

MARIANNA RODRIGUES SANTOS

**FILOGENIA MOLECULAR, TAXONOMIA, BIOGEOGRAFIA E
CONSERVAÇÃO DE *DISCOCACTUS* PFEIFF. (CACTACEAE)**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de *Doctor Scientiae*.

VIÇOSA
MINAS GERAIS – BRASIL
2013

**Ficha catalográfica preparada pela Biblioteca Central da Universidade
Federal de Viçosa - Câmpus Viçosa**

T

S237f
2013 Santos, Marianna Rodrigues, 1984-
Filogenia molecular, taxonomia, biogeografia e
conservação de *Discocactus* Pfeiff. (Cactaceae) / Marianna
Rodrigues Santos. – Viçosa, MG, 2013.
xv, 120f. : il. (algumas color.) ; 29 cm.

Texto em português e inglês.

Inclui apêndices.

Orientador: Flávia Cristina Pinto Garcia.

Tese (doutorado) - Universidade Federal de Viçosa.

Inclui bibliografia.

1. Cactaceae. 2. *Discocactus*. 3. Filogenia. 4. Fitogeografia.
5. Biodiversidade. 6. Plantas raras. 7. América do Sul.
I. Universidade Federal de Viçosa. Departamento de Biologia
Vegetal. Programa de Pós-Graduação em Botânica. II. Título.

CDD 22. ed. 583.56

MARIANNA RODRIGUES SANTOS

**FILOGENIA MOLECULAR, TAXONOMIA, BIOGEOGRAFIA E
CONSERVAÇÃO DE *DISCOCACTUS* PFEIFF. (CACTACEAE)**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de *Doctor Scientiae*.

APROVADA: 13 de novembro de 2013.

Lidyanne Yuriko Saleme Aona

Luiz Menini Neto

Christiano Franco Verola

Pedro Bond Schwartzburd

Cássio van den Berg
(Presidente da Banca)

Para Márcio e minha família,
por todo apoio recebido
durante esta jornada.

“Talvez não tenha conseguido
fazer o melhor, mas lutei para
que o melhor fosse feito. Não
sou o que deveria ser, mas graças
a Deus, não sou o que era antes”
(Marthin Luther King)

“A persistência é o menor caminho para o êxito.”
(Charles Chaplin)

AGRADECIMENTOS

Agradeço,

A Deus por ter me dado força para enfrentar todas as dificuldades, que foram muitas, durante este doutorado, obrigada Senhor!

Ao Márcio pelo amor, paciência e pela compreensão devido às inúmeras viagens em que o deixei sozinho na companhia apenas da nossa June;

À minha família pelo suporte incondicional;

À Universidade Federal de Viçosa, ao Programa de Pós-graduação em Botânica e à Profa. Flávia pela oportunidade de ter ingressado em uma Universidade ímpar como a UFV;

Ao Dr. Cássio Van den Berg, pela orientação e logística na realização da parte laboratorial no LAMOL, pela disponibilidade e, principalmente, pela paciência!

À Dra. Daniela Zappi e, especialmente ao Dr. Nigel Taylor pela orientação. Saibam que o amor que vocês têm pelo que fazem é que me inspirou a estudar as cactáceas!

À CAPES pela bolsa concedida;

À FAPEMIG pelo custeio do projeto;

Ao British Cactus and Succulent Society e Cactus and Succulent Society of America pela aprovação de projetos que viabilizaram algumas viagens de campo, muito úteis no início do projeto;

Ao Herbário VIC, pela infraestrutura e aos funcionários Fernanda, Sr. Maurício e Celso por toda ajuda dispensada durante estes anos;

Aos herbários que me receberam, emprestaram materiais e também aqueles que disponibilizam virtualmente as fotos das exsicatas (todos estão citados neste trabalho);

Ao Dr. Urs Eggli e o gerente de coleções botânicas Peter Audiffred por terem me recebido nos herbários ZSS e L, respectivamente, onde pude consultar os tipos de *Discocactus* e ao Programa de Pós-graduação em Botânica da UFV pela ajuda de custo para esta visita;

Ao IEF e ICMBIO pelas licenças;

Aos parques que visitei e seus funcionários pelo pronto atendimento e disponibilidade logística durante as visitas;

Ao Sr. Eustáquio (ICMBio) por ter me acompanhado durante muitos campos realizados;

À Suelma e ao Plano Nacional para a Conservação de Cactáceas (ICMBio) pela logística e pela oportunidade de fazer parte deste grande projeto;

Às minhas amigas Rúbia e Paty pela força;

Aos meus amigos Marlon, Ariane pela amizade e por toda a ajuda no LAMOL e com as análises;

Ao Dr. Ary de Oliveira, meu orientador de mestrado, que passou a ser um amigo. Com suas sábias palavras e sua vasta experiência me acalmou para este dia.

Aos funcionários, Rick e Dissinho, pelo apoio durante as minhas temporadas no LAMOL-UEFS;

Aos muitos amigos que fiz durante as temporadas bahianas em Feira de Santana e que direta ou indiretamente me ajudaram na “lamolação” em dias de semana, finais de semana e feriados;

Aos meus irmãos da Primeira Igreja Batista de Viçosa pelo apoio espiritual;

Ao Dr. Christiano Verolla e à Raisa pela ajuda com as análises da parte biogeográfica deste estudo;

À Lidy pela força;

Aos colegas e amigos que contribuíram com coletas e fotos de exsiccatas: Bernarda de Souza, Fábila Silva, Gerardus Olsthorn, Juliana Rando, Marcelo Telles, Marcos Meiado, Marlon Machado e Thiago Stefanello;

Aos colegas do programa de Pós-graduação em Botânica;

Ao nosso querido secretário Ângelo por ter sido tão prestativo durante todos estes anos;

Ao Reinaldo pelas ilustrações;

Enfim, agradeço a todos que direta ou indiretamente contribuíram para a realização deste trabalho, e desde já peço desculpas àqueles que injustamente não tiveram seus nomes citados.

A todos, o meu sincero,

OBRIGADA!

SUMÁRIO

LISTA DE TABELAS.....	viii
LISTA DE FIGURAS.....	viii
RESUMO.....	xii
ABSTRACT.....	xiv
INTRODUÇÃO GERAL.....	1
REFERÊNCIAS.....	8

CAPÍTULO 1- A phylogenetic study of *Discocactus* Pfeiff. (Cactaceae) based on nuclear and plastid DNA sequences

Abstract.....	14
Introduction.....	15
Material and Methods.....	16
Results.....	20
Discussion.....	26
Acknowledgements.....	31
References.....	31
Appendix.....	37

CAPÍTULO 2 – Taxonomy distribution and conservation of *Discocactus* Pfeiff. (Cactaceae)

Abstract.....	44
Introduction.....	45
Material and Methods.....	47
Results.....	48

Conclusions.....	87
Acknowledgements.....	87
References.....	88
Appendix.....	91

**CAPÍTULO 3 – Biogeography and conservation status assessment of
Discocactus Pfeiff. (Cactaceae): an endemic genus of South America**

Abstract.....	98
Introduction.....	99
Material and Methods.....	100
Results.....	101
Discussion.....	108
Conclusions.....	113
Acknowledgements.....	114
References.....	115
CONCLUSÕES GERAIS.....	119

LISTA DE TABELAS

CAPÍTULO 1

- Table 1.** Primers designed and used to amplify and sequence the plastid and nuclear loci. All primers were used to amplify and sequence, except ITS which was sequenced using other primers.....17
- Table 2.** PCR conditions for the amplification of different genome regions used in this study.....18
- Table 3.** DNA data sets used in this study, in relation to one of the most parsimonious trees resulting from combined analysis and evolutive models selected to Bayesian inference.....21

CAPÍTULO 3

- Table 1.** Conservation status and geographical distributions patterns of *Discocactus* Pfeiff.102
- Table 2.** Occurrence of *Discocactus* Pfeiff. in Full Protected Conservation Units (FPCUs) in Brazil.109

LISTA DE FIGURAS

CAPÍTULO 1

- Figure 1.** Distribution area of *Discocactus* species.....16
- Figure 2.** Strict consensus of 861 most parsimonious trees for *Discocactus* and outgroups based on combined plastid and nuclear sequences. Bootstrap percentages are given above branches. CI=0.74 RI=0.80. Morphological features related to ribs morphology are shown by symbols.....23

Figure 3. Strict consensus of 340 most parsimonious trees for <i>Discocactus</i> and outgroups based on combined plastid sequences. Bootstrap percentages are given above branches. CI=0.74 RI=0.90.	24
Figure 4. Majority-rule consensus of 7000 trees obtained in the Bayesian analysis with algorithm Markov chain Monte Carlo and the model of evolution=GTR+I+G in a combined analysis of seven DNA regions in <i>Discocactus</i> . Displayed as a phylogram (A) and cladogram for better visualization (B). Numbers above branches are posterior probabilities for clades estimated by the proportion of occurrence in the tree set.....	25
Figure 5. <i>Discocactus bahiensis</i> (A, B); <i>D. boliviensis</i> (C, D); <i>D. catingicola</i> (E,F); <i>D. diersianus</i> (G, H); <i>D. ferricola</i> (I, J); <i>D. hartmannii</i> (K, L); <i>D. horstii</i> (M, N); <i>D. heptacanthus</i> (O); <i>D. placentiformis</i> (P, Q); <i>D. pseudoinsignis</i> (R); <i>D. zehntneri</i> subsp. <i>zehntneri</i> (S,T); <i>D. zehntneri</i> subsp. <i>boomianus</i> (U,V); <i>D. zehntneri</i> subsp. <i>petr-halfarii</i> (X,Z). Photo: Basarab Popa (N, O); Gerardus Olsthorn (Z); Marianna Santos (C, D, E, H, I, J, K, L, M, P, Q, R); Marcelo Telles (A, S, T); Marlon Machado (B, U,V,X); Pierre Braun (G).....	30

CAPÍTULO 2

Figure 1. Map showing geographic distribution of <i>Discocactus</i> species that are widely distributed in the central-W and NE regions of Brazil.....	56
Figure 2. A-G. <i>Discocactus bahiensis</i> . A. Habit showing shape of stem and ribs. B-C. Different types of areole. D. Longitudinal section of flower and perianth-segments. E. Stigma-lobes. F. Stamen. G. Seed. H-M. <i>Discocactus boliviensis</i> . H. Habit showing shape of stem and ribs. I. Areole. J. Longitudinal section of flower and perianth-segments. K. Stigma-lobes. L. Stamen. M. Seed.	57
Figure 3. Map showing geographic distribution of <i>Discocactus</i> species that occur in the NE and W regions of Brazil and Eastern parts of of Paraguay and Bolivia.....	61
Figure 4. A-G. <i>Discocactus catingicola</i> . A. Habit showing stem and rib shape. B. Areole. C. Longitudinal section of flower and perianth-segments. D. Detail of curled hairs at top of nectar-chamber. E. Stigma. F. Stamen. G. Seed. H-M. <i>Discocactus</i>	

diersianus. H. Habit showing stem and rib shape. I. Areole. J. Longitudinal section of flower and perianth-segments. L. Areole. K. Stigma. M. Seed.....62

Figure 5. A-F. *Discocactus ferricola*. A. Habit showing stem and rib shape. B. Areole. C. Longitudinal section of flower and perianth segments. D. Stigma. E. Stamen. F. Seed. G-M. *Discocactus hartmanniii*. G. Habit showing stem and rib shape. H. Areole. I. Longitudinal section of flower and perianth segments. J. Stigma. K. Stamen. L. Fruit. M. Seed. N-Q. *Discocactus heptacanthus*. N. Habit showing stem and rib shape. O. Areole. P. Fruit. Q. Seed.71

Figure 6. Map showing geographic distribution of *Discocactus* species that are endemic to Minas Gerais State, Brazil.76

Figure 7. A-F. *Discocactus horstii*. A. Habit showing stem and rib shape. B. Areole. C. Longitudinal section of flower and perianth segments. D. Stigma. E. Stamen. F. Seed form. G-O. *Discocactus pseudoinsignis*. G. Habit showing stem and rib shape. H-I. different types of areole. J. Longitudinal section of flower and perianth segments. K. Stigma. L. Stamen. M. Ovules. N. Fruit. O. Seed.77

Figure 8. *Discocactus placentiformis*. A. Habit showing stem and rib shape. B. Plant body variation depending on substrate (on rocks and sand). C. Different types of areole. D. Longitudinal section of flower and perianth segments. E. Stigma. F. Stamen. G. Fruit. H. Seed form variation.78

Figure 9. Map showing geographic distribution of *Discocactus* species that are restricted to the NE region of Brazil.84

Figure 10. A-G. *Discocactus zehntneri* subsp. *zehntneri*. A. Habit showing stem and rib shape. B. areole. C. Longitudinal section of flower and perianth-segments. D. Stigma-lobes. E. Stamen. F. Fruit. G. Seed. H-K. *Discocactus zehntneri* subsp. *boomianus*. H. Habit showing stem and rib shape. I. areole. J. Fruit. K. Seed.85

Figure 11. A-B. Habit showing stem, rib shape and areole morphology of *Discocactus zehntneri* subsp. *petr-halfarii* subsp. *nov.* C. Longitudinal section of flower and perianth-segments. D. Stigma. E. Stamen. F. Seed.86

CAPÍTULO 3

Figure 1. Records of <i>Discocactus</i> species in Brazil, Bolivia and Paraguay. Brazilian biomes are shown.	104
Figure 2. Richness of <i>Discocactus</i> using 1°×1° grid squares (A), diversity of the genus calculated by Shannon index (B). The numbers represent the grid squares used UPGMA analysis.	105
Figure 3. Dendrogram obtained in similarity analysis (UPGMA, Jaccard's index), showing formation of floristic regions (vertical bars). Cophenetic correlation coefficient=0.9877. Bootstrap percentages are given above branches.	106
Figure 4. Comparison of <i>Discocactus</i> records with the distribution of priority areas for biodiversity conservation, and federal and state conservation units, according to MMA (2007).	108

RESUMO

SANTOS, Marianna Rodrigues, D.Sc. Universidade Federal de Viçosa, novembro de 2013. **Filogenia molecular, taxonomia, biogeografia e conservação de *Discocactus* Pfeiff. (Cactaceae)**. Orientadora: Flávia Cristina Pinto Garcia. Coorientadores: Cássio van den Berg e Nigel Paul Taylor.

O número de espécies de *Discocactus* Pfeiff. pode variar de sete a 52 táxons, dependendo da classificação. Esta divergência entre os sistemas de classificação reflete a dificuldade na delimitação de suas espécies e subespécies e, conseqüentemente, na elaboração de políticas de conservação que possam proteger estes táxons. Este trabalho teve como objetivo realizar estudos filogenético, taxonômico e biogeográfico de *Discocactus*, com intuito de aumentar o conhecimento científico acerca deste gênero e, conseqüentemente, subsidiar outros estudos sobre sua biologia e ecologia. Além disto, a delimitação filogenética, taxonômica e geográfica de suas espécies poderá auxiliar no estabelecimento de estratégias de conservação das espécies em seu habitat natural e inclusão de táxons não contemplados nas listas brasileiras de espécies ameaçadas de extinção. Para a realização do estudo filogenético foram utilizadas 69 amostras, sendo 62 de *Discocactus*, seis de *Arrojadoa* Britton & Rose e uma de *Melocactus* (L.) Link & Otto e destas foram obtidas sequências de cinco regiões de DNA plastidial e duas nucleares. Os dados foram analisados através dos métodos de Máxima Parcimônia e Bayesiana. O tratamento taxonômico, chaves de identificação, avaliação do risco de extinção e distribuição geográfica foram baseados em um trabalho de campo extensivo, conduzido em toda a área de distribuição do gênero, um estudo minucioso da literatura disponível e análise de espécimes depositados em herbários do Brasil e exterior. O *status* de conservação foi revisado através de critérios de avaliação da IUCN (International Union for Conservation of Nature and Natural Resources). O estudo biogeográfico foi conduzido através das análises de riqueza e diversidade de espécies pelo método de quadrículas. Padrões de distribuição foram definidos e o método de UPGMA foi utilizado para verificar a existência de blocos florísticos. Além disto, foi realizado um levantamento de espécies do gênero em Unidades de Conservação (UCs) para avaliar se as espécies consideradas ameaçadas estão devidamente protegidas. Apesar do número de regiões do DNA utilizadas, a resolução das árvores em geral foi baixa, no entanto, pequenos grupos foram bem suportados. Esses grupos se correlacionam com padrões biogeográficos e morfológicos, o que ajudou nas decisões

taxonômicas em comparação com sistemas de classificação anteriores. O *status* de *Discocactus petr-halfarii* Zachar é revisto como subespécie de *D. zehntneri* Britton & Rose, e alguns sinônimos foram reposicionados. As regiões do DNA *trnS-trnG*, *rpl16*, ITS e *phyC* exibiram os maiores níveis de variação e são indicados como boa opção para futuros estudos filogenéticos envolvendo outros gêneros de Cactaceae. Os estudos taxonômico, filogenético e de distribuição geográfica sugere que *Discocactus* abriga 11 espécies e três subespécies. Em relação ao estudo biogeográfico, foi constatado que a maioria das espécies do gênero é conhecida em menos de dez localidades e dois táxons são micro-endêmicos. Quase todas as espécies foram consideradas criticamente ameaçadas, vulneráveis ou ameaçadas, principalmente pela destruição do *habitat* e coleta ilegal. Contudo, a maioria destas espécies não está protegida em UC. A maior riqueza e diversidade de *Discocactus* estão concentradas em duas regiões: no norte da Bahia (próximo à Chapada Diamantina e Morro do Chapéu), incluindo *D. bahiensis* Britton & Rose e táxons de *D. zehntneri* e na região central da Cadeia do Espinhaço em Minas Gerais (Planalto de Diamantina e Grão Mogol), onde *D. placentiformis* (Lehm.) K. Schum., *D. horstii* Buining & Brederoo e *D. pseudoinsignis* N.P. Taylor & Zappi são encontrados.

ABSTRACT

SANTOS, Marianna Rodrigues, D.Sc. Universidade Federal de Viçosa, November, 2013. **Molecular phylogeny, taxonomy, biogeography and conservation of *Discocactus* Pfeiff. (Cactaceae).** Adviser: Flávia Cristina Pinto Garcia. Co-Advisers: Cássio van den Berg and Nigel Paul Taylor.

The number of *Discocactus* species may vary from seven to 52 depending on the classification employed. This divergence among classification systems reflects the difficulty in the delimitation of its species and subspecies, and consequently in the elaboration of conservation policies to protect these taxa. This study aims to understand the phylogeny, taxonomy and biogeography of the genus *Discocactus* Pfeiffer., in order to increase scientific knowledge about this group and therefore assist other studies on its biology and ecology. Moreover, a better genetic delimitation, taxonomic and geographic understanding of its species may assist in establishing strategies for conservation in their natural habitat and inclusion of taxa not included in the list of Brazilian endangered species. To perform the phylogenetic analysis 69 samples, 62 of *Discocactus*, six of *Arrojadoa* Britton & Rose and one of *Melocactus* (L.) Link & Otto were used and sequences were obtained from five DNA regions including plastid and two nuclear. Data were analyzed using Bayesian and Maximum Parsimony methods. The taxonomic treatment, identification keys, assessment of extinction risk and geographic distribution of *Discocactus* taxa were based on extensive field research conducted throughout the area of distribution of the genus, a detailed study of the available literature and analysis of specimens deposited in herbaria both in Brazil and abroad. The conservation status was determined by applying IUCN (International Union for Conservation of Nature and Natural Resources) evaluation criteria. The biogeographic study was conducted by the analysis of richness and species diversity by the grid squares method. Distribution patterns were defined and the UPGMA method was used to verify the existence of floristic blocks. In addition, a survey about species of the genus found within Conservation Units (CUs) was conducted to assess whether the species considered threatened are properly protected. Although a high number of DNA regions was used, the resolution of phylogenetic trees was generally low, however, smaller groups were well supported. These groups are correlated with morphological and biogeographic patterns, which helped in taxonomic decisions comparing with previous classification systems. The status of *Discocactus petr-halfarii*

Zachar is revised as a subspecies of *D. zehntneri* Britton & Rose and some synonyms were repositioned. The DNA regions *trnS* - *trnG*, *rpl16*, ITS and *phyC* exhibited the highest levels of variation and are indicated as good options for future phylogenetic studies involving other genera of Cactaceae. The study of morphology, phylogeny and geographical distribution suggest that *Discocactus* Pfeiffer can be divided into 11 species and three subspecies. Regarding the biogeographic study, it was found that the majority of species of the genus occurs in less than ten locations and two taxa are micro-endemics. Almost all species are critically endangered, vulnerable or threatened mainly by *habitat* destruction and illegal collection. However, unfortunately, most of the species of the genus are not protected in CUs. The highest richness and diversity of *Discocactus* is concentrated in the northern region of Bahia (near the Chapada Diamantina and Morro do Chapéu) including *D. bahiensis* Britton & Rose and taxa of *D. zehntneri* and in the centre of the Espinhaço mountain range (Planalto de Diamantina and Grão Mogol), where *D. placentiformis* (Lehm.) K.Schum, *D. horstii* Buining & Brederoo and *D. pseudoinsignis* N.P. Taylor & Zappi are found.

INTRODUÇÃO GERAL

Estudos filogenéticos em Cactaceae

Nos primeiros trabalhos com enfoque filogenético para a família Cactaceae, o estabelecimento do parentesco entre as linhagens foi realizado através da morfologia (Metzing & Kiesling, 2008). Contudo, nestes estudos não foram utilizados os métodos cladísticos que só foram incorporadas aos estudos com a família a partir da década de 80. Entretanto, nestes trabalhos não foram englobados representantes da família como um todo, sendo os estudos realizados com grupos menores, em nível de tribo (Taylor & Zappi, 1989) e gêneros (Zimmerman, 1985; Taylor, 1991). Terranzas & Arias (2003) realizaram o estudo cladístico para toda a subfamília Cactoideae utilizando trabalhos que detalharam a morfologia interna e externa da subfamília ao longo dos anos.

O uso de sequências de DNA para a reconstrução filogenética em Cactaceae, iniciou-se com o teste do monofiletismo para a subfamília Cactoideae e neste estudo foram utilizados dados dos marcadores de cloroplasto *rpoC1*, *trnT-trnL* e *rpl16* (Wallace & Cotta, 1996; Aplenquist & Wallace, 2002; Butterworth et al., 2002). Posteriormente, Nyffeler (2002), reconstruiu a filogenia para a família como um todo com base em sequências combinadas dos marcadores de cloroplasto *trnK/matK* e *trnL-F*. Com este estudo foi reconhecido o monofiletismo de Cactaceae e, principalmente, das subfamílias Opuntioideae e Cactoideae, estabelecendo o parafiletismo da subfamília Pereskioideae.

Recentemente, Griffith & Porter (2009) reconstruíram a filogenia de Opuntioideae com base em marcadores de cloroplasto (*trnL-F*) e nuclear (ITS) identificando duas linhagens principais para a subfamília: (1) uma linhagem incluindo as Opuntioideae com caule cilíndrico, e (2) linhagem incluindo as com caule aplanado, demonstrando o valor taxonômico do caráter “forma do caule” para a taxonomia do grupo.

Mais recentemente, Calvente (2010), reconstruiu a filogenia de Rhipsalidae usando marcadores *trnQ-rps16*, *rpl32-trnL*, *psbA-trnH* e ITS. Este estudo forneceu evidências importantes para a redefinição de limites genéricos em Rhipsalidae, especialmente em *Hatiora* Britton & Rose e *Schlumbergera* Lem.

A maioria dos estudos que aborda a filogenia em Cactaceae foi elaborada para gêneros de Cactoideae: (1) *Lophocereus* (Berger) Britton & Rose, com base nos marcadores *trnL-F*, *trnC-trnD* e *trnS-trnfM* (Hartmann et al., 2002); (2) *Pachycereus* (A. Berger) Britton & Rose, com base nos marcadores *rpl16*, *trnL-F* e ITS (Arias et al., 2003); (3) *Micranthocereus* Backeb., com base no marcador *trnL-F* (Aona, 2003); (4) *Mammillaria* Haw., com base nos marcadores *rpl16* e *psbA-trnH* (Butterworth & Wallace, 2004); (5) *Peniocereus* (Berger) Britton & Rose, com base nos marcadores *rpl16* e *trnL-F* (Arias et al., 2005); (6) *Rebutia* K. Schum., com base nos marcadores *atpB-rbcL*, *trnL-F* e *trnK-rps16* (Ritz et al., 2007); (7) *Rhipsalis* Gaertn., com base nos marcadores de cpDNA, *trnQ-rps16*, *rpl32-trn-L*, *psbA-trnH*, além do nDNA (ITS) e o gene nuclear malato sintase (MS) (Calvente, 2010).

Até o momento, as filogenias de Cactaceae produzidas utilizaram 13 marcadores de cloroplasto (*rpl16*, *rpoC1*, *trnT-trnL*, *trnL-F*, *psbA-trnH*, *trnK-matK*, *rbcL*, *atpB-rbcL*, *trnL-rps16*, *trnC-trnD*, *trnS-trnfM*, *trnQ-rps16*, *rpl32-trn-L*), três nucleares (ITS, MS e *phyC*) e um mitocondrial (*cox3*). Estes estudos estabeleceram o parentesco entre os grandes grupos e linhagens da família, especialmente em Opuntioideae e Pereskioideae, ressaltando a necessidade de mais estudos em Cactoideae, uma vez que esta subfamília inclui a maior diversidade de cactos da família e um grande número de tribos e gêneros que ainda permanecem pouco investigados.

A família Cactaceae

Cactaceae pertence à ordem Caryophyllales que atualmente possui 34 famílias, entre elas, Aizoaceae, Caryophyllaceae, Amaranthaceae, Droseraceae, Nyctaginaceae, Polygonaceae e Portulacaceae (Stevens, 2010). Cactaceae está dividida em quatro subfamílias: Maihuenoideae, Pereskioideae, Opuntioideae e Cactoideae. Cactoideae apresenta o maior número de gêneros e espécies sendo subdividida em nove tribos: Hylocereeae, Cereeae, Echinocereeae, Trichocereeae, Notocactaeae, Rhipsalidae, Browningeae, Pachycereeae e Cactaeae (Hunt & Taylor, 1990). Dentre estas, as tribos Trichocereeae e Cereeae predominam em número de gêneros e espécies no Brasil (Taylor & Zappi, 2004).

Trata-se de uma família botânica composta por espécies arborescentes, arbustivas, trepadeiras ou epífitas, geralmente suculentas e perenes, sendo quase todas

endêmicas das Américas, onde se distribuem desde o Canadá até a Argentina (Gibson & Nobel, 1986; Montes *et al.*, 1997). Apenas três subespécies de *Rhipsalis baccifera* (J.S.Muell.) Stearn (*Rhipsalis baccifera* subsp. *mauritina* (DC.) Barthlott, subsp. *erythrocarpa* (K. Schum.) Barthlott e subsp. *shaferi* (Britton & Rose) Barthlott & N.P.Taylor) são encontradas no Velho Mundo (Maxwell 1998, 1999 a, b). O maior centro de diversidade da família encontra-se no México e sudoeste dos Estados Unidos, seguido pelas cordilheiras Andinas, abrangendo o Peru, Bolívia, nordeste do Chile e da Argentina e, posteriormente, o leste do Brasil (Hernández & Godínez, 1994; Boyle & Anderson, 2002; Taylor & Zappi, 2004).

As Cactaceae ocorrem entre as latitudes 35° Norte e Sul, tanto ao nível do mar como até mais de 5000m de altitude (Boyle & Anderson, 2002; Taylor & Zappi, 2004). Desta forma, as espécies desta família possuem a capacidade de crescer em diferentes ecossistemas, no entanto, a maior diversidade é encontrada em regiões áridas e semi-áridas, onde são importantes fontes de recursos para diversas espécies de vertebrados e invertebrados, principalmente, na estação seca, época em que há grande escassez de recursos (Taylor & Zappi, 2004). Adicionalmente, algumas espécies de cactos representam importante fonte de recursos alimentares também para seres humanos e seus rebanhos, além de possuírem enorme valor ornamental (Ortega-Baes & Godínez-Alvarez, 2006).

Em toda área de ocorrência, a família Cactaceae compreende 124 gêneros e 1.438 espécies (Hunt *et al.*, 2006), representando a segunda família com maior número de espécies endêmicas das Américas, sendo que Bromeliaceae ocupa o primeiro lugar (Taylor & Zappi, 2004). No Brasil é registrado até o momento, 37 gêneros, 233 espécies (174 endêmicas), 94 subespécies (80 endêmicas) e uma variedade (Zappi *et al.*, 2013).

Recentemente foi aprovado pelo ICMBio-MMA (Instituto Chico Mendes, Ministério do Meio Ambiente) o Plano de Ação Nacional para a Conservação de Cactáceas que visa a ampliação do conhecimento sobre as cactáceas no Brasil, além de desenvolver ações que irão contribuir para o manejo de espécies da família que estão no Anexo I da Lista Oficial das Espécies da Flora Brasileira Ameaçadas de Extinção (MMA, 2008).

Histórico de *Discocactus* Pfeiff.

Discocactus foi descrito como gênero em 1837 por Louis Pfeiffer que descreveu *D. insignis* e outras duas espécies em 1839, *D. lehmannii* e *D. linkii*. Pfeiffer usou os seguintes caracteres para distinguir as espécies do gênero: “O tubo do cálice aderido ao ovário, nu na base, não dividido, sépalas conadas, livres no topo. Pétalas numerosas que formam uma corola tubulosa em forma de funil. Estames filiformes, os externos no nível das pétalas, os internos mais curtos e aderidos ao cálice do tubo. Estilete clavado, 5 lobos, alongados, lienares. Frutos muito simples, discóides, com costelas e espinhosos. Um cefálio falso no topo com lâ macia e tuberculos sem pêlos (como em *Melocactus*). Flores surgem através das lâs no topo, longo tubo glabro, floração noturna, decíduas, receptáculo persistente”. Esta descrição não é perfeita porque a forma do fruto nunca é discoide, não possui costelas e não é espinhoso. Estes caracteres são referentes ao cefálio ou caule que são discóides e espinhosos.

Anteriormente em 1826, Lehmann havia publicado *Cactus placentiformis* que, posteriormente foi incorporada ao gênero. As três espécies descritas por Pfeiffer assim como *Cactus placentiformis* Lehm. foram combinadas por Schumann em *D. placentiformis* (Lehmann) Karl Schumann em 1894. Em 1846 e 1850, *D. alteolens* Lemaire ex Albert Dietrich e *D. tricornis* Monville ex Pfeiffer foram descritas, respectivamente. Todas as espécies citadas acima se tratam de formas variáveis de *D. placentiformis* que é endêmica do Estado de Minas Gerais (Taylor & Zappi, 2004; Machado, 2004).

Em 1898 *Malacocarpus heptacanthus* Barbosa Rodrigues foi descrita e em 1900, *Echinocactus hartmannii* Karl Schumann, ambos os táxons referentes a *Discocactus* e que posteriormente foram incluídas no gênero em 1922 por Britton & Rose (1922). Neste estudo os autores descreveram três espécies novas para o Estado da Bahia, *D. bahiensis*, *D. zehntneri* e *D. subnudus*. Segundo Buining (1980), esta última, provavelmente se trata de uma planta que foi danificada pelo fogo e que, conseqüentemente ficou sem seus espinhos, o que inspirou o epíteto dado à espécie.

Backeberg em 1960 descreveu *D. paranaensis* para o Estado do Paraná, desde então esta espécie não foi recoletada sendo conhecida apenas pela descrição original. Em 1963, Backeberg descreveu *D. boliviensis* e o tipo foi coletado no leste da Bolívia próximo à fronteira com o Brasil. Entre 1971 e 1980, um número considerável de

espécies foram descritas para o gênero, principalmente por Buining & Brederoo que publicaram 32 espécies e 2 subespécies sendo 26 espécies e 1 subespécie novas (Buining, 1980) (Tabela). Outras espécies foram descritas no período entre 1978 a 2001 por Diers, Esteves, Brederoo & Broeck, Pierre Josef Braun entre outros (Machado, 2004) (Tabela). Entretanto, a maioria das espécies foram sinonimizadas por Hunt *et al.* (2006) que consideraram apenas 11 espécies e duas subespécies (Tabela). Em 2008, Zachar descreveu *D. petr-halfari*, no mesmo ano foi considerada subespécie de *D. bahiensis* por P.J. Braun & Esteves (2008).

Tabela. Histórico de *Discocactus*.

Autor/ano	Espécies e subespécies publicadas e outras informações
Pfeiffer 1837	Publicou <i>Discocactus</i> como gênero e <i>D. insignis</i>
Pfeiffer 1839	Publicou <i>D. lehmannii</i> e <i>D. linkii</i>
Lemaire ex Albert Dietrich 1846	<i>D. alteolens</i>
Monville ex Pfeiffer 1850	<i>D. tricornis</i>
Schumann 1894	Combinou <i>D. insignis</i> , <i>D. lehmannii</i> e <i>D. linkii</i> assim como <i>Cactus placentiformis</i> descrita por Lehmann em 1826 em <i>D. placentiformis</i> .
Barbosa Rodrigues 1898	<i>Malacocarpus heptacanthus</i>
Schumann 1900	<i>Echinocactus hartmannii</i>
Britton & Rose 1922	Incluiu <i>Malacocarpus heptacanthus</i> e <i>Echinocactus hartmannii</i> no gênero e publicaram <i>D. bahiensis</i> , <i>D. zehntneri</i> e <i>D. subnudus</i>
Backeberg 1960	<i>D. paranaensis</i>
Backeberg 1963	<i>D. boliviensis</i>
Buining & Brederoo 1971	<i>D. boomianus</i>
Buining & Brederoo 1973	<i>D. horstii</i>
Buining & Brederoo 1974	<i>D. albispinus</i> , <i>D. catingicola</i> , <i>D. magnimammus</i> , <i>D. mamillosus</i> e <i>D. patulifolius</i> .
Buining & Brederoo 1975	Publicaram <i>D. cephaliaciculosus</i> , <i>D. ferricola</i> , <i>D. griseus</i> , <i>D. pachytele</i> , <i>D. rapirhizus</i> , <i>D. semicampaniflorus</i> e <i>D. silicicola</i> .
Diers 1978	<i>D. estevesii</i>
Esteves 1979	<i>D. diersianus</i>
Buining & Brederoo 1980	Publicaram <i>D. araneispinus</i> , <i>D. flavispinus</i> , <i>D. latispinus</i> , <i>D. magnimammus</i> subesp. <i>bonitoensis</i> , <i>D. nigrisaetosus</i> , <i>D. melanochlorus</i> , <i>D. nigrisaetosus</i> , <i>D. pugionacanthus</i> , <i>D. pulvinicapitatus</i> , <i>D. silvaticus</i> , <i>D. spinosior</i> , <i>D. squamibaccatus</i> e <i>D. subviridigriseus</i> .

Cont. Tabela. Histórico de *Discocactus*.

Autor/ano	Espécies e subespécies publicadas e outras informações
Diers & Esteves 1980	<i>D. cangaensis</i> , <i>D. goianus</i> e <i>D. subterrâneo-proliferans</i>
Buining & Broek 1980	<i>D. woutersianus</i>
Diers & Esteves 1981	<i>D. lindanus</i>
P.J. Braun & Brederoo 1981	<i>D. multicolorispinus</i>
W.R. Abraham 1987	<i>D. buenekeri</i>
Diers & Esteves 1987	<i>D. pseudolatispinus</i>
Diers & Esteves 1988	<i>D. proeminentegibus</i>
P.J. Braun & Esteves 1990	<i>D. zehntneri</i> var. <i>horstiorum</i>
N.P. Taylor & Zappi 1991	<i>D. pseudoinsignis</i> e <i>D. zehntneri</i> subesp. <i>boomianus</i>
P.J. Braun & Esteves 1994	<i>D. crassispinus</i> , <i>D. magnimammus</i> subesp. <i>setosiflorus</i>
P.J. Braun & Esteves 1995	<i>D. cephaliaciculosus</i> subesp. <i>nudicephalus</i> , <i>D. piauiensis</i>
P.J. Braun & Esteves 1996	<i>D. crassispinus</i> subesp. <i>araguaensis</i> , <i>D. hartmannii</i> subesp. <i>giganteus</i>
P.J. Braun & Esteves 2001	<i>D. bahiensis</i> subesp. <i>gracilis</i>
Hunt <i>et al.</i> (2006)	Reconheceram apenas 11 espécies, duas subespécies e sinonimizaram, principalmente, a maioria das espécies publicadas por Buining & Brederoo, Diers & Esteves e P.J. Braun & Esteves. <i>Discocactus bahiensis</i> Britton & Rose, <i>Discocactus boliviensis</i> Backeb., <i>Discocactus catingicola</i> Buining & Brederoo, <i>Discocactus diersianus</i> Esteves Pereira, <i>Discocactus hartmannii</i> (K.Schum.) Britton & Rose, <i>Discocactus ferricola</i> Buining & Brederoo, <i>Discocactus heptacanthus</i> (Barb.Rodr.) Britton & Rose, <i>Discocactus horstii</i> Buining & Brederoo, <i>Discocactus placentiformis</i> (Lehm.) K.Schum., <i>Discocactus pseudoinsignis</i> N.P. Taylor & Zappi, <i>Discocactus zehntneri</i> Britton & Rose: <i>Discocactus zehntneri</i> ssp. <i>zehntenri</i> Britton & Rose e <i>Discocactus zehntneri</i> ssp. <i>boomianus</i> (Buining & Brederoo) N.P. Taylor & Zappi.
Zachar 2008	<i>D. petr-halfari</i>
P.J. Braun & Esteves 2008	<i>D. bahiensis</i> subesp. <i>petr-halfari</i>

O gênero *Discocactus* Pfeiff.

O gênero *Discocactus* está posicionado na subfamília Cactoideae e antes pertencia à tribo Trichocereae (Taylor & Zappi, 2004), entretanto, sempre foi associado ao gênero *Melocactus* Link & Otto (tribo Cereae) devido ambos apresentarem hábito globoso e cefálio terminal, sendo tal similaridade vegetativa motivo para supor que este seja um caso de convergência (Machado, 2004). Atualmente, dados moleculares corroboram tal hipótese, pois, de acordo com Crozier

(2005), estes gêneros formam um grupo irmão, pertencente à mesma tribo (Cereeae) e subtribo (Cactanae).

Discocactus reúne plantas com hábito globoso-achatado ou discóide, da qual a região florífera é diferenciada em cefálio (região onde as aréolas são dispostas de forma mais compacta, sendo reprodutivamente ativas) posicionado no ápice do caule, de onde surgem flores noturnas, brancas, apresentando a esfingofilia como síndrome de polinização (Machado, 2004).

O número de espécies de *Discocactus* é algo que tem causado muita divergência entre os especialistas e sistemas de classificação. Segundo Buining (1980), o gênero possui 32 espécies e 2 subespécies. Braun & Esteves-Pereira (1993 a, b, c, 1995, 2002) reconhecem 51 táxons, sendo 25 espécies, 22 subespécies heterotípicas e 4 variedades. Já Taylor & Zappi (2004) reconheceram 10 táxons: 7 espécies e três subespécies. Entretanto, apenas a região leste do Brasil foi o objeto de estudo destes autores, sendo assim, eles não incluíram os táxons que ocorrem na região oeste do país e nos países vizinhos (Bolívia e Paraguai). Hunt *et al.* (2006) consideraram 11 espécies e 2 subespécies em toda a área de ocorrência do gênero (Brasil, Bolívia e Paraguai), e, com isto, sinonimizaram muitas espécies e subespécies reconhecidas por outros autores.

No Catálogo de Plantas e Fungos do Brasil (Flora do Brasil), o gênero está representado por 10 espécies (8 endêmicas) e 2 subespécies (2 endêmicas) (Zappi *et al.*, 2013), entretanto, ainda há muito o que discutir sobre o número real de espécies e subespécies em *Discocactus*. Sendo assim, a divergência na classificação infraespecífica do gênero reflete a dificuldade na delimitação de espécies de *Discocactus*, apenas pela morfologia e distribuição geográfica.

Em relação à conservação, de acordo com a IUCN (União Mundial para a Conservação da Natureza) (2011), todas as espécies de *Discocactus* se encontram ameaçadas de extinção podendo ser em maior ou menor grau. Contudo, tal avaliação da IUCN foi realizada apenas para sete espécies, não considerando os outros táxons que ocorrem na região oeste do Brasil, Bolívia e Paraguai. Esta situação traz à tona a importância de uma melhor delimitação de espécies do gênero através do maior conhecimento sobre sua morfologia, relações filogenéticas e distribuição geográfica, que possibilitará a inclusão de um maior número de táxons em listas de espécies ameaçadas de extinção.

