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# Systematics of the *Gymnocalycium paraguayense-fleischerianum* group (Cactaceae): morphological and molecular data

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## ABSTRACT

MEREGALLI, M., D. METZING, R. KIESLING, S. TOSATTO & R. CARAMIELLO (2002). Systematics of the *Gymnocalycium paraguayense-fleischerianum* group (Cactaceae): morphological and molecular data. *Candollea* 57: 299-315. In English, English and French abstracts.

Six populations of *Gymnocalycium paraguayense* (K. Schum.) Hosseus and *G. fleischerianum* Backeb. (Cactaceae), endemics to Paraguay and until present considered as two different species, were studied using macromorphology, micromorphology and molecular data based on RAPD methods. Results were very homogeneous and suggest that all populations should be referred to a single species, composed of two taxa at forma rank. *Gymnocalycium paraguayense* is typified and *G. paraguayense* f. *fleischerianum* Mereg., Metzing & R. Kiesling is described; a list of synonyms is added.

## RÉSUMÉ

MEREGALLI, M., D. METZING, R. KIESLING, S. TOSATTO & R. CARAMIELLO (2002). Systématique du groupe *Gymnocalycium paraguayense-fleischerianum* (Cactaceae): données morphologiques et moléculaires. *Candollea* 57: 299-315. En anglais, résumés anglais et français.

Ce travail présente une étude conduite sur *Gymnocalycium paraguayense* (K. Schum.) Hosseus et *G. fleischerianum* Backb., Cactaceae du Paraguay jusqu'à présent considérées comme deux espèces distinctes. Les données macromorphologiques, micromorphologiques et moléculaires, obtenues avec les méthodes RAPD, ont été prises en considération pour chaque population. Les résultats ont permis d'assigner à une seule espèce tous les exemplaires étudiés. *Gymnocalycium paraguayense* est typifié et *G. paraguayense* f. *fleischerianum* Mereg., Metzing & R. Kiesling est décrite; une liste des synonymes est fournie.

**KEY-WORDS:** CACTACEAE – *Gymnocalycium* – Paraguayan flora – Systematics – RAPD.

## Introduction

The genus *Gymnocalycium* Pfeiffer ex Mittler, a group of globular cacti, is represented in Paraguay by about nine species (METZING, 1994; HUNT, 1999a). Infrageneric classifications of the genus are mainly based on seed characters (KREUZINGER, 1935; SCHÜTZ, 1962, 1969; BUXBAUM, 1968). One of the subgenera in the Fric-Schütz system is *Macrosemineum* Metzing (1992). This taxon is represented in Paraguay by two endemic entities, *Gymnocalycium paraguayense* (K. Schum.) Hosseus (1939) and *Gymnocalycium fleischerianum* Backeb. [nom. inval.] (for nomenclatural status see below). Their status as distinct species has not been questioned in taxonomic treatments or popular publications (e.g. BACKEBERG, 1966;

MEREGALLI, 1985; SCHÜTZ, 1986; SWALES & HUNT, 1989; PILBEAM, 1995). However, a comparative study of the *G. paraguayense-fleischerianum* complex (shortened as *pfc* in this work), based on several populations observed in recent field investigations, showed that the macromorphological traits used to differentiate the two taxa are neither constant nor always discernable. The status of the two taxa is thus not yet entirely clear (cf. METZING, 1994: 16).

The plants grow in extra-zonal habitats, such as low, usually isolated, rocky outcrops scattered in semi-evergreen forest or presently cultivated areas. Each population is thus more or less isolated from the others, and today populations are often very small, in extreme cases numbering only a few tens of individuals (METZING, 1994). In these cases, founder effects and/or genetic drift enhanced by reduced, possibly sometimes absent, gene flow among the populations may lead to the stabilization of peculiar characters. Thus simple macromorphological analysis does not clarify the taxonomic relationships among the forms.

The differentiating traits are essentially the acute ribs with prominent tubercles, and a rather deep transverse furrow between the areoles in *G. paraguayense*. *Gymnocalycium fleischerianum* has wider, more rounded ribs lacking the transverse furrow and has longer and thinner spines. Examination of a large number of plants revealed a high intra- and interpopulation level of variability, so that usually only the furrow on the ribs allowed the taxa to be separated; however, in a few single plants even this character is not reliable (METZING, unpublished pers. obs.). Often the plants attributed to *G. paraguayense* grow in less shallow soil and have a rather flat stem, while those referred to *G. fleischerianum* are found in shallow soil at the margin of rocky outcrops, where they have a more rounded stem.

To evaluate the taxonomic status of these plants, seed morphology and molecular data were investigated. In *Gymnocalycium* seed morphology determines the subgeneric classification but may also be used to infer relationships at species or even sometimes subspecies level. This is particularly true for some subgenera (MEREGALLI, 1985, 1998).

In subgenus *Macrosemineum* Schütz, seed morphology allows easy differentiation of all the known species, or at least it gives very useful information. Sometimes significant characters are

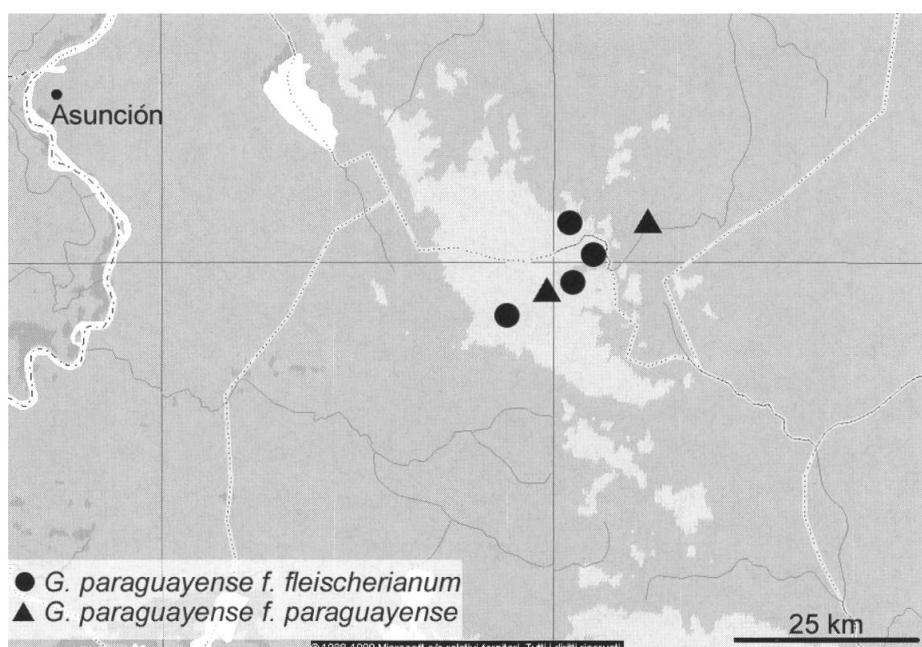


Fig. 1. – Distribution of *Gymnocalycium paraguayense* (K. Schum.) Hosseus f. *paraguayense* and *G. paraguayense* f. *fleischerianum* Mereg. & al.

typical of a single population [MEREGALLI, unpublished observations on *G. denudatum* (Link & Otto) Mittler and *G. uruguayense* (Arechav.) Britton & Rose]. Molecular analyses were recently applied to *Cactaceae*, mainly through the investigation of chloroplast genes (WALLACE, 1995). These studies proved to be highly informative, particularly in the evaluation of taxonomy and phylogeny at generic and suprageneric level. However, for investigating genetic polymorphism at the level of single populations, RAPD methods seem to give better information (METZ & al., 1997).

