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Karyotypic studies in *Lycium* section Mesocope (Solanaceae) from South America

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Abstract - Mitotic chromosome numbers and karyotypes were determined for South American species of *Lycium* L. section Mesocope Miers (Tribe Lycieae). Twenty populations from nine species were examined. All of them have $2n = 2x = 24$, a fact that confirms the basic number x = 12 suggested for the tribe and the subfamily Solanoideae. The chromosome numbers of *L. chanar*, *L. minutifolium*, *L. stenophyllum*, and *L. vimineum* are reported for the first time and data on karyotype composition are all new. Chromosome measurements were statistically compared and a phenogram was obtained with those data. Results suggest that although these species are morphologically different, their differentiation was not followed by variations in chromosome number or structure. The karyotype of all the studied taxa is composed of 11 m pairs + 1 sm pair (pair #1 bears a microsatellite on the short arm) showing slight differences in size among the species. Thus, during the diversification within the section, no major visible chromosomal rearrangements have occurred, although cryptic structural changes, as paracentric inversions or reciprocal translocations of segments of similar length may have taken place.

Key words: chromosome number, karyotype, *Lycium* sect. Mesocope, phenetics, South America.

INTRODUCTION

The woody genus *Lycium* L. is Cosmopolitan with more than 80 spp. (HUNZIKER 2001) mostly adapted to arid and semiarid regions of the world. It is included in the subfamily Solanoideae, tribe Lycieae. Within the tribe, it is considered primitive and older than the other two genera (*Grabowskia* Schltdl. and *Phrodus* Miers) with a great morphological diversity (BERNARDELLO 1986a; BERNARDELLO and CHIANG-CABRERA 1998). The American continent has the major species concentration (51), being the U.S.A. in the north and Argentina in the south its centres of diversification (HITCHCOCK 1932; CHIANG-CABRERA 1981; BERNARDELLO 1986a). South America is regarded as the region where the tribe (BERNARDELLO 1986a; BERNARDELLO and CHIANG-CABRERA 1998) and the family as a whole (RAVEN and AXELROD 1974; D'ARCY 1991; HUNZIKER 2001) have originated.

The infrageneric classification of *Lycium* recognises four sections: Lycium, Schistocalyx Dunal, Mesocope Miers, and Sclerocarpellum C. Hitchc. (CHIANG 1983a; BERNARDELLO 1986a), although some species are not easily placed in them (BERNARDELLO and CHIANG-CABRERA 1998). Section Mesocope is close to section Lycium, but it differs basically because their members have a prominent red or orange ovarian nectary (BERNARDELLO 1986b, 1987; BERNARDELLO and LEIVA-GONZÁLEZ 1993).

The cytological knowledge of this genus is meagre. Most of the published papers just report chromosome numbers from meiotic analyses. The majority of the taxa are diploid with $n = 12$ (e.g., FEDOROV 1969; BERNARDELLO 1982; CHIANG

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1982; GHAFFARI 1987), being x = 12 the basic number for the genus and the tribe as well (BERNARDELLO 1985; CHIANG 1983b; DI FULVIO 1977). This base number is common in Solanoideae (cf. HUNZIKER 2001).

On the other hand, there are few karyotypic studies in *Lycium*. They have been examined eight South American species (BERNARDELLO *et al.* 1995; STIEFKENS and BERNARDELLO 1996, 2000) and six Iranian species (SHEIDAI *et al.* 1999). Thus, the goal of this work is to study the mitotic chromosomes of South American representatives of *Lycium* section Mesocope, with emphasis in qualitative and quantitative analyses of their karyotypes applying numerical techniques. With the obtained data, we try to understand their systematic relationships and evolutionary trends.

MATERIAL AND METHODS

The nine studied species, the 20 populations and the 76 individuals used are included in Table 1. Vouchers at deposited at the Herbarium of the "Museo Botánico de Córdoba" (CORD) and were determined by G. Bernardello.

Chromosomes were prepared from root-tip mitoses from germinating seeds. To enhance the percentage of seed germination, seeds were soaked for 1 day in running water, put in sterile petri dishes on filter paper embedded in giberellic acid (GA_3,1000 ppm) to break the seed dormancy (ELLIS *et al.* 1985), and stored in a stove at 30°C in the dark. The adequate size of the germinating roots to visualise abundant metaphases was between 2-10 mm. Fresh root tips were pretreated for 2 hours in a saturated solution of paradichloro-benzene in water at room temperature (MEYER 1945), rinsed in distilled water, and fixed in freshly made ethanol: acetic acid (3:1) for 24 hours. Then, they were placed in alcoholic hydrochloric acid-carmine (SNOW 1963) for 5-7 days. Stained root tips were stored in 50% acetic acid until the squash was made. Meristem cells were squashed in a drop of 50% acetic acid and heated gently. Slides were made permanent in Rhenohistol by means of BRADLEY's method (1948). Satellites were classified after BATTAGLIA (1955) and chromosomes after LEVAN *et al.* (1964). STEBBINS's asymmetry categories (1971) were calculated as well.