As espécies de *Discocactus* possuem um número reduzido de populações, além disto, estas populações são pequenas e possuem grande especificidade de *habitat* ocorrendo em áreas bastante restritas o que as torna muito vulneráveis à perturbação e destruição de seu *habitat*. Outro fator que ameaça a conservação destas espécies é a coleta e comercialização desenfreada realizada por colecionadores ou simplesmente pelos moradores de povoados carentes. Devido aos problemas mencionados, todo o gênero está listado no apêndice da CITES (Convenção Internacional sobre o Comércio de Espécies Silvestres, Lüthy, 2001). Algumas também estão incluídas na Lista de Espécies Ameaçadas de Extinção da Flora do Estado de Minas Gerais (*D. horstii*, *D. placentiformis* e *D. pseudoinsignis*) (Biodiversitas, 2006) e no Apêndice I da Lista Oficial das Espécies da Flora Brasileira Ameaçadas de Extinção (apenas *D. horstii*) (MMA, 2008). Outras estão no Apêndice II desta lista (*D. bahiensis* Britton & Rose, *D. catingicola* Buining & Brederoo, *D. ferricola* Buining & Brederoo, *D. placentiformis* (Lehm.) K. Schum. e *D. zehntneri* Britton & Rose), que engloba espécies com dados insuficientes.

Diante do exposto, este trabalho teve como objetivo realizar estudos filogenético, taxonômico e biogeográfico do gênero *Discocactus*, com intuito de aumentar o conhecimento científico acerca deste grupo. Além disto, poderá auxiliar futuramente o estabelecimento de estratégias de conservação das espécies em seu habitat natural e inclusão de táxons não contemplados nas listas brasileiras de espécies ameaçadas de extinção.

REFERÊNCIAS

- AONA, L.Y.S. **Caracterização e delimitação do gênero *Micranthocereus* Backeb. (Cactaceae) baseadas em caracteres morfológicos e moleculares.** 115 p. Dissertação de Mestrado, UNICAMP, Campinas, SP.
- ARIAS, S., T. TERRAZAS & K. CAMERON. Phylogenetic analysis of *Pachycereus* (Cactaceae, Pachycereeae) based on chloroplast and nuclear DNA sequences. **Systematic Botany**, vol. 28, p.547-557, 2003.

- APPLEQUIST, W. L. & R. S. WALLACE. Deletions in the plastid trnT-trnL intergenic spacer define clades within Cactaceae subfamily Cactoideae. **Plant Systematics and Evolution**, vol. 231, p. 153-162, 2002.
- BIODIVERSITAS. 2006. **Lista oficial de espécies ameaçadas de extinção no Brasil**. Disponível em: <<http://www.biodiversitas.org.br/florabr/grupo3fim.asp> >. Acesso em: 23 março. 2010.
- BOYLE, T. H.; ANDERSON, E. Biodiversity and conservation. In: NOBEL, P. S. (Ed.). **Cacti: biology and uses**. Los Angeles: University of California, 2002. p. 125-141.
- BRAUN, P.J.; ESTEVES PEREIRA, E. Aggregationen in *Discocactus* Pfeiffer (Cactaceae). Teil 1. **Kakteen und andere Sukkulenten**, vol. 44, p. 62-65, 1993a.
- BRAUN, P.J.; ESTEVES PEREIRA, E. Aggregationen in *Discocactus* Pfeiffer (Cactaceae). Teil 2. **Kakteen und andere Sukkulenten**, vol. 44, p.103-105, 1993b.
- BRAUN, P.J.; ESTEVES PEREIRA, E. Aggregationen in *Discocactus* Pfeiffer (Cactaceae). Teil 3. **Kakteen und andere Sukkulenten**, vol. 44, p.112-117, 1993c.
- BRAUN, P.J.; ESTEVES PEREIRA, E. Aggregationen in *Discocactus* (Nachträge). **Kakteen und andere Sukkulenten**, vol. 46, p. 64, 1995.
- BRAUN, P.J.; ESTEVES PEREIRA, E. Kakteen und andere Sukkulenten in Brasilien. **Schumannia**, vol. 3, p. 1–235, 2002.
- BRAUN, P.J.; ESTEVES PEREIRA, E. *Discocactus bahiensis* Britton & Rose subsp. *petr-halfari* (Zachar) P.J.Braun & Esteves. **Kakteen und Andere Sukkulenten**, vol. 57, n. 9, p. 184, 2008.
- BUINING, A.F.H. The genus *Discocactus* Pfeiffer. Buining-fonds, **Succulenta**, The Netherlands. 223 p., 1980.
- BUTTERWORTH, C. A., J. H. COTA-SANCHEZ & R. S. WALLACE. Molecular systematics of tribe Cactaeae (Cactaceae: Cactoideae): A phylogeny based on rpl16 intron sequence variation. **Systematic Botany**, vol. 27, p. 257-270, 2002.

- BUTTERWORTH, C. A. & R. S. WALLACE. Phylogenetic studies of *Mammillaria* (Cactaceae) - insights from chloroplast sequence variation and hypothesis testing using the parametric bootstrap. **American Journal of Botany**, vol. 91, p. 1086-1098, 2004.
- CROZIER, B.S. **Systematics of Cactaceae Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw**, 2005. 154 p. PhD. Dissertation- The University of Texas, Austin.
- GIBSON, A. C.; NOBEL, P. S. **The cactus primer**. Cambridge, Massachusetts: Harvard University, 286 p. 1986.
- GRIFFITH, M. P. & J. M. PORTER. Phylogeny of Opuntioideae. **International Journal of Plant Sciences**, vol. 170, p. 107-116, 2009.
- HARTMANN, S., J. D. NASON & D. BHATTACHARYA. Phylogenetic origins of *Lophocereus* (Cactaceae) and the senita cactus-senita moth pollination mutualism. **American Journal of Botany**, vol. 89, p. 1085-1092, 2002.
- HERNÁNDEZ, H. M.; GODÍNEZ, H. Contribución al conocimiento de las cactáceas mexicanas amenazadas. **Acta Botánica Mexicana**, México, v. 26, p. 33-52, 1994.
- HUNT, D. R. & TAYLOR. N.P. The genera of Cactaceae: progress towards consensus. **Bradleya**, vol. 8, p. 85–107, 1990.
- HUNT, D.; TAYLOR, N. P.; CHARLES, G. **The new cactus lexicon**. Milborne Port.: DH Books, 2 vols., 900p. 2006.
- IUCN 2011. IUCN: **Red List of Threatened Species. Version 2011.1**. Disponível em: <www.iucnredlist.org>. Acesso em: 09 Julho, 2011.
- LÜTHY, J. 2001. **The Cacti of CITES Appendix I**. Bundesamt für Veterinärwesen, Switzerland.
- MACHADO, M.C. **O gênero *Discocactus* Pfeiff. (Cactaceae) no estado da Bahia, Brasil: variabilidade morfológica, variabilidade genética, taxonomia e**

- conservação**, 2004. 113p. M.Sc. Thesis- Universidade Estadual de Feira de Santana, Feira de Santana.
- MMA (Ministério do Meio Ambiente). **Lista Oficial das Espécies da Flora Brasileira Ameaçadas de Extinção**. Brasília, 55 p., 2008.
- MAXWELL, P. The *Rhipsalis riddle* - or the day the cacti came down from the trees: **part 1**, 1998. Disponível em: <<http://www.rhipsalis.com/maxwell.htm>>. Acesso em: 03 dez. 2007.
- MAXWELL, P. The *Rhipsalis riddle* - or the day the cacti came down from the trees: **part 2**, 1999a. Disponível em: <<http://www.rhipsalis.com/maxwell2.htm>>. Acesso em: 03 dez. 2007.
- MAXWELL, P. The *Rhipsalis riddle* - or the day the cacti came down from the trees: **part 3**, 1999b. Copyright. Disponível em: <<http://www.rhipsalis.com/maxwell3.htm>>. Acesso em: 03 dez. 2007.
- METZING, D. & R. KIESLING. The study of the cactus evolution: The pre-DNA era. **Heseltonia**, vol. 14, p. 6-25, 2008.
- MONTES, S. A.; LÓPEZ, S. G.; CRUZ, L.U.G. **Flora Del Valle de Tehuacán-Cuicatlán: Cactaceae A.L. Juss.** México: UNAM - Instituto de Biología, fascículo 14, 146 p., 1997.
- NYFFELER, R. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from trnK/matK and trnL-trnF sequences. **American Journal of Botany**, vol. 89, p. 312–326, 2002.
- ORTEGA-BAES, P.; GODÍNEZ-ALVAREZ, H. Global diversity and conservation priorities in the Cactaceae. **Biodiversity and Conservation**, Netherlands, vol. 15, p. 817-827. 2006.
- RITZ, C. M., L. MARTINS, R. MECKLENBURG, V. GOREMYKIN & F. H. HELLWIG. The molecular phylogeny of *Rebutia* (Cactaceae) and its allies

- demonstrates the influence of paleogeography on the evolution of South American mountain cacti. **American Journal of Botany**, vol. 94, p.1321-1332, 2007.
- STEVENS, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 9, 2010. Disponível em: <<http://www.mobot.org/MOBOT/research/APweb/>>. Acesso em: 23 março. 2010.
- TAYLOR, N. P. & D. C. ZAPPI. An alternative view of generic delimitation and relationships in tribe Cereeae (Cactaceae). **Bradleya**, vol 7, p. 13-40, 1989.
- TAYLOR, N. P. The genus *Melocactus* (Cactaceae) in Central and South America. **Bradleya**, vol. 9, p. 1-80, 1991.
- TAYLOR, N.P.; ZAPPI, D.C. **Cacti of eastern Brazil**. England: Richmond, Surrey: Royal Botanic Gardens, Kew, 499 p., 2004.
- TERRAZAS, T. & S. ARIAS. Comparative stem anatomy in the subfamily Cactoideae. The **Botanical Review**, vol. 68, pag. 444-473, 2003.
- WALLACE, R. S. & J. H. COTA. An intron loss in the chloroplast gene *rpoC1* supports a monophyletic origin for the subfamily Cactoideae of the Cactaceae. **Current Genetics**, vol. 29, p. 275-281, 1996.
- ZACHAR, M. Nowość z Brazylii - *Discocactus petr-halfarii*. **Kaktusy i Inne** 2: 43-47, 2008.
- ZAPPI DC, TAYLOR N, MACHADO M, SANTOS, MR. **2013**. *Discocactus* in Lista de espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. (<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB1482>).
- ZIMMERMAN, A. D. **Systematics of the genus *Coryphantha* (Cactaceae)**. University of Texas, Austin, EUA, PhD thesis. 1985.

CAPITULO I

A PHYLOGENETIC STUDY OF *DISCOCACTUS* PFEIFF. (CACTACEAE) BASED ON NUCLEAR AND PLASTID DNA SEQUENCES

This Chapter is formatted for submission to **Systematic Botany**

A phylogenetic study of *Discocactus* Pfeiff. (Cactaceae) based on nuclear and plastid DNA sequences

Marianna R. Santos^{1,4}, Flavia C.P. Garcia¹, Marlon C. Machado² Ariane R. Barbosa² Nigel P. Taylor³ and Cássio Van Den Berg²

¹ Programa de Pós-Graduação em Botânica, Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Av. P.H. Rolfs, s.n., 36570-000, Viçosa, Minas Gerais, Brazil.

² Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Transnordestina, s.n., 44036-900, Feira de Santana, Bahia, Brazil.

³ Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, Singapore 259569.

⁴ Author for correspondence (mrodriguesantos@gmail.com)

Abstract — The present study uses regions of cpDNA and nuDNA to infer the phylogeny of the genus *Discocactus* aiming to understand the relationship among its species. For this, we included 69 samples representing all known species of *Discocactus* and selected outgroups, then we performed parsimony and Bayesian phylogenetic analyses of DNA sequences from seven DNA regions (nuclear ITS, *phyC*; plastid *matK* gene, *psbA-trnH* and *trnS-trnG* intergenic spacers, *rpl16* and *rps16* introns). Despite the number of DNA regions, the overall resolution was low, but several small, well-supported groups were recovered. These groups correlate to biogeographic and macro-morphological patterns, and helped in taxonomic decisions assessing previous classification systems. The status of *Discocactus petr-halfarii* Zachar is revised as a subspecies of *D. zehntneri* Britton & Rose. The DNA regions *trnS-trnG*, *rpl16*, ITS and *phyC* displayed the higher levels of variation, and are indicated as good choices for to help in future phylogenetic studies involving other genera of Cactaceae.

Key words: cacti, phylogeny, South America, nuDNA, cpDNA.

INTRODUCTION

Cactaceae is assigned to the order Caryophyllales (Cantino et al. 2007; Stevens 2012), comprises 127 genera, 1438 species and is divided into four subfamilies: Cactoideae, Pereskioideae, Maihuenioideae and Opuntioideae (Hunt et al. 2006). The family is distributed from Canada to Southern Patagonia in Chile and Argentina (Speirs 1982; Kiesling 1988). Only *Rhipsalis baccifera* (J.S.Muell.) Stearn occurs in Africa, Madagascar and Asia (Thorne 1973; Barthlott 1983). The greatest species richness is concentrated in Mexico, southwestern Andean region and in eastern Brazil (Taylor and Zappi 2004).

Cactoideae is the most diverse subfamily of cacti and constitutes a monophyletic group, characterized by a complete reduction of leaves (Nyffeler 2002). Although phylogenetic studies have been conducted for some genera and tribes of Cactoideae: Hartmann et al. (2002) (*Lophocereus* (Berger) Britton & Rose); Arias et al. (2003, 2006) (*Pachycereus* (Berger) Britton & Rose); Butterworth and Wallace 2004; Crozier (2004, 2005); Harpke and Peterson (2006) (*Mammillaria* Haw.); Arias et al. (2005) (*Peniocereus* (Berger) Britton & Rose); Ritz et al. (2007) (*Rebutia* K. Schum.); Calvente et al. (2011) (Rhipsalideae); Schlumpberger and Renner (2012) (*Echinopsis* Zucc.), the relationships within this subfamily are still poorly understood.

Discocactus Pfeiff. is a genus of globose cacti belonging to subfamily Cactoideae and tribe Cereeae, having *Melocactus* Link & Otto as sister group (Crozier 2005). This genus is characterized by having a discoid to depressed-globose, single or caespitose habit, the fertile part of the stem differentiated into a terminal cephalium bearing nocturnal, white flowers pollinated by moths and fruits dehiscing by lateral slits, the seeds with a strongly tuberculate testa (Barthlott and Hunt 2000; Anderson 2001; Taylor and Zappi 2004; Machado 2004). The genus is largely restricted to Brazil with only two taxa occurring in bordering areas of Bolivia and Paraguay. In Brazil its distribution comprises the Central-Western, Northeastern and Southeastern regions of the country (Fig.1).

The number of *Discocactus* species is controversial and the divergence in its classification amongst modern authors (Buining 1980; Taylor 1981; Anderson 2001; Taylor and Zappi 2004; Hunt et al. 2006) reflects the difficulty of species delimitation

in the genus. According to Hunt et al. (2006), the most recent taxonomic treatment, *Discocactus* includes 11 species and two heterotypic subspecies.

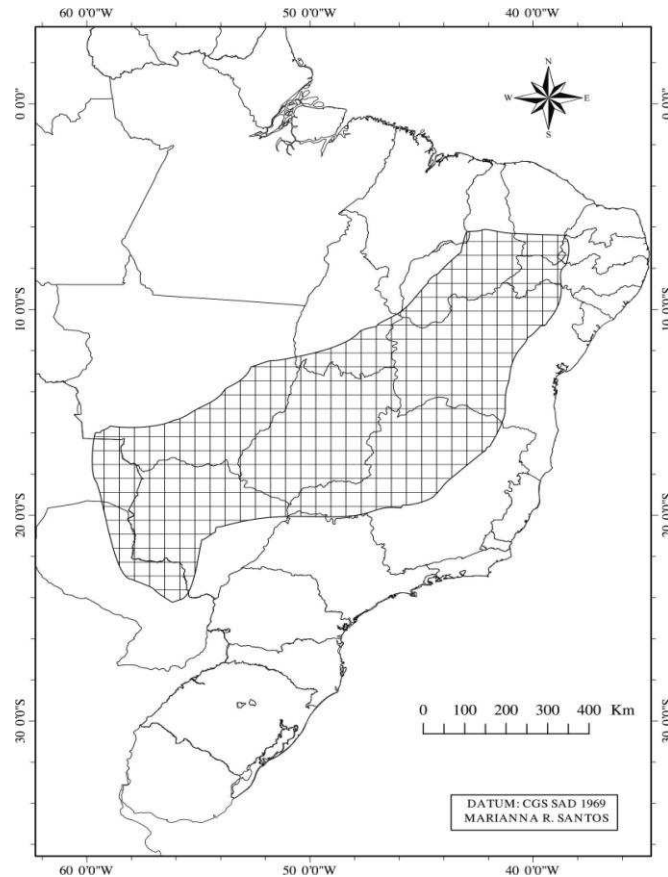


Figure 1. Distribution area of *Discocactus* species.

Therefore, this work aims to do a phylogenetic study for the genus *Discocactus* in order to understand better the relationships among its species, thus throwing some light on and hopefully elucidating the divergences among the current classification systems that have been proposed for the genus. A clear classification of *Discocactus* species will foster our knowledge of the evolution of the genus, and it will also serve to accurately assess the actual conservation status of each distinct taxon.

MATERIAL AND METHODS

Taxon sampling— A total of 69 samples was collected of which 62 belong to *Discocactus*, six are *Arrojadoa* Britton & Rose and one *Melocactus*. All species (11) and two infraspecific taxa currently attributed to *Discocactus* (Hunt et al. 2006), besides one more infraspecific taxa (*D. zehntneri* subsp. *petr-halfarii*), recently recognized (Santos et al., 2013 chapter 2) were sampled. We tried to sample the overall

morphological variations of species throughout the distribution area. All voucher specimens are deposited at the Herbarium of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (VIC). Sampling details and GenBank accession numbers are presented in Appendix 1.

DNA extraction and purification— The CTAB protocol of Doyle and Doyle (1987) with modifications was used to extract the total DNA from slices of the stems previously dried with a hairdryer and preserved in silica gel. The purification of the DNA extracted was performed using chloroform/isoamyl alcohol (24:1) as in the original protocol, adapted for 2 mL microtubes. The pellet was washed twice with 70% ethanol and subsequently resuspended in TE buffer. The concentration of the extracted DNA was estimated on 1% agarose gels, by comparison with a 100 bp DNA ladder.

Amplification and sequencing— Double-stranded amplification of the target sequences was done using the polymerase chain reaction (PCR) in a PCR System 9700 GeneAmp thermal cycler. After initial tests seven regions were selected (ITS, *matK*, *phyC*, *psbA-trnH*, *rpl16*, *rps16* and *trnS-trnG*). Primer sequences used for amplification and sequencing primers are shown in Table 1.

Table 1. Primers designed and used to amplify and sequence the plastid and nuclear loci. All primers were used to amplify and sequence, except ITS which was sequenced using other primers*.

Regions	Primer name:sequence (5' to 3')	Reference
ITS	17SE: ACGAATTCATGGTCCGGTGAAGTGTTTCG 26SE: TAGAATTCCTCCGGTTCGCTCGCCGTTAC	Sun et al., 1944
ITS*	92: AAGGTTTCCGTAGGTGAAC ITS4: TCCTCCGCTTATTGATATGC	Desfeux et al., 1996 White et al. 1990
<i>matK</i>	1326: TCTAGCACACGAAAGTCGAAGT 390F: CGATCTATTCATTCAATATTTTC	Cuénoud et al., 2002
<i>phyC</i>	F: AGCTGGGGCTTTCAAATCTT R: TCCTCCACTTGACCACCTCT	Helsen et al., 2009
<i>psbA-trnH</i>	psbA: GTTATGCATGAACGTAATGCTC trnH: CGCGCATGGTGGATTCACAAATC	Sang et al., 1997
<i>rpl16</i>	F71: GCTATGCTTAGTGTGTGACTCGTTG R1516: CCCTTCATTCCTCTATGTTG	Small et al., 1998
<i>rps16</i>	1F:5'-GTGGTAGAAAGCAACGTGCGACTT 2R:5'-TCGGGATCGAACATCAAT TGCAAC	Oxelman et al., 1997
<i>trnS-trnG</i>	S: GCCGCT TTAGTCCACTCAG G:GAACGAATCACACTT TTACCAC	Hamilton, 1999

The reactions were performed using the Top Taq Master Mix kit (Qiagen) in a total volume of 10-12 μ l containing the following components: 5-6.25 μ l of Top Taq 2 \times Master Mix, 0.24-0.75 μ M of each primer. For ITS reactions were used as additives 1M Betaine, 1 μ g BSA and 2% DMSO. The parameters of the amplification reactions for each region are shown in Table 2.

Table 2. PCR conditions for the amplification of different genome regions used in this study.

Genomic region	Initial melting °C/Time	Denaturing °C/Time	Anneling °C/Time	Extension °C/Time	Final extension °C/Time	N° of Cycles
ITS	94/3min	94/1 min	50/ 1 min	72/3 min	72/ 7 min	28
<i>matK</i>	94/1 min	94/ 45 s	48/30seg	72/1min	72/7 min	26
<i>phyC</i>	94/5 min	94/1 min	55/1:30	72/2 min	72/ 9 min	35
<i>psbA-trnH</i>	80/5 min	95/1 min	66/4min	-	66/10min	30
<i>rpl16</i>	94/5 min	94/30 s	52/1 min	72/1:30	72/7 min	35
<i>rps16</i>	94/5 min	94/30 s	52/1 min	72/1:30	72/7 min	35
<i>trnSG</i>	80/5 min	95/1 min	66/4min	-	66/10min	30

All reactions were maintained at 4°C after final extension.

The PCR products were quantified using agarose gel electrophoresis (1%) with Sodium Borate buffer (Brody and Kern 2004), for 40 min at 100V, 62W, 70 mA in electrophoretic source (Consort-E-832). The gel was stained with ethidium bromide (5 μ g/mL) and visualized on a UV light transilluminator (UV). The concentrations (in ng) of PCR products were estimated by comparing the intensity of bands produced by PCR with the intensity of bands DNA ladder marker through Kodak 1D software 3.6.

All PCR products were purified by precipitation with polyethylene glycol (PEG 8000) at 11% and resuspended in the same volume of PCR with ultrapure water. The sequencing reactions were performed in 10 μ l, with the Big Dye Terminator Kit version 3.1 (Applied Biosystems), 2.5 pmol primer, and 0.5-1 μ l PCR product. The amplification for sequencing was performed for 35 cycles (except for ITS that did not exceed 28 cycles) of 10s at 96°C, followed by 5s at 50°C and a final extension for 4 min at 60°C. Before sequencing, the reactions were purified by the following steps: 40 μ l of Isopropanol 80% (incubated during 15 minutes at room temperature in the dark, then centrifuged for 15 minutes in maximum speed and after the supernatant was discarded), twice with 150 μ l of Ethanol 70% (centrifuged for 5 minutes in maximum speed and after the supernatant was discarded). After these three steps, the samples were allowed

to dry and then re-suspended with 10 µl of formamide used in sequencing process. The samples were sequenced in both directions on an automated sequencer (Applied Biosystems ABI3130XL) at UEFS.

Sequence editing and alignment— The electropherograms obtained were edited using the programs PREGAP4 and GAP4 of the Staden Package (Staden et al. 1998) and the sequences obtained were submitted to BLASTn search on the NCBI. The edited sequences were aligned automatically with the software MUSCLE (Edgar 2004) through the software MEGA 5.0 (Tamura et al. 2011). The resulting alignments were manually revised using BIOEDIT (Hall et al. 2009), following the guidelines of Kelchner (2000) for the plastid regions. Ambiguous regions were excluded from the analysis and indels were coded by the method of Simple coding (Simmons and Ochoterena 2000) using the GAPCODER (Young and Healy 2003).

Congruence testing—The markers sampled for this study are located in the chloroplast (cp) and nuclear (n) DNA. For this reason, congruence between the primer data sets was tested using the incongruence length difference (ILD) test (Farris et al. 1995). The tests were conducted in PAUP *version 4.0b10a (Swofford 2002) using the criterion of Maximum Parsimony (MP), heuristic search with 100 replicates, 50 random additions replicates were run using tree-bisection-reconnection (TBR) branch swapping, Multrees activated and saving 20 trees in each replication. The tests were made initially congruence between the regions pair to pair of each genome (nuclear and plastid) and then between the data sets of the two genomes.

Parsimony analyses— The Maximum Parsimony (MP) analyses were performed using PAUP* version 4.0b10 (Swofford 2002) with Fitch parsimony criteria, where characters have equal weight and are unordered (Fitch 1971). Gaps corresponding to insertions-deletions (indels) were coded as missing in the nucleotide matrix, but added as binary characters in the end. For each heuristic search, 1,000 random sequence addition replicates were run using tree bisection-reconnection (TBR) branch-swapping, limiting the number of trees saved per replicate up to 10 to reduce time spent in swapping on large islands of trees. The shortest trees identified from this search were then used as starting trees for a second search, swapping on them up to 10,000 trees using TBR. Internal support was evaluated with 1,000 bootstrap replicates (Felsenstein 1985) with simple taxon-addition and the TBR algorithm, saving up to 10 trees per replicate. The

character state optimization used was the Accelerated Transformation (ACCTRAN). For assessment of bootstrap percentages (BP) we considered 85–100% as strong, 75–84% as moderate, and 50–74% as weak support (Kress et al. 2002).

Bayesian analyses—For Bayesian inference, we first selected evolutionary models for each DNA region using Akaike information criterion (AIC) (Akaike 1974) in MrModeltest 2.3 (Nylander 2008). The substitution models selected for each region can be seen in Table 3. Bayesian analysis was performed using MrBayes 3.1 (Ronquist *et al.* 2003, 2005) with a mixed-model approach. The algorithm consisted of two independent runs with four chains for 10,000,000 generations, sampling one every 1000 trees. After discarding the first 8,500 (70.8%) trees as burn-in, the remaining trees (3000) were used to assess topology and posterior probabilities (PP) in a majority-rule consensus in MrBayes to provide posterior probabilities (PP). Because PP in Bayesian analyses are not equivalent to BP and are generally much higher (Erixon et al. 2003), we used >0.95 PP as well supported.

RESULTS

Missing data and sequence alignment — Our combined data sets include 69 samples, but not all taxa were sequenced for all seven regions (Table 3; Appendix 1). Sequence length of the studied regions varied considerably among taxa. Aligned length for all regions is shown in Table 3.

Data partition combinability and phylogenetic analyses —The ILD test detected significant incongruence at the 5% level but not at 1% between nuclear and plastid DNA regions ($p = 0.02$), thus they were analyzed both combined and separately to compare their topologies. Because the topology of nuDNA tree did not show good resolution, here we will present for parsimony results, trees with all regions combined and plastid combined (cpDNA) (Figs. 2 and 3).

The combined data matrix was 6705 bp long, of which 6091 characters are constant, 369 parsimony-uninformative and 245 parsimony-informative. General features of the DNA regions are presented in Table 3. The alignment of *trnS-trnG* and ITS plus indels were the longest and provided the majority of variable and potentially parsimony-informative sites (Table 3). The parsimony analysis for the seven loci matrix resulted in 861 most parsimonious trees (CI=0.74, RI=0.80) (Fig. 2). The

Table 3. DNA data sets used in this study, in relation to one of the most parsimonious trees resulting from combined analysis and evolutive models selected to Bayesian Inference (BI).

DNA regions	N° taxa	Aligned Length	N° of variables Sites	N° of potentially Parsimony informative sites	Number of changes/variable site	Fitch tree Length	N° de Indels	CI	RI	Evolutive Model to BI
ITS region	59	853	64 (7.5%)	19 (2.2%)	1.70	109	-	0.31	0.27	GTR+I
ITS1	-	213	10 (4,7%)	2 (0.9%)	1.30	13	-	0.50	0.50	F81
5.8S/18S/26S	-	394	29 (7.4%)	6 (0.7%)	1.24	36	-	0.50	0.33	GTR+I
ITS2	-	246	25 (10.2%)	11 (4.5%)	2.40	60	-	0.24	0.24	HKY+G
Indels included	-	1096	307(28.0%)	75 (6.8%)	1.46	449		0.35	0.18	-
Indels only		243	243	56 (23.0%)	1.13	338	243	0.71	0.18	-
<i>matK</i> gene	66	915	19 (2.0%)	7 (0.8%)	1.53	29		0.41	0.50	HKY
Indels included	-	934	37 (4.0%)	28 (3.0%)	1.27	47		0.47	0.58	-
Indels only	-	19	19	2 (10.5%)	1	19	19	1	1	-
<i>phyC</i> gene	63	999	63 (6.3%)	29 (2.9%)	1.81	114	-	0.45	0.68	HKY+I+G
<i>psbA-trnH</i> gene	67	484	24 (4.96%)	9 (1.9%)	2.83	68		0.25	0.56	GTR+I+G
Indels included	-	491	30 (6.1%)	11(2.2%)	2.53	76		0.29	0.59	-
Indels only	-	7	7	2(28.6%)	1	13	7	0.54	0.62	-
<i>rpl16</i> intron	65	915	40 (4.37%)	17(1.9%)	1.10	44		0.90	0.96	F81+I
Indels included	-	925	48 (5.19%)	21 (2.3%)	1.15	55		0.82	0.93	-
Indels only	-	10	9	4 (44.4%)	2	18	10	0.50	0.73	-
<i>rps16</i> intron	68	928	24 (2.6%)	10 (1.0%)	1.92	46		0.31	0.51	GTR+I
Indels included	-	937	33 (3.6%)	17 (1.8%)	2.42	80		0.27	0.46	-
Indels only		9	9	7(77.8%)	2.44	22	9	0.41	0.70	
<i>trnS-trnG</i> spacer	69	1312	89 (6.8%)	68 (5.2%)	2.16	192		0.44	0.80	GTR+I
Indels included	-	1323	96 (7.3%)	75 (5.7%)	2.47	237		0.37	0.76	-
Indels only		11	11	8 (72.7%)	1.72	19	11	0.58	0.88	-
nuDNA	-	1852	127 (6.9%)	48 (2.6%)	1.75	223	-	0.39	0.66	-
cpDNA	-	4554	196 (4.3%)	117 (2.6%)	1.93	379	-	0.42	0.90	-
All data combined	-	6705	614 (9.2%)	245 (0.4%)	1.40	861	-	0.74	0.80	GTR+I+G

Note: CI= Consistency Index , calculated excluding uninformative characters; RI= Retention Index, calculated excluding uninformative characters.

strict consensus trees of the parsimony combined and cpDNA, and Bayesian are shown in Figure 2, 3 and 4, respectively.

The CI and RI values of the *trnS-G*, *rps16*, *psbA-trnH* and *matK* indels show that they contributed relatively more to tree resolution than their corresponding nucleotide, unlike of ITS and *rpl16* indels, which therefore contain a great deal of noise (Table 3).

Phylogenetic relationships among species— The samples of the species *D. catingicola* Buining & Brederro, *D. diersianus* Esteves, *D. hartmannii* (K. Schum.) Britton & Rose, *D. heptacanthus* (Barb. Rodr.) Britton & Rose and *D. placentiformis* (Lehm.) K. Schum. do not had sufficient variation to understand their relations. With it, they will be discussed according with the small groups formed in the topologies (Fig. 2, 3 and 4). The relationships among other species (*D. pseudoinsignis* N.P. Taylor & Zappi, *D. horstii* Buining & Brederoo, *D. boliviensis* Backeb., *D. ferricola* Buining & Brederoo and *D. zehntneri* Britton & Rose) will be related below.

Discocactus zehntneri comprises one species and three heterotypic subspecies (subsp. *boomianus* (Buining & Brederoo) N.P. Taylor & Zappi, subsp. *petr-halfarii* (Zachar) M.R. Santos & Machado and subsp. *zehntneri*). The monophyly of this species group was supported by parsimony consensus trees (BP=74%), cpDNA (BP=86%) and Bayesian (PP=1). It is represented by clade A in all trees showed (Figs. 2, 3 and 4). Only in the Bayesian tree, the specimen *D. zehntneri* subsp. *zehntneri* MM2 did not group with other taxa of the group (Fig. 4).

Samples of *Discocactus placentiformis* could not be grouped in a species for lack of variation. However, some specimens clustered with good support, e.g. in the parsimony consensus tree (Fig. 2) the group D is formed by the specimens *D. placentiformis* MRS 110 and 112 (BP=74%). Already in the Bayesian consensus tree group E includes the already mentioned species, and *D. placentiformis* MRS 100 with 0.72 of PP (Fig. 4).

The close relationship between *D. pseudoinsignis* and *D. horstii* is pointed out by good support of the group represented by L in the parsimony consensus tree (BP=72%) and group F in the Bayesian consensus tree (PP=1) (Figs 2 and 4). In the cpDNA consensus tree this relationship is showed as group D with 69% of BS. The affinity between *D. ferricola* and *D. boliviensis* indicated by groups E (parsimony) and H

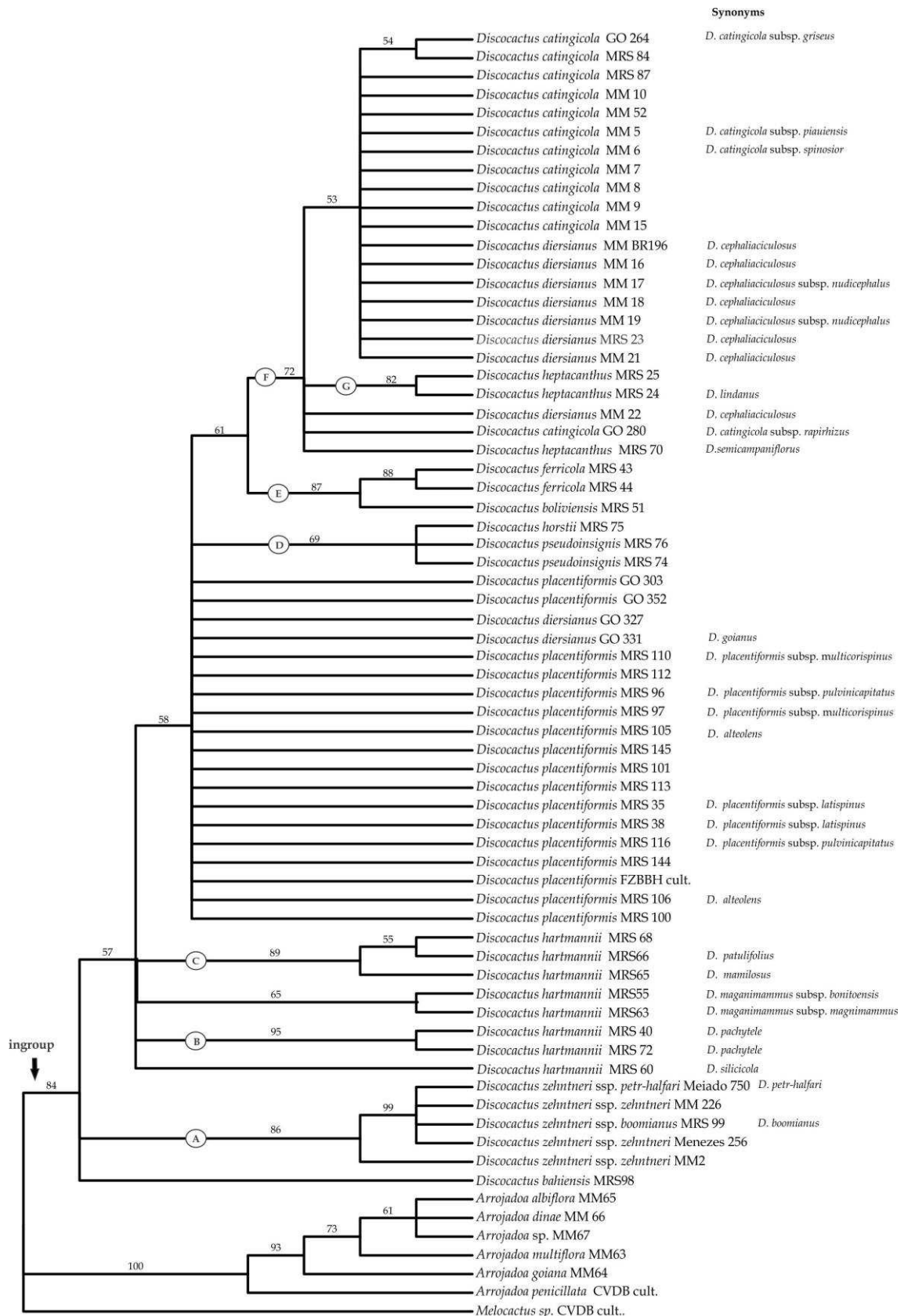


Figure 3. Strict consensus of 340 most parsimonious trees for *Discocactus* and outgroups based on combined plastid sequences. Bootstrap percentages are given above branches. CI=0.74 RI=0.90.

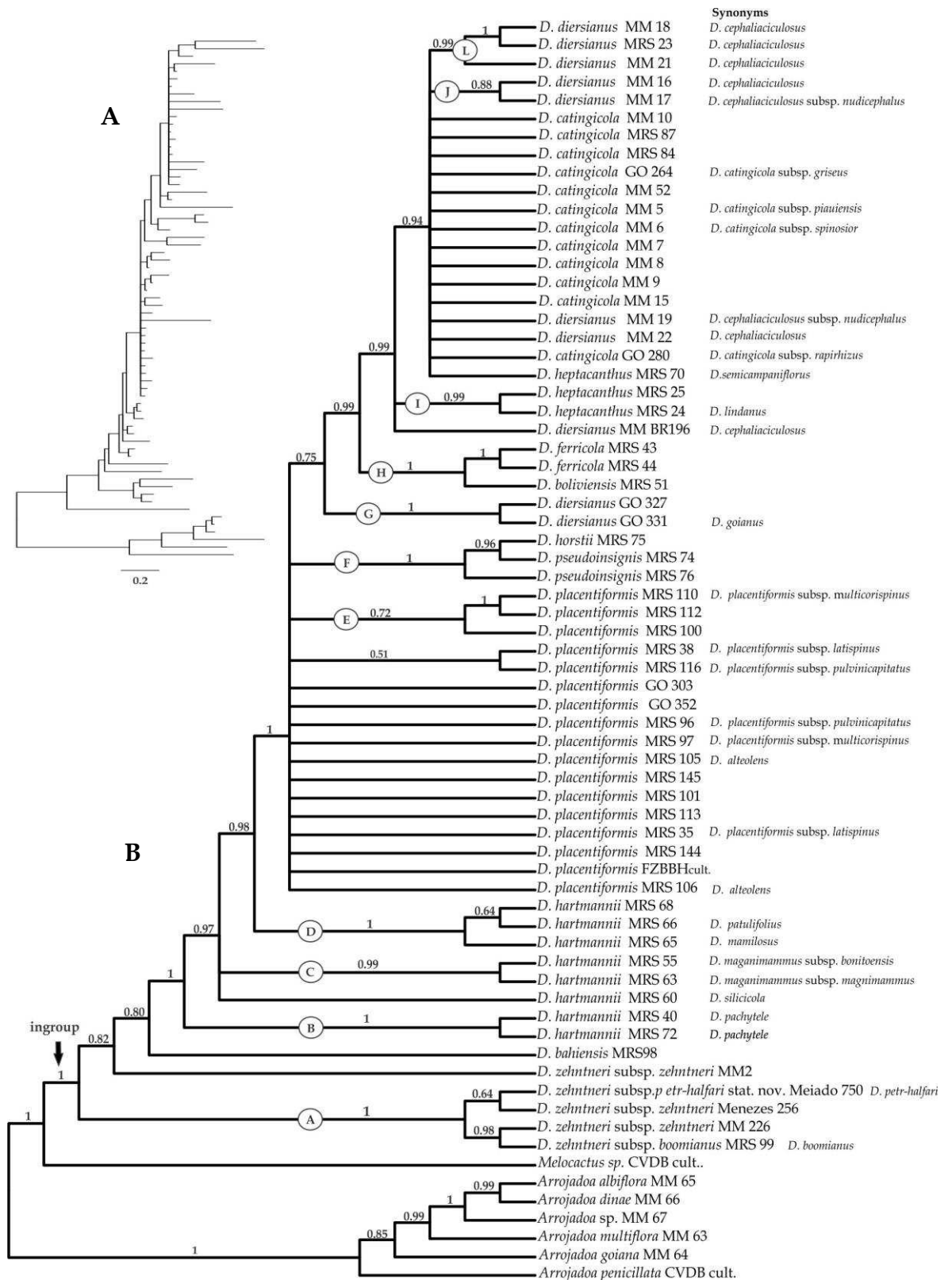


Figure 4. Majority-rule consensus of 3000 trees obtained in the Bayesian analysis with algorithm Markov chain Monte Carlo and the model of evolution=GTR+I+G in a combined analysis of seven DNA regions in *Discocactus*. Displayed as a phylogram (A) and cladogram for better visualization (B). Numbers above branches are posterior probabilities for clades estimated by the proportion of occurrence in the tree set.

