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Chromosomes of some argentine angiosperms and their taxonomic significance

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Abstract — Meiotic or mitotic chromosomes of eleven species of angiosperms native in Argentina, belonging to seven families, were studied in order to be used in cytotaxonomic studies and gain some insights on the evolutive relationships of the different groups. Most of our data were the first cytological information for the species. The genus *Alvaradoa* Liebm. was investigated cytologically for the first time. The novel karyologically studied species, all diploid, were: *Bomarea macrocephala* Pax and *Bomarea stans* Kränz. 2n = 18, *Loasa bergii* Hieron. 2n = 26, *Alvaradoa subovata* Cronquist 2n = 16, *Schizanthus grahamii* Gillies 2n = 20, *Viola montagnei* Gay and *Viola roigii* Rossow 2n = 14. The same numbers to the ones previously reported in the literature were found from different populations of *Cajophora chuquitensis* (Meyen) Urb. & Gilg. 2n = 18, *Ramorinoa girolae* Speg. 2n = 20, and *Schizanthus grahamii* Gillies 2n = 20. The presence of supernumerary chromosomes, 0 to 6, in *Ligaria cuneifolia* (Ruiz & Pav.) Tiegh., 2n = 20, was recorded. Data are compared with previously published results and chromosome figures of all studied species are provided.

Key words: *Alvaradoa*, argentine angiosperms, *Bomarea*, chromosomes, *Loasa*, *Schizanthus*, *Viola*.

INTRODUCTION

Chromosome studies on native angiosperm species from Argentina were carried out in order to be used in cytotaxonomic studies and gain some insights on the evolutive relationships of the different groups. Most of our data are new counts but three of them had the same numbers to ones before now reported for different populations. Data are compared with previously published results and briefly discussed.

MATERIALS AND METHODS

Plant material was collected from wild populations between 2000 and 2003, except one sample from Lagunas de Epulauquen which was collected in 1997.

A complete list of studied species, chromosome numbers and voucher specimens is given in Table 1. Species vouchers were deposited at the Instituto de Botánica Darwinion (SI). Collections were alphabetically ordered by family, genus and species and discussed below in that order.

For meiotic studies, flowers buds were fixed in ethanol-chloroform-glacial acetic acid (6:3:1) for at least 24 h, and then transferred into 70% ethanol and stored at 4-5° C until required. Immature anthers were squashed directly in propionic acid haematoxylin (2%) using ferric citrate as mordant. Meiosis was studied at least in 5-10 PMCs (pollen mother cells) per plant.

Mitotic chromosomes of *Ramorinoa girolae* Gillies were obtained from root tips meristems from germinating seeds and those of *Cajophora chuquitensis* (Meyen) Urb. et. Gilg. from mitosis of pollen grains, these last ones without pretreatment. Ripe seeds of *Ramorinoa girolae* were placed on wet filter paper in Petri dishes. The root tips meristems were pretreated in 2mM 8-hydroxyquinoline for 4 h, fixed in absolute ethanol and glacial acetic acid (3:1) at room temperature and stored in the fixative at 4-5° C. Root tips were hydrolyzed in 5N HCl for 15 minutes at room temperature, and squashed as previously described. Photographs were taken using a photomicroscope Leica DMLB, a Leitz camera and Agfa APX25 film.

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Table 1 — Species, origins, chromosome numbers and type of studied chromosomes.

