ISSN 0373-580 X

Bol. Soc. Argent. Bot. 35 (3-4): 237 - 244. 2000

KARYOTYPES AND DNA CONTENT IN DIPLOID AND POLYPLOID *LYCIUM* (SOLANACEAE)*

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Summary: Karyotypes and nuclear DNA content of *Lycium* species from Argentina and Chile are analyzed, being the first report for the genus. *Lycium elongatum*, *L. infaustum*, and *L. chilense* var. *vergarae* and var. *minutifolium* are diploid (2n=24), whereas *L. chilense* var. *chilense*, var. *confertifolium*, and var. *descolei* are tetraploid (2n=48). For the diploid taxa, karyotypes, total chromosome length, and mean chromosome lengths and ratios were obtained. All these taxa have identical karyotype formula (11 *m* pairs + 1 *sm* pair). The range of DNA content in the diploids was 3.22-3.84 pg, while in the tetraploids was 6.50-6.60 pg, i.e. about twice the amount of the diploids. Significant differences were detected between diploid and tetraploid taxa, and among the diploids, but tetraploids showed no significant differences among them. Because of the constancy of karyotypic features, it was not possible to determine relationships between karyotypes and DNA content variations. Speciation at the diploid level in *Lycium* has not produced great differences in DNA content per basic genome and in the karyotype formula.

Key words: *Lycium*, Solanaceae, karyotype, DNA content, polyploidy.

Resumen: Cariotipos y contenido de ADN en *Lycium* diploides y poliploides (Solanaceae). Se analizan los cariotipos y el contenido de ADN nuclear de especies argentinas y chilenas de *Lycium*, siendo el primer informe para el género. *Lycium elongatum*, *L. infaustum*, *L. chilense* var. *vergarae* y var. *minutifolium* son diploides (2n= 48), mientras que *L. chilense* var. *chilense*, var. *confertifolium*, y var. *descolei* son tetraploides (2n=48). Para los diploides se obtuvieron cariotipos, largo cromosómico total y longitud cromosómica e índice braquial promedios. Todas estas entidades tienen idéntica fórmula cariotípica (11 pares *m* + 1 par *sm*). El rango observado de contenido de ADN en los taxa diploides fue 3.22-3.84 pg, mientras que en los tetraploides fue 6.50-6.60 pg, o sea alrededor del doble de la cantidad de los diploides. Se detectaron diferencias significativas entre los diploides y los tetraploides, y entre los diploides entre sí, pero los tetraploides no mostraron diferencias entre ellos. Debido a la constancia cariotípica, no se pudieron determinar relaciones entre cariotipos y contenido de ADN. La especiación a nivel diploide en *Lycium* no ha producido grandes diferencias en los cariotipos ni en el contenido de ADN por genoma básico.

Palabras clave: *Lycium*, Solanaceae, cariotipos, contenido de ADN, poliploidía.

INTRODUCTION

The cosmopolitan genus *Lycium* L. contains ca. 75 shrubby species that mainly grow in arid or semiarid environments (Bernardello, 1986a). It is included in Tribe Lycieae Hunz., subfam. Solanoideae, considered monophyletic and derived (Olmstead & Palmer, 1992). Within this tribe, composed only by three woody genera (Hunziker, 1979), *Lycium* is regarded as primitive and older (Bernardello, 1987; Bernardello & Chiang-Cabrera, 1998). The American continent has the highest concentration of species with two centers of diversification: Arizona (U.S.A.) in the North and Argentina in the South (Hitchcock, 1932; Bernardello & Chiang-Cabrera, 1998). The morphological variation in the genus is extensive, with a wide range of variation in sizes, forms, and colors in the flowers (Chiang, 1981; Bernardello, 1986a; Bernardello & Chiang-Cabrera, 1998).

There are several articles on American representatives concerning different aspects of these plants, like anatomy and morphology, embryology, reproductive biology, and systematics (e.g., Bernardello, 1983a, b, 1986a, b, 1987; Bernardello & Bonzani, 1991; Bernardello & Leiva-González, 1993; Bernardello & Chiang-Cabrera, 1998). However, their cytological knowledge is meager. Data available indicate that most of the species are diploid with n=x=12 (cf. Bernardello, 1982; Chiang, 1982; Hunziker *et al.*, 1985; Chiang *et al.*, 1989; Stiefkens & Bernardello, 1996), although a few

* Dedicated to Prof. Dr. Juan H. Hunziker on the ocassion of his 75th anniversary.

