KARYOTYPE, HETEROCHROMATIN, AND PHYSICAL MAPPING OF 5S AND 18-5.8-26S rDNA GENES IN *SETIECHINOPSIS* (CACTACEAE), AN ARGENTINE ENDEMIC GENUS

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Abstract: *Setiechinopsis* is a monotypic Argentine endemic genus (*S. mirabilis*) of the tribe Trichocereeae. It is one of the most difficult cacti to find and grows in lowlands and brackish soils. Our aim was to analyze for the first time its cytogenetic features in two populations. It presented $2n = 22$ with small chromosomes (mean chromosome length = 2.87 µm; mean haploid genome length = 31.60 µm). The karyotype was symmetrical: $10m + 1sm$. The first *m* pair (No. 1) had nucleolar organizing regions and terminal microsatellites on the short arms. The ratio of the length of the largest *m* chromosome pair (No. 1) to that of the smallest *m* chromosome pair (No. 10) was 1.55. Banding patterns showed $CMA^+/DAPI^-$ constitutive NOR-associated heterochromatin in *m* chromosome pair No. 1, comprising the distal satellite and a small proximal band. Additionally, four *m* chromosome pairs showed $CMA^+/DAPI^-$ pericentromeric bands. The percentage of $CMA^+/DAPI^-$ heterochromatin was 11.22% of the total karyotype length. No $CMA^-/DAPI^+$ bands were detected. The signal of the 18-5.8-26S gene was located in the satellite and the terminal portion of the short arm of pair No. 1. The signals of the 5S rDNA gene were located in pericentromeric regions in *m* chromosome pairs Nos. 2–5. The locations of the 18-5.8-26S sites coincided with the NOR-associated $CMA^+/DAPI^-$ bands, whereas 5S sites coincided with the pericentromeric $CMA^+/DAPI^-$ bands. Sizes, numbers, and intensities of both rDNA signals had a great similarity between the homologs. Comparisons with the few studies made in the Cactaceae suggest that morphological variation in the family was not followed by major modifications in karyotype formulae and chromosome size, but that the occurrence and distribution of different repetitive DNA fragments tend to vary among the different taxa so far analyzed.

The majority of cytological studies in cacti provide chromosome counts and indicate that their base chromosome number is $x = 11$ (cf. Pinkava et al. 1977, 1985, 1998; Powell and Weedin 2001; Pinkava 2002). On the other hand, there are comparatively few detailed karyotypic studies available (e.g., Johnson 1980; Palomino et al. 1988; Cota and Wallace 1995; Das and Mohanty 2006, 2008; Las Peñas et al. 2008, 2009), probably due to the small chromosome size and the presence of mucilage in cactus tissues, which hinders the separation of cells and chromosomes and interferes with their observation (Cota and Wallace 1995).

The CMA/DAPI technique determines the distribution of heterochromatin (e.g., Moscone et al. 1996; Guerra et al. 2000; Urdampilleta et al. 2006). More informative markers are often provided by fluorescence in situ hybridization (FISH), a method that allows hybridization of known labeled marker sequences (probes) to homologous chromosomal targets (e.g., Adams et al. 2000; Schwarzacher and Heslop-Harrison 2000; Schwarzacher 2003). FISH enables the physical mapping of sequences to their location within the genome, in particular repetitive sequences that cannot be mapped easily by any other method (Schwarzacher 2003). These repetitive sequences change rapidly during evolution, providing excellent markers for the identification of chromosomes and chromosome segments, and for detecting rearrangements. The 5S and 18-5.8-26S rDNA genes, in particular, have been extensively used to establish possible chromosomal homologies (e.g., Moscone et al. 1999; Adams et al. 2000; Taketa et al. 2005; Cai et al. 2006). Both techniques have rarely been applied to Cactaceae (Las Peñas et al. 2008, 2009).

The cacti of tribe Trichocereeae (subfamily Cactoideae) are arborescent, columnar or globular, being mainly found in arid and semiarid biomes in subequatorial South America. In large part, the taxonomic difficulty found in members of this tribe, and in the family Cactaceae as a whole, is the result of their extensive morphological variability. This variability has been attributed to environmental gradients (Gibson and Nobel 1986), as well as to changes associated with hybridization and genome doubling (polyploidy) (Arakaki et al. 2007).

