SABRINA MOTA LAMBERT

# Variabilidade genética e morfológica inter e intra populacional em *Melocactus paucispinus* G. Heimen & R. Paul e *Melocactus glaucescens* Buining & Brederoo (Cactaceae), espécies ameaçadas de extinção da Chapada Diamantina, Bahia, Brasil.

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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*.

ORIENTADOR: PROF. DR. EDUARDO LEITE BORBA (UFMG/UEFS)

FEIRA DE SANTANA – BA
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Orientador e Presidente da Banca

Feira de Santana – BA

2006

“**Os detalhes da natureza são revelados àqueles que têm olhos para ver, paciência para observar e capacidade para analisar**.”

(Carl P. Suanson)

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# SUMÁRIO

# RESUMO

*Melocactus paucispinus* (Cactaceae) é endêmico do estado da Bahia, Brasil, e devido a sua raridade e apreciação de coletores tem sido considerada como ameaçada de extinção. Esta espécie é usualmente simpátrica e inter-fértil com *M. concinnus*, e em algumas populações é visível evidencias morfológicas de hibridação entre elas. Foram acessados os níveis de variabilidade genética e morfológica e a sub-estruturação entre populações destas espécies, tentando ainda verificar a ocorrência de hibridação natural entre elas. A variabilidade genética foi analisada usando aloenzimas (12 locos) e a variabilidade morfológica usando análise morfométrica multivariada (17 caracteres vegetativos) em dez populações de *M. paucispinus* e três de *M. concinnus* ocorrentes na Chapada Diamantina, Bahia. A variabilidade genética é baixa em ambas as espécies ($P$ = 0.0 – 33.3, A = 1.0 – 1.6, $H_e$ = 0.000 – 0.123 em *M. paucispinus*; $P$ = 0.0 – 25.0, A = 1.0 – 1.4, $H_e$ = 0.000 – 0.104 em *M. concinnus*). Foi encontrado deficit de heterozigotos dentro das populações para as espécies, com altos valores de $F_{IS}$ (0.732 e 0.901 em *M. paucispinus* e *M. concinnus*, respectivamente). Evidência de hibridação foi detectada pela freqüência relativa no segundo loco da diaforase. Foram encontrados altos níveis de estruturação genética ($F_{ST}$ = 0.504 em *M. paucispinus* e 0.349 em *M. concinnus*) e morfológica ($A$ = 0.20 em *M. paucispinus* e 0.17 em *M. concinnus*) entre populações. Ambas as espécies de *Melocactus* dispõem de níveis de variabilidade genética menores que os valores reportados para outras espécies de cactos. Evidências indicam a ocorrência de introgressão para ambas as espécies em duas áreas. Os elevados valores de $F_{ST}$ não podem ser explicados por sub-estruturação geográfica, mas são consistentes com hibridação. Ao contrário, diferenciação morfológica em *M. paucispinus*, mas não em *M. concinnus*, é provavelmente devido a isolamento por distância.

*Melocactus glaucescens* é endêmico da Chapada Diamantina, Nordeste do Brasil, e é criticamente ameaçada de extinção. Esta espécie cresce simpatricamente com outras espécies congenéricas, e há evidências de hibridação entre elas. Foram avaliados os níveis de variabilidade genética e morfológica e a sub-estruturação entre as populações de *M. glaucescens* e espécies simpátricas, e testamos a ocorrência de hibridação natural. A variabilidade genética foi investigada usando aloenzimas (12 locos em nove sistemas enzimáticos), e a variabilidade morfológica através de análises morfométricas multi e univariadas de 18 caracteres em nove populações naturais de *M. glaucescens*, *M.*

*ernestii*, *M. concinnus* e dois distintos morfos de supostos híbridos. A variabilidade genética foi baixa em todas as populações ($P$ = 7.7–41.7, A = 0.3–1.7, $H_e$ = 0.009–0.096), e todos os taxas dispõem de déficit de heterozigotos. Baixos níveis de estruturação genética e moderados níveis de estruturação morfológica foram encontrados para *M. glaucescens* ($F_{ST}$ = 0.045, $A$ = 0.16) e *M. concinnus* ($F_{ST}$ = 0.022, $A$ = 0.11). Os resultados obtidos com o marcador genético usado são inconclusivos para confirmar a hipótese de ocorrência de hibridação, devido a ausência de loco diagnóstico nas possíveis espécies parentais. No entanto, essa hipótese tem suporte em outros resultados. A presença de alelos raros nos supostos híbridos pode ser uma indicação que essas populações já são estáveis e estão em processo de diferenciação em relação aos seus parentais. O grande número de alelos exclusivos em *M. glaucescens* é um fator importante a ser considerado na delimitação de estratégias de conservação para a espécie.

# ABSTRACT

*Melocactus paucispinus* (Cactaceae) is endemic to the Bahia state, Brazil, and due to its rarity and desirability to collectors it has been considered threatened with extinction. This species is usually sympatric and inter-fertile with *M. concinnus*, and morphological evidence for hybridization between them is present in some populations. We assessed levels of genetic and morphological variation and sub-structuring in populations of these species, and tried to verify the occurrence of natural hybridization between them. We surveyed genetic variability using allozymes (12 loci) and morphological variability using multivariate morphometric analyses (17 vegetative characters) in 10 populations of *M. paucispinus* and three of *M. concinnus* occurring in the Chapada Diamantina, Bahia state. Genetic variability was low in both species ($P$ = 0.0 – 33.3, A = 1.0 – 1.6, $H_e$ = 0.000 – 0.123 in *M. paucispinus*; $P$ = 0.0 – 25.0, A = 1.0 – 1.4, $H_e$ = 0.000 – 0.104 in *M. concinnus*). Deficit of heterozygotes within the populations was detected in both species, with high values of $F_{IS}$ (0.732 and 0.901 in *M. paucispinus* and *M. concinnus*, respectively). Evidence of hybridization was detected by the relative allele frequency in the two diaphorase loci. High levels of genetic ($F_{ST}$ = 0.504 in *M. paucispinus* and 0.349 in *M. concinnus*) and morphological ($A$ = 0.20 in *M. paucispinus* and 0.17 in *M. concinnus*) structuring among populations were found. Both the *Melocactus* species displayed levels of genetic variability lower than the values reported for other cactus species. The evidence indicates the occurrence of introgression in both species at two sites. The high $F_{ST}$ values cannot be explained by geographical substructuring, but are consistent with hybridization. Conversely, morphological differentiation in *M. paucispinus*, but not in *M. concinnus*, is probably due to isolation by distance.

*Melocactus glaucescens* is endemic to the Chapada Diamantina, Northeastern Brazil, and is critically endangered. This species grows in sympatry with other congeneric species, and there is evidence of hybridation among them. We evaluated the levels of genetic and morphological variability and their sub-structuring between the populations of *M. glaucescens* and sympatric species, and we tested for the occurrence of natural hybridation. The genetic variability was investigated using allozymes (12 loci in nine enzymatic systems), and the morphological variability was investigated using multi- and univariate morphometric analyses of 18 vegetative characters in nine natural

populations of *M. glaucescens*, *M. ernestii*, *M. concinnus* and two distinct morphs of supposed hybrids. The genetic variability was low in all populations ($P$ = 7.7–41.7, A = 0.3–1.7, $H_e$ = 0.009–0.096), and all taxa displayed a deficit in heterozygotes. Low levels of genetic structuring and moderate levels of morphological structuring were found for *M. glaucescens* ($F_{ST}$ = 0.045, $A$ = 0.16) and *M. concinnus* ($F_{ST}$ = 0.022, $A$ = 0.11). The results obtained with the genetic marker used are inconclusive to confirm the hypothesis of occurrence of hybridation due to an absence of diagnostic loci in the presumed parental species. However, this hypothesis cannot be refuted by the results either. The presence of rare alleles in the supposed hybrids may be an indication that these populations are already stable and in the process of differentiating from their parental species. The large number of exclusive alleles and the levels of morphological structuring in the populations of *M. glaucescens* are important factors to be considered in the definition of strategies for the conservation of the species.

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## CAPÍTULO 1



## CAPÍTULO 2

# INTRODUÇÃO GERAL

A família Cactaceae apresenta cerca de 1800 espécies (Anderson, 2001), representando, na biodiversidade Neotropical, o segundo maior grupo de plantas endêmicas (Taylor, 2000). A família é caracterizada pela presença de caule especializado (cladódio), usualmente suculento, apresentando pequenas gemas denominadas aréolas, onde comumente desenvolvem-se espinhos (Taylor, 1997; Judd *et al*., 1999, 2002). Os cactos distribuem-se, com algumas exceções, nas regiões tropicais e subtropicais do continente americano, especialmente em ambientes áridos e semi-áridos (Montes, 1997), sendo mais freqüente nas zonas de climas quentes e secos, apesar de ocorrem desde desertos até regiões úmidas de florestas tropicais, além de ocorrerem nas mais diversas altitudes (Taylor, 1997).

De acordo com Taylor (2000), existem quatro grandes centros de diversidade das Cactaceae nas Américas. O primeiro é o México e o sudoeste dos Estados Unidos, onde 27% de todos os gêneros de cactos são endêmicos (Taylor, 1997), com o México retendo a maior diversidade tanto em nível genérico (54) quanto específico (850). O segundo corresponde à cadeia Andina, compreendendo os países do Peru e Bolívia, com quase 18% de todos os gêneros de cactos e 420 espécies e variedades endêmicos para esta região (Montes, 1997; Taylor, 1997). O terceiro é o leste do Brasil, compreendendo as regiões Nordeste e Sudeste e o leste dos estados de Goiás e Tocantins, possuindo 30 gêneros, 134 espécies e 43 subespécies, das quais 161 (91%) são táxons nativos (espécies e subespécies) e destes 122 são endêmicos para a região. O quarto centro de diversidade corresponde ao Paraguai, Uruguai e Argentina e as regiões Centro-oeste e Sul do Brasil (Taylor, 1997).

O leste do Brasil, segundo Taylor (2000), é uma vasta área tropical conhecida pela diversidade e endemismo de cactos. A maior biodiversidade de cactos nesta área está concentrada nos estados da Bahia e Minas Gerais, cada um possuindo mais de 90 táxons, entre os quais 30 e 36 são considerados endêmicos, respectivamente.

A família Cactaceae pertence à ordem Caryophyllales, juntamente com mais 29 famílias, entre elas Amaranthaceae, Aizoaceae, Caryophyllaceae, Nyctaginaceae, Phytolacaceae, Plumbaginaceae, e Portulacaceae (APG, 2003; Soltis *et al*., 2005). Cactaceae, Portulacaceae e Aizoaceae formam um clado dentro da ordem, baseado na presença de fitoferritina no floema parenquimático, metabolismo ácido das Crassuláceas

(CAM) e hábito suculento (Judd *et al*., 1999). Cactaceae é monofilética, sendo seu monofiletismo suportado por numerosas características morfológicas e uma inversão de 6 Kb no cpDNA (Judd *et al*., 2002). A família Cactaceae divide-se em quatro subfamílias: Maihuenoideae e Pereskioideae, que apresentam numerosos caracteres plesiomórficos, tanto vegetativos quanto reprodutivos; Opunthioideae, subfamília monofilética sustentada principalmente pela presença de tufos de espinhos curtos denominados gloquídeos nas aréolas, presença de sementes envolvidas por um arilo e caracteres de cpDNA; e Cactoideae, que é morfologicamente a mais complexa das subfamílias, e por isso menos sustentada por sinapomorfias, mas sempre possuindo espécies suculentas com minúsculas folhas vestigiais subentendendo cada aréola, ou completamente ausentes, e muitas das suas espécies apresentam costelas no caule. O monofiletismo de Cactoideae é suportado por uma redução extrema ou uma completa ausência de folhas e pela deleção do íntron *rpo*C1 do genoma do cloroplasto (Wallace, 1995; Judd *et al*., 2002). A subfamília Cactoideae apresenta o maior número de gêneros e espécies (cerca de 85% das espécies da família), subdividindo-se em 9 tribos – Echinocereae, Hylocereae, Cereae, Trichonocereae, Notocacteae, Rhipsalideae, Browningieae, Pachicereae e Cacteae – das quais Cereae e Trichonocereae são as mais representativas para o leste do Brasil (Hunt & Taylor, 1990).

Os cactos apresentam numerosas especializações para sobreviver em áreas secas, tais como caule diferenciado, geralmente fotossintetizante, possuem tecido de estoque de água, folhas modificadas em espinhos para diminuir a perda de água pela transpiração e metabolismo CAM. São plantas que possuem flores extremamente variáveis em cor e forma, sendo visitadas por diversos tipos de polinizadores (insetos, pássaros, morcegos) e geralmente são alogâmicas (Judd *et al*., 2002).

A família Cactaceae apresenta grande potencial econômico, principalmente no que se refere ao seu valor ornamental. Segundo Hollis (1997), as formas apresentadas pelos cactos fazem com que estes sejam muito requisitados como peças ornamentais. Além disto, propriedades alimentícias de seus frutos (Viñas *et al*., 1997) e cladódios (Taylor, 1991), assim como as diversas propriedades medicinais atribuídas a alguns membros da família, contribuem, em muito, para tal ênfase econômica (Taylor, 1991; Pimenta, 1997).

De acordo com Taylor (2000), alguns aspectos econômicos relacionados às Cactaceae podem levar a degradação ambiental e, em longo prazo, à extinção de espécies. Neste sentido, é dada atenção especial ao extrativismo de indivíduos da

natureza para fins comerciais, principalmente como ornamentais. Este tipo de coleta exploratória põe em risco alguns táxons, a exemplo de algumas espécies de *Melocactus* (e.g., *M. glaucescens* Buining & Brederoo, *M. paucispinus* G. Heimen & R. Paul, *M. conoideus* Buining & Brederoo, *M. deinacanthus* Buining & Brederoo) que apresentam distribuição restrita.

Diversas espécies de *Melocactus*, conhecidas popularmente como coroa-de-frade, são coletadas e comercializadas por várias comunidades. As consequências destas coletas em relação aos *Melocactus* são mais sérias do que em relação aos diversos outros gêneros de Cactaceae também comercializados, pois neste caso em particular, os indivíduos são sempre retirados inteiros da natureza por causa da impossibilidade de propagação por parte do cladódio. Uma vez que a coleta e a comercialização das espécies acontecem de forma indistinta, espécies que apresentam distribuição restrita, ocupando áreas muito reduzidas e com número de indivíduos também muito baixo, podem ser extintas através de uma única coleta (Taylor, 2000).

Devido ao forte extrativismo de alguns grupos vegetais dos campos rupestres, prática esta que favorece a extinção de espécies com distribuição restrita, como é o caso de algumas espécies de *Melocactus*, foi desenvolvido um projeto intitulado "Conservação e manejo de espécies de Eriocaulaceae, Orchidaceae e Cactaceae, da Chapada Diamantina ameaçadas de extinção", do qual este estudo é parte integrante. Este projeto envolveu estudos de dados biológicos e ecológicos de espécies das famílias Cactaceae (*Melocactus glaucescens* e *M. paucispinus*), Eriocaulaceae (*Syngonathus mucugensis* Giul. e *S. curralensis* Moldenke) e Orchidaceae (*Cattleya tenuis* Campacci & Vedovello e *Laelia sincorana* Schltr.), que são de fundamental importância para a elaboração de um plano de manejo detalhado para as espécies envolvidas.

O gênero *Melocactus* (L.) Link & Otto, compreende 32 espécies (Anderson, 2001) ocorrentes no sudeste e nordeste do Brasil, na região Amazônica, no norte do Caribe, oeste dos Andes e América Central. Embora apresente ampla distribuição, o centro de diversidade de *Melocactus* é o leste do Brasil, especialmente a Bahia, onde 18 das 22 espécies e sub-espécies reconhecidas para a área são endêmicas (Taylor, 1999). O gênero abarca plantas de hábito depresso-globoso, de caules altamente suculentos podendo ter mucilagem abundante ou ausente; a parte reprodutiva é diferenciada em um cefálio terminal de onde surgem flores tubulares, diurnas, de coloração indo desde o branco aos diferentes tons de rosa e vermelho, de síndrome ornitófila e frutos indeiscentes.

*Melocactus* pertence à tribo Cereae (subfamília Cactoideae), sendo o membro mais derivado desta, caracterizando-se pela produção de um cefálio terminal (Taylor & Zappi, 1989). Os cactos que produzem cefálio possuem duas fases de crescimento: uma juvenil e não reprodutiva e uma adulta que resulta numa mudança na aparência do indivíduo pela produção do cefálio, uma parte compactada do caule, formada por um adensamento de aréolas reprodutivamente ativas, de onde surgem as flores e os frutos (Taylor, 2000; Anderson, 2001).

O gênero *Melocactus* é monofilético, tendo como sinapomorfias, além do cefálio terminal, frutos delgados-clavados, pólen com perfurações tectais simples e sementes com poucas células-testa (Taylor & Zappi, 1989; Taylor, 1991; Taylor, 2000), sendo todas estas características únicas dentro da tribo Cereae. *Melocactus* (tribo Cereae) e *Discocactus* (tribo Trichonocereeae) são gêneros fortemente convergentes e por isso tradicionalmente associados; ambos apresentam indivíduos com pouco crescimento, e compartilham a presença do cefálio terminal e de frutos delgados (Taylor, 2000), no entanto estes gêneros diferem quanto ao nível de cerdas no cefálio (mais abundante em *Melocactus*), e caracteres florais (as flores de *Discocactus* são brancas e noturnas) e de frutos (os frutos de *Discocactus* são lateralmente deiscentes).

Ao contrário do que se conhece sobre o sistema reprodutivo de cactos (Boyle, 1997; Negron-Órtiz, 1998; Boyle & Idnurm, 2001), a maioria das espécies de *Melocactus* parece ser auto-compatível, apesar de poucos estudos terem sido realizados (Locatelli & Machado, 1999; Nassar & Ramírez, 2004; Colaço *et al*., submetido) e alguns ainda parecem ser cleistogâmicos – *M. lanssensianus* P.J. Braun (Taylor, 1991, 2000). O mesmo acontece com relação à citologia, onde nas poucas contagens cromossômicas feitas para Cactaceae há indícios que a maioria são diplóides, enquanto que alguns trabalhos apontam evidências de poliploidia para espécies de *Melocactus* (Das *et al*., 1998; Assis *et al*., 2003).

*Melocactus paucispinus* e *M. glaucescens* são espécies endêmicas da Bahia, que, devido a sua distribuição restrita e populações com reduzido número de plantas, estão na lista de espécies ameaçadas de extinção. Tanto *M. glaucescens* quanto *M. paucispinus* são espécies de áreas de campo rupestre (Taylor, 1991, 2000). Campo rupestre é muitas vezes descrito como um mosaico de diferentes tipos vegetacionais, refletindo uma mistura de diferentes topografias, substratos e microclimas, combinando elementos de caatinga e cerrado; ocorre nas regiões Nordeste e Sudeste do Brasil, principalmente nos estados de Bahia e Minas Gerais, caracterizando-se pela presença de

vegetação herbácea em solo arenoso-pedregoso e afloramentos rochosos de quartzito, arenito, gnaisse e canga de vegetação herbáceo-arbustiva (Joly, 1970; Giulietti *et al*., 1987; Giulietti & Pirani, 1988; Harley, 1995). A principal área de campo rupestre do Brasil é a cadeia do Espinhaço, a qual é formada por algumas serras, com duas áreas principais: a da Chapada Diamantina, na Bahia, e a segunda formada pela Serra do Cipó e pelo Planalto da Diamantina, no centro de Minas Gerais (Giulietti & Pirani, 1988). Devido à descontinuidade das cadeias de montanhas e dos afloramentos rochosos dos campos rupestres, muitas espécies, principalmente as rupícolas, estão distribuídas em populações disjuntas. Esta característica tem sido sugerida como um dos mais importantes fatores responsáveis pela grande diversidade e elevado endemismo desta formação (Joly, 1970; Giulietti & Pirani, 1988).

Na revisão feita por Taylor (1991) para o gênero *Melocactus*, eram conhecidas apenas cinco populações de *Melocacatus paucispinus*, com duas destas possuindo menos de 50 indivíduos. Segundo o autor, a espécie ocorria nos municípios de Seabra, Abaíra e Rio de Contas, possuindo uma extensão de ocorrência igual a 6585 $Km^2$, porém a área de ocupação era menor do que 500 $Km^2$. Recentemente esta distribuição foi ampliada, tendo sido descobertas cinco novas populações localizadas no município de Morro do Chapéu, e uma em Umburanas, formando uma pequena disjunção na distribuição da espécie. Apesar desta descoberta ser significativa, estas populações também não apresentam um número muito grande de indivíduos, o que faz com que a preocupação com sua coleta e comercialização permaneça. Como se trata de uma espécie rara, *M. paucispinus* está listada no Apêndice I do CITES (Convenção sobre Comércio Internacional de Espécies Ameaçadas), que torna proibida a comercialização internacional de plantas coletadas na natureza, independente da autorização do país de origem, desde junho de 1992 e possui o *status* de ameaçada de extinção na Lista Vermelha da IUCN – União Internacional para Conservação da Natureza (Taylor, 1997).

*Melocactus glaucescens* é uma espécie endêmica do município de Morro do Chapéu. Segundo Taylor (2000), a espécie apresenta distribuição extremamente restrita, sendo conhecida, até então, uma única população da espécie, com área de ocupação menor do que 10 $Km^2$, tendo sido proposta a criação de uma reserva como forma de preservá-la. O recente levantamento das populações de *Melocactus* feito para o projeto de manejo e conservação permitiu a descoberta de três novas populações de *M. glaucescens*. Estas são disjuntas, estando todas localizadas em Morro do Chapéu, o que

mantém seu *status* de espécie endêmica do município. São populações pequenas, também com um número reduzido de indivíduos, mas com diferenças morfológicas acentuadas entre si, quanto à forma do cladódio, dimensões de espinhos e coloração de frutos. Devido ao seu cefálio surpreendentemente branco, suas pequenas flores lilás-magenta e seus frutos vermelhos, esta espécie é uma das mais distintas dentro do gênero (Taylor, 1991). *Melocactus glaucescens* também figura no Apêndice I da CITES desde junho de 1992, e é considerada como criticamente ameaçada na Lista Vermelha da IUCN (Taylor, 1997).

Em uma das populações de *M. glaucescens* conhecidas, esta ocorre simpatricamente com outra espécie congenérica, *M. ernestii* Vaupel, hibridizando naturalmente. Os indivíduos oriundos destes cruzamentos são reconhecidos atualmente como *M.* ×*albicephalus*. Nesta área de hibridação, é possível reconhecer dois morfos distintos entre os híbridos, havendo indícios de hibridação introgressiva. Taylor (1991, 2000) em sua revisão para o gênero, relata ainda a existência de uma zona híbrida entre *M. glaucescens* e outra espécie, *M. concinnus* Buining & Brederoo, não havendo nesta área mais nenhum indivíduo puro de *M. glaucescens*.

Para *M. paucispinus* também foram encontrados alguns indivíduos em uma das populações recentemente descobertas no município de Morro do Chapéu, que apresentam características morfológicas intermediárias entre esta espécie e *M. concinnus*, além de ter sido citada por Taylor (2000) a existência de uma população da espécie no município de Piatã (BA), na qual estão presentes plantas que demonstram possível introgressão com este mesmo táxon.

O processo de hibridação é freqüente em plantas, sendo estimado quase 70 mil plantas híbridas interespecíficas ocorrendo na natureza (Stace, 1984). Hibridação é algo comum em cactos, podendo ocorrer até entre espécies de diferentes gêneros (Rowley, 1994; Boyle & Idnurm, 2003). Alguns gêneros dentro de Cactaceae são de origem híbrida como ×*Haagespostoa*, ×*Hyrtgerocactus* e ×*Pacherocactus* (Anderson, 2001). De acordo com Taylor (2000) estudos com Cactaceae indicam que taxas relacionados, e especialmente taxas irmãos, raramente são simpátricos sugerindo que especiação na família ocorre especialmente por alopatria. Espécies congenéricas quando simpátricas tendem a hibridizar, como ocorre com espécies de *Opuntia* (Grant & Grant, 1979), *Arrojadoa* (Taylor & Zappi, 2004), *Cipocereus* (Taylor & Zappi, 2004) e *Melocactus* (Taylor, 1991, 2000; Taylor & Zappi, 2004).

De acordo com Rhymer & Simberloff (1996), hibridação é o intercruzamento de indivíduos de populações distintas geneticamente, enquanto introgressão compreende o fluxo gênico entre populações de indivíduos híbridos com uma ou ambas as espécies parentais. Rieseberg (1995) expõe que para que haja avanços evolutivos, um alto grau de variabilidade genética é requerido. Por causa da baixa taxa de mutação, argumenta-se que a recombinação genética, maximizada por processos de hibridação, é provavelmente a maior fonte de variação. De fato, uma das conseqüências do processo de hibridação é o aumento da diversidade biológica, à medida que esta permite a evolução de novas espécies. No entanto, outra conseqüência advinda deste processo é justamente o contrário, quando a hibridação provoca a destruição da biodiversidade, ao permitir a fusão de duas espécies diferentes através do fluxo gênico interespecífico.

Para Rhymer & Simberloff (1996), uma espécie pode ser extinta por hibridação ou introgressão com outras, principalmente se esta ocorrer simpatricamente com espécies congenéricas. Isto é particularmente importante se estivermos tratando de espécies raras ou se o número de indivíduos de uma população for muito pequeno. Neste último caso, é possível que a população desapareça totalmente devido a processos de introgressão, podendo-se eliminar o genoma de muitos indivíduos e permitir uma "reconstrução" parcial das espécies. Em casos onde introgressão ocorre por várias gerações em uma determinada população, pode haver a formação de zonas híbridas, podendo nestas ocorrer o desaparecimento dos taxa parentais e a substituição destes por híbridos. Para *M. glaucescens*, Taylor & Zappi (2004) relatam a suposta formação de zona híbrida em uma das populações conhecidas, na qual já não se tem mais indivíduos puros de *M. glaucescens*.

Observações morfológicas podem sugerir que hibridação ou introgressão tenham ocorrido, mas na grande maioria das vezes, a identificação de híbridos apenas por características morfológicas é difícil, particularmente depois de algumas gerações de retrocruzamento. O advento de técnicas moleculares foi um passo importante para averiguar a existência e extensão destes problemas. Análises de genética molecular podem permitir o conhecimento, não só do grau de hibridação e introgressão entre populações, como também do grau de indivíduos hibridizados por população (Rhymer & Simberloff, 1996).

Entre as técnicas moleculares utilizadas para estudo de variabilidade genética e hibridação, a eletroforese de isoenzimas tem demonstrado bons resultados em diversos trabalhos para diferentes espécies de cactos (Parker & Hamrick, 1992; Neel *et al*., 1996;

Colunga-GarcíaMarín *et al.*, 1999; Nassar, 1999; Nassar *et al*., 2001, 2002, 2003; O´Leary & Boyle, 2000; Hamrick *et al*., 2002; Clark-Tapia & Molina-Freaner, 2003; Moraes *et al*., 2005; Machado *et al*., submetido). De acordo com Crawford (1989), dados obtidos através de eletroforese de isoenzimas diferem fundamentalmente de outras informações rotineiramente empregadas em sistemática de plantas, pois os padrões de bandas formados nos géis são produzidos por enzimas específicas, podendo ser interpretados em termos genéticos, e permitindo a quantificação da similaridade e diferença entre populações, grupos de populações, espécies, etc.

