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KARYOTYPE STUDIES IN *LYCIUM* SECTIONS *SCHISTOCALYX* AND *SCLEROCARPELLUM* (*SOLANACEAE*)

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Mitotic chromosome numbers and karyotypes of species in two sections of *Lycium* (*Solanaceae*) from the American continent were determined in 23 populations. Both species in the small South American section *Schistocalyx* were examined: *Lycium ciliatum* and three varieties of *L. chilense* had diploid ($2n=24$) as well as tetraploid ($2n=48$) populations. *Lycium ameghinoi* from the small American section *Sclerocarpellum* was diploid with $2n=24$. The basic number $x=12$ for the genus was confirmed. The karyotypes of these taxa were highly symmetrical: the chromosomes were metacentric or submetacentric with the formula: 11 *m* + 1 *sm*. Microsatellites were present in chromosome pair no. 1 and were attached to the short arms. As in other *Lycium* taxa already investigated, karyotypic features suggest that morphological differentiation in the group has not been accompanied by karyotype divergence.

Keywords. Chromosome number, karyotypes, *Lycium*, polyploidy, South America.

Introduction

The cosmopolitan genus *Lycium* L. (*Solanaceae–Solanoideae*) has c.80 species adapted to arid and semiarid conditions (Hunziker, 2001). Within tribe *Lycieae* Hunz., it is regarded as primitive and older than the other two genera: *Grabowskia* Schltdl. with four South American species (one reaching Mexico) and *Phrodus* Miers which is monotypic and endemic to northern Chile (Hunziker, 2001). These three genera are typically woody, mostly being shrubs or small trees. *Lycium* also has great morphological diversity (Bernardello, 1986; Bernardello & Chiang-Cabrera, 1998). Most species inhabit the American continent, with the arid regions of the USA and Argentina being centres of diversification (Hitchcock, 1932; Chiang-Cabrera, 1981; Bernardello, 1986). South America is considered to be the region where both the family (Hunziker, 2001) and tribe *Lycieae* have originated (Bernardello, 1986; Bernardello & Chiang-Cabrera, 1998).

The basic number for *Lycium* and for tribe *Lycieae* is $x=12$, a widespread number in the *Solanoideae* where most of the species are diploid with $2n=24$ (Fedorov, 1969; Bernardello, 1982; Chiang, 1982; Moscone, 1989a; cf. Hunziker, 2001).

In this paper, we analyse the somatic chromosomes and karyotypes of some *Lycium* species from Chile and Argentina belonging to sections *Schistocalyx* Dun. and *Sclerocarpellum* C.L.Hitchc., to clarify the taxonomic relationships of the species and possibly assess evolutionary relationships.

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We examined both species in section *Schistocalyx*, an exclusively southern South American section considered monophyletic (Miller, 2002), and characterized as having flowers with short corolla tubes, long spreading lobes, and much exserted stamens with enlarged fringed bases. This section is composed of two polymorphic species (*L. chilense* Miers ex Bertero and *L. ciliatum* Schltdl.) that have some reported cases of polyploidy (Bernardello, 1982; Stiefkens & Bernardello, 2000). Two of the varieties of *L. chilense* studied (var. *chilense* and var. *filifolium* (Miers) Bernardello) grow in Chile and Argentina, whereas var. *descolei* F.A.Barkley is an Argentinian Patagonia endemic. *Lycium ciliatum*, on the other hand, occurs from southern Bolivia to central Argentina and Uruguay (Bernardello, 1986). We also investigated *L. ameghinoi* Speg., a Patagonian endemic from Neuquén to Santa Cruz provinces in Argentina. It is one of the three South American members of the small American section *Sclerocarpellum*. This section is considered derived because of the presence of several synapomorphies, such as the two 1-ovuled locules of the ovary and drupaceous fruits with two 1-seeded pyrenes (Bernardello & Chiang-Cabrera, 1998) but is not monophyletic according to Miller (2002).

Materials and Methods

Table 1 lists the five taxa and the 23 populations studied. Vouchers were deposited at the Herbarium of the Museo Botánico de Córdoba (CORD).

Cytological preparations were made from root-tip mitoses in germinating seeds. To enhance the germination percentage, seeds were soaked for one day in running water, put in sterile Petri dishes on filter paper soaked in gibberellic acid (GA_3, 1000ppm) to break the seed dormancy, and stored in an oven at 30°C in the dark. Young roots 2–10mm long provided preparations with abundant metaphases. Fresh root tips were pretreated for 2 hours in a saturated solution of paradichloro-benzene in water at room temperature, rinsed in distilled water, fixed in freshly made ethanol:acetic acid (3:1) for 24 hours, and placed in alcoholic hydrochloric acid-carmine (Snow, 1963) for 5–7 days. Stained root tips were stored in 50% acetic acid until needed. Root tips were squashed in a drop of 50% acetic acid and heated gently. Slides were made permanent in Euparal by means of Bradley's method (1948). Satellites were classified after Battaglia (1955) and chromosomes after Levan *et al*. (1964).

