

DNA CONTENT AND CYTOGENETIC CHARACTERISTICS OF *Gymnocalycium quehlianum* (CACTACEAE) ALONG AN ALTITUDINAL GRADIENT



CONTENIDO DE ADN Y CARACTERÍSTICAS CITOGÉNÉTICAS DE *Gymnocalycium quehlianum* (CACTACEAE) A LO LARGO DE UN GRADIENTE ALTITUDINAL

Martino P.¹, Gurvich E.D.^{1,2}, Las Peñas M.L.^{1,2}

¹Instituto Multidisciplinario de Biología Vegetal (UNC- CONICET).

²Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina.

Corresponding author:

M. Laura Las Peñas

lauralaspensas@unc.edu.ar

 ORCID 0000-0003-4244-7807

Cite this article as:

Martino P., Gurvich E.D., Las Peñas M.L. 2022. DNA CONTENT AND CYTOGENETIC CHARACTERISTICS OF *Gymnocalycium quehlianum* (CACTACEAE) ALONG AN ALTITUDINAL GRADIENT. BAG. Journal of Basic and Applied Genetics XXXIII (1): 51-59.

Received: 11/05/2021

Revised version received: 02/04/2022

Accepted: 03/15/2022

General Editor: Elsa Camadro

DOI: 10.35407/bag.2022.33.01.04

ISSN online version: 1852-6233

ABSTRACT

Important changes in vegetation types occur along elevational gradients. The genus *Gymnocalycium* is endemic to southern South America, and its species are distributed along elevational gradients. In particular, *Gymnocalycium quehlianum* is a globular cactus endemic to the Sierras de Córdoba. Studying cytogenetic aspects and DNA content in populations throughout their distribution is key to understanding the species. DNA content and cytogenetic characteristics were analyzed in four populations of *G. quehlianum* (615, 744, 948 and 1257 masl). The genome size in the four populations varied between 3.55 and 4.30 pg. The populations were diploid ($2n = 22$). All populations showed the karyotype formula of 10 metacentrics (m) + 1 submetacentric (sm). The species presented symmetrical karyotypes and constitutive heterochromatin CMA+/DAPI- associated with nucleolar organizing regions, always found in the first pair of m chromosomes. The 18-5.8-26S rDNA locus is found in the terminal regions of the first pair of chromosomes m , and the 5S locus is adjacent to the 18-5.8-26S locus. A tendency for DNA content to decrease with increasing altitude was observed.

Key words: *Gymnocalycium quehlianum*, chromosome number, cytogenetic, DNA content, altitudinal gradient

RESUMEN

A lo largo de los gradientes altitudinales se producen cambios importantes en los tipos de vegetación. El género *Gymnocalycium* es endémico del sur de América del Sur y sus especies se distribuyen en gradientes altitudinales. En particular, *Gymnocalycium quehlianum* es un cactus globular endémico de las Sierras de Córdoba. Estudiar aspectos citogenéticos y de contenido de ADN en las poblaciones a lo largo de su distribución es clave para comprender a la especie. En cuatro poblaciones de *G. quehlianum* (615, 744, 948 y 1257 msnm) se analizaron el contenido de ADN y las características citogenéticas. El tamaño del genoma en las cuatro poblaciones varió entre 3,55 y 4,30 pg. Las poblaciones resultaron diploides ($2n=22$). Todas las poblaciones presentaron la fórmula del cariotipo de 10 metacéntricos (m) + 1 submetacéntrico (sm). La especie presentó cariotipos simétricos y heterocromatina constitutiva CMA+/DAPI- asociados con regiones organizadoras nucleolares, que siempre se encontraban en el primer par de cromosomas m . El locus de ADNr 18-5.8-26S se encuentra en las regiones terminales del primer par de cromosomas m y el locus de 5S está adyacente al locus 18-5.8-26S. Se observó una tendencia del contenido de ADN a disminuir con el aumento de la altitud.

Palabras clave: *Gymnocalycium quehlianum*, número cromosómico, citogenética, contenido de ADN, gradiente altitudinal

INTRODUCTION

Gymnocalycium (subfamily Cactoideae) is a genus endemic to southern South America and comprises about 50 species, most of them with a narrow geographical distribution (Charles, 2009). In Argentina, it is the Cactaceae genus with the highest number of species (41), representing 18% of the total richness of the subfamily in the country (Kiesling *et al.*, 2008). *Gymnocalycium* is divided into subgenera based on the characteristics of seeds, floral anatomy and fruits (Schütz, 1986; Demaio *et al.*, 2011). *G. quehlianum* (subgenus *Trichomosemineum*) is endemic to Córdoba province (Argentina) and is distributed along the Sierra Chica up to the Sierra Norte (Figure 1). It is abundant in mountain environments between 500 and 1200 m a.s.l. (Charles, 2009; Gurvich *et al.*, 2014). It has depressed grayish green conical stems, ribs consisting of a hump with small radial spines, and white flowers with a reddish throat (Charles, 2009, Kiesling and Ferrari, 2009)

The altitudinal gradient is the main factor that influences vegetation patterns in mountain environments. As altitude increases, temperature decreases and solar radiation becomes more intense. However, the effects of precipitation are variable, depending on the different mountain ranges (Körner, 2007). These environmental changes condition the presence of different species along the gradient (Cabido *et al.*, 2010), although many species have wide distribution ranges (Knight and Ackerly, 2002, Bauk *et al.*, 2015). Climate change will lead to changes in distribution range of many species and, therefore, to the loss of genetic diversity within each species. When addressing the impacts of climate change on biological diversity, each species is considered as one unit in most studies, neglecting intraspecific genetic variations (Thuiller *et al.*, 2008). Maintaining genetic diversity within a species is crucial for adaptation in the short- and long-term (Jump *et al.*, 2009).