*New records

Species	Provenance	Chromosome number n/2n	Chromo-somes
Alstromeriaceae			
Bomarea macrocephala Pax *	Tucumán, Dpto. Tafí, Quebrada de Carapunco, 13-XI-2002, Wulff 938 (SI).	2n = 18 9 II	Meiotic
Bomarea stans Kränz. *	Salta, Dpto. Santa Victoria, Rodeopampa, 3000 m, 6-II-2003, Sanso & Xifreda 234 (SI).	2n = 18 9 II	Meiotic
Fabaceae			
Ramorinoa girolae Speg.	San Luis, Dpto. Belgrano, Sierra de las Quijadas, 1-II-2001, Sanso & Pereyra 74b (SI).	2n = 20	Mitotic
Loasaceae			
Loasa bergii Hieron. *	Neuquen, Dpto. Ñorquín, Puerta de Trolope, 4-II-2002, Sanso & Pereyra 123 (SI).	2n = 26 13 II	Meiotic
Cajophora chuquitensis (Meyen) Urb. & Gilg.	Salta, Dpto. Santa Victoria, Abra de Patahuasi, 7-II-2003, Sanso & Xifreda 254 (SI).	n = 8	Mitotic
Loranthaceae			
Ligaria cuneifolia (Ruiz & Pav.) Tiegh.	Salta, Dpto. Anta, en proximidades del dique Campo Alegre, 10-III-2000, Xifreda & Sanso 2083 (SI).	2n = 20 10 II + 0-6 Bs	Meiotic
Simaroubaceae			
Alvaradoa subovata Cronquist *	Salta, Dpto. Chicoana, Quebrada de Escoipe, 2-II-2003, Sanso & Xifreda 160 (SI).	2n = 16 8 II	Meiotic
Solanaceae			
Schizanthus grahamii Gillies	Mendoza, Dpto. Malargüe, Valle de las Leñas, de las Leñas a Valle Hermoso, 22-I-2001, Sanso & Pereyra 58 (SI).	2n = 20 10 II	Meiotic
Schizanthus hookeri Gillies *	Neuquen, Dpto. Minas, Lagunas de Epulauquen, 13-XII-1997, Xifreda & Sanso 2011 (SI).	2n = 20 10 II	Meiotic
Violaceae			
Viola montagnei Gay *	San Juan, Dpto. Ullún, Sierra del Tigre, 3380 m, 10-II-2000, Kiesling, Wulff & Tombesi 9439 (SI).	2n = 14 7 II	Meiotic
Viola roigii Rossow *	San Juan, Dpto. Angaco, Pie de Palo, 3100 m, 07-II-2000, Kiesling, Wulff & Tombesi 9385 (SI).	2n =14 7 II	Meiotic

The following indexes of plant chromosomes numbers were consulted: Bolkhovskikh *et al.* (1969), Moore (1973, 1974, 1977), Goldblatt (1981, 1984, 1985, 1988), Goldblatt and Johnson (1990, 1991, 1994, 1996, 1998, 2000).

RESULTS AND DISCUSSION

The studied species were unknown cytogenetically except *Cajophora chuquitensis* (Meyen) Urb. & Gilg., *Ligaria cuneifolia* (Ruiz & Pav.) Tiegh., *Schizanthus grahamii* Gillies and *Ramorinoa girolae* Speg. (see Table 1 and text below). The novel karyological reports were for: *Bomarea macrocephala* Pax, *Bomarea stans* Kränz., *Loasa bergii* Hieron., *Alvaradoa subovata* Cronquist, *Schizanthus grahamii* Gillies, *Viola montagnei* Gay and *Viola roigii* Rossow

In the species meiotically studied, their behaviour was regular with formation of bivalents (table 1), except by the presence of numerical polymorphism for B-chromosomes in *Ligaria cuneifolia* (see below).

Alstroemeriaceae - Bomarea Mirb. with about 100 species is found from Cuba and Central Mexico to Argentina and Chile and is best represented in the Andean regions of South America (Sanso and Xifreda 2001). *Bomarea macrocephala* (subgenus *Wichuraea*), 2n=18 (figs. 1 and 2) is an erect species, endemic to northern Argentina, provinces of Salta, Jujuy, Catamarca and Tucumán, occurring in “quebradas” at 1800-3000 m above sea level meanwhile *B. stans* (subgenus *Sphaerine*), 2n=18 (fig. 3) inhabits at 2000-3000 m above sea level in Bolivia and Santa Victoria department, Salta, Argentina (Sanso and Xifreda 1995). Both species showed at diakinesis or metaphases I, nine bivalents of different morphologies, in general with a minimum of 2 chiasmata each one. Their size was quite similar and two formed close bivalents with at least, three chiasmata.

