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ORIGINAL ARTICLE

# Classical and molecular cytogenetics and DNA content in *Maihuenia* and *Pereskia* (Cactaceae)

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**Abstract** We studied cacti species of the subfamilies Pereskioideae (five species of the southern clade) and both species of Maihuenioideae using molecular cytogenetic techniques and DNA content. Mitotic chromosomes were analyzed for *Pereskia aculeata, P. bahiensis, P. grandifolia, P. nemorosa, P. sacharosa, Maihuenia poeppigii*, and *M. patagonica,* using the Feulgen stain, CMA/DAPI fluorescent chromosome banding, fluorescence in situ hybridization (FISH, probes of 5S rDNA and p*Ta*71 for 18-5.8-26S rDNA), and DNA content by flow cytometry technique. The karyotypes were highly symmetrical, most of the pairs being metacentric (*m*). CMA/DAPI banding revealed the presence of $CMA^{+}/DAPI^{-}$ bands associated with NORs in the first *m* pair of all species. The co-localization of 18-5.8-26S rDNA loci with $CMA^{+}/DAPI^{-}$/NORs blocks allowed the identification of homeologous chromosome pairs between species of both subfamilies. FISH using probe 5S rDNA was applied for the first time in both subfamilies. Diploid species had always one *m* pair carrying 5S rDNA genes, with pericentromeric location in different chromosome pairs. In the tetraploid cytotype of *M. patagonica,* the 5S rDNA probe hybridized to two pairs. The 2C DNA content obtained by FC varied twofold (from 1.85 to 2.52 pg), with significant differences between species. Mean chromosome length, karyotype formula, percentage of heterochromatin position of 5S rDNA locus, and nuclear Cx DNA content vary among *Maihuenia* and *Pereskia* species and allowed to differentiate them. Both genera are closely related and that the differences found are not strong enough to separate Maihuenioideae from Pereskioideae.

**Keywords** Karyotype · Heterochromatin · Physical mapping · C-DNA · *Pereskia* · *Maihuenia*

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## Introduction

Cactaceae comprises ca. 1,500 species that are mostly spiny succulent plants with photosynthetic stems (Anderson 2001; Hunt et al. 2006). Cacti are further characterized by morphological and physiological adaptations to drought (Barthlott and Hunt 1993; Hunt et al. 2006), the presence of betalains, crassulacean acid metabolism, and sieve-element plastids of the centrospermous type lacking starch inclusions (Barthlott and Hunt 1993; Mauseth and Plemons 1995; Mauseth 2006). They are conspicuous components of the New World's arid regions and represent one of the world's most spectacular desert radiations (Mandujano et al. 1996; Godínez-Álvarez et al. 2003).

At the molecular level and based on different loci, the family is characterized by the inversion of a chloroplast genetic region including *atp*E, *atp*B, and *rbc*L genes (Downie and Palmer 1994); some authors pointed out that it is monophyletic (e.g., Applequist and Wallace 2002; Cuénoud et al. 2002; Nyffeler 2002; Bárcenas et al. 2011, Hernández-Hernández et al. 2011), but others paraphyletic (Arakaki et al. 2011). There are two monophyletic lineages with many extant species: Opuntioideae (Anderson 2001)

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and Cactoideae (Cuénoud et al. 2002; Bárcenas et al. 2011; Hernández-Hernández et al. 2011), which include most of the species of the family.

In addition, there are two small genera: *Pereskia* Mill. and *Maihuenia* Phil. in subfamily Pereskioideae (Leuenberger 1986; Barthlott 1988), although other authors recognized Maihuenioideae as a distinct subfamily (Leuenberger 1997; Anderson 2001; Edwards et al. 2005; Hunt et al. 2006; Metzing and Kiesling 2008). Molecular phylogenies indicated that Pereskioideae is paraphyletic and two groups were delimited: one with the northern species and the other with southern species (Wallace 1995; Butterworth and Wallace 2005; Edwards et al. 2005; Bárcenas et al. 2011; Hernández-Hernández et al. 2011). *Maihuenia* was recently placed among the branches of the *Pereskia* grade (Bárcenas et al. 2011; Hernández-Hernández et al. 2011).

Karyotypic features have proven useful in understanding evolutionary trends and systematic relationships in several angiosperm groups (e.g., Shan et al. 2003; Weiss-Schneeweiss et al. 2003; Bernardello et al. 2008). Most cytogenetic studies in cacti provided chromosome counts and indicated that their basic number is $x = 11$ (cf. Pinkava et al. 1985, 1998; Powell and Weedin 2001; Pinkava 2002; Goldblatt and Johnson 2013). On the other hand, there are comparatively few detailed karyotypic studies available (e.g., Das and Mohanty 2008; Las Peñas et al. 2008, 2009, 2011), mainly for North American cacti (Johnson 1980; Palomino et al. 1988; Cota and Philbrick 1994; Cota and Wallace 1995; Bandyopadhyay and Sharma 2000).