(Bayesian) is tightly supported (cpDNA BP=87%; PP=1), but in the parsimony consensus tree only the two specimens of *D. ferricola* grouped with BP=100%. Other important groups formed are those related to *D. hartmannii* specimens, where B grouped the same samples in all trees (*D. hartmannii* MRS 40 and 72) with great support BP=93%, cpDNA BP=95%, PP=1. The group C, comprising *D. hartmannii* MRS55 and MRS63 presented a PP=0.99, but in parsimony analyses it was not recovered due to low support. In cpDNA parsimony and Bayesian trees the groups G and I are formed by two specimens of *D. heptacanthus* (BP=82%; PP=0.99, respectively). On the other hand, in the parsimony consensus tree this group was not well supported. The relationship between specimens of *D. catingicola* was not well supported, but the affinity among samples of *D. diersianus* is confirmed and it is represented by groups G (*D. diersianus* GO331 and GO327), J (*D. diersianus* MM16 and MM17) and L (*D. diersianus* MM 18, MM21 and MRS23) in the Bayesian consensus tree (PP=1, 0.88 and 0.99, respectively) and by group F (*D. diersianus* MM 19 and MM 21) in the strict consensus tree of parsimony (BP=84%).

DISCUSSION

Phylogenetic analyses — Despite the fact we included five plastid and two nuclear regions in data analyses, this was not sufficient to resolve many of the phylogenetic relationships among *Discocactus* species. However, we could resolve relationships in several small groups of samples (Figs. 2, 3 and 4).

Furthermore, the low resolution of the trees could be associated with the low genetic variation of *Discocactus* species, for which the DNA regions used in this study were not sufficiently diverse to detect it. The low variation is also a problem in others groups of Cactaceae (Hartmann et al. 2002, Arias et al. 2003, Butterworth and Wallace 2004, Helsen et al. 2009). The inclusion of more DNA regions will be necessary to solve this problem, following a trend in molecular systematics reported by Degnan and Rosenberg (2009) and Santos (2011). Besides, regions that have informative indels such as *trnS-G*, *rps16*, *psbA-trnH* and *matK* (Table 3) must be researched because it is likely that they also can help in improving the resolution of *Discocactus* phylogeny.

However, maybe also it will be necessary to carry out studies focusing genetic and morphological variation using populational samples of these species, as done by Machado (2004). Based on isozyme and morphometric data with populations of

Discocactus species that occur in Bahia, Brazil, he confirmed the subdivision of *D. zehntneri* into two subspecies as proposed by Taylor and Zappi (1991; 2004), and also suggested the lack of differentiated subspecies within *D. bahiensis* and *D. catingicola*. However, that study did not sample the endemic population of *D. zehntneri* subsp. *petr-halfarii*, which was not known at the time.

The presence of homoplasy within groups with lower taxonomic levels is not unusual and many of the morphological characters used in the classification can be particularly homoplastic, in other words, very susceptible to rapid changes (Santos, 2011). One example of this in *Discocactus* is the features related to ribs, because they are physiologically adapted to the expansion and contraction depending on the availability of water in the environment (Buxbaum 1950; Gibson and Nobel 1986). Rib type, grooves between areoles and tubercles shape may influence the adaptability of each species regarding to water availability in the environment. This great variation in traits related to rib morphology can be observed in Figure 2 (Santos et al. 2013a).

Biogeographic and morphological relationships — The species *D. horstii*, *D. placentiformis* and *D. pseudoinsignis* are narrow endemics to northern Minas Gerais (Taylor and Zappi 2004; Zappi et al. 2013). They share some macro-morphological characters like straight ribs with grooves between areole sinuses and tubercles of regular shape, but each has particular features which distinguish them (Santos et al. 2013a). They occur in the southern half of the Espinhaço mountain range and east of São Francisco River in the *cerrado-campo rupestre* element on pure quartz sand and gravel or on arenitic rocks (*D. placentiformis*) (Taylor and Zappi 2004). This broad region can be subdivided into the Diamantina-Serra do Cipó region (Santos et al. 2013b) where the samples of *D. placentiformis* (MRS100, 110 and 112) occur and their affinity is confirmed by BP values (Figs. 2 and 4).

The Serra do Cabral and Engenheiro Dolabela subregion is a disjunct area of the Espinhaço range (Santos et al. 2013b), where the largest plants of the genus are represented by samples MRS 38 and 116 that grouped only in Bayesian tree with low PP. Other specimens of *D. placentiformis* belong to this subregion, but the relationship among them is not well resolved phylogenetically. Another subregion is Grão Mogol (Santos et al. 2013b), separated from Diamantina, Serra do Cabral and Engenheiro Dolabela subregion by the Jequitinhonha River, mainly by its tributaries Itacambiruçu

and Macaúbas rivers, where *D. horstii* and *D. pseudoinsignis* occupy similar habitats. *D. pseudoinsignis* occurs also in Cristália and Botumirim municipalities separated from Grão Mogol by the Itacambiruçu River (IBGE 1997). These species show a very close phylogenetic relationship and probably have a common ancestor (Figs. 2, 3 and 4).

Discocactus catingicola, *D. diersianus* and *D. heptacanthus* are geographically widespread and morphologically variable (Fig. 2; Santos et al. 2013a, b). These species occur to the West of the São Francisco River, Espinhaço range and the Chapada Diamantina (Bahia). The distribution pattern of *D. catingicola*, *D. heptacanthus* and *D. diersianus* is Midwestern Brazil - Western Bahia and in the state of Tocantins. The samples of *D. heptacanthus* (MRS23 and 24) included in this study grouped with good support in the Bayesian and cpDNA trees and present a shared distribution in the *cerrado* vegetation zone on gravelly or sandy soil (Taylor and Zappi 2004), around the Chapada dos Veadeiros National Park in Goiás State and the Chapada dos Guimarães in Mato Grosso State (personal observation).

Some groups of *Discocactus diersianus* had good support in the trees (Figs. 2, 3 and 4). This species occurs in eastern Goiás and southern Tocantins states on quartz and granitic rocks surrounded by deciduous dry land vegetation (personal observation; Santos et al. 2013a). *Discocactus catingicola* occurs in a central area that includes Southern Piauí, Western Bahia and Northern and Western Minas Gerais states, on sandy soil west of the São Francisco River in *Cerrado* vegetation (Taylor and Zappi 2004). It is also sometimes found on rocks composed of iron-manganese, known locally as “canga”, in extreme Western Minas Gerais (the “Triângulo Mineiro and Alto Paranaíba”) and in upper drainage of the São Francisco River (personal observation).

Discocactus ferricola and *D. boliviensis* are closely related phylogenetically (Fig. 2), probably sharing a common ancestor confirmed by significant support in the molecular trees. They occur in a subregion that can be termed the Corumbá/Eastern Bolivia area, on “canga” and limestone outcrops, respectively. *Discocactus hartmannii* has a morphology well differentiated with ribs strongly spiralled, with pronounced grooves between the areoles and conical teeth-like tubercles (Buxbaum 1950; Santos et al. 2013a). This species is distributed in the Central-Southern part of Mato Grosso do Sul state and in Southeastern Paraguay, on sandy or gravelly quartz soil. The groups well

supported in the trees correspond to those closest geographically (Figs. 2, 3 and 4; Appendix 1).

The *D. zehntneri* group is well supported in all molecular trees and its representatives likely therefore shared a common ancestor. The single species and two subspecies occur in the *caatinga* vegetation of northern Bahia, northern Piauí and Ceará (Taylor and Zappi 2004; Zappi et al. 2013) in a subregion belonging to lower drainage of the São Francisco River. The representatives of this group share the same distribution with another group represented by the taxon *D. bahiensis* (Taylor and Zappi 2004). This coexistence may have given rise to *D. zehntneri* subsp. *petr-halfarii* (Zachar) M.R. Santos & Machado through an historic process of hybridization. Today, its population is very well established reproductively, and it is best classified as a subspecies of *D. zehntneri*, because they share several morphological characters (Santos et al. 2013a).

Taxonomic changes— Compared to the classification of Hunt et al. (2006), we recognize one extra subspecies of *Discocactus*, (Fig. 5). The synonyms names of species according to Hunt et al. (2006) are not in all cases confirmed by the present phylogenetic study, being the taxonomy study very important helping to take some taxonomic decisions (Fig. 2; Santos et al. 2013a). For example, *Discocactus silicicola* Buining & Brederoo which was previously subsumed in *D. catingicola*, we now place as a synonym of *D. hartmannii* (Figs. 2, 3 and 4; Santos et al. 2013a) due to presence of ribs with deep grooves between areoles, conical and elongated tubercles. *Discocactus lindanus* Diers & Esteves Pereira, previously allied with *D. diersianus*, is now placed here as synonym of *D. heptacanthus* because it grouped with good support in this study with other sample of *D. heptacanthus* and has morphological features shared that confirm this change like, ribs forming nipple-like tubercles compressed, flowers up to 110 mm, nectar chamber with curled hairs at the apex (Santos et al. 2013a).

Discocactus petr-halfarii Zachar, initially considered as a new species (Zachar 2008), does not show sufficient genetic divergence from the others taxa of *D. zehntneri* to be assigned species status (this study; Figs. 2, 3 and 4). Hence, *D. petr-halfarii* is treated here as subspecies of *D. zehntneri* also due to some morphological characters



Figure 5. *Discocactus bahiensis* (A, B); *D. boliviensis* (C, D); *D. catingicola* (E,F); *D. diersianus* (G, H); *D. ferricola* (I, J); *D. hartmannii* (K, L); *D. horstii* (M, N); *D. heptacanthus* (O); *D. placentiformis* (P, Q); *D. pseudoinsignis* (R); *D. zehntneri* subsp. *zehntneri* (S,T); *D. zehntneri* subsp. *boomianus* (U,V); *D. zehntneri* subsp. *petr-halfarii* (X,Z). Photo: Basarab Popa (N, O); Gerardus Olsthorn (Z); Marianna Santos (C, D, E, H, I, J, K, L, M, P, Q, R); Marcelo Telles (A, S, T); Marlon Machado (B, U,V,X); Pierre Braun (G).

shared like ribs broken into rounded tubercles well developed and prominent areoles not sunken. However *D. petr-halfarii* has some morphologic traits that distinguish it from other subspecies of this group like the absence of central spine, while the other subspecies have 1-2 central spines (Santos et al. 2013a).

Based on the present study, the DNA regions *trnS-trnG*, *rpl16*, ITS and *phyC* were indicated to be a good choice to help studies involving other genera of Cactaceae due to a good number of informative sites. Indels of some regions like *trnS-G*, *rps16*, *psbA-trnH* and *matK* also contribute to tree resolution. Various former synonyms are to be referred to alternative synonymy based on the molecular and morphological data presented here. The selection of more DNA regions and carrying out studies about genetic and morphological variations, seem to be the way for solving the remaining doubts in the relationships and species delimitation within *Discocactus*.

ACKNOWLEDGMENTS

We would like to thank the Instituto Estadual do Meio Ambiente of Minas Gerais (IEF) and Instituto Chico Mendes (ICMBIO-MMA) for collecting permits and all directors of parks visited. This study was supported by grants from CAPES (PhD scholarship), FAPEMIG (TO APQ-01314-11-CC8034), FAPESB (PNX0014/2009) and Cactus Succulent Society of America (CSSA) and the British Cactus and Succulent Society (BCSS). Finally, we would like to thank the Instituto Chico Mendes (ICMBIO-MMA) for financing some fieldwork.

REFERENCES

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716–723.
- Anderson, E. F. 2001. The cactus family. Timber Press, Portland, Oregon, USA.
- Arias, S. and T. Terrazas. 2006. Análisis cladístico del género *Pachycereus* (Cactaceae) con caracteres morfológicos. *Brittonia* 58:197-216.
- Arias, S., T. Terrazas and K. Cameron. 2003. Phylogenetic analysis of *Pachycereus* (Cactaceae, Pachycereeae) based on chloroplast and nuclear DNA sequences. *Systematic Botany* 28:547-557.

- Arias, S., T. Terrazas, H.J. Arreola-Nava, M. Vázquez-Sánchez and K. Cameron. 2005. Phylogentic relationships in *Peniocereus* (Cactaceae) inferred from plastid DNA sequence data. *Journal of Plant Research* 118: 317-328.
- Barthlott. W. 1983. Biogeography and evolution in Neo and Paleotropical Rhipsalinae (Cactaceae). In: KYBITZKI, K. (ED.). *Dispersal and Distribution: An international Symposium, Sonderbande des Naturwissenschaftlichen Vereins, Hamburg*, pp. 241-248.
- Barthlott. W. and D. Hunt. 2000. Seed diversity in the Cactaceae: subfamily Cactoideae. *Succulent Plant Research* 5, 173 p.
- Brody, J.R. and S.E. Kern. 2004. Sodium boric acid: a tris-free, cooler conductive medium for DNA electrophoresis. *Biotechniques* 36:2-4.
- Buining, A.F.H. 1980. *The genus Discocactus Pfeiffer*. Buining-fonds, Succulenta, The Netherlands, p. 223.
- Butterworth, C.A. and R.S. Wallace. 2004. Phylogenetic studies of *Mammillaria* (Cactaceae) – insights from chloroplast sequence variation and hypotheses testing using the parametric bootstrap. *American Journal of Botany* 91: 1086-1098.
- Buxbaum, F. 1950. *Morphology of cacti*. Abbey Garden Press, Pasadena, California, USA.
- Calvente, A., D.C. Zappi, F. Forest and L.G. Lohmann. 2011a. Molecular phylogeny of tribe Rhipsalidae (Cactaceae) and taxonomic implications for *Schlumbergera* and *Hattiora*. *Molecular Phylogenetics and Evolution* 58: 456-468.
- Cantino P. D., Doyle J.A., Graham S. W., Judd W. S., Olmstead R. G. Soltis D. E., Soltis P. S. and Donoghue M. J. 2007. Towards a phylogenetic nomenclature of Tracheophyta. *Taxon* 56: 822–846.
- Crozier, B.S. 2004. Subfamilies of Cactaceae Juss., including Blossfeldioideae subfam. nov. *Phytologia* 86: 52–64.
- Crozier, B.S. 2005. Systematics of Cactaceae Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw. 154 p. PhD. Dissertation- The University of Texas, Austin.
- Cuénoud, P., V. Savolainen, L. W. Chatrou, M. Powell, R. J. Grayer, and M. W. Chase. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. *American Journal of Botany* 89: 132–144.
- Degnan, J.H. and N.A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology and Evolution*. 24: 332-340.

- Desfeux, C., S. Maurice, J.P. Henr, B. Lejeune and P.H. Gouyon. 1996. The evolution of reproductive system in the genus *Silene*. *Proceedings of the Royal Society B: Biological Sciences* 263: 409-414.
- Doyle, J.J. and J.L.Doyle. 1987. A rapid isolation procedure for small quantities of fresh tissue. *Phytochemical Bulletin* 19:11-15.
- Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*. 32(5):1792-1797.
- Erixon, P., B. Svenblad, T. Britton and B. Oxelman. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology*. 52: 665-673.
- Farris, J.S., M. Källersjö, A.G. Kluge and C. Bult. 1995. Testing significant of incongruence. *Cladistics* 10:315-319.
- Felsenstein, J. 1985. Confidence limit on phylogenies: An approach to using bootstrap. *Evolution* 39: 783-791.
- Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology*. 20:406-416.
- Gibson, A. C. and P. S. Nobel. 1986. The cactus primer. Cambridge, Massachusetts: Harvard University, 286 p.
- Givnish, T.J, M.H.J. Barfuss, V.Ee Benjamin, R. Riina, K. Schulte, R. Horres, P.A. Gonsiska, R.S. Jabaily, D.M. Crayn, A.C. Smith, K. Winter, G.K. Brown, T.M. Evans, B.K. Holst, H. Luther, W. Till, G. Zizka, P.E. Berry and K.J. Sytsma. 2011. Phylogeny, adaptive radiation, and historical biogeography in Bromeliaceae: insights from an eight-locus plastid phylogeny. *American Journal of Botany* 98:872–895
- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95-98.
- Hamilton, M.B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology*. 8:521–523.
- Harpke, D. and A. Peterson. 2006. Non-concerted ITS evolution in *Mammillaria* (Cactaceae). *Molecular Phylogenetics and Evolution* 41:579-593.
- Hartmann, S., J.D. Nason and D. Bhattacharya. 2002. Phylogenetics origins of *Lophocereus* (Cactaceae) and the senita cactus-senita moth pollination mutualism. *American Journal of Botany* 89:1085-1092.

- Helsen P, R.A. Browne, D.J. Anderson, P. Verdyck and S. Dongen. 2009. Galapagos' *Opuntia* (prickly pear) cacti: extensive morphological diversity, low genetic variability. *Biological Journal of the Linnean Society*. 96: 451-461.
- Hunt, D., N. P. Taylor and G. Charles. 2006. The new cactus lexicon, vols. I, II. DH Books, Milborne Port.
- IBGE. 1997. Diagnóstico Ambiental da Bacia do Rio Jequitinhonha: Divisão Geográfica da Bacia do Rio Jequitinhonha. Available: <http://www.ibge.gov.br/home/geociencias/recursosnaturais/diagnosticos_levantamentos/jequitinhonha/images/fig_09.jpg> Accessed October 10th, 2013.
- Kelchner, S.A. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* 87: 482-498.
- Kiesling, R. 1988. Cactaceae. In: CORREA, M.N. (ed.). Flora Patagónica 5, Dicotiledoneas dialipétalas (Oxalidaceae a Cornaceae). Colección Científica del INTA 8, Buenos Aires, Argentina. Pp. 218-243.
- Kress, W.J., L.M. Prince and K.J. Williams. 2002. The phylogeny and a new classification of the gingers (Zigiberaceae): evidence from molecular data. *American Journal Of Botany* 89: 1682-1696.
- Machado, M.C. 2004. O gênero *Discocactus* Pfeiff. (Cactaceae) no estado da Bahia, Brasil: variabilidade morfológica, variabilidade genética, taxonomia e conservação. 113p. M.Sc. Thesis- Universidade Estadual de Feira de Santana, Feira de Santana.
- Nyffeler, R. 2002. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from trnK/matK and trnL-trnF sequences. *American Journal of Botany* 89: 312–326.
- Nylander, J.A., 2008. MrModeltest v.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Simmons, M. P., and H. Ochoterena. 2000. Gaps as characters in sequence based phylogenetic analyses. *Systematic Biology* 49: 369–381.
- Oxelman, B., M. Lidén, and D. Berglund. 1997. Chloroplast rps16 intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.
- Ritz, C.M., L. Martins, R. Mecklenburg, V. Goremykin and F.H. Hellwig. 2007. The molecular phylogeny of *Rebutia* (Cactaceae) and its allies demonstrates the influence of paleogeography on the evolution of South American mountain cacti. *American Journal of Botany* 94: 1321-1332.

- Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Ronquist, F., J.P. Huelsenbeck, and P. Van Der Mark. 2005. Mr. Bayes 3.1 Manual. Florida State University: School of Computational Science. <http:// mrbayes.csit.fsu.edu>, retrieved 29 August 2011.
- Sang, T., D.J. Crawford and T.F. Stuessy. 1997. Chloroplast phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84, 1120-1136.
- Santos, T.M. 2011. Eventos antigos de especiação híbrida no grupo bifoliado do gênero *Cattleya* Lindl. (Orchidaceae) inferidos a partir de uma filogenia baseada em 16 regiões de DNA nuclear e plastidial. 66p. M.Sc. Thesis- Universidade Estadual de Feira de Santana, Feira de Santana.
- Santos, M.R., Garcia, F.C.P., Machado, M.C. and Taylor, N.P. 2013a. Taxonomy, distribution and conservation of *Discocactus* Pfeiff. (Cactaceae) In: Santos, M.R. 2013. Filogenia molecular, taxonomia, biogeografia e conservação de *Discocactus* Pfeiff. 114 p. PhD thesis – Universidade Federal de Viçosa.
- Santos, M.R., Garcia, F.C.P., Taylor, N.P. and Verola, C.F. 2013b. Biogeography and conservation status assessment of *Discocactus* Pfeiff. (Cactaceae): an endemic genus of South America. In: Santos, M.R. 2013. Filogenia molecular, taxonomia, biogeografia e conservação de *Discocactus* Pfeiff. 114 p. PhD thesis – Universidade Federal de Viçosa.
- Schlumpberger, B.O. and S. S. Renner. 2012. Molecular phylogenetics of *Echinopsis* (Cactaceae): polyphyly at all levels and convergent evolution of pollination modes and growth forms. *American Journal of Botany* 99: 1335-1349.
- Small, R.L., J.A. Ryburn, R.C. Cronn, T. Seelanan, and J. F. Wendel. 1998. The tortoise and the hare: choosing between noncoding plastome and nuclear Adh sequences for phylogenetic reconstruction in a recently diverged plant group. *American Journal of Botany* 85:1301–1315.
- Speirs, D.C. 1982. The cacti of western Canada (part 3). *National Cactus and Succulent Journal* 37:53-54.
- Staden, R., K.F. Beal and J.K. Bonfiel. 1998. The Staden Package. *Methods Mol. Biol.* 132: 115-130.
- Stevens, P.F. (2001 onwards). Angiosperm Phylogeny Website. Version 12, 2012. Disponível em: <<http://www.mobot.org/MOBOT/research/APweb/>>. Accessed: March 23rd, 2013.

- Sun, Y., D. Z. Skinner, G. H. Liang and S. H. Hulbert. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26-32.
- Swofford, D.L. 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods) v.4 beta 10. Sinauer Associates, Inc., Sunderland, Massachusetts, EUA.
- Tamura, K.; D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* 28: 2731-2739.
- Taylor, N.P. 1981. Reconsolidation of *Discocactus* Pfeiff. *Cact. Succ. J. Gr. Brit.* 43: 37–40.
- Taylor, N.P. and D.C. Zappi. 2004. *Cacti of eastern Brazil*. England: Richmond, Surrey: Royal Botanic Gardens, Kew, 499 p.
- Thorne, R.F. 1973. Floristic relationships between tropical Africa and tropical America. In: MEGGARS, B.J., E.S. AYENSU AND W.D. DUCKWORTH (eds.). *Smithsonian Institution Press, Washington, D.C.* Pp. 27-47.
- White, T.J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315–322 in M. Innis, D. Gelfand, J. Sninsky, and T. White (eds.). *PCR: A Guide to Methods and Applications*. Academic Press, San Diego, California.
- Young N. and J. Healy. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4:6.
- Zachar, M. 2008. Nowość z Brazylii - *Discocactus petr-halfarii*. *Kaktusy i Inne* 2: 43-47.
- Zappi D.C, Taylor N, Machado M. and Santos, MR. 2013. *Discocactus* in Lista de espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. (<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB1482>).

Appendix 1. Species and loci sequenced for this study, vouchers and provenance, herbarium code and GenBank accession numbers.

Species	Vouchers	Geographic origin of sample or distribution	ITS	<i>matK</i>	<i>phyC</i>	<i>psbA-trnH</i>	<i>rpl16</i>	<i>rps16</i>	<i>trnS-trnG</i>
<i>Arrojadoa albiflora</i>	MM 65 (HUEFS)	?	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Arrojadoa dinae</i> Buining & Brederoo	MM 66 (HUEFS)	Monte Azul-MG	-	XXX	XXX	XXX	XXX	XXX	XXX
<i>Arrojadoa</i> sp.	Cultivated	----	-	XXX	XXX	XXX	XXX	XXX	XXX
<i>Arrojadoa multiflora</i> F.Ritter	MM63 (HUEFS)	Jacaraci-BA	-	XXX	XXX	XXX	XXX	XXX	XXX
<i>Arrojadoa goiana</i> M.C. Machado	MM 64 (HUEFS)	?	XXX	XXX	XXX	XXX	-	XXX	XXX
<i>Arrojadoa penicillata</i> (Gurke) Britton & Rose	CVDB cultivated	----	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus bahiensis</i> Britton & Rose	MRS 98 (VIC)	Morro do Chapéu-BA	XXX	XXX	XXX	-	XXX	XXX	XXX
<i>Discocactus boliviensis</i> Backeb.	MRS 51 (VIC)	Bolivia	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i> Buining & Brederoo	MM 5 (HUEFS)	Corrente-PI	-	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	MM 6 (HUEFS)	Barreiras-BA	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	MM 7 (HUEFS)	Gilbués-PI	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	MM 8 (HUEFS)	Barreiras-BA	XXX	XXX	XXX	XXX	-	XXX	XXX
<i>Discocactus catingicola</i>	MM 9 (HUEFS)	Aurora do Tocantins-TO	XXX	XXX	XXX	XXX	XXX	XXX	XXX

Appendix 1. Continued.

Species	DNA source	Geographic origin of sample or distribution	ITS	matK	phyC	psbA-trnH	rpl16	rps16	trnS-trnG
<i>Discocactus catingicola</i>	MM 10 (HUEFS)	Sítio Grande-BA	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	MM 15 (HUEFS)	Natividade-TO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	MM 52 (HUEFS)	Terra Ronca-GO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	MRS 84 (VIC)	Cônego Marinho-MG	XXX	XXX	XXX	XXX	-	-	XXX
<i>Discocactus catingicola</i>	MRS 87 (VIC)	Pandeiros-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	GO 264 (VIC)	João Pinheiro-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus catingicola</i>	GO 280 (VIC)	Correntina-BA	-	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i> Esteves Pereira	MM 16 (HUEFS)	Almas-TO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MM 17 (HUEFS)	Conceição do Tocantins-TO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MM 18 (HUEFS)	Arraias-TO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MM 19 (HUEFS)	?	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MM 21 (HUEFS)	Teresina de Goiás_GO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MM 22 (HUEFS)	Monte Alegre-GO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MRS 23 (VIC)	Funil do Paranã-GO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	GO 327 (VIC)	Marzagão-GO	XXX	-	-	XXX	XXX	XXX	XXX

Appendix 1. Continued.

Species	DNA source	Geographic origin of sample or distribution	ITS	<i>matK</i>	<i>phyC</i>	<i>psbA-trnH</i>	<i>rpl16</i>	<i>rps16</i>	<i>trnS-trnG</i>
<i>Discocactus diersianus</i>	GO 331 (VIC)	Piracanjuba - GO	XXX	XXX	-	XXX	XXX	XXX	XXX
<i>Discocactus diersianus</i>	MM BR196 (HUEFS)	Arraias-TO	XXX	XXX	XXX	-	XXX	XXX	XXX
<i>Discocactus ferricola</i> Buining & Brederoo	MRS 43 (VIC)	Corumbá-MS	-	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus ferricola</i>	MRS 44 (VIC)	Porto da Manga-MS	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i> (K.Schum.) Britton & Rose	MRS 40 (VIC)	Aquidauana-MS	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 55 (VIC)	Bonito-MS	XXX	XXX	-	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 60 (VIC)	Porto Murтинho-MS	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 63 (VIC)	Caracol-MS	-	XXX	-	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 65 (VIC)	Antônio João-MS	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 66 (VIC)	Cel. Sapucaia-MS	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 68 (VIC)	Paraguay	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus hartmannii</i>	MRS 72 (VIC)	Cipolândia-MS	XXX	XXX	XXX	XXX	XXX	-	XXX
<i>Discocactus heptacanthus</i> (Barb.Rodr.) Britton & Rose	MRS 24 (VIC)	Alto Paraíso-GO	XXX	XXX	XXX	XXX	XXX	XXX	XXX

Appendix 1. Continued.

Species	DNA source	Geographic origin of sample or distribution	ITS	matK	phyC	psbA-trnH	rpl16	rps16	trnS-trnG
<i>Discocactus heptacanthus</i>	MRS 25 (VIC)	São Jorge-GO	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus heptacanthus</i>	MRS 70 (VIC)	Coxim-MS	XXX	XXX	-	XXX	XXX	XXX	XXX
<i>Discocactus horstii</i> Buining & Brederoo	MRS 75 (VIC)	Grão Mogol-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Melocactus</i> sp.	CVDB Cultivated	---	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i> (Lehm.) K.Schum.	MRS 38 (VIC)	Joaquim Felício-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 35 (VIC)	Serra do Cabral-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	FZBBH-Cultivated	Santana do Pirapama-MG	-	XXX	-	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 96 (VIC)	Eng. Dolabela-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 97 (VIC)	Cons. Mata-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 100 (VIC)	P.E. Biribiri-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 101 (VIC)	Sopa-Diamantina-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 105 (VIC)	PARNA Sempre Vivas-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 106 (VIC)	Mendanha-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX

Appendix 1. Continued.

Species	DNA source	Geographic origin of sample or distribution	ITS	matK	phyC	psbA-trnH	rpl16	rps16	trnS-trnG
<i>Discocactus placentiformis</i>	MRS 110 (VIC)	Monjolos-MG	XXX	-	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 112 (VIC)	Chapada PERP-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 113 (VIC)	P.E. Rio Preto-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 116 (VIC)	Sítio-Engenheiro Dolabela-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 144 (VIC)	Diamantina-MG	-	XXX	XXX	XXX	-	XXX	XXX
<i>Discocactus placentiformis</i>	MRS 145 (VIC)	Barão do Guaicuí-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	GO 303 (VIC)	Fruta do Leite-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus placentiformis</i>	GO 352 (VIC)	Rio Pardo de Minas- MG	XXX	-	XXX	XXX	XXX	XXX	XXX
<i>Discocactus pseudoinsignis</i> N.P. Taylor & Zappi	MRS 74 (VIC)	Grão Mogol-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus pseudoinsignis</i>	MRS 76 (VIC)	Cristália-MG	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus zehntneri</i> subsp. <i>boomianus</i> (Buining & Brederoo) N.P. Taylor & Zappi	MRS 99 (VIC)	P.E. Morro do Chapéu - BA	XXX	XXX	XXX	XXX	XXX	XXX	XXX

Appendix 1. Continued.

Species	DNA source	Geographic origin of sample or distribution	ITS	matK	phyC	psbA-trnH	rpl16	rps16	trnS-trnG
<i>Discocactus zehntneri</i> subsp. <i>petr-halfarii</i> (Zachar) M.R. Santos & M.C. Machado	Meiado 750 (HVASF)	Juazeiro-BA	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus zehntneri</i> subsp. <i>zehntenri</i> Britton & Rose	MM 2 (HUEFS)	Sento Sé-BA	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus zehntneri</i> subsp. <i>zehntenri</i>	Menezes 256	Guaraciaba-CE	XXX	XXX	XXX	XXX	XXX	XXX	XXX
<i>Discocactus zehntneri</i> subsp. <i>zehntenri</i>	MM BR226	Casa Nova-BA	-	XXX	XXX	XXX	XXX	XXX	XXX

Abbreviations: PERP (Parque Estadual do Rio Preto) P.E. (Parque Estadual) PARNA (Parque Nacional) FZBBH (Fundação Zoo Botânica de Belo Horizonte) BA(Bahia) CE (Ceará) GO (Goiás) MG (Minas Gerais) MS (Mato Grosso do Sul) PI (Piauí) TO (Tocantins).

CAPITULO II

TAXONOMY, DISTRIBUTION AND CONSERVATION OF *DISCOCACTUS* PFEIFF. (CACTACEAE)

This Chapter is formatted for submission to **Phytotaxa**

Taxonomy, distribution and conservation of *Discocactus* Pfeiff. (Cactaceae)

MARIANNA R. SANTOS^{1,4}, FLAVIA C.P. GARCIA¹, MARLON C. MACHADO²
AND NIGEL P. TAYLOR³

¹ Programa de Pós-graduação em Botânica, Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Av. P.H. Rolfs, s/n CEP 36570-000, Viçosa, Minas Gerais, Brazil.

² Departamento de Ciências Biológicas, Universidade Estadual de Feira de Santana, Av. Transnordestina, s/n, 44036-900 Feira de Santana, Bahia, Brazil.

³ Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, Singapore 259569.

⁴ Author for correspondence : mrodriguessantos@gmail.com.

Abstract

The *Discocactus* Pfeiff. taxonomy is disputed and all species are considered threatened or endangered. Then, this study aims increase information about the genus through the taxonomic study of the species providing descriptions, identification keys, illustrations, besides geographic distribution and conservation status. For this, field collections and examination of herbarium material were made and the conservation status was evaluated according to IUCN criteria. Analysis of morphological traits, phylogeny and geographic distribution suggest that *Discocactus* can be divided into 11 species and 3 heterotypic subspecies, giving a total of 14 taxa. Most species of the genus are threatened, mainly by habitat destruction and illegal collection. Thus, there is a great need for studies of the phenology, pollination and reproductive biology of *the genus* to understand more about the biology and ecology of its species. Moreover, due to the great variation of *Discocactus* species, studies that consider their genetic and morphological variability are also of utmost importance.

Key words: Cereae, conservation status, South America, cacti.

Introduction

Discocactus is a genus of family Cactaceae, belonging to subfamily Cactoideae and tribe Cereeae (Crozier 2005). This cactus genus is characterized as having a low-growing, discoid to depressed-globose, single or caespitose habit (Machado *et al.* 2004). The fertile part of stem is differentiated into a terminal cephalium that produces nocturnal white flowers, which are tubular or funnel-shaped, sweet scented and pollinated by moths (hawkmoth syndrome). The fruits are globose-clavate to oblong, dehiscent by lateral slits, and the seeds are helmet-shaped to globular, with a strongly tuberculate testa. The genus is restricted to South America, almost all species being restricted to Brazil, just two taxa occurring in bordering areas of Bolivia and Paraguay. In Brazil its distribution comprises the Central-Western, Northeastern and Southeastern regions of the country (Taylor & Zappi 2004, Machado *et al.* 2004).

Discocactus was described as a genus in 1837 by Louis Pfeiffer where the author wrote on the species *D. insignis* Pfeiff. Pfeiffer used the following characters to distinguish the species of the genus: “the tube of the calyx adhering to the ovary, naked at the base, undivided, connate form sepals which are free at the top. Numerous petals which form a funnel-shaped corolla. Stamens thread-like, the outer ones level with the petals, the inner ones shorter and attached to the tube calyx. Style clavate, 5 stigma lobes, elongated, linear. Fruits very simple, disc-like, ribbed and prickly. A false cephalium at the top, existing of soft wool and not of hairy tubercles (as on *Melocactus*). The flowers come through the wool at the top, with long protract glabrous tube, nightflowering, deciduous, receptacle persistent” (Pfeiffer 1837). This description is not perfect because the shape of the fruit is never disc-like, not ribbed or spiny. It is indeed the cephalium or stem that are disc-like and spiny.

Modern estimates of species numbers vary greatly depending upon circumscription at specific level. *Discocactus* is an example of the divergence among classification systems that characterizes the Cactaceae. The taxonomy of *Discocactus* is disputed: 53 taxa have been described for the genus, and the currently proposed classification systems are rather divergent. Buining accepted 35 species and one subspecies (Buining 1980). Braun & Esteves Pereira (1993a-c, 1995, 2001) recognize 51 taxa: 25 species, 22 subspecies and four varieties. Taylor & Zappi (2004) recognize only ten taxa: seven species and three subspecies. Nowadays, Hunt *et al.* (2006) acknowledge 11 species and 2 subspecies. All these circumscriptions preceded

publication of the species *D. petr-halfarii* Zachar (Zachar 2008) which is analysed in this study.

The tribal placement of *Discocactus* has also been controversial. *Discocactus* has traditionally been associated with *Melocactus* (L.) Link & Otto (Britton & Rose 1919-1923; Buxbaum 1958; Backeberg 1958-1962; Endler & Buxbaum 1974) because of their similar morphology - both share a globular body and develop a terminal cephalium, although flower, fruit and seed characters are different (*Melocactus* possess diurnal, hummingbird-pollinated flowers, indehiscent fruits and seeds usually without a strongly tuberculate testa, while *Discocactus* possess nocturnal, moth-pollinated flowers, dehiscent fruits and seeds with a strongly tuberculate testa). More recently *Discocactus* has been placed in tribe Trichocereae (Taylor & Zappi 2004, Hunt *et al.* 2006), in the belief that the similarities of body and cephalium between *Discocactus* and *Melocactus* are due to convergent evolution, *Discocactus* supposedly being closer to *Gymnocalycium* Pfeiff. ex Mittler. However, a recent family-wide phylogenetic investigation (Crozier 2005) places *Discocactus* as the sister group of *Melocactus*, thus reinstating *Discocactus* in tribe Cereeae.

All species of *Discocactus* are considered threatened or endangered (IUCN 2013, Lüthy 2001). The intrinsic reasons for this are the small number of populations per species, the generally small population sizes, and habitat specificity leading to very localized populations. These factors make *Discocactus* populations extremely vulnerable to habitat modification and destruction, and to collecting. Many species inhabit areas of *Cerrado*, a vegetation type that covers areas with nutrient deficient soil, which, nevertheless has been systematically destroyed, the areas occupied by this vegetation being progressively converted to agriculture, especially for soybean plantations. This process has already destroyed a number of *Discocactus* populations. *Discocactus* species are very ornamental and the commercial or hobbyist collection of plants and seeds is another factor that endangers the natural populations. As a result, the whole genus is placed in Appendix I of CITES (Lüthy 2001). Moreover, some species are also included in federal (MMA 2008) and regional (Biodiversitas 2006) lists of threatened species within Brazil.

Therefore, this study aims to achieve a taxonomic revision of *Discocactus*, providing descriptions, identification keys, illustrations, besides noting geographic distribution and conservation status. The phylogeny of the genus *Discocactus* made

previously will be used to help circumscribe the species and to better understand the relationships among them. Thus we hope to resolve the divergent classification systems hitherto proposed for the genus. In addition, a clear circumscription of *Discocactus* species will aid in assessing the actual conservation status of each species and its conservation needs.

Materials and Methods

Taxon Sampling

Discocactus species were sampled as listed by Hunt *et al.* (2006), including localities from where some synonyms of currently accepted species of the genus were described. We collected *Discocactus* throughout the distribution area of the genus in Brazil, Paraguay and Bolivia. From each population visited, voucher specimens were collected and deposited at VIC (Herbarium of the Universidade Federal de Viçosa, Brazil). A methodology of collect and herborization were adapted to *Discocactus* from Reyes-Aguero *et al.* (2007).

Taxonomic Treatment

The taxonomic treatment, identification keys and geographical distribution of taxa are based on extensive field research conducted in three countries of South America (Brazil, Paraguay and Bolivia) where *Discocactus* species occur. A detailed examination of the literature and of collections from the following herbaria was realized: BHCB, CGMS, CPAP, EAC, HUEFS, HVASF, K, L, NY, MBM, R, RB, SPF, U, UB, UC, UFMT, UFG, US, VIC and ZSS (Thiers 2013).

The description of the genus was based on traits, followed by a key to all species according to morphological characters and geography. Live plants are also cited when observed during visits to botanic gardens. Besides nomenclatural and diagnostic/descriptive details, phylogeographical and ecological data are provided, likewise distribution records. The additional specimens examined are listed in Appendix 1.

Geographical distribution and conservation assessment

The geographical distribution of species was obtained from the literature, field trips and from an extensive herbarium survey. When the coordinates were not available

in herbaria records, the informations were acquired from the nearest municipality. For preparation of the maps we utilized the software ArcGIS 10.1 (ESRI 2012). The conservation status of the species was revised through the IUCN evaluation criteria (IUCN 2001). When a taxon was not assessed by IUCN we discuss the importance of the species evaluation according to observations in the field, regarding its distribution and habitat conservation.

Results

Taxonomic Treatment

Analysis of morphological traits and geographic distribution suggest that *Discocactus* Pfeiffer can be divided into 11 species and 3 heterotypic subspecies, giving a total of 14 taxa, including one new subspecies.

Discocactus Pfeiff. (1837:241)

Type: — *D. insignis* Pfeiff (= *D. placentifformis* (Lehm.) K. Schum.)