At least five individuals and 25 cells per taxon were examined (Table 1); from them, 10 metaphases were photographed with phase contrast optics on Kodak Panatomic X film. Karyograms were constructed by arranging the chromosomes in two groups according to arm ratio (metacentric, *m*, or submetacentric, *sm*) and ordering them in decreasing size. Idiograms were based on the mean values recorded for each taxon (Table 2). The parameters used were:

- Mean total chromosome length of each pair (c_1–c_{12})
- Mean arm ratio of each pair (r_1–r_{12})
- Mean total haploid chromosome length of the complement (tl)

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TABLE 1. Accession data for *Lycium* populations studied. The data given are: collector code and number, country, province, department, locality, year, and in parentheses, number of individuals sampled and number of cells examined

Taxon	Collection data
L. chilense Miers ex Bertero var. *chilense*	**B 757**. Argentina, Córdoba, San Justo, Miramar, 1991 (10, 30)
	B 780. Argentina, La Pampa, Toya, Bajo Giuliani, 1992 (10, 40)
	B 845. Chile, Coquimbo, near Rivadavia, 1994 (20, 50)
	B 865. Chile, Coquimbo, La Serena, 1994 (5, 25)
L. chilense var. *descolei* F.A.Barkley	**B 786**. Argentina, Chubut, Biedma, near Puerto Pirámide, 1992 (8, 35)
	B 785. Argentina, Chubut, Biedma, Punta Pardela, 1992 (12, 50)
L. chilense var. *filifolium* (Miers) Bernardello	**B 756**. Argentina, Córdoba, San Justo, Miramar, 1991 (10, 45)
	G 238. Argentina, La Pampa, Limay, between Chacharramendi and La Reforma, 1990 (10, 30)
	B 111. Argentina, La Pampa, Toay, Parque Luro, 1992 (8, 35)
	AC 502. Argentina, La Pampa, Valle Argentino, 1993 (10, 30)
L. ciliatum Schltdl.	**S 7**. Argentina, Córdoba, Capital, Córdoba, 1990 (12, 50)
	RS s.n. Argentina, Córdoba, Capital, Pilar, 1990 (10, 30)
	D s.n. Argentina, Córdoba, Tercero arriba, Los Cóndores, 1990 (10, 45)
	S 8. Argentina, Córdoba, Cruz del Eje, Canteras Quilpo, 1991 (12, 50)
	AC 488. Argentina, Córdoba, Punilla, Los Terrones, 1991 (10, 60)
	B 760. Argentina, Córdoba, San Justo, Miramar, 1991 (8, 25)
	S 9. Argentina, Córdoba, Colón, between Jesús María and Sinsacate, 1991 (8, 35)
	B 823. Argentina, Córdoba, Colón, Río Carnero before Jesús María, 1991 (10, 40)
	B 273. Argentina, Córdoba, Colón, near Río Pinto, 1991 (10, 35)
	B 827, **B 828**. Argentina, Córdoba, San Justo, Miramar, 1992 (7, 25)
	GB 87. Argentina, Catamarca, Pomán, 1994 (5, 25)
L. ameghinoi Speg.	**B 783**. Argentina, Chubut, Biedma, Punta Pardela, 1992 (15, 70)

Collector abbreviations: **AC**, A. Cocucci; **B**, G. Bernardello; **D**, Dominguez; **DB**, D. Burckhardt; **G**, L. Galetto; **GB**, G. Barboza; **S**, L. Stiefkens; **RS**, R. Subils.

- Mean chromosome length of the complement (C)
- Mean arm ratio of the complement (r)
- Ratio between the longest and the shortest chromosome lengths of the complement (R)
- Asymmetry indices of Romero Zarco (1986):
  – Intrachromosomic, $A_1 = 1 - \left(\sum_{i=1}^{n} \frac{b_i}{B_i} \right) / n$, where n is the number of homologous chromosome pairs, b_i is the average length for short arms in every homologous chromosome pair, and B_i is the average length for long arms in every homologous chromosome pair

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TABLE 2. Karyotype data for diploid *Lycium* taxa studied

Taxon	Haploid karyotype formulae	tl	C	r	A_1	A_2	St	R
Lycium chilense								
var. *chilense*	11 *m** + 1 *sm*	20.0	1.7	1.14	0.10	0.13	1A	1.52
var. *descolei*	11 *m** + 1 *sm*	23.9	2.0	1.19	0.14	0.12	1A	1.46
var. *filifolium*	11 *m** + 1 *sm*	30.0	2.5	1.20	0.15	0.12	1A	1.51
L. ciliatum	11 *m** + 1 *sm*	21.2	1.7	1.21	0.15	0.11	2A	1.40
L. ameghinoi	11 *m** + 1 *sm*	20.2	1.7	1.23	0.17	0.16	2A	1.70

tl, mean total haploid chromosome length; C, mean chromosome length; r, mean arm ratio. Mean asymmetry indices: A_1, intrachromosomic; A_2, interchromosomic; St, Stebbins' (1971) category of asymmetry; R, ratio between largest and smallest chromosomes in complement. Lengths in μm. *m*, metacentric chromosome; *sm*, submetacentric chromosome. An asterisk indicates that the first chromosome pair has a satellite on the short arm.

