

MARLON CÂMARA MACHADO

**O gênero *Discocactus* Pfeiff. (Cactaceae) no estado da Bahia,
Brasil: variabilidade morfológica, variabilidade genética,
taxonomia e conservação**

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PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

**O gênero *Discocactus* Pfeiff. (Cactaceae) no estado da Bahia,
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taxonomia e conservação**

MARLON CÂMARA MACHADO

Dissertação apresentada ao Programa de Pós-Graduação em Botânica da Universidade Estadual de Feira de Santana como parte dos requisitos para a obtenção do título de *Mestre em Botânica*.

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RESUMO

De forma a elucidar as relações taxonômicas entre os táxons de *Discocactus* Pfeiff. (Cactaceae) descritos para o estado da Bahia, foram investigadas a variabilidade morfológica e a variabilidade genética de 337 indivíduos em 17 populações naturais, nove das quais localidades-tipo para táxons descritos. Os padrões de variação morfológica foram analisados com o uso de métodos estatísticos e multivariados, e métodos para delimitação de espécies baseados em caracteres e em árvores filogenéticas. O protocolo utilizado para delimitação de espécies com base em caracteres foi modificado de forma a também poder ser utilizado na distinção de táxons infra-específicos. Os métodos empregados diferem no número de espécies reconhecidas, porém o desacordo ocorre principalmente no nível de resolução taxonômica alcançado. Os resultados obtidos são comparados de forma crítica, e três espécies são reconhecidas: *D. bahiensis* Britton & Rose, *D. catiungicola* Buining & Brederoo e *D. zehntneri* Britton & Rose, a última consistindo de duas subespécies, *D. zehntneri* subsp. *zehntneri* e *D. zehntneri* subsp. *boomianus* (Buining & Brederoo) N.P.Taylor & Zappi. A variabilidade genética das populações foi investigada utilizando-se marcadores enzimáticos. A diversidade de aloenzimas em dez loci revelou um alto grau de diversidade genética quando comparado a outras espécies vegetais, porém similares aos valores médios obtidos para outras espécies de Cactaceae. Foi detectada deficiência de heterozigotos nas populações de *D. bahiensis* e *D. zehntneri*. Níveis baixos a moderados de diferenciação genética foram detectados entre as populações de *D. bahiensis* e *D. catiungicola*, enquanto que níveis mais elevados são encontrados em *D. zehntneri*, o que é atribuído a uma partição geográfica das populações desta espécie. Evidência para introgressão com *D. zehntneri* foi encontrada em uma população de *D. bahiensis*. Ausência de subgrupos e elevadas identidades genéticas entre populações co-específicas de *D. bahiensis* e *D. catiungicola* não sustentam o reconhecimento de táxons infra-específicos nestas espécies, sendo congruente com as circunscrições taxonômicas obtidas com base nas análises morfométricas. Para *D. zehntneri*, caracteres morfológicos permitem a divisão da espécie em duas subespécies, enquanto que aloenzimas repartem as populações em uma base geográfica. Com base nos resultados obtidos a partir das análises morfológicas e genéticas, modificações nos sistemas de classificação atuais são propostas. Descrições expandidas para os táxons reconhecidos também são apresentadas e o estado de conservação das populações é discutido, sendo propostas a criação de reservas para a manutenção da variabilidade morfológica e genética destes táxons.

ABSTRACT

In order to elucidate the taxonomic relationships among the taxa of the genus *Discocactus* Pfeiff. (Cactaceae) described for the state of Bahia, an investigation of morphologic and genetic variability was conducted on 337 individuals belonging to 17 populations, nine of which being type-localities for described taxa. The patterns of morphologic variation were analyzed with statistic and multivariate methods, and protocols for species delimitation based on characters and phylogenetic trees. The character-based protocol was modified in order to be utilized in the distinction of taxa at the infraspecific level. The methods employed disagree on the number of species recognized; however, disagreement occurs mostly on the level of resolution achieved. The results obtained are critically compared, and three species are thus recognized: *D. bahiensis* Britton & Rose, *D. catingicola* Buining & Brederoo and *D. zehntneri* Britton & Rose, the latter with two subspecies, *D. zehntneri* subsp. *zehntneri* and *D. zehntneri* subsp. *boomianus* (Buining & Brederoo) N.P.Taylor & Zappi. The genetic variability of the populations was investigated using allozyme markers. Allozyme diversity at ten loci reveals a high degree of genetic diversity compared to other plant taxa, but similar to average values for other cactus species. Within-population heterozygosity deficit was detected in *D. bahiensis* and *D. zehntneri*. Moderate to low levels of genetic differentiation were detected among populations of *D. bahiensis* and *D. catingicola*, while high levels are found in *D. zehntneri*, which are attributed to a north and south geographic partition of the populations. Evidence for introgression with *D. zehntneri* was found in one population of *D. bahiensis*. Absence of subgroups and high genetic identities among conspecific populations of *D. bahiensis* and *D. catingicola* do not support recognition of infraspecific taxa within these species, being congruent with their taxonomic circumscriptions based on the morphometric analyses. For *D. zehntneri*, morphology supports a division of the species in two subspecies, while allozymes partition the populations on a geographical basis. Modifications are proposed to the current taxonomic circumscriptions of the taxa occurring in the state, with basis on the results obtained from the morphologic and genetic analyses. Expanded descriptions of the recognized taxa are presented, and the conservation status of the populations is discussed. The creation of local reserves is proposed in order to maintain the morphologic and genetic variability of the taxa.

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INTRODUÇÃO GERAL

Cactaceae Juss., com cerca de 100 gêneros e mais de 1300 espécies (Cronquist 1981, Barthlott & Hunt 1993, Hunt 1999), é a segunda maior família de plantas superiores endêmicas da região Neotropical (Taylor & Zappi 2004). Os cactos se distribuem nas Américas desde o oeste e sul do Canadá ao sul do Chile (Patagônia), sendo mais frequentes nas zonas de climas quentes e secos situados entre as latitudes 35° N e 35° S, excetuando-se a zona úmida equatorial (Taylor 1997). A única exceção é o gênero *Rhipsalis*, cuja distribuição atual se estende a regiões tropicais do continente Africano e Madagascar, e ao sul da Índia e Sri Lanka no continente Asiático (Wallace & Gibson 2002). A família Cactaceae ocorre em um amplo espectro de condições ecológicas e climáticas, desde desertos onde quase não chove, como em partes do deserto de Atacama no noroeste do Chile, até ambientes úmidos de floresta tropical que recebem mais de 2000 mm de chuva por ano, além de encontrarem-se distribuídos desde o nível do mar até cerca de 5200 m de altitude nos Andes (Taylor 1997).

A família possui quatro centros de diversidade principais nas Américas (Taylor 1997), dos quais o primeiro em ordem de importância é o México e sudoeste dos Estados Unidos, que abrigam cerca de 27% dos gêneros. O segundo centro de diversidade, abrangendo cerca de 18% dos gêneros, ocorre na cadeia dos Andes, compreendendo Peru, Bolívia, sul do Equador e nordeste do Chile. O terceiro centro em ordem de importância é o leste do Brasil, definido como as regiões Nordeste e Sudeste, e o leste dos estados de Goiás e Tocantins. A região abriga cerca de 11% dos gêneros da família, e aproximadamente 80% das espécies são endêmicas. O quarto centro de diversidade compreende Paraguai, Uruguai e Argentina e as regiões Centro-oeste e Sul do Brasil (Taylor 1997).

As espécies de Cactaceae são em geral xerófitas e suculentas, perenes, adaptadas às regiões semi-áridas das Américas. Os cactos possuem hábitos diversos: arbóreo, arbustivo, subarbustivo, trepador, epífito ou geófito; apresentam raiz fibrosa ou tuberosa. O caule em geral assume formas colunares, cilíndricas, globulares, aladas ou achatadas, sendo frequentemente segmentado, e na maioria das vezes sem folhas típicas. As folhas, quando presentes, são dispostas em espiral ao longo do caule, sendo simples e inteiras, porém mais frequentemente modificadas em espinhos. As gemas axilares se desenvolvem em ramos muito curtos e comprimidos, cobertos com um indumento persistente de espinhos e/ou pêlos, de onde se originam novos ramos ou botões florais (uma característica única das

Cactaceae, denominada aréola). A subfamília Opuntioideae K.Schum. se caracteriza por apresentar um tipo de espinho denominado gloquídeo, que são curtos e fortemente farpados (Barthlott & Hunt 1993). A base da folha subentendendo cada aréola é modificada em um tubérculo, sendo que os tubérculos podem se fundir verticalmente, formando costelas. As flores são solitárias, raramente surgindo em cachos, geralmente sésseis, mais raramente sendo pediceladas ou surgindo em inflorescências do tipo panícula ou cimosa; as flores são em geral conspícuas, bissexuais, actinomorfas ou mais raramente zigomorfas. O receptáculo inclui a zona em torno do ovário (“pericarpelo”) e prolonga-se entre o ovário e o perianto (hipanto perigínico), sendo nu ou coberto por escamas tipo brácteas e/ou aréolas. As tépalas são freqüentemente numerosas. Os estames estão presentes em grande número, com antera 2-locular, tetraesporangiada, de deiscência longitudinal. O ovário é ínfero (exceto em algumas espécies de *Pereskia* Mill.), unilocular, 3 – 20 carpelar, com óvulos numerosos, e o estilete é longo, com estigma 3 – 20 lobado. Os frutos são suculentos ou secos, nus, escamados, pilosos, eriçados ou espinescentes, indeiscentes ou variavelmente deiscentes. As sementes são numerosas, algumas vezes estrofioladas, na subfamília Opuntioideae envoltas em um envelope funicular (ariladas), e possuem testa variavelmente esculpada. O embrião é em geral fortemente curvado, mais raramente quase reto, e os cotilédones são bastante reduzidos ou vestigiais, raramente foliáceos (Barthlott & Hunt 1993).

A família Cactaceae pertence à ordem Caryophyllales, que possui cerca de 15 famílias, entre as quais se incluem Caryophyllaceae, Aizoaceae, Portulacaceae, Nyctaginaceae e Amaranthaceae (Judd et al. 1999). As famílias dessa ordem possuem um conjunto de sinapomorfias distintas que confirmam o status monofilético da ordem. São caracteres florais, vegetativos, embriológicos, químicos e fisiológicos distintos, entre os quais se destacam: sementes contendo embriões bastante curvos, dispostos na periferia de um perisperma nutritivo central, diferindo das outras espécies vegetais que em geral possuem endosperma como tecido nutritivo; e presença de betalaínas, uma classe de pigmentos nitrogenados derivados de tirosina, que substitui os pigmentos antocianinas derivadas de flavonóides geralmente encontrados em outras plantas. Estudos recentes de seqüências de DNA de cloroplastos (cpDNA) mostram que as famílias de Caryophyllales apresentam a perda do intron *rpl2* (Wallace & Gibson 2002).

De acordo com evidências recentes, Cactaceae se encontra mais proximamente relacionada às famílias Portulacaceae, Didieraceae e Basellaceae, com as quais formam a subordem Portulacineae Thorne (Cronquist & Thorne 1994). Análises obtidas de

sequenciamento de genes confirmam este posicionamento, e indicam que a família Cactaceae evoluiu de uma linhagem de Portulacaceae que atualmente inclui os gêneros *Talinum* e *Portulaca*, o que torna a família Portulacaceae parafilética em relação a Cactaceae (Wallace & Gibson 2002). Cactaceae é uma família considerada monofilética, opinião baseada no seu conjunto único de caracteres, destacando-se a sinapomorfia de gemas axilares modificadas em ramos curtos, as aréolas; esta hipótese é confirmada de forma conclusiva por análises recentes de seqüências de DNA (Wallace & Gibson 2002).

A família Cactaceae encontra-se atualmente dividida em quatro subfamílias: Maihuenoideae e Pereskioideae, que possuem caracteres vegetativos e reprodutivos considerados plesiomórficos; Opuntioideae, definida dentre outras sinapomorfias pela presença de tufo de gloquídeos nas aréolas e sementes envolvidas em um envelope funicular (arilo) de coloração clara ou acastanhada, fino, tricomatoso, alveolado ou alado; e Cactoideae, que abarca cerca de 85% das espécies da família, sendo morfológicamente mais complexa e por isso menos facilmente delimitada por sinapomorfias, porém todas as espécies da subfamília possuem em geral caule suculento e folhas vestigiais minúsculas, geralmente microscópicas, subentendendo cada aréola. Estudos de variação do DNA de cloroplastos fornecem forte suporte para a monofilia das subfamílias Opuntioideae e Cactoideae, assim como para a separação entre Pereskioideae e Maihuenoideae (Wallace 1995, Wallace & Gibson 2002).

Cactoideae apresenta o maior número de gêneros e espécies, e é subdividida em nove tribos: Echinocereeae, Hylocereeae, Cereae, Trichocereeae, Notocacteeae, Rhipsalideae, Browningieae, Pachycereeae e Cacteeae (Hunt & Taylor 1990). Dentre essas tribos, predominam no leste do Brasil representantes de Cereae e Trichocereeae, tanto em número de gêneros como de espécies. A tribo Cereae é caracterizada por apresentar flores nuas ou dotadas de poucas escamas diminutas e raramente com aréolas tricomas ou espinescentes, enquanto que em Trichocereeae a presença de aréolas tricomas ou espinescentes no pericarpelo e mesmo no tubo floral é bastante comum.

O gênero *Discocactus* Pfeiff. (Trichocereeae) agrega plantas com hábito globoso-achatado ou discóide, cuja região florífera é diferenciada em um cefálio (região onde as aréolas são dispostas de forma mais compacta, sendo reprodutivamente ativas) posicionado no ápice do caule, de onde emergem flores noturnas, brancas, de síndrome esfingófila, e posteriormente frutos que apresentam deiscência lateral. O gênero *Discocactus* tem sido associado tradicionalmente ao gênero *Melocactus* Link & Otto (Cereae), devido a ambos apresentarem hábito globoso e desenvolverem um cefálio terminal. Porém, a estrutura do

cefálio em *Melocactus* difere da encontrada em *Discocactus*, sendo mais cerdoso e desenvolvido. Também as flores e frutos são diferentes, *Melocactus* possuindo flores diurnas e coloridas em tons de vermelho ou rosa, adaptadas para polinização por beija-flores, e frutos indeiscentes. Assim, a similaridade vegetativa entre *Discocactus* e *Melocactus* pode ser o resultado de convergência. Taylor & Zappi (2004) posicionam o gênero *Discocactus* na tribo Trichocereae, próximo ao gênero *Gymnocalycium* Pfeiff. ex Mittler. Estes autores levantam a hipótese de que *Discocactus* tenha evoluído a partir de *Gymnocalycium*, estando mais proximamente relacionado a algumas seções deste gênero que a outras, o que tornaria *Gymnocalycium* parafilético. *Gymnocalycium* difere de *Discocactus* por não possuir um cefálio, por possuir flores diurnas e coloridas, e por possuir uma maior variabilidade na morfologia de suas sementes.

Todas as espécies de *Discocactus* se encontram ameaçadas de extinção em maior ou menor grau. Razões intrínsecas para isso incluem o reduzido número de populações conhecidas para cada espécie, o tamanho em geral pequeno destas populações, e especificidade de hábitat fazendo com que as populações ocorram em áreas bastante restritas. Estes fatores tornam as populações de *Discocactus* extremamente vulneráveis à modificação e destruição de seu hábitat e à coleta de plantas para satisfazer o comércio de plantas ornamentais. Como resultado, o gênero inteiro foi listado no Apêndice I de CITES (Convenção Internacional sobre o Comércio de Espécies Silvestres; Lüthy 2001).

Discocactus foi descrito em 1837 por Pfeiffer, tendo como tipo *D. insignis* Pfeiff. Porém, a primeira espécie hoje identificável como pertencendo ao gênero *Discocactus* foi descrita em 1826 como *Cactus placentiformis* Lehm. Pfeiffer descreveu outras duas espécies para o novo gênero *Discocactus*, sendo elas *D. lehmannii* Pfeiff. e *D. linkii* Pfeiff., ambas descritas em 1839. As três espécies descritas por Pfeiffer foram subsequentemente combinadas por Schumann em *D. placentiformis* (Lehm.) K.Schum. Outras espécies descritas no mesmo período foram *D. alteolens* Lem. ex A.Dietr. (1846), e *D. tricornis* Monv. ex Pfeiff. (1850). A tipificação de todas estas espécies é problemática, e provavelmente todos os nomes publicados referem-se a formas do variável *D. placentiformis*, que ocorre na região central do estado de Minas Gerais.

A próxima entidade distinta de *Discocactus* foi descrita em 1898 como *Malacocarpus heptacanthus* Barb.Rodr., originário do estado de Mato Grosso. Em 1900 Karl Schumann descreveu *Echinocactus hartmannii* K.Schum., um novo táxon de *Discocactus*, com origem no Paraguai. Britton & Rose (1922) combinaram *M. heptacanthus* e *E. hartmannii* em *Discocactus*, e descreveram três novas espécies, *D.*

bahiensis Britton & Rose, *D. zehntneri* Britton & Rose e *D. subnudus* Britton & Rose, as duas primeiras originárias da região noroeste do estado da Bahia, e a terceira proveniente da costa do mesmo estado. No entanto, a identidade de *D. subnudus* é questionável, já que o tipo foi baseado em uma planta claramente danificada por fogo, e na costa baiana nenhuma espécie de *Discocactus* foi encontrada desde então.

A próxima espécie de *Discocactus* a ser descrita foi *D. paranaensis* Backeb. em 1960, originária de material cultivado supostamente coletado no estado do Paraná, porém esta espécie não foi recoletada desde então, sendo conhecida apenas da descrição original. Em 1963, Backeberg descreveu um novo táxon, *D. boliviensis* Backeb., originário do leste da Bolívia, próximo à fronteira com o Brasil. No período entre 1971 e 1980, 26 novos táxons de *Discocactus* foram descritos por Buining & Brederoo, resultado das expedições de Buining ao Brasil durante a década de 60, quando ele e o brasileiro Leopoldo Horst exploraram muito do interior do país: *D. boomianus* (1971), *D. horstii* (1973), *D. albispinus* (1974), *D. catingicola* (1974), *D. magnimammus* (1974), *D. mamillosus* (1974), *D. patulifolius* (1974), *D. cephaliaciculosus* (1975), *D. ferricola* (1975), *D. griseus* (1975), *D. pachythele* (1975), *D. rapirhizus* (1975), *D. semicampaniflorus* (1975), *D. silicicola* (1975), *D. araneispinus* (1980), *D. flavispinus* (1980), *D. latispinus* (1980), *D. magnimammus* ssp. *bonitoensis* (1980), *D. melanochorus* (1980), *D. nigrisaetosus* (1980), *D. pugionacanthus* (1980), *D. pulvinicapitatus* (1980), *D. silvaticus* (1980), *D. spinosior* (1980), *D. squamibaccatus* (1980) e *D. subviridigriseus* (1980). Boa parte destes novos táxons foi publicada no livro “The Genus *Discocactus* Pfeiff.” (Buining 1980), porém muitas destas novas entidades são fracamente definidas e delimitadas, sendo possivelmente coespecíficas.

No mesmo período, entre 1978 e 1981, outros oito táxons de *Discocactus* foram descritos por outros autores: *D. estevesii* Diers (1978), *D. diersianus* Esteves (1979), *D. cangaensis* Diers & Esteves (1980), *D. goianus* Diers & Esteves (1980), *D. subterraneo-proliferans* Diers & Esteves (1980), *D. woutersianus* Brederoo & Broek (1980), *D. lindanus* Diers & Esteves (1981), e *D. multicolorispinus* P.J.Braun & Brederoo (1981). Desta época aos dias de hoje, novos táxons foram descritos: *D. buenekeri* W.R.Abraham (1987), *D. pseudolatispinus* Diers & Esteves (1987), *D. proeminentigibus* Diers & Esteves (1988), *D. zehntneri* var. *horstiorum* P.J.Braun (1990), *D. pseudoinsignis* N.P.Taylor & Zappi (1991), *D. crassispinus* P.J.Braun & Esteves (1994), *D. magnimammus* ssp. *setosiflorus* P.J.Braun & Esteves (1994), *D. cephaliaciculosus* ssp. *nudicephalus* P.J.Braun & Esteves (1995), *D. piauiensis* P.J.Braun & Esteves (1995), *D. crassispinus* ssp.

araguaiensis P.J.Braun & Esteves (1996), *D. hartmannii* ssp. *giganteus* P.J.Braun & Esteves, e por último, *D. bahiensis* ssp. *gracilis* P.J.Braun & Esteves (2001), elevando para cinquenta e dois o número de táxons e quarenta e cinco o número de espécies descritas em *Discocactus*.

Esquemas de classificação para os táxons descritos foram propostos por Braun & Esteves Pereira (1993a-c, 1995, 2001b) e Taylor & Zappi (2004). Porém, estes esquemas diferem consideravelmente, sendo que Taylor é bem mais conservador, reconhecendo apenas sete espécies: *D. bahiensis*, *D. ferricola*, *D. heptacanthus*, *D. horstii*, *D. placentiformis*, *D. pseudoinsignis* e *D. zehntneri*. No conceito de Taylor, *D. heptacanthus* é uma espécie bastante variável, de distribuição ampla, que engloba muitos dos binômios anteriormente publicados; *D. placentiformis* inclui as espécies encontradas na região central do estado de Minas Gerais, de onde também são provenientes *D. pseudoinsignis* e *D. horstii*; *D. bahiensis* inclui *D. subviridigriseus* e *D. bahiensis* subsp. *gracilis*, e *D. zehntneri* inclui *D. albispinus*, *D. araneispinus*, *D. boomianus*, *D. bueneckeri* e *D. zehntneri* var. *horstiorum*. Braun & Esteves reconhecem vinte e cinco espécies e as classificam em dez grupos distintos, além de discutir a irradiação do gênero a partir da região central do Brasil e leste da Bolívia, definindo hipóteses de linhas de evolução e relacionamentos entre os grupos propostos de *Discocactus*.

Para o estado da Bahia foram descritos doze táxons, compreendendo dez espécies: *D. albispinus*, *D. araneispinus*, *D. bahiensis*, *D. bahiensis* ssp. *gracilis*, *D. boomianus*, *D. bueneckeri*, *D. catingicola*, *D. nigrisaetosus*, *D. spinosior*, *D. subviridigriseus*, *D. zehntneri* e *D. zehntneri* var. *horstiorum*. O esquema de classificação proposto por Braun & Esteves Pereira (1993a-c, 1995, 2001b) reconhece apenas três espécies: *D. catingicola* com uma variedade, *nigrisaetosus*, e duas subespécies, *rapirhizus* e *griseus*; *D. bahiensis* com duas subespécies, *subviridigriseus* e *gracilis*; e *D. zehntneri* com cinco subespécies, *albispinus*, *araneispinus*, *bueneckeri*, *boomianus* e *horstiorum*. Taylor também reconhece apenas três espécies: *D. heptacanthus*, *D. bahiensis*, e *D. zehntneri*. No entanto, o número de táxons infra-específicos no esquema de classificação proposto por Taylor é bem menor: *D. heptacanthus* está representando no estado por uma única subespécie, *catingicola*; *D. bahiensis* não apresenta táxons infra-específicos, e *D. zehntneri* possui duas subespécies, *zehntneri* e *boomianus*.

A maioria das descrições de táxons em *Discocactus* baseiam-se em um conceito fortemente tipológico, e praticamente cada nova população descoberta foi descrita como um novo táxon. A divergência na classificação infra-específica entre os dois sistemas de

classificação atualmente propostos para o gênero salienta as dificuldades de delimitação dos diversos táxons existentes, sendo que ambos esquemas estão fortemente baseados na distribuição dos táxons e em características morfológicas que em geral se sobrepõem bastante neste grupo. Estudos empregando grande número de indivíduos e de populações, levando em consideração a variabilidade dentro e entre as populações de cada táxon são inexistentes em *Discocactus*. Da mesma forma, trabalhos empregando outras técnicas além da taxonomia tradicional são raros em Cactaceae (e.g., Baker & Johnson 2000, Baker & Pinkava 1987, Casas et al. 1999, Chamberland 1997, Cruz & Casas 2002) e completamente ausentes para espécies brasileiras.

Neste trabalho objetiva-se delimitar e descrever os táxons de *Discocactus* que ocorrem no estado da Bahia, e os padrões de distribuição da variabilidade morfológica e da variabilidade genética destes. Para tal, os padrões de variação morfológica das populações são analisados utilizando-se protocolos para delimitação de espécies baseados em caracteres (ajustado para a aplicação em níveis infra-específicos) e baseados em árvores filogenéticas, sendo também aplicados métodos de análise multivariada. Secundariamente objetiva-se testar a congruência entre os diferentes métodos utilizados, e discutir os pontos fortes e limitações de cada método. Espera-se que o uso de métodos desenvolvidos especificamente para delimitação de espécies assegure um tratamento objetivo e reprodutível, e com isso ajude a resolver a taxonomia deste grupo. A variabilidade genética das populações também é analisada utilizando-se marcadores enzimáticos, de forma a testar a delimitação das espécies obtida com as análises morfológicas, e auxiliar na determinação de áreas prioritárias para a preservação das espécies e definição de estratégias para a conservação ex-situ. As descrições das espécies reconhecidas são ampliadas, e o estado de conservação das populações é discutido.

Capítulo 1. Analyses of quantitative morphological data employing multivariate, character-based and tree-based methods: specific and infraspecific delimitation of taxa in the genus *Discocactus* Pfeiff. (Cactaceae) from the state of Bahia, Brazil

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RESUMO

De forma a testar a consistência dos sistemas de classificação propostos para as espécies do gênero *Discocactus* que ocorrem no estado da Bahia, Brasil, foram analisados os padrões de variação de vinte e dois caracteres morfológicos quantitativos, amostrados em 337 indivíduos pertencentes a dezessete populações naturais, nove das quais localidades-tipo para táxons descritos. Foram visitadas populações englobando a maioria dos táxons descritos para o estado, com mais de uma população de cada táxon sendo visitada quando possível.

Vários procedimentos para delimitação de táxons foram empregados no conjunto de dados obtido. A existência de caracteres diagnósticos para cada espécie foi verificada com a aplicação de PAA (Análise de Agrupamento de Populações; Davis & Nixon, 1992), um método para delimitação de espécies com base em caracteres. PAA foi ajustado para a avaliação de limites infra-específicos, sendo que uma diferença de 80% na frequência de ocorrência de valores dos caracteres foi considerada diagnóstica para o nível infra-específico. Também foi aplicado o método para delimitação de espécies com base em árvores filogenéticas de Wiens & Penkrot (2002), com uma análise cladística sendo efetuada utilizando as populações como terminais, e a consistência da topologia das árvores obtidas sendo verificada com bootstrap, de forma a testar se populações supostamente coespecíficas formariam clados exclusivos e com elevado suporte. Os padrões de agrupamento dos indivíduos e populações no espaço multivariado foram examinados com métodos de análise multivariada englobando técnicas de ordenação (PCA, CVA) e agrupamento (UPGMA).

Os resultados obtidos a partir das análises efetuadas foram comparados de forma a auxiliar na delimitação taxonômica de limites específicos e infra-específicos entre os táxons do gênero *Discocactus* que ocorrem no estado da Bahia. O número de táxons reconhecidos pelos diferentes métodos utilizados é discordante, com três, quatro, sete ou oito espécies sendo delimitadas por métodos diferentes; no entanto, o desacordo ocorre principalmente no nível de resolução taxonômica obtida. Com base nos resultados obtidos a partir das análises dos dados morfológicos, foram reconhecidos como distintos os táxons detectados por todos os métodos de delimitação empregados. Desta forma, são reconhecidas para o estado da Bahia três espécies de *Discocactus*: *D. bahiensis*, *D. catingicola* e *D. zehntneri*, a última consistindo de duas subespécies, *zehntneri* e *boomianus*.