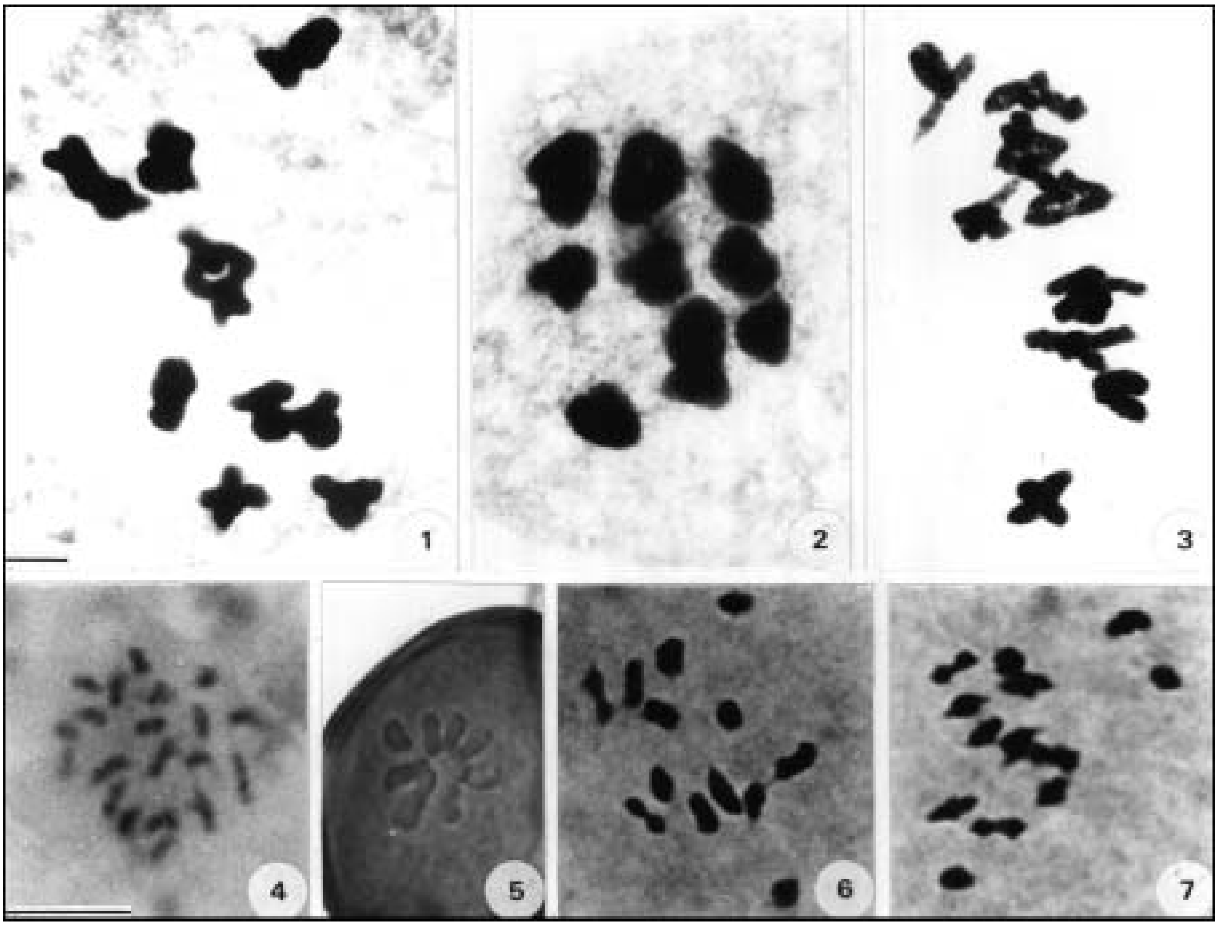
With these new reports, now there are available chromosome data for the four species of this genus growing in Argentina. The remaining two taxa, *B. edulis* (Tuss.) Herb. and *B. boliviensis* Baker were previously studied by SANSO and HUNZIKER (1998).

Up to now, nine species were cytologically studied and all were diploid 2n= 2x= 18, except *B. hirtella* (HBK) Herb. which has been reported as tetraploid (CAVE 1967). The genus may be considered chromosomally stable with a constancy of the chromosome number. The basic chromosome number x= 9, among other characters, distinguishs *Bomarea* from the related genus *Alstroemeria* (SANSO and XIFREDA 2001).