TABLE 3. Results of ANOVA ($P<0.05$) on nine karyological variables in the *Lycium* taxa studied

Variable	df	*F*	*P*
A_1	49	9.21	0.00*
A_2	49	4.39	0.005*
tl	49	13.53	0.00*
C	49	12.65	0.00*
R	49	3.93	0.009*
c_1	49	12.35	0.00*
r_1	49	2.52	0.06
c_{12}	49	15.73	0.00*
r_{12}	49	1.77	0.15

df, degrees of freedom; * denotes 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 largest and smallest chromosomes of complement; c_1, c_{12}, mean chromosome lengths of pairs 1 and 12; r_1, r_{12}, mean arm ratios of pairs 1 and 12.

– Interchromosomic, $A_2 = s/x$, the ratio between standard deviation and mean chromosome length for each sample. Stebbins' classification (1971) was also employed.

A statistical analysis was performed among nine variables (Table 3), five of which (A_1, A_2, tl, C and R) included genomic data. Only data from chromosome pairs nos 1 and 12 were used (c_1, r_1, c_{12}, r_{12}), because they can be clearly distinguished. The parameters were compared with ANOVA and Bonferroni's test, using the program SPSS (release 6.0 for Windows, 1993, SPSS Inc., Chicago, USA).

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Results

Lycium ciliatum and the varieties of *L. chilense* analysed had diploid ($2n=24$) as well as tetraploid ($2n=48$) populations (Figs 1, 2A, 3). The tetraploid populations found were: *L. ciliatum* (S 8, B 273), *L. chilense* var. *chilense* (B 780, B 845, B 865), var. *descolei* (B 785), and var. *filifolium* (B 756): see Table 1. The single population of *L. ameghinoi* studied was diploid with $2n=24$ (Fig. 2B).

Table 2 gives karyotype features of the diploid populations and Fig. 4 shows the idiograms obtained from the mean data for each taxon. The complete raw data set is given in the Appendix. We did not analyse karyotypes of the polyploids because the similar morphology of most chromosomes made it difficult to match homologues.

In general, the chromosomes were small (Table 2; Figs 1–3), 2.0μm being the mean chromosome length for all taxa. The range for individual species was also quite small: 1.75–2.5μm. Accordingly, the overall haploid genome length was relatively homogeneous among the different species (range 20.0–30.0μm, mean 23.0μm; Table 2). The shortest chromosome pair was no. 11 in *L. ameghinoi* (0.8μm) and the longest was pair no. 1 in *L. chilense* var. *filifolium* (3.5μm). *Lycium chilense* var. *filifolium* had the longest total genome length (30.0μm), while *L. chilense* var. *chilense* had the shortest at 20.0μm.

All taxa analysed shared the same karyotype formula: 11 *m* pairs + 1 *sm* pair, with the first *m* pair having a satellite on the short arm (Figs 1, 2, 4; Table 2). Pairs nos 2 to 11 (all *m*) were quite similar with minor size differences among them (Fig. 4), and thus comparatively difficult to recognize. However, the single satellited pair (no. 1) was easily identified and to a lesser extent the only *sm* pair (no. 12). Terminal microsatellites were found in 72% of the cells examined.

According to A_1 and A_2 indices, the karyotypes were symmetrical (Table 2; Fig. 4). Using Stebbins' (1971) classification, they all were in category A: highly symmetrical karyotypes (Table 2).

The results indicate that no differences were found in the mean arm ratio of pairs nos 1 and 12 among the taxa analysed, but that there were some significant differences in the remaining variables which distinguish some taxa (Table 3).

Table 4 contains the Bonferroni's test results, which show that the variables tl, C, c_1 and c_{12} can distinguish some taxa. These variables, related to chromosome length, separate *L. chilense* var. *chilense*, *L. ciliatum* and *L. ameghinoi*, with shorter genome lengths, from *L. chilense* var. *filifolium* and var. *descolei* that have longer genomes. The A_1 index shows significant differences only between *L. chilense* var. *chilense* and the other taxa studied. The A_2 index, together with the ratio between the longest and the shortest chromosome lengths of the complement (R), separate *L. ameghinoi* from *L. chilense* var. *descolei* and *L. ciliatum*.

Discussion

The *Solanaceae* as a whole show a dysploid series from $x=7$ to $x=13$, with $x=12$ being the most frequent (around 50% of the samples studied; cf. Fedorov, 1969;