Analyses of quantitative morphological data employing multivariate, character-based and tree-based methods: specific and infraspecific delimitation of taxa in the genus *Discocactus* Pfeiff. (Cactaceae) from the state of Bahia, Brazil

RUNNING TITLE: Morphological studies on *Discocactus* from Bahia, Brazil

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ABSTRACT

Discocactus is a taxonomically difficult genus where morphological characters – which are traditionally utilized to delimit species in the group – often overlap. Studies employing a large number of populations and sampling many individuals per population in order to assess intra and interpopulational patterns of variation are absent in the genus. In this study, the variation of 22 quantitative morphological characters was analyzed among 337 individuals from 17 populations, 9 of which were type localities for described taxa. We employed character-based (adjusted for application in the evaluation of infraspecific limits) and tree-based species delimitation protocols and compared their results with those obtained from multivariate and statistical analysis in order to make taxonomic decisions on the specific and infraspecific boundaries of taxa in the genus *Discocactus* (Cactaceae) that occur in the state of Bahia, Brazil. The methods employed disagree on the number of species recognized, with three, four, seven or eight species delimited by different methods; however, disagreement occur mostly on the level of resolution achieved. The results obtained are critically compared, and three species are recognized: *D. bahiensis*, *D. catingicola* and *D. zehntneri*, the latter with two subspecies, *zehntneri* and *boomianus*.

ADDITIONAL KEYWORDS: Population Aggregation Analysis (PAA) –

Morphometrics – Endangered species – Northeastern Brazil – Caatinga – Cerrado – Campo Rupestre – Neotropics

INTRODUCTION

Discocactus is a genus of small cacti characterized by having a discoid to depressed-globose, single or cespitose habit, the fertile part of stem differentiated in a terminal cephalium, nocturnal white flowers of sphingophilous syndrome, fruits dehiscing by lateral slits, and seeds with strongly tuberculated testa. The genus is almost completely 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.

All *Discocactus* species are considered threatened or endangered; intrinsic reasons for this are the small number of populations per species, the generally small population sizes, and habitat specificity leading to restricted populations. These factors make *Discocactus* populations extremely vulnerable to habitat modification and destruction, and to overcollecting. As a result, the whole genus is placed in Appendix I of CITES (Lüthy 2001).

The taxonomy of *Discocactus* is disputed: 52 taxa have been described for the genus, and the currently proposed classification systems are rather divergent. Braun & Esteves Pereira (1993a-c, 1995, 2001b) recognizes 51 taxa: 25 species, 22 heterotypic subspecies and four heterotypic varieties, while Taylor & Zappi (2004) recognize only ten taxa: seven species and three heterotypic subspecies. For the state of Bahia in Northeastern Brazil twelve *Discocactus* taxa have been described, ten of which as distinct species (Table 1). These species fall more or less in three groups, recognized with basis in morphological traits and ecological preferences: the complex of *D. bahiensis* Britton & Rose, the complex of *D. catingicola* Buining & Brederoo, and the complex of *D. zehntneri* Britton & Rose.

In the current classification systems proposed by Braun & Esteves Pereira (1993a-c, 1995, 2001b) and Taylor & Zappi (2004) only three species are recognized, each of which corresponding to one of the three complexes; however, the classification systems differ considerably in the infraspecific classification of the species (Table 1). Taylor & Zappi (2004) also hypothesize that *D. bahiensis* could be conspecific with *D. heptacanthus*, being linked to *D. heptacanthus* subsp. *catingicola* via a population somewhat intermediate between the two taxa, located in the municipality of Paramirim, Bahia.

The divergence in the infraspecific classification of the two proposed systems reflects the difficulty of delimitation of these plants, and both systems are strongly based in the geographical distribution of the taxa and in morphological characters which often

overlap. Studies employing a large number of populations and a bigger population sampling in order to assess intra- and interpopulational patterns of variation are absent in the genus, and are rare in the Cactaceae (e.g., Baker & Johnson, 2000; Baker & Pinkava, 1987; Casas et al., 1999; Chamberland, 1997; Cruz & Casas, 2002). Moreover, explicit protocols for species delimitation (Sites & Marshall, 2003, 2004) have never been applied to species of Cactaceae.

In this study we analyze the patterns of morphological variation in *Discocactus* using the protocols for species delimitation outlined by Davis & Nixon (1992) and Wiens & Penkrot (2002). The character-based method is modified in order to be applied in the evaluation of infraspecific limits. We also analyze the data using multivariate and statistical techniques. The results obtained from the various methods are compared in order to determine the specific and infraspecific boundaries among the *Discocactus* taxa that occur in the state of Bahia, Brazil. The use of explicit protocols will assure an objective and repeatable treatment and hopefully resolve the controversial classification schemes as proposed so far in this group of plants.

The analyses conducted should answer the following questions: how many *Discocactus* species occur in the state of Bahia? How many infraspecific taxa can be recognized under each species? What differentiates the species and their infraspecific taxa from each other? Do the different methods applied agree in their results? How the morphological variation found between the taxa correlates with geography and ecology?

MATERIAL AND METHODS

Studied taxa

All taxa described for the state of Bahia were surveyed at their type localities (Table 2), except for *D. zehntneri*, *D. bahiensis* and *D. buenekeri* Abraham for which precise locality information could not be obtained. Buining (1980) has designated a population of *D. bahiensis* occurring near the village of Abreus, municipality of Juazeiro as being a close match to the type; this population and two other populations found nearby were investigated. Based on examination of the original descriptions and type specimens, the population described as *D. albispinus* Buining & Brederoo is assumed to represent the same taxon as *D. zehntneri*. For *D. buenekeri* no populations have been studied.

In addition to the taxa from Bahia it was studied a further taxon, *D. rapirhizus* Buining & Brederoo, that occur in the state Goiás near the border with Bahia; this taxon

was included in the current investigation because it is regarded as conspecific with *D. catingicola* in the classification system proposed by Braun & Esteves Pereira (1993a-c, 1995, 2001b), and it occurs relatively close to the area of distribution of the latter. The taxon described as *D. spinosior* Buining & Brederoo is considered to be synonym with *D. catingicola*; the type locality of *D. spinosior* has not been visited, but a neighboring population in the municipality of Barreiras was investigated. The population of *D. catingicola* from the municipality of Paramirim, considered by Taylor & Zappi (2004) to represent a somewhat intermediate form between *D. catingicola* and *D. bahiensis*, could not be studied because it was destroyed with the construction of a dam in the valley where it occurred, and attempts to locate other populations in the vicinity failed. It was not possible to verify if *D. catingicola* is conspecific with *D. heptacanthus* as advocated by Taylor & Zappi (2004), because the latter was not investigated at its type locality in the state of Mato Grosso as its location lies well outside the study area.

Sampling of populations

Seventeen populations were visited, nine of which being type localities for described taxa (Table 2). More than one population of a given taxon has been sampled when possible, but most of the described taxa are known only from the type locality and thus only one population could be sampled. Sampled individuals constituted of mature plants (assessed by the presence of a well-developed cephalium) spaced by at least five meters. A total of 337 individuals were sampled. Ecological conditions, habitat features, soil and vegetation types were recorded from each population.

Morphological characters analyzed

Twenty two vegetative morphological characters were used for the present study. Table 3 contains a list of the characters used and the means and standard deviations for the measurements from each population. Characters chosen inform about body dimensions (1-2), rib shape (4-7) and number (3), areole number (8), spine number (9, 12) and dimensions (10-11, 13-18), cephalium dimensions (19-20) and dimensions of the bristles of the cephalium (21-22). The characters examined include many which are traditionally used in the taxonomy of the genus.

All measurements of continuous quantitative characters were taken with the aid of a vernier caliper. The height of stems was measured from the ground level, thus not including underground portions of the stems. The values for the spine characters represent

an average of the measurements of three areoles located in different ribs and disposed in the middle portion of the stem. Spine diameters were measured at the middle of the spine. Reproductive characters could not be utilized in this study because most populations were not in flower when surveyed.

Multivariate and statistical analyses

Patterns of morphological similarity/difference were analyzed by multivariate statistical methods using the software package STATISTICA for Windows, Release 5.5 A (StatSoft 2000). The analyses included Principal Component Analysis (PCA), Canonical Variate Analysis (CVA) and Cluster Analysis for the calculation of variability parameters and morphological structuring. A basic data matrix was constructed with the morphological characters considered as variables. CVAs were performed with population as the categorical variable (individuals were grouped according to the population to which they belonged). A CVA was conducted using all individuals, and separate CVAs were conducted using only the individuals assigned to each of the three complexes (Table 2). The eigenvectors resulting from PCA and the standardized coefficients for canonical variables resulting from CVAs were used to identify the characteristics that most significantly contribute to the resulting patterns observed. Cluster Analysis was conducted on a matrix of morphological distance between populations calculated using Mahalanobis Generalized Distance as distance coefficient, and UPGMA was used as clustering algorithm.

Species are distinguished in multivariate space if no overlap occurs between clusters. The criterion for delimitation of infraspecific taxa is less objective, as it is based on a subjective assessment of low overlap between clusters, and a threshold frequency of intermediates is not provided; overlap between clusters are assessed with a verification of how many cluster elements are misidentified as members of other clusters (i.e., how many elements are closer to centroids of other clusters), and with visual inspection of clustering patterns. Given these caveats, infraspecific taxa are recognized for groups of clusters with little overlap between constituent elements.

The pair-wise geographical distances between the populations was computed with geodetic distances on WGS84 earth ellipsoid calculated using the INVERSE 2.0 program (National Geodetic Survey, 2002). Mantel tests using the randomization (Monte Carlo) method were applied to the matrices of morphological and geographical distances in order to test for significant correlations between morphologic and geographic distances.

Character-based species delimitation

Character-based species delimitation was performed using the method of Population Aggregation Analysis (PAA) devised by Davis and Nixon (1992). This method is a formalization of the traditional methodology for delimiting species based on diagnostic character differences. PAA is based in two assumptions: all individuals sampled from a population are conspecific, and character attributes shared among different populations are evidence that these populations are conspecific. PAA operates as follows: character values are compared between all population samples, and populations are aggregated if they possess identical character attributes or overlapping ranges of values for all characters surveyed; the population aggregates that remain at the end of the process are those found to have at least one diagnostic character, i.e. fixed for different character attributes or with non-overlapping ranges of values, and these population aggregates are regarded as belonging to distinct species.

The matrix of morphological measurements was used as basis for estimates of relationships among populations. In order to analyze the data, the continuous quantitative characters present in the matrix were converted into meristic quantitative characters according to the following procedure: first, the original values were converted into discrete values in steps of 0.5cm and 0.2mm for characters measured in centimeters and millimeters respectively, by rounding the original value to the nearest discrete value; next, a scale of discrete values was created by selecting the minimum and maximum values assumed by the character in the entire data set (all individuals from all populations), and all the intermediate values; then, for every value in the scale it was computed for each population the number of individuals with the same value, and the number of individuals with this value was divided by the total number of individuals of the population and multiplied by 100 in order to get the frequency of occurrence of that value in the population. Relationships among populations were assessed with basis on the transformed matrix, by comparing the ranges of values of different populations. Characters were considered diagnostic at the species level if a group of populations differed from other such groups by possessing non-overlapping ranges of characters values, or if the characters were invariant for alternative character attributes.

In order to apply PAA at the infraspecific level the method has been modified to take in account differences in frequency of occurrence of character values among groups of populations that display overlapping ranges of values in the characters. A character was considered diagnostic at the infraspecific level if a cutoff value could be found in the scale

of values of the character so that two groups of populations differ in having 80% or more of their individuals with values located at opposite sides of the cutoff value; the two groups of populations are thus considered to represent distinct infraspecific taxa.

We acknowledge that an 80% difference in frequency of occurrence of character values is an arbitrary measure of dissimilarity, but we take this difference to imply that two groups of populations are diverging, or have retained different proportions of character values after a range fragmentation event, or that one group originated from a dispersal event and have only a subset of the character values found in the other group; in any case, whatever be the origin of the different proportions of occurrence of the character values between the populations, this difference in frequency of occurrence is here understood as implying an absence of gene flow between the two groups of populations, or case gene flow is present, that it occur at levels insufficient to homogenize the different proportions of occurrence of character values between the two groups of population.

Of course, this is not the only possible explanation for the differences in frequency of occurrence of the character values: the local prevalence of a subset of the species' range of values for a given character can be a result of local environmental conditions favoring phenotypes with that subset of values. Thus, a difference in the distribution of character values can exist between two populations that meet the criterion adopted here for recognizing infraspecific taxa, in spite of these populations being engaged in extensive gene flow. However, in the absence of evidence indicating this scenario as a possible reason for the differences in frequency of occurrence of character values between two groups of populations, it is reasonable to conclude that these differences are a result of limited or absent gene flow.

Tree-based species delimitation

Tree-based species delimitation was conducted following the method described by Wiens & Penkrot (2002) with basis in the matrix of morphological measurements. The method requires a cladistic analysis to be performed on populations of a putative species under test (the focal species) and populations of closely related species, with relative support for individual branches assessed via nonparametric bootstrapping. Strongly supported branches and concordance of population-level clades with geographic proximity are considered as evidence for species-level differentiation, whereas weak support and discordance with geography are taken as evidence for gene flow and conspecificity. Species can be either exclusive or non-exclusive (when other species are nested within its

populations); in the last case, specific status is warranted when the basal clades of populations appear on adjacent branches of the tree and are weakly supported and not concordant with geography, suggesting gene flow among the basal clades.

For the cladistic analysis, meristic and continuous quantitative morphological characters were coded using the step-matrix gap-weighting coding method described by Wiens (2001; see also Wiens & Etheridge [2003] for an application of the method). Means of characters values were calculated for each population (Table 3) and range-standardized by applying the formula $((\text{mean}_{\text{pop}} - \text{min}_{\text{character}})/(\text{max}_{\text{character}} - \text{min}_{\text{character}})) * 999$ for each population mean value, where mean_{pop} is the population's mean value and $\text{max}_{\text{character}}$ and $\text{min}_{\text{character}}$ are the maximum and minimum population mean values for the character. Each population with a unique standardized mean value for a character was given a unique character state in the data matrix; step matrices were created for each character by calculating the difference between each pair of population's standardized mean values, these differences denoting the cost of transition between the populations' character states.

In addition to the characters derived from the morphological measurements, one qualitative binary character was added to the data matrix, presence of tuberous roots, state 1 being assigned for the *D. bahiensis* populations and state 0 for the populations of the remaining species. A transition cost of 999 was given to this qualitative character in order to make it equivalent in weight to the quantitative characters. Cladistic analyses of the data matrix were performed using PAUP* (Swofford 1998). Heuristic search was performed using the maximum-parsimony criterion with tree-bisection-reconnection (TBR) algorithm and 1000 random taxon-addition sequence replicates with swapping limited to 15 trees per replicate. Relative support for individual branches was assessed using nonparametric bootstrapping (Felsenstein 1985), with 1000 bootstrap pseudoreplicates. Each bootstrap pseudoreplicate used a heuristic search with simple taxon-addition sequence replicates and swapping limited to 15 trees per replicate in order to find the shortest tree for that matrix. Branches with bootstrap values > 70% were considered to be strongly supported, following Hillis & Bull (1993).

RESULTS

Habitat preferences

Each of the three complexes of species is found in different types of soil and within different vegetation types. Plants of the *D. bahiensis* complex are found in gravely areas

close to rivers with limestone-derived loamy soil, within the *caatinga* vegetation type; plants of the *D. catingicola* complex usually grow in sandy areas close to rivers, within the *cerrado* vegetation type; plants of the *D. zehntneri* complex usually inhabit sandy and gravelly areas found over 700m in altitude, in ecotones between *caatinga* and *cerrado de altitude* vegetation types, except for plants of the population ZA_BG which grow as rupicolous on a granite rock outcrop at a lower elevation (500m), within the *caatinga* vegetation type.

Multivariate and statistical analyses

The scatter plot of the individuals' scores on the first two PCA factors is shown in Fig. 1. The first two Principal Components accounted for a large proportion of the variance, respectively 42.77% and 19.55%, summing up to 62.32% of the morphological variation. There is extensive overlap between the individuals belonging to the *D. bahiensis* and *D. catingicola* complexes, but there is a clear separation between the cluster formed by these complexes and the cluster formed by the individuals of the *D. zehntneri* complex, this separation occurring mainly on the first Principal Component, with the second Principal Component separating populations of the *D. bahiensis* and *D. zehntneri* complexes (mainly BG_SR and ZA_BG, respectively) that otherwise overlap on Principal Component 1. Overall, PCA did not provide a good separation between the three supposed species and their infraspecific taxa, owing mostly to an ample overlap of characters values between individuals of the *D. bahiensis* and *D. catingicola* complexes.

The scatter plots of the individuals' scores on the first two CVA canonical roots, and on the first and third CVA canonical roots are shown in Fig. 2. The first, second and third canonical roots explained respectively 68.78%, 16.34% and 4.75% of the morphological variation, summing up 89.87% of the existing variability. Complete separation of the *D. zehntneri* complex from the other complexes was achieved along the first canonical root, while the second canonical root discriminated between the *D. bahiensis* and *D. catingicola* complexes. The third canonical root separated the population ZA_BG from the remaining populations of the *D. zehntneri* complex. Table 4 displays the coefficients for canonical variables on the first three canonical roots.

The scatter plots of the first two canonical roots from CVAs where only the populations of each species complex were included are shown in Fig. 3. In the CVA of the *D. bahiensis* complex, three groups of populations can be distinguished along the first canonical root (that explains 69.93% of the morphological variation), in spite of some

overlap: a group formed by populations BS_JU and BS_RO, a group formed by populations BB_AB, BB_GO and BB_OL, and the population BG_SR. These groups display little overlap, with respectively 97.37%, 97% and 100% of individuals correctly classified within each group (Table 5). The CVA of the *D. catingicola* complex displays extensive overlap between populations, with only CR_PO being somewhat separated along the first canonical root (that explains 42.4% of the morphological variation), even so having a high degree of overlap with 15.79% of its individuals being incorrectly classified as belonging to other populations (Table 5). In the CVA of the *Discocactus zehntneri* complex, a clear spatial separation occurs between ZA_BG and the remaining populations along the first canonical root (that explains 59.48% of the morphological variation), while two groups of populations can be distinguished along the second canonical root (that explains 22.5% of the morphological variation), in spite of some overlap: a group formed by populations ZH_CL and ZR_LI, and a group formed by populations ZB_EC, ZB_LA and ZB_VS. These groups display little overlap, with respectively 97.50% and 98.67% of individuals correctly classified within each group (Table 5).

The UPGMA dendrogram resulting from the cluster analysis of morphological distances between the populations is shown in Fig. 4. Three major groups can be recognized with basis in the morphology, each of which corresponds to one of the three complexes. Overall, within each species the geographically closer populations were the most similar morphologically, except for the population BS_JU which is more similar to population BS_RO but geographically closer to populations BB_AB, BB_GO, and BB_OL, and the population ZA_BG which is close to the population ZR_LI but quite distinct morphologically (Fig. 4).

The Mantel tests performed on the matrices of morphological and geographical distances of populations within each species complex indicated that there are positive significant correlations between geography and morphology in all three complexes, with populations of the *D. catingicola* complex having the most significant correlations ($r = 0.673468$, $p = 0.008$), followed by populations of the *D. zehntneri* complex ($r = 0.214549$, $p = 0.04$), and the *D. bahiensis* complex ($r = 0.637237$, $p = 0.048$). Populations of the *D. catingicola* complex are well separated geographically from populations of the other complexes, while there is a superposition in the geographical distribution of the *D. bahiensis* and *D. zehntneri* complexes (see Fig. 1 in Machado et al., submitted).

Population Aggregation Analysis

The application of this character-based protocol to the morphological data resulted in support for the recognition of the three complexes as distinct species (Table 6). A single fixed diagnostic character was found for populations of the *D. bahiensis* complex, and this was the absence of bristles in the cephalium. A single polymorphic diagnostic character distinguish populations of the *D. zehntneri* complex, the height of the rib between the areoles, whose range of values do not overlap with the ranges of values found in the other complexes (Table 3). No character was diagnostic for the *D. catingicola* complex, and it is recognized as a species on the basis of the lack of the diagnostic characters present in the other species. No group of populations within the *D. catingicola* complex had differences in frequency of occurrence of character values significant enough to enable them to be recognized as distinct at the infraspecific level.

Within the *D. bahiensis* complex, the population BG_SR differ from the others in the following polymorphic diagnostic characters: smaller stem diameter (85% of the individuals of BG_SR with stem less than 10cm in diameter against 94% of the individuals of the other populations with stem more than 10cm in diameter) and stem height (90% of the individuals of BG_SR with stem up to 3cm in height against 87% of the individuals of the other populations with stem more than 3cm in height), ribs narrower at the areole (100% of the individuals of BG_SR with ribs up to 2cm in diameter at the areole against 85% of the individuals of the other populations with ribs more than 2cm in diameter at the areole), and thinner spines (for all measured spines, 100% of the individuals of BG_SR with spines up to 1mm in diameter against more than 80% of the individuals of the other populations with spines more than 1mm in diameter). The other populations of the complex did not exhibit significant differences in character frequencies to be regarded as diagnostic.

Within the *D. zehntneri* complex, the population ZA_BG differ from the others in the following polymorphic characters: presence of central spines (85% of the individuals of ZA_BG having at least one central spine against 80% of the individuals of the other populations lacking a central spine), smaller number of radial spines (81% of the individuals of ZA_BG having less than 13 radial spines per areole against 82% of the individuals of the other populations having 13 or more radial spines per areole), radial spines longer (for all measured radial spines, an average of 82% of the individuals of ZA_BG with spines more than 3cm in length against an average of 86% of the individuals of the other populations with spines up to 3cm in length) and thicker (for all measured

spines, an average of 97% of the individuals of ZA_BG with spines more than 0.8mm in diameter against an average of 96.5% of the individuals of the other populations with spines up to 0.8mm in diameter). The other populations of the complex did not exhibit significant differences in character frequencies to be regarded as diagnostic.

Wiens-Penkrot tree-based species delimitation method

The application of the tree-based protocol devised by Wiens & Penkrot (2002) to the morphological data resulted in a single most parsimonious tree of length 43341.38, which is shown in Fig. 5A. The populations of the *Discocactus bahiensis* complex failed to group together in the cladogram. Five clades had high bootstrap support and a strict observance of the method results in their recognition as distinct exclusive species (Table 6); additionally, two sets of populations are recognized as non-exclusive species, one comprising the populations CC_SG and CC_AV, and the other comprising the populations BB_AB, BB_GO, BB_OL and BG_SR.

Due to introgression in the population BG_SR (see discussion), a cladistic analysis was made with the exclusion of this population from the data set in order to test its influence in the topology of the tree. Fig. 5B shows the resulting single most parsimonious tree of length 39281.51. In this tree all three complexes are exclusive and well-supported, demonstrating that the overall intermediacy of character values of the population BG_SR between the *D. bahiensis* and *D. zehntneri* complexes is the responsible for making the *D. bahiensis* complex non-exclusive in the analyses including the population BG_SR. Seven clades had high bootstrap support and a strict observance of the Wiens-Penkrot method results in their recognition as distinct exclusive species (Table 6); additionally, one set of populations is recognized as non-exclusive species, comprising the populations BB_AB, BB_GO and BB_OL.

DISCUSSION

There are gaps in the geographical area covered by the populations sampled (see Fig. 1 in Machado et al., submitted), mainly the region between the populations of the *D. catiungicola* complex and the remaining complexes. No *Discocactus* populations are recorded for this region, both in the literature and in herbaria, with the exception of a population of *D. catiungicola* from the municipality of Paramirim, considered by Taylor & Zappi (2004) to represent a somewhat intermediate form between the *D. catiungicola* and *D.*

bahiensis complexes, that could not be studied because it was destroyed. For the *D. zehntneri* complex, no record of populations exists for the area between northern and southern populations – it is possible that intermediate populations occur, but this area was not investigated due to the difficulty of access to it. The population BG_SR of the *D. bahiensis* complex is fairly removed from the remaining surveyed populations of this species, but although reports exist in the literature (Braun & Esteves Pereira 2001a), other populations have not been located in the intervening area by the authors. In spite of these caveats, no strong geographic bias was detected in the analyses conducted.

The specific and infraspecific classifications obtained from each of the delimitation methods employed are summarized in Table 6. There is disagreement regarding the number of species recognized, with different methods recognizing three, four, seven or eight species. The methods differ mostly on the level of resolution achieved, with some methods recovering a higher number of distinct species than others, but with all taxa being disposed along similar hierarchies; the major conflict comes from the Wiens-Penkrot method, where inclusion of the population BG_SR makes the *D. bahiensis* complex non-exclusive, due to the intermediacy of characteristics of this population relative the *D. bahiensis* and *D. zehntneri* complexes.

The population BG_SR is suspected to be undergoing introgression of genes from populations of the *D. zehntneri* complex found nearby – it is located only three kilometers away from a population of *D. zehntneri* subsp. *boomianus* (Buining & Brederoo) N.P.Taylor & Zappi. Morphological evidence include smaller stature, tendency for having a higher number of spines in the areoles, thinner spines, and increased tendency to offset, all characteristics usually found in plants of the *D. zehntneri* complex. One plant of apparently F1 hybrid origin has been observed by the first author in the population BG_SR, this plant possessing a more globose body and bristles around the cephalium, being strongly reminiscent of *D. zehntneri* subsp. *boomianus*. Allozyme markers also provide evidence of introgression (Machado et al., submitted).

The higher number of potential species resolved by the Wiens-Penkrot method relative to the other methods lies in the different kind of entities that the different methods are designed to recover: the Wiens-Penkrot method is designed to discover distinct lineages, whereas the other methods are designed to detect interbreeding populations irrespective of the temporal scale (i.e., both ongoing and historical gene flow). Distinct lineages can coexist within a species, and a strong support for a clade cannot be automatically taken as justification for specific status of this clade, even if their constituent

populations are concordant with geography; other delimitation methods must be applied to the same data set in order to corroborate or reject the hypothesis that the clade is a distinct species.

An example of the taxonomic over-resolution of the Wiens-Penkrot method was the recognition of *D. nigrisaetosus* Buiining & Brederoo and *D. catingicola* as possibly distinct species, while none of the other methods distinguish these taxa – no diagnostic characteristics being found with PAA even at infraspecific level to support the recognition of two distinct entities, and extensive overlap can be observed in CVA, with only 82.86% of the individuals belonging to *catingicola* being correctly classified, 14.28% being erroneously classified as *nigrisaetosus* and the remaining as *rapirhizus*, and only 79.31% of the individuals belonging to *nigrisaetosus* being correctly classified, 17.24% being classified as *catingicola* and the remaining as *rapirhizus* (see Table 4). An allozyme survey of the same populations and individuals shows that these populations have high degrees of genetic identities (Machado et al., submitted). Thus, species limits should be inferred with caution if the Wiens-Penkrot method is the single method applied to the data.

The Wiens-Penkrot method has its main strength in the assumption that weak support and discordance of population-level clades with geographical proximity are strong evidence for conspecificity – this is the most plausible explanation if such a pattern is detected in a clade. The method has also the ability to detect patterns that may not be identified by other methods, such as the non-exclusivity of the *D. bahiensis* complex with the inclusion of the population BG_SR in the analyses.

CVA can be very powerful in identifying species limits as morphological variation between populations can be examined directly in the multivariate space, making it easier to assess clustering patterns and relationships among clusters of populations. The criterion adopted here delimits species as mutually exclusive clusters; however, this study reveals that mutually exclusive clusters may not always possess diagnostic characteristics to differentiate them – the population ZA_BG proved to be distinct in the multivariate space, but none of the characters examined was useful for diagnosing this population at the species level – it could only be distinguished at the subspecies level by PAA.

The reverse situation can also happen, with species diagnosed by PAA and not forming mutually exclusive clusters in multivariate space, but the worst scenario occur when both methods fail to reveal the existence of distinct species, as rejection of the multiple-species hypothesis is not automatic evidence of the presence of a single species – this is the most likely cause, but alternative explanations exists, such as the presence of

cryptic species not revealed by morphology, or extensive hybridization between species so as to blur species boundaries. Under these circumstances the majority of the methods employed in this study will fail, and data-sets other than morphology must be examined if there is suspicion that more than one species may be present.