### Taxonomic history

The Paraguayan cactus flora was first explored in the last years of the 19th century (cf. METZING, 1994). The commercial collector Hermann Grosse exported a large number of cacti to the German nursery Haage (Erfurt), and the first specimens of *Gymnocalycium* from the Cordillera de los Altos were also sent to Europe at this time. The morphological resemblance to *Echinocactus denudatus* Link & Otto [= *Gymnocalycium denudatum* (Link & Otto) Mittler] led the new collections from Paraguay to be named as varieties of this species (MUNDT, 1897; HAAGE, 1898), and the first illustrations (engravings) were published in the plant lists of Haage nursery (e.g. HAAGE, 1899: 12-13). Subsequently, these plants were attributed to *Echinocactus paraguayensis* and *Gymnocalycium fleischerianum*: *Echinocactus paraguayensis* was described by SCHUMANN (1903) and combined to *Gymnocalycium* by HÖSSEUS (1939); *Gymnocalycium fleischerianum* was described by Backeberg in 1936 (nom. inval. Art. 36.1) and 1959 (nom. inval. Art. 37.1) (cf. EGGLI & TAYLOR, 1992; METZING & al., 1995).

### Materials and methods

Samples of six populations of the complex, nearly 80 specimens in all, were examined (see Fig. 1 for geographic distribution). These collections included material from most of the presently known and documented populations. As outgroups, samples of five species belonging to the same subgenus *Macrosemineum* were selected (Table 1). Macromorphological observations [DM, RK] were based on study of mature cultivated plants as well as on investigations in habitat. Differences in rib number, spine number and spine length were tested for significance by means of ANOVA, with Tukey test as a post-hoc test, performed with Systat: Statistics, version 5.2, Evanston, IL [MM].

#### MICROMORPHOLOGICAL ANALYSIS [MM, ST]

Seeds were collected from cultivated plants whose flowers were hand-pollinated. Samples were cleaned from mucilage by gently washing in water; no detergent was used to avoid damaging epicuticular structures. After drying, they were examined and measured by light microscopy, then mounted, coated with gold and examined by SEM on a Cambridge S 360. Terminology for seed structures follows BARTHLOTT & HUNT (2000). Pollen was collected from flowers in full anthesis, prepared with critical point and, after coating with gold, examined at SEM on the same Cambridge S 360.

#### RAPD ANALYSIS [MM, ST, RC]

DNA was extracted from 29 samples from 27 plants belonging to the 6 *pcf* populations and the outgroups (Table 2). Source material was either 2 week-old seedlings, thus reducing problems related to presence of mucilage in adult plants, or directly seeds obtained from cultivated habitat-collected plants whose flowers were hand-pollinated, after rehydration for 24 hours and discarding the integument. In some cases DNA was also obtained from small vegetative propagules of habitat-collected plants.

DNA was extracted according to the hot CTAB protocol modified according to METZ & al. (1997). When vegetative propagules were used a further purification with QIAEX II Gel Extraction Kit (Qiagen) followed the extraction. PCR was performed in a volume of 25 ml containing

<b>Table 1. – List of material.</b>		
<b>Present identification</b>	<b>Collection</b>	<b>Locality</b>
<i>G. paraguayense</i>	Metzing, Piltz & Bercht M35	Dept. Cordillera, S of Itacurubí, Cerro Peró
<i>G. paraguayense</i>	Metzing, Piltz & Bercht M40	Dept. Paraguarí, S of Piribebuy, Yhaguy Guazú
<i>G. fleischerianum</i>	Metzing, Piltz & Bercht M4	Dept. Cordillera, Oyopóí
<i>G. fleischerianum</i>	Metzing, Piltz & Bercht M9	Dept. Cordillera, Piraretá
<i>G. fleischerianum</i>	Metzing, Piltz & Bercht M32	Dept. Cordillera, Saltos Amambay
<i>G. fleischerianum</i>	Metzing, Piltz & Bercht M41	Dept. Paraguarí, Chololó
OUTGROUPS		
<i>G. uruguayense</i> s. l.	Horst HU1284	Uruguay, Artigas, ruta 30
<i>G. uruguayense</i> s. l.	Horst HU1353	Uruguay, Minas, Zapican
<i>G. denudatum</i>	Gerloff Gf18	Brasil, RGS, Minas Camaqua
<i>G. mesopotamicum</i>	Piltz P241	Argentina, Corrientes, Mercedes
<i>G. horstii</i>	Horst HU79	Brasil, RGS, Minas Camaqua
<i>G. buenekeri</i>	Gerloff Gf260	Brasil, RGS, Sao Francisco de Asis

<b>Table 2. – List of material for DNA analysis (29 samples from 27 plants).</b>			
<b>Collection</b>	<b>Source of DNA</b>	<b>Collection</b>	<b>Source of DNA</b>
M 35-1	seeds from a single fruit	M 4-4	vegetative propagule
M 35-2	seedlings from seeds of a single fruit	M 9-1	seeds from a single fruit
M 35-3	seeds from a single fruit	M 9-2	seeds from a single fruit
M 35-4	vegetative propagule	M 9-2	vegetative propagule
M 40-1	seeds from a single fruit	M 9-3	seeds from a single fruit
M 40-1	vegetative propagule	M 9-4	seeds from a single fruit
M 40-2	vegetative propagule	M 9-5	seedlings from seeds of a single fruit
M 40-3	vegetative propagule	M 41-1	seedlings from seeds of a single fruit
M 32	vegetative propagule	M 41-2	seeds from a single fruit
M 4-1	seeds from a single fruit	M 41-3	seeds from a single fruit
M 4-2	seeds from a single fruit	M 41-4	seeds from a single fruit
M 4-3	seeds from a single fruit		
OUTGROUPS			
HU1284	seeds from a single fruit	P241	seeds from a single fruit
HU1353	seeds from a single fruit	HU79	seeds from a single fruit
Gf18	seeds from a single fruit	Gf260	seeds from a single fruit

AmpliTaq polymerase, 0.4 mM dNPTs, 2.5 mg BSA and buffer (3 mM MgCl<sub>2</sub>, 30 mM KCl, 10 mM Tris, pH 8.3) (Amersham Pharmacia “Ready-to-go” RAPD Analysis Beads), plus 1 ml of the primer and 1 ml (50 ng) of the sample DNA, according to the protocol suggested by Amersham.

A control PCR tube containing all components excluding genomic DNA was run with each primer to check for contamination, while a tube with *Escherichia coli* genome was run as positive control.

A panel of six ten-mer primers (Amersham Pharmacia “Ready-to-go” RAPD primers, see Table 3 for sequence) was used in two replicate analyses. The thermal sequence was: a first cycle of 5 min at 95°C followed by 45 cycles of 1 min at 95°C, 1 min at 36°C, and 2 min at 72°C.