Objetivando averiguar a real viabilidade e atual situação das espécies de *Melocactus* em questão, foi proposto um estudo de variabilidade genética baseado na técnica de eletroforese de isoenzimas, a partir do qual se pretendeu determinar a variabilidade genética intra-populacional e intra-específica, assim como a partição desta variabilidade entre populações co-específicas e o grau de endogamia nas populações das espécies em estudo. Foi também realizada uma análise morfométrica multivariada das espécies de *M. glaucescens* e *M. paucispinus*, na qual se pretendeu determinar a variabilidade morfológica, também intra-populacional e intra-específica, assim como a partição desta entre populações co-específicas, e a correlação entre estes dois marcadores (genético e morfológico). Tais dados deram suporte também para analisar as inferências sobre hibridação levantadas através das análises de campo e em literatura. Os resultados obtidos aqui constituíram importante recurso para compor o plano de manejo e conservação destas espécies. Neste sentido, a dissertação ora apresentada foi organizada em dois capítulos, em forma de artigos. O primeiro trazendo os dados de análises genética e morfológica para *M. paucispinus* e o segundo para *M. glaucescens*.

**CAPÍTULO 1**

# Allozyme diversity and morphometrics of *Melocactus paucispinus* (Cactaceae) and evidence for hybridization with *M. concinnus* in the Chapada Diamantina, Northeastern Brazil

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## RESUMO

Foram investigados os níveis de variabilidade genética e morfológica em 10 populações e 256 indivíduos de *Melocactus paucispinus*, a sub-estruturação destas dentro e entre populações e a possível ocorrência de hibridação natural com *M. concinnus* (três populações, 64 indivíduos), usando dados isoenzimáticos e morfométricos. Entre as populações de *M. concinnus*, uma delas (CM03) apresenta indivíduos morfologicamente intermediários entre as duas espécies.

A diversidade de isoenzimas em 12 loci revela baixa variabilidade genética para ambas as espécies, sendo menores que as reportadas para outras espécies do gênero (Nassar *et al*., 2001), assim como para outras espécies de Cactaceae (Parker & Hamrick, 1992; Hamrick *et al*., 2002; Nassar *et al*., 2002, 2003; Clark-Tapia & Molina-Freaner, 2003; Moraes *et al*., 2005; Machado *et al*., submetido) e para diversas espécies vegetais com características similares (Hamrick & Godt, 1992; Colunga-GarcíaMarín *et al.*, 1999; Martínez-Palacios *et al*., 1999). A baixa variabilidade apresentada pelas espécies de *Melocactus* em estudo pode estar associada a um recente efeito gargalo sofrido pelas populações, aliado à exploração destas por coletores e comerciantes, o que afeta negativamente sua diversidade.

Foram encontrados deficit de heterozigotos em praticamente todos os loci em pelo menos uma das populações, sendo refletido nos elevados valores de $F_{IS}$ encontrados, possivelmente resultante de endogamia ou sub-estruturação das populações, ambos diretamente relacionados ao sistema de polinização e dispersão nas espécies.

Os níveis de variabilidade morfológica analisados por meio de 17 caracteres morfométricos também são baixos para ambas as espécies, sendo estes menores que os apresentados para outras espécies de cactos (*Discocatus* spp. – Machado *et al*., submetido). As maiores variabilidades foram encontradas para a população de *M. paucispinus* de Seabra (população tipo) e para as três populações de *M. concinnus*. Os caracteres comprimento e largura do caule, altura das costelas, diâmetro do espinho radial e comprimento e largura do cefálio foram os caracteres mais variáveis, enquanto que o número de espinhos radiais foi o caracter com menor variação para ambas as espécies.

Tanto *M. paucispinus* quanto *M. concinnus* apresentam altos níveis de estruturação genética ($F_{ST}$) e morfológica ($A$). Para *M. paucispinus*, análise hierárquica

mostra que estruturação genética é maior em escala local que entre populações de diferentes áreas, podendo ser resultante do limitado fluxo gênico entre populações, combinado com ação de seleção natural. As populações de *M. paucispinus* de Delfino e Seabra apresentaram as maiores diferenciações morfológicas.

Análises de agrupamento por dados genéticos e morfológicos mostram que a população de *M. paucispinus* de Delfino é mais próxima das populações de *M. concinnus* podendo se tratar de um morfo de *M. concinnus* com características convergentes com *M. paucispinus* ou mesmo indivíduos resultantes de forte hibridação e/ou introgressão entre essas duas espécies. Em termos genéticos, tal agrupamento se dá pela alta freqüência do alelo 94 do loco da DIA-2; morfometricamente, as características que mais contribuem para tal agrupamento são a largura do caule, a altura e largura das costelas na região mediana e o número de aréolas por costela.

A população de *M. concinnus* com características híbridas (CM03) é geneticamente mais próxima de *M. paucispinus*, isto pela inversão na freqüência relativa do alelo da DIA-2 em relação as populações da sua espécie, sendo este um forte indício de hibridação. Como tal população, no entanto, apresenta monomorfismo para todos os locos, o agrupamento desta população com *M. paucispinus* pode ser meramente coincidente, uma vez que a freqüência do alelo da DIA-2 pode também estar associado a fixação deste alelo por ação de deriva genética agindo sobre a população. As duas explicações, no entanto, não são mutuamente excludentes, sendo plausível que hibridação e deriva genética tenham agido para produzir este cenário. *M. concinnus* também apresentou elevada estruturação morfológica a qual não pode ser explicada por sub-estruturação geográfica, mas somente por hibridação e/ou introgressão.

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# Allozyme Diversity and Morphometrics of *Melocactus paucispinus* (Cactaceae) and Evidence for Hybridization with *M. concinnus* in the Chapada Diamantina, North-eastern Brazil

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• *Background and Aims Melocacatus paucispinus* (Cactaceae) is endemic to the state of Bahia, Brazil, and due to its rarity and desirability to collectors it has been considered threatened with extinction. This species is usually sympatric and inter-fertile with *M. concinnus*, and morphological evidence for hybridization between them is present in some populations. Levels of genetic and morphological variation and sub-structuring in populations of these species were assessed and an attempt was made to verify the occurrence of natural hybridization between them.

• *Methods* Genetic variability was surveyed using allozymes (12 loci) and morphological variability using multivariate morphometric analyses (17 vegetative characters) in ten populations of *M. paucispinus* and three of *M. concinnus* occurring in the Chapada Diamantina, Bahia.

• *Key Results* Genetic variability was low in both species ($P$ = 0·0–33·3, $A$ = 1·0–1·6, $H_e$ = 0·000–0·123 in *M. paucispinus*; $P$ = 0·0–25·0, $A$ = 1·0–1·4, $H_e$ = 0·000–0·104 in *M. concinnus*). Deficit of heterozygotes within the populations was detected in both species, with high values of $F_{IS}$ (0·732 and 0·901 in *M. paucispinus* and *M. concinnus*, respectively). Evidence of hybridization was detected by the relative allele frequency in the two diaphorase loci. High levels of genetic ($F_{ST}$ = 0·504 in *M. paucispinus* and 0·349 in *M. concinnus*) and morphological ($A$ = 0·20 in *M. paucispinus* and 0·17 in *M. concinnus*) structuring among populations were found.

• *Conclusions* The *Melocactus* spp. displayed levels of genetic variability lower than the values reported for other cactus species. The evidence indicates the occurrence of introgression in both species at two sites. The high $F_{ST}$ values cannot be explained by geographical substructuring, but are consistent with hybridization. Conversely, morphological differentiation in *M. paucispinus*, but not in *M. concinnus*, is probably due to isolation by distance.

**Key words:** Allozymes, Cactaceae, campo rupestre, Chapada Diamantina, genetic diversity, *Melocactus concinnus*, *Melocactus paucispinus*, morphological variability, morphometrics.

## INTRODUCTION

The genus *Melocactus* consists of 36 species (Anderson, 2001), being common in arid and semi-arid regions of tropical and subtropical zones of the western hemisphere. Although it has a wide distribution, the greatest concentration of taxa and the centre of diversity lie in eastern Brazil, especially in the state of Bahia. Taylor and Zappi (2004) recognized 22 species and subspecies in eastern Brazil, of which 18 are endemic. Plants in the genus are characterized by a small, globose to slightly elongated, unbranched stem, the fertile part differentiated into a terminal cephalium. Flowers are diurnal, small, and embedded within the cephalium with only the perianth segments visible. According to Taylor (1991), most species are self-compatible, but floral adaptations promote hummingbird-mediated cross-pollination. The fruits are small turbinate berries, with small black seeds embedded in a watery pulp; the seeds are locally dispersed by lizards (Taylor, 1991; Fonseca, 2004; Taylor and Zappi, 2004).

*Melocactus* spp. are collected and sold by local communities because of their ornamental value. Since this occurs indiscriminately, those species with a more restricted distribution, occupying specific areas and having a small number of individuals in the populations, are at risk of becoming extinct as a result of a single collection (Taylor and Zappi, 2004).

*Melocacatus paucispinus* is endemic to Bahia, and due to its rarity and desirability to collectors, the species has been listed on Appendix I of CITES. It has been listed as Endangered in the IUCN Red List of Threatened Species (IUCN, 2004) due to its erratic distribution and generally small population sizes. In the revision of the genus by Taylor (1991) only five populations were known for *M. paucispinus*, two of these possessing less than 50 individuals. The species was known to occur in the

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FIG. 1. (A) *Melocactus paucispinus*, an individual from Seabra population. (B) *M. concinnus*, an individual from Morro do Chapéu population. (C–D) *M. concinnus*, individuals from CM03 population in Morro do Chapéu (possible introgressants or hybrids with *M. paucispinus*).

municipalities of Seabra, Rio de Contas and Abaíra (adjoining to Rio de Contas), with an area of occurrence of 6585 $km^2$, but with an area occupied smaller than 500 $km^2$. More recently the species has been found further north than the previously known populations, in the municipality of Morro do Chapéu (Machado, 1999; Taylor and Zappi, 2004), where it occurs as several small populations, and again further north in Delfino, in the municipality of Umburanas (Machado and Charles, 2004). *Melocactus concinnus* occurs in the states of Bahia and Minas Gerais, and is widespread geographically.

The distribution of both species is fragmented, with isolated and often widely disjunct populations. Both are elements of the campo rupestre vegetation of the Cadeia do Espinhaço mountain range (Taylor, 1991). Campo rupestre is a vegetation type that occurs in mountainous areas of the north-eastern and south-eastern regions of Brazil, mainly in Bahia and Minas Gerais. It is characterized by open, herbaceous vegetation on sandy, stony soils mixed with herbs and shrubs growing on outcropping islands of quartzite, sandstone, gneiss or 'canga' rocks (Giulietti and Pirani, 1988; Borba and Semir, 1998). Because of the discontinuity of these mountain ranges and outcrops, many species, especially the rupicolous ones, are distributed in disjunct populations. It has been suggested that this characteristic is responsible for the differentiation of plant populations, leading to the great diversity and high levels of endemism in the campo rupestre vegetation, one of the highest among the vegetation types of Brazil (Joly, 1970; Giulietti and Pirani, 1988; Harley, 1988; Borba *et al.*, 2001; Jesus *et al.*, 2001).

*Melocactus paucispinus* and *M. concinnus* (Fig. 1) may be distinguished by their habit, depressed in *M. paucispinus* and globose in *M. concinnus*, by the presence of both glaucousness in the epidermis and a central spine in *M. concinnus* (never present in *M. paucispinus*), and by a small depression between the areoles on the ribs, also present only in *M. concinnus*. These two species are sympatric and inter-fertile (M. A. S. Colaço *et al.*, unpubl. data). Morphological evidence for hybridization between them is present in some of the populations (Fig. 1), already reported by Taylor (1991) and Taylor and Zappi (2004). According to Taylor (1991), *M. concinnus* is supposed to be the most promiscuous *Melocactus* sp., frequently hybridizing when sympatric with other species, and usually generates hybrid swarms, e.g. with *M. glaucescens*, *M. zehntneri* and *M. oreas*. However, such statements are conjectural, arising from observation of morphologically intermediate individuals in the field.

Hybridization is frequent in plants, with approx. 70 000 distinct interspecific hybrids having been estimated in nature (Stace, 1984), and it has been considered one of the main problems in conservation (Ellstrand, 1992). According to Barton (2001), in the wide scale, hybridization has been very important for evolution, being suggested as one of the processes that contributes to genetic recombination,

TABLE 1. *Populations of* Melocactus paucispinus *and* M. concinnus *occurring in the Chapada Diamantina, Bahia, Brazil, used in this study*

| Species | Name | *N** | Municipality | Location | |
|---|---|---|---|---|---|
| *M. paucispinus* | PM01 | 28 | Morro do Chapéu | Morrão | 11°33′51·4″S, 41°10′38·1″W |
| | PM02 | 22 | Morro do Chapéu | Tabuleiro dos Tigres | 11°36′02·6″S, 41°09′53·9″W |
| | PM03 | 26 | Morro do Chapéu | Fazenda 2 Irmãs | 11°33′35·2″S, 41°17′50·3″W |
| | PM04 | 24 | Morro do Chapéu | Margens do rio Jacuipe | 11°33′34·1″S, 41°07′35·7″W |
| | PM05 | 26 | Morro do Chapéu | Cachoeira do Ferro Doido | 11°36′56·7″S, 41°00′20·7″W |
| | PD01 | 25 | Umburanas | Delfino | 10°21′58·7″S, 41°11′54·4″W |
| | PR01 | 29 | Rio de Contas | Campo dos Gerais | 13°29′23·2″S, 41°57′18·7″W |
| | PR02 | 21 | Rio de Contas | Campo do Alto da Cruz | 13°25′28·7″S, 41°54′40·4″W |
| | PR03 | 25 | Rio de Contas | Brumadinho | 13°31′00·3″S, 41°54′30·4″W |
| | PS01 | 30 | Seabra | Palmeira dos Mendes | 12°25′40·4″S, 41°59′45·3″W |
| *M. concinnus* | CM01 | 24 | Morro do Chapéu | Orchidário | – |
| | CM02 | 20 | Morro do Chapéu | Barrigudas | 11°30′23·0″S, 41°18′17·5″W |
| | CM03 | 20 | Morro do Chapéu | Morrão | 11°34′10·9″S, 41°10′36·6″W |

Vouchers are deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS).
* $N$ = number of individuals sampled.

increasing the levels of variability existing in nature (Stebbins, 1959; Rieseberg, 1995) and consequently acting as an important resource for adaptation and speciation (Lewontin and Birch, 1966; Arnold, 1996). Nevertheless, hybridization can impoverish biodiversity by allowing the fusion of two different species via interspecific gene flow, which Rhymer and Simberloff (1996) considered to be maximized if the species are sympatric and congeneric. Hybridization may promote the extinction of species by inhibiting the population growth and negatively affecting effective reproduction, competitive status and ecological interactions (Levin *et al.*, 1996), which are particularly important in rare species (Rhymer and Simberloff, 1996). In some cases, hybridization is associated with introgression, which may lead to the complete disappearance of populations through formation of hybrid swarms, the parental species being substituted by hybrids (Forbes and Allendorf, 1991). Taylor and Zappi (2004) reported the occurrence of a hybrid swarm between two *Melocactus* spp. (*M. glaucescens* and *M. concinnus*), in which there were no pure individuals of *M. glaucescens*.

The objective of this study was to assess levels of genetic and morphological variation and sub-structuring within and between populations of *M. paucispinus*, and to verify the occurrence of natural hybridization with *M. concinnus*, using allozyme markers and morphometric data. This study is part of a project for the conservation and management of species of Cactacteae and other endangered plant groups in the Chapada Diamantina, Bahia, involving studies of demography, biology, variability, propagation and ethnobotany of the plants; the results obtained here will help in the determination of actions and priority areas for conservation of the species.

## MATERIALS AND METHODS

### *Populations sampled*

Samples were taken from ten populations of *Melocactus paucispinus* (256 individuals) and from three populations of *M. concinnus* (64 individuals) (Table 1; Fig. 2). Geographic distances between the Morro do Chapéu populations range from 5·5 to 32 km, and those between the Rio de Contas populations range from 5 to 10 km. The complete matrix of geographical distances can be obtained from the first author on request. All individuals sampled were mature, as evidenced by the presence of a well-developed cephalium. Vouchers for each species are deposited at the herbarium of the Universidade Estadual de Feira de Santana (HUEFS) (*M. paucispinus–S. M. Lambert et al. 01, S. M. Lambert et al. 03*; *M. concinnus–S. M. Lambert et al. 02*).

### *Electrophoretic procedures*

Small sections of stem tissue were crushed in 0·5 mL of grinding buffer [100 mL Tris–HCl 0·1 mol $L^{-1}$ pH 7·0, 6·846 g sucrose, 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 and Ganders, 1990]. Extracts were absorbed in 1·0 × 0·3 cm Whatman number 3 paper wicks, which were loaded into 8·5 % starch gels (Sigma hydrolyzed potato starch).

For the electrodes and gels, four buffer systems were used: (1) electrode: histidine 0·065 mol $L^{-1}$ 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 $L^{-1}$, boric acid 0·0935 mol $L^{-1}$, EDTA 0·0059 mol $L^{-1}$, pH 8·0; gel: electrode solution diluted 1 : 10; modified from Ridgway *et al.* (1970); (3) electrode: citric acid 0·04 M adjusted to pH 6·1 with N-(3-aminopropyl)-morpholine; gel: electrode solution diluted 1 : 20; modified from Clayton and Tretiak (1972); and (4) electrode: boric acid 0·3 mol $L^{-1}$, NaOH 0·06 mol $L^{-1}$, pH 8·0; gel: Tris 0·01 mol $L^{-1}$, pH 8·5; modified from Shaw and Prasad (1970).

Standard horizontal electrophoresis was performed until the inner marker (bromophenol blue) reached 9 cm from the application site using the following running conditions: system 1: 150 V; systems 2, 3 and 4: 25 mA. Nine enzymatic systems gave enough resolution for reading


FIG. 2. Map of the state of Bahia, north-eastern Brazil, showing the localities of the populations of *Melocactus paucispinus* (circles) and *M. concinnus* (squares).

and were used. System 1 was used for malate dehydrogenase locus 1 (MDH; EC 1.1.1.37); system 2 was used for MDH locus 2, phosphoglucomutase (PGM; EC 2.7.5.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), and shikimate dehydrogenase (SKDH; EC: 1.1.1.25); system 3 was used for diaphorase (DIA; EC 1.8.1.4); and system 4 was used for acid phosphatase (ACPH; EC 3.1.3.2), leucine aminopeptidase (LAP; EC 3.4.11.1), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and phosphoglucose isomerase (PGI; EC. 5.3.1.9). The staining procedures were similar to but slightly adjusted from Brune *et al.* (1998; ACPH, LAP, DIA, SKDH, G6PDH), 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 ascending order from the locus with lowest mobility. The alleles were numbered according their mobility relative to the allele with the highest mobility of a standard individual present in all gels and designated as 100.

*Analysis of allozyme data*

The allele 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

TABLE 2. *Morphological characters used in the morphometric analysis of ten populations of* M. paucispinus *and three populations of* M. concinnus *occurring in the Chapada Diamantina, Brazil*

| Characters | *M. paucispinus* | *M. concinnus* |
|---|---|---|
| Stems | | |
| 01. Length (mm) | 69·21 ± 14·27 (21·00–133·10) | 84·07 ± 14·65 (34·10–107·30) |
| 02. Width (mm) | 155·41 ± 20·15 (16·80–201·10) | 129·96 ± 9·90 (84·10–146·90) |
| Ribs | | |
| 03. Number | 9·64 ± 0·77 (8·0–15·0) | 8·52 ± 0·71 (8·0–11·0) |
| 04. Height (mm) | 15·36 ± 8·44 (0·40–39·30) | 24·70 ± 8·56 (9·60–42·30) |
| 05. Width at 1/4 (mm)* | 43·46 ± 5·79 (27·60–61·40) | 46·40 ± 5·34 (30·0–56·0) |
| 06. Width at 2/4 (mm) | 37·16 ± 4·94 (22·10–50·40) | 37·09 ± 5·58 (19·30–50·30) |
| 07. Width at 3/4 (mm) | 29·99 ± 4·85 (5·0–46·20) | 26·10 ± 4·75 (14·80–35·90) |
| 08. Number of areoles | 5.33 ± 0,75 (3·0–9·0) | 6·66 ± 0·74 (5·0–8·0) |
| Spines (mean of four areoles of different ribs) | | |
| 09. Lower number of radials | 4·21 ± 0·90 (3·0–6·0) | 5·98 ± 0·99 (5·0–7·0) |
| 10. Higher number of radials | 4·52 ± 0·75 (3·0–6·0) | 6·42 ± 0·91 (5·0–7·0) |
| 11. Length of right radial (mm) | 20·26 ± 3·30 (11·36–28·65) | 20·60 ± 2·40 (16·88–26·98) |
| 12. Length of lower radial (mm) | 22·36 ± 3·61 (12·45–33·28) | 22·91 ± 3·38 (15·65–30·30) |
| 13. Length of left radial (mm) | 20·52 ± 4·70 (12·78–60·60) | 21·04 ± 2·45 (16·83–28·20) |
| 14. Diameter of the lower radial (mm) | 1·77 ± 0·99 (1·0–17·0) | 1·43 ± 1·39 (1·0–12·20) |
| 15. Angle between right and left radials | 166·32 ± 14·28 (111·0–182·0) | 160·41 ± 15·73 (130·0–188·0) |
| Cephalium | | |
| 16. Length (mm) | 32·18 ± 15·00 (4·90–78·20) | 33·97 ± 16·40 (8·70–68·10) |
| 17. Width (mm) | 70·75 ± 10·41 (18·60–97·30) | 70·87 ± 16·06 (29·40–102·50) |

Values shown are means ± s.d., with minimum and maximum recorded values in brackets.
*Width of ribs at one-, two- and three-quarters along their length.

expected ($H_e$) mean heterozygosity per locus. Deviations from the expected mean heterozygosity under Hardy–Weinberg (HW) equilibrium were tested using $\chi^2$ with a correction for small samples according to Levene (1949). A test for linkage disequilibrium was performed using GENEPOP software (Raymond and Rousset, 1995). Default program settings were used: 100 batches of 1000 iterations per batch with 1000 dememorization steps. As multiple tests enhance type 1 errors, the sequential Bonferroni procedure (Rice, 1989) was applied to each population.

Partitioning of genetic diversity among conspecific populations was estimated by $F$ statistics ($F_{IS}$, the inbreeding coefficient, measures the reduction in heterozygosity due to non-random mating within a population; $F_{ST}$ measures the differentiation among populations; Wright, 1978). Two large regions (Morro do Chapéu and Rio de Contas) were sampled for *M. paucispinus*, which gave an opportunity to test if this species exhibits hierarchical partitioning of genetic variability and if it conforms to the expectations of the 'stepping stone' model, where only adjacent populations exchange genes (Kimura and Weiss, 1964). A hierarchical analysis of $F$ was carried out within and among major sampling regions (Morro do Chapéu and Rio de Contas): $F_{SR}$, variation among populations within regions; $F_{RT}$, total variation among regions; $F_{ST}$, total variation among the eight populations. Values of fixation indexes and their respective components of variance ($\sigma^2$) were calculated following Wright (1978).

Gene flow ($N_m$) among populations was estimated indirectly from the population genetic structure using Wright's (1951) equation as modified by Crow and Aoki (1984): $F_{ST} = 1/(1 + 4N_m\alpha)$, where $\alpha = [n/(n-1)]^2$, where $n$ is the number of populations analysed for each species. This formula establishes two properties of $F_{ST}$ for neutral alleles: it is nearly independent of both mutation rate and the number of the demes (Slatkin and Barton, 1989). A second indirect estimate of gene flow was made based on the mean frequency of alleles found exclusively in single populations ('private alleles', Barton and Slatkin, 1986), with correction for sample size. The basic rationale underlying the latter method is that private alleles are likely to show a high frequency only when $N_m$ is low. $N_m$(W) refers to Wright's gene flow estimate, and $N_m$(S) to Slatkin's estimate.

Matrices of genetic distances (Nei's unbiased genetic distance; 1978) and genetic identities (Nei's unbiased genetic identity; 1978) were calculated for populations and species. Cluster analysis was performed with the genetic distance matrix of the populations with UPGMA as grouping algorithm (Sneath and Sokal, 1973). All analyses were made using the BIOSYS 1.0 software package (Swofford and Selander, 1989), except for the cluster analysis, which was performed in the software package STATISTICA for Windows, Release 5.5 A (StatSoft, 2000), and the linkage disequilibrium and $N_m$(S) analyses, both performed with GENEPOP software (Raymond and Rousset, 1995).

### *Morphometric analysis*

The individuals sampled for the allozyme analysis were also used in an analysis of morphological variability, in which 17 vegetative morphological characters were used (Table 2). The characters were chosen based on both previous fieldwork and the literature regarding the taxonomy of the group and morphometrics of cacti species (Backer and Pinkava, 1987; Chamberland, 1997; Casas *et al.*, 1999; Backer and Johnson, 2000; Thomson, 2002). All measurements of continuous quantitative characters were taken

with the aid of a vernier caliper. The values for the spine characters represent an average of the measurements of four areoles located on different ribs, the measurements always being made on the fourth areole along the rib from the apex of the plant.

Patterns of morphological similarity/difference were analysed by multivariate statistical methods using the software package STATISTICA for Windows, Release 5.5A (StatSoft, 2000). The analyses included 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. CVA was performed with the population as the categorical variable (individuals were grouped according to the population to which they belonged). The standardized coefficients for canonical variables resulting from CVA were used to identify the characteristics that contribute most significantly to the resulting patterns observed. The morphological matrix was analysed using discriminant analysis with population as the grouping variable in order to obtain a matrix of squared Mahalanobis distances of individuals to the centroid of the group (D2); the morphological variability of populations was calculated as the median of these distances ($D2_m$) (Goldman *et al.*, 2004). We used the median of the squared Mahalanobis distances instead of an average of these distances because of the non-normal distribution of the data. The non-parametric test of Kruskal–Wallis was applied to verify the occurrence of significant differences between medians of conspecific populations. Cluster analysis was carried out on a matrix of morphological distance among populations calculated using Mahalanobis Generalised Distance as the distance coefficient, and UPGMA was used as the clustering algorithm (Sneath and Sokal, 1973).

A multi-response permutation procedure (MRPP) analysis made with the PC-ORD 4.10 program (McCune and 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 ($F_{ST}$) (Borba *et al.*, 2002). The average Euclidian distance (ED) between the individuals of each population resulting from the MRPP analysis was also utilized as a measure of variability within populations (Borba *et al.*, 2002). The two indices of morphological variability are essentially different, as $D2_m$ is more affected by form and ED is more affected by size of the characters.

### *Correlation analyses*

For *M. paucispinus*, the matrix of squared Mahalanobis distances between populations was compared with the matrix of genetic distances (Nei, 1978), and both were also compared with the matrix of geographical distances between populations, using Mantel tests with the randomization (Monte Carlo) method (1000 randomizations) in the PC-ORD 4.10 program (McCune and Mefford, 1999), in order to test for significant correlations between morphological, genetic and geographic distances. This procedure was not used for *M. concinnus* due to the low number of populations sampled for this species. The pair-wise geographical distances between the populations were computed with geodetic distances on WGS84 earth ellipsoid calculated using the INVERSE 2.0 program (National Geodetic Survey, 2002). A Spearman rank correlation analysis between the morphological (ED and $D2_m$) and genetic ($H_e$) variability of populations was also carried out using the software package STATISTICA for Windows, Release 5.5A (StatSoft, 2000).

## RESULTS

### *Intra-populational variability*

Using nine enzymatic systems, 12 loci were obtained with good resolution and were used in this study. One locus was monomorphic for all populations studied (IDH). The remaining loci displayed a low degree of polymorphism, with 66·6 % of the loci only having two alleles per locus. SKDH was the most polymorphic locus, with four alleles (Table 3).

