

Alstroemeria presliana HERB. (ALSTROEMERIACEAE) IN CHILE FROM A CYTOGENETIC PERSPECTIVE

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ABSTRACT

Alstroemeria (Alstroemeriaceae) is an endemic genus of South America with two major distribution centers in the continent: Chile and Brazil. In Chile the genus is distributed from the North, near Iquique (20°13' S, 70°09' W) to the Chilean and Argentine Patagonia (53°10' S, 70°54' W). The central zone of Chile presents the highest number of species. *A. presliana* Herb. grows from Curicó (34°59' S, 71°14' W) to Cautín (38°45' S, 72°34' W) in Chile and Neuquén (36°50' S, 71°05' W), Argentina. A comparative karyotype study was made between a population of *A. presliana* subsp. *presliana* and a population of *A. presliana* subsp. *australis* Ehr. Bayer. Both populations presented asymmetric karyotypes, with $2n = 2x = 16$ chromosomes, but with different chromosome formulae: *A. presliana* subsp. *presliana* has a haploid formula with $4m + 1sm\text{-sat} + 1st\text{-sat} + 2t$, i.e., four pairs of metacentric chromosomes, one submetacentric pair with satellite, one subtelocentric pair with satellite, and two telocentric pairs. *A. presliana* subsp. *australis* has a formula with $2m + 1m\text{-sat} + 1sm + 4t$ chromosomes, i.e., two pairs of metacentric chromosomes, one metacentric pair with satellite, one submetacentric pair, and four telocentric chromosomes. These results indicated that the karyotype of the subspecies is very different, and it would be possible to recognize *A. presliana* subsp. *australis* as a new species.

Kew words: Alstroemeriaceae, karyotype, chromosome numbers, Chile.

INTRODUCTION

Alstroemeria is a South American genus of Alstroemeriaceae that includes around 50 species found from Brazil to the Patagonian Region of Argentina and Chile, in highly diverse habitats ranging from sea level to 4000 m altitude (Bayer, 1987; Ravenna, 1988; Sanso, 2002; Aagesen and Sanso, 2003). Central Chile is recognized as a center of diversity of the genus (Bayer, 1987), with satellite distribution in central and eastern Brazil. Approximately 32 species grow in Chile (Bayer, 1987; Muñoz and Moreira, 2003) and are found between 20° and 53° S lat, with the major number of taxa between 28° and 37° S (Muñoz and Moreira, 2003).

The Chilean species of *Alstroemeria* have acquired worldwide importance as ornamental plants, due to the beauty of their flowers (Buitendijk *et al.*, 1997). Many of the species have acquired considerable commercial value and they are cultivated in different countries, i.e., Holland, Great Britain, Japan and the USA (Baeza *et al.*, 2007a). The main factors for this attention are the harvest durability of the flowers and the attractiveness of the perigonium. There are new cultivars, such as the hybrid of *Alstroemeria*, which are produced by controlled hybridization, mutations and artificial selection (Sanso, 2002).

Alstroemeria presliana Herb. subsp. *presliana* has a very restricted distribution in the Andes, from Curicó (35°27' S lat) to Antuco (37°25' S lat), from 1500 to 2000 m of altitude. It is also present in Neuquén Province (36°50' S, 71°05' W) in Argentina. On the other hand, *A. presliana* Herb. subsp. *australis* Ehr.

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Bayer only grows in Chile, with a narrow geographic distribution from Curanilahue (37°23' S lat) to the South of the Cautín river (38°29' S lat) from 200 to 1500 m altitude. (Muñoz and Moreira, 2003). These two subspecies have beautiful pink flowers that are basically differentiated by their size and the intensity of the color, as well as their geographic distribution. Without doubt, this species has an enormous potential as an ornamental plant and it is probable that in the future it will be considered an appropriate species for genetic improvement.

The objective of this research was to analyze and compare the morphology, symmetry and size of the chromosomes of *A. presliana* subsp. *australis* and *A. presliana* subsp. *presliana*, with the perspective that the detailed analysis of the chromosomes of related taxa can provide valuable information related to its evolution and their taxonomy (Dimitrova and Greilhuber, 2000).

MATERIALS AND METHODS

Vegetal material

A. presliana samples were collected in two locations in Chile: Termas de Chillán (36°54' S, 71°24' W, 1720 m.a.s.l.), Ñuble Province, Bío-Bío Region, 2 January 2003 by C. Baeza N° 4192 (*A. presliana* subsp. *presliana*), and Piedra del Águila (37°49' S, 73°08' W, 1350 m.a.s.l.), Nahuelbuta National Park, Malleco Province, La Araucanía Region, 15 December 2005 by C. Baeza N° 4250 c (*A. presliana* subsp. *australis*). The material collected was deposited at the Herbarium of the Universidad de Concepción, Concepción, Chile (CONC).

Obtaining karyotypes

Root apexes of 5-10 mm length, obtained from plants grown in greenhouses, were pre-treated with a solution of 8-hydroxyquinoline (2 mM) for 24 h at 4 °C, then fixed in a solution of ethanol/acetic acid (3:1) for 24 h and stored at -20 °C. The root of the population of *A. presliana* subsp. *presliana* (C. Baeza 4192) were washed twice in distilled water for 10 min prior to maceration

and then were digested in a mixture of enzymes of 4% cellulase (Onozuka R-10, Serva, Heidelberg, Germany) and 1% pectolyase Y-23 (Seishin Pharmaceutical, Chiba-Ken, Japan) and 1% pectolyase Y-23 (Seishin Pharmaceutical, Chiba-Ken, Japan) in KCl 75 mM, at pH 4.0 for 25 min at 37 °C. Following a brief washing in distilled water, the roots were placed in acetic acid 45% for 1 min and then were squashed on slides. The prepared samples were stored in a freezer at -84 °C and after 2 h the cover glass were taken out. The samples were dried for 24 h at room temperature and stored at -20 °C. The chromosomes were dyed with DAPI (1 ng L⁻¹ 4', 6-diamidino-2-phenylindole) (Baeza *et al.*, 2007b).

With the population of *A. presliana* subsp. *australis* (C. Baeza 4250 c), after fixation an acid hydrolysis was done with HCl 0.5 N for 25 min