Genome size and ploidy level, two important variables that determine genetic diversity, have been related to ecological characteristics (Knight and Ackerly, 2002; Ramsey and Ramsey, 2014). Genome size (C-value) is a feature that may change between populations, varying between 0.05 and 127.4 pg in Angiosperms; however, this variation is not necessarily related to ploidy level (Bennett and Leitch, 2005). Genome size has been related to different features, such as minimum cell generation time, life history, plant phenology, and some important parameters for plant breeders, such as frost resistance, biomass production, ecological adaptations, cellular cycle time, and DNA synthesis, all of which could affect plant growth rate (Ohri, 1998; Burton and Husband, 2001). Some genetic traits, like DNA content and ploidy level, may vary along environmental gradients (Knight and Ackerly, 2002). Numerous studies have related

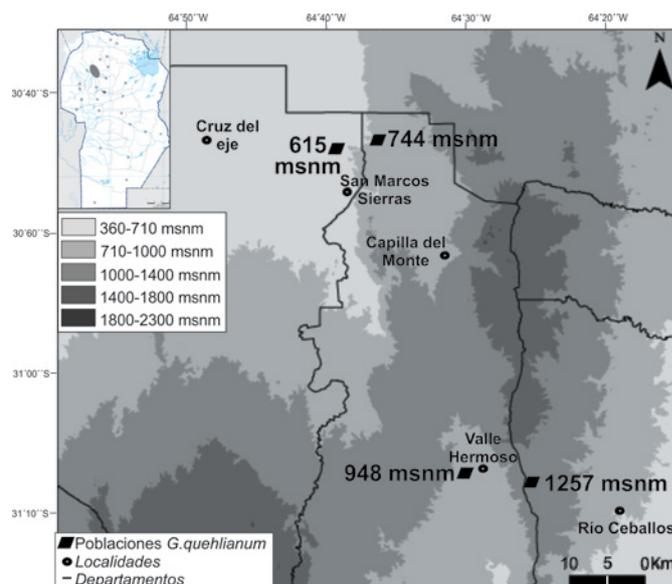


Figure 1. Location of the four collection sites (altitudinal provenances: 615, 744, 948, 1257 m a.s.l.) of *G. quehlianum* along an altitudinal gradient in Córdoba Mountains (Córdoba province, Argentina). Map obtained with DIVA-GIS 2 (Hijmans *et al.* 2002).

C-value changes to morphological characters, habitat and distribution (Bennett, 1976; Knight and Ackerly, 2002; Bennett and Leitch, 2005; Slovák *et al.*, 2009). However, the functional significance of this variation and the mechanisms of these changes are diverse. When comparing species, Knight and Ackerly (2002) found that those having a higher 2C content were more frequent at intermediate latitudes and altitudes. Cx values, representing the DNA content of one non-replicated monoploid genome with the chromosome number x (Greilhuber *et al.* 2005) There are few cacti examined to date, presenting a range of Cx- values from 1.53 to 8.94 pg (Palomino *et al.*, 1999; Zonneveld *et al.*, 2005; Del Angel *et al.*, 2006; Negron-Ortiz, 2007; Las Peñas *et al.*, 2014, 2017; Bauk *et al.*, 2016).

Eupolyploidy refers to the possession of three or more complete sets of chromosomes representing the haploid genome (Ramsey and Schemske, 1998; Soltis *et al.*, 2003). Ploidy level has played significant roles in diversification and speciation processes in flowering plants (Stebbins, 1971; Grant, 1981; Leitch and Bennett, 1997; Levin, 2002; Coghlan *et al.*, 2005; Leitch and Leitch, 2008; Soltis *et al.*, 2009; Soto-Trejo *et al.*, 2013). Chromosome counts have been performed only in a few of the 1,400 species of Cactaceae (of which 26.2% are diploid, 13.4% are both diploid and polyploid, and 60.4% are polyploid), confirming that the frequency of genome duplication in the group is far more common than diploidy (Pinkava, 2002; Las Peñas *et al.*, 2019).

The cytogenetic data available for *G. quehlianum* indicate that it is diploid ($2n=22$) and its DNA content

Table 1. Content DNA and cytogenetic characteristic in four populations of *G. quehlianum*. Voucher data (all from Argentina, Córdoba province), 2C (diploid DNA amount), Cx (basic DNA amount), 2n (somatic chromosome number), FK (karyotype formulae), TLH (mean total haploid chromosome length), C (mean chromosome length), A₁ (intrachromosomal asymmetry index), A₂ (interchromosomal asymmetry index), FISH (fluorescent *in situ* hybridization, 45S: number of rDNA 18–5.8–26S loci, 5S: number of rDNA 5S loci).

Population	Voucher specimens	Content DNA (pg)					2n	KF	TLH (µm)	C (µm)	A ₁	A ₂	FISH	
		2C	4C	8C	Cx	45S							5S	
<i>G. quehlianum</i>														
San Marcos Sierras (615 msnm)	Las Peñas <i>et al.</i> 835	4.3	9.12	17.52	2.15	22	10 m*+1sm	29.02	2.64	0.19	0.13	2	4	
Capilla del monte (744 msnm)	Las Peñas <i>et al.</i> 836	3.84	7.78	15.63	1.92	22	10 m*+1sm	45.26	4.11	0.19	0.18	-	-	
Valle Hermoso (948 msnm)	Las Peñas <i>et al.</i> 837	3.89	7.77	15.43	1.94	22	10 m*+1sm	34.65	3.15	0.2	0.16	2	4	
Camino del Cuadrado (1257 msnm)	Las Peñas <i>et al.</i> 838	3.55	7.23	14.68	1.78	22	10 m*+1sm	34.65	3.15	0.19	0.14	2	4	

is 2C=6.46 pg (Das and Das, 1998). Ploidy level is an important characteristic that may be affecting speciation and patterns of species diversity. Chromosome number of a species may vary with altitude (Grant, 1981; Levin, 2002; Morales Nieto *et al.*, 2007). The main aim of this work was to explore possible relationships between altitudinal range, cytogenetic characteristics, and DNA content in *G. quehlianum*.

MATERIALS AND METHODS

Species and study area

Gymnocalycium quehlianum occurs in rocky outcrops from 600 to 1200 m a.s.l. (Demaio *et al.*, 2011; Gurvich *et al.*, 2014). We studied four populations from Córdoba (Argentina) located along an altitudinal gradient between the localities of San Marcos Sierra (31° 28' S, 64° 34' W) and Camino del Cuadrado (31° 41' S, 64° 50' W), at 615, 744, 948, and 1257, m a.s.l. (Figure 1). Vegetation varies from subtropical dry forest to temperate grasslands at the extreme sites (Giorgis *et al.*, 2011).