Chromosome banding and fluorescence in situ hybridization (FISH) have provided valuable tools for studies on genome organization and evolution in plants as a whole (e.g., Sumner 1990; Jiang and Gill 2006; Raskina et al. 2008). The physical mapping of tandem repeat genes (rDNA), and other genomic landmarks, with FISH resulted in useful markers to identify chromosome and fine karyotype comparisons for cytoevolution and cytotaxonomy (Jiang and Gill 2006; Raskina et al. 2008). The use of these data in systematics and evolution contributes to the evaluation of relationships among species and populations to better understand their divergence (Jiang and Gill 2006). The 5S and 18-5.8-26S rDNA genes have been extensively used to establish possible chromosomal homeologies (e.g., Moscone et al. 1999; Adams et al. 2000; Taketa et al. 2005). Both techniques have rarely been applied to Cactaceae (Las Peñas et al. 2008, 2009, 2011).

The nuclear DNA content of *Maihuenia* and *Pereskia* and of most members of the cacti family is mostly unknown. Up to now, only the DNA content of 37 species was reported (Bennett and Leitch 2010). This is unfortunate because the nuclear DNA content of organisms is a valuable source of information (Bennett and Leitch 2005; Gregory 2005). It has been the subject of intense research since the term C-value for unreplicated haploid nuclei was coined. Comparative DNA content studies in angiosperms have shown that C-values are correlated with some features, such as minimum generation time, life history, plant phenology, and important parameters for plant breeders, including frost resistance, biomass production, and ecological adaptations (Ohri 1998). Moreover, nuclear DNA amount was a useful tool to study phylogenetic relationships between taxonomically related groups (Ohri 1998; Zonneveld 2001) and to expand the understanding of genome size evolution (Bennett and Leitch 2005).

*Maihuenia* comprises cushion- or mat-forming shrubs confined to southern South America, mainly to Patagonia, including two species with persistent leaves (Leuenberger 1997, 2008). On the other hand, *Pereskia* comprises tropical trees or shrubs (Leuenberger 1986). It is paraphyletic, with a clade of eight species centered around the Caribbean basin, and another with nine species mostly distributed in the southern half of South America; the latter is part of a major clade comprising *Maihuenia* plus Cactoideae and Opuntioideae (Edwards et al. 2005).

Species of *Pereskia* have been recognized as morphologically plesiomorphic (Gibson and Nobel 1986; Metzing and Kiesling 2008), displaying characters such as broad, flattened leaves with C3 photosynthesis, areoles with leaf production, and non-succulent tissues (Leuenberger 1986, 2008).

The overall aim of this work was to study the mitotic chromosomes of *Maihuenia* and South American *Pereskia* using conventional staining, CMA/DAPI fluorescent chromosome banding, FISH, and DNA content by flow cytometry to determine their karyotypes and therefore elucidate evolutionary relationships of those taxa.

## Materials and methods

Seeds were collected from plants in natural populations. The study material and voucher specimens are detailed in Table 1.

Mitotic chromosomes were examined in root tips obtained from germinated seeds. Seeds were put in petri dishes with moistened filter paper. When the primary roots were 2–10 mm long, root tips were cut and pretreated in 8-hydroxyquinoline at 4 °C for 24 h. Root tips were rinsed in distilled water and fixed in freshly made ethanol:glacial acetic acid (3:1) at room temperature for 24 h. After fixation, root tips were hydrolyzed with 5 N HCl at room temperature for 40 min and put in Feulgen solution at room temperature in the dark for 2 h (Jong 1997). Root-tip meristem cells were isolated on a slide and squashed.

**Table 1** *Maihuenia* and *Pereskia* species studied, collection data, chromosome numbers here detected and previous reports

| Species | Voucher information | 2*n* | Previous reports |
|---|---|---|---|
| *M. patagonica* (Phil.) Britton & Rose | Argentina: Neuquén, Barrancas. Barboza et al., 1247 (CORD) | 22 | Leuenberger (1997)<br>Las Peñas et al. (2009) |
| | Argentina: Rio Negro, Valcheta. Steibel and Troiani, 16213 (CORD) | 44 | Las Peñas et al. (2009) |
| *M. poeppigii* (Otto & Pfeiff.) F.A.C. Weber ex K. Schum | Argentina: Neuquén, Zapala. Las Peñas et al., 521 (CORD)<br>Argentina, Neuquén, Chenque Pehuen. Barboza et al., 2421 (CORD) | 22 | Leuenberger (1997) |
| *P. aculeata* Haw | Argentina: Misiones, Guaraní. Barboza et al., 1036 (CORD) | 22 | Leuenberger (1986)<br>Las Peñas et al. (2009)<br>Lombello and Forni-Martins (1998) |
| *P. bahiensis Gürke* | Brazil: Bahia, Rio de Contas. Urdampilleta et al., 432 (UEC) | 22 | Leuenberger (1986) |
| *P. grandifolia* Haw. | Brazil: Município Campinas, Barão Geraldo. Urdampilleta and Las Peñas, 431 (UEC) | 22 | Leuenberger (1986)<br>Del Angel et al. (2006)<br>Negron Ortiz 2007 |
| *P. nemorosa* Rojas acosta | Moreno SN (CORD) | 22 | Leuenberger (1986) |
| *P. sacharosa* Griseb. | Argentina: Salta, Gral. Güemes. Las Peñas et al., 395 (CORD) | 22 | Leuenberger (1986) |

