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Karyotypes and fluorescent chromosome banding in *Pyrrhocactus* (Cactaceae)

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Summary. Karyotypes of the seven *Pyrrhocactus* species were studied for the first time with Feulgen staining and CMA/DAPI banding. All showed $2n = 22$ ($x = 11$). The karyotypes were symmetrical with $9m + 2sm$ pairs, excepting *P. catamarcensis* with $8m + 2sm + 1st$ pairs. They had a terminal microsatellite on short arms of pair #1. *Pyrrhocactus bulbocalyx* possessed a second satellited pair (#2) exclusively detected with Feulgen. Increasing asymmetry was associated with a decline in karyotype size. Fluorochrome banding, applied for the first time in Cactaceae, revealed that nucleolar chromosome pair #1 had one $CMA^+/DAPI^-$ terminal band in all species related to the nucleolar organizing region; additional pericentromeric bands were found. A pattern of homogeneous sized chromosomes with median and submedian centromeres is conserved in the genus. However, karyotypes can be distinguished by a combination of cytogenetic features. Species diversification in *Pyrrhocactus* has not been associated with large chromosome rearrangements or polyploidy, but with cumulative small and cryptic structural changes.

Keywords: *Pyrrhocactus*; Cactaceae; CMA/DAPI banding; karyotype evolution; karyosystematics; somatic chromosome number; South America

Introduction

Among the ca. 30 families of the order Caryophyllales (Cuénoud et al. 2002, APGII 2003), Cactaceae is typical for a number of features. The most noticeable is that these plants are mostly spiny succulents with photosynthetic stems and scarcely developed leaves; these organs are associated with highly modified axillary buds or shoots – i.e. areoles – that bear spines. Cacti are further characterized by the presence of betalains, crassulacean acid metabolism, specialized xylem cells helping in water storage, and sieve-element plastids of the centrospermous type lacking starch inclusions (Behnke 1981, Cronquist 1981, Barthlott and Hunt 1993, Mauseth and Plemons 1995, Mauseth 2006). Their flowers are mostly solitary, hermaphroditic, and actinomorphic, having commonly numerous tepals in a graded series; the ovary is inferior and there are usually numerous stamens (Cronquist 1981, Barthlott and Hunt 1993, Anderson 2001).

The family of cacti has been hypothesized to be of relatively recent origin (Gibson and Nobel

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1986, Mauseth 1990). It comprises about 100 genera and 1,500–1,800 species native to temperate and tropical regions of the New World, especially in warm, dry environments (Barthlott and Hunt 1993, Anderson 2001). Many species are cultivated as ornamentals, while others are edible or have varied local uses (Barthlott and Hunt 1993). The family is considered monophyletic, after morphological and molecular evidences (Wallace and Forquer 1995, Nyffeler 2002). It is likely that *Pereskia* is sister to all other Cactaceae (Wallace and Forquer 1995, Nyffeler 2002, Butterworth and Wallace 2005, Edwards and Donoghue 2006). Within the family there is a high level of diversity in habit and vegetative and reproductive traits (Mauseth 2006). Thus, the determination of systematic relationships at different taxonomic levels has proven to be problematic. The situation is further complicated by extensive parallel evolution in various structures, i.e. the resultant homoplasy has rendered phylogenetic analyses difficult (Cota and Wallace 1995, Edwards et al. 2005). Three subfamilies have been traditionally recognized: Pereskioideae, Opuntioideae, and Cactoideae (Anderson 2001, Wallace and Gibson 2002). In addition, molecular evidences suggested the recognition of another monogeneric subfamily: Maihuenioideae (Wallace 1994, Nyffeler 2002, Hunt et al. 2006, Mauseth 2006).

The largest and most complex subfamily Cactoideae – with approximately 90% of the species diversity – shows the greatest morphological extremes in habit and stem structure (Mauseth 2006). Their interstitially pitted or cratered seed-testa is probably unique in angiosperms (Barthlott and Hunt 1993). Relationships among its genera are poorly understood (Applequist and Wallace 2002) and the arrangement of genera into tribes is likewise disputed (Gibson and Nobel 1986, Barthlott and Hunt 1993, Anderson 2001).

Among its problematic genera, *Pyrrhocactus* is an outstanding example. It belongs to the South American tribe Notocacteae, typical for the extreme cuticular ornamentation of the seed testa (Barthlott and Hunt 1993). It was included in *Neoporteria*, together with the genera *Islaya*, *Horridocactus*, *Neochilenia*, and *Thelocephala* (Donald and Rowley 1966, Barthlott and Hunt 1993). Alternatively, all these genera, together with *Chileosyce*, were integrated into *Eriosyce*, in a complex system with two sections and several subsections each one (Kattermann 1994). Unfortunately, as there are limited molecular studies of the tribe, their inter-generic relationships are still not well understood (Wallace and Gibson 2002).

Pyrrhocactus sensu stricto is characterized by a dry fruit when ripe and seeds with large often deeply sunken hilum (Kattermann 1994, Kiesling and Meglioli 2003). It comprises seven species endemic to Western Argentina, being the province of San Juan its main center of diversification (Kattermann 1994, Kiesling 1999, Kiesling and Meglioli 2003). Among other unknown features of this genus, its chromosomes have never been studied. In Cactaceae, the majority of cytological studies only provide chromosome counts, which show that its basic chromosome number is $x = 11$ (e.g. Ross 1981, Pinkava et al. 1985, 1992, Parfitt 1987, Cota and Philbrick 1994, Cota and Wallace 1995, Bandyopadhyay and Sharma 2000, Powell and Weedin 2001). On the other hand, there are very few detailed karyotypic studies available (Johnson 1980, Palomino et al. 1988, Cota and Wallace 1995), which are even rarer for South American cacti (Das and Mohanty 2006). This fact may be related to the relatively small chromosome size (ca. 2 μm); in addition, mucilage is usually present in their tissues, which hinders the separation of cells and chromosomes and interferes with their observation (Cota and Wallace 1995). Nevertheless, cytogenetic studies are needed because chromosome numbers and karyotype analyses have been helpful in addressing systematic and evolutionary problems in many angiosperm families (e.g. Bernardello et al. 1994, Shan et al. 2003, Weiss-Schneeweiss et al. 2003), including Cactaceae (Palomino et al. 1988, Cota and Wallace 1995, Bandyopadhyay and Sharma 2000, Das and Mohanty 2006).