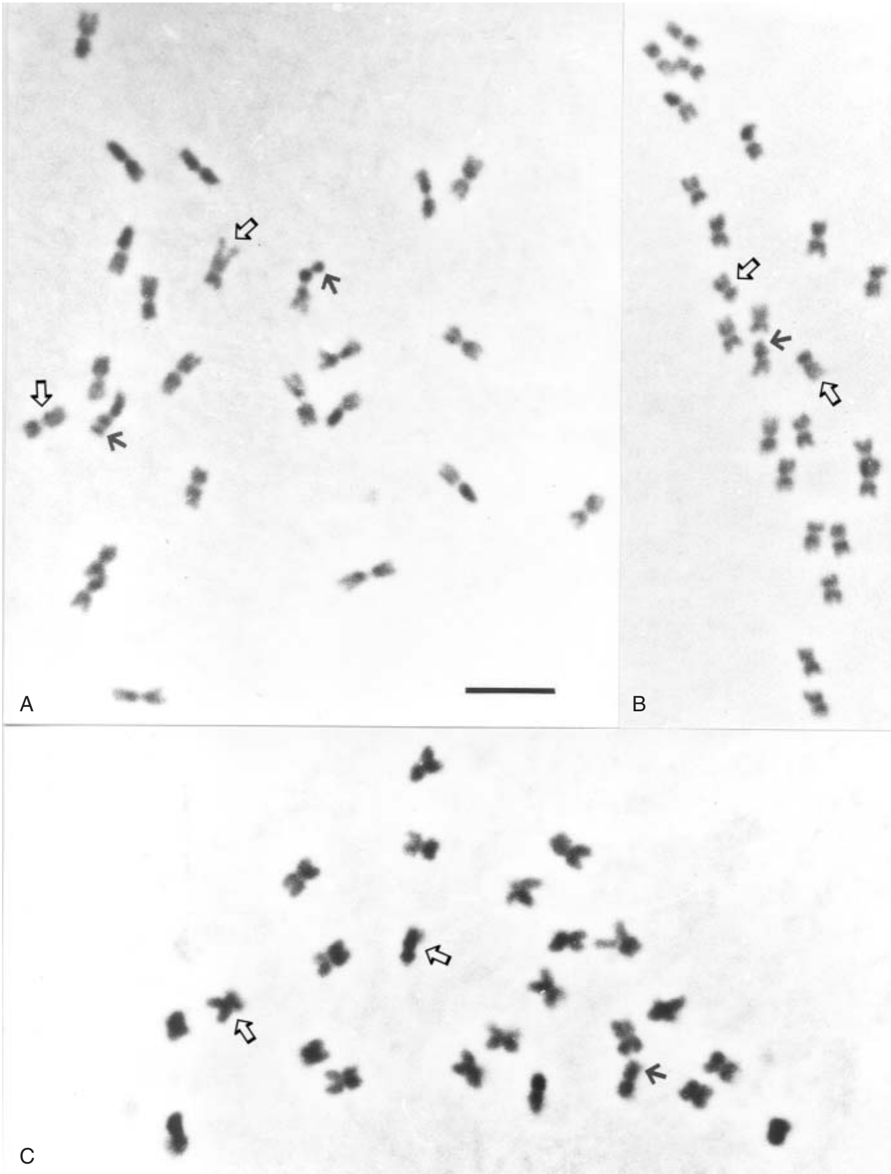

FIG. 1. Photomicrographs of mitotic metaphase in *Lycium* taxa with $2n = 24$. A, *L. chilense* var. *filifolium* (B 111); B, *L. chilense* var. *chilense* (B 845); C, *L. chilense* var. *descolei* (B 785). Scale bar = 5μm, all at same scale. Solid arrows indicate satellites, and hollow arrows the submetacentric (*sm*) chromosomes.

Hunziker, 2001). It is considered the ancestral basic number (Raven, 1975; Grant, 1982). Subfamily *Solanoideae* also has $x = 12$, with the exception of tribe *Nicandreae* Wettst. which has $x = 10$, 11 (Hunziker, 2001). Published data also suggest that $x = 12$ is the basic number for tribe *Lycieae* (Stiefkens & Bernardello, 2002).