At least four cells per individual and 26 per species were examined. Ten metaphases of each species were photographed with phase contrast optics and Kodak Panatomic X film. Karyograms were constructed organising the chromosomes in two groups after their arm ratio (*m* or *sm*) and ordering them within each one after their decreasing size. Idiograms were based on the mean values for each taxon. The measured variables were: mean total chromosome length of each pair (c_1-c_{12}), mean arm ratio of each pair after LEVAN *et al.* (1964) (r_1-r_{12}), mean total haploid chromosome length of the complement (tl), mean chromosome length of the complement (C), mean arm ratio (r), ratio between the longest and the shortest chromosome lengths of the complement (R), asymmetry indices of ROMERO ZARCO (1986): intrachromosomic (A_1) and interchromosomic (A_2).

Variables were statistically compared with ANOVA and TUKEY's test using the program SPSS (release 6.0 for Windows, SPSS Inc., 1993). In addition, phenograms were generated as follows: the studied taxa were the OTUs and the data matrix included nine variables (A_1, A_2, tl, c, R, c_1, r_1, c_{12}, r_{12}), the taxonomic distance coefficient and the UPGMA method were used with the program SYSTAT (release 7.0 for Windows, SPSS Inc., 1997).

Table 1 – Populations studied of *Lycium* section Mesocope species, including the used code and collection data (country, province, Department, date, collector and number). The number within brackets indicates: (the number of individuals studied for each accession, the number of studied cells). Collector's abbreviations: DB = D. Burckhardt, B = G. Bernardello, G = L. Galetto.

Taxon	Code	Populations
L. cuneatum Dammer	cunea	Argentina, Santa Fe, Gral. Obligado, near Berna, 25-1-92, B 793 (5, 26)
L. chanar Phil.	chana	Argentina, Mendoza, Las Heras, Puente del Inca, 20-1-94, G 268 (4, 25), 271 (3, 15), 272 (4, 20).- San Juan, Calingasta, Quebrada Arroyo Cabeceras, 23-1-95, Apochian 189 (6, 30)
L. fuscum Miers	fuscu	Argentina, San Juan, Iglesias, near Arrequintín, 9-1-94, B 841 (7, 35)
L. gilliesianum Miers	gillie	Argentina, La Pampa, Toay, Parque Luro, 7-11-92, B 832 bis (7, 30)
L. minutifolium Remy	minut	Chile, Coquimbo, near Rivadavia, 11-1-94, B 850 (2, 15); near Algarrobal bridge, 11-1-94, B 851 (6, 35); near Gabriela Mistral bridge, 12-1-94, B 862 (4, 20).- Atacama, Incahuasi, Panamerican road Km 575, 14-1-94, B 854 (4, 25)
L. morongii Britton	moron	Argentina, Corrientes, Itatí, Corsa Cué, B 804 (6, 30)
L. nodosum Miers	nodos	Argentina, Córdoba, Río seco, Paso de la Cina, 5-2-79, B 180 (6, 37)
L. stenophyllum Remy	steno	Chile, IV Región, Elqui, 15 Km SW of Viñita Baja, 4-12-93, DB 6 (3, 14); Huasco, DB 14 (2, 10); Elqui, Vicuña, DB 10 (2, 8); Coquimbo, near Algarrobal bridge, 11-1-94, B 848 (3, 18)
L. vimineum Miers	vimin	Argentina, Entre Ríos, Victoria, Charigüé, 18-2-94, G 276 (3, 10), 277 (3, 15), 278 (3, 10)

RESULTS

The nine analysed *Lycium* species were diploid with $2n = 24$ (Figs. 1-4). The chromosome numbers of *L. chanar*, *L. minutifolium*, *L. stenophyllum*, and *L. vimineum* are reported for the first time (Figs. 2-3). Table 2 includes new information on the karyotype features of the examined taxa, whereas in Fig. 4 idiograms are drawn with the mean data for each taxon.

The mitotic chromosomes are small (Table 2; Figs. 1-3). The mean chromosome length for all taxa is 1.83 µm, while the range for individual species was quite small (1.67-2.07 µm). Consequently, the overall haploid genome length was relatively homogeneous among the different species (range = 20.01-24.93 µm, $\bar{x} = 21.94$ µm; Table 2). The shortest measured chromosome pair was #11 *L. vimineum* (1 µm) and the longest one was pair #1 in a cell of *L. stenophyllum* (2.9 µm). Considering the total genome length, *L. minutifolium* had the longest while *L. vimineum* the shortest one.