Voucher specimens were deposited in Museo Botánico de Córdoba, Argentina (CORD) and Universidade Estadual de Campinas, Brazil (UEC) herbaria

Slides were made permanent in Euparal after removing the cover slips with liquid nitrogen. Ten metaphases per population were photographed with a phase contrast optic Zeiss Axiophot microscope (Jena, Germany) and a Leica DFC300FX camera (Wetzlar, Germany). Photographs were taken to measure the following features for each chromosome pair: s (short arm), l (long arm), and c (total chromosome length); the length of the satellite was added to the respective chromosome arm. The arm ratio ($r = l/s$) was then calculated and used to classify the chromosomes as recognized by Levan et al. (1964). In addition, mean chromosome length (C), mean total haploid chromosome length of the karyotype based on mean chromosome length (tl), and mean arm ratio (R) were calculated. Idiograms were based on mean values. Chromosomes were arranged first into groups according to their increasing arm ratio and then according to the decreasing length within each group. Karyotype asymmetry was estimated using the intrachromosomal ($A_1 = 1 - [\sum(b/B)/n]$) and interchromosomal ($A_2 = s/x$) indices of Romero Zarco (1986), where b and B are the mean length of short and long arms of each pair of homologues, respectively, $n$ is the number of homologues, s is the standard deviation, and $x$ the mean chromosome length.

To prepare slides for fluorochrome banding and FISH, root tips were washed twice in distilled water (10 min each), digested with a 2 % cellulase and 20 % pectinase solution for 45 min at 37 °C, and squashed in a drop of 45 % acetic acid (Las Peñas et al. 2008). After coverslip removal in liquid nitrogen, the slides were stored at −20 °C.

## CMA/DAPI banding

Slides were stained with a drop of 0.5 mg/ml chromomycin $A_3$ (CMA) in McIlvaine buffer, pH 7.0 and distilled water (1:1) containing 2.5 mM $MgCl_2$ for 90 min and subsequently stained with 2 μg/ml 4′-6-diamidino-2-phenylindole (DAPI) for 30 min, and finally mounted in McIlvaine's buffer-glycerol v/v 1:1 (Schweizer and Ambros 1994). The amount of heterochromatin was expressed as percentage of the total length of the haploid karyotype.

## FISH

The protocols for fluorescent in situ hybridization were developed using the methodology described by Schwarzacher and Heslop-Harrison (2000). To identify the rDNA 18S-5.8S-26S loci, the p*Ta*71 probe was used (Gerlach and Bedbrook 1979), labeled with biotin-14-dUTP by nick translation (Bionick, Invitrogen) and subsequently detected with avidin-FITC (Sigma). For the analysis of the 5S rDNA loci, a specific probe was obtained from the genome of *Pereskia aculeata* Mill. by PCR (Las Peñas et al. 2011). The 5S fragments were labeled with Digoxigenin-11-dUTP (DIG Nick translation mix, Roche) and detected with Anti-DIG-Rhodamine (Roche). The slides were mounted with

antifade Vectashield (Vector Laboratories) containing DAPI.

DNA content estimations by flow cytometry

Plants to be used for flow cytometry were grown from seeds collected in the field. For every species, 1–3 individuals were measured, three runs each. DNA content was measured by obtaining nuclear suspensions according to Doležel et al. (2007) with minor modifications. Briefly, small pieces of fresh leaves from the sample and the appropriate standard were co-chopped with a sharp razor blade in a glass petri dish containing 0.5 ml of Otto I solution (0.1 M citric acid + 0.5 % Tween 20) and 0.2 ml of 1 % PVP (PVP 10, Sigma-Aldrich). Nuclear suspensions were then filtered through a 45-μm mesh nylon membrane and kept at room temperature for 10–60 min. After the inclusion of 0.5 mL of Otto II buffer (0.4 M $Na_2HPO_4{\cdot}12H_2O$), the nuclear suspensions were filtered again. Propidium iodide (50 μg $mL^{-1}$) and RNAse (50 μg $mL^{-1}$) were added to stain DNA and avoid the labeling of double-stranded RNA. Samples were kept at room temperature and analyzed after 10 min in a FACSVantage Flow Cytometer (Becton–Dickinson, San José, USA) equipped with a water-cooled argon ion LASER Innova 304 (Coherent, USA) tuned to emit light at 488 nm. Laser power was set to 100 mW and the fluorescence emitted from PI was collected in FL2 using a 575/26 band pass filter. A 70 μm nozzle was selected to perform flow cytometric measurements. Chicken red blood cells (CRBC) and DNA QC particles (BD) were used to calibrate the flow cytometer and to optimize fluorescence detection as well as to check instrument linearity. Dot plots of FL2-A vs time were used as a control of fluorescence emission during sample analysis. Doublets were excluded from the analysis using dot plots of FL2-A vs FL2-W. Three DNA estimations were carried out for each plant (5,000 nuclei per analysis) on three different days. *Zea mays* L. CE-777 (2C = 5.43 pg) was used as internal standard for all species except for *M. patagonica* (Phil.) Britton & Rose $2n = 44$, for which *Solanum lycopersicum* L. was used (2C = 1.96). Nuclear DNA content was calculated as: (Sample peak mean/Standard peak mean) × 2C DNA content of the standard (in pg). *Cx* values, representing the DNA content of one non-replicated monoploid genome with the chromosome number *x* (Greilhuber et al. 2005), were calculated as the 2C nuclear DNA amount divided by ploidy level.