**Table 3. – Primers used.**

PRIMER 1: 5' - d(GGTGCGGGAA) - 3'	PRIMER 4: 5' - d(AAGAGCCGT) - 3'
PRIMER 2: 5' - d(GTTTCGCTCC) - 3'	PRIMER 5: 5' - d(AACGCGCAAC) - 3'
PRIMER 3: 5' - d(GTAGACCCGT) - 3'	PRIMER 6: 5' - d(CCCGTCAGCA) - 3'

Amplification products were electrophoresed on 2% agarose gel supplemented with 0.5 ng/ml ethidium bromide. Two different amounts of DNA (5 ng/ml and 20 ng/ml) for each product were run contiguously. PUC18 and 1 viral DNA were used as molecular weight markers. Gels were photographed under UV light with Polaroid film.

The RAPD bands for each primer were scored for presence/absence. No quantitative scoring based on the brightness of the bands was used, as this proved to be subjective. Data were coded by presence/absence into a matrix for each primer and a general matrix including all the bands obtained for the six primers. Each matrix was analysed according to several distance methods (Jaccard, Sorensen, Baroni-Urbani), using the Syn-tax program, and dendograms were constructed.

### Results and discussion

#### STEM MORPHOLOGY (Fig. 2, a-d)

Body flat-globular to globular (during the dry season sometimes sunken into the soil); epidermis glossy green; stems with 5-11(-13) ribs, usually 6-8; diameter of mature plants about 4-8(-10) cm; older plants producing offshoots from base, building small groups or mats; areoles approximately circular with white to pale brownish wool; ribs rounded, with no or little undulation from tubercles on vertical clefts and lacking very prominent tubercles; spines curved towards the body or prominent, spreading and straight to slightly curving.

The furrow on the rib between areoles (Fig. 2, a-b) is the main diagnostic character to separate *G. paraguayense* from *G. fleischerianum*. Indeed plants showed nearly no intrapopulation variability concerning this character, which was present in all examined specimens from Yhaguy Guazú and Cerro Peró, referred to *G. paraguayense*. It was usually absent in the plants from Oyo-poí, Piraretá, Saltos Amambay and Chololó (Fig. 2, c-d), although a weak furrow was occasionally found on specimens from these populations.

The general aspect of the plants from each population is usually more or less characteristic. Specimens from Yhaguy Guazú have more prominent tubercles and narrower ribs than those from Cerro Peró. This last is also very variable in number of ribs, from 5 to 11.

The population from Chololó is characterised by strong clustering habit and denser, prominent spination. The population from Saltos Amambay has plants with more prominent tubercles in comparison to other populations referred to *G. fleischerianum*. The highest variability was found at Piraretá (cf. ESSER, 1982), and this can be related to the large number of individuals present in this area.

The mean number and length of the marginal spines is smaller in the forms referred to *G. paraguayense* than in those belonging to *G. fleischerianum*, the population from Saltos Amambay lying between these.

This is confirmed by analyses of variance for rib number, spine number and spine length (Table 4). Spine number and in part spine length significantly differentiate the populations referred to *G. fleischerianum* from those referred to *G. paraguayense*, with the exception of Saltos Amambay, which is set apart from the other populations of *G. fleischerianum* for spine number (but not for spine length).

BACKEBERG (1959) mentioned up to 20 spines per areole in *G. fleischerianum*, but such a high number was not confirmed in our study. ESSER (1982) noted a spine length of up to 60 mm.

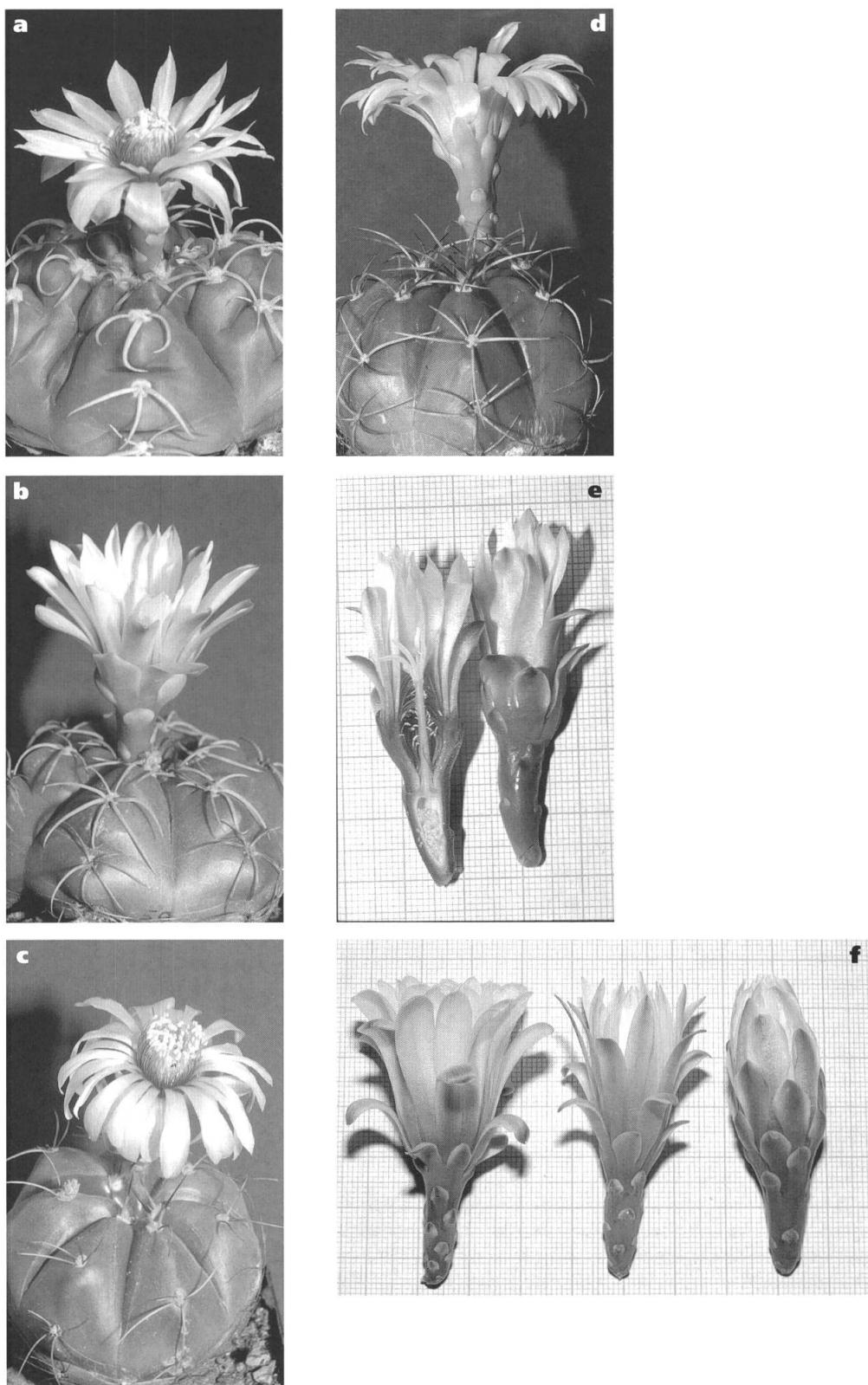


Fig. 2. – *Gymnocalycium paraguayense* (K. Schum.) Hosseus f. *paraguayense*, Cerro Peró (a); Yhagui Guazú (b). *G. paraguayense* f. *fleischerianum* Mereg. & al., Piraretá (c); Chololó (d). Flower of *G. paraguayense* (K. Schum.) Hosseus f. *paraguayense*, Cerro Peró (e). Flowers of *G. paraguayense* f. *fleischerianum* Mereg. & al. (f): Oyopoí (left), Piraretá (centre), Saltos Amambay (right).