Setiechinopsis (Backeb.) de Haas is among the Trichocereeae genera with delimitation problems. Some authors considered it as monotypic [with *S.*

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Figure 1. *Setiechinopsis mirabilis,* cultivated plant in flower. The common name of *Setiechinopsis mirabilis* according to C. Spegazzini is "la flor de la oracion", which can not be translated literally ("the flower of the preaching") but as "the flower that opens at the preaching time", referring to the sunset, the time after the day's work and the evening meal. The pure white perianth attracts nocturnal pollinators. Tepals end in a thin point, as do the scales on the receptacle; hence the name "Seti-echinopsis", i.e., "Echinopsis with bristles".

mirabilis (Speg.) de Haas] and endemic to Argentina (Kiesling 1999; Kiesling et al. 2008). However, other authors included it under *Echinopsis* sensu lato (as *E. mirabilis* Speg.), together with the genera *Chamaecereus*, *Helianthocereus*, *Hymenorebutia*, *Lobivia*, *Pseudolobivia*¸ *Soehrensia*, and *Trichocereus* (Anderson 2001; Hunt et al. 2006).

Such differences of opinion correspond to different concepts about the breadth of the genera, but especially to the relative importance attached to morphological characters, basically the flower structure. To analyze this, in the following paragraphs, when we refer to a genus, we are taking it in the narrow sense; specifically this means considering *Echinopsis* sensu stricto, such that *Trichocereus*, *Lobivia* and *Setiechinopsis* have full generic status of equal taxonomic rank with *Echinopsis* (sensu stricto).

Setiechinopsis stems are small, only (3–)20 cm high, rarely branched. The stem has the shape of a spindle, that is, a greater diameter at the center and a lesser diameter at the extremes. That shape of stem does not appear in any other genus of known affinity such as *Echinopsis*, *Lobivia* or *Trichocereus*.

The stem color of *Setiechinopsis* is mostly dark (mauve), even growing in the shade in cultivation (Fig. 1); that means the red pigments are produced more easily, in a greater degree than virtually any other cacti of the region. Such pigment production is not so frequent in *Echinopsis*.

Several flowers of *Setiechinopsis* may open simultaneously at the apex of the stem, and flowering occurs several times a year during the warm season. The perianth is relatively short, and absolutely white—an attraction to nocturnal pollinators, in contrast with the completely dark plant and receptacle. The flower receptacle is extremely thin, which is also compatible with moth pollination, although there is no reported study or direct observation of pollination. The length of the flower is 7–15 cm, and this large variation corresponds to the state of hydration of the plant, and thus to the availability of water.

The number of stamens is relatively low for most species of the cactus family (except for some phylogenetically distant genera, such as *Rhipsalis* or *Yavia*), and the stamens are unusually short (2 mm) in relation to the complete length of the flower. Most (ca. 66) of them are borne very high on the tube, and a few (ca. 10) lower along the tube—another unusual character, evidently an adaptation to specific pollinators. This may be seen as a consequence of the intercalary growth of the lower part of the receptacle, whereas in closely related genera this intercalary growth is uniform or at the upper/middle part of the receptacle, forming two separate groups of numerous, very long stamens. The style, including the stigma, is about 1.2 cm long, which means it does not reach the level of the lower stamens; this arrangement allows self-pollination. Without doubt these plants are self-fertile, based on the observation that when individual plants are isolated in cultivation, all the flowers set fruit with numerous fertile seeds (Las Peñas, pers. obs.). This should be confirmed by controlled experiments. In contrast, *Echinopsis* spp. in similar conditions in cultivation do not produce fruit, according to our observations.

Setiechinopsis seeds are ca. 1.5 mm in diameter, truncate-globular, dark-brown, with the cuticle partially separated and rugose. These are similar to the seeds of many other species of the same tribe, Trichocereeae, suggesting a basal lineage.