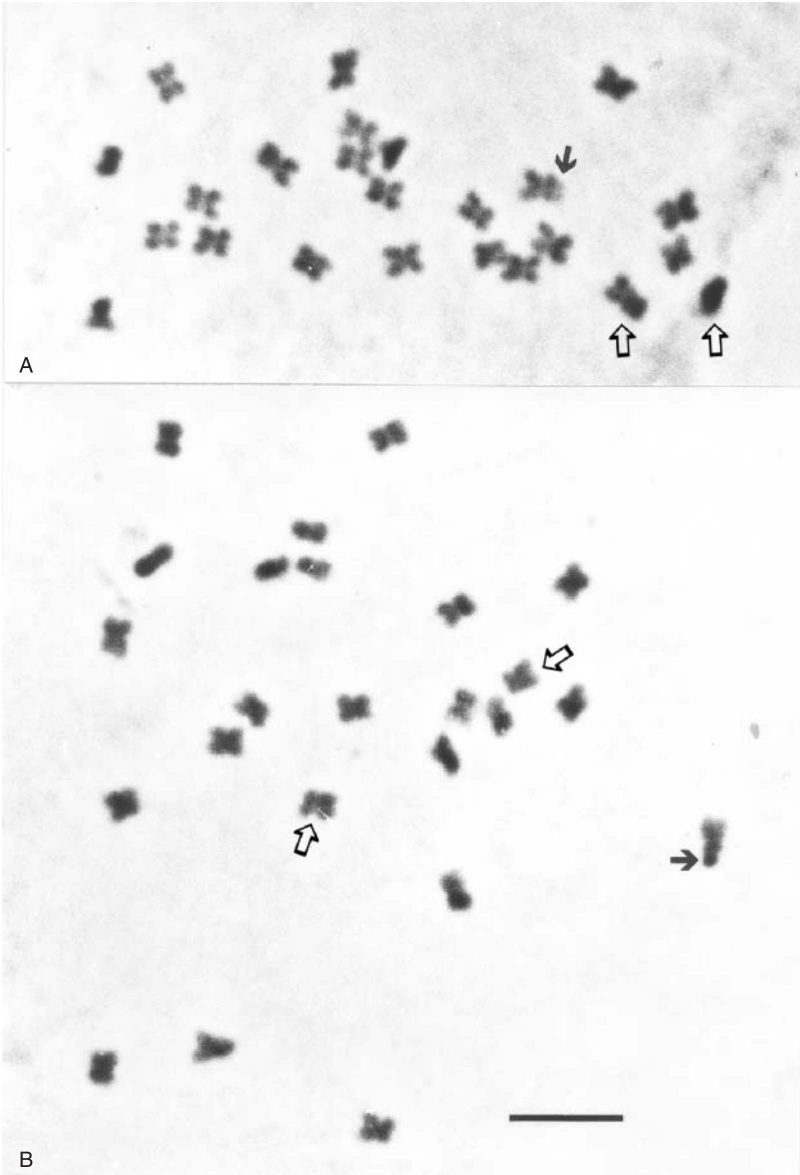

FIG. 2. Photomicrographs of mitotic metaphase in *Lycium* taxa with $2n=24$. A, *L. ciliatum* (S 7); B, *L. ameghinoi* (B 783). Scale bar = 5µm, both at same scale. Solid arrows indicate satellites, and hollow arrows the submetacentric (*sm*) chromosomes.

Polyploidy is known in various species of *Lycium* world-wide, with 3*x*, 4*x*, 6*x*, 8*x* and 10*x* recorded (Lewis, 1961; Baquar *et al*., 1965; Spies *et al*., 1993; Minne *et al*., 1994). In some populations of *L. ciliatum* and *L. chilense*, tetraploids have been detected (Bernardello, 1982; Stiefkens & Bernardello, 2000), and with the new data

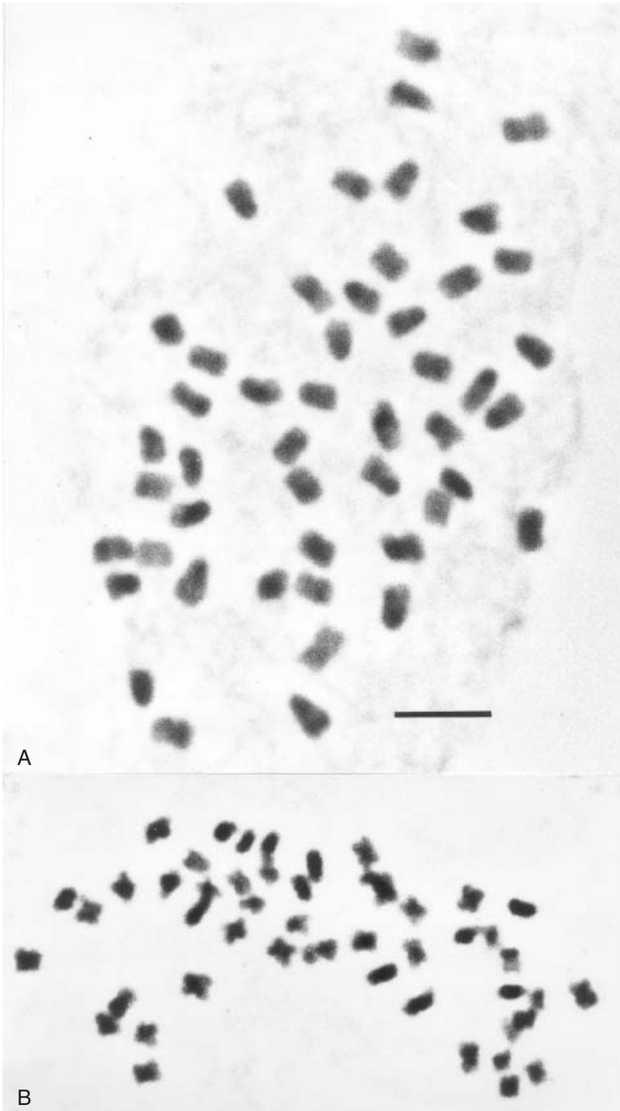

FIG. 3. Photomicrographs of mitotic metaphase in *Lycium* taxa with $2n=48$. A, *L. chilense* var. *filifolium* (B 756); B, *L. ciliatum* (S 8). Scale bar = 5μm, both at same scale.

obtained here it seems that they are not rare in section *Schistocalyx*. Previous meiotic studies on the tetraploids (Bernardello, 1982) indicated that they formed normal bivalents. As both diploid and polyploid populations observed grow in arid and semiarid environments, no correlation can be drawn between the ploidy level and aridity, as reported in other cases (Stebbins, 1985; Poggio *et al.*, 1989).