We take a conservative view and regard as distinct taxa only those which are resolved by all methods employed; this leads to recognition of *D. bahiensis* and *D. catingicola* as distinct species without infraspecific taxa, and *D. zehntneri* with two subspecies, *zehntneri* and *boomianus* – we decided to maintain the last two taxa within a single species because there are no diagnostic characters distinguishing them. This circumscription is concordant with habitat preferences of the taxa, and is similar to the circumscription adopted by Taylor & Zappi (2004), with the difference that these authors consider *D. catingicola* to be conspecific with *D. heptacanthus*, a taxon described for the state of Mato Grosso that was not surveyed at its type locality because its location lies outside the study area; thus, there is still doubt if *D. catingicola* is a distinct species.

In the classification system proposed by Taylor & Zappi (2004), *D. catingicola* is recognized as a subspecies of *D. heptacanthus*, but one population (CR_PO) of the taxon described as *D. rapirhizus*, which Taylor & Zappi (2004) consider to represent *D. heptacanthus* subsp. *heptacanthus*, displayed no differences in relation to *D. catingicola*. This result needs to be interpreted with caution, because it does not necessarily implies that there are not differences between *D. heptacanthus* and *D. catingicola* – it can be that *D. rapirhizus* was wrongly assigned to *D. heptacanthus* by Taylor & Zappi (2004), and that its true affinities are indeed with *D. catingicola*, as suggested by the classification system of Braun & Esteves Pereira (1993a-c, 1995, 2001b) and demonstrated in this study. In order to clarify the taxonomic position of *D. catingicola*, future studies need to include *D. heptacanthus*, *D. catingicola*, and as many populations as possible in the intervening area between the type localities of these two taxa.

The lack of strong morphological distinction between *D. bahiensis* and *D. catingicola* as indicated by the results of the PCA analysis was due to Principal Components 1 and 2 being basically size-related axes, demonstrating that the two taxa are very similar in quantity of elements and dimensions of the characters used in this study. Their morphological similarity is also evidenced by the smaller linkage distance between the two groups when compared to their linkage distance to *D. zehntneri*.

In spite of the morphological similarity, *D. bahiensis* and *D. catingicola* grow in distinct types of soil and are found in different vegetation types, although both are

generally found close to rivers. The populations of these two species are also completely allopatric, the closest populations from each species being separated by more than 400km. Besides the fixed diagnostic character of possessing no bristles in the cephalium, *D. bahiensis* also differ from *D. catingicola* in possessing tuberous taproots, another fixed diagnostic character that was not included in the analyses. The results of the analyses and the exclusive presence of tuberous roots in *D. bahiensis* support the recognition of this species as distinct from *D. catingicola*. Unfortunately the population of *D. catingicola* from the municipality of Paramirim, considered by Taylor & Zappi (2004) to represent a somewhat intermediate form between *D. catingicola* and *D. bahiensis*, could not be studied because it was destroyed with the construction of a dam in the valley where it occurred, and attempts to locate other populations in the vicinity failed.

Only one of the methods employed in this study – the tree-based delimitation method – supports the recognition of other taxa within the *D. catingicola* complex: *D. rapirhizus* for the population CR_PO, and *D. nigrisaetosus* for the populations CN_PN and CN_CO. PAA did not find any diagnostic differences between any groups of populations within the *D. catingicola* complex, and there was extensive overlap of individuals from all the populations in the multivariate analyses. An allozyme survey of the same populations and individuals reveals low genetic differentiation between these populations (Machado et al., submitted).

Two populations of *D. bahiensis* (BS_JU and BB_OL) are assigned by Braun & Esteves Pereira (1993a-c, 1995, 2001b) to the taxon *D. bahiensis* subsp. *subviridigriseus* (Buining & Brederoo) P.J.Braun & Esteves. However the morphological divergence of this taxon is not big, mostly owing to its thicker spines. Only one of the methods employed in this study – the multivariate analyses – supports the recognition of this subspecies: PAA showed that it had no diagnostic differences from the other *D. bahiensis* populations, and although subspecies *subviridigriseus* formed a strongly supported clade in the Wiens-Penkrot tree-based delimitation method, suggesting that perhaps it is a good lineage, it cannot be satisfactorily distinguished from the remaining *D. bahiensis*.

The population BG_SR has been described as *D. bahiensis* subsp. *gracilis* P.J.Braun Esteves. In the analyses conducted this population differ from the remaining *D. bahiensis* basically in size, and thus the results should be interpreted with caution: in the field experience of the authors with Cactaceae, individuals from populations located at extremes of the distribution range of a species tend to be smaller than individuals derived from populations located at the core of the distribution range, probably due to the less than

optimal conditions found at the extremes of distribution. However, environmental conditions are not very different between BG_SR and the other *D. bahiensis* populations. This population appears to be undergoing introgression with *D. zehntneri*, and this is probably the reason for its smaller stature.

In spite of the CVA analyses supporting *D. zehntneri* as a taxon distinct from *D. boomianus* in the multivariate space, none of the characters examined was useful for diagnosing the two taxa at the species level – they were only distinguished at infraspecific level by PAA, as subspecies of a single taxon, *D. zehntneri*. The morphological distinction between subspecies *zehntneri* and *boomianus* meets with ecological differences in the habitats of both subspecies, with *D. zehntneri* subsp. *zehntneri* being found at lower altitudes, growing as a rupicolous on granite rock outcrops, while *D. zehntneri* subsp. *boomianus* generally being found at higher altitudes growing in sandy to gravelly soils of sandstone origin (with exception of one population, ZH_CL, which is found in gravelly soils upon a iron and manganese-rich conglomerate rock outcrop). The two subspecies occur in very close proximity in one area, where the surveyed population of *D. zehntneri* subsp. *zehntneri* (ZA_BG) is separated by only 8 km from a population of *D. zehntneri* subsp. *boomianus* (ZR_LI). *Discocactus* flowers display the typical features of hawk moth pollination syndrome, including nocturnal anthesis, long and narrow flower tube, strong sweet scent, and white color. Sphingid moths are reported to travel great distances during feeding bouts (Proctor, Yeo & Lack, 1996), thus gene flow between these populations is theoretically possible, although it was not confirmed by an allozyme survey of the same populations and individuals, where it was found that the degree of genetic identity between these populations is only moderate (Machado et al., submitted), providing stronger evidence for the separation of the two taxa.

CONCLUSIONS

We recognize three species for the state of Bahia, Brazil, based on the results of the methods utilized: *D. bahiensis*, *D. catingicola*, and *D. zehntneri*, the last with two subspecies, *zehntneri* and *boomianus*. These taxa were well-resolved by all methods employed, and the species circumscriptions adopted are also supported by an investigation of genetic variability with the same populations and individuals utilized in this study (Machado et al., submitted). The publication of a formal taxonomic treatment for these taxa has been submitted elsewhere (Machado et al., unpubl. data).

In summary, the use of different methods to specific and infraspecific delimitation of taxa, especially when based on the same data set, can provide stronger evidence of true specific and infraspecific boundaries where the methods are congruent, and when the different methods give conflicting results, the most likely causes for the disagreement can often be sorted out, further strengthening taxonomic decisions.

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Table 1. Classification systems proposed for the *Discocactus* taxa that occur in the state of Bahia, Brazil, including the taxa described as *D. rapirhizus*, *D. griseus*, and *D. heptacanthus*, from the states of Goiás (GO), Minas Gerais (MG), and Mato Grosso (MT), respectively. Taxa marked with an asterisk have not been studied in the field.

Original name	Braun & Esteves Pereira (1993a-c, 1995, 2001b)	Taylor & Zappi (2004)
<i>D. bahiensis</i> Britton & Rose	<i>D. bahiensis</i> subsp. <i>bahiensis</i>	<i>D. bahiensis</i>
<i>D. subviridigriseus</i> Buining & Brederoo	<i>D. bahiensis</i> subsp. <i>subviridigriseus</i> (Buining & Brederoo) P.J.Braun & Esteves	
<i>D. bahiensis</i> subsp. <i>gracilis</i> P.J.Braun & Esteves	<i>D. bahiensis</i> subsp. <i>gracilis</i>	
<i>D. catingicola</i> Buining & Brederoo	<i>D. catingicola</i> subsp. <i>catingicola</i> var. <i>catingicola</i>	<i>D. heptacanthus</i> subsp. <i>catingicola</i>
<i>D. spinosior</i> Buining & Brederoo		
<i>D. nigrisaetosus</i> Buining & Brederoo	<i>D. catingicola</i> subsp. <i>catingicola</i> var. <i>nigrisaetosus</i> (Buining & Brederoo) P.J.Braun & Esteves	
<i>D. rapirhizus</i> Buining & Brederoo	<i>D. catingicola</i> subsp. <i>rapirhizus</i> (Buining & Brederoo) P.J.Braun & Esteves (GO)	<i>D. heptacanthus</i> subsp. <i>heptacanthus</i> (MT) *
<i>D. griseus</i> Buining & Brederoo *	<i>D. catingicola</i> subsp. <i>griseus</i> (Buining & Brederoo) P.J.Braun & Esteves (MG) *	
<i>D. zehntneri</i> Britton & Rose	<i>D. zehntneri</i> subsp. <i>zehntneri</i>	<i>D. zehntneri</i> subsp. <i>zehntneri</i>
<i>D. albispinus</i> Buining & Brederoo	<i>D. zehntneri</i> subsp. <i>albispinus</i> (Buining & Brederoo) P.J.Braun & Esteves	
<i>D. boomianus</i> Buining & Brederoo	<i>D. zehntneri</i> subsp. <i>boomianus</i> (Buining & Brederoo) N.P.Taylor & Zappi	<i>D. zehntneri</i> subsp. <i>boomianus</i>
<i>D. araneispinus</i> Buining & Brederoo	<i>D. zehntneri</i> subsp. <i>araneispinus</i> (Buining & Brederoo) P.J.Braun & Esteves	
<i>D. zehntneri</i> var. <i>horstiorum</i> P.J.Braun	<i>D. zehntneri</i> subsp. <i>horstiorum</i> (P.J.Braun) P.J.Braun & Esteves	
<i>D. buenekeri</i> Abraham *	<i>D. zehntneri</i> subsp. <i>buenekeri</i> (Abraham) P.J.Braun & Esteves *	

Table 2. Locality data for 17 *Discocactus* populations surveyed in the states of Bahia (BA) and Goiás (GO), Brazil, with number of individuals sampled and an acronym for each population, information about the species complex to which the population belongs, if the location represents a type locality for a described taxon, and the voucher specimens deposited at HUEFS. Precise locality information was withheld for conservation reasons.

Complex	Location	Municipality and State	Type locality for described taxon	Acronym	Sample size	Voucher specimens (HUEFS)
<i>Discocactus bahiensis</i>	Abreus	Juazeiro (BA)		BB_AB	20	M. Machado 440 – 443
	Goiabeira	Juazeiro (BA)		BB_GO	20	M. Machado 430 – 433
	Olhos D'Água	Juazeiro (BA)		BB_OL	20	M. Machado 420 – 423
	São Rafael	Morro do Chapéu (BA)	<i>D. bahiensis</i> subsp. <i>gracilis</i>	BG_SR	20	M. Machado et al. 390 – 393
	Rodeadouro	Juazeiro (BA)	<i>D. subviridigriseus</i>	BS_RO	21	M. Machado et al. 370 – 373
	Juremal	Juazeiro (BA)		BS_JU	17	M. Machado et al. 380 – 383
<i>Discocactus catingicola</i>	Sítio Grande	São Desidério (BA)	<i>D. catingicola</i>	CC_SG	18	M. Machado et al. 300 – 303
	Acaba Vidas	Barreiras (BA)		CC_AV	17	M. Machado et al. 310 – 313
	Porto Novo	Santana (BA)	<i>D. nigrisaetosus</i>	CN_PN	17	M. Machado et al. 340 – 344
	Coribe	Coribe (BA)		CN_CO	12	M. Machado et al. 330 – 333
	Posse	Posse (GO)	<i>D. rapirhizus</i>	CR_PO	19	M. Machado et al. 320 – 323
<i>Discocactus zehntneri</i>	Brejinho Grande	Sento Sé (BA)	<i>D. albispinus</i>	ZA_BG	21	M. Machado 410 – 415
	Lages	Morro do Chapéu (BA)	<i>D. boomianus</i>	ZB_LA	30	M. Machado et al. 450 – 454
	Entroncamento de Cafarnaum	Morro do Chapéu (BA)		ZB_EC	25	M. Machado et al. 470 – 474
	Volta da Serra	Morro do Chapéu (BA)		ZB_VS	20	M. Machado et al. 460 – 464
	Limoeiro	Sento Sé (BA)	<i>D. araneispinus</i>	ZR_LI	20	M. Machado 400 – 403
	Campo Largo	Sento Sé (BA)	<i>D. zehntneri</i> subsp. <i>horstiorum</i>	ZH_CL	20	M. Machado et al. 350 – 356

Table 3. List of morphological vegetative characters investigated, with means and standard deviation values for the measurements from each of the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil, grouped by species complex. For population acronyms see Table 2.

	<i>Discocactus bahiensis</i> complex						<i>Discocactus catingicola</i> complex					<i>Discocactus zehntneri</i> complex					
	BB_OL	BB_AB	BB_GO	BG_SR	BS_JU	BS_RO	CC_AV	CN_CO	CN_PN	CR_PO	CC_SG	ZB_LA	ZB_EC	ZB_VS	ZA_BG	ZH_CL	ZR_LI
Stem																	
01. Height (cm)	4,38 ± 0,78	4,04 ± 0,73	4,14 ± 1,12	2,37 ± 0,58	5,18 ± 0,59	5,23 ± 0,97	5,50 ± 0,59	6,07 ± 0,66	5,57 ± 1,31	5,23 ± 0,63	5,55 ± 1,13	4,90 ± 0,62	5,02 ± 0,66	4,82 ± 0,84	5,03 ± 1,11	2,48 ± 0,56	4,21 ± 0,93
02. Width (cm)	13,35 ± 2,86	13,32 ± 1,51	12,93 ± 2,28	8,90 ± 0,94	14,64 ± 1,48	13,52 ± 2,19	13,07 ± 2,07	15,15 ± 1,63	15,37 ± 1,67	12,42 ± 1,09	13,08 ± 2,07	6,97 ± 0,93	7,02 ± 0,88	6,36 ± 0,82	7,99 ± 1,67	5,32 ± 0,92	7,93 ± 1,30
Ribs																	
03. Number	13,40 ± 0,88	12,40 ± 0,82	13,95 ± 1,50	13,65 ± 1,09	12,88 ± 0,33	13,00 ± 0,55	10,18 ± 0,88	10,83 ± 1,11	10,41 ± 0,94	12,00 ± 1,56	10,22 ± 0,65	17,37 ± 2,33	16,88 ± 1,92	14,85 ± 1,56	13,86 ± 1,80	17,10 ± 3,37	19,95 ± 2,37
04. Height at the areole (cm)	1,14 ± 0,25	1,34 ± 0,21	1,17 ± 0,28	0,94 ± 0,22	1,60 ± 0,42	1,20 ± 0,28	1,78 ± 0,42	1,89 ± 0,37	2,10 ± 0,37	1,36 ± 0,29	1,80 ± 0,35	0,97 ± 0,15	0,86 ± 0,10	0,94 ± 0,15	0,50 ± 0,20	0,73 ± 0,10	0,73 ± 0,16
05. Height between areoles (cm)	1,01 ± 0,30	1,22 ± 0,23	1,13 ± 0,30	0,75 ± 0,16	1,59 ± 0,41	1,21 ± 0,29	0,96 ± 0,26	1,00 ± 0,30	1,20 ± 0,36	0,97 ± 0,19	1,13 ± 0,26	0,03 ± 0,11	0,02 ± 0,10	0,20 ± 0,28	0 ± 0,28	0 ± 0,28	0 ± 0,28
06. Width at the areole (cm)	2,82 ± 0,65	3,00 ± 0,42	2,57 ± 0,54	1,56 ± 0,31	2,99 ± 0,42	2,97 ± 0,47	3,38 ± 0,66	3,66 ± 0,52	3,67 ± 0,63	2,68 ± 0,44	3,31 ± 0,37	1,32 ± 0,14	1,30 ± 0,14	1,19 ± 0,14	1,86 ± 0,36	0,97 ± 0,14	1,18 ± 0,22
07. Width between areoles (cm)	1,90 ± 0,52	2,20 ± 0,41	1,77 ± 0,44	1,07 ± 0,25	2,31 ± 0,53	2,16 ± 0,45	2,59 ± 0,53	2,99 ± 0,49	3,05 ± 0,68	2,14 ± 0,44	2,64 ± 0,40	0,78 ± 0,11	0,74 ± 0,09	0,73 ± 0,12	1,02 ± 0,26	0,56 ± 0,11	0,63 ± 0,15
08. Number of areoles along the rib	3,35 ± 0,59	2,80 ± 0,52	3,10 ± 0,30	3,10 ± 0,72	2,94 ± 0,24	2,43 ± 0,51	3,53 ± 0,72	4,75 ± 0,75	4,41 ± 0,94	3,58 ± 0,61	4,06 ± 0,42	4,83 ± 0,65	5,32 ± 0,69	5,65 ± 0,99	3,86 ± 0,73	3,95 ± 0,51	5,15 ± 1,09
Spines (average of three areoles)																	
09. Number of centrals	0	0,40 ± 0,50	0	0,10 ± 0,31	0	0	0,12 ± 0,33	0,33 ± 0,49	0,35 ± 0,49	0,32 ± 0,48	0	0,30 ± 0,47	0,08 ± 0,28	0,50 ± 0,51	0,95 ± 0,50	0,10 ± 0,31	0
10. Length of the main central (cm)	0	0,78 ± 1,01	0	0,07 ± 0,24	0	0	0,20 ± 0,57	0,57 ± 0,85	0,50 ± 0,72	0,40 ± 0,61	0	0,18 ± 1,19	0,04 ± 0,90	0,29 ± 1,26	0,83 ± 1,55	0,04 ± 0,59	0
11. Diameter of the main central (mm)	0	0,58 ± 0,73	0	0,040 ± 0,141	0	0	0,13 ± 0,36	0,37 ± 0,55	0,38 ± 0,54	0,29 ± 0,44	0	0,18 ± 0,27	0,04 ± 0,14	0,29 ± 0,31	0,83 ± 0,38	0,04 ± 0,12	0
12. Number of radials	8,15 ± 1,24	8,45 ± 0,97	8,05 ± 1,23	10,03 ± 1,29	7,14 ± 0,83	6,63 ± 1,17	7,45 ± 0,92	7,36 ± 1,15	8,12 ± 1,40	7,42 ± 0,72	6,65 ± 1,03	14,06 ± 1,39	13,87 ± 1,12	13,38 ± 1,17	11,46 ± 1,54	13,17 ± 1,19	16,25 ± 1,88
13. Length of lower right radial (cm)	2,76 ± 0,45	3,17 ± 0,37	2,66 ± 0,43	2,02 ± 0,34	2,85 ± 0,45	2,66 ± 0,48	2,57 ± 0,40	2,79 ± 0,58	2,55 ± 0,55	2,59 ± 0,41	2,21 ± 0,35	2,70 ± 0,45	3,11 ± 0,53	2,29 ± 0,35	3,91 ± 0,88	2,16 ± 0,32	2,43 ± 0,34
14. Diameter of lower right radial (mm)	1,38 ± 0,19	1,59 ± 0,20	1,14 ± 0,20	0,64 ± 0,12	2,31 ± 0,48	2,02 ± 0,23	1,64 ± 0,34	1,99 ± 0,33	1,62 ± 0,27	1,67 ± 0,23	1,54 ± 0,28	0,52 ± 0,06	0,44 ± 0,05	0,45 ± 0,09	1,01 ± 0,23	0,26 ± 0,05	0,40 ± 0,08
15. Length of lowermost radial (cm)	2,66 ± 0,49	3,18 ± 0,28	2,59 ± 0,43	2,00 ± 0,33	2,92 ± 0,43	2,77 ± 0,39	2,76 ± 0,43	3,15 ± 0,63	2,59 ± 0,64	2,79 ± 0,47	2,46 ± 0,37	2,63 ± 0,45	3,14 ± 0,61	2,31 ± 0,36	4,27 ± 1,00	2,10 ± 0,36	2,30 ± 0,42
16. Diameter of lowermost radial (mm)	1,40 ± 0,22	1,61 ± 0,20	1,13 ± 0,18	0,64 ± 0,13	2,39 ± 0,38	1,99 ± 0,23	1,84 ± 0,30	2,38 ± 0,44	1,94 ± 0,28	1,88 ± 0,25	1,83 ± 0,35	0,50 ± 0,07	0,44 ± 0,06	0,46 ± 0,09	0,98 ± 0,20	0,23 ± 0,05	0,36 ± 0,08
17. Length of lower left radial (cm)	2,76 ± 0,50	3,17 ± 0,33	2,60 ± 0,44	1,98 ± 0,28	2,95 ± 0,42	2,65 ± 0,42	2,59 ± 0,45	2,75 ± 0,56	2,56 ± 0,58	2,56 ± 0,37	2,25 ± 0,28	2,71 ± 0,46	3,14 ± 0,54	2,31 ± 0,38	3,92 ± 0,86	2,16 ± 0,33	2,43 ± 0,35
18. Diameter of lower left radial (mm)	1,38 ± 0,20	1,60 ± 0,18	1,11 ± 0,22	0,65 ± 0,10	2,25 ± 0,45	2,01 ± 0,27	1,67 ± 0,34	1,94 ± 0,36	1,64 ± 0,22	1,67 ± 0,22	1,51 ± 0,26	0,52 ± 0,06	0,45 ± 0,05	0,45 ± 0,09	1,02 ± 0,21	0,25 ± 0,05	0,39 ± 0,07
Cephalium																	
19. Height (cm)	1,51 ± 0,41	1,28 ± 0,40	1,55 ± 0,67	1,17 ± 0,58	1,23 ± 0,29	1,85 ± 0,72	1,74 ± 0,83	1,70 ± 0,38	1,40 ± 0,49	1,72 ± 0,74	1,51 ± 0,56	2,15 ± 0,69	2,58 ± 0,63	2,33 ± 0,60	1,64 ± 0,39	1,44 ± 0,48	1,70 ± 0,76
20. Width (cm)	3,02 ± 0,86	2,91 ± 0,70	3,05 ± 1,22	2,75 ± 0,55	2,72 ± 0,33	3,67 ± 1,19	3,07 ± 1,11	3,25 ± 0,75	4,03 ± 1,11	2,89 ± 0,73	2,77 ± 0,83	2,75 ± 0,58	3,25 ± 0,71	3,04 ± 0,81	2,51 ± 0,62	2,04 ± 0,46	2,30 ± 0,76
21. Length of peripheral bristles (cm)	0	0	0	0	0	0	0	1,90 ± 0,67	1,79 ± 0,45	1,68 ± 0,44	1,84 ± 0,56	5,31 ± 1,19	5,62 ± 1,10	4,12 ± 0,61	2,45 ± 0,86	4,08 ± 0,82	3,26 ± 0,92
22. Diameter of peripheral bristles (mm)	0	0	0	0	0	0	0	0,36 ± 0,14	0,45 ± 0,10	0,40 ± 0,09	0,35 ± 0,10	0,39 ± 0,14	0,40 ± 0,09	0,34 ± 0,06	0,31 ± 0,08	0,17 ± 0,07	0,23 ± 0,05

Table 4. Coefficients for canonical variables on the three first roots of a CVA using all individuals from the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil, with population as the grouping variable. For description of the variables see Table 3.

Variable	Root 1	Root 2	Root 3
1	-0.172	-0.144	0.645
2	0.417	0.065	-0.305
3	-0.074	0.173	-0.135
4	-1.735	-2.046	-0.175
5	1.791	1.380	-1.344
6	-0.155	0.237	0.574
7	0.133	-1.066	-0.763
8	-0.377	-0.058	-0.054
9	0.956	-0.129	-1.350
10	-0.218	-0.173	1.214
11	-0.745	-0.163	-0.155
12	-0.413	0.115	-0.043
13	0.372	0.477	-0.346
14	-0.607	1.337	-0.759
15	-0.797	-1.109	0.959
16	1.238	-1.620	0.413
17	0.400	1.253	0.223
18	1.586	-0.703	1.430
19	0.053	0.029	0.249
20	-0.142	0.164	-0.016
21	-0.697	-0.316	-0.299
22	-2.806	-8.551	-2.521
Eigenval	40.218	9.557	2.780

Table 5. Classification matrix from a CVA using all individuals from the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil, with population as the grouping variable. Shaded areas correspond to classifications of populations belonging to the same species complex. For population acronyms see Table 2.

	Percent of correct classifications	<i>Discocactus bahiensis</i> complex						<i>Discocactus catingicola</i> complex					<i>Discocactus zehntneri</i> complex					
		BB_AB	BB_GO	BB_OL	BG_SR	BS_JU	BS_RO	CC_AV	CC_SG	CN_CO	CN_PN	CR_PO	ZA_BG	ZB_EC	ZB_LA	ZB_VS	ZH_CL	ZR_LI
BB_AB	70.00	14	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0
BB_GO	90.00	0	18	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
BB_OL	85.00	0	3	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BG_SR	100.00	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0
BS_JU	88.24	0	0	1	0	15	1	0	0	0	0	0	0	0	0	0	0	0
BS_RO	100.00	0	0	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0
CC_AV	64.71	0	0	0	0	0	0	11	3	1	1	1	0	0	0	0	0	0
CC_SG	72.22	0	0	0	0	0	0	2	13	2	1	0	0	0	0	0	0	0
CN_CO	75.00	0	0	0	0	0	0	1	0	9	1	1	0	0	0	0	0	0
CN_PN	76.47	0	0	0	0	0	0	2	2	0	13	0	0	0	0	0	0	0
CR_PO	78.95	0	0	0	0	0	0	2	2	0	0	15	0	0	0	0	0	0
ZA_BG	100.00	0	0	0	0	0	0	0	0	0	0	0	21	0	0	0	0	0
ZB_EC	84.00	0	0	0	0	0	0	0	0	0	0	0	0	21	3	1	0	0
ZB_LA	83.33	0	0	0	0	0	0	0	0	0	0	0	0	3	25	2	0	0
ZB_VS	85.00	0	0	0	0	0	0	0	0	0	0	0	0	1	1	17	1	0
ZH_CL	100.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0
ZR_LI	85.00	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	17
		p=.05935	p=.05935	p=.05935	p=.05935	p=.05045	p=.06231	p=.05045	p=.05341	p=.03561	p=.05045	p=.05638	p=.06231	p=.07418	p=.08902	p=.05935	p=.05935	p=.05935

Table 6. Comparison of the specific and infraspecific classifications obtained from the delimitation methods employed in this study on the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. Populations resolved for each taxon by the different methods are shown following the name of the taxon. For population acronyms see Table 2.