## Results

*Maihuenia poeppigii*, *Pereskia bahiensis*, *P. grandifolia*, *P. nemorosa*, and *P. sacharosa* were always diploid with $2n = 22$ (Fig. 1). Data on karyotypes with conventional techniques are summarized in Table 2. Most karyotype formulae were symmetrical, mainly with *m* chromosomes (*M. poeppigii* and *P. sacharosa*), or only one submetacentric (*sm*) pair (*P. bahiensis, P. grandifolia* and *P. nemorosa*). This symmetry was also evident with the ranges of mean arm ratio (1.17–1.30) and asymmetry indices ($A_1$: 0.13–0.30, $A_2$: 0.11–0.26) (Table 2).

After Feulgen staining, the presence of satellites was a remarkably constant character; indeed, all studied species showed satellites in a terminal position on the short arms of the longest pair of the karyotype.

The species studied differed slightly in chromosome size. In general, the chromosomes were small, ranging from 1.92 to 2.70 μm. The average chromosome length for *Pereskia* was 2.31 μm, with the smallest value corresponding to *P. bahiensis* (1.92 μm), and the highest one to *P. sacharosa* (2.70 μm) (Table 2).

The fluorescent chromosome banding pattern obtained by CMA/DAPI staining in *M. poeppigii*, *Pereskia bahiensis*, *P. grandifolia*, *P. nemorosa*, and *P. sacharosa* showed $CMA^+/DAPI^-$ heterochromatin in the chromosome pair #1 associated with NORs (CG-rich), the satellite and a small proximal part of the short arm where it is attached. The total amount of the $CMA^+/DAPI^-$ heterochromatin ranged from 3.30 to 8.04 % of the total karyotype length (Table 2, Figs. 1, 3). No $CMA^-/DAPI^+$ bands were detected. *P. nemorosa* had in addition a $CMA^+/DAPI^-$ pericentromeric heterochromatic bands in one *m* pair.

In the species studied, the 18-5.8-26S rDNA sites coincided with $CMA^+/DAPI^-$ bands described above (Figs. 2, 3), i.e., one site per haploid genome. With the 5S rDNA probe, signals in pericentromeric regions were observed in all species: two or four signals according to the ploidy level, one locus in diploids and two loci in the tetraploid *M. patagonica*. The size, number and intensity of 5S rDNA signals were highly similar between the homologues (Fig. 3). The locations were variable: in *P. aculeata*, *P. bahiensis*, *P. grandifolia*, and *P. nemorosa* signals were on *m* pair #10; in *P. sacharosa*, on *m* pair #2; in *M. poeppigii*, on *m* pair #11, and in *M. patagonica*, on *m* pair #4. All 5S rDNA signals were non-syntenic with 18-5.8-26S rDNA.

In all the *Pereskia* species and in the two *Maihuenia* species, 2C DNA content obtained by FC varied twofold (from 1.85 to 2.52 pg; Table 2). *Pereskia aculeata* exhibited the smallest value, whereas *P. sacharosa* exhibited the highest one (Cx = 0.95–1.36). An ANOVA test was performed with the C-DNA values of *Maihuenia* and *Pereskia* species, and then the values were compared by means of a Tukey's test (Table 2). Comparisons showed significant differences among species, except for *P. bahiensis* and *P.*