## FLOWER MORPHOLOGY (Fig. 2, e-f)

Flowers white with purplish-pink throat, funnel-shaped, 4-6 cm long; pericarpel 1-2 cm long, 5-7 mm in diameter; pericarpel scales with light margin and sometimes pinkish tip; perianth segments numerous, rounded, truncate, cuspidate or apiculate; style whitish, with pale yellow stigma 20-35 mm long; stamens purple-pinkish, to whitish at the apex, pollen straw-coloured.

The form of the perianth segments showed high intrapopulational variability, and the form of the flower - broad or slender – also varied within the populations. Most plants were dioecious, this is the rule in the two populations referred to *G. paraguayense*. All plants were autosterile. There was no floral character typical for a single or for a group of populations.

## FRUIT MORPHOLOGY

Fruits club-shaped berries, light to dark green, sometimes bluish (colour depends on the amount of epidermal wax), 2-4 cm long. After ripening, the epidermal tissue becomes very soft and dissolves when touched. This apomorph character distinguishes the *pfc* from all other species of subgenus *Macrosemineum*. As with flower characters, variability was rather high and not correlated to the populations.

## SEED MORPHOLOGY (Fig. 6)

Seed is clearly distinct from that of all the other species of the subgenus and allows prompt identification of *pfc*.

Seed oval to broadly oval, reddish to dark brown or nearly black, 1-1.6 mm from top of testa to hilum, periphery often with a weakly distinct keel formed by enlarged and more convex cells; border slightly to greatly expanded around hilum, curvilinear; testa cells irregularly polygonal (often hexagonal), weakly to moderately convex, anticlinal boundaries channelled, straight, with or without visible pits at the intersections of cells; cuticle generally uniform, with rare striations in central field; striations sometimes more developed in anticlinal field; hilum large, 1-1.5 mm long, basal, impressed, moderately convex, with a thin uniform and regular strophiolar pad; testa border at hilum, in lateral view, slightly to moderately expanded and curvilinear; a distinct impression is usually present above the hilum.

*Intrapopulation variability.* – Quite in contrast with what is observed in other species of the subgenus (MEREGLI, unpublished pers. obs.), intrapopulation variability was relatively high. Seed characters were typical of the single individual: seeds collected from the same plant but

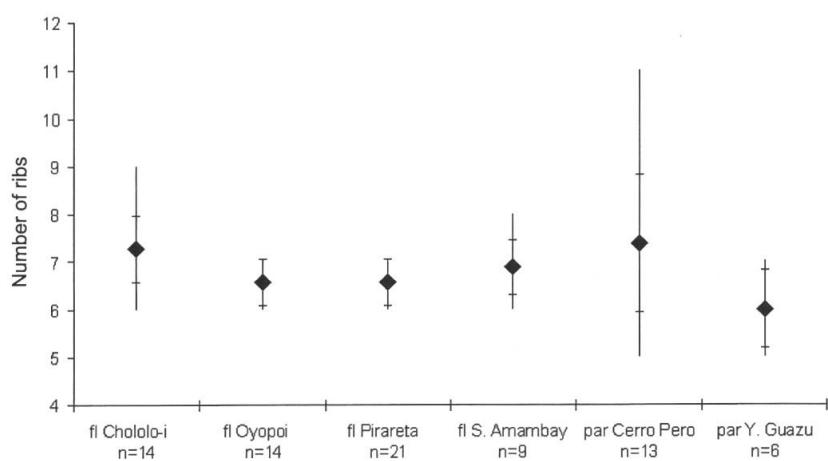


Fig. 3. – Number of ribs in six populations of *pfc*. Mean ± standard deviation and minimum-maximum. **n**: number of specimens. **par** = *Gymnocalycium paraguayense* (K. Schum.) Hosseus f. *paraguayense*, **fl** = *G. paraguayense* f. *fleischerianum* Mereg. & al.

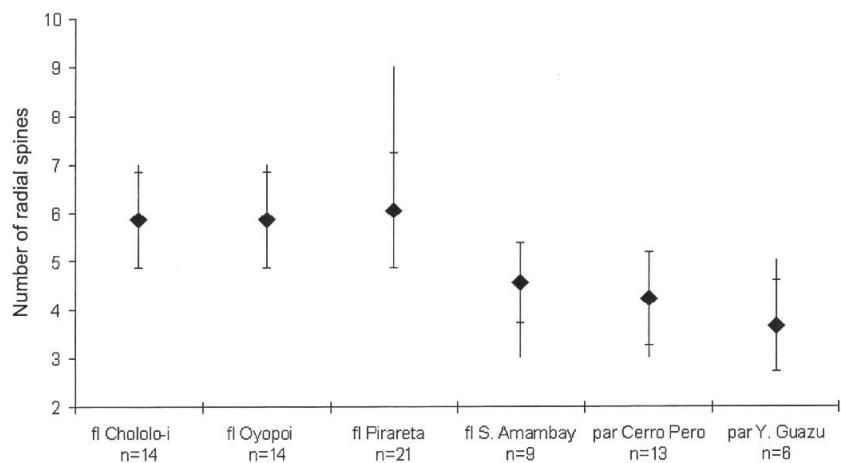


Fig. 4. – Number of radial spines in six populations of *pfc*. Mean  $\pm$  standard deviation and minimum-maximum. **n**: number of specimens. **par** = *Gymnocalycium paraguayense* (K. Schum.) Hosseus f. *paraguayense*, **fl** = *G. paraguayense* f. *fleischerianum* Mereg. & al.

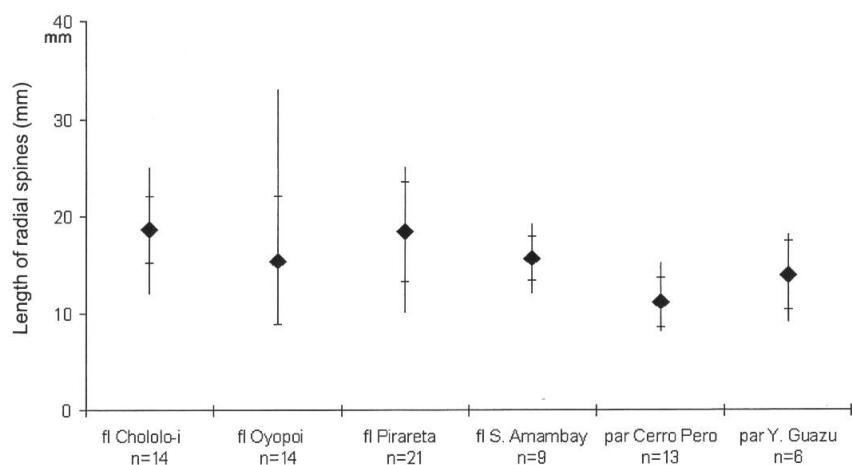


Fig. 5. – Length of radial spines in six populations of *pfc*. Mean  $\pm$  standard deviation and minimum-maximum. **n**: number of specimens. **par** = *Gymnocalycium paraguayense* (K. Schum.) Hosseus f. *paraguayense*, **fl** = *G. paraguayense* f. *fleischerianum* Mereg. & al.