	Wiens-Penkrot method (BG_SR included)	Wiens-Penkrot method (BG_SR excluded)	PAA	CVA (multivariate)	Taxa resolved by all methods	
<i>D. bahiensis</i> complex	<i>D. bahiensis</i> (BB_AB, BB_GO, BB_OL, BG_SR)	<i>D. bahiensis</i> (BB_AB, BB_GO, BB_OL, BG_SR)	<i>D. bahiensis</i> subsp. <i>bahiensis</i> (BB_AB, BB_GO, BB_OL, BS_JU, BS_RO)	<i>D. bahiensis</i> subsp. <i>bahiensis</i> (BB_AB, BB_GO, BB_OL)	<i>D. bahiensis</i>	
	<i>D. subviridigriseus</i> (BS_JU, BS_RO)	<i>D. subviridigriseus</i> (BS_JU, BS_RO)		<i>D. bahiensis</i> subsp. <i>subviridigriseus</i> (BS_JU, BS_RO)		
				<i>D. bahiensis</i> subsp. <i>gracilis</i> (BG_SR)		<i>D. bahiensis</i> subsp. <i>gracilis</i> (BG_SR)
<i>D. catingicola</i> complex	<i>D. catingicola</i> (CC_AV, CC_SG)	<i>D. catingicola</i> (CC_AV, CC_SG)	<i>D. catingicola</i> (CC_AV, CC_SG, CN_CO, CN_PN, CR_PO)	<i>D. catingicola</i> (CC_AV, CC_SG, CN_CO, CN_PN, CR_PO)	<i>D. catingicola</i>	
	<i>D. nigrisaetosus</i> (CN_CO, CN_PN)	<i>D. nigrisaetosus</i> (CN_CO, CN_PN)				
	<i>D. rapirhizus</i> (CR_PO)	<i>D. rapirhizus</i> (CR_PO)				
<i>D. zehntneri</i> complex	<i>D. zehntneri</i> (ZA_BG)	<i>D. zehntneri</i> (ZA_BG)	<i>D. zehntneri</i> subsp. <i>zehntneri</i> (ZA_BG)	<i>D. zehntneri</i> (ZA_BG)	<i>D. zehntneri</i> subsp. <i>zehntneri</i>	
	<i>D. boomianus</i> (ZB_EC, ZB_LA, ZB_VS, ZH_CL, ZR_LI)	<i>D. boomianus</i> (ZB_EC, ZB_LA, ZB_VS)		<i>D. zehntneri</i> subsp. <i>boomianus</i> (ZB_EC, ZB_LA, ZB_VS, ZH_CL, ZR_LI)		<i>D. boomianus</i> subsp. <i>boomianus</i> (ZB_EC, ZB_LA, ZB_VS)
		<i>D. araneispinus</i> (ZH_CL, ZR_LI)				<i>D. boomianus</i> subsp. <i>araneispinus</i> (ZH_CL, ZR_LI)

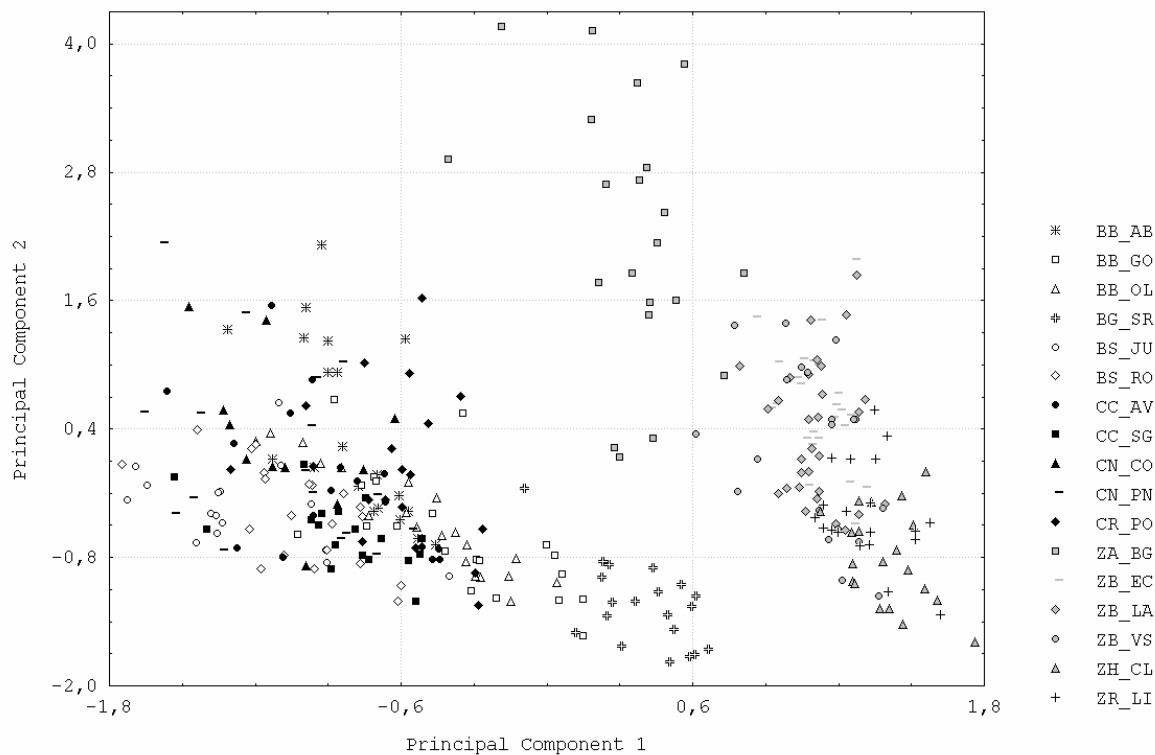


Figure 1. Scatter plot of the first two factors from a PCA using the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. The percentage of variance explained by Principal Component 1 and 2 are 42.77% and 19.55% respectively. For population acronyms see Table 2.

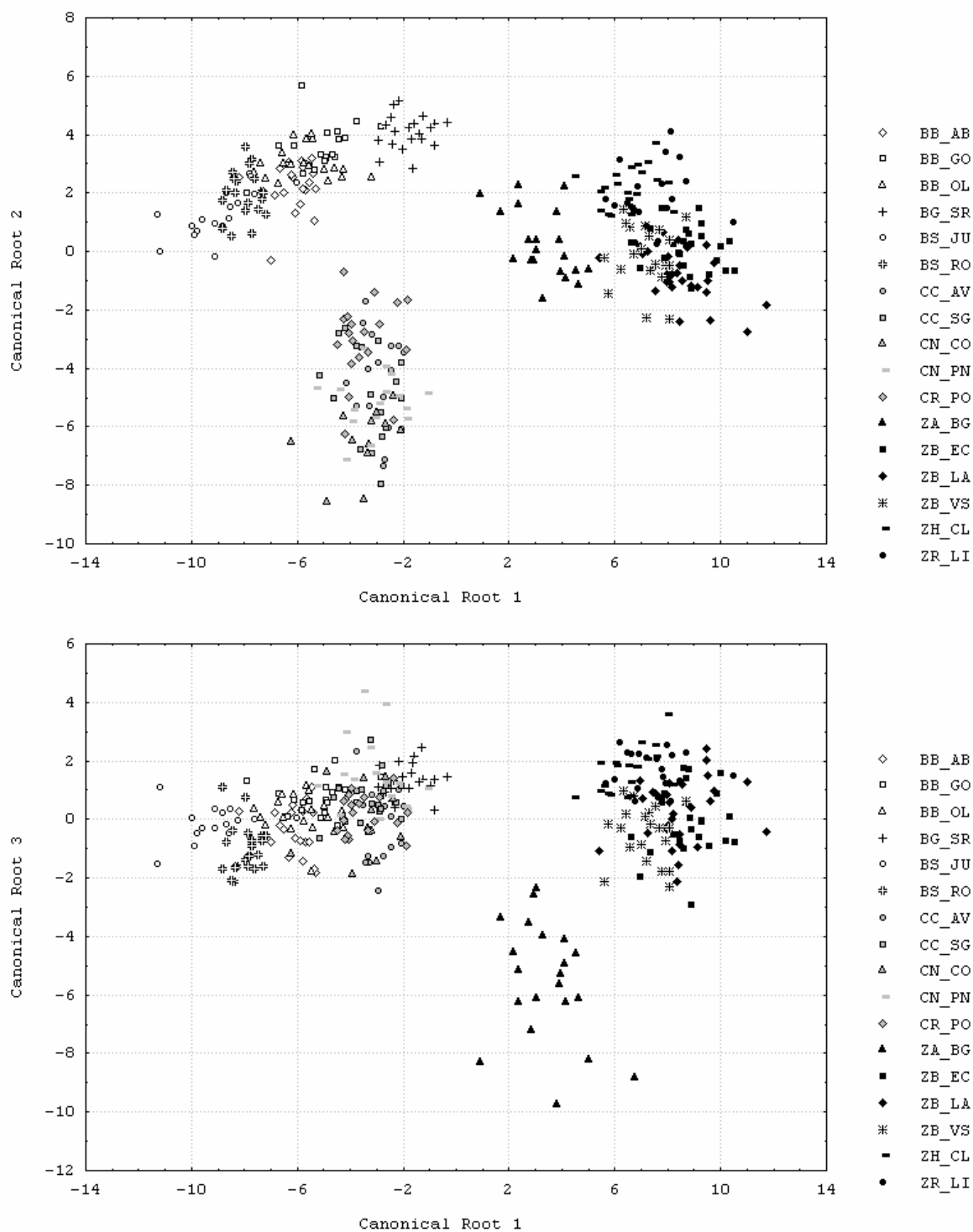


Figure 2. Scatter plot of individuals' scores on the first three canonical roots from a CVA using the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil, with population as the grouping variable. The first, second and third canonical roots explained respectively 68.78%, 16.34% and 4.75% of the morphological variation. For population acronyms see Table 2.

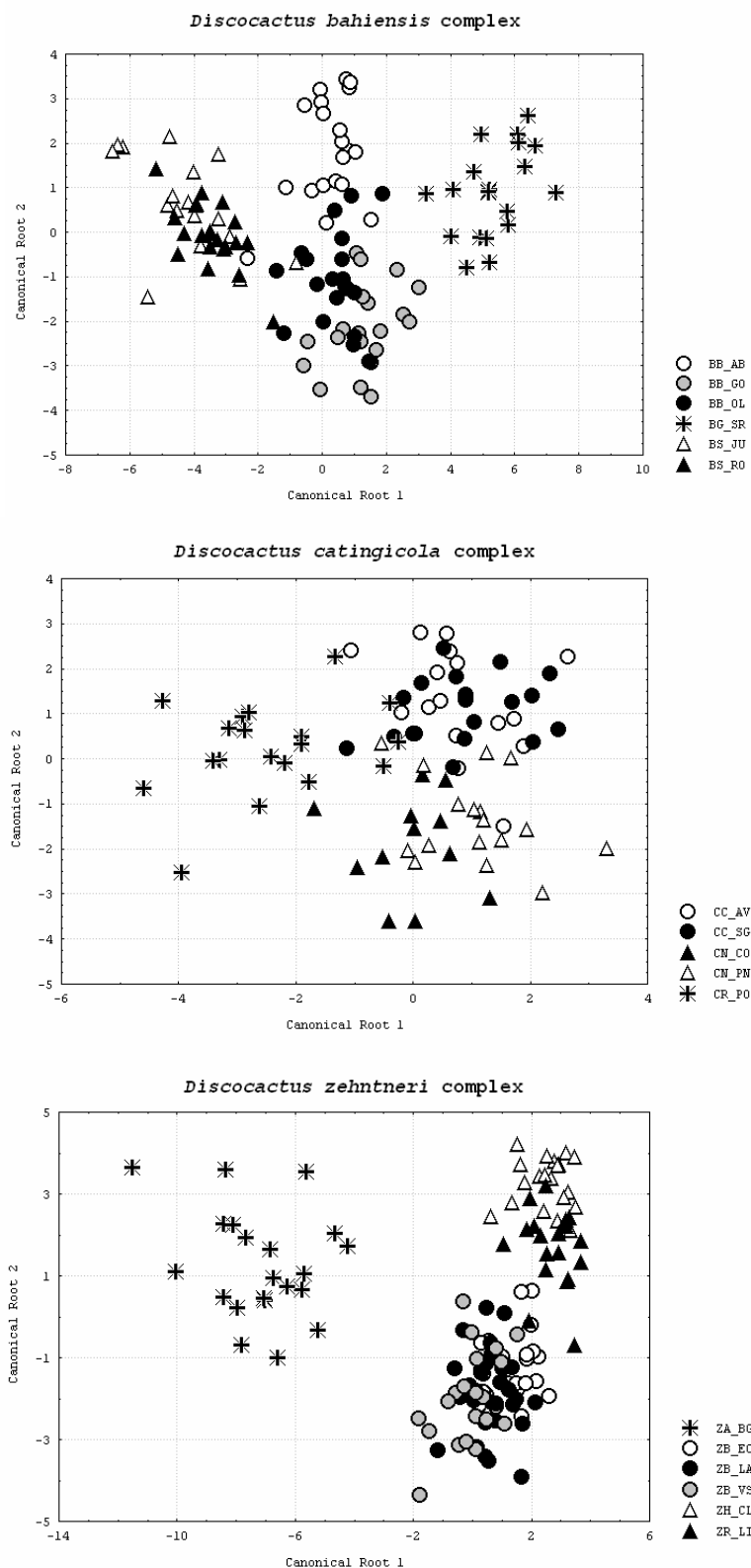


Figure 3. Scatter plot of individuals' scores on the first two canonical roots from CVA analyses conducted for each species complex separately and having population as the grouping variable, for the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. For population acronyms see Table 2.

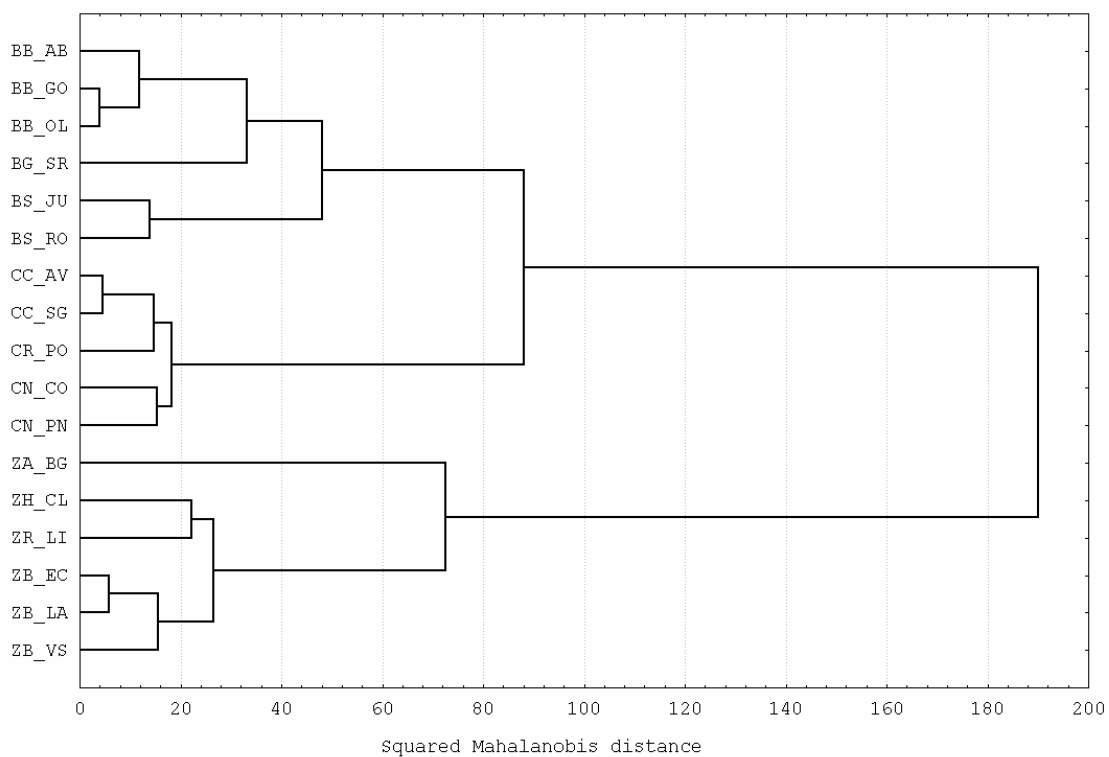


Figure 4. UPGMA dendrogram of morphological distances among the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. For population acronyms see Table 2.

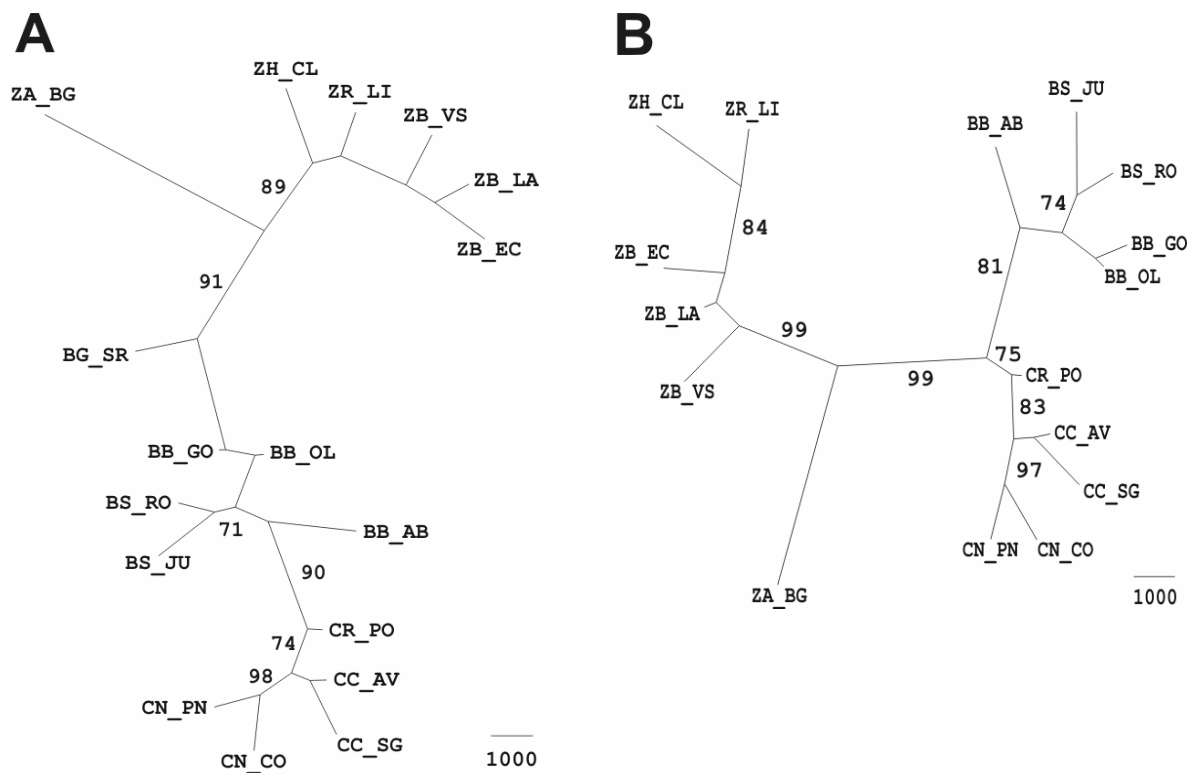


Figure 5. Shortest unrooted trees obtained from a cladistic analysis of morphological quantitative data of the 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. **A** – tree with all populations included, **B** – tree obtained by excluding the population BG_SR. Numbers indicate bootstrap support > 70%. For population acronyms see Table 2.

Capítulo 2. Genetic variability of three *Discocactus* Pfeiff. (Cactaceae) species from the state of Bahia, Brazil

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RESUMO

A variabilidade genética de três espécies de *Discocactus* Pfeiff. (Cactaceae) do estado da Bahia, Brasil, foi investigada em 17 populações e 337 indivíduos. A diversidade de aloenzimas em 10 loci revela elevados graus de diversidade genética nas espécies de *Discocactus* estudadas quando comparados aos valores médios para outras espécies vegetais (Hamrick et al., 1992), porém sendo similares ou superiores aos valores médios encontrados em outras espécies de cactáceas (Hamrick et al., 2002) e outras espécies vegetais com ciclos de vida e outras características similares (Hamrick et al., 1992).

Déficit de heterozigotos foi detectado em *D. bahiensis* e *D. zehntneri*. Níveis baixos a moderados de diferenciação genética foram detectados entre as populações de *D. bahiensis* e *D. catingicola*, enquanto que elevados níveis de diferenciação são encontrados em *D. zehntneri*, o que é atribuído a uma partição geográfica das populações. Foram detectadas evidências de introgressão de genes de *D. zehntneri* em uma população de *D. bahiensis*, com a ocorrência nesta população de dois alelos exclusivos de *D. zehntneri*.

A ausência de subgrupos e as elevadas identidades genéticas encontradas entre populações co-específicas de *D. bahiensis* e *D. catingicola* não provêm subsídios para o reconhecimento de táxons infra-específicos nestas espécies, estando em congruência com as circumscrições taxonômicas adotadas com base em um estudo de variabilidade morfológica utilizando os mesmos indivíduos e populações (CAPÍTULO 1). Em *D. zehntneri* os resultados obtidos a partir das análises de dados morfológicos particionam a espécie em duas subespécies – *zehntneri* e *boomianus*, enquanto que os resultados obtidos da análise de variabilidade genética subdividem geograficamente as populações da espécie em dois grupos, com um grupo mais ao sul constituído de populações da subespécie *boomianus*, e um grupo mais ao norte constituído de populações de ambas subespécies. Estes resultados não comprometem a interpretação de duas subespécies em *D. zehntneri*, existindo diferenciação genética moderadamente alta entre a subespécie *zehntneri* e as populações da subespécie *boomianus* que ocorrem no norte, associada a um elevado grau de diferenciação morfológica da subespécie *zehntneri*. Uma origem única para a subespécie *boomianus* é bem sustentada pela análise de dados morfológicos e genéticos, e o elevado grau de diferenciação entre as populações dessa subespécie é interpretado como sendo derivado de sua origem no norte, com o posterior estabelecimento de populações mais ao sul, seguido de isolamento e divergência genética, enquanto que as populações do norte retiveram mais características em comum com a subespécie típica.

Genetic variability of three *Discocactus* Pfeiff. (Cactaceae) species from the state of Bahia, Brazil

RUNNING TITLE: Genetic variability of *Discocactus* from Bahia, Brazil

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ABSTRACT

The genetic variability of three *Discocactus* (Cactaceae) species from the state of Bahia, Brazil, was investigated in 17 populations and 337 individuals. Allozyme diversity at ten loci reveals a high degree of genetic diversity compared to other plant taxa, but similar to average values for other cactus species. Within-population heterozygosity deficit was detected in *D. bahiensis* and *D. zehntneri*. Moderate to low levels of genetic differentiation were detected among populations of *D. bahiensis* and *D. catingicola*, while high levels are found in *D. zehntneri*, which are attributed to a north and south geographic partition of the populations. Evidence of introgression with *D. zehntneri* was found in one population of *D. bahiensis*. Absence of genetic substructure and high genetic identities among conspecific populations of *D. bahiensis* and *D. catingicola* do not support recognition of infraspecific taxa within these species, being congruent with their taxonomic circumscriptions as adopted by the authors with basis on a study of morphological variability using the same populations and individuals. For *D. zehntneri*, morphology supports a division of the species in two subspecies, while allozymes partition the populations on a geographical basis.

ADDITIONAL KEYWORDS: Allozymes – Genetic diversity – Endangered species – Northeastern Brazil – Caatinga – Cerrado – Campo Rupestre – Neotropics

INTRODUCTION

The state of Bahia is located in the Northeastern region of Brazil. A number of vegetation types occur within the state, but most prominent is the *caatinga*, formation characteristic of the semiarid region of the northeast of the country (Rizzini, 1976; Machado, 1998). The most common physiognomy is that of a low and deciduous, open scrub forest, with xerophytic, often thorny plants. Centuries of human occupation resulted in extensive disturbance and fragmentation of the *caatinga*, with several plant species becoming rare and patchy in occurrence. In the western region of the state predominates *cerrado*, a savannah-like vegetation type which occupy most of the central region of Brazil (Eiten, 1972; Rizzini, 1976). The *cerrado* has experienced much destructive change in recent years due to increased agricultural development in the regions where this vegetation occurs, and nowadays the *cerrado* is a highly fragmented formation.

In the mountainous areas of the central part of the state (Chapada Diamantina) occurs the vegetation type termed *campo rupestre*, a formation is characterized by open, herbaceous vegetation on sandy, stony soils mixed with herbs and shrubs growing on outcropping islands of quartzite, sandstone, gneiss or “pedra canga” rocks (Giulietti & Pirani, 1988; Borba & Semir, 1998). Because of the discontinuity of these mountain ranges and outcroppings, many species, especially the rupicolous ones, are distributed in disjunct populations. It has been suggested that this characteristic is responsible for the great diversity and high endemism of the *campo rupestre* vegetation, one of the highest among the vegetation types of Brazil (Joly, 1970; Giulietti & Pirani, 1988).

Discocactus is a genus of small cacti almost completely confined to Brazil. Number of species ranges from seven to twenty five, depending on the classification system adopted. Populations of the genus are often small, inhabiting restricted, often isolated areas within the *caatinga*, *cerrado* and *campo rupestre* formations. These factors make *Discocactus* populations extremely vulnerable to habitat modification and destruction, and to overcollecting. All species in the genus are considered threatened or endangered, and the whole genus is placed in Appendix I of CITES (Lüthy, 2001).

The taxonomy of *Discocactus* is disputed. Several taxa have been described for the state of Bahia (see Table 1 in Machado et al., submitted), and these taxa fall more or less in three groups, recognized with basis in morphological traits and ecological preferences: the complex of *D. bahiensis* Britton & Rose, the complex of *D. catiungicola* Buining & Brederoo, and the complex of *D. zehntneri* Britton & Rose. In the current classification

systems proposed by Braun & Esteves Pereira (1993a-c, 1995, 2001b) and Taylor & Zappi (2004) only three species are recognized, each of which corresponding to one of the three species complexes; however, the classification systems differ considerably in the infraspecific classification of the species.

The divergence of the classification systems proposed for *Discocactus* reflects the difficulty of delimitation of these taxa, whose morphological characters – traditionally utilized to delimit taxa in the genus – often overlap. For the current study, species circumscriptions are based on a more comprehensive morphological investigation of 22 vegetative characters using multivariate techniques and character-based and tree-based species delimitation protocols (Machado et al., submitted), conducted on the same populations and individuals surveyed for the assessment of genetic variability in this study. The species recognized for the state are *D. bahiensis*, *D. catingicola* and *D. zehntneri*, the latter with two subspecies, *zehntneri* and *boomianus*.

The objective of this study was to assess levels of genetic variation and sub-structuring within and between the *Discocactus* species and populations that occur in the state of Bahia using isozyme markers, in order to test the species circumscriptions proposed by Machado et al. The results obtained can also aid in the determination of priority areas for conservation of the species in the state, and also in the delineation of sampling strategies for ex situ conservation.

MATERIAL AND METHODS

Species and populations surveyed

In order to cover the whole range of variation within each species, the majority of the taxa described for the state of Bahia were surveyed at their type localities (Table 1, Fig. 1); this also enabled species limits to be analyzed. Precise information about type localities was obtained for all taxa except for *Discocactus zehntneri* Britton & Rose, *D. bahiensis* Britton & Rose and the taxon described as *D. buenekeri* Abraham, for which exact locality information was not provided in the original descriptions nor in the herbarium sheets of the type specimens. In addition to the populations from Bahia it was included a population from the state Goiás that represents the taxon described as *D. rapirhizus* Buining & Brederoo, because it occurs relatively close to the area of distribution of *D. catingicola* Buining & Brederoo and was regarded as conspecific with the later taxon in the classification system proposed by Braun & Esteves Pereira (1993a-c, 1995, 2001b); we

also found it hardly distinguishable from *D. cattingicola* on morphological grounds (Machado et al., submitted).

A total of 337 individuals were sampled in 17 natural populations (*D. bahiensis* - six populations, 118 individuals; *D. cattingicola* - five populations, 83 individuals; *D. zehntneri* - six populations, 136 individuals) in Bahia and Goiás states (Table 1, Fig. 1). Sampled individuals constituted of mature plants (assessed by the presence of a well-developed cephalium) spaced by at least five meters. The number of individuals sampled at each population varied from 12 to 30; in the smallest populations the largest possible number of plants was sampled, but still taking the required distance between them into consideration. A small stem piece (1cm³) was collected from each individual, labeled, and kept on ice during the collecting day. At the end of the day the samples were frozen in liquid nitrogen. On returning to the laboratory the samples were transferred to a -80°C ultrafreezer, where they were kept until electrophoresis was performed. About three plants from each population were collected and deposited as vouchers at the herbarium of the Universidade Estadual de Feira de Santana (UEFS; Table 1).