Among the modern banding techniques, staining with base-specific fluorochromes has been recognized as a reliable method to distinguish some types of heterochromatin in plants (Vosa 1970, 1976; Schweizer 1976). Some fluorochromes – e.g. CMA – stain GC rich

regions, whereas others – e.g. DAPI – AT rich regions allowing the identification of different types of heterochromatin (Schweizer 1976, Sumner 1990). These procedures have been applied with success in several plant families to identify the distribution of the heterochromatin in related species for both systematic and evolutionary comparisons (e.g. Guerra 2000, Souza and Benko-Iseppon 2004, Gitaí et al. 2005, Urdampilleta et al. 2006). However, they have not been applied in any Cactaceae up to now.

Upon this background, in the present contribution a detailed morphometric karyotype analysis has been performed in all seven *Pyrrhocactus sensu stricto* species with the aims of: (1) report chromosome numbers and karyotype data for the first time for the genus, (2) contribute to the cytogenetic characterization of the species with CMA/DAPI banding, which is applied for the first time for the family, and (3) cast light on the taxonomic relationships and patterns of chromosomal differentiation in the genus.

Materials and methods

Plant material. Seven species were collected from different localities of Argentina (Table 1). *Pyrrhocactus umadeave* has the northernmost range (provinces of Salta and Jujuy), whereas *P. strausianus* the southernmost (provinces of La Pampa, Mendoza, Neuquén). The remainder species grow in Western Argentina: *P. catamarcensis* and *P. sanjuanensis* are endemic to San Juan, *P. pachacoensis* to San Juan and Mendoza, *P. bulbocalyx* to La Rioja, San Juan, and San Luis, and *P. kattermannii* to La Rioja and San Juan (Kiesling 1999, Kiesling and Meglioli 2003). Vouchers are kept in the herbarium of the Museo Botánico de Córdoba (CORD).

Specimens were planted in earthenware pots in an equal parts mixture of sand and soil. In addition, seeds were put in Petri dishes with moistured filter paper. For mitotic counts, root tips, from either seedling radicles or adventitious roots from stems, were used.

Feulgen staining. The protocol used to obtain mitotic chromosomes was fixing the roots in a 3:1 ethanol:glacial acetic acid mixture after pretreatment in a 2 mM saturated solution of 8-hydroxyquinoline for 8 h. For slide preparation, root tips were hydrolyzed with HCl 5 N for 30 min at room temperature and then washed, stained with Schiff reagent for 2 h (Jong 1997), and squashed in a drop of 2% acetic carmine. Permanent mounts were made following Bowen's method (1956).

The number of individuals and cells examined for each species are included in Table 1. At least ten metaphases per species (one per individual) except for *P. kattermannii* were photographed with a phase contrast optic Zeiss Axiophot microscope and a Leica DFC300FX camera. Photographs were used to take measurements of 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). Satellites were classified according to Battaglia (1955). In addition, mean chromosome length (C), mean total haploid chromosome length of karyotype based on the mean chromosome length (tl), and mean arm ratio (R) were calculated. Idiograms were based on the mean values for each species. The 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) and interchromosomal (A_2) indices of Romero Zarco (1986). These data were taken for all species except *P. kattermannii*, for which very few individuals and cells were available (Table 1).

CMA/DAPI banding. Root tips were washed twice in distilled water (10 min each), digested with a 2% cellulase–20% pectinase solution (30 min), and squashed in a drop of 45% acetic acid. Only one root tip was used in each slide. After coverslip removal in liquid nitrogen, the slides were aged for three days, stained with chromomycin A3 (CMA) for 90 min and subsequently with 4′-6-diamidino-2-phenylindole (DAPI) for 30 min, and finally mounted in McIlvaine's buffer–glycerol v/v 1:1 (Schweizer 1976).

Results

Pyrrhocactus species investigated were diploid and showed $2n = 22$ in all cells examined. Figures 1 and 2 illustrate examples of the chromosomes encountered. Karyotype formulae and means of measurements taken are included in Table 2.

Table 1. *Pyrrhocactus* species studied (all from Argentina) and collection data: collector and number, province, locality, date, and, in brackets, number of individuals, number of cells studied, respectively

Species	Collection data
P. bulbocalyx (Werderm.) Backeb.	Las Peñas and Chiarini 229, San Juan, Marayes, 11-12-2005 (10, 25)
P. catamarcensis (Speg.) F. Ritter	Las Peñas 151, San Juan, Ullum, 03-01-2004 (15, 40)
P. kattermannii Kiesling	Las Peñas 355, La Rioja, Sierra de Famatina, 05-03-2007 (1, 3)
P. pachacoensis Rausch	Las Peñas 353, San Juan, Pachaco, 19-01-2007 (8, 32)
P. sanjuanensis (Speg.) Backeb.	Las Peñas 90, San Juan, Villicum, 06-01-2004 (10, 35)
P. strausianus (K. Schum.) A. Berger	Kiesling 10234, Mendoza, Dique de Potrerillos, 20-12-2005 (7, 20)
P. umadeave (Werderm.) Backeb.	Las Peñas 264, Salta, Santa Rosa de Tastil, 16-01-2006 (10, 40)

Table 2. Karypotype data for *Pyrrhocactus* taxa studied

Species	Haploid karyotype formulae	*tl*	*C*	*r*	A_1	A_2	*R*	*St*	Heterochromatin
P. bulbocalyx	9*m*** + 2*sm*	33.70	3.10	1.30	0.21	0.17	1.88	1A	4.5 CMA^+
P. catamarcensis	8*m** + 2*sm* + 1*st*	22.00	2.00	1.29	0.26	0.25	1.27	2A	13.35 $CMA^+/DAPI^+$ 0.35 $CMA^-/DAPI^+$
P. kattermannii	9*m** + 2*sm*	35.30	3.10	1.20	0.15	0.24	2.50	1A	6.5 CMA
P. pachacoensis	9*m** + 2*sm*	29.50	2.95	1.30	0.25	0.20	1.87	1A	11.5 CMA^+
P. sanjuanensis	9*m** + 2*sm*	29.50	2.68	1.40	0.29	0.23	1.25	2A	6.5 CMA^+
P. strausianus	9*m** + 2*sm*	28.80	2.60	1.25	0.16	0.24	1.65	1A	8.5 CMA^+
P. umadeave	9*m** + 2*sm*	25.00	2.20	1.35	0.24	0.24	1.18	1A	7.0 CMA^+