Some alleles were exclusive to a one species: PGM-1 90, PGM-2 88, PGI 113, LAP 107, SKDH 109, SKDH 120, G6PD 118, MDH-2 111 and DIA-1 80 to *M. paucispinus*; SKDH 85, MDH-I 119, and MDH-2 80 to *M. concinnus*. A few of these alleles were exclusive to single populations: PGM-1 90, PGM-2 88, SKDH 109, SKDH 120 and DIA-1 80 (PS01), MDH-2 111 (PD01) and MDH-1 119 (CM02). However, neither of the species was fixed for alternative alleles at any locus, and thus no locus was diagnostic for either species (Table 3).

The percentage of polymorphic loci ($P$; 0·95 criterion) ranged from 0·0–33·3 %, the mean number of alleles per locus was between 1·0 and 1·6, and mean heterozygosity ($H_e$) ranged from 0·0 to 0·123 (Table 4). The populations PM05, PR01 and CM03 did not have any polymorphic loci. The populations PS01 and CM01 possessed the greatest genetic variability.

Of the 13 populations, ten showed significant deviations from the expected values in HW equilibrium for at least one locus; six of these did not have any locus in equilibrium (PM02, PM04, PM06, PR02, PR03 and PS1). Of the 11 polymorphic loci, ten were not in HW equilibrium in at least one population (except MDH-1) and six were not in HW equilibrium in any population in which they were polymorphic (PGM-1, PGM-2, LAP, G6PD, DIA-1, DIA-2). The reason for disequilibrium was a deficit of heterozygotes in all loci except for SKDH, ACPH, MDH-1 and MDH-2 in one population each. The high positive values for $F_{IS}$ (Table 5) reflect the deficit of heterozygotes in the populations.

After Bonferroni correction, populations PM01 and PS01 presented significant associations between loci LAP/G6PD ($P = 0{\cdot}006$; critical $\alpha = 0{\cdot}008$ for six tests) and DIA-1/DIA-2 ($P = 0{\cdot}00001$; $\alpha = 0{\cdot}005$ for ten tests), respectively. None of the ten linkage disequilibrium tests in *M. concinnus* were significant after the correction ($\alpha = 0{\cdot}005$).

In both morphological analyses (discriminant analysis and MRPP), the population with the highest variability

TABLE 3. *Allele frequencies at 12 allozymic loci in ten populations of* M. paucispinus *and three populations of* M. concinnus *occurring in the Chapada Diamantina, Brazil*

| | | *Melocactus paucispinus* | | | | | | | | | | *Melocactus concinnus* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Allele* | PM01 | PM02 | PM03 | PM04 | PM05 | PD01 | PR01 | PR02 | PR03 | PS01 | CM01 | CM02 | CM03 |
| PGM-1 | 90 | – | – | – | – | – | – | – | – | – | 0·231 | – | – | – |
| | 100 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0·769 | 1 | 1 | 1 |
| | *N* | *24* | *18* | *18* | *21* | *26* | *24* | *23* | *15* | *24* | *26* | *22* | *20* | *20* |
| PGM-2 | 88 | – | – | – | – | – | – | – | – | – | 0·043 | – | – | – |
| | 100 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0·957 | 1 | 1 | 1 |
| | *N* | *24* | *20* | *16* | *21* | *26* | *24* | *23* | *15* | *24* | *23* | *21* | *19* | *20* |
| PGI | 100 | 1 | 1 | 0·886 | 0·833 | 1 | 1 | 1 | 1 | 0·833 | 1 | 1 | 1 | 1 |
| | 113 | – | – | 0·114 | 0·167 | – | – | – | – | 0·167 | – | – | – | – |
| | *N* | *18* | *23* | *22* | *24* | *27* | *24* | *24* | *19* | *24* | *22* | *21* | *20* | *20* |
| LAP | 100 | 0·931 | 1 | 1 | 1 | 1 | 1 | 1 | 0·960 | 1 | 1 | 1 | 1 | 1 |
| | 107 | 0·069 | – | – | – | – | – | – | 0·050 | – | – | – | – | – |
| | *N* | *29* | *17* | *28* | *23* | *26* | *22* | *19* | *20* | *24* | *26* | *22* | *18* | *14* |
| SKDH | 85 | – | – | – | – | – | – | – | – | – | – | 0·063 | 0·063 | – |
| | 100 | 0·977 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0·727 | 0·938 | 0·938 | 1 |
| | 109 | – | – | – | – | – | – | – | – | – | 0·159 | – | – | – |
| | 120 | 0·023 | – | – | – | – | – | – | – | – | 0·114 | – | – | – |
| | *N* | *22* | *10* | *18* | *23* | *17* | *23* | *19* | *12* | *22* | *22* | *16* | *16* | *13* |
| IDH | 100 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | *N* | *25* | *23* | *27* | *23* | *27* | *14* | *19* | *20* | *22* | *27* | *14* | *19* | *14* |
| G6PD | 100 | 0·895 | 0·656 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | 118 | 0·105 | 0·344 | – | – | – | – | – | – | – | – | – | – | – |
| | *N* | *19* | *16* | *27* | *23* | *19* | *21* | *21* | *21* | *22* | *27* | *20* | *19* | *20* |
| MDH-1 | 100 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0·971 | 1 |
| | 119 | – | – | – | – | – | – | – | – | – | – | – | 0·029 | – |
| | *N* | *24* | *17* | *26* | *21* | *18* | *18* | *17* | *15* | *21* | *23* | *18* | *17* | *15* |
| MDH-2 | 80 | – | – | – | – | – | – | – | – | – | – | 0·026 | 0·028 | – |
| | 100 | 1 | 1 | 1 | 1 | 1 | 0·955 | 1 | 1 | 1 | 1 | 0·974 | 0·972 | 1 |
| | 111 | – | – | – | – | – | 0·045 | – | – | – | – | – | – | – |
| | *N* | *28* | *18* | *21* | *23* | *26* | *22* | *24* | *13* | *24* | *18* | *19* | *18* | *19* |
| ACPH | 83 | 0·036 | – | 0·037 | – | – | – | – | – | – | – | 0·026 | – | – |
| | 100 | 0·964 | 1 | 0·963 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0·974 | 1 | 1 |
| | *N* | *28* | *22* | *27* | *24* | *27* | *22* | *23* | *19* | *24* | *21* | *19* | *17* | *17* |
| DIA–1 | 80 | – | – | – | – | – | – | – | – | – | 0·103 | – | – | – |
| | 96 | – | – | – | – | – | – | – | – | – | 0·069 | 0·667 | 0·714 | – |
| | 100 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0·828 | 0·333 | 0·286 | 1 |
| | *N* | *24* | *18* | *28* | *23* | *14* | *2* | *20* | *13* | *22* | *29* | *6* | *7* | *6* |
| DIA-2 | 94 | – | – | – | – | – | 0·857 | – | – | – | 0·167 | 0·5 | 0·714 | – |
| | 100 | 1 | 1 | 1 | 1 | 1 | 0·143 | 1 | 1 | 1 | 0·833 | 0·5 | 0·286 | 1 |
| | *N* | *28* | *17* | *28* | *21* | *25* | *7* | *22* | *15* | *23* | *30* | *8* | *7* | *15* |

See Table 1 for the names of the populations.
* *N* = sample size.

scores was PS01 of *M. paucispinus* from Seabra, followed by the populations CM01, CM02, CM03 of *M. concinnus*. The population with the lowest variability was PM03 (Table 4). Among the characters analysed, those that showed the highest variation for both species are the length and width of the stem (variables 1 and 2, respectively), height of the ribs (variable 4), diameter of the radial spine (variable 14) and length and width of the cephalium (variables 16 and 17, respectively). The least variable characters were the numbers of radial spines (variables 9 and 10).

Spearman rank correlation analysis between morphologic and genetic variability resulted in a statistically significant correlation between $H_e$ and $D2_m$ ($r = 0{\cdot}581$, $P = 0{\cdot}037$), but not between $H_e$ and ED ($r = 0{\cdot}191$, $P = 0{\cdot}532$). The population CM03 displayed one of the highest scores for morphological variability in both morphological analyses, but it did not display any genetic variability.

*Structuring of the variability*

Both species displayed high average values of $F_{ST}$ (0·504 for *M. paucispinus* and 0·349 for *M. concinnus*), interpreted as a high level of genetic structuring (Table 5). By excluding the populations PD01 of *M. paucispinus* and CM03 of *M. concinnus* the average values of $F_{ST}$ drop to 0·158 and 0·022, respectively, due to an inversion in the relative frequency of the alleles of DIA-2 (PD01) and DIA-1 (CM03) in these populations.

The hierarchical analysis showed a high $F_{SR}$ among populations (0·338, $\sigma^2 = 0{\cdot}101$), an $F_{RT}$ of 0·253 ($\sigma^2 = 0{\cdot}067$) and an $F_{ST}$ of 0·140 ($\sigma^2 = -0{\cdot}034$), showing that there is more genetic structuring within regions than between them or among all populations.

The $N_m$ values estimated from a mean frequency of private alleles of 0·117 in *M. paucispinus* was 0·571, and the $N_m$(W) was 0·199 (Table 5). The mean frequency of

TABLE 4. *Genetic variability at 12 allozymic loci and morphological variability ($D2_m$ and ED) based on the morphometric analysis of 17 morphological characters in ten populations of* M. paucispinus *and three populations of* M. concinnus *occurring in the Chapada Diamantina, Brazil*

| Population | *N* | A | *P* | $H_o$ | $H_e$ | $D2_m$* | ED |
|---|---|---|---|---|---|---|---|
| *M. paucispinus* | | | | | | | |
| PM01 | 24·4 (1·0) | 1·3 (0·1) | 16·7 | 0·004 (0·004) | 0·037 (0·019) | $12·21^{ac}$ | 3·99 |
| PM02 | 18·3 (1·0) | 1·1 (0·1) | 8·3 | 0·016 (0·016) | 0·039 (0·039) | $12·94^{ac}$ | 4·07 |
| PM03 | 23·8 (1·3) | 1·2 (0·1) | 8·3 | 0·011 (0·011) | 0·023 (0·018) | $8·54^{ac}$ | 3·65 |
| PM04 | 22·5 (0·3) | 1·1 (0·1) | 8·3 | 0·014 (0·014) | 0·024 (0·024) | $9·15^{bc}$ | 3·90 |
| PM05 | 23·2 (1·4) | 1·0 (0·0) | 0·0 | 0·000 (0·000) | 0·000 (0·000) | $8·62^{bc}$ | 4·69 |
| PD01 | 18·6 (2·1) | 1·2 (0·1) | 8·3 | 0·000 (0·000) | 0·029 (0·023) | $10·33^{bc}$ | 3·73 |
| PR01 | 21·2 (0·7) | 1·0 (0·0) | 0·0 | 0·000 (0·000) | 0·000 (0·000) | $10·00^{bc}$ | 3·54 |
| PR02 | 16·4 (0·9) | 1·1 (0·1) | 8·3 | 0·000 (0·000) | 0·008 (0·008) | $10·59^{ac}$ | 4·64 |
| PR03 | 23·0 (0·3) | 1·1 (0·1) | 8·3 | 0·014 (0·014) | 0·024 (0·024) | $11·89^{ac}$ | 3·91 |
| PS01 | 24·5 (1·0) | 1·6 (0·2) | 33·3 | 0·022 (0·016) | 0·123 (0·050) | $17·11^{a}$ | 4·78 |
| Mean | 21·59 | 1·17 | 9·98 | 0·008 | 0·031 | 11·14 | 4·09 |
| *M. concinnus* | | | | | | | |
| CM01 | 17·2 (1·5) | 1·4 (0·1) | 25·0 | 0·009 (0·006) | 0·104 (0·056) | $13·10^{ac}$ | 3·78 |
| CM02 | 16·4 (1·3) | 1·4 (0·1) | 25·0 | 0·010 (0·006) | 0·093 (0·048) | $14·54^{ac}$ | 4·47 |
| CM03 | 16·1 (1·2) | 1·0 (0·0) | 0·0 | 0·000 (0·000) | 0·000 (0·000) | $15·00^{ac}$ | 4·05 |
| Mean | 16·56 | 1·27 | 16·67 | 0·006 | 0·066 | 14·21 | 4·10 |

See Table 1 for the names of the populations. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0·95

* Different letters in conspecific populations indicate statistically different median values in the Kruskal–Wallis test.

*N* = mean sample size per locus; A = mean number of alleles per locus; *P* = percentage of polymorphic loci; $H_o$ = observed and $H_e$ = expected mean heterozygosity per locus (Nei, 1978; unbiased estimate); $D2_m$ = median of the Mahalanobis generalized distance of the individuals to the centroid of the population; ED = mean of the Euclidean distance between the individuals of the population. Numbers in parentheses are s.d.

TABLE 5. F *statistics (Wright, 1978) and* $N_m(W)$ *at 12 allozymic loci in ten populations of* M. paucispinus *and three populations of* M. concinnus *occurring in the Chapada Diamantina, Brazil*

| | | | *M. paucispinus* | | *M. concinnus* | |
|---|---|---|---|---|---|---|
| Locus | *M. paucispinus* $F_{IS}$ | *M. concinnus* $F_{IS}$ | $F_{ST}$ | *N*m(W) | $F_{ST}$ | *N*m(W) |
| PGM-1 | 0·783 | – | 0·213 | 0·75 | – | |
| PGM-2 | 1·000 | – | 0·039 | 4·99 | – | |
| PGI | 0·38 | – | 0·114 | 1·57 | – | |
| LAP | 1·000 | – | 0·050 | 3·85 | – | |
| SKDH | 0·524 | 1·000 | 0·174 | 0·96 | 0·022 | 4·94 |
| G6PD | 0·707 | – | 0·254 | 0·59 | – | |
| MDH-1 | – | –0·03 | – | | 0·02 | 5·44 |
| MDH-2 | 1·000 | –0·028 | 0·041 | 4·73 | 0·009 | 12·23 |
| ACPH | 1·000 | –0·027 | 0·029 | 6·78 | 0·018 | 6·06 |
| DIA-1 | 1·000 | 1·000 | 0·857 | 0·03 | 0·428 | 0·15 |
| DIA-2 | 1·000 | 1·000 | 0·716 | 0·08 | 0·372 | 0·19 |
| Mean | 0·732 | 0·901 | 0·504 | 0·199 | 0·349 | 0·207 |
| *A*-value | | | 0·2 | | 0·17 | |

*A*-values of the MRPP analysis of 17 morphological characters in all populations analysed are also presented.

private alleles was lower in *M. concinnus* (0·027), resulting in a $N_m(S)$ of 12·17, while $N_m(W)$ was 0·207. These dissimilar values could be attributed to the low number of private alleles in this species, because the $N_m(S)$ method requires that a reasonable number of private alleles be present (Slatkin and Barton, 1989), and only two were found in this species. There is a large variation concerning $N_m(W)$ values among loci, especially in *M. concinnus* (Table 5).

High levels of morphological structuring were also found in both species (Table 5), with *A* values of 0·20 and 0·17 for *M. paucispinus* and *M. concinnus*, respectively, these values being correlated with the high values of genetic differentiation ($F_{ST}$) found in the species. When the populations PD01 and PS01 are removed from the MRPP analysis the *A* value still remains high (0·18).

### *Phenetic relationships*

The genetic identities between conspecific populations ranged from 0·842 to 1·000 for *M. paucispinus* and from 0·914 to 1·000 for *M. concinnus*. PD01 has the lowest values of genetic identities among the conspecific populations of *M. paucispinus* (0·842–0·880). This population is genetically more similar to the populations CM01 and CM02 of *M. concinnus* (0·983 and 0·995, respectively), due to allele frequencies of the DIA-2 (Table 3). If PD01 is removed,

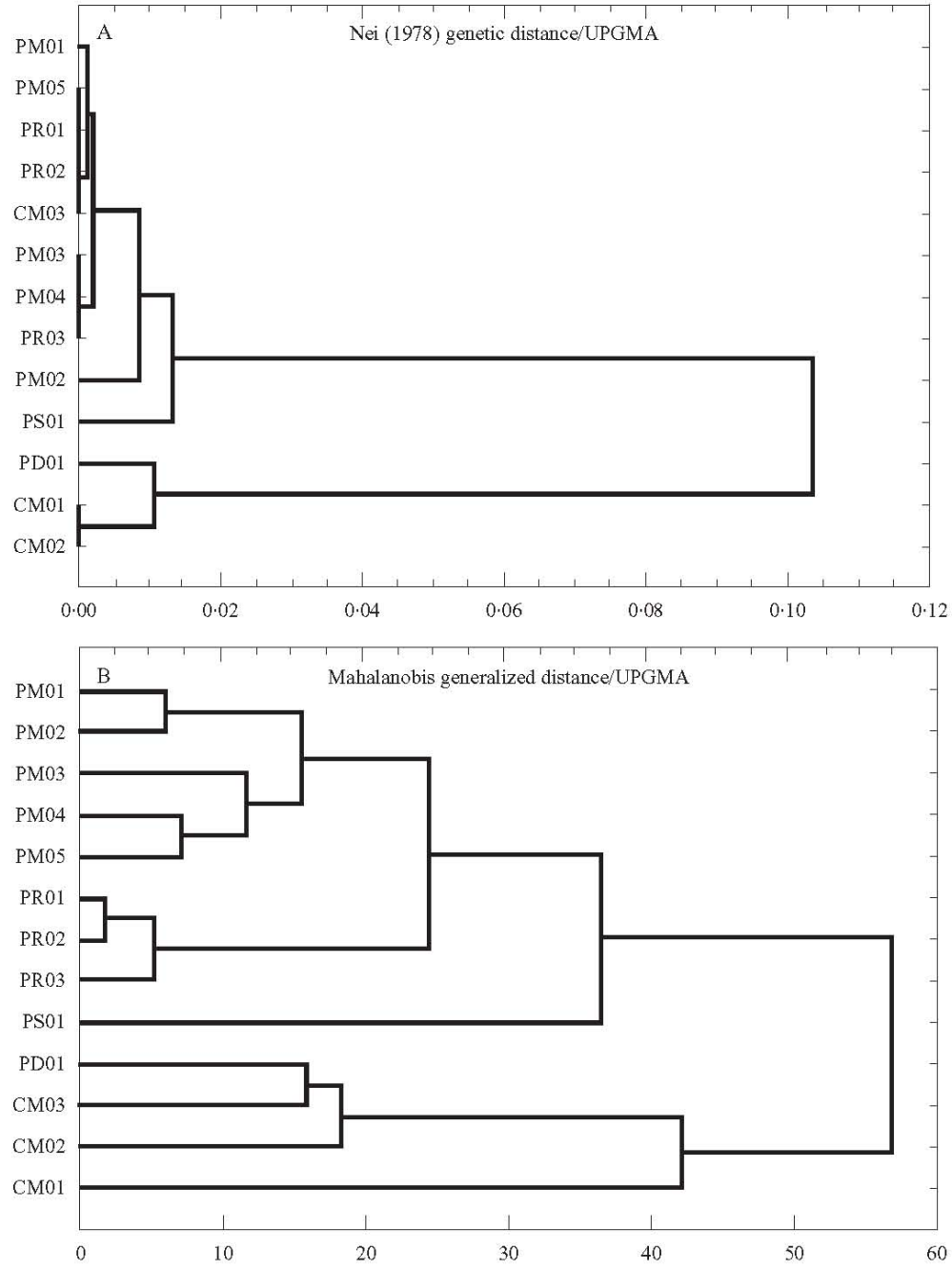


FIG. 3. Dendrograms showing the phenetic relationships among ten populations of *Melocactus paucispinus* and three populations of *M. concinnus* occurring in the Chapada Diamantina, Brazil, constructed using the matrix of genetic distances (Nei, 1978, unbiased estimate; cophenetic correlation = 0·886) based on 12 allozymic loci (A), and using the matrix of Mahalanobis generalized distance based on 17 morphological characters (B) with UPGMA as the clustering algorithm. See Table 1 for the names of the populations.

the mean genetic identity among the populations of *M. paucispinus* increases to 0·994, ranging from 0·978 to 1·000. The population CM03 is genetically more similar to the populations of *M. paucispinus* (0·989–1·000)—except for the population PD01 (0·854)—than to its two conspecific populations (CM01, 0·943; CM02, 0·914), mainly due to the allele frequencies of DIA-1 and DIA-2 (Table 3). The genetic identity between the species ranged from 0·854 to 1·000.

The UPGMA dendrogram obtained from the cluster analysis of Nei (1978) unbiased genetic distances (Fig. 3) reveals the formation of two main groups: one composed of nine of the ten populations of *M. paucispinus* (except PD01) plus population CM03 of *M. concinnus*, and the

TABLE 6. *Matrix of classification of the individuals in the discriminant analysis of 17 morphological characters in ten populations of* Melocactus paucispinus *and three populations of* M. concinnus *occurring in the Chapada Diamantina, Brazil*

| | | *Melocactus paucispinus* | | | | | | *Melocactus concinnus* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Percent correct | PM1 | PM2 | PM3 | PM4 | PM5 | PD1 | PR1 | PR2 | PR3 | PS1 | CM1 | CM2 | CM3 |
| PM1 | 71·43 | 20 | 1 | – | 2 | 5 | – | – | – | – | – | – | – | – |
| PM2 | 77·27 | 3 | 17 | – | – | – | – | 1 | – | – | 1 | – | – | – |
| PM3 | 92·31 | – | – | 24 | 2 | – | – | – | – | – | – | – | – | – |
| PM4 | 70·83 | 1 | 1 | 2 | 17 | 2 | – | – | – | – | – | – | – | 1 |
| PM5 | 96·15 | – | – | – | 1 | 25 | – | – | – | – | – | – | – | – |
| PD1 | 100·00 | – | – | – | – | – | 25 | – | – | – | – | – | – | – |
| PR1 | 82·76 | – | – | – | – | – | – | 24 | 3 | 2 | – | – | – | – |
| PR2 | 57·14 | – | 1 | – | – | – | – | 7 | 12 | 1 | – | – | – | – |
| PR3 | 76·00 | – | 1 | – | – | – | – | 5 | – | 19 | – | – | – | – |
| PS1 | 93·33 | – | – | – | – | 2 | – | – | – | – | 28 | – | – | – |
| CM1 | 100·00 | – | – | – | – | – | – | – | – | – | – | 24 | – | – |
| CM2 | 95·00 | – | – | – | – | – | 1 | – | – | – | – | – | 19 | – |
| CM3 | 85·00 | – | – | – | – | – | 3 | – | – | – | – | – | – | 17 |
| Total | 84·69 | 24 | 21 | 26 | 22 | 34 | 29 | 37 | 15 | 22 | 29 | 24 | 19 | 18 |
| *P* = | | 0·087 | 0·068 | 0·081 | 0·075 | 0·081 | 0·078 | 0·090 | 0·065 | 0·078 | 0·093 | 0·075 | 0·062 | 0·062 |

See Table 1 for the names of the populations.

other composed of population PD01 grouped with the remaining two populations of *M. concinnus* (CM01 and CM02), with a genetic distance between these groups of 0·10.

Two groups of populations (PM05, PR01, PR02, CM03; and PM03, PM04, PR03) did not display any genetic differentiation. The population from Seabra (PS01) was the most distant within the group due to its higher levels of polymorphism, with four alleles exclusive for this population, some of them at high frequencies (PGM-1 90, SKDH 109, DIA-1 80).

The UPGMA dendrogram obtained from the cluster analysis of morphological distances resulted in the formation of two main groups (Fig. 3): one uniting the populations of *M. paucispinus* except for PD01 (Delfino), and the other containing the populations of *M. concinnus* plus PD01. Within the group of *M. paucispinus* the population PS01 (Seabra) displayed the greatest differentiation, and the remaining populations were divided into two subgroups, one grouping the populations from Morro do Chapéu and the other grouping the populations from Rio de Contas. Smaller genetic distances were found among the populations from Rio de Contas than among the populations from Morro do Chapéu.

Table 6 shows the classification matrix of the individuals analysed. The percentage of correct classifications ranged from 57 to 100 %. The incorrect classifications mostly occurred between conspecific populations, except for one individual each from PM04 and CM02, and three individuals from CM03. Within *M. paucispinus* the percentage of incorrect classifications was higher among populations from the same locality, with the exception of populations PM02, PR02 and PR03, each of which had one individual incorrectly classified as belonging to a population from a different locality. The populations of *M. paucispinus* with a higher degree of morphological differentiation (PS01 and PD01) displayed the highest values of correct classifications.

The scatterplots of the scores of individuals on the first two CVA canonical axes, and on the first and third CVA canonical axes are shown in Fig. 4. The first, second and third canonical axes explained 48·81 %, 27·67 % and 8·82 % of the morphological variation, respectively; in total 85·3 % of the observed variability. On the first canonical axis there is a separation between the populations of *M. concinnus* plus PD01 from the remaining populations of *M. paucispinus*, mainly due to the higher height of the ribs (variable 4), higher width at the base of ribs (variable 5) and higher number of areoles per rib (variable 8) in those populations. Within *M. paucispinus* there is separation of the population PS01 in the same axis due to the higher width of the individuals (variable 2) and the higher number of ribs (variable 3) in this population. Such separation also occurs in the third canonical axes, the greater length of the individuals (variable 1) being the most significant character for this separation. In the second canonical axes there is a slight separation between the populations of Rio de Contas and Morro do Chapéu in the form of a gradient; the individuals from Rio de Contas are mainly distributed in the uppermost region of the scatterplot, with some individuals overlapping with individuals from the Morro do Chapéu populations, which are mostly located in the lower region of the scatterplot.

For *M. paucispinus*, Mantel tests did not produce statistically significant results for pair-wise correlations between genetic and morphological distances ($r$ = 0·527, $P$ = 0·064), nor between genetic and geographical distances ($r$ = 0·356, $P$ = 0·084) of conspecific populations; however, there was a significant correlation between morphological and geographical distances ($r$ = 0·364, $P$ = 0·022).