Collection data of the studied populations are presented in Table 1. 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 in the Experimental Garden of Museo Botánico (Córdoba, Argentina) to obtain adventitious roots.

Nuclear DNA content analyses

The amount of DNA was measured by flow cytometry in three individuals per population and three runs per individual. DNA content was measured by obtaining nuclear suspensions, according to Doležal *et al.*, (2007), with minor modifications. Small pieces of fresh leaves from each sample individual and from *Zea mays* L. CE-777 (2C = 5.43 pg), which was used as internal 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 5 % PVP (PVP 40, Sigma-Aldrich). Nuclear suspensions were then filtered through a 45µm mesh nylon membrane and maintained at room temperature for 10–60 min. After that, 0.5 mL of Otto II buffer (0.4 M Na₂HPO₄·12 H₂O), 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 Bd Accuri™ C6 Flow Cytometer equipped with a 488 nm and a 633 nm Laser. Three DNA estimations were made for each plant (5,000 or 10,000 nuclei per analysis) on three days. Nuclear DNA content was calculated as (Sample peak mean/Standard peak mean) *2C DNA content of the standard (in pg). Cx values, were calculated as the 2C nuclear DNA amount divided by ploidy level (Greibhuber *et al.*, 2005).

1.1. Cytogenetic analyses

Metaphase chromosomes were prepared from adventitious root tips pretreated with 2 mM 8-hydroxyquinoline for 24 h at 4°C and fixed in 3:1 ethanol:acetic acid. For slide preparation, root tips were washed twice in distilled water (10 min each), digested with a pectinex solution for 45 min at 37°C, and squashed in a drop of 45% acetic acid. The coverslip was removed in liquid nitrogen and then the slides were stored at -20°C.

Karyotype analysis. Slide preparations were stained with Giemsa and permanent mounts were made with Entellan® (Merck, Germany). Ten metaphases of different individuals per population were photographed with a phase contrast optic Olympus BX61 with software Cytovision® (Leica Biosystems) and camera JAI® model CV-M4+ CL monochromatic. The following measurements were taken: length of the short arm (s) and long arm (l), and total chromosome length (c) for each pair. The arm ratio (r = l/s) was calculated and used to classify chromosomes and determine homologs, according

to Levan *et al.*, (1964). Karyograms were constructed by organizing the chromosomes into groups according to their arm ratio and ordering them by decreasing length within each category. The resulting idiograms were based on the mean values obtained from the measurements of all individuals of each population. Karyotype asymmetry was estimated using the intrachromosomal ($A_1 = 1 - [P(b/B)/n]$) 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.

Chromosome banding. Slides for fluorescent banding were stained with a drop of 0.5 mg/ml Chromomycin A₃ (CMA) in McIlvaine's buffer, pH 7.0, and distilled water (1:1) containing 2.5 mM MgCl for 90 min, subsequently stained with 2 µg/ml 4'-6-diamidino-2-phenylindole (DAPI) (both Sigma-Aldrich, Austria) for 30 min, and finally mounted in McIlvaine's buffer-glycerol v/v 1:1 (Schweizer, 1976; Schweizer and Ambros, 1994). The relative lengths of short and long chromosome arms (data not shown) and bands were calculated (considering haploid karyotype length = 100%) in five metaphases per population, each from a different individual. The amount of heterochromatin was expressed as percentage of the total length of the haploid karyotype.

Fluorescent *in situ* hybridization (FISH). The protocol of Schwarzacher and Heslop-Harrison (2000) was used with the pTa71 probe to identify the 18S-5.8S-26S rDNA loci (Gerlach and Bedbrook, 1979) labeled with biotin-14-dUTP by nick translation (Bionick, Invitrogen) and subsequently detected with avidin-FITC (Sigma). For analysis of the 5S rDNA loci, a specific probe from *Pereskia aculeata* was used (Las Peñas *et al.*, 2011). These fragments were labeled with Digoxigenin-11-dUTP (DIG Nick translation mix, Roche) and detected with Anti-DIG-Rhodamine (Roche, USA). The slides were mounted with Vectashield antifade (Vector Laboratories) containing DAPI.

1.2. Statistical analyses

The analysis of variance or its nonparametric equivalent Kruskal Wallis was used for the analysis of DNA content and cytogenetic variables, followed by a comparison of means (Tukey, $p < 0.05$). The analyses were performed using the INFOSTAT statistical package (Di Rienzo *et al.*, 2012).

RESULTS

Genome size of *G. quehlianum* varied between 3.55 pg and 4.30 pg in the four populations, with no significant differences ($p = 0.11$) among them (Table 1). However, a

trend of DNA content decrease with increasing altitude was observed. In addition, endopolyploidy with peaks for 4C and 8C (Table 1) was observed in all the analyzed populations.

Regarding the cytogenetic characteristics, all the populations presented $2n = 22$ (Table 1; Figure 2). No differences were found in the 10 m and 1 sm karyotype formula; the first m pair had a terminal satellite on the short arms, which was detected in 75% of the examined cells (Figure 2).

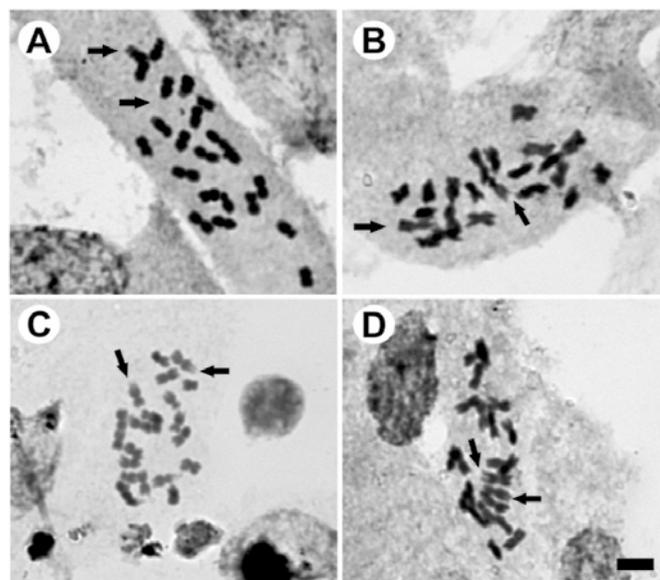


Figure 2. Somatic metaphases of *G. quehlianum* with Giemsa staining. **A** San Marcos Sierra (615 m a.s.l.), **B** Capilla del Monte Km 101 (744 m a.s.l.), **C** Valle Hermoso (948 m a.s.l.), **D** Camino del Cuadrado (1257 m a.s.l.). Arrows indicate satellites. Bar = 5 µm.