Lengths are in μm. Chromosome nomenclature after Levan et al. (1964). An asterisk indicates that the first chromosome pair has a satellite on the short arm and two asterisks mean two satellited pairs (1 and 2)
tl Mean total haploid chromosome length, *C* mean chromosome length, *r* mean arm ratio, A_1 mean intrachromosomic asymmetry index, A_2 mean interchromosomic asymmetry index, *St* Stebbins' (1971) category of asymmetry, *R* ratio between the largest and the smallest chromosomes in the complement, *Heterochromatin* amount and type expressed as percentage of the haploid karyotype length

In general, the chromosomes were small, being 2.6 μm the average chromosome length for all taxa (Table 2); *P. kattermannii* seems to have longer chromosomes, but as very few cells were available it was excluded from comparisons. The species showed a total karyotype length ranging from 22 μm (in *P. catamarcensis*) to 33.7 μm (in *P. bulbocalyx*) (Fig. 1). The smallest chromosome was found in a cell of *P. catamarcensis* (pair #11 with 1.3 μm) and the longest in a cell of *P. bulbocalyx* (pair #1 with 3.6 μm).

With Feulgen staining, there was a remarkable constancy in the presence of microsatellites. Effectively, in the karyotypes of all species there was one in a terminal position located on the short arms of the *m* pair #1 – the longest of the karyotype – (Figs. 1, 2, 3). Additionally, *P. bulbocalyx* possessed a second satellited *m* pair (#2, the second longest pair; Figs. 1a, 2, 3). The frequency of appearance of the satellites was also similar: they were observed in the 70–90% of the examined cells in both homologues in all taxa.

All karyotypes were symmetrical considering both centromere position and chromosome size variation (Table 2). There was a slight difference among the size of the different chromosomes and most of them had equivalent arms. The mean arm ratio for all the species ranged from 1.29 to 1.40, since karyotypes are composed of nine *m* pairs and two *sm* pairs; the only exception was *P. catamarcensis* with eight *m* pairs, two *sm* pairs, and one small *st* pair (Fig. 1b). Values obtained for the intrachromosomal asymmetry index (A_1; range = 0.21–0.29) indicated that chromosome arms are quite similar in length. On the other hand, the interchromosomal asymmetry index (A_2) varied

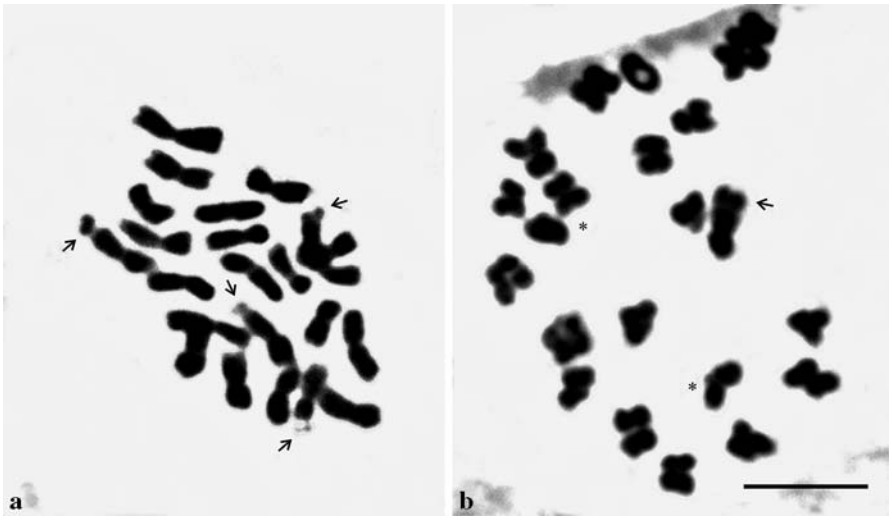

Fig. 1. Somatic metaphases of *Pyrrhocactus* species with Feulgen staining ($2n = 22$). **a** *P. bulbocalyx*. **b** *P. catamarcensis*. *Arrows* indicate satellites and the *asterisks* in **b** indicate an *st* pair. *Bar* 5 μm

between 0.17 and 0.25, indicating that there is little variation among the size of the different chromosomes in each species; *P. bulbocalyx* showed more variation according to the difference in size between the longest and smallest chromosomes. In *P. kattermannii*, the karyotype showed the most common formula, but quantitative data to compare with the other species are missing because very few cells were available for measurements.

The fluorescent chromosome banding patterns obtained by CMA/DAPI staining in all the studied species showed constitutive heterochromatin $CMA^+/DAPI^-$ and *P. catamarcensis* also had in addition a $CMA^-/DAPI^+$ band (Fig. 2). The total amount of the CMA^+ heterochromatin ranged from 4.5 to 13.7% of the total karyotype length. *Pyrrhocactus catamarcensis* and *P. pachacoensis* were the species with the highest heterochromatin amount (Table 2). All species showed a NOR-associated heterochromatin in the satellited chromosome pair #1; it comprised the distal satellite and a small proximal band on the short arm where it is attached (Figs. 2, 3). Although in *P. bulbocalyx* there were no additional fluorescent bands (Fig. 2e), there was another secondary constriction revealed with Feulgen staining on chromosome pair #2 (Fig. 1a).

Additional $CMA^+/DAPI^-$ pericentromeric heterochromatic bands were found in different numbers and positions: one in *P. sanjuanensis* and *P. umadeave* (on pair #2 in both species), two in *P. strausianus* and *P. kattermannii* (on pairs #2–3), four in *P. catamarcensis* (on pairs #2–5), and five in *P. pachacoensis* (on pairs #1–5). The latter species is outstanding because it was the only one with a pericentromeric band on pair #1 (Figs. 2, 3). A $CMA^-/DAPI^+$ band located on pair #6 was exclusive of *P. catamarcensis* (Figs. 2a, 3).