Fabaceae - *Ramorinoa* Speg. (Sub. Papilionoideae, Tribu Dalbergiae) is a monotypic genus, endemic from Argentina growing in sandy and rocky places of San Juan, San Luis and La Rioja provinces, between 2000 and 3200 m above sea level. Its species, *Ramorinoa girolae*, "the chica", includes from leafless shrubs of 2-3 meters to trees of 10 meters of high with the branches finishing in dry spines (GÓMEZ-SOSA 1994). In this species, twenty chromosomes at mitotic metaphases were observed (fig. 4). The chromosomal size was small, between 1.7 and 3.5 μm. Previously, SUBILS (1982) reported the same chromosome number and showed a drawing with 10 II in PCMs.

Loasaceae - Our observations done on pollen grains of *Cajophora chuquitensis* showed eight somatic chromosomes: one large, six of medium size and one small (fig. 5). BRÜCHER (1986) observed in diploid cells (sub *C. heptamera* (Wedd.) Urb. & Gilg.) four large, eight of medium size and four small chromosomes.

Cajophora C. Presl. comprises about 34 species with its major distribution center in South America, andine regions of Chile, Bolivia and Argentina (WEIGEND 1997). The genus is quite uni-



Figures 1-7. — 1-2, *Bomarea macrocephala* Pax, diakinesis, 9 II; 3, *Bomarea stans* Kränz., diakinesis, 9 II; 4, *Ramorinoa girolae* Speg., mitotic metaphase, 2n = 20; 5, *Cajophora chuquitensis* (Meyen) Urb. & Gilg., pollen grain mitosis, n = 8; 6-7, *Loasa bergii* Hieron., diakinesis, 13 II. Scale bar = 10 μm. Figs. 1-3 and 4-7 with the same scales.

form karyologically, all the studied species with 2n = 16 chromosomes, except *C. buraeavii* Urb. & Gilg., 2n = 14 (Huynh cited by Bolkhovskikh *et al.* 1969) and *C. silvestris* (Poepp.) Urb. & Gilg., n = 26 (Grau 1988).

The remaining species whose chromosomes have been previously studied, all with 2n = 16, are: *Cajophora carduifolia* K. Presl - sub *C. sepiaria* (Ruiz & Pav. ex G. Don) Macbr.- (Fernández Casas 1982), *C. cernua* (Griseb.) Urb. & Gilg. (Poston and Thompson 1977; Brücher 1986; Brücher 1989; Bernardello *et al.* 1990), *C. aconquijae* Sleumer, *C. clavata* Urb. & Gilg., *C. coronata* (Gillies ex Arn.) Hook. & Arn. (Huynh cited by Bolkhovskikh *et al.* 1969), *C. hibiscifolia* (Griseb.) Urb. & Gilg., *C. lateritia* Klotzsch (Poston and Thompson 1977; Brücher 1986, Brücher 1989 and other authors cited by Bolkhovskikh *et al.* 1969), *C. macrocarpa* Urb. & Gilg., *C. rosulata* (Wedd.) Urb. & Gilg. (Brücher 1986; Brücher 1989). *C. nivalis* Lillo (Brücher 1986) and *C. rusbyana* Urban & Gilg. (Diers 1961).

Loasa bergii Hieron. showed at diakinesis and metaphases I 13 II (figs. 6 and 7).