The obtained data showed that all these species have the same karyotype formula: 11 m pairs + 1 sm pair, with the first m pair having a microsatellite on the short arm (Fig. 4; Table 2). This satellited pair and the sm pair were easily identified in the complement. On the other hand, from pair 2 to pair 11 (all m) chromosomes were very similar with slight size differences among them (Fig. 4).

The observed satellites were terminal microsatellites because they are small and spheroidal, having a similar diameter (or smaller) than the chromosome that bears it and being located at its end. Their presence was quite constant because they were detected in the 75% of the studied cells, and in these cells, they were seen in both homologues the 52% of the cases.

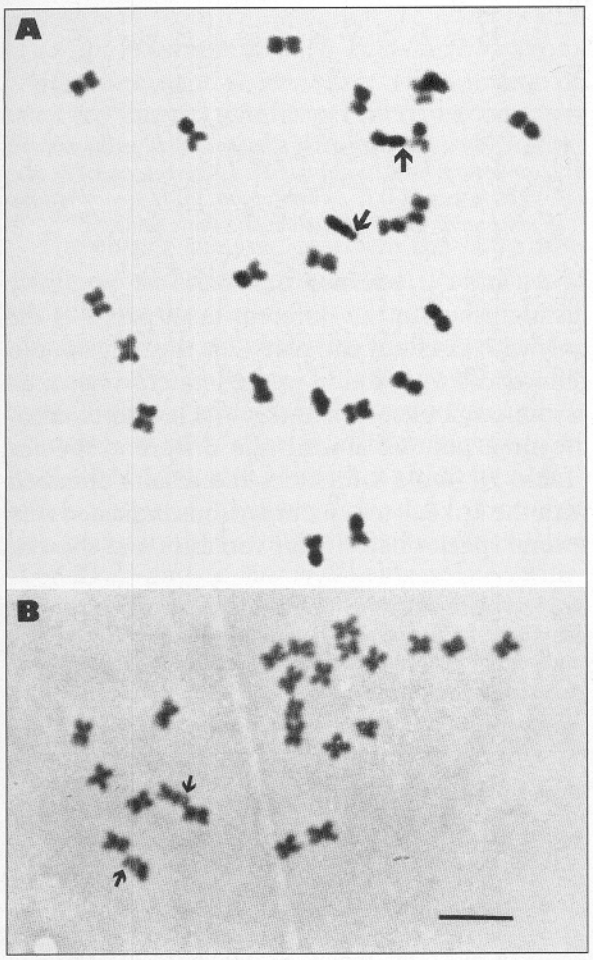

Fig. 1 – Photomicrographs of mitotic metaphases of *Lycium* sect. Mesocope species. A) *L. gillesianum*. B) *L. cuneatum*. Bar 5 µm, all at the same scale. Arrows point satellites.

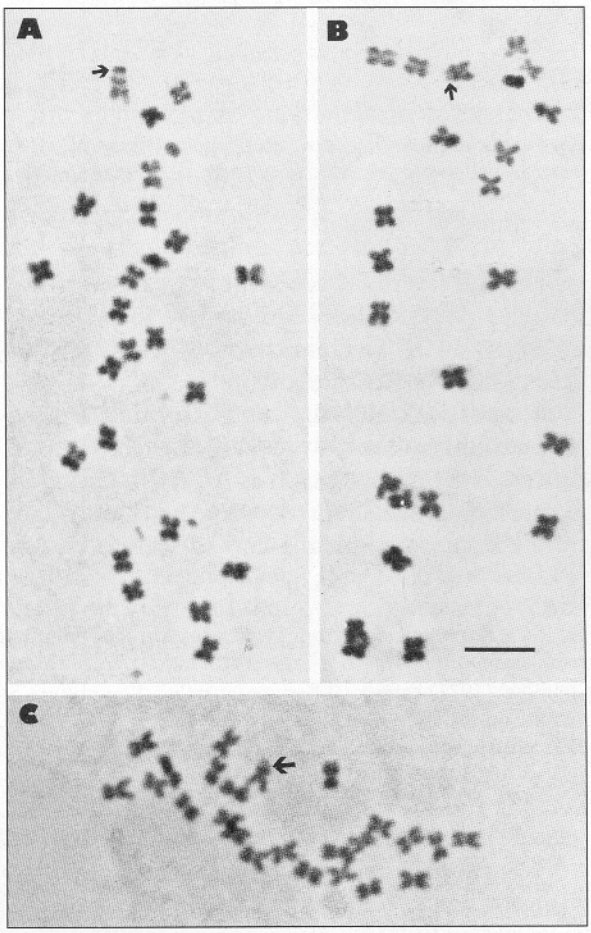

Fig. 2 – Photomicrographs of mitotic metaphases of *Lycium* sect. Mesocope species. A) *L. chanar*. B) *L. nodosum*. C) *L. stenophyllum*. Bar 5 µm, all at the same scale. Arrows point satellites.