## DISCUSSION

The *Melocactus* spp. studied here displayed levels of genetic variability lower than the average values reported for another *Melocactus* species, *M. curvispinus* (*P* = 89·5 %,

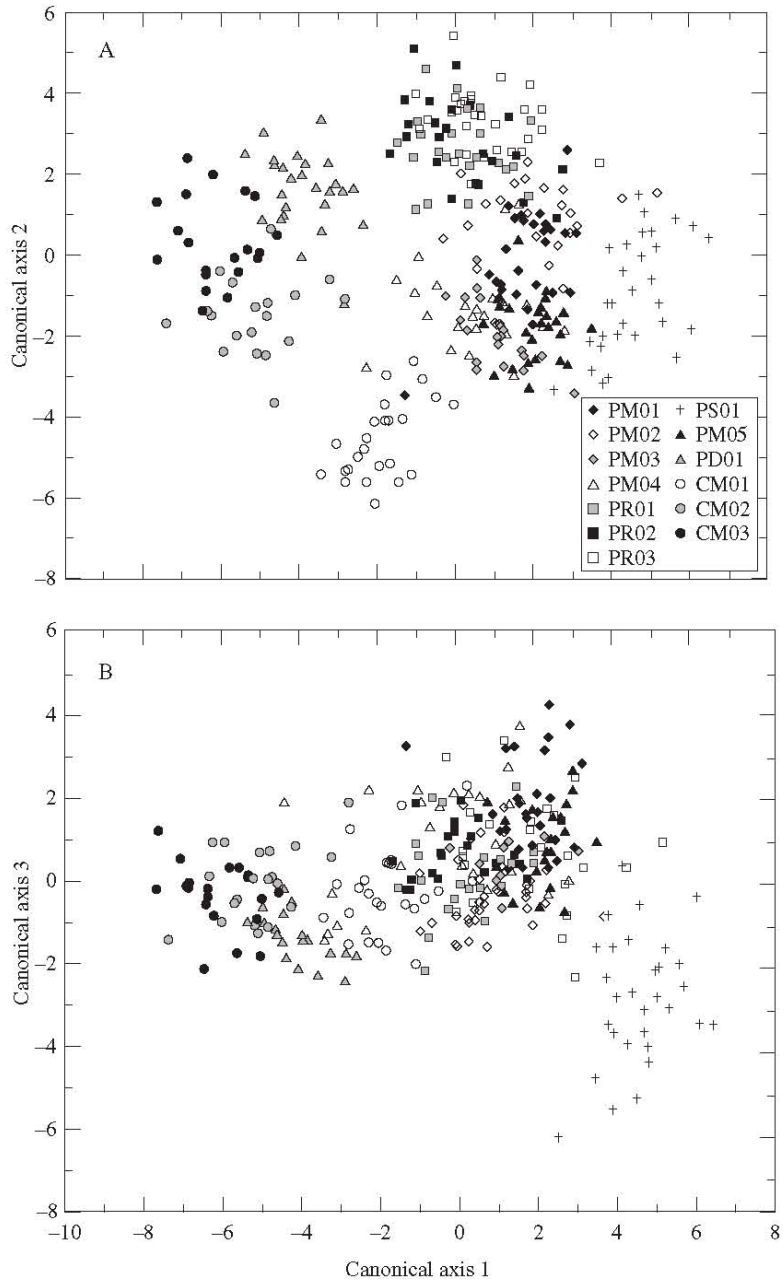


FIG. 4. Representation of the scores on the three first canonical axes of the CVA using 17 morphological characters in ten populations of *Melocactus paucispinus* and three populations of *M. concinnus* occurring in the Chapada Diamantina, Brazil. (A) Canonical axes 1 and 2. (B) Canonical axes 1 and 3. See Table 1 for the names of the populations.

$A$ = 3·82, $H_e$ = 0·145; Nassar *et al.*, 2001) and for other cactus species (Parker and Hamrick, 1992; Hamrick *et al.*, 2002; Nassar *et al.*, 2002, 2003; Clark-Tapia and Molina-Freaner, 2003; Moraes *et al.*, 2005) and lower than the average values reported for plant species with similar characteristics, namely woody, long-lived, animal-pollinated plants (Hamrick and Godt, 1992; Colunga-GarcíaMarín *et al.*, 1999; Martínez-Palacios *et al.*, 1999). The low genetic variability displayed by the species surveyed is in sharp contrast with the high levels found in species of the genus *Discocactus* (Cactaceae) that occur in the same general area, occupy similar habitats, and also have a globose habit (M. C. Machado *et al.*, unpubl. res.). The low variability presented by these *Melocactus* species may be associated with recent bottleneck effects experienced by the populations, as they occur in disturbed areas, generally next to roads, in agricultural areas or in areas of sand extraction. Moreover, these populations

are being exploited by collectors and traders, and this could be adversely affecting the genetic diversity. The same has been observed in populations of *Discocactus bahiensis*, a species that grows in similar sites and is subject to similar threats (M. C. Machado *et al.*, unpubl. res.).

Studies of levels of intra- and inter-populational morphologic variability are absent in *Melocactus* and rare in Cactaceae (Cassas *et al.*, 1999; Backer and Johnson, 2000; Schmalzel *et al.*, 2004). The levels of morphological variability in these *Melocactus* spp. are lower than those observed by M. C. Machado *et al.* (unpubl. res.) in 17 populations of three *Discocactus* species (*D. bahiensis*: $D2_m$ = 11·53–17·71; *D. catingicola*: $D2_m$ = 20·39–32·88; *D. zehntneri*: $D2_m$ = 10·64–32·18).

In *M. paucispinus*, the indirect migration estimates gave values lower than one, suggesting restricted long-term gene flow among populations (Slatkin, 1987; Slatkin and Barton, 1989), possibly due to historical factors, indicating environmental fragmentation. *Melocactus concinnus* also showed a low value of $N_m$(W), providing further evidence for genetic drift having played a prominent role in these populations. The population presenting both the highest genetic and morphological diversity was the *M. paucispinus* population from Seabra (PS01), which possessed exclusive or private alleles, and presented the highest average values for the majority of the morphological characters. In general, allozyme differentiation among populations is a result of genetic drift or directional selection. The presence of four exclusive alleles in this population can be interpreted as a result of its geographic isolation: gene flow between the PS01 population and the remaining *M. paucispinus* populations is possibly non-existent, and genetic drift could be responsible for the differentiation observed. This kind of process can quickly lead to speciation in small, geographically isolated populations (Levin, 2000). The main issue raised by the distinctness of this population would be taxonomic, since the type specimen of the species comes from this population. However, it cannot be excluded that selection is also occurring, especially since genetic drift affects all loci in the same way, but natural selection does not. The balance of selection and gene flow could be different from the balance between drift and gene flow, where gene flow might be weaker than selection in some loci and stronger than genetic drift at other loci (Slatkin, 1987). This could explain the differences observed in $N_m$ values among the different loci.

The smaller genetic distance between the population CM03 of *M. concinnus* and populations of *M. paucispinus* than to its remaining conspecific populations could be explained by the possibility of gene flow occurring between the species, since they are intercompatible (M. A. S. Colaço *et al.*, unpubl. res.). The frequencies of alleles of the two DIA loci in this population strengthens this hypothesis. The population CM03 occurs sympatrically with *M. paucispinus* in Morro do Chapéu and individuals displaying intermediate characteristics between the two species have been observed in this population, as noted earlier by Taylor (1991) and Taylor and Zappi (2004). Morphologically this population shows characters intermediate between the two species, including the presence of depressed-globose individuals, with diameter of the spines being similar to that found in *M. paucispinus* individuals. However, they are usually glaucous, have more radial spines per areole than individuals of *M. paucispinus* and show re-entrance in the ribs: typical characteristics of *M. concinnus* individuals.

The population CM03 is monomorphic for an allele (DIA-1 100) that is a low-frequency allele in other *M. concinnus* populations and which is monomorphic in all populations of *M. paucispinus* (except PS01). This strengthens the hypothesis of gene flow between the two species (see Fig. 1). However, the grouping of CM03 with populations of *M. paucispinus* could also be merely incidental: CM03 is monomorphic for all loci sampled, as are the populations PM05, PR01 and PR02 of *M. paucispinus* with which CM03 is grouped. The lack of variation thus cannot be taken uncritically as an indication of close relationship. Since CM03 is monomorphic for all loci, another explanation is that the small size of the CM03 population coupled with genetic drift led to it becoming fixed for an allele that occurs in low frequency in the species as a whole, and that happens to be the most frequent allele in *M. paucispinus*. The above two explanations are not mutually exclusive, and the most plausible hypothesis is that hybrization and genetic drift have acted together.

The population PD01 groups with populations of *M. concinnus* in the morphological and genetic analyses. The characteristics that contribute most significantly to the grouping of PD01 with the populations of *M. concinnus* in the morphological analysis are the stem width (variable 2), rib height (variable 4), rib width at mid-region (variable 6) and number of areoles on the ribs (variable 8), all of them with higher values in PD01. In genetic terms, the grouping of PD01 with populations of *M. concinnus* is due to the higher frequency of the allele 94 at the locus DIA-2 (Table 3). These results could be a consequence of gene flow between *M. paucispinus* and *M. concinnus* at the location where PD01 occurs, since the latter species is widely distributed and possibly occurs in that region (Taylor, 1991; Taylor and Zappi, 2004). Thus, hybridization and/or introgression processes could be generating the genetic and morphological differentiation of the population PD01. Another explanation for grouping of PD01 with populations of *M. concinnus* is that this population in fact represents a *M. concinnus* morph that is superficially similar to *M. paucispinus*. The most important morphological characters in diagnosing it as a *M. paucispinus* population are the relative lack of glaucousness in the epidermis (plants of *M. paucispinus* are never glaucous, whereas plants of *M. concinnus* are glaucous, often intensely so), the depressed habit, and the lack of a central spine in the areoles (characters typical of *M. paucispinus*). However, other morphological characteristics strongly suggest that the plants from the PD01 population are more akin to *M. concinnus*. Thus, PD01 can be interpreted either as an *M. paucispinus* population that has undergone extensive introgression with *M. concinnus* or as an *M. concinnus* population that displays morphological convergence with *M. paucispinus*.

Hybridization and introgression may have significant influence in the conservation of species as they can promote the extinction of pure populations and consequently compromise the survival of rare species (Levin *et al.*, 1996; Rhymer and Symberloff, 1996). Arnold (1997) argued that the greatest barrier to gene flow between some species of plants is the non-formation of F1 hybrids; if these are formed, generally introgression is verified. Thus, rare species could have numerical disadvantages due to the proliferation of these fertile hybrids, which promote a reduction in the proportional representation of pure individuals and inhibit the growth of populations of the rare species (Levin *et al.*, 1996).

The high average values of $F_{IS}$ observed in both species (Table 5) are much higher than those found in *M. curvispinus* by Nassar *et al.* (2001). However, several works have demonstrated moderate to high levels of endogamy in Cactaceae, such as in *Stenocereus griseus* ($F_{IS}$ = 0·145; Nassar *et al.*, 2003), *Cereus repandus* ($F_{IS}$ = 0·182; Nassar *et al.*, 2003), *Pilosocereus lanuginosus* ($F_{IS}$ = 0·176; Nassar *et al.*, 2003), *Stenocereus gummosus* ($F_{IS}$ = 0·608; Clark-Tapia and Molina-Freaner, 2003), *Pereskia guamacho* ($F_{IS}$ = 0·301; Nassar *et al.*, 2002), and, in Brazil, *Pilosocereus machrisii* and *P. euchlorus* ($F_{IS}$ ranged from 0·025 to 0·569 and from −0·276 to 0·529, respectively; Moraes *et al.* 2005).

The high values of $F_{IS}$ observed indicate a strong heterozygote deficit, which could be the result of endogamy or sub-structuring of the populations. Local subdivision could also explain the significant associations among different loci observed in two populations of *M. paucispinus.* Hummingbirds are the main pollinators of *Melocactus* spp. (Taylor, 1991). The most frequent floral visitor to *M. paucispinus* is the hummingbird *Chlorostilbon aureoventris* (M. A. S. Colaço *et al.*, unpubl. res.), a territorial species. The behaviour of this pollinator may contribute to genetic subdivision of the populations, since hummingbirds promote gene flow only among the individual plants within their feeding territories. Endogamy could also be a factor responsible for lack of heterozygotes in *M. paucispinus* because this species is self-compatible and autogamous (M. A. S. Colaço *et al.*, unpubl. res.). In spite of being autogamous, studies of reproductive biology for the species indicate a low level of fruit production in spontaneous autopollination experiments, suggesting the possibility of inbreeding depression resulting from recent endogamy within the populations. Endogamy could also occur because of crosses between closely related individuals, since seed dispersal is extremely local, being mediated by lizards (Taylor, 1991; Fonseca, 2004). The limited dispersal ability of the lizards could contribute to an increase both in endogamy and genetic sub-structuring within the *Melocactus* populations (Nassar *et al.*, 2001). Inbreeding depression is generally associated with a decrease of fitness of the individuals, affecting viability, fecundity, development and susceptibility to environmental stress, therefore increasing the probability of extinction of small populations (Hauser and Loescheke, 1995; Bijlsma *et al.*, 2000). This is especially important for species threatened with extinction, in which the disappearance of one population may affect the survival the whole species.

The genetic identity values found between conspecific populations are similar to those reported for other plant species (Thorpe, 1982; Crawford, 1989; Borba *et al.*, 2001; Jesus *et al.*, 2001). The values of genetic differentiation found for both species are similar to those observed by Nassar *et al.* (2001) for different populations of *M. curvispinus*, and this may be conservative in the genus, due to similar pollination and seed dispersal mechanisms. In spite of the lack of correlation between the genetic variability and geographic distance, the latter may be a factor influencing the differentiation of population PD01. This population is geographically isolated and its differentiation is reflected in the high $F_{ST}$ value (0·504) observed, indicating a geographic sub-structuring of the species. After removing this population from the analysis, the $F_{ST}$ reduces dramatically (to 0·158), a value lower than that found in *M. curvispinus* ($F_{ST}$ = 0·193; Nassar *et al.*, 2001) and close to the values found in for other cacti (*Lophocereus schottii*: $F_{ST}$ = 0·130, Parker and Hamrick, 1992; *Pereskia guamacho*: $F_{ST}$ = 0·112, Nassar *et al.*, 2002; *Stenocereus gummosus*: $F_{ST}$ = 0·10, Clarkia-Trapia and Molina-Freaner, 2003). However, it should be also noticed that there is no evidence of a 'stepping-stone' pattern in *M. paucispinus* and that there is more structuring at a local scale ($F_{SR} > F_{RT} > F_{ST}$) than at a large scale. Limited gene flow among close populations combined with a low level of natural selection (Linhart and Grant, 1996) could explain these results.

Geographic sub-structuring of populations is one of the factors responsible for the high $F_{ST}$ values reported for other plant species with disjunct populations occurring in the mountainous regions of the Espinhaço Range, such as Orchidaceae (Borba *et al.*, 2001), Asteraceae (Jesus *et al.*, 2001), Eriocaulaceae (A. C. S. Pereira *et al.*, unpubl. res.) and other Cactaceae (M. C. Machado *et al.*, unpubl. res.). A major contribution to the observed differentiation among populations is probably the restricted gene flow and local dispersion of seeds in these species of cactus, as in some bat-pollinated species (Nassar *et al.*, 2002, 2003). However, as stated earlier, the differentiation of PD01 may be due to hybridization/introgression or it may be a *M. concinnus* morph. This is clearly the case in *M. concinnus*, in which the high $F_{ST}$ value cannot be explained by geographic sub-structuring, but only by hybrization and/or introgression in CM03.

Besides the population PD01 from Delfino, which clusters with populations of *M. concinnus*, the remaining populations of *M. paucispinus* differ morphologically on a geographical basis, with the populations of each municipality grouping together, resulting in the high *A*-value for this species. However, the lack of correlation between geographical and morphological distances is probably a result of the higher differentiation displayed by the population PS01 from Seabra, mainly due to characters of the stem and ribs. The sets of populations from Morro do Chapéu and Rio de Contas are morphologically more similar to each other. However the population from Seabra is geographically located between Morro do Chapéu and

Rio de Contas. As in the $F_{ST}$ analysis, the high *A*-value for *M. concinnus* cannot be explained by geographic substructuring, but only by hybridization and/or introgression.

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## LITERATURE CITED

**Anderson EF. 2001.** *The cactus family*. Portland, OR: Timber Press.

**Arnold ML. 1996.** *Natural hybridization and introgression*. Princeton, NJ: Princeton University Press.

**Arnold ML. 1997.** *Natural hybridization and evolution*. Oxford: Oxford University Press.

**Backer MA, Johnson RA. 2000.** Morphometric analysis of *Escobaria sneedii* var. *sneedii*, *E. sneedii* var. *leei*, and *E. guadalupensis* (Cactaceae). *Systematic Botany* **25**: 577–587.

**Backer MA, Pinkava DJ. 1987.** A cytological and morphometric analysis of a triploid apomict, *Opuntia* × *kelvinensis* (subgenus *Cylindropyntia*, Cactaceae). *Brittonia* **39**: 387–401.

**Barton NH. 2001.** The role of hybridization in evolution. *Molecular Ecology* **10**: 551–568.

**Barton NH, Slatkin M. 1986.** A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity* **56**: 409–415.

**Bijlsma R, Bundgaard J, Boerema AC. 2000.** Does inbreeding affect the extinction risk of small populations? Predictions from *Drosophila*. *Journal of Evolutionary Biology* **13**: 502–514.

**Borba EL, Semir J. 1998.** Wind-assisted fly pollination in three *Bulbophyllum* (Orchidaceae) species occurring in the Brazilian 'campos rupestres'. *Lindleyana* **13**: 203–218.

**Borba EL, Felix JM, Solferini VN, Semir J. 2001.** Fly-pollinated *Pleurothallis* (Orchidaceae) species have high genetic variability: evidence from isozyme markers. *American Journal of Botany* **88**: 419–428.

**Borba EL, Shepherd GJ, van den Berg C, Semir J. 2002.** Floral and vegetative morphometrics of five *Pleurothalis* (Orchidaceae) species: correlation with taxonomy, phylogeny, genetic variability and pollination systems. *Annals of Botany* **90**: 219–230.

**Brune W, Alfenas AC, Junghans TG. 1998.** Identificações específicas de enzimas em géis. In: Alfenas AC, ed. *Eletroforese de isoenzimas e proteinas afins: fundamentos e aplicações em plantas e microorganismos*. Viçosa: Universidade Federal de Viçosa, 201–328.

**Casas A, Caballero J, Valiente-Banuet A, Soriano JA, Dávila P. 1999.** Morphological variation and the process of domestication of *Stenocereus stellatus* (Cactaceae) in Central México. *American Journal of Botany* **86**: 522–533.

**Chamberland M. 1997.** Systematics of the *Echinocactus polycephalus* complex (Cactaceae). *Systematic Botany* **22**: 303–313.

**Clark-Tapia R, Molina-Freaner F. 2003.** The genetic structure of a columnar cactus with a disjunct distribution: *Stenocereus gummosus* in the Sonoran desert. *Heredity* **90**: 443–450.

**Clayton JW, Tretiak DN. 1972.** Amine-citrate buffers for pH control in starch gel eletrophoresis. *Journal of the Fisheries Research Board of Canada* **29**: 1169–1172.

**Colunga-GarcíaMarín P, Coello-Coello J, Eguiarte LE, Piñero D. 1999.** Isozymatic variation and phylogenetic relationships (*Agave fourcroydes*) and its wild ancestor *A. angustifolia* (Agavaceae). *American Journal of Botany* **86**: 115–123.

**Corrias B, Rossi W, Arduino P, Cianchi R, Bullini L. 1991.** *Orchis longicornu* Poiret in Sardinina: genetic, morphological and chorological data. *Webbia* **45**: 71–101.

**Crawford DJ. 1989.** Enzyme eletrophoresis and plant systematics. In: Soltis DE, Soltis PS, eds. *Isozymes in plant biology*. Portland, OR: Dioscorides Press, 146–164.

**Crow JF, Aoki K. 1984.** Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences USA* **81**: 6073–6077.

**Ellstrand NC. 1992.** Gene flow by pollen: implication for plant conservation genetics. *Oikos* **63**: 77–86.

**Fonseca RBS. 2004.** *Fenologia reprodutiva e dispersão de* Melocactus glaucescens *Buining & Brederoo e* M. paucispinus *G. Heiman & R. Paul (Cactaceae) no município de Morro do Chapéu, Chapada Diamantina—Bahia—Brasil*. Masters Thesis, Universidade Estadual de Feira de Santana, Brazil.

**Forbes SH, Allendorf FW. 1991.** Mitochondrial genotypes have no detectable effects on meristic traits in cutthroat trout hybrid swarms. *Evolution* **45**: 1350–1359.

**Giulietti AM, Pirani JR. 1988.** Patterns of geographic distribution of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. In: Heyer WR, Vanzolini PE, eds. *Proceedings of a workshop on Neotropical distribution patterns*. Rio de Janeiro: Academia Brasileira de Ciências, 39–63.

**Goldman DH, van den Berg C, Griffith MP. 2004.** Morphometric circumscription of species and infraspecific taxa in *Calopogon* R.Br. (Orchidaceae). *Plant Systematics and Evolution* **274**: 37–60.

**Hamrick JL, Godt MJ. 1992.** Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and genetic resources*. Sunderland, MA: Sinauer, 43–63.

**Hamrick JL, Nason JD, Fleming TH, Nassar JM. 2002.** Genetic diversity in columnar cacti. In: Fleming TH, Valiente-Banuet A, eds. *Columnar cacti and their mutualisms: evolution, ecology and conservation*. Tucson, AR: University of Arizona Press, 122–133.

**Harley RM. 1988.** Evolution and distribution of *Eriope* (Labiatae) and its relatives, in Brazil. In: Heyer WR, Vanzolini PE, eds. *Proceedings of a workshop on Neotropical distribution patterns*. Rio de Janeiro: Academia Brasileira de Ciências, 71–120.

**Hauser TP, Loescheke V. 1995.** Inbreeding depression in *Lychnis floscuculi* (Caryophyllaceae): effects of different levels of inbreeding. *Journal of Evolutionary Biology* **8**: 589–600.

**IUCN. 2004.** *2004 IUCN Red List of Threatened Species*. www.redlist.org. 17 Apr. 2005.

**Jesus FF, Solferini VN, Semir J, Prado PI. 2001.** Local genetic differentiation in *Proteopsis argentea* (Asteraceae), a perennial herb endemic in Brazil. *Plant Systematics and Evolution* **226**: 59–68.

**Joly AB. 1970.** *Conheça a vegetação brasileira*. São Paulo: EDUSP.

**Kimura M, Weiss GH. 1964.** The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* **49**: 561–576.

**Levene H. 1949.** On a matching problem arising in genetics. *Annals of Mathematical Statistics* **20**: 91–94.

**Levin DA. 2000.** *The origin, expansion, and demise of plant species*. New York: Oxford University Press.

**Levin DA, Francisco-Ortega J, Jansen RK. 1996.** Hybridization and the extinction of rare plant species. *Conservation Biology* **10**: 10–16.

**Lewontin RC, Birch LC. 1966.** Hybridization as a source of variation for adaptation to new environments. *Evolution* **20**: 315–336.

**Linhart YB, Grant MC. 1996.** Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **27**: 237–277.

**McCune B, Mefford MJ. 1999.** *PCOrd—Multivariate analysis of ecological data*, version 4.10. Gleneder Beach: MjM Software.

**Machado MC. 1999.** The cacti of Morro do Chapéu, Bahia, Brazil. *British Cactus and Succulent Journal* **17**: 201–213.

**Machado MC, Charles, G. 2004.** *Pilosocereus bohlei* Hofacker—a remarkable new species from Brazil. *British Cactus and Succulent Journal* **22**: 188–192.

**Martínez-Palacios A, Eguiarte LE, Furnier G. 1999.** Genetic diversity of the endangered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahuan desert. *American Journal of Botany* **86**: 1093–1098.

**Moraes EM, Abreu AG, Andrade SCS, Sene FM, Solferini VN. 2005.** Population genetic structure of two columnar cacti with a patchy distribution in eastern Brazil. *Genetica* **125**: 311–323.

**Nassar JM, Hamrick JL, Fleming TH. 2001.** Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). *Heredity* **87**: 69–79.

**Nassar JM, Hamrick JL, Fleming TH. 2002.** Allozyme diversity and genetic structure of the leafy cactus (*Pereskia guamacho* [Cactaceae]). *The American Genetic Association* **93**: 193–200.

**Nassar JM, Hamrick JL, Fleming TH. 2003.** Population genetic structure of Venezuelan chiropterophilous columnar cacti (Cactaceae). *American Journal of Botany* **90**: 1628–1637.

**National Geodetic Survey. 2002.** *INVERSE*—Version 2.0. Silver Spring: 1315 East-West Highway, Silver Spring, MD 20910–3282. http://ngs.noaa.gov/. 18 Jan. 2005.

**Nei M. 1978.** Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

**Parker KC, Hamrick JL. 1992.** Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schotii*. *American Journal of Botany* **79**: 86–96.

**Raymond M, Rousset F. 1995.** GENEPOP (version 1·2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248–249.

**Rhymer JM, Simberloff D. 1996.** Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**: 83–109.

**Rice WR. 1989.** Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

**Ridgway GJ, Sherburne SW, Lewis RD. 1970.** Polymorphism in the esterases of Atlantic herring. *Transactions of the American Fisheries Society* **99**: 147–151.

**Rieseberg LH. 1995.** The role hybridization in evolution: old wine in new skins. *American Journal of Botany* **82**: 944–953.

**Schmalzel RJ, Nixon RT, Best AL, Tress Jr JA. 2004.** Morphometric variation in *Coryphantha robustipina* (Cactaceae). *Systematic Botany* **29**: 553–568.

**Shaw CR, Prasad R. 1970.** Starch gel eletrophoresis of enzymes—a compilation of recipes. *Biochemical Genetics* **4**: 297–320.

**Slatkin M. 1987.** Gene flow and the geographic structure of natural populations. *Science* **236**: 787–792.

**Slatkin M, Barton NH. 1989.** A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**: 1349–1368.

**Sneath PHA, Sokal RR. 1973.** *Numerical taxonomy*. San Francisco: Freeman and Co.

**Soltis DE, Haufler CH, Darrow DC, Gastony GJ. 1983.** Starch gel eletrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedule. *American Fern Journal* **73**: 9–27.

**Stace CA. 1984.** *Plant taxonomy and biosystematics*. London: Edward Arnold.

**StatSoft INC. 2000.** *Statistica for windows (computer program manual)*. Tulsa: Salt Soft Inc.

**Stebbins GL. 1959.** The role of hybridization in evolution. *Proceedings of the American Philosophical Society* **103**: 231–251.

**Stuber CW, Goodman MM, Johnson FM. 1977.** Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.). *Biochemical Genetics* **15**: 383–394.

**Sun M, Ganders FR. 1990.** Outcrossing rates and allozymes variation in rayed and rayless morphs of *Bidens pilosa*. *Heredity* **64**: 139–143.

**Swofford DL, Selander RB. 1989.** *BIOSYS-1: computer program for the analysis of allelic variation in population genetics and biochemical systematics*. Champaign: Illinois Natural History Survey.

**Taylor NP. 1991.** The genus *Melocactus* (Cactaceae) in Central and South America. *Bradleya* **9**: 1–80.

**Taylor NP, Zappi DC. 2004.** *Cacti of Eastern Brazil*. Richmond, Surrey, UK: Royal Botanic Gardens, Kew.

**Thomson G. 2002.** A re-evaluation of the taxonomic status of the genus *Melocactus* in Aruba, Netherlands Antilles. *Bradleya* **20**: 29–44.

**Thorpe JP. 1982.** The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics* **13**: 139–168.

**Wright S. 1951.** The genetical structure of populations. *Annals of Eugenics* **15**: 323–354.

**Wright S. 1978.** *Evolution and the genetics of populations—Variability within and among natural populations. Vol. 4*. Chicago: University of Chicago Press.

**CAPÍTULO 2**

# Allozyme diversity and morphometrics of *Melocactus glaucescens* Buining & Brederoo (Cactaceae) and evidence for hybridization with congeneric species in the Chapada Diamantina, Northeastern Brazil

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## RESUMO

A análise isoenzimática de 12 locos polimórficos para populações de *Melocacatus glaucescens*, *M. ernestii*, *M. concinnus* e duas populações de híbridos (uma de *M.* ×*albicephalus* e outra de possíveis híbridos e/ou introgressantes) revelou baixo nível de variabilidade genética para todos os táxons, valores estes menores que as médias reportadas para outras espécies de Cactaceae (Parker & Hamrick, 1992; Nassar *et al*., 2001, 2002, 2003; Hamrick *et al*., 2002; Clark-Tapia & Molina-Freaner, 2003; Machado *et al*., submetido) e mesmo para plantas com hábitos de vida similares (Hamrick & Godt, 1992; Colunga-GarcíaMarín *et al.*, 1999; Martínez-Palacios *et al*., 1999). Entretanto, os valores apresentados por *M. glaucescens* condizem com aqueles reportados para espécies endêmicas (Hamrick & Godt, 1992) e são maiores que os observados para outra espécie do gênero – *M. paucispinus* (Lambert *et al.*, no prelo). Assim como evidenciado para *M. paucispinus*, a espécie pode ter passado de um recente efeito gargalo, o que associado à atividade de coleta e vulnerabilidade das populações estaria levando a diminuição da variabilidade na mesma.