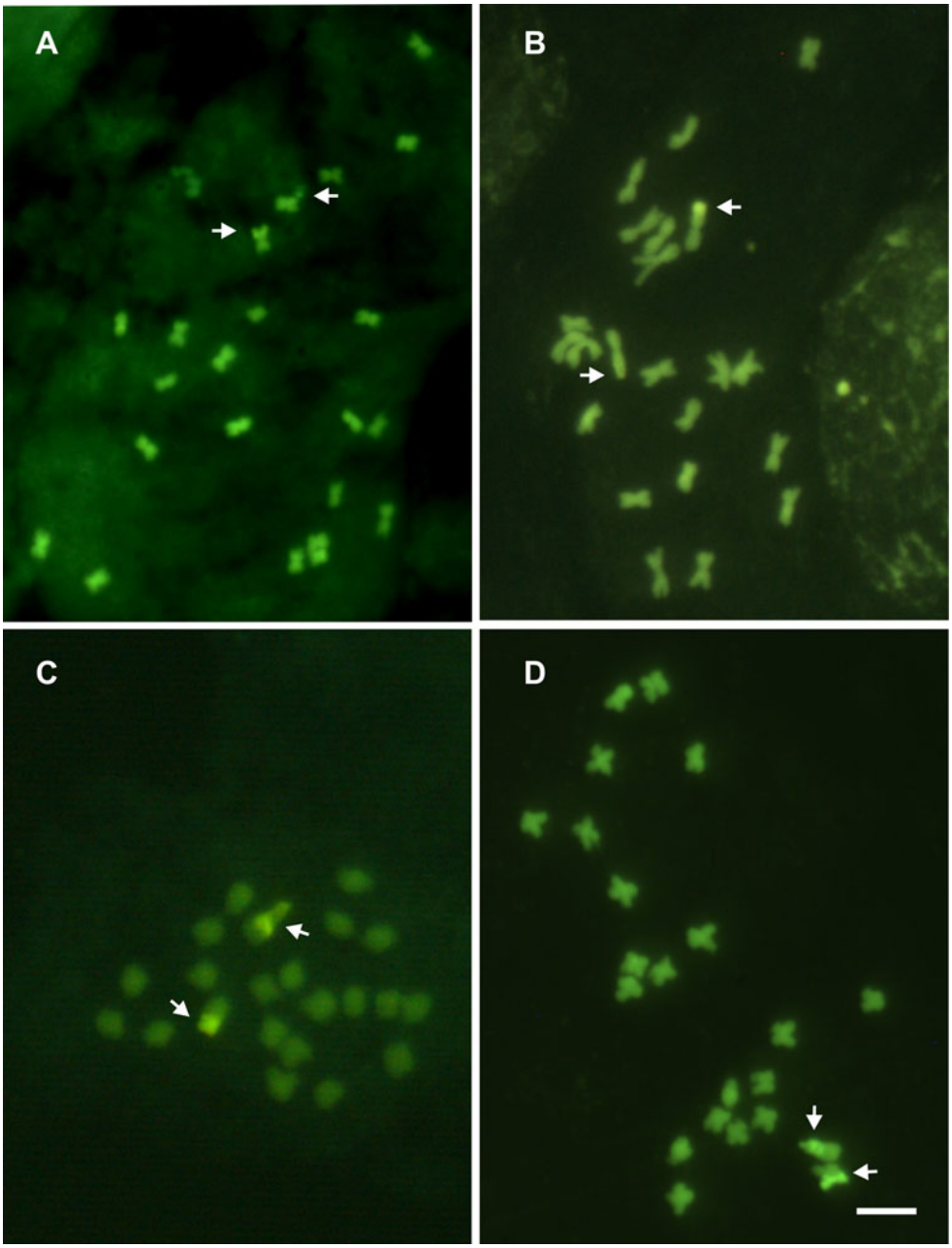


**Fig. 1** Fluorochrome chromosome banding in **a** *Maihuenia poeppigii*, **b** *Pereskia bahiensis*, **c** *P. grandiflora*, **d** *P. sacharosa*. *Arrows* indicate $CMA^{+}/DAPI^{-}$ NOR-associated heterochromatin. *Bar* 5 μm

*grandifolia*, which were not significantly different from each other.

## Discussion

Our results confirm that the basic chromosome number for the family is $x = 11$ (e.g., Pinkava et al. 1985, 1998; Cota and Wallace 1995; Bandyopadhyay and Sharma 2000; Das and Mohanty 2006; Las Peñas et al. 2009, 2011). Data available for 17 *Pereskia* species (Leuenberger 1986; Lombello and Forni-Martins 1998; Las Peñas et al. 2009; this work) and for *M. poeppigii* (Leuenberger 1997; this work) indicate that they are diploid. On the other hand, *M. patagonica* showed diploid and tetraploid cytotypes (Las Peñas et al. 2009). Polyploidy has been reported for ca. 25 % of all the cacti investigated so far (cf. Palomino et al. 1988; Cota and Wallace 1995; Pinkava 2002; Arakaki et al. 2007); particularly, polyploidy seems to have played an important role in the evolution of Opuntioideae, in which it

**Table 2** Karyotype data for *Maihuenia* and *Pereskia* species

| Species | KF | tl | C | r | $A_1$ | $A_2$ | % Ht ($CMA^+$/$DAPI^-$/NORs) | Mean 2Cvalue in pg (±sd) | Mean C = X | Tukey's grouping |
|---|---|---|---|---|---|---|---|---|---|---|
| *M. patagonica* | 9 m* + 2 sm | 29.71 | 2.47 | 1.38 | 0.27 | 0.26 | 3.30 | 2.68 (0.01) | 1.34 | – |
| | 18 m** + 4 sm | 56.00 | 2.45 | 1.33 | 0.30 | 0.26 | 6.20 | 4.98 (0.08) | 1.245 | – |
| *M. poeppigii* | 11 *m** | 22.17 | 2.01 | 1.20 | 0.16 | 0.13 | 5.03 | 2.41 (0.08) | 1.205 | – |
| *P. aculeata* | 10 m* + 1 sm | 28.80 | 2.60 | 1.23 | 0.16 | 0.24 | 4.96 | 1.85 (0.04) | 0.925 | a |
| *P. bahiensis* | 10 *m** + 1 *sm* | 21.14 | 1.92 | 1.30 | 0.20 | 0.16 | 5.04 | 2.35 (0.02) | 1.175 | b |
| *P. grandifolia* | 10 *m** + 1 *sm* | 22.96 | 2.09 | 1.28 | 0.20 | 0.11 | 8.04 | 2.26 (0.14) | 1.13 | b |
| *P. nemorosa* | 10 *m** + 1 *sm* | 23.75 | 2.45 | 1.31 | 0.26 | 0.18 | 7.45 | 2.72 (0.01) | 1.36 | b |
| *P. sacharosa* | 11 *m** | 29.62 | 2.69 | 1.17 | 0.13 | 0.12 | 6.59 | 2.52 (0.20) | 1.26 | c |

