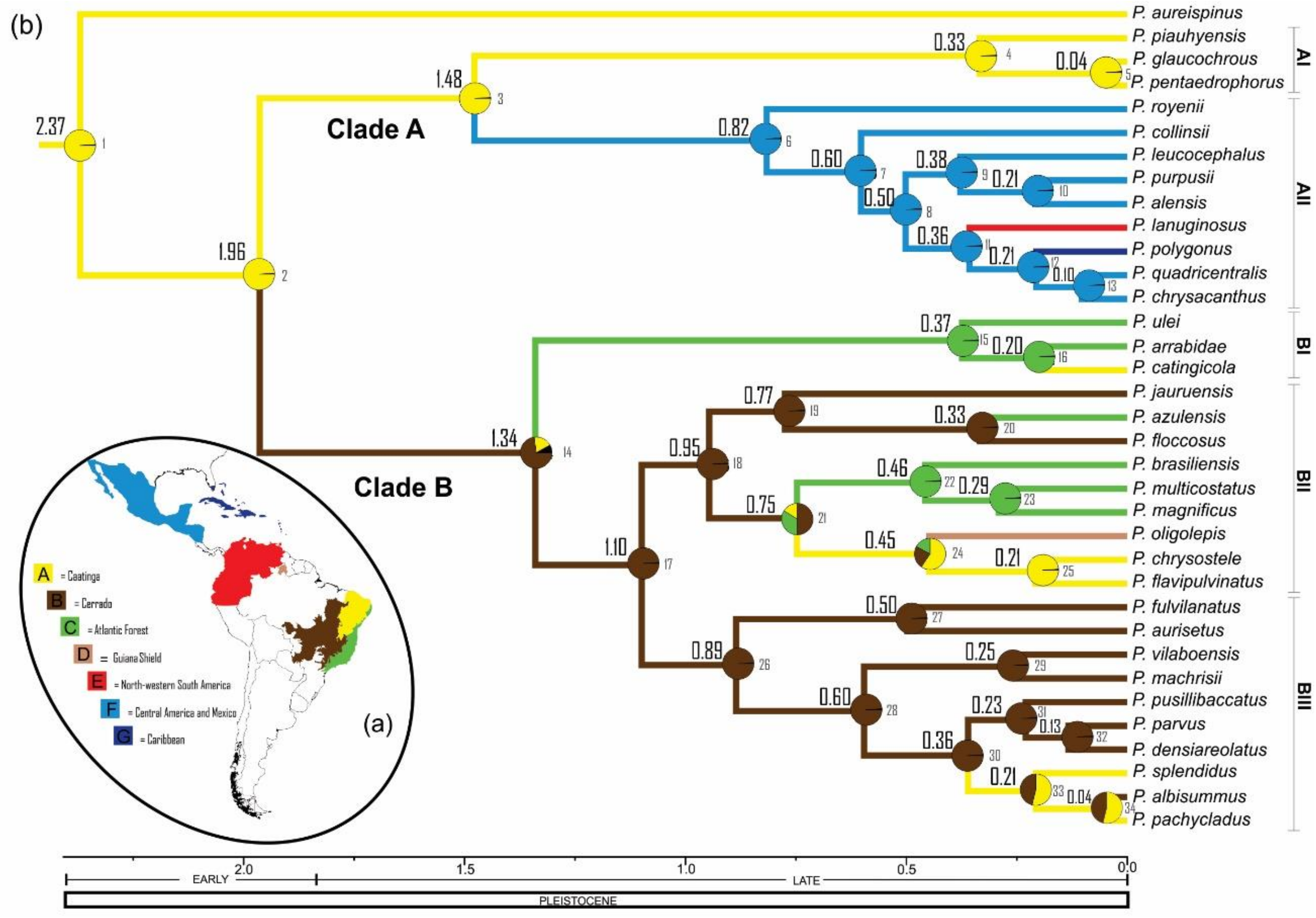
Appendix S2, Table S1. Results of the mean divergence age (95% HPD) in Ma from different calibrations for *Pilosocereus* data set (number of the node in Figure 2).

Nodes	Analysis AE1	Analysis AE2	Analysis AE3
1	27.59 (24.00-31.25)	20.52 (13.08-28.85)	18.03 (11.37-26.01)
2	22.30 (19.15-25.40)	17.21 (11.81-22-54)	15.07 (10-16-20.21)
3	18.41 (13.18-22.87)	14.02 (8.55-19.40)	11.35 (6.88-16.41)
4	12.09 (7.35-17.14)	9.10 (4.77-14.03)	6.29 (4.30-8.39)
5	10.70 (6.29-15.40)	8.08 (4.11-12.54)	5.61 (3.64-7.62)
6	4.62 (1.44-9.10)	3.51 (0.89-7.26)	2.52 (0.70-4.80)
7	8.27 (4.63-12.46)	6.28 (3.07-10.15)	4.43 (2.68-6.36)
8	6.56 (3.42-10.00)	4.98 (2.35-8.21)	3.54 (2.07-5.27)
9	2.06 (0.40-4.56)	1.55 (0.31-3.60)	1.14 (0.25-2.47)
10	5.54 (2.87-8.62)	4.19 (1.95-7.04)	2.99 (1.66-4.52)
11	4.93 (2.54-7.79)	3.73 (1.70-6.31)	2.67 (1.47-4.09)
12	3.09 (1.24-5.44)	2.31 (0.84-4.29)	1.71 (0.71-2.86)
13	1.64 (0.48-3.37)	1.23 (0.32-2.61)	0.91 (0.26-1.81)
14	3.57 (1.74-5.81)	2.70 (1.17-4.71)	1.95 (1.02-3.11)
15	3.02 (1.47-4.94)	2.28 (1.0-4.02)	1.65 (0.82-2.64)
16	2.28 (1.05-3.91)	1.72 (0.67-3.14)	1.25 (0.59-2.14)
17	0.52 (0.05-1.45)	0.38 (0.03-1.11)	0.28 (0.03-0.78)
18	0.06 (0.0-0.35)	0.05 (0.0-0.26)	0.04 (0.0-0.19)
19	1.20 (0.48-2.26)	0.90 (0.31-1.77)	0.66 (0.26-1.22)
20	0.93 (0.40-1.72)	0.69 (0.25-1.37)	0.51 (0.22-0.94)
21	0.79 (0.31-1.46)	0.59 (0.21-1.17)	0.43 (0.18-0.80)
22	0.62 (0.21-1.22)	0.46 (0.14-0.96)	0.34 (0.14-0.67)
23	0.37 (0.08-0.82)	0.27 (0.05-0.64)	0.20 (0.05-0.45)
24	0.53 (0.17-1.08)	0.40 (0.11-0.84)	0.29 (0.09-0.58)
25	0.32 (0.06-0.75)	0.24 (0.04-0.59)	0.18 (0.03-0.41)
26	0.18 (0.01-0.52)	0.13 (0.01-0.41)	0.10 (0.0-0.28)
27	2.05 (0.98-3.54)	1.54 (0.63-2.81)	1.12 (0.55-1.89)
28	0.53 (0.10-1.32)	0.40 (0.07-1.02)	0.29 (0.06-0.70)
29	0.28 (0.03-0.78)	0.21 (0.03-0.61)	0.16 (0.02-0.42)
30	1.75 (0.85-3.04)	1.31 (0.54-2.40)	0.95 (0.46-1.60)
31	1.43 (0.63-2.49)	1.09 (0.43-1.99)	0.78 (0.36-1.34)
32	1.17 (0.42-2.15)	0.88 (0.29-1.71)	0.64 (0.25-1.16)
33	0.56 (0.13-1.28)	0.42 (0.10-0.99)	0.31 (0.08-0.68)
34	1.16 (0.48-2.13)	0.88 (0.33-1.68)	0.64 (0.28-1.16)
35	0.70 (0.21-1.39)	0.58 (0.17-1.19)	0.42 (0.14-0.83)
36	0.34 (0.08-0.79)	0.38 (0.08-0.86)	0.28 (0.06-0.61)
37	0.77 (0.24-1.52)	0.53 (0.15-1.10)	0.38 (0.12-0.77)
38	0.51 (0.12-1.10)	0.26 (0.05-0.63)	0.19 (0.04-0.44)
39	1.36 (-)	1.02 (-)	0.74 (-)
40	0.89 (-)	0.67 (-)	0.48 (-)
41	0.93 (0.32-1.85)	0.71 (0.22-1.46)	0.51 (0.18-0.99)
42	0.41 (0.07-1.01)	0.31 (0.05-0.80)	0.22 (0.04-0.54)
43	0.50 (0.15-1.08)	0.38 (0.10-0.84)	0.28 (0.08-0.58)
44	0.32 (-)	0.24 (-)	0.17 (-)
45	0.22 (-)	0.17 (-)	0.12 (-)
46	0.23 (0.04-0.59)	0.18 (0.03-0.46)	0.13 (0.02-0.32)
47	0.03 (0.0-0.15)	0.02 (0.0-0.11)	0.01 (0.0-0.08)

Appendix S2, Figure S2. Numbers of lineages through time to *Pilosocereus sensu stricto* data set.



Appendix S2, Figure S3. Ancestral range reconstruction analysis using the MCMC Bayesian discrete phylogeographic approach (DPA) in BEAST applied to the *Pilosocereus sensu stricto* data set (A2 settings) with migration rates constrained by geographic distance.



CAPITULO 2

PHYLOGENETIC RELATIONSHIPS OF *PILOSOCEREUS* (CACTACEAE) AND TAXONOMIC IMPLICATIONS

Manuscrito a ser submetido a *Systematic Botany*

Lavor et al.: PHYLOGENY AND TAXONOMY OF *PILOSOCEREUS*

Phylogenetic relationships of *Pilosocereus* (Cactaceae) and taxonomic implications

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Abstract*—**The aim of this paper was to investigate the phylogenetic relationships within *Pilosocereus* (Cactaceae, Cactoideae), one of the most emblematic genera of cacti of the neotropical dry woodlands. The genus includes 42 species and 8 subspecies placed in two subgenera: *Pilosocereus* subg. *Pilosocereus* and *Pilosocereus* subg. *Gounellea*. We used an expanded sampling and a broad coverage of genomic regions (*psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E, *trn*S-*trn*G, *ycf*1 and *Phy*C) resulting in an aligned concatenated matrix of 4563 bp length. Maximum parsimony and Bayesian analyses recovered a non-monophyletic *Pilosocereus* as well as four consistently non-monophyletic species. We propose taxonomic changes to accommodate para- or polyphyletic taxa including the resurrection of three species, three new synonyms, two new names and a new circumscription for *Pilosocereus* with 42 species and four subspecies. ***Xiquexique is proposed as new genus, composed of species formally positioned in the *P*. subg. *Gounellea* (3 sp.), and is characterized by molecular and morphological characters.

***KeyWords*—**Cacti, Dry woodland, Molecular phylogenetics, Xerophytes, *Xique-xique*.

Monophyly, the direct relationship of an ancestor and all his descendent lineages forming a clade, has been considered a key point in delimiting groups in contemporary systematics. Although initially questioned by some authors, that considered necessary to stretch the monophyletic requirement in order to retain the very natural and useful grouping of organisms in the last century (see Cronquist 1968), currently many taxonomical rearrangements are proposed following such rule (Clark et al. 2000; Bartish et al. 2002; Hardy and Faden 2004; Baker et al. 2006; Metzgar et al. 2008; Su et al. 2010; Middleton and Livshultz 2012; Xue et al. 2014; Ortiz-Rodriguez et al. 2016). As more phylogenetic hypothesis emerges at the generic level using broad species sampling, particularly in speciose taxa from high diversity tropical areas, many species traditionally accepted may appear as para- or polyphyletic. Such results may be a consequence of biological processes (e. g. hybridization, incomplete lineage sorting) or mere artifacts of the earlier interpretation and taxonomy of the species. In the present work, we a use a case of genus, *Pilosocereus* Byles & Rowley (Cactaceae), with high species density across the Neotropical region to test for species delimitation using six molecular regions.

Cactaceae is one of the most representative families in the Neotropics, with 130 genera and 1,850 species (Nyffeler and Eggli 2010). Striking features of the group include: the presence of succulent photosynthetic stems; CAM metabolism; specialized root system (with high water absorption capacity) (Edwards and Diaz 2006); reduced or absent leaves; areoles (axillary meristematic regions where distinct structures, like branches, bristles, flowers and fruit arise) commonly displaying a great amount of spines, hairs and bristles (Gibson and Nobel 1986; Nyffeler 2002), as well as a great diversity of growth forms and habits (Hernández-Hernández et al. 2011). The group is highly variable and with many conspicuous, dominant and relevant taxa in arid and deserted areas in the Americas (Nyffeler 2002; Hernández-Hernández et al. 2011; Hernández-Hernández et al. 2014).

Among the four subfamilies (Cactoideae, Opuntioideae, Pereskioideae and Maihuenioideae) Cactoideae is the largest with six tribes and about 1,530 species (Nyffeler and Eggli 2010), which make up about 83% of the total family species richness. Cereeae is the largest of Cactoideae tribes, with 41 genera and 589 species (subdivided into the subtribes Rebutiinae, Cereinae and Trichocereinae), presenting a great variety of growth forms (Nyffeler and Eggli 2010). With the exception of *Harrisia* Britton, *Melocactus* Link & Otto and *Pilosocereus*, Cereeae could be recognized as an exclusively South American tribe (Nyffeler and Eggli 2010). In phylogenetic studies encompassing the whole family or for large groups (such as subfamilies and tribes) Cereeae (sensu Nyffeler and Eggli 2010, corresponding to the BCT clade) is nested within the RNBCT clade (core Rhipsalideae, core Notocacteae, plus BCT) (Nyfeller 2002; Bárcenas et al. 2011).

Pilosocereus is one of the largest genera of the tribe Cereeae and subtribe Cereinae, in number of species and range of distribution (Hunt et al. 2006). It currently includes 42 species and 8 subspecies distributed from USA to the east of Brazil (Hunt et al. 2006; Zappi and Taylor 2011). It comprises columnar cacti with long or short areolar hairs (reproductive or non-reproductive); absent cephalium or with a pseudocephalium in some species (such as *P. densiareolatus* and *P. chrysostele*) (Zappi 1994). The flowers are usually nocturnal and the fruits are dehiscent by transversal slits, depressed-globose, with persistent floral remnants (erect or pendent) and with white or colored funicular pulp (Zappi 1994). *Pilosocereus* species may be shrubs or tree-like, densely branched above or at the base level (or unbranched), and with a wide variation in number and color of spines and number of ribs (Zappi 1994).

A single taxonomic revision published for the genus (Zappi 1994) proposed the division of *Pilosocereus* into two subgenera: *Pilosocereus* subg. *Pilososcereus* and *Pilosocereus* subg. *Gounellea*. *Pilosocereus* subg. *Gounellea* is recognized by the candelabriform branching pattern; sinuate ribs sinuses with conspicuous podaria beneath the areoles; and erect to pendent (not immersed at the apex of the fruit) floral remnants. It includes *P. gounellei*, *P. tuberculatus* and, more recently, *P. frewenii* Zappi & Taylor (Zappi and Taylor 2011). *Pilosocereus* subg. *Pilosocereus* includes all the remaining species of the genus (39 spp.) and is characterized by the erect branching pattern, straight rib sinuses and pendent floral remnants (immersed in the apex of the fruit) (Fig. 1). In addition, Zappi (1994) proposed the delimitation of five informal groups within *P.* subg. *Pilosocereus* based on floral morphology, spine and habit of the species.

Some studies have been developed for groups of species within the genus, especially for the group *P. aurisetus* (sensu Zappi 1994), which is composed of a complex of species with great morphological variety, overlapping diagnostic characters and taxonomic uncertainty (Jesus 2010; Moraes et al. 2012; Bonatelli et al. 2013, 2014, 2015; Menezes et al. 2016; Perez et al. 2016). However, the great majority of the studies involving *Pilosocereus* focused on isolated species and on various aspects such as morphology (e. g. Godofredo 2009; Menezes and Loiola, 2015), reproductive biology (e. g. Locatelli et al. 1997; Rivera-Marchand and Ackerman 2006; Lucena 2007; Rocha et al. 2007a,b; Meiado et al. 2008; Munguia-Rosas et al. 2009; Abud et al. 2010; Martins et al. 2012), and population genetics (e. g. Nassar et al. 2003; Moraes et al. 2005; Figueredo et al. 2010; Kattab et al. 2014; Monteiro et al. 2015).

Calvente et al. (2016) recently published the only comprehensive molecular phylogeny focused in *Pilosocereus* to date and demonstrated the non-monophyly of the genus. Despite presenting fairly well-resolved trees, Calvente et al. (2016) suggested that additional studies (encompassing more species and different markers) are still necessary for a better resolution of relationships within and outside the genus. Hunt et al. (2006) mentioned that the “great battle” of delimitation in Cactaceae occurs at the generic level, since it is in this rank that the greatest uncertainties and taxonomic instabilities occur. Many cacti genera harbor a large number of species with morphological similarity that can be only result of convergent evolution, a process thought to be common in the family (Hernández-Hernández et al. 2011). Consequently, hindered by their superficial similarities, many studies of molecular phylogenetics have been recovering non-monophyletic taxa, requiring further investigation with increased taxa sampling and various genomic regions and the reevaluation of the traditional circumscriptions (Bárcenas et al. 2011).

Thus, to improve the knowledge of the relationships within *Pilosocereus* and among the lineages of the genus with other groups of the subtribe Cereinae, we added new taxa, samples and molecular data for *Pilosocereus* and outgroups to the data set used by Calvente et al. (2016) and present a broader and more robust phylogeny in this paper. We use this phylogenetic backbone to evaluate the monophyly of taxa in the group and propose taxonomic changes.

MATERIALS AND METHODS

Taxon Sampling **—** In order to better define and confirm relationships of lineages of *Pilosocereus* in subtribal and tribal levels we used an outgroup sampling with ten taxa from two distinct subfamilies and from main clades in Cactoideae (according to the phylogenetic hypothesis presented in Hernández-Hernández et al. 2014): *Pereskia grandifolia* (subfamily Pereskioideae); *Copiapoa cinerea*, *Rhipsalis baccifera*, *Browningia microsperma*, *Cleistocactus* sp., *Oreocereus hempelianus, Arrojadoa rhodantha*, *Cereus jamacaru*, *Melocactus zehntneri* and *Stephanocereus leucostele* (subfamily Cactoideae) (Table 1). For *Pilosocereus*, we include 44 ingroup taxa reaching nearly 88% of the species of the group (36 sp. and 8 subsp. out of 41 sp. and 9 subsp. according to Hunt et al. (2006) classification). Appendix 1 includes voucher information and GenBank accession numbers.

DNA Extraction, Amplification and Sequencing **—** Genomic DNA was either extracted from silica dried stems or roots, or from herbarium specimens, using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) or the Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) in accordance to manufacturers protocols.

Amplification reactions conditions and primers for six regions, four non-coding intergenic spacers of chloroplast DNA (cpDNA): *trn*S-*trn*G, *psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E, one nuclear low-copy gene, Phytochrome C (*Phy*C), followed protocols described in Calvente et al. (2016). Furthermore, we added sequences for one plastid gene – *ycf*1 – in this work (Appendix 2). Amplifications for *ycf*1 were conducted in a 20 µl reaction containing the following reagents (adding water to complete the final volume) and conditions: 4 µl of 5X GoTaq Buffer, 2.4 µl of 3mM MgCl2, 1.6 µl of each primer (120ng), 0.2 µl of GoTaq, 0.5 µl of 250µM dNTPs, 1 µl of template DNA; 94°C for 3 min, followed by 40 cycles of 94°C for 45 sec, 45ºC for 45 sec, 72°C for 2 min and 30 sec and finishing at 72°C for 5 min. Compared to Calvente et al. (2016), the present work expanded coverage of taxa and genomic regions including: 51 taxa were added for *ycf*1; 32 taxa for *psb*D-*trn*T; 29 taxa for *pet*L-*psb*E; 27 taxa for *Phy*C; 25 taxa for *trn*S-*trn*G and 24 taxa for *trn*L-*trn*T (added taxa are discriminated in Appendix 1). For all cases, we coded absent data as missing characters (-). The amplification products were purified using the NucleoSpin Gel or PCR clean-up Kit (Macherey-Nagel, Düren, Germany) and QIAquick PCR Purification Kit (QIAGEN, Crawley, UK), following the manufacturer’s protocol. Automated sequencing was performed by Macrogen Inc. Korea and Netherlands.

Alignment and phylogenetic analyses — Complementary sequences were assembled in Sequencher 4.1.4 (Gene Codes, Ann Arbor, Michigan, USA) and aligned manually in Mesquite v. 3.04 (Maddison and Maddison 2015). Indels were coded using the simple indel coding method (Simmons and Ochoterena 2000) and included in phylogenetic analyses as presence/absence data. *Pereskia grandifolia* was used as outgroup to root the trees in all analyses based on previous knowledge of phylogenetic relationships in Cactaceae and on its position near the primary node of divergence within subfamily, Pereskioideae (Arakaki et al. 2011; Hernández-Hernández et al. 2014).

We performed Maximum Parsimony (MP) analyses for all regions individually as well as for a total evidence approach including all plastid markers and *Phy*C (a concatenated dataset) using heuristic searches with 1,000 replicates of random taxon addition (retaining 20 trees at each replicate), tree bisection reconnection (TBR) branch swapping and equal weighting of all characters in PAUP* v4.0a147 (Swofford 2002). Support was assessed with non-parametric bootstrap analysis (BS) using 1000 replicates of random-taxon addition and TBR branch swapping. Clades with bootstrap percentages of 50–74% are described as weakly supported, 75–89% as moderately supported and 90–100% as strongly supported.

We also inferred phylogenetic relationships using Bayesian Inference (BI) in MrBayes 3.2.2 (Ronquist et al. 2011), hosted on the CIPRES Science Gateway (Miller et al. 2010). Choice of substitution models used the Akaike Information Criterion implemented in MrModelTest 2.2 (Nylander 2004) and run in PAUP* v4.0a147 (Swofford 2002). Bayesian analyses performed on individual genes used molecular substitution models selected for each marker: GTR for *psb*D-*trn*T, GTR+I for *pet*L-*psb*E, GTR+G for *ycf*1, GTR+I+G for *trn*S-*trn*G, F81+I for *trn*L-*trn*T and HKY+G for *Phy*C. For the total evidence approach we built a concatenated matrix with two partitions (all plastid/*Phy*C), using GTR+I+G and HKY+G as the best-fit model for each partition, respectively, and the overall substitution rate unlinked between partitions. MrBayes was set to build two independent runs of four chains each, for 10 million generations, sampling every 1000th generation. We assessed convergence between runs by monitoring the standard deviation of split frequencies (< 0.01), and using the Potential Scale Reduction Factor (PSRF). After discarding the first 25% samples as burnin, we pooled the remaining trees to construct a 50% majority rule consensus tree. We describe clades as weakly supported when the Posterior Probability (PP) < 0.8; as moderately supported when PP ranged from 0.8 to 0.95, and as strongly supported when PP > 0.95.

RESULTS

In this work, we assess phylogenetic relationships for *Pilosocereus* based on 220 new sequences produced here and added to the data set used by Calvente et al. (2016). In total, we used 429 sequences for 46 species and 8 subspecies (total 75 specimens) from six markers (Appendix 1). Statistics for each genomic region studied are summarized in Table 3. Phylogenetic relationships recovered with MP (Fig. 2) and BI (Fig. 3) analyses were generally similar with majority of nodes receiving moderate (BS of 75-89% and PP>0.8) to strong support values (BS of 90-100% and PP > 0.95).

The tribe Cereeae emerges strongly supported as monophyletic (BS=100, PP=1) sister of *Rhipsalis baccifera* (BS=98, PP=1). The position of *Browningia microsperma* (subtribe Rebutiinae) and the clade representing subtribe Trichocereinae (*Cleistocactus* sp., and *Oreocereus hempelianus*) is controversial; *Browningia microsperma* appears more closely related to Cereinae in the MP topology (BS=80.25), whereas the clade

(*Cleistocactus* sp., *Oreocereus hempelianus*) is more closely related to Cereinae in the BI topology (BS=0.81). Subtribe Cereinae is monophyletic in a strongly supported clade in both analyses (BS=100, PP=1).

The genus *Pilosocereus* as circumscribed in Hunt et al. (2006) (*Pilosocereus* sensu lato) was recovered as paraphyletic in both MP and BI analyses as the clade (*M. zehntneri* (*A. rhodantha*, *C. jamacaru*)) is nested inside the genus. *Pilosocereus* species are distributed in three strongly supported main clades: (I) containing *P. gounellei* and *P. tuberculatus* (*P.* subg. *Gounellea* clade; BS=100, PP=1); (II) *P. bohlei* isolated (BS=100, PP=1); and (III) the remaining of *Pilosocereus* species (*Pilosocereus* sensu stricto (s. s.); BS=100, PP=1). A closer relationship of the (*M. zehntneri* (*A. rhodantha*, *C. jamacaru*)) clade with the *Pilosocereus* s. s. clade is recovered in both MP and BI results although not strongly supported (BS=57.09, PP =0.86).

In *Pilosocereus* s. s. species are clustered in three main clades: (1) *P. aureispinus* isolated; (2) a clade (BS=80.04, PP=1) including all non-Brazilian species clustered (BS=99.8, PP=1) (the relationships are better resolved in the BI topology, although poorly supported) and a clade of the Brazilian species *P. glaucochorus*, *P. pachycladus* subsp. *pernambucoensis*, *P. pentaedrophorus* subsp. *pentaedrophorus* and *P. piauhyensis* (BS=99.39, PP=1); (3) a major strongly supported clade (BS=94.32, PP=1) clustering all remaining Brazilian species, but with poor resolution in more inclusive nodes. In the MP topology (Fig. 2) *P. aureispinus* is the first diverging lineage inside *Pilosocereus* s. s. (BS=100), however in the BI topology the relationships between clades 1, 2 and 3 are uncertain (Fig. 3).

The relationships within clade 3 are not fully resolved, however six weakly to strongly supported multispecies clades are recovered both in MP and BI analyses: (A) *P. ulei*, *P. arrabidae*, *P. catingicola* subsp. *catingicola* and *P. catingicola* subsp. *salvadorensis* (BS=98.09, PP=1); (B) *P. oligolepis, P. chrysostele* subsp. *cearensis* and *P. flavipulvinatus* (BS=95.33, PP=1); (C) *P. brasiliensis* subsp. *brasiliensis, P. brasiliensis* subsp. *ruschianus*, *P. magnificus* and *P. multicostatus* (BS=84.7, PP=0.80); (D) *P. fulvilanatus* subsp. *fulvilanatus* and *P. pentaedrophorus* subsp. *robustus* (BS=78.62; PP=0.99); (E) *P. azulensis* and *P. floccosus* subsp. *quadricostatus* (BS=100; PP=1); (F) *P. fulvilanatus* subsp. *rosae* and *P. aurisetus* subsp. *aurilanatus* (BS=96.24, PP=1); (G) *P. densiareolatus*, *P. parvus*, *P. pussilibaccatus*, *P. spendidus*, *P. pachycladus* subsp. *pachycladus* e *P. albissumus* (BS=95.94, PP=1); and (H) *P. pachycladus* subsp. *pachycladus* and *P. albisummus* (BS=92.47; PP=1). In the BI topology (Fig. 2) clade A is the first diverging lineage inside *Pilosocereus* s. s. (BS=100) while B and C are sister clades, however in the MP topology the relationships between clades A, B and C are uncertain (Fig. 3).

Inside *Pilosocereus* s. s., four species are consistently non-monophyletic in their current delimitation in both MP and BI topologies since their respective subspecies are clustered separately in distinct strongly supported clades: *P. pachycladus*, *P. pentaedrophorus*, *P. fulvilanatus* and *P. aurisetus*. *Pilosocereus aurisetus* subsp. *aurilanatus* is more closely related o *P. fulvilanatus* subsp. *rosae* than to *P. aurisetus* subsp. *aurisetus*. *Pilosocereus fulvilanatus* subsp. *fulvilanatus* is closer to *P. pentaedrophorus* subsp. *robustus* than to *P. fulvilanatus* subsp. *rosae*. *Pilosocereus* *pentaedrophorus* subsp. *pentaedrophorus* and *P. pachycladus* subsp. *pernambucoensis* and are nested in clade 2 while *P. pentaedrophorus* subsp. *robustus* and *P. pachycladus* subsp. *pachycladus* and are nested in clade D and H, respectively. The BI results also indicate the non-monophyly of *P. leucocephalus, P. collinsii, P. arrabidae, P. magnificus* and *P. multicostatus* under moderate to low PP support. However, among those, only *P. leucocephalus*, P. *magnificus* and *P. multicostatus* are non-monophyletic under moderate to low BS support on the MP topology.

DISCUSSION

***Relationships within Cactoideae* —** The results from this work corroborate some relationships found in previous works treating the whole family (Arakaki et al. 2011; Hernández-Hernández et al. 2011, 2014). Despite the controversial positioning of *Browningia microsperma*, the relationships of subtribes within Cereeae (sensu Nyffeler and Eggli 2010) agree with previous results. We found differences on relationships within subfamily Cactoideae, mainly due to the inclusion of *Pilosocereus* subg. *Gounellea* and *P. bohlei,* which had not yet been sampled in any major Cactaceae or Cactoideae molecular phylogenetic hypothesis published so far.

Our results show that *Arrojadoa rhodantha* and *Stephanocereus leucostele* do not group together in the same clade, supporting their positioning in separate genera. Due to conspicuous morphological similarities, such as the presence of an apical cephalium when young, supplanted by continuous vegetative growth, then forming cephalium rings, their segregation into two genera have always raised questions on whether these genera would or not form a single group, despite their different pollination mechanisms and floral morphology (Taylor and Zappi 1989). Hunt et al. (2006) even claimed that, based on the circumscription they make for *Arrojadoa*, and on preliminary molecular evidences, they considered including *Stephanocereus leucostele* within *Arrojadoa*. Our results refute such hypothesis.

We recovered a paraphyletic *Pilosocereus* sensu lato (s. l.) corroborating the results found in Calvente et al. (2016), as other genera of tribe Cereinae clustered within the group. We found a clade with *Melocactus*, *Arrojadoa* and *Cereus* species more closely related to *Pilosocereus* s. s. than *P. bohlei*, *P. gounellei* and *P. tuberculatus* (the two latter belong to *P.* subg. *Gounellea*). Although this relationship is not strongly supported in neither MP nor BI topologies (BS=57.09, PP=0.86), the clade corresponding to subgenus *Gounellea* is strongly supported as a distinct lineage from a clade including all the others in the BI results (PP=1, BS=50.88). These results indicate that the subgenus *Gounellea* represents a distinct lineage from the remaining species of *Pilosocereus*.

Pilosocereus subg. *Gounellea* differ morphologically from *Pilosocereus* s. s. because of its candelabriform branching, sinuate ribs and fruits with floral remnant frequently erect, not sunken into the apex of the pericarp (Zappi 1994). *Pilosocereus frewenii* (not sampled in our study) more recently described and included in *P.* subg. *Gounellea*, has the same morphological features common to *P. gounellei* and *P. tuberculatus*, however it is smaller in habit and seed sizes and bears flowers with different coloration (Zappi and Taylor 2011). Zappi (1994) states that the remarkable features of the subgenus *Gounellea* segregating it from *P.* subg. *Pilosocereus* would be plesiomorphic within the genus. Such hypothesis was raised by the presence of features such as ribs with marked hexagonal podaria, which are also found in some species of the subtribe Trichocereinae (e.g. *Rauhocereus* Backeb., *Weberbauerocereus* Backeb. and *Haageocereus* Backeb.) (Zappi, 1994). Based on our results we believe that morphological characters shared by both subgenera are possibly homoplasious and developed independently in both groups.

According to Taylor and Zappi (2004), *P. bohlei* shares similarities with *P. gounellei* (such as the narrower apical part of the fertile stem), but has fruits with floral remnants inserted in the pericarp (which is a diagnostic character of *P.* subg. *Pilosocereus*). However, the flowers in *P. bohlei* are smaller and S-shaped, different from all the species of the genus. We consider that we cannot find satisfying evidence in this work to support either the inclusion or the exclusion of *P. bohlei* from *Pilosocereus*. Further studies are needed to elucidate the positioning of *P. bohlei* using additional molecular and morphological data. We find support in our results and on overall morphological diagnostic characters to propose a generic status for *Pilosocereus* subg. *Gounellea*. This new genus accommodates *P. gounellei, P. tuberculatus* and *P. frewenii*. We believe the positioning of *P. bohlei* in another new genus is still premature. Further investigation using a broader sampling for Cereinae is currently under course and may lighten its positioning.

Relationship in Pilosocereus s. s. — *P. aureispinus* appears isolated from the remaining species of *Pilosocereus* s. s. corroborating other studies using molecular data (Bonatelli et al. 2013, 2014). We cannot find a conspicuous overall morphological differentiation between *P aureispinus* and the remainder of *Pilosocereus* s.s. species. The only consistent difference is the globose to depressed-globose fruit and the seed coat (Zappi 1994). In general, the major clades formed within *Pilosocereus* s. s. (clades 1-3; Fig. 2, 3) apparently do not reflect easily perceived diagnostic characters. As discussed by Calvente et al. (2016) it is not easy to find diagnostic characters (even through ancestral states reconstruction analyses) in this group or explain phylogenetic relationships recovered. However, we discuss morphological similarities for well supported clades.

Clade 2 includes a subclade of Brazilian species. Zappi (1994) reports that *P. pentaedrophorus* is a species related to *P. glaucochorous* because of the shared glabrous flowering areoles, curved flowers and stem epidermis striking blue colored, glaucous. Some populations of *P. pachycladus* subsp. *pernambucoensis* also present glaucous epidermis and these three taxa occur in close (but not sympatric) localities in the Brazilian state of Bahia. Because *P. pachycladus* subsp. *pernambucoensis* has a wide distribution in the northeastern region of Brazil and is also found in localities close to *P. piauhyensis*, there is a misleading history of collections in the state of Piauí for *P. pachycladus* subsp. *pernambucoensis*, however the material referred to this taxa there actually belongs under *P. piauhyensis* (Zappi 1994).

The second subclade in clade 2 includes non-Brazilian species. *Pilosocereus collinsii* and *P. purpusii* occur in sympatry in Mexico and Zappi (1994) indicated the first as a synonym of the second. However, Hunt et al. (2006) included both as distinct and accepted species. Main morphological differences among them are the number of ribs (7-10 in the first and 12 in the second) and sizes of the flowers (5 and 7 cm, respectively). Our results corroborate Hunt et al (2006) decision, since *P. collinsii* does not emerge as a sister species of *P. purpusii* in any of our analyses.

Other species apparently require further studies at the population level and possibly delimitation reevaluation are *P. royenii* and *P. polygonus*. *Pilosocereus royenii* occurs in Mexico and in the Caribbean Islands, but some authors believe that Mexican populations (specifically in the Yucatán peninsula) are morphologically distinct from the Caribbean ones (these usually identified as *P. gaumeri* (Britton & Rose) Backeb., a synonym of *P. royenii* according to Hunt et al. 2006). However, the Caribbean populations are sympatric with *P. polygonus,* and these two species are differentiated only by the abundant presence of hairs in the areoles in *P. royenii* and absent in *P. polygonus* (Zappi 1994). Hunt et al. (2006) argue that perhaps the populations for these two species occurring in the Caribbean could be the same species that present a variation in the amount of hairs in the areoles. Our results do not serve to refute this premise, since our sample of *P. royenii* is from a population that occurs in the Yucatán peninsula (Mexico).

On the large clade of Brazilian species (clade 3), clade "A" presents three taxa included in the *P. arrabidae* group (sensu Zappi 1994): *P. catingicola* subsp. *catingicola*, *P. catingicola* subsp. *salvadorensis* and *P. arrabidae*. All three present large flowers, fruits and seeds, small numbers of ribs, and are found in forest formations or sand dunes (*restinga*) on the Brazilian coast. The other species belonging to this clade, *P. ulei*, although presenting a completely different morphology from the species mentioned above is also found restricted to areas of *restinga*, where it grows in sympatry with *P. arrabidae* (Zappi 1994).

Clade "B" presents three taxa: *P. flavipulvinatus*, *P. oligolepis* and *P. chrysostele* subsp. *cearensis*. Zappi (1994) suggested a close relationship between the first two based on their morphology. This relationship would be a confirmation of a phytogeographical linkage between vegetation in the past (Taylor and Zappi 2004) since *P. flavipulvinatus* is found in ecotonal areas of Caatinga/Cerrado and Carnaubais (i.e. a *Copernicia* palm dominated vegetation between Amazon and Caatinga in Brazil) in the Brazilian northeast (Menezes et al. 2013), while *P. oligolepis* is found in rock outcrops in savanna areas in the north of Brazil (Lavor et al. 2016). Endemic to the state of Ceará *P. chrysostele* subsp. *cearensis* occurs in savanna formations and rock outcrops (Menezes et al. 2013) in localities close to those of *P. flavipulvinatus*. The similar habitat connection seems to be the link between these closely related taxa. *Pilosocereus chrysostele* subsp. *cearensis* is treated by Taylor and Zappi (2004) and Hunt et al. (2006) as a synonym of *P. piauhyensis*, what is refuted by our results.

Clade "C" is composed by *P. brasiliensis*, *P. magnificus* and *P. multicostatus*. Previous studies based on morphology and habitat evidences have not linked them. *Pilosocereus magnificus* and *P. multicostatus* present sympatric and restricted distribution in the state of Minas Gerais, while P. *brasiliensis* subsp. *ruschianus* has a wider distribution (occurring in Minas Gerais, Bahia and Espirito Santo states) and *P. brasiliensis* subsp. *brasiliensis* occurs only in the states of Espirito Santo and Rio de Janeiro (Zappi 1994). The geographical proximity seems to be the only connection among these species, however further studies focusing on this clade can elucidate more aspects of its complexity.

We assume the same for clades "D" and "E". Species on these clades although not morphologically similar occur in close or sympatric localities. We find *P. fulvilanatus* subsp. *fulvilanatus* to the north of Minas Gerais state and *P. pentaedrophorus* subsp. *robustus* in the middle-east and south of Bahia state and northeast of Minas Gerais, Brazil. Also in Minas Gerais, *P. azulensis* and *P. floccosus* subsp. *quadricostatus* both occur in the municipality of Pedra Azul (northern Minas Gerais), however *P. floccosus* subsp. *quadricostatus* expands to northeastern Minas Gerais, along the drainage basins of the Pardo and Jequitinhonha Rivers (Zappi 1994). The species of clade "F", *P. fulvilanatus* subsp. *rosae* and *P. aurisetus* subsp. *aurilanatus*, are also distinct morphologically but do not occur sympatrically. Both are found in rocky outcrops in the center-north of the Minas Gerais State with restricted distributions (Zappi 1994; Taylor and Zappi 2004).

The species *P. parvus* and *P. pusillibaccatus*, which make up the clade "G", belong to the species complex *P. aurisetus* (sensu Zappi 1994), and have already been synonymized (together with *P. jauruensis*) in Zappi (1994) as *P. machrisii*, but they were considered validly accepted by Hunt et al. (2006), delimitation corroborated by our results. *Pilosocereus aurisetus* group is composed of *P. aureispinus, P. aurisetus, P. bohlei, P. jauruensis, P. machrisii, P. parvus, P. pusillibaccatus* and *P. vilaboensis* presenting great morphological variation. It is distributed throughout the central region of the Brazil and presents a history of controversial classification (Zappi 1994; Taylor and Zappi 2004; Bonatelli et al. 2013). In our results this group did not appear as monophyletic.

Also in clade G, *P. splendidus* is considered by Zappi (1994) as a synonym for *P. pachycladus*, but is accepted by Hunt et al. (2006). It appears as sister of clade H, composed of *P. pachycladus* subsp. *pachycladus* and *P. albisummus*, corroborating the classification of Hunt et al. (2006). The species *P. albisummus* is reported by several authors as a poorly known taxon (e.g. Zappi 1994; Taylor and Zappi 2004; Hunt et al. 2006). The close relationship found in our work for *P. pachycladus* subsp. *pachycladus* and *P. albisummus*, may give a hint to the history of this species.

Intraspecific monophyly — Among the species tested we have evidence for the monophyly of *P. catingicola*, *P. brasiliensis*, *P. gounellei*, *P. bohlei*, *P. aureispinus*, *P. oligolepis*, *P. flavipulvinatus*, *P. jauruensis*, *P. pusillibaccatus* and *P. parvus* (corresponding to ≈23% of the species of *Pilosocereus* included in this study). Among those, we included samples of different subspecies for the first two. *Pilosocereus catingicola* subsp. *catingicola* and *P. catingicola* subsp. *salvadorensis* are grouped in our phylogeny in clade A. Although both subspecies are distinct morphologically, they are not found in sympatry as the dry vegetation along the valley of the São Francisco river basin (Brazil) is separating them (Zappi, 1994). Similar fact occurs with *P. brasiliensis* subsp. *brasiliensis* and *P. brasiliensis* subsp. *ruschianus* (clade C). Few morphological differences may be noted between these two latter subspecies, which according to Zappi (1994) would reflect the adaptation to distinct habitats (*P. brasiliensis* subsp. *brasiliensis* occurs in rock outcrops inside restinga formations of the

Atlantic Forest domain in Brazil, while *P. brasiliensis* subsp. *ruschianus* occurs in areas of dry forest rock outcrops also in Brazil).

Among the four consistently non-monophyletic species highlighted in this study, *P. aurisetus*, *P. fulvilanatus*, *P. pachycladus* and *P. pentaedrophorus* have two subspecies each, one homotypic and the other heterotypic. For the first three species, the heterotypic subspecies arose from synonyms and lowering of rank of other species of *Pilosocereus* to subspecies level by Zappi (1994). Only *P. pentaedrophorus* subsp. *robustus* was described initially as a subspecies. As an efford to integrate phylogenetic evidence in the taxonomy of *Pilosocereus* and to recognize monophyletic species we propose the elevation of these four heterotypic subspecies to the species level.

To propose *P. aurisetus* subsp. *aurilanatus*, Zappi (1994) downgraded *P. aurilanatus* Ritter to the category of subspecies under *P. aurisetus*, based on both taxa morphological similarities (as their very distinct fruit that divides in the whole apex breaking the floral remnant, and by the very smooth seed coats). According to Zappi's (1994) understanding they would compose a continuous distribution for *P. aurisetus* as they occur isolated from each other in rocky outcrops in Minas Gerais (Brazil). Due to the non-monophyly and morphological differentiation between the two taxa (differing in the flower-bearing areoles, with dark golden hairs in *P. aurisetus* subsp. *aurilanatus* and with white hairs in *P. aurisetus* subsp. *aurisetus*; and stems size 4.5-7.0 cm diam in *P. aurisetus* subsp. *aurilanatus* and < 5.5 in *P. aurisetus* subsp. *aurisetus*; Zappi 1994; Hunt et al. 2006), we propose to resurrect *Pilosocereus aurilanatus* Ritter.

Zappi (1994) and Taylor and Zappi (2004) state that the two subspecies of *P. fulvilanatus* are very similar morphologically, differing in the less robust habit of *P. fulvilanatus* subsp. *rosae*, wich also has branches smaller than 6 cm in diameter (8-12 cm in *P. fulvilanatus* subsp. *fulvilanatus*) and more ribs (6-8 compared to 4-7 in *P. fulvilanatus* subsp. *fulvilanatus*) (Hunt et al. 2006). These taxa occur separated on different sides of the Espinhaço mountain Range (Minas Gerais state, Brazil). The proposition of *P. fulvilanatus* subsp. *rosae* by Zappi (1994) is based on the lowering of *Pilosocereus rosae* P. J. Braun to the subspecies rank. Due to the consistent morphological and geographical differentiation among them and to the non-monophyly of *P. fulvilanatus* found here, we propose the resurrection of *Pilosocereus rosae*.

Pilosocereus pachycladus is one of the most widely distributed species in Northeastern Brazil, with great morphological diversity. The many heterotypic synonyms recognized for this species indicate an extensive history of nomenclatural and taxonomic confusion (Zappi 1994). Zappi (1994) proposed *P. pachycladus* subsp. *pernambucoensis*, synonymizing and lowering *Pilosocereus pernambucoensis* Ritter to the subspecies rank, based on the existence of morphologically intermediate populations in the region of Juazeiro and Sento Sé (Brazil). These intermediate populations occur to the north of the São Francisco river basin presenting 10-15 ribs and relatively fine spination (Zappi 1994; Taylor and Zappi 2004). However, *P. pachycladus* subsp. *pernambucoensis* differs from *P. pachycladus subsp. pachycladus* by a considerable number of features as the greater number of ribs (13–19 in the first and 5-12 in the latter), which are lower and closer to each other; the overall smaller spines (reaching 1,8 in the first and 3 cm in the latter); and the flower-bearing areoles (with scarce wool in the first and with abundant wool in the latter) (Taylor and Zappi 2004). We also propose to resurrect *Pilosocereus pernambucoensis* Ritter, however further investigation at the population level using molecular and morphological characters is needed to elucidate the processes influencing morphological variation in intermediate populations.

Zappi (1994) described *Pilosocereus pentaedrophorus* subsp. *robustus* based on observed morphological discontinuities among the populations of *P. pentaedrophorus*. *Pilosocereus pentaedrophorus* subsp. *pentaedrophorus* occurs from the northern of Bahia state to Pernambuco (Brazil) and *P. pentaedrophorus* subsp. *robustus* occurs in the center-south of Bahia to the northeast of Minas Gerais (mainly in the drainage basin of the Contas and Pardo rivers in Brazil). Besides the geographical separation, *P. pentaedrophorus* subsp. *robustus* differs from *P. pentaedrophorus* subsp. *pentaedrophorus* for the stouter branches (never leaning) up to 7.5 cm diam. (up to 4.5 cm in the latter), with (5-) 6-10 ribs acute (4-6 in the latter) (Zappi 1994). Based on the phylogenetic evidence found here and on the consistency of morphological and geographical characters we propose to elevate the heterotypic subspecies to the species rank. As the specific epithet “robustus” is already used in *Pilosocereus* for a different taxa, we propose a new name.

TAXONOMIC TREATMENT

We propose a new monophyletic genus including species formerly included in *Pilosocereus* subg. *Gounellea*, and the elevation of four heterotypic subspecies to the species rank. Following these changes *Pilosocereus* is now composed by the species grouped in *Pilosocereus* s.s. clade plus *P. bohlei*, including a total of 42 species and four heterotypic subspecies.

Xiquexique Lavor, Calvente & Versieux **nom. nov.** *Pilosocereus* subg. *Gounellea* Zappi, Succ. Pl. Res., 3:36 (1994)—TYPE: *Pilosocereus gounellei* (F. A. C. Weber) Byles & G. D. Rowley in Cact. Succ. J. Gr. Brit. 19: 67 (1957). (=X*iquexique gounellei* (F. A. C. Weber) Lavor & Calvente).

Description – Tree-like to shrubby cacti, main stem upright, mature branches arched, running more or less parallel to the ground, apices ascending, new axes arising subapically (branching candelabriform); ribs 4-15, sinuses sinuate with conspicuous podaria beneath the areoles; fruit with floral remnant frequently erect, not sunken into apex of the pericarp, circular at point of attachment.

Etimology **–** Named in reference to the vernacular name of *Pilosocereus gounellei*, the xique-xique, broadly known in Brazil and one of the most emblematic cacti of the Brazilian semi-arid Northeastern region.

Distribution **–** Eastern Brazil (along the entire Northeast region and in the central-north portion of Minas Gerais state).

We publish this new name as a replacement name for *Pilosocereus* subg. *Gounellea* to avoid a generic name derived from one of the species of the genus (recommendation 20A1 from the Melbourne code; McNeill et al. 2012a). We kept the generic name in masculine to maintain the gender of *Pilosocereus* (recommendation 62A from the Melbourne code; McNeill et al. 2012b).

Three species and one heterotypic subspecies that were included within *Pilosocereus* subgenus *Gounellea*, are now transferred to *Xiquexique*.

Xiquexique gounellei (F. A. C. Weber) Lavor & Calvente, **comb. nov**. *Pilocereus gounellei* F. A. C. Weber in K. Schum., Gesamtbeschr. Kakt.: 188 (1897). *Pilosocereus gounellei* (F. A. C. Weber) Byles & G. D. Rowley in Cact. Succ. J. Gr. Brit. 19: 67 (1957), **syn. nov**.—TYPE: Brazil, Pernambuco, 'Certão', *Gounelle* s.n. (P†). Neotype (Zappi 1994): Brazil, Paraíba, Várzia, São Gonçalo (Várzea de Souza), Jan. 1936, *P. Luetzelburg* 26921 (M!; photo: K, IPA).

Xiquexique gounellei subsp. zehntneri (Britton & Rose) Lavor & Calvente **comb. nov.** *Cephalocereus zehntneri* Britton & Rose, Cact. 2: 35 (1920). *Pilosocereus gounellei* subsp. *zehntneri* (Britton & Rose) Zappi in Succ. Pl. Res. 3: 43 (1994), **syn. nov.**—Type (Zappi 1994): Brazil, Bahia, district of Chique-Chique (Xique-Xique), Serra de Tiririca, Nov. 1917, *Zehntner* s. n. (US; K, photo ex US; lectoparatype: NY).

Xiquexique frewenii (Zappi & Taylor) Lavor & Calvente **comb. nov.** *Pilosocereus frewenii* Zappi & Taylor in Bradleya 29: 131 – 136 (2011), **syn. nov.**—Type: Brazil, Minas Gerais, Mun. Santana de Pirapama, distrito de Coberto, north of Inhame, Bambuí limestone outcrop in dry forest at the western foot of the Serra do Cipó, 777 meters above sea level, 19 July 2009, *Zappi & Taylor* 2208 (holotype: SPF; isotype: RB).

Xiquexique tuberculatus (Werderm.) Lavor & Calvente **comb. nov.** *Pilocereus tuberculatus* Werderm., Bras. Säulenkakt.: 101 (1933). *Pilosocereus tuberculatus*

(Werderm.) Byles & G. D. Rowley in Cact. Succ. J. Gr. Brit. 19(3): 69 (1957), **syn. nov.**—TYPE: Brazil, Pernambuco, Serra Negra, ‘900 m’, Mar. 1932, Werdermann (B†). Lectotype (Zappi 1994): Werdermann, l. c. infra, photograph, p. 21.

New names and synonyms for Pilosocereus

***Pilosocereus* aurilanatus** F. Ritter, Kakt. Südamer. 1: 77–78, Abb. 50 (1979). *Pilosocereus aurisetus* subsp. *aurilanatus* (Ritter) Zappi in Succ. Pl. Res. 3: 123 (1994), **syn. nov.**—TYPE: Brazil, Minas Gerais, Joaquim Felício, 1964, Ritter 1325 (holotype: U).

***Pilosocereus* pernambucoensis** F. Ritter, Kakt. Südamer. 1:65 (1979). *Pilosocereus pachycladus* subsp. *pernambucoensis* (F. Ritter) Zappi in Succ. Pl. Res. 3: 109 (1994), **syn. nov.**—TYPE: Brazil, Pernambuco, Araripina, 1963, *Ritter* 1219 (holotype: U).

***Pilosocereus* rosae** P. J. Braun in Kakt. and. Sukk. 35(8): 178–181 (1984). *Pilosocereus fulvilanatus* subsp. *rosae* (P. J. Braun) Zappi in Succ. Pl. Res. 3: 100 (1994), **syn. nov.**—TYPE: Brazil. Minas Gerais: Mun. Augusto de Lima, near Santa Bárbara, 6 km from road BR 135, west slopes of the Serra do Espinhaço, 800 m, 1983, *Horst & Uebelmann 546* (ZSS; isotype: K!).

***Pilosocereus* zappiae** Lavor & Calvente **nom. nov**. *Pilosocereus pentaedrophorus* subsp. *robustus* Zappi in Succ. Pl. Res. 3: 74 (1994), **syn. nov.**—HOLOTYPE: Brazil. Bahia, Mun. Livramento do Brumado, 11 km S of town on road to Brumado, 450 m, 13°45'S, 41°49'W, 23 Nov. 1988, *Taylor & Zappi* in *Harley 25544* (SPF; isotype: CEPEC!, K!).

***Etimology* –** Named in honor of the botanist Daniela Zappi. Her extensive taxonomic work with Brazilian cacti and *Pilosocereus*, in particular, have pushed the limits of the knowledge of the genus and of the Brazilian Cactaceae, as whole.

KEY TO GENERA *PILOSOCEREUS* AND XIQUEXIQUE

1. Branching pattern candelabriform (subacrotonic, main stem upright, new shoots running more or less parallel to the ground, apices ascending,); ribs rounded, sinuses sinuate with conspicuous podaria beneath the areoles; fruit depressed to globose, with floral remnant erect or pendent, not sunken into apex the of the pericarp, forming a circular insertion point ……………………………..…………… *Xiquexique*

Branching pattern erect (basi- to mesotonic, new shoots erect to suberect not parallel to the ground); ribs sinuses straight; fruit depressed-globose, with floral remnant pendent, sunken into apex the of the pericarp, forming a linear insertion) point ……………………………………………………………………… *Pilosocereus*

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TABLE 1. Species sampled in this study classification following Nyffeler and Eggli (2010) (outgroups are printed in bold). Type species of *Pilosocereus* subgenera are underlined (Zappi 1994).

Subfamily	Tribe	Subtribe	Species and subspecies
Pereskioideae			1. ***Pereskia grandifolia*** Haw.
Cactoideae			2. ***Copiapoa cinerea*** (Phil.) Britton & Rose
	Rhipsalideae		3. ***Rhipsalis baccifera*** (J. S. Mueller) Stearn.
	Cereeae	Rebutiinae	4. ***Browningia microsperma*** (Werderm. & Backeb.) W. T. Marshall.
			5. ***Cleistocactus*** sp. Lem.
		Trichocereinae	6. ***Oreocereus hempelianus*** (Gurke) D. R. Hunt
		Cereinae	7. ***Arrojadoa rhodantha*** Britton & Rose
			8. ***Cereus jamacaru*** D. C.
			9. ***Melocactus zehntneri*** (Britton & Rose) Luetzelb.
			10. ***Stephanocereus leucostele*** (Gurke) A.
			Pilosocereus subg. *Pilosocereus*:
			11. *Pilosocereus albisummus* P. J. Braun & Esteves
			12. *Pilosocereus alensis* (F. A. C. Weber) Byles & G. D. Rowley
			13. *Pilosocereus arrabidae* (Lem.) Byles & G. D. Rowley
			14. *Pilosocereus aureispinus* (Buining & Brederoo) F. Ritter
			15. *Pilosocereus aurisetus* (Werderm.) Byles & G. D. Rowley subsp. *aurisetus*
			16. *Pilosocereus aurisetus* subsp. *aurilanatus* (F. Ritter) Zappi
			17. *Pilosocereus azulensis* (F. A. C. Weber ex Rol. Goss) Byles & G. D. Rowley
			18. *Pilosocereus bohlei* Hofacker
			19. *Pilosocereus brasiliensis* (Britton & Rose) Backeb subsp. *brasiliensis*
			20. *Pilosocereus brasiliensis* subsp. *ruschianus* (Buining & Brederoo) Zappi
			21. *Pilosocereus catingicola* (Gurke) Byles & G. D. Rowley subsp. *catingicola*
			22. *Pilosocereus catingicola* subsp. *salvadorensis* (Werderm.) Zappi
			23. *Pilosocereus chrysacanthus* (F. A. C. Weber) Byles & G. D. Rowley
			24. *Pilosocereus chrysostele* subsp. *cearensis* P. J. Braun & Esteves

Table 1 (Continue)

Subfamily	Tribe	Subtribe	Species and Subspecies
Cactoideae	Cereeae	Cereinae	25. *Pilosocereus collinsii* (Britton & Rose) Byles & G. D. Rowley
			26. *Pilosocereus densiareolatus* F. Ritter
			27. *Pilosocereus flavipulvinatus* (Buining & Brederoo) F. Ritter
			28. *Pilosocereus floccosus* subsp. *quadricostatus* (F. Ritter) Zappi
			29. *Pilosocereus fulvilanatus* (Buining & Brederoo) F. Ritter subsp. *fulvilanatus*
			30. *Pilosocereus fulvilanatus* subsp. *rosae* (P. J. Braun) Zappi
			31. *Pilosocereus glaucochrous* (Werderm.) Blyles & G. D. Rowley
			32. *Pilosocereus jauruensis* (Buining & Brederoo) P. J. Braun
			33. *Pilosocereus leucocephalus* (Poselg.) Byles & G. D. Rowley
			34. *Pilosocereus machrisii* (E. Y. Dawson) Backeb.
			35. *Pilosocereus magnificus* (Buining & Brederoo) F. Ritter
			36. *Pilosocereus multicostatus* F. Ritter
			37. *Pilosocereus oligolepis* (Vaupel) Byles & G. D. Rowley
			38. *Pilosocereus pachycladus* F. Ritter subsp. *pachycladus*
			39. *Pilosocereus pachycladus* subsp. *pernambucoensis* (F. Ritter) Zappi
			40. *Pilosocereus parvus* (Diers & Esteves) P. J. Braun
			41. *Pilosocereus pentaedrophorus* (Labour.) Byles & G. D. Rowley subsp. *pentaedrophorus*
			42. *Pilosocereus pentaedrophorus* subsp. *robustus* Zappi
			43. *Pilosocereus piauhyensis* (Gurke) Byles & G. D. Rowley
			44. *Pilosocereus polygonus* (Lam.) Byles & G. D. Rowley
			45. *Pilosocereus purpusii* (Britton & Rose) Byles & G. D. Rowley
			46. *Pilosocereus pusillibaccatus* P. J. Braun & Esteves
			47. *Pilosocereus quadricentralis* (E. Y. Dawson) Backeb.
			48. *Pilosocereus royenii* (L.) Byles & G. D. Rowley
			49. *Pilosocereus splendidus* F. Ritter
			50. *Pilosocereus ulei* (K. Schum.) Byles & G. D. Rowley
			51. *Pilosocereus vilaboensis* (Diers & Esteves) P. J. Braun

Table 1 (Continue)

Subfamily	Tribe	Subtribe	Species
Cactoideae	Cereeae	Cereinae	*Pilosocereus* subg. *Gounellea*:
			52. *Pilosocereus gounellei* (F. A. C. Weber ex K. Schum.) Byles & G. D. Rowley
			53. *Pilosocereus tuberculatus* (Werderm.) Byles & G. D. Rowley

TABLE 2. Summary statistics from the maximum parsimony analyses of *Pilosocereus* (ingroup and outgroup) for plastid and nuclear regions used in this study (CI = consistency index; RI = retention index).

	*trn*L-*trn*T	*psb*D-*trn*T^{GGU}	*pet*L-*psb*E	*trn*S-*trn*G	*ycf*1	*Phy*C	Combined dataset
Size of aligned matrix	308	616	555	1327	751	1006	4563
Informative sites (bp)	22	41	42	139	163	48	454
Length of best tree	48	76	103	473	534	136	1418
Number of most parsimonious trees	3974	386	2	120	8732	210	19338
CI	0.79	0.94	0.87	0.78	0.71	0.88	0.75
RI	0.85	0.96	0.93	0.79	0.83	0.90	0.82

APPENDIX 1**.** Vouchers and GenBank accession numbers for species used in the phylogenetic analyses of *Pilosocereus* (new sequences generated in this study are printed in bold).

Species: collector/voucher herbarium/location (country) - accession numbers *trn*S-*trn*G, *psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E, *PhyC and ycf1* (Abbreviations — BHCB: Herbário Universidade Federal de Minas Gerais, Brazil; CCTS: Herbário da Universidade Federal de São Carlos (Campus Sorocaba), Brazil; CEPEC: Herbário CEPEC, Brazil; HUEFS: Herbário Universidade Federal de Feira de Santana, Brazil; QCNE: Museo Ecuatoriano de Ciencias Naturales del Instituto Nacional de Biodiversidad, Ecuador; SORO: Herbário do Centro de Ciências e Tecnologia para Sustentabilidade, Universidade Federal de São Carlos (Campus Sorocaba), Brazil; UFRN: Herbário Universidade Federal do Rio Grande do Norte, Brazil; MA: Vascular Plant Herbarium, Real Jardín Botánico de Madrid, Espanha).

Arrojadoa rhodantha: Machado M. 777/HUEFS107367/Bahia (Brazil) – KX301205, KX30108, KX301167, KX301129, KX301244, **XXXXXXX**; ***Browningia microsperma***: Madsen JE. 7311/MA752144/Loja (Ecuador) – **KX387798, KX387705, KX387770, KX387739, XXXXXXX, XXXXXXX**; ***Cereus jamacaru***: Calvente A. 461/UFRN/Bahia (Brazil) – KX301200, KX301076, KX301162, KX301119, KX301238, **XXXXXXX**; ***Cleistocactus* sp**: Aedo C. 14512/MA759900/Cochabamba (Bolivia) – **KX387795, KX387701, KX387766, KX387735, XXXXXXX, XXXXXXX**; ***Copiapoa cinerea***: Rodrigues R. 3120/MA702058-11019/Antofagasta (Chile) – -, **KX387702, KX387767, KX387736, XXXXXXX, XXXXXXX**; ***Melocactus zehntneri***: Calvente A. 462/UFRN/ Rio Grande do Norte (Brazil) – KX301198, KX301074, KX301160, KX301117, KX301236, **XXXXXXX**; ***Oreocereus hempelianus***: Aedo C. 11369/MA728565/Moquegua (Peru) – **KX387796, KX387703, KX387768, KX387737, XXXXXXX, XXXXXXX**; ***Pereskia grandifolia***: Damaso P./UFRN 7796/Rio Grande do Norte (Brazil) – -, **KX387707, KX387772, KX387741, XXXXXXX, XXXXXXX**; ***Pilosocereus albisummus***: Moraes E.M. S141/SORO4530 /Minas Gerais (Brazil) – KX301216, KX301097, KX301178, KX301140, KX301255, **XXXXXXX**; ***P. alensis***: Sanchez-Mejorada H. 4449/MEXU/Jalisco (Mexico) – KX301188, KX301064, KX301150, KX301107, KX301226, **XXXXXXX**; ***P. arrabidae*** (1): Machado MC. S35M1/SORO4488/Bahia (Brazil) – **KX387801, KX387714, KX387774, KX387747, XXXXXX, XXXXXX, XXXXXXX**; ***P. arrabidae* (2)**: Franco FF S79B1/SORO 2656/ Rio de Janeiro (Brazil) – KX301222, KX301103, KX301184, KX301146, KX301261, **XXXXXXX**; ***P. aureispinus* (1)**: Moraes EM. S21/HUFS642/Bahia (Brazil) – KX301201, **KX387712**, JN035609, -, KC779308.1, **XXXXXXX**; ***P. aureispinus* (2)**: Moraes EM. S21/HUFS642/Bahia (Brazil) – JN035414/JN035456, KX301080, KX301163, KX301123, KX301240, -; ***P. aurisetus* subsp. *aurisetus* (1)**: Moraes EM. S11/HUFS646/ Minas Gerais (Brazil) – JN035403 /JN035437, **KX387709**, JN035585, **KX387743**, KC779292.1, **XXXXXXX**; ***P. aurisetus* subsp. *aurisetus* (2)**: Moraes EM. S30/ SORO2651/Minas Gerais (Brazil) – KC779380.1, KX301082, KC779380.1, KX301125, KX301241, **XXXXXXX; *P. aurisetus* subsp. *aurilanatus* (1)**: Moraes EM. S7/HUFS639/Minas Gerais (Brazil) – KC779423.1, **KX387708**, KC621246.1, **KX387742**, **XXXXXX, XXXXXX**; ***P. aurisetus* subsp. *aurilanatus* (2)**: Moraes EM. S7/HUFS639/Minas Gerais (Brazil) – KC779425.1, KX301077, KC621248.1, KX301120, KC779288, **XXXXXXX; *P. azulensis***: Olsthoorn G. 253/ SORO 4531/ Minas Gerais (Brazil) – KX301214, KX301095, KX301176, KX301138, KX301253, **XXXXXXX**; ***P. bohlei* (1)**: Moraes EM. S51/CCTS3000/Bahia (Brazil) – **KX387802, KX387715, KX387775, KX387748, XXXXXX, XXXXX**; ***P. bohlei* (2)**: Moraes EM. S51/CCTS3000/Bahia (Brazil) – KX301211, KX301092, KX301173, KX301135, KX301250, **XXXXXX**; ***P. brasiliensis* subsp. *brasiliensis* (1)**: Franco FF. S86/SORO 4568/Espirito Santo (Brazil) – **KX387810, KX387723, KX387782, KX387755, XXXXXXX, XXXXXXX**; ***P. brasiliensis* subsp. *brasiliensis* (2)**: Franco FF. S79E /SORO2654/Espirito Santo (Brazil) – KX301223, KX301104, KX301185, KX301147, KX301262, **XXXXXXX**; ***P. brasiliensis* subsp. *ruschianus***: Olsthoorn G. 188/SORO 4540/Minas Gerais (Brazil) – **KX387809, KX387722, KX387781**, -, **XXXXXXX, XXXXXXX**; ***P. catingicola* subsp. *catingicola***: Olsthoorn G. 1026/SORO4532/Bahia (Brazil) – KX301217, KX301098, KX301179, KX301141, KX301256, **XXXXXX**; ***P. catingicola*** **subsp.** ***salvadorensis*** **(1)**: Menezes MOT. 378/EAC57091/Bahia (Brazil) – **KX387803, KX387716**, -, **KX387749, XXXXXXX, XXXXXXX**; ***P. catingicola*** **subsp.** ***salvadorensis*** **(2)**: Menezes MOT. 152/EAC44189/Ceará (Brazil) – KX301218, KX301099, KX301180, KX301142, KX301257, **XXXXXXX**; ***P. chrysacanthus***: Arias S. 858/ MEXU/Oaxaca (Mexico) – KX301190, KX301066, KX301152, KX301109, KX301228, **XXXXXXX**; ***P. chrysostele*** **subsp.** ***cearenses*** **(1)**: Lavor P. 36/ UFRN17127/Ceará (Brazil) – **KX387789, KX387695, KX387761, KX387729**, -, **XXXXXXX**; ***P. chrysostele*** **subsp.** ***cearenses*** **(2)**: Menezes MOT. 161/ EAC44385/ Ceará (Brazil) – **KX387804, KX387717, KX387776, KX387750, XXXXXXX, XXXXXX**; ***P. collinsii*** **(1)**: Arias S. 1658/MEXU/Chiapas (Mexico) – **KX387784, KX387690, KX387756, KX387724**, -, **XXXXXXX**; ***P. collinsii*** **(2)**: Arias S. 1635/_ – KX301191, KX301067, KX301153, KX301110, KX301229, **XXXXXXX**; ***P. densiareolatus***: Moraes EM. S43/ SORO2650/Minas Gerais (Brazil) – KX301208, KX301089, KX301170, KX301132, KX301247, **XXXXXXX**; ***P. flavipulvinatus*** **(1)**: Lavor P. 71/_/Ceará (Brazil) – **KX387799, KX387706, KX387771, KX387740**, -, **XXXXXXX**; ***P. flavipulvinatus*** **(2)**: Menezes MOT. 259/EAC48762/Ceará (Brazil) – KX301224, KX301105, KX301186, KX301148, KX301263, -; ***P. floccosus*** **subsp.** ***quadricostatus***: Olsthoorn G. 42/SORO4558/Minas Gerais (Brazil) – KX301220, KX301101, KX301182, KX301144, KX301259, **XXXXXXX**; ***P. fulvilanatus*** **subsp.** ***fulvilanatus***: Moraes EM. S42/SORO2655/Minas Gerais (Brazil) – KX301207, KX301088, KX301169, KX301131, KX301246, **XXXXXXX**; ***P. fulvilanatus*** **subsp.** ***rosae***: Olsthoorn G. 263/ SORO 4534/Minas Gerais (Brazil) – KX301215, KX301096, KX301177, KX301139, KX301254, **XXXXXXX**; ***P. glaucochrous***: Machado MC. S35M2/SORO 4536/Bahia (Brazil) – KX301202, KX301083, KX301164, KX301126, -, -; ***P. gounellei*** **(1)**: Lavor P. 18/UFRN16223/Piauí (Brazil) – **KX387787, KX387693, KX387759, KX387727, XXXXXXX, XXXXXXX**; ***P. gounellei*** **(2)**: Lavor P. 22/ UFRN16227/Piauí (Brazil) – **KX387788, KX387694, KX387760, KX387728, XXXXXXX, XXXXXXX**; ***P. jauruensis*** **(1)**: Moraes EM. S23/HUFS 638/Mato Grosso do Sul (Brazil) – KC779348.1, KX387713, KC779348.1, **KX387746, XXXXXXX, XXXXXXX**; ***P. jauruensis*** **(2)**: Moraes EM. S25/SORO 2646/Mato Grosso do Sul (Brazil) – KC779358.1, KX301081, KC779358.1, KX301124, KC779302, **XXXXXXX**; ***P. lanuginosus***: Mero 96/QCNE/_ – KX387791, **KX387697, KX387763, KX387731, XXXXXXX, XXXXXX, XXXXXXX**; ***P. leucocephalus*** **(1)**: Arias S. 1621/_/_ – **KX387785, KX387691, KX387757, KX387725, XXXXXXX, XXXXXXX**; ***P. leucocephalus*** **(2)**: Arias S. 1654/MEXU/Chiapas (Mexico) – KX301193, KX301069, KX301155, KX301112, KX301231, -; ***P. machrisii*** **(1)**: Moraes EM. S18/HUFS 648/Goiás (Brazil) – KC779332.1, **KX387710**, KC621149.1, **KX387744**, **XXXXXXX, XXXXXXX**; ***P. machrisii*** **(2)**: Moraes EM. S17/HUFS 645/Goiás (Brazil) – KC779262.1/JN035400, KX301078, JN035602, KX301121, KX301239, **XXXXXXX**; ***P. magnificus*** **(1)**: Moraes EM. S37/SORO4550/Minas Gerais (Brazil) – **KX387805, KX387718, KX387777, KX387751, XXXXXXX, XXXXXXX**; ***P. magnificus*** **(2)**: Taylor NP. & Zappi DC. 755/BHCB20954/ Minas Gerais (Brazil) – KX301204, KX301085, KX301166, KX301128, KX301243, **XXXXXXX**; ***P. multicostatus*** **(1)**: Moraes EM. S41/SORO2649/Minas Gerais (Brazil) – **KX387806, KX387719, KX387778, KX387752, XXXXXXX, XXXXXXX**; ***P. multicostatus*** **(2)**: Moraes EM. S39/SORO2653/Minas Gerais (Brazil) – KX301206, KX301087, KX301168, KX301130, KX301245, **XXXXXXX**; ***P. oligolepis*** **(1)**: Lavor et al. 60/UFRN 18663/Roraima (Brazil) – **KX387792, KX387698, KX387764, KX387732, XXXXXXX, XXXXXXX**; ***P. oligolepis*** **(2)**: Lavor P. & Lavor J. 69/UFRN18670/ Roraima (Brazil) – **KX387793, KX387699, KX387765, KX387733, XXXXXXX, XXXXXXX**; ***P. pachycladus*** **subsp.** ***pachycladus*** **(1)**: Taylor NP. 1434/ CEPEC 50888/Bahia (Brazil) – **KX387807, KX387720, KX387779, KX387753, XXXXXXX, XXXXXX**; ***P. pachycladus*** **subsp.** ***pachycladus*** **(2)**: Moraes EM. S45/SORO2647/ Minas Gerais (Brazil) – KX301209, KX301090, KX301171, KX301133, KX301248, **XXXXXX**; ***P. pachycladus*** **subsp.** ***pernambucoensis***: Lavor P. 23/UFRN16228/Piauí (Brazil) – KX301197, KX301073, KX301159, KX301116, KX301235, **XXXXXXX**; ***P. parvus*** **(1)**: Moraes EM. S47/SORO2648/Goiás (Brazil) – **KX387808, KX387721, KX387780, KX387754, XXXXXXX, XXXXXXX**; ***P. parvus*** **(2)**: Moraes EM. S47/SORO2648/Goiás (Brazil) – KX301210, KX301091, **KX301172**, KX301134, KX301249, -; ***P. pentaedrophorus*** **subsp.** ***pentaedrophorus***: Calvente A. 409/UFRN 13193/Bahia (Brazil) – **KX387783, KX387689**, -, -, -, **XXXXXXX**; ***P. pentaedrophorus*** **subsp.** ***robustus***: Olsthoorn G. 172/SORO4538/Bahia (Brazil) – KX301212, KX301093, KX301174, KX301136, KX301251, **XXXXXX**; ***P. piauhyensis***: Lavor P. 14/UFRN16219/Piauí (Brazil) – **KX387786, KX387692, KX387758, KX387726, XXXXXXX**, -; ***P. polygonus***: DNA Bank Kew 45353/_/_ – **KX387794, KX387700**, -, **KX387734, XXXXXXX, XXXXXXX**; ***P. purpusii***: Blancas Vazquez JJ. 119/MEXU/Nayarit (Mexico) – KX301189, KX301065, KX301151, KX301108, KX301227, **XXXXXXX**; ***P. pusillibaccatus* (1)**: Lavor P. 20/UFRN16225/ Piauí (Brazil) – KX301195, KX301071, KX301072, KX301157, KX301114, KX301233, **XXXXXXX**; ***P. pusillibaccatus* (2)**: Lavor P. 21/ UFRN 16226/Piauí (Brazil) – KX301196, KX301072, KX301158, KX301115, KX301234, **XXXXXXX**; ***P. quadricentralis***: Arias S. 2180/MEXU/Oaxaca (Mexico) – KX301187, KX301063, KX301149, KX301106, KX301225, **XXXXXXX**; ***P. royenii***: S Arias 1098/MEXU/ Yucatán (Mexico) – KX301192, KX301068, KX301154, KX301111, KX301230, -; ***P. splendidus***: Moraes EM. S139/ SORO4539/Bahia (Brazil) – KX301213, KX301094, KX301175, KX301137, KX301252, **XXXXXXX**; ***P. tuberculatus***: Lavor P. 47/UFRN18650/Pernambuco (Brazil) – **KX387790, KX387696, KX387762, KX387730, XXXXXXX, XXXXXXX**; ***P. ulei***: Franco FF. S79/SORO4557/Rio de Janeiro (Brazil) – KX301221, KX301102, KX301183, KX301145, KX301260, **XXXXXXX**; ***P. vilaboensis* (1)**: Moraes EM. S20/HUFS 641/Goiás (Brazil) – **KX387800, KX387711, KX387773, KX387745, XXXXXXX, XXXXXXX**; ***P. vilaboensis* (2)**: Moraes EM. S19/CCTS3001/Goiás (Brazil) – KC779340.1, KX301079, KC621157.1, KX301122, KC779305.1, **XXXXXXX**; ***Rhipsalis baccifera***: Rodriguez A. 5318/MA733603/Limón (Costa Rica) – **KX387797, KX387704, KX387769, KX387738, XXXXXXX, XXXXXXX**; ***Stephanocereus leucostele***: Calvente A. 413/UFRN 13195/Bahia (Brazil) – KX301199, KX301075, KX301161, KX301118, KX301237, **XXXXXXX**.

APPENDIX 2. Primers of the different genomic regions used in this study.

Region name	Primers	Source
*pet*L-*psb*E	petL: AGTAGAAAACCGAAATAACTAGTT A psbE: TATCGAATACTGGTAATAATATCAGC	Shaw et al. 2007
*psb*D-*trn*T^{GGU}	psbD: CTCCGTARCCAGTCATCCATA trnT(GGU)-R: CCCTTTTAACTCAGTGGTAG	Shaw et al. 2007
*trn*L-*trn*T	5’trnLUAAR(TabB): TCTACCGATTTCGCCATATC trnT^{UGU}F (TabA): CATTACAAATGCGATGCTCT	Taberlet et al. 1991
*trn*S-*trn*G	5’trnG2S: TTTTACCACTAAACTATACCCGC SGFwd2: CACCCATGGTTCCCATTAGA	Shaw et al. 2005
	trnS^{GCU}: AGATAGGGATTCGAACCCTCGGT SGRev2: TCCGCTCATTAGCTCTCCTC	Bonatelli et al. 2013
*Phy*C	PhyF: AGCTGGGGCTTTCAAATCTT PhyR: TCCTCCACTTGACCACCTCT	Helsen et al. 2009
*ycf*1	ycf1-4182F*: AAATAYRRATAGAAAATATTTKGATT ycf1-5248R*: GAATTCTYAATTCTCTACGACG	Franck et al. 2012

FIGURE LEGENDS

FIG. 1. Main differences between *Pilosocereus* subgenus *Pilosocereus* and *Pilosocereus* subgenus *Gounellea*, respectively, relative to the branching (a – candelabriform pattern; b – erect pattern); ribs (c,d – sinuses sinuate with conspicuous podaria beneath the areoles; e,f – sinuses straight) and fruit (g – floral remnant erect or pendent, not sunken into apex the of the pericarp, circular at point of attachment; h – floral remnant pendent, sunken into apex the of the pericarp, forming a linear insertion point) (Species: a,c – *P. gounellei* subsp. *gounellei*; b, e – *P. pachycladus* subsp. *pernambucoensis*; d,g – *P. tuberculatus*; f – *P. flavipulvinatus*; h – *P. oligolepis*).

FIG. 2. Strict consensus tree from Maximum Parsimony analyses of the combined dataset of (*trn*S-*trn*G, *psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E, *Phy*C and *ycf*1) produced for *Pilosocereus* (ingroup and outgroups). Bootstrap values are shown above branches.

FIG. 3. Majority rule consensus tree derived from Bayesian analyses of the combined dataset (from *trn*S-*trn*G, *psb*D-*trn*T, *trn*L-*trn*T, *pet*L-*psb*E, *ycf*1 and *Phy*C) produced for *Pilosocereus* and outgroups. Posterior Probability values are shown above branches.

FIGURES

FIGURE 1

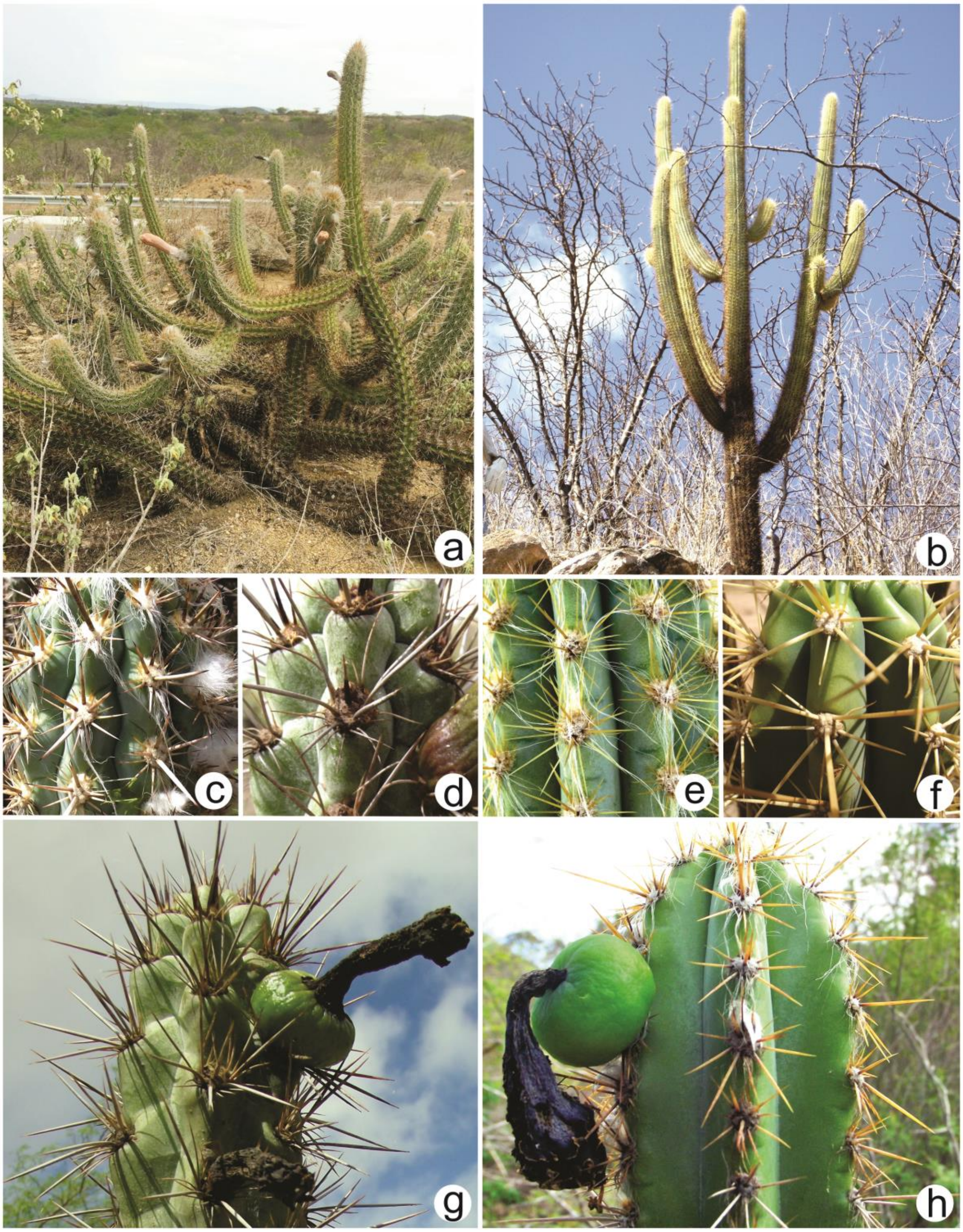


FIGURE 2

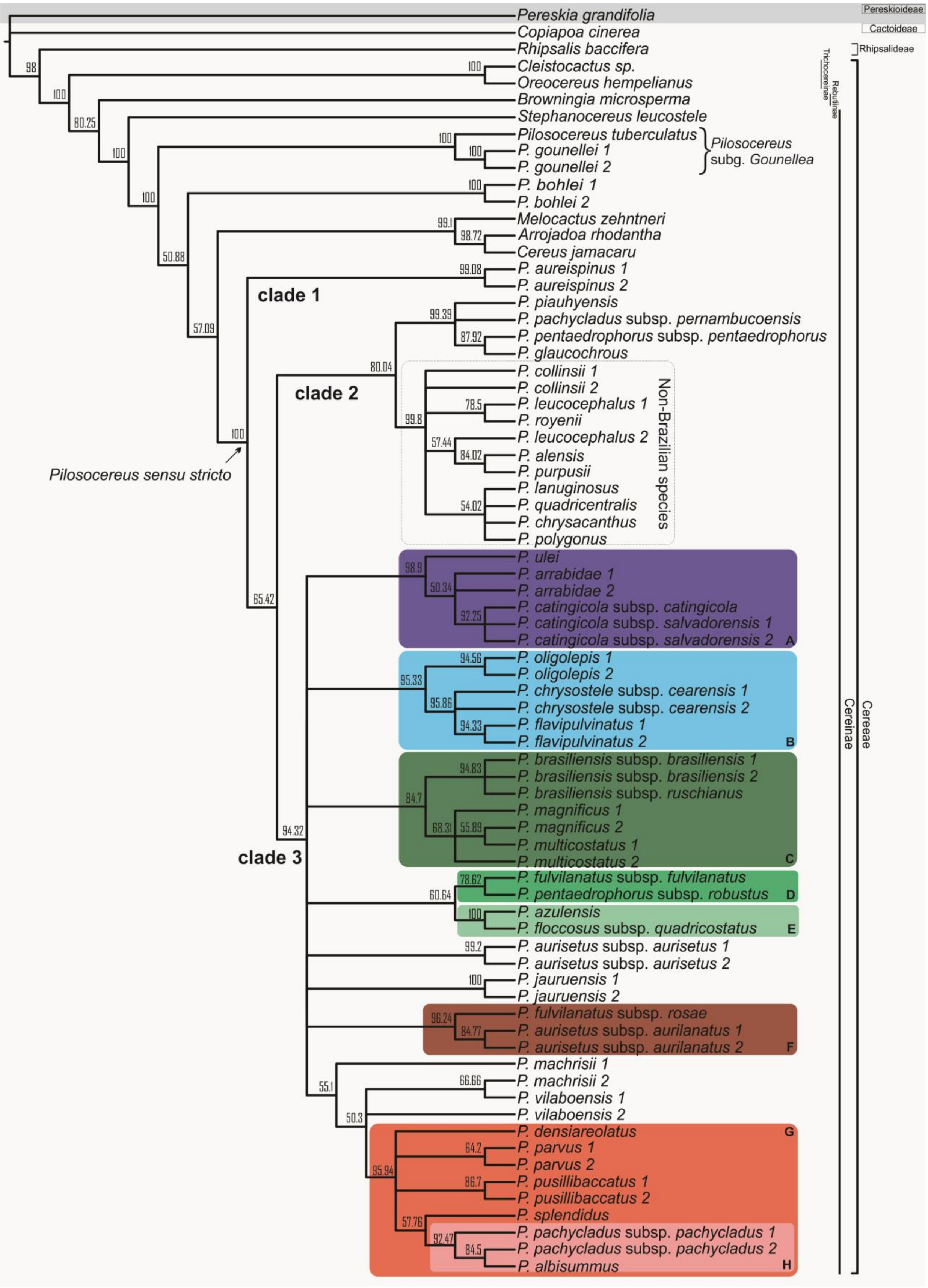
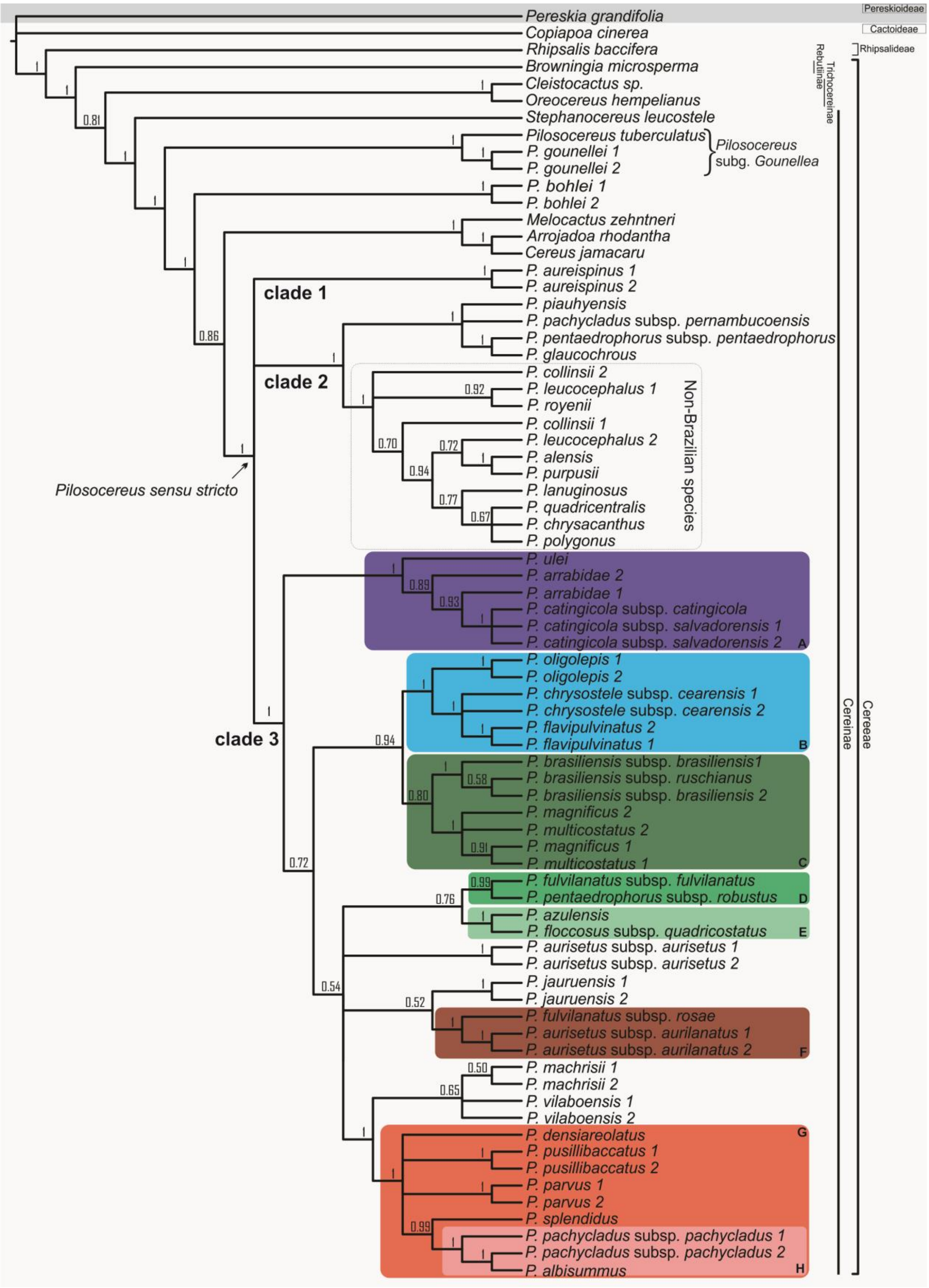

Pereskia grandifolia
Pereskioideae
Copiapoa cinerea
Cactoideae
Rhipsalis baccifera
Rhipsalideae
98
100
Cleistocactus sp.
Oreocereus hempelianus
Trichocereinae
Rebutiinae
100
Browningia microsperma
80.25
Stephanocereus leucostele
100
Pilosocereus tuberculatus
100
100
P. gounellei 1
P. gounellei 2
Pilosocereus subg. Gounellea
100
100
P. bohlei 1
P. bohlei 2
99.1
Melocactus zehntneri
50.88
98.72
Arrojadoa rhodantha
Cereus jamacaru
99.08
P. aureispinus 1
P. aureispinus 2
clade 1
57.09
99.39
P. piauhyensis
P. pachycladus subsp. pernambucoensis
87.92
P. pentaedrophorus subsp. pentaedrophorus
P. glaucochrous
80.04
P. collinsii 1
clade 2
P. collinsii 2
78.5
P. leucocephalus 1
P. royenii
99.8
100
P. leucocephalus 2
57.44
84.02
P. alensis
Pilosocereus sensu stricto
P. purpusii
P. lanuginosus
54.02
P. quadricentralis
P. chrysacanthus
P. polygonus
Non-Brazilian species
P. ulei
98.9
P. arrabidae 1
50.34
P. arrabidae 2
65.42
92.25
P. catingicola subsp. catingicola
P. catingicola subsp. salvadorensis 1
P. catingicola subsp. salvadorensis 2
A
94.56
P. oligolepis 1
P. oligolepis 2
95.33
P. chrysostele subsp. cearensis 1
95.86
P. chrysostele subsp. cearensis 2
94.33
P. flavipulvinatus 1
P. flavipulvinatus 2
B
Cereinae
Cereeae
94.83
P. brasiliensis subsp. brasiliensis 1
P. brasiliensis subsp. brasiliensis 2
P. brasiliensis subsp. ruschianus
84.7
P. magnificus 1
68.31
55.89
P. magnificus 2
94.32
P. multicostatus 1
clade 3
P. multicostatus 2
C
78.62
P. fulvilanatus subsp. fulvilanatus
60.64
P. pentaedrophorus subsp. robustus
D
100
P. azulensis
P. floccosus subsp. quadricostatus
E
99.2
P. aurisetus subsp. aurisetus 1
P. aurisetus subsp. aurisetus 2
100
P. jauruensis 1
P. jauruensis 2
96.24
P. fulvilanatus subsp. rosae
84.77
P. aurisetus subsp. aurilanatus 1
P. aurisetus subsp. aurilanatus 2
F
P. machrisii 1
55.1
66.66
P. machrisii 2
P. vilaboensis 1
50.3
P. vilaboensis 2
P. densiareolatus
G
64.2
P. parvus 1
95.94
P. parvus 2
86.7
P. pusillibaccatus 1
P. pusillibaccatus 2
P. splendidus
57.76
92.47
P. pachycladus subsp. pachycladus 1
84.5
P. pachycladus subsp. pachycladus 2
P. albisummus
H

FIGURE 3


Pereskioideae
Cactoideae
Rhipsalideae
Trichocereinae
Rebutiinae
Cereinae
Cereeae
Pereskia grandifolia
Copiapoa cinerea
Rhipsalis baccifera
Browningia microsperma
Cleistocactus sp.
Oreocereus hempelianus
Stephanocereus leucostele
Pilosocereus tuberculatus
P. gounellei 1
P. gounellei 2
Pilosocereus subg. Gounellea
P. bohlei 1
P. bohlei 2
Melocactus zehntneri
Arrojadoa rhodantha
Cereus jamacaru
P. aureispinus 1
P. aureispinus 2
clade 1
P. piauhyensis
P. pachycladus subsp. pernambucoensis
P. pentaedrophorus subsp. pentaedrophorus
P. glaucochrous
clade 2
P. collinsii 2
P. leucocephalus 1
P. royenii
P. collinsii 1
P. leucocephalus 2
P. alensis
P. purpusii
P. lanuginosus
P. quadricentralis
P. chrysacanthus
P. polygonus
Non-Brazilian species
Pilosocereus sensu stricto
P. ulei
P. arrabidae 2
P. arrabidae 1
P. catingicola subsp. catingicola
P. catingicola subsp. salvadorensis 1
P. catingicola subsp. salvadorensis 2
A
P. oligolepis 1
P. oligolepis 2
P. chrysostele subsp. cearensis 1
P. chrysostele subsp. cearensis 2
P. flavipulvinatus 2
P. flavipulvinatus 1
B
clade 3
P. brasiliensis subsp. brasiliensis1
P. brasiliensis subsp. ruschianus
P. brasiliensis subsp. brasiliensis 2
P. magnificus 2
P. multicostatus 2
P. magnificus 1
P. multicostatus 1
C
P. fulvilanatus subsp. fulvilanatus
P. pentaedrophorus subsp. robustus
D
P. azulensis
P. floccosus subsp. quadricostatus
E
P. aurisetus subsp. aurisetus 1
P. aurisetus subsp. aurisetus 2
P. jauruensis 1
P. jauruensis 2
P. fulvilanatus subsp. rosae
P. aurisetus subsp. aurilanatus 1
P. aurisetus subsp. aurilanatus 2
F
P. machrisii 1
P. machrisii 2
P. vilaboensis 1
P. vilaboensis 2
G
P. densiareolatus
P. pusillibaccatus 1
P. pusillibaccatus 2
P. parvus 1
P. parvus 2
P. splendidus
P. pachycladus subsp. pachycladus 1
P. pachycladus subsp. pachycladus 2
P. albisummus
H
0.81
0.86
0.92
0.70
0.72
0.94
0.77
0.67
0.89
0.93
0.94
0.58
0.80
0.91
0.72
0.99
0.76
0.54
0.52
0.50
0.65
0.99

CAPITULO 3

PATTERNS OF DISTRIBUTION, RICHNESS, ENDEMISM AND CONSERVATION OF *PILOSOCEREUS* CACTI IN THE NEOTROPICS

Manuscrito a ser submetido na *Biodiversity and Conservation*

ORIGINAL PAPER

Patterns of distribution, richness, endemism and conservation of *Pilosocereus* cacti in the Neotropics

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Abstract The Neotropics are one of the richest floristic regions in the Americas and demands urgently strategies for its biodiversity conservation. Data on distribution, richness, diversity, and areas of endemism are important sources for assessing conservation status and proposing protection programs for taxa at risk, such as most cacti, a group strongly affected by disturbances and loss of habitat by human actions. *Pilosocereus* is one of the largest and most widely distributed generain the family, containing 42 species and 9 subspecies. They occurr throughout the Neotropical region, in different habitats, always associated with xeric environments. *Pilosocereus* species have significant ecological importance and are widely used in popular culture as a source of human and animal food. Despite that many species are currently ranked in main threat categories of IUCN. Thus, here we study patterns of distribution, richness, and endemism for the genus in order to assist in the management and conservation planning for the group. We found that some taxa present restrictions on the type of vegetation, but the clear majority were widely distributed in different environmental gradients. The greatest species richness and phylogenetic diversity are found in the states of Bahia and Minas Gerais (eastern Brazil). Areas of endemism are highlighted in eastern Brazil and Mexico. We suggest further studies, in situ and ex situ conservation strategies, taking into account that a relevant number of taxa in *Pilosocereus* are threatened, have restricted distribution or still lack basic information regarding their biology.

Keywords Conservation distribution richness *Pilosocereus* Cactaceae Neotropics

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Introduction

The Neotropical region is one of the biodiversity richest places on Earth, however plant species are not evenly distributed in this region. Conservation of this unique and unevenly distributed biota have always been a concern of different authors, denouncing habitat loss and a severe biodiversity crisis along diverse environments (e.g., Mori et al. 1981; Davis et al. 1997 and references therein; Singh 2002; Ceballos et al. 2009; Martinelli and Moraes 2013). To cope with these crisis, private and government agencies have tried to developed strategies to set conservation priorities, but one of the critical issues in environmental policies is indeed the choice of priorities (Oliveira 2003).

Many different criteria exist to select species, environments or even areas as priority targets. Studies focused in mapping distribution patterns of species are an important source of data to draw conservation strategies because they can determine areas with high concentration of species (diversity criterion); areas with high concentration of species with restricted distribution (endemism criterion); and areas with high concentration of endangered species (threat criterion) (Gentry 1992; Oliveira 2003). Basic knowledge about how and where species are distributed is the first step to understand the dynamics and life history of species / populations, and all this information serves to set up plans for the management and conservation of possibly threatened species (Godinez-Alvarez et al. 2003).

Cacti are one of the groups of plants that are exposed to major threats by human action, mainly by deforestation, reduction of natural habitats and illegal commerce (Hernández and Godinez 1994). The family is nearly confined to the Neotropical region where it is centered in Mexico, Brazil and the Andean region, commonly associated to xeric environments (Taylor and Zappi 2004). In those places, common challenges to set strategies for its conservation are the lack of complete distributional data of many taxa (Hernández and Godinez 1994).

Research have shown that efforts to develop protection measures need to be greater in countries such as Mexico, Argentina, Peru, Brazil, Bolivia, USA, Chile, Cuba, Costa Rica and Paraguay, since they concentrate around 94% of all cacti species in the Americas (Ortega-Baes and Godinez-Alvarez 2006). For some countries with a large territorial extension, such as Brazil, conservation measures for cacti are still scarce considering that the Brazilian eastern region is the third largest center of diversity for the family. Numerous conservation reserves exist in the region, but the current knowledge on the number of protected taxa within these areas is still uncertain (Zappi et al. 2011).

Pilosocereus is a widely distributed genera, which contains 42 species and 9 subspecies (Hunt et al. 2006; Zappi and Taylor 2011) divided into two subgenera: *Gounellea* and *Pilosocereus* (Zappi 1994). It occurs throughout the Neotropical region, from USA to eastern Brazil, in many different habitats, always associated with xeric environments (Zappi 1994; Taylor and Zappi 2004). The genus is mostly abundant and diverse in semi-arid regions of eastern Brazil (Taylor and Zappi 2004). There the species have a significant ecological importance acting as dominants in the habitat where they occur and providing the main source of floral resources to pollinators or visitors (Rocha et al. 2007). Few species of *Pilosocereus* are also relevant by their uses. For instance, *P. gounellei* is used for animal and human nutrition as supplementary fodder to sheep, goats and cattle during severe droughts and to produce flour, sweets and biscuits, or medicinal usage (Andrade et al. 2006; Lucena et al. 2013; Monteiro et al. 2015). *Pilosocereus pachycladus* is also used for cooking and for construction (Lucena et al. 2013).

Many species of *Pilosocereus* are currently under major conservation threat due to human activities as habitat loss due to agriculture and livestock; fires for pasture renewal; and illegal collection (Silva et al. 2011). As a result, 13 taxa of *Pilosocereus* are listed under threat categories in the IUCN Red List of Threatened Species (IUCN 2017) (as critically endangered, endangered and vulnerable). Moreover, four species (*P. albisummus, P. mollispinus, P. oligolepis* and *P. splendidus)* are classified under the Data Deficient (DD) category and three taxa have not been assessed (*P. chrysostele* subsp. *cearensis, P. gounellei* subsp. *zehntneri* and *P. pachycladus* subsp. *pernambucoensis*). Many of these taxa have a small number of records and are not currently found in any protected areas (table 1).

We designed this study to provide a general overview on the patterns of distribution, richness, and endemism of *Pilosocereus* and to survey current available data to aid in the management and conservation planning for the group and for the areas where it occurs. This work specifically aims to: (1) present general patterns of geographic distribution of *Pilosocereus*; (2) highlight areas with high species richness, endemism and phylogenetic diversity; (3) evaluate if protected areas currently established throughout the occurrence of taxa assure the proper conservation of taxonomic and phylogenetic diversity of the group and (4) indicate areas where special attention is needed to maximize the proper conservation of the group.

Material and methods

Geographic distribution patterns

A matrix containing occurrence data for *Pilosocereus* included 2624 records from the online databases SpeciesLinks (CRIA 2016), the Global Biodiversity Information Facility (GBIF 2016) and the Virtual Herbarium REFLORA (Flora do Brasil 2016) and also included records listed in literature monographs (Zappi 1994; Taylor and Zappi 2004; Hunt et al. 2006). Application of names and synonyms in this work followed Hunt et al. (2006). We excluded records unidentified to species level, with imprecise locality data or duplicated. The species occurrence data obtained was then visualized in Quantum Gis v2.14.0 (QGis 2011) and compared to literature records (Zappi 1994; Taylor and Zappi 2004; Hunt et al. 2006) to search for incongruence. The incongruent records were then individually examined for consistency on the identifications and we preferred records identified by group specialists. To analyze distribution patterns of the studied species we obtained occurrence maps in Quantum Gis v2.14.0 (QGis 2011) using three environmental layers: (1) Biomes (Olson et al. 2001) (due to great territorial extension, the Brazilian ecoregions, Caatinga, Cerrado and Atlantic Forest, were encoded separately) (see figure 2); (2) climate types by Koppen-Geiger (Peel et al. 2007); and (3) soil types (FAO 2003) (see Appendix 1-3).

We performed a similarity analysis to examine the distribution of floristic composition throughout the geographic space. A matrix containing presence or absence data for each species of *Pilosocereus* in 1° x 1° grid cells was recorded and the Jaccard's distance was calculated using the group average with the package Vegan v. 2.4-1 (Oksanen et al. 2016) applied in in R v. 3.2.4 (R core team 2013). Only cells containing a minimum of two species were included for comparisons.

Richness and phylogenetic diversity

We analyzed richness, collection effort, and phylogenetic diversity using 1° x 1° grid cells (distinguished by an alpha-numeric code in the figures). Collection effort and richness were assessed through the total number of records and the total number of species registered in each cell, respectively, using complement Tom.Bio v. 2.5.0 (Burkmar 2016) in QGis. The phylogenetic diversity index was calculated for each cell based on a phylogram of *Pilosocereus* (Lavor et al. in prep) assessed with package Picante v. 1.6-2 (Kembel et al. 2014) applied in R v. 3.2.4 (R core team 2013).

Endemism and conservation

To build a Parsimony Analysis of Endemicity (PAE) following Morrone (1994), we used numbered grid cells to produce a presence/absence matrix in Mesquite v. 3.04 (Maddison and Maddison 2015). To root the trees, we included a hypothetical cell with all species absent. We performed the parsimony analyses on TNT (Goloboff et al. 2008) using trees generated on a traditional search performing 100 replicates of TBR Branch Swapping, starting from a Wagner's tree and retaining 10 trees per replicate. A strict consensus tree summarized equally parsimonious trees. Clusters of cells that shared at least two species were considered as representing endemism areas. As the size of the grid cells can influence the results, we tested two cell sizes: 1º×1° cells (299 grid) and 2º×2º cells (147 grid) and selected the case resulting in the highest number of endemism areas.

We obtained data on the conservation status of taxa and presences within protect areas from the IUCN Red List of Threatened Species (IUCN 2017). Protected areas were assessed from The World Database on Protected Areas (WDPA) (IUCN and UNEP-WCMC 2016). To evaluate if protected areas assure the proper conservation of taxonomic and phylogenetic diversity we performed a gap analysis using distribution data in Quantum Gis v2.14.0 (QGis 2011).

RESULTS

Geographic distribution patterns

Pilosocereus presents a disjunct distribution in the Americas, ranging from USA (Florida) and Mexico to Eastern Brazil (Fig. 1 and 2a). Only ten species (~20%) occur outside Brazil: *P. alenis*, *P. chrysacanthus*, *P. collinsii*, *P. leucocephalus, P. purpusii* and *P. quadricentralis* are distributed in Mexico and Central America; *P. royenii* is found in Mexico and Caribbean islands; *P. polygonus* in USA (Florida) and Caribbean islands; and *P. lanuginosus* is the only species to occur in northwestern South America (Fig.1a, b). *Pilosocereus oligolepis* is the only species of genus to occur outside (Guyana) and inside (Roraima state) Brazil. In Brazil 32 species and 9 subspecies of *Pilosocereus* occur. The greatest species richness is found in eastern Brazil, especially in Bahia and Minas Gerais States (Fig.1c) and in central Brazil (Fig. 1d).

Inside Brazil, taxa are distributed in: (1) northeastern region (Fig. 1e, c) (*P. catingicola*, *P. chrysostele*, *P. chrysostele* subsp. *cearensis*, *P. falvipulvinatus*, *P. piauhyensis* and *P. tuberculatus*); (2) coastal southeastern region (Fig. 1f) (*P. arrabidae*, *P. brasiliensis*, *P. brasiliensis* subsp. *ruschianus* and *P. ulei*); (3) widely in eastern region (Fig. 1g) (*P. gounellei, P. pentaedrophorus* and *P. pachycladus* subsp. *pernambucoensis).* A few taxa are restrictedly distributed and microendemic to specific areas (Fig. 1h) in: (1) eastern Brazil (*P. albisummus, P. aureispinus, P. aurisetus* subsp. *aurilanatus*, *P. azulensis*, *P. bohlei*, *P. diersianus*, *P. flexibilispinus*, *P. frewenii*, *P. fulvilanatus* subsp. *rosae*, *P. magnificus, P. mollispinus, P. multicostatus*, *P. parvus, P. pusillibaccatus* and *P. splendidus*); or (2) northern Brazil (*P. oligolepis*).

The analyses of the distribution of *Pilosocereus* in different biomes (Fig. 2b) show an equal number of taxa restricted to Caatinga (≈16%), and to Cerrado (≈16%); fewer taxa restricted to Atlantic Forest (≈6%) and to Tropical and subtropical broadleaf Dry Forest (≈4%). Considering taxa widely distributed (≈59%), the highest number is found in both Cerrado and Atlantic Forest biomes (≈18%), followed by those distributed in Caatinga, Cerrado and Atlantic Forest (≈14%), while the remaining taxa are distributed in two or more biomes in unequal numbers (table 2). For climate types (Fig. 2c) the greatest number of taxa of *Pilosocereus* is found with wide distribution in two or more climate types (≈61%) and the fewer number of taxa are restricted to Tropical Savannah climate (≈37%) or to Tropical Rainforest climate (only *P. ulei*) (table 3). A similar situation is found for soil type (Fig. 2d), as the greatest number of species is found in two or more soil types (wide distribution ≈86%) and fewer species showed a restricted soil preference (only *P. flexibilispinus* and *P. pusillibaccatus* in Ferralsols; *P. mollispinus* and *P. splendidus* in Lithosols; *P. aureispinus* and *P. fulvilanatus* subsp. *rosae* in Luvisols; and *P. parvus* in Arenosols) (table 4).

The similarity analysis resulted in two large clusters of cells outside Brazil, in Caribbean islands (blue) and Mexico (red) (Fig. 3a). Inside Brazil, we observe two main clusters: central (orange); and eastern (pink and green). Inside the eastern cluster, we noticed a subdivision in the core northeast (pink) and the core southeast (green) (Fig. 3b).

Richness and phylogenetic diversity

Outside Brazil, most cells have a small number of taxa (80,4% of the cells hold only 1 taxa; 18,1% hold 2-3 taxa and 1,39% hold 4 taxa). Cells B3 (covered by Arid Steppe Hot, Temperate Dry Winter Hot and Tropical Savannah climates; and Xerosols and Kastanozems soil type) and O13 (covered by Tropical Savannah climate and Cambisols, Luvisols, Phaeozems and Vertisols soil type) are the richest (4 taxa), followed by cells F8 (covered by Temperate Dry Winter Hot climate; and Andosols, Luvisols and Regosols soil type) and N13 (covered by Arid Desert Hot, Arid Steppe Hot and Tropical Savannah climates; and Cambisols, Luvisols and Vertisols soil type) with 3 taxa each (Fig. 4a). All these cells are located in Mexico covering areas of Tropical and Subtropical Coniferous Forests and Tropical and Subtropical Dry Broadleaf Forests biomes.

Inside Brazil, more than half of the cells hold 1 to 3 taxa: 39,7% hold 2-3 taxa; 32% hold 1 taxa; 23% hold 4-5 taxa; 4,4 % hold 6-7 taxa and 0,6% hold 8 taxa. Cell T16 is the richest with 8 taxa, followed by U16, V14, W14 with 7 each; and R21, S19, T21, U14 with 6 each (Fig. 4b). The higher richness occurs in the eastern region of the country (especially in Bahia and north Minas Gerais states), in Caatinga biome (cells T16, U14, U16, V14, W14); in ecotonal areas between Caatinga and Cerrado (R21, S19) and Atlantic Forest and Cerrado (T21). Those areas are under Tropical Savannah (R21, S19, U16); Tropical Savannah and Arid Steppe Hot (S19, T16); Arid Steppe Hot and Arid Desert Hot (U14, V14, W14) climates; and the grid cell T21 is covered by Ferralsols and Luvisols; R21 and U16 Ferralsols, Lithosols and Luvisols; S19 and V14 by Lithosols and Luvisols; T16 by Lithosols, Luvisols and Phaeozems; U14 by Lithosols, Luvisols and Vertisols; W14 by Arenosols and Luvisols soil type. The remaining grid cells with greater richness (4-5 taxa) are still in the same eastern region of Brazil, but in the states of Pernambuco, Paraíba, Rio Grande do Norte, Ceará and Rio de Janeiro.

Collection effort analysis resulted in Mexico, Ecuador and the region of Puerto Rico and Bahamas holding greatest number of records outside Brazil (Fig. 4c). Brazilian eastern region is better sampled, particularly in the states of Bahia, Sergipe, Pernambuco and Paraíba (Fig. 4d).

The Phylogenetic Diversity index range from 0.009 to 0.044 in this study (Fig. 5). The lowest values are outside Brazil, with many cells ranging from 0.009-0.017 and only a few cells ranging from 0.017-0.024 (in Mexico). In Brazil, the northeastern region shows the greater variation and higher values. Inside this area, the state of Bahia reaches the highest values (range from 0.009 to 0.044).

Endemism and conservation

PAE analysis revealed seven endemism areas for *Pilosocereus* (Fig. 6). Two are in Mexico: in Sonora, Sinaloa and Nayarit states (group 1), and Guerrero, Oaxaca and Chiapas states (group 2); five areas are in eastern Brazil: in Ceará state (group 3), Bahia state (groups 4, 5 and 6) and Minas Gerais state (group 7). These endemism areas are not fully contained within any protected area, but 1 grid cell of groups 1 and 2 contains significant portions of conservation units of sustainable use in Mexico and the group 5 encompasses parts in conservation units of sustainable use inside Brazil.

Among the total of number of records analyzed (2624), only 339 (≈13%) were collected inside conservation units: 192 records (≈7%) within Sustainable Use Units and 114 records (≈4%) within Integral Protection Units. Nevertheless, 33 species are protected within Conservation Units (≈65%) (of total number of species included in this study). It is controversial whether *P. collinsii* and *P. pusillibaccatus* occur or not within protected areas. According to IUCN *P. collinsii* occurs in the National Park Cañón del Sumidero (in Chiapas State, Mexico) and *P. pusillibaccatus* occurs in the State Park of Jalapão (in Tocantins State, Brazil), both areas of integral protection. However, we did not find evidence of their occurrence in those areas either by herbarium records or in the literature. Thus, in this work we consider these species as "not recorded in any conservation unit."

Outside Brazil, cells B3 and O13 have small fragments of native vegetation protected inside reserves, as the Biosphere reserve “Serra de Álamos” in B3. Inside Brazil, we highlight T16 in northern Bahia state, encompassing a small area protected within State Park “Morro do Chapéu” and environmental protection area “Gruta dos Brejões”. Considering phylogenetic diversity, T16 is also among the five cells with the highest values (0.40-0.44), other two cells have small areas of reserves, namely environmental protection area “Lago de Sobradinho” and “Serra Branca”, and ecological station “Raso Catarina” but the remaining two cells among the top five for phylogenetic diversity values do not harbor any conservation unit.

Discussion

General Patterns of distribution of *Pilosocereus*

Studies have pointed that precipitation, temperature and geologic history are key elements to explain temporal and spatial variation in the occurrence of Cactaceae (Godínez-Álvarez et al.

2003; Ortega-Baes et al. 2010). *Pilosocereus* presents a Neotropical disjunct general distribution pattern with two core areas of occurrence: (1) Eastern Brazil with most of the species and (2) Mexico and Caribbean region. Those two nuclear regions are distant apart but offer similar habitats, particularly dry biomes (namely Caatinga in Brazil, and a different dry forests types in Central America). Considering climatic preferences, *Pilosocereus* is primarily distributed in Tropical Savanna and Arid Hot Steppe (≈ 61%) climates, both offering predominantly warm temperatures and lower temperatures reaching 18°C (annual mean) (Peel et al. 2007).

Mourelle and Ezcurra (1996, 1997) highlighted that the diversity patterns for different life-forms in the family vary according to environmental conditions. In general, columnar cacti[1] as *Pilosocereus*, are richer in places with more frost-free days, while opuntioids[2] are more frequent in warm climates with rain distributed during the summer. Globoids[3] prefer areas with low rain (distributed during the summer only) and rocky soil. The patterns of occurrence observed here for *Pilosocereus* are in accordance with the general pattern described for columnar cacti.

Pilosocereus species can be either widely or restrictedly distributed in different biomes. In the Caatinga species occur more commonly widespread throughout the region whereas in Cerrado and Atlantic Forest the restricted pattern of distribution (microendemic) is more frequent among the species. The vegetation in Caatinga is a predominantly low-stature, dry woodland, often with thorns and shrubs, well adapted to seasonality with a marked long period of draught and with soils that vary from shallow to deep and from stony to sandy, with numerous rock outcrops (Taylor and Zappi 2004). These conditions favor the occurrence of

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[1] Cactus with column stems (cladodes), with ribs presenting an arrangement of areolas in longitudinal lines.
[2] Cactus with flattened cladodes and glochids.
[3] Cactus with globular shaped stem.

taxa well adapted to aridity, as *Pilosocereus*. On the other hand, the general environmental conditions in Cerrado and Atlantic Forest biomes do not favor the massive occurrence of *Pilosocereus*. Instead, the occurrence of *Pilosocereus* species there is restricted to several scattered patches offering optimal conditions.

The conditions in Cerrado are more xeric (in relation to the Atlantic Forest) with marked seasonality, seasonal fires and mild temperatures throughout the year (average between 22ºC and 27ºC) (Klink and Machado 2005). There the arboreal-shrub vegetation, mainly of low size, dominates the landscape covered by a dense herbaceous stratum. The Atlantic Forest is a rainforest under higher annual precipitation, lower seasonality and warm and humid climate (Ribeiro et al. 2009). In both biomes rock outcrops form micro xeric habitats (“xeric islands”) for *Pilosocereus*. In the Cerrado, rock outcrops act as a refuge away from the fires, which cacti cannot tolerate (Taylor and Zappi 2004). In the Atlantic Forest, rock outcrops (inselbergs) and habitats known as "restinga" (coastal sandy plains and dunes covered by herbaceous and shrubby tree vegetation) (Rocha et al. 2007), act as small "islands" of xeric conditions favoring the occurrence of *Pilosocereus* species (Taylor and Zappi 2004).

In Mexico and Central America region *Pilosocereus* is more commonly found in Tropical and Subtropical Coniferous Forests, Dry Broadleaf Forests and Moist Broadleaf Forests. A mosaic of landscapes marks this region (Fig. 1a-b and 2b) where these biomes form long and narrow strips (Olson et al. 2001). In common, they offer high climatic seasonality and abundant rocky substrates (raised from intense geological modification taking place in this region during the last millions of years; Pindell and Kennan 2009), which lead to the configuration of favorable xeric habitats for the genus. No evident soil preference is noticed in our analysis and this may be related to the evolution of adaptations to tolerate any rocky and oligotrophic soils.

Reproductive biology features may be influencing the distribution patterns particularly by dispersal agents (Mourelle and Ezcurra 1996, 1997; Ortega-Baes et al. 2010). Columnar cacti can be widely distributed due efficient pollinators and long distance dispersers, such as birds and bats, aiding in the pollen and seeds flow (Proctor et al. 1996). *Pilosocereus* has typical quiropterophylous flowers and its fleshy fruits are eaten and dispersed by birds or bats (Zappi 1994). These agents may be correlated to widely distributed species while other narrowly distributed species may have a distinct reproductive / dispersal system. However, few studies have been done so far exploring the reproductive biology or ecology of *Pilosocereus* (Nassar et al. 1997; Valiente-Banuet et al. 1997; Rivera-Marchand and Ackerman 2006; Rocha et al. 2007; Munguía-Rosas and Sosa 2010; Munguía-Rosas et al. 2010). The works available were not focused on elucidating long-distance dispersion patterns, remaining many hypotheses relating distribution vs. natural history of species open to investigation.

Species richness, phylogenetic diversity and endemism of *Pilosocereus*

The northeastern Brazilian state of Bahia, which presented the greatest species richness, phylogenetic diversity and areas of endemism, shows a great environmental complexity. It harbors large extensions of land as well as the ecotonal areas of important Brazilian biomes (Caatinga, Cerrado and Atlantic Forest); the Espinhaço mountain range (one of the floristically richest regions in the entire world – Giulietti et al. 1997); one of the largest Brazilian river basins (the São Francisco) and a marked geological/topographical differentiation.

Bahia has, besides the vegetation and climate characteristic of the semi-arid Caatinga, transitional ecotonal areas between the Caatinga, Cerrado and Atlantic Forest evergreen forests biomes, which increases its landscape heterogeneity and creates complex vegetation. These transition areas among biomes form a landscape mosaic with a combined biota composed of two or more types of communities what results in areas with high species richness (Kark and Rensburg 2006). This importance of Bahia state may also be related to its large area, nearly reaching the equivalent to 1/3 of Mexican territory. All these elements that create this differentiation in the landscape could also act as barriers to dispersion aiding in the process of diversification for *Pilosocereus*, and even other groups found there. In fact, this whole region is floristically singular and particularly diverse for the whole family, integrating the third largest diversity center for Cactaceae (Taylor and Zappi 2004).

The other states that also presented high rates of richness and phylogenetic diversity (but smaller than Bahia), such as Minas Gerais (north), Pernambuco, Paraíba, Rio Grande do Norte and Ceará show typical vegetation of Caatinga (except for Minas Gerais that also presents transition areas between the Caatinga, Cerrado and Atlantic Forest biomes) and arid to semi-arid climate with the presence of rocky outcrops. The state of Ceará has towards the north an area of endemism (group 3). This is also a state with great landscape mosaics, with coastal, mountain and inland (typically semiarid climate) areas interspersed (Moro et al. 2015). There the greatest endemism is in a region of Caatinga that is bounded by the crystalline formations of the Uruburetama, Machado and Baturité mountains (west, south and east respectively), with a notable topographic variation; and limited towards the north by the coastal plains. These mountains have a particularly rich flora, with many recently described species or narrowly-endemic ones (see Versieux et al. 2013; Moro et al. 2015).

The results found in our floristic analysis of similarity shows that the two eastern Brazilian subgroups are divided along northern Minas Gerais, close to the drainage basin of São Francisco River (to the west) and Pardo and Jequitinhonha rivers (between the two subclusters). The role of river basins in shaping genetic distribution of a rupicolous species of Bromeliaceae endemic to rocky outcrops of Minas Gerais has been demonstrated (Lavor et al.

2014). Similarly, rivers may be acting as barriers to *Pilosocereus* species distribution in this region.

Past and future: proper conservation of taxonomic and phylogenetic diversity of *Pilosocereus*

Our results show that the genus has been extensively sampled not only on sites with recognized species richness (such as Mexico and the states of Bahia and Minas Gerais in Brazil), but also in other regions such as the Caribbean and Northwest of South America and northeast Brazil (extra-Bahia). Considering the overall records, only ≈33% comes from either partial or integral protection units (parks, reserves, and other types of conservation units), demonstrating that collections for *Pilosocereus* are not restricted to protected areas. However, additional collection efforts are still necessary to better sample species with small populations or that have an apparent microendemic distribution pattern. The data on geographic distribution, richness, diversity, and endemism is important to assess the conservation status and to propose protection action plans for at-risk taxa, such as Cacti (Ortega-Baes et al. 2010).

Of the total of species and subspecies studied here, 33 (≈65%) are protected in conservation units. In Brazil, only two of those species (*P. aurisetus* subsp. *aurilanatus* and *P. oligolepis*) have a small number of records and a microendemic distribution, whereas the rest (31 spp.) present more than 25 records and a wider geographic distribution. Outside Brazil, only *P. quadricentralis* and *P. collinsii* are not represented within any conservation unit (this latter with controversial records in the National Park Cañón del Sumidero - in Chiapas State, Mexico - not found in our data collection).

Considering the cells that presented the higher phylogenetic diversity values or species richness, although some might have conservation units, their protected areas could be enhanced or, in many cases, new reserves could be created. For phylogenetic diversity, among the top five cells, only three of them harbor reserves, suggesting that the conservation plans are not taking full advantage of precise selection of areas to maximize *Pilosocereus* protection.

The great problem for the conservation of Brazilian species comes from the number of species with endemic or microendemic distribution within the genus (37%) that are vulnerable due to their small population size. Usually, such taxa also lack studies on their basic natural history. Some of the restricted-distributed *Pilosocereus* are currently reported as "deficient data" by the IUCN due to information gaps and currently know from the type or by a few collections. One example is *P. oligolepis*, only collected in two sites in 1927 in the state of Roraima (northern Brazil) since its description. A field expedition to search for present-day populations took place in 2014 and found three additional populations, increasing the knowledge of the species in terms of number of individuals, populations, and habitat (Lavor et al. 2016). We suggest that a greater collection effort be employed for other data deficient species occurring in remote areas or in places with difficult access such as *P. albisummus, P. mollispinus* and *P. splendidus*. In addition, the species *P. azulensis* and *P. diersianus* are poorly known (Hunt et al 2006) and require further field and taxonomic studies.

Other species still awaiting more detailed characterization includes *P. aureispinus, P. aurisetus* subsp. *aurilanatus, P. flexibilispinus, P. frewenii, P. fulvilanatus* subsp. *rosae, P. multicostatus* and *P. pusillibaccatus* to better know aspects of their life history and biology. Secondly, we suggest ex situ measures of preservation for species in the main categories of threat and restricted distribution, with the rescue and transplantation of specimens for protected areas (such as botanical gardens or conservation units near the localities where these species are found) and even the creation of germplasm banks.

Finally, we suggest that to assure the proper conservation of the taxonomic and phylogenetic diversity of the genus new conservation units could be created. Those areas are in Mexico (especially in the states of Sonora, Sinaloa, Guerrero, and Oaxaca) and in Brazil (which mainly contemplate areas in the central / northern portion of Minas Gerais and Bahia), which were the areas of greatest richness, phylogenetic diversity and endemism for *Pilosocereus*, but which are also areas of great diversity for the entire Cactaceae family (Taylor and Zappi 2004; Ortega-Baes and Godinez-Alvarez 2006).

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Figure captions

Figure 1. Distribution of all species of *Pilosocereus*, outside (a-b) and inside (c-h) Brazil.

Figure 2. Distribution of all species of *Pilosocereus* in different environment layers: geographic range (a); biomes (b); climate type (c) and soil type (d) (pie chart showing the largest numbers of taxa restrict by each layer).

Figure 3. Floristic similarity of grid cells outside (a) and inside (b) Brazil for *Pilosocereus* species.

Figure 4. Richness (a-b) and collection effort (c-d) of *Pilosocereus* species outside and inside Brazil (respectively).

Figure 5. Results of the Phylogenetic Diversity (PD) analyses for *Pilosocereus* species.

Figure 6. Results of the Parsimony Analysis of Endemicity (PAE) showing the different endemism areas superposed upon a conservation unit background (Left: Mexico; Rigth: Brazil).

Figures

Figure 1

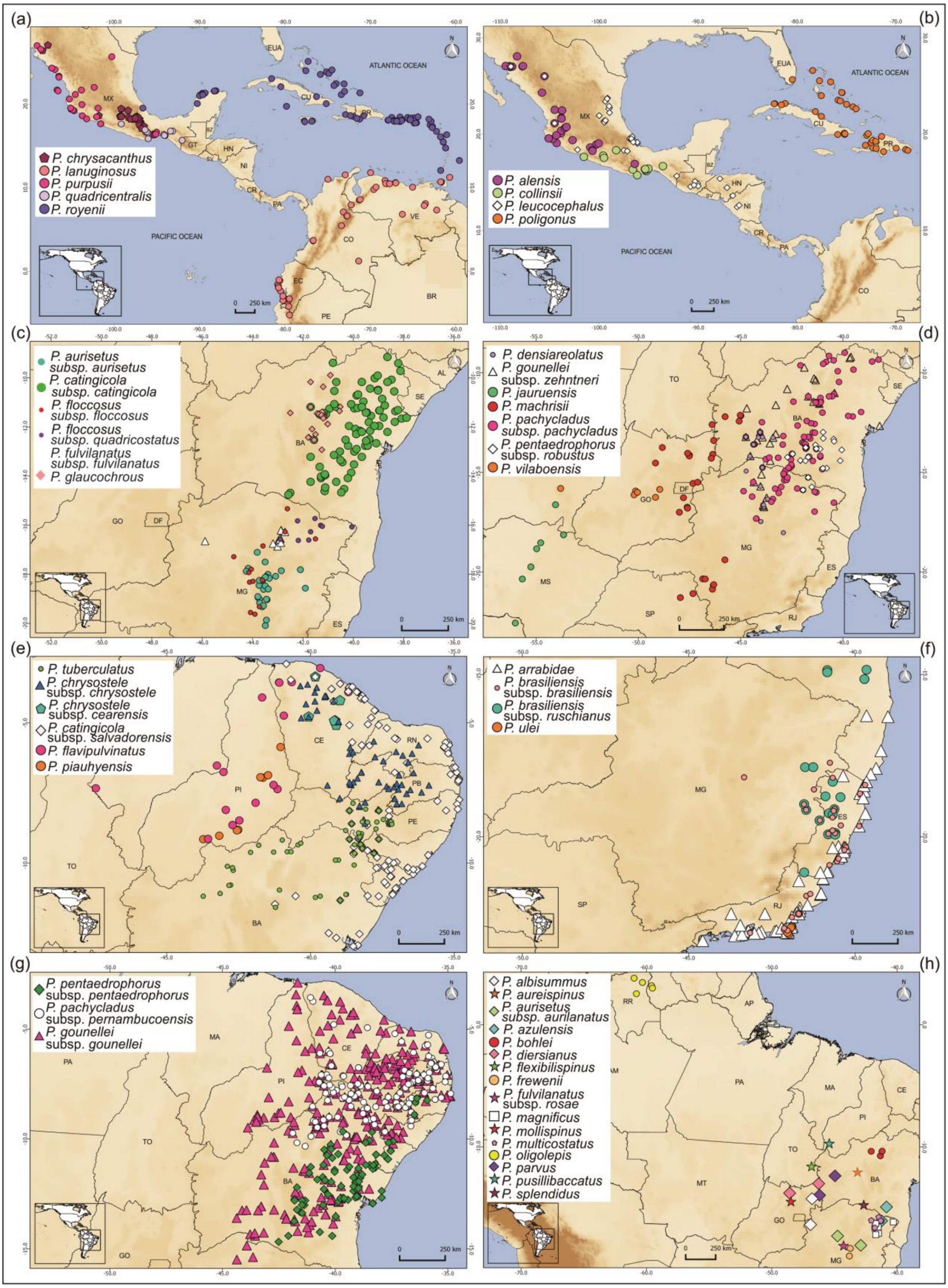


Figure 2

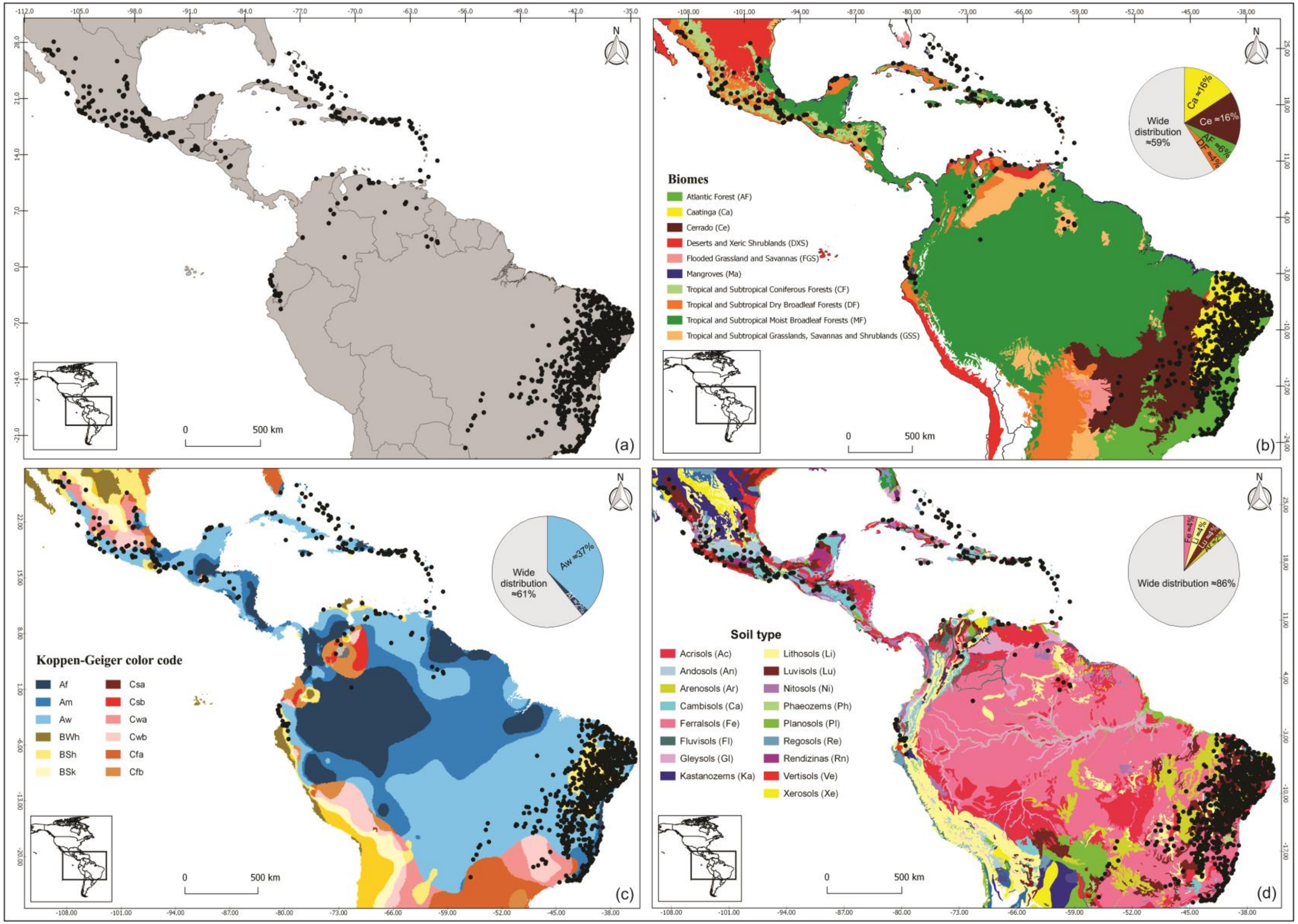

N
0 500 km
(a)
Biomes
Atlantic Forest (AF)
Caatinga (Ca)
Cerrado (Ce)
Deserts and Xeric Shrublands (DXS)
Flooded Grassland and Savannas (FGS)
Mangroves (Ma)
Tropical and Subtropical Coniferous Forests (CF)
Tropical and Subtropical Dry Broadleaf Forests (DF)
Tropical and Subtropical Moist Broadleaf Forests (MF)
Tropical and Subtropical Grasslands, Savannas and Shrublands (GSS)
Wide distribution ≈59%
Ca ≈16%
Ce ≈16%
AF ≈6%
DF ≈4%
(b)
Kopppen-Geiger color code
Af
Am
Aw
BWh
BSh
BSk
Csa
Csb
Cwa
Cwb
Cfa
Cfb
Wide distribution ≈61%
Aw ≈37%
(c)
Soil type
Acrisols (Ac)
Andosols (An)
Arenosols (Ar)
Cambisols (Ca)
Ferralsols (Fe)
Fluvisols (Fl)
Gleysols (Gl)
Kastanozems (Ka)
Lithosols (Li)
Luvisols (Lu)
Nitosols (Ni)
Phaeozems (Ph)
Planosols (Pl)
Regosols (Re)
Rendizinas (Rn)
Vertisols (Ve)
Xerosols (Xe)
Wide distribution ≈86%
(d)

Figure 3

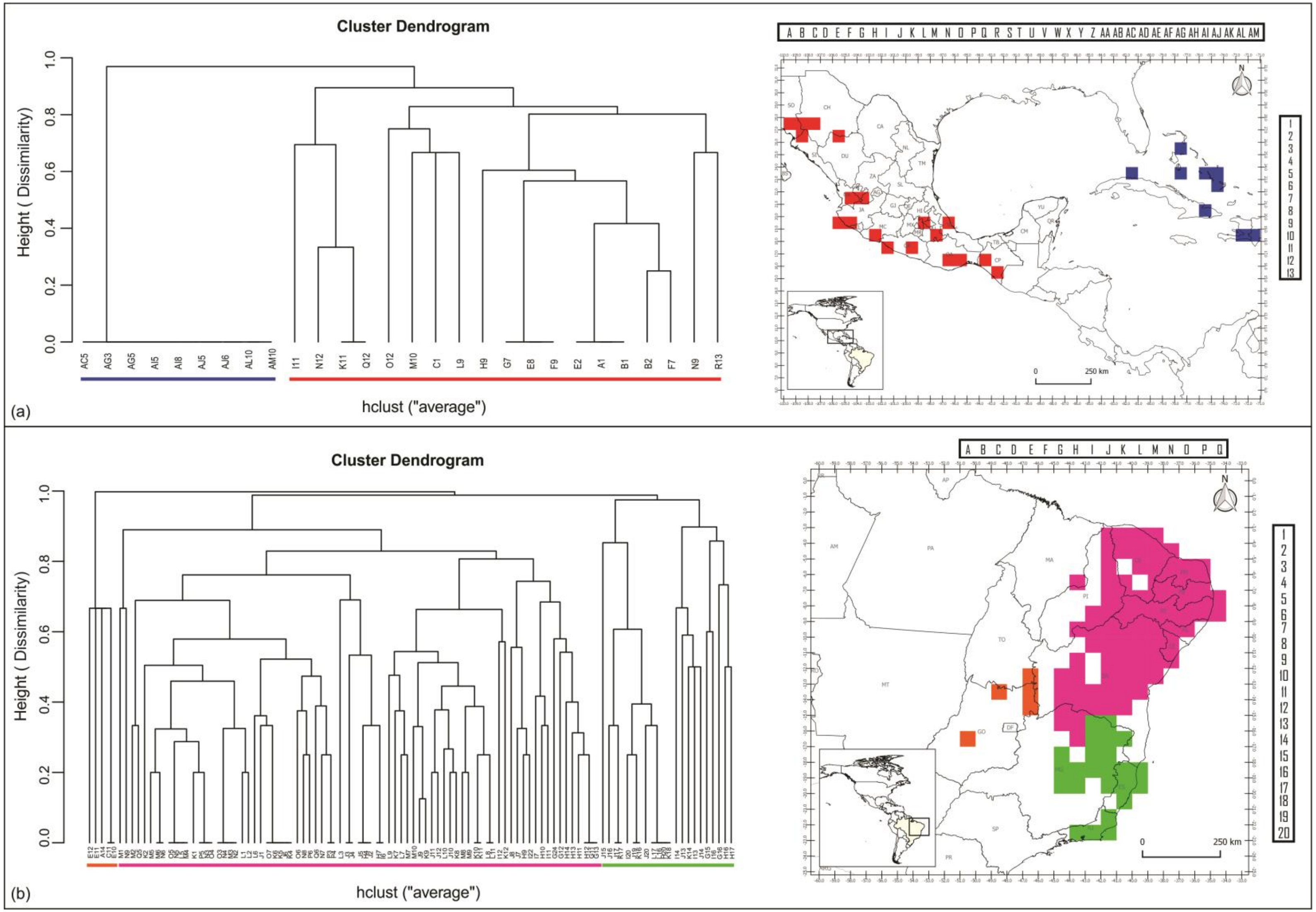

Cluster Dendrogram
Height (Dissimilarity)
0.0
0.2
0.4
0.6
0.8
1.0
AC5
AG3
AG5
AI5
AI8
AJ5
AJ6
AL10
AM10
I11
N12
K11
Q12
O12
M10
C1
L9
H9
G7
E8
F9
E2
A1
B1
B2
F7
N9
R13
hclust ("average")
(a)
A B C D E F G H I J K L M N O P Q R S T U V W X Y Z AA AB AC AD AE AF AG AH AI AJ AK AL AM
1 2 3 4 5 6 7 8 9 10 11 12 13
N
0 250 km
Cluster Dendrogram
Height (Dissimilarity)
0.0
0.2
0.4
0.6
0.8
1.0
hclust ("average")
(b)
A B C D E F G H I J K L M N O P Q
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
N
0 250 km

Figure 4

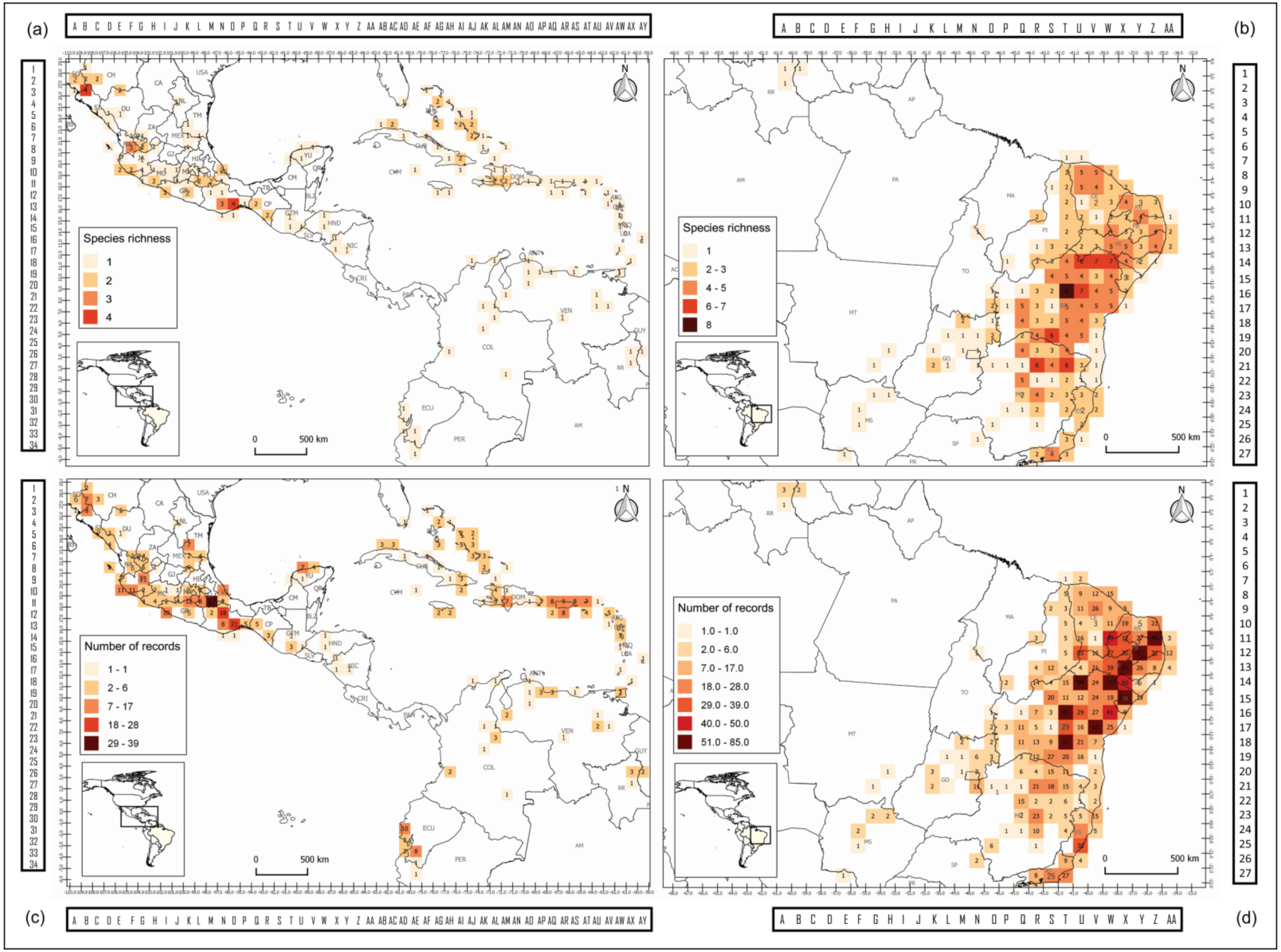
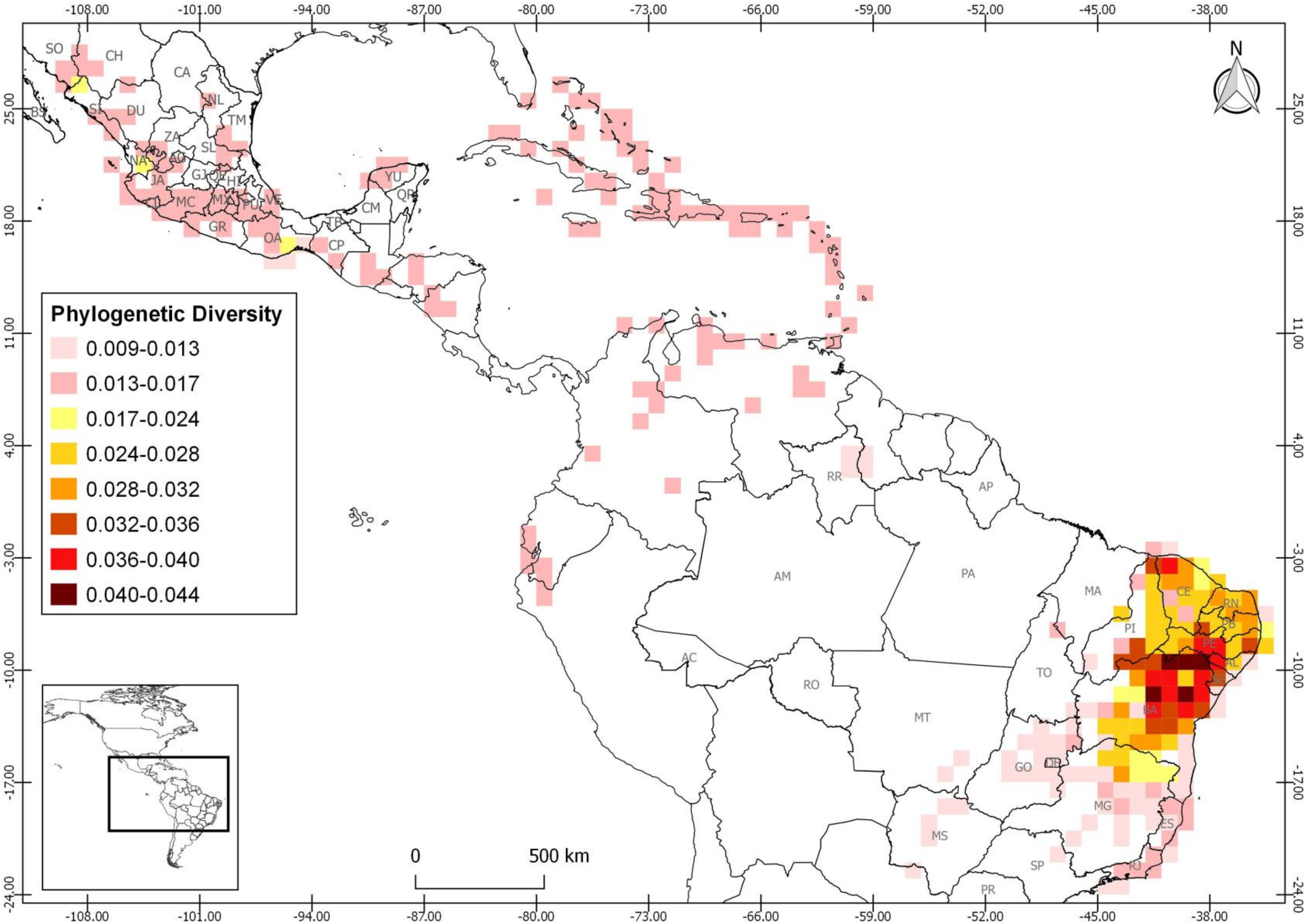

(a)
(b)
(c)
(d)
Species richness
1
2
3
4
Species richness
1
2 - 3
4 - 5
6 - 7
8
Number of records
1 - 1
2 - 6
7 - 17
18 - 28
29 - 39
Number of records
1.0 - 1.0
2.0 - 6.0
7.0 - 17.0
18.0 - 28.0
29.0 - 39.0
40.0 - 50.0
51.0 - 85.0
N
0
500 km
USA
CA
CH
DU
ZA
NL
TM
MEX
GJ
MC
HID
YU
QR
CM
CP
BLZ
GTM
HND
SLV
NIC
CRI
PAN
CUB
DOM
ANT
VEN
COL
GUY
ECU
PER
AM
RR
AP
PA
MA
PI
TO
MT
GO
MS
MG
SP
PR

Figure 5


-108.00
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-66.00
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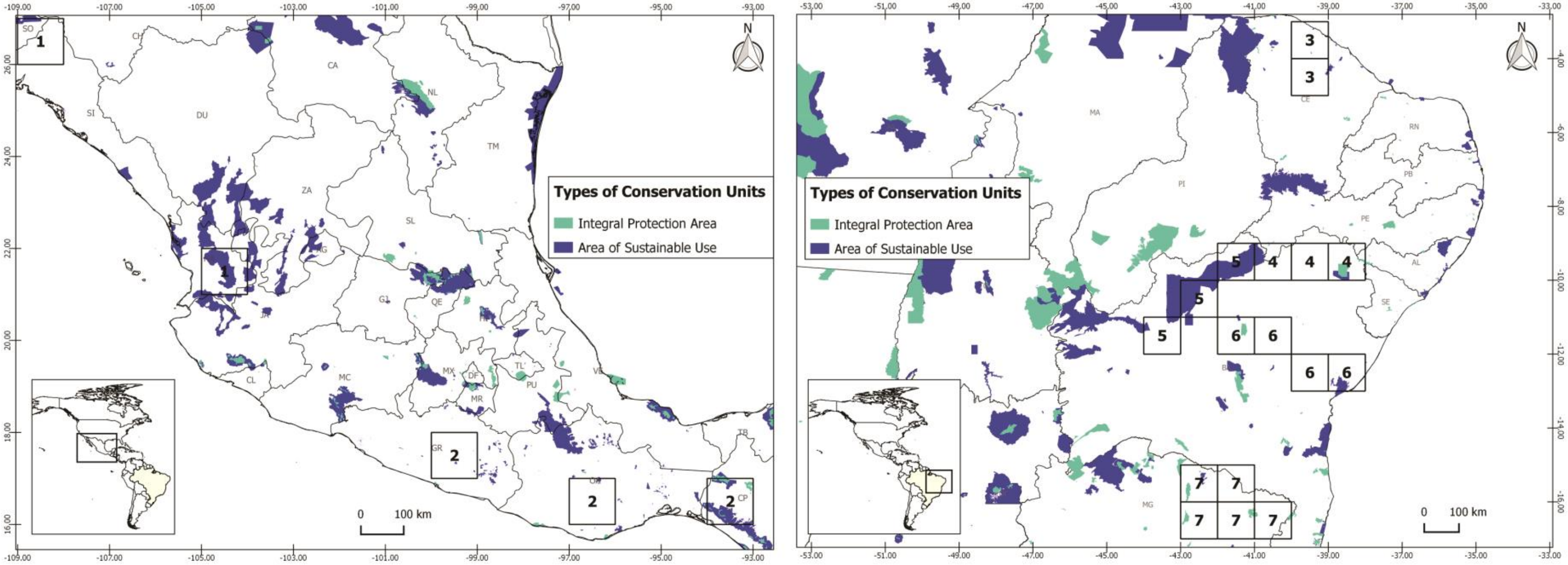
25.00
18.00
11.00
4.00
-3.00
-10.00
-17.00
-24.00
N
SO
CH
BS
SI
DU
CA
NL
TM
ZA
AG
SL
NA
JA
GJ
QE
HI
CL
MC
MX
PU
VE
GR
OA
YU
QR
CM
TB
CP
RR
AP
AM
PA
MA
CE
RN
PB
PI
PE
AL
SE
BA
AC
TO
RO
MT
GO
DF
MG
ES
MS
SP
RJ
PR
Phylogenetic Diversity
0.009-0.013
0.013-0.017
0.017-0.024
0.024-0.028
0.028-0.032
0.032-0.036
0.036-0.040
0.040-0.044
0
500 km

Figure 6


Types of Conservation Units
🟩 Integral Protection Area
🟦 Area of Sustainable Use
N
0 100 km
1
1
2
2
2
SO
CH
SI
DU
CA
NL
TM
ZA
SL
AG
GJ
QE
HI
JA
CL
MC
MX
DF
TL
MR
PU
VE
GR
OA
CP
TB
-109.00
-107.00
-105.00
-103.00
-101.00
-99.00
-97.00
-95.00
-93.00
26.00
24.00
22.00
20.00
18.00
16.00
Types of Conservation Units
🟩 Integral Protection Area
🟦 Area of Sustainable Use
N
0 100 km
3
3
4
4
4
5
5
5
6
6
6
6
7
7
7
7
7
MA
PI
CE
RN
PB
PE
AL
SE
BA
MG
-53.00
-51.00
-49.00
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-39.00
-37.00
-35.00
-33.00
-4.00
-6.00
-8.00
-10.00
-12.00
-14.00
-16.00

Tables

Table 1 IUCN conservation status to *Pilosocereus* species followed by total number of records and occurrence within protected areas.

IUCN conservation status	Species	Total number of records	Occurrence in protected areas
Critically Endangered (CR)	*Pilosocereus azulensis* (F.A.C.Weber ex Rol.-Goss) Byles & G.D.Rowley	2	n
	P. diersianus (Esteves) P.J.Braun	2	n
	P. frewenii Zappi & N.P.Taylor	2	n
	P. fulvilanatus subsp. *rosae* (P.J.Braun) Zappi*	1	n
Endangered (EN)	*P. aurisetus* subsp. *aurilanatus* (F.Ritter) Zappi*	2	y
	P. magnificus (Buining & Brederoo) F.Ritter	6	n
	P. multicostatus F.Ritter	8	n
	P. quadricentralis (E.Y.Dawson) Backeb.	17	n
	P. ulei (K.Schum.) Byles & G.D.Rowley	11	y
Vulnerable (VU)	*P. aureispinus* (Buining & Brederoo) F.Ritter	2	n
	P. brasiliensis subsp. *ruschianus* (Buining & Brederoo) Zappi*	26	n
	P. floccosus subsp. *quadricostatus* (F.Ritter) Zappi*	10	y
	P. parvus (Diers & Esteves) P.J.Braun	2	n
Near Threatened (NT)	*P. arrabidae* (Lem) Byles & G.D.Rowley	88	y
	P. catingicola subsp. *salvadorensis* (Werderm.) Zappi*	224	y
	P. chrysostele (Vaupel) Byles & G.D.Rowley subsp. *chrysostele*	88	y
	P. densiareolatus F.Ritter	20	y
	P. flexibilispinus P.J.Braun & Esteves	1	n
	P. fulvilanatus (Buining & Brederoo) F.Ritter subsp. *fulvilanatus*	17	y
	P. pentaedrophorus subsp. *robustus* Zappi*	27	y
Least Concern (LC)	*P. alensis* (F.A.C.Weber) Byles & G.D.Rowley	55	y
	P. aurisetus (Werderm.) Byles & G.D.Rowley subsp. *aurisetus*	34	y
	P. bohlei Hofacker	5	n
	P. brasiliensis (Britton & Rose) Backeb subsp. *brasiliensis*	54	y
	P. catingicola (Gurke) Byles & G.D.Rowley subsp. *catingicola*	151	y
	P. chrysacanthus (F.A.C.Weber) Byles & G.D.Rowley	81	y
	P. collinsii (Britton & Rose) Byles & G.D.Rowley	25	n
	P. flavipulvinatus (Buining & Brederoo) F.Ritter	20	y
	P. floccosus (Backeb. & Voll) Byles & G.D.Rowley subsp. *floccosus*	16	y
	P. glaucochrous (Werderm.) Blyles & G.D.Rowley	42	y

Table 1 continued

IUCN conservation status	Species	Total number of records	Occurrence in protected areas
Least Concern (LC)	*P. gounellei* (F.A.C.Weber ex K.Schum.) Byles & G.D.Rowley subsp. *gounellei*	546	y
	P. jauruensis (Buining & Brederoo) P.J.Braun	9	n
	P. lanuginosus (L.) Byles & G.D.Rowley	57	y
	P. leucocephalus (Poselg.) Byles & G.D.Rowley	60	y
	P. machrisii (E.Y.Dawson) Backeb.	41	y
	P. pachycladus F.Ritter subsp. *pachycladus*	146	y
	P. pentaedrophorus (Labour.) Byles & G.D.Rowley subsp. *pentaedrophorus*	132	y
	P. piauhyensis (Gurke) Byles & G.D.Rowley	8	y
	P. polygonus (Lam.) Byles & G.D.Rowley	39	y
	P. purpusii (Britton & Rose) Byles & G.D.Rowley	56	y
	P. pusillibaccatus P.J.Braun & Esteves	2	n
	P. royenii (L.) Byles & G.D.Rowley	112	y
	P. tuberculatus (Werderm.) Byles & G.D.Rowley	84	y
	P. vilaboensis (Diers & Esteves) P.J.Braun	7	y
Data Deficient (DD)	*P. albisummus* P.J.Braun & Esteves	2	n
	P. mollispinus P.J.Braun & Esteves	1	n
	P. oligolepis (Vaupel) Byles & G.D.Rowley	6	y
	P. splendidus F.Ritter	1	n
Not assessed	*P. chrysostele* subsp. *cearensis* P.J.Braun & Esteves	7	y
	P. gounellei subsp. *zehntneri* (Britton & Rose) Zappi	36	y
	P. pachycladus subsp. *pernambucoensis* (F.Ritter) Zappi	234	y

***** Asterisk indicate species assessment in 2002 and currently have been excluded from Red List.

Table 2 Patterns of distribution of *Pilosocereus* in different biomes following Olson et al. (2001) (total percent of taxa).

Patterns / Biome		**Taxa**
Restrict	**Ca**	*P. aureispinus, P. bohlei, P. chrysostele, P. chrysostele* subsp. *cearenses, P. glaucochrous, P. piauhyensis, P. splendidus, P. tuberculatus* (≈16%)
	Ce	*P. albisummus, P. diersianus, P. flexibilispinus, P. fulvilanatus, P. fulvilanatus* subsp. *rosae, P. mollispinus, P. parvus, P. pusillibaccatus* (≈16%)
	AF	*P. azulensis, P. brasiliensis* subsp. *ruschianus, P. ulei* (≈6%)
	DF	*P. collinsii, P. quadricentralis* (≈4%)
Wide	**Ce, AF**	*P. aurisetus, P. aurisetus subsp. aurilanatus, P. brasiliensis, P. floccosus subsp. quadricostatus, P. frewenii, P. jauruensis, P. machrisii, P. multicostatus, P. vilaboensis* (≈18%)
	Ce, Ca	*P. flavipulvinatus* (≈2%)
	AF, Ma	*P. arrabidae* (≈2%)
	DF, CF	*P. alensis* (≈2%)
	GSS, MF	*P. oligolepis* (≈2%)
	Ca, Ce, AF	*P. densiareolatus, P. floccosus, P. gounellei subsp. zehntneri, P. magnificus, P. pachycladus, P. pentaedrophorus, P. pentaedrophorus* subsp. *robustus,* (≈14%)
	Ca, AF, Ma	*P. catingicola, P. catingicola* subsp. *salvadorensis, P. pachycladus* subsp. *pernambucoensis* (≈6%)
	DF, CF, Ma	*P. purpusi* (≈2%)
	DF, CF, DSX	*P. chrysacanthus* (≈2%)
	DF, CF, DXS, MF	*P. leucocephalus* (≈2%)
	Ca, Ce, AF, Ma	*P. gounellei* (≈2%)
	DF, DXS, MF, GSS, Ma	*P. lanuginosus* (≈2%)
	DF, DXS, MF, Ma, CF, FGS	*P. polygonus, P. royenii* (≈4%)

Abreviations. **AF**: Atlantic Forest; **Ca**: Caatinga; **Ce**: Cerrado; **CF**: Tropical and Subtropical Coniferous Forests; **DF**: Tropical and Subtropical Dry Broadleaf Forests; **DXS**: Deserts and Xeric Shrublands; **FGS**: Flooded Grasslands and Savannas; **GSS**: Tropical and Subtropical Grasslands, Savannas and Shrublands; **Ma**: Mangrove; **MF**: Tropical and Subtropical Moist Broadleaf Forests.

Table 3 Patterns of distribution of *Pilosocereus* in different climate types following Peel et al. (2007) (total percent of taxa).

Patterns / Climate type		**Taxa**
Restrict	**Aw**	*P. albisummus, P. aureispinus, P. aurisetus, P. aurisetus* subsp. *aurilanatus, P. densiareolatus, P. diersianus, P. flexibilispinus, P. floccosus, P. floccosus* subsp. *quadricostatus, P. frewenii, P. fulvilanatus, P. fulvilanatus* subsp. *rosae, P. magnificus, P. mollispinus, P. multicostatus, P. parvus, P. pusillibaccatus, P. splendidus, P. vilaboensis* (≈37%)
	Af	*P. ulei* (≈2%)
Wide	**Aw, BSh**	*P. azulensis, P. bohlei, P. chrysostele, P. chrysostele* subsp. *cearenses, P. flavipulvinatus, P. glaucochrous, P. gounellei* subsp. *zehntneri, P. pachycladus* subsp. *pernambucoensis, P. pentaedrophorus* subsp. *robustus, P. piauhyensis, P. quadricentralis* (≈22%)
	Aw, Cfa	*P. jauruensis* (≈2%)
	Aw, Am	*P. oligolepis* (≈2%)
	Aw, BSh, BWh	*P. collinsii, P. pachycladus, P. tuberculatus* (≈6%)
	Aw, Af, Am	*P. brasiliensis* (≈2%)
	Aw, Cwa, Cwb	*P. machrisii* (≈2%)
	Aw, Am, BSh	*P. polygonus* (≈2%)
	Aw, Af, Am, BSh	*P. brasiliensis* subsp. *ruschianus, P. pentaedrophorus* (≈4%)
	Aw, Am, BSh, BWh	*P. catingicola, P. gounellei* (≈4%)
	Aw, Af, Am, Cwa	*P. arrabidae* (≈2%)
	Aw, BSh, BSk, Cwa	*P. purpusii* (≈2%)
	Aw, BSh, BSk, Cwa, Cwb	*P. alensis* (≈2%)
	Aw, Af, Am, BSh, BWh	*P. catingicola subsp. salvadorensis* (≈2%)
	Aw, Af, Am, BSh, Cfa	*P. royenii* (≈2%)
	Aw, Af, Am, BSh, Cfa, Csa, Cwb	*P. chrysacanthus* (≈2%)
	Aw, Af, Am, BSh, BWh, Cfb, Csb	*P. lanuginosus* (≈2%)
	Aw, Af, Am, BSh, BSk, Cfa, Cfb, Cwa, Cwb	*P. leucocephalus* (≈2%)

Abreviations. **Af**: Tropical Rainforest; **Am**: Tropical Monsoon; **Aw**: Tropical Savannah; **BWh**: Arid Desert Hot; **BSh**: Arid Steppe Hot; **BSk**: Arid Steppe Cold; **Csa**: Temperate Dry Summer Hot; **Csb**: Temperate Dry Summer Warm; **Cfa**: Temperate Without dry season Hot; **Cfb**: Temperate Without dry season Warm; **Cwa**: Temperate Dry Winter Hot; **Cwb**: Temperate Dry Winter Warm.

Table 4 Patterns of distribution of *Pilosocereus* in different Soil types following FAO (2003) (total percent of taxa).

Patterns / Soil type		**Taxa**
Restrict	**Fe**	*P. flexibilispinus, P. pusillibaccatus* (≈4%)
	Li	*P. mollispinus, P. splendidus* (≈4%)
	Lu	*P. aureispinus, P. fulvilanatus* subsp. *rosae* (≈4%)
	Ar	*P. parvus* (≈2%)
Wide	**Fe, Lu**	*P. azulensis, P. floccosus* subsp. *quadricostatus, P. magnificus, P. multicostatus* (≈8%)
	Fe, Li	*P. aurisetus* subsp. *aurilanatus*, *P. fulvilanatus* (≈4%)
	Li, Lu	*P. bohlei, P. frewenii* (≈4%)
	Ac, Fe	*P. albisummus* (≈2%)
	Ac, Gl	*P. ulei* (≈2%)
	Ac, Li	*P. diersianus* (≈2%)
	Ar, Fe	*P. piauhyensis* (≈2%)
	Ac, Li, Lu	*P. chrysostele* subsp. *cearenses* (≈2%)
	Ar, Fe, Lu	*P. jauruensis* (≈2%)
	Ar, Gl, Li	*P. oligolepis* (≈2%)
	Fe, Li, Lu	*P. pentaedrophorus subsp. robustus* (≈2%)
	Ar, Fe, Li,	*P. vilaboensis* (≈2%)
	Ac, Fe, Li, Lu	*P. aurisetus, P. floccosus* (3.9%)
	Ar, Fe, Li, Lu	*P. densiareolatus* (≈2%)
	Ac, Fe, Li, Gl	*P. brasiliensis* (≈2%)
	Fe, Li, Lu, Ph	*P. glaucochrous* (≈2%)
	Ac, Ar, Fe, Li, Lu	*P. flavipulvinatus, P. machrisii, P. pentaedrophorus* (≈6%)
	Ac, Fe, Li, Lu, Ph	*P. gounellei subsp. zehntneri* (≈2%)
	Ac, Ar, Li, Lu, Re	*P. tuberculatus* (≈2%)
	Ar, Fe, Li, Lu, Re	*P. pachycladus subsp. pernambucoensis* (≈2%)
	Ac, Ar, Fe, Li, Lu, Ph	*P. catingicola* (≈2%)
	Ac, Ar, Fe, Li, Lu, Re	*P. catingicola subsp. salvadorensis* (≈2%)
	Ar, Fe, Li, Lu, Re, Pl	*P. chrysostele* (≈2%)
	Lu, Ni, Ph, Pl, Rn, Ve	*P. collinsii* (≈2%)
	Ac, Fe, Gl, Lu, Ni, Ph,	*P. brasiliensis subsp. ruschianus* (≈2%)
	Ar, Fe, Li, Lu, Ph, Ve	*P. pachycladus* (≈2%)
	Ac, Ar, Ca, Fe, Gl, Ni, Pl	*P. arrabidae* (≈2%)
	Ca, Ka, Lu, Na, Re, Ve, Xe	*P. alensis* (≈2%)

Table 4 Continued

Patterns / Soil type		**Taxa**
Wide	**Ac, Ar, Fe, Li, Lu, Ph, Re**	*P. gounellei* (≈2%)
	Ca, Gl, Lu, Ph, Pl, Rn, Ve	*P. quadricentralis* (≈2%)
	Ca, Li, Lu, Na, Ph, Re, Ve, Xe	*P. chrysacanthus* (≈2%)
	Ca, Fl, Gl, Lu, Ni, Pl, Re, Rn	*P. polygonus* (≈2%)
	Gl, Lu, Ka, Na, Ph, Re, Ve, Xe	*P. purpusii* (≈2%)
	Ac, Ca, Fe, Fl, Li, Lu, Ka, Re, Xe	*P. lanuginosus* (≈2%)
	Ca, Fl, Li, Lu, Ka, Na, Ph, Rn, Ve, Xe	*P. leucocephalus* (≈2%)
	Ac, Ca, Fe, Fl, Li, Lu, Na, Ni, Ph, Pl, Re, Rn	*P. royenii* (≈2%)

Abreviations. **Ac**: Acrisols; **An**: Andosols; **Ar**: Arenosols; **Ca**: Cambisols; **Fe**: Ferralsols; **Fl**: Fluvisols; **Gl**: Gleysols; **Ka**: Kastanozems; **Li**: Lithosols; **Lu**: Luvisols; **Ni**: Nitosols; **Ph**: Phaeozems; **Pl**: Planosols; **Re**: Regosols; **Rn**: Rendzinas; **Ve**: Vertisols; **Xe**: Xerosols.

Appendix

Appendix 1 The coding by Biomes name following Olson et al. (2001): 1. Atlantic Forest (AF) (Alto Paraná Atlantic forest, Atlantic coast restinga, Bahia coast forest, Bahia interior forest, Pernambuco interior forest, Pernambuco coast forest, Serra do Mar coast forest); 2. Caatinga (Ca) (Atlântica dry forest, Caatinga); 3. Cerrado (Ce) (Campos rupestre montane savanna, Cerrado); 4. Tropical and Subtropical Coniferous Forests (CF); 5.Tropical and Subtropical Dry Broadleaf Forests (DF); 6. Deserts and Xeric Shrublands (DXS); 7. Flooded Grasslands and Savannas (FGS); 8. Tropical and Subtropical Grasslands, Savannas and Shrublands (GSS); 9. Mangrove (Ma); 10. Tropical and Subtropical Moist Broadleaf Forests (MF).

Appendix 2 A description of the climate types of the Koppen-Geiger following Peel et al. (2007): 1. Tropical Rainforest (Af); 2. Tropical Monsoon (Am); 3. Tropical Savannah (Aw); 4. Arid Desert Hot (BWh); 5. Arid Steppe Hot (BSh); 6. Arid Steppe Cold (BSk); 7. Temperate Dry Summer Hot (Csa); 8. Temperate Dry Summer Warm (Csb); 9. Temperate Without dry season Hot (Cfa); 10. Temperate Without dry season Warm (Cfb); 11. Temperate Dry Winter Hot (Cwa); 12. Temperate Dry Winter Warm (Cwb).

Appendix 3 The coding by major major soil groupings of the FAO/UNESCO soil classification system (FAO, 2003): 1. Acrisols (Ac); 2. Andosols (An); 3. Arenosols (Ar); 4. Cambisols (Ca); 5. Ferralsols (Fe); 6. Fluvisols (Fl); 7. Gleysols (Gl); 8. Kastanozems (Ka); 9. Lithosols (Li); 10. Luvisols (Lu); 11. Nitosols (Ni); 12. Phaeozems (Ph); 13. Planosols (Pl); 14. Regosols (Re); 15. Rendzinas (Rn); 16. Vertisols (Ve); 17. Xerosols (Xe).

4. CONSIDERAÇÕES FINAIS

O gênero *Pilosocereus* é um dos maiores e mais diversos gêneros dentro da família Cactaceae, sendo em contrapartida um dos menos estudados em proporção a essa riqueza. Isto talvez se deva ao fato deste gênero apresentar tamanho número de espécies, com diferenciações morfológicas e ampla distribuição nas áreas xéricas do Novo Mundo, que estudos globais, abrangendo todas as espécies, tenham sido dificultados até hoje. A taxonomia complexa, com diversos complexos de espécies também pode ter contribuído para essa falta de conhecimento sobre o grupo como um todo. Devido a este conjunto de fatores, o entendimento de aspectos da história natural, principalmente em um contexto histórico, era desconhecido até o momento para o gênero. Muito do sucesso alcançado na conclusão deste trabalho vem do status que a ciência se encontra atualmente, onde um crescente aumento ao acesso de diferentes tipos de informações tem possibilitado pesquisas cada vez mais integrativas.

Pilosocereus se mostra como um grupo relativamente jovem, mas com uma alta taxa de diversificação, já que em menos de 2 Ma conseguiu apresentar um grande número de linhagens distribuídas pelos neotrópicos. No entanto, a despeito do que já se sabia para outros gêneros na família, este é o primeiro gênero a ter sua origem reportada para uma das áreas xéricas de maior extensão e destaque na América do Sul, que é a Caatinga. Isto adiciona um novo grau de importância a este bioma exclusivamente brasileiro, que é um dos mais negligenciados em termos de pesquisas e proteção no Brasil.

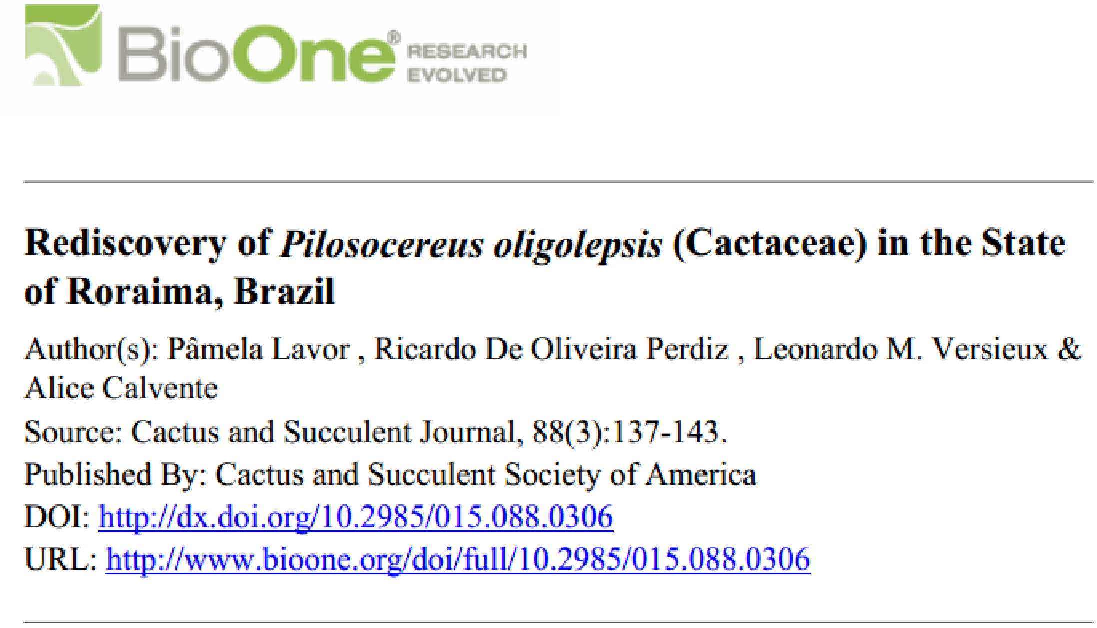
Assim como a história biogeográfica para a família Cactaceae é marcada por diferentes episódios de expansão e dispersão a longa distância (explicando sua ampla distribuição nas Américas), *Pilosocereus* também apresenta este mesmo componente histórico, mas que também sofre a influência dos ciclos climáticos do Pleistoceno, que teriam criado oportunidades para colonização e estabelecimento em outros nichos semelhantes ao ancestral. Consequentemente, atualmente as espécies do gênero podem ser encontradas nos mais diferentes biomas na região neotropical, mas comumente associados a afloramentos rochosos, onde micro habitats xéricos (com vegetação e clima característicos) são um fator determinante para sua distribuição. Talvez por isso os centros de diversidade, riqueza e endemismo para as espécies do gênero estejam em localidades que apresentam áreas tipicamente xéricas, como o México e a região leste do Brasil, nos estados da Bahia e norte de Minas Gerais.

Em termos sistemáticos, o gênero, assim como algumas de suas espécies heterotípicas, se mostra não monofilético em sua atual circunscrição (semelhante ao encontrado para muitos gêneros em Cactaceae). No entanto, este fato não é surpreendente quando se leva em consideração que as espécies do sugênero *Gounellea* apresentam uma marcante diferenciação morfológica. Dessa forma, a melhor alternativa para solucionar esse problema é a proposição de mudanças taxonômicas a fim de se recircunscrever o gênero e torna-lo monofilético.

Por fim, neste trabalho levantou-se evidências para auxiliar na conservação de todo o gênero, com o intuito de se avaliar seu real grau de ameaça e de sugerir medidas para assegurar a conservação da sua diversidade taxonômica e filogenética. Como resultado percebeu-se que a maior ameaça à *Pilosocereus* são as lacunas de conhecimento, que levam ao descaso das políticas públicas, à ignorância de agentes destrutivos, e à geração de medidas conservacionistas errôneas, já que só é possível preservar de forma adequada aquilo que se conhece em sua totalidade.

5. ANEXOS

5.1 ARTIGOS PUBLICADOS



Rediscovery of *Pilosocereus oligolepsis* (Cactaceae) in the State of Roraima, Brazil

Author(s): Pâmela Lavor , Ricardo De Oliveira Perdiz , Leonardo M. Versieux & Alice Calvente

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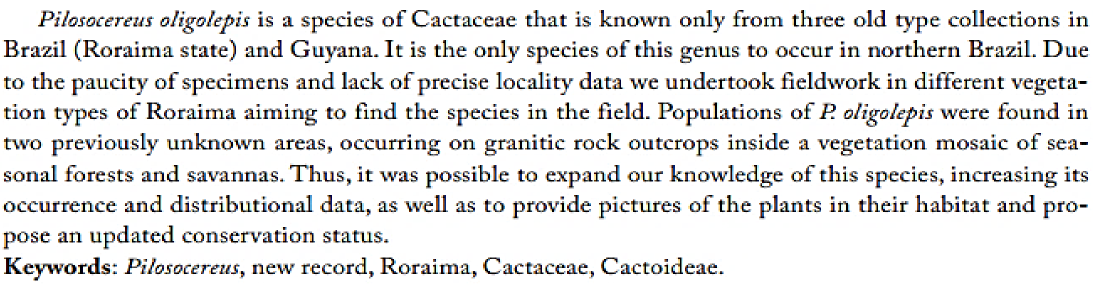
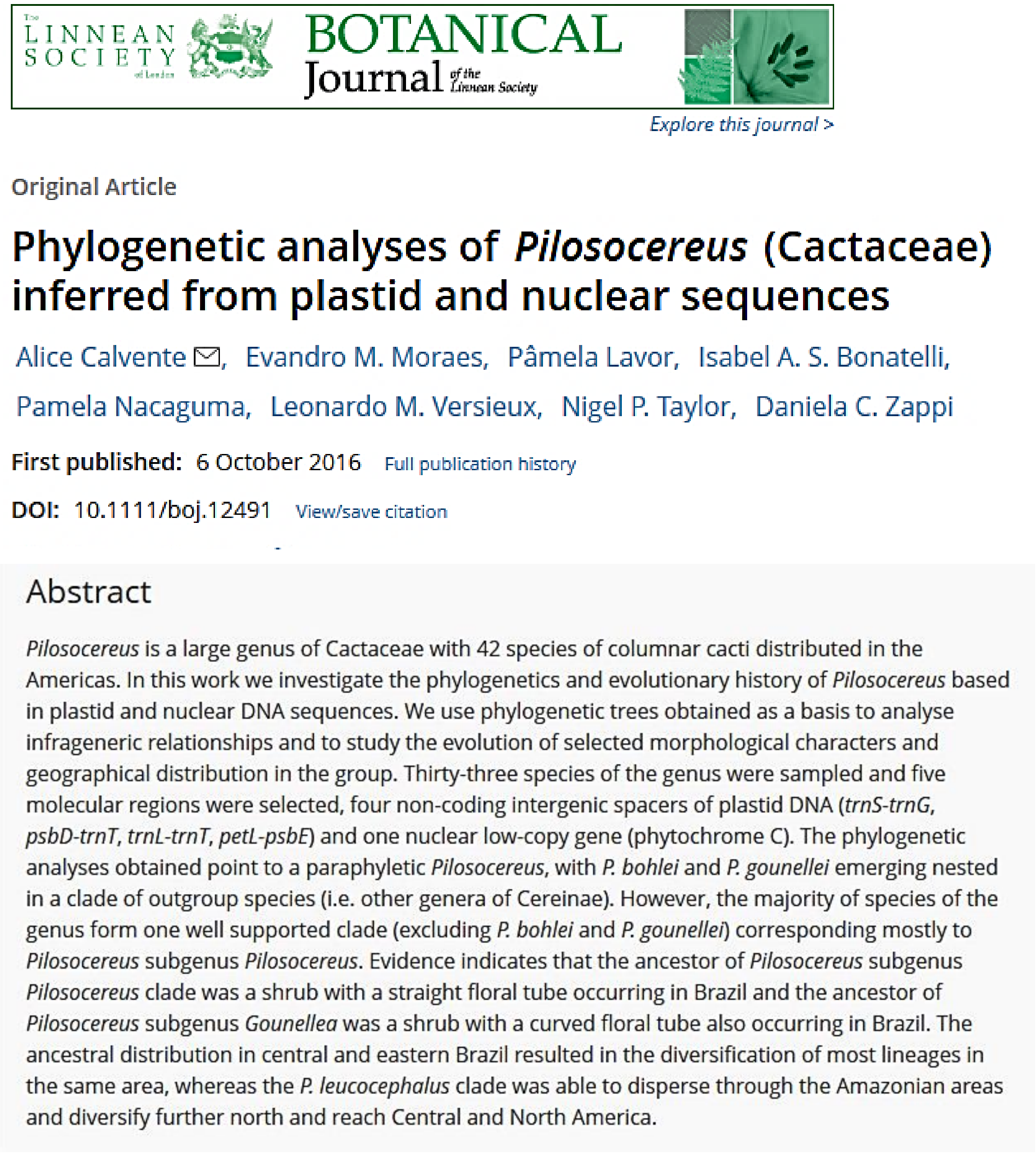
DOI: http://dx.doi.org/10.2985/015.088.0306
URL: http://www.bioone.org/doi/full/10.2985/015.088.0306



Pilosocereus oligolepis is a species of Cactaceae that is known only from three old type collections in Brazil (Roraima state) and Guyana. It is the only species of this genus to occur in northern Brazil. Due to the paucity of specimens and lack of precise locality data we undertook fieldwork in different vegetation types of Roraima aiming to find the species in the field. Populations of *P. oligolepis* were found in two previously unknown areas, occurring on granitic rock outcrops inside a vegetation mosaic of seasonal forests and savannas. Thus, it was possible to expand our knowledge of this species, increasing its occurrence and distributional data, as well as to provide pictures of the plants in their habitat and propose an updated conservation status.

Keywords: *Pilosocereus*, new record, Roraima, Cactaceae, Cactoideae.



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Phylogenetic analyses of *Pilosocereus* (Cactaceae) inferred from plastid and nuclear sequences

Alice Calvente ✉, Evandro M. Moraes, Pâmela Lavor, Isabel A. S. Bonatelli, Pamela Nacaguma, Leonardo M. Versieux, Nigel P. Taylor, Daniela C. Zappi

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Abstract

Pilosocereus is a large genus of Cactaceae with 42 species of columnar cacti distributed in the Americas. In this work we investigate the phylogenetics and evolutionary history of *Pilosocereus* based in plastid and nuclear DNA sequences. We use phylogenetic trees obtained as a basis to analyse infrageneric relationships and to study the evolution of selected morphological characters and geographical distribution in the group. Thirty-three species of the genus were sampled and five molecular regions were selected, four non-coding intergenic spacers of plastid DNA (*trnS-trnG*, *psbD-trnT*, *trnL-trnT*, *petL-psbE*) and one nuclear low-copy gene (phytochrome C). The phylogenetic analyses obtained point to a paraphyletic *Pilosocereus*, with *P. bohlei* and *P. gounellei* emerging nested in a clade of outgroup species (i.e. other genera of Cereinae). However, the majority of species of the genus form one well supported clade (excluding *P. bohlei* and *P. gounellei*) corresponding mostly to *Pilosocereus* subgenus *Pilosocereus*. Evidence indicates that the ancestor of *Pilosocereus* subgenus *Pilosocereus* clade was a shrub with a straight floral tube occurring in Brazil and the ancestor of *Pilosocereus* subgenus *Gounellea* was a shrub with a curved floral tube also occurring in Brazil. The ancestral distribution in central and eastern Brazil resulted in the diversification of most lineages in the same area, whereas the *P. leucocephalus* clade was able to disperse through the Amazonian areas and diversify further north and reach Central and North America.

5.2 NORMAS DAS REVISTAS

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Prentice, I.C., Guiot, J., Huntley, B., Jolly, D. & Cheddadi, R. (1996) Reconstructing biomes from palaeoecological data; a general method and its application to European pollen data at 0 and 6 ka. *Climate Dynamics*, **12,** 185-194.

Cox, C. B. & Moore, P. D. (1999) *Biogeography: an ecological and evolutionary approach*, 6th edn. Blackwell Science Ltd, Oxford.

Guo, Q. (1994) *Dynamic desert* Puccinellia maritima *plant community ecology: changes in space and time*. PhD Thesis, University of New Mexico, Albuquerque.

May, R.M. (1994) The effects of spatial scale on ecological questions and answers. *Large-scale ecology and conservation biology* (ed. by P.J. Edwards, R.M. May and N.R. Webb), pp. 1-17. Blackwell Scientific Publications, Oxford.

StatSoft Inc. (2003) *STATISTICA (data analysis software system), version 6.1*. StatSoft, Inc., Tulsa, OK.

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If the OnlineOpen option is not selected the corresponding author will be presented with the copyright transfer agreement (CTA) to sign. The terms and conditions of the CTA can be previewed in the samples associated with the Copyright FAQs below: CTA Terms and Conditions http://authorservices.wiley.com/bauthor/faqs_copyright.asp

Colour figures

Charges apply for the reproduction of colour figures in the hard copy of the journal. So, if your paper contains colour figures, the Colour Work Agreement form (available here), which outlines the charges, must be completed by the corresponding author and sent to Wiley Blackwell at acceptance. If using a limited colour palette we ask that authors avoid using red with green as this is a common colour-blindness combination. If you are not prepared to pay for colour in print, figures will be produced in colour in electronic versions of the paper, but black and white in the print copy. For the convenience of readers, we ask that you design your colour artwork so that it can be understood as best as possible in greyscale. Note that the same figure file must be used for both the print and online versions (we do not accept differing colour and black-and-white versions of the same figure). Authors must complete the Colour Work Agreement form even if they opt for colour online/black and white in print. Articles received by Wiley Blackwell with colour work will not be published until the form has been received. Please send a scanned copy of the form to our production editor (jbi@wiley.com) for information and, if paying for colour, post the hard copy of the form to: Customer Services (OPI), John Wiley & Sons Ltd, European Distribution Centre, New Era Estate, Oldlands Way, Bognor Regis, West Sussex, PO22 9NQ.

5.2.2 Systematic Botany

INFORMATION FOR AUTHORS

General: Members of the American Society of Plant Taxonomists (ASPT) are encouraged to submit manuscripts pertinent to plant systematics and closely related disciplines for publication in *Systematic Botany*. Membership in ASPT is required for at least one author from time of submission to publication (Not a member? Join ASPT here).

Manuscripts considered to be significantly lacking in depth, originality, or quality of English grammar or syntax may be returned without review. Acceptance of papers for publication depends on merit as judged by each of two or more referees. Manuscripts must not have previously been published in whole or in part (including other languages) and must not be in consideration for publication in another journal at the time of submission.

Language: Manuscripts must be in English although we publish a second abstract in another language. We strongly urge authors who are not native speakers of English to have either a native speaker who is familiar with botany critically evaluate the manuscript or hire an English editing service before submitting. Authors will only be allowed two revisions to ensure that the English grammar and style are correct before the manuscript is rejected. It is not the duty or responsibility of the Editorial staff to edit English.

Specifics: Authors are strongly urged to format their manuscripts by comparing their work to similar papers published recently in Systematic Botany and follow formatting meticulously. Failure to format will result in the manuscript being returned and the authors asked to make the corrections. These take time from both authors and editors and causes delays in publication. Spending time to correctly format the manuscript in the beginning is a speedier process. More specific details can be found in the Checklist for Preparation of Manuscripts and Illustrations. For review copy, keep manuscript file size down by using jpeg format and reduced pixel density for figures (keep good quality figure files for later submission of final revised manuscript).

Descriptions of new taxa (species and below): *Systematic Botany* does publish new taxa, but only when such taxa are placed in a broader context, such as (this list is not meant to be complete) a key to all species, or species in the area, demonstration of how the new taxa alter the generic concept, or demonstrate that the new taxon is distinct from published ones (via phylogenetic or morphometric analyses). These papers need to include an illustration clearly showing the diagnostic characters, but a line drawing is not required. Authors are encouraged to include information on conservation status, if available.

Data: Data should be submitted to Dryad (http://datadryad.org/) and cited as such in the text in the revision and DNA sequences must be submitted to GenBank. Data files should be provided for review purposes at the time of submission.

Figures: Final, publication-quality figures must be tiff files with a resolution of 1200 ppi (473 pixels per cm) or better (line drawings) or 350 ppi (138 pixels per cm) or better (continuous tone) for photographs. Figures can be full page width (7 inches = 178 mm wide) or single column width (3.375 inches = 86 mm wide), and no more than 9.5 inches (241 mm) high. Please size your image and calculate your resolution for these dimensions. See the checklist for more details regarding figures.

Color Figures: Figures may be submitted in full color and will be available online in color and authors will be assessed a non-waivable fee of $30 per color figure. Authors that do not wish any figure to be in color should submit only black and white or grayscale figures.

Submit: Submit manuscripts to http://www.editorialmanager.com/sysbot/. If this will be your first submission of a manuscript to the Systematic Botany Editorial Manager website, you must first register by clicking "register now" and following the instructions. Authors are welcome to include names, addresses, and email addresses of possible objective reviewers. We will give serious consideration to authors' recommendations for reviewers, but we do not guarantee to follow them.

Page Charges: Members of ASPT are not assessed page charges; however, members are strongly encouraged to
contribute to the cost of these pages. Authors are assessed charges for alterations made after type has been set. The following are charges that are not waived.

$5.00 each for author corrections above the five free allowed
$20.00 each for BW line figure replacements

$30.00 each for color figures, online only
$500.00 A payment of $500 above any special charges makes the article eligible for open access. This fee is prorated for articles less than 10 pages.

Publication online ahead of print: Papers will be made available online ahead of print unless authors specify
otherwise. The online version will be the publication date of record and each article will receive a date stamp stating the official publication date and assigned a doi that will be retained when the article is printed.

Papers longer than 50 printed pages: should be sent to Editor-in-Chief of Systematic Botany Monographs.

SYSTEMATIC BOTANY

CHECKLIST FOR PREPARATION OF MANUSCRIPTS AND ILLUSTRATIONS

August 2013

I. General Instructions

Membership in ASPT is required for at least one author from date of manuscript submission through to publication. Not a member? Contact the ASPT Business Office to join now (aspt@uwyo.edu).

Consult current issues for guidance on format.

Read Information for Authors on inside back cover of most recent issue or the web site.

Double-space throughout. Do not justify right margin.

Either American or international spelling is acceptable.

Use line numbering on initial submission to facilitate reviews of electronic manuscripts (do not use on revised manuscripts submitted for final acceptance).

Font formatting in manuscript corresponds to that used in the journal (e.g., italics for genus and species names; SMALL CAPITALS for primary headings and ALL CAPITALS for the short title on title page; ***Bold***
Italics for second level headings, etc.).

Do not italicize common Latin or non-English words or phrases (e.g., et al., i.e., sensu, etc.).

Include surname(s) of author(s) and page number as a header on all manuscript pages.

Assemble manuscript in this order: 1) Title page, 2) Abstract page, 3) Text, 4) Literature Cited, 5) Tables, 6) Appendices, if any, 7) Figure legends. A tiff file for each figure must be submitted separately, prepared following the instructions in section IX, below.

II. Title Page (Page 1)

Running head 6–8 lines below top of page, in all capital letters, no italics, and right justified. Include author(s) surname(s), if more than 2 use first author et al., colon, and a short title (total characters including
spaces must not exceed 70).

Center title, in upper and lower case, capitalize all major words, bold. Omit authors of scientific names. Include family in parentheses unless the genus is the type for the family. Below title, list all author names in bold upper and lower case in one centered paragraph. Author names are followed by author addresses starting on next line. Each address is a separate, centered paragraph. Addresses are written out in full without abbreviation. Include country in address, including those in the U. S. A.

Use superscript numbers following author names and preceding addresses to associate each author and the
appropriate address. Commas between author names precede superscripts. Example: John J. Jones,1,3Amy
A. Anderson,2 and Steve S. Staley1. Superscript number(s) following author(s) name(s) are also used to indicate any new addresses. New addresses are numbered sequentially after all author primary addresses.

Author for correspondence may be designated using a superscript number. The "Author for correspondence" follows on a new line following author addresses and should be the final superscript number used. Include email address in parentheses.

III. Abstract Page (Page 2)

Abstract must be one paragraph and begins with the word "Abstract" followed by an em-dash (—). For example, ***Abstract***—Morphology and molecular data….

Do not cite references, taxonomic authorities, or use abbreviations in the abstract.

Be concise (usually not more than 200 words), but include brief statements about the paper's intent, materials and methods, results, and findings. Include all new taxonomic names and new combinations, in boldface Italics.

Below abstract, as a separate paragraph, include up to six non-title keywords (or short phrases such as

‘adaptive radiation’) in alphabetical order, with the first word capitalized, separated by commas, and with a
period following the final term.

This section should begin with ‘Keywords’ in bold Italics. The keywords themselves should not be in bold.

For example, ***Keywords***—Adaptive radiation, chloroplast DNA, nuclear nitrate reductase gene, phylogeography, *Ulmus*.

IV. Text (Page 3, etc.)

Cite each figure and table in the text. Number figures and tables such that they are cited in numerical order.

Use “Figure” only to start a sentence; otherwise, “Fig.” or “Figs.”

Use these abbreviations without spelling out or punctuation: hr, min, sec, yr, mo, wk, d, diam, m, cm, mm,
µm; designate temperature as 30°C.

Write out other abbreviations first time used in the text; abbreviate thereafter. “Transmission electron microscopy (TEM) was used....”

Numbers: Write out one to nine unless a measurement or in taxonomic descriptions (e.g., four samples, 3 mm, 35 sites, 6 yr). Use 1,000 instead of 1000; 0.13 instead of .13; % instead of percent. Number ranges should be separated by an en-dash (–).

If three or more words are joined by a conjunction, use a comma after each word except the last. Example:
red, black, and white.

Each reference cited in the text must be listed in Literature Cited section, and vice versa.

Literature citations in the text are as follows: One author: Jones (1990) or (Jones 1990). No comma is used.

Two authors: Jones and Jackson (1990) or (Jones and Jackson 1990). No comma is used.

Three or more authors: Jones et al. (1990) or (Jones et al. 1990). No comma is used.

Multiple references for same author: Jones (1990, 1994) or (Jones 1990, 1994). Jones and Smith (in press) or (Jones and Smith in press) J. Jones (unpubl. data); J. Jones (in mss.); (J. Jones pers. obs.); or J. Jones (pers. comm.). No comma is used.

Within parentheses, use a semicolon to separate different types of citation (Fig. 4; Table 2) and (Felix and Smith 1988; Jones and Anderson 1989). Cite several references within parentheses by year, with the oldest
one first.

Main headings are large and small capital letters and centered on one line. The following are main headings: MATERIALS AND METHODS, RESULTS, DISCUSSION, TAXONOMIC TREATMENT, KEY (no Introduction, Conclusion, or Summary sections). Summary or conclusions must be incorporated in
discussion. Do not use main heading formatting for other headings such as Excluded Species, these should
be formatted as second level headings. Do not generate small capital letters by using all capitals and changing font size.

Second level headings are ***Bold Italics*** with normal indentation. Capitalize first letter of each major word and use Italics for all plant names. Headings are followed by an em-dash (—).

Third level headings are LARGE AND SMALL CAPITALS followed by an em-dash (—), with normal indentation.

Taxonomic authorities should be cited for all taxon names at generic rank and below at their first usage in the text, or referenced in a table or appendix.

Use a space after all initials including s. n., s. l., etc.

ACKNOWLEDGEMENTS follows discussion section.

Style is same as third level heading - the paragraph begins with ACKNOWLEDGEMENTS in large and small
capitals followed by an emdash (—), indent first line.

V. Taxonomic Treatment

For nomenclatural matter (i.e., synonymy, typification) use one paragraph per homotypic basionym (see recent Systematic Botany. Heterotypic basionyms are in separate paragraphs.

New names and new combinations should be in bold (not italicized). All other names of accepted taxa should be in large and small capitals (not italicized).

Names of synonyms are italicized in upper and lowercase.

Use authors of plant names as posted on The International Plant Names Index website (http://www.ipni.org/) for authors of botanical names. Please use a space after all initials, even if not done in IPNI. Authors should be given the first time a name is mentioned, or alternately in a table where all relevant names are listed (e.g., table of voucher specimens). References cited only as part of nomenclatural matter and not elsewhere are not included in literature cited; use TL-2 for abbreviations.

Use Index Herbariorum acronyms for designations of herbaria.

If specimens are cited, use the following forms:

TYPE: MEXICO. Nuevo León: 24 km S of San Roberto Jct., 26 Sep 1970, *Turner 6214* (holotype: TEX!; isotype: UC!).

Representative Specimens Examined— U. S. A. Michigan: Lapeer Co., along Flint River, 1.5 mi NE Columbiaville, 5 Jul 1955, *Beal s. n.* (NCSC). Ohio: Wood Co., just W Scotch ridge, 7 Jun 1955, *Beal 1073* (US).

Each country begins a new paragraph.

Abbreviate subspecies as subsp.

VI. Literature Cited

(Continue page numbering, include in same file as text. Not a separate file.)

Verify all entries against original sources, especially journal titles, volume and page numbers, accents, diacritical marks, and spelling in languages other than English. Capitalize all nouns in German. Cite references in strict alphabetical order by first author's surname. References by a single author precede multiauthored works by same senior author, regardless of date. Of those multiauthored works, 1) references with two authors precede all other multiauthored works and are listed in alphabetical order, and 2) references with three or more authors are listed in alphabetical order of authors, regardless of the number of authors involved.

List works by the same author(s) chronologically, beginning with earliest date of publication. Write out all authors' names, even if the first author is the same for succeeding citations.

"In press" citations must have been accepted for publication and the name of the journal or publisher included.

Insert a period and space after each initial of an author's name.

Leave one space between the colon following the volume number and the page number(s).

Write out journal titles in full using italics font. **Do not**use abbreviations.

Write author's names in upper and lower case.

Citations should be in the format:

Journal: Authors. Year. Title. *Journal Name* Volume: first page–last page.

Book: Authors. Year. *Title*. City: Publisher.

Edited book:Authors. Year. Title. Pp. no.–no. in *Book title,* ed.Editor. City: Publisher.

Examples of various citations:

Kim, S.-C., D. J. Crawford, J. Francisco-Ortega, and A. Santos-Guerra. 1996. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proceedings of the National Academy of Sciences USA* 93: 7743–7748.

Specht, C. D. and D. W. Stevenson. In press. A new generic taxonomy for the monocot family Costaceae (Zingiberales). *Taxon*.

Smith, C. F. 1998. *A flora of the Santa Barbara region, California*. Ed. 2. Santa Barbara: Santa Barbara Botanic Garden.

Nooteboom, H. P. 2003. Symplocaceae. Pp. 443–449 in *The families and genera of vascular plants* vol. 6, ed. K. Kubitzki. Berlin: Springer Verlag.

Swofford, D. L. 1998. PAUP* Phylogenetic analysis using parsimony (*and other methods), v. 4.0 beta 10. Sunderland: Sinauer Associates.

Bauml, J. A. 1979. *A study of the genus* Hymenocallis *(Amaryllidaceae) in Mexico*. M. S. thesis. Ithaca, New York: Cornell University.

DO NOT USE TABS TO MAKE HANGING INDENTS. Use paragraph formatting command.

VII. Tables and Appendices

(Continue page numbering, include in manuscript file following literature cited.)

Each table must start on a separate page, doublespaced. Include tables in manuscript file, use page or section breaks and landscape layout as necessary to fit the table on the page.

The title should be indented and begin with the word TABLE (large and small caps.) and number (in Arabic) followed by a period.

Do not use footnotes; instead, add notes to the end of the table caption.

Do not use vertical lines in tables.

DO NOT use tabs or spaces to align columns. Use the table building and formatting tools in your word processing package. Use left justification and place all text to the top of each cell, not centered.

Lists of voucher specimens, GenBank numbers, character lists, and any material that is long enough to disrupt the readability of the manuscript should be an appendix, not a table and ultimately will be formatted as comma-delimited paragraphs.

VIII. Figure Legends

(Continue page numbering, include in same file as text. Not a separate file)

Double-space legends and group them according to figure arrangements. Quadruple space between groups.

Do not use a separate page for each group.

Type legends in paragraph form, starting with statement of inclusive numbers:

FIGS. 3–5. Seeds of orchids. 3. At germination. 4. 2 wk after germination. 5. Seedlings.

FIG. 6. *Ipomopsis spicata* subsp. *robruthii*. A. Habit. B. Flower.

IX. Preparation of Illustrations

Important: Illustrations are either black and white half-tones (photographs), drawings, or graphs. Figures can be submitted in color and appear in full color for online versions at $30 per figure. Authors that wish to avoid all color charges should only submit black and white or grayscale figures.

Prepare illustrations using professional standards.

Lines should meet in sharp corners without inappropriate gaps or irregularities, Latin plant names should be italicized, letters and objects should be sharp and not evidently pixellated. Proofread figures carefully. They are the most difficult part of the paper to revise on short notice, or in proof. The Printer and Editors cannot edit or otherwise alter digital figure files in any way.

Final figures should be submitted as tiff files. All resolution requirements are for figures when sized at either full page or single column width (see below). Do not adjust resolution by shrinking the size of the figure. Line art (e.g., cladograms, botanical illustrations) **must** be at least 1200 pixels per inch (473 pixels per cm). Photographs (grayscale or color) **must** be a minimum of 350 dpi (138 pixels per cm). Images with mixed line art and grayscale **must** be at least 900 pixels per inch (354 pixels per cm). Be sure to check resolution when the figure is printed **at the appropriate size** for the journal.

Two widths are possible for figures: a full-page width figure is **177** mm wide, and a one-column width figure is **85** mm wide. Full page height is **240** mm (9.5 inches), but allow space for the caption if possible.

Files must be rasterized or scanned at the full resolution. Rasterizing at a low resolution and later resaving at a higher resolution will NOT improve the image quality. If you are scanning a paper illustration, make sure the hardcopy is sharp and clear, and both it and the scanning glass are clean. Dust removal/image editing is the author's responsibility.

Color graphics **must** be CMYK mode (**not** RGB).

Illustrations of highly magnified areas require a scale bar; a numerical magnification may also be included in the caption. Be sure to calculate magnification accordingly if reproduction is not at 100%. Include a scale and references to latitude and longitude on each map.

Group several drawings to form a plate of drawings, in the same order as discussed in the text. If several photos are included, group them into one or more plates.

Be sure to save black and white images as grayscale or bitmap, not color. **Do not** save layers! (in Photoshop, choose "Flatten Image" from the Layer menu). Crop the image so the image extends from edge to edge, there should be **no** blank white margins. Save as a tiff file using LZW compression (an option in Photoshop). (Do not use jpeg, which degrades images; line art is especially badly degraded in jpegs). Consult with editor if uncertain whether image file will be acceptable.

X. Data

All sequences used as data must be deposited in one of the international nucleotide sequence databases, preferably GenBank. Post-review final manuscript will not be accepted until sequence database accession numbers are included. Newly reported sequences **must** be documented by an herbarium specimen. Previously published sequences may cite the voucher or a literature reference where voucher information is given.

All data sets for phylogenetic and other analyses must be submitted to Dryad (http://datadryad.org/). Do not submit data prior to submitting your article. Dryad is integrated into the Editorial Manager system and you will be notified to submit your data once your manuscript is submitted.

Be certain to cite Dryad in your manuscript as a source for the data and any supplemental files and include the citation in the literature cited using the submitting author's name and date and including the title of the article.

For example:

Wang, X. 2014. Data from: ITS1: a DNA barcode better than ITS2 in eukaryotes? Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.n56t9

Italicize the full name of a gene, e.g., *rbcL, matK.*

In addition to character state distributions, consistency index, and retention index (where appropriate), some measure of support for clades (e.g., bootstrap values, decay indices ["Bremer support"], jackknife, etc.) must be provided for phylogenetic analyses.

When the data matrix is not part of the manuscript the data file must be provided with submitted manuscript for use by reviewers.

Voucher specimens should be cited in an appendix to document sources of morphological and molecular data. Vouchers are herbarium specimens, not living plant accession numbers from botanical gardens or DNA tube numbers, etc. Final versions of appendices must be submitted as comma delimited text, not tables although tabular format is acceptable for review purposes.

Additional analyses or bulky data sets should be placed on Dryad (http://datadryad.org/) and cited as such in the text. Online posting should be used sparingly, and data and analyses essential to the conclusions in the paper should appear in the published manuscript unless the length is prohibitive. Online supplemental material should not duplicate materials available on GenBank, or other online sources. Materials for online posting should usually be pdf files.

XI. What and Where to Submit

Before submission, have all coauthors read the manuscript critically. Papers longer than 50 printed pages should be sent to Editor-in-Chief of *Systematic Botany Monographs*.

Initial Submission

Microsoft Word format is preferred; contact the Editor in Chief if you are unable to submit in Word format.

Ensure that all files are free of hidden comments or tracked changes.

For review copy, keep file sizes down by using jpeg format and reduced pixel density for figures (keep good quality figure files for later submission of final revised manuscript). File name must include the surname of the first author and date of submission (e.g., Clark20Nov02.doc)

Cover letter. This should include any special instructions, any address change during the next several months, and phone and fax number and email address for the corresponding author. Names, addresses, and email addresses of possible objective reviewers should also be included. The cover letter must also include a statement that the manuscript has not been published in any portion or form (including another language) and is not in consideration for publication in any other journal.

Submit cover letter, manuscript file, data file(s), tables, and figures, to the Systematic Botany Editorial Manager website (see below). The author will receive an email message acknowledging receipt of the

new
submission. The manuscript will be forwarded to an Associate Editor for review.
Revised Manuscript
Final revised manuscript is submitted to the Systematic Botany Editorial Manager website. File name takes
the form: “Clark MS02–80 Revision1.doc” [the manuscript number is assigned when a new manuscript is received]. The final version must be submitted as a word processing file. Do not send PDF files.
Proofread figures carefully. They are the most difficult part of the paper to revise on short notice, or in proof. Editors and publisher cannot edit figures, author must provide revised files. The full cost of illustration changes in proof will be billed to the author. Please remember to remove line numbering, remove figures from manuscript file, and update information for "in press" citations.
Final revised manuscripts requiring significant editing by the Managing Editor to conform to *Systematic Botany* style will be returned to authors causing significant delay in publication.
Final revised manuscripts must use grammatically and stylistically correct English. The Editorial staff is not responsible for correcting English. Non-native speakers should request proofreading by a native speaker prior to submitting the revision or hire an editing service. If the revised manuscript retains inadequate English language, the authors will be allowed only one more revision. Poor English in the second revision will result in rejection.
Proofs and reprint order forms are sent to authors via email attachment as PDF files. Authors send corrected proof to Managing Editor and reprint orders to printer. Authors should make only necessary changes in proof. There is a mandatory charge for more than five changes made in proof.
Cover Illustrations Authors of accepted manuscripts may submit illustrations relevant to their manuscript to
be considered for the cover as digital files directly to the Managing Editor for consideration. Cover illustrations should be square, a minimum of 750 x 750 pixels (8-bit color in CMYK or 8-bit grayscale for black and white photographs) or 2,250 x 2,250 pixels (black and white line drawings). The name of the species, family, manuscript author names, and manuscript number should be included with the file. Permission of copyright holders is required for any files submitted.
Submit manuscripts to http://www.editorialmanager.com/sysbot/. If this will be your
first submission of a manuscript to the Systematic Botany Editorial Manager website, you
must first register by clicking "register now" and following the instructions.
Note: All manuscript submissions are promptly acknowledged via email. If you do not
receive an acknowledgement you should inquire to be sure it was received!
Questions? Contact the Editorial Office: systbot@gmail.com

5.2.3 Biodiversity and Conservation

GENERAL

Language

The journal‘s language is English. British English or American English spelling and terminology may be used, but either one should be followed consistently throughout the article. Authors are responsible for ensuring the language quality prior to submission.

Spacing

Please double–space all material, including notes and references.

Nomenclature

This is not a taxonomic journal and does not publish new scientific names of species or other ranks except in exceptional circumstances. The correct names of organisms conforming with the international rules of nomenclature must be used, but author citations of names are to be omitted except in exceptional cases where full bibliographic references to the original publication are justified.

Online Submission

Please follow the hyperlink “Submit online” on the right and upload all of your manuscript files following the instructions given on the screen.

Article Types

Original Research (9,000):

Manuscripts which are based on newly generated data which has not previously been published or new analyses of existing data sets. Topics which are likely to be of interest to a wide range of biodiversity scientists and conservationists are given priority, although local studies or ones restricted to one or a few species may be considered if they serve as case studies or include some novel approach. Articles dealing with several groups of organisms and wide geographical areas are generally welcome. Ecological or genetic papers will be considered only where they contribute to the core themes of the journal. Also, this is not a taxonomic journal, and papers which describe new species or propose new systematic arrangements will not normally be considered. In addition, author citations of scientific names are not to be included. The title page should be organized as in the section "Title page". This should be followed by an Abstract (150-250 words) and Key words (ones not in the title). The Introduction should place the work in a broader context and make the objectives clear. Methods and Results sections normally follow, and articles close with a Discussion of the results. Subheadings and alternative headings may be used where appropriate. References must follow the style given in "References", and be followed by Figure captions, Figures, and Tables (in that order).

Review Article (12,000):

Unsolicited reviews are encouraged, generally should have a global or regional perspective, and may concern particular groups of organisms or methodologies. They are generally prepared by experienced researchers with special in-depth knowledge of the topic. Extensive lists of references are expected. The general guidance given for Original Research submissions should be followed, but the system of headings and subheadings generally varies depending on the topic. Reviews generally include indications of outstanding issues to be addressed, and directions future work could take to elucidate those issues. If in doubt whether a review topic might be suitable, please contact the Editor-in-Chief prior to preparation and submission.

Invited Reviews (12,000):

Invited Reviews are ones which the Review Editor has invited, and are generally on subjects of wide or topical interest, or which may be controversial. The Reviews Editor makes invitations on the basis of her own experience with inputs from the journal's Associate Editors. Otherwise, the guidance given under "Review Article" above applies.

Book Review (12,000):

The journal no longer publishes individual book reviews as separate items, but combines book reviews and notices into batches which are issued one or two times each year. Authors wishing to submit reviews of books they have received should first check with the Editor-in-Chief whether the titles are already being covered. Publishers wishing to have titles considered for inclusion should send them to the Editor-in-Chief.

Commentary (2,000):

Remarks on particular topical issues or criticisms of published work in this or other journals, often controversial and bringing attention to matters of concern. They should follow the general guidance under "Original Articles", and require an Abstract, but the internal structure will depend on the topic. Commentaries do not generally include original previously unpublished data.

Letter to the Editor (1,000):

Opinions or criticisms drawing attention to issues of concern, or pointing out errors or inadequacies in Original Research articles published either in this journal or in other journals, are now welcome. They can be controversial, but need to cite supporting evidence for views expressed. No Abstract is required, no headings or subheadings are generally necessary, and References should normally not exceed 10-15.

The word count should include title, abstract, keywords, body of the text, figures, and tables but excluding authors affiliations, references and on-line supplementary material.

Title Page

The title page should include:

The name(s) of the author(s)

A concise and informative title

The affiliation(s) and address(es) of the author(s)

The e-mail address, telephone and fax numbers of the corresponding author

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.
Use tab stops or other commands for indents, not the space bar.
Use the table function, not spreadsheets, to make tables.
Use the equation editor or MathType for equations.
Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).
Manuscripts with mathematical content can also be submitted in LaTeX.
LaTeX macro package (zip, 182 kB)

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.
Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Citation

Cite references in the text by name and year in parentheses. Some examples:
Negotiation research spans many disciplines (Thompson 1990).
This result was later contradicted by Becker and Seligman (1996).
This effect has been widely studied (Abbott 1991; Barakat et al. 1995a, b; Kelso and Smith 1998; Medvec et al. 1999, 2000).

Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text. Do not use footnotes or endnotes as a substitute for a reference list.
Reference list entries should be alphabetized by the last names of the first author of each work. Order multi-author publications of the same first author alphabetically with respect to second, third, etc. author. Publications of exactly the same author(s) must be ordered chronologically.

Journal article

Gamelin FX, Baquet G, Berthoin S, Thevenet D, Nourry C, Nottin S, Bosquet L (2009) Effect of high intensity intermittent training on heart rate variability in prepubescent children. Eur J Appl Physiol 105:731-738. doi: 10.1007/s00421-008-0955-8
Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:
Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329
Article by DOI
Slifka MK, Whitton JL (2000) Clinical implications of dysregulated cytokine production. J Mol Med. doi:10.1007/s001090000086

Book

South J, Blass B (2001) The future of modern genomics. Blackwell, London

Book chapter

Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257

Online document

Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

Dissertation

Trent JW (1975) Experimental acute renal failure. Dissertation, University of California
Always use the standard abbreviation of a journal’s name according to the ISSN List of Title Word Abbreviations, see
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Supply all figures electronically.
Indicate what graphics program was used to create the artwork.
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Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

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All lines should be at least 0.1 mm (0.3 pt) wide.
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