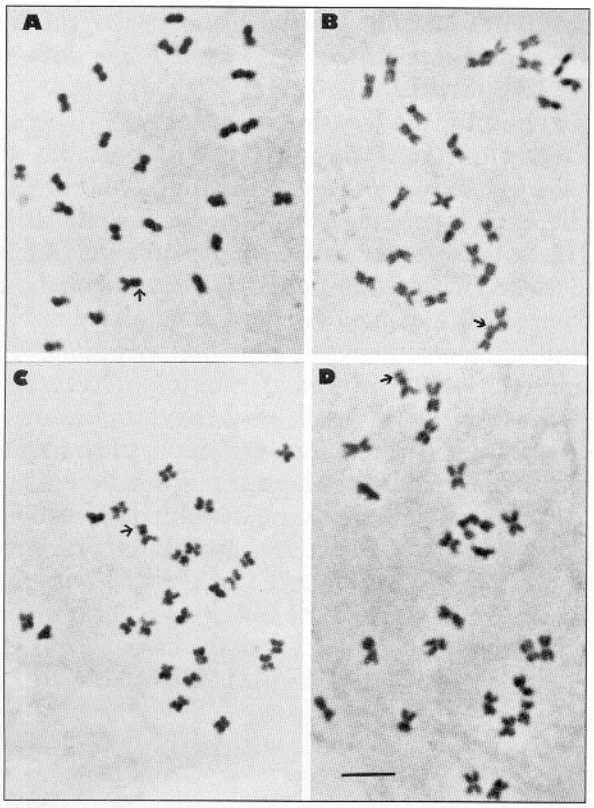

Fig. 3 – Photomicrographs of mitotic metaphases of *Lycium* sect. Mesocope species. A) *L. vimineum*. B) *L. minutifolium*. C) *L. fuscum*. D) *L. morongii*. Bar 5 µm, all at the same scale. Arrows point satellites.

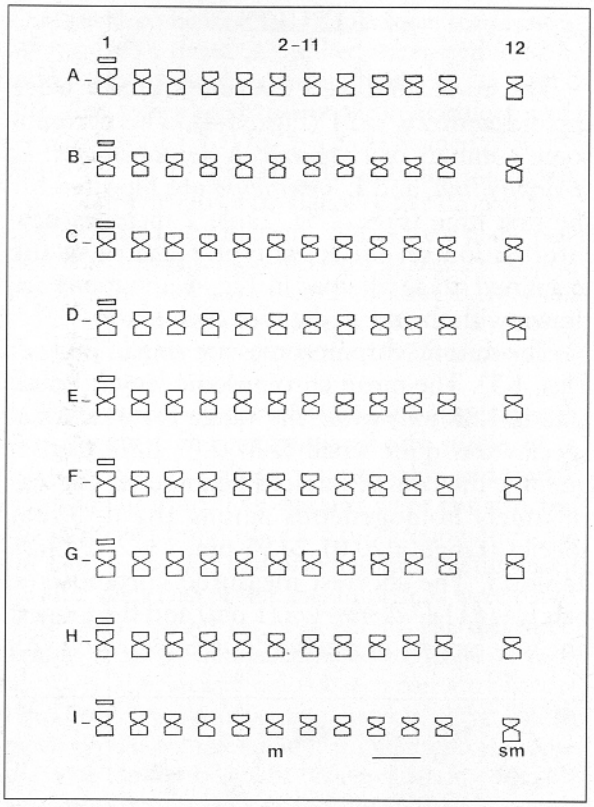

Fig. 4 – Idiograms for each species. A) *L. gilliesianum*. B) *L. nodosum*. C) *L. morongii*. D) *L. cuneatum*. E) *L. chanar*. F) *L. fuscum*. G) *L. minutifolium*. H) *L. stenophyllum*. I) *L. vimineum*. Bar 5 µm, all at the same scale.

The karyotypes were symmetrical, after the ROMERO ZARCO (1986) indices (A_1 and A_2) and STEBBINS's (1971) classification (Table 2), all belonging to the A category.