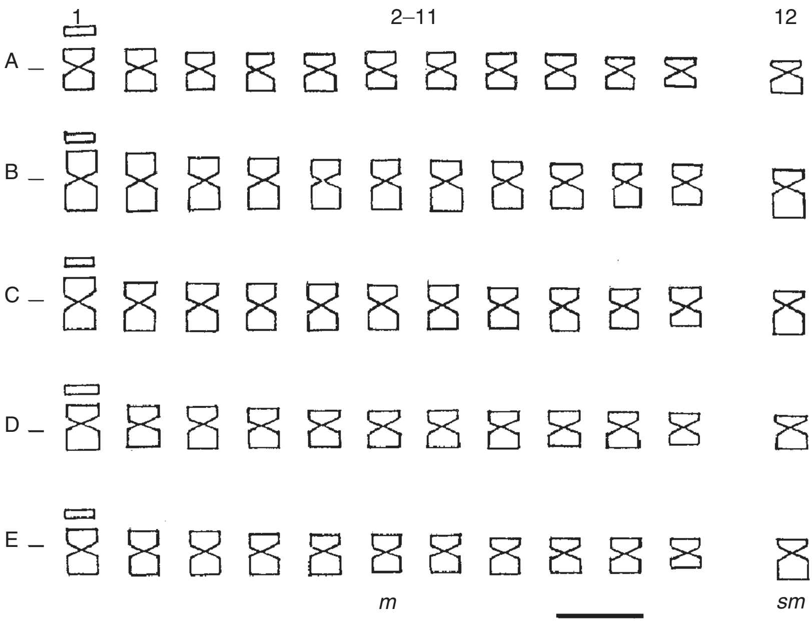

FIG. 4. Idiograms for each taxon based on mean values. A, *L. chilense* var. *chilense*; B, *L. chilense* var. *filifolium*; C, *L. chilense* var. *descolei*; D, *L. ciliatum*; E, *L. ameghinoi*. Scale bar = 5µm, all at same scale; *m*, metacentric; *sm*, submetacentric.

TABLE 4. Results of Bonferroni's test on the karyological variables analysed in the diploid *Lycium* taxa studied. The variables included for each pair of species are statistically significant

L. chilense var. *descolei*	A_1, tl, C, c_{12}			
L. chilense var. *filifolium*	A_1, tl, C, c_1, c_{12}	tl, C, c_1, c_{12}		
L. ciliatum	A_1	c_{12}	tl, C, c_1, c_{12}	
L. ameghinoi	A_1	A_2, R	tl, C, c_1, c_{12}	A_2, R
	L. chilense var. *chilense*	*L. chilense* var. *descolei*	*L. chilense* var. *filifolium*	*L. ciliatum*

A_1, intrachromosomal asymmetry index; A_2, interchromosomal asymmetry index; tl, total haploid chromosome length; C, mean chromosome length; R, ratio between largest and smallest chromosomes of complement; c_1, c_{12}, mean chromosome lengths of pairs 1 and 12.

As reported for *Solanum* L. and other *Solanaceae* (Stebbins, 1971; Moscone, 1989a,b; Bernardello & Anderson, 1990; Bernardello *et al.*, 1994), the *Lycium* karyotypes examined are constant and symmetrical. The existence of one satellited chromosome pair is common in the family (Moscone, 1989a), usually in the shorter arms of *m* pairs. At the same time, *m* and *sm* chromosomes are very frequent (Moscone, 1989a,b, 1990; Bernardello & Anderson, 1990; Bernardello *et al.*, 1994).

In *Magnoliophyta*, symmetrical karyotypes have been correlated with ancestral taxa (Stebbins, 1971). Molecular studies have shown subfamily *Solanoideae* to be

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monophyletic and derived (Olmstead & Palmer, 1992; Olmstead *et al*., 1999). Within it, tribe *Lycieae* has a basal position (Olmstead *et al*., 1999) with some plesiomorphic features, such as woody habit and highly symmetrical karyotypes.

Woody perennials, in contrast with annuals, frequently have constant, less diversified karyotypes (Brandham, 1983; Ehrendorfer, 1983), a trend supported by our results and by data on other woody *Solanaceae* such as *Capsicum* L. (Moscone *et al*., 1993). Previous studies in other South American *Lycium* species from several sections (Bernardello *et al*., 1995; Stiefkens & Bernardello, 1996, 2000, 2002) indicate that although the taxa are morphologically different (Bernardello, 1986; Bernardello & Chiang-Cabrera, 1998), this was not accompanied by variation in chromosome morphology. Cryptic structural changes (i.e. paracentric inversions or reciprocal translocations of segments of similar length; Stebbins, 1958) could have taken place, as these changes cannot be detected with the staining methods used. However, earlier meiotic studies, which included a hybrid (Bernardello, 1982; Chiang, 1982; Bernardello *et al*., 1995), found normal formation of bivalents, suggesting that large inversions or translocations have not occurred.