Plants low growing. *Stem* depressed-globose to globose, usually solitary (very small or to c. 10 × 25 cm) or eventually in some species branching by offsets at lateral areoles. *Ribs* 9-26, well-defined or somewhat broad basally and often comprising distinct tubercles. *Areoles* small to large, oval or circular, 1.5-9 (-15) × 1.0-8(-12) mm, sometimes sunken, 3-7 visible per rib above ground level; felted when young, but soon becoming glabrous. *Spines* stiff, woody, sometimes pectinate, white to yellowish, grey, reddish, brownish or black, curved, turned-up or straight, somewhat thickened at the base, terete, circular or flattened in cross-section; radial spines 4-18 per areole, centrals 0-2. *Cephalium* terminal, bearing flowers and fruit, 0.5-9 × 2-11 cm, sometimes depressed, densely woolly, with or without bristly, reddish to black spines. *Flowers* solitary or 2-10 developing rapidly at the same time, borne on the periphery and centre of the cephalium, tubular-funnel-shaped to salverform, white, anthesis nocturnal, sweetly scented (hawkmoth syndrome); scales of the floral tube with apex olive green-brown to brown or yellowish to white; pericapel ovoid, basally naked, with scales above; tube (including nectar-chamber) elongate, bearing leafy bract-scales glabrous in their axils; perianth-segments oblanceolate to lanceolate or spatulate, margin irregularly undulate and dentate; the outermost often greenish, brownish or even deep pink, inner

series white, rarely pale pinkish; flowers removed from cephalium bearing adherent wool and bristles; stamens numerous, inserted along the inside of the tube, and at its apex, filaments short, sometimes the lowermost inserted above a whorl of hairs, which protect the nectar-chamber, with the nectariferous tissue different in colour to the inner region of the floral tube, this differentiated tissue terminating irregularly below the first whorl of stamens; style long or short, stigma-lobes 5-6, slender, whitish. *Fruits* globose-clavate to oblong, naked, slightly fleshy, red, orange-yellow, greenish or white to pink, sometimes only the apex coloured; dehiscent by vertical slits in the pericarpel, pulp scanty, but very attractive to ants, perianth remains persistent. *Seeds* helmet-shaped to globular, 1-2 mm long (hilum to apex) and 0.8-2.2 mm wide; the outer testa dark brown to shining black ornamented with tubercules, the papillae pointed or elongate.

Etymology: from Greek *diskos*, a disc or quoit, and *cactus* alluding to the flattish stem.

Vernacular names: cabeça-de-frade, roseta-do-diabo, coroa-do-diabo, coroa-de-frade, Frade-de-cavalo, pelota-del-tigre.

Key to the species of *Discocactus* Pfeiff.

1. Plant with ribs not tuberculate or with only sinuosities in the space between areoles.
 2. Areoles with spines pectinate tightly adhered to stem, no central spines.
.....**8. *D. horstii*.**
 2. Areoles with woody spines, straight or curved down or up, 0-1 central spines.
 3. Ribs slightly spiraled, with sinuosities and shallow grooves well marked (0.2 mm) or narrowing in thickness in the space between areoles (plants of Serra do Cabral); flower 85×75-85 mm, nectar chamber 11-25 mm length; style 30-45 mm.....**9. *D. placentiformis*.**
 3. Ribs straight, no grooves and without narrowing in thickness in the space between areoles, with sinuosities just below the areoles; flower 75×60 mm, nectar chamber 34mm of length, style 54 mm.....**10. *D. pseudoinsignis*.**
1. Plants with ribs tuberculate

4. Roots napiform.....**1. *D. bahiensis***
4. Roots branched.
5. Ribs tightly spiraled.
6. Ribs tuberculate, nipple like form (conical and elongated), grooves in the space between areoles up to 2 cm depth.....**6. *D. hartmannii***
6. Ribs tuberculate with other forms, grooves between areoles < 2 cm.
7. Tubercles globular or rhomboid.
8. Tubercles globular**11. *D. zehntneri*.**
8. Tubercles rhomboid**5. *D. ferricola*.**
7. Tubercles pentagonal or rounded**2. *D. boliviensis*.**
5. Ribs weakly spiraled
9. Style length 15-22 mm, included inside of nectar chamber; cephalium bristles 40-50×1-2mm, brown-reddish.....**4. *D. diersianus***
9. Style length 36-76 mm, exserted; cephalium bristles 1.5-50×1 mm, grey to black.
10. Tubercles flattened, grooves between areoles 1.5-2 cm depth; apex of the inner perianth segments cuspidate; style exserted the last external whorl of stamens**7. *D. heptacanthus*.**
10. Tubercles not flattened , grooves between areoles 0.4-1.2 cm depth; apex of the inner perianth segments acute; style of the same size of the last external whorl of stamens**3. *D. catingicola*.**

1. *Discocactus bahiensis* Britton & Rose (1922: 220), Figure 2:A-G.

Type: — BRAZIL. Bahia, Juazeiro, 2-6 June 1915, *Rose, J.N.* 19.783 (holotype US!).

Plants low growing, solitary or producing small offsets. *Roots* somewhat napiform. *Stem* 3-7×5.5-18 cm, pale green, flattened-globular, half or more buried in the ground. *Ribs* 10-16, well-defined, regular or spiralled, forming distinct tubercles, with ridges between areoles. *Areoles* 2-5 visible per rib above ground level, oval to elliptic, 3-10×3-8 mm, sometimes sunken; when young with white to cream trichome-like wool, but soon becoming glabrous. *Spines* stiff, woody, cream to grey-brown, curved, terete or flattened in cross-section; radial spines 5-14, 6-45×0.5-3 mm, 2-4 pairs of spines at each side of the areole; central spine 0. *Cephalium* 0.9-2.1×2.2-4.7 cm, sometimes depressed, densely woolly; bristles yellow to black, to 30 mm. *Flowerbud* greenish to brown; flower funnelshaped, 40-70×23-54 mm; pericarpel 4.5×3.5 mm, ovoid, basally naked, with scales naked in their axils above; receptacle-tube 43×10 mm, covered with fleshy scales 1.5-5×1-5 mm, olive green to brown, lanceolate, apex rounded or irregular, margin undulating; transitional perianth-segments in 3-4 whorls, lanceolate, 20×5 mm, fleshy, greenish brown, margin lightly undulated, smooth or with irregular form; inner perianth-segments 8-20×5-7 mm, lanceolate to elliptic, margin irregular, apex slightly cuspidate, white; outer perianth-segments 18×5 mm, lanceolate to elliptic, margin and tip irregular, greenish white; nectar-chamber tubular, 14-16×2-6 mm, open (without hairs at apex), with glands; stamen filaments 3-11 mm, anthers 1-2 mm, yellow; style 32-40 mm, golden yellow, reaching to the half of the receptacle-tube, stigma-lobes 5-8, 4 mm, cream to white, with papillae; ovules in clusters of 1-5, funicle with fine hairs. *Fruits* 12-50×7-10 mm, clavate, naked, white to greenish, to reddish at apex; cupula (fruit apex) 3.5-5.0 mm; perianth remains persistent. *Seeds* helmet-shaped, 1.2-1.8×1.5-1.9 mm, shiny black, testa with pointed tubercles.

Distribution and habitat: — BRAZIL. Endemic to Northeast region and to *Caatinga* Biome, occurs in Bahia, Ceará, Pernambuco and Piauí States (Figure 1). *Discocactus bahiensis* inhabits stony and loamy soil (red to orange). Northern *caatinga* element on exposed, gravelly river terraces amongst limestone or iron-stained quartzite, and seasonally inundated river flood plain, under and between jurema-preta (*Mimosa tenuiflora* (Willd.) Poir.) and carnaúba (*Copernicia prunifera* (Mill.) H.E.Moore) within the caatinga (Taylor & Zappi 2004).

IUCN conservation assessment: — Previously this species was considered Endangered (Taylor & Zappi 2004). It is now regarded as Vulnerable (VU A2c) based on an estimated past population decline of greater than 30% as a result of the ongoing decline in area and quality of the species habitat. The species has a very patchy distribution; and its area of occupancy is less than 500 km². Part of its range was eliminated by permanent inundation from the Represa de Sobradinho (BA/PE) in the 1970s and the remainder has been heavily impacted by agriculture and road/house construction during the past 20 years. Some of the remaining few sites are accessible by road and visited by collectors. Found at 380–650 m, in the Rio São Francisco drainage of northern Bahia (probably in adjacent Pernambuco), Ceará (Rio Salgado) and north-western Piauí (Rio Canindé) (IUCN 2013).

Notes: — In the phylogenetic study, this species was considered sister of *Discocactus zehntneri* (Santos *et al.*, 2013). According to Machado *et al.* (2005) there is evidence of introgression of genes from *D. zehntneri* in a population of *D. bahiensis* (São Rafael-BA), with the occurrence of two alleles exclusive to *D. zehntneri*. In plants of this place, there are differences in areoles and spines (thinner and more numerous), where areoles are more elongated, radial spines organization 3-4 pairs of spines at each side of the areole (Fig. 2 C). *Discocactus piauiensis* P.J. Braun & E. Esteves Pereira before considered synonymous of *D. catingicola* can be placed as *D. bahiensis* synonymous after observation of morphological characters (irregular margin of the perianth segments of flowers that are characteristic of *D. bahiensis* Fig. 2 D) in holotype material deposited in UFG (14761). Flowering occurs most probably during November to February with a peak probably in January, when most plants were observed with flowers. Usually 1-5 flowers arise at a time.

Specimens selected: — BRAZIL. Bahia: Mun. Juazeiro, *Rose & Russell* 19742 (NY, US); l.c., 2–6 June 1915, *Rose & Russell* 19781 (NY, US); l.c. 2–6 June 1915, *Rose & Russell* 19783 (US, NY); l.c., 3 Dec. 2011, *M.V. Meiado et al.*, 751 (HVASF); 12 - 13 km W of Juazeiro, 19 Sep. 1985, *Horst & Uebelmann* 438 (U, ZSS); l.c., between Juremal and Curral Velho, s.d., *Horst & Uebelmann* 633 (ZSS); l.c., south of town, 29 Mar. 2004, *M. C. Machado et al.* 370 – 373 (HUEFS); l.c., ca. 12km from the village of Juremal on road to Abreus, 30 Mar. 2004, *M. C. Machado et al.* 380 – 383 (HUEFS); l.c., near the village of Olhos D'Água, 29 Apr. 2004, *M. Machado* 420 – 423 (HUEFS);

l.c., besides the village of Goiabeira, 29 Apr. 2004, *M. C. Machado* 430 – 433 (HUEFS); l.c., surroundings of the village of Abreus, 30 Apr. 2004, *M.C. Machado* 440 – 443 (HUEFS); Mun. Campo Formoso, W of Abreus, W bank of the Rio Salitre, s.d., *Horst* 437 (U, ZSS); Mun. Morro do Chapéu, s.d., *Braun & Esteves Pereira* s.n. (UFG); 21 Apr. 2004 *M.C. Machado* 204 (HUEFS); l.c., São Rafael, 21 Apr. 2004, *M.C. Machado et al.* 390 – 393 (HUEFS); l.c., 16 Apr. 2011, *Santos, M.R.* 98 (VIC). Ceará: Mun. Lavras de Mangabeira, 25 Jan. 2009, *Menezes, M.O.T. et al.* 195 (EAC). Piauí: Teresina, Mun. Francisco Ayres, 8 Sept. 1988, *Horst & Uebelmann* 943 (K, ZSS); l.c. divisa dos estados da Bahia e Piauí, arredores da cidade de Corrente, June 1993, *Esteves* 114 (UFG).

2. *Discocactus boliviensis* Buining & Brederoo (1977:258), Figure 2: H-M.

Type: — BOLIVIA. E Santa Cruz, San Cyrilo, 280m, *Horst* 457 (U!).

Plants somewhat flattened globular, forming groups by offsetting and seedlings. *Roots* branched and thickened. *Stem* 15×25-29 cm, light to dark green. *Ribs* 12-13, regular or spiralled, forming rounded to pentagonal tubercles. *Areoles* 2-5 visible per rib above ground level, oval, 4-8×3-6 mm, sunken. *Spines* at first yellow, later brown to grey, straight, or turned up or down, rounded in cross-section; radial spines 5-8, 16-35 mm, central spine 0. *Cephalium* 1.5-7.0×3.5-6.0 cm; bristles brownish. *Flowerbud* slender with olive-brown scales; flower funnel-shaped, when open 38-60×43-55 mm; pericarpel 4.5×4.5 mm, globular; receptacle-tube 32×12 mm, covered with fleshy scales 1-1.5×1-3 mm, white inside, olive to light brown outside; transitional perianth-segments in 5-6 whorls, lanceolate, 17×4 mm, fleshy, margin entire, white with an olive-brown and rounded to acute tip; inner perianth-segments lanceolate, apex acute, margin entire, white; outer perianth-segments 16×3 mm, lanceolate, margin smooth, apex rounded to acute, white; nectar-chamber tubular, 11×1.5 mm, open (without hairs at apex); stamen filaments 3-4 mm, anthers 1-5 mm, yellow; style 13 mm, shorter than stamens, not exceeding nectar-chamber, stigma-lobes 5, 1 mm, cream coloured, adpressed; ovary cavity oval-shaped, 2.5×1 mm, ovules in clusters of 3, funicles naked. *Fruits* 28-30×9 mm, clavate, light brown to red; perianth remains persistent. *Seeds* helmet-shaped, 1.7-2.2×1.7-2 mm, shiny black; testa with many nipple-like tubercles.

Distribution and habitat: —BOLIVIA, E Santa Cruz (Figure 3). Endemic to Bolivia, occurs on limestone outcrops between bromeliads, surrounded by deciduous forest, altitude 280m.

IUCN conservation assessment: — This species is listed as Vulnerable because it has a very restricted area of occupancy of 4 to 10 km², and is known from only two locations. Although there are no major threats affecting the species at present, it is predicted that livestock farming may impact the species in the near future which would likely result in it becoming Critically Endangered or even Extinct in a very short time period. The potential mining of limestone is another threat. The species is known from two sites; there are less than 2,000 mature individuals in total. The population trend is stable, but the sites are not protected (IUCN 2013).

Notes: — In the phylogenetic study this species was considered as sister of *D. ferricola* (Santos *et al.* 2013). The morphological difference between them is related to the shape of their tubercles where in *D. boliviensis* are pentagonal to round and in *D. ferricola* has a rhomboid form. Last species always occur always on outcrops “lajedos” constituted mainly by iron and manganese. *D. boliviensis* was found in flower in January, but probably its flowering begins before this, because there were vestiges of old flowers and also fruits observed.

Specimens examined: — BOLIVIA. Santa Cruz: E Santa Cruz, San Cyrilo, s.d., Horst & Uebelmann 457, 458 (U); Mun. Mota Cucito, 18 Jan. 2011, M.R. Santos in Huaylla, H. 3683 (VIC).

3. *Discocactus catingicola* Buining & Brederoo (1974: 265–267), Figure 4: A-G.

Holotype: — BRAZIL. W Bahia, Mun. São Desidério, 1974, Horst 392 (U!).

Plants simple, flattened globular, not forming groups by offsetting. *Roots* branched. *Stem* 3-9×8-20 cm, dark olive green. *Ribs* 9-13, straight or slightly spiralled, not forming flattened tubercles with deep grooves (0.4-1.2 cm) between successive areoles. *Areoles* 3-6 visible per rib above ground level, oval to elongated, 3-9×2-8 mm, sunken. *Spines* yellow to grey, sometimes with darker transverse bands, straight or slightly curved turned-up or down, rounded to flattened in cross-section; radial spines 3-10, 2-

36×1-3 mm, central spine 0-1, 8-20 mm. *Cephalium* 0.5-4.5×2-7.5 cm; bristles 1.5-50 mm with vivid or dark colours. *Flowerbud* olive green; flower tubular, when open 48-70×35-60 mm; pericarpel 5-9×4.5-5 mm, ovoid; receptacle-tube 38-45×6-14 mm, covered with lanceolate to spatulate scales 6.2-30×1.5-8 mm, fleshy, uppermost with apex retuse or irregular, ciliate, without light olive green vein and tip, white within; inner perianth-segments spatulate 16-25×4-8 mm, fleshy, apex acute, margin smooth or irregular, white; outer perianth-segments 16-30×5-8 mm, fleshy, white, spatulate, margin smooth or irregular, apex obtuse-dentate; nectar-chamber tubular, 18-22×2.5-4 mm, closed by a whorl of curled hairs; stamen filaments 5-17 mm, anthers 1-2.5 mm, yellow; style 36-45 mm (reach the last whorl of stamens), stigma-lobes 4-6, 3-5 mm; ovary cavity heart-shaped, 3-7×2.5-3 mm, ovules in bundles of 1-5, funicles naked. *Fruit* 25-45×6-8 mm, clavate, white to greenish at apex; perianth remains persistent. *Seeds* oval to helmet-shaped, 1.1-2.0×1-2 mm, shiny black; testa with nipple-like tubercles.

Distribution and habitat:—BRAZIL. Central-S and W Bahia, E Goiás, N Minas Gerais and S Piauí (Figure 1). Western *cerrado-caatinga* ecotone on exposed sandy soils or rocks (iron, “canga”), usually close to rivers, 450–700 m, western and central-southern Bahia and eastern Goiás. In Minas Gerais State occurring to the west of the Rio São Francisco.

IUCN conservation assessment: — Previously considered as Vulnerable, it is now listed as Least Concern and is one of the most common cacti of this genus. It is widespread and occurs in several protected areas. There are population declines in part of its range as the area is transformed into agricultural land at a very fast rate. In need of regular monitoring, since the *cerrado* habitats are undergoing much destructive change. Low morphological and genetic differentiation was observed among its populations. The species habitat is declining due to large-scale agriculture including soy bean and cotton crops, and *Eucalyptus* plantations. It is a widespread and common plant but with a declining population trend, especially in Minas Gerais, Bahia, and Piauí. The population is relatively stable in the western part of its range, where habitat loss has not been as severe as in the east. Population monitoring is therefore recommended.

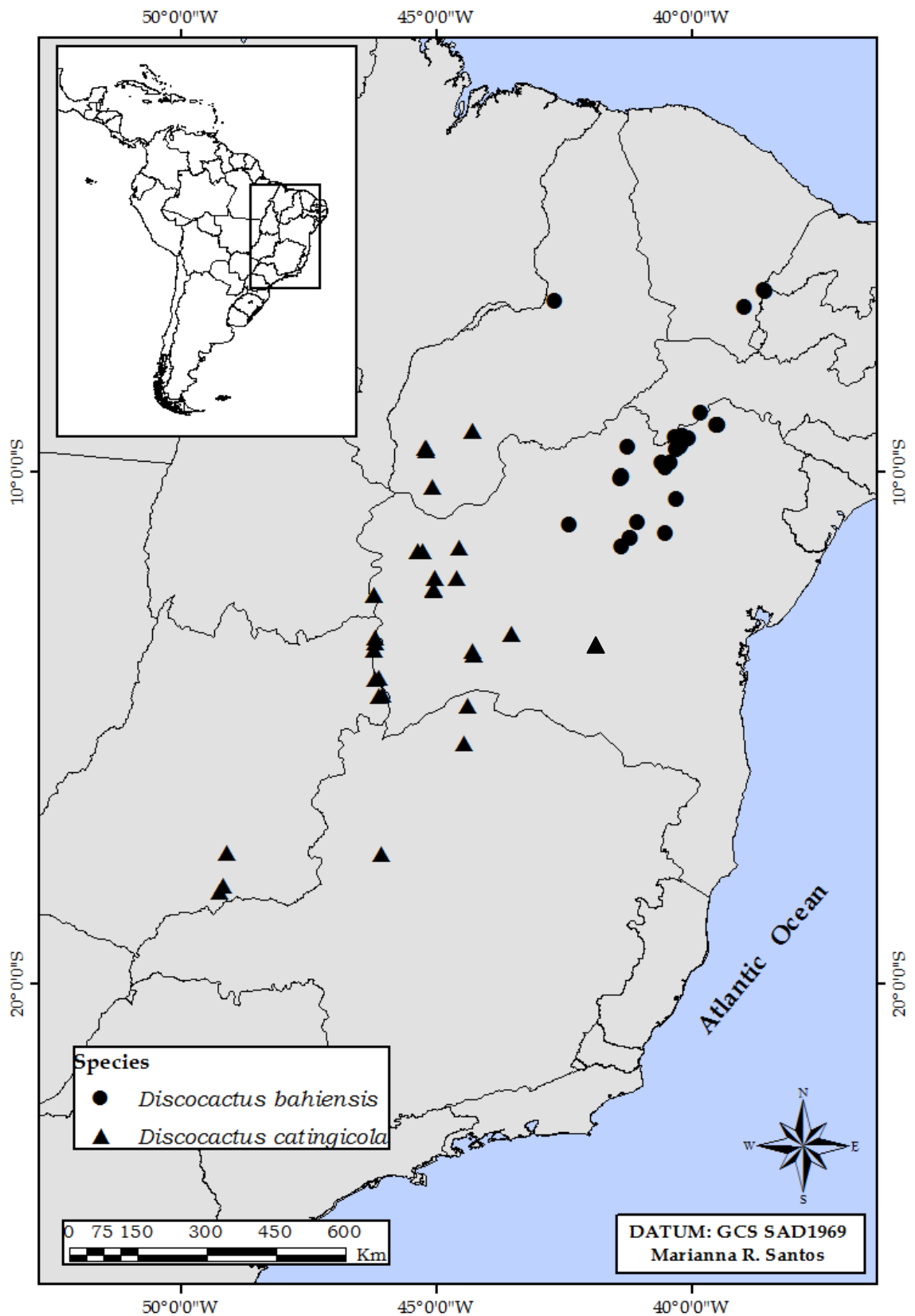


Figure 1. Map showing geographic distribution of *Discocactus* species that are widely distributed in the central-W and NE regions of Brazil.

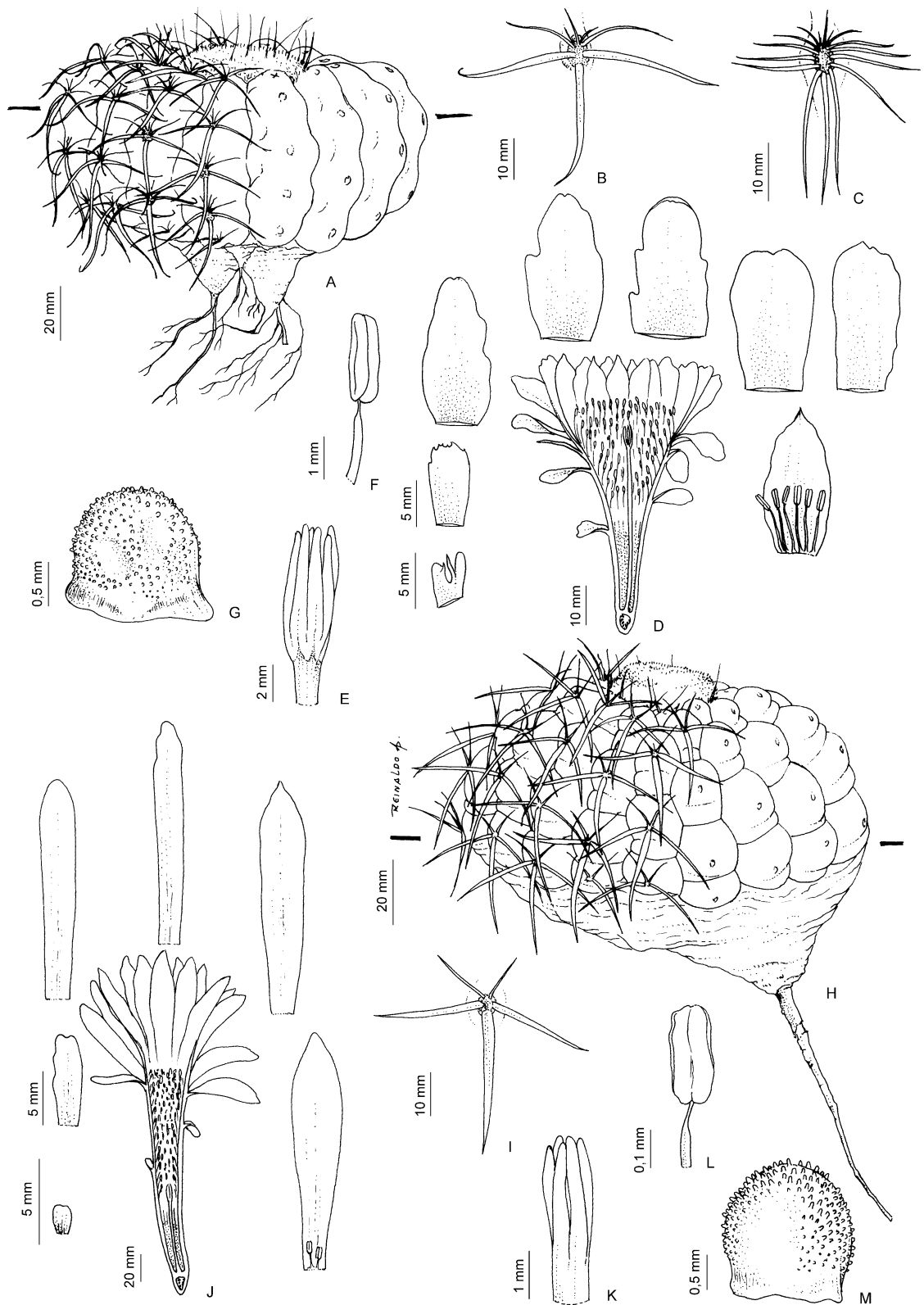


Figure 2. A-G. *Discocactus bahiensis*. A. Habit showing shape of stem and ribs. B-C. Different types of areole. D. Longitudinal section of flower and perianth-segments. E. Stigma-lobes. F. Stamen. G. Seed. H-M. *Discocactus boliviensis*. H. Habit showing shape of stem and ribs. I. Areole. J. Longitudinal section of flower and perianth-segments. K. Stigma-lobes. L. Stamen. M. Seed.

Notes: — In our phylogenetic study this species grouped with other species (*D. heptacanthus* and *D. diersianus*) with bootstrap support of 94% (Santos *et al.* 2013). *Discocactus catingicola* is largely confused with *D. heptacanthus* once they are morphologically similar and both possess a broad distribution. But some characters can help to distinguish them like tubercles shape, where in *D. heptacanthus* they are nipple-like, flattened with grooves (1.5-2 cm of depth) between areoles while it not occurs in *D. catingicola* that not possess tubercles flattened and the grooves between areoles are less deep (0.4-1.2 cm). Some synonyms before not related to *D. catingicola* was recognized by Hunt *et al.* (2006) (*D. prominentigibbus* Diers & Esteves, *D. silicicola* Buining & Brederoo and *D. silvaticus* Buining & Brederoo). However *D. silicicola* is more related to *D. hartmannii* due to characters like ribs shapes that possess tubercles well developed, nipple-like, conical and vertically positioned (Fig. 5G).

The flowering of *D. catingicola* was observed in cultivated specimens and the first flowers appear in the second half of September after the first rains. In Central-S Bahia (Paramirim) it was observed by N.P. Taylor flowering in November. Its flowers are durable, opening fully around 18h with a slight fragrance and remaining open until the next day with limited fragrance. In the morning of the second day, their pinkish inner perianth-segments form a tube and stay partially open until 12h p.m. when they completely close. With this behaviour of maintaining the flowers open until the next day could mean that this species is adapting to another pollinator such as hummingbirds, but this needs careful study to determine its biology and ecology.

Specimens selected: — BRAZIL. Bahia: W Bahia, Mun. Barreiras, 1974, *Horst & Uebelmann* 205A (U, K, ZSS); 11-12 Sept. 1988, *Horst & Uebelmann* 949 (K, ZSS); l.c., Redondo waterfall, 14 Jan. 2004, *M.C. Machado et al.* 310 – 313 (HUEFS); Mun. São Desidério, *Horst & Uebelmann* 392 (U); Mun. Santana, *Horst* 448 (U, ZSS); 17 Jan. 2004, *M. Machado et al.* 340 – 344 (HUEFS); Mun. Santa Maria da Vitória, 4 Oct. 1985 *Horst & Uebelmann* 760 (K, ZSS); Mun. Coribe, *Horst & Uebelmann* 760A (ZSS); 16 Jan. 2004 *M.C. Machado* 146 (HUEFS); l.c. cent.-S Bahia, Mun. Paramirim, 28 Nov. 1988, *Taylor in Harley* 25558 (K). Goiás: E Goiás, Mun. Posse, 15 Jan. 2004, *M. Machado et al.* 320 (HUEFS); near Rio Araguaia, 1975, *Esteves* 50 (UFG); Mun. Gurupi, 1980, *Esteves* 151 (UFG). Mun. Porto Novo, 1975, *Horst & Uebelmann* 448 (U) (ZSS) Ex. Cult. (ZSS). Minas Gerais: N Minas Gerais, Mun. Cônego Marinho, 19

Mar. 2011, *Santos, M.R.* 84 (VIC); l.c., 19 Mar. 2011, *Santos, M.R.* 85 (VIC); Mun. Januária, 19 Mar. 2011, *Santos, M.R.* 87 (VIC); l.c.; 19 Mar. 2011, *Santos, M.R.* 88 (VIC); Mun. João Pinheiro, 13 May, 2011, *G. Olsthoorn* 264 (VIC); Mun. Rio Paranaíba, 16 Mar. 2013, *Fonseca, R.S. & Arruda, D.M.* 1081 (VIC); l.c. Serra dos Aegres, *Horst & Uebelmann* 343 (U, ZSS).

4. *Discocactus diersianus* Esteves Pereira (1979:179-186), Figure 4: H-M.

Type: — BRAZIL: Goiás: ‘SE region of the state of Goiás’, 650 m, s.d., *Esteves Pereira* 87 (KOELN). Paratype: S of Goiânia. May 2005, *Esteves* 10, *P.J. Braun* 304 (ZSS!)

Plants simple, single or offsetting when damaged by fire, disc-shaped to flattened globose. *Roots* branched. *Stem* 10-13×25-26 cm, olive green to dark green. *Ribs* 13-18 arranged vertically or slightly spiralled; with a V-shaped depression or furrow just below each areole making the ribs appear to be more or less dissolved in rows of rounded tubercles and forming a sinuous groove between adjacent ribs. *Areoles* 4-6 visible per rib above ground level, more or less oval, 1-1.2×0.7 mm, sunken. *Spines* grey to brownish or blackish grey, tips dark brown to black, curved strongly or hooked, round or quadrangular in section; radial spines 4-10, 60-80×4-5 mm, central spine 0-1, 40×2-3 mm. *Cephalium* 9×10 cm; bristles to 40-50×1-2 mm, brownish to reddish. *Flower bud* white to greenish; flower tubular to slender funnel-shaped, when open 40-60×30-60 mm; pericarpel 4-7×3-4 mm, ovoid, with very few rudimentary pointed scales, slightly constricted between pericarpel and receptacle-tube, the latter 50×4-5 mm, flared somewhat in its upper part, white to greenish; scales 1.5-7.0×1-2 mm, lanceolate to spatulate, light greenish to white and sometimes with a fine red to brownish tip, margin smooth, at its reddish apex, sometimes slightly dentate or crenate; inner perianth-segments lanceolate 25×3 mm, with acute to acuminate tip, very thin, margin entire but often somewhat undulate, white; outer perianth-segments 30×4 mm, lanceolate with retuse to acute apex, margin entire but very often undulate, apex red; nectar-chamber tubular, 20×1.5-2.5 mm, closed by a whorl of curled hairs; stamen filaments 0.5-3 mm, anthers 1.4-1.8 mm, cream; style 15-22 mm, short, reaching only to beneath the stamens, i.e. inside nectar-chamber, stigma-lobes 4-6, 4-5 mm, long, white, finely papillose; ovary cavity oval to heart-shaped, 3-4×1.5-2.5, ovules in

bundles of 2-3, funiculi often branched at base, with fine hairs. *Fruit* 45×7-8 mm, slender clavate, white to cream-coloured, greenish to brownish near apex, with persistent perianth remains; when ripe dehiscing lengthwise; larger fruits containing 70-80 seeds. *Seeds* oval to helmet-shaped, 1.2-1.7×1.4-1.55 mm, testa shiny black with more or less elongated tubercles, these becoming shorter and nearly flat towards the hilum margin.

Distribution and habitat: — BRAZIL. SE, N Goiás and S Tocantis (Figure 3). *Discocactus diersianus* occurs at an altitude of about 650 m, surrounded by dry forests. The plants grow in small groups together with Bromeliaceae on exfoliating rocks of granite outcrops interspersed with humus. They are exposed to full sunlight.

IUCN conservation assessment: — This species was not assessed until 2010 for the IUCN Red List. It is listed as Endangered because it occurs in fragmented patches of crystalline rock in Goiás and Tocantins, Brazil. It is found at five to ten locations, which add up to an area of occupancy of less than 500 km². There has been a population decline of more than 50 percent in the last 60 years (generation length is 20 years), and it is estimated that no more than 5,000 mature individuals remain in total, with the largest subpopulation consisting of less than 1,000 individuals. The main threats to the species are quarrying, cattle ranching, and the removal of plants by farmers to avoid cattle getting injured by the cactus spines. These threats are understood, but have not ceased and are, at least in part, not reversible. Collection for ornamental trade is another threat that has wiped out complete subpopulations (IUCN 2013).

Notes: — With morphology studies we saw that the synonymous *D. estevesii* before belonging to *D. catingicola* according with Hunt *et al.* (2006), probably can be placed as *D. diersianus* synonymous due to traits related to stem and flower morphology, where the plants of *D. estevesii* do not have deep grooves between areoles and have flower size and the reddish apex in outer perianth-segments like *D. diersianus*. But the results about phylogeny not show good resolution, then we need more researches to understand the phylogenetic relation between *D. diersianus* and *D. estevesii*. Its flowering begins in September and was observed in cultivated specimens. *Discocactus diersianus* flowers open totally at 19h p.m. and close around 5-6h a.m. of the next day.

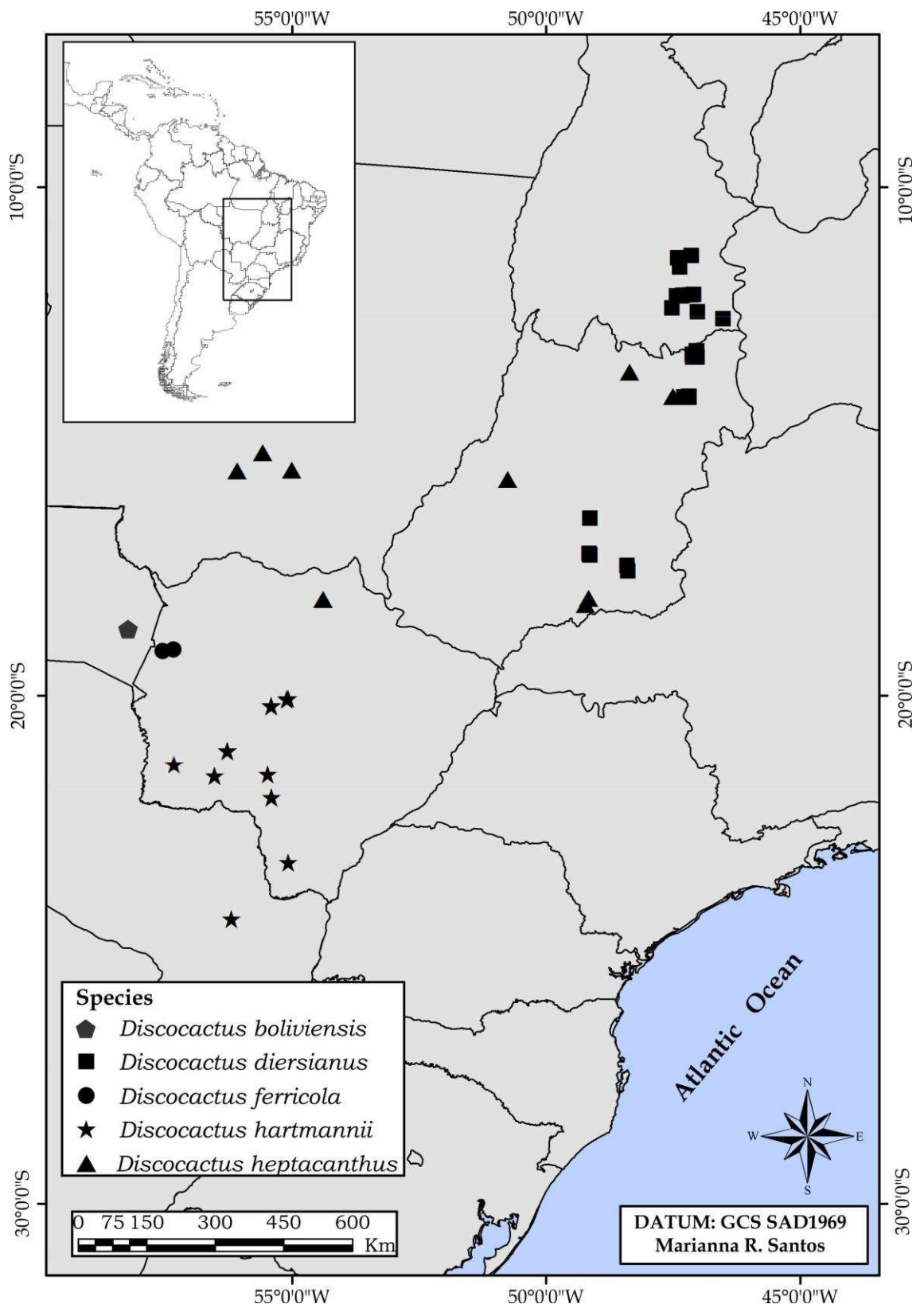


Figure 3. Map showing geographic distribution of *Discocactus* species that occur in the NE and W regions of Brazil and Eastern parts of of Paraguay and Bolivia.

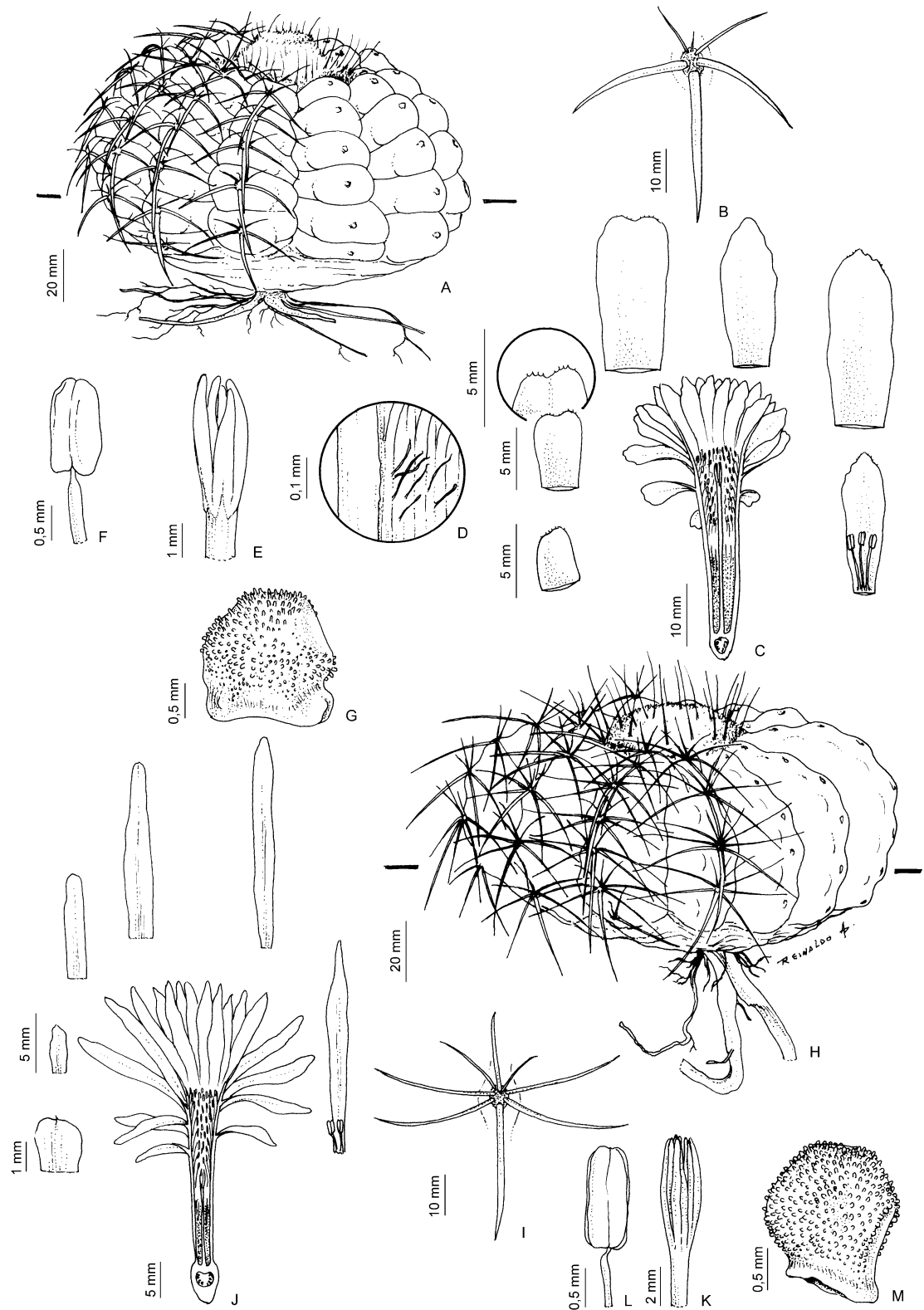


Figure 4. A-G. *Discocactus catingicola*. A. Habit showing stem and rib shape. B. Areole. C. Longitudinal section of flower and perianth-segments. D. Detail of curled hairs at top of nectar-chamber. E. Stigma. F. Stamen. G. Seed. H-M. *Discocactus diersianus*. H. Habit showing stem and rib shape. I. Areole. J. Longitudinal section of flower and perianth-segments. L. Areole. K. Stigma. M. Seed.