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polyploid taxa have been reported (cf. Bernardello, 1982; Chiang, 1982). Most cytological studies in the genus report chromosome numbers based on the analysis of meiotic material (cf. Bernardello, 1982; Chiang, 1982), whereas there are few karyotypic studies of South American taxa (Bernardello *et al.*, 1995; Stiefkens & Bernardello, 1996). These articles have pointed out a high constancy in the karyotype of the seven species studied.

Among flowering plants, there is a wide range of variation in nuclear DNA content (cf. Bennett & Leitch, 1995, 1997). Bennett (1976, 1987) has suggested that interspecific variation in DNA content has adaptive significance and is correlated with the environment and the geographical distribution. Within the Solanaceae, this kind of studies include a few genera: *Capsicum*, *Cyphomandra*, *Hyosciamus*, *Lycopersicon*, *Nicotiana*, *Petunia*, *Solanum*, and *Withania* (cf. Bennett & Leitch, 1995, 1997; Belletti *et al.*, 1998; Bennett *et al.*, 1998) and no data are available regarding *Lycium* and tribe Lycieae. In this paper, we report and compare DNA contents, somatic chromosome numbers, and karyotypes of the diploid taxa in three species and five varieties of *Lycium* from Argentina and Chile, for a better understanding of the systematic and evolutionary relationships within the genus. The species studied are included in sect. Schistocalyx (*L. chilense*) and sect. Lycium (*L. elongatum*, *L. infaustum*), although the sectional treatment of the genus was recently considered artificial (Bernardello & Chiang-Cabrera, 1998).

Material and Methods

Table 1 includes the taxa studied and its collection data.

Studies of somatic chromosomes were done as follows. Mitosis in root tip cells was studied from squashes from primary roots of germinating seeds. Seeds were soaked for 1-2 days in running water, put in petri dishes on moist filter paper, and stored at room temperature in the dark. Fresh root tips were pretreated for 2 hrs in a saturated solution of paradichloro-benzene in water at room temperature (±20°C), rinsed in distilled water, and fixed in freshly made ethanol:acetic acid (3:1) at room temperature (±20°C) for 12-24 hrs. Then, they were placed in alcoholic acid-carmine (Snow, 1963) for one week. Meristem cells were isolated in a drop of 50% acetic acid on a slide, macerated, squashed, and heated gently. Slides were made permanent in Euparal by means of Bradley's method (1948). At least four cells per individual and 15 per species were examined. Ten metaphases of each species were photographed with phase contrast optics and Kodak Panatomic X film. The photographs were used to take measurements of short arm, long arm, and total chromosome length for each chromosome pair. Centromeric indices and arm ratios were calculated and used to classify the chromosomes after Levan *et al.* (1964). Satellites were classified according to Battaglia (1955). Total haploid chromosome length of the karyotype (tl), based on the mean chromosome lengths for each species, average chromosome length, and average arm ratio were calculated. In each cell, 12 pairs of chromosomes were identified as homologous based on similarity in size and centromere position. Karyograms were constructed by organising the chromosomes into groups according to their arm ratio, ordering them by decreasing length within each category, and numbering them using this same scheme. Idiograms are based on the mean values for each taxon. Karyotype asymmetry was estimated using the indices of Romero Zarco (1986) and Stebbins' classification (1971).

DNA content was measured in telophase nuclei (2C) at the root apex of germinating seeds (Tito *et al.*, 1991). Seeds were germinated and fixed as for the previous method but without pretreatment. Maize flint (*Zea mays* L. spp. *mays*) "opaque 2" line was used as standard to calculate genome size in picograms; its genome size (2C = 6.658 pg) was calibrated according to Bennett & Smith (1976) using *Allium cepa* L.'Ailsa Craig' (Rosato *et al.*, 1997). After fixation, the roots were rinsed 30 minutes in distilled water. Hydrolysis was carried out with 5 N HCl at 20°C. Different times of hydrolysis were tested and the optimum period determined was 40 minutes. After hydrolysis, the roots were rinsed three times with distilled water for 15 minutes. Staining was done with Feulgen at pH 2.2 for 2 hrs in the dark. Then, the material was rinsed three times in SO_2 water for 10 minutes each rinse, then rinsed again with distilled water 10 minutes and squashed in 45% acetic acid. The cover slip was removed after freezing with CO_2 and the material was dehydrated in absolute alcohol, mounted in Euparal, and maintained in the dark until measurements were made. The amount of Feulgen

Table 1. *Lycium* taxa studied. If not specified, they were collected in ARGENTINA. Data include: province (in italics), collection site, collector, and number. Herbarium samples are deposited at CORD. All populations were studied cytologically. An asterisk indicates populations analyzed in DNA content.