A statistical analysis was performed among nine variables (Table 3). Some of them include data from the genome, such as A_1, A_2, tl, C, and R. Regarding particular chromosome pairs, only data from chromosome pairs 1 and 12 were used (c_1, r_1, c_{12}, and r_{12}), because these pairs can be clearly distinguished in the different taxa: pair 1 is the only with satellites and pair 12 is the only *sm*. No differences were found in the interchromosomic asymmetry index (A_2) and in the length of chromosome pair 12 among the different species (Table 3). Table 4 includes the results obtained with the Tukey's test. Comparisons indicated that several species had similar variables and showed

Table 2 – Karyotype data from *Lycium* section Mesocope species: karyotype formulae, total haploid chromosome length (tl), mean chromosome length (c), mean arm ratio (r), mean asymmetry indices (intrachromosomic: A_1, interchromosomic: A_2) and Stebbins' (1971) category of asymmetry (St). Lengths are in µm. An asterisk indicates that the first chromosomic pair has a satellite on the short arm.

Taxon	Karyotype formulae	tl	c	r	A_1	A_2	St
L. cuneatum	11 m* + 1 sm	20.34	1.69	1.18	0.12	0.14	2A
L. chanar	11 m* + 1 sm	22.68	1.89	1.17	0.11	0.14	2A
L. fuscum	11 m* + 1 sm	22.70	1.89	1.22	0.16	0.14	2A
L. gillesianum	11 m* + 1 sm	20.66	1.72	1.25	0.18	0.17	2A
L. minutifolium	11 m* + 1 sm	24.93	2.07	1.21	0.16	0.08	1A
L. morongii	11 m* + 1 sm	23.23	1.94	1.20	0.14	0.12	1A
L. nodosum	11 m* + 1 sm	20.03	1.67	1.27	0.19	0.13	2A
L. stenophyllum	11 m* + 1 sm	22.90	1.90	1.17	0.12	0.13	1A
L. vimineum	11 m* + 1 sm	20.01	1.67	1.24	0.17	0.13	2A

Table 3 – Comparison of nine karyological variables from the studied species of *Lycium* section Mesocope by ANOVA ($p<0.05$). df = degrees of freedom, * = statistically significant differences. A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index, tl: total haploid chromosome length, c: mean chromosome length, R: ratio between the largest and the smallest chromosomes of the complement, c_1: mean chromosome length of pair 1, r_1: mean arm ratio of pair 1, c_{12}: mean chromosome length of pair 12, r_{12}: mean arm ratio of pair 12.

Variable	df	F	p
A_1	89	4.92	0.0001*
A_2	89	2.65	0.14
lt	89	3.01	0.006*
c	89	3.03	0.006*
R	89	2.85	0.009*
c_1	89	2.46	0.021*
r_1	89	3.05	0.005*
c_{12}	89	1.38	0.21
r_{12}	89	2.15	0.04*

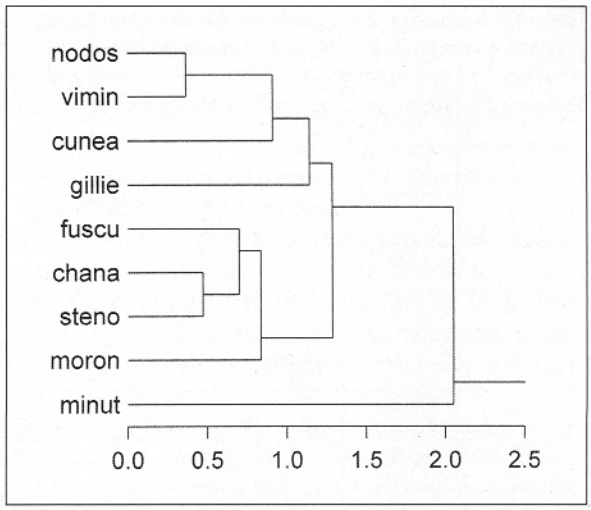

Fig. 5 – UPGMA phenogram derived from average taxonomic distance (9 variables, cf. material and methods). Species codes are given in Table 1.

no differences in them. A_1 is useful to distinguish *L. nodosum* from *L. cuneatum, L. chanar*, and *L. stenophyllum*, and also *L. cuneatum* from *L. gilliesianum* and *L. vimineum*. The arm ratio of pair 1 (r_1) showed significant differences between *L. minutifolium* and *L. gilliesianum*, *L. chanar*, and *L. stenophyllum*. The variables R and A_2 were useful in a few instances.