The average chromosomal length was 3.26 µm. The highest chromosome length value (5.50 µm) was found in pair 1 from Capilla del Monte (744 m a.s.l.), and the lowest value (2.27 µm) was found in pair 10 from Valle Hermoso (948 m a.s.l.). Statistically significant differences were found in C values ($p < 0.0001$), with the shortest chromosomes (2.64 µm) being detected in the population from San Marcos Sierra (615 m a.s.l.), and the longest ones (4.11 µm), at Capilla del Monte (744 m a.s.l.). The length of the haploid genome (TLH) varied between 29.02 µm (615 m a.s.l.) and 45.26 µm (744 m a.s.l.). No significant differences in intra- and inter-chromosomal asymmetry indices (A_1 and A_2), respectively, were observed (Table 1).

The populations of *G. quehlianum* at 615, 948, and 1257 m a.s.l were analyzed with the banding technique and FISH. All of them presented constitutive heterochromatin bands CMA⁺/DAPI⁻ associated with nucleolar

organizing regions (NORs) in the first pair of *m* chromosomes (Figure 3). On the other hand, FISH showed that the probe for 18-5.8-26S ribosomal genes hybridized in the terminal regions of the first *m* pair, coinciding with the CMA⁺/DAPI⁻ bands described above (Figure 3). The 5S locus was located in two chromosome pairs: in the first *m* pair below the 18-5.8-26S gene and in the last *m* pair in a paracentromeric position (Figure 3, 4). Both genes had similar sizes, being homomorphic. No differences between populations were observed when using either the banding or the FISH techniques (Figure 4).

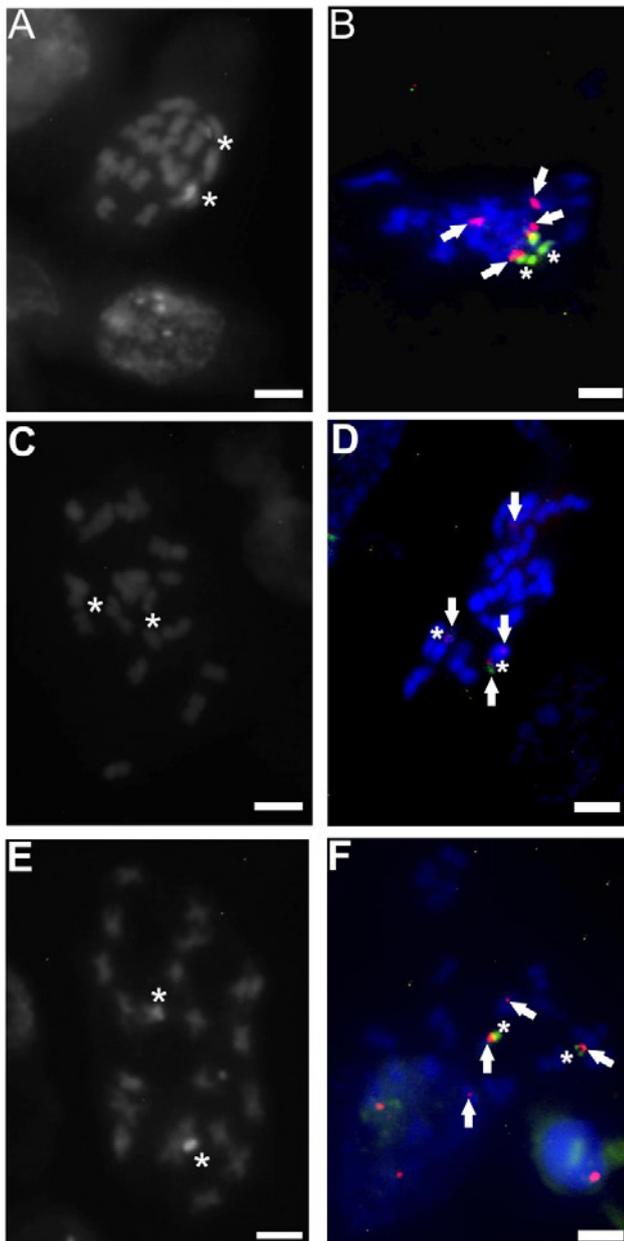


Figure 3. Populations of *G. quehlianum*: **A** and **B**. San Marcos Sierras (615 m a.s.l.); **C** and **D** Valle Hermoso (948 m a.s.l.); **E** and **F**: Camino del Cuadrado (1257 m a.s.l.). **A**, **C** and **E** Fluorochrome chromosome banding CMA/DAPI; **B**, **D** and **F**. FISH using 18-5.8-26S (green) and 5S rDNA (red) probes. Arrows: rDNA 18-5.8-26S and CMA⁺/DAPI⁻/NORs, Asterisks: rDNA 5S. Bar= 5 μm.