L. chilense Miers ex Bertero

var. *confertifolium* (Miers) Barkley: *Río Negro*, Ruta 251 Km 104-105, A. A. Cocucci 441. *San Juan*, Ruta 436, Km 189, Bernardello 837. CHILE, Coquimbo, Ovalle, Bernardello 860*.

var. *chilense*: *Córdoba*, Miramar, Bernardello 757. CHILE, Coquimbo, antes de Rivadavia, Bernardello 845*; Coquimbo, La Serena, Bernardello 865.

var. *descolei* Barkley: *Chubut*, Punta Pardela, Bernardello 785*; Puerto Pirámide, Bernardello 786.

var. *minutifolium* (Miers) Barkley: *La Pampa*, Parque Luro, Bernardello 257*. *Chubut*, Península de Valdéz, Bernardello 252. *Santa Cruz*, Lago Cadriel, A. A. Cocucci 450.

var. *vergarae* (Phil.) Bernardello: *San Juan*, Arrequintín, Bernardello 839*.

L. elongatum Miers: *Córdoba*, Tulumba, Bernardello 721*.

L. infaustum Miers: *Córdoba*, Serrezuela, A. T. Hunziker *et al.* 25389*.

staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 nm using the scanning method in a Zeiss Universal Microspectrophotometer (UMSP 30) in the Instituto Fitotécnico Santa Catalina. Sixty nuclei per taxon were measured and data were compared using a *t* test. The differences in DNA content between taxa (varieties and species) were tested through an ANOVA and comparisons between means using the Tukey´s test.

Results and Discussion

Chromosome numbers (Table 2) indicate that *L. elongatum*, *L. infaustum*, and two varieties of *L. chilense* (*vergarae* and *minutifolium*) are diploid with 2n=24 (Fig. 1), whereas the remaining varieties of *L. chilense* studied (*chilense*, *confertifolium*, and *descolei*) are tetraploid with 2n=48 (Fig. 2). Our data on *L. elongatum* and *L. infaustum* agree with an earlier article (Stiefkens & Bernardello, 1996). For *L. chilense* as a species, previous counts indicated n=12, 24 (Bernardello, 1982) without specification of the varieties examined. Thus, all these counts are new.

These findings agree with previous data showing that x=12 is the basic number for the genus and tribe Lycieae (Bernardello, 1982, 1985; Chiang, 1982, 1983), as happens in most genera and tribes in subfam. Solanoideae (cf. Hunziker, 1979; Moscone, 1992).

As diploids and polyploids naturally grow in arid and semiarid environments, no correlation can be drawn between the level of ploidy and aridity, as found in other cases (e.g., Stebbins, 1985; Poggio *et al.*, 1989).

Table 2. *Lycium* taxa studied, ploidy level, somatic chromosome numbers, karyotype formula, total haploid chromosome length (tl) in µm, mean chromosome length (c) in µm, mean arm ratio (r), mean intrachromosomal asymmetry index (A_1), mean interchromosomal asymmetry index (A_2), nuclear DNA content in picograms ($\bar{x} \pm$ standard deviation), and DNA per basic genome in picograms. An asterisk indicates that the first chromosome pair bears a satellite in the short arm. Data on *L. elongatum* and *L. infaustum* are taken from Stiefkens & Bernardello (1996). The letters in the DNA content column indicate the results of the Tukey´s test.

Taxon	Ploidy level	2*n*	Karyotype formula	tl	c	r	A_1	A_2	DNA content (2C)	DNA per basic genome
L. elongatum	2x	24	11 m* + 1 sm	25.01	2.08	1.22	0.16	0.12	3.22 ± 0.24^a	1.61
L. infaustum	2x	24	11 m* + 1 sm	21.52	1.79	1.25	0.18	0.13	3.84 ± 0.22^b	1.92
L. chilense										
var. *minutifolium*	2x	24	11 m* + 1 sm	21.04	1.75	1.20	0.14	0.16	3.68 ± 0.24^c	1.84
var. *vergarae*	2x	24	11 m* + 1 sm	20.94	1.75	1.19	0.14	0.14	3.55 ± 0.16^c	1.77
var. *confertifolium*	4x	48							6.60 ± 0.45^d	1.65
var. *chilense*	4x	48							6.50 ± 0.36^d	1.62
var. *descolei*	4x	48							6.57 ± 0.46^d	1.64