*KF* karyotype formulae, *tl* mean total haploid chromosome length, *C* mean chromosome length, *r* mean arm ratio, $A_1$, $A_2$ mean asymmetry indices (intrachromosomic: $A_1$, interchromosomic: $A_2$), *% Ht* heterochromatin amount expressed as percentage of the karyotype length. Tukey's test (*Same letters* indicate of taxa that not significantly difference using ±0.05). Lengths are in µm. *m* metacentric chromosome, *sm* submetacentric chromosome. An *asterisk* indicates that the first chromosome pair has a satellite on the short arms. *Two asterisks* indicate that the first and second chromosome pair have satellites on the short arms. Karyotypic data of *M. patagonica* ($2n = 22$; 44) and *P. aculeata* published in Las Peñas et al. (2009)

reaches 64 %. However this mechanism of chromosome evolution seems to be absent in Pereskioideae (0 %).

*Maihuenia* and *Pereskia* species have small chromosomes and symmetrical karyotypes mostly composed of *m* and *sm* chromosomes, as it is the rule in Cactaceae (e.g., Cota and Philbrick 1994; Cota and Wallace 1995; Bandyopadhyay and Sharma 2000; Das et al. 2000; Das and Mohanty 2006; Las Peñas et al. 2008, 2009, 2011). Subtelocentric (*st*) chromosomes are rare in cacti and were reported only for two species (Johnson 1980; Las Peñas et al. 2008). Telocentric (*t*) chromosomes have been detected in *Rebutia* by Mosti et al. (2011), a genus with admittedly peculiar (relatively mesic) growing conditions for a globular cactus.

In *Maihuenia* and *Pereskia*, the number, type, and position of NORs is consistent in all species confirmed with molecular techniques: attached to the short arms of the longest *m* pair. In other South American cacti, this fact was also reported (Las Peñas et al. 2008, 2009, 2011). In other Cactaceae, only with classical techniques, from two to four satellites per haploid genome in different positions have been described (Cota and Wallace 1995; Palomino et al. 1999; Bandyopadhyay and Sharma 2000; Das and Mohanty 2006).

CMA/DAPI banding has been widely used in angiosperms to identify heterochromatic bands with respect to their highly repeated DNA composition. The banding patterns of heterochromatin can be relatively conserved (e.g., Galasso et al. 1996; Moscone et al. 1996; Marcon et al. 2005) or variable (e.g., Miranda et al. 1997; Guerra et al. 2000; Fregonezi et al. 2006) at the generic level. The scarce data available for cacti (Las Peñas et al. 2008, 2009, 2011) and our data on *Maihuenia* and *Pereskia* showed that $CMA^+$/$DAPI^-$ heterochromatin blocks were always present on a terminal position associated with the chromosome pair with secondary constrictions. These results indicate that this character could be highly conserved in Cactaceae.

Both fluorochrome banding and rDNA gene mapping have been valuable in the identification of homeologous chromosome pairs among plant species (e.g., Moscone et al. 1996, 2007). Although more data are needed, in Cactaceae NOR-bearing pair #1(with locus 18-5.8-26S) seems to be homeologous in all genera examined so far: *Acanthocalycium*, *Cumulopuntia*, *Maihuenia, Pereskia, Pyrrhocactus*, *Setiechinopsis*, and *Trichocereus* (Las Peñas et al. 2008, 2009, 2011, this study).

The location of 5S rDNA in *Pereskia* and *Maihuenia* is here reported for the first time. 5S rDNA is located in a centromeric region, a frequent location in angiosperms (e.g., Kulak et al. 2002; Besendorfer et al. 2005). A correlation was found between the number of 5S rDNA loci and ploidy level in many plant species (Adams et al. 2000; Taketa et al. 2005); those results are consistent with the present findings in the tetraploid *M. patagonica*, in which 18-5.8-26S (Las Peñas et al. 2009) and 5S rDNA loci were duplicated. Polyploidy is frequently associated with changes in the genome structure, which leads to the loss or gain of DNA sequences (Leitch and Leitch 2008). The exact duplication of the diploid karyotype formula and the number of ribosomal genes (18-5.8-26S and 5S) in *M. patagonica* suggest that this cytotype could have been recently originated by autopolyploidy. The similar intensity of FISH signals of both rDNA genes may be an indicator that there are no differences among the number of their copies (Appels et al. 1980; Weiss-Schneeweiss et al. 2003).