Studies in the Cactaceae about salt tolerance or adaptation are scarce (see Nobel 2002; Schuch and Kelly 2008). *Setiechinopsis* grows at the borders of salt lakes, in soils with high levels of salinity. The salt concentration varies seasonally at a given location, as the salt in solution ascends to the surface with water by capillary action during the 10-month annual dry period, and forms a white layer of salt on the surface. During the wet season, rain dissolves the salt and during that season it descends to lower layers of the soil. This process may explain the more or less regular disappearance of complete populations, which are literally killed by the excess of salt at the level of the roots during some unfavorable years. One of us (RK) visited a population of this cactus briefly in October 2010 and found eight dead plants and only two that were alive. This process merits a deeper study examining the osmotic process and salt accumulation.

The distribution of this species is extensive, from 29°51′S, 61°40′W to 34°47′S, 68°27′W, encompassing about 1,200 km in NE–SW distance, but as the plants are difficult to find due their size and color and because they are hidden by the nurse plants, the range may be even more extensive. The

nurse plants are mostly of the genus *Atriplex* (perhaps several species, but certainly including *A. lithophila*), of the Chenopodiaceae. One of the common names of this shrub is "cachi yuyo", which translates literally as "salty weed" or "salty plant".

Several other cacti (e.g., *Pterocactus tuberosus*, *Echinopsis leucantha* and *Stetsonia coryne*) share the localities with *Setiechinopsis* to some extent, but all of them occur also in less salty environments, whereas *Setiechinopsis* seems to occur *exclusively* in highly saline soils. (The same exclusive preference for saline soils is also observed with *Gymnocalycium ragonesei*, but with regard to a much smaller area of exclusive habitat). *Cereus validus* (= *C. forbesii*) also grows in the same general area, as reported by Nobel and Bobich (2002), but (a) it occurs in soils of lower salinity, and (b) it also occurs on hills, where soils can be salty but not to the extreme degree as in the salt flats. Another species that typically occurs with *Setiechinopsis* is *Grahamia bracteata*, a succulent member of the Portulacaceae.