The cluster analysis, based on the same nine cytological variables, grouped the species mainly according to the chromosome length (Fig. 5): 1) *L. minutifolium* isolated with the longest chromosomes (tl = 24.93 µm), 2) a group formed by *L. nodosum*, *L. vimineum*, *L. cuneatum*, and *L. gilliesianum* with shorter chromosomes (tl = 20.01-20.66 µm), and 3) another group by *L. fuscum*, *L. chanar*, *L. stenophyllum*, and *L. morongii* with comparatively longer chromosomes (tl = 22.68-23.23 µm), *L. morongii* being isolated within it because it is the species with the longest genome, after *L. minutifolium*.

DISCUSSION

Solanaceae has a dysploid series with x = 7 to x = 13. The basic number x = 12 is the most common (ca. 50% of the studied species; cf. FEDOROV 1969; HUNZIKER 2001) and is considered the ancestral basic number for the family as well (RAVEN 1975; GRANT 1982). The members of subfam. Solanoideae also have x = 12, except the tribe Nicandreae with x = 10, 11 (cf. HUNZIKER 2001). Data published so far, suggest that x = 12 is the base number for the tribe Lycieae (e.g., CHIANG 1982; BERNARDELLO 1982; HUNZIKER *et al.* 1985; CHIANG *et al.* 1989).

In Solanaceae, it is common the presence of a chromosome pair with satellites (MOSCONE 1989a), mainly in shorter arms of m pairs. Regarding the chromosome type in the family, m and sm chromosomes are very frequent (e.g., PICKERSGILL 1977; MAIZONNIER 1984; PRINGLE and MURRAY 1988; MOSCONE 1989a, b, 1990; BERNARDELLO and ANDERSON 1990; BERNARDELLO *et al.* 1994). Symmetrical karyotypes, as found in *Lycium,* have been correlated in Magnoliophyta with a primitive condition of the taxa (STEBBINS 1950, 1971). In general, woody perennial species in contrast to annual species, usually have constant, less diversified karyotypes (BRANDHAM 1983; EHRENDORFER 1983), a trend that our results support and that was suggested for other woody Solanaceae, as *Capsicum* (MOSCONE *et al.* 1993).

Subfam. Solanoideae is considered as monophyletic and derived (OLMSTEAD and PALMER 1992). Lycieae also seems to be monophyletic (BERNARDELLO and CHIANG-CABRERA 1998) and derived, but with some plesiomorphic features, as woody habit and high symmetric karyotypes.

In spite of the fact that the studied taxa are morphologically diverse (BERNARDELLO 1986a; BERNARDELLO and CHIANG-CABRERA 1998), their differentiation was not accompanied by variation either in chromosome number or morphology.

Table 4 – Results of the Tukey test for the karyological variables analysed of *Lycium* section Mesocope species. Code = 1. *L. gilliesianum*, 2. *L. nodosum*, 3. *L. morongii*, 4. *L. cuneatum*, 5. *L. chanar*, 6. *L. fuscum*, 7. *L. minutifolium*, 8. *L. stenophyllum*, 9. *L. vimineum*. A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index, R: ratio between the largest and the smallest chromosomes of the complement, r_1: mean arm ratio of pair 1.

1									
2									
3	R								
4	A_1	A_1							
5		A_1							
6									
7	A_2, R, r_1				r_1				
8		A_1					r_1		
9				A_1					
	1	2	3	4	5	6	7	8	9

Effectively, their karyotypes are constant and symmetrical, as happens in *Solanum* and other Solanaceae (e.g., STEBBINS 1971; MOSCONE 1989a, b; BERNARDELLO and ANDERSON 1990; BERNARDELLO *et al.* 1994). Identical results were detected studying other South American members of the genus belonging to sections Lycium and Schistocalyx (STIEFKENS and BERNARDELLO 1996, 2000). Nevertheless, cryptic structural changes, such as paracentric inversions or reciprocal translocations of segments of similar length (STEBBINS 1958), could have occurred since these changes can not be detected with the techniques used. Previous meiotic studies (cf. BERNARDELLO 1982; CHIANG 1982) have reported the formation of normal bivalents suggesting that large inversions or translocations have not happened.

Interestingly enough, SHEIDAI *et al.* (1999) reported the karyotypes of six Iranian *Lycium* species and recorded for two of them (*L. depressum* and *L. shawii*) the same karyotype formula as here found. The remaining species showed a very close pattern with most m chromosomes and a few or no sm chromosomes. Regarding the chromosome size, they indicated longer chromosomes for the taxa they have examined, but the discrepancy may be due to the different pretreatment used in both works. Finally, they found no regularity in the presence of satellited are we here report.