Although the karyotypes of the examined species were of very similar morphology, they can be distinguished by a combination of karyotype formula, karyotype length, number of satellites, and patterns of fluorochrome banding (Fig. 3; Table 2).

Discussion

General karyotype features. All species were examined cytologically for the first time and resulted diploid with $x = 11$. Certainly, this basic number is the most frequent for the family, considering the ca. 650 taxa known so far (e.g. Fedorov 1969; Pinkava et al. 1977, 1985, 1992; Parfitt 1987; Cota and Wallace 1995; Bandyopadhyay and Sharma 2000; Das and

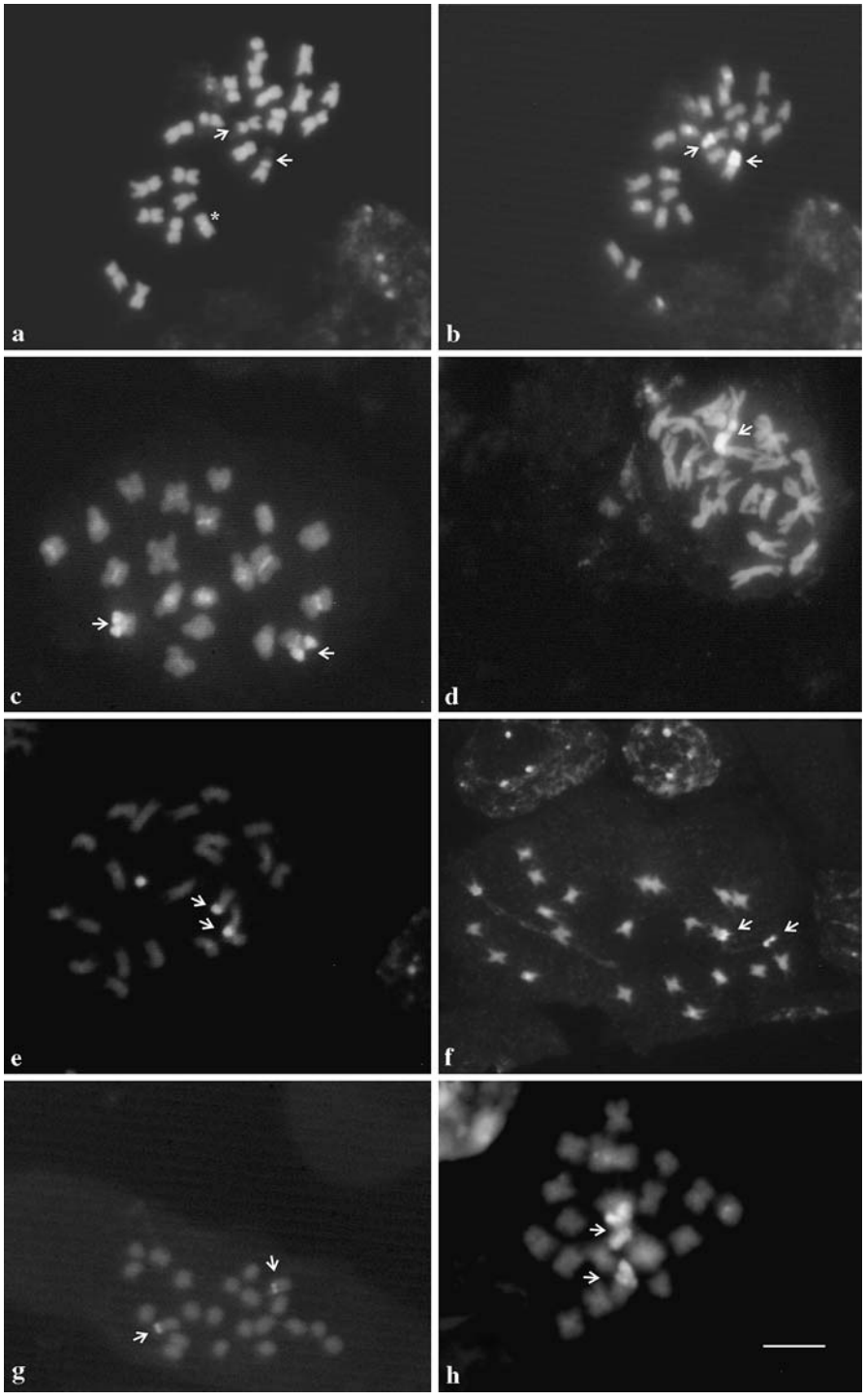

Fig. 2. Fluorochrome chromosome banding in *Pyrrhocactus* species. **a**, **b** *P. catamarcensis*. **c** *P. pachacoensis*. **d** *P. kattermannii.* **e** *P. bulbocalyx.* **f** *P. strausianus*. **g** *P. sanjuanensis.* **h** *P. umadeave*. **a** DAPI fluorescence. **b–h** CMA fluorescence. *Arrows* indicate $CMA^{+}/DAPI^{-}$ NOR-associated heterochromatin and the *asterisk* in **a** indicates a $DAPI^{+}$ band. Bar 5 μm