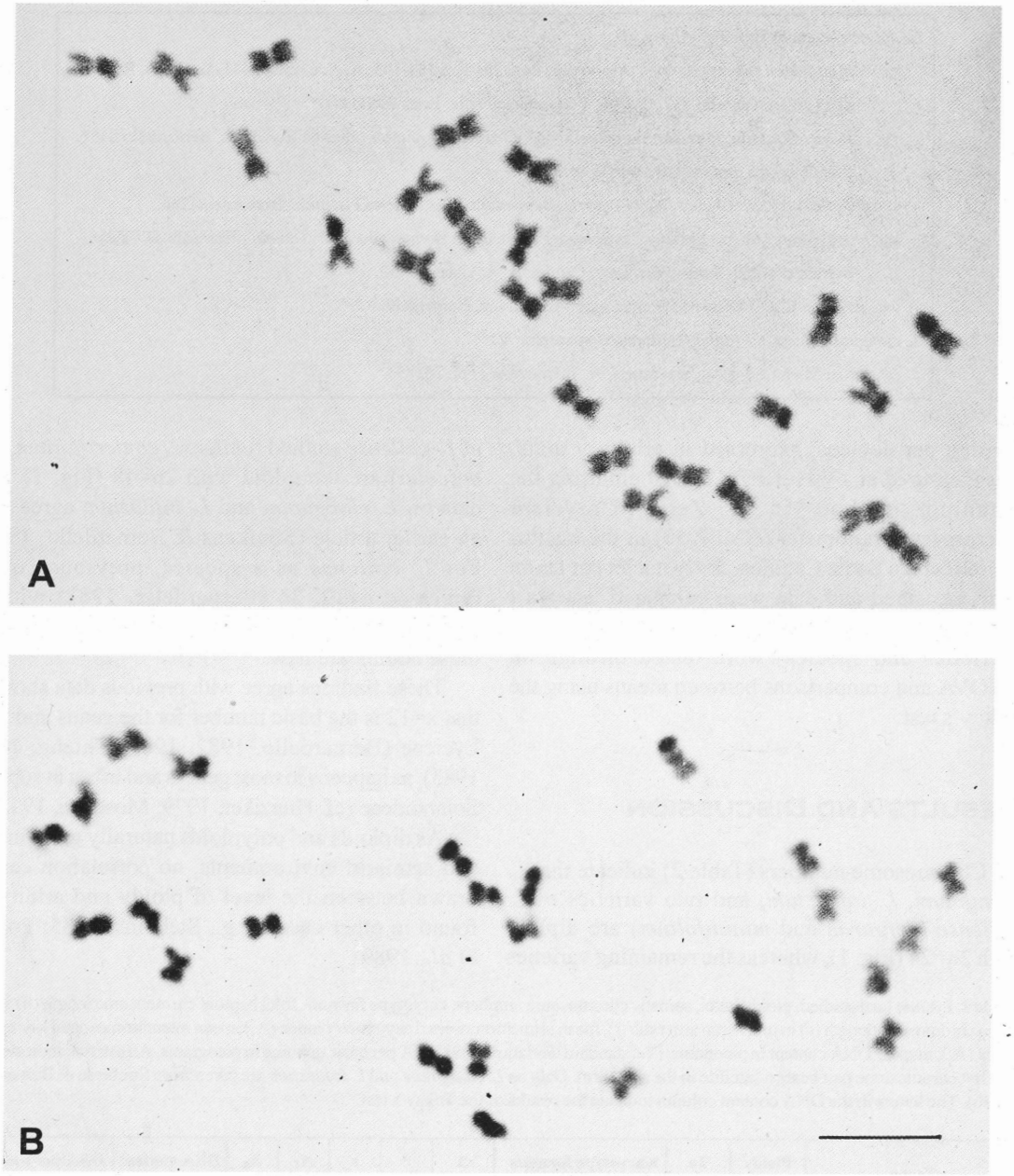

Fig. 1. Photomicrographs of mitotic metaphases of *Lycium*. **A**: *L. chilense* var. *minutifolium*, **B:** *L. chilense* var. *vergarae*. Bar = 5 μm, both at the same scale.