Em uma das populações de *M. glaucescens* analisadas foi evidenciado um loco duplicado (ACP) encontrado para praticamente todos os indivíduos sendo que em dois deles este foi silenciado ou não foi duplicado. A duplicação foi observada apenas para este loco e por isto não analisamos o fato com um evento de poliploidia, apesar de haverem indícios disto em trabalho de citogenética com a espécie (Assis *et al*., 2003). Caso esta duplicação tenha algum valor adaptativo, este evento pode levar á diferenciação desta população e eventual especiação.

Os maiores valores de variabilidade genética foram encontrados para as duas populações de *M. concinnus*, seguidos pelas populações de *M. glaucescens* de Brejões e da Cidade das Pedras, e a população com menor variabilidade foi a de *M. ernestii*. Alelos exclusivos foram observados para todas as populações, exceto as de *M. concinnus*, sendo os maiores números de alelos encontrados para as populações dos possíveis híbridos e as populações G03 e G01 de *M. glaucescens*.

Análise morfométrica revela alta variabilidade morfológica para as espécies analisadas, sendo maiores que as observadas para *M. paucispinus* (Lambert *et al*., no prelo) e próximas às reportadas para espécies de *Discocactus* (Machado *et al*., submetido). Em ambas as análises morfológicas feitas, a população de *M. ernestii* apresentou a maior variabilidade (contrastando com os dados de variabilidade genética),

enquanto que as populações de *M. concinnus* apresentaram os menores índices de variabilidade.

Nenhuma das populações analisadas encontra-se em equilíbrio de HW, sendo observado déficit e/ou excesso de heterozigotos para todos os doze loci em ao menos uma das populações, o que reflete em elevados valores de $F_{IS}$ para *M. glaucescens* e *M. concinnus*, podendo ser resultado de endogamia (para *M. glaucescens* uma endogamia biparental, visto que a espécie é auto-incompatível) e/ou sub-estruturação das populações, ambos em virtude do sistema de polinização e dispersão das mesmas.

Tanto *M. glaucescens* quanto *M. concinnus* apresentaram baixos índices de estruturação genética, indicando que as maiores variabilidades são encontradas dentro e não entre populações. Para *M. glaucescens*, entretanto, há um alto número de alelos exclusivos que evidenciam diferenciação genética entre suas população, no entanto estes estão em baixa freqüência. A presença de alelos exclusivos é um importante fator a ser levado em conta para a conservação das espécies.

Ao contrário da estruturação genética, moderados valores de estruturação morfológica foram encontrados tanto para *M. glaucescens* quanto *M. concinnus*. Em *M. glaucescens* esta estruturação deve-se à significante diferença entre os indivíduos das distintas populações, principalmente no que se refere ao comprimento e largura de espinhos e número e largura de costelas, aliado ainda aos diferentes tons de coloração dos frutos de cada população. Tal estruturação morfológica evidencia a necessidade de se conservar todas as populações de *M. glaucescens* para que se possa preservar a diversidade existente para a espécie.

Análises de agrupamento demonstram que *M. glaucescens* não forma um grupo isolado nem genética nem morfologicamente. As populações de *M. concinnus* separam-se das demais na análise genética, sendo tal fato explicado pela inversão na freqüência relativa nos alelos dos locos Dia-I e Dia-II. No agrupamento obtido por dados morfológicos, há diferenciação da população de *M. ernestii*. Apesar de estarem inseridas no grupo das populações de *M. glaucescens* e *M. concinnus*, as populações de *M. ×albicephalus* e dos possíveis híbridos estão mais próximos entre si, dando suporte para o reconhecimento dos híbridos também como *M. ×albicephalus*. Tais relações são todas evidenciadas na análise de CVA.

A ausência de loco diagnóstico não permitiu a confirmação da hipótese de hibridação testada neste estudo. A presença de alelos exclusivos nas populações de híbridos enfraquece nossa suposição, no entanto por se tratar de alelos raros os mesmo

podem não ter sido detectados nos parentais por artefato de amostragem ou ainda pela possibilidade de outras espécies estarem participando na formação destes híbridos (Taylor & Zappi, 2004). É possível admitir ainda que estes indivíduos (*M.* ×*albicephlaus*) sejam híbridos de gerações posteriores, já estabilizados e diferenciando-se dos parentais, apresentando alelos próprios, e assim o sendo haveria suporte para que os mesmos voltem a ser reconhecidos como espécie distinta. Características morfológicas e ecológicas intermediárias sustentam a hipótese de hibridação.

Com relação à conservação de *M. glaucescens*, o baixo número de populações associado ao reduzido número de indivíduos é motivo suficiente para reafirmarmos seu *status* de espécie criticamente ameaçada de extinção (Taylor & Zappi, 2004) e o fato destas ocorrerem sempre próximas a áreas antrópicas ou a rodovias tornam-nas ainda mais susceptíveis.

Elevado número de alelos exclusivos nas populações associado à diferenciação morfológica entre elas reforça a necessidade de preservação de todas as populações da espécie. Presença de sub-estruturação nas populações pode levar a diferenciação dentro das mesmas, tornando prioritária a conservação destas como um todo.

# Allozyme diversity and morphometrics of *Melocactus glaucescens* Buining & Brederoo (Cactaceae) and evidence for hybridization with congeneric species in the Chapada Diamantina, Northeastern Brazil

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Running title: Variability in *Melocactus* (Cactaceae) species

**Abstract**

*Melocactus glaucescens* (Cactaceae) is endemic to the Chapada Diamantina, Northeastern Brazil, and is crictically endangered. This species grows in sympatry with other congeneric species, and there is evidence of hybridation among them. We evaluated the levels of genetic and morphological variability and their sub-structuring between the populations of *M. glaucescens* and sympatric species, and we tested for the occurrence of natural hybridation. The genetic variability was investigated using 12 allozyme loci, and the morphological variability was investigated using multi- and univariate morphometric analyses of 18 vegetative characters in nine natural populations of *M. glaucescens*, *M. ernestii*, *M. concinnus* and two distinct morphs of supposed hybrids. The genetic variability was low in all populations ($P$ = 7.7–41.7, A = 0.3–1.7, $H_e$ = 0.009–0.096), and all taxa displayed a deficit in heterozygotes. Low levels of genetic structuring and moderate levels of morphological structuring were found for *M. glaucescens* ($F_{ST}$ = 0.045, $A$ = 0.16) and *M. concinnus* ($F_{ST}$ = 0.022, $A$ = 0.11). The results obtained with the genetic marker used are inconclusive to confirm the hypothesis of occurrence of hybridation due to an absence of diagnostic loci in the presumed parental species. However, this hypothesis cannot be refuted by the results either. The presence of rare alleles in the supposed hybrids may be an indication that these populations are already stable and in the process of differentiating from their parental species. The large number of exclusive alleles and the levels of morphological structuring in the populations of *M. glaucescens* are important factors to be considered in the definition of strategies for the conservation of the species.

*Keywords*: Cactaceae, *Melocactus*, genetic diversity, morphological variability, morphometrics.

## Introduction

The genus *Melocactus* Link & Otto comprises 36 species (Anderson 2001) with a wide distribution in the Americas, ranging from Southeastern and Northeastern Brazil and the Amazonian region, northwards to the Caribbean (up to Cuba) and Mexico, and westwards to the Andes and Central America (Taylor 2000). Although of wide distribution, the greatest concentration of taxa and centre of diversity lies within eastern Brazil, especially in the state of Bahia – of a total of 22 species and subspecies recognized by Taylor & Zappi (2004) to occur in eastern Brazil, 18 are endemic to the core area. Plants in the genus are characterized by having a small globose to slightly elongated, unbranched stem, the fertile part of the stem differenced in a terminal cephalium. Flowers are diurnal, small, embedded within the cephalium with only the perianth segments visible; according to Taylor (1991) most species are self-compatible, but floral adaptations promote hummingbird-mediated cross-pollination. Fruits are small turbinate berries, with small black seeds embedded in the watery pulp; seeds are locally dispersed by lizards and ants (Taylor 1991; Taylor & Zappi 2004; Fonseca 2004).

Species of Melocactus are collected and commercialized by local communities due to their ornamental value. Since the collection and commercialization of plants occur indiscriminately, those species with a more restricted distribution, occupying specific areas and having a small number of individuals in the populations, are at risk of being extinct with a single collection (Taylor 1991; Taylor & Zappi, 2004).

*Melocactus glaucescens* Buining & Brederoo is a species endemic to the municipality of Morro do Chapéu, Chapada Diamantina, Bahia, Brazil. Only four pure populations occupying an area of less than 10 $km^2$ are known for the species (Taylor & Zappi 2004). Due to its rarity and desirability to collectors, since 1992 the species has been listed in Appendix I of CITES (Taylor & Zappi 2004); the species has also been listed in the IUCN Red List of Threatened Species (IUCN 2004) with the status of Critically Endangered, due to its quite local distribution and generally small population sizes.

This species is one of the most distinctive in the genus due to the presence of a whitish to creamy-yellow cephalium entirely composed of wool (other species in the genus develop reddish to brownish bristles in the cephalium, making the cephalium appear reddish in color), small magenta flowers and entirely red or magenta fruits, this

combination of traits not being found in any other species of the genus (Taylor 1991). Plants of *M. glaucescens* differ morphologically from population to population, mainly in the level of glaucousness of the epidermis, the number, height and width of the ribs, and the size and diameter of the spines. These characteristics helps in distinguishing the different populations of the species and can be used to identify the population of provenance of plants based on their appearance (Fig. 1A-D).

In one of its known populations *M. glaucescens* grows in sympatry with *M. ernestii* Vaupel (Fig. 1E). The latter species is also well-characterized and distinctive within the genus due to the reddish to yellowish color and great length of its spines, particularly the lowermost. *M. ernestii* has a wide distribution in Brazil, having been recorded from the states of Alagoas, Bahia, Minas Gerais, Paraíba, Pernambuco and Sergipe. *Melocactus glaucescens* and *M. ernestii* seem to hybridize in nature, and the resulting hybrid taxon was even described as a distinct species, *M. albicephalus* Buining & Brederoo. Nowadays its status as natural hybrid is generally accepted, the taxon being accordingly renamed as *Melocactus* ×*albicephalus* (Taylor & Zappi 2004).

Several individuals with characteristics intermediate between *M. glaucescens* and *M. ernestii* can be found in the area where the two species grow in sympatry, and it is possible to recognize two different morphs among these individuals. One of the morphs possess markedly intermediate characteristics, being composed in the majority of glaucous individuals very similar to *M. glaucescens*, but with smaller bodies, thicker spines of greater length and usually straw-yellow in color, and a whitish cephalium with reddish bristles mainly restricted to its periphery. Individuals with these characteristics are traditionally recognized as *M.* ×*albicephalus* (Fig. 1G), and are treated as such in this work. Individuals of the second morph are more similar to *M. glaucescens*, being characterized by the large size of their bodies, the often not glaucous epidermis, whitish to creamy-yellow cephalium but with reddish bristles present, variable spines but generally with smaller diameter than those of *M.* ×*albicephalus*, and ribs less prominent than the latter. These individuals are a priori regarded as possible first generation hybrids and / or displaying introgression with *M. glaucescens* (Fig. 1H-I).

Evidence of hybridization and introgression of *M. glaucescens* are found in another area of occurrence of the species in the municipality of Morro de Chapéu, where it grows in sympatry with *M. concinnus* Buining & Brederoo (Fig. 1F). According to Taylor and Zappi (2004) there is a hybrid swarm in this area, being no longer possible to find pure individuals of *M. glaucescens*, only introgressant

individuals. Also according to these authors, the level of variation found in this hybrid population suggest that other species besides *M. glaucescens* and *M. concinnus* may have been involved in the hybridization process.

Hybridization and introgression are recognized among the processes that can lead to an increase in the genetic variability in natural populations via the genetic recombination that they promote, and thus these processes are very important for evolution (Lewontin & Birch 1966; Arnold 1997; Barton 2001). However, these processes can also cause the impoverishment of biodiversity by allowing the fusion of two different species via interspecific gene flow, what for Rhymer & Simberloff (1996) is maximized if these species occur sympatrically and are congeneric; the creation of hybrid swarms is one of the most extreme effects of introgressive hybridization (Forbes & Allendorf 1991) and can lead to the local extinction of one or both parental species by inhibiting the growth of the populations and negatively affecting its effective reproduction, its competitive status and its ecological interactions (Levin *et al*. 1996). This effect is particularly important when concerning rare species or when the number of individuals in a population is very small. In this last scenario it is possible for a species to disappear completely due to introgression – if introgression occurs for several generations in a given population, the resulting hybrid swarms can lead to the total disappearance of the parental taxa and their replacement by the hybrid individuals. In this way, hybridization and introgression can affect the genetic, morphological and ecological integrity of a taxon (Allendorf & Leary 1988; Hayes *et al*. 1996), and can also lead to inbreeding depression (Templeton 1986).

The objective of this study was to assess levels of genetic and morphological variation and sub-structuring within and between populations of *Melocactus glaucescens*, and to verify the occurrence of natural hybridization with *M. ernestii* and *M. concinnus*, via the utilization of allozyme markers and morphometric data. This study is part of a project for the conservation and management of species of Cactacteae and other endangered plant groups in the Chapada Diamantina, Bahia state, involving studies on the demography, biology, variability, propagation and ethnobotany of the plants; the results obtained here can aid in the determination of priority areas for conservation of the species in the state.

## Materials and methods

### *Populations sampled*

A total of 278 individuals were sampled in nine natural populations of *M. glaucescens* (4 pop., 116 individuals), *M. ernestii* (1 pop., 25 individuals), *M. concinnus* (2 pop., 44 individuals), *M.* ×*albicephalus* (1 pop., 40 individuals) and possible hybrids of first generations and/or introgressants (1 pop., 36 individuals) occurring in the Chapada Diamantina, Northeastern Brazil (Table 1; Fig. 1). All individuals sampled consisted of mature individuals as evidenced by the presence of a well-developed cephalium. Vouchers of the species are deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS).

### *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 four 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 *et al*. (1970); 3) electrode: citric acid 0.04 M adjusted to pH 6.1 with N-(3-aminopropyl)-morpholine; gel: electrode solution diluted 1:20; modified from Clayton and Tretiak (1972); 4) electrode: boric acid 0.3 mol/L, NaOH 0.06 mol/L, pH 8.0; gel: Tris 0.01 mol/L, pH 8.5; modified from Shaw and Prasad (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 - 130 V; systems 2, 3 and 4 - 25 mA. Nine enzymatic systems gave enough resolution for reading and were used. System 1 was used for malate dehydrogenase locus 1 (*Mdh*; EC 1.1.1.37); system 2 was used for malate

dehydrogenase locus 2, phosphoglucomutase (*Pgm*; EC 2.7.5.1), isocitrate dehydrogenase (*Idh*; EC 1.1.1.42), and shikimate dehydrogenase (*Skdh*; EC: 1.1.1.25); system 3 was used for diaphorase (*Dia*; EC 1.8.1.4); and system 4 was used for acid phosphatase (*Acp*; EC 3.1.3.2), leucine aminopeptidase (*Lap*; EC 3.4.11.1), glucose-6-phosphate dehydrogenase (*G6pdh*; EC 1.1.1.49) and phosphoglucose isomerase (*Pgi*; EC. 5.3.1.9). The staining procedures were similar to but slightly adjusted from Brune *et al*. (1998; *Acp*, *Lap*, *Dia*, *Skdh*, *G6pdh*), 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 ascending order from the locus with lowest mobility. The alleles were numbered according their mobility relative to the allele with highest mobility of a standard individual present in all gels and designated as 100.

***Analyses of allozyme data***

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 $\chi^2$ 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). All analyses were made using the BIOSYS 1.0 software package (Swofford & Selander 1989), except for the cluster analysis that was performed in the software package STATISTICA, Release 5.5 A (StatSoft 2000).

***Morfometric analyses***

The individuals sampled for the allozyme analysis were also used in analyses of morphological variability, where measures of 18 vegetative morphological characters were utilized (Table 2). The characters were chosen based on both previous fieldwork and the literature regarding the taxonomy of the group and morphometrics of cacti species (Baker & Pinkava 1987; Chamberland 1997; Casas *et al*. 1999; Baker & Johnson 2000; Thomson 2002). All measurements of continuous quantitative characters were taken with the aid of a vernier caliper. The values for the spine characters represent an average of the measurements of four areoles located on different ribs, the measurements being made always on the fourth areole along the rib, counting from the apex of the plant.

Patterns of morphological similarity/difference were analyzed by multivariate statistical methods using the software package STATISTICA, Release 5.5 A. The analyses included 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. CVA was performed with population as the categorical variable (individuals were grouped according to the population to which they belonged). The standardized coefficients for canonical variables resulting from CVA were used to identify the characteristics that most significantly contribute to the resulting patterns observed. The measures of the 18 vegetative morphological characters were analyzed using discriminant analysis with population as the grouping variable in order to obtain a matrix of squared Mahalanobis distances of individuals to the centroid of the group (D2); the morphologic variability of populations was calculated as the median of these distances ($D2_m$). We used the median of the squared Mahalanobis distances instead of an average of these distances because of the non-normal distribution of the data. We carried out a variance analysis of all taxons, for each variable separating (ANOVA ONE-WAY). The significance of the differences between the means was tested using the Tukey's test, with $p<0.05$. Cluster Analysis was carried out on a matrix of morphological distance among populations using Mahalanobis Generalized Distance as the distance coefficient, and UPGMA was used as clustering algorithm (Sneath & Sokal 1973).

A multi-response permutation procedure (MRPP) analysis was carried out with the PC-ORD 4.10 program (McCune & Mefford 1999) to calculate the chance-corrected

within-group agreement (*A*) among populations of every species. The *A* values were compared with the indexes of genetic differentiation among conspecific populations ($F_{ST}$) (Borba *et al*. 2002). The average Euclidian distance (ED) between the individuals of each population resulting from the MRPP analysis was also utilized as a measure of variability within populations (Borba *et al*. 2002). The two indices of morphological variability are essentially different, as $D2_m$ is more affected by form and ED is more affected by size of the characters.

A Spearman rank correlation analysis between the morphological (ED and $D2_m$) and genetic ($H_e$) variability of populations was carried out using the software package STATISTICA, Release 5.5 A.

## Results

### *Intra-populational variability*

Using nine enzymatic systems 12 loci were obtained with good resolution and were used in this study (Table 3). The population G01 of *M. glaucescens* displayed a duplicate locus for *Acp*, with 31 out of 33 individuals sampled having more than one allele in this enzymatic system (fixed heterozigosity). This duplication was not found in any other population, and in no other enzymatic system for this population.

All the loci were polymorphic. *Pgi* and *G6pd* displayed low polymorphism with only two alleles in each of these systems. The most polymorphic locus was *Lap* with six alleles (Table 3). Some alleles were exclusive to a given species: *Pgm-1* 71, *Pgm-1* 95, *Pgm-2* 98, *Lap* 94, *Lap* 96, *Lap* 107, *Lap* 112, *Lap* 116, *G6pd* 110, *Mdh-1* 130, *Mdh-2* 80, *Acp* 92, *Dia-1* 104 and *Dia-2* 107 for *M. glaucescens; Pgm-2* 107 for *M. ernestii; Idh* 79 for *M. ×albicephalus; Pgi-1* 93, *Skdh* 85, *Idh* 84, *Acp* 96, *Dia-1* 93 and *Dia-2* 92 for the populations of the supposed hybrids; and *Skdh* 89 and *Dia-1* 96 for *M. concinnus*. Many alleles were exclusive to single populations: *Pgm-1* 95, *Pgm-2* 98, *Lap* 96, *Mdh-2* 80 (G01), *Lap* 116, *Dia-1* 104 (G02), *Pgm-1* 71, *Lap* 112, *Mdh-1* 130 (G03), *Acp* 92 (G04), *Pgm-2* 107 (E01), *Idh* 79 (A01) E *Pgm-1* 93, *Skdh* 85, *Idh* 84, *Acp-2* 96, *Dia-1* 93, *Dia-2* 92 (H01). However, none of the species were fixed for alternative alleles at any locus, and thus no locus was diagnostic for any of the species (Table 3).

Percentage of polymorphic loci (*P*; 0.95 criterion) ranged from 7.7 to 41.7%, the

mean number of alleles per locus (A) varied from 0.3 to 1.7, and mean heterozygosity ($H_e$) ranged from 0.009 to 0.096 (Table 4). The populations C01, C02, G03 and G01 presented the greatest genetic variability, while the population E01 presented the smallest genetic variability.

All of the nine populations showed significant deviations from the expected values in HW equilibrium in at least one locus, and three of these did not have any locus in equilibrium (G01, E01 and A01). All of the 12 polymorphic loci investigated were not in HW equilibrium in at least one population, and seven were not in HW equilibrium in all populations were they were polymorphic (*Pgm-1*, *Pgm-2*, *Pgi*, *Lap*, *Skdh*, *Dia-1*, *Dia-2*). The reason for disequilibrium was deficit or excess of heterozygotes: all polymorphic loci (except *Idh* and *Mdh-1*) displayed deficit of heterozigotes in at least one population, four displayed deficit of heterozygotes in all populations were they were polymorphic (*Pgm-2*, *Skdh*, *Dia-1*, *Dia-2*), and six displayed excess of heterozygotes in at least one population (*Pgm-1*, *Pgi*, *Lap*, *Idh*, *Mdh-1* and *Acp*). The high values for $F_{IS}$ (Table 5) reflect the deficit or excess of heterozygotes in the populations.

In the discriminant analysis, the population with the highest score of morphological variability ($D2_m$) was E01 of *M. ernestii* (that had the lowest score of genetic variability), followed by the populations G01 and G04 of *M. glaucescens*. The population with lowest score of morphological variability was C02 of *M. concinnus*, that displayed the second highest score of genetic variability (Table 4). All the morphologic characters analyzed displayed a large amount of variation for all the taxa investigated. In the MRPP analysis, the population with the highest score of morphological variability (ED) was A01, followed by the populations G01, G04 and H01; the population C01 of *M. concinnus* had the lowest score of morphological variability. Spearman rank correlation analysis between morphological and genetic variability resulted in an absence of statistically significant correlation between these data ($H_e$ and $D2_m$ , r= 0.550, p=0.125; $H_e$ and DE, r= 0.486, p=0.329).

*M. glaucescens* and *M. concinnus* presented low values for $F_{ST}$ (0.048 and 0.022, respectively), indicating a low level of genetic structuring for the species. When the duplicated locus of *Acp* is added to the analysis, the value of $F_{ST}$ for *M. glaucescens* is raised to 0.317, forcing a higher genetic structuring. Moderate levels of morphologic structuring were found for both species, with *A* values of 0.16 for *M. glaucescens* and 0.11 for *M. concinnus* (Table 5).

***Phenetic relationships***

The genetic identity between cospecific populations ranged from 0.996 to 0.999 for *M. glaucescens*, and in *M. concinnus* it was 1.000 (Table 6). Among the conspecific populations of *M. glaucescens* the highest genetic identity was between the populations G01 and G04, and the lowest was between the populations G03 and G04. Between the not conspecific populations, *M. ×albicephalus* (A01) displayed highest genetic identity with G04 (1.000), and the supposed hybrids (H01) displayed highest genetic identity with E01 (1.000). The genetic identity between A01 and H01 was 0.999. The lowest genetic identities were found between the populations of *M.* concinnus and the populations of the others taxas. The genetic identity between species ranged from 0.916 (*M. glaucescens* with *M. concinnus*) to 1.000 (supposed hybrids with *M. ernestii*).

The dendrogram obtained from the matrix of Nei's (1978) genetic distance (Fig. 2A) shows that two groups are formed with a distance of 0.075 between them. One group comprises the two populations of *M. concinnus* without any genetic distance between them, and the other group comprises the populations of *M. glaucescens*, *M. ernestii*, *M. ×albicephalus* and the supposed hybrids. In this second group, the pairs of populations A01 + G04 and E01 + H01 also did not have any genetic distance between them. The populations of *M. glaucescens* do not cluster together, having included among them the populations of *M. ×albicephalus*, *M. ernestii* and of the supposed hybrids. When the duplicated locus of *Acp* is included in the analysis there is a separation of the population G01 of *M. glaucescens* from the remaining populations of all taxa.

The dendrogram obtained from the matrix of squared Mahalanobis distances of the discriminant analysis (Fig. 2B) resulted in the separation of *M. ernestii* from a bigger group containing the populations of all the remaining taxa. This bigger group is subdivided in two groups: the first comprises *M. ×albicephalus*, the supposed hybrids and the population G03 of *M. glaucescens*; the second comprises the populations of *M. concinnus* and the remaining populations of *M. glaucescens*.

The percentage of correct classifications ranged from 84 to 100% (Table 7). The incorrect classifications mostly occurred between conspecific populations, except for one individual each from populations G02 and G04 (*M. glaucescens*) that were classified as C01 (*M. concinnus*), and three individuals of C01 incorrectly classified as G02. The high percentage of incorrect classifications of individuals from C01 in G02

explains the proximity of these populations in the cluster analysis.

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. 3. The first, second and third canonical roots from the CVA analysis explained respectively 71.38%, 10.34% and 8.24% of the morphological variation, summing up 89.96% of the observed variability. In the first canonical root there is a separation between *M. ernestii* (population E01), the hybrid populations (A01 and H01) and the populations of *M. glaucescens* and *M. concinnus*. The separation of the population E01 was mainly a result of the higher length of the central (variable 9) and lowermost radial (variable 13) spines, and the higher number of ribs (variable 3) and radial spines (variable 11). In the second canonical root the populations of all taxa are distributed along a gradient (Fig. 3B), with the populations G01 and G03 positioned in the extremities. The separation of the population G01 was due to its low number of ribs (variable 3), the smaller length of the lowermost radial spine (variable 13) and the taller cephalium (variable 17). The separation of the population G03 was due for higher values of widtht at 2/4 of the rib (variable 6), length of central spine (variable 9) and length of the left radial spine (variable 14). All the morphometric measures of A01 and H01 were intermediate between those of *M. glaucescens* and *M. ernestii*.

## Discussion

### *Variability*

In the analysis of genetic variability conduced, the *Melocactus* species displayed levels of genetic variability higher than those obtained for *M. paucispinus* (*P*= 9.98%, A= 1.17, $H_e$= 0.031; Lambert *et al*. in press.), a species that occur in the same region in the Chapada Diamantina. However, the levels of genetic variability of the *Melocactus* species investigated are lower than those found for *M. curvispinus* (*P*= 89.5%, A= 3.82, $H_e$= 0.145; Nassar *et al*. 2001) and lower than the average values reported for other cactus species (Parker & Hamrick 1992; Nassar *et al*. 2001, 2002, 2003; Hamrick *et al*. 2002; Clark-Tapia & Molina-Freaner 2003; Machado M. C. *et al*. unpublished data); the values were also lower than the average values reported for other plant species with similar characteristics – woody, long-lived, animal-pollinated plants (Hamrick & Godt 1990; Colunga-GarcíaMarín *et al.* 1999; Martínez-Palacios *et al*. 1999). The genetic variability displayed by *M. glaucescens* is close to the average value reported for

endemic species ($H_e$= 0.063; Hamrick & Godt 1992), this species may have passed by a genetic bottleneck in the recent past, what in association to the colection of individuals and general vulnerability of the populations could be leading to a decrease in the genetic variability of the species.