A pattern of homogeneously sized chromosomes with median and submedian centromeres is conserved in Cactaceae (e.g., Palomino et al. 1988; Cota and Philbrick 1994; Cota and Wallace 1995). Thus, karyotypic orthoselection might have occurred, preserving rather similar complements throughout a higher taxon because they seem to be more stable, a situation also reported in other groups (cf.

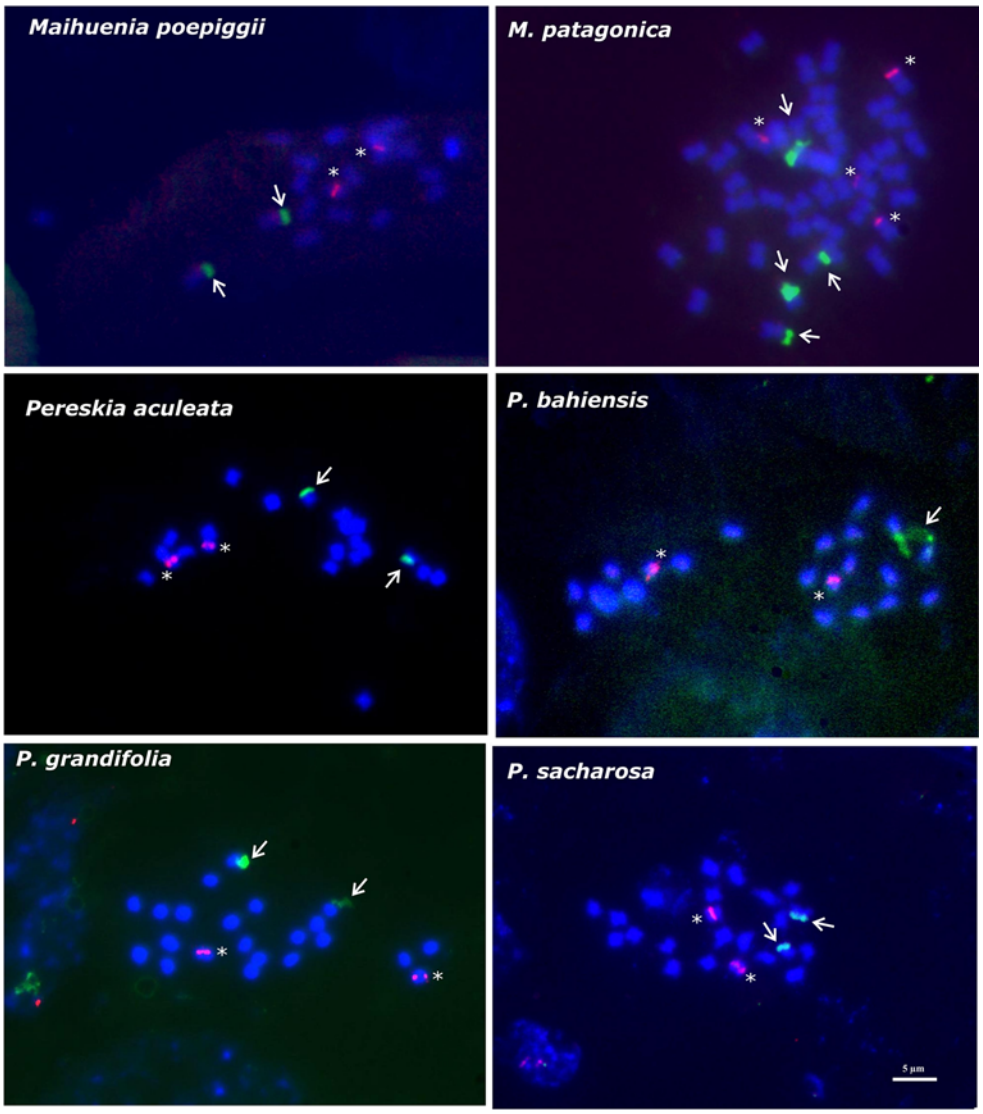


**Fig. 2** Somatic chromosomes detected by FISH using 18-5.8-26S and 5S rDNA probes in *Maihuenia* and *Pereskia* species. *Arrows* show 18-5.8-26S rDNA sites and *asterisks* show 5S rDNA site hybridization

Stiefkens and Bernardello 2002; Moscone et al. 2003; Acosta et al. 2006; Bernardello et al. 2008).

Analysis of the nuclear DNA content by flow cytometry is useful for exploring ploidy level, detecting intraspecific hybrids and apomixis, determining genome size and endoreduplication, and identifying sex (Costich et al. 1991; Bennett et al. 2000). In Cactaceae, this method has been exclusively used in some species of Cactoideae (*Rebutia*, *Mammillaria* and *Escobaria*), Opuntioideae (*Opuntia*) and *Pereskia grandifolia* (Palomino et al. 1999; Zonneveld et al. 2005; Del Angel et al. 2006; Negron-Ortiz 2007). However, nuclear DNA content of species of the subfamily Maihuenioideae is unknown.