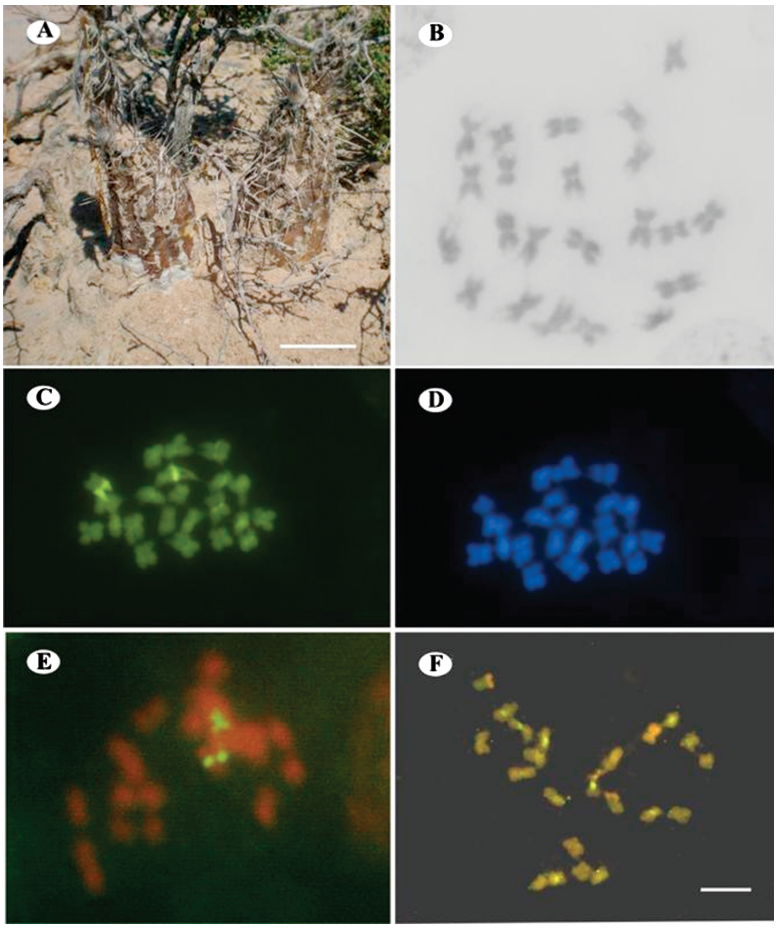

Figure 2. *Setiechinopsis mirabilis.* **A.** A plant from La Rioja. **B.** Somatic metaphase (2n = 22) with Feulgen staining. **C.** Fluorochrome banding with CMA fluorescence. **D.** Fluorochrome banding with DAPI fluorescence. **E.** FISH using 18S-5.8-26S rDNA probe. **F.** FISH using 5S rDNA probe. Bar = 5 cm for A and 5 µm for B–F.

Upon this background, the aim of this study was to analyze for the first time the cytogenetic features in two populations of *S. mirabilis*. Karyotypes were determined using the Feulgen technique; the number and position of heterochromatin bands was determined through CMA/DAPI fluorescent chromosome banding; and the location of the sequences of 5S and 18-5.8-26S rDNA was determined using fluorescence in situ hybridization (FISH). Thus, we hope to cast light on the evolutionary relationships of *Setiechinopsis* and contribute to the understanding of its systematic position.

Materials and Methods

Plant material

Setiechinopsis mirabilis. Argentina: Santiago del Estero province, Loreto, Salar de Atamisqui, Las Peñas 443; La Rioja province, Chamical, Salina La Antigua, Las Peñas 503 (Fig. 2A). Voucher specimens were deposited in the herbarium of the Museo Botánico de Córdoba (CORD). Living plants were placed in earthenware pots in an equal part mixture of sand and potting soil.

Karyotype analysis

The preparation of metaphase chromosomes was done from adventitious roots pretreated with 2 mM 8-hydroxyquinoline for 8 h at 4°C and fixed in 3:1 ethanol:acetic acid. For slide preparation, root tips were hydrolyzed with 5 N HCl for 30 min at room temperature and then washed, stained with Feulgen for 2 h, and squashed in a drop of 2% acetic carmine (as per Jong 1997). Permanent mounts were made following Bowen's (1956) method. Ten metaphases from 10 individuals per population were photographed with a phase-contrast optic Zeiss Axiophot microscope (Jena, Germany) and a Leica DFC300FX camera (Wetzlar, Germany). 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 that of its

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 the mean chromosome lengths (tl), and mean arm ratio (R) were calculated. Idiograms were based on the mean values. The chromosomes were arranged first into groups according to their increasing arm ratio and then according to decreasing length within each group. Karyotype asymmetry was estimated using the intrachromosomal (A_1) and interchromosomal (A_2) indices of Romero Zarco (1986) and Stebbins's classification (1971).

For the preparation of slides for fluorochrome banding and FISH, root tips were washed twice in distilled water (10 min each), digested with a solution of 2% cellulase (Sigma-Aldrich, Vienna, Austria) and 20% pectinase (from *Aspergillus niger*; Sigma-Aldrich, Vienna, Austria) for 45 min at 37°C, and squashed in a drop of 45% acetic acid (Schwarzacher et al. 1980). Only one root tip was used per slide. 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 by volume) containing 2.5 mM $MgCl_2$ for 90 min, then stained with 2 µg/ml 4′-6-diamidino-2-phenylindole (DAPI) for 30 min (both stains from Sigma-Aldrich, Vienna, Austria), and finally mounted in McIlvaine's buffer-glycerol (1:1) (Schweizer 1976; Schweizer and Ambros 1994). The amount of heterochromatin was expressed as a percentage of the total length of the haploid karyotype.

FISH

The probe p*Ta* 71 containing the 18S-5.8S-26S rDNA was used (Gerlach and Bedbrook 1979). For the 5S rDNA, a probe was obtained from the genome of *Pereskia aculeata* by PCR using the primers 5L1 (5′-CGGTGCATTAATGCTGGTAT-3′) and 5L2 (5′-CCATCAGAACTCCGCAGTTA-3′) (Shibata and Hizume 2002).