Electrophoretic procedures

Small sections of stem tissue were crushed in 0.5 mL of grinding buffer (100 mL Tris-HCl 0.1 mol/L pH 7.0, 6.846 g saccharose, 0.6 g PVP [polyvinylpyrrolidone], 0.0292 g EDTA [ethylenediaminetetraacetic acid], 0.145 g BSA [bovine albumin], 0.13 g DIECA [sodium diethylcarbamate], 0.6 g Borax, and 100 µL β-mercaptoethanol; modified from Sun & Ganders, 1990). Extracts were absorbed in 1.0 X 0.3 cm Whatman number 3 paper wicks, which were loaded into 8.5% starch gels (Sigma hydrolyzed potato starch).

For electrode and gels two buffer systems were used: 1) electrode: histidine 0.065 mol/L adjusted to pH 6.5 with citric acid; gel: electrode buffer diluted 1:4; modified from Stuber et al. (1977); 2) electrode: lithium hydroxide 0.05 mol/L, boric acid 0.0935 mol/L, EDTA 0.0059 mol/L, pH 8.0; gel: electrode solution diluted 1:10; modified from Ridgway, Sherburne & Lewis (1970). Standard horizontal electrophoresis was performed until the inner marker (bromophenol blue) reached nine cm from the application site using the following running conditions: system 1 - 25 mA; system 2 - 200 V. Seven enzymatic systems gave enough resolution for reading and were used. System 1 was used for diaphorase (DIA; EC 1.8.1.4), phosphoglucomutase (PGM; EC 2.7.5.1), and malate dehydrogenase (MDH; EC 1.1.1.37); system 2 was used for isocitrate dehydrogenase

(IDH; EC 1.1.1.42), shikimate dehydrogenase (SKDH; EC 1.1.1.25), phosphoglucose isomerase (PGI; EC 5.3.1.9), and leucine aminopeptidase (LAP; EC 3.4.11.1).

The staining procedures were similar to but slightly adjusted from Alfenas et al. (1991; DIA, SKDH), Brune, Alfenas & Junghans (1998; LAP), Corrias et al. (1991; IDH, PGI), and Soltis et al. (1983; PGM, MDH). Modifications were mainly in the amounts of the components used; the exact recipes can be obtained on request. Enzymatic systems showing more than one locus were numbered in descending order from the locus with highest mobility. The alleles were numbered according their mobility relative to the allele with highest mobility of a standard individual of *D. zehntneri* present in all gels and designated as 100.

Data analysis

The allelic frequencies were determined by manually counting the banding patterns of the homozygotes and heterozygotes stained in the gels. Genetic variability for every population was estimated by the following parameters: proportion of polymorphic loci (P ; 0.95 criterion), mean number of alleles per locus (A), observed (H_o) and expected (H_e) mean heterozygosity per locus. Deviations from the expected mean heterozygosity under Hardy-Weinberg (HW) equilibrium were tested using χ^2 with a correction for small samples according to Levene (1949). Partitioning of genetic diversity among conspecific populations was estimated by F statistics (F_{IS} , the inbreeding coefficient measures the reduction in heterozygosity due to nonrandom mating within a population; F_{ST} , measures the differentiation among populations; Wright, 1978). Matrices of genetic distances (Nei's [1978] unbiased genetic distance) and genetic identities (Nei's [1978] unbiased genetic identity) were calculated for populations and species. Cluster analysis was performed with the genetic distances matrix of the populations with UPGMA as grouping algorithm (Sneath & Sokal, 1973), using the software package STATISTICA for Windows, Release 5.5 A (StatSoft, 2000). A matrix of geographic distances between populations was produced with geodetic distances on WGS84 earth ellipsoid calculated using the INVERSE 2.0 program (National Geodetic Survey, 2002). The genetic and geographic distances matrices were compared by a Mantel test with 5000 randomized runs, using the PC-ORD 4.10 program (McCune & Mefford, 1999). All analyses were made using the BIOSYS 1.0 software package (Swofford & Selander, 1989) except for the Mantel tests.

The individuals sampled for the allozyme analysis were also used in a study of morphological variability of the populations, and measures of 22 vegetative morphological

characters were analyzed using discriminant analysis with population as the grouping variable in order to obtain a matrix of Mahalanobis distances of individuals to group centroid (D_2); the morphologic variability of populations was calculated as the median of these distances (D_{2m} ; Machado et al., submitted; not shown). The non-parametric test of Kruskal-Wallis was applied to verify the occurrence of significant differences between medians of conspecific populations. A Spearman rank correlation analysis between the morphologic (D_{2m}) and genetic (H_o) variability of populations was also conducted using . A multi-response permutation procedure (MRPP) analysis made with the PC-ORD 4.10 program (McCune & Mefford, 1999) was used to calculate the chance-corrected within-group agreement (A) among populations of every species, and the A values were compared with the indexes of genetic differentiation among populations (F_{ST}).

RESULTS

Intrapopulation alzyme variability

Using seven enzymatic systems 10 loci were obtained with good resolution and were used in this study (Table 2). All loci were polymorphic in at least two species. PGI was the most polymorphic, with 11 alleles. A high percentage of loci polymorphic in *D. bahiensis* (40%) and *D. catingicola* (33.34%) had a very common allele with frequency of occurrence of 95% or higher and one or more rarer alleles with frequency of occurrence smaller than 5%.

Some alleles were exclusive to a given species: MDH-1 92, MDH-1 116, MDH-2 108, PGM-2 84, LAP 60, PGI 64, PGI 72, and PGI 76 for *D. bahiensis*; PGI 80 for *D. catingicola*; and DIA 104, MDH-3 108, PGM-1 100, PGM-2 108, and LAP 100 for *D. zehntneri*. A few of these alleles were exclusive to single populations: DIA 104 (ZR_LI), MDH-1 92 and PGM-2 84 (BB_OL), PGM-2 108 (ZA_BG) and PGI 64 (BB_AB). However, none of the species were fixed for alternative alleles at any locus, and thus no locus was diagnostic for any of the species.

Percentage of polymorphic loci (P ; 0.95 criterion) ranged from 40 to 70%, the mean number of alleles per locus was between 1.6 and 2.8, and mean heterozygosity (H_e) ranged from 0.103 to 0.382 (Table 3). In general, populations of *D. bahiensis* displayed the highest variability. Within *D. zehntneri*, northern populations (ZA_BG, ZH_CL and ZR_LI) displayed higher genetic diversity than southern populations (ZB_EC, ZB_LA and ZB_VS).

No significant differences in morphological variability were present among the populations of *D. bahiensis* ($H=12.0294$, $p=0.0344$). The populations with the highest values of morphological variability were CN_CO and CN_PN for *D. catingicola* ($H=16.3892$, $p=0.0025$), and ZA_BG for *D. zehntneri* ($H=24.6064$, $p=0.0002$). Spearman rank correlation analysis between morphologic and genetic variability of conspecific populations and of all populations pooled did not result in statistically significant correlations (all populations: $R=-0.137$, $p=0.599$; *bahiensis* only: $R=0.086$, $p=0.871$; *catingicola* only: $R=-0.100$, $p=0.873$; *zehntneri* only: $R=-0.200$, $p=0.704$; Fig. 3).

Of the 17 populations, 15 showed significant deviation from the expected values in HW equilibrium in at least one locus; the exceptions were the populations BS_RO and ZB_LA. *Discocactus bahiensis* and *D. zehntneri* were the species with largest proportion of loci presenting deviations from HW equilibrium (*D. bahiensis*: 33.34 - 71.43%; *D. zehntneri*: 33.34 - 66.67%) while *D. catingicola* displayed a smaller proportion of loci deviating from HW equilibrium (16.67 - 25%). Overall, all loci had significant deviations from HW equilibrium in at least one of the populations, with MDH-3 not in equilibrium in nine populations, SKDH in seven, MDH-2 in six, DIA in five, and the remaining loci not in equilibrium in three populations or less. The reason for disequilibrium was deficit of heterozygotes in all loci except for DIA in four populations, and PGI in one population, which presented excess of heterozygotes. Mean F_{IS} was low in *D. catingicola*, while *D. bahiensis* and *D. zehntneri* displayed higher F_{IS} values (Table 4), reflecting a deficit of heterozygous in these populations.

In all species, the loci MDH-2 and MDH-3 were interpreted as resulting from a duplication event. In the majority of individuals of *D. bahiensis*, *D. catingicola* and *D. zehntneri* subsp. *zehntneri*, a band of higher intensity was observed in an intermediate position between the alleles of the two loci, suggestive of fixed heterozygosity; since this pattern was found only in the MDH enzymatic system, single gene duplication is a more likely explanation than polyploidy. The majority of individuals of *D. zehntneri* subsp. *boomianus* (Buining & Brederoo) N.P.Taylor & Zappi did not display the intermediate band, suggesting a higher divergence between the MDH-2 and MDH-3 loci – the populations of this subspecies are nearly fixed for an allele of higher mobility in the locus MDH-3 (Table 2). In the northern populations of *D. zehntneri* subsp. *boomianus* (ZH_CL and ZR_LI), further differentiation occurs, with a loss of expression of the locus MDH-2 in the majority of the individuals in these populations, as reflected by the low sample size of alleles observed in this locus (Table 2).

Species genetic structure

Differences between populations accounted for 6.8 to 36% of species variability (Table 4). *Discocactus bahiensis* and *D. catingicola* displayed moderate to low average values of F_{ST} , interpreted as a moderate level of genetic structuring. *D. zehntneri* displayed a very high average value of F_{ST} (0.360). Including only the populations assigned to subspecies *boomianus* in the analysis of genetic structuring within *D. zehntneri* still led to a high value of F_{ST} (0.307), while separating northern (ZH_CL and ZR_LI; Fig 1) and southern (ZB_EC, ZB_LA, and ZB_VS; Fig. 1) populations of subspecies *boomianus* resulted in low F_{ST} values among the populations of each set (0.069 and 0.068, respectively). Including among the northern populations of subspecies *boomianus* the geographically close population of subspecies *zehntneri* (ZA_BG) increased F_{ST} only moderately (0.169). Even the species with low F_{ST} (*D. catingicola*) presented exclusive alleles in some populations (Table 2).

Values of chance-corrected within-group agreement (A) among populations of *D. bahiensis* and *D. zehntneri* were similar (0.24 and 0.29, respectively) and correlated to the higher values of genetic differentiation (F_{ST}) found in these species (0.127 and 0.360, respectively; Table 4); *D. catingicola* presented the lowest values for both A (0.075) and F_{ST} (0.068).

Phenetic relationships

Genetic identities among conspecific populations of *Discocactus bahiensis* and *D. catingicola* were all above 0.890 (Table 5, Fig. 2). Within *D. zehntneri*, the lowest levels of genetic identities (0.689 - 0.747) are between subsp. *zehntneri* and southern populations of subsp. *boomianus*; the identities between northern and southern populations of subsp. *boomianus* ranged from 0.776 - 0.889, while the identities between subsp. *zehntneri* and northern subsp. *boomianus* is slightly higher (0.882 - 0.898).

Genetic identities among species are presented in Table 5. *D. bahiensis* and *D. catingicola* had pair values above 0.836, and are grouped in the dendrogram (Fig. 2). *D. zehntneri* shared many alleles with *D. bahiensis*, specially its northern populations (ZA_BG, ZH_CL, and ZR_LI; Table 2, Fig. 1), with pair values genetic identities between 0.617 - 0.814, while the southern populations of *D. zehntneri* (ZB_EC, ZB_LA, and ZB_VS) diverged more from *D. bahiensis* (0.550 - 0.703). Genetic identities between populations of *D. zehntneri* and *D. catingicola* were usually lower (Table 5).

The UPGMA dendrogram obtained from the cluster analysis of Nei (1978) unbiased genetic distances (Fig. 2) reveals a differentiation between *D. zehntneri* and a group formed by *D. bahiensis* and *D. catingicola*, with a distance of approximately 0.43 between these groups. The next separation occur within *D. zehntneri*, where two groups separate by a distance of approximately 0.24, reflecting a north and south geographic partition of the populations (Fig. 1). It is caused by an inversion in the relative frequencies of the most common alleles in the loci PGM-1, PGM-2, and LAP (Table 2). Locus MDH-3 also influences the large F_{ST} value found in *D. zehntneri*, but reflects a partition between subspecies *zehntneri* and *boomianus* (Table 2). A low genetic distance (approximately 0.13) separates *D. bahiensis* and *D. catingicola*; no subgroups are found within these species – their populations are linked successively in the dendrogram.

Mantel tests for pairwise correlations between genetic and morphologic, and genetic and geographic distances of conspecific populations did not produce statistically significant results, except for the correlation of genetic and morphologic distances in populations of *D. zehntneri*, which had a statistically significant negative correlation ($r = -0.342126$, $p < 0.0328$).

DISCUSSION

Genetic variability

For all parameters of genetic variability analyzed, the *Discocactus* species surveyed possessed in general values similar or higher than the means reported for other cactus species (Hamrick et al., 2002) and other plant species with similar characteristics (Hamrick et al., 1992; Table 6), except for the parameters mean number of alleles per locus, which was somewhat smaller in *D. catingicola* and *D. bahiensis* than the values of the other classes, and total genetic diversity, for which *D. catingicola* had the smallest value. In *D. catingicola* and *D. bahiensis*, a high proportion of loci polymorphic had a very common allele with frequency of occurrence of 95% or higher (Table 6, columns P* and P**) and several rare alleles per locus, a pattern concordant with the observed for other cactus species surveyed (Hamrick et al., 2002). The high variability of the *Discocactus* species is in sharp contrast to the very low levels found in species of the genus *Melocactus* (Cactaceae) occurring in the same general area and having similar habits (Lambert et. al., submitted).

Within *D. bahiensis*, the populations which had the lowest genetic diversities (BG_SR and BS_RO) are located within highly disturbed areas (close to a village and near a road, respectively), besides being known for a longer time by plant collectors and thus having suffered more from collection; as a result, these populations may have experienced genetic bottlenecks, adversely affecting their genetic diversities. In *D. catinicola*, all populations had similar genetic diversity except for the population CN_CO which was also the smallest; this population was located within a disturbed agricultural area, and its lower genetic diversity may also have resulted from bottlenecking effects.

A possible explanation for the smaller genetic diversity of the southern populations of *D. zehntneri* relative to its northern populations, in spite of the southern populations being generally much larger, is that the southern population may have been originated from a small number of individuals carrying only a subset of the genetic diversity of the species (founder effect); this and subsequent genetic drift may also have led to the observed inversion in the relative frequencies of the most common alleles in the loci PGM-1, PGM-2, and LAP, generating the low values of genetic identities among the two sets of populations. A northern origin of the southern populations is supported by morphological analyses (Machado et al., submitted).

The population BG_SR of *D. bahiensis* appear to be experiencing introgression of genetic material from a nearby population of *D. zehntneri*, a hypothesis suggested by morphological analyses (Machado et al., submitted) and by the presence in this populations of alleles (LAP 92 and SKDH 108) otherwise only found in *D. zehntneri* (Table 2).

The somewhat high mean F_{IS} observed in *D. bahiensis* and *D. zehntneri* indicate a deficit of heterozygous individuals in these species. A deficit of heterozygous individuals can be due either to inbreeding, or to population subdivision. In the particular case of *Discocactus* species, population subdivision is a less likely reason for excess homozygosity because flowering is synchronous in the genus (Machado, pers. obs.; Charles, 2003: 81). Inbreeding due to selfing is not possible because *Discocactus* species are self-incompatible (Machado, unpubl. data) and thus obligate outcrossers; but biparental inbreeding (Heywood, 1991) between related individuals is likely to occur, since seed dispersal is mostly local, mediated by ants (Machado, pers. obs.; Taylor & Zappi, 2004). In the case of *D. zehntneri*, which also propagates asexually by lateral sprouting, chances of crosses among close relatives is even higher. Biparental inbreeding is thus the most plausible reason for the high mean F_{IS} values observed in *D. bahiensis* and *D. zehntneri*.

Intraspecific phenetic relationships and genetic structure

The genetic identities found among conspecific populations were similar to those reported in other plant species (Thorpe, 1982; Crawford, 1989; van der Bank et al., 2001). Only the identities between northern and southern populations of *D. zehntneri* were lower than the values usually found for allopatric conspecific populations (Crawford, 1989; Hartl 2000; Thorpe, 1982; van der Bank et al., 2001). The low values of chance-corrected within-group agreement (A) and F_{ST} in *D. catingicola* reflect a low genetic and morphologic differentiation among its populations. Although A values of *D. bahiensis* and *D. zehntneri* were similar, the F_{ST} value for *D. zehntneri* was almost three times higher than the F_{ST} value for *D. bahiensis*, what can be explained by the fact that only one population of *D. zehntneri* is more divergent morphologically (Machado et al., submitted), while in genetic terms there is a large divergence between the populations of this species – the large F_{ST} value (0.360) observed for *D. zehntneri* is basically the result of the geographic subdivision of this species in two sets of divergent populations in the northern and southern ends of its distribution range. This F_{ST} value of *D. zehntneri* is very high (Hartl, 2000), and indicate that its northern and southern populations must have been isolated for a very long period of time in order to achieve the observed level of divergence, of the order of several thousands of generations (Hartl, 2000), what could translate in a period of a few hundred thousand years of isolation since these plants are long-lived with a generation time of several years.

Geographical sub-structuring of populations is also the factor responsible for the very high F_{ST} value (0.431) reported for the cactus *Lophocereus schottii* (Engelm.) Britton & Rose (Nason, Hamrick & Fleming, 2002), reflecting a separation of Baja California and Sonora populations during the formation of the Sea of Cortez during the late Pliocene (~ 3 - 5 million years ago). Other plant species occurring in the same general area as *D. zehntneri* are also recorded as possessing high levels of differentiation among their populations, such as *Acianthera teres* (Lindl.) Borba (Orchidaceae) found in disjunct populations of the Espinhaço Range ($F_{ST} = 0.205$; Borba et al., 2001), and *Proteopsis argentea* Mart. & Zucc. (Asteraceae) from the same mountain range ($F_{ST} = 0.300$; Jesus et al., 2001), that displayed marked differentiation even among populations separated by just a few kilometers.

The northern populations of *D. zehntneri* are ascribed to two taxa: subspecies *zehntneri* and subspecies *boomianus*. In spite of the higher genetic identities between subspecies *zehntneri* and northern populations of subspecies *boomianus* than the genetic

identities of either group to southern populations of subspecies *boomianus*, morphology clearly distinguishes subspecies *zehntneri*, while no clear distinction is possible among populations of subspecies *boomianus*. One hypothesis that could explain the higher similarity of subspecies *zehntneri* and northern populations of subspecies *boomianus* is that the latter subspecies is polytypic, with distinct origins for its northern and southern populations and their morphological similarities being a result of convergence. However, this hypothesis is not supported by a cladistic analysis of the populations using morphological characters (Machado et al., submitted) and by a Wagner tree built with the allozyme data (not shown). The alternative hypothesis is that both sets of populations of subspecies *boomianus* do indeed have a common origin. Thus, the degree of genetic differentiation observed between northern and southern populations of this subspecies could be a result of either: a) introgression between subspecies *zehntneri* and subspecies *boomianus* in the north, or b) a northern origin of subspecies *boomianus*, with subsequent migration and divergence of the southern populations while the northern populations diverged less from the common ancestor with subspecies *zehntneri*.

Against hypothesis (a) is the fact that relatively high differentiation occurs between subspecies *zehntneri* and northern populations of subspecies *boomianus* – genetic identities between the two taxa are slightly lower than the mean values usually found for conspecific populations (Crawford, 1989; Thorpe, 1982; van der Bank et al., 2001), and F_{ST} value between them is moderate, suggesting that gene flow among the subspecies is negligible or absent, as evidenced by the inversion in the relative frequencies of the most common alleles in the locus MDH-3; moreover, a high level of morphologic divergence occurs between the subspecies (Machado et al., submitted). Hypothesis (b) provides the most plausible explanation given the data currently available: a northern origin for subspecies *boomianus*, followed by migration and genetic divergence of the southern populations, which probably were established with a subset of the alleles present in the subspecies as a whole – northern populations possess higher genetic diversity, including five exclusive alleles (DIA 96, DIA 104, PGM-2 108, LAP 76 and LAP 84), while southern populations only possess two exclusive alleles (DIA 84 and SKDH 108). Also, due to founder effect and genetic drift, relative frequencies for the most common alleles were inverted in some loci (PGM-1, PGM-2, and LAP; Table 2), further contributing for the differentiation of the two sets of populations of subspecies *boomianus*.

Overall, the results of the allozyme survey of the *Discocactus* species are congruent with the taxonomic circumscriptions adopted by the authors, which are based on a detailed

morphological investigation of 22 vegetative characters using multivariate techniques and character-based and tree-based species delimitation protocols (Machado et al., submitted), conducted on the same populations and individuals surveyed for the assessment of genetic variability in this study. The absence of subgroups and the high genetic identities among conspecific populations of *D. bahiensis* and *D. catingicola* do not support recognition of infraspecific taxa within these species, in accordance with the results obtained from morphology. The major source of disagreement occurs on the infraspecific relationships of the *D. zehntneri* populations, where morphology supports a division of the species in two subspecies – *zehntneri* and *boomianus*, while allozymes partition the populations on a geographical basis. However, this result do not compromises the interpretation of two subspecies occurring within *D. zehntneri* as discussed earlier, and we maintain this taxonomic circumscription of *D. zehntneri*.

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Table 1. Locality data for 17 *Discocactus* populations surveyed in the states of Bahia (BA) and Goiás (GO), Brazil, with number of individuals sampled and an acronym for each population, information if the location represents a type locality for a synonymous taxon, and the voucher specimens deposited at HUEFS. Precise locality information was withheld for conservation reasons.

Taxon	Location	Municipality and State	Type locality for synonymous taxon	Acronym	Sample size	Voucher specimens (HUEFS)
<i>Discocactus bahiensis</i>	Abreus	Juazeiro (BA)		BB_AB	20	M. Machado 440 – 443
	Goiabeira	Juazeiro (BA)		BB_GO	20	M. Machado 430 – 433
	Olhos D'Água	Juazeiro (BA)		BB_OL	20	M. Machado 420 – 423
	São Rafael	Morro do Chapéu (BA)	<i>D. bahiensis</i> subsp. <i>gracilis</i>	BG_SR	20	M. Machado et al. 390 – 393
	Rodeadouro	Juazeiro (BA)	<i>D. subviridigriseus</i>	BS_RO	21	M. Machado et al. 370 – 373
	Juremal	Juazeiro (BA)		BS_JU	17	M. Machado et al. 380 – 383
<i>Discocactus catingicola</i>	Sítio Grande	São Desidério (BA)	<i>D. catingicola</i>	CC_SG	18	M. Machado et al. 300 – 303
	Acaba Vidas	Barreiras (BA)		CC_AV	17	M. Machado et al. 310 – 313
	Porto Novo	Santana (BA)	<i>D. nigrisaetosus</i>	CN_PN	17	M. Machado et al. 340 – 344
	Coribe	Coribe (BA)		CN_CO	12	M. Machado et al. 330 – 333
	Posse	Posse (GO)	<i>D. rapirhizus</i>	CR_PO	19	M. Machado et al. 320 – 323
<i>Discocactus zehntneri</i> subsp. <i>zehntneri</i>	Brejinho Grande	Sento Sé (BA)	<i>D. albispinus</i>	ZA_BG	21	M. Machado 410 – 415
<i>Discocactus zehntneri</i> subsp. <i>boomianus</i>	Lages	Morro do Chapéu (BA)	<i>D. boomianus</i>	ZB_LA	30	M. Machado et al. 450 – 454
	Entroncamento de Cafarnaum	Morro do Chapéu (BA)		ZB_EC	25	M. Machado et al. 470 – 474
	Volta da Serra	Morro do Chapéu (BA)		ZB_VS	20	M. Machado et al. 460 – 464
	Limoeiro	Sento Sé (BA)	<i>D. araneispinus</i>	ZR_LI	20	M. Machado 400 – 403
	Campo Largo	Sento Sé (BA)	<i>D. zehntneri</i> subsp. <i>horstiorum</i>	ZH_CL	20	M. Machado et al. 350 – 356

Table 2. Allele frequencies for 10 polymorphic loci of 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. Alleles are identified by relative mobility; N = sample size. For population acronyms see Table 1.