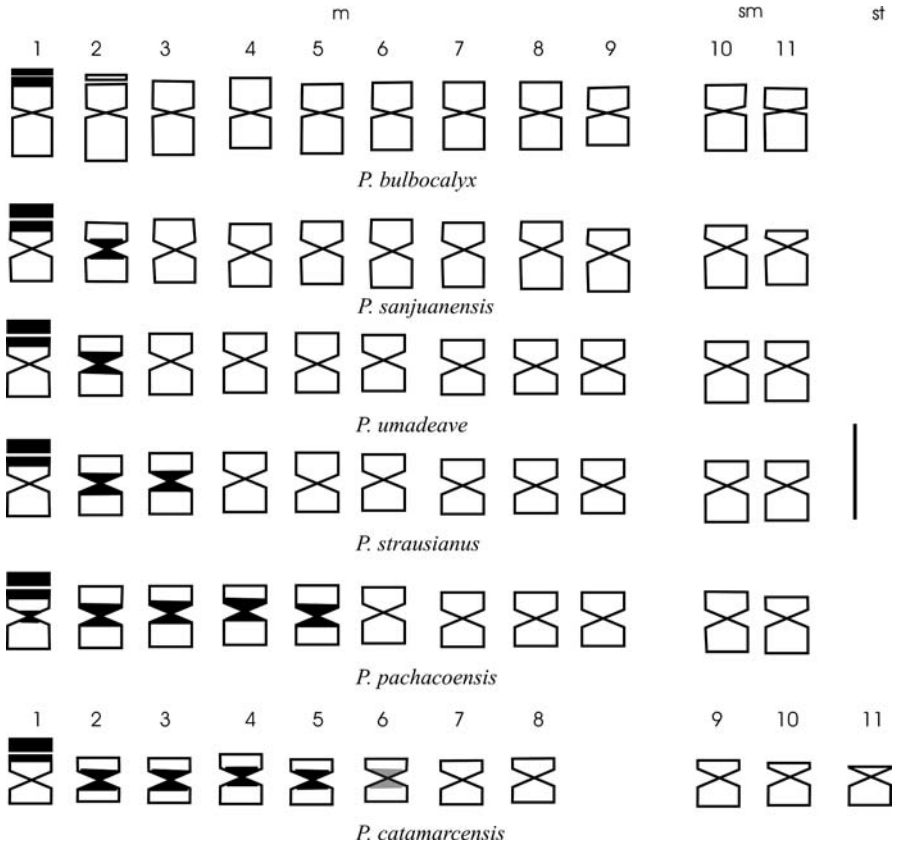

Fig. 3. Idiograms of *Pyrrhocactus* species showing heterochromatic fluorochrome banding paterns. *Black square* $CMA^{+}/DAPI^{-}$, *grey square* $CMA^{-}/DAPI^{+}$. Bar 5 μm

Mohanty 2006; Goldblatt and Johnson 2006 and other volumes of the chromosome number index series). Aneuploidy is extremely rare in Cactaceae, whereas polyploidy occurs in about 25% of the cacti investigated (cf. Pinkava 2002), being an important factor in the evolution in Opuntioideae and, to a lesser extent, in Cactoideae (Palomino et al. 1988, Cota and Wallace 1995, Pinkava 2002, Das and Mohanty 2006).

Pyrrhocactus species had small 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). Effectively, the largest chromosomes reached 4.5 μm (in *Echinocereus*: Cota and Wallace 1995) and the smallest ca. 1.5 μm (in *Mammilaria*, *Echinopsis*, and *Opuntia*, among other genera; Pinkava et al. 1977, Bandyopadhyay and Sharma 2000, Das et al. 2000).

As a whole, karyotypes in the genus are conserved, regarding the correspondence of chromosome size and morphology. The predominance of symmetric karyotypes composed mainly of *m* and *sm* chromosomes, as reported here for *Pyrrhocactus*, is regular for Cactaceae where no bimodal karyotypes have been reported (e.g. *Echinocereus*, *Echinopsis*, *Mammilaria*, *Nyctocereus*, *Opuntia*; Johnson 1980, Palomino et al. 1988, Cota and Philbrick 1994, Bandyopadhyay and Sharma 2000, Das and Mohanty 2006). This trend is also frequent in other Caryophyllales, such as Halophytaceae, Polygonaceae, and Plumbaginaceae (Bernardello 1989, Hunziker et al. 2000, Yildiz and Gücel 2006), and in other angiosperms as well (e.g. Boraginaceae: Luque

1995; Leguminosae: Seijo and Fernández 2003; Sapindaceae: Ferrucci 2000; Solanaceae: Bernardello et al. 1994). Chromosomes with unequal arms are rare in Cactaceae. Effectively, *t* chromosomes have never been detected and *st* chromosomes are very rare (one pair found here for *P. catamarcensis* and five pairs for a tetraploid cytotype of *Mamillaria prolifera*; Johnson 1980).

The presence of NORs attached to the short arms of one (or two) long *m* pair, as documented here, is common in Cactaceae when traditional stains are used (Cota and Wallace 1995, Palomino et al. 1999, Bandyopadhyay and Sharma 2000, Das and Mohanty 2006). In *Pyrrhocactus*, the number, type, and position of NORs is consistent in all species. Thus, there is no variability, other than in *Echinocereus* where satellites can be used as cytogenetic markers to characterize particular species (Cota and Wallace 1995). Only *P. bulbocalyx* had a second satellited pair which is exclusively detected with conventional staining.

Chromosome banding. Base-specific fluorochromes for chromosome staining has been widely used to characterize heterochromatin bands with respect to their highly repeated DNA composition (e.g. Schweizer 1979, Moscone et al. 1996, Schweizer and Ambros 1994, Marcon et al. 2005), known also as satellite DNA. Nevertheless, no previous reports were found regarding CMA/DAPI banding for Cactaceae.

Even though there is a great variability in heterochromatin distribution patterns, discontinuous and extreme changes within a related group of species are not common (Hoshi and Kondo 1998, Guerra 2000). In most genera in which data are available for at least five species, the numbers of bands and the heterochromatin amount varied, but the general patterns were relatively conserved [e.g. *Vigna* (Fabaceae; Galasso et al. 1996), *Citrus* (Rutaceae; Miranda et al. 1997), *Clivia* (Amaryllidaceae; Ran et al. 1999), *Cestrum* (Solanaceae; Fregonezi et al. 2006)]. In *Pyrrhocactus* species, terminal $CMA^+/DAPI^-$ bands were observed, revealing GC-blocks in chromosome pair #1 that are associated with the NORs regions, a fact suggesting that they might be restricted to this location in the genus. Fluorochrome banding has been of great help in the identification of homeologous chromosome pairs between species (Moscone et al. 1995, 1996), and this NOR-bearing pair #1 may certainly be homeologous in all species examined (Figs. 2, 3). CG-rich composition of NORs and NOR-associated heterochromatin, as found in *Pyrrhocactus*, is the rule in plants as a whole (Sinclair and Brown 1971, Schweizer 1979, Morawetz 1986, Benko-Iseppon and Morawetz 2000, Urdampilleta et al. 2006). The NOR-heterochromatin is frequently, but not always (as here reported for *P. bulbocalyx*), positively stained by C- and fluorochrome banding with high affinity for GC-rich chromatin (Guerra 2000).