Both probes were labeled with biotin-14-dATP (BioNick, Invitrogen Carlsbad, USA). The FISH protocol was that of Schwarzacher and Heslop-Harrison (2000), with minor modifications. The preparations were incubated in 100 µg/ml RNase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series, and air-dried. On each slide, 30 µl of hybridization mixture was added (4–6 ng/µl of probe, 50% formamide, 10% dextran sulfate, 3.3 ng/µl of salmon DNA, 2x SSC and 0.3% SDS), previously denatured at 70°C for 10 min. Chromosome denaturation/ hybridization was done at 90°C for 10 min, 48°C for 10 min, and 38°C for 5 min, using a thermal cycler (Mastercycler®, Eppendorf, Hamburg), and slides were placed in a humidity chamber at 37°C overnight. The probe was detected with avidin-FITC conjugate and counterstained and mounted with 25 µl antifading agent (VECTASHIELD®, Vector Laboratories, Burlingame, CA, USA) containing 1 µl of a 100 µg/ml solution of propidium iodide.

Results

Setiechinopsis mirabilis presented a somatic chromosome number of 2n = 22, observed in 100 metaphase plates of 6 individuals (Fig. 2B). Chromosomes were small (Table 1; Fig. 2B), the mean chromosome length being 2.87 µm with a range of 2.31 (pair No. 9) to 3.56 µm (pair No. 1). The mean haploid genome length was 31.60 ± 0.36 µm.

The karyotype formula was: 10 *m* + 1 *sm*. The first *m* pair had nucleolar organizing regions and terminal microsatellites on the short arms (Fig. 2B). Table 1 includes the karyotypic characteristics calculated for each chromosome pair used to generate the idiogram (Fig. 3).

Table 1. Measurements in µm (mean ± standard error) of somatic chromosome pairs in *Setiechinopsis mirabilis (*short arms: s; long arms: l; total length: t; arm ratios: r; and centromeric indices: i). Abbreviations after Levan et al. (1964).

Pair	s	l	t	r	i	Chromosome type
1	1.60 ± 0.08	1.87 ± 0.05	3.47 ± 0.13	1.17	46.05	*m*
2	1.53 ± 0.00	1.78 ± 0.02	3.32 ± 0.03	1.16	46.24	*m*
3	1.44 ± 0.05	1.73 ± 0.01	3.17 ± 0.04	1.20	45.46	*m*
4	1.37 ± 0.01	1.64 ± 0.08	3.01 ± 0.07	1.19	45.56	*m*
5	1.30 ± 0.15	1.67 ± 0.06	2.97 ± 0.21	1.29	43.71	*m*
6	1.32 ± 0.00	1.53 ± 0.03	2.85 ± 0.03	1.16	46.29	*m*
7	1.30 ± 0.08	1.40 ± 0.00	2.69 ± 0.07	1.08	48.11	*m*
8	1.18 ± 0.01	1.32 ± 0.04	2.50 ± 0.05	1.12	47.24	*m*
9	1.00 ± 0.05	1.41 ± 0.04	2.41 ± 0.09	1.41	41.45	*m*
10	1.07 ± 0.02	1.29 ± 0.09	2.36 ± 0.08	1.21	45.32	*m*
11	1.00 ± 0.05	1.85 ± 0.03	2.86 ± 0.08	1.85	35.14	*sm*

The ratio of the length of the largest chromosome pair (No. 1) to that of the smallest (No. 10) was 1.55. The karyotype was symmetrical due to the high percentage of *m* pairs and the existence of small differences between the chromosome pairs, as indicated by the asymmetry indices of Romero Zarco (1986): A_1 = 0.19 and A_2 = 0.13 and Stebbins's classification (1971): category "1A".