Specimens examined: —BRAZIL. Goiás: Mun. Maripotaba/Cromínia, 1975, *Esteves* 10 (UFG); S of Goiânia, 17 Oct. 1987, *P. J. Braun* 304 (K, ZSS); between Mun. Goiatuba and Mun. Cachoeira Alta, 1973, *Esteves* 2 (UFG); Mun. Teresina de Goiás, 15 Aug. 2010, *M.R. Santos* 23 (VIC); l.c. Rio Paranã, 1973, 1985, *Horst & Uebelmann* 431 (U, ZSS) Ex cult. (ZSS); Mun. de Itumbiara, 2012, *G. Olsthoorn* 330 (VIC); Mun. Marzagão, road GO 139, 2012, *G. Olsthoorn* 327 (VIC); Mun. Natividade, 1985, *Horst & Uebelmann* 645 (K, ZSS) Ex cult. (ZSS). Tocantins: c. 50 km N of Arraias, 1986. *P. J. Braun* 716 (ZSS); Campo Cerrado near Natividade, 1984 *P. J. Braun* 547 (ZSS); Mun. Paranã, 11 Nov. 1991, *Hatschbach* 56072 (MBM); between Mun. Paranã and Teresina de Goiás-GO, 21 Apr. 1994, *Esteves* 402 (UFG); Mun. Conceição, 1990, *Esteves* 148 (UFG).

5. *Discocactus ferricola* Buining & Brederoo (1975: 2-5), Figure 5: A-F.

Type: — BRAZIL, Mato Grosso do Sul, Corumbá, 1975, *Horst & Uebelmann* 195 (U!).

Plants simple, flattened globular to globular, forming groups by offsetting and with seedlings. *Roots* branched. *Stem* 8-9×7-25 cm, pale to dark green. *Ribs* 11-15, running spirally, tubercles with globular to rhomboid form. *Areoles* 3-6 visible per rib, oval, 15×7 mm, sunken. *Spines* at first yellowish, when older brown-reddish to grey, arranged radially, more or less recurved; radial spines 5-9, 15-50×1 mm, central spine 0-1, 30-35 mm. *Cephalium* 1-7×1.9-6.5 cm; bristles to 50 mm, dark coloured. *Flower-bud* greenish brown; flower tubular, when open 55×35 mm; pericarpel 5×3.5mm, ovoid; receptacle-tube 27×12 mm, bearing few small scales 4×2 mm, lanceolate, white within, outside and apex greenish brown; transitional perianth-segments in several whorls, lanceolate to spatulate, 20×4 mm, fleshy, margin crenate, white, olive-brown along the midvein; inner perianth-segments 18×6 mm, spatulate, acute to cuspidate at apex, white; outer perianth-segments 23×5 mm, lanceolate, acute to cuspidate; nectar-chamber tubular, 14×2 mm, open (without hairs at apex); stamen filaments 3-6 mm, anthers 1 mm, yellow; style 17 mm, extending to between nectar-chamber and first whorl of stamens, stigma lobes 4, 1 mm, white to cream coloured, with papillae; ovary cavity heart-shaped, 2.5×1.5 mm, ovules in bundles of 2-3, funicles naked. *Fruit* 30-40×5-9 mm, clavate, white to light brown; perianth remains persistent. *Seeds* globular, 1.5×1.5 mm, shiny black; testa with short nipple-like tubercles.

Distribution and habitat: — BOLIVIA. (E Santa Cruz, border with Brazil). BRAZIL. NW Mato Grosso do Sul (Figure 3). Plants endemic to Pantanal biome, growing in clusters of seedlings and with offsets, between bromeliads, mosses and very small shrubs on flattened acid rocks containing iron and manganese called *canga*.

IUCN conservation assessment: — This species was not assessed until 2010 for the IUCN Red List. It is listed as Endangered (EN (B2ab(iii, v))), because it occurs only in the region of Corumbá and along the border with Bolivia and has an area of occupancy of no more than 20 km². The species habitat is degrading due to mining activities and urbanization, which has led to a population decline of 30% over the last 30 years. The threats have not ceased, and thus continue causing decline in mature individuals and suitable habitat. Generation length is 10 years. The main threat to the species is mining, especially in Bolivia, and human settlements. The flat rocky areas are cleaned of any remaining vegetation during the urbanization process.

Notes: — In the phylogenetic study *D. ferricola* was considered as sister of *D. boliviensis* with 100% bootstrap support (Santos *et al.*, 2013). Morphological differences between these species were stated in Notes of *D. boliviensis* description. *Discocactus ferricola* forms a carpet of adult plants and their plantlets (mainly derived from vegetative reproduction that occurs with the emergence of new plants from areolas) on the iron rocks. Often accumulate around the mother plant and is possible to observe the plants piled up one over another. *Discocactus ferricola* was found in flower in January, but probably its flowering begins before this because there were vestiges of old flowers and also fruits.

Specimens examined: — BRAZIL. Mato Grosso do Sul: Corumbá, 17 Jan. 2011, Santos, M.R. 43 (VIC); l.c., 17 Jan. 2011, Santos, M.R. 44 (VIC); l.c. Parque do Pantanal, 12 Nov. 2006, J.S. Garcia 55 (CGMS); l.c. surrounding Corumbá, 1974, Horst & Uebelmann 195 (U, ZSS); l.c. Corumbá, 16 Nov. 1989, V.J. Pott 1157 (MBM). BOLIVIA. Santa Cruz: E Santa Cruz, 18 Jan. 2011, Santos, M.R. 52 (VIC); l.c., 18 Jan. 2011, Santos, M.R. 53 (VIC).

6. *Discocactus hartmannii* (K. Schum.) Britton & Rose (1922: 217), Figure 5: G-M.

Type: — PARAGUAY, N of Paraguay, Capivary, San Pedro, *Grosse* (B, destroyed).

Lectotype: — designated by Taylor & Zappi in *Bradleya* 9 (1991:86).

D. silicicola Buining & Brederoo *syn. nov.* Holotype: — Mato Grosso do Sul, Porto Murtinho, 1975, *Horst* 325 (U!).

Plants simple, disc-shaped to globular. *Roots* branched. *Stem* 4-7×7-19 cm, dark green. *Ribs* 14-22, with deep grooves between ribs (>2 cm), without ridges, and comprising conical (acute) and elongated tubercles, tightly spiralled. *Areoles* oval to elongated, 3-7 visible per rib, 2-6×1.75-4 mm, sunken. *Spines* at first yellow to horn-coloured, later grey to brown, arranged radially, rounded in cross-section, more or less recurved, apical spines decurved; radial spines 3-12, 2-20 mm, central spine 0-1, 1-8 mm. *Cephalium* 1.3-3.5×2.7-6.0 cm; bristles 2-40 mm, vivid to dark coloured. *Flower bud* light brown to olive green; flower tubular, when open 70-76×42-55 mm; pericarpel 4.5-8×4-5 mm, ovoid; receptacle-tube 45-58×8-9 mm, bearing lanceolate scales, 1.6-28×0.5-6.5 mm, white to light-olive green within, light-brown without, tip emarginate to cleft, sometimes with a fingernail-like tip; inner perianth-segments 23-26×3-6.5, white, spatulate, acute to cuspidate, sometimes with fingernail-like tip, margin undulate or dentate; outer perianth-segments 22-28×4-7 mm, lanceolate, acute, margin undulate or dentate; nectar-chamber tubular, 20-34×3-5 mm, closed by hairs at apex; stamen filaments 4-11 mm, anthers 1-2 mm, yellow; style 37-51 mm, extending to the midpoint or upper part of the floral tube, stigma-lobes 5-8, 4.5-7 mm, with papillae; ovary cavity oval, 3-6×2-3 mm, ovules in bundles of 2-4, funicles naked or with hairs. *Fruit* 38-44×9-11 mm, clavate, white with pinkish apex; perianth remains persistent. *Seeds* 1.6-2.2×1.2-1.8 mm helmet-shaped, shiny black; testa with many elongate tubercles.

Distribution habitat: — BRAZIL. SW Mato Grosso do Sul. PARAGUAY. SE Paraguay (Figure 3). *Discocactus hartmannii* is endemic to Chaco Vegetation, occurring on stony hills composed of loam, sand, gravel or rocky soil containing much mica, between low shrubs, 280-700m altitude. In Paraguay occurring on sandy soil, with palms, near places similar to the Brazilian Veredas.

IUCN conservation assessment: — This species is listed as Critically Endangered (A2ac), because in the past *Discocactus hartmannii* was widespread and common, but now it only occurs in fragmented patches due to severe habitat loss. As a result of deforestation for large-scale agriculture, the populations have declined by an estimated 80% in the last 30 years (generation length is 10 years) and this decline is continuing. The main threat to this species is agriculture. In the past, small-scale agriculture was destroying the habitat, which has now been replaced with extensive agro-industry farming in the last ten to 20 years, mainly consisting of *Eucalyptus* plantations, crops for renewable energy and cattle ranching (IUCN 2013). In particular the former habitat in Mato Grosso do Sul and the border area with Paraguay is completely deforested. In Paraguay, the main threat to the species is livestock ranching.

Notes: — According to Hunt *et al.* (2006), *D. silicicola* is a synonymous belonging to *D. heptacanthus*, but in morphological studies (due mainly to ribs with tubercles shape conical and elongated) *D. silicicola* was resolved as related to *D. hartmannii*. Hunt *et al.* (2006) recognized some synonyms before not related to *D. hartmannii* (*D. patulifolius*, *D. mamillosus*, *D. pachytele*, *D. magnimammus*, *D. magnimammus* subsp. *bonitoensis*, *D. hartmannii* subsp. *setosiflorus*, *D. hartmannii* subsp. *giganteus*) and we confirm these synonymizations because the specimens analyzed shared morphological characters determinants to identify *D. hartmannii* that is the form of tubercles cited above and the ribs tightly spiralled. The specie was found in flower in January, but probably its flowering begins before this, because there were vestiges of old flowers and also fruits.

Specimens examined: — BRAZIL. Mato Grosso do Sul: Mun. Bonito, 20 Jan. 2011, *M.R. Santos* 55 (VIC); lc. near Bonito, 1983, *P. J. Braun* 216 (ZSS); 15 km W Bonito, 22 June 1975, *Horst & Uebelmann* 193 (U, ZSS) Ex cult. (ZSS); Mun. Caracol, 22 Jan. 2011, *M.R. Santos* 63 (VIC), *P. J. Braun* 220 (ZSS); l.c. 21 km E of Caracol, Bela Vista, 1971, 29 May 1972, *Horst & Uebelmann* 324 (U, ZSS); Mun. Antônio João towards to Capão Bonito, 23 Jan. 2011, *M.R. Santos* 65 (VIC); W and N of Rio Apa, 28 May 1973, *Horst & Uebelmann* 191 (U, ZSS); Road leaving Coronel Sapucaia, 25 Jan. 2011, *M.R. Santos* 66 (VIC); lc. near Iguatemi, 1983, *P. J. Braun* 208 (ZSS); Serra Maracaju, 1983, *P. J. Braun* 214 (ZSS); along Rio Iguatemi and along Rio Ibaré, 26 May 1972, *Horst & Uebelmann* 190 (U, ZSS); Mun. Aquidauana, Aldeia Córrego Seco, 16 Jan. 2011, *M.R.Santos* 72, 73 (VIC); road living Aquidauana towards Cipolândia, 16

Jan. 2011, *M.R. Santos* 40 (VIC); l.c. NE of Aquidauana, *Horst & Uebelmann* 198 (U); Mun. Porto Murtinho, 21 Jan. 2011, *M.R. Santos* 60 (VIC); near Porto Murtinho, 30 May 1972, *Horst & Uebelmann* 325 (U, ZSS) Ex. Cult. (ZSS). PARAGUAY. San Pedro: Mun. San Estanislao, 26 Jan. 2011, *M.R. Santos* 68 (VIC); l.c. near Yhu, 1983, *P. J. Braun* 204 (ZSS); Amambay, Bela Vista, *Horst & Uebelmann* HU 556 (ZSS).

7. *Discocactus heptacanthus* (Barb.Rodr.) Britton & Rose (1922: 218), Figure 5: N-Q.
Type: —BRAZIL, Mato Grosso, near Cuiabá, *Barb.Rodr.* (Britton & Rose, l.c.).
Lectotype: — (designated in Taylor & Zappi, 2004): Rodrigues, Pl. Matto Grosso (1898: tab. 11).

Discocactus lindanus L. Diers & Esteves Pereira *syn. nov.* Isotype: — Brazil: Goiás: E to NE region of the state of Goiás, 1973, *Esteves Pereira* 9 (UFG!).

Plants simple, flattened globular, to almost globular. *Roots* branched. *Stem* 3-9×8-16 cm, pale to olive green. *Ribs* 9-16, straight or slightly spiralled, forming nipple-like tubercles flattened with grooves between areoles (0.4-1.2 cm depth). *Areoles* 2-6 visible per rib above ground level, oval, 3-8×2-7 mm, sunken. *Spines* yellow, pale yellow to grey to brown, straight or slightly curved, turned-up or down, rounded in section; radial spines 3-9, 3-35×1-2 mm, central spine 0-1, 15-24×2 mm. *Cephalium* 1-2.7×2.5-6 cm; bristles 20-50 mm vivid or dark coloured. *Flower bud* olive green; flower tubular to funnel-shaped, when open 52-110×45-70 mm; pericarpel 4-10×4-7 mm, ovoid; receptacle-tube 44-70×12-20 mm, bearing lanceolate to fimbriate scales 3-30×1.5-10 mm, brown-green along the midvein and at apex, otherwise white; inner perianth-segments spatulate 22-27×6-12 mm, cuspidate, margin dentate, white; outer perianth-segments 23-30×6-11 mm, white, spatulate, margin smooth, acute or obtuse; nectar-chamber tubular, 11-28×2.5-5 mm, closed by curled hairs; stamen filaments 2.5-20 mm, anthers 1-2 mm, yellow; style 44-76 mm, more or less exceeding the upper whorl of stamens, stigma-lobes 4-7, 4-12 mm; ovary cavity heart-shaped, 2.5-5×2.5-3 mm, ovules in bundles of 4-5. *Fruit* 25-42×6-12 mm, clavate, white with greenish-pink apex; perianth remains persistent. *Seeds* elongate to helmet-shaped, 1.1-2.0×1.1-1.8 mm, shiny black; testa with dense nipple-like tubercles.

Distribution and habitat: — BRAZIL. N and W of Goiás, S Mato Grosso and N Mato Grosso do Sul (Figure 3). The specie occurs in western *cerrado* vegetation, on exposed gravel or sand, *cerrado* and *cerrado-caatinga* ecotone.

IUCN conservation assessment: — This species is listed as Near Threatened according to criteria A2ac+A4ac. *Discocactus heptacanthus* is widespread in part of Brazil and adjacent Bolivia, but only occurs in fragmented patches. It has suffered from a population decline in the range of 20 to 30 % over the last 20 years due to large-scale deforestation for agriculture as well as mining and urbanization. The decline is presumed to continue at an even higher rate, as the region is under ongoing pressure from agro-industrial and urban development. The main threat is deforestation of the species habitat for cattle ranching and renewable energy crops. Mining is another important threat in areas where the species grows on iron-rich rock known as “canga”. Urbanization is threatening the population in the surroundings of Cuiabá. In the Pocone region, pollution from mining activities is also affecting the plants (IUCN 2013).

Notes: — At the locality where the synonymous *D. squamibaccatus* Buining & Brederoo was described, little red scales are found to be persistent on the reddish fruit. According to Hunt *et al.* (2006) *D. lindanus* L. Diers & Esteves is a synonymous of *D. diersianus*, but in our phylogenetic study this taxon grouped with *D. heptacanthus* (Santos *et al.*, 2013 Chapter 1) and morphological characters also can comprove this proximity like the flattened tubercles and the pistil exceeding the corolla aperture. Others synonyms are cited by Hunt *et al.* (2006) related to *D. heptacanthus* as *D. flavispinus* Buining & Brederoo, *D. melanochlorus* Buining & Brederoo, *D. semicampaniflorus* Buining & Brederoo and *D. subterraneo-proliferans* L. Diers & Esteves that also share morphological traits as mentioned above.

Specimens selected: — BRAZIL. Goiás: Mun. Posse, 15 Jan. 2004, *M.C. Machado* 131b (HUEFS); 1973, *Horst & Uebelmann* HU 200 (U, ZSS); Mun. Goiânia, 1 dec. 1988, *Horst* 585 (K); NE Goiás, 17 Sep. 1987 *Horst & Uebelmann* 526 (K); *sin loco*, s.d., *Horst & Uebelmann* 632 (K); 3km S of Rio Paranã, s.d., *Horst & Uebelmann* 431 (K); *sin loco*, 1 Jan. 1989, *Horst & Uebelmann* 588 (K); *sin loco* *Horst & Uebelmann* 649 (K); *sin loco*, 1985 *Horst & Uebelmann* 720 (K); 1985, *Horst & Uebelmann* 631 (K); *sin loco*, May 1865, *Burchell* 7215 (K); *sin loco*, 1985, *Horst & Uebelmann* 648

(K); Mun. Alto Paraíso, 15 Aug. 2010, *M. R. Santos* 25 (VIC); lc. 10 Km from Alto Paraíso, 15 Aug. 2010, *M.R.Santos* 24 (VIC); *sin loco*, 1972, *Horst & Uebelmann* HU 392 (U). Mato Grosso: Mun. Nova Xavantina, 24. Sep. 1967, *Ramos et al.* 6503 (K); l.c. near Nova Xavantina, May 1949, *Sick, H.* B436 (RB); Mun. Chapada dos Guimarães, 17 Sep. 1979, *P.I. Oliveira* 84 (MBM, NY); l.c., 5 Sept. 1993, *Guarim Neto, G.* (UFMT); Mun. Cuiabá, 1972, *Horst & Uebelmann* HU 326 (U, ZSS); near Cuiabá, Chapada dos Guimarães, 1974, *Horst & Uebelmann* 453 (U, ZSS); Mun. Jaciara, *Horst & Uebelmann* 326a (ZSS); lc. Córrego do Surucucu, 10 Oct. 1968, *Fonsêca, S.G.* 1365 (UB). Mato Grosso do Sul: Mun. Corguinho, 10 Feb. 2007, *J.S.Garcia* 60 (CGMS); Mun. Coxim, 6 June 1972, *Horst & Uebelmann* HU 198a (U) (ZSS); l.c. 40 km Coxim, 29 Jan. 2011, *M.R. Santos* 70 (VIC); l.c., 29 Jan. 2011, *M.R. Santos* 71 (VIC); l.c. hills along Rio Juaru, *Horst & Uebelmann* 455 (ZSS).

8. *Discocactus horstii* Buining & Brederoo 1973, Figure 7: A-F.

Holotype: — BRAZIL. Minas Gerais, Mun. Grão Mogol, *Horst* 360 (U!).

Plants simple, depressed globular. *Roots* branched. *Stem* 2×6 cm, purplish brown to green. *Ribs* 15-22, straight, not tuberculate. *Areoles* oval to elongate, 4-6 visible per rib, 1-1.5×0.25-1 mm, not sunken. *Spines* brown with grey coating, pectinate, adpressed to stem; radial spines 9-11, 3-3.5×0.5-0.75 mm, central spine 0. *Cephalium* 1-1.5×1.8-2 cm; bristles 20 mm, vivid to dark coloured. *Flower-bud* tinged yellow-brown; flower tubular, when open 60-75×60 mm; pericarpel 5×4.5 mm, ovoid; receptacle-tube 36×4-7 mm, bearing oblong, scattered, raised scales, 15-24×1 mm, yellow-brown without, white within; inner perianth-segments 20-24×5.5-9 mm, white, lanceolate to oblong, cuspidate and slightly crenate at apex; outer perianth-segments 30-35×3.5-8 mm, lanceolate to oblong, acute and crenate at tip; nectar-chamber tubular, 17×2-2.5 mm, open; stamen filaments 3-4 mm, anthers 0.7 mm, yellow; style 37×0.5 mm, extending to midpoint of the floral tube, stigma-lobes 6, 3 mm, with papillae; ovary cavity ovoid to rounded, 3×2 mm, ovules in bundles of 2-4, funicles naked or with hairs. *Fruit* 30×4 mm, tubular clavate, white; perianth remains persistent. *Seeds*, 1-1.1×0.9-1 mm helmet-shaped, shiny black; testa with nipple-like tubercles arranged in rows.

Distribution and habitat: — BRAZIL. N Minas Gerais (Figure 6). Endemic to South-eastern *campo rupestre* (Grão Mogol) element, in quartz gravel and sand beneath shrubs in *campo rupestre*, c. 1000 m, Serra do Barão, northern Minas Gerais.

IUCN conservation assessment: — Previously *D. horstii* was considered Endangered, but now is listed as Vulnerable (VU D2). This species has a very restricted range; its extent of occurrence is less than 100km² and its area of occupancy is around 6km², and it is known from only three locations. It declined substantially in the past due to collection for the international horticultural trade as well as quartz extraction, but populations are stable at present, as the entire population is under strict protection and included in the Parque Estadual de Grão Mogol. However, the threat of mining could potentially return, which would bring the species to the brink of extinction in a very short time. Its survival depends on the continuing protection of its sites. This remarkable Brazilian cactus was heavily collected in the 1970's for the European horticultural market (Buining 1974: 70). In the past mining (or digging) for quartz crystals destroyed large parts of the habitat (IUCN 2013).

Notes: — This species was observed flowering mainly in December and January. In our phylogenetic study this species is considered as sister of *D. pseudoinsignis* with strong support (Santos *et al.*, 2013). These species are sympatric, but there are no studies that prove gene flow between them. The pectinate spines of *D. horstii* seem to have another function besides protection as cited Rizzini (1987) because they are porous and able to absorb water like a sponge. Its outer casing of thorns has walls with parietal organization just described for this species.

Specimens examined: — BRAZIL. Minas Gerais: Mun. Grão Mogol, 1971, *Horst & Uebelmann* 360 (U); l.c., 1987, P.J. *Braun* 851 (ZSS); l.c., Jan. 2006, *Machado, M.C.* 765 (HUEFS); l.c., 16 Mar. 2011, *M. R. Santos* 75 (VIC).

9. *Discocactus placentiformis* (Lehm.) K. Schum (1894: 190), Figure 8.

Type: — ‘Brasilia meridionali’, apparently not preserved. Neotype: — (designated by Taylor & Zappi, 2004): Lehmann in Nov. Act. Nat. Cur. 16(1) (1832: tab. 16).

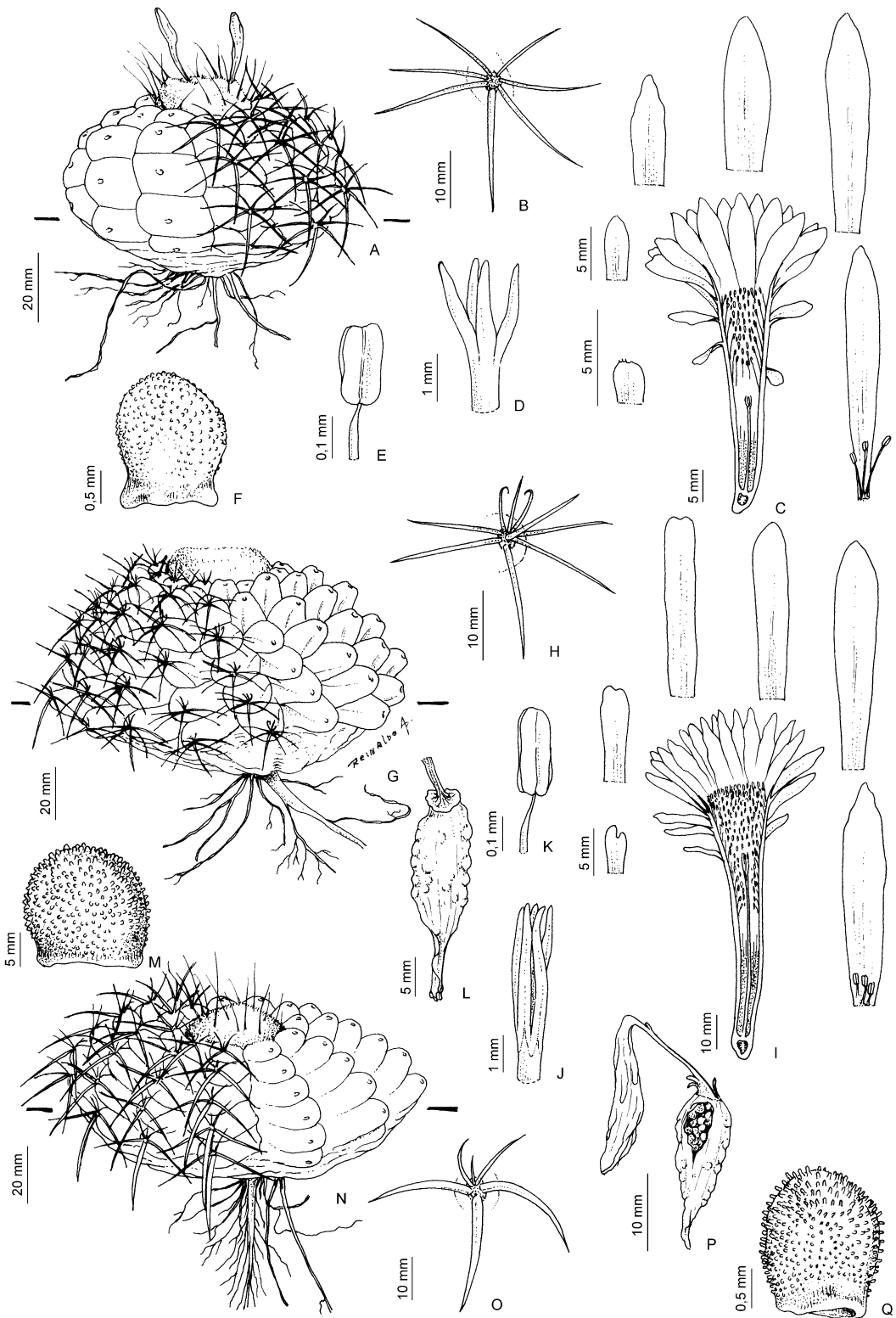


Figure 5. A-F. *Discocactus ferricola*. A. Habit showing stem and rib shape. B. Areole. C. Longitudinal section of flower and perianth-segments. D. Stigma. E. Stamen. F. Seed. G-M. *Discocactus hartmannii*. G. Habit showing stem and rib shape. H. Areole. I. Longitudinal section of flower and perianth-segments. J. Stigma. K. Stamen. L. Fruit. M. Seed. N-Q. *Discocactus heptacanthus*. N. Habit showing stem and rib shape. O. Areole. P. Fruit. Q. Seed.

Plants depressed-globose, solitary. *Roots* branched. *Stem* 3.5-13.4×6-29 cm, dark green. *Ribs* 9-26, well-defined, vertical or weakly spiralled, forming distinct tubercles, with shallow grooves (0.2 mm) between successive areoles. *Areoles* 3-9 visible per rib above ground level, oval to elongated, 3-15×2-12 mm, sunken. *Spines* grey to brown, flattened or rounded in cross-section; radial spines 3-10, 5-45×1-4 mm, central spine 0-1, 9-20×1 mm. *Cephalium* 0.5-7×1-11 cm, sometimes depressed, densely woolly; bristles vivid and dark coloured, 10-45 mm. *Flower bud* olive green to brownish; flower slender funnel-shaped, to 85×75-85 mm, borne on the periphery and centre of the cephalium, white, sweetly scented; pericarpel 5-7×4-5 mm, ovoid, white and red-brown to pinkish; receptacle-tube 34-50×7-12 mm, bearing fleshy scales 1-24×1.5-6 mm, olive green to brown, lanceolate, apex retuse, margin entire; inner perianth-segments 15-30×2-57 mm, white, lanceolate to spatulate, acute to cuspidate, sometimes cleft; outer perianth-segments 18-35×3-7 mm, lanceolate, rounded to acute, greenish-white; nectar-chamber tubular, 11-25×2-3.5 mm, open, with glands; stamen filaments 4-5 mm, anthers 0.75-2 mm, yellow; style 30-45 mm, golden yellow, extending beyond the midpoint of the receptacle-tube, stigma-lobes 4-8, 3.5-8 mm, cream to white, with papillae; ovary cavity ovoid 4-5.5×2.5-4 mm, ovules in bundles of 1-5. *Fruit* 30-50×5-15 mm, clavate, naked, white to reddish at apex; cupula 3.5 mm; perianth remains persistent. *Seeds* helmet-shaped but somewhat variable in form, 1.2-1.9×1-2 mm, shiny black; testa with nipple-like tubercles irregularly distributed.

Distribution and habitat: — BRAZIL. N Minas Gerais (Figure 6). Endemic to the core area within Minas Gerais, widespread South-eastern *campo rupestre/cerrado* element on arenitic rocks, quartz sand and gravel, *cerrado/campo rupestre*, rarely within the southern limits of the *caatinga*, 550–1450 m, east of the Rio São Francisco, central and northern Minas Gerais..

IUCN conservation assessment: — Previously considered Vulnerable, but now listed as Least Concern. *Discocactus placentiformis* is widespread in central and northern Minas Gerais, Brazil. In parts of its range it is locally very abundant, with several thousand mature individuals, while in other areas the subpopulations are smaller and have suffered from exploitation for making candy as well as habitat loss due to agricultural development. The populations are still declining, but not fast enough to

qualify for any of the IUCN threatened categories in the near future. When located close to villages, formerly, this species was affected by exploitation for the production of candies. The major threats are due to conversion of its habitat into pasture land for cattle ranching, for crops, or for *Eucalyptus* plantations and collecting for horticultural (IUCN 2013).

Notes: 2-16 flowers arise at the same time. Many morphological characters vary according to substrate (personal observations), the plant form, size, and spines being the most variables traits when plants occur on rocks, sand or gravelly soil (Figure 8). On rocks the plant body is more globose and elongated, whereas on gravel or sandy soil it is more depressed. The population reported by Heringer in Oct. 1959 in Mun. Sete Lagoas, MG, at Lagoa Grande is locally extinct. The population reported by Martius at Ouro Branco, Minas Gerais was not found and may have been recorded in error, so that the populations at Santana do Pirapama are considered the southernmost extant records of its range.

Specimens selected: — BRAZIL. Minas Gerais: Mun. Grão Mogol, 31 Jan. 1991, *Taylor et al.* 1512 (K, ZSS, BHCB); Mun. Claros dos Poções, June 1979, *E. Esteves Pereira* 111 (UFG); Mun. Jequitaí, 27 Jan. 1991, *Taylor et al.* 1450 (K, BHCB); Mun. Francisco Dumont, 7 Aug. 1988, *Eggl* s.n. (ZSS); Mun. Bociáúva, s.d., Faz. Laginha, *Horst & Uebelmann* 548 (ZSS, K); l.c. Engenheiro Dolabela, 24 Mar. 2011, *M.R. Santos* 92-96 (VIC); Mun. Buenópolis, 13 Oct. 1988, *Taylor & Zappi* in *Harley* 24988, 24993 (K); Mun. Augusto de Lima, 31 Aug. 1985, *Horst & Uebelmann* 356 (K, ZSS); Mun. Joaquim Felício, 20 Oct. 1999, *Hatschbach et al.* 69382 (MBM); l.c. Parque Estadual da Serra do Cabral, 22 Oct. 2010, *M.R. Santos* 38 (VIC); l.c., 22 Oct. 2010, *M.R. Santos* 39 (VIC); Mun. Diamantina, 1982, *Horst & Uebelmann* 548 (K, ZSS); *ibid.*, c. 15 km NE of Diamantina on road to Mendanha, 30 Jan. 1969, *Irwin et al.* (K, NY, UB); *ibid.*, estrada para Conselheiro Mata, KM 165, 18 July 1987, *Zappi et al.* in *CFCR* 11297 (SPF); l.c. Sopa, 14 May 2011, *M.R. Santos* 101 (VIC); Mun. Mendanha, 15 May 2011, *M.R. Santos* 107 (VIC); Mun. Couto de Magalhães de Minas, 3 Aug. 1988, *Eggl* 1056 (ZSS), *Horst & Uebelmann* 232 (K) (U) (ZSS); l.c. Parque Estadual do Biribiri, 14-15 May 2011, *M.R. Santos* 100 (VIC); l.c.; 14-15 May 2011, *M.R. Santos* 102 (VIC); Mun. Datas, 17 Feb. 1988, *Supthut* 8835 (ZSS); l.c. Barão do Guaicuy,

1973, 1985, 16 Aug. 1988, *Horst & Uebelmann* 357 (K, ZSS); Mun. Sete Lagoas, 16 Oct. 1959, *Heringer* 7228 (UB); Mun. São Gonçalo do Rio Preto, 16 May 2011, *M.R. Santos* 113 (VIC); Mun. Serro, 2 Sep. 2002, *R.C.Mota* 1626 (BHCB); Mun. Conselheiro Mata, 17 May 2011, *M.R. Santos* 108 (VIC); l.c., 17 May 2011, *M.R. Santos* 109 (VIC); l.c., 17 May 2011, *M.R. Santos* 110 (VIC); Mun. Santana do Pirapama, 21 July 2009, *Zappi*. D.C. 2210 (RB).

10. *Discocactus pseudoinsignis* N. P. Taylor & Zappi (1991:86), Figure 7: G-O.

Holotype: — Minas Gerais, Mun. Cristália, *Zappi et al.* in CFCR 12045 (SPF!).

Plants depressed-globose, solitary. *Roots* branched. *Stem* 4.9-10.5×10-21 cm, pale to light green. *Ribs* 12-13(-15), well-defined, straight or slightly spiralled, weakly to non-tuberculate, with elevated ridges between adjacent areoles. *Areoles*: 3-6 visible per rib above ground level, oval to elongated, 1.2-8.0×2-6 mm, not sunken. *Spines* grey to blackish, rounded in cross-section, short, straight, curved down or turned-up, dagger shaped; radial spines 4-9, 3-42×1-2 mm, central spine 0-1, 9-30×0.5-2 mm. *Cephalium* 0.5-5×2-9 cm, more or less globular, with white to greyish wool; bristles dark coloured, 32-40 mm. *Flower bud* brownish to olive green; flower tubular to funnel-form, 75×60 mm, borne mainly in centre of the cephalium, white, naked, sweetly scented; pericarpel 7.5×4 mm, ovoid, cream-coloured to white; receptacle-tube 60-68×4-5 mm, bearing lanceolate to spatulate scales, 1-24×1.5-6 mm, cream to olive green without, white within, apex obtuse and irregularly dentate, margin entire; inner perianth-segments 22×2 mm, white, lanceolate to spatulate, acute to cuspidate, sometimes cleft; outer perianth-segments 30×4 mm, lanceolate, acute, cream-green without, white olive green within, midvein and tip brown; nectar-chamber tubular, 34×2.5mm, open, with glands; stamen filaments 5-8 mm, anthers 1.5 mm, yellow; style 54 mm, yellow to orange, extending to the midpoint of the receptacle-tube, stigma-lobes 7, 6 mm, cream to white, with papillae; ovary cavity elongate to heart-shaped, 5.5×2 mm, ovules in bundles of 5. *Fruit* 32-45×5-9 mm, clavate, naked, cream-white to yellow- orange at apex; perianth remains persistent. *Seeds* helmet-shaped, 1-1.4×1-2 mm, shiny black; testa with nipple-like tubercles regularly distributed.

Distribution and habitat:— BRAZIL. N Minas Gerais (Figure 6). Endemic to South-eastern *cerrado-campo rupestre* (Mun. Cristália, Botumirim and Grão Mogol) element in pure quartz sand or sand between arenitic rocks, *campo rupestre*, 700–1200 m.

IUCN conservation assessment: — Previously evaluated as Endangered [EN B1ab(iii) + 2ab(iii)] (3) (Taylor & Zappi 2004) and here also considered Endangered but based upon different criteria (EN (B1ab (iii, v))). *Discocactus pseudoinsignis* is endemic to northern Minas Gerais, Brazil. It has an extent of occurrence of approximately 1,000 km² and it is found in few locations. Two subpopulations occur in the Parque Estadual de Grão Mogol, but those subpopulations outside of the protected area are declining due to habitat loss from quarrying, urbanization, and fires. The causes of decline are understood, but have not ceased and are, at least in part, not reversible. They are therefore expected to continue reducing the number of mature individuals as well as the extent of occurrence and área of occupancy of this cactus (IUCN 2013). The major threats are habitat modification, mainly the quarrying of sand and urbanization. Botumirim region, it has been severely damaged by fire and *Eucalyptus* plantations.

Notes: — In our phylogenetic study (Santos *et al.*, 2013) it was shown to be sister of *D. horstii* with strong support. *Discoactus pseudoinsignis* and *D. horstii* have ribs not tubercled (what distinguishes them from other *Discocactus*) but can be distinguished from each other mainly by presence of spines pectinate only in *D. horstii*. The flowering period of this species begins in November and it was also observed with few flowers until March in Cristália, MG.

Specimens examined: — BRAZIL. Minas Gerais: Mun. Grão Mogol, 1972, 1974, 1985, Horst & Uebelmann 347 (U, K, ZSS) Ex cult. (ZSS); l.c., 10 km NW of the town, 22 Oct. 1978, Hatschbach 41634 (MBM); l.c., Vale do Ribeirão das Mortes, 4 Sep. 1986, I. Cordeiro & R.Mello-Silva in CFCR 10088 (SPF); l.c., Córrego Escurona, 13 May 1990, Zappi *et al.* in CFCR 12901 (SPF); l.c., Várzeas Escuras, 6 Nov. 1997, F. Fernandes (K); in Parque Estadual de Grão Mogol, Serra do Barão, 16 March 2011, Santos, M.R. 74 (VIC); Mun. Cristália, 28 May 1988, Zappi *et al.* in CFCR 12045 (SPF); l.c. around the city, 17 March 2011, Santos, M.R. 76 (VIC); l.c., 17 March 2011, Santos, M.R. 77 (VIC); l.c., 17 March 2011, Santos, M.R. 78 (VIC).