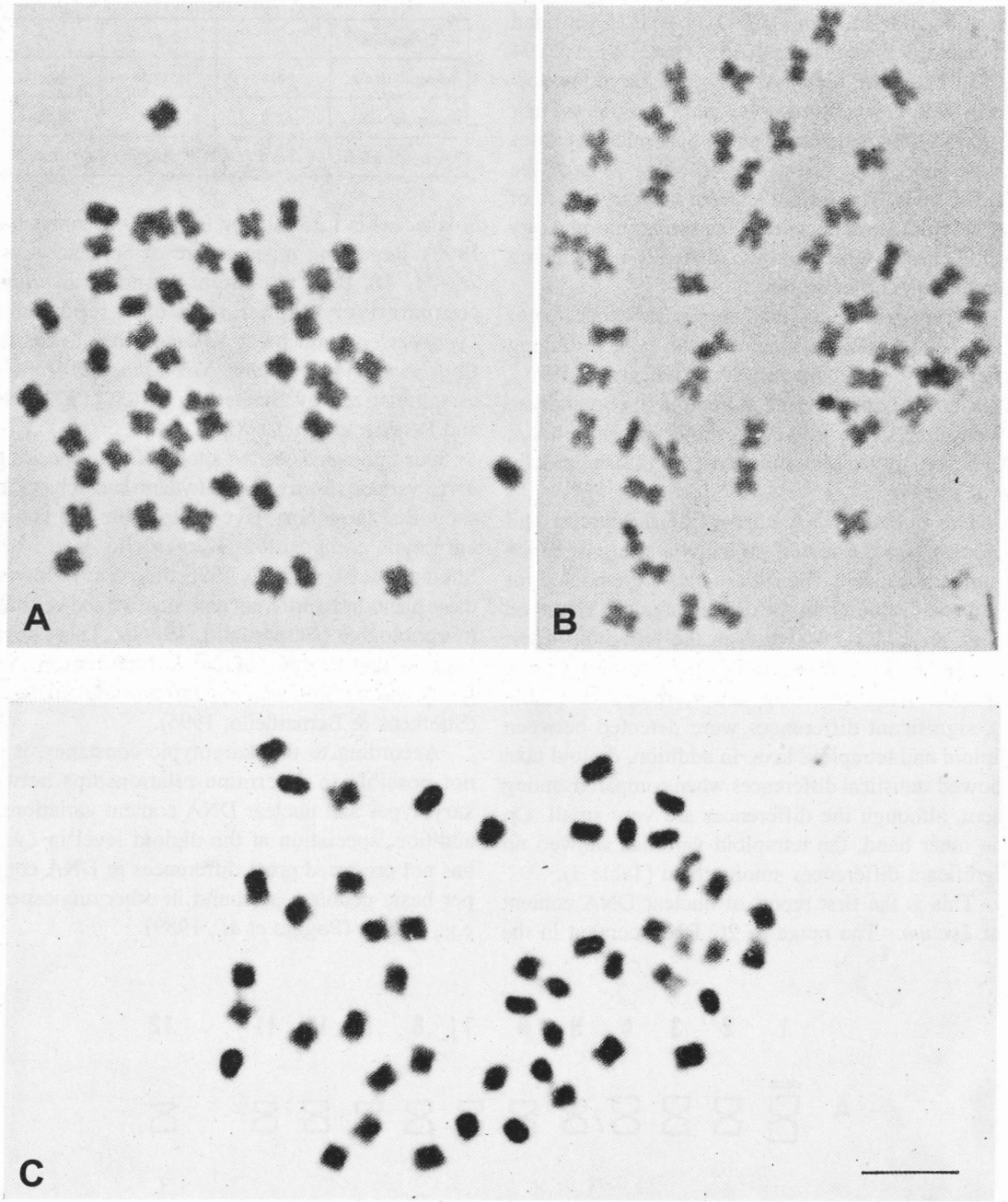

Fig. 2. Photomicrographs of mitotic metaphases of *Lycium*. **A**: *L. chilense* var *confertifolium*. **B:** *L. chilense* var. *descolei*. **C**: *L. chilense* var. *chilense*. Bar=5 μm, all at the same scale.