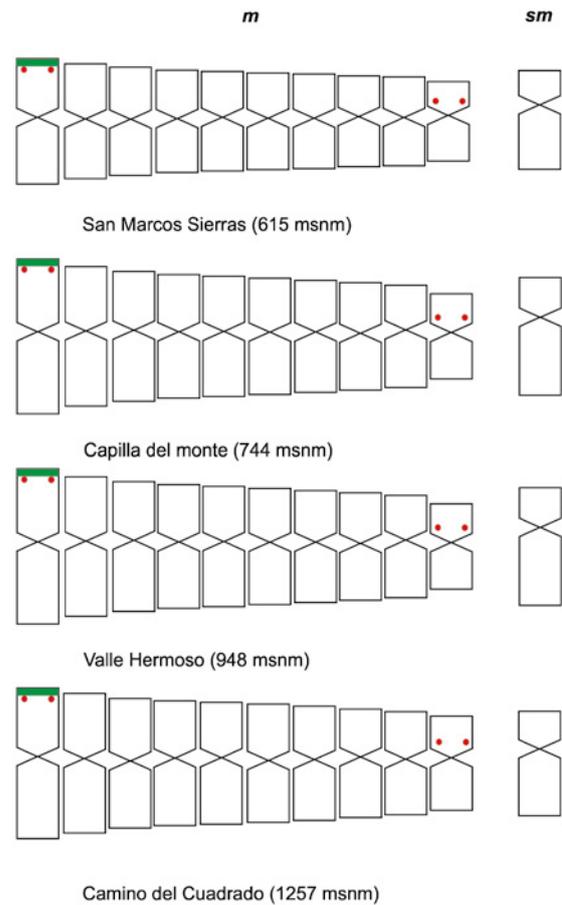


Figure 4. Idiograms with physical location of repetitive segments in *G. quehlianum* rDNA 18S-5.8S-26S /CMA⁺/DAPI⁻ /NORs (green), and 5S rDNA (red). Bar = 2 μm.

DISCUSSION

The DNA content has been related to ecological features along altitudinal gradients in some Angiosperms (Knight and Ackerly, 2002; Šmarda and Bureš, 2006). In previous data on DNA content in *G. quehlianum*, Das and Das (1998) obtained a value of 6.46 pg for 2C using the Feulgen densitometry method. This value is higher than the one obtained in this study, which yielded an average of 3.86 pg among the four populations. These differences in the results could be due to the use of different methodological techniques to obtain the values of DNA content. The Feulgen densitometry method may not be as precise as flow cytometry (Dolezer and Bartos, 2005).

Species of several genera of New Zealand grasslands (*Agrostis*, *Festuca*, *Poa*, *Puccinellia*) with the highest C values were found in extreme environments, such as the sub-Antarctic region (Murray *et al.*, 2005). Knight and Ackerly (2002) analyzed the correlation between nuclear DNA content and environmental gradients in

different species of California, USA. Those with a higher content were more frequent in intermediate locations of the gradients, with lower contents being found at both extremes. In *G. quehlianum*, a decrease in DNA content values with increasing altitude was observed (4.30 pg at 615 m a.s.l., and 3.55 pg at 1257 m a.s.l.). Non significant statistical differences were found in the DNA content values among populations. Furthermore, in the analysis of the DNA content of all populations, a mixture of nuclei with three peaks, 2C, 4C and 8C, was observed, which suggests endopolyploidy. This process is considered an important mechanism of adaptation to high temperatures and water scarcity (Nagl, 1978; Negrón-Ortiz, 2007; Leitch and Leitch, 2013). This pattern occurs in species with a small genome, which also gives them advantages in arid environments (Palomino *et al.*, 1999; Del Angel *et al.*, 2006). In Cactaceae, endopolyploidy was found in the subfamily Opuntioideae (Negrón-Ortiz, 2007; Segura *et al.*, 2007) as well as in some species of Cactoideae (Palomino *et al.*, 1999; Del Angel *et al.*, 2006; Bauk *et al.*, 2016). However, it has not been observed in *Gymnocalycium*. The processes of endoduplication of the genome can vary among individuals of the same species in response to the effects of different environmental conditions (Barow, 2006; Leitch and Leitch, 2013).

The most common basic chromosome number in Cactaceae is $x=11$ (Pinkava, 2002; Goldblatt and Johnson, 2006; Das and Mohanty, 2006; Las Peñas *et al.*, 2009, 2014). In all populations, the chromosome number of *G. quehlianum* was $2n=2x=22$, except for one individual of the population at 948 m a.s.l., indicating that a greater number of individuals should be analyzed in each population in order to explore whether the different chromosome numbers are associated with the altitudinal gradient. The chromosome numbers reported here coincide with meiotic studies that included the determination of pollen stem cells (Das and Das, 1998). Furthermore, no significant differences in the cytogenetic variables were detected among populations. Thus, *G. quehlianum* chromosomes had an average length of 2.98 μm and a symmetric karyotype, with most of the chromosomal pairs being metacentric. These characteristics coincide with the karyotypic homogeneity presented for the family (Cota and Philbrick, 1994; Cota and Wallace, 1995; Das *et al.*, 2000; Das and Mohanty, 2006; Las Peñas *et al.*, 2008, 2009, 2014).

Results of the CMA/DAPI chromosome banding and FISH techniques show that *G. quehlianum* presented the 18-5.8-26S loci in the terminal regions of the first pair of metacentric chromosomes, coinciding with the CMA⁺/DAPI⁻ band pattern. No differences among populations were observed along the altitudinal gradient. The location of this gene is highly conserved in Cactaceae (Las Peñas *et al.*, 2009, 2014, 2017). On the other hand, the 5S probe hybridized in the first *m* pair next to the

18-5.8-26S, and in the smallest *m* pair. In most of Cactoideae, a pair of 18-5.8-26S and 5S loci is present in a haploid genome (Bauk *et al.*, 2016; Las Peñas *et al.*, 2016, 2017). In the genus *Pfeiffera*, the 5S gene is duplicated in a chromosome different from the one carrying the 18-5.8-26S gene (Moreno *et al.*, 2015), as it was found in this work. The 5S rDNA unit is located independently of other rDNA sequences, which can be related to the fact that it is transcribed by RNA polymerase III, whereas the polycistronic 45S rDNA uses RNA polymerase I (García and Kovařík, 2013; Brasileiro-Vidal *et al.*, 2007).

In most Angiosperms, the 18-5.8-26S and 5S sites are found in different chromosomes (Roa and Guerra 2015). However, in this work, the 5S locus was located adjacent to the 18-5.8-26S gene and in the last *m* pair.