Locus	allele	<i>Discocactus bahiensis</i>						<i>Discocactus catingicola</i>					<i>Discocactus zehntneri</i>						
		BB_AB	BB_GO	BB_OL	BG_SR	BS_JU	BS_RO	CC_AV	CC_SG	CN_CO	CN_PN	CR_PO	ZA_BG	ZB_EC	ZB_LA	ZB_VS	ZH_CL	ZR_LI	
DIA	76	0.139	0	0.1	0	0.235	0.024	0	0.083	0	0	0.026	0.263	0.357	0.25	0.263	0.225	0.225	
	84	0.167	0.289	0.25	0.342	0.176	0.167	0.265	0.083	0	0.147	0.105	0	0	0.018	0	0	0	
	96	0.139	0	0	0	0	0	0	0	0.083	0	0	0.053	0	0	0	0	0.05	
	100	0.556	0.711	0.65	0.658	0.588	0.81	0.735	0.833	0.917	0.853	0.868	0.684	0.643	0.732	0.737	0.775	0.5	
	104	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.225	
	N	18	19	20	19	17	21	17	18	12	17	19	19	21	28	19	20	20	
MDH-1	92	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	100	0.8	0.45	0.5	0.45	0.647	0.357	0	0	0	0	0	1	0.976	0.925	0.925	1	0.95	
	108	0.175	0.425	0.275	0.3	0.353	0.5	1	1	1	1	0	0.024	0.075	0.075	0	0.05		
	116	0.025	0.125	0.175	0.25	0	0.143	0	0	0	0	0	0	0	0	0	0	0	
	N	20	20	20	20	17	21	17	18	12	17	19	21	21	20	20	20	20	
	MDH-2	96	0	0.105	0.55	0	0.235	0	0.118	0	0	0.118	0	0	0	0	0	0	0
100		0.9	0.842	0.4	1	0.676	1	0.882	1	1	0.882	1	1	1	1	1	1	1	
108		0.1	0.053	0.05	0	0.088	0	0	0	0	0	0	0	0	0	0	0	0	
N		20	19	20	16	17	21	17	18	12	17	19	21	19	20	20	4	1	
MDH-3		96	0	0	0	0	0.176	0	0.133	0.056	0	0.071	0.158	0	0	0	0	0	0
		100	1	1	1	1	0.824	1	0.867	0.944	1	0.929	0.842	0.737	0.053	0	0	0.15	0.05
	108	0	0	0	0	0	0	0	0	0	0	0	0.263	0.947	1	1	0.85	0.95	
	N	20	19	19	16	17	21	15	18	12	14	19	19	19	20	20	20	20	
	PGM-1	84	0	0	0.025	0	0	0	0	0.028	0.042	0	0	0	0	0	0	0	0
		92	1	1	0.975	1	1	1	1	0.972	0.958	1	1	0.105	1	1	0.8	0.206	0.278
100		0	0	0	0	0	0	0	0	0	0	0	0.895	0	0	0.2	0.794	0.722	
N		20	19	20	16	17	21	16	18	12	17	19	19	18	28	20	17	18	
PGM-2		84	0	0	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		92	0	0	0	0	0	0	0.265	0.25	0	0.235	0.053	0.048	1	0.981	0.85	0.395	0.25
	100	1	1	0.975	1	1	1	0.735	0.75	1	0.765	0.947	0.905	0	0.019	0.15	0.605	0.75	
	108	0	0	0	0	0	0	0	0	0	0	0	0.048	0	0	0	0	0	
	N	20	20	20	20	17	21	17	18	12	17	19	21	20	27	20	19	20	

Table 2. (continued)

Locus	allele	<i>Discocactus bahiensis</i>						<i>Discocactus catingicola</i>					<i>Discocactus zehntneri</i>					
		BB_AB	BB_GO	BB_OL	BG_SR	BS_JU	BS_RO	CC_AV	CC_SG	CN_CO	CN_PN	CR_PO	ZA_BG	ZB_EC	ZB_LA	ZB_VS	ZH_CL	ZR_LI
IDH	92	0.053	0	0.125	0	0	0	0	0.056	0.083	0	0.026	0	0	0	0	0	0
	100	0.947	1	0.775	1	1	1	0.941	0.889	0.917	0.971	0.789	1	1	1	1	1	1
	108	0	0	0.1	0	0	0	0.059	0.056	0	0.029	0.184	0	0	0	0	0	0
	<i>N</i>	19	19	20	19	17	20	17	18	12	17	19	21	21	28	20	20	20
LAP	60	0.1	0.105	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	68	0.275	0.342	0.125	0	0.294	0.214	0.029	0.056	0.125	0.029	0.026	0	0	0	0	0	0
	76	0.575	0.263	0.45	0.25	0.353	0.214	0.882	0.944	0.875	0.882	0.921	0	0	0	0	0	0
	84	0.05	0.263	0.35	0.4	0.353	0.571	0.088	0	0	0.088	0.053	0.15	0	0	0	0.925	0.475
	88	0	0.026	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	92	0	0	0	0.35	0	0	0	0	0	0	0	0.3	1	1	0.95	0.075	0.525
	100	0	0	0	0	0	0	0	0	0	0	0	0.55	0	0	0.05	0	0
	<i>N</i>	20	19	20	20	17	21	17	18	12	17	19	20	21	28	20	20	20
	PGI	64	0.025	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
72		0.15	0.075	0	0	0	0.024	0	0	0	0	0	0	0	0	0	0	0
76		0.125	0.325	0	0	0	0.071	0	0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0.235	0	0.417	0.059	0	0	0	0	0	0	0
84		0.05	0.1	0.6	0	0.441	0.452	0	0.083	0.042	0	0.079	0	0	0	0	0	0
88		0	0	0.05	0	0.088	0	0.088	0.25	0.208	0	0.026	0	0	0	0	0	0
92		0.175	0.225	0	0	0	0	0.294	0.083	0.208	0.294	0.105	0	0	0	0	0	0
96		0	0	0	0	0	0	0	0.167	0	0	0.211	0.262	0.368	0.175	0.525	0.25	0.425
100		0.475	0.275	0.35	1	0.471	0.381	0.382	0.389	0.083	0.647	0.447	0.643	0.632	0.825	0.375	0.75	0.575
104		0	0	0	0	0	0	0	0.028	0.042	0	0.132	0	0	0	0	0	0
108		0	0	0	0	0	0.071	0	0	0	0	0	0.095	0	0	0.1	0	0
<i>N</i>		20	20	20	16	17	21	17	18	12	17	19	21	19	20	20	20	20
SKDH	92	0.325	0.1	0.275	0.063	0.029	0	0.029	0.056	0	0.059	0	0	0.139	0	0	0.133	0
	100	0.675	0.875	0.725	0.938	0.971	1	0.971	0.944	1	0.941	1	1	0.833	0.925	1	0.867	1
	108	0	0.025	0	0	0	0	0	0	0	0	0	0	0.028	0.075	0	0	0
	<i>N</i>	20	20	20	16	17	21	17	18	12	17	19	21	18	20	19	15	20

Table 3. Genetic variability at 10 loci and morphological variability of 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. For population acronyms see Table 1. N = mean sample size; A = mean number of alleles per locus; P = proportion of polymorphic loci (%); H_o = observed mean heterozygosity per locus; H_e = expected mean heterozygosity per locus; $D2_m$ = median Squared Mahalanobis distance (measure of morphological variability; see text for explanation). Standard deviations in parentheses.

Population	N	A	P^*	H_o	H_e^{**}	$D2_m^{***}$
<i>D. bahiensis</i>						
BB_AB	19.7 (0.2)	2.6 (0.5)	70.0	0.171 (0.081)	0.303 (0.090)	16.70 ^{ns}
BB_GO	19.4 (0.2)	2.5 (0.5)	60.0	0.281 (0.108)	0.308 (0.100)	11.98 ^{ns}
BB_OL	19.9 (0.1)	2.8 (0.4)	70.0	0.240 (0.083)	0.382 (0.081)	11.87 ^{ns}
BG_SR	17.8 (0.6)	1.6 (0.3)	40.0	0.173 (0.089)	0.192 (0.091)	11.53 ^{ns}
BS_JU	17.0 (0.0)	2.1 (0.3)	60.0	0.247 (0.095)	0.319 (0.089)	17.71 ^{ns}
BS_RO	20.9 (0.1)	2.0 (0.4)	40.0	0.190 (0.082)	0.219 (0.094)	15.91 ^{ns}
mean	19.12	2.27	56.7	0.217	0.287	14.28
<i>D. catiungicola</i>						
CC_AV	16.7 (0.2)	2.1 (0.3)	70.0	0.229 (0.093)	0.237 (0.071)	27.20 ^{AB}
CC_SG	18.0 (0.0)	2.4 (0.5)	70.0	0.183 (0.085)	0.204 (0.074)	21.39 ^{AB}
CN_CO	12.0 (0.0)	1.9 (0.5)	40.0	0.108 (0.060)	0.139 (0.074)	30.56 ^A
CN_PN	16.7 (0.3)	2.0 (0.2)	70.0	0.182 (0.072)	0.188 (0.051)	32.88 ^A
CR_PO	19.0 (0.0)	2.3 (0.5)	60.0	0.163 (0.073)	0.186 (0.074)	20.39 ^B
mean	16.48	2.14	62.0	0.173	0.191	26.48
<i>D. zehntneri</i>						
ZA_BG	20.3 (0.3)	2.0 (0.3)	60.0	0.106 (0.055)	0.237 (0.076)	32.18 ^a
ZB_EC	19.7 (0.4)	1.6 (0.2)	40.0	0.129 (0.077)	0.139 (0.063)	14.42 ^b
ZB_LA	23.9 (1.3)	1.6 (0.2)	40.0	0.102 (0.055)	0.103 (0.046)	14.14 ^b
ZB_VS	19.8 (0.1)	1.7 (0.2)	60.0	0.118 (0.061)	0.182 (0.065)	16.26 ^{ab}
ZH_CL	17.5 (1.6)	1.7 (0.2)	70.0	0.136 (0.055)	0.221 (0.056)	10.64 ^b
ZR_LI	17.9 (1.9)	1.9 (0.3)	70.0	0.221 (0.084)	0.267 (0.080)	14.55 ^{ab}
mean	19.85	1.77	56.7	0.135	0.191	17.03

* A locus was considered polymorphic if the frequency of the most common allele did not exceed 95%

** Unbiased estimate (Nei, 1978)

*** Different letters in conspecific populations indicate statistically different median values in Kruskal-Wallis test; ns = not statistically significant

Table 4. Summary of F-statistics (Wright, 1978) per locus and species for the populations of *Discocactus* surveyed in the states of Bahia and Goiás, Brazil.

Locus	F_{IS}			F_{ST}		
	<i>D. bahiensis</i>	<i>D. catingicola</i>	<i>D. zehntneri</i>	<i>D. bahiensis</i>	<i>D. catingicola</i>	<i>D. zehntneri</i>
DIA	-0.314	-0.077	-0.203	0.043	0.048	0.038
MDH-1	0.317	-	-0.068	0.069	-	0.028
MDH-2	0.812	1	-	0.258	0.074	-
MDH-3	1	1	1	0.152	0.042	0.493
PGM-1	-0.026	-0.037	0.672	0.021	0.023	0.581
PGM-2	-0.026	-0.221	0.246	0.021	0.092	0.552
IDH	0.894	-0.010	-	0.116	0.049	-
LAP	0.246	-0.103	0.355	0.102	0.020	0.571
PGI	-0.057	-0.054	0.253	0.206	0.093	0.074
SKDH	0.750	0.353	0.754	0.129	0.023	0.080
Mean	0.223	0.063	0.274	0.127	0.068	0.360

Table 5. Matrix of genetic similarity between populations of the *Discocactus* species that occur in the state of Bahia, Brazil (Nei [1978] unbiased genetic identity). Minimum and maximum values are in parenthesis.

species	number of populations	<i>D. bahiensis</i>	<i>D. catingicola</i>	<i>D. zehntneri</i>
<i>D. bahiensis</i>	6	0.944 (0.890 - 0.977)		
<i>D. catingicola</i>	5	0.880 (0.836 - 0.911)	0.987 (0.964 - 0.999)	
<i>D. zehntneri</i>	6	0.680 (0.550 - 0.814)	0.602 (0.523 - 0.683)	0.851 (0.689 - 0.996)

Table 6. Comparisons of mean levels of genetic diversity for the *Discocactus* species that occur in the state of Bahia, Brazil with other cactus species and plant species with similar life-history traits. A = mean number of alleles per locus; P = proportion of polymorphic loci; H_T = species-level genetic diversity; H_S = mean within-population genetic variability; G_{ST} = proportion of genetic diversity accounted for differentiation among populations [$G_{ST}=(H_T-H_S)/H_T$].

	P*	P**	A	H_T	H_S	G_{ST}
<i>Discocactus bahiensis</i>	60.0	100.0	3.70	0.324	0.287	0.114
<i>Discocactus cattingicola</i>	60.0	90.0	2.70	0.202	0.191	0.054
<i>Discocactus zehntneri</i>	70.0	80.0	2.50	0.287	0.191	0.334
Cactus species ^a		91.5	3.38	0.222	0.195	0.120
Animal-pollinated, outcrossing woody plants ^b		63.2	2.87	0.268	0.241	0.099
Long-lived woody plants ^b		65.0	2.88	0.253	0.232	0.084
All plant species ^b		51.3	2.89	0.224	0.173	0.228

* 95% criterion (see Table 3 for explanation)

** No criterion

^a From Hamrick et al. (2002)

^b From Hamrick et al. (1992)

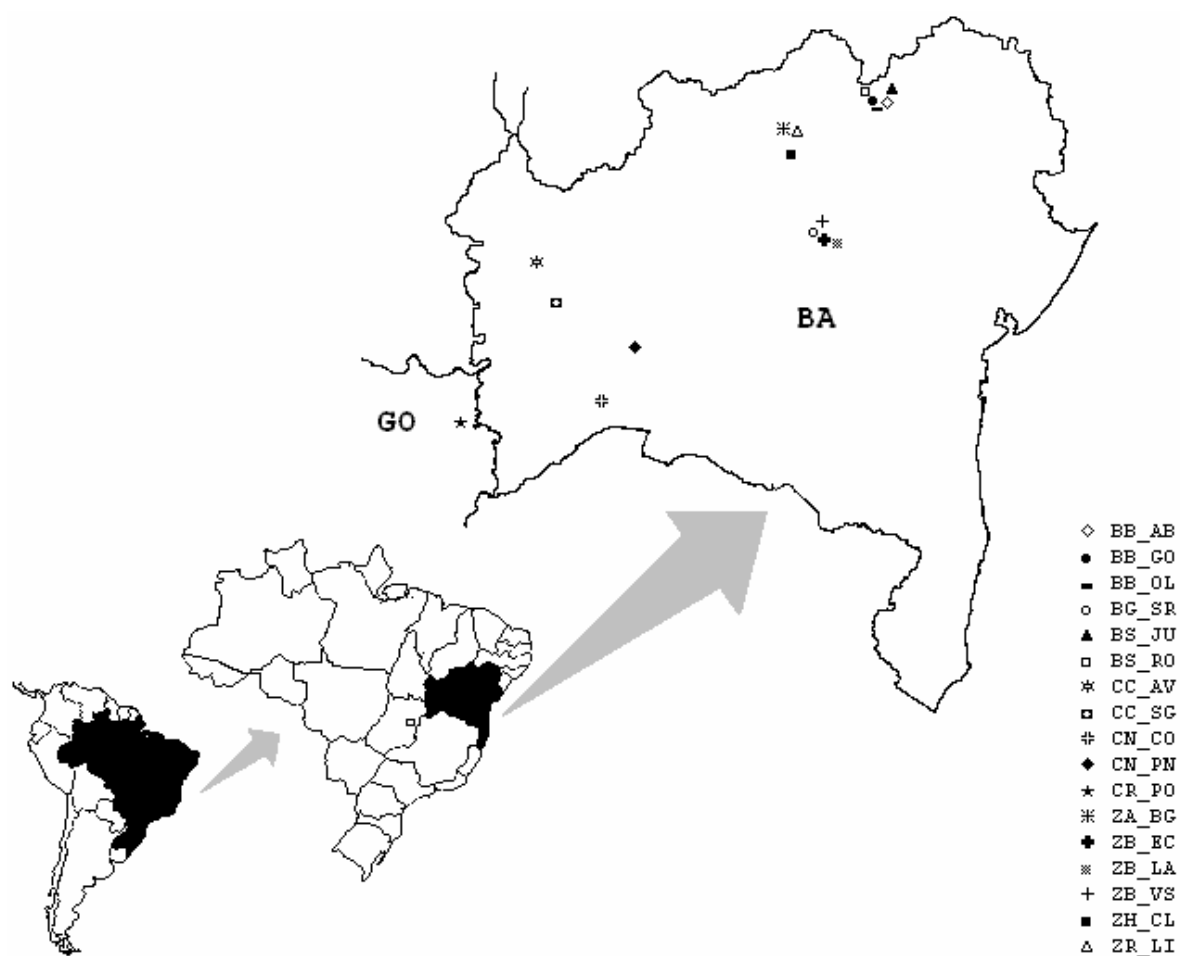


Figure 1. Geographic locations of 17 *Discocactus* populations surveyed in the states of Bahia (BA) and Goiás (GO), Brazil. For population acronyms see Table 1.

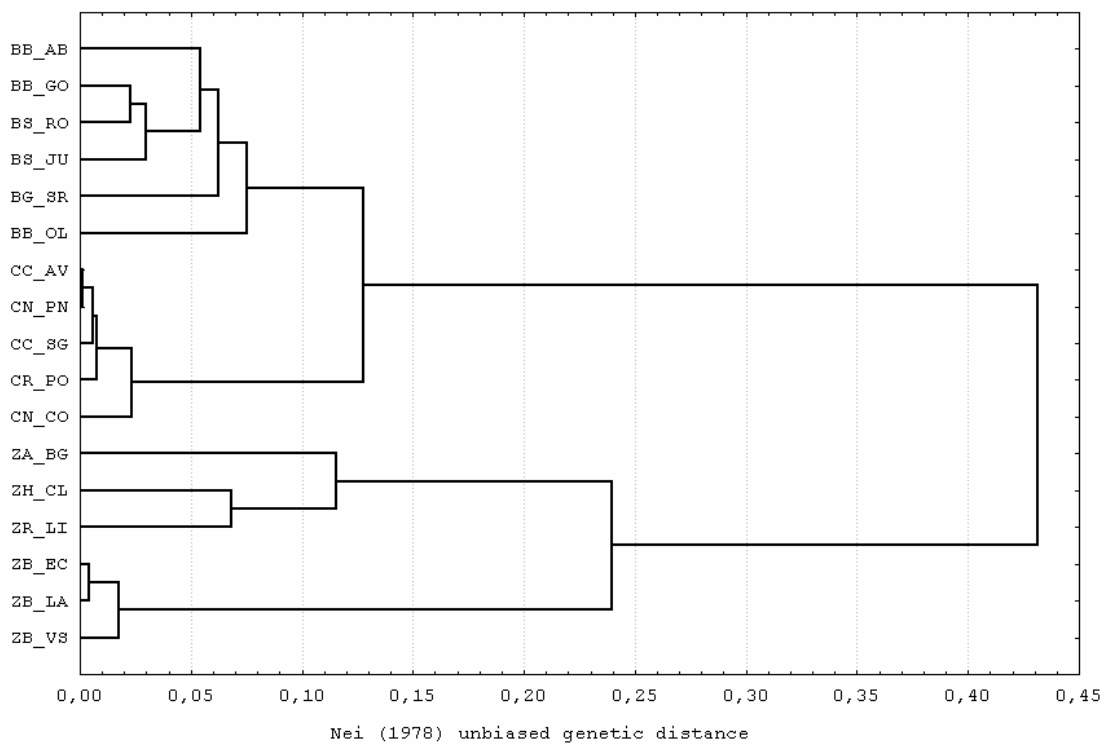


Figure 2. UPGMA cluster dendrogram calculated using Nei (1978) genetic distances between 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil; cophenetic correlation = 0.932. For population acronyms see Table 1.

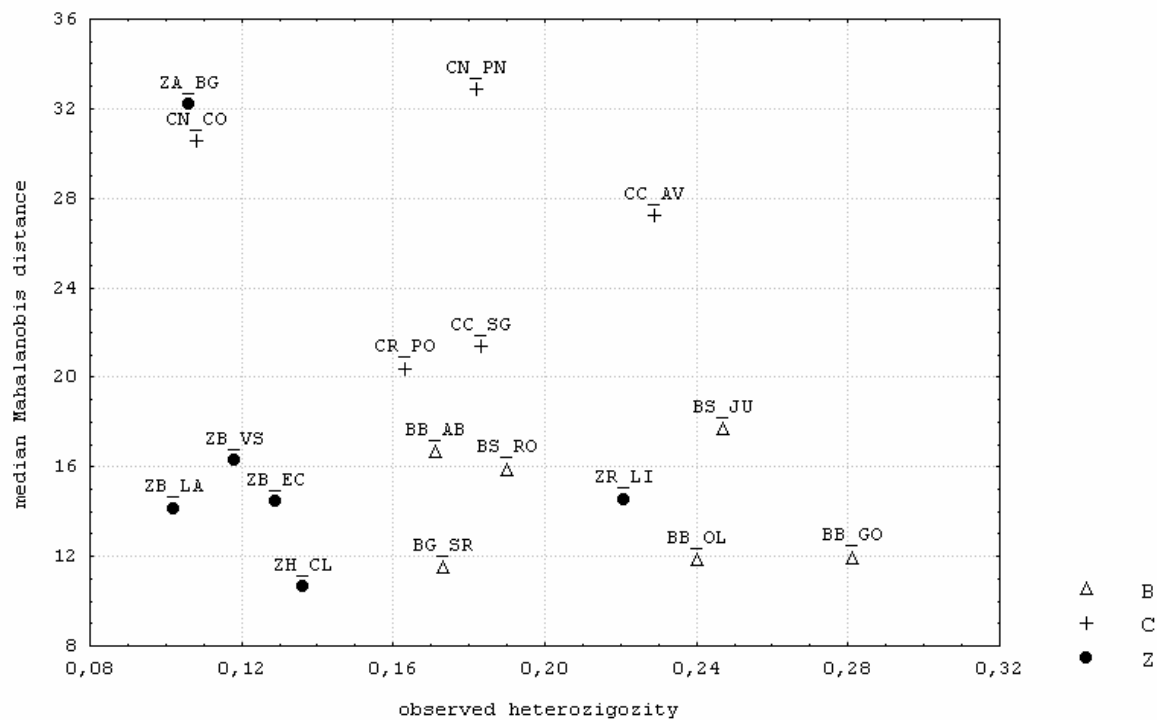


Figure 3. Plot of pairwise morphologic and genetic variability of 17 *Discocactus* populations surveyed in the states of Bahia and Goiás, Brazil. For population acronyms see Table 1. B = *Discocactus bahiensis*, C = *D. catingicola*, Z = *D. zehntneri*.

Capítulo 3. Taxonomy and conservation of the *Discocactus* Pfeiff. (Cactaceae) species occurring in the state of Bahia, Brazil

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RESUMO

A taxonomia das espécies de *Discocactus* (Cactaceae) que ocorrem no estado da Bahia, Brasil, é revista com base nos resultados de uma investigação das relações morfológicas (CAPÍTULO 1) e genéticas (CAPÍTULO 2) entre 17 populações, abrangendo a maioria dos táxons descritos para o estado e totalizando 337 indivíduos analisados. Evidência morfológica e genética justifica o reconhecimento de três espécies para a Bahia: *D. bahiensis*, *D. catingicola* e *D. zehntneri*, a última com duas subespécies, *zehntneri* e *boomianus*. São apresentadas descrições morfológicas com base em uma revisão da bibliografia relevante, no exame do material depositado em herbários, e em medidas de 337 indivíduos estudados em hábitat. As descrições representam uma ampliação do conhecimento atual sobre a variação morfológica encontrada em cada táxon, porém não englobam todos os aspectos dos mesmos, pois possuem como principal objetivo apenas a identificação dos táxons.

Os níveis de ameaça de extinção foram determinados para todos os táxons estudados. Todas as espécies de *Discocactus* são consideradas ameaçadas de extinção, devido ao pequeno número de populações conhecido para cada espécie, em geral baixo número de indivíduos em cada população, e especificidade de hábitat que faz com que as populações ocorram em áreas restritas. Estes fatores fazem com que as populações de *Discocactus* sejam extremamente vulneráveis à modificação e destruição de seus hábitats e à remoção de indivíduos das populações. De todas as populações de *Discocactus* estudadas, apenas duas estão inclusas em áreas de preservação. *D. bahiensis* e *D. catingicola* vem experimentando um rápido declínio na qualidade de seus hábitats devido ao desenvolvimento agrícola e urbano das áreas onde populações destas espécies ocorrem. A criação de reservas é proposta para áreas que contém populações com elevado grau de variabilidade genética e morfológica. Atenção especial é sugerida para a população BG_SR de *D. bahiensis*, devido à sua distinção morfológica e ao fato de que esta população está se diferenciando geneticamente pela ocorrência de introgressão com *D. zehntneri*. Em relação ao último táxon, sugere-se que esforços sejam direcionados à tentativa de se encontrar novas populações da subespécie *zehntneri*. É proposta a criação de reservas para proteger as populações da subespécie *boomianus* que ocorrem no limite norte da área de distribuição deste táxon, em virtude da diversidade genética mais elevada destas populações, e grau de diferenciação em relação às populações que ocorrem no limite sul da área de distribuição da subespécie.

Taxonomy and conservation of the *Discocactus* Pfeiff. (Cactaceae) species occurring in the state of Bahia, Brazil

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ABSTRACT

The taxonomy of the *Discocactus* (Cactaceae) species that occur in the state of Bahia, Brazil, is revised with basis on the results of an investigation of morphologic and genetic relationships among 17 populations embracing the majority of the taxa described for the state, summing up 337 individuals analysed. Morphologic and genetic evidence justify the recognition of three species for the state: *D. bahiensis*, *D. catingicola* and *D. zehntneri*, the latter with two subspecies, *zehntneri* and *boomianus*. Current threats and the conservation status of the populations surveyed are discussed.

RESUMO

A taxonomia das espécies de *Discocactus* (Cactaceae) que ocorrem no estado da Bahia, Brasil, é revista com base nos resultados de uma investigação das relações morfológicas e genéticas entre 17 populações, abarcando a maioria dos táxons descritos para o estado e totalizando 337 indivíduos analisados. Evidências morfológicas e genéticas justificam o reconhecimento de três espécies para o estado: *D. bahiensis*, *D. catingicola* e *D. zehntneri*, a última com duas subespécies, *zehntneri* e *boomianus*. São discutidos os estados de conservação das populações investigadas e as ameaças às quais estas populações estão submetidas.

INTRODUCTION

Discocactus is a genus of small cacti characterized by having a discoid to depressed-globose, single or cespitose habit, the fertile part of stem differenced in a terminal cephalium, nocturnal white flowers of sphingophilous syndrome, fruits dehiscing by lateral slits, and seeds with strongly tuberculated testa. The genus is almost completely restricted to Brazil with only two taxa occurring in bordering areas of Bolivia and Paraguay. In Brazil its distribution embraces the Central-Western, Northeastern and Southeastern regions of the country.

All *Discocactus* species are considered threatened or endangered; intrinsic reasons for this are the small number of populations per species, the generally small population sizes, and habitat specificity leading to quite localized populations. These factors make *Discocactus* populations extremely vulnerable to habitat modification and destruction, and to collecting. As a result, the whole genus is placed in Appendix I of CITES (Lüthy 2001).

The taxonomy of *Discocactus* is disputed: 52 taxa have been described for the genus, and the currently proposed classification systems are rather divergent. Braun & Esteves Pereira (1993a-c, 1995, 2001b) recognizes 51 taxa: 25 species, 22 heterotypic subspecies and 4 heterotypic varieties, while Taylor & Zappi (2004) recognizes only 10 taxa: 7 species and 3 heterotypic subspecies. For the state of Bahia in Northeastern Brazil 12 *Discocactus* taxa have been described, ten of which as distinct species (Table 1). These species fall more or less in three groups, recognized with basis in morphological traits and ecological preferences: the complex of *D. bahiensis* Britton & Rose, the complex of *D. catingicola* Buining & Brederoo, and the complex of *D. zehntneri* Britton & Rose.

In the current classification systems proposed by Braun & Esteves Pereira (1993a-c, 1995, 2001b) and Taylor & Zappi (2004) only three species are recognized, each of which corresponding to one of the three species complexes; however, the classification systems differ considerably in the infraspecific classification of the species (Table 1). Taylor & Zappi (2004) also hypothesize that *D. bahiensis* could be conspecific with *D. heptacanthus*, being linked to *D. heptacanthus* subsp. *catingicola* via a population somewhat intermediate between the two taxa, located in the municipality of Paramirim, Bahia.

The divergence in the infraspecific classification of the two proposed systems reflects the difficulty of delimitation of these plants, and both systems are strongly based in the geographical distribution of the taxa and in morphological characters which often

overlap. Due to these problems conducted a more throughout investigation of the genus, using a larger number of populations and a bigger population sampling in order to analyze the patterns of morphologic and genetic variation within and between the *Discocactus* species and populations that occur in the state of Bahia, in order to expand the knowledge of intra and interspecific relationships within the genus. The results of these investigations have been published elsewhere (Machado et al., submitted). In this article we provide expanded descriptions of the species accepted for the state with basis on the results from the morphologic and genetic investigations, and in light of these results we discuss the current threats and conservation status of the populations surveyed.

MATERIAL AND METHODS

All taxa described for the state of Bahia were surveyed at their type localities (Table 2, Fig. 1), except for *D. zehntneri*, *D. bahiensis* and *D. buenekeri* Abraham for which precise locality information could not be obtained. Three to six specimens were collected at each population and deposited as vouchers at the herbarium of the Universidade Estadual de Feira de Santana (HUEFS; Table 2). The morphological descriptions are based on a revision of the relevant bibliography and on examinations of the material collected and other collections deposited in the following herbaria: CEPEC, HRCB, HUEFS, K, SPF, U, UFG, US, and ZSS. In addition to the material examined in herbaria, measurements of 337 individuals sampled in 17 natural populations in the states of Bahia and Goiás (Table 2, Fig. 1) were utilized for preparing the morphological descriptions (from Machado et al., submitted). The descriptions do not aim in principle to account for all aspects of the plant, but are meant to be primarily diagnostic, supplementing details in the dichotomous keys to facilitate identification.

All materials cited have been seen unless indicated by ‘n.v.’ (non vidi). The cited records are organized by state and municipality (abbreviated to ‘Mun.’), and mostly arranged from north to south and west to east. Apart from herbarium exsiccata, whose locations are indicated by the standard Index Herbariorum codes, other field observations by the authors (or from reliable sources, even if unsupported by vouchers) are mentioned in some cases. A bracketed questionmark (?) preceding a record’s entry indicates uncertainty about the collection’s identity, whereas after the name of the municipality it indicates doubt about the correct municipal attribution of the locality cited. Besides

nomenclatural and diagnostic/descriptive details, bibliographical, phytogeographical and ecological data are provided, likewise distribution records and conservation assessments.