The physical positioning of GC-rich heterochromatic bands appeared in most *Pyrrhocactus* species in centromeric regions, corresponding to an equilocal pattern distribution. This situation agrees with the heterochromatin dispersion model suggested by Schweizer and Loidl (1987), which proposes transfer of heterochromatin between non-homologous members of a chromosome set at equilocal positions and between chromosome arms of similar lengths, as favored by chromosome proximity in the polarized mitotic interphase nucleus.

In the studied genus, the banding pattern was useful to differentiate the species. There is a general correlation reported in several families between karyotype length and number and length of heterochromatin bands (Greilhuber 1984, Pringle and Murray 1993, Moscone et al. 1996, Benko-Iseppon and Morawetz 2000). Nevertheless, in *Pyrrhocactus* species, the opposite correlation is observed: *P. catamarcensis* with the smallest karyotype length showed more heterochromatin and *P. bulbocalyx* with the largest karyotype had only one band. The biological significance of these contradictory trends is not understood. Within Cactaceae, more data are needed to know if the trend detected in *Pyrrhocactus* is widespread in the family or it is a peculiarity of the genus.

Karyotypes and systematics. Karyotype features allowed individual species to be distinguished. *Pyrrhocactus catamarcensis* is

outstanding, being the species with most changes: one *st* pair, six pairs with bands (including a unique $CMA^{-}/DAPI^{+}$ band), and the smallest karyotype. On the other hand, *P. bulbocalyx* differs in having the longest karyotype, two satellited pairs (one of them only detected with Feulgen), and the smallest number of bands. The remaining species show equivalent karyotypes in terms of formula and length. Among them, *P. pachacoensis* is unique in having a centromeric band in pair #1 together with four other banded pairs and *P. strausianus* and *P. kattermannii* have a total of three banded pairs; these two species can be differentiated because of their karyotype length and the relationship between the largest and the smallest chromosomes. Although *P. umadeave* and *P. sanjuanensis* are morphologically different and geographically isolated, they are karyotypically very closely related showing slight differences in karyotype length.

According to these results, some morphological chromosome variation has accompanied evolutionary divergence of the taxa, which has occurred in sympatric conditions. In *Pyrrhocactus*, species diversification seems not to have been associated with large chromosome rearrangements or with polyploidy, but with cumulative small and cryptic structural changes, as suggested for other Cactaceae (Cota and Wallace 1995) and other angiosperms (e.g. Bernardello et al. 1994, Acosta et al. 2005, Gitaí et al. 2005).

A pattern of homogeneously sized chromosomes with median and submedian centromeres is conserved in *Pyrrhocactus* and is common in Cactaceae (e.g. Palomino et al. 1988, Cota and Philbrick 1994, Cota and Wallace 1995). Therefore, a karyotypic orthoselection might have occurred in it, which preserves rather similar complements throughout a higher taxon because they seem to be more stable, a circumstance also reported in a few other genera (cf. Brandham and Doherty 1998, Stiefkens and Bernardello 2000, Moscone et al. 2003, Acosta et al. 2005).

Cactaceae is probably an exception to Stebbins' (1971) hypothesis concerning increasing karyotype asymmetry in specialized taxa, which was proved in several angiosperms (Cox et al. 1998, Souza and Benko-Iseppon 2004, Acosta et al. 2005). Levin (2002) proposed an alternative hypothesis for increasing asymmetry associated with a decline in karyotype size, as observed in some genera (e.g. *Vicia*: Raina and Rees 1983; *Papaver*: Srivastava and Lavinia 1991), including *Pyrrhocactus* where *P. catamarcensis* possessed the most asymmetrical karyotype and the shortest karyotype.

As a consequence of our results, it is clear that karyotype data are useful to understand species differentiation within Cactaceae. It would be interesting to continue them in the related group of genera that comprise the genus *Eriosyce sensu lato*. These data would be valuable to better understand the evolution and systematics of the group.

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References

Acosta MC, Bernardello G, Guerra M, Moscone EA (2005) Karyotype analysis in several South American species of *Solanum* and *Lycianthes rantonnei* (Solanaceae). Taxon 54: 713–723

Anderson EF (2001) The cactus family. Timber Press, Portland

Angiosperm Phylogeny Group II (APG) (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot J Linn Soc 141: 399–436

Applequist WL, Wallace RS (2002) Deletions in the plastid *trn*T-*trn*L intergenic spacer define clades within Cactaceae subfamily Cactoideae. Pl Syst Evol 231: 153–162

Bandyopadhyay B, Sharma A (2000) The use of multivariate analysis of karyotypes to determine relationships between species of *Opuntia*. Caryologia 53: 121–126

Barthlott W, Hunt D (1993) Cactaceae. In: Kubitzki K, Rohwer JG, Bittrich V (eds) The families and

genera of vascular plants. Vol II. Flowering plants dicotyledons, Springer, Berlin

Battaglia E (1955) Chromosome morphology and terminology. Caryologia 8: 179–187

Behnke HD (1981) Sieve-element characters. Nord J Bot 1: 381–400

Benko-Iseppon AM, Morawetz W (2000) Cytological comparison of Calyceraceae and Dipsacaceae with special reference to their taxonomic relationships. Cytologia 65: 123–128

Bernardello L (1989) The chromosomes of *Grahamia* (Portulacaceae). Pl Syst Evol 163: 127–131

Bernardello LM, Heiser CB, Piazzano M (1994) Karyotypic studies in *Solanum* section *Lasiocarpa* (Solanaceae). Amer J Bot 81: 95–103

Bowen C (1956) Freezing by liquid carbon dioxide in making slides permanent. Stain Technol 31: 87–90