The dispersion of 5S sites here reported for *G. quehlianum* may be attributed to several factors, including structural chromosomal rearrangements, such as translocations (Hayashi *et al.*, 2001); dispersion of rDNA repeats; and amplification of new minor loci and deletion or not of original major loci (Pedrosa-Harand *et al.*, 2006). The latter two factors were proposed as the mechanisms by which 5S rDNA loci repeatedly changed position during the radiation of species, without changing the co-linearity of other markers. These position changes can be mediated by mobile elements (Kalendar *et al.*, 2008; Raskina *et al.*, 2008).

This work makes one of the first contributions of information about DNA content and cytogenetic characteristics (karyotype, heterochromatin distribution and position of ribosomal genes) of *G. quehlianum* along an altitudinal gradient. Furthermore, we previously reported greater variation in ecological characters (Martino *et al.*, 2021) than in cytogenetic ones among the four populations; this result may be attributed to the great phenotypic plasticity of morphological traits in response to environmental differences of this species. At the same time, our results indicate that changes in DNA content as well as cytogenetic changes are due to cryptic chromosome rearrangements for the species adaptation to the altitudinal gradient.

Climate change is affecting organisms throughout the world. Therefore, understanding the relationships between species characteristics and environment would help predict species responses to climate change (Gurvich *et al.*, 2002; Aragón-Gastélum *et al.*, 2014). Species with wide distributions along climate gradients would be less affected by climate than species with more limited distribution, such as *G. quehlianum*. In order to conserve this species, it is important to know its genetic characteristics all across its distribution range. Further research of these aspects is necessary to predict species responses to environmental changes.

ACKNOWLEDGEMENTS

This work was supported Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCyT, PICT 2016-0077), SECyT- FCEfYn-UNC (Universidad Nacional de Córdoba, Argentina) for financial support. and the Research Committee of the Cactus and Succulent Society of America. D. E. Gurvich and M. L. Las Peñas are researches at IMBIV-UNC, CONICET. Sergio García provided some samples. Roberto Kiesling kindly identified the vouchers.

AUTHOR CONTRIBUTIONS

Design of the research: Martino P.; Gurvich G.; Las Peñas M. L.; Performance of the research: Martino P.; Gurvich G.; Las Peñas M. L.

BIBLIOGRAPHY

- Aragón-Gastélum J.L., Flores J., Yáñez-Espinosa L., Badano E., Ramírez-Tobías H.M., Rodas-Ortiz J.P., González-Salvatierra C. (2014) Induced climate change impairs photosynthetic performance in *Echinocactus platyacanthus*, an especially protected Mexican cactus species. *Flora-Morphology, Distribution, Functional Ecology of Plants*. 209(9): 499-503.
- Barow M. (2006) Endopolyploidy in seed plants. *BioEssays*. 28: 271-281.
- Bauk K., Zeballos S.R., Las Peñas M.L., Flores J., Gurvich D.E. (2015) Are seed mass and seedling size and shape related to altitude? Evidence in *Gymnocalycium monvillei* (Cactaceae). *Botany*. 93: 1-5.
- Bauk K., Santiñaque F., Lopez-Carro B., Las Peñas M.L. (2016) Contenido de ADN y patrón de genes ribosomales en el género monotípico *Stetsonia* (Cactaceae). *Bol. Soc. Argent. Bot.* 51: 323-330.
- Bennett M.D., Smith J.B. (1976) Nuclear DNA amounts in angiosperms. *Philos Trans R Soc Lond B Biol Sci*. 274: 227-276.
- Bennett M.D., Leitch I.J. (2005). Genome size evolution in plants. In: Gregory TR, ed. *The evolution of the genome*. San Diego, CA, USA: Elsevier.
- Brasileiro-Vidal A.C., Dos Santos-Serejo J.A., Soares Filho W.D.S., Guerra M. (2007) A simple chromosomal marker can reliably distinguish *Poncirus* from *Citrus* species. *Genetica*. 129: 273-279.
- Burton T.L., Husband B.C. (2001) Fecundity and offspring ploidy in matings among diploid, triploid and tetraploid *Chamerion angustifolium* (Onagraceae): consequences for tetraploid establishment. *Heredity*. 87: 573-579.
- Cabido M., Giorgis M., Tourn M. (2010) Guía para una excursión botánica en las Sierras de Córdoba. *Bol. Soc. Argent. Bot.* 45: 209-219.
- Charles G. (ed.) (2009) *Gymnocalycium* in habitat and culture. Charles, Bank, Bridge, Stamford, England.
- Coghlan A., Eichler E.E., Oliver S.G., Paterson A.H., Stein L. (2005) Chromosome evolution in eukaryotes: a multi-kingdom perspective. *Trends in Genet.* 21: 673-682.
- Cota J.H., Philbrick C.T. (1994) Chromosome number variation and polyploidy in the genus *Echinocerus* (Cactaceae). *Am. J. Bot.* 81: 1054-1062.
- Cota J.H., Wallace R.S. (1995) Karyotypic studies in the *Echinocerus* (Cactaceae) and their taxonomic significance. *Caryologia*. 48: 105-122.
- Das A.B., Das P. (1998) Nuclear DNA content and chiasma behavior in six species of *Gymnocalycium* Pfeiff. of the family Cactaceae. *Caryologia*. 51: 159-165.
- Das A.B., 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.
- Das A.B., Mohanty S. (2006) Karyotype analysis and in situ nuclear DNA content in seven species of *Echinopsis* Zucc. of the family Cactaceae. *Cytologia*. 71: 79.
- Del Angel C., Palomino G., García A., Méndez I. (2006) Nuclear genome size and karyotype analysis in *Mammillaria* species (Cactaceae). *Caryologia*. 59: 177-186.
- Demaio P.H., Barfuss M.H., Kiesling R., Till W., Chiappella J.O. (2011) Molecular phylogeny of *Gymnocalycium* (Cactaceae): assessment of alternative infrageneric systems, a new subgenus, and trends in the evolution of the genus. *Amer. J. Bot.* 98: 1841-1854.
- Di Rienzo J.A., Casanoves F., Balzarini M.G., González L., Tablada M., Robledo C. W. 2012. InfoStat. version (2012) Universidad Nacional de Córdoba, Argentina: Grupo InfoStat.
- Doležel J., Greilhuber J., Suda J. (2007) Estimation of nuclear DNA content in plants using flow cytometry. *Nat. Protoc.* 2: 2233-2244.
- Doležel J., Bartos J. (2005) Plant DNA flow cytometry and estimation of nuclear genome size. *Ann. Bot.* 95: 99-110.
- García S., Kovařík A. (2013) Dancing together and separate again: gymnosperms exhibit frequent changes of fundamental 5S and 35S rRNA gene (rDNA) organisation. *Heredity*. 11: 23-33.
- Gerlach W.L., Bedbrook J.L. (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acids Res.* 7: 1869-1885.
- Giorgis M.A., Tecco P.A., Cingolani A.M., Renison D., Marcora P., Paiaro V. (2011) Factors associated with woody alien species distribution in a newly invaded mountain system of central Argentina. *Biol. Invas.* 13: 1423-1434.
- Goldblatt P., Johnson E.D. (2006) Index to Plant Chromosome numbers 2001-2003. *Monogr. Syst. Bot. Missouri Bot. Gard.* 106. St. Louis, Missouri.
- Grant V. (1981) *Plant speciation*. New York: Columbia University Press.
- Greilhuber J. (2005) Intraspecific variation in genome size in angiosperms: identifying its existence. *Ann. Bot.* 95: 91-98.
- Gurvich D.E., Zeballos S.R., Demaio P.H. (2014) Diversity and composition of cactus species along an altitudinal gradient in the Sierras del Norte Mountains (Córdoba, Argentina). *S. Afr. J. Bot.* 93: 142-147.
- Gurvich D.E., Diaz S., Falczuk V., Harguindeguy N.P., Cabido M., Thorpe P.C. (2002) Foliar resistance to simulated extreme temperature events in contrasting plant functional and chorological types. *Global Change Biol.* 8: 1139-1145.
- Hayashi M., Miyahara A., Sato S., Kato T., Yoshikawa M., Taketa M., Hayashi M., Pedrosa A., Onda R., Imaizumi-Anraku H., Bachmair A., Sandal N., Stougaard J., Murooka Y., Tabata S., Kawasaki S., Kawaguchi M., Harada K. (2001) Construction of a genetic linkage map of the model legume *Lotus japonicus* using an intraspecific F2 population. *DNA Res* 8: 301-310
- Hijmans, R.J., Guarino, L., Bussink, C., Barrantes, I. & Rojas, E. (2002) DIVA-GIS, version 2. Sistema de información geográfica para el análisis de datos de biodiversidad. Lima: International Potato Center.