Criteria for the identification of priority taxa

The internationally agreed process for assessing the level of threat of extinction to living organisms is that established by IUCN, whose system aims, as far as is possible, to provide an objective means of determining Categories of Threat, or 'Red List Categories' (IUCN 2001). The principal categories employed in the IUCN system are 'Extinct (EX)', 'Extinct in the Wild (EW)', 'Critically Endangered (CR)', 'Endangered (EN)', 'Vulnerable (VU)' and 'Least Concern (LC)'. The series of standard criteria that lead to their determination should be stated when an assessment is published, in order that the category can be easily verified or reassessed in the future, as circumstances change. The Categories CR, EN & VU are defined by any one of 5 sets of criteria, which are of a consistent type throughout, but differ in degree for each Category. Besides the categories for threatened taxa listed above, there is also 'Near Threatened (NT)' for taxa that are close to qualifying as VU and 'Data Deficient (DD)' where there is inadequate information to make a direct or indirect assessment of risk of extinction based on geographical distribution and/or population status.

While the standard methodology embodied in the published IUCN system has been employed here, it has recently been noted by Farjon & Page (1999) that its application can result in rather long lists of equally threatened taxa. This suggests that further prioritization is desirable if the limited resources currently applied to nature conservation are not to be spread too thinly. Farjon & Page (1999) have devised a novel additional formula for achieving such prioritization. This formula calculates a score based on the Category of Threat, where arbitrarily assigned values of CR=4, EN=3, VU=2 and LC/NT=1, are multiplied by the sum of 3 other criteria, namely 'Phylogenetic Distinction', 'Ecological Importance' and 'Genetic Diversity', whose ranges of values are given below. The resulting total score effectively short-lists and prioritizes between taxa with the same IUCN rating. The Farjon & Page criteria are defined as follows:

Phylogenetic distinction (PD). This is a measure of the relative taxonomic isolation of an organism and recognizes that not all taxa of the same rank are equal. Thus, if the taxon is representative (a) of a monotypic genus, it scores 4; (b) of a species or infraspecific rank of a small genus (2–5 species) or monotypic infrageneric rank within any

genus, it scores 3; (c) of a species of a larger genus (>5 species), it scores 2; and (d) of an infraspecific rank of a species of a larger genus, it scores 1.

Ecological importance (EI). If a taxon is co-dominant in a distinct vegetation type, it scores 2; if it is only a more minor constituent of the vegetation, it scores 1.

Genetic diversity (GD). This criterion distinguishes between taxa known to be unusually rich in regional diversity, often expressed in high levels of morphological variation or varied ecological adaptation, from those whose genetic diversity is less. Taxa displaying genetic diversity within and/or between regional populations receive a score of 2, less diverse taxa scoring 1.

The short-list formula is, therefore, calculated as follows: IUCN category of threat (score 1–4) X (PD [score 1–4] + EI [score 1–2] + GD [score 1–2]).

TAXONOMIC INVENTORY

Discocactus Pfeiff.

in Allg. Gartenzeitung 5: 241 (1837). Type: *D. insignis* Pfeiff. (= *D. placentifformis* (Lehm.) K.Schum.).

VERNACULAR NAMES. Roseta-do-diabo, Coroa-do-diabo, Coroa-de-frade, Cabeça-de-frade.

Stems depressed-globose, often half buried in the ground, non-mucilaginous, lacking woody tissues, very small or up to 30 cm in diameter, remaining solitary or sprouting offsets; ribs 9–26, well-defined or broken up into tubercles; areoles small to large but soon becoming glabrous, indeterminate growth absent. Spines relatively few, usually 3–21 per areole, sometimes very stout or minute. At maturity stem apex transformed into a white-woolly and often bristly cephalium, bearing flowers and fruit from its deeply sunken apex. Flowers salverform, nocturnal, sweetly scented (hawkmoth syndrome), 1–6 or more rapidly developing at the same time; pericarpel ovoid, usually quite naked, tube (including the nectar-chamber) elongate, bearing leafy bract-scales glabrous in their axils; perianth-segments narrowly oblanceolate, the outermost often greenish, brownish or even deep pink, inner series white, rarely pale pinkish; stamens numerous, inserted along the inside of the tube, and at its apex, on short filaments, sometimes the lowermost inserted above a whorl of hairs which protect the nectar-chamber; style long or short, stigma-lobes c. 5–6, slender, whitish. Fruit clavate, usually naked, dehiscing by longitudinal splits in the pericarp, red,

orange-yellow, greenish or white, pulp scanty, but very attractive to ants. Seeds 0.8–2.0 mm in length, 0.8–2.2 mm in diameter, hat-shaped, black, very shiny, testa tuberculate, the tubercles sometimes elongated and pointed.

The following species are recognized for the state of Bahia, Brazil:

1. Cephalium lacking bristles, at least at the periphery, root tuberous ----- *D. bahiensis*
1. Cephalium bristly thoroughly or with long bristles at the periphery, root not tuberous ---- 2
2. Stem 11 – 18 cm in diameter, ribs broken into more or less vertical tubercles, height of rib between tubercles 8 – 16 mm, width of rib between tubercles 18 – 40 mm, spines not interwoven and not hiding the stem ----- *D. catiugicola*
2. Stem 4 – 9 cm in diameter, ribs broken into more or less spiralled tubercles, height of rib between tubercles 0 – 4 mm, width of rib between tubercles 4 – 12 mm, spines interwoven and almost completely hiding the stem ----- 3
3. Spines 0.8 – 1.2 mm in diameter, diameter of rib at areole 18 – 22 mm -----
----- *D. zehntneri* subsp. *zehntneri*
3. Spines 0.2 – 0.6 mm in diameter, diameter of rib at areole 8 – 15 mm -----
----- *D. zehntneri* subsp. *boomianus*

1. *Discocactus bahiensis* Britton & Rose, Cact. 3: 220 (1922); Buining, Gen. *Discocactus*: 123–129 (1980). Holotype: Bahia, *Zehntner* in *Rose* 19783 (US).

Echinocactus bahiensis (Britton & Rose) Lützelb., *Estud. Bot. Nordeste* 3: 69 (1926).

Discocactus subviridigriseus Buin. *et al.* in *Succulenta* 56: 262 (1977); Buining, Gen.

Discocactus: 115 (1980). *D. bahiensis* subsp. *subviridigriseus* (Buin. *et al.*) P.J.

Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 44: 63 (1993). Holotype: Bahia,

Mun. Juazeiro, *Horst* 438 (U).

D. bahiensis subsp. *gracilis* P. J. Braun & E. Esteves Pereira in *ibid.* 52: 286–290, illus. (2001). Holotype: Bahia, Vereda do Romão Gramacho (Rio Jacaré), São Rafael, s.d., Braun & Esteves Pereira s.n. (UFG 22432, n.v.).

[*D. placentiformis* sensu Britton & Rose, *Cact.* 3: 220, fig. 233 (1922) non (Lehm.) Schumann.]

VERNACULAR NAME. Frade-de-cavalo.

Solitary or freely caespitose in age, stem 4.3 cm in height (1.4 – 7.2), 13.0 cm in diameter (7.2 – 20.5), grey- to light or yellow-green; ribs 13 (11 – 18), sometimes composed of tubercles, height of rib at the areole 1.2 cm (0.6 – 2.7), between areoles 1.1 cm (0.3 – 2.7), width of rib at the areole 2.7 cm (1.0 – 4.5), between areoles 1.9 (0.5 – 3.4); areoles oval, 8.0 mm in width (6.0 – 10.0), 5 mm in length (3.0 – 8.0), 1.0 – 2.3 cm apart, 3 visible above ground on each rib (2 – 5). Spines strongly recurved, sometimes hooked at apex, in some populations interwoven with those of adjacent areoles, pale yellow to pinkish brown at first, later greyish or dark brown to blackish, central spine absent or rarely 1 to 2.5 cm in length and 1.7 mm in diameter, radial spines 8 (5 – 13), 2.7 cm in length (1.5 – 4.3), 1.5 mm in diameter (0.5 – 3.4). Cephalium low, with soft whitish wool, 1.3 cm in height (0.5 – 3.8), 2.9 cm in diameter (0.7 – 6.6), bristles on the periphery of the cephalium absent. Flowers 4.0 – 7.2 cm in length, 3.0 – 5.4 cm in width; outer perianth-segments green, yellow or pinkish brown, inner segments 0.8 – 2.0 cm in length, 0.5 – 0.7 cm in width, white. Fruit greenish white, sometimes red, 2.5 – 5.0 cm in length, 0.8 – 1.0 cm in diameter. Seed 1.2 – 1.8 mm in length, 1.5 – 1.9 mm in diameter, testa with sparse to dense patches of pointed tubercles.

Northern *caatinga* element: on exposed, gravelly river terraces amongst limestone or iron-stained quartzite, and seasonally inundated river flood plain, under and between juremapreta (*Mimosa tenuiflora*) and carnaúba (*Copernicia prunifera*) within the *caatinga*, 380–650 m, Rio São Francisco drainage of northern Bahia. Figs. 2 – 4.

BAHIA: N Bahia, Mun. Sento Sé, reported from S of the original town by Buining (1980: 121, photo); Mun. Juazeiro, near the town, 2–6 June 1915, *Rose & Russell* 19742, 19783 (US); l.c., SSW of town, 0.5 km S of Rodeadouro, 10 Jan. 1991, *Taylor et al.* 1387 (K, ZSS, HRCB, CEPEC), *Horst* 438 (U, ZSS), 9°28'S, 40°33'W, 7 April 2000, *E.A. Rocha*

et al. (K, photos); l.c., E of town, 2–6 June 1915, *Rose & Russell* 19764 (US); l.c., between Juremal and Curral Velho, *Horst & Uebelmann* 633 (ZSS T02127 & TP58-114/163/164); l.c., south of town, road to Sento Sé, then ca. 1km on road to Rodeadouro, 29 Mar. 2004, *M. Machado et al.* 370 – 373 (HUEFS); l.c., ca. 12km from the village of Juremal on road to Abreus, 30 Mar. 2004, *M. 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, near the river Salitre, 29 Apr. 2004, *M. Machado* 430 – 433 (HUEFS); l.c., surroundings of the village of Abreus, 30 Apr. 2004, *M. Machado* 440 – 443 (HUEFS); Mun. Campo Formoso, c. 60 km W of Maçaroca (Massaroca), W of Abreus, W bank of the Rio Salitre, *Horst* 437 (U, ZSS); *ibid.*, reported from Vargem Grande, Laje, Panelas, Delfino and [Faz.] Vargem do Sal ['Sol'] by Braun & Esteves Pereira, l.c. (2001a); Mun. Itaguaçu da Bahia, Serra Azul, reported by Braun & Esteves Pereira, l.c. (2001a); Mun. Ourulândia (Ouro Branco), reported by Braun & Esteves Pereira, l.c. (2001a); Mun. Jacobina, Caatinga do Moura, reported by Braun & Esteves Pereira, l.c. (2001a); Mun. Morro do Chapéu, Vereda do Romão Gramacho (Rio Jacaré), São Rafael, s.d., *Braun & Esteves Pereira* s.n. (UFG 22432); l.c., behind the village of São Rafael, 21 Apr. 2004, *M. Machado et al.* 390 – 393 (HUEFS).

CONSERVATION STATUS. Endangered [EN B2ab(i, ii, iii, iv, v)] (3); area of occupancy < 500 km²; PD=2, EI=1, GD=2. Short-list score (3x5) = 15. 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. Some of its sites are accessible by road and have been visited by hobbyist collectors. The most impacted populations (BG_SR and BS_RO) have the lowest levels of genetic diversity; one population (BG_SR) is undergoing genetic differentiation due to introgression with *D. zehntneri*. The most genetically diverse populations are those located between the villages of Abreus, Goiabeira, Olhos D'Água and Juremal (all within the municipality of Juazeiro, Table 2; Machado et al., submitted).

2. *Discocactus catingicola* Buining & Brederoo in *Kakt. and. Sukk.* 25: 265–267 (1974).

Holotype: W Bahia, Mun. São Desidério, *Horst* 392 (U).

D. spinosior Buin. *et al.* in *Succulenta* 56: 261 (1977); Buining, Gen. *Discocactus*: 146 (1980). Holotype: W Bahia, Mun. Barreiras, *Horst* 205A (U? [labelled with number only]).

D. nigrisaetosus Buin. *et al.* in *Succulenta* 56: 260 (1977); Buining, tom. cit. 129 (1980). *D. catingicola* var. *nigrisaetosus* (Buin. *et al.*) P.J. Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 44: 112 (1993). Holotype: W Bahia, Mun. Santana, *Horst* HU 448 (U? [labelled with number only]).

D. piauiensis P.J. Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 46: 57–62, with figs (1995). Holotype: S Piauí, 600 m, *E. Esteves Pereira* 114 (UFG 14761, n.v.; B?, iso., n.v.), l.c., 1993, *Braun* 1652, 1655 (B, paratypi, n.v.).

D. rapirhizus Buin. & Brederoo in *Ashingtonia* 2: 44–47 (1975). *D. catingicola* subsp. *rapirhizus* (Buin. & Brederoo) P.J. Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 44: 112 (1993). Holotype: Goiás, surroundings of Posse, 19 June 1974, *Buining & Horst* HU 200 (U).

Solitary or sparingly caespitose in age, stem 5.4 cm in height (2.6 – 9.1), 13.2 cm in diameter (10.0 – 18.8), plain green; ribs 10 (9 – 16), usually composed of tubercles, height of rib at the areole 1.7 cm (0.8 – 2.8), between areoles 1.0 cm (0.4 – 1.9), width of rib at the areole 3.2 cm (2.1 – 4.8), between areoles 2.6 (1.6 – 4.6); areoles positioned slightly below and sunken into tubercle apex, oval, 7.0 mm in width (6.0 – 8.0), 4 mm in length (3.5 – 5.0), 1.1 – 2.0 cm apart, 4 visible above ground on each rib (3 – 6). Spines weakly recurved, pale yellow then grey to brownish in age, central spine absent or rarely 1 to 1.9 cm in length and 1.4 mm in diameter, radial spines 7 (5 – 12), 2.5 cm in length (1.4 – 4.6), 1.7 mm in diameter (1.0 – 3.3). Cephalium low, with soft whitish wool, 1.5 cm in height (0.7 – 4.3), 3.1 cm in diameter (1.6 – 6.7), bristles on the periphery of the cephalium yellow to dark brownish or blackish, 1.7 cm in length (0.7 – 3.4) and 0.4 mm in diameter (0.1 – 0.6). Flowers 5.0 – 6.0 cm in length, 4.0 – 6.0 cm in width, light brown to olive-green in bud; outer perianth-segments white to greenish, 2.2 – 2.4 cm in length, 0.7 – 0.8 cm in width, inner segments 1.8 – 2.0 cm in length, 0.5 – 0.7 cm in width, white. Fruit white or faintly pinkish, 4.0 – 4.5 cm in length, 0.8 – 1.3 cm in diameter. Seed 1.5 – 2.0 mm in length, 1.5 – 2.0 mm in diameter, testa with with irregularly spaced, elongate, pointed tubercles.

Western *cerrado* element: on exposed sand soils, usually close to rivers, 450–700 m, western and central-southern Bahia and eastern Goiás. Figs. 5 – 6.

BAHIA: W Bahia, Mun. Barreiras, near old airport, *Horst* 205A (U, ZSS, K); l.c., Redondo waterfall, near the margins of the river Rio de Janeiro, ca. 20 km in dust road

after the Acaba Vidas waterfall, 14 Jan. 2004, *M. Machado et al.* 310 – 313 (HUEFS); Mun. São Desidério, west of Sítio Grande at waterfall, *Horst* 392 (U); l.c., west of Sítio Grande, locality known as Jatobás, 13 Jan. 2004, *M. Machado et al.* 300 – 303 (HUEFS); Mun. Santana, near Porto Novo, *Horst* 448 (U, ZSS); l.c. (?), *Braun* 341 (ZSS); l.c., ca. 1 km from the village of Porto Novo on road to Santana, then ca. 5 km in side road, 17 Jan. 2004, *M. Machado et al.* 340 – 344 (HUEFS); Mun. Santa Maria da Vitória, airport, *Horst & Uebelmann* 760 (ZSS, K); Mun. Coribe, 8 km south of Coribe, *Horst & Uebelmann* 760A (ZSS); l.c., ca. 10 km to the south of the town on road BR 136, 16 Jan. 2004, *M. Machado et al.* 330 – 333 (HUEFS); cent.-S Bahia, Mun. Paramirim, 28 Nov. 1988, *Taylor* in *Harley* 25558 (K, SPF, CEPEC).

GOIÁS: E Goiás, Mun. Posse, surroundings of the town, 15 Jan. 2004, *M. Machado et al.* 320 (HUEFS).

CONSERVATION STATUS. Vulnerable [VU B2ab(iii)] (2); area of occupancy estimated to be < 2000 km²; PD=1, EI=1, GD=2. Short-list score (2x4) = 8. Vulnerable due to the fragmented nature of its distribution and small population size. In need of regular monitoring, since the *cerrado* habitats are undergoing much destructive change. Low morphologic and genetic differentiation is observed among its populations (Machado et al., submitted).

3. *Discocactus zehntneri* Britton & Rose, *Cact.* 3: 218 (1922); N. P. Taylor in *Cact. Succ. J. Gr. Brit.* 43: 40 (1981). Type: Bahia, Mun. Sento Sé, *Zehntner* in *Rose & Russell* 19779.

Echinocactus zehntneri (Britton & Rose) Lützelb., *Estud. Bot. Nordeste* 3: 69 (1926).
D. albispinus Buin. & Brederoo in *Cact. Succ. J. (US)* 46: 252–257 (1974). *D. zehntneri* f. *albispinus* (Buin. & Brederoo) Riha in *Kaktusy* 19: 26 (1983). *D. zehntneri* var. *albispinus* (Buin. & Brederoo) P.J. Braun in *Succulenta* 69: 215 (1990). *D. zehntneri* subsp. *albispinus* (Buin. & Brederoo) P.J. Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 46: 64 (1995). Holotype: Bahia, Mun. Sento Sé, *Horst* 390 (U).

Sparingly or strongly caespitose, stem 4.7 cm in height (1.5 – 8.0), 7.0 cm in diameter (4.2 – 13.6), light to dark green; ribs 16 (10 – 26), broken up into more or less spiralled tubercles, height of rib at the areole 0.9 cm (0.3 – 1.3), between areoles absent or rarely to 0.7 cm, width of rib at the areole 1.3 cm (0.7 – 3.0), between areoles 0.7 (0.3 – 1.5); areoles positioned slightly below and sunken into tubercle apex, oval, 3.0 mm in width

(1.5 – 6.0), 6 mm in length (3.0 – 10.0), 1.1 – 1.5 cm apart, 5 visible above ground on each rib (2 – 7). Spines creamy white to pinkish to grey, interwoven and almost completely hiding the stem, central spine absent or 1 – 2, if present 3.5 cm in length (2.7 – 5.4) and 0.9 mm in diameter (0.6 – 1.3), radial spines 13 (9 – 21), 2.7 cm in length (1.3 – 6.3), 0.5 mm in diameter (0.1 – 1.6). Cephalium white-woolly, 1.8 cm in height (0.6 – 5.1), 2.6 cm in diameter (1.4 – 5.4), bristles on the periphery of the cephalium yellow to brownish-yellow, 4.3 cm in length (1.8 – 9.1) and 0.3 mm in diameter (0.1 – 0.5). Flowers 3.3 – 7.7 cm in length, 3.5 – 5.5 cm in width, greenish in bud; outer perianth-segments green to yellowish, 1.9 – 2.8 cm in length, 0.4 – 0.6 cm in width, inner segments 1.7 – 2.5 cm in length, 0.4 – 0.6 cm in width, white. Fruit commonly bright red or magenta, rarely yellowish to greenish-white, 1.8 – 4.6 cm in length, 0.6 – 0.9 cm in diameter. Seed 0.8 – 2.0 mm in length, 0.8 – 2.2 mm in diameter, testa densely covered by elongate tubercles.

This species is divisible into two subspecies:

3a. *Discocactus zehntneri* subsp. *zehntneri*

Sparingly caespitose, stem 4.9 cm in height (3.0 – 8.0), 8.0 cm in diameter (5.5 – 13.6), ribs 13 (10 – 18), height of rib at the areole 1.0 cm (0.4 – 1.2), between areoles absent, width of rib at the areole 1.8 cm (1.2 – 3.0), between areoles 1.0 (0.6 – 1.5), 4 areoles along each rib (3 – 5), central spine 1 or 2, 3.4 cm in length (2.7 – 5.4) and 0.9 mm in diameter (0.6 – 1.3), radial spines 11 (9 – 14), 3.9 cm in length (2.7 – 6.3), 1.0 mm in diameter (0.7 – 1.6), cephalium 1.6 cm in height (1.0 – 2.6), 2.5 cm in diameter (1.5 – 4.3), bristles on the periphery of the cephalium 2.2 cm in length (1.8 – 4.0) and 0.2 mm in diameter (0.1 – 0.3).

Northern Rio São Francisco *caatinga* element: on exposed gneiss rocks and gravelly soil in *caatinga*, 450–500 m, north of the Chapada Diamantina, Mun. Sento Sé, northern Bahia. Endemic to Bahia. Figs. 7 – 8.

BAHIA: Mun. Sento Sé, *Zehntner* in *Rose & Russell* 19779 (US), 29 km SE of the [old] town, nr ‘Brejo Grande’, July 1974, *Horst* 441 (U, ZSS); l.c., Serra do Francisco, *Horst* 390 (U, ZSS); l.c., near the village of Brejo Grande, gneiss rock outcrop to the east of the village, 28 Apr. 2004, *M. Machado* 410 – 415 (HUEFS).

CONSERVATION STATUS. Data Deficient [DD]. Part of its former habitat is believed to have been submerged beneath the Represa (Lago) de Sobradinho leaving, perhaps, only a single locality intact (type of the synonymous *D. albispinus*). Detailed knowledge of its extent of occurrence and area of occupancy is currently lacking. However, it is likely to be Endangered (EN). *D. zehntneri* subsp. *zehntneri* is a quite distinct taxon with high levels of morphological and genetic variability, and displaying elevated degree of differentiation from the following subspecies (Machado et al., submitted). These facts alone provide compelling justification for its preservation.

3b. *Discocactus zehntneri* subsp. *boomianus* (Buining & Brederoo) N. P. Taylor & Zappi in *Bradleya* 9: 86 (1991). Holotype: Bahia, Mun. Morro do Chapéu, *Horst* 222 (U).

Discocactus boomianus Buin. & Brederoo in *Succulenta* 50: 26 (1971). *D. zehntneri* var. *boomianus* (Buin. & Brederoo) P.J. Braun in *ibid.* 69: 218 (1990).

D. araneispinus Buin. *et al.* in *Succulenta* 56: 258 (1977); *Gen. Discocactus*: 39 (1980). *D. zehntneri* var. *araneispinus* (Buin. *et al.*) P.J. Braun in *Succulenta* 69: 215 (1990). *D. zehntneri* subsp. *araneispinus* (Buin. *et al.*) P.J. Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 46: 64 (1995). Holotype: Bahia, Mun. Sento Sé, *Horst* 440 (U).

? *D. buenekeri* W.R. Abraham in *Kakt. and. Sukk.* 38: 284, with illus. (1987); P.J. Braun in *Succulenta* 69: 219–221 (1990). *D. zehntneri* subsp. *buenekeri* (W.R. Abraham) P.J. Braun & E. Esteves Pereira in *Kakt. and. Sukk.* 44: 64 (1993). Holotype: N Bahia, sine loc., *CWRA* 27 (KOELN [Succulentarium?], n.v.).

D. zehntneri var. *horstiorum* P.J. Braun in *ibid.* 69: 218 (1990). *D. zehntneri* subsp. *horstiorum* (P.J. Braun) P.J. Braun & E. Esteves Pereira in *ibid.* 46: 64 (1995). Holotype: Bahia, *K.I. Horst* 667 (ZSS; K, iso.).

Strongly caespitose, stem 4.6 cm in height (1.5 – 6.4), 6.6 cm in diameter (4.2 – 9.7), ribs 16 (13 – 26), height of rib at the areole 0.9 cm (0.3 – 1.3), between areoles absent or rarely to 0.7 cm, width of rib at the areole 1.2 cm (0.7 – 1.6), between areoles 0.7 (0.3 – 1.2), 5 areoles along each rib (2 – 7), central spine absent or rarely 1 to 3.6 cm in length and 0.8 mm in diameter, radial spines 14 (11 – 21), 2.5 cm in length (1.3 – 4.4), 0.4 mm in diameter (0.1 – 0.7), cephalium 2.1 cm in height (0.6 – 5.1), 2.7 cm in diameter (1.3 – 5.4), bristles on the periphery of the cephalium 4.4 cm in length (1.4 – 9.1) and 0.3 mm in diameter (0.1 – 0.5).

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. Figs. 9 – 10.

BAHIA: Mun. Sento Sé, E part of Serra do Mimoso, N of Limoeiro, *Horst* 440 (U, ZSS); l.c., serra near Minas do Mimoso, [‘Moreno’ *fide* Uebelmann (1996): HU 667], 1988, *K.I. Horst* 667 (K, ZSS); l.c., near the village of Campo Largo, a few km from the village on the road to Minas do Mimoso, 26 Mar. 2004, *M. Machado et al.* 350 – 356 (HUEFS); l.c., near the village of Limoeiro, mountain ca. 10 km to the east of the village, 28 Apr. 2004, *M. Machado* 400 – 403 (HUEFS); Mun. Morro do Chapéu, 21–22 km W of town on road BA 052, 11°28’S, 41°20’W, 25 Dec. 1988, *Taylor & Zappi* in *Harley* 27395 (K, SPF, CEPEC), 23 July 1988, *Eggl* 1274 (ZSS); l.c. (?), cult. ZSS, *Horst (& Uebelmann)* 222 (K); 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, then ca. 18km to the north on road passing by the villages of São Rafael and Tareco until the locality known as Volta da Serra, arenitic rock outcrops in the western slopes of the mountain, 12 Jun. 2004, *M. Machado et al.* 460 – 464 (HUEFS); l.c., ca. 25 km to the west of the town, arenitic rock outcrops in the western slopes of the mountain, north of the turn to Cafarnaum, 12 Jun. 2004, *M. Machado et al.* 470 – 474 (HUEFS).

CONSERVATION STATUS. Vulnerable [VU D2] (2); PD=1, EI=1, GD=2. Short-list score (2x4) = 8. Habitats fragmented and subject to collection, at least one being directly accessible from a main road, although designated as a protected area. This subspecies is partitioned on a geographical and genetic basis in two sets of populations with elevated degree of genetic differentiation among them, a northern set located in mun. Sento Sé, genetically more diverse, and a southern set located in mun. Morro do Chapéu. None of the northern populations is currently included within protected areas.

DISCUSSION

Both a more thoroughly morphological investigation (Machado et al. 2005a, submitted) and a survey of genetic variability (Machado et al. 2005b, submitted) indicate moderate to low levels of differentiation among populations of *D. bahiensis*. The taxon described as *D. subviridigriseus* is usually distinguished from *D. bahiensis* because of its more robust habit and stouter spines; however, it could not be satisfactorily separated from other forms of *D. bahiensis* on our morphological investigation. The same applies to the taxon described as *D. bahiensis* subsp. *gracilis*, which is distinguished by its smaller size, higher number of spines in the areole, thinner spines, and increased tendency to offset. These same characteristics were interpreted as deriving from introgression of genes from nearby populations of *D. zehntneri*, and this hypothesis is corroborated on our survey of allozyme diversity. Overall, the allozyme survey indicates an absence of genetic substructure and high genetic identities among populations of *D. bahiensis*, thus lending further support for not recognizing infraspecific taxa within this species.