The average nuclear DNA content of *Maihuenia* and *Pereskia* species studied was 2C = 2.18 pg, indicating a small genome size according to the ranges defined for angiosperms of 1.4–3.5 pg (Bennett et al. 2000). Species with small genome sizes seem to be more evolutionarily flexible, allowing them to colonize new and more diverse environments (Leitch and Leitch 2008). In the cacti studied, the small genome size as well as their morphological and physiological features, may have allowed the plants to adapt to arid and semiarid sites (Del Angel et al. 2006).

For the diploid *P. grandifolia*, nuclear 2C DNA content = 2.26 pg is reported in this study. This value is comparatively high relative to that obtained by Del Angel et al. (2006) and Negron-Ortiz (2007), with 1.96 pg. These small differences probably reflect variations related to the use of different DNA reference standards that may result in different estimates of the same material (Doležel et al. 1998).

In this work, DNA content of the two *Maihuenia* species was studied for the first time. The values found are within

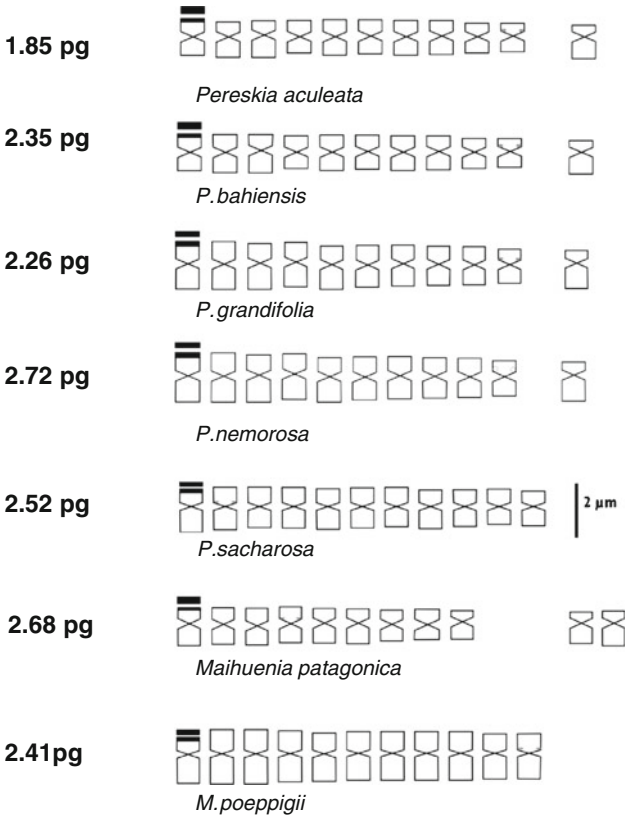


**Fig. 3** Idiogram with physical location of repetitive segments in *Maihuenia* and *Pereskia* species. *Black* locus of 18-5.8-26S rDNA ($CMA^{+}/DAPI^{-}$, NOR-associated), *gray* loci of 5S rDNA

the 2C range for the family (*Cx* values 0.54–3.46 pg) (Palomino and Heras 2001; Zonneveld et al. 2005; Negron-Ortiz 2007; Segura et al. 2007). In tetraploid *M. patagonica*, Cx values were not exactly the same of the diploid cytotype (1.24 vs. 1.34), which suggests a reduction of 7 % in genome size of the base numbers (Leitch and Bennett 2004; Parisod et al. 2009; Scaldaferro et al. 2012).

Cytogenetic results (Las Peñas et al. 2009, this work) and data on DNA content obtained for the two *M. patagonica* cytotypes are not enough to separate the populations into varieties or subspecies. Studies of the reproductive systems in diploid and tetraploid cytotypes may be useful to determine possible differences between them; likewise, the analysis of a greater number of populations may help to elucidate the distribution of diploids and tetraploids.

Mean chromosome length, karyotype formula, percentage of heterochromatin position of 5S rDNA locus, and nuclear Cx DNA content vary among species of *Maihuenia* and *Pereskia* and were valuable features to differentiate them.

Several Cactaceae phylogenies have been published (Nyffeler 2002; Bárcenas et al. 2011; Hernández-Hernández et al. 2011). In them, *Pereskia* resulted paraphyletic and the placement of *Maihuenia* is among the branches of the *Pereskia* grade. In addition, there other studies that attempted to resolve phylogenetic relationships within and between *Pereskia* (Edwards et al. 2005; Butterworth and Wallace 2005; Butterworth and Edwards 2008). They also indicate the paraphyly of *Pereskia* and support the hypothesis of *Pereskia* representing the earliest members of cacti, particularly a clade of Caribbean-basin-centered *Pereskia*.

Our cytogenetic data show that both genera are closely related and that the differences found are not strong enough to separate Maihuenioideae from Pereskioideae. Unfortunately, no cytogenetic data are available on members of the basal Caribbean-basin-centered clade which will be helpful to understand its value in the diversification of the group.

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