- Jump A.S., Mátyás C., Peñuelas J. (2009) The altitude-for-latitude disparity in the range retractions of woody species. *Trends in ecol. Evol.* 24: 694–701.
- Kalendar, R., Tanskanen, J., Chang, W., Antonius, K., Sela, H., Peleg, O., & Schulman, A. H. (2008) Cassandra retrotransposons carry independently transcribed 5S rRNA. *Proc. Nat. Aca. Sci.* 105: 5833–5838.
- Kiesling R., Larrocca L., Faúndez J., Metzging D., Albesiano S. (2008) Cactaceae. In: *Catálogo de las Plantas Vasculares del Cono Sur* (F.O. Zuolaga, O. Morrone, M. J. Belgrano, eds.) *Monogr. Syst. Bot. Missouri Bot. Gar* 107, Saint Louis, EUA. Vol 2, Pp: 1715–1830.
- Kiesling R., Ferrari O. (2009) 100 cactus argentinos. Editorial Albatros, Buenos Aires, Argentina.
- Knight C., Ackerly D. (2002) Variation in nuclear DNA content across environmental gradients: a quantile regression analysis. *Ecol. Letters.* 5: 66–76.
- Körner C. (2007) The use of altitude in ecological research. *Trends. Ecol. Evol.* 22: 569–574.
- Las Peñas M.L., Bernardello G., Kiesling R. (2008) Karyotypes and fluorescent chromosome banding in *Pyrrhocactus* (Cactaceae). *Plant. Syst. Evol.* 272: 211–222.
- Las Peñas M.L., Urdampilleta J.D., Bernardello G., Forni-Martins E.R. (2009) Karyotypes, heterochromatin, and physical mapping of 18S–26S rDNA in Cactaceae. *Cytogenet. Genome Res.* 124: 72–80.
- Las Peñas M.L., Kiesling R., Bernardello G. (2011) Karyotype, Heterochromatin, and physical mapping of 5S and 18–5.8–26S rDNA Genes in *Setiechinopsis* (Cactaceae), an Argentine Endemic Genus. *Haseltonia.* 9: 83–90.
- Las Peñas M.L., Bernardello G., Kiesling R. (2014) Classical and molecular cytogenetics and DNA content in *Maihuenia* and *Pereskia* (Cactaceae). *Plant. Syst. and Evol.* 300: 549–558.
- Las Peñas M.L., Santiñaque F., López-Carro B., Stiefkens L. (2016) Estudios citogenéticos y de contenido de ADN en *Brasiliopuntia schulzii* (Cactaceae). *Gayana Bot.* 73: 414–420.
- Las Peñas M.L., Oakley L., Moreno N.C., Bernardello G. (2017) Taxonomic and cytogenetic studies in *Opuntia* ser. *Armatae* (Cactaceae). *Botany.* 95: 101–120.
- Las Penas M.L., Kiesling R., Bernardello G. (2019) Phylogenetic reconstruction of the genus *Tephrocactus* (Cactaceae) based on molecular, morphological, and cytogenetical data. *Taxon: Taxon* 68: 714–730.
- Leitch I.J., Bennett M.D. (1997) Polyploidy in angiosperms. *Trends Plant Sci.* 2:470–476.
- Leitch A.R., Leitch I.J. (2008) Genomic plasticity and the diversity of polyploid plants. *Science.* 320: 481–483.
- Leitch I.J., Leitch A.R. (2013) Genome size diversity and evolution in land plants. In: I.J. Leitch, J. Greilhuber, J. Dolezel J. Wendel (eds.), *Plant Genome Diversity.* Vol. 2, pp. 307–320. Springer-Verlag, Vienna, Austria.
- Levan A., Fredga K., Sandberg A. (1964) Nomenclature for centromeric position on chromosomes. *Hereditas.* 52: 201–220.
- Levin D.A. (2002) The role of chromosome changes in plant evolution. Oxford University Press, New York.
- Martino P.A., Las Peñas M.L. y Gurvich D.E. (2021) Asociaciones entre las características reproductivas y la abundancia en *Gymnocalycium quehlianum* (Cactaceae) a lo largo de un gradiente altitudinal. *Ciencias Botánicas,* 99: 514–524.
- Morales Nieto C.R., Quero Carrillo A.R., Avendaño Arrazate C.H. (2007) Caracterización de la diversidad nativa del zacate banderita [*Bouteloua curtipendula* (Michx.) Torr.], mediante su nivel de ploidía. *Téc Pecu Méx.* 45: 263–278.
- Moreno N.C., Amarilla L.D., Las Peñas M.L., Bernardello G. (2015) Molecular cytogenetic insights into the evolution of the epiphytic genus *Lepismium* (Cactaceae) and related genera. *Bot. J. Linn. Soc.* 177: 263–277.
- Murray B., De Lange P.J., Ferguson A.R. (2005) Nuclear DNA variation, chromosome numbers and polyploidy in the endemic and indigenous grass flora of New Zealand. *Ann. Bot.* 96: 1293–1305.