Brandham PE, Doherty MJ (1998) Genome size variation in the Aloaceae, an Angiosperm family displaying karyotypic orthoselection. Ann Bot 82: 67–73

Butterworth CA, Wallace RS (2005) Molecular phylogenetics of the leafy cactus genus *Pereskia* (Cactaceae). Syst Bot 30: 800–808

Cota JH, Philbrick CT (1994) Chromosome number variation and polyploidy in the genus *Echinocerus* (Cactaceae). Amer J Bot 81: 1054–1062

Cota JH, Wallace RS (1995) Karyotypic studies in the *Echinocerus* (Cactaceae) and their taxonomic significance. Caryologia 48: 105–122

Cox AV, Abdelnour GJ, Bennett MD, Leitch IJ (1998) Genome size and karyotype evolution in the slipper orchids (Cypripedioidae: Orchidaceae). Amer J Bot 85: 681–687

Cronquist A (1981) An integrated system of classification of flowering plants. Columbia University Press, New York

Cuénoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ, Chase MW (2002) Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbc*L, *atp*B, and matK DNA sequences. Amer J Bot 89: 132–144

Das AB, Mohanty S (2006) Karyotype analysis and in situ nuclear DNA content in seven species of *Echinopsis* Zucc. of the family Cactaceae. Cytologia 71: 75–79

Das AB, Mohanty S, Das P (2000) Cytophotometric estimation of 4C DNA content and chromosome analysis in four species of *Astrophytum* Lem. of the family Cactaceae. Cytologia 65: 141–148

Donald J, Rowley GD (1966) Reunion of the genus *Neoporteria*. Cactus Succ J 28: 54–58

Edwards EJ, Donoghue MJ (2006) *Pereskia* and the origin of the cactus life-form. Amer Naturalist 167: 777–793

Edwards EJ, Nyffeler R, Donoghue MJ (2005) Basal cactus phylogeny: implication of *Pereskia* (Cactaceae) paraphyly for the transition to the cactus life form. Amer J Bot 92: 1177–1188

Fedorov AA (1969) Chromosome numbers of flowering plants. Reprint 1974 by O. Koeltz Sci. Publ., Koenigstein

Ferrucci MS (2000) Cytotaxonomy of Sapindaceae with special reference to the tribe Paullinieae. Genet Molec Biol 23: 941–946

Fregonezi JN, Fernandes T, Domingues Torezan JM, Vieira O, Vanzela ALL (2006) Karyotype differentiation of four *Cestrum* species (Solanaceae) based on the physical mapping of repetitive DNA. Genet Molec Biol 29: 97–104

Galasso I, Saponetti LS, Pignone D (1996) Cytotaxonomic studies in *Vigna* III. Chromosomal distribution and reacting properties of the heterochromatin in five wild species of the section *Vigna*. Caryologia 49: 311–319

Gibson AC, Nobel PS (1986) The cactus primer. Harvard University Press, Cambridge

Gitaí J, Horres R, Benko-Iseppon AM (2005) Chromosomal features and evolution of Bromeliaceae. Pl Syst Evol 253: 65–80

Goldblatt P, Johnson ED (2006) Index to plant chromosomes numbers 2001–2002. Monogr Syst Bot Missouri Bot Gard 106. Missouri Bot Garden, St Louis

Greilhuber J (1984) Chromosomal evidence in taxonomy. In: Heywood VH, Moore DM (eds) Current concepts in plant taxonomy, Academic, London

Guerra M (2000) Patterns of heterochromatin distribution in plant chromosome. Genet Molec Biol 23: 1029–1041

Hoshi Y, Kondo K (1998) A chromosome phylogeny of the Droseraceae by using CMA-DAPI fluorescent banding. Cytologia 63: 329–339

Hunt D, Taylor N, Charles G (2006) The new cactus lexicon. vols I, II reprint. DH Books, Milborne Port

Hunziker JH, Pozner R, Escobar A (2000) Chromosome number in *Halophytum ameghinoi* (Halophytaceae). Pl Syst Evol 221: 125–127

Johnson MA (1980) Further cytological investigations in *Mammillaria prolifera* and other *Mammillaria* species. Cactus Succ J 42: 43–47

Jong J (1997) Laboratory manual of plant cytologycal techniques. Royal Botanical Garden, Edinburgh

Kattermann F (1994) *Eryosyce* (Cactaceae). The genus revised and amplified. Royal Botanic Gardens, Kew

Kiesling R (1999) Cactaceae. In: Zuloaga FO, Morrone O (eds) Catálogo de las Plantas Vasculares de la República Argentina II. Monogr. Syst. Bot. Missouri Bot. Gard., vol 74, Missouri Bot Garden Press, St Louis, pp 423–489.

Kiesling R, Meglioli S (2003) Cactaceae. In: Kiesling R (ed) Flora de San Juan, vol II reprint Estudio Sigma, Buenos Aires, pp 161–193

Levan A, Sandberg A, Fredga K (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220

Levin DA (2002) The role of chromosome change in plant evolution. Oxford University Press, New York

Luque T (1995) Karyology of *Nonea medicus* (Boraginaceae) in Spain; relationships between genera of Boraginaceae Barbier & Mathez (Anchuseae DC.). Bot J Linn Soc 117: 321–331

Marcon BA, Barros ICL, Guerra M (2005) Variation in chromosome numbers, CMA bands and 45S rDNA sites in species of *Selaginella* (Pteridophyta). Ann Bot 95: 271–276

Mauseth JD (1990) Continental drift, clime and the evolution of Cacti. Cactus Succ J (US) 62: 302–308

Mauseth JD (2006) Structure–function relationships in highly modified shoots of Cactaceae. Ann Bot 98: 901–926

Mauseth JD, Plemons BJ (1995) Developmentally variable, polymorphic woods in cacti. Amer J Bot 82: 1199–1205

Miranda M, Ikeda F, Endo T, Morigucki T, Omura M (1997) Comparative analysis on the distribution of heterochromatin in *Citrus*, *Poncirus* and *Fortunella* chromosomes. Chrom Res 5: 86–92

Morawetz W (1986) Remarks on karyological differentiation patterns in tropical woody plants. Pl Syst Evol 152: 49–100