The banding patterns showed $CMA^+/DAPI^{\cdot\cdot}$ constitutive heterochromatin associated with NOR (GC-rich) in the satellited chromosome pair No. 1; it comprised the distal satellite and a small proximal band on the short arm where it is attached (Fig. 2C, D). In addition, four *m* chromosome pairs showed $CMA^+/DAPI^{\cdot\cdot}$ pericentromeric bands (Fig. 2C). The percentage of $CMA^+/DAPI^{\cdot\cdot}$ heterochromatin was 11.22% of the total karyotype length. On the other hand, no $CMA^{\cdot\cdot}/DAPI^+$ bands were detected in this species.

Regarding the cytogenetic mapping of rDNA genes, the signal of the 18-5.8-26S gene was located in the satellite and the terminal portion of the short arm of pair No. 1 (Fig. 2D). The signals of the 5S rDNA gene were located in pericentromeric regions in *m* pairs No. 2 to 5 (Fig. 2E). The locations of the 18-5.8-26S sites coincided with the NOR-associated $CMA^+/DAPI^{\cdot\cdot}$ bands, whereas 5S sites coincided with the pericentromeric $CMA^+/DAPI^{\cdot\cdot}$ bands described above (Fig. 3). The sizes, numbers, and intensity of both rDNA signals showed great similarity between the homologues.

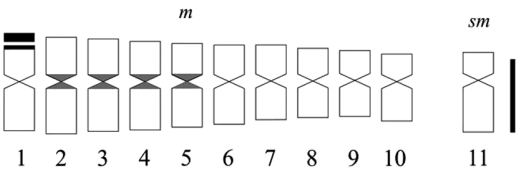

Figure 3. Idiogram with physical location of repetitive segments in *Setiechinopsis mirabilis*. Black indicates locus of 18-5.8-26S rDNA ($CMA^+/DAPI^{\cdot\cdot}$, NOR-associated). Gray indicates loci of 5S rDNA ($CMA^+/DAPI^{\cdot\cdot}$). Bar = 2 µm.

Discussion

Setiechinopsis mirabilis proved to be diploid with x = 11 and had small chromosomes, both typical features of Cactaceae (e.g., Cota and Philbrick 1994; Pinkava 2002; Das and Mohanty 2006; Ortolandi et al. 2007; Las Peñas et al. 2008, 2009). Its karyotype was symmetrical, with only one *sm* chromosome pair and no marked differences in size between the pairs of the complement. As a whole, *st* chromosomes are rare in Cactaceae (Johnson 1980; Cota and Philbrick 1994; Las Peñas et al. 2008), and *t* chromosomes have been never found.

Regarding the satellites, there is variability among the few karyotypically studied cactus species: from one (Las Peñas et al., 2008, 2009), as here found for *S. mirabilis*, to 2–4 pairs (e.g., Cota and Philbrick 1994; Cota and Wallace 1995; Bandyopadhay and Sharma 2000; Das et al. 1999, 2000; Das and Mohanty 2008).

In many plant species, the centromere is associated with blocks of heterochromatin containing highly repetitive DNA sequences in tandem, representing a significant fraction of the total DNA (Schwarzacher 2003). The CMA/DAPI fluorescence banding technique here used to detect heterochromatin, has been frequently applied to other plant families to determine the distribution of heterochromatin, e.g., Solanaceae (Moscone et al. 1996), Rutaceae (Guerra et al. 2000), and Sapindaceae (Urdampilleta et al. 2006); nevertheless, it has rarely been applied to cacti (Las Peñas et al. 2008, 2009). *S. mirabilis* had one chromosome pair with $CMA^+/DAPI^{\cdot\cdot}$ NOR-associated bands, as reported in several angiosperms (e.g., Sinclair and Brown 1971; Schweizer 1976; Morawetz 1986; Guerra 2000) and in the few Cactaceae studied (Las Peñas et al. 2008, 2009). In addition, *S. mirabilis* showed four chromosome pairs with pericentromeric $CMA^+/DAPI^{\cdot\cdot}$ bands. Guerra (2000) pointed out that species with small chromosomes (less than 3 µm) have higher numbers of proximal bands than species with larger chromosomes, which has been observed in several families (e.g., Sheikh and Kondo 1995; Galasso et al. 1996; Lengerova et al. 2004). In regard to the Cactaceae, only in the seven species of the genus *Pyrrhocactus* were five chromosome pairs with pericentromeric $CMA^+/DAPI^{\cdot\cdot}$ bands reported (Las Peñas et al. 2008). Thus, results suggest that in cacti there is variability in the distribution of heterochromatin and that more taxa should be analyzed to elucidate the evolutionary and systematic value of the presence (and number) or absence of such bands.

