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

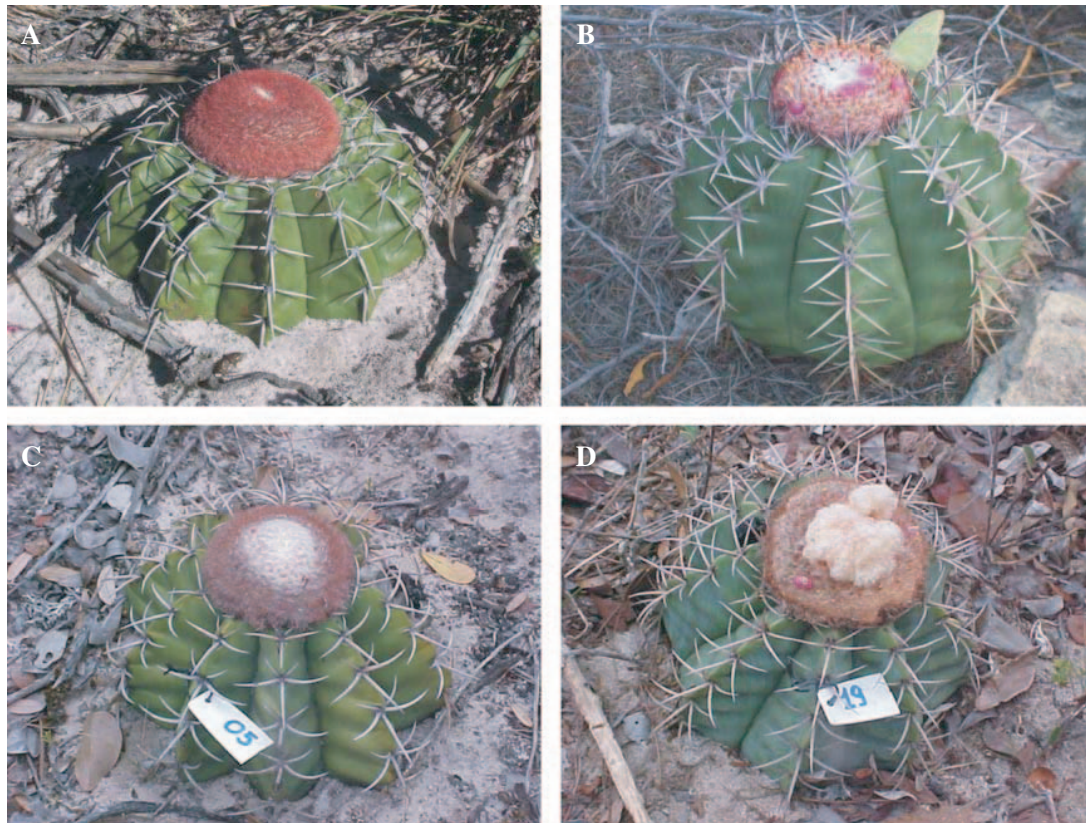* For correspondence. E-mail sabrina.lambert@bol.com.br; borba@gmx.net

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 Cactaceae 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 χ^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 (σ^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 ($P = 0·006$; critical $\alpha = 0·008$ for six tests) and DIA-1/DIA-2 ($P = 0·00001$; $\alpha = 0·005$ for ten tests), respectively. None of the ten linkage disequilibrium tests in *M. concinnus* were significant after the correction ($\alpha = 0·005$).