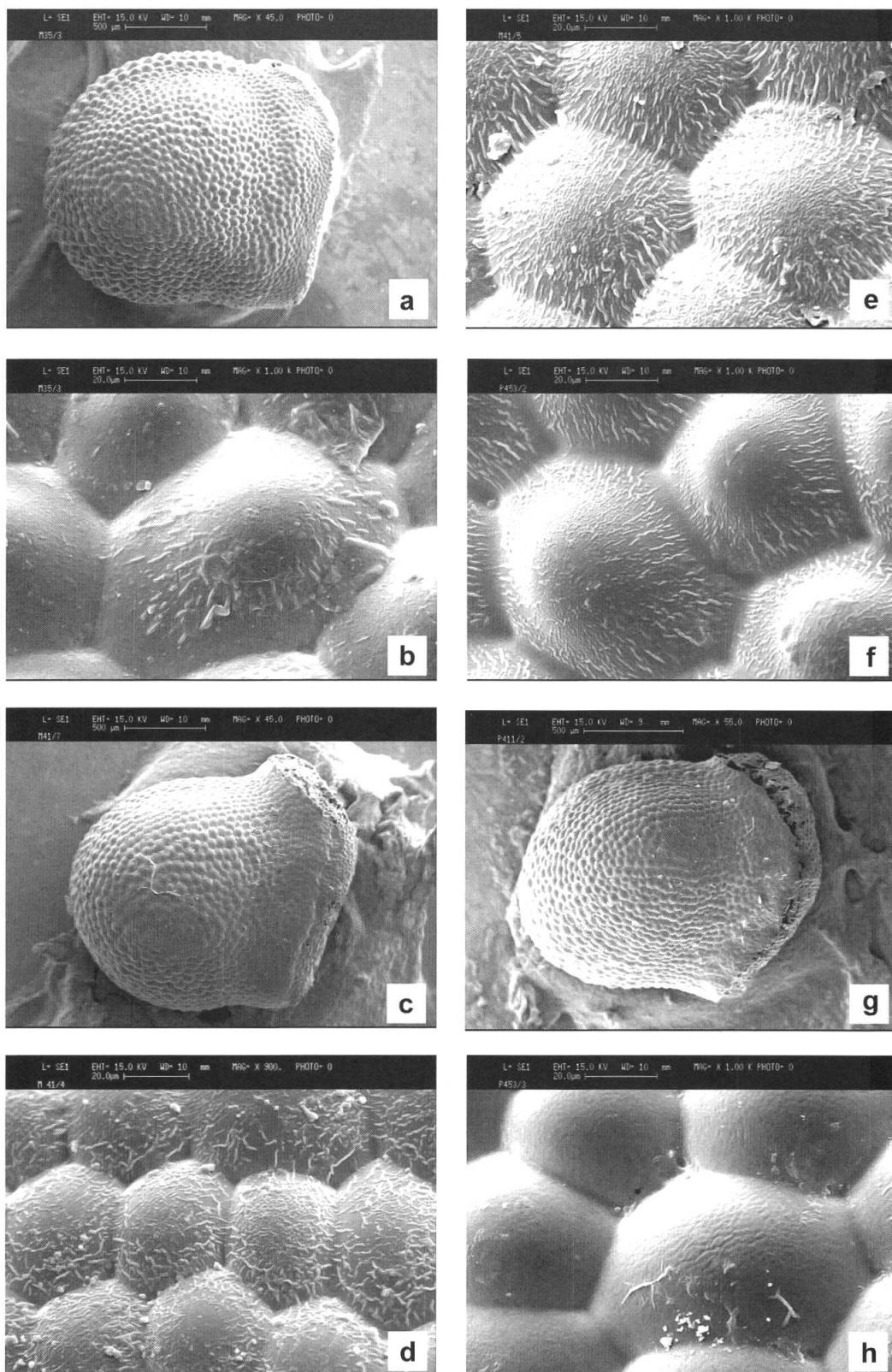


Fig. 6. – Seed of *Gymnocalycium paraguayense* (K. Schum.) Hosseus f. *paraguayense*, Cerro Peró (a, b); *G. paraguayense* f. *fleischerianum* Meregl. & al., Chololó (c, d, e, f); Oyopoí (7); Piraretá (g).

**Table 4. – Analysis of variance for rib number, spine number and spine length. ANOVA with Tukey test as post-hoc test. n.s. = not significant.**

		<i>fleischerianum</i> Chololó	<i>fleischerianum</i> Oyopoí	<i>fleischerianum</i> Piraretá	<i>fleischerianum</i> Saltos Amambay	<i>paraguayense</i> Cerro Peró
<i>fleischerianum</i> Oyopoí	rib #	<b>n.s.</b>				
	spine #	<b>n.s.</b>				
	sp. length	<b>n.s.</b>				
<i>fleischerianum</i> Piraretá	rib #	<b>n.s.</b>	<b>n.s.</b>			
	spine #	<b>n.s.</b>	<b>n.s.</b>			
	sp. length	<b>n.s.</b>	<b>n.s.</b>			
<i>fleischerianum</i> Saltos Amambay	rib #	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>		
	spine #	<b>p &lt; 0.005</b>	<b>p &lt; 0.005</b>	<b>p &lt; 0.005</b>		
	sp. length	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>		
<i>paraguayense</i> Cerro Peró	rib #	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	
	spine #	<b>p &lt; 0.005</b>	<b>p &lt; 0.005</b>	<b>p &lt; 0.005</b>	<b>n.s.</b>	
	sp. length	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	
<i>paraguayense</i> Yhaguy Guazú	rib #	<b>p &lt; 0.005</b>	<b>n.s.</b>	<b>n.s.</b>	<b>n.s.</b>	<b>p &lt; 0.005</b>
	spine #	<b>p &lt; 0.005</b>	<b>p &lt; 0.005</b>	<b>p &lt; 0.005</b>	<b>n.s.</b>	<b>n.s.</b>
	sp. length	<b>p &lt; 0.005</b>	<b>n.s.</b>	<b>p &lt; 0.005</b>	<b>n.s.</b>	<b>n.s.</b>

from different fruits or in different years had identical morphology, suggesting that these traits are genetically determined. Polymorphism was higher in plants from Oyopoí and Piraretá, and mainly concerned dimensions and form; cells were sometimes larger (a feature not related to seed dimension) and their convexity varied (usually the smaller cells were also more convex); in some specimens, often in those with more convex cells, the peripheral keel was more pronounced.

In all the other populations variation was more limited, particularly in seeds of plants from Cerro Peró. Most plants from the other populations produced more or less isodiametric seeds, with scarcely convex cells. Cuticle striation was usually more constant within a single population.

*Interpopulation variability.* – Plants from Cerro Peró usually had somewhat larger seed, higher than wide, with more convex cells, and peripheral keel nearly always present. Occasionally, however, these characters were also found in seeds from Oyopoí.