Thus, and from the chromosomic point of view, the differences among the studied species of *Lycium* so far are slight which indicates that the karyotype structure is a conservative character for the genus. This phenomenon was observed in other plant groups, as tribe Aloinae (Liliaceae, genera *Aloe*, *Gasteria*, and *Haworthia*; BRANDHAM 1976). Perhaps, most morphological and physiological features of *Lycium* depend on the stability of their genic characters within a particular chromosome form that was adopted and fixed for their members.

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REFERENCES

BATTAGLIA E., 1955 – *Chromosome morphology and terminology*. Caryologia, 8: 179-187.

BERNARDELLO L., 1982 – *Estudios en Lycium (Solanaceae). II. Recuentos cromosómicos en entidades argentinas*. Hickenia, 1: 321-328.

–, 1985 – *El número cromosómico de Phrodus microphyllus (Solanaceae)*. Boletín de la Sociedad Argentina de Botánica, 24: 196-197.

–, 1986a – *Revisión taxonómica de las especies sudamericanas de Lycium (Solanaceae)*. Boletín de la Academia Nacional de Ciencias, Córdoba, 57: 173-356.

–, 1986b – *Estudios en Lycium (Solanaceae) V. El gineceo de Lycieae*. Kurtziana, 18: 23-45.

–, 1987 – *Comparative floral morphology in Lycieae (Solanaceae).* Brittonia, 39: 112-129.

BERNARDELLO L. and ANDERSON G.J., 1990 – *Karyotypic studies in Solanum section Basarthrum (Solanaceae).* American Journal of Botany, 72: 420-431.

BERNARDELLO L., HEISER C.B. and PIAZZANO M., 1994 – *Karyotypic studies in Solanum section Lasiocarpa (Solanaceae).* American Journal of Botany, 81: 95-103.

BERNARDELLO L. and LEIVA-GONZÁLEZ S., 1993 – *Relaciones fenéticas entre las especies sudamericanas de Lycium (Solanaceae).* Anales del Instituto de Biología de la UNAM, ser. Botánica, 64: 33-47.

BERNARDELLO L., RODRÍGUEZ I., STIEFKENS L. and GALETTO L., 1995 – *The hybrid nature of Lycium ciliatum x cestroides (Solanaceae): experimental, anatomical, an cytological evidence.* Canadian Journal of Botany, 73: 1995-2005.

BERNARDELLO L. and CHIANG-CABRERA F., 1998 – *A cladistic study on the American species of Lycium (Solanaceae) based on morphological variation.* Monographs in Systematics Botany of the Missouri Botanical Garden, 68: 33-46.

BRADLEY M.V., 1948 – *A method for making acetocarmine squashes permanent without removal of cover slip.* Stain Technology, 23: 41-44.

BRANDHAM P.E., 1976 – *The frecuency of spontaneous structural change.* In: K. Jones and P.E. Brandham (Eds), "Current Chromosome Research", pp. 77-87, Elsevier, Amsterdam.

–, 1983 – *Evolution in a stable chromosome system.* In: P.E. Brandham and M.D. Bennett (Eds), "Kew Chromosome Conference II", pp. 251-260, G. Allen & Unwin, London.

CHIANG F., 1982 – *Estudios cromosómicos en Lycium (Solanaceae) de Norteamérica.* Boletín de la Sociedad Botánica de México, 43: 9-23.

–, 1983a – *Nomenclatural changes for new sectional delimitation in Lycium (Solanaceae).* Taxon, 32: 456-458.

–, 1983b – *El número cromosómico de Grabowskia geniculata.* Boletín de la Sociedad Botánica de México, 45: 141-142.

CHIANG-CABRERA F., 1981 – *A taxonomic study of the North American species of Lycium (Solanaceae).* Ph. D. Dissertation. The University of Texas, Austin.

D'ARCY W.G., 1991 – *The Solanaceae since 1976 with a review of its biogeography.* In: J.G. Hawkes, R.N. Lester, M. Nee and N. Estrada (Eds), " Solanaceae III: Taxonomy, Chemistry and Evolution, pp. 75-137, Royal Botanic Gardens, Kew, and Linnean Society of London, Richmond.

DI FULVIO T.E., 1977 – *Recuentos cromosómicos en Angiospermas argentinas III.* Kurtziana, 10: 69-72.

ELLIS R.H., HONG T.D. and ROBERTS E.H., 1985 – *Handbook of seed technology for genebanks. Vol. II. Compendium of specific germination, information and test recomendation.* Handbook for genebanks 3. IBPGR, Rome.

EHRENDORFER F., 1983 – *Quantitative and qualitative differentiation of nuclear DNA in relation to plant systematics and evolution.* In: U. Jensen & D.E. Fairbrothers (Eds), "Proteins and nucleic acids in plant systematics", pp. 3-35, Springer Verlag, Berlin.