Some *Lycium* species from Iran (Sheidai *et al*., 1999) and China (Dongli *et al*., 2000) studied karyotypically showed either the same karyotype formula as found here or a very similar one. Thus, karyotype structure seems to be a conservative character in the genus. The same phenomenon was detected, for instance, in *Aloaceae* tribe *Aloinae* (Brandham, 1976).

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Appendix

Chromosome measurements in µm, range and means ± standard deviation, and arm ratios of Lycium *taxa analysed*

S, short arm; l, long arm; c, total chromosome length; r, arm ratio.

L. chilense var. *chilense*

Pair	s	l	c	r
1	0.9–1.1	0.9–1.15	1.1–2.25	1.02
	0.98 ± 0.005	1.01 ± 0.006	2.0 ± 0.11	
2	0.8–1.0	0.9–1.1	1.7–2.1	1.04
	0.93 ± 0.007	0.98 ± 0.006	1.91 ± 0.12	
3	0.8–1.0	0.8–1.0	1.6–2.0	1.05
	0.88 ± 0.006	0.92 ± 0.008	1.81 ± 0.14	

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(Cont'd)

Pair	s	l	c	r
4	0.8–1.0	0.8–1.0	1.6–2.0	1.02
	0.87 ± 0.006	0.9 ± 0.007	1.77 ± 0.13	
5	0.7–0.9	0.8–1.0	1.5–1.9	1.11
	0.81 ± 0.007	0.90 ± 0.005	1.72 ± 0.11	
6	0.7–0.9	0.8–1.0	1.5–1.9	1.10
	0.8 ± 0.008	0.88 ± 0.007	1.68 ± 0.14	
7	0.65–0.9	0.8–1.0	1.45–1.9	1.12
	0.77 ± 0.007	0.86 ± 0.006	1.63 ± 0.11	
8	0.65–0.9	0.7–0.9	1.35–1.8	1.06
	0.76 ± 0.007	0.81 ± 0.06	1.58 ± 0.12	
9	0.65–0.85	0.7–0.95	1.35–1.8	1.08
	0.74 ± 0.006	0.80 ± 0.008	1.54 ± 0.14	
10	0.6–0.8	0.6–0.8	1.2–1.6	1.06
	0.69 ± 0.004	0.74 ± 0.007	1.43 ± 0.11	
11	0.5–0.8	0.6–0.8	1.1–1.6	1.05
	0.64 ± 0.009	0.67 ± 0.007	1.31 ± 0.15	
12	0.4–0.6	0.9–1.25	1.3–1.85	1.95
	0.49 ± 0.004	0.97 ± 0.10	1.46 ± 0.15	

L. chilense var. *descolei*

Pair	s	l	c	r
1	0.9–1.3	0.95–1.45	1.85–2.75	1.11
	1.12 ± 0.11	1.25 ± 0.13	2.37 ± 0.23	
2	0.8–1.2	0.9–1.3	1.7–2.5	1.08
	1.07 ± 0.11	1.16 ± 0.12	2.23 ± 0.23	
3	0.8–1.20	0.8–1.4	1.6–2.6	1.09
	1.03 ± 0.10	1.13 ± 0.15	2.16 ± 0.24	
4	0.75–1.2	0.85–1.35	1.6–2.55	1.07
	1.03 ± 0.11	1.10 ± 0.13	2.13 ± 0.23	
5	0.7–1.15	0.8–1.3	1.5–2.45	1.13
	0.96 ± 0.12	1.1 ± 0.15	2.06 ± 0.26	
6	0.7–1.1	0.8–1.3	1.5–2.4	1.10
	0.96 ± 0.11	1.06 ± 0.16	2.02 ± 0.27	
7	0.7–1.05	0.8–1.35	1.5–2.4	1.10
	0.93 ± 0.10	1.03 ± 0.17	1.97 ± 0.27	
8	0.65–1.10	0.8–1.2	1.45–2.3	1.12
	0.89 ± 0.14	1.0 ± 0.13	1.9 ± 0.26	
9	0.7–1.05	0.7–1.2	1.4–2.25	1.11
	0.86 ± 0.11	0.95 ± 0.14	1.81 ± 0.25	
10	0.6–1.0	0.7–1.2	1.3–2.2	1.22
	0.76 ± 0.12	0.93 ± 0.16	1.70 ± 0.26	
11	0.6–1.0	0.65–1.1	1.25–2.1	1.12
	0.76 ± 0.11	0.85 ± 0.15	1.62 ± 0.26	
12	0.5–0.85	1.0–1.55	1.5–2.4	1.94
	0.64 ± 0.009	1.25 ± 0.15	1.9 ± 0.24	