The duplication of the *Acp* locus in the population G01 was present in all the individuals analysed, except in two individuals, where the duplication seemed either to be absent or was silenced. In spite of studies which indicate that some individuals of this population are polyploid (Assis *et al*. 2003), the duplication of only one locus suggests that in this case single gene duplication is a more likely explanation than polyploidy. The most common allele in this locus (allele 100) is fixed for the remaining populations of the species, and the other allele (allele 81) is rare, indicating that a duplication event has occurred. If this duplication has any adaptative value, this event can lead to the differentiation of this population and eventually to speciation (Kreitman & Akashi 1995).

The morphometric analyses show that all taxa possess high levels of variability, generally higher than those found for *M. paucispinus* ($D2_m$= 11.14, ED= 4.09; Lambert *et al.* in press) and close to those reported to species in the genus *Discocatus* (*D. bahiensis* – $D2_m$= 14.28, *D. catingicola* - $D2_m$= 26.48, *D. zehntneri* - $D2_m$= 17.03; Machado M. C. *et al*. unpublished data). Similar studies regarding intra and inter-populational morphological variability are rare in the family Cactaceae (Casas *et al*. 1999; Baker & Johnson 2000; Schmalzel *et al*. 2004).

The separation of the populations of *M. concinnus* from the populations of the other species in the cluster analysis of the genetic data can be explained by an inversion in the relative frequencies of the most common alleles in the loci *Dia-1* and *Dia-2*. *Melocactus concinnus* is quite different genetically from all the other taxa investigated, however it is morphologically very similar to *M. glaucescens*. The opposite occurs with *M. ernestii* – it is morphologically very distinct from the other taxa, but genetically it differs little from *M. glaucescens* and the hybrids. This can be more a result of the general lack of variability than an indication of close proximity, since *M. ernestii* is regarded as belonging to a species group different than that of *M. glaucescens*. It is important to point out that *M. glaucescens* is considered very close to *M. concinnus* (Taylor 1991; Taylor 2000; Taylor & Zappi 2004), although these species are genetically more distinct than *M. ernestii* and *M. glaucescens*. The populations G04, E01, A01 and H01 are those with the highest number of polymorphic loci and this seem

to be the reason for their clustering in the genetic analyses.

The values of $F_{IS}$ reveal a high level of endogamy. In spite of being smaller than those found for *M. paucispinus* ($F_{IS}$ = 0.732; Lambert *et al*. in press.), they are higher than those observed for *M. curvispinus* (Nassar *et al*. 2001) and for other cactus species (*Pereskia guamacho* - $F_{IS}$= 0.301, Nassar *et al*. 2002; *Stenocereus griseus* - $F_{IS}$=0.145, Nassar *et al.* 2003; *Cereus repandus* - $F_{IS}$= 0.182, Nassar *et al*. 2003; *Pilosocereus lanuginosus* - $F_{IS}$= 0.176, Nassar *et al*. 2003; *Stenocereus gummosus* - $F_{IS}$= 0.608, Clark-Tapia & Molina-Freaner 2003; *Pilosocereus machrisii* - $F_{IS}$= 0.025 - 0.569, Moraes *et al.* 2005; *P. euchlorus* - $F_{IS}$=-0.276 - 0.529, Moraes *et al.* 2005). This data indicate moderate to high levels of endogamy for the family Cactaceae as a whole.

The high deficit of heterozigotes can be the result of genetic sub-structuring of the populations and/or endogamy. The behavior of the pollinators of the species can be the cause of the genetic sub-structuring, because *Melocactus* species are pollinated by hummingbirds (Taylor 1991), with *Clorostilbon aureoventris* being the most common visitor for *M. glaucescens* (Colaço M. A. *et al.* unpublished data). This hummingbird species is territorialist, and due to this behavior it may promote gene flow only among the plants belonging to its territory, what would contribute for the genetic sub-structuring of the *Melocactus* species. Because *M. glaucescens* is a self-incompatible species (Colaço M. A. *et al.* unpublished data), endogamy could not occur directly. However, biparental endogamy – crosses between related individuals – can occur. Biparental endogamy is an important factor for reducing the heterozygosity in relatively small populations and raises their probability of extinction because it affects negatively the fitness of their individuals, decreasing their viability and fecundity and raising their susceptibility to environmental stress (Handel 1983; Heywood 1991; Hauser & Loescheke 1995; McCauley *et al*. 1996; Bijlsma *et al*. 2000). Seed dispersal in *Melocactus* species is mostly local, with lizards and ants being the main dispersers (Taylor 1991; Fonseca 2004), and according to Nassar *et al*. (2001), the limited dispersal ability of these animals may contribute to the processes of endogamy and sub-structuring within the populations.

Values of genetic identity between conspecific populations were similar to those observed in *M. paucispinus* (Lambert *et al.*, in press) and also to those reported for other plant species (Thorpe 1982; Crawford 1989; Borba *et al*. 2001; Jesus *et al*. 2001). Nassar *et al* (2001) found similar values of genetic differentiation among populations of *M. curvispinus*. Similarity in the pollination and dispersal mechanisms suggests that

they are conserved in the genus as a whole. The low values of $F_{ST}$ found for *M. glaucescens* and *M. concinnus* reflect a low genetic structuring for the species, and lower than those found for other cactus species (*Lophocereus schottii* - $F_{ST}$= 0.130, Parker & Hamrick 1992; *Melocactus curvispinus* - $F_{ST}$ = 0.193, Nassar *et al*. 2001; *Pereskia guamacho* - $F_{ST}$= 0.112, Nassar *et al*. 2002; *Stenocereus gummosus* - $F_{ST}$= 0.10, Clark-Tapia & Molina-Freaner 2003).

The presence of exclusive alleles in all populations of *M. glaucescens* indicates genetic differentiation among its populations, but the frequency of the alleles is low. The moderate *A* values found in both *M. glaucescens* and *M. concinnus* indicate some morphological structuring in these species. In the case of *M. glaucescens* this morphological structuring can be explained by the significative morphological differences between individuals belonging to each population sampled, mainly related to the length and width of the spines, and number and width of the ribs. Besides these morphometric differences, fruit color varies from population to population (deep red in G01 and different hues of magenta in the other populations), strengthening the morphological structuring of the species.

In the dendrogram obtained from the cluster analysis of morphological distances, the population G03 of *M. glaucescens* clusters with the supposed hybrids (H01) and *M.* ×*albicephalus* (A01). This result can be explained by the fact of plants from G03 having longer spines, what makes it more similar to the hybrid plants which also have longer spines, possibly due to the influence of *M. ernestii*. In this dendrogram *M. concinnus* populations (C01 and C02) are placed among the *M. glaucescens* populations; this indicates the great morphological similarity of these species, in spite of the high genetic differences separating them. The clustering of A01 and indicate that there is strong similarity between these populations, and support that individuals from H01 may also be identified as *M.* ×*albicephalus*.

**Hybridization**

Of the *M. glaucescens* populations, the one that co-occurs with *M. ernestii* and apparently hybridizes with it is G01. In all the remaining *M. glaucescens* populations there is either no sympatry with other *Melocactus* species, or no evidence of hybridization among them. In the area where G01 occur also grows *M. ernestii* and plants that have been identified as *M.* ×*albicephalus*. Actually, the plants of all these

taxa grow more or less mingled, the only pattern that can be observed being the different ecological preferences of each taxon – *M. ernestii* is rupicolous, while *M. glaucescens* is arenicolous and the hybrids are found in gravelly areas or growing in the same environment as one of the presumed parents. In this area (Lages or Cidade das Pedras) we collected individuals of *M. glaucescens* (grouped in the analyses as population G01), individuals of *M. ernestii* (grouped in the analyses as population E01), individuals that most closely resemble what has been described as *M.* ×*albicephalus* (grouped in the analyses as population A01) and supposed hybrid individuals that were considered first generation hybrids and/or introgressants with *M. glaucescens* (grouped in the analyses as population H01). The other populations of *M. glaucescens* (G02, G03, G04) did not grow in sympatry with any other *Melocactus* species.

There is support for the hybridization hypothesis tested in this study, but it cannot be confirmed with basis in the genetic marker utilized, since no diagnostic loci were found in the presumed parental populations. The presence of seven exclusive alleles in the supposed hybrids or introgressants and one exclusive allele for *M.* ×*albicephalus* weaken such assumption since these alleles should also be present in the parental species. These exclusive alleles were rare, and may have not been detected in the parental species due to a sampling artifact; alternatively, other species may have been involved in the formation of these hybrids (Taylor & Zappi 2004). Another possible explanation is that these hybrid individuals, including *M.* ×*albicephalus*, may represent hybrids of later generations which are already stabilized and differenced from the parental species with the development of exclusive alleles. If this is the case, these supposed hybrids could be recognized as comprising an already distinct species.

In spite of some individuals from both hybrid populations (A01 and H01) display morphological characteristics close to those of *M. glaucescens* (as evidenced in the CVA analysis), the occurrence of the two taxa in sympatry weaken the idea that the supposed hybrid populations could in fact be differenced *M. glaucescens* populations. Another evidence that supports the hybridization hypothesis is that the hybrids individuals have an ecological niche which is intermediate between those of *M. glaucescens* (arenicolous, shaded places) and *M. ernestii*. (rupicolous, fully exposed places): the supposed hybrids investigated are found in gravelly places at the edges of the rock outcrops, and sometimes also in the rocks and in the sand, with exposure to the sun varying from shaded areas to fully exposure.

**Conservation of *Melocactus glaucescens***

The low number of populations of *M. glaucescens* as well as the small quantity of individuals in each of these populations are already good reasons for a higher attention be given to this species, and we reaffirm its status of critically endangered (Taylor & Zappi 2004). The fact that all populations are located in places of easy access, usually close to roads or in agricultural areas, make them susceptible to be decimated quickly either by criminous collection of plants or agricultural use of the areas where they occur.

The high number of exclusive alleles present in the populations of *M. glaucescens* is an important factor to be considered for the conservation of the species, since the loss of any population mean a loss in the genetic variability of the species, which as a whole is already low. Besides the genetic variability present in the populations there is an evident morphological differentiation among them, what reinforces the need to preserve both *in situ* and *ex situ* all the populations of *M. glaucescens*.

Among the populations of *M. glaucescens* the one with highest variability is G01, which have the highest number of polymorphic loci (eight) and exclusive alleles (four), besides having a duplicated locus, all of which summed up are a very important source of variability for the species. From this population comes the type of the species, and however it is the most degraded population of all populations of *M. glaucescens*. During the field work for this study we observed that a high number of individuals on this population were destroyed by domestic animals that graze in the area, that had eaten the plants during dry spells when little other vegetation was available. A plan of management for this population must consider a possible need to control the populations of *M. ×albicephalus* and *M. ernestii*, otherwise the outnumbered population of *M. glaucescens* can be extinct via hybridization (Rhymer & Simberloff 1996), as seems to have happened in another area where this species occurred (Taylor 1991).

The occurrence of population sub-structuring is another fact to be considered since these populations are not panmictic, what can cause the differentiation within them. This implies that the populations should be preserved in their whole area of occupancy, with special attention to be given to the edges of the population where the individuals are more exposed to the action of fire and animals. Also, special attention should be given to isolated groups of individuals, where the chances of genetic differentiation to be occurring are even higher. The absence of correlation between the

genetic and morphologic data points out that the dissociated use of one or another of these sets of information to make decisions on the conservation of the species would not cover the totality of the existing variation, thus it is crucial that these data sets be analyzed in conjunction.

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**References**

Allendorf F. W. & Leary R. F. (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* **2**: 170-184.

Anderson E. F. (2001) *The Cactus Family*. Timber Press, Portland.

Arnold M. L. (1997) *Natural Hybridization and Evolution*. Oxford University Press, Oxford.

Assis J. G. A., Oliveira A. L. P. C., Resende S. V., Senra J. F. V. & Machado M. C. (2003) Cromossome numbers in Brazilian *Melocactus* (Cactaceae). *Bradleya* **21**: 1-6.

Baker M. A. & Pinkava D. J. (1987) A cytological and morphometric analysis of a triploid apomict, *Opuntia* ×*kelvinensis* (subgenus Cylindropuntia, Cactaceae). *Brittonia* **39**: 387-401.

Baker M. A. & Johnson R. A. (2000) Morphometric analysis of *Escobaria sneedii* ver. s*needii*, *E. sneedii* var. *leei,* and *E. guadalupensis* (Cactaceae). *Systematic Botany* **25**: 577-587.

Barton N. H. (2001) The role of hybridization in evolution. *Molecular Ecology* **10**: 551-568.

Bijlsma R., Bundgaard J. & Boerema A. C. (2000) Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. *Journal of EvolutionyBiology* **13**: 502-514.

Borba E. L., Felix J. M., Solferini V. N. & Semir J. (2001) Fly-pollinated *Pleurothallis* (Orchidaceae) species have high genetic variability: evidence from isozyme markers. *American Journal of Botany* **88**: 419-428.

Borba E.L., Shepherd G. J., van den Berg C. & Semir J. (2002) Floral and vegetative morphometrics of five *Pleurothalis* (Orchidaceae) species: correlation with taxonomy, phylogeny, genetic variability and pollination systems. *Annals of Botany* **90**: 219-230.

Brune W., Alfenas A. C. & Junghans T. G. (1998) Identificações específicas de enzimas em géis. In: Alfenas A. C. (ed). *Eletroforese de Isoenzimas e Proteinas Afins: Fundamentos e Aplicações em Plantas e Microorganismos*. Universidade Federal de Viçosa, Viçosa, pp.201-328.

Casas A., Caballero J., Valiente-Banuet A., Soriano J. A. & Dávila P. (1999) Morphological variation and the process of domestication of *Stenocereus stellatus* (Cactaceae) in Central México. *American Journal of Botany* **86**: 522-533.

Chamberland M. (1997)**.** Systematics of the *Echinocactus polycephalus* complex (Cactaceae). *Systematic Botany* **22**: 303-313.

Clark-Tapia R. & Molina-Freaner F. (2003) The genetic structure of a columnar cactus with a disjunct distribution: *Stenocereus gummosus* in the Sonoran desert. *Heredity* **90**: 443-450.

Clayton J. W. & Tretiak D. N. (1972) Amine-citrate buffers for pH control in starch gel eletrophoresis. *Journal of Fisheries Research of Board Canada* **29**: 1169-1172.

Colunga-GarcíaMarín P., Coello-Coello J., Eguiarte L. E. & Piñero D. (1999) Isozymatic variation and phylogenetic relationships between henequen (*Agave fourcroydes*) and its wild ancestor *A. angustifolia* (Agavaceae). *American Journal of Botany* **86**: 115-123.

Corrias B., Rossi W., Arduino P., Cianchi R. & Bullini L. (1991) *Orchis longicornu* Poiret in Sardinina: genetic, morphological and chorological data. *Webbia* **45**: 71-101.

Crawford D. J. (1989) Enzyme eletrophoresis and plant systematics. In: Soltis E. D. & Soltis P. S. (eds). *Isozymes in Plant Biology*. Dioscorides Press, Portland, pp.146-164.

Fonseca R. B. S. (2004) *Fenologia Reprodutiva e Dispersão de Melocactus glaucescens Buining & Brederoo e M. paucispinus G. Heiman & R. Paul (Cactaceae) no Município de Morro do Chapéu, Chapada Diamantina – Bahia – Brasil*. Master

Thesis. Universidade Estadual de Feira de Santana, Feira de Santana, Brazil.

Forbes S. H. & Allendorf F. W. (1991) Mitochondrial genotypes have no detectable effects on meristic traits in cutthroat trout hybrid swarms**.** *Evolution* **45**: 1350-1359.

Hamrick J. L. & Godt M. J. (1992) Allozyme diversity in plant species. In: Brown A. H. D., Clegg M. T., Kahler A. L. & Weir B. S. (eds). *Plant Population Genetics, Breeding and Genetic Resources*. Sinauer, Sunderland, pp.43-63.

Hamrick J. L., Nason J. D., Fleming T. H. & Nassar J. M. (2002) Genetic diversity in columnar cacti. In: Fleming T. H. & Valiente-Banuet A. (eds). *Columnar Cacti and their Mutualisms: Eevolution, Ecology and Conservation*. University of Arizona Press, Tucson, pp.22-133.

Handel S. N. (1983) Polination ecology, plant population structure, and gene flow**.** In: Real L. (ed). *Polination Biology*. Academic Press, Orlando, pp.163-211.

Hauser T.P., Loescheke V. (1995) Inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae): effects of different levels of inbreeding. *Journal of Evolutionary Biology* **8**: 589-600.

Hayes J. P., Guffley S. Z., Kriegler F. J., McCracken G. F. & Parker C. R. (1996) The genetic diversity of native, stocked, and hybrid populations of brook trout in the southern Appalachians. *Conservation Biology* **10**: 1403-1412.

Heywood J. S. (1991) Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* **22**: 335-355.

IUCN (2004) *2004 IUCN Red List of Threatened Species.* [17 Apr. 2005]. Available from URL: http://www.redlist.org.

Jesus F. F., Solferini V. N., Semir J. & Prado P. I. (2001) Local genetic differentiation in *Proteopsis argentea* (Asteraceae), a perennial herb endemic in Brazil. *Plant Systematics and Evolution* **226**: 59-68.

Kreitman N. & Akashi H. (1995) Molecular evidence for natural selection. *Annual Review of Ecology and Systematics* **26**: 403-422.

Lambert S. M., Borba E. L., Machado M. C. & Andrade S. C. S. (In press) Allozyme diversity and morphometrics of *Melocactus paucispinus* (Cactaceae) and evidence for hybridization with *M. concinnus* in the Chapada Diamantina, North-eastern Brazil. *Annals of Botany*.

Levene H. (1949) On a matching problem arising in genetics. *Annals of Mathematical Statistics* **20**: 91-94.

Levin D. A., Francisco-Ortega J. & Jansen R. K. (1996) Hybridization and the

extinction of rare plants species. *Conservation Biology* **10**: 10-16.

Lewontin R. C. & Birch L. C. (1966) Hybridization as a source of variation for adaptation to new environments. *Evolution* **20**: 315-336.

Martínez-Palacios A., Eguiarte L. E. & Furnier G. (1999) Genetic diversity of the endangered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahuan desert. *American Journal of Botany* **86**: 1093-1098.

McCauley D. E., Stevens J. E., Peroni P. A. & Ravelli J. A. (1996) The spatial distribution of clorosplast DNA and allozyme polymorphisms within a population of *Silene alba* (Caryophyllaceae). *American Journal of Botany* **83**: 727-731.

McCune B. & Mefford M. J. (1999) *PCOrd – Multivariate analysis of ecological data*, version 4.10. MjM Software, Gleneder Beach.

Moraes E. M., Abreu A. G., Andrade S. C. S., Sene F. M. & Solferini V. N. (2005) Population genetic structure of two columnar cacti with a patchy distribution in eastern Brazil. *Genetica* **125**: 311-323.

Nassar J. M., Hamrick J. L. & Fleming T. H. (2001) Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). *Heredity* **87**: 69-79.

Nassar J. M., Hamrick J. L. & Fleming T. H. (2002) Allozyme diversity and genetic structure of the leafy cactus (*Pereskia guamacho* [Cactaceae]). *The American Genetic Association* **93**: 193-200.

Nassar J. M., Hamrick J. L. & Fleming T. H. (2003) Population genetic structure of Venezuelan chiropterophilous columnar cacti (Cactaceae). *American Journal of Botany* **90**: 1628-1637.

Nei M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583-590.

Parker K. C. & Hamrick J. L. (1992) Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schotii*. *American Journal of Botany* **79**: 86-96.

Rhymer J. M. & Simberloff D. (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**: 83-109.

Ridgway G. J., Sherburne S. W. & Lewis R. D. (1970) Polymorphism in the esterases of Atlantic herring. *Transactions of the American Fisheries Society* **99**: 147-151.

Schmalzel R. J., Nixon R. T., Best A. L. & Tress Jr J. A. (2004) Morphometric variation in *Coryphantha robustipina* (Cactaceae). *Systematic Botany* **29**: 553-568.

Shaw C. R. & Prasad R. (1970) Starch gel eletrophoresis of enzymes – a compilation of

recipes. *Biochemical Genetics* **4**: 297-320.

Sneath P. H. A. & Sokal R. R. (1973) *Numerical Taxonomy*. Freeman and Co., San Francisco.

Soltis D. E., Haufler C. H., Darrow D. C. & Gastony G. J. (1983) Starch gel eletrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedule. *American Fern Journal* **73**: 9-27.

Statsoft Inc. (2000) *Statistica for Windows (Computer Program Manual)*. Salt Soft Inc, Tulsa.

Stuber C. W., Goodman M. M. & Johnson F. M. (1977) Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.). *Biochemical Genetics* **15**: 383-394.

Sun M. & Ganders F. R. (1990) Outcrossing rates and allozymes variation in rayed and rayless morphs of *Bidens pilosa*. *Heredity* **64**: 139-143.

Swofford D. L. & Selander R. B. (1989) *BIOSYS-1: Computer Program for the Analysis of Allelic Variation in Population Genetics and Biochemical Systematics*. Illinois Natural History Survey, Champaign.

Taylor N. P. (1991) The genus *Melocactus* (Cactaceae) in Central and South America. *Bradleya* **9**: 1-80.

Taylor N. P. (2000) *Taxonomy and Phytogeography of the Cactaceae of Eastern Brazil*. PhD thesis. The Open University and Royal Botanic Gardens, Kew.

Taylor N. P. & Zappi D. C. (2004) *Cacti of Eastern Brazil*. Royal Botanic Gardens, Kew.

Templeton A. R. (1986) Coadaptation and outbreeding depression. In: Soulé M. E. (ed). *Conservation Biology: the Science of Scarcity and Diversity*. Sinauer Associates, Massachusetts, pp.105-116.

Thomson G. (2002) A re-evaluation of the taxonomic status of the genus *Melocactus* in Aruba, Netherlands Antilles. *Bradleya* **20**: 29-44.

Thorpe J. P. (1982) The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. *Annual Review of Ecology and Systematics* **13**: 139-168.

Wright S. (1978) *Evolution and the Genetics of Populations - Variability Within and Among Natural Populations – Vol. 4.* University of Chicago Press, Chicago.

Table 1 Populations of *Melocactus glaucescens*, *M. ernestii*, *M. concinnus*, *M. ×albicephalus* and putative hybrids occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil, used in this study. Vouchers are deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS). *N*= number of individuals sampled.

| Species | Name | *N* | Location | |
|---|---|---|---|---|
| *M. glaucescens* | G01 | 33 | Lajes (Cidade das Pedras) | 11º29’38.4”S, 41º20’22.5”W |
| | G02 | 28 | Lajedo Bordado | 11º16’24.1”S, 41º05’04.7”W |
| | G03 | 30 | Brejões | 11º00’27.9”S, 41º20’35.9”W |
| | G04 | 25 | Volta da Serra | 11º19’06.7”S, 41º22’27.9”W |
| *M. ernestii* | E01 | 25 | Lajes (Cidade das Pedras) | 11º29’37.3”S, 41º20’21.5”W |
| *M. ×albicephalus* | A01 | 40 | Lajes (Cidade das Pedras) | 11º29’21.6”S, 41º20’21.6”W |
| putative hybrids | H01 | 36 | Lajes (Cidade das Pedras) | 11º29’28.3”S, 41º20’36.0”W |
| *M. concinnus* | C01 | 24 | Orquidário | - |
| | C02 | 20 | Barrigudas | 11º30’23.0”S, 41º18’17.5”W |

Table 2 Morphological characters used in the morphometric analyses of four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M. ×albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. Values showed as means ± standard deviations (minimum – maximum). Different letters in the same line indicate statistically different values for $p < 0.05$ in the Tukey's test.

| Characters | *M. glaucescens* | *M. ernestii* | *M.×albicephalus* | Hybrids | *M. concinnus* |
|---|---|---|---|---|---|
| Stem | | | | | |
| 01- Length (mm) | 113.6±21.2 [a] | 112.5±19.4 [ab] | 153.5±189.6 [b] | 117.0±11.3 [ab] | 80.3±15.0 [a] |
| | (70.9-180.6) | (85.2-178.3) | (98.9-131.9) | (96.5-151.9) | (34.1-107.3) |
| 02- Width (mm) | 144.1±14.7 [a] | 114.6±11.5 [b] | 150.3±12.2 [a] | 142.5±16.8 [a] | 128.6±10.3 [c] |
| | (82.1-176.8) | (92.9-137.9) | (107.2-178.3) | (113.6-198.0) | (84.1-144.3) |
| Ribs | | | | | |
| 03- Number | 8.8±1.1 [a] | 12.2±0.9 [c] | 10.3±0.6 [b] | 9.7±0.8 [b] | 8.4±0.7 [a] |
| | (7-12) | (10-14) | (9-12) | (9-12) | (8-11) |
| 04- Height (mm) | 19.7±9.7 [ac] | 18.4±2.5 [b] | 23.4±5.3 [c] | 17.6±3.2 [ab] | 20.6±6.7 [ac] |
| | (4.3-35.2) | (10.3-18.4) | (14.6-37.0) | (9.8-22.1) | (9.6-32.7) |
| 05- Width at 1/4 (mm) | 49.6±9.5 [a] | 28.7±4.4 [b] | 43.2±6.7 [c] | 45.4±7.8 [ac] | 47.1±5.8 [ac] |
| | (19.8-68.6) | (17.5-35.3) | (28.9-57.5) | (29.0-65.6) | (30.0-56.0) |
| 06- Width at 2/4 (mm) | 42.1±7.5 [b] | 23.6±2.8 [c] | 36.7±6.4 [a] | 37.6±6.6 [a] | 37.1±6.3 [a] |
| | (22.6-55.4) | (18.0-28.7) | (22.8-53.9) | (25.7-55.8) | (19.3-50.3) |
| 07- Width at 3/4 (mm) | 33.7±6.5 [c] | 18.2±2.2 [d] | 29.3±5.8 [ab] | 30.2±4.7 [a] | 26.0±5.2 [b] |
| | (18.0-47.0) | (13.6-22.1) | (13.9-39.8) | (19.4-40.7) | (18.4-35.9) |
| 08- Number of areoles | 8.7±1.6 [a] | 8.7±1.1 [a] | 8.2±0.9 [a] | 8.6±0.5 [a] | 6.9±0.7 [b] |
| | (5.0-13.0) | (7-13) | (7-11) | (8-9) | (5-8) |
| Spines (mean of four areoles of different ribs) | | | | | |
| 09- Length of the main central (mm) | 18.4±7.0 [b] | 42.6±7.2 [c] | 25.8±3.8 [a] | 27.0±3.7 [a] | 14.4±4.0 [d] |
| | (7.8-35.9) | (23.9-59.4) | (18.9-40.3) | (18.4-37.2) | (5.9-23.1) |