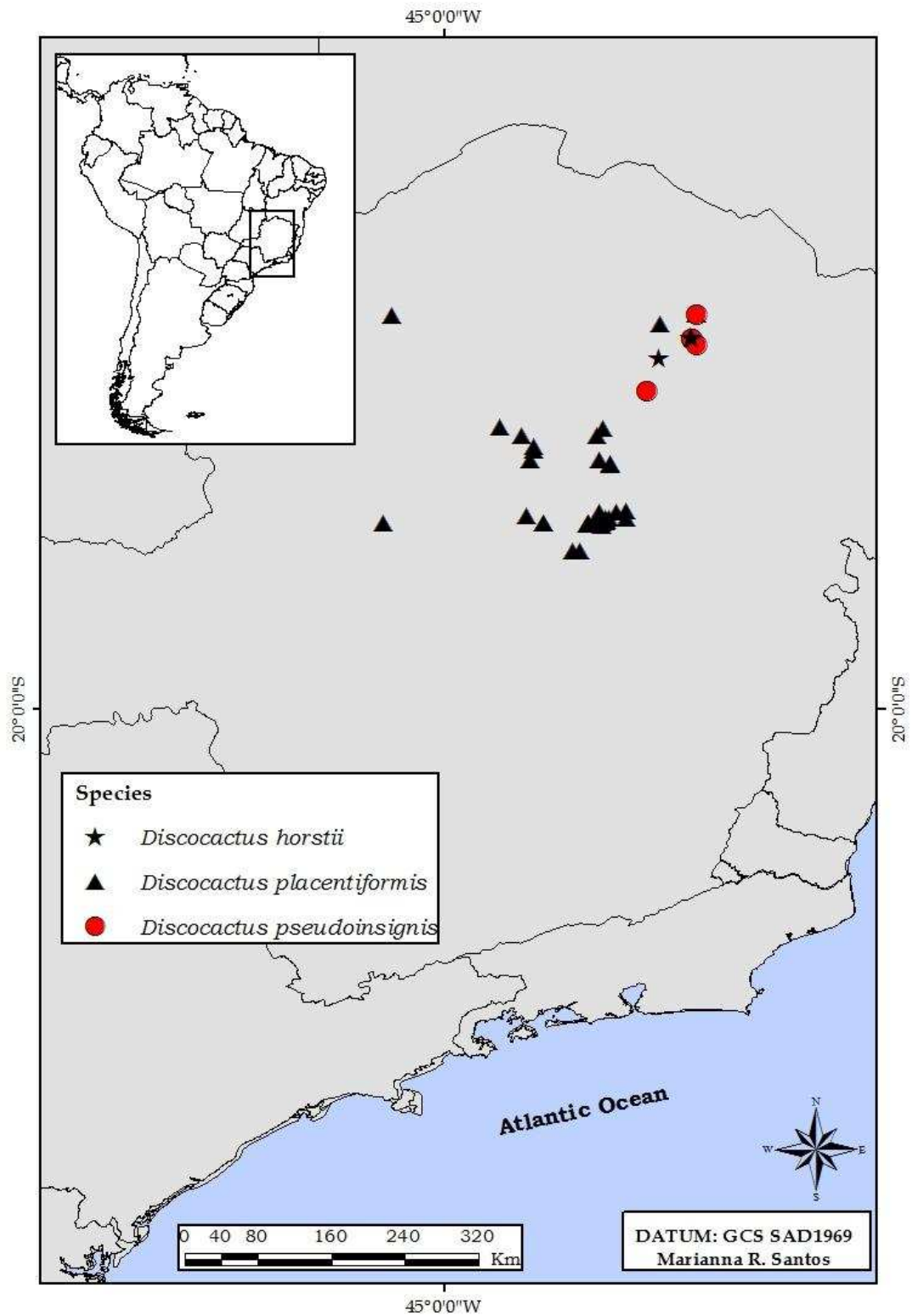


Figure 6. Map showing geographic distribution of *Discocactus* species that are endemic to Minas Gerais State, Brazil.

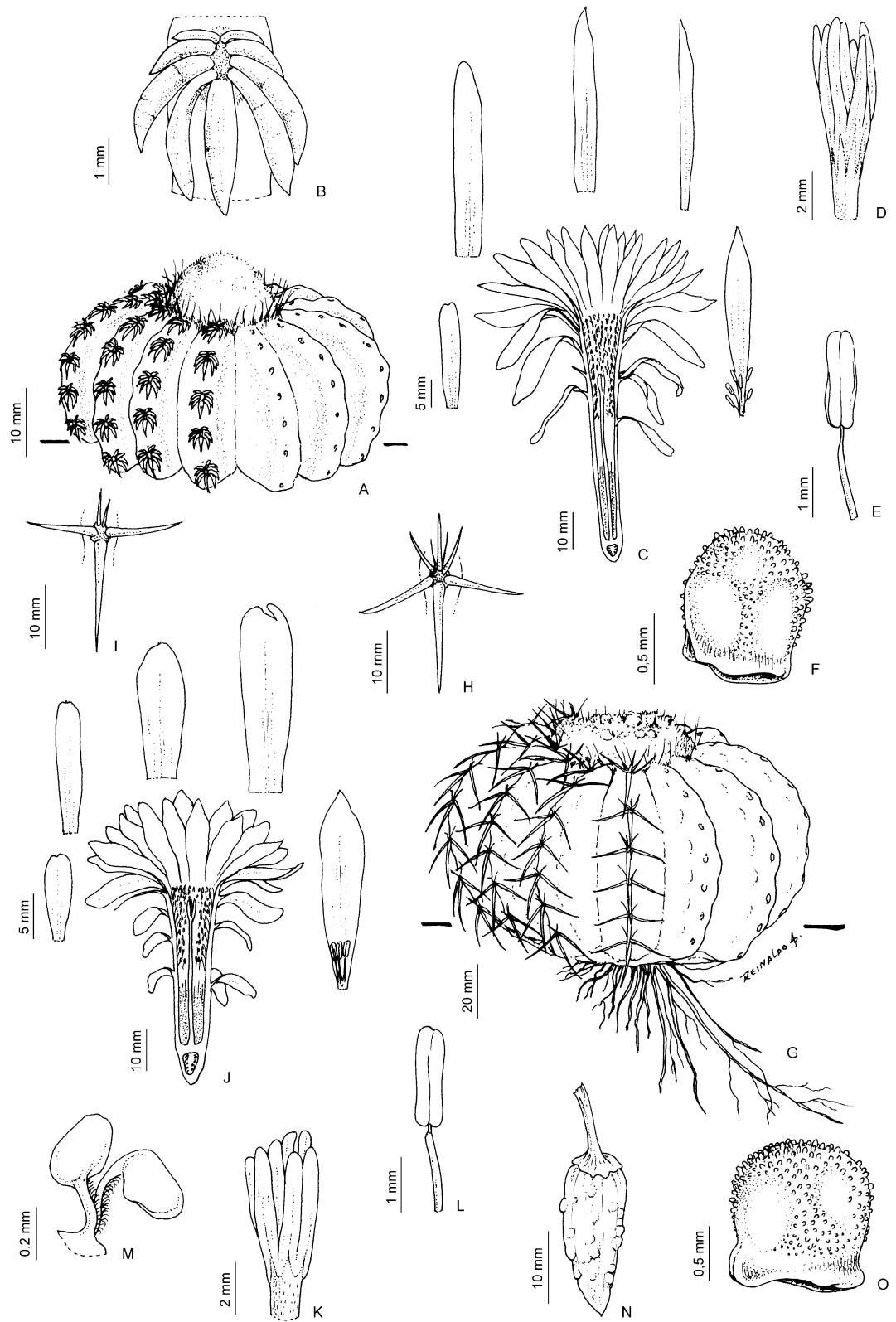


Figure 7. A-F. *Discocactus horstii*. A. Habit showing stem and rib shape. B. Areole. C. Longitudinal section of flower and perianth-segments. D. Stigma. E. Stamen. F. Seed form. G-O. *Discocactus pseudoinsignis*. G. Habit showing stem and rib shape. H-I. different types of areole. J. Longitudinal section of flower and perianth-segments. K. Stigma. L. Stamen. M. Ovules. N. Fruit. O. Seed.

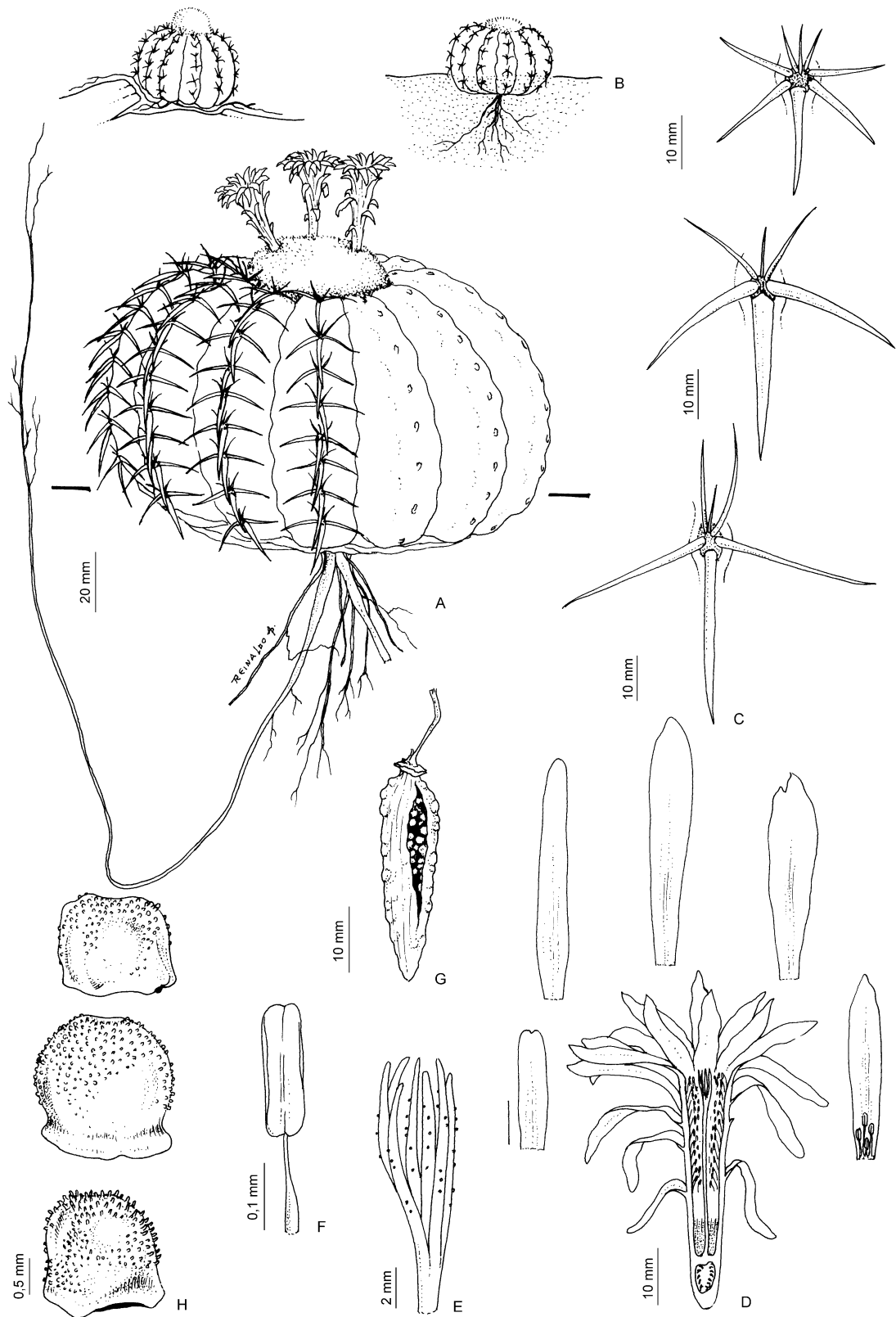


Figure 8. *Discocactus placentiformis*. A. Habit showing stem and rib shape. B. Plant body variation depending on substrate (on rocks and sand). C. Different types of areole. D. Longitudinal section of flower and perianth segments. E. Stigma. F. Stamen. G. Fruit. H. Seed form variation.

11. *Discocactus zehntneri* Britton & Rose (1922: 218).

Type: — BRAZIL. Bahia, Mun. Sento Sé, 1915, *Zehntner* in *Rose & Russell* 19779 (US!).

Plants depressed-globose to globose, producing offsets at the base. *Roots* branched. *Stem* 1-7×2-15 cm, pale green. *Ribs* 11-26, broken into rounded tubercles spirally arranged. *Areoles*: 5-7 visible per rib above ground level, oval to elliptic, 0.5-13×0.4-10 mm, not sunken. *Spines* grey to brown, rounded in cross-section, long, straight and spreading on both sides, almost hiding the stem; radial spines 7-18, 4-40×1-2 mm, central spines 0-2, 26-70×1-2 mm. *Cephalium* 0.6-3.5×2-4.5 cm, with white to greyish wool; with or without bristles, when present with vivid and dark colours, 7-60 mm. *Flower-bud* greenish; flower funnel-shaped, 33-77×35-55 mm, white, naked, sweetly scented; pericarpel 2.5-4×2-4 mm, ovoid, cream-coloured to white; receptacle-tube 5-35×4-8 mm bearing lanceolate to spatulate scales, 8-25×2-5 mm, white, green or reddish without, white within, apex rounded to acuminate, green to reddish; inner perianth-segments 17-25×4-6 mm, lanceolate to spatulate, acute to cuspidate, white; outer perianth-segments 19-35×3.5-6 mm, green to yellowish, lanceolate to spatulate, rounded to acute; nectar-chamber tubular, 12×3-3.5mm; style 23-30 mm, extending beyond the midpoint of the receptacle-tube, stigma-lobes 5-6, 5 mm. *Fruit* 18-46×5-9 mm, pink to reddish; perianth remains persistent. *Seeds* oval to cap-shaped, 0.8-2×0.8-2.3 mm testa with small to elongate tubercles.

D. zehntneri is divided into three subspecies, one being recognized here for the first time:

Key to the subspecies of *D. zehntneri*

1. Plant body globose to depressed-globose; central spine 0-2; stem not hidden by spines (up to 2 mm thickness).

2. Spines straight, central spines 0-2; style 26-30 mm.....**11.1. *D. zehntneri* subsp. *zehntneri***

2. Spines curved, central spines 0; style 12-18 mm11.3. *D. zehntneri* subsp. *petr-halfarii*

1. Plant body globose to elongate, caespitose; central spine 0-1; stem obscured by spines that are like bristles (up to 1 mm thickness).....11.2. *D. zehntneri* subsp. *boomianus*.

11.1. *Discocactus zehntneri* subsp. *zehntneri* Figure 10: A-G.

Plants globose to depressed, caespitose. *Roots* branched. *Stem* 3-8×5.5-13.6 cm, light to dark green. *Ribs* 12-13(-21), broken into rounded to elongated, spiralled tubercles. *Areoles* 3-5 visible per rib, oval, 3-13×2-10 mm, not sunken. *Spines* grey to brown; radial spines 7-14, 22-75×1-2 mm; central spines 0-2, 35-70×1-2 mm, erect. *Cephalium* 1-3.5×2-4.5 cm, with white to greyish wool; bristles on the periphery of the cephalium, grey with light brown to orange tip, 7-60×1-5 mm. *Flower* tubular, 33-77×35-55 mm, naked, sweetly scented; receptacle-tube scales 7-22×1-5 mm, lanceolate, fleshy, white to light brown, margin finely dentate, tip acute; inner perianth-segments 17-25×4-6 mm, lanceolate to spatulate, acute to cuspidate, white; outer perianth-segments 19-35×3.5-6 mm, lanceolate, white to light green, apex rounded to acute; nectar-chamber tubular 12 mm; receptacle-tube 24-35×5-6 mm; style 26-30 mm, its apex positioned a little above the middle of the receptacle-tube; stigma-lobes 5, 5 mm; ovary cavity 2×2 mm; stamens 1-5 mm. *Fruit* 18-46×6-9 mm, bright red, white or greenish. *Seeds* helmet-shaped, 0.8-2.0×0.8-2.3 mm, testa with small to elongate tubercles.

Distribution and habitat : — BRAZIL. N Bahia and NW Ceará (Figure 9). Northern *caatinga* element on exposed gneissic rocks and gravelly soil in *caatinga*, 450–500 m, north of the Chapada Diamantina, Mun. Sento Sé, northern Bahia. Endemic to Bahia and Ceará.

IUCN conservation assessment: — In 2002 the species was considered Vulnerable, but is now assessed as Near Threatened according to criteria B1ab (iii), because its extent of occurrence is less than 20,000 km². The subpopulations in the lowland and in some high altitude localities are declining, while other high altitude subpopulations occur in a protected area and are stable. The main threat is quarrying of the rock, sand

and iron ore on which this cactus thrives, and this is expected to lead to further population decline. Part of the species range was flooded as a consequence of the construction of the Sobradinho dam in the 1970s (Taylor & Zappi 2004, IUCN 2013).

Notes: — *D. zehntneri* sens. lat. shown to be monophyletic (Santos *et al.*, 2013) with a good support in Bayesian and Parsimony analysis. Morphologically this subspecies can be differentiated from others by presence of until two central spines that is perpendicular to the areola and its radial spines are larger (up to 75 mm), not curved. The flowering occurs mainly between November to February. Usually only a single flower develops at a time.

Specimens examined: — BRAZIL. Bahia: Mun. Sento Sé, 5-6 June 1915, *Zehntner* in *Rose & Russell* 19779 (US, NY); 29 km SE of the old town, July 1974, *Horst* 441 (U, ZSS); l.c., Serra do Francisco, s.d., *Horst & Uebelmann* 390 (U, ZSS) Ex cult. ZSS; l.c., near the village of Brejo Grande, 28 Apr. 2004, *M. Machado* 206 (HUEFS); *ibid.* 1973, *Horst & Uebelmann* 441 (ZSS) Ex cult. ZSS. Ceará: Guaraciaba do Norte, 22 Jan., 2011, *Menezes, M.O.T.* 256 (EAC).

11.2. *Discocactus zehntneri* subsp. *boomianus* (Buining & Brederoo) N.P. Taylor & Zappi (1991: 86), Figure 10: H-K.

Holotype: — BRAZIL. Bahia, Mun. Morro do Chapéu, *Horst* 222 (U!).

Plants globose to elongate, caespitose. *Roots* branched. *Stem* 1-7×3-12 cm, light to dark green. *Ribs* 13-26, broken into rounded, spiralled tubercles. *Areoles* 4-7 visible per rib, oval, 2-7×1-3 mm, not sunken. *Spines* grey to brown; radial spines 9-18, 4-40×1 mm, central spine 0-1, 30-42 (60)×1 mm; ± 5 pairs of spines pectinately arranged at each side of the areole, adpressed; stem almost obscured by dense spination. *Cephalium* 0.6-5.1×1.3-5.4 cm, with white to greyish wool; bristles on the periphery of the cephalium 14-44(-91) ×1-5. *Flower* tubular, 33-77×35-55 mm, naked, sweetly scented; receptacle-tube scales 5-28×2-4 mm, lanceolate to lineate, white to reddish, midrib and apex red, margin smooth but slightly undulate, acuminate; inner perianth-segments 17-25×4-6 mm, lanceolate to spatulate, acute to cuspidate, white; outer perianth-segments 19-35×3.5-6 mm, lanceolate to spatulate, rounded to acute, light green to yellowish; nectar

-chamber tubular 12×3 mm; receptacle-tube 5-26×4-8 mm; style 23-25 mm, apex reaching the whorls of stamens, stigma-lobes 6, 5 mm; ovary cavity cordate in long section, 2×2 mm, ovules in fascicles of 4, funicles bare glabrous; stamens 1-2 mm. *Fruit* 18-46×6-9 mm, pink to reddish. *Seeds* globose to helmet-shaped, 0.8-2.0×0.8-2.2 mm, testa with small to elongate tubercles.

Distribution and habitat: — BRAZIL. N Bahia (Figure 9). Northern *campo rupestre* (Chapada Diamantina) element: on exposed arenitic rocks or ‘pedra canga’ (iron- and manganese rich conglomerate rock), often with an accumulation of gravel, or in pure quartz sand, *caatinga / campo rupestre*, c. 700–1000 m, Mun. Morro do Chapéu and Mun. Sento Sé, northern Bahia. Endemic to Bahia.

IUCN conservation assessment: — This subspecies received the same assessment as *D. zehntneri* subsp. *zehntneri*, and is listed as Near Threatened according to criteria B1ab (iii), because its extent of occurrence is less than 20,000 km² (IUCN 2013).

Notes: — *Discocactus zehntneri* subsp. *boomianus* grouped with others *D. zehntneri* subspecies with good support in Bayesian and Parsimony analysis showing that the *D. zehntneri* group is monophyletic (Santos *et al.*, 2013). *D. zehntneri* subsp. *boomianus* can be differentiated morphologically from others subspecies by cephalium with more bristles and these are thinner as well as their spines. Their spines (radials 4-5 pairs in each side of areole may reach up to 18 radial spines if we include those who are at the bottom and top of the areola) are like bristles more adhered to the stem covering it and sometimes is not possible to see easily the plant body being difficult to visualize the number of ribs. The flowering occurs mainly during November to February and the peak seems to be in December when most plants have flowers, but this needs to be further investigated. Usually, 1-6 flowers develop at a time.

Specimens examined: — BRAZIL. Bahia: Mun. Sento Sé, *Horst & Uebelmann* 440 (U) (ZSS) Ex cult. ZSS; l.c., Mimoso, 1 Jan. 1988, *K.I. Horst* 667 (K, ZSS, HUEFS); l.c., near the village of Campo Largo, 26 Mar. 2004, *M. Machado et al.* 350 – 356 (HUEFS); l.c., near the village of Limoeiro, 28 Apr. 2004, *M. Machado* 400 – 403 (HUEFS); 28 Apr. 2004, *M.C. Machado* 205c (HUEFS); 26 Mar. 2004, *M.C. Machado* 201 (HUEFS); Mun. Morro do Chapéu, 25 Dec. 1988, *Taylor & Zappi* in *Harley* 27395

(K); 11 Out. 2008, *M.C. Machado* 1167 (HUEFS); 23 July 1988, *Eggl* 1274 (ZSS); l.c. Cafarnaum towards Morro do Chapéu, ZSS, *Horst & Uebelmann* 222 (U, K, ZSS); l.c., ca. 21 km to the west of town on road BA 052, 12 Jun. 2004, *M. Machado et al.* 450 – 454 (HUEFS); l.c., ca. 26 km to the west of town on road BA 052, 12 Jun. 2004, *M. Machado et al.* 460 – 464 (HUEFS); l.c., ca. 25 km to the west of the town, 12 Jun. 2004, *M. Machado et al.* 470 – 474 (HUEFS); sin loco, 1980, *J.Y. Clark*, cult. (K); l.c. BA052, 16 Apr. 2011, *Santos, M.R.* 99 (VIC).

11.3. *Discocactus zehntneri* subsp. *petr-halfarii* (M. Zachar) M.R. Santos & M.C. Machado *stat. nov.*, Figure 11.

Holotype: —BRAZIL, Bahia, Mun. Juazeiro, 2007, *M. Zachar* (UFG!).

Plants depressed-globose to globose. *Roots* branched. *Stem* 4-5×2-15 cm, green. *Ribs* 10-14, broken into rounded tubercles. *Areoles* 2-5 visible per rib, oval to elliptic, 0.5-1.4×0.4 mm, not sunken, with much beige to white indumentum. *Spines* grey to yellow; radial spines 7-12, 10-25×1.5 mm, arranged in c. 3 pairs on each side, central spine 0. *Cephalium* 0.6-2.0×2.6-3 cm, with white to greyish wool; with little or no bristles. *Flower* funnel-shaped, 34-36×23-26 mm, white, naked, perianth-segments with ciliate margins (when observed under microscope), sweetly scented; receptacle-tube scales 2-8×0.5-4.0 mm, cream to white, apex acuminate to acute; inner perianth-segments 15-22×3-6 mm, white lanceolate to spatulate, acute to cuspidate; outer perianth-segments 10-24×4-6 mm, lanceolate to spatulate, acute, cream to white; style 12-18 mm, apex upper half of corolla, stigma-lobes 5, 3-4 mm; stamens 1-2 mm. *Fruit* 35×5 mm, pink to reddish, about 100 seeds per fruit; perianth remains persistent. *Seeds* helmet-shaped or more elongated, c. 1×1 mm, testa with small to elongate tubercles.

Distribution and habitat: — BRAZIL. N Bahia (Figure 9), occurs in “Caatinga”, on stony clay soil at an altitude of 350-550 m.

IUCN conservation assessment: — this taxon has not yet been assessed for the IUCN Red List. However, it could receive an assessment as Critically Endangered, because it is known from only one population occurring in area <100 km².

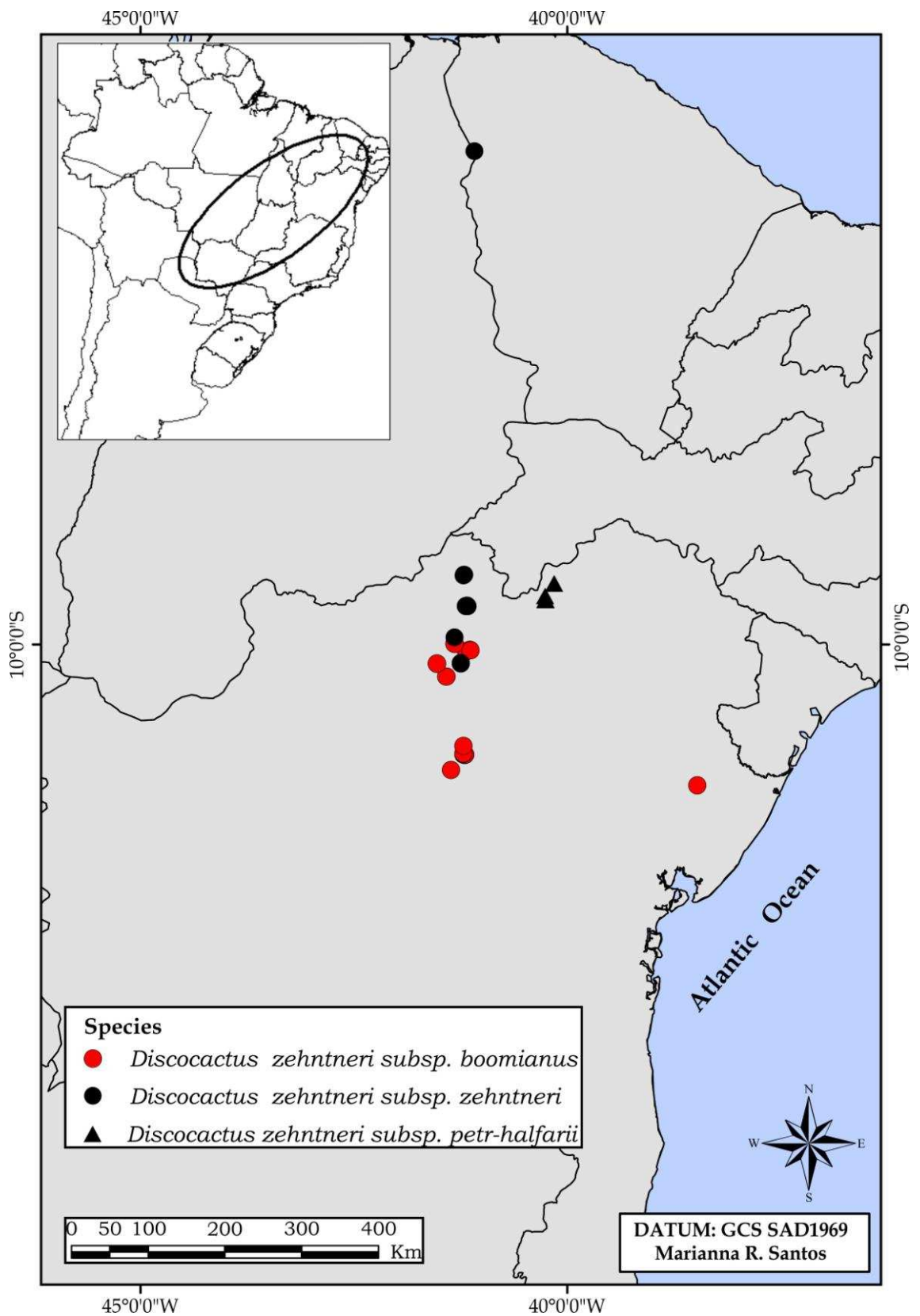


Figure 9. Map showing geographic distribution of *Discocactus* species restricted to the NE region of Brazil.

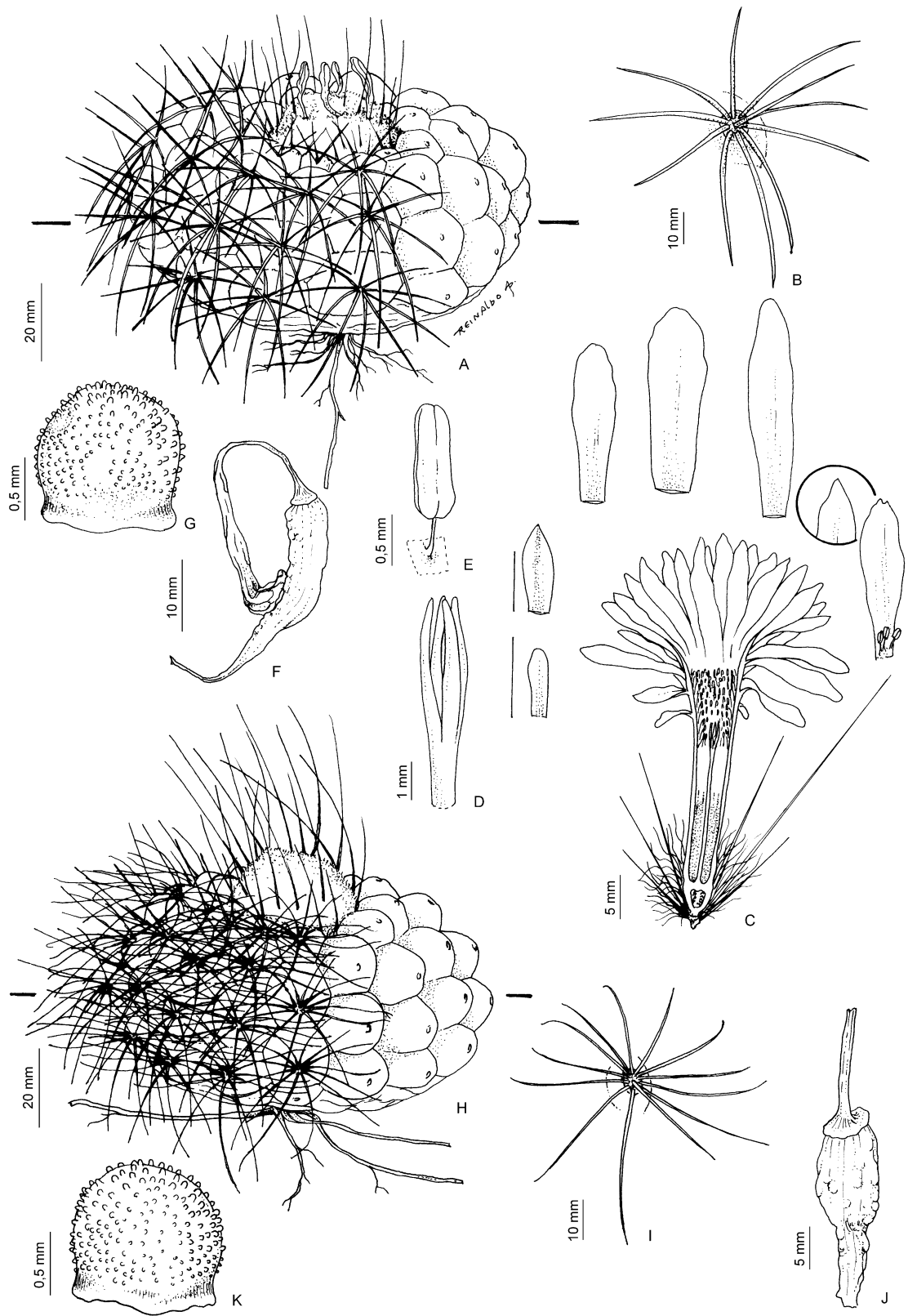


Figure 10. A-G. *Discocactus zehntneri* subsp. *zehntneri*. A. Habit showing stem and rib shape. B. areole. C. Longitudinal section of flower and perianth-. D. Stigma-lobes. E. Stamen. F. Fruit. G. Seed. H-K. *Discocactus zehntneri* subsp. *boomianus*. H. Habit showing stem and rib shape. I. areole. J. Fruit. K. Seed.

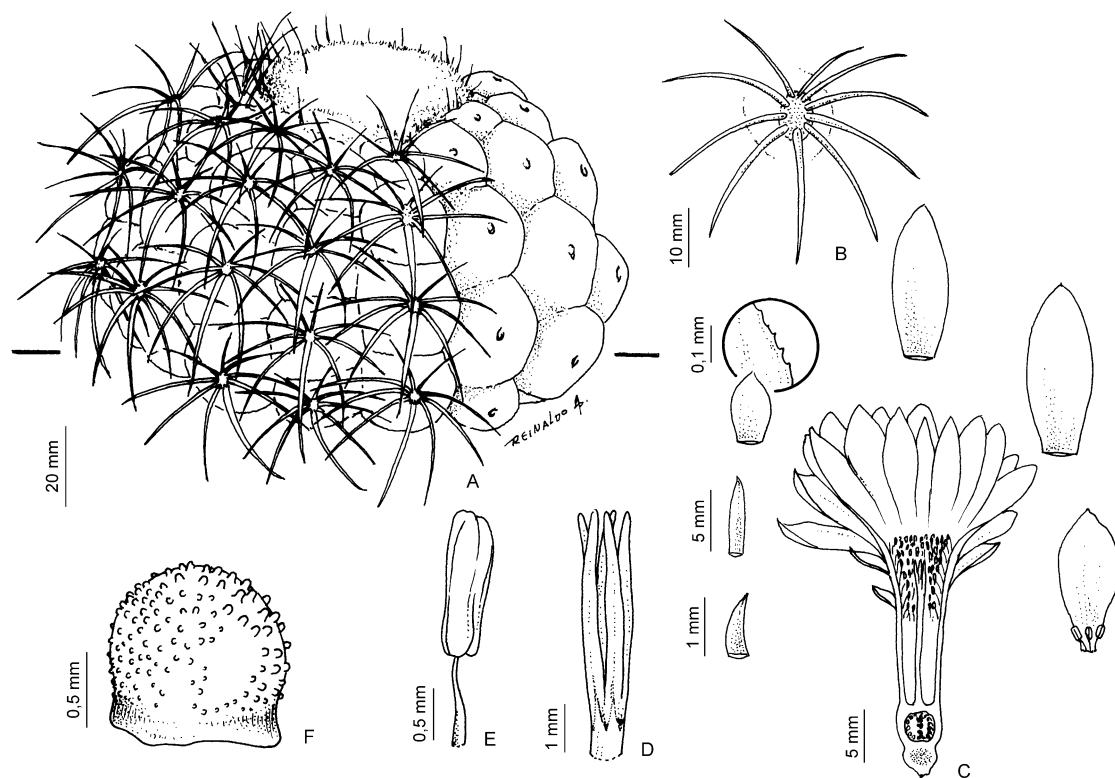


Figure 11. A-B. Habit showing stem, rib shape and areole morphology of *Discocactus zehntneri* subsp. *petr-halfarii* subsp. nov. C. Longitudinal section of flower and perianth segments. D. Stigma. E. Stamen. F. Seed.

Notes: — Previously regarded as a synonym and subspecies of *D. bahiensis*, *D. petr-halfarii* Zachar (Zachar 2008) is now placed as a subspecies of *D. zehntneri*. In phylogenetic study of the genus *Discocactus* this taxon grouped with other samples of *D. zehntneri* group with good Bootstrap support and Probability Posterior (Santos *et al.* 2013). Morphologically this taxon is also very related with *D. zehntneri* group due to the plant body globose, ribs with globular tubercles and can be differentiated from others subspecies mainly for central spines absent, radial spines curved and areole well developed. *Discocactus zehntneri* subsp. *petr-halfarii* is an established taxon, occurring as a large population that is morphologically uniform and which is self-sustaining reproductively. The flowering occurs between November to March, and fruits can be found until June.

Specimens examined: — BRAZIL. Bahia: Juazeiro, 4 March 2007, Zachar, M. (UC) (UFG); Frade, 26 km of Juazeiro in BR-235, Dec. 3, 2011, M.V. Meiado et al. 750 (HVASF, VIC).

Conclusions

According to previous phylogenetic study and morphological studies made here we recognize 11 species and three heterotypic subspecies of *Discocactus*. The results related to the phylogenetic study (Chapter 1) and taxonomic (this chapter) led to some changes in taxonomy *Discocactus*, as the new status of *D. petr-halfarii* now being considered as subspecies of *D. zehntneri*. Synonyms before related to other species were repositioned: *D. silicicola*, for example, considered related as a synonym of *D. catingicola*, this work has been repositioned as a synonym of *D. hartmannii*. *Discocactus lindanus*, which was synonymous with *D. diersianus*, was more related to *D. heptacanthus*.

Only two species of the genus (*D. catingicola* and *D. placentiformis*) were considered Least Concern by IUCN Red List assessments. With this most species and subspecies of the genus (84%) are threatened, mainly by habitat destruction and illegal collection.

There is a great need for studies of phenology, pollination and reproductive biology of *Discocactus* to understand more about the ecology of their species. Moreover, due to the great variation of its species, studies that consider genetic and morphological variability are also of utmost importance, especially for species that have wide distributions and grow in different types of soil and rocks.

Acknowledgements

We thank Urs Egli (ZSS), Peter Audiffred (L, U) for receiving the first author during her visit to European herbaria. The curators of the Brazilian herbaria are thanked for arranging loans and facilitating visits (BHCB, EAC, HUEFS, HVASF, MBM, R, RB, UFG e UFMT) and to others for making available scans of vouchers (K, NY, US, UC). Juliana Gastaldello Rando, Bernarda de Souza, Fábila Silva and Thiago Stefanello kindly sent pictures from SPF, UB and CGMS collections, respectively. Gerardus

Olsthoorn for seeds, flowers and photos. M.V. Meiado and M.O.T. Menezes for collected plants and Reinaldo Pinto for illustrations. We also are grateful to CAPES, FAPEMIG, CSSA and BCSS for funding this research.

References

- Reyes-Aguero, J.A.; Carlín-Castelán, F; Aguirrer, J.R. & Hernandez, H. M. (2007) Preparation of *Opuntia* herbarium specimens. *Haseltonia* 13: 76–82.
- Backeberg, C. (1958–1962). *Die Cactaceae. Handbuch der Kakteenkunde*, 1–6. VEB Gustav Fischer Verlag, Jena.
- Biodiversitas. (2006) Lista oficial de espécies ameaçadas de extinção no Brasil. Disponível em: <<http://www.biodiversitas.org.br/florabr/grupo3fim.asp>>. Accessed: 23 Mar. 2010.
- Braun, P.J. & Esteves Pereira, E. (1993a). Aggregationen in *Discocactus* Pfeiffer (Cactaceae). Teil 1. Kakteen und andere Sukkulenten, 44: 62-65.
- Braun, P.J.& Esteves Pereira, E. (1993b). Aggregationen in *Discocactus* Pfeiffer (Cactaceae). Teil 2. Kakteen und andere Sukkulenten, 44:103-105.
- Braun, P.J.& Esteves Pereira, E. (1993c) Aggregationen in *Discocactus* Pfeiffer (Cactaceae). Teil 3. Kakteen und andere Sukkulenten 44:112-117.
- Braun, P.J.& Esteves Pereira, E. (1995) Aggregationen in *Discocactus* (Nachträge). Kakteen und andere Sukkulenten. 46: 64.
- Braun, P.J.& Esteves Pereira, E. (2002). Kakteen und andere Sukkulenten in Brasilien. *Schumannia*, 3:1–235.
- Britton, N. L. & Rose, J. N. (1919–1923). *The Cactaceae*, 1–4. Carnegie Institution, Washington, D.C. (Vol. 1, 1919, vol. 2, 1920, vol. 3, 1922, vol. 4, 1923.)
- Buining, A. F. H. (1980) The genus *Discocactus* Pfeiffer. Buining-fonds, Succulenta, The Netherlands. 224 pp.
- Buining, A. F. H.& Brederoo, A.J. (1973) *Discocactus horstii*. Krainz, Die Kakteen 15, 1, 3 pp.
- Buining, A. F. H.& Brederoo, A.J. (1974) *Discocactus catingicola*. Kakteen und andere Sukkulenten. 25:265–267.
- Buining, A. F. H.& Brederoo, A.J. (1975) *Discocactus ferricola*. Kakteen und andere Sukkulenten, 26: 2-3

- Buining, A. F. H. & Brederoo, A.J. (1977) *Discocactus boliviensis*. *Succulenta* 56(11): 258.
- Buxbaum, F. (1958) The phylogenetic division of the subfamily Cereoideae [*sic*], Cactaceae. *Madroño* 14: 177–206.
- Crozier, B.S. (2005) Systematics of Cactaceae Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw, 154 pp. PhD. Dissertation- The University of Texas, Austin.
- Endler, J. & Buxbaum, F. (1974) Die Pflanzenfamilie der Kakteen, ed. 3. A. Philler, Minden.
- ESRI. (2012) ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.
- Esteves Pereira, E. (1979) *Discocactus diersianus*. *Cactus & Succulent Journal (US)*, 51: 179-183.
- Hunt, D.; Taylor, N. P. & Charles, G. (eds.) (2006) *The New Cactus Lexicon*. Milborne Port.: dh Books, 2 vols.
- IUCN (2013) IUCN Red List of Threatened Species. Version 2013.1. <www.iucnredlist.org>. Downloaded on 28 March 2013.
- Lüthy, J. (2001) *The Cacti of CITES Appendix I*. Bundesamt für Veterinärwesen, Switzerland.
- Machado, M.C. (2004) O gênero *Discocactus* Pfeiff. (Cactaceae) no estado da Bahia, Brasil: variabilidade morfológica, variabilidade genética, taxonomia e conservação, 113p. M.Sc. Thesis- Universidade Estadual de Feira de Santana, Feira de Santana.
- MMA (Ministério do Meio Ambiente). (2008) *Lista Oficial das Espécies da Flora Brasileira Ameaçadas de Extinção*. Brasília, 55 pp.
- Pfeiffer, L. (1837) *Enumeratio diagnostica cactearum hucusque cognitarum*: 43–46. L. Oehmigke, Berlin.
- Santos, M.R.; Garcia, F.C.P.; Machado, M.C.; Barbosa, A.R.; Taylor, N.P. & Van Den Berg, C. (2013) A phylogenetic study of *Discocactus* Pfeiff. (Cactaceae) based on nuclear and plastid DNA sequences. In: Santos, M.R. (2013) *Filogenia molecular, taxonomia, biogeografia e conservação de Discocactus Pfeiff.* 114 p. PhD thesis – Universidade Federal de Viçosa.
- Schumann, K. M. (1894) Cactaceae. In: Engler, A. & Prantl, K., *Das Pflanzenfamilien*, 3: 156–205.