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*

Locus	Allele*	Melocactus paucispinus										Melocactus concinnus		
		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 $D2_m$ ($r = 0.581$, $P = 0.037$), but not between H_e and ED ($r = 0.191$, $P = 0.532$). 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, $\sigma^2 = 0.101$), an F_{RT} of 0·253 ($\sigma^2 = 0.067$) and an F_{ST} of 0·140 ($\sigma^2 = -0.034$), 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*	*M. paucispinus*		*M. concinnus*	
Locus	F_{IS}	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,
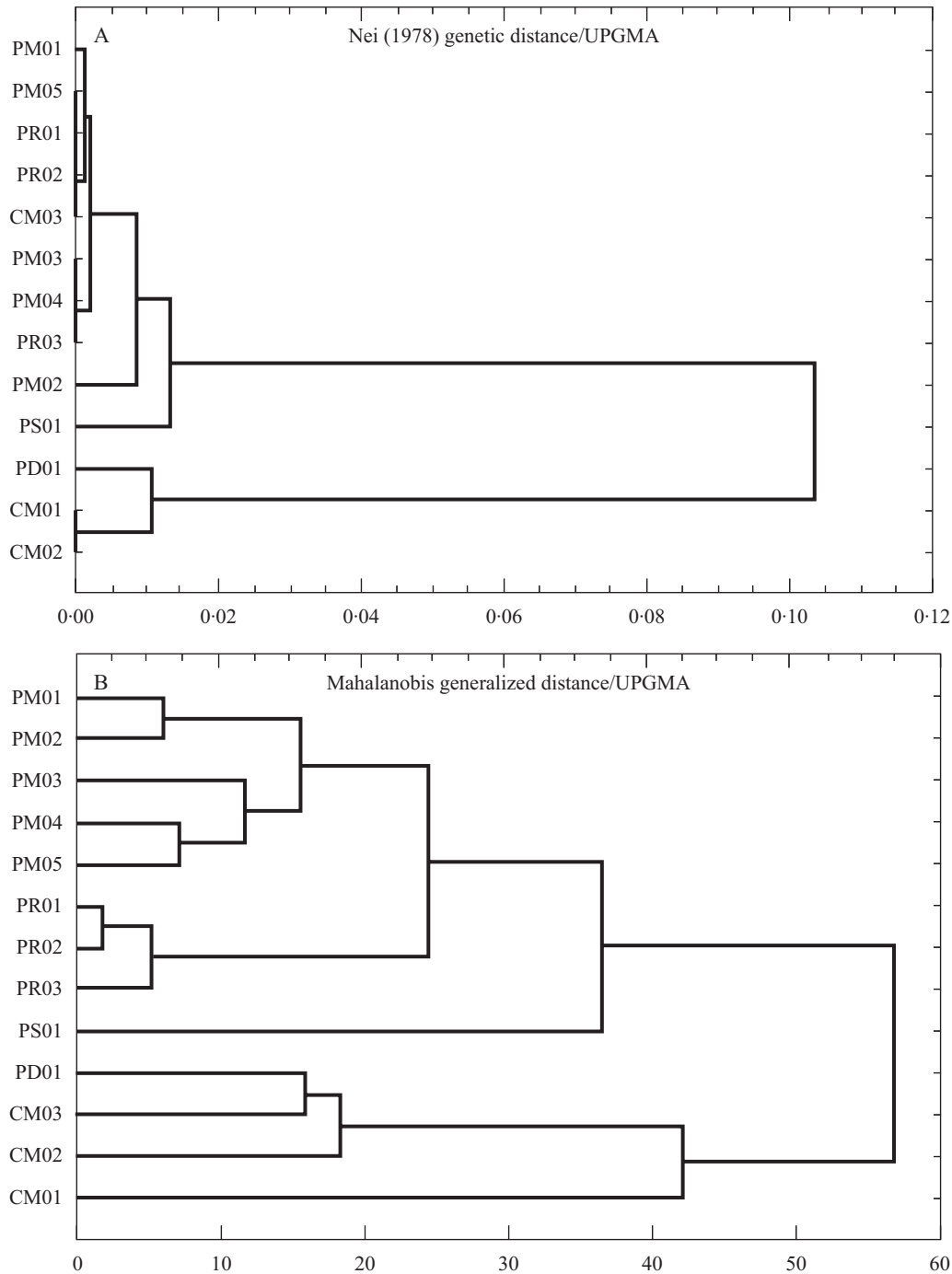