Table 2 (Continued)

| Characters | *M. glaucescens* | *M. ernestii* | *M.×albicephalus* | Hybrids | *M. concinnus* |
|---|---|---|---|---|---|
| 10- Lower number of radials | 6.2±1.2 [a] | 10.5±1.1 [c] | 8.5±0.6 [b] | 7.8±0.6 [b] | 6.2±1.0 [a] |
| | (3.0-11.0) | (8.0-12.0) | (7.0-9.0) | (7.0-9.0) | (5.0-7.0) |
| 11- Higher number of radials | 6.4±1.1 [a] | 10.8±0.7 [b] | 8.6±0.6 [c] | 7.9±0.5 [d] | 6.5±0.9 [a] |
| | (5.0-11.0) | (9.0-12.0) | (7.0-10.0) | (7.0-9.0) | (5.0-7.0) |
| 12- Length of right radial (mm) | 22.0±8.9 [ab] | 28.1±3.9 [c] | 24.7±2.8 [ac] | 25.9±3.2 [c] | 20.3±2.7 [b] |
| | (7.8-57.8) | (22.52-39.0) | (17.3-31.8) | (15.9-31.3) | (16.9-27.0) |
| 13- Length of lower radial (mm) | 21.6±6.6 [a] | 73.2±9.6 [b] | 31.5±3.8 [c] | 32.6±4.4 [c] | 21.9±3.7 [a] |
| | (11.2-42.2) | (58.9-98.6) | (22.8-42.5) | (24.0-44.4) | (15.6-30.3) |
| 14- Length of left radial (mm) | 21.2±7.5 [a] | 26.7±3.5 [b] | 24.1±2.6 [bc] | 26.0±3.1 [b] | 20.9±2.8 [ac] |
| | (9.3-42.5) | (20.2-33.1) | (17.3-31.4) | (19.0-31.4) | (16.8-28.2) |
| 15- Diameter of the lower radial (mm) | 1.5±0.3 [a] | 1.4±0.2 [ab] | 1.7±0.2 [c] | 1.4±0.2 [ab] | 1.3±0.2 [b] |
| | (0.9-2.0) | (1.2-1.8) | (1.2-2.1) | (1.0-1.9) | (1.0-1.7) |
| 16- Angle between right and left radials | 151.9±15.5 [a] | 129.9±17.8 [bc] | 123.2±14.3 [b] | 134.2±15.1 [c] | 153.5±13.1 [a] |
| | (113.0-180.0) | (93.0-158.0) | (94.0-154.0) | (92.0-164.0) | (130.0-180.0) |
| Cephalium | | | | | |
| 17- Length (mm) | 42.1±18.6 [a] | 30.6±11.5 [bc] | 41.8±19.0 [ab] | 43.4±18.6 [a] | 26.5±12.6 [c] |
| | (14.8-112.1) | (17.1-54.2) | (12.1-93.6) | (19.2-83.8) | (8.7-53.9) |
| 18- Width (mm) | 68.6±9.6 [a] | 62.5±10.0 [a] | 77.8±12.7 [b] | 78.3±11.7 [b] | 63.8±12.4 [a] |
| | (7.7-91.8) | (35.2-74.1) | (45.1-95.9) | (58.2-129.0) | (29.4-81.9) |

Table 3 Allele frequencies at 12 allozymic loci in four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M. ×albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. See Table 1 for the names of the populations. *N*= sample size.

| Locus | Allele | *M. glaucescens* | | | | *M. ernestii* | *M. ×albic.* | Hybrids | *M. concinnus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G01 | G02 | G03 | G04 | E01 | A01 | H01 | C01 | C02 |
| *Pgm-1* | 71 | - | - | 0.040 | - | - | - | - | - | - |
| | 93 | - | - | - | - | - | - | 0.028 | - | - |
| | 95 | 0.030 | - | - | - | - | - | - | - | - |
| | 100 | 0.970 | 1.000 | 0.960 | 1.000 | 1.000 | 1.000 | 0.972 | 1.000 | 1.000 |
| | *N* | *33* | *25* | *25* | *23* | *25* | *38* | *36* | *22* | *20* |
| *Pgm-2* | 98 | 0.030 | - | - | - | - | - | - | - | - |
| | 100 | 0.970 | 1.000 | 1.000 | 1.000 | 0.940 | 1.000 | 1.000 | 1.000 | 1.000 |
| | 107 | - | - | - | - | 0.060 | - | - | - | - |
| | *N* | *33* | *24* | *25* | *24* | *25* | *36* | *35* | *21* | *19* |
| *Pgi* | 89 | 0.061 | - | - | - | - | - | 0.028 | - | - |
| | 100 | 0.939 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.972 | 1.000 | 1.000 |
| | *N* | *33* | *26* | *24* | *24* | *25* | *40* | *36* | *21* | *20* |
| *Lap* | 94 | - | 0.038 | 0.043 | - | - | - | - | - | - |
| | 96 | 0.030 | - | - | - | - | - | - | - | - |
| | 100 | 0.848 | 0.865 | 0.739 | 0.958 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | 107 | 0.121 | - | 0.109 | 0.042 | - | - | - | - | - |
| | 112 | - | - | 0.109 | - | - | - | - | - | - |
| | 116 | - | 0.096 | - | - | - | - | - | - | - |
| | *N* | *33* | *26* | *23* | *24* | *25* | *36* | *36* | *22* | *18* |

Table 3 (Continued)

| Locus | Allele | *M. glaucescens* | | | | *M. ernestii* | *M. ×albic.* | Hybrids | *M. concinnus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G01 | G02 | G03 | G04 | E01 | A01 | H01 | C01 | C02 |
| *Skdh* | 85 | - | - | - | - | - | - | 0.029 | - | - |
| | 89 | - | - | - | - | - | - | - | 0.063 | 0.063 |
| | 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.971 | 0.938 | 0.938 |
| | *N* | *29* | *21* | *25* | *24* | *25* | *36* | *34* | *16* | *16* |
| *Idh* | 79 | - | - | - | - | - | 0.027 | - | - | - |
| | 84 | - | - | - | - | - | - | 0.014 | - | - |
| | 100 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.973 | 0.986 | 1.000 | 1.000 |
| | *N* | *30* | *26* | *20* | *24* | *25* | *37* | *36* | *14* | *19* |
| *G6pd* | 100 | 1.000 | 0.981 | 0.960 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| | 110 | - | 0.019 | 0.040 | - | - | - | - | - | - |
| | *N* | *27* | *26* | *25* | *22* | *24* | *33* | *36* | *20* | *19* |
| *Mdh-1* | 100 | 0.933 | 0.882 | 0.978 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.971 |
| | 119 | 0.067 | 0.118 | - | - | - | - | - | - | 0.029 |
| | 130 | - | - | 0.022 | - | - | - | - | - | - |
| | *N* | *30* | *17* | *23* | *18* | *25* | *37* | *33* | *18* | *17* |
| *Mdh-2* | 80 | 0.065 | - | - | - | - | - | - | - | - |
| | 92 | - | - | - | 0.022 | - | - | 0.014 | 0.026 | 0.028 |
| | 100 | 0.935 | 1.000 | 1.000 | 0.978 | 1.000 | 1.000 | 0.986 | 0.974 | 0.972 |
| | *N* | *31* | *26* | *25* | *23* | *25* | *40* | *36* | *19* | *18* |
| *Acp* | 81 | - | 0.022 | - | 0.083 | - | 0.026 | - | 0.026 | - |
| | 88 | - | - | 0.040 | - | - | 0.064 | - | - | - |
| | 92 | - | - | - | 0.042 | - | - | - | - | - |

Table 3 (Continued)

| Locus | Allele | *M. glaucescens* | | | | *M. ernestii* | *M. ×albic.* | Hybrids | *M. concinnus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G01 | G02 | G03 | G04 | E01 | A01 | H01 | C01 | C02 |
| | 96 | - | - | - | - | - | - | 0.014 | - | - |
| | 100 | 1.000 | 0.978 | 0.960 | 0.875 | 1.000 | 0.910 | 0.986 | 0.974 | 1.000 |
| | *N* | *31* | *23* | *25* | *24* | *25* | *39* | *36* | *19* | *17* |
| *Dia-I* | 93 | - | - | - | - | - | - | 0.032 | - | - |
| | 96 | - | - | - | - | - | - | - | 0.667 | 0.714 |
| | 100 | 1.000 | 0.929 | 1.000 | 1.000 | 1.000 | 1.000 | 0.968 | 0.333 | 0.286 |
| | 104 | - | 0.071 | - | - | - | - | - | - | - |
| | *N* | *20* | *14* | *24* | *16* | *24* | *22* | *31* | *6* | *7* |
| *Dia-2* | 86 | - | - | - | - | - | - | 0.028 | 0.500 | 0.714 |
| | 92 | - | - | - | - | - | - | 0.028 | - | - |
| | 100 | 1.000 | 0.944 | 0.854 | 1.000 | 1.000 | 1.000 | 0.944 | 0.500 | 0.286 |
| | 107 | - | 0.056 | 0.146 | - | - | - | - | - | - |
| | *N* | *26* | *18* | *24* | *20* | *25* | *34* | *36* | *8* | *7* |

Table 4 Genetic variability at 12 allozymic loci and morphological variability based on the morphometric analysis of 18 morphological characters in four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M. ×albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. *N*= mean sample size per locus; A= mean number of alleles per locus; *P*= percentage of polymorphic loci (%); $H_o$= observed and $7H_e$= expected mean heterozygosity per locus (Nei 1978; unbiased estimate); $D2_m$= median of the Mahalanobis generalized distance of the individuals to the centroid of the population; ED= mean of the Euclidean distance between the individuals of the population. Standard deviations in parentheses. See Table 1 for the name of the populations. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95.

| Population | *N* | A | *P* | $H_o$ | $H_e$ | $D2_m$ | ED |
|---|---|---|---|---|---|---|---|
| *M. glaucescens* | | | | | | | |
| G01 | 29.6 (1.1) | 1.7 (0.27) | 41.7 | 0.021 (0.014) | 0.073 (0.024) | 18.81 | 59.46 |
| G02 | 22.7 (1.2) | 1.6 (0.20) | 33.3 | 0.036 (0.021) | 0.066 (0.026) | 11.54 | 38.87 |
| G03 | 24.0 (0.4) | 1.7 (0.30) | 16.7 | 0.035 (0.016) | 0.081 (0.039) | 12.17 | 39.42 |
| G04 | 22.2 (0.8) | 0.3 (0.19) | 8.3 | 0.011 (0.008) | 0.030 (0.020) | 14.15 | 54.06 |
| *M. ernestii* | | | | | | | |
| E01 | 23.0 (1.8) | 1.1 (0.10) | 7.7 | 0.003 (0.003) | 0.009 (0.009) | 20.41 | 47.62 |
| *M. ×albicephal.* | | | | | | | |
| A01 | 33.0 (3.0) | 1.2 (0.20) | 7.7 | 0.018 (0.014) | 0.017 (0.013) | 13.35 | 105.44 |
| Hybrids | | | | | | | |
| H01 | 32.5 (2.7) | 1.7 (0.20) | 7.7 | 0.006 (0.003) | 0.032 (0.009) | 9.81 | 47.67 |
| *M. concinnus* | | | | | | | |
| C01 | 15.9 (1.9) | 1.4 (0.10) | 23.1 | 0.008 (0.005) | 0.096 (0.052) | 11.83 | 32.58 |
| C02 | 15.2 (1.7) | 1.4 (0.10) | 23.1 | 0.009 (0.006) | 0.086 (0.045) | 9.05 | 43.32 |

Table 5 – *F* statistics (Wright 1978) at 12 allozymic loci and *A* value of the MRPP analysis of 18 morphological characters in four populations of of *M. glaucescens* and two populations of *M. concinnus*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil.

| Locus | $F_{IS}$ | | $F_{ST}$ | |
|---|---|---|---|---|
| | *M. glaucescens* | *M. concinnus* | *M. glaucescens* | *M. concinnus* |
| Pgm-1 | 0.410 | - | 0.023 | - |
| Pgm-2 | 1.000 | - | 0.023 | - |
| Pgi | -0.650 | - | 0.046 | - |
| Lap | 0.632 | - | 0.049 | 0.000 |
| Skdh | - | 1.000 | - | - |
| G6pd | 0.664 | - | 0.019 | - |
| Mdh-1 | -0.100 | -0.030 | 0.047 | 0.015 |
| Mdh-2 | 0.734 | -0.028 | 0.037 | 0.000 |
| Acp | 0.737 | -0.027 | 0.027 | 0.013 |
| Dia-1 | 1.000 | 1.000 | 0.055 | 0.003 |
| Dia-2 | 0.882 | 1.000 | 0.074 | 0.048 |
| *mean* | *0.579* | *0.901* | *0.045* | *0.022* |
| *A* (MRPP) | | | 0.16 | 0.11 |

Table 6 Matrix of mean genetic identities (Nei [1978] unbiased estimate) between four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M. ×albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. Minimum and maximum values are in parentheses.

| Species | No. of populations | *M. glaucescens* | *M. ernestii* | *M. ×albicephalus* | Hybrids | *M. concinnus* |
|---|---|---|---|---|---|---|
| *M. glaucescens* | 4 | 0.997(0.996 – 0.999) | | | | |
| *M. ernestii* | 1 | 0.997 (0.995 – 0.999) | ---- | | | |
| *M.×albicephalus* | 1 | 0.998 (0.995 – 1.000) | 0.999 | --- | | |
| Hybrids | 1 | 0.997 (0.995 – 0.999) | 1.000 (1.000 – 1.000) | 0.999 | --- | |
| *M. concinnus* | 2 | 0.927 (0.908 – 0.945) | 0.928 (0.914 – 0.943) | 0.927 (0.913 – 0.942) | 0.933 (0.919 – 0.947) | 1.000 |

Table 7 Matrix of classification of the individuals in the discriminant analysis of 18 morphological characters in four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M. ×albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. See Table 1 for the name of the populations.

| Pop. | Correct (%) | *M. glaucescens* | | | | *M. ernestii* | *M. ×albicephalus* | Hybrids | *M. concinnus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | G01 | G02 | G03 | G04 | E01 | A01 | H01 | C01 | C02 |
| G01 | 90.91 | 30 | - | - | 3 | - | - | - | - | - |
| G02 | 96.43 | - | 27 | - | - | - | - | - | 1 | |
| G03 | 96.66 | - | - | 29 | 1 | - | - | - | - | - |
| G04 | 84.00 | 3 | - | - | 21 | - | - | - | 1 | - |
| E01 | 100.00 | - | - | - | - | 25 | - | - | - | - |
| H01 | 87.50 | - | - | - | - | - | 35 | 5 | - | - |
| H02 | 90.91 | - | - | - | - | - | 3 | 30 | - | - |
| C01 | 87.5 | - | 3 | - | - | - | - | - | 21 | - |
| C02 | 100.00 | - | - | - | - | - | - | - | - | 20 |
| Total | 92.25 | 30 | 30 | 28 | 26 | 25 | 38 | 35 | 23 | 20 |

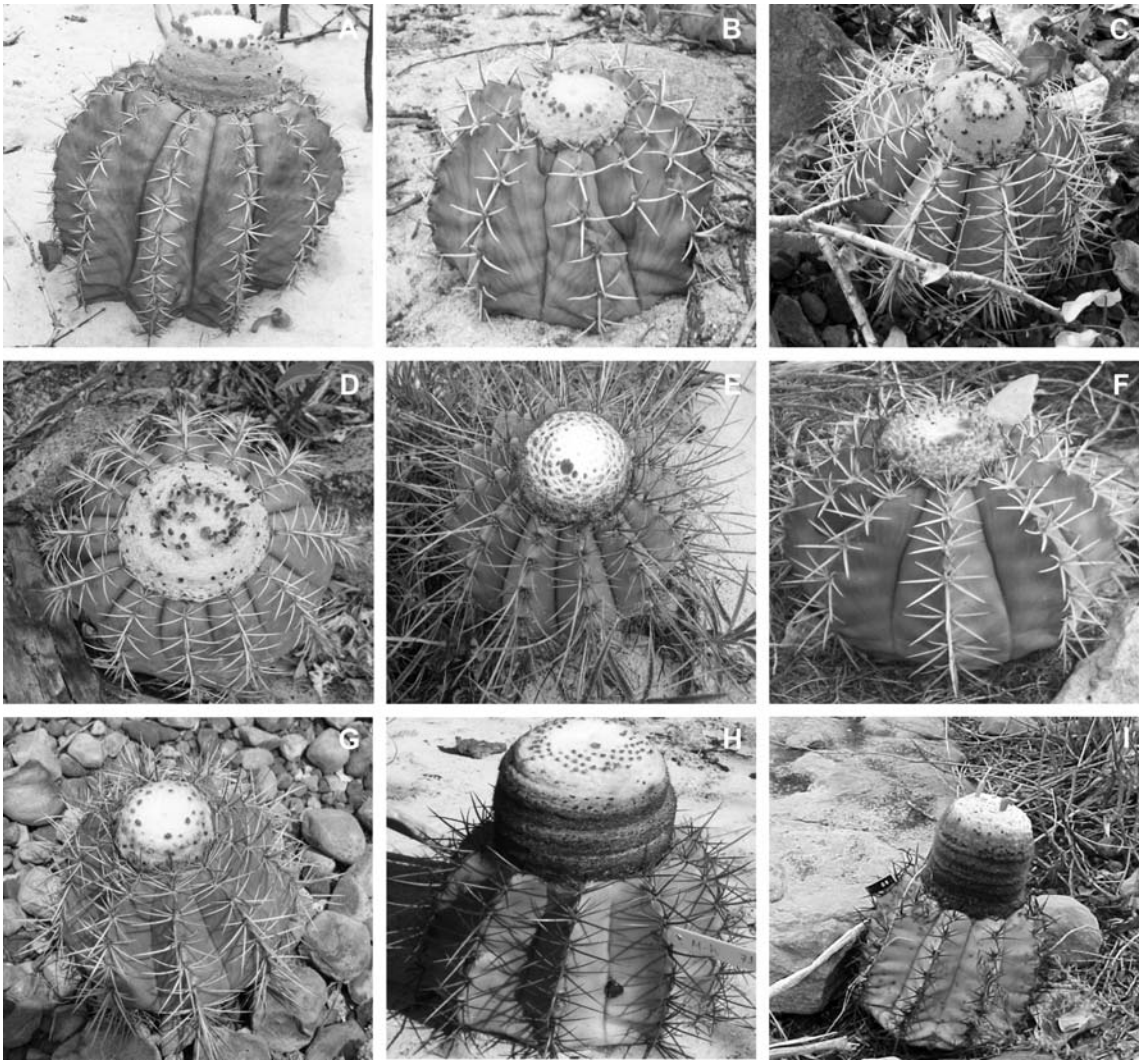


Fig 1. Individuals of *Melocactus glaucescens* from the populations G01 (A), G02 (B), G03 (C) and G04 (D), *M. concinnus* (E), *M. ernestii* (F), *M.* ×*albicephalus* (G), and putative hybrids between *M. glaucescens* and *M. ernestii* (H, I), occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil.

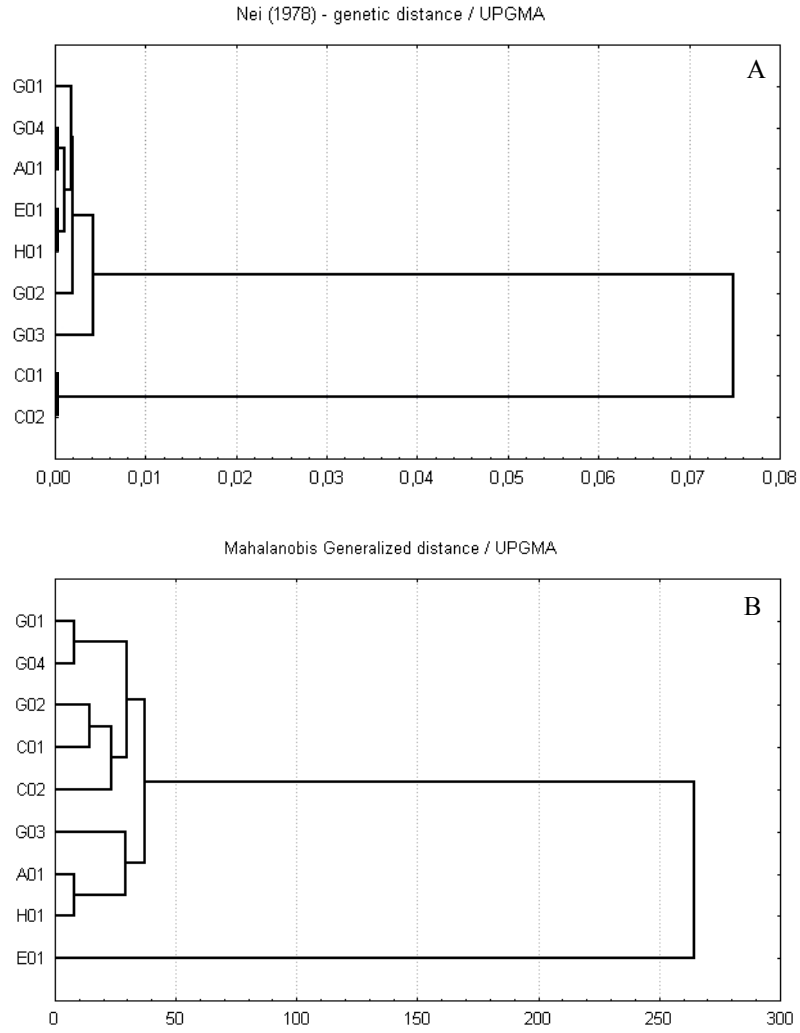


Fig. 2 Dendrogram showing the phenetic relationships among four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M.* ×*albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. Constructed using the matrix of genetic distances (Nei 1978; umbiased estimate) based on 12 allozymic loci (A) and using the matrix Mahalanobis generalized distance based on 18 morphological characters (B) with UPGMA as clustering algorithm. See Table 1 for the name of the populations.

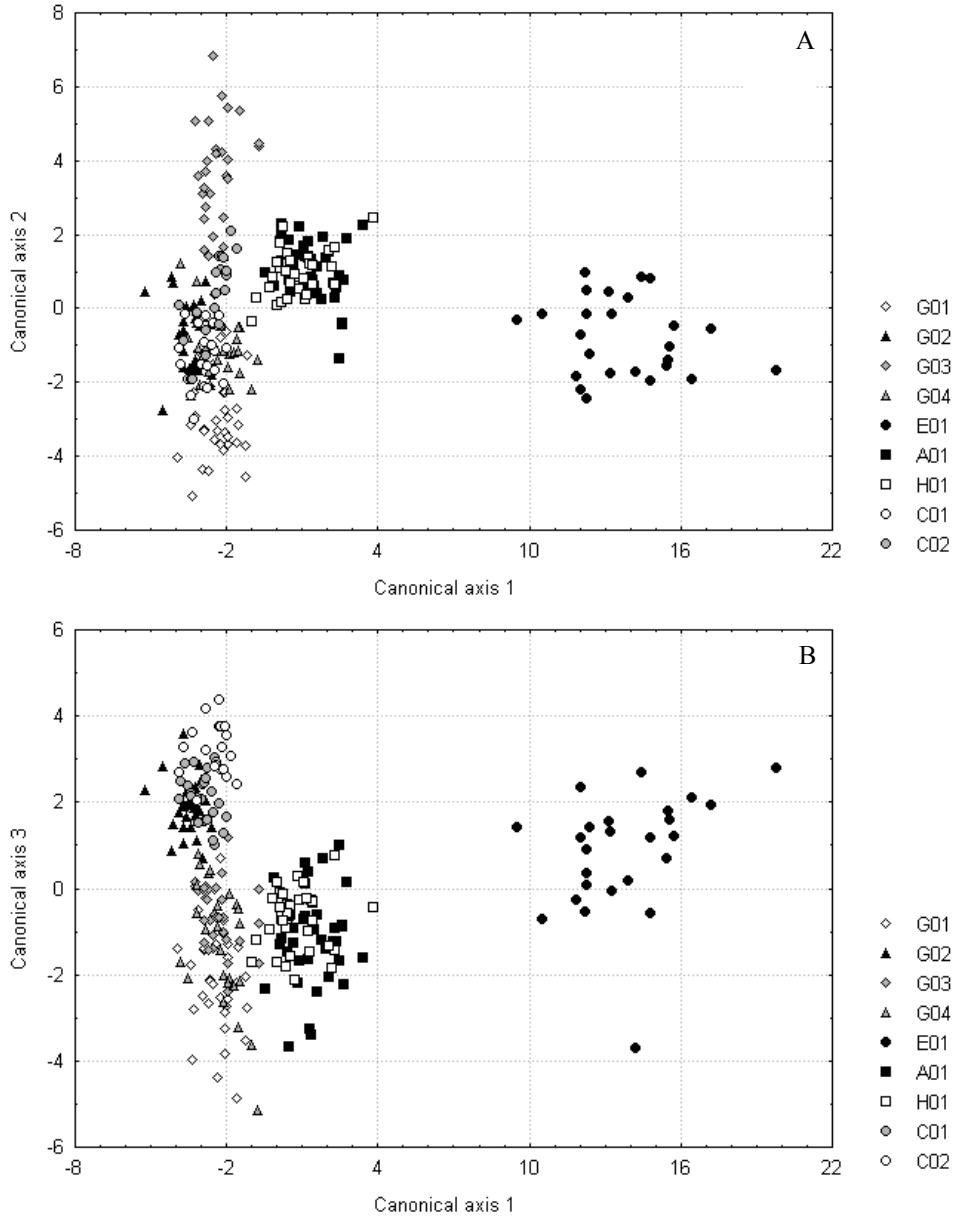


Fig. 3 Representation of the scores on the three first canonical axes of the CVA using 18 morphological characters in four populations of *M. glaucescens*, one population of *M. ernestii*, two populations of *M. concinnus*, one population of *M.* ×*albicephalus* and one population of putative hybrids between *M. glaucescens* and *M. ernestii*, occurring at the municipality of Morro do Chapéu, Chapada Diamantina, Northeastern Brazil. (A) – Canonical axes 1 (71.38%) and 2 (10.34%); (B) – Canonical axes 1 and 3 (8.24%). See Table 1 for the name of the populations.

## CONCLUSÕES GERAIS

As espécies de *Melocactus* estudadas possuem baixa diversidade genética, sendo inferior a observada para outra espécie do gênero, *M. curvispinus* (Nassar *et al.*, 2002), assim como para outras espécies de cactos (Parker and Hamrick, 1992; Hamrick *et al*., 2002; Nassar *et al*., 2002, 2003; Clark-Tapia and Molina-Freaner, 2003; Moraes *et al.*, 2005) e de plantas com características de vida similares (Hamrick and Godt, 1992; Colunga-GarcíaMarín *et al.*, 1999; Martínez-Palacios *et al*.,1999). Acredita-se que esta baixa variabilidade seja decorrente de um efeito gargalo sofrido recentemente por estas espécies, associado à atividade de coleta e degradação das áreas de ocorrência das mesmas, tornando suas populações vulneráveis e conseqüentemente levando a uma redução na variabilidade.

Os altos índices de endogamia encontrados para as espécies é algo importante e que deve ser considerado, visto que endogamia tende a afetar negativamente a viabilidade de populações pequenas (Allendorf & Leary, 1988; Forbes and Allendorf, 1991; Hayes *et al*., 1996; Levin *et al*., 1996). Em se tratando de espécies ameaçadas como as aqui estudadas este fator pode contribuir para a extinção das mesmas (Rhymer & Simberloff, 1996).

Elevados índices de diferenciação genética indicam ausência de panmixia e uma sub-estruturação nas populações, sendo essa estruturação maior para *M. paucispinus* que para *M. glaucescens*. Em *M. paucispinus* a estruturação dentro das populações sendo maior que entre populações de diferentes regiões revela que deve haver uma certa conservação genética para o gênero como um todo, visto que tendo fluxo gênico restrito devido à pequena capacidade de polinização e dispersão apresentada pelos seus agentes, a diferença entre populações distantes deveria ser maior que dentro de populações. Esta alta estruturação dentro das populações é outro fator que deve ser levado em conta na hora de se propor áreas de preservação para as espécies, sendo necessária a conservação das populações como um todo, visto que processos de sub-estruturação podem levar a diferenciação dentro das mesmas.

A baixa variabilidade encontrada, associada à ausência de locos diagnósticos não permitiram que as hipóteses de hibridação entre *M. paucispinus* e *M. concinnus* e entre *M. glaucescens* e *M. ernestii* levantadas aqui fossem confirmadas. Para tanto um estudo complementar utilizando outro marcador genético com maior taxa de variação poderá auxiliar na resolução desta questão.