The populations of *D. bahiensis* surveyed were all relatively small, each comprising a few dozen up to a few hundred plants; this species seems to inhabit on very specific habitats, consisting of open and barren, flat gravelly areas usually close to rivers. The smaller populations were those located close to settlements or roads, and these populations also displayed lower levels of genetic diversity. The populations BS_RO (type locality for the synonymous *D. subviridigriseus*) and BG_SR (type locality for the synonymous *D. bahiensis* subsp. *gracilis*) presented higher degree of disturbance, but the other populations also displayed signs of being impacted by human activities, all being located within agricultural areas, BB_GO and BB_AB very close to villages. Collection was evident in some populations, especially in the population BS_JU where a large number of holes and some uprooted plants were observed. Effects of cattle trampling and livestock foraging (chiefly by goats) have also been observed, principally in the population BB_GO. On the whole, the populations of *D. bahiensis* occur within regions suitable for agriculture which are undergoing intense development, threatening the continuing survival of this species.

D. catiugicola was the taxon that presented the lowest levels of both morphological and genetic differentiation among its populations, in spite of a number of these having been described as distinct taxa (*D. nigrisaetosus*, *D. rapirhizus*, and *D. spinosior*). The results of our morphologic and genetic investigations do not support the elaborate

intraspecific classification proposed by Braun & Esteves Pereira (1993a-c, 1995, 2001b) for this species due to an absence of genetic substructure and high genetic identities among its populations, coupled with a high degree of morphological similarity (Machado et. al., submitted). We did not evaluate the relationships between *D. heptacanthus* and *D. catingicola*, which is regarded as being a subspecies of the former in the classification system proposed by Taylor & Zappi (2004), because the former was not surveyed at its type locality; however, the population from the state of Goiás described as *D. rapirhizus*, although ascribed by Taylor & Zappi (2004) to *D. heptacanthus* subsp. *heptacanthus*, is hardly distinguishable from typical *D. catingicola* both morphologically and genetically. However, this result needs to be interpreted with caution, because it does not necessarily implies that there are not differences between *D. heptacanthus* and *D. catingicola* – it can be that *D. rapirhizus* was wrongly assigned to *D. heptacanthus* by Taylor & Zappi (2004), and that its true affinities are indeed with *D. catingicola*, as suggested by the classification system of Braun & Esteves Pereira (1993a-c, 1995, 2001b) and demonstrated in our morphologic and genetic investigations.

D. catingicola presented the largest area of distribution among the species surveyed. Despite Buining's use of the epithet *catingicola*, this species is more typical of the savannah-like vegetation domain known as *cerrado*, and probably occurs within the *caatinga* domain only at its eastern limits. Even in these areas, the species seems to inhabit enclaves of *cerrado*, sharing its habitat with other species typical of this vegetation domain. All the surveyed populations of *D. catingicola* were small, ranging from a few dozen up to a few hundred plants; this species inhabits sandy phases of the *cerrado*, often close to rivers. Its habitat is more continuous than that of the previous species, but the *cerrado* as a whole has undergone much destructive change in the past few decades, with large-scale establishment of agricultural crops such as soy-bean, cotton, and maize plantations, resulting in extensive habitat fragmentation and possibly in the extinction of a number of *D. catingicola* populations.

Of the *D. catingicola* populations surveyed, the most affected was CN_CO, which was very small – we had difficulty in finding enough mature plants for our survey. This population is located within a disturbed agricultural area, where cattle trampling was evident; the local people also informed that the plants are usually destroyed when found, in order to avoid the cattle to be crippled on its spines. As a reflection of the reduced size, CN_CO presented the lowest genetic diversity among all surveyed populations of *D. catingicola*. The population CN_PN is experiencing the same threat, the area where it

occurs having been fenced for cattle raising. Vicinal roads were cut through the populations CC_SG and CC_AV, and the original *cerrado* vegetation of areas neighboring to these populations are being replaced by agricultural fields; CC_AV is also being affected by repeated burning of the *cerrado* vegetation. The least affected population was CR_PO, since the *cerrado* area where it occurs is large and seems to be less disturbed; this population accordingly presented the highest genetic diversity among all surveyed populations of *D. catiungicola*. However, it is located near an expanding town, and is close to a garbage dump.

A high level of genetic differentiation was observed among the populations of *D. zehntneri*, which were partitioned on a geographical basis in northern and southern sets of populations. Morphology however is not in fully agreement with genetic patterns, and it divides the species in two subspecies – *zehntneri* and *boomianus*, with the northern group including populations of both subspecies and the southern group comprising populations of subspecies *boomianus* only. Notwithstanding the results of the genetic survey, the interpretation of two subspecies occurring within *D. zehntneri* is not prejudiced since the typical subspecies and the northern populations of subspecies *boomianus* have moderate levels of genetic divergence, and there is evidence for a single origin of subspecies *boomianus*, the southern populations possibly originating via migration from the north with subsequent isolation and divergence over a long period of time leading to the high degree of genetic differentiation between northern and southern populations of the subspecies, while the northern populations diverged less from the common ancestor with subspecies *zehntneri*. The northern populations, although having been described as distinct taxa (*D. araneispinus* and *D. zehntneri* var. *horstiorum*), cannot be satisfactorily separated from *D. boomianus* based on morphology.

D. zehntneri is the only species endemic to the state of Bahia. This species occur in gneiss rock outcrops within the *caatinga* or in montane habitats, usually ecotone areas between *caatinga* and montane *cerrado* in sandy or gravelly soil. The only location visited for the typical subspecies (ZA_BG) was a gneiss rock outcrop near the small village of Brejo Grande, municipality of Sento Sé – this is the type locality of the synonymous *D. albispinus*. In this habitat there are several hundreds of plants, and evidence of cattle trampling was the only threat observed. At present this is the only known location for the subspecies – precise locality information was not provided in the original description of *D. zehntneri*, and part of its range is believed to have been submerged beneath the Represa (Lago) de Sobradinho; the location reported by Buining (1980) for this taxon could not be located. Detailed knowledge

of its extent of occurrence and area of occupancy is currently lacking, and the region where it occurs should be explored more thoroughly in order to try to find more populations of this taxon, so that a better assessment of its conservation status can be made. Nevertheless, the status of the population ZA_BG should be regularly monitored so that any changes could be promptly detected and appropriate actions taken. *D. zehntneri* subsp. *zehntneri* is a morphologically distinct taxon with high genetic variability and elevated degree of genetic differentiation from subspecies *boomianus* (Machado et al., submitted), and these facts alone provide compelling justification for the preservation of its only known population.

Within *D. zehntneri* subsp. *boomianus* there are two geographical sets of populations, a northern one in the municipality of Sento Sé and a southern one in the municipality of Morro do Chapéu. These two sets of populations also differ significantly in genetic composition (Machado et al., submitted). Of the northern populations, one (ZH_CL) is very small, with a few dozens of plants only; this population occurs on a ‘pedra canga’ (iron- and manganese rich conglomerate rock) outcrop, that has been prospected for ore extraction; the location is being offered by Bahian Company of Mineral Research (CBPM) to private companies for commercial exploration. The second northern population investigated (ZR_LI) occur in a little disturbed mountainous area that seems to be visited only by the local people when they go hunting; the plants are found scattered over a large area. The only observed threat was the occasional burning of the vegetation by the local people. On our visit to the location we were informed by our local guide that there are other areas in the neighboring mountains where the plants can be found. Two of the three southern populations of subspecies *boomianus* surveyed (ZB_EC, ZB_LA, and ZB_VS) are large both in extent of habitat and number of mature individuals, which are estimated to range from 50,000 to at least 150,000. One population (ZB_VS) occupied a smaller patch of suitable habitat, and was likewise small. Another large population is known from Morro do Chapéu, but it has not been investigated because it occurs in very close proximity to other two (ZB_EC and ZB_LA). Overall, the southern *boomianus* populations occur in little disturbed areas, and are generally large; threats observed include cattle trampling, and collecting – the three largest populations are accessible by main roads.

Of the *Discocactus* populations surveyed, only two are included within protected areas – the *D. zehntneri* population ZB_LA, which occur within the Morro do Chapéu State Park, and the *D. catingicola* CC_AV, which occur within the Environmental Protection Area (APA) of Acaba Vidas. However, the Morro do Chapéu State Park has not been properly enforced yet, and the APA of Acaba Vidas belongs to a category of protected area that does

not preclude the use of the land by the local people, as long as it is carried out on a sustainable way. No population of *D. bahiensis* is currently under any sort of protection; this species is experiencing a fast decline in habitat quality, and priority must be given for the protection of representative samples of the range of morphological and genetic variation that occur within the species. We recommend the establishment of local reserves in the region between the villages of Abreus, Goiabeira, Olhos D'Água and Juremal (all within the municipality of Juazeiro) because this area holds an expressive number of *D. bahiensis* populations with high degree of genetic and morphologic diversity. Special attention should also be given to the population BG_SR in virtue of its morphological distinctiveness and the fact that it is undergoing genetic differentiation due to introgression with *D. zehntneri*. New protected areas including populations of *D. catingicola* need to be created in order to better preserve this species; a good candidate is the area surrounding the town of Posse in the eastern part of Goiás state where the population CR_PO is located, since this area is extensive and little disturbed.

The southern populations of *D. zehntneri* subsp. *boomianus* seems to be adequately preserved at present in spite of only one of these be located within a protected area, because the majority of the populations comprise large numbers of plants and occupy places unsuitable for agriculture. However, regular monitoring of the status of these populations is necessary, so that any changes in the current status may be promptly detected and appropriate actions taken. In order to preserve a representative sample of the range of variation within *D. zehntneri*, one or more reserves should be created in the northern limit of distribution of this taxon so as to include populations of both subspecies *zehntneri* and northern populations of subspecies *boomianus*, this way accounting for the morphologic and genetic differentiation that occurs between southern and northern sets of populations, and the higher genetic variability of the northern populations. In the particular case of the population ZH_CL, ex-situ conservation may be the only viable alternative for its preservation case mining activities are initiated in the area where it occurs. We recommend the establishment of ex-situ gene banks to enable future re-introductions, and a detailed investigation of the region where the population ZH_CL is located with the aim of locating other populations.

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Table 1. Classification systems proposed for the *Discocactus* taxa that occur in the state of Bahia, Brazil, including the taxa described as *D. rapirhizus*, *D. griseus*, and *D. heptacanthus*, from the states of Goiás (GO), Minas Gerais (MG), and Mato Grosso (MT), respectively. Taxa marked with an asterisk have not been studied in the field.

Original name	Braun & Esteves Pereira (1993a-c, 1995, 2001b)	Taylor & Zappi (2004)	This study
<i>D. bahiensis</i> Britton & Rose	<i>D. bahiensis</i> subsp. <i>bahiensis</i>	<i>D. bahiensis</i>	<i>D. bahiensis</i>
<i>D. subviridigriseus</i> Buining & Brederoo	<i>D. bahiensis</i> subsp. <i>subviridigriseus</i> (Buining & Brederoo) P.J.Braun & Esteves		
<i>D. bahiensis</i> subsp. <i>gracilis</i> P.J.Braun & Esteves	<i>D. bahiensis</i> subsp. <i>gracilis</i>		
<i>D. catingicola</i> Buining & Brederoo	<i>D. catingicola</i> subsp. <i>catingicola</i> var. <i>catingicola</i>	<i>D. heptacanthus</i> subsp. <i>catingicola</i>	<i>D. catingicola</i>
<i>D. spinosior</i> Buining & Brederoo			
<i>D. nigrisaetosus</i> Buining & Brederoo	<i>D. catingicola</i> subsp. <i>catingicola</i> var. <i>nigrisaetosus</i> (Buining & Brederoo) P.J.Braun & Esteves		
<i>D. rapirhizus</i> Buining & Brederoo	<i>D. catingicola</i> subsp. <i>rapirhizus</i> (Buining & Brederoo) P.J.Braun & Esteves (GO)	<i>D. heptacanthus</i> subsp. <i>heptacanthus</i> (MT) *	
<i>D. griseus</i> Buining & Brederoo *	<i>D. catingicola</i> subsp. <i>griseus</i> (Buining & Brederoo) P.J.Braun & Esteves (MG) *		
<i>D. zehntneri</i> Britton & Rose	<i>D. zehntneri</i> subsp. <i>zehntneri</i>	<i>D. zehntneri</i> subsp. <i>zehntneri</i>	<i>D. zehntneri</i> subsp. <i>zehntneri</i>
<i>D. albispinus</i> Buining & Brederoo	<i>D. zehntneri</i> subsp. <i>albispinus</i> (Buining & Brederoo) P.J.Braun & Esteves		
<i>D. boomianus</i> Buining & Brederoo	<i>D. zehntneri</i> subsp. <i>boomianus</i> (Buining & Brederoo) N.P.Taylor & Zappi	<i>D. zehntneri</i> subsp. <i>boomianus</i>	<i>D. zehntneri</i> subsp. <i>boomianus</i>
<i>D. araneispinus</i> Buining & Brederoo	<i>D. zehntneri</i> subsp. <i>araneispinus</i> (Buining & Brederoo) P.J.Braun & Esteves		
<i>D. zehntneri</i> var. <i>horstiorum</i> P.J.Braun	<i>D. zehntneri</i> subsp. <i>horstiorum</i> (P.J.Braun) P.J.Braun & Esteves		
<i>D. buenekeri</i> Abraham *	<i>D. zehntneri</i> subsp. <i>buenekeri</i> (Abraham) P.J.Braun & Esteves *		

Table 2. Locality data for 17 *Discocactus* populations surveyed in the states of Bahia (BA) and Goiás (GO), Brazil, with number of individuals sampled and an acronym for each population, information about the species complex to which the population belongs, if the location represents a type locality for a described taxon, and the voucher specimens deposited at HUEFS. Precise locality information was withheld for conservation reasons.

Taxon	Location	Municipality and State	Type locality for synonymous taxon	Acronym	Sample size	Voucher specimens (HUEFS)
<i>Discocactus bahiensis</i>	Abreus	Juazeiro (BA)		BB_AB	20	M. Machado 440 – 443
	Goiabeira	Juazeiro (BA)		BB_GO	20	M. Machado 430 – 433
	Olhos D'Água	Juazeiro (BA)		BB_OL	20	M. Machado 420 – 423
	São Rafael	Morro do Chapéu (BA)	<i>D. bahiensis</i> subsp. <i>gracilis</i>	BG_SR	20	M. Machado et al. 390 – 393
	Rodeadouro	Juazeiro (BA)	<i>D. subviridigriseus</i>	BS_RO	21	M. Machado et al. 370 – 373
	Juremal	Juazeiro (BA)		BS_JU	17	M. Machado et al. 380 – 383
<i>Discocactus catingicola</i>	Sítio Grande	São Desidério (BA)	<i>D. catingicola</i>	CC_SG	18	M. Machado et al. 300 – 303
	Acaba Vidas	Barreiras (BA)		CC_AV	17	M. Machado et al. 310 – 313
	Porto Novo	Santana (BA)	<i>D. nigrisaetosus</i>	CN_PN	17	M. Machado et al. 340 – 344
	Coribe	Coribe (BA)		CN_CO	12	M. Machado et al. 330 – 333
	Posse	Posse (GO)	<i>D. rapirhizus</i>	CR_PO	19	M. Machado et al. 320 – 323
<i>Discocactus zehntneri</i> subsp. <i>zehntneri</i>	Brejinho Grande	Sento Sé (BA)	<i>D. albispinus</i>	ZA_BG	21	M. Machado 410 – 415
<i>Discocactus zehntneri</i> subsp. <i>boomianus</i>	Lages	Morro do Chapéu (BA)	<i>D. boomianus</i>	ZB_LA	30	M. Machado et al. 450 – 454
	Entroncamento de Cafarnaum	Morro do Chapéu (BA)		ZB_EC	25	M. Machado et al. 470 – 474
	Volta da Serra	Morro do Chapéu (BA)		ZB_VS	20	M. Machado et al. 460 – 464
	Limoeiro	Sento Sé (BA)	<i>D. araneispinus</i>	ZR_LI	20	M. Machado 400 – 403
	Campo Largo	Sento Sé (BA)	<i>D. zehntneri</i> subsp. <i>horstiorum</i>	ZH_CL	20	M. Machado et al. 350 – 356

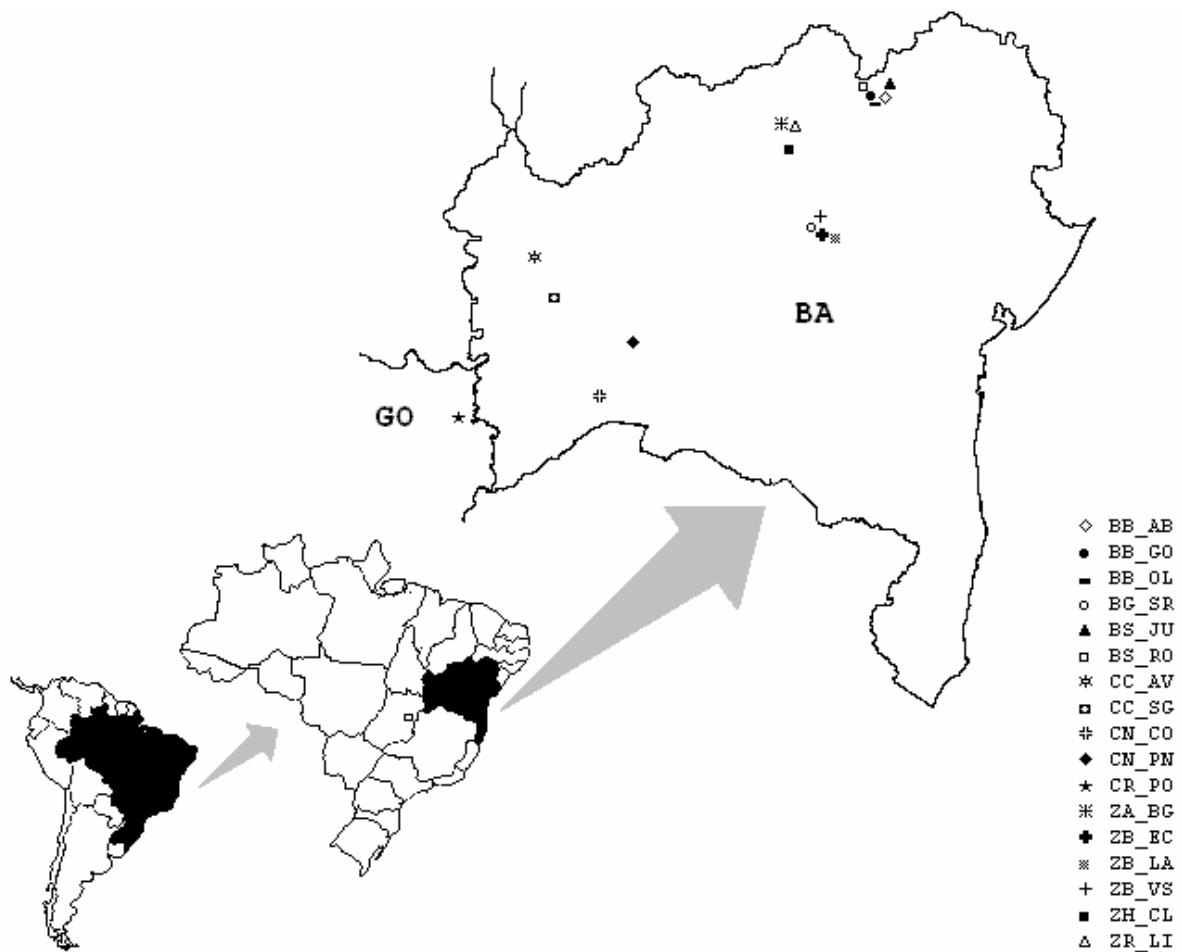


Figure 1. Geographic locations of the 17 *Discocactus* populations surveyed in the states of Bahia (BA) and Goiás (GO), Brazil. For population acronyms see Table 2.



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Figures 2 – 10. *Discocactus* taxa occurring in the state of Bahia, Brasil: 2 – 4, *D. bahiensis* (2 – BB_GO, 3 – BS_JU, 4 – BG_SR); 5 – 6, *D. catingicola* (5 – CC_AV, 6 – CR_PO); 7 – 8, *D. zehntneri* subsp. *zehntneri* (ZA_BG); 9 – 10, *D. zehntneri* subsp. *boomianus* (9 – ZB_LA, 10 – ZH_CL). For population acronyms see Table 2.

CONCLUSÕES GERAIS

São reconhecidas para o estado da Bahia três espécies de *Discocactus*: *D. bahiensis*, *D. catingicola* e *D. zehntneri*, a última consistindo de duas subespécies, *zehntneri* e *boomianus*. A circumscrição adotada baseia-se nos resultados obtidos a partir das análises dos dados morfológicos, onde foram reconhecidos como distintos os táxons detectados por todos os métodos de delimitação empregados. Foram utilizados métodos de análise multivariada englobando técnicas de ordenação (PCA, CVA) e agrupamento (UPGMA), e métodos para a delimitação de espécies com base em caracteres (Análise de Agrupamento de Populações – PAA; Davis & Nixon, 1992) e árvores filogenéticas (método de Wiens & Penkrot, 2002), sendo que o método PAA foi modificado de forma a ser utilizado na identificação de táxons infraespecíficos.

As circumscrições adotadas são concordantes com as preferências ecológicas dos táxons reconhecidos, com cada táxon ocupando habitats distintos: *D. bahiensis* ocorre em áreas abertas com solo argiloso e cascalhento que constituem antigas planícies fluviais, dentro do domínio do bioma caatinga; *D. catingicola*, apesar de seu epífeto, ocorre em solos arenosos de áreas de domínio do bioma cerrado; *D. zehntneri* subsp. *zehntneri* cresce como rupícola em afloramentos rochosos de gnáiss em meio à caatinga, enquanto que *D. zehntneri* subsp. *boomianus* cresce em solos arenosos ou cascalhentos, derivados de arenito ou pedra canga em áreas montanhosas do norte da Chapada Diamantina.

Os resultados obtidos a partir da análise de variabilidade genética, onde foram empregados os mesmos indivíduos e populações utilizadas para as análises morfológicas, são em geral bastante congruentes com a delimitação proposta para os táxons de *Discocactus* que ocorrem no estado da Bahia. A ausência de subgrupos e as elevadas identidades genéticas entre as populações de *D. bahiensis* e *D. catingicola* não sustentam nenhum particionamento infraespecífico para estes táxons. Em *D. zehntneri* existe uma certa discrepância entre os resultados obtidos a partir das análises de dados morfológicos, que sustentam a divisão da espécie em duas subespécies – *zehntneri* e *boomianus*, e os resultados obtidos da análise de variabilidade genética, que subdivide geograficamente as populações da espécie em dois grupos, com um grupo mais ao sul constituído de populações da subespécie *boomianus*, e um grupo mais ao norte constituído de populações de ambas subespécies. No entanto, estes resultados não comprometem a interpretação de duas subespécies em *D. zehntneri*, pois existe diferenciação genética entre a subespécie *zehntneri* e as populações da subespécie *boomianus* que ocorrem no norte, embora não tão

elevada quanto a diferenciação observada entre os grupos de populações com ocorrência no norte e no sul. Além disso, subespécie *zehntneri* apresenta um elevado grau de diferenciação morfológica em relação à subespécie *boomianus*. Uma origem única para a subespécie *boomianus* é sustentada pela análise cladística dos dados morfológicos, e por uma árvore de Wagner construída a partir dos dados genéticos. Sendo assim, o elevado grau de diferenciação entre as populações dessa subespécie é interpretado como sendo derivado de uma origem da subespécie no norte, com o posterior estabelecimento de populações mais ao sul, seguido de isolamento e divergência genética, enquanto que as populações do norte retiveram mais características em comum com a subespécie *zehntneri*.

Evidências morfológicas e genéticas indicam que está ocorrendo introgressão de genes de *D. zehntneri* na população BG_SR de *D. bahiensis*. Evidências morfológicas incluem tamanho reduzido, maior número de espinhos por aréola, espinhos com menor diâmetro, e tendência a ser mais cespitoso. As evidências genéticas são a ocorrência na população BG_SR de dois alelos que de outra forma seriam exclusivos de *D. zehntneri*.

As espécies de *Discocactus* estudadas possuem graus elevados de diversidade genética, sendo similares ou superiores aos valores médios encontrados em outras espécies de cactos (Hamrick et al., 2002) e outras espécies de plantas com ciclos de vida e outras características similares (Hamrick et al., 1992). A alta variabilidade genética observada nas espécies de *Discocactus* é altamente contrastante com os valores extremamente baixos encontrados em espécies do gênero *Melocactus* (Cactaceae), que ocorrem na mesma área geral e possuem hábitos similares (Lambert et. al., dados não publicados).

Todas as espécies de *Discocactus* são consideradas ameaçadas de extinção, devido ao pequeno número de populações conhecido para cada espécie, em geral baixo número de indivíduos em cada população, e especificidade de hábitat que faz com que as populações ocorram em áreas restritas. Estes fatores fazem com que as populações de *Discocactus* sejam extremamente vulneráveis à modificação e destruição de seus habitats e à remoção de indivíduos das populações. De todas as populações de *Discocactus* estudadas, apenas duas estão inclusas em áreas de preservação – a população de *D. zehntneri* ZB_LA, que ocorre dentro do Parque Estadual de Morro do Chapéu, e a população de *D. catingicola* CC_AV, que ocorre dentro da Área de Proteção Ambiental (APA) da Cachoeira do Acaba Vidas. Nenhuma população de *D. bahiensis* encontra-se sob proteção, e esta espécie vem experimentando um rápido declínio na qualidade de seus habitats devido ao desenvolvimento agrícola e urbano das áreas onde populações dessa espécie ocorrem. É recomendada a criação de reservas na região entre as vilas de Abreus, Goiabeira, Olhos D'Água e Juremal

(todas no município de Juazeiro), pois essa região possui um número expressivo de populações de *D. bahiensis* com elevado grau de variabilidade genética e morfológica. Atenção especial também precisa ser dada à população BG_SR devido à sua distinção morfológica e ao fato de que esta população está se diferenciando geneticamente devido à ocorrência de introgressão com *D. zehntneri*. Mais áreas de preservação que incluam populações de *D. catiungicola* devem ser criadas de forma a preservar melhor esta espécie, pois as regiões de Cerrado onde *D. catiungicola* ocorre estão sendo bastante modificadas com o desenvolvimento agrícola e urbano destas regiões. Uma área sugerida aqui é a região ao redor da cidade de Posse no leste do estado de Goiás devido às extensas áreas de hábitat adequado e ao relativamente alto grau de conservação da região.

D. zehntneri subsp. *zehntneri* é um táxon bastante distinto, com elevada variabilidade morfológica e genética, além de apresentar um alto grau de diferenciação morfológica e genética em relação à subespécie *boomianus*. Apenas uma população é conhecida para este táxon. Em adição à preservação desta população, esforços devem ser direcionados à tentativa de se encontrar novas populações para este táxon. As populações sulinas de *D. zehntneri* subsp. *boomianus* se encontram relativamente bem preservadas no momento, pois estas populações possuem em geral um grande número de indivíduos, ocupam áreas que não são apropriadas para o desenvolvimento agrícola, e uma das populações ocorre dentro de uma área de preservação. No entanto, uma ou mais reservas precisam ser criadas para proteger as populações desta subespécie que ocorrem mais ao norte de forma a abarcar a totalidade da variação genética que ocorre na subespécie. No caso específico da população ZH_CL, conservação ex-situ pode ser a única alternativa viável para a sua preservação caso a área onde esta população ocorre comece a ser minerada.

Em resumo, a utilização de diversos métodos para delimitação de espécies, especialmente quando os métodos são aplicados ao mesmo conjunto de dados, constituem forte evidência dos verdadeiros limites específicos e infraespecíficos quando os resultados obtidos a partir dos métodos empregados são congruentes, e quando existe conflito entre os resultados obtidos as causas mais prováveis para o desacordo em geral podem ser determinadas, reforçando ainda mais as decisões taxonômicas adotadas. As espécies de *Discocactus* investigadas possuem elevado grau de variabilidade genética, e devido à modificação e destruição dos hábitats restritos em que ocorrem, estas espécies encontram-se vulneráveis e ameaçadas de extinção. Novas áreas de preservação abarcando a totalidade da variação morfológica e genética das populações precisam ser criadas de forma a assegurar a existência continuada das espécies.

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