FIG. 3. Dendrograms showing the phenetic relationships among ten populations of *Melocactus paucispinus* and three populations of *M. concinnus* occurring in the Chapada Diamantina, Brazil, constructed using the matrix of genetic distances (Nei, 1978, unbiased estimate; cophenetic correlation = 0·886) 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* ($P = 89·5$ %,
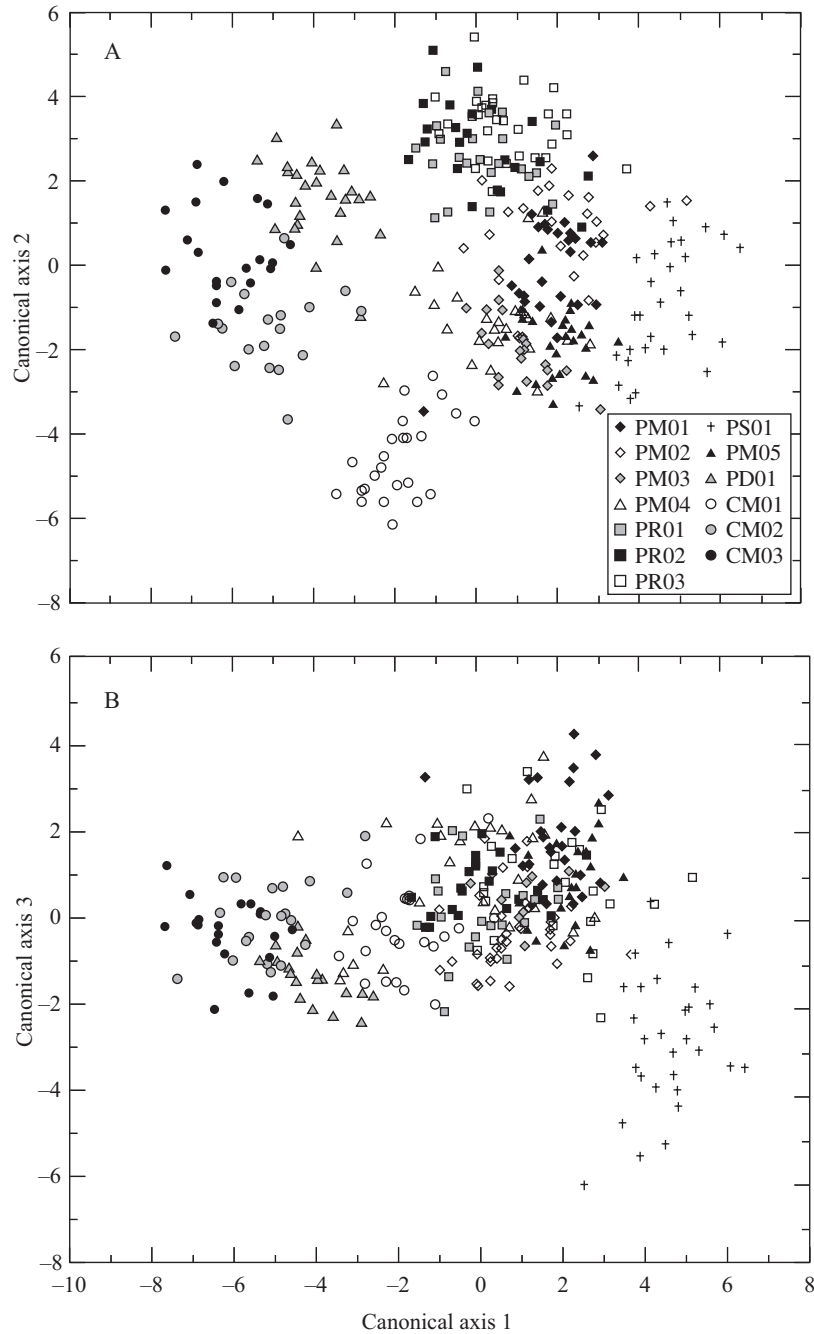
FIG. 4. Representation of the scores on the three first canonical axes of the CVA using 17 morphological characters in ten populations of *Melocactus paucispinus* and three populations of *M. concinnus* occurring in the Chapada Diamantina, Brazil. (A) Canonical axes 1 and 2. (B) Canonical axes 1 and 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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