Loasa Adans exhibits as basic chromosome numbers x= 12, 13 and 14 although other different numbers have been found. The species cytologically known are: *Loasa acanthifolia* Desr., 2n= 26 (Tschischow 1956; Grau 1988) and n = 19 (Poston and Thompson 1977), *L. arnottiana* Gay, *L. artemisiifolia* Poepp. ex Urb. & Gilg., *L. lateritia* Gillies ex Arn., *L. pallida* Gillies ex Arn., 2n = 26 (Grau 1988), *L. tricolor* Ker- Gawl., 2n = 26 (Poston and Thompson 1977; Grau 1988), *L. malesherbioides* Phil., 2n = 12, *L. triloba* Domb. ex Juss., 2n = 36, *L. urmenetae* Phil., 2n = 24 (Grau 1988), *L. rupestris* Gardner, n = 12 (Coleman and Smith 1969), *L. triphylla* Juss., n = 14 (Poston and Thompson 1977 and other authors cited by Bolkhovskikh *et al.* 1969), *L. ferruginea* Urban & Gilg., n = 14 and n = 15, *L. rudis* Benth., n = 14, *L. speciosa* J. D. Smith and *L. aurantiaca* Urban & Gilg., n = 12, *L. macrantha* Urb. & Gilg., n = 14, *L. urens* Jacq. n = 14 and n = 15 (sub *L. hispida L.*), *L. poissoniana* Urban & Gilg., n = 14, *L. erinus*?, 2n = 40 and *L. vulcanica* Andre, n= 14 (several authors cited by Bolkhovskikh *et al.* 1969).

Loranthaceae - *Ligaria* Tiegh. is a monotypic genus with a very polymorphic species occurring in arid-subtemperate areas of South America (Barlow and Wiens 1971). Cells of *Ligaria cuneifolia* showed numerical polymorphism for B-chromosomes. The observed configuration at diakinesis and metaphases I was 10 II + 0-6 B-chromosomes and 20 A-chromosomes plus a variable number of B-chromosomes at anaphases I (fig. 8). The number of A-chromosomes confirms previous reports obtained by Covas and Schnack (1946) - sub *Psittacanthus cuneifolius* (Ruiz & Pav.) Blume and Barlow and Wiens (1971), but the presence of supernumerary chromosomes has not been reported before. They were smaller than A-chromosomes and at metaphases I they tended to be non-congressed. In several species, interpopulational differences with respect to B-chromosome frequencies were recorded and correlations between these and different variables such as latitude, altitude, climatic factors or genome size were pointed out (Rosato *et al.* 1998). Due to the high number of B-chromosomes found in *Ligaria cuneifolia* and its large chromosome size, this species constitutes an interesting model to take into account in order to study these kinds of correlations.

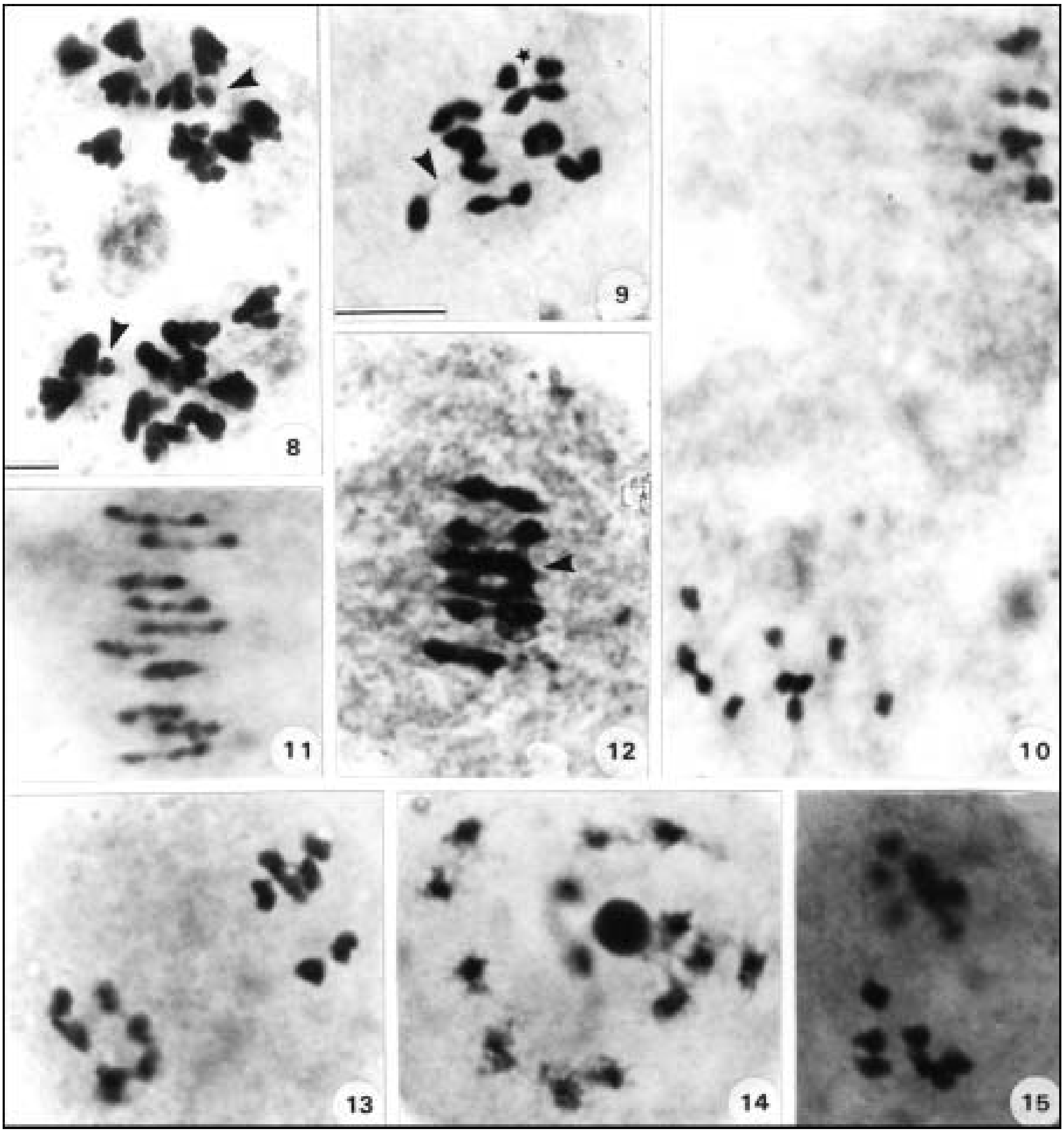
B-chromosomes were observed in other genera of the family as *Phoradendron*. In *P. californicum* Nutt. up to 8 supernumerary chromosomes were present (Wiens 1964).