A presença de alelos exclusivos entre os híbridos de *M. glaucescens* com *M. ernestii* leva-nos a crer que estes já estejam se estabelecendo e diferenciando-se dos parentais. Isto é um fator importante no auxílio da delimitação deste taxa, de forma que os híbridos possa vir a serem reconhecidos como espécie distinta. Elevada estruturação morfológica entre populações de *M. paucispinus* e *M. concinnus* é outra evidência de hibridação.

A formação de uma zona híbrida em uma das populações de *M. glaucescens* pode levá-la a extinção local, como já evidenciado para outra população desta mesma espécie (Taylor & Zappi, 2004). Para *M. glaucescens* isto é bastante grave devido à pequena quantidade de populações existentes. Para a manutenção desta população deve ser considerada a possibilidade de remoção dos indivíduos de *M. ernestii* e dos possíveis híbridos da área.

A variabilidade morfológica apresentada pelas espécies de *Melocactus* é, no geral, menor que as apresentadas por espécies do gênero *Discocactus* (Cactaceae; Machado *et al*., submetido). A ausência de estudos morfológicos a nível populacional é uma das grandes carências para Cactaceae, assim como para diversas espécies vegetais, sendo os mesmos, no entanto de grande importância para o entendimento da diversidade e da real viabilidade de espécies. A análise morfométrica demonstrou uma grande variação para os diversos caracteres analisados, podendo expressar uma dificuldade na utilização destes para delimitação das espécies. Características qualitativas como a presença/ausência de espinhos, glaucosidade, cor de frutos, flores e cerdas do cefálio são características que conjuntamente podem auxiliar para tal fim. Estudos de variabilidade populacional como este constituem importantes ferramentas no auxílio para delimitação de caracteres taxonômicos e podem ser úteis na resolução de problemas nesta área.

Apesar da baixa variabilidade morfológica encontrada, uma elevada estruturação foi observada tanto para *M. paucispinus* quanto para *M. glaucescens*, evidenciando diferenciação entre suas populações. Para *M. glaucescens* os elevados valores de estruturação morfológica refletem a diferença entre os indivíduos das quatro populações, sendo possível reconhecer a origem dos mesmos pela aparência. Tais índices de estruturação morfológica são importantes na hora de se analisar a conservação de espécies, sendo para *M. glaucescens* em particular, imprescindível à preservação de todas as populações existentes para que se possa abranger ao máximo a variabilidade existente para a espécie.

A pequena quantidade de populações naturais destas espécies, associado ao número reduzido de indivíduos nas mesmas leva-nos a reafirmar o *status* destas como espécies ameaçadas de extinção. O fato de todas as populações estarem em áreas de fácil acesso, geralmente próximo a rodovias e/ou em regiões agropecuárias tornam-nas susceptíveis a rápida extinção por ação de coletores e pelo uso de suas áreas de ocorrência para pastagem de animais. Para *M. glaucescens* a situação é ainda mais acentuada pela presença de hibridação e introgressão com *M. ernestii*, e esta ocorrendo na área tipo da espécie. Muitas outras espécies de *Melocactus* apresentam distribuição restrita (e.g., *M. deinacanthus* Buining & Brederoo, *M. conoideus* Buining & Brederoo, *M. pahcyacanthus* Buining & Brederoo, *M. lanssensianus* P.S. Braun, *M. estevesii* P.S. Braun) e estudos de variabilidade genética e morfológica como este podem auxiliar na preservação destas e de diversas outras espécies. Além do mais, tais estudos podem ser muito importantes para o entendimento de padrões biogeográficos do gênero e até mesmo da família em geral. O mesmo pode ser aplicado para as diversas espécies de plantas.

Presença de alelos exclusivos em diversas populações de ambas as espécies (acentuadamente em *M. glaucescens*) é fato importante quando se fala em conservação. Deixando de preservar uma destas populações podemos ter perda significativa de variabilidade para a espécie. Para ambas as espécies foi observada a ausência de correlação entre dados genéticos e morfológicos indicando, portanto que o uso dissociado destes critérios para conservação implicaria na perda de informação sobre a variabilidade existente, sendo fundamental a análise conjunta destes dados.

Por fim, a presença de alelos raros e de diferenciação morfológica elevada faz necessária a preservação *in situ* e *ex situ* de todas as populações de *M. glaucescens* e de populações para cada localidade de ocorrência de *M. paucispinus*, sendo priorizadas aquelas com maior índice de variabilidade, como as populações de Seabra (PS01) e Delfino (PD01), as populações PR02 e PR03 de Rio de Contas e as populações PM01, PM02, PM04 de Morro do Chapéu. Entre as ações para conservação *ex situ*, a criação de um banco de sementes e de plântulas para cada uma das populações das espécies são medidas que podem ser adotadas. É necessária a investidas na procura de novas populações de ambas as espécies e urgente a criação de áreas de conservação como medidas para que se possa preservar as populações conhecidas.

Estudos de variabilidade genética e morfológica como estes são de fundamental importância para avaliarmos a situação atual de espécies, especialmente quando

associados a estudos de demografia das populações e biologia da espécie, sendo possível delimitar seus reais *status* de conservação e detectar onde a manutenção das mesmas pode estar sendo comprometida, dando fortes subsídios para ações práticas de manejo que virão a assegurar a preservação destas. A integração de todos estes conjuntos de dados, conforme proposto no projeto "Conservação e manejo e espécies de Eriocaulaceae, Orchidaceae e Cactaceae da Chapada Diamantina ameaçadas de extinção", do qual o presente estudo é parte integrante, permitirá uma nova perspectiva na conservação destas espécies, e poderá servir de modelo metodológico para estudos futuros de conservação de outras espécies destes grupos de plantas nestas e em demais áreas dos campos rupestres brasileiros.

## REFERÊNCIAS BIBLIOGRÁFICAS


ALLENDORF, F.W.; LEARY, R.F. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. Conservation Biology, 2: 170-184.

ANDERSON, E.F. 2001. The Cactus Family. Portland, Oregon, USA: Timber Press.

AGIOSPERME PHYLOGENY GROUP. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society, 141: 399-436.

ARNOLD, M. 1996. Natural Hybridization and Introgression. Princeton: Princeton University Press.

ARNOLD, M.L. 1997. Natural Hybridization and Evolution. Oxford: Oxford University Press.

ASSIS, J.G.A.; OLIVEIRA, A.L.P.C.; RESENDE, S.V.; SENRA, J.F.V.; MACHADO, M. 2003. Cromossome numbers in Brazilian *Melocactus* (Cactaceae). Bradleya, 21: 1-6.

BACKER, M.A.; PINKAVA, D.J. 1987. A cytological and morphometric analysis of a triploid apomict, *Opuntia* ×*kelvinensis* (subgenus Cylindropyntia, Cactaceae). Brittonia, 39: 387-401.

BACKER, M.A.; JOHNSON, R.A. 2000. Mophometric analysis of *Escobaria sneedii* var. s*needii*, *E. sneedii* var. *leei,* and *E. guadalupensis* (Cactaceae). Systematic Botany, 25: 577-587.

BARTON, N.H.; SLATKIN, M. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity, 56: 409-415.

BARTON, N.H. 2001. The role of hybridization in evolution. Molecular Ecology, 10: 551-568.

BIJLSMA, R.; BUNDGAARD, J.; BOEREMA, A.C. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. Journal of Evolutiony Biology, 13: 502-514.

BORBA, E.L.; SEMIR, J. 1998. Wind-assisted fly pollination in three *Bulbophyllum* (Orchidaceae) species occurring in the Brazilian 'campos rupestres'. Lindleyana, 13: 203-218.

BORBA, E.L.; FELIX, J.M.; SOLFERINI, V.N.; SEMIR J. 2001. Fly-pollinated *Pleurothallis* (Orchidaceae) species have high genetic variability: evidence from isozyme markers. American Journal of Botany, 88: 419-428.

BORBA, E.L.; SHEFHERD, G.J; VAN DEN BERG, C.; SEMIR, J. 2002. Floral and vegetative morphometrics of five *Pleurothalis* (Orchidaceae) species: correlation with taxonomy, phylogeny, genetic variability and pollination systems. Annals of Botany, 90: 219-230.

BOYLE, T.H. 1997. The gentics of self-incompatibility in the genus *Schlumbergera* (Cactaceae). The Journal of Heredity, 88: 209-214.

BOYLE, T.H.; IDNURM, A. 2001. Physiology and genetics of self-incompatibility in *Echinopsis chamaecereus* (Cactaceae). Sexual Plant Reproduction, 13: 323-327.

BOYLE, T.H.; IDNURM, A. 2003. Intergeneric hybridization between *Schlumbergera* Lem. and *Hatiora* Britt. & Rose (Cactaceae). Journal of the American Society for Horticultural Science, 128: 724-730.

BRUNE, W.; ALFENAS, A.C.; JUNGHANS, T.G. 1998. Identificações específicas de enzimas em géis. In: ALFENAS, A.C. (ed.) Eletroforese de isoenzimas e proteínas afins: fundamentos e aplicações em plantas e microorganismos. Viçosa: Universidade Federal de Viçosa. p.: 201-328.

CASAS, A.; CABALLERO, J.; VALIENTE-BANUET, A.; SORIANO, J.A.; DÁVILA, P. 1999. Morphological variation and the process of domestication of *Stenocereus stellatus* (Cactaceae) in Central México. American Journal of Botany, 86: 522-533.

CHAMBERLAND, M. 1997. Systematics of the *Echinocactus polycephalus* complex (Cactaceae). Systematic Botany, 22: 303-313.

CLARK-TAPIA, R.; MOLINA-FREANER, F. 2003. The genetic structure of a columnar cactus with a disjunct distribution: *Stenocereus gummosus* in the Sonoran desert. Heredity, 90: 443-450.

CLAYTON, J.W.; TRETIAK, D.N. 1972. Amine-citrate buffers for pH control in starch gel eletrophoresis. Journal of Fisheries Research of Board Canada, 29: 1169-1172.

COLAÇO, M.A.S.; FONSECA, R.B.S.; LAMBERT, S.M.; COSTA, C.; BORBA, E.L.; MACHADO, C.G. Submitted manuscript. Reproductive biology of the *Melocactus glaucescens* and *M. paucispinus* (Cactaceae), of the stat of Bahia, Brazil. Revista Brasileira de Botânica.

COLUNGA-GARCÍAMARÍN, P.; COELLO-COELLO, J.; EGUIARTE, L.E.; PIÑERO, D. 1999. Isozymatic variation and phylogenetic relationships (*Agave fourcroydes*) and its wild ancestor *A. angustifolia* (Agavaceae). American Journal of Botany, 86: 115-123.

CORRIAS, B.; ROSSI, W.; ARDUINO, P.; CIANCHI, R.; BULLINI, L. 1991. *Orchis longicornu* Poiret in Sardinina: genetic, morphological and chorological data. Webbia, 45: 71-101.

CRAWFORD, D.J. 1989. Enzyme eletrophoresis and plant systematics. In: SOLTIS, E.D.; SOLTIS, P.S. (eds.) Isozymes in Plant Biology. Portland: Dioscorides Press. p.: 146-164.

CROW, J.F.; AOKI, K. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proceedings of the National Academy of Sciences USA, 81: 6073–6077.

DAS, A.B.; MOHANTY, S.; DAS, P. 1998. Variation in karyotype and 4C DNA content in six species of *Melocactus* of the family Cactaceae. Cytologia, 63: 9-16.

ELLSTRAND, N.C. 1992. Gene flow by pollen: implication for plant conservation genetics. Oikos, 63: 77-86.

FONSECA, R.B.S. 2004. Fenologia reprodutiva e dispersão de *Melocactus glaucescens* Buining & Brederoo e *M. paucispinus* G. Heiman & R. Paul (Cactaceae) no município de Morro do Chapéu, Chapada Diamantina – *Bahia – Brasil*. Tese de mestrado - Universidade Estadual de Feira de Santana. Feira de Santana.

FORBES, S.H.; ALLENDORF, F.W. 1991. Mitochondrial genotypes have no detectable effects on meristic traits in cutthroat trout hybrid swarms. Evolution, 45: 1350-1359.

GIULIETTI, A.M.; MENEZES, N.L.; PIRANI, J.R.; MEGURO, M.; WANDERLEY, M.G.L. 1987. Flora da Serra do Cipó, Minas Gerais: caracterização e lista de espécies. Boletim de Botânica (Universidade de São Paulo), 9: 1-151.

GIULIETTI, A.M.; PIRANI, J.R. 1988. Patterns of geographic distribution of some plant species from the Espinhaço Range, Minas Gerais and Bahia, Brazil. In: HEYER, W.R.; VANZOLINI, P.E. (eds.) Proceedings of a Workshop on Neotropical Distribution Patterns. Rio de Janeiro: Academia Brasileira de Ciências. p.: 39-63.

GOLDMAN, D.H.; VAN DEN BERG, C.; GRIFFITH, M.P. 2004. Morphometric circumscription of species and infraspecific taxa in *Calopogon* R.Br. (Orchidaceae). Plant Systematics and Evolution, 274: 37-60.

GRANT, V.; GRANT, K.A. 1979. Hybridization and variation in the *Opuntia phaeacantha* group in Central Texas. Botanic Gazette, 140: 208-215.

HAMRICK, J.L.; GODT, M.J. 1992. Allozyme diversity in plant species. In: BROWN, A.H.D.; CLEGG, M.T.; KAHLER, A.L.; WEIR, B.S. (eds.) Plant Population Genetics, Breeding and Genetic Resources. Sunderland: Sinauer. p.: 43-63.

HAMRICK, J.L.; NASON, J.D.; FLEMING, T.H.; NASSAR, J.M. 2002. Genetic diversity in columnar cacti. In: FLEMING, T.H.; VALIENTE-BANUET, A. (eds.) Columnar Cacti and their Mutualisms: Evolution, Ecology and Conservation. Tucson: University of Arizona Press. p.: 122-133.

HANDEL, S.N. 1983. Polination ecology, plant population structure, and gene flow. In: REAL, L. (ed.) Polination Biology. Orlando: Academic Press. p.:163-211.

HARLEY, R.M. 1988. Evolution and distribution of *Eriope* (Labiatae) and its relatives, in Brazil. In: HEYER, W.R.; VANZOLINI, P.E. (eds.) Proceedings of a workshop on Neotropical distribution patterns. Rio de Janeiro: Academia Brasileira de Ciências. p.: 71-120.

HARLEY, R.M. 1995. Introduction. In: STANNARD B.L (ed.). Flora of the Pico das Almas, Chapada Diamantina – Bahia, Brazil. Kew: Royal Botanic Gardens. p.: 43-76.

HAUSER, T.P.; LOESCHEKE, V. 1995**.** Inbreeding depression in *Lychnis flos-cuculi* (Caryophyllaceae): effects of different levels of inbreeding. Journal of Evolutionary Biology, 8: 589-600.

HAYES, J.P.; GUFFLEY, S.Z.; KRIEGLER, F.J.; MCCRACKEN, G.F.; PARKER, C. R. 1996. The genetic diversity of native, stocked, and hybrid populations of brook trout in the southern Appalachians. Conservation Biology, 10: 1403-1412.

HEYWOOD, J.S. 1991. Spatial analysis of genetic variation in plant populations. Annual Review of Ecology and Systematics, 22: 335-355.

HOLLIS, H.B. 1997. Introducción y semblanza histórica. In: SEPTIÉN, C.V.; PÉREZ, L.R. (orgs.) Suculentas Mexicanas Cactáceas. Cidade do México: CVS Publicações. p.: 10-15.

HUNT, D.R.; TAYLOR, N.P. 1990. The genera of Cactaceae: progress toward consensus. Bradleya, 8: 85-107.

IUCN. 2004. 2004 IUCN Red List of Threatened Species**.** www.redlist.org. 17 Abr. 2005.

JESUS, F.F.; SOLFERINI, V.N.; SEMIR, J.; PRADO, P.I. 2001. Local genetic differentiation in *Proteopsis argentea* (Asteraceae), a perennial herb endemic in Brazil. Plant Systematics and Evolution, 226: 59-68.

JOLY, A.B. 1970. Conheça a Vegetação Brasileira. São Paulo: EDUSP.

JUDD, W.S.; CAMPBELL, C.S.; KELLOG, E.A.; STEVENS, P.F. 1999. Plant Systematic. 1 ed. Sunderland: Sinauer Associates.

JUDD, W.S.; CAMPBELL, C.S.; KELLOGG, E.A.; STEVENS, P.F.; DONOGUE, M.J. 2002. Plant Systematic. 2 ed. Sunderland: Sinauer Associates.

KIMURA, M.; WEISS, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics, 49: 561- 576.

KREITMAN, N.; AKASHI, H. 1995. Molecular evidence for natural selection. Annual Review of Ecology and Systematics, 26: 403-422.

LAMBERT, S.M.; BORBA, E.L.; MACHADO, M.C.; ANDRADE, S.C.S. In press. Allozyme diversity and morphometrics of *Melocactus paucispinus* (Cactaceae) and evidence for hybridization with *M. concinnus* in the Chapada Diamantina, Northeastern Brazil. Annals of Botany.

LEVENE, H. 1949. On a matching problem arising in genetics. Annals of Mathematical Statistics, 20: 91-94.

LEVIN, D.A.; FRANCISCO-ORTEGA, J.; JANSEN, R.K. 1996. Hybridization and the extinction of rare plants species. Conservation Biology, 10: 10-16.

LEVIN, D.A. 2000. The Origin, Expansion, and Demise of Plant Species. New York: Oxford University Press.

LEWONTIN, R.C.; BIRCH, L.C. 1966. Hybridization as a source of variation for adaptation to new environments. Evolution, 20: 315-336.

LINHART, Y.B.; GRANT, M.C. 1996. Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics, 27: 237-277.

LOCATELLI, E.; MACHADO, I.C.S. 1999. Comparative study of the floral biology in two ornithophilous species of Cactaceae: *Melocactus zehntneri* and *Opuntia palmadora*. Bradleya, 17: 75-85.

MACHADO, M.C. 1999. The cacti of Morro do Chapéu, Bahia, Brazil. British Cactus and Succulent Journal, 17: 201-213.

MACHADO, M.C.; CHARLES, G. 2004. *Pilosocereus bohlei* Hofacker – a remarkable new species from Brazil. British Cactus and Succulent Journal, 22: 188-192.

MACHADO, M.C.; ZAPPI, D.C.; BORBA, E.L.; LAMBERT, S.M. Submitted manuscript. Genetic variability of three *Discocactus* Pfeiff. (Cactaceae) species from the state of Bahia, Brazil. Botanical Journal of the Linnean Society.

MARTÍNEZ-PALACIOS, A.; EGUIARTE, L.E.; FURNIER, G. 1999. Genetic diversity of the engendered endemic *Agave victoriae-reginae* (Agavaceae) in the Chihuahua desert. American Journal of Botany, 86: 1093-1098.

MCCAULEY, D.E.; STEVENS, J.E.; PERONI, P.A.; RAVELLI, J.A. 1996. The spatial distribution of clorosplast DNA and allozyme polymorphisms within a population of *Silene alba* (Caryophyllaceae). American Journal of Botany, 83: 727-731.

MCCUNE. B.; MEFFORD, M.J. 1999. PCOrd – Multivariate analysis of ecological data, version 4.10. Gleneder Beach: MjM Software.

MONTES, S.A. 1997. Distribución, grupos taxonómicos y formas de vida: distribución general**.** In: SEPTIÉN, C.V.; PÉREZ, L.R. (orgs.) Suculentas Mexicanas Cactáceas. México: CVS Publicações. p.: 17-25.

MORAES, E.M.; ABREU, A.G.; ANDRADE, S.C.S.; SENE, F.M.; SOLFERINI, V.N. 2005. Population genetic structure of two columnar cacti with a patchy distribution in eastern Brazil. Genetics, 125: 311-323.

NASSAR, J.M. 1999**.** Comparative population genetic structure of Venezuelan cacti and estimates of their mating systems. PhD dissertation. Coral Gables, FL University of Miami.

NASSAR, J.M.; HAMRICK, J.L.; FLEMING, T.H. 2001. Genetic variation and population structure of the mixed-mating cactus, *Melocactus curvispinus* (Cactaceae). Heredity, 87: 69-79.

NASSAR, J.M.; HAMRICK, J.L.; FLEMING, T.H. 2002. Allozyme diversity and genetic structure of the leafy cactus (*Pereskia guamacho* [Cactaceae]). The American Genetic Association, 93: 193-200.

NASSAR, J.M.; HAMRICK, J.L.; FLEMING, T.H. 2003. Population genetic structure of Venezuelan chiropterophilous columnar cacti (Cactaceae). American Journal of Botany, 90: 1628-1637.

NASSAR, J.M.; RAMÍREZ, N. 2004. Reproductive biology of the melon cactus, *Melocactus curvispinus* (Cactaceae). Plant Systematic Evolution, 248: 31-44.

NATIONAL GEODETIC SURVEY. 2002. INVERSE – Version 2.0. Silver Spring: 1315 East-West Highway, Silver Spring, MD 20910-3282. http://ngs.noaa.gov/. 18 Jan. 2005.

NEEL, M.C.; CLEGG, J.; ELLSTRAND, N.C. 1996 Isozime variation in *Echinocereus enelmannii* var. *munzii* (Cactaceae). Conservation Biology, 10: 622-631.

NEGRON-ÓRTIZ, V. 1998. Reproductive biology of rare cactus, *Opuntia spinosíssima* (Cactaceae), in the Florida Keys: why is seed set very low? Sexual Plant Reproduction, 11: 208-212.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

O´LEARY, M.C.; BOYLE, T.H. 2000. Divesity and distribuition of isozymes in a *Schlumbergera* (Cactaceae) clonal germoplasm collection. Journal of the American Society for Horticultural Science, 125: 81-85.

PARKER, K.C.; HAMRICK, J.L. 1992. Genetic diversity and clonal structure in a columnar cactus, *Lophocereus schotii*. American Journal of Botany, 79: 86-96.

PEREIRA, A.C.S.; BORBA, E.L.; GIULIETII, A.M. Submitted manuscript. Genetic and morphological variability of the Endangered *Syngonanthus mucugensis* Giul. (Eriocaulaceae), from the Chapada Diamantina, Brazil: implications for conservation and taxonomy. Botanical Journal of Linnean Society.

PIMENTA, E. 1997. Usos y conservación: el nopal en México y el mundo. In: SEPTIÉN, C.V.; PÉREZ, L.R. (orgs.) Suculentas Mexicanas Cactáceas. Cidade do México: CVS Publicações. p.: 87-95.

RAYMOND, M.; ROUSSET, F. 1995**.** GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86: 248-249.

RHYMER, J.M.; SIMBERLOFF, D. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics, 27: 83-109.

RICE, W.R. 1989. Analyzing tables of statistical tests. Evolution, 43: 223–225.

RIDGWAY, G.J.; SHERBURNE, S.W.; LEWIS, R.D. 1970. Polymorphism in the esterases of Atlantic herring. Transactions of the American Fisheries Society, 99: 147-151.

RIESEBERG, L.H. 1995. The role of hybridization in evolution: old wine in new skins. American Journal of Botany, 82: 944-953.

ROWLEY, G.D. 1994. Spontaneous bigeneric hybrids in Cactaceae. Bradleya, 12: 2-7.

SCHMALZEL, R.J.; NIXON, R.T.; BEST, A.L.; TRESS, J.R.J.A. 2004. Morphometric variation in *Coryphantha robustipina* (Cactaceae). Systematic Botany**,** 29: 553-568.

SHAW, C.R.; PRASAD, R. 1970. Starch gel eletrophoresis of enzymes – a compilation of recipes. Biochemical Genetics, 4: 297-320.

SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. Science, 236: 787-792.

SLATKIN, M.; BARTON, N.H. 1989**.** A comparison of three indirect methods for estimating average levels of gene flow. Evolution, 43: 1349-1368.

SNEATH, P.H.A.; SOKAL, R.R. 1973. Numerical taxonomy. San Francisco: Freeman and Co.

SOLTIS, D.E.; HAUFLER, C.H.; DARROW, D.C.; GASTONY, G.J. 1983. Starch gel eletrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedule. American Fern Journal 73: 9-27.

SOLTIS, D.E.; SOLTIS, P.S.; ENFRESS, P.K.; CHASE, M.W. 2005. Phylogeny and Evolution of Angiosperms. Sunderland: Sinauer Associates.

STACE, C.A. 1984. Plant Taxonomy and Biosystematics. London: Edward Arnold.

STATSOFT, I.N.C. 2000. Statistica for Windows (Computer program manual). Tulsa: Salt Soft Inc.

STEBBINS, G.L. 1959. The role of hybridization in evolution. Proccedings of the American Philosophical Society, 103: 231-251.

STUBER, C.W.; GOODMAN, M.M.; JOHNSON, F.M. 1977. Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.). Biochemical Genetics, 15: 383-394.

SUN, M.; GANDERS, F.R. 1990. Outcrossing rates and allozymes variation in rayed and rayless morphs of *Bidens pilosa*. Heredity, 64: 139-143.

SWOFFORD, D.L.; SELANDER, R.B. 1989. BIOSYS-1: computer program for the analysis of allelic variation in population genetics and biochemical systematics. Champaign: Illinois Natural History Survey.

TAYLOR, N.P.; ZAPPI, D.C. 1989. An alternative view of generic delimitation and relationships in tribe Cereeae (Cactaceae). Bradleya, 7: 13-40.

TAYLOR, N.P. 1991. The genus *Melocactus* (Cactaceae) in Central and South America. Bradleya, 9: 1-80.

TAYLOR, N.P. 1997. Cactaceae. In: OLDFIELD, S. (org.). Cactus and Succulent Plants – Status Survey and Conservation Action Plan. Cambridge: IUCN/SSC. p.: 17-20.

TAYLOR, N.P. 2000. Taxonomy and Phytogeography of the Cactaceae of Eastern Brazil. Tese de Doutorado. The Open University and Royal Botanic Gardens. Kew.

TAYLOR, N.P.; ZAPPI, D.C. 2004. Cacti of Eastern Brazil. Richmond, Surrey: Royal Botanic Gardens, Kew.

TEMPLETON, A.R. 1986. Coadaptation and outbreeding depression. In: SOULÉ, M.E. (ed). Conservation Biology: the Science of Scarcity and Diversity. Massachusetts: Sinauer Associates. p.:105-116.

THOMSON, G. 2002. A re-evaluation of the taxonomic status of the genus *Melocactus* in Aruba, Netherlands Antilles. Bradleya, 20: 29-44.

TORPE, J.P. 1982. The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annual Review of Ecology and Systematics, 13: 139-168.

VIÑAS, R.A.; GARCÍA, P.S.; BAUTISTA, P.B. 1997. Usos y conservación: agroindustria de algunos frutos. In: SEPTIÉN, C.V.; PÉREZ, L.R. (orgs.) Suculentas Mexicanas Cactáceas. Cidade do México: CVS Publicações. p.: 79-85.

WALLACE, R.S. 1995. Molecular systematic study of the Cactaceae: using chloroplast DNA variation to elucidate Cactus Phylogeny. Bradleya, 13: 1-12.

WRIGHT, S. 1951. The genetical structure of populations. Annals of Eugenics, 15: 323-354.

WRIGHT, S. 1978. Evolution and the Genetics of Populations - Variability within and among Natural Populations. Vol. 4. Chicago: University of Chicago Press.

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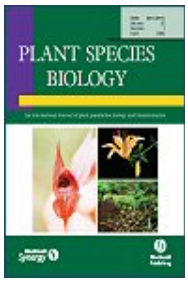


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***Chapter in a book***

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