Plants from Yhaguy Guazú, referred to *G. paraguayense*, had isodiametrical seeds, with small, slightly convex cells. They could not be differentiated from the seeds of some plants from the nearby Chololó, referred to *G. fleischerianum*. In seeds of plants from Chololó the cuticle striation in central field was often denser (Fig. 6 e), rendering these seeds slightly more mat as seen in the light microscope. However, this feature was not accompanied by any other constant difference, and also appeared elsewhere occasionally.

The above observations indicated that seed morphology does not differentiate any one population, with the partial exception of that from Cerro Peró, which was very uniform and slightly better characterised. It must be noted that this last location is geographically isolated from all the others, and is also the one whose stem morphology is more differentiated.

#### POLLEN MORPHOLOGY (Figs. 7, 8)

As typical for the genus, pollen is in monads, grains of middle size, 50-55  $\mu\text{m}$  in diameter, spheroidal, trizonocolpate; exine tectate, with spinula, puncta and foveola (Fig. 7, 8). It was not

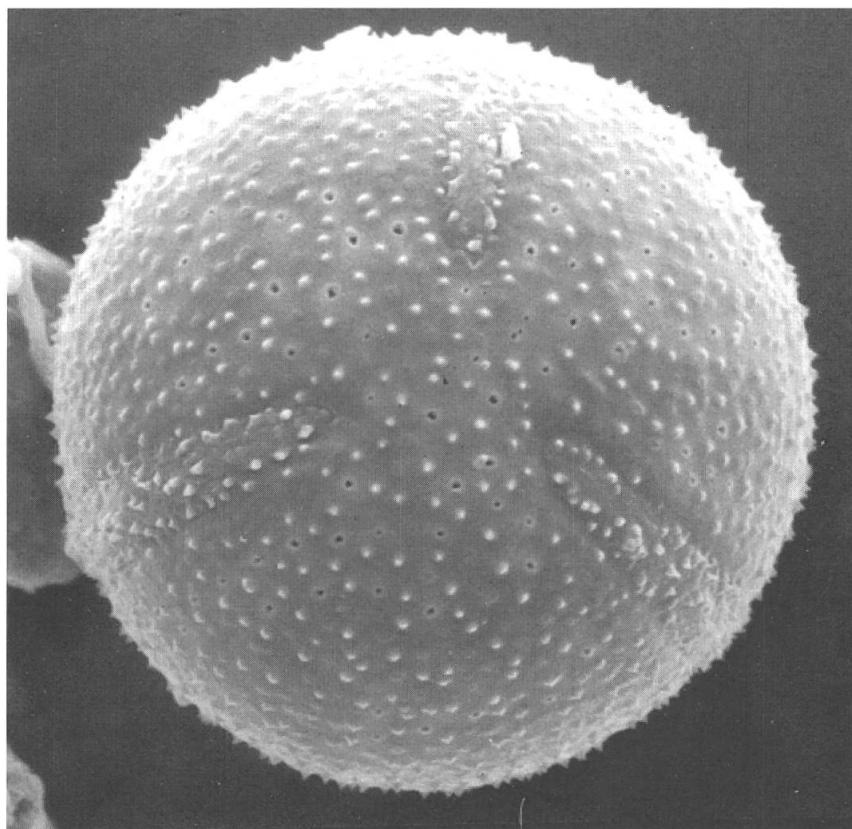


Fig. 7. – Pollen grain of *Gymnocalycium paraguayense* f. *fleischerianum* Mereg. & al., Piraretá.

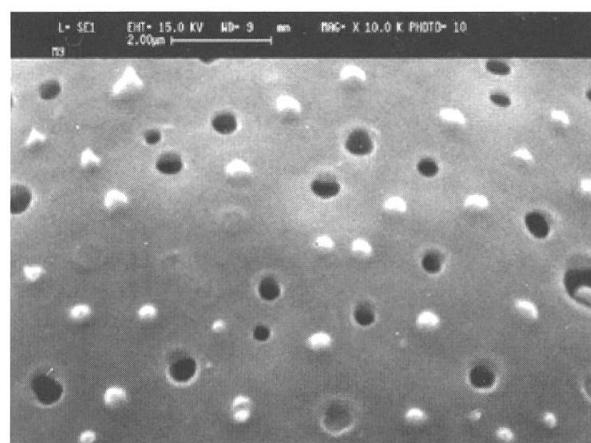


Fig. 8. – Pollen of *Gymnocalycium paraguayense* f. *fleischerianum* Mereg. & al., Piraretá: exine.

possible to examine material from all the populations. However, the samples checked by SEM were relatively uniform, and showed only a slight difference in density of spinula between Piraté and Cerro Peró.

#### RAPD ANALYSIS (Figs. 9, 10)

Preliminary purification of DNA did not result in any significant improvement in results when DNA was extracted from seeds or small seedlings. However, it proved indispensable when working with DNA extracted from vegetative propagules, probably due to presence of mucilage. Electrophoresis patterns showed no difference between DNA extracted from 2-week-old seedlings, seeds from the same mother plant, or vegetative propagules of that plant. Replicates also usually yielded reproducible results. The two dilutions of the same sample were highly similar, except that in a few cases some bands were missing from one of the two dilutions; such bands were excluded from the analysis. The different distance methods use to analyze data gave analogous clusters.

On each gel some bands common to all samples were observed: these are considered as sequences typical of the genus *Gymnocalycium*, or subgenus *Macrosemineum* at least. In all dendograms, with the exclusion of p5, *pfc* was separated from the outgroups. Only in p5 two of the outgroups, *G. denudatum* and *G. mesopotamicum*, fell within the *pfc* cluster; this might have been due to the presence of non-homologous bands running in similar positions. This is indeed one of the main interpretative difficulties in RAPD analysis (ADAMS & TURUSPEKOV, 1998). However, the failure of p5 to segregate *pfc* may also be a consequence of the close relationships shared by all the species examined in subgenus *Macrosemineum*.

Different samples of the same population usually showed the same, or nearly the same pattern, suggesting rather high genetic uniformity within each population. Patterns for the six populations usually showed small variations, limited to 2-3 bands for each primer; the single popula-