- Nagl W. (1978) Endopolyploidy and polyteny in differentiation and evolution: towards an understanding of quantitative and qualitative variation of nuclear DNA in ontogeny and phylogeny. North-Holland Publishing Company, Amsterdam.
- Negron-Ortiz V. (2007) Chromosome numbers, nuclear DNA content and polyploidy in *Consolea* (Cactaceae), an endemic cactus of the Caribbean Islands. *Am. J. Bot.* 94: 1360–1370.
- Ohri D. (1998) Genome size variation and plant systematics. *Ann. Bot.* 82: 75–83.
- Palomino G., Doležel J., Cid R., Brunner I., Méndez I., Rubluc A. (1999) Nuclear genome stability of *Mammillaria san-angelensis* (Cactaceae) regenerants induced by auxins in long term in vitro culture. *Plant Science.* 141: 191–200.
- Pedrosa-Harand A., De Almeida C.S., Mosiolek M., Blair M.W., Schweizer D., Guerra M. (2006) Extensive ribosomal DNA amplification during Andean common bean (*Phaseolus vulgaris* L.) evolution. *Theor. Appl. Genet.* 112: 924–933.
- Pinkava D.J. (2002) On the evolution of the North American Opuntioideae. In: Hunt D. N. Taylor (eds.), *Studies in the Opuntioideae,* pp. 78–99. Royal Botanic Gardens, Kew.
- Ramsey J., Ramsey T.S. (2014) Ecological studies of polyploidy in the 100 years following its discovery. *Phil. Trans. R. Soc. B.* 369: 20130352.
- Ramsey J., Schemske D.W. (1998) Pathways, mechanisms, and rates of polyploidy formation in flowering plants. *Ann. Rev. Ecol. Syst.* 29: 467–501.
- Raskina, O., Barber, J. C., Nevo, E., & Belyayev, A. (2008) Repetitive DNA and chromosomal rearrangements: speciation-related events in plant genomes. *Cytog. Gen. Res.* 120: 351–357.
- Roa F., Guerra M. (2015) Non-random distribution of 5S rDNA sites and its association with 45S rDNA in plant chromosomes. *Cytogen. Gen. Res.* 146: 243–249.
- Schütz B. (1986) Monografie rodu *Gymnocalycium*. KK Astrophytum Brno.
- Schwarzacher T., Heslop-Harrison P. (2000) Practical *in situ* hybridization. Bios Scientific Publishers Limited, Oxford.
- Schweizer D., Ambros P. (1994) Chromosome banding. In: J.R. Gosden (ed.), *Methods in Molecular Biology. Chromosome analysis protocols,* pp. 97–112 Humana Press, Totowa, United States of America.
- Schweizer D. (1976) Reverse fluorescent chromosome banding with chromomycin and DAPI. *Chromosoma.* 58: 307–324.
- Segura S., Scheinvar L., Olalde G., Leblanc O., Filardo S., Muratalla A., Gallegos C., Flores S. (2007) Genome sizes and ploidy levels in Mexican cactus pear species *Opuntia* (Tourn.) Mill. Series *Streptacanthae* Britton et Rose, *Leucotrichae* DC., *Heliabravoanae* Scheinvar and *Robustae* Britton et Rose. *Genet. Res. Crop Evol.* 54: 1033–1041.
- Slovák M., Vít P., Urfus T., Suda J. (2009) Complex pattern of genome size variation in a polymorphic member of the Asteraceae. *J. Biogeo.* 36: 372–384.
- Šmarda P., Bureš P. (2006) Intraspecific DNA content variability in *Festuca pallens* on different geographical scales and ploidy levels. *Ann. Bot.* 98: 665–678.
- Soltis D.E., Soltis P.S., Bennett M.D., Leitch I.J. (2003) Evolution of genome size in the angiosperms. *Am J Bot.* 90: 1596–1603.
- Soltis D.E., Albert V.A., Leebens Mack J., Bell D., Paterson A.H., Zheng C., Sankoff D., De Pamphilis C.W., Wall P.K., Soltis P.S. (2009) Polyploidy and angiosperm diversification. *Am J Bot.* 96: 336–348.

Soto-Trejo, F. Palomino G., Villaseñor J.L., Crawford D.J. (2013) Polyploidy in Asteraceae of the xerophytic scrub of the ecological reserve of the Pedregal of San Angel. México City. Bot. J. Linn. Soc. 173:211–229.

Stebbins G.L. (1971) Chromosomal evolution in higher plants. London: Edward Arnold.

Thuiller W., Albert C., Araujo M.B., Berry P.M., Cabeza M., Guisan A., Sykes M.T. (2008) Predicting global change impacts on plant species' distributions: future challenges. Perspec. Plant Ecol. Evol. Syst. 9: 137-152.

Zonneveld B.J.M., Leitch I.J., Bennett M.D. (2005) First nuclear DNA amounts in more than 300 angiosperms. Ann. Bot. 96: 229-244.

—