L. chilense var. *filifolium*

Pair	s	l	c	r
1	0.95–1.8	1.05–2.0	2.0–3.8	1.14
	1.39±0.31	1.59±0.37	2.98±0.66	
2	1.0–1.55	1.0–1.9	2.0–3.45	1.18
	1.3±0.21	1.54±0.35	2.84±0.56	
3	1.0–1.5	1.0–1.8	2.0–3.3	1.13
	1.29±0.18	1.46±0.32	2.75±0.50	
4	1.0–1.5	1.0–1.7	2.0–3.2	1.08
	1.28±0.19	1.39±0.26	2.67±0.45	
5	0.9–1.4	1.1–1.7	2.0–3.1	1.14
	1.21±0.19	1.39±0.23	2.6±0.41	
6	0.9–1.4	1.0–1.6	1.2–3.0	1.09
	1.21±0.18	1.32±0.23	2.53±0.42	
7	0.85–1.4	0.9–1.6	1.75–3.0	1.14
	1.15±0.19	1.32±0.26	2.47±0.46	
8	0.8–1.3	1.0–1.5	1.8–2.8	1.14
	1.11±0.18	1.27±0.19	2.38±0.37	
9	0.8–1.2	0.9–1.3	1.7–2.5	1.11
	1.04±0.15	1.16±0.15	2.2±0.29	
10	0.8–1.2	0.8–1.3	1.6–2.5	1.10
	1.02±0.14	1.13±0.19	2.15±0.33	
11	0.7–1.0	0.8–1.2	1.5–2.2	1.18
	0.9±0.12	1.07±0.15	1.97±0.27	
12	0.6–1.0	1.1–1.9	1.7–2.9	1.95
	0.85±0.14	1.66±0.32	2.51±0.46	

L. ciliatum

Pair	s	l	c	r
1	0.85–1.15	0.9–1.25	1.75–2.4	1.10
	0.98±0.008	1.09±0.10	2.08±0.17	
2	0.75–1.05	0.9–1.32	1.65–2.37	1.13
	0.93±0.10	1.06±0.12	2.0±0.20	
3	0.8–1.05	0.85–1.2	1.65–2.25	1.06
	0.94±0.10	1.0±0.09	1.95±0.18	
4	0.8–1.0	0.8–1.15	1.6–2.15	1.09
	0.90±0.009	0.99±0.10	1.90±0.17	
5	0.75–1.0	0.8–1.1	1.55–2.1	1.09
	0.87±0.008	0.96±0.009	1.83±0.17	
6	0.7–1.0	0.8–1.2	1.5–2.2	1.15
	0.84±0.009	0.96±0.11	1.80±0.18	
7	0.57–1.0	0.82–1.05	1.4–2.05	1.15
	0.81±0.11	0.93±0.007	1.75±0.18	
8	0.55–0.92	0.8–1.05	1.35–1.97	1.16
	0.78±0.11	0.91±0.008	1.69±0.17	

(Cont'd)

Pair	s	l	c	r
9	0.5–0.92	0.8–1.0	1.3–1.92	1.16
	0.75 ± 0.11	0.88 ± 0.008	1.63 ± 0.18	
10	0.6–0.9	0.75–1.0	1.35–1.9	1.16
	0.73 ± 0.11	0.85 ± 0.07	1.58 ± 0.16	
11	0.5–0.9	0.7–0.95	1.2–1.85	1.17
	0.69 ± 0.10	0.81 ± 0.008	1.51 ± 0.17	
12	0.3–0.6	0.85–1.20	1.15–1.8	2.09
	0.48 ± 0.008	1.0 ± 0.009	1.48 ± 0.16	

L. ameghinoi

Pair	s	l	c	r
1	0.9–1.1	0.9–1.35	1.8–2.45	1.06
	1.01 ± 0.006	1.08 ± 0.16	2.09 ± 0.21	
2	0.8–1.1	0.9–1.2	1.7–2.3	1.09
	0.95 ± 0.10	1.03 ± 0.11	1.98 ± 0.21	
3	0.7–1.1	0.9–1.2	1.60–2.3	1.12
	0.90 ± 0.11	1.01 ± 0.12	1.91 ± 0.23	
4	0.6–1.05	0.8–1.3	1.4–2.35	1.17
	0.84 ± 0.13	0.99 ± 0.16	1.83 ± 0.28	
5	0.6–1.0	0.7–1.2	1.3–2.2	1.12
	0.81 ± 0.13	0.91 ± 0.14	1.72 ± 0.27	
6	0.6–1.0	0.7–1.2	1.3–2.2	1.11
	0.8 ± 0.13	0.89 ± 0.15	1.69 ± 0.27	
7	0.5–1.0	0.7–1.0	1.2–2.1	1.22
	0.72 ± 0.15	0.88 ± 0.14	1.61 ± 0.29	
8	0.5–0.9	0.7–1.1	1.2–2.0	1.28
	0.68 ± 0.14	0.88 ± 0.12	1.56 ± 0.25	
9	0.5–0.9	0.6–1.0	1.1–1.9	1.24
	0.66 ± 0.15	0.82 ± 0.12	1.48 ± 0.28	
10	0.4–0.9	0.6–1.0	1.0–1.9	1.16
	0.63 ± 0.15	0.74 ± 0.13	1.38 ± 0.28	
11	0.4–0.75	0.4–0.9	0.8–1.65	1.11
	0.58 ± 0.12	0.65 ± 0.17	1.23 ± 0.29	
12	0.4–0.7	0.9–1.1	1.3–2.1	2.04
	0.55 ± 0.14	1.12 ± 0.22	1.67 ± 0.36	