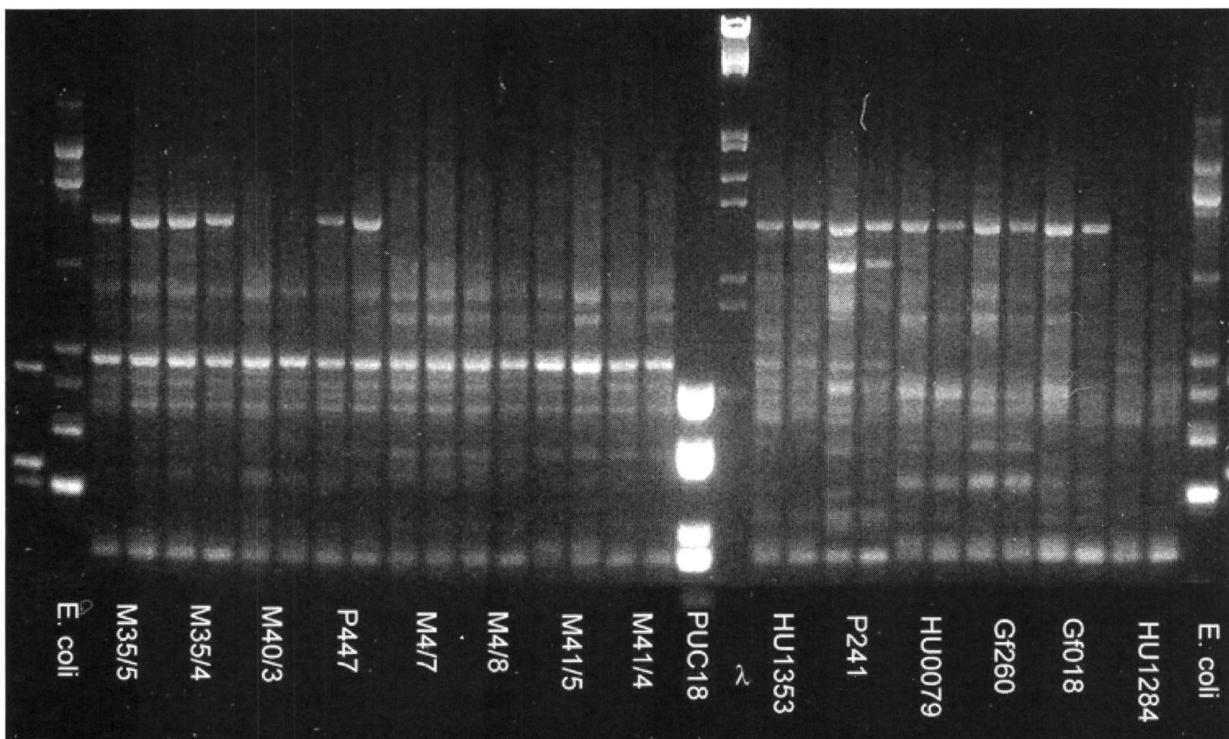


Fig. 9. – Electrophoretic gel (primer 4).

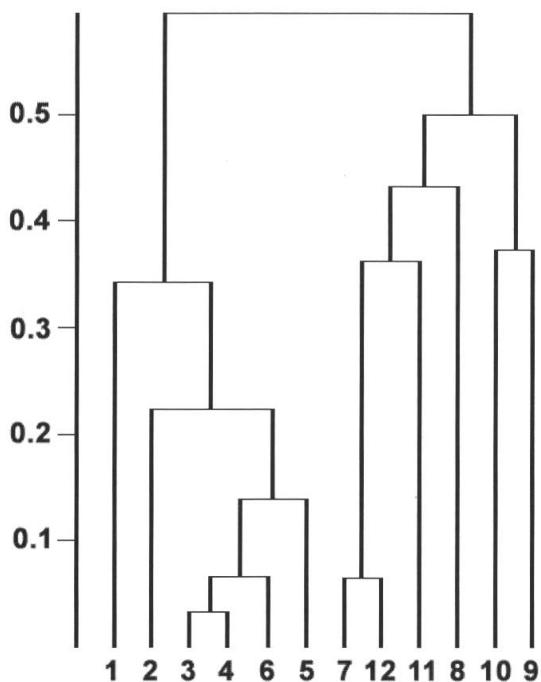


Fig. 10. – Dendrogram constructed from the molecular data according to the Jaccard distance index. Populations: 1, Cerro Peró; 2, Yhaguy Guazú; 3, Oyopoi; 4, Piraretá; 5, Chololó; 6, Saltos Amambay; 7-12: outgroups: 7, *Gymnocalycium uruguayense* s.l., Artigas; 8, *G. mesopotamicum*; 9, *G. horstii*; 10, *G. buenekeri*; 11, *G. denudatum*; 12, *G. uruguayense* s.l., Zapican.

tions could not be segregated, with the exclusion of the Cerro Peró form, which showed a constant difference, though this was determined by the presence or absence of very few bands. Particular attention was given to the Yhaguy Guazú population, currently attributed to *G. paraguayense* according to its morphological characters and living in sympatry with populations referred to *G. fleischerianum*. According to DNA analysis this population seemed intermediate between the Cerro Peró form and all the other populations.

### Conclusions

According to literature, segregation of the Paraguayan plants into two different species is determined by only very few morphological traits.

Macromorphological analysis showed that the transversal furrow on the ribs cannot be used as a discriminating factor. Statistical analyses demonstrated a significant difference between populations ascribed to *G. fleischerianum* and *G. paraguayense* for spine number only, and with the exclusion of the form from Saltos Amambay.

Micromorphological analyses did not confirm any constant difference between them.

Molecular data provided evidence for the monophyly of these Paraguayan cacti in respect to other species in subgenus *Macrosemineum* and did not support any statistically significant possibility of differentiating any population on a genetic basis.

The population living near Cerro Peró is slightly but consistently differentiated from all the others, particularly regarding seed structure and molecular pattern. This might be related to the rather isolated geographical position of Cerro Peró, whereas the other sites are rocky outcrops not far from each other, and suggests that the limited differences shown by plants from Cerro

Peró may be derived by the fixation of a few distinct characters in this small population by genetic isolation. Particularly significant in this respect is the fact that the main distinguishing morphological character of these plants, the transverse furrow on the ribs, is occasionally also present in some plants of the populations from the main distribution area, with particular regard to the form native to Yhaguy Guazú, a population otherwise scarcely differentiated for morphological traits such as seed structure, but placed in an intermediate position by DNA analysis and stem morphology.

Attribution of these plants to two different species does not seem to be justified. The category of *subspecies*, a rank used in cactus taxonomy only in the last 10-15 years, is not applicable as the populations are sympatric or nearly so. We prefer not to use the rank of *variety*, following suggestions by the IOS Cactaceae working group (HUNT, 1999b). The most appropriate taxonomic treatment, which takes into account the slight morphological and genetic differences, may be to assign the rank of *forma* to plants lacking the transverse furrow below the areoles. The epithet *fleischerianum*, at present used for this taxon at *species* rank, can be maintained at *forma* rank.

Our data suggest some hypothesis on colonization of the Paraguayan areas where these plants live. Climate and vegetation in the southern part of Brazil and nearby regions changed during the last thousands years, shifting from a drier and colder climate after the late glacial period to a warmer and more humid one (cf. PRANCE, 1982; BEHLING, 1995; PENNINGTON & al., 2000). It may be assumed that in Southern Paraguay a similar change occurred, explaining the now scattered distribution of several globular cacti in a region now dominated by semi-evergreen forest (ESSER, 1982). Globular cacti, among them species of *Gymnocalycium*, were outcompeted by vegetation adapted to higher moisture, and became confined to relict habitats in rocky outcrops, where the soil is rather shallow and the microclimate more arid. When these habitats occur in marginal and isolated areas the reduced gene flow allows independent evolution of small isolated populations, with fixation of mutations and/or distinct characters determined by the founder effect. This may have happened for the population of Cerro Peró, more differentiated and less variable than the other populations.

The now scattered distribution of small isolated populations must also in part be of anthropogenic origin, caused by the long use of central Paraguay for agriculture (ESSER, 1982, 1984).