Covas and Schnack (l. c.) recorded the presence of polyploidy individuals and suggested that the genus would possess the largest chromosomes in the dicots.

Simaroubaceae - Alvaradoa subovata, the "pichi blanco" is a dioecious tree 3-6 m height growing in the inferior soil of the "yungas", from department of Santa Cruz, S Bolivia, to the argentine NW provinces of Jujuy, Salta and Tucumán (Xifreda and Sanso 1998). Meiotic studies revealed 8 bivalents at diplotene and diakinesis (fig. 9). At least one of them, appeared to be heteromorphic and presented desynapsis and, one pair was associated to the nucleolus at diplotene. The chromosomes were small, with a size ranged between 1.5 and 2.5 *μm.*

In Argentina there are other 7 species of Simaroubaceae pertaining to 5 genera. However, cytological information about them has to our knowledge been restricted only to *Ailanthus altissima* (Mill.) Swingle, 2n= 64 (Murin 1978), 2n =80 (Desai cited by Bolkhovskikh *et al.* 1969) and *Castela coccinea* Griseb., 2n = 26 (Bernardello *et al.* 1990).

Other Simaroubaceae species which chromosome numbers have been reported are: *Ailanthus excelsa* Roxb., 2n = 62 (Pathak *et al.* cited by Bolkhovskikh *et al.* 1969), 2n= 86 (Ghosh cited by Moore 1973), *A. grandis* Prain, n = 31 (Me-



Figures 8-15. — 8, *Ligaria cuneifolia* (Ruiz & Pav.) Tiegh., anaphase I, 2n = 20 + 2 B chromosomes. The arrowheads point B-chromosomes; 9, *Alvaradoa subovata* Cronquist, diakinesis, 8 II. Two pairs presenting desynapsis are pointed out, the arrowhead shows the heteromorphic one; 10, *Schizanthus hookeri* Gillies, late anaphase I, 10 chromosomes at each pole; 11, *Schizanthus grahamii* Gillies, metaphase I, 10 II; 12-13, *Viola montagnei* Gay, 12, metaphase I, 7 II, the arrowhead points one sneaky II, 13, late anaphase I, 7 chromosomes at each pole; 14-15, *Viola roigii* Rossow, 14, diplotene, 7 II, 15, late anaphase I, 7 chromosomes at each pole. Scale bar = 10 µm. Figs. 9-15 with the same scale.