Moscone EA, Loidl J, Ehrendorfer F, Hunziker AT (1995) Analysis of active nucleolus organizing regions in *Capsicum* (Solanaceae) by silver staining. Amer J Bot 82: 276–287

Moscone EA, Lambrou M, Ehrendorfer F (1996) Fluorescent chromosome banding in the cultivated species of *Capsicum* (Solanaceae). Pl Syst Evol 202: 37–63

Moscone EA, Baranyi M, Ebert I, Greihuber J, Ehrendorfer F, Hunziker AT (2003) Analysis of nuclear DNA content in *Capsicum* (Solanaceae) by flow cytometry and feulgen densitometry. Ann Bot 92: 21–29

Nyffeler R (2002) Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trn*K/*mat*K and *trn*L-*trn*F sequences. Amer J Bot 89: 312–326

Palomino G, Socorro ZL, Scheinvar L (1988) Estudios citogenéticos de dos especies y una variedad del género *Nyctocereus* (Cactaceae). Bol Soc Bot Mex 48: 75–80

Palomino G, Dolezer J, Cid R, Brunner I, Mendez I, Rubluo A (1999) Nuclear genome stability of *Mamillaria san-angelensis* (Cactaceae) regenerants induced by auxins in long-term *in vitro* culture. Pl Sci 141: 191–200

Parfitt BD (1987) *Echinocereus nicholii* (L. Benson) Cactaceae. Phytologia 63: 157–158

Pinkava DJ (2002) On the evolution of the North America Opuntioideae. In: Hunt D, Taylor N (eds) Studies in the Opuntioideae, Royal Botanic Gardens, Kew, pp 78–99

Pinkava DJ, McGill LA, Reeves T (1977) Chromosome number in some cacti of western North America. Bull Torrey Bot Club 104: 105–110

Pinkava DJ, Baker MA, Parfitt BD (1985) Chromosome numbers in some cacti of western North America. V. Syst Bot 10: 471–483

Pinkava DJ, Parfitt BD, Baker MA, Worthington RD (1992) Chromosome numbers in some cacti of western Noth America. VI. Madroño 39: 98–113

Powell AM, Weedin JF (2001) Chromosome numbers in Chihuahuan Desert Cactaceae. III. Trans-Pecos Texas. Amer J Bot 88: 481–485

Pringle GJ, Murray BG (1993) Karyotypes and C-banding patterns in species of *Cyphomandra* Mart. ex Sendtn. (Solanaceae). Bot J Linn Soc 111: 331–342

Raina SN, Rees H (1983) DNA variation between and within chromosome complements of *Vicia* species. Heredity 51: 335–346

Ran Y, Murray BG, Hammett KRW (1999) Karyotype analysis of the genus *Clivia* by Giemsa and fluorochrome banding and in situ hybridization. Euphytica 106: 139–147

Romero Zarco C (1986) A new method for estimating karyotype asymmetry. Taxon 35: 556–530

Ross R (1981) Chromosome counts, cytology, and reproduction in the Cactaceae. Amer J Bot 68: 463–470

Schweizer D (1976) Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma (Berlin) 58: 307–324

Schweizer D (1979) Fluorescent chromosome banding in plants: applications, mechanisms, and implica-

tions for chromosome structure. In: Davies DR, Hopwood DA (eds) Proceedings of the Fourth John Innes Symposium. Norwich, Crowe

Schweizer D, Loidl J (1987) A model for heterochromatin dispersion and the evolution of C-band patterns. Chrom Today 9: 61–74

Schweizer D, Ambros P (1994) Chromosome banding. In: Gosden JR (ed) Methods in molecular biology. Chromosome analysis protocols, Humana Press, Totowa

Seijo G, Fernández A (2003) Karyotype analysis and chromosome evolution in South American species of *Lathyrus* (Leguminosae). Amer J Bot 90: 980–987

Shan F, Yan G, Plummer JA (2003) Karyotype evolution in the genus *Boronia* (Rutaceae). Bot J Linn Soc 142: 309–320

Sinclair JH, Brown DD (1971) Retention of common nucleotide sequences in the ribosomal deoxyribonucleic acid of eukaryotes and some of their physical characteristics. Biochemistry 10: 10–20

Souza M, Benko-Iseppon AN (2004) Cytogenetics and chromosome banding patterns in Caesalpinioideae and Papilionioideae species of Pará, Amazonas, Brazil. Bot J Linn Soc 144: 181–191

Srivastava S, Lavania UC (1991) Evolutionary DNA variation in *Papaver*. Genome 34: 763–768

Stebbins GL (1971) Chromosomal evolution in higher plants. E. Arnold, London

Stiefkens L, Bernardello G (2000) Karyotype and DNA content in diploid and poliploid *Lycium* (Solanaceae). Bol Soc Argent Bot 35: 237–244

Sumner AT (1990) Chromosome banding. Unwin Hyman, London

Urdampilleta JD, Ferrucci MS, Torezan JMD, Vanzela ALL (2006) Karyotype relationships among four South American species of *Urvillea* (Sapindaceae; Paullinieae). Pl Syst Evol 258: 85–95

Vosa CG (1970) Heterochromatin recognition with fluorochromes. Chromosome 30: 366–372

Vosa CG (1976) Heterochromatin classification in *Vicia faba* and *Scilla sibirica*. Chrom Today 5: 185–192

Wallace RS (1994) Phylogenetic analysis. Succ Pl Res 1: 125–130

Wallace RS, Forquer ED (1995) Molecular evidence for the systematic placement of *Echinocerus pensilis*. Haseltonia 3: 71–76

Wallace RS, Gibson AC (2002) Evolution and systematics. In: Nobel PS (ed) Cacti: biology and uses. University of California Press, CA, pp 1–21

Weiss-Schneeweiss H, Stuessy TF, Siljak-Yakovlev S, Baeza CM, Parker J (2003) Karyotype evolution in South American species of *Hypochaeris* (Asteraceae, Lactuceae). Pl Syst Evol 241: 171–184

Yildiz K, Gücel S (2006) Chromosome numbers of 16 endemic plant taxa from Northern *Cyprus*. Turkey J Bot 30: 181–192