For the diploid taxa, we obtained karyotype formulae, total haploid chromosome length, and mean chromosome lengths and ratios (Table 2). The chromosomes are small ($\bar{x}$ = 1.84 ± 0.16 µm) and the haploid chromosome length ranges from 20.94 to 25.01 µm. All taxa have identical karyotype formula, with 11 *m* chromosome pairs and one *sm* pair (Fig. 3). Both members of pair 1 bear microsatellites in the short arm, clearly observed in 78% of the studied cells. We did not analyze the karyotypes of the polyploid taxa because of the similar morphology of the chromosomes and the difficulty in matching homologue chromosomes.

Karyotypes are highly symmetrical according to the A_1 and A_2 indices obtained (Table 2), belonging to category 1A in Stebbins' classification (1971). This agrees with the general trend in the Solanaceae (Stebbins, 1971; Moscone,1989; Bernardello & Anderson, 1990; Moscone *et al.*, 1992; Bernardello *et al.*, 1994).

The nuclear DNA content of the nuclei and samples tested showed no statistical significance within each taxon. Thus, those data were pooled for each one (Table 2). In the diploid taxa, the observed range was 3.22-3.84 while in the tetraploid ones: 6.50-6.60, i.e., about twice the amount of the diploids. When statistical tests were applied (Table 3), significant differences were detected between diploid and tetraploid taxa. In addition, diploid taxa showed statistical differences when compared among them, although the differences are very small. On the other hand, the tetraploid varieties showed no significant differences among them (Table 3).

This is the first report of nuclear DNA content for *Lycium*. The range of 2C DNA content in the Solanaceae is 1.25-30.6 pg (x = 6.49; Bennett *et al.*, 1998). Regarding other genera of Solanaceae with 2n=24, 48, the 2C content found in *Lycium* is comparatively higher than data reported for *Lycopersicon* and most *Solanum*, but lower than *Capsicum*, *Cyphomandra*, *Nicotiana*, and *Withania*, as summarized by Bennett & Leitch (1995, 1997) and Bennett *et al.* (1998).

Table 3. Comparisons among DNA content measurements by ANOVA at P< 0.05. df= degrees of freedom. * Statistically significant differences.

Comparison	df	*F*	*P*
Among all taxa	419	1587.50	0.001*
Among diploids	239	88.51	0.001*
Among tetraploids	179	0.82	0.40

Our findings based on a total of nine species and seven varieties from sections Lycium and Schistocalyx show that these taxa have comparable and constant karyotype composition (Bernardello *et al.*, 1995; Stiefkens & Bernardello, 1996, this paper). However, these plants have different reproductive and vegetative morphologies (Bernardello, 1986a). These results suggest that morphological differentiation in the group was not followed by chromosomal divergence (Stiefkens & Bernardello, 1996).

According to this karyotypic constancy, it was not possible to determine relationships between karyotypes and nuclear DNA content variations. In addition, speciation at the diploid level in *Lycium* has not produced great differences in DNA content per basic genome, as found in other angiosperms, e.g., *Larrea* (Poggio *et al.*, 1989).

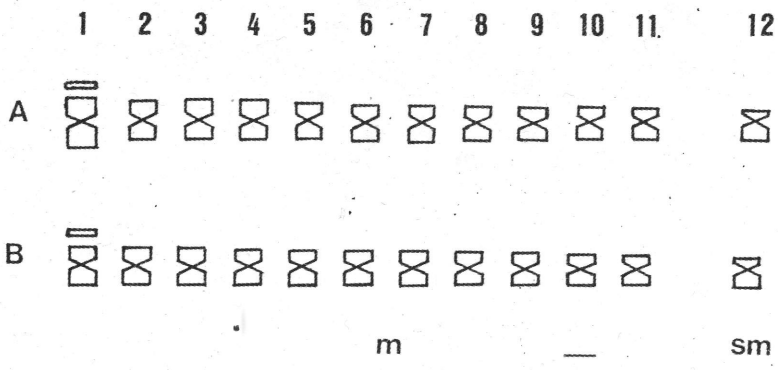

Fig. 3. Idiograms of *Lycium*. **A**: *L. chilense* var. *minutifolium*. **B**: *L. chilense* var. *vergarae*. Bar = 2 µm, both at the same scale.

ACKNOWLEDGEMENTS

We thank CONICET, SECYT-UNC, and CONICOR for financial support. Lidia Poggio, Carlos Naranjo, and Marcela Rosato are sincerely acknowledged for helping in many ways.

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Recibido el 19 de Septiembre de 2000, aceptado el 08 de Noviembre de 2000.