FEDOROV A. (ed.), 1969 – *Chromosome numbers of flowering plants.* V. L. Komarov. Bot. Inst., Leningrad (Reprinted by O. Koeltz Sci. Publ., Koeningstein, 1974).

GHAFFARI S.M., 1987 – *Chromosome counts of some Angiosperms from Iran, II.* Iranian Journal of Botany, 3: 183-188.

GRANT V., 1982 – *Periodicities in the chromosome numbers of the Angiosperms.* Botanical Gazette, 143: 379-389.

HITCHCOCK C.L., 1932 – *A monographic study of the genus Lycium of the western hemisphere.* Annals of the Missouri Botanical Garden, 19: 179-374.

HOWARD H.W., 1970 – *Genetics of the potatoes.* Springer Verlag, New York.

HUNZIKER A.T., 2001 – *Genera Solanacearum. The genera of Solanaceae Illustrated, arranged according to a new system.* A. R. G. Gantner Verlag, Ruggell.

HUNZIKER J.H., XIFREDA C.C. and WULFF A.F., 1985 – *Estudios cromosómicos en Angiospermas de Sudamérica.* Darwiniana, 26: 7-14.

LEVAN A., FREDGA K. and SANDBERG A., 1964 – *Nomenclature for centromeric position on chromosomes.* Hereditas, 52: 201-220.

MAIZONNIER D., 1984 – *Cytology.* In: H.C. Sink, (Ed), "Petunia", Monographs in Theoretical and Applied Genetics, 9: 21-33.

MEYER J.R., 1945 – *Prefixing with paradichlorobenzene to facilitate chromosome study.* Stain Technology, 20: 121-125.

MOSCONE E.A., 1989a – *Estudios citotaxonómicos en las tribus Solaneae y Nicotianeae (Solanaceae) de América del Sur.* Tesis Doctoral. Universidad Nacional de Córdoba.

–, 1989b – *Karyotype analyses in three Patagonian and S. Andean endemic genera of Nicotianeae (Solanaceae).* Plant Systematics and Evolution, 166: 31-39.

MOSCONE E.A., HUNZIKER A.T. and EHRENDORFER F., 1993 – *Giemsa C-banded karyotypes in Capsicum (Solanaceae).* Plant Systematics and Evolution, 186: 213-229.

OLMSTEAD R. and PALMER J., 1992 – *Chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution.* Annals of the Missouri Botanical Garden, 79: 346-360.

PICKERSGILL B., 1977 – *Chromosomes and evolution in Capsicum.* In: E. Pochard (Ed), "Capsicum 77", pp. 27-37, I.N.R.A., Montfavret Avignon.

PRINGLE G.J. and MURRAY B. G., 1988 – *Karyotype diversity and nuclear DNA variation in Cyphomandra.* In: J.G. Hawkes, R.N. Lester, M. Nee and N. Estrada, (Eds), "Solanaceae III: Taxonomy, Chemistry and Evolution", pp. 247-252, Royal Bot. Gardens, Kew and Linnean Soc. London, Richmond.

RAVEN P.H., 1975 – *The bases of Angiosperm phylogeny: cytology.* Annals of the Missouri Botanical Garden, 62: 724-764.

RAVEN P.H. and AXELROD D.I., 1974 – *Angiosperm biogeography and past continental movement.* Annals of the Missouri Botanical Garden, 61: 539-673.

ROMERO ZARCO C., 1986 – *A new method for estimating karyotype asymmetry.* Taxon, 35: 526-530.

SHEIDAI M., NARENGI Z. and KHATAMSAZ M., 1999 – *Karyotype and seed protein analyses of Lycium (Solanaceae) in Iran.* Edinburg Journal of Botany, 56: 253-264.

SNOW R., 1963 – *Alcoholic hydrochloric acid carmine as stain for chromosome in squash preparations.* Stain Technology, 38: 9-13.

STEBBINS G.L., 1950 – *Variation and evolution in plants.* Columbia University Press, New York.

–, 1958 – *Longevity, habitat, and release of genetic variability in the higher plants.* Cold Spring Harbor Symposia on Quantitative Biology, 23: 365-378.

–, 1971 – *Chromosomal evolution in higher plants.* E. Arnold, London.

STIEFKENS L. and BERNARDELLO G., 2000 – *Karyotypes and DNA content in diploid and polyploid Lycium (Solanaceae).* Boletín de la Sociedad Argentina de Botánica, 35: 237-244.

STIEFKENS L. and BERNARDELLO L., 1996 – *Karyotypic studies in South American Lycium (Solanaceae).* Cytologia, 61: 395-402.

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