HRA, KHOSLA cited by GOLDBLATT 1981), n = 32 (GILL and SINGHAL 1979), *A. pubescens* Benth., 2n = 36 (MORTON 1962), *Brucea mollis* Wall, n = 12 (MEHRA and KHOSLA 1969; KHOSLA cited by GOLDBLATT 1981), *Castela polyandra* Moran & Felger, n = 13 (MORAN and FELGER cited by MOORE 1973), *Picraena excelsa* Lindl. (= *Picrasma excelsa Planch.*), ca. 60 (JANAKI cited by BOLKHOVSKIKH *et al.* 1969), *Picrasma javanica* Bl., n = 12 (MEHRA and KHOSLA 1969; KHOSLA cited by GOLDBLATT 1981), *P. nepalensis* Benn., n = 12 (MEHRA, KHOSLA cited by GOLDBLATT 1981) *P.*

quassioides Benn. (= *P. ailanthoide Planch.*), 2n = 50 (Nakajima cited by Bolkhovskikh *et al.* 1969), n = 12 (Sandhu and Mann cited by Goldblatt and Johnson 1991), *Simaruba glauca* DC., n = 16 (Bawa 1973).

The basic chromosome number found in *Alvaradoa* Liebm., and investigated here for the first time, x = 8, suggests that several of the taxa previously cited would be polyploidy species.

A close relationship between *Alvaradoa* Liebm. and *Picramnia* Sw. has been suggested by several authors and discussed by Fernando *et al.* (1995). Fernando & Quinn (1995) proposed the pertaining of *Alvaradoa* and *Picramnia* to a new family, Picramniaceae (Engl.) Fernando & Quinn. At present, the chromosome number for *Picramnia* keeps unknown and therefore since the karyological point of view is not possible to support or reject such closely put forward relationship between both genera.

Solanaceae - The observed chromosome number 2n= 20 = 10 II in PCMs of a population of *Schizanthus grahamii* Gillies (fig. 11) from Valle de las Leñas, Mendoza, agrees with a previous report from a population of Cuesta de Rahue, Neuquén done by Moscone (1992) also on microsporocytes. Most of the bivalents, nine out ten, presented only one terminal chiasma.

S. hookeri, the other argentine species, from Lagunas de Epulauquen, Neuquén, just as *S. grahamii* exhibited at diakinesis and metaphases I 10 II and 10 chromosomes at each pole at late anaphases I (fig. 10).

Concerning the basic chromosome number in *Schizanthus* Ruiz & Pav., x = 10 seems to be the most represented. Other cytologically studied species as *S. pinnatus* Ruiz & Pav., *S. wisetonensis* Hort. and *S. retusus* Hook. presented the same chromosome number (2n= 20) although for *S. retusus* 2n = 22 was also recorded (several authors cited by Bolkhovskikh *et al.* 1969).

Violaceae - *Viola montagnei* inhabits in Chile and in Argentina, from Catamarca to Mendoza (figs. 12 and 13). *V. roigii* is endemic in Sierra Pie de Palo, San Juan, Argentina (figs. 14 and 15). Both of them, perennial species with their leaves forming rosettes, belong to *Viola* section *Andinium* W. Becker and grow in high mountains. PCMs showed 7 II at diplotene (fig. 14), diakinesis and metaphases I and, 14 chromosomes at anaphases I (figs. 13 and 15). The chiasmata, only one per bivalent, were observed in terminal positions (figs. 12 and 14).

The genus probably arose in the Andes, and South America groups seem to be the most primitive in *Viola* L. (Clausen 1929; Valentine 1962). Parsimony and maximum likelihood approaches using molecular data also support an Andean origin for the genus and show all near basal groups retaining chromosome numbers based on x = 6 (Ballard *et al.* 1999). However, the species studied in this survey presented x = 7. The absence of karyological information on South American species prevents further comments on taxonomic aspects but it is known that in *Viola* karyological features contribute significantly to a better understanding of the systematics.

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