### Conservation

Populations of *G. paraguayense* are endangered by livestock grazing, agriculture and expanding settlements, and there is also a potential threat from overcollecting (METZING, 1994; TAYLOR & al., 1997). To determine conservation status in the natural habitats further field studies are necessary (cf. OLDFIELD, 1997).

### Nomenclature

*Gymnocalycium paraguayense* (K. Schum.) Hosseus in Arch. Esc. Farm. Fac. Ci. Med. Cordoba 9: 106. 1939.

≡ *Echinocactus paraguayensis* K. Schum. in Bull. Herb. Boissier ser. 2, 3: 252. 1903.

**Lectotype (designated here): PARAGUAY, "inter rupes prope Chololó, in valle fluminis Y-aca, ex cult. Vallertes", 4.IV.1902, E. Hassler 6693 (G!).**

**Epitype: PARAGUAY, Dept. Paraguarí:** "SE of Piribebuy, near the Yhaguy Guazú, on rocks", 7.VIII.1988, D. Metzing & al. 40 (SI).

### *Gymnocalycium paraguayense* f. *paraguayense*

= *Gymnocalycium paraguayense* var. *roseiflorum* (K. Schum.) Schütz in Friciana 6(40): 11. 1966. ≡ *Echinocactus denudatus* var. *roseiflorus* K. Schum., Gesamtbeschr. Kakt.:

414. 1898.  $\equiv$  *Gymnocalycium denudatum* var. *roseiflorum* (K. Schum.) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art 33.2)].

**Type not designated.**

- = *Gymnocalycium paraguayanum* var. *scheidelianum* (F. Haage) Schütz in Friciana 6(40): 11. 1966.  $\equiv$  *Echinocactus denudatus* var. *scheidelianus* F. Haage in Monatsschr. Kakteenk. 8: 37. 1898.  $\equiv$  *Gymnocalycium denudatum* var. *scheidelianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art 33.2)].

**Type not designated.**

- = *Gymnocalycium paraguayanum* var. *wagnerianum* (F. Haage) Schütz in Friciana 6(40): 11. 1966.  $\equiv$  *Echinocactus denudatus* var. *wagnerianus* F. Haage in Monatsschr. Kakteenk. 8: 37. 1898.  $\equiv$  *Gymnocalycium denudatum* var. *wagnerianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art 33.2)].

**Type not designated.**

- = *Gymnocalycium paraguayanum* var. *wieditzianum* (F. Haage) Schütz in Friciana 6(40): 11. 1966.  $\equiv$  *Echinocactus denudatus* var. *wieditzianus* F. Haage in Monatsschr. Kakteenk. 8: 36. 1898.  $\equiv$  *Gymnocalycium denudatum* var. *wieditzianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art 33.2)].

**Type not designated.**

- = *Echinocactus denudatus* var. *anisitsii* Hort. ex Fric, Kakteenjäger [trade catalogue A. V. Fric]: 6. 1929 (cited according to BACKEBERG, 1959: 1705-1706).

**Type not designated.**

- = *Echinocactus denudatus* var. *bruennowianus* F. Haage in Monatsschr. Kakteenk. 8: 37. 1898.

**Type not designated.**

- = *Echinocactus denudatus* var. *delaetianus* F. Haage in Monatsschr. Kakteenk. 8: 36-37. 1898.  $\equiv$  *Gymnocalycium denudatum* var. *delaetianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art 33.2)].

**Type not designated.**

- = *Echinocactus denudatus* var. *golzianus* Mundt in Monatsschr. Kakteenk. 7: 197. 1897 (based on description in Monatsschr. Kakteenk. 7: 169. 1897).  $\equiv$  *Gymnocalycium denudatum* var. *golzianum* (Mundt) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art 33.2)].

**Type not designated.**

- = *Gymnocalycium denudatum* var. *paraguayanum* F. Haage, Kakteen-Preisverzeichnis 106: 14. 1928 [nom. inval. (ICBN Art. 32.1)].  $\equiv$  *Gymnocalycium denudatum* var. *paraguayanum* (F. Haage) Y. Itô, Cacti: 85. 1952 [nom. inval. (ICBN Art. 36.1)].  $\equiv$  *Echinocactus denudatus* var. *paraguayanus* F. Haage, Trade Catalogue Fa. F. A. Haage jr.: 105. 1923 [nom. inval. (ICBN Art. 32.1)].

**Type not designated.**

***Gymnocalycium paraguayanum* f. *fleischerianum* Mereg., Metzing & R. Kiesling, f. nova**

**Holotype:** PARAGUAY, Dept. Paraguarí: “SE of Piribebuy, Oyopó, on rocky plateau”, 17.VII.1988, D. Metzing & al. 4 (SI).

*A G. paraguayanum f. paraguayanum costis rotundatis, sine sulco transverso, spinis 5-9, longioribus, sine spina centrale differt.*

Distinguishable from *G. paraguayense* f. *paraguayense* by the more rounded ribs, usually lacking transversal furrow, the in average more numerous (5-9) and longer spines (up to at least 35 mm), the missing central spine.

The epithet *fleischerianum* is adopted from *Gymnocalycium fleischerianum* Backeb., nom. inval., and is named after the Czech cactus grower Zdenek Fleischer (1905-1978).

SYNONYMS:

- = *Echinocactus denudatus* var. *andersohnianus* F. Haage in Monatsschr. Kakteenk. 8: 36. 1898. = *Gymnocalycium denudatum* var. *andersohnianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art. 33.2)]. = *Gymnocalycium fleischerianum* var. *andersohnianum* (F. Haage) Schütz in Friciana 6(40): 11. 1966 [nom. inval. (ICBN Art. 43.1)].

**Type not designated.**

- = *Echinocactus denudatus* var. *heuschkelianus* F. Haage in Monatsschr. Kakteenk. 8: 36. 1898. = *Gymnocalycium denudatum* var. *heuschkelianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art. 33.2)]. = *Gymnocalycium fleischerianum* var. *heuschkelianum* (F. Haage) Schütz in Friciana 6(40): 11. 1966 [nom. inval. (ICBN Art. 43.1)].

**Type not designated.**

- = *Echinocactus denudatus* var. *meiklejohnianus* F. Haage in Monatsschr. Kakteenk. 8: 36. 1898. = *Gymnocalycium denudatum* var. *meiklejohnianum* (F. Haage) Y. Itô, Expl. Diagram Austroechinocactinae: 170. 1957 [nom. inval. (ICBN Art. 33.2)]. = *Gymnocalycium fleischerianum* var. *meiklejohnianum* (F. Haage) Schütz in Friciana 6(40): 11. 1966 [nom. inval. (ICBN Art. 43.1)].

**Type not designated.**

- = *Gymnocalycium fleischerianum* Backeb. in Backeb. & Knuth, Kaktus-ABC: 288-289. 1936 [nom. inval. (ICBN Art. 36.1)]. = *Gymnocalycium fleischerianum* Backeb., Cactaceae 3: 1703. 1959 [nom. inval. (ICBN Art. 37.1)].

**Type not designated.**

- = *Gymnocalycium fleischerianum* var. *anoplum* Y. Itô, Cactaceae: 380. 1981 [nom. inval. (ICBN Art. 8.2, 37.1, 43.1)].

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