The location of the signals of the 18-5.8-26 S rDNA in *S. mirabilis* coincided with the findings in six species of different subfamilies of cacti (Las Peñas et al. 2009). Although more data are needed to confirm the trend, it seems that in Cactaceae these signals are highly conserved, as reported, for instance, in the family Asteraceae (Fregonezi et al. 2004; Ruas et al. 2005). On the other hand, the location of the 5S rDNA gene was here reported for the first time in a species of the Cactaceae. Its location was in a centromeric region, a frequent location for the 5S rDNA gene in both gymnosperms and angiosperms (e.g., Kulak et al. 2002; Besendorfer et al. 2005). Generally, 5S sites are more numerous than 18-5.8-26S sites (e.g., Hemleben and Werts 1988; Sastri et al. 1992; Moscone et al. 1999).

The similar intensity of FISH signals of both rDNA genes may be an indication that there are no differences among the number of copies of genes (Appels et al. 1980; Weiss-Schneeweiss et al. 2003). The co-location of 5S rDNA and heterochromatin here observed was described for a few species: *Solanum lycopersicum* (Solanaceae) (Xu and Earle 1996), *Hypochaeris* spp. (Asteraceae) (Ruas et al. 2005), and *Cestrum* spp. (Solanaceae) (Fernandes et al. 2009).

Data available showed that conventional karyotypes in Cactaceae have slight differences among the

studied species, mainly in regard to the length of the genome and asymmetry indices (e.g., Palomino et al. 1988; Cota and Wallace 1995; Bandyopadhyay and Sharma 2000; Das et al. 1999; Das and Mohanty 2006, 2008; Las Peñas et al. 2008, 2009). This tendency was also observed in the karyotypes of the *Echinopsis* (sensu lato) group: *Acantocalycium spiniflorum* and *Echinopsis* spp. (Das and Mohanty 2006; Las Peñas et al. 2009). Thus, karyotypic features suggest that morphological differentiation in cacti was not followed by chromosomal divergence, as reported in other plant families (e.g., Bernardello et al. 1994; Cox et al. 1998; Acosta et al. 2005; Chiarini and Bernardello 2006).

Nevertheless, fluorescent chromosome banding showed cytogenetic variability, at least among the examined species (*S. mirabilis*, this work; *Acantocalycium spiniflorum* and *Echinopsis tubiflora*, Las Peñas et al. 2009). Additionally, this technique was helpful to chromosomically differentiate all seven *Pyrrhocactus* species (Las Peñas et al. 2008).

Setiechinopsis is cytogenetically differentiated from the studied members of *Echinopsis* sensu lato regarding their fluorescent banding pattern (Las Peñas et al. 2009; Las Peñas 2009). Accordingly, more species should be explored with this technique to understand its systematic value, mainly in genera with taxonomic problems such as *Lobivia* and *Trichocereus*. Biologically, *Setiechinopsis* is differentiated from *Echinopsis*: the saline soils where it grows, the typical color of the stem, its short life, and its morphologically inferred nocturnal pollination (Kiesling 2003). Some cacti (*Echinocactus grusonii* and *Carnegiea gigantea*) have proved to be tolerant of different levels of salinity and *C. gigantea* roots responded positively in root growth to increasing salinity (Schuch and Kelly 2008). Experimental salinity studies should be performed in *Setiechinopsis* to understand its salt tolerance.

Data available in Cactaceae suggest that morphological variation was not followed by major modifications in karyotype formulae and chromosome size, but that the occurrence and distribution of different repetitive DNA fragments tends to vary among the different taxa so far analyzed.

Based on the previous morphological, ecological and biological characters, we are inclined to keep *Setiechinopsis* as a proper genus, and the conclusions of the chromosome research support this opinion.

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