- Taylor, N.P. & Zappi, D.C. (1991) *Discocactus pseudoinsignis* and *Discocactus zehntneri* subsp. *boomianus*. *Bradleya* 9: 86.
- Taylor, N.P. & Zappi, D.C. (2004) *Cacti of Eastern Brazil*. England: Richmond, Surrey: Royal Botanic Gardens, Kew, 499 pp.
- Thiers B. (2012) [continuously updated]. *Index Herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available at: <http://sweetgum.nybg.org/ih/> Accessed September 2010.
- Zachar, M. (2008) Nowość z Brazylii - *Discocactus petr-halfarii*. *Kaktusy i Inne*, 2: 43-47.
- Zappi, D.; Taylor, N.; Machado, M. & Santos, M.R. (2013) *Discocactus* in Lista de espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. (<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB1482>)

Appendix 1. List of examined specimens.

Species and source/voucher

Discocactus bahiensis. Brazil. Bahia: *sine loco*, 23 Aug. 1988, *Horst & Uebelmann* HU 902 (ZSS); Mun. Juazeiro, 2–6 June 1915, *Rose & Russell* 19742 (US, NY); l.c., 2–6 June 1915, *Rose & Russell* 19781 (US, NY); l.c., 2–6 June 1915, *Rose & Russell* 19783 (US, NY); *M.V. Meiado et al.*, 751 (HVASF); l.c., 29 Apr. 2004, *M.C. Machado* 208c (HUEFS); l.c., 24 Apr. 2004, *M.C. Machado* 207b (HUEFS); l.c., 30 Mar. 2004 *M.C. Machado* 203 (HUEFS); l.c., 10 Jan. 1991, *Taylor et al.* 1387 (K); 12 - 13 km W of Juazeiro, Fazenda Seca, 19 Sep. 1985, *Horst & Uebelmann* 438 (U, ZSS); l.c., 7 April 2000, *E.A. Rocha 87 et al.* (K); l.c., E of town, 2–6 June 1915, *Rose & Russell* 19764 (US); l.c., between Juremal and Curral Velho, s.d., *Horst & Uebelmann* 633 (ZSS); l.c., south of town, 29 Mar. 2004, *M. C. Machado et al.* 370 – 373 (HUEFS); l.c., ca. 12km from the village of Juremal on road to Abreus, 30 Mar. 2004, *M. C. Machado et al.* 380 – 383 (HUEFS); l.c., near the village of Olhos D'Água on road to Abreus, 29 Apr. 2004, *M. Machado* 420 – 423 (HUEFS); l.c., besides the village of Goiabeira, 29 Apr. 2004, *M. C. Machado* 430 – 433 (HUEFS); l.c., surroundings of the village of Abreus, 30 Apr. 2004, *M.C. Machado* 440 – 443 (HUEFS); Mun. Campo Formoso, *Horst* 437 (U, ZSS) Ex cult. ZSS; Mun. Morro do Chapéu, s.d., *Braun & Esteves Pereira* s.n. (UFG); l.c. 21 Apr. 2004 *M.C. Machado* 204 (HUEFS); l.c., 21 Apr. 2004, *M.C. Machado et al.* 390 – 393 (HUEFS); l.c., 16 Apr. 2011, *Santos, M.R.* 98 (VIC); l.c., Febr. 1978, *Horst & Uebelmann* HU 485, Ex cult. ZSS. Ceará: Mun. Lavras de Mangabeira, 25 Jan. 2009, *Menezes, M.O.T. et al.* 195 (EAC). Piauí: Teresina, Mun. Francisco Ayres, 8 Sept. 1988, *Horst & Uebelmann* 943 (K, ZSS).

Discocactus boliviensis. Bolivia. Santa Cruz: E Santa Cruz, San Cyrilo, s.d., *Horst & Uebelmann* 457 (U); l.c., s.d., *Horst & Uebelmann* 458 (U); Mun. Mota Cucito, Santa Cruz, 18 Jan. 2011, *M.R. Santos in Huaylla, H.* 3683 (VIC).

Discocactus catiingicola. Brazil. Bahia: W Bahia, Mun. Barreiras, 1974, *Horst & Uebelmann* 205A (U, K, ZSS) Ex cult. ZSS; 11-12 Sept. 1988, *Horst & Uebelmann* 949 (K, ZSS); l.c., Redondo waterfall, 14 Jan. 2004, *M.C. Machado*

et al. 310 – 313 (HUEFS); l.c., 14 Jan. 2004, *M.C. Machado* 125c (HUEFS); Mun. São Desidério, *Horst & Uebelmann* 392 (U); l.c., 13 Jan. 2004, *M. Machado et al.* 300 – 303 (HUEFS), 13 Jan. 2004 *M.C. Machado* 105 (HUEFS); Mun. Santana, *Horst* 448 (U, ZSS) Ex. Cult. ZSS; l.c., *Braun* 341 (ZSS); l.c., 17 Jan. 2004, *M. Machado et al.* 340 – 344 (HUEFS); l.c., 17 Jan. 2004, *M.C. Machado* 160b (HUEFS); Mun. Santa Maria da Vitória, 4 Oct. 1985 *Horst & Uebelmann* 760 (K, ZSS) Ex cult. ZSS; Mun. Coribe, *Horst & Uebelmann* 760A (ZSS); l.c., 16 Jan. 2004, *M. Machado et al.* 330 – 333 (HUEFS); 16 Jan. 2004 *M.C. Machado* 146 (HUEFS); Mun. Paramirim, 28 Nov. 1988, *Taylor in Harley* 25558 (K). Goiás: E Goiás, Mun. Posse, 15 Jan. 2004, *M. Machado et al.* 320 (HUEFS); Mun. Porto Novo, 1975, *Horst & Uebelmann* 448 (U, ZSS) Ex. Cult. (ZSS). Minas Gerais: N Minas Gerais, Mun. Cônego Marinho, Parque Estadual Veredas do Peruaçu, 19 Mar. 2011, *Santos, M.R.* 84 (VIC); l.c., 19 Mar. 2011, *Santos, M.R.* 85 (VIC); Mun. Januária, Refúgio Pandeiros, 19 Mar. 2011, *Santos, M.R.* 87 (VIC); l.c., 19 Mar. 2011, *Santos, M.R.* 88 (VIC); Mun. João Pinheiro, 13 May, 2011, *G. Olsthoorn* 264 (VIC); Mun. Rio Paranaíba, Serra do Salitre, 16 Mar. 2013, *Fonseca, R.S. & Arruda, D.M.* 1081 (VIC); l.c. Serra dos Alegres, *Horst & Uebelmann* 343 (U, ZSS) Ex. Cult. ZSS.

Discocactus diersianus. Brazil: Goiás: S of Goiânia. type locality of *Esteves* 10, 17 Oct. 1987, *P. J. Braun* 304 (K, ZSS); Mun. Teresina de Goiás, 15 Aug. 2010, *M.R. Santos* 23 (VIC); l.c. Rio Paranã, 1973, 1985, *Horst & Uebelmann* 431 (U, ZSS) Ex cult. ZSS; Mun. de Itumbiara, 2012, *G. Olsthoorn* 330 (VIC); Mun. Marzagão, 2012, *G. Olsthoorn* 327 (VIC); Mun. Natividade, 1985, *Horst & Uebelmann* 645 (K, ZSS) Ex cult. ZSS. Tocantins: c. 50 km N of Arraias, 1986, *P. J. Braun* 716 (ZSS); Mun. Natividade, 1984 *P. J. Braun* 547 (ZSS); Mun. Paranã, Road to Caldas, 11 Nov. 1991, *Hatschbach* 56072 (MBM).

Discocactus ferricola. Brazil. Mato Grosso Do Sul: Mun. Corumbá, 17 Jan. 2011, *Santos, M.R.* 43 (VIC); l.c., 17 Jan. 2011, *Santos, M.R.* 44 (VIC); l.c. Parque do Pantanal, 12 Nov. 2006, *J.S. Garcia* 55 (CGMS); l.c., surrounding Corumbá, 1974, *Horst & Uebelmann* 195 (U, ZSS); l.c. Corumbá, 16 Nov. 1989, *V.J. Pott* 1157 (MBM, CPAP). Bolivia. Santa Cruz: E Santa Cruz, 18 Jan. 2011, *Santos, M.R.* 52 (VIC); l.c., 18 Jan. 2011, *Santos, M.R.* 53 (VIC).

Discocactus hartmannii. Brazil. Mato Grosso do Sul: Mun. Bonito, 20 Jan. 2011, *M.R. Santos* 55 (VIC); lc. near Bonito, 1983, *P. J. Braun* 216 (ZSS); 15 km W Bonito, 22 June 1975, *Horst & Uebelmann* 193 (U, ZSS) Ex cult. ZSS; Mun. Caracol, bridge over the Caracol River, 22 Jan. 2011, *M.R. Santos* 63 (VIC), *P. J. Braun* 220 (ZSS); l.c. 21 km E of Caracol, Bela Vista, 1971, 29 May 1972, *Horst & Uebelmann* 324 (U, ZSS); Mun. Antônio João, 23 Jan. 2011, *M.R. Santos* 65 (VIC); W and N of Rio Apa, 28 May 1973, *Horst & Uebelmann* 191 (U, ZSS); l.c., Mun. Coronel Sapucaia, 25 Jan. 2011, *M.R. Santos* 66 (VIC); lc. near Iguatemi, 1983, *P. J. Braun* 208 (ZSS); Serra Maracaju, locus classicus near Maneco, 1983, *P. J. Braun* 214 (ZSS); along Rio Iguatemi and along Rio Ibaré, 26 May 1972, *Horst & Uebelmann* 190 (U, ZSS); Mun. Aquidauana, 16 Jan. 2011, *M.R.Santos* 72 (VIC); l.c., 16 Jan. 2011, *M.R.Santos* 73 (VIC); l.c., road living Aquidauana towards Cicolândia, 16 Jan. 2011, *M.R. Santos* 40 (VIC); Mun. Porto Murtinho, 21 Jan. 2011, *M.R.Santos* 60 (VIC); near Porto Murtinho, 30 May 1972, *Horst & Uebelmann* 325 (U, ZSS), Ex. Cult. ZSS. Paraguay. San Pedro: Mun. San Estanislao, Estancia Apepu, 26 Jan. 2011, *M.R. Santos* 68 (VIC); lc. near Yhu, 1983, *P. J. Braun* 204 (ZSS); Amambay, Bela Vista, s.d., *Horst & Uebelmann* HU 556 (ZSS).

Discocactus heptacanthus. Brazil. Goiás: Mun. Posse, 15 Jan. 2004, *M.C. Machado* 131b (HUEFS); l.c., 1973, *Horst & Uebelmann* HU 200 (U, ZSS); Mun. Goiânia, 1 dec. 1988, *Horst* 585 (K), Ex. Cult. ZSS; NE Goiás, 17 Sep. 1987 *Horst & Uebelmann* 526 (K); *sin loco* *Horst & Uebelmann* 632 (K) Ex. Cult. ZSS; 3km S of Rio Paranã, *Horst & Uebelmann* 431 (K); *sin loco* 1 Jan. 1989, *Horst & Uebelmann* 588 (K), Ex. Cult. ZSS; *sin loco* *Horst & Uebelmann* 649 (K); *sin loco* 1985 *Horst & Uebelmann* 720 (K), Ex. Cult. ZSS; l.c., 1985, *Horst & Uebelmann* 631 (K) Ex. Cult. ZSS; *sin loco*, May 1865, *Burchell* 7215 (K); *sin loco*, 1985, *Horst & Uebelmann* 648 (K), Ex. Cult. ZSS; Mun. Alto Paraíso, around the Parque Nacional Chapada dos Veadeiros, 15 Aug. 2010, *M.R. Santos* 25 (VIC); lc. 10 Km from Alto Paraíso, 15 Aug. 2010, *M.R.Santos* 24 (VIC); *sin loco*, 1972, *Horst & Uebelmann* HU 392 (U); l.c. Mata Azul, *Horst & Uebelmann* (U) (Holotype). Mato Grosso: Mun. Nova Xavantina, near Olaria, 24. Sep. 1967, *Ramos et al.* 6503 (K); l.c. near Nova Xavantina, May 1949, *Sick, H.* B436 (RB); Mun. Chapada dos Guimarães, 17 Sep. 1979, *P.I. Oliveira* 84

(MBM, NY); Mun. Cuiabá, 1972, *Horst & Uebelmann* HU 326 (U, ZSS) Ex. cult. ZSS; l.c., near Cuiabá, Chapada dos Guimarães, 1974, *Horst & Uebelmann* 453 (U, ZSS) Ex. Cult. ZSS; Mun. Jaciara, *Horst & Uebelmann* 326a (ZSS) Ex cult. ZSS; l.c., Córrego do Surucucu, 10 Oct. 1968, *Fonsêca, S.G.* 1365 (UB). Mato Grosso do Sul: Mun. Corguinho, 10 Feb. 2007, *J.S.Garcia* 60 (CGMS); Mun. Coxim, 6 June 1972, *Horst & Uebelmann* HU 198a (U, ZSS); l.c., 40 km Coxim, 29 Jan. 2011, *M.R. Santos* 70 (VIC); l.c., 29 Jan. 2011, *M.R. Santos* 71 (VIC); l.c., hills along Rio Juaru, E of Coxim, *Horst & Uebelmann* 455 (ZSS) Ex. Cult. ZSS.

Discocactus horstii. Brazil. Minas Gerais: Mun. Grão Mogol, Serra do Barão, 1971, *Horst & Uebelmann* 360 (U); l.c., 1987, *P.J. Braun* 851 (ZSS); l.c., Jan. 2006, *Machado, M.C.* 765 (HUEFS); l.c., 16 Mar. 2011, *M. R. Santos* 75 (VIC).

Discocactus placentiformis. Brazil. Minas Gerais: Mun. Grão Mogol, 31 Jan. 1991, *Taylor et al.* 1512 (K, ZSS, BHCB); Mun. Claros dos Poções, June 1979, *E. Esteves Pereira* 111 (UFG); l.c., 1986, *P.J. Braun* 707 (ZSS); Mun. Jequitaiá, 27 Jan. 1991, *Taylor et al.* 1450 (K, BHCB); Mun. Montes Claros, 8 Sep. 1982, *Horst & Uebelmann* 543 (K, ZSS), Ex cult. ZSS; Mun. Francisco Dumont, 7 Aug. 1988, *Eggl* s.n. (ZSS); Mun. Bocaiúva, *Horst & Uebelmann* 548 (ZSS, K); l.c., Faz. Olho d'Água, 1978, *Horst & Uebelmann* 461 (ZSS); l.c. near Engenheiro Dolabela, 1985, 18 Aug. 1988, *Horst & Uebelmann* 640 (K, ZSS) Ex cult. ZSS; l.c., near Rio Jequitaiá, 3 Sept. 1985, *Horst & Uebelmann* 425 (U, K, ZSS) Ex cult. ZSS; l.c. Engenheiro Dolabela, 24 Mar. 2011, *M.R. Santos* 92-96 (VIC); Mun. Buenópolis, Serra do Cabral, 13 Oct. 1988, *Taylor & Zappi* in *Harley* 24988 (K); l.c., 13 Oct. 1988, *Taylor & Zappi* in *Harley* 24993 (K); l.c., 5 Oct. 1985, *Horst & Uebelmann* 639 (K, ZSS); *ibid.* Serra do Cabral, 1978, 1985, *Horst & Uebelmann* 146 (U, ZSS) Ex cult. ZSS; Mun. Augusto de Lima, 31 Aug. 1985, *Horst & Uebelmann* 356 (K, ZSS) Ex cult. ZSS; *ibid.*, near road BR 135, 4 km from the border of Mun. Corinto, 6 Aug. 1988, *Eggl* 1096 (ZSS); Mun. Joaquim Felício, 20 Oct. 1999, *Hatschbach et al.* 69382 (MBM); l.c. inside Parque Estadual da Serra do Cabral, 22 Oct. 2010, *M.R.Santos* 38 (VIC); l.c., 22 Oct. 2010, *M.R.Santos* 39 (VIC); Mun. Diamantina, 1982, *Horst & Uebelmann* 548 (K, ZSS) Ex cult. ZSS; *ibid.* 10 Dec. 1992, *J.A. Lombardi* 181 (BHCB); l.c.,

near Senador Mourão, 26 Aug. 1985, *Horst & Uebelmann* 704, 707 (K, ZSS) Ex cult. ZSS; l.c., 29 Aug. 1985, *Horst & Uebelmann* 105 (K, U) Ex. Cult. ZSS; *ibid.*, c. 15 km NE of Diamantina on road to Mendanha, 30 Jan. 1969, *Irwin et al.* (K, NY, UB); from Diamantina to Mendanha, 26 Sep. 1994, *Splett* 700 (K,UB); *ibid.*, between Diamantina and Biribiri, 8 Mar. 1995, *Splett* 808 (UB); *ibid.*, estrada para o vilarejo de Três Barras, 15 Apr. 1987, *Zappi* in *CFCR* 10540 (SPF); *ibid.*, estrada para Conselheiro Mata, 18 July 1987, *Zappi et al.* in *CFCR* 11297 (SPF); 1 km from Conselheiro Mata, *Horst & Uebelmann* 542 (ZSS); l.c. near Diamantina, 31 Aug. 1985, 1988, *Horst & Uebelmann* 603 (ZSS) Ex cult. ZSS; l.c. Sopa, 14 May 2011, *M.R. Santos* 101 (VIC); l.c. from Diamantina to Monjolos, 17 May 2011, *M.R. Santos* 108 (VIC); l.c. between Mendanha and Inhaí inside Parque Nacional das Sempre Vivas, 15 May 2011, *M.R. Santos* 105 (VIC); l.c., 15 May 2011, *M.R. Santos* 106 (VIC); Mun. Mendanha, 15 May 2011, *M.R. Santos* 107 (VIC); Mun. Couto de Magalhães de Minas, 3 Aug. 1988, *Eggl* 1056 (ZSS); l.c., s.d., *Horst & Uebelmann* 232 (K, U, ZSS) Ex. cult. ZSS; l.c. Parque Estadual do Biribiri, 14-15 May 2011, *M.R. Santos* 100 (VIC); l.c., 14-15 May 2011, *M.R. Santos* 102 (VIC); Mun. Datas, 5.5 km N of Datas, 17 Feb. 1988, *Supthut* 8835 (ZSS); l.c. Barão do Guaicuy, 1973, 1985, 16 Aug. 1988, *Horst & Uebelmann* 357 (K, ZSS) Ex cult. ZSS; l.c., 18 July 2011, *M.R. Santos* 144 (VIC); l.c., 18 July 2011, *M.R. Santos* 145 (VIC); Mun. Gouveia, just east of Ponte de Paraúna (E of Pres. Juscelino), 16 Oct. 1959, *Heringer* 7228 (UB); Mun. São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, 16 May 2011, *M.R. Santos* 113 (VIC); l.c. Parque Estadual do Rio Preto, Chapada region, 18 May 2011, *M.R. Santos* 112 (VIC); *ibid.* 24 Feb. 2007, *N.F.O Mota et al.* 908 (BHCB); Mun. Serro, 2 Sep. 2002, *R.C.Mota* 1626 (BHCB); Mun. Conselheiro Mata, 17 May 2011, *M.R. Santos* 108 (VIC); l.c., 17 May 2011, *M.R. Santos* 109 (VIC); l.c., 17 May 2011, *M.R. Santos* 110 (VIC); l.c. 54 Km from Ituiutaba, 26 Mar. 1959, *Leg. Castellanos* 22413 (R); Mun. Santana do Pirapama, 21 July 2009, *Zappi. D.C.* 2210 (RB).

Discocactus pseudoinsignis. Brazil. Minas Gerais: Mun. Grão Mogol, 1972, 1974, 1985, *Horst & Uebelmann* 347 (U, K, ZSS) Ex cult. ZSS; l.c., 10 km NW of the town, 22 Oct. 1978, *Hatschbach* 41634 (MBM); l.c., Vale do Ribeirão das Mortes, 4 Sep. 1986, *I. Cordeiro & R.Mello-Silva* in CFCR 10088 (SPF); l.c., Córrego Escurona, 13 May 1990, *Zappi et al.* in CFCR 12901 (SPF); l.c., Várzeas Escuras, 6 Nov. 1997, *F. Fernandes* (K); in Parque Estadual de Grão Mogol, Serra do Barão, 16 March 2011, *Santos, M.R.* 74 (VIC); Mun. Cristália, 28 May 1988, *Zappi et al.* in CFCR 12045 (SPF).; l.c. around the city, 17 March 2011, *Santos, M.R.* 76 (VIC); l.c., 17 March 2011, *Santos, M.R.* 77 (VIC); l.c., 17 March 2011, *Santos, M.R.* 78 (VIC).

Discocactus zehntneri subsp. *boomianus*. Brazil. Bahia: Mun. Sento Sé, *Horst & Uebelmann* 440 (U, ZSS) Ex cult. ZSS; l.c., Mimoso, 1 Jan. 1988, *K.I. Horst* 667 (K, ZSS, HUEFS); l.c., near the village of Campo Largo, 26 Mar. 2004, *M. Machado et al.* 350 – 356 (HUEFS); l.c., near the village of Limoeiro, 28 Apr. 2004, *M. Machado* 400 – 403 (HUEFS); l.c., 28 Apr. 2004, *M.C. Machado* 205c (HUEFS); l.c., 26 Mar. 2004, *M.C. Machado* 201 (HUEFS); Mun. Morro do Chapéu, 25 Dec. 1988, *Taylor & Zappi* in *Harley* 27395 (K); l.c., 11 Oct. 2008, *M.C. Machado* 1167 (HUEFS); l.c., 23 July 1988, *Eggl* 1274 (ZSS); l.c. Cafarnaum towards Morro do Chapéu, s.d., *Horst & Uebelmann* 222 (U, K, ZSS); l.c., 12 Jun. 2004, *M. Machado et al.* 450 – 454 (HUEFS); l.c., 12 Jun. 2004, *M. Machado et al.* 460 – 464 (HUEFS); l.c., 12 Jun. 2004, *M. Machado et al.* 470 – 474 (HUEFS); sin loco, 1980, *J.Y. Clark*, cult. (K); l.c., BA052, 16 Apr. 2011, *Santos, M.R.* 99 (VIC).

Discocactus zehntneri subsp. *petr-halfarii*. Brazil. Bahia: Juazeiro, March 2007, *Zachar, M.* (UFG); l.c., Frade, Dec. 3, 2011, *M.V. Meiado et al.* 750 (HVASF, VIC).

Discocactus zehntneri subsp. *zehntneri*. Brazil. Bahia: Mun. Sento Sé, 5-6 June 1915, *Zehntner* in *Rose & Russell* 19779 (US, NY); l.c., July 1974, *Horst* 441 (U, ZSS); l.c., Serra do Francisco, *Horst & Uebelmann* 390 (U, ZSS) Ex cult. ZSS; l.c., near the village of Brejo Grande, 28 Apr. 2004, *M. Machado* 206 (HUEFS 84844); *ibid.* 1973, *Horst & Uebelmann* 441 (ZSS), Ex cult. ZSS. Ceará: Guaraciaba do Norte, 22 Jan., 2011, *Menezes, M.O.T.* 256 (EAC).

CAPÍTULO III

BIOGEOGRAPHY AND CONSERVATION STATUS ASSESSMENT OF *DISCOCACTUS* PFEIFF. (CACTACEAE): AN ENDEMIC GENUS OF SOUTH AMERICA

This Chapter is formatted for submission to **Botanical Journal of Linnean Society**

Biogeography and conservation status assessment of *Discocactus* Pfeiff. (Cactaceae): an endemic genus of South America

MARIANNA R. SANTOS^{1,4}, FLÁVIA C.P. GARCIA¹, NIGEL P. TAYLOR² AND
CHRISTIANO F. VEROLA³

¹ Programa de Pós-graduação em Botânica, Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Av. P.H. Rolfs, s/n CEP 36570-000, Viçosa, Minas Gerais, Brazil.

² Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, Singapore 259569.

³ Universidade Federal do Ceará, Centro de Ciências, Departamento de Biologia, Campus do PICI, Bloco 906, Fortaleza, Ceará, Brazil.

⁴ Corresponding author: Email: mrodriguesantos@gmail.com

ABSTRACT

Discocactus is a genus of Cactaceae endemic to South America, with 11 species and two heterotypic subspecies recognized, many of them considered threatened, occurring in *Cerrado*, *Caatinga* and *Pantanal* biomes. The aims of this study were characterize the biogeographic patterns, richness, diversity and endemism of *Discocactus* spp.; compare their distribution with Full Protected Conservation Units (FPCUs) and priority areas for biodiversity conservation; and determine conservation status, indicating important areas for conservation. For analysis of the biogeography of the genus studies of herbarium records, field work, parsimony analysis and geographic information systems were undertaken. The conservation status was defined according to IUCN categories. The majority of the species are known from less than ten sites, and two taxa are micro-endemics, being founded in only one place. Almost all species were considered Critically Endangered, Vulnerable or Endangered, but, unfortunately, most of them are not protected in FPCUs. The highest richness and diversity of *Discocactus* is concentrated in the northern region of Bahia (near the Chapada Diamantina and Morro do Chapéu) including *D. bahiensis* and taxa of *D. zehntneri*, and in the Middle of the Espinhaço mountain range (Planalto de Diamantina and Grão Mogol) where *D. placentiformis*, *D. horstii* and *D. pseudoinsignis* are found. The local and regional endemism of *Discocactus* species needs further study, especially in relation to the creation of new protected areas or amplification of the existing Conservation Units.

KEYWORDS: cacti – UPGMA – diversity – richness–geographic distribution.

INTRODUCTION

Discocactus Pfeiff. is a genus of Cactaceae, belonging to subfamily Cactoideae, tribe Cereeae (Crozier, 2005) and is characterized as having low-growing, discoid to depressed-globose, single or caespitose habit (Machado *et al.*, 2005). The fertile part of stem is differentiated into a terminal cephalium, that produces nocturnal white, tubular or funnelshaped flowers, which are sweet scented and pollinated by moths. Their fruits are globose-clavate to oblong, dehiscent by lateral slits, and seeds are helmet shaped to globular, with a strongly tuberculate testa (Buining, 1980; Barthlott & Hunt, 2000).

The genus is endemic to South America, almost all species being restricted to Brazil, just two taxa occurring in bordering areas of Bolivia and Paraguay. In Brazil the genus occurs in *Cerrado*, *Caatinga* and *Pantanal* biomes and its distribution comprises the Midwest, Northeastern and Southeastern (Minas Gerais State) regions of the country (Taylor & Zappi, 2004; Machado *et al.*, 2005; Zappi *et al.*, 2013).

All species of *Discocactus* are considered threatened or endangered (Lüthy, 2001; IUCN, 2012). The intrinsic reasons for this are the small number of populations per species, the generally small population sizes and habitat specificity leading to very localized populations. These factors make *Discocactus* populations extremely vulnerable to habitat modification and destruction and to collecting. Many species inhabit areas of *Cerrado*, a vegetation type that cover areas with poor soil (agronomic point of view), yet it has been systematically converted to agriculture, mainly for soybean plantations.

However, according to Haridasan (2008), the concept of nutritional deficiency well established in agriculture, should not be extended indiscriminately to native plants in natural ecosystems (ecological point of view). The high plant species richness in *Cerrado* is a factor which reinforces the view that the soils of this ecosystem are not poor in nutrients in relation to the needs of native plants. Currently, they are described and cataloged more than 12,300 native species of plants (Mendonça *et al.*, 2008) and the percentage of endemic species (that do not occur elsewhere) reaches 44% (Silva & Bates, 2002). These factors contributed to the *Cerrado* biome be included among the 34 priority ecosystems for conservation on Earth (Mittermeier *et al.* 2004).

Discocactus species as well as other cacti occur on rocky outcrops such as granite, quartzite and limestone that can be affected by the mining. They are very ornamental and commercial collection of plants and seeds is another factor that

endangers the natural populations. As a result, the whole genus is placed in Appendix I of CITES (Lüthy, 2001). Moreover, some species are also included in federal (MMA, 2008) and regional (Biodiversitas, 2006) lists of threatened species within Brazil.

Thus, this study aims to: 1) characterize geographic distribution patterns, richness, diversity and endemism of *Discocactus*; 2) compare the distribution records of the genus with Conservation Units of Full Protection and areas indicated for biodiversity conservation; 3) indicate important areas for the conservation of *Discocactus*; 4) define the conservation status of the species according to the categories and their criteria proposed by IUCN (Lüthy, 2001).

MATERIAL AND METHODS

ANALYSIS OF THE GEOGRAPHICAL DISTRIBUTION PATTERNS, RICHNESS AND DIVERSITY

The data of geographical distribution of 11 species and three heterotypic subspecies of *Discocactus* accepted in the taxonomic revision of the genus (Santos *et al.*, chapter 2) were obtained through examination of specimens in the following herbaria: BHCB, CGMS, EAC, HUEFS, HVASF, K, L, NY, MBM, R, RB, SPF, U, UB, UC, UFMT, UFG, US, VIC and ZSS (Thiers, 2013), besides consulting specialized literature and observation of natural populations.

All distribution data were converted into geographical coordinates (decimal degrees) and localities were plotted on America's Base Map (Bletter *et al.* 2004). Grids of $1^{\circ} \times 1^{\circ}$ were superimposed on the distribution area of the species, representing Operational Geographic Units (OGUs). Analyses were performed for richness (total number of species per grid) and diversity (calculated using the Shannon index, which takes into account the contribution of each species per square). The maps of the distribution of richness and diversity were made using the program DIVA GIS 7.5 (Hijmans *et al.*, 2012).

The distribution patterns were defined according to Menini Neto & Forzza (2013) which are considered species with: (1) widespread distribution, when found in more than ten grid squares; (2) intermediate distribution, when found in two to ten grid squares; (3) restricted distribution, when found in only one grid square, but with more than one known site of occurrence; and (4) micro-endemic, when found in only one grid

square and with only one known site of occurrence (Table 1). We performed a cluster analysis (UPGMA, Unweighted Pair-Group Method with Arithmetic Mean) with set squares, using the Jaccard index in the program Past version 2.17c (Hammer *et al.*, 2001). This analysis aims to verify the existence of floristic blocks that could help in understanding the relationship between species and the environments in which they occur. The methodology was adapted from studies accomplished by Verola (2008) and Menini Neto & Forzza (2013).

DISTRIBUTION OF SPECIES IN PROTECTED AREAS AND INDICATION OF PRIORITY AREAS FOR CONSERVATION

The information about the distribution of the species was overlapped on occurrence of the Full Protection Conservation Units (FPCUs), at both state and federal levels, and the priority areas for conservation of Brazilian biodiversity (Administrative Rule no. 9 of the Ministry of the Environment, 23 January 2007), available on the Ministry of the Environment (Ministerio de Meio Ambiente) website <http://mapas.mma.gov.br/i3geo/datadownload.htm>). The indication of priority areas for conservation of *Discocactus* was based on the previously mentioned analysis and by superimposing their distribution on existing conservation units.

CONSERVATION STATUS

Through observations of populations in the field and analysis of herbarium material, the conservation status was defined for each *Discocactus* species and according to the categories of IUCN (2013), as presented in Table 1. The results were compared with existing lists of threatened plant species both in Brazil (MMA, 2008) and in Minas Gerais State (Biodiversitas, 2007), and then the conservation status of each species was updated.

RESULTS

GEOGRAPHICAL DISTRIBUTION PATTERNS

The species of *Discocactus* are restricted to South America, more specifically to Brazil, Bolivia and Paraguay (Fig. 1) with the following distribution limits: north, Guaraciaba municipality, Ceará state (-4.13 S, -41.05 W), where *Discocactus zehntneri*

Table 1. Conservation status and geographical distributions patterns of *Discocactus*.

Species*	Conservation status			Geographical distribution Patterns
	This study†	Biodiversitas (2007)	MMA‡ (2008)	
<i>Discocactus bahiensis</i>	VU (A2c)	-	Anexo II	Widespread distribution
<i>Discocactus boliviensis</i>	VU (D2)	-	-	Restricted distribution
<i>Discocactus catingicola</i>	LC	-	Anexo II	Widespread distribution
<i>Discocactus diersianus</i>	EN (A2ac)	-	-	Intermediate distribution
<i>Discocactus ferricola</i>	EN (B2ab(iii, v))	-	Anexo II	Restricted distribution
<i>Discocactus hartmannii</i>	CR (A2ac)	-	-	Intermediate distribution
<i>Discocactus heptacanthus</i>	NT	CR	-	Intermediate distribution
<i>Discocactus horstii</i>	VU (D2)	CR	Anexo I	Micro-endemic
<i>Discocactus placentiformis</i>	LC	-	Anexo II	Intermediate distribution
<i>Discocactus pseudoinsignis</i>	EN (B1ab(iii, v))	-	-	Restricted distribution
<i>Discocactus zehntneri</i> subsp. <i>boomianus</i>	VU (B1ab(iii,iv)+D2)) ⁺⁺	-	-	Intermediate distribution
<i>Discocactus zehntneri</i> subsp. <i>petr-halfarii</i> **	CR (A3c; B1ab(iii,v)+2ab(iii,v))	-	-	Micro-endemic
<i>Discocactus zehntneri</i> subsp. <i>zehntneri</i>	NT	-	Anexo II	Intermediate distribution

**This subspecies was evaluated previously as *Discocactus petr-halfarii*, but according to Santos et al (in prep.) it is to be treated as a subspecies of *D. zehntneri*.

† CR, Critically Endangered; EN, Endangered; VU, Vulnerable; LC, Least Concern; NT, Near Threatened.; NE, Not Evaluated. The items in parentheses are the adopted criteria by IUCN (2001) regarding Red List status. ⁺⁺ In the current evaluation, this taxon is not evaluated (NE), but informally may merit this status. It was evaluated previously as Vulnerable by Taylor & Zappi 2004.

‡In MMA list, the species included in Anexo I and Anexo II are considered, respectively, 'threatened' and 'data deficient'.

§Geographical distribution patterns are explained in text.

subsp. *zehntneri* Britton & Rose occurs; *D. hartmannii* (K.Schum.) Britton & Rose reaches further south in San Estanislao, Paraguay (-24.40 W, -56.19 S); east, Lavras de Mangabeira municipality, Ceará (-6.44 S, 38.59 W), where *Discocactus bahiensis* Britton and Rose has been recorded, and west, San Cyrilo, Depto Santa Cruz, Bolivia (-19.02 S, -57.51 W), where *Discocactus boliviensis* Buining & Brederoo is located.

Three species of *Discocactus* occur in more than two Brazilian states, namely: *Discocactus bahiensis* (Bahia, Ceará and Piauí) and *Discocactus catingicola* Buining & Brederoo (Bahia, Goiás, Minas Gerais, Piauí and Tocantins), which are considered widely distributed according to the patterns adopted in this study. *Discocactus heptacanthus* (Barb.Rodr.) Britton & Rose was considered to have an intermediate scale of distribution and occurs in Goiás, Mato Grosso and Mato Grosso do Sul. The other species and their respective distribution patterns are listed in Table 1 and shown in Fig. 1.

RICHNESS AND DIVERSITY PATTERNS AND FORMATION OF FLORISTIC REGIONS

The region with greatest richness is in Eastern Brazil, represented by grid squares 8, 12, 18 and 40. Each of these grids has three species and is located in the Espinhaço mountain range, where the first three comprise the northern part of the Chapada Diamantina in Bahia State and grid 40 corresponds to region of Grão Mogol in Minas Gerais State (Fig. 2A). The diversity map (Fig. 2B) shows that the main centre of diversity of *Discocactus* is formed by grid squares 12, 18 and 40. The secondary centre of diversity of the genus is in grid squares 22 and 48, followed by 8, 9, 41 and 46.

The cluster analysis obtained a high cophenetic correlation coefficient (0.9877) and the following groups were identified: (A) central-south Mato Grosso do Sul and southeastern Paraguay; (B) mid Espinhaço range (northern Minas Gerais); (C) west side of the São Francisco River in Bahia, northern Piauí and Tocantins, Brazil Midwest and (D) lower reaches of the São Francisco River in northern Bahia (Chapada Diamantina), western and northeastern Piauí, and southeastern Ceará. However, the groups C and D do not show a good Bootstrap support (Fig.3).

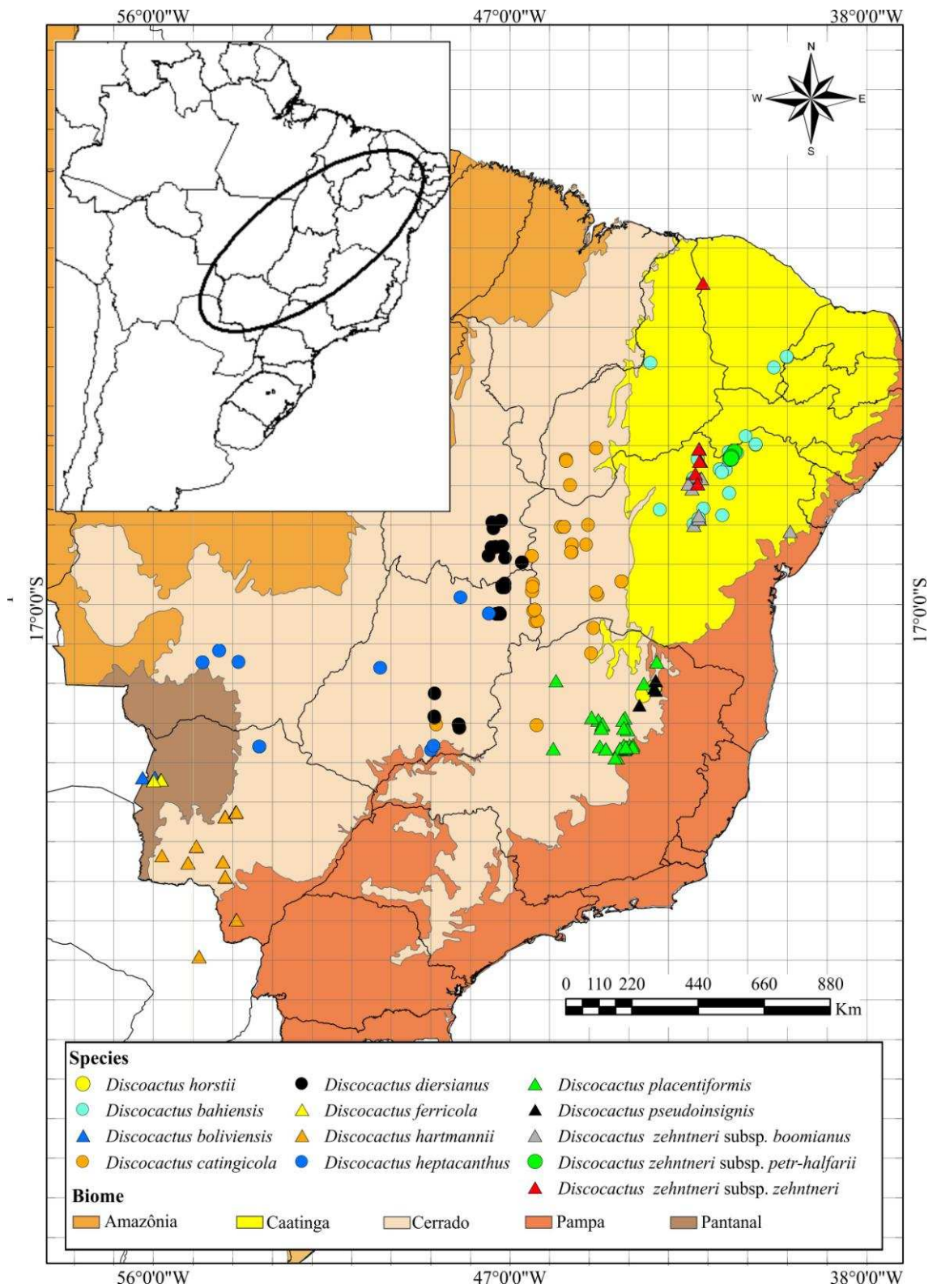


Figure 1. Records of *Discocactus* species in Brazil, Bolivia and Paraguay. Brazilian biomes are shown.

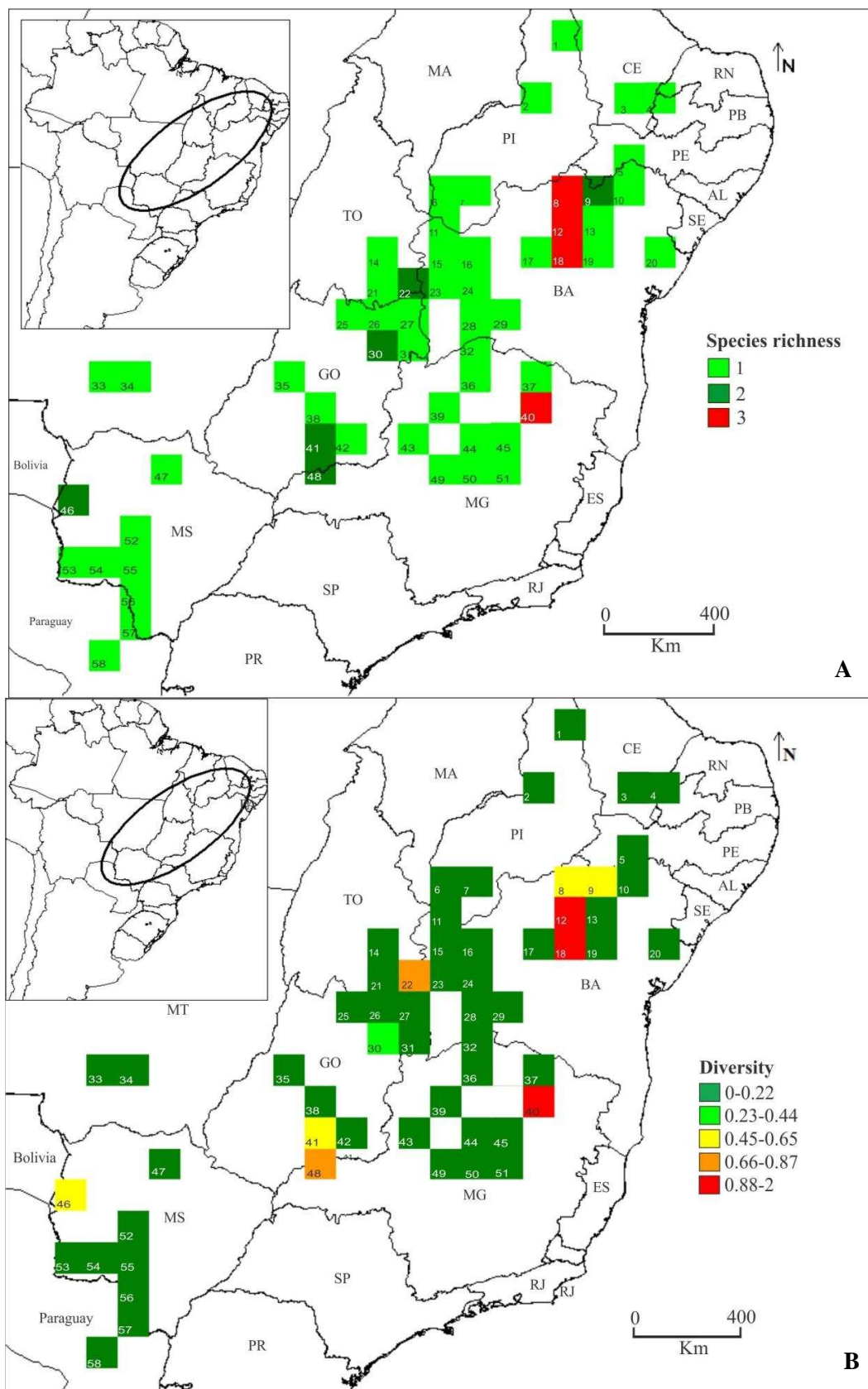


Figure 2. Richness of *Discocactus* using 1°x1° grid squares (A), diversity of the genus calculated by Shannon index (B). The numbers represent the grid squares used in UPGMA analysis.

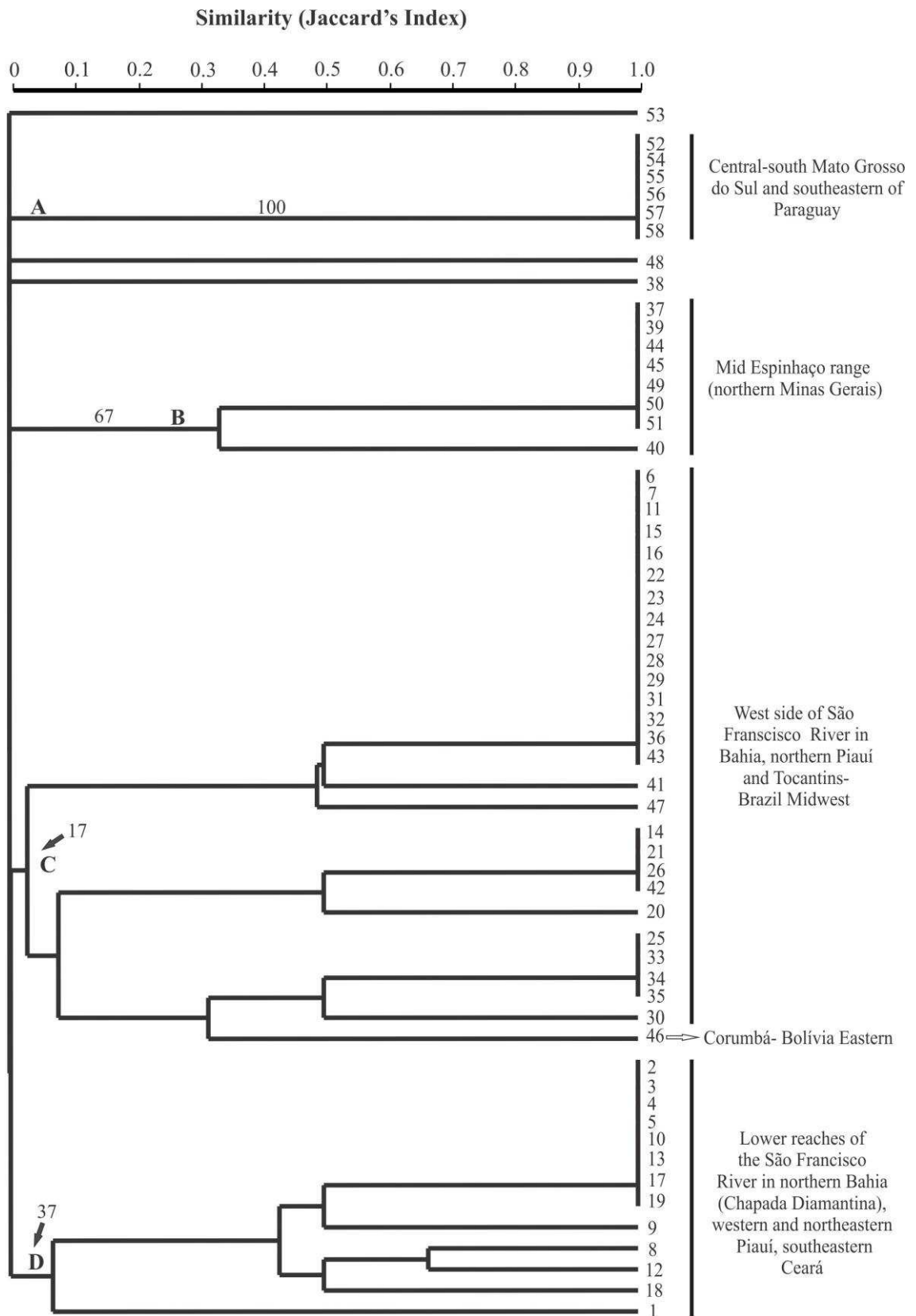


Figure 3. Dendrogram obtained in similarity analysis (UPGMA, Jaccard's index), showing formation of floristic regions (vertical bars). Cophenetic correlation coefficient=0.9877. Bootstrap percentages are given above branches.

DISTRIBUTION OF SPECIES VERSUS CONSERVATION UNITS

The Figure 5 and Table 2 show that only four species of *Discocactus* are recorded in state or federal Full Protection Conservation Units (FPCUs), being all registered in Minas Gerais State in vegetation types of *Cerrado*. Thus, all species of *Discocactus* that occur in this state are protected in one or more CUs shown in Table 2. Most of areas where populations of *Discocactus* species occur (mainly those considered the more threatened (Table 1) is outside of full protected areas or in other types of Conservation Units that not have an effective protection.

CONSERVATION STATUS

The conservation *status* of each *Discocactus* species is presented in Table 1 and compared with the previously published lists of threatened species, showing that the knowledge about conservation status of the genus has been updated. Two taxa are currently placed in the Critically Endangered category (*D. hartmannii* and *D. zehntneri* subsp. *petr-halfarii* (Zachar) M.R. Santos & M.C. Machado for different reasons. *Discocactus hartmannii* occurs in fragmented patches due to severe habitat loss caused by agriculture, and its population has declined. *Discocactus zehntneri* subsp. *petr-halfarii* is CR category because its only population occupies an area less than 10 Km² and is suffering decline due to illegal collection by horticultural interest. Four taxa (*D. bahiensis*, *D. boliviensis*, *D. horstii* and *D. zehntneri* subsp. *boomianus*) are classed in the Vulnerable category, mainly because of a decline in the quality of their habitats and fragmented distribution. *D. horstii*, specifically, occurs in only one place in the Serra do Barão in Grão Mogol municipality, Minas Gerais, but is fortunately protected within a State Conservation Unit. *Discocactus zehntneri* subsp. *boomianus* was not evaluated separately in current IUCN study (IUCN, 2013) but we believe its status may be Vulnerable (Table 1), because is subject to illegal collection and its habitat is fragmented.

Three species (*Discocactus diersianus* Esteves Pereira, *Discocactus ferricola* and *Discocactus pseudoinsignis*) are designated in the Endangered Category, attributed mainly to reduction in extent of occurrence, fragmented distribution and degradation of habitat quality. *Discocactus heptacanthus* and *D. zehntneri* subsp. *zehntneri* are scored as Near Threatened and finally, *D. catingicola* and *D. placentiformis* are judged as of Least Concern, because they have various known populations, generally formed of

many mature individuals and have a wide distribution. *D. catiingicola* is widely distributed in four states, but even though *D. placentiformis* has an intermediate distribution pattern (Table 1), it is broadly distributed in the northern part of Minas Gerais state (Fig.1).

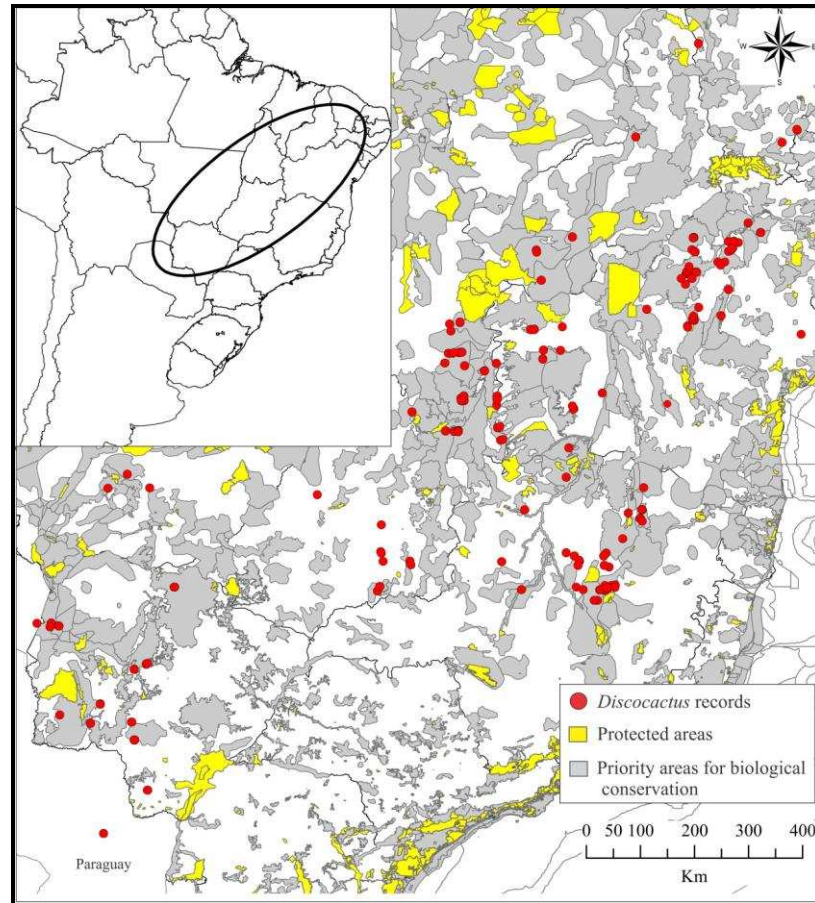


Figure 4. Comparison of *Discocactus* records with the distribution of priority areas for biodiversity conservation, and federal and state conservation units, according to MMA (2007).

DISCUSSION

PATTERNS OF GEOGRAPHICAL DISTRIBUTION, DIVERSITY, RICHNESS AND ENDEMISM

This is the first study focusing on the biogeography of a single genus of Cactaceae in this geographical area, but researches with Cactaceae in general and other plant families, such as Orchidaceae, Bromeliaceae and Eriocaulaceae have been made (Giulietti & Hensold, 1990; Costa *et al.*, 2008; Taylor & Zappi, 2004; Zappi & Taylor, 2008; Verola, 2008; Versieux *et al.*, 2008; Menini Neto & Forzza, 2013).

Table 2. Occurrence of *Discocactus* in Full Protected Conservation Units (FPCUs) in Brazil.

Species	Conservation Units	Sphere	Vegetation Type*
<i>Discocactus catingicola</i>	P.E. Veredas do Peruaçu-MG	State	Campo limpo - next to Veredas
	Refúgio Pandeiros-MG	State	
<i>Discocactus horstii</i>	P.E. de Grão Mogol –MG	State	Campo rupestre
<i>Discocactus placentiformis</i>	P.E. do Biribiri-MG	State	Campo rupestre
	P.E. do Rio Preto-MG	State	
	P.E. Serra do Cabral-MG	State	
	PARNA Sempre Vivas-MG	Federal	
<i>Discocactus pseudoinsignis</i>	P. E. de Grão Mogol-MG	State	Campo rupestre

P.E.= Parque Estadual; PARNA=Parque Nacional * All vegetation type belong to Cerrado Biome.

The patterns of geographical distribution diversity, richness and endemism are similar in part with *Discocactus* species, mainly when the focus is Espinhaço mountain range (in Minas Gerais and Bahia) and disjunct areas that have the *campo rupestre* vegetation type (Giulietti & Hensold, 1990; Giulietti *et al.*, 1997; Verola, 2008; Versieux *et al.*, 2008; Menini Neto & Forzza, 2013).

Verola (2008) studied the biogeographical patterns of *Hoffmannseggella* H.G. Jones, a genus of Orchidaceae, and found as important richness and diversity areas the Planalto da Diamantina and Serra do Cipó region that belong to Espinhaço range. Studies of Eriocaulaceae (Giulietti & Hensold, 1990; Costa *et al.*, 2008) also recognize these areas as having the most diversity, richness and endemism of species of the family, the Chapada Diamantina (Bahia) being in second place.

Already recognized in the Cactaceae, are both aforementioned areas as being characterized by greater diversity, richness and endemism in the family (Taylor & Zappi, 2004; Zappi & Taylor, 2008). For *Discocactus* is not different, because the grid squares 8, 12, 18 (with *D. bahiensis* and *D. zehntneri* subspecies) and 40 (with *D. placentiformis*, *D. pseudoinsignis* and *D. horstii*) (Fig. 2), and groups B and D clustered in UPGMA correspond to these areas. The Figure 3 includes the grids mentioned above

and they form different floristic blocks showing that there is maintenance of the diversity in the Espinhaço mountain range through the substitution of species.

The secondary regions of richness and diversity are outside the Espinhaço range and is formed by grid squares 22 and 48, where *D. catingicola*, which is always associated with *cerrado* vegetation, except *campo rupestre*, *D. heptacanthus* and *D. diersianus* on different types of rock outcrops (arenitic, granitic and iron outcrops known as “canga”) and soils commonly times associated with the dry forest vegetation known as “Mata Seca”.

DEFINITION OF THE STATUS AND INDICATION OF PRIORITY AREAS FOR THE CONSERVATION THROUGH ANALYSIS OF GEOGRAPHICAL DISTRIBUTION

The analysis of geographical distribution has played an important role in determining the natural distribution patterns of organisms (Posadas & Miranda-Esquivel, 1999), evaluating biological affinities between distinct areas (Trejo-Torres & Ackerman, 2001; Sigrist & Carvalho, 2008) and defining areas of endemism and high biodiversity (Smidt *et al.*; 2007; Verola, 2008; Chiron, 2009; Menini Neto & Forzza, 2013). Consequently, it also becomes important from the point of view of conservation, recognizing regions with a distinct biotic composition and high degree of endemism (Posadas & Miranda-Esquivel, 1999).

Discocactus populations are extremely vulnerable to habitat modification and destruction. Moreover, their species are very ornamental and commercial collection of plants and seeds is another factor that endangers the natural populations (Lüthy, 2001). The analysis of the geographical distribution of the species, superimposed on the Conservation Units and priority areas for biodiversity conservation, portrays a negative picture regarding the effective conservation of *Discocactus* species (Fig.4).

We know that the existence of a Conservation Unit is not a guarantee of biodiversity protection, at least in Brazil (because of funding issues and lack an appropriate supervision), but the importance of the protected areas is undeniable, if they are established and administrated properly (Rylands & Brandon, 2005), as they are internationally considered to be the main tool for the conservation of species and ecosystems (UNEP-WCMC, 2008).

Only four species of *Discocactus* were recorded in state and federal Conservation Units, all them in Minas Gerais state, occurring in different vegetation types of *Cerrado* (Table 2). Three of them (*D. placentiformis*, *D. horstii* and *D. pseudoinsignis*) show significant endemism in the southern parts of the Espinhaço range (Planalto Diamantina and Grão Mogol).

With the exception of those species protected in UCs in Minas Gerais and recorded from *cerrado* vegetation, other taxa of the genus that are considered Vulnerable (*D. bahiensis*, *D. boliviensis*, *D. zehntneri* subsp. *boominaus*), Endangered (*D. diersianus*, *D. ferricola*) and Critically Endangered (*D. hartmannii*, *D. zehntneri* subsp. *petr-halfarii*) do not have any populations registered until now inside FPCUs. Some of these species like the *D. zehntneri* subspecies and *D. bahiensis* occur in areas near the Parque Estadual do Morro do Chapéu and PARNA Chapada Diamantina, Bahia, respectively. In this case it may be desirable to expand these units to include such taxa with the reserves. However, for *Discocactus zehntneri* subsp. *petr-halfarii*, which occurs in *caatinga* vegetation, this would require the creation of a new Conservation Unit in Juazeiro, Bahia state, where the only known population is found and with it, some adjacent populations of *D. bahiensis* would benefit.

The two *Discocactus* species that occur in Mato Grosso do Sul are in Pantanal Biome and none is within any Full Protection Conservation Unit. They suffer major threats from human actions. *Discocactus ferricola* occurs on outcrops of “canga” in the region of Corumbá, on private properties where cattle are grazing, as well as exploitation of these outcrops by mining companies. This region needs urgently of implementation of a FPCU, because besides *Discocactus ferricola* that is endemic, occur other cactus species and bromeliads. *Discocactus hartmannii* occurs in a type of Chaco vegetation that is very similar to *cerrado* in Brazil and Paraguay and corresponds to the group of grid squares clustered in UPGMA in the group A (Fig. 3). It shows the importance of conservation, in spite of not being endemic to a only one grid square, since this species is endemic to central-southern Mato Grosso do Sul state and southeastern Paraguay.

Inside Brazil, *D. hartmannii* is recorded in some municipalities like Bonito, Jardim and Porto Murtinho that are included in area of the PARNA da Serra da Bodoquena, but their populations are have not been registered up till now within the

park's boundary. Here, a study is recommended to consider whether these areas of occurrence can be included with the park or, if this is not possible, due to the great fragmentation of remaining natural habitats found in the region (soybean cultivation and livestock), its populations should be included in protected areas of municipal status, for protection in each municipality where it occurs.

The conservation unit nearest to populations of *Discocactus heptacanthus* and *D. diersianus* is PARNA Chapada dos Veadeiros, Goiás, which is within the *Cerrado* Biome. Until now no population of either species was found inside the limits of the park. Therefore, it is necessary to perform a complete survey throughout the park area, to determine whether these taxa occur inside the boundary of PARNA.

DISCOCACTUS AND HABITAT ADAPTATION

Cerrado or Brazilian Savanna is an ecosystem adapted to fire (Eiten, 1972). The fires have occurred with historic frequency in *Cerrado* for several years and even severe events do not destroy this vegetation. There are records of fire in *Cerrado* since the late Pleistocene, 32.400 years ago (Miranda *et al.*, 2002). Therefore, the fire influences the distribution and floristic composition of *Cerrado*, affecting vegetation structure and acting positively on groups adapted and negatively in non-adapted groups (Moreira, 1996, 2000; Neves *et al.*, 2011). The widespread occurrence of *Discocactus* in *cerrado* areas (Fig. 1), confirms that this genus among many of Cactaceae in South America, is a group belonging to species adapted to the fire dynamics of this biome.

Morphological characteristics such as the flattened stem, epidermis coated by a layer of wax, plus the ability to grow on different types of substrates (sand, gravel, and rocky outcrops), where fire can often be avoided, are some of the adaptations that may be allowing the colonization of *Discocactus* species in vegetation types of *Cerrado*. These characteristics make the genus evolutionarily successful.

Unlike other taxa allied to the genus (*Melocactus* (L.) Link & Otto, *Coleocephalocereus* Backeb. and *Arrojadoa* Britton & Rose), *Discocactus* species survive fires, where the vegetative parts are partially damaged (in the region of the areole, the apex of the ribs and cephalium). However, there is a high survival rate (personal observation). *Arrojadoa dinae* Buining & Brederoo and *Cipocereus crassissepalus* Buining & Brederoo are an exception, but they have a different survival

mechanism, because they produce tuberous stems or roots, and with fire, the shoot dies. Following the plant regenerates from the underground storage structure but may take years to reach reproductive capability again. *Discocactus* has a stem and cephalium that ensure that reproduction, even after this type of damage, can continue. Species with such adaptations to fire are those with a broad distribution, this fact was observed for this study in *D. catingicola*, *D. diersianus*, *D. placentiformis*, *D. hartmannii* and *D. heptacanthus* (Table 1, Fig. 1), the latter being found more on limestone outcrops that occur in areas of transition between Cerrado and dry forest.

However, not all species of the genus have such capacity. Those species that occur in the Caatinga biome that are older groups (*D. bahiensis*, *D. zehntneri*) and are also occasionally subject to fire, cannot resist burning, unless they occur on rocky outcrops, which is the case of *D. zehntneri*, except *D. zehntneri* subsp. *petr-halfarii* and *D. bahiensis*, that occur in a site with gravelly and loamy soil.

CONCLUSIONS

The highest richness and diversity of *Discocactus* is concentrated in the northern region of Bahia (near Chapada Diamantina and Morro do Chapéu) where there occur *D. bahiensis* and *D. zehntneri*, *sens. lat.*, and in the Middle of the Espinhaço mountain range (Planalto de Diamantina and Grão Mogol) where occur *D. placentiformis*, *D. horstii* and *D. pseudoinsignis*. This study found two types of endemism, i.e. at local and at regional level. The first is represented by the occurrence of *D. horstii* in Grão Mogol, Minas Gerais and *D. zehntneri* subsp. *petr-halfarii* near Juazeiro, Bahia. The regional endemism is represented by the occurrence of *D. ferricola* and *D. boliviensis* near Corumbá and in adjacent eastern Bolivia and by *D. hartmannii* that occurs in the south-central region of Mato Grosso do Sul and southeastern Paraguay. Other regional endemism is represented by the occurrence of *Discocactus placentiformis* in the Planalto de Diamantina and Serra do Cabral, and *D. pseudoinsignis* distributed throughout the region of Grão Mogol.

Two species of the genus (*D. catingicola* and *D. placentiformis*) were considered Least Concern, so most of the species and subspecies of the genus (84%) are threatened, mainly by habitat destruction and illegal collection. Only FPCUs in the State of Minas Gerais are effectively protecting four species of *Discocactus*, it being desirable that a thorough survey of Conservation Units is made to determine the presence or absence of

other *Discocactus* species that require protection. If they are not found within the limits of protected areas, it will be necessary to study the expansion of existing Conservation Units or create new CUs, especially where locally and regionally endemic taxa occur. In addition, there is a great need for partnership between federal, state and municipal governments and also among neighbour countries for the creation of a conservation strategy for *Discocactus* species and other cacti that are under threat, but until now are not effectively protected.

It is noteworthy that the evolutionary success of *Discocactus* species relates to the colonization of *Cerrado* environments, where the fire regime prevents the occurrence of other cacti that are not adapted to its dynamics. Studies on reproductive biology, physiology and anatomy are indispensable for understanding more about the success of this genus.

Moreover, it is important to highlight that conservation of the areas where *Discocactus* occur is an opportunity to protect several threatened species belonging to other families, including Bromeliaceae, Eriocaulaceae, Gesneriaceae, Orchidaceae and Velloziaceae. Many of these are important in the context of ornamental horticulture, and endangered because of pressures created by collection and destruction of their habitat, especially outside the CUs. Therefore, the creation of FPCUs incorporating the areas containing different types of substrate (rock outcrops, sand and gravel) is of extreme importance to the preservation of the richness and endemism of *Discocactus*, cacti in general and other botanical families.

ACKNOWLEDGEMENTS

We would like to thank the Instituto Estadual do Meio Ambiente of Minas Gerais (IEF) and Instituto Chico Mendes (ICMBIO-MMA) for provision of collecting permits and all directors of the park reserves visited. This study was supported by grants from CAPES (PhD scholarship), FAPEMIG (TO APQ-01314-11-CC8034), the Cactus Succulent Society of America (CSSA), the British Cactus and Succulent Society (BCSS). Finally, we would like to thank the Instituto Chico Mendes (ICMBIO-MMA) for financing some field trips and Itayguara Ribeiro da Costa for help in the analyzes.

REFERENCES

- Barthlot W, Hunt D. 2000.** Seed-diversity in the Cactaceae: subfamily Cactoideae. *Succulent Plant Research* 5.
- Bletter N, Janovec J, Brosi B, Douglas CD. 2004.** A digital base map for studying the Neotropical flora. *Taxon* 53: 46–477.
- Biodiversitas. 2007.** Revisão das listas das espécies da flora e da fauna ameaçadas de extinção do estado de Minas Gerais, Vol. 2. Belo Horizonte: Fundação Biodiversitas.
- Buining AFH. 1980.** The genus *Discocactus* Pfeiffer. Buining-fonds, Succulenta, The Netherlands. 224 pp.
- Chiron GR. 2009.** Riqueza e endemismo de espécies de *Baptistonia* (Orchidaceae) no Brasil. *Hoehnea* 36: 459–477.
- Costa FN, Trovó M, Sano PT. 2008.** Eriocaulaceae na Cadeia do Espinhaço: riqueza, endemismo e ameaças. *Megadiversidade* 4: 89–97
- Crozier BS. 2005.** Systematics of Cactaceae Juss.: phylogeny, cpDNA evolution, and classification, with emphasis on the genus *Mammillaria* Haw, 154 pp. PhD. Dissertation- The University of Texas, Austin.
- Eiten, G.1972.** The cerrado vegetation of Brazil. *The Botanical Review* 38:201-341.
- Giulietti AM, Hensold N. 1990.** Padrões de distribuição geográfica dos gêneros de Eriocaulaceae. *Acta Botanica Brasilica* 4: 133–158.
- Giulietti AM, Pirani JR, Harley RM. 1997.** Espinhaço Range region – eastern Brazil. The Americas. In: Davis SD, Heywood VH, Herrera-MacBryde O, Villa-Lobos J, Hamilton AC, eds. *Centres of plant diversity, Vol. 3*. London: World Wild Fund for Nature and World Conservation Union, 397–404.

- Hammer Ø, Harper DAT, Ryan PD. 2001.** PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 1–9.
- Haridasan M. 2008.** Nutritional adaptations of native plants of the cerrado biome in acid soils. *Brazilian Journal of Plant Physiology* **20**: 183-195.
- Hijmans RJ, Guarino L, Mathur P. 2012.** DIVA-GIS Version 7.5. Developed by Bioversity International, the UC Berkeley Museum of Vertebrate Zoology, the International Potato Center (CIP), SINGER/SGRP, FAO, and USDA. Available for download in < <http://www.diva-gis.org/>>.
- Hunt D, Taylor NP, Charles G. 2006.** The new cactus lexicon, vols. I, II. dh Books, Milborne Port, UK.
- IUCN 2013.** IUCN Red List of Threatened Species. Version 2013.1. <www.iucnredlist.org>. Downloaded on 11 August 2013.
- Lüthy J. 2001.** The Cacti of CITES Appendix I. Bundesamt für Veterinärwesen, Switzerland.
- Machado, M.C. 2004.** O gênero *Discocactus* Pfeiff. (Cactaceae) no estado da Bahia, Brasil: variabilidade morfológica, variabilidade genética, taxonomia e conservação, 113p. Unpublished M.Sc. Thesis- Universidade Estadual de Feira de Santana, Feira de Santana.
- Mendonça RC, Felfili JM, Walter BMT, Silva Júnior MC, Rezende AV, Filgueiras TS, Nogueira PE & Fagg CW. 2008.** Flora Vascular do Bioma Cerrado. In: Sano, S.M.; Almeida, S.P. & Ribeiro, J.F. (Eds.). Cerrado: ecologia e flora. Brasília: Embrapa Informação Tecnológica. p.421-1.279.
- Menini Neto L, Forzza RC. 2013.** Biogeography and conservation status assessment of *Pseudolaelia* (Orchidaceae). *Botanical Journal of the Linnean Society* **171**:191–200.
- Miranda HS, Bustamante MMC, Miranda AC. 2002.** The fire factor. In: Oliveira PS, Marquis RJ (ed.). *The Cerrados of Brazil: ecology and natural history of a neotropical savanna*. New York: Columbia University Press. Pp. 51-68.

- Ministério do Meio Ambiente. 2008.** Instrução normativa nº6 de 23 de setembro de 2008.
- Mittermeier RA, Gil PR, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J & Fonseca GAB. 2004.** Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. Mexico City: CEMEX. 392p.
- Moreira AG. 1996.** Proteção contra o fogo e seu efeito na distribuição e composição de cinco fitofisionomias do cerrado. In: Miranda HS, Saito CH, Dias BRS (ed.). *Impactos de queimadas em áreas de cerrado e restinga*. Brasília, Dept. Ecologia, Universidade de Brasília. Pp. 112-121.
- Moreira AG. 2000.** Effects of fire protection on savanna structure in central Brazil. *Journal of Biogeography* **27**:1021-1029.
- Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kents J. 2000.** Biodiversity hotspots for conservation priorities. *Nature* **403**: 853–858.
- Neves ACO, Bedê LC, Martins RP. 2011.** Revisão sobre os Efeitos do Fogo em Eriocaulaceae como Subsídio para a sua Conservação. ICMBIO: *Número Temático: Ecologia e Manejo de Fogo em Áreas Protegidas*.
- Posadas P, Miranda-Esquivel DR. 1999.** El PAE (parsimony analysis of endemism) como una herramienta en la evaluación de la biodiversidad. *Revista Chilena de Historia Natural* **72**: 539–546.
- Rylands AB, Brandon K. 2005.** Unidades de conservação brasileiras. *Megadiversidade* **1**: 27–35.
- Santos MR, Garcia FCP, Machado MC, Taylor NP.** Taxonomy, distribution and conservation of *Discocactus* Pfeiff. (Cactaceae). In: Santos, M.R. (2013) Filogenia molecular, taxonomia, biogeografia e conservação de *Discocactus* Pfeiff. 114 p. PhD thesis – Universidade Federal de Viçosa.
- Sigrist MS, Carvalho CJB. 2008.** Detection of areas of endemism on two spatial scales using parsimony analysis of endemism (PAE): the Neotropical region and the Atlantic Forest. *Biota Neotropica* **8**: 33–42.

- Silva JMC & Bates JM. 2002.** Biogeographic Patterns and Conservation in the South American Cerrado: A Tropical Savanna Hotspot. *BioScience* 52(3): 225-233.
- Smidt EC, Silva-Pereira V, Borba EL, van den Berg C. 2007.** Richness, distribution and important areas to preserve *Bulbophyllum* in the Neotropics. *Lankesteriana* 7: 107–113.
- Taylor NP, Zappi DC. 2004.** Cacti of eastern Brazil. England: Richmond, Surrey: Royal Botanic Gardens, Kew, 499 p.
- Thiers B. 2013.** [continuously updated]. *Index Herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available at: <http://sweetgum.nybg.org/ih/> Accessed September 2010.
- Trejo-Torres JC, Ackerman JD. 2001.** Biogeography of the Antilles based on a parsimony analysis of orchid distributions. *Journal of Biogeography* 28: 775–794.
- UNEP-WCMC. 2008.** *State of the world's protected areas: an annual review of global conservation progress*. Cambridge: UNEP-WCMC.
- Verola CF. 2008.** Estudos biosistemáticos em espécies de *Hoffmannseggella* H. G. Jones (Orchidaceae: Laeliinae) ocorrentes nos complexos rupestres de altitude. Unpublished Doctoral Thesis, Universidade Estadual de Campinas.
- Versieux LM, Wendt T, Louzada RB, Wanderley MGL. 2008.** Bromeliaceae da Cadeia do Espinhaço. *Megadiversidade* 4: 98–110.
- Zappi DC, Taylor N. 2008.** Diversidade e endemismo das Cactaceae na Cadeia do Espinhaço. *Megadiversidade* 4: 111–116.
- Zappi DC, Taylor N, Machado M, Santos, MR. 2013.** *Discocactus* in Lista de espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. (<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB1482>).

CONCLUSÕES GERAIS

São reconhecidas 11 espécies e 3 subespécies de *Discocactus*: *D. bahiensis*, *D. boliviensis*, *D. catingicola*, *D. diersianus*, *D. hartmannii*, *D. heptacanthus*, *D. horstii*, *D. placentiformis*, *D. pseudoinsignis*, *D. zehntneri* e suas três subspécies heterotípicas: subesp. *boomianus*, subesp. *petr-halfarii* e subesp. *zehntneri*. A circumscrição adotada baseia-se nos resultados obtidos na literatura e a partir de dados morfológicos e filogenéticos apresentados neste estudo.

As sete regiões do DNA (4 plastidiais e 2 nucleares) apresentaram baixa variação em comparação a outros estudos, o que culminou na baixa resolução das árvores construídas pelos métodos de parsimônia e Bayesiana. No entanto, algumas regiões como *trnS-trnG*, *rpl16*, *ITS* e *phyC* apresentaram o maior número de sítios informativos, podendo ser indicados como uma boa escolha para estudos futuros envolvendo outros grupos em Cactaceae. Os indels de algumas regiões (*trnS-trnG*, *rps16*, *psbA-trnH* e *matK*) também mostraram-se bastante informativos, contribuindo com bons resultados de CI e RI e, conseqüentemente, com a resolução das árvores apresentadas.

Os resultados relacionados ao estudo filogenético (Capítulo 1) e taxonômico (Capítulo 2) geraram algumas mudanças na taxonomia de *Discocactus*, como: o novo status de *D. petr-halfarii* que passou a ser considerado como subspécie de *D. zehntneri*. Sinônimos antes relacionados a outras espécies foram reposicionados: *D. silicicola*, por exemplo, consideradorelacionada como sinônimo de *D. catingicola*, neste trabalho foi reposicionado como sinônimo de *D. hartmannii*; *D. lindanus*, que era sinônimo de *D. diersianus*, mostrou-se mais relacionado à *D. heptacanthus*.

O estudo biogeográfico e de conservação de *Discocactus*, mostrou que a grande riqueza e diversidade do gênero estão concentradas em duas regiões: uma ao norte da Bahia (Chapada da Diamantina e Morro do Chapéu), onde ocorrem *D. bahiensis* e *D. zehntneri sens. lat.*; e na região central da Cadeia do Espinhaço em Minas Gerais

(Planalto de Diamantina e Grão Mogol), onde ocorrem *D. placentiformis*, *D. horstii* e *D. pseudoinsignis*.

Foram identificados dois tipos de endemismo, em nível local, representado por *D. horstii* em Grão Mogol, MG e *D. zehntneri* subesp. *petr-halfarii* em Juazeiro, BA e em nível regional, representado por *D. ferricola* e *D. boliviensis* em Corumbá/oeste da Bolívia, *D. hartmannii* que ocorre no centro-sul do Mato Grosso do Sul e Sudeste do Paraguai, *D. placentiformis* (Planalto de Diamantina e Serra do Cabral) e *D. pseudoindignis* que está distribuída em toda região de Grão Mogol no Vale do Jequitinhonha. É importante ressaltar o sucesso evolutivo de *Discocactus* na colonização de ambientes de Cerrado, onde o regime de queimadas impede a ocorrência de outros cactos não adaptados à dinâmica deste tipo de vegetação.

Quase todas as espécies do gênero são consideradas ameaçadas de extinção em menor ou em maior grau, principalmente, devido à fragmentação do habitat e coleta ilegal. A proteção efetiva de espécies ameaçadas de extinção ainda deixa a desejar, pois apenas quatro espécies de *Discocactus* (*D. placentiformis*, *D. catingicola*, *D. pseudoinsignis* e *D. horstii*) estão protegidas em Unidades de Conservação de Proteção Integral, sendo todas as UCs localizadas no Estado de Minas Gerais. A maioria dos registros de ocorrência das demais espécies em outros estados se encontram no entorno de UCs.

Com isto, há uma grande necessidade de parcerias entre governos federal, estadual, municipal e também entre governos de países vizinhos para elaboração de políticas e estratégias de conservação para que as espécies de *Discocactus* e de outras cactáceas ameaçadas sejam devidamente protegidas, seja expandindo o limite de UCs já existentes ou criando novos parques.

Como perspectivas para novos estudos, devido a grande variação morfológica e baixa variação genética encontrada para *Discocactus*, a seleção de mais regiões de DNA e estudos que abordem a variabilidade genética e morfológica são imprescindíveis para uma melhor delimitação de suas espécies assim como a resolução de relação filogenética entre elas. Além disto, estudos sobre a fenologia, polinização e biologia reprodutiva são importantes para entendermos como é o comportamento ecológico e biológico das espécies do gênero frente a todas as ameaças que vem sofrendo, e que podem estar comprometendo a manutenção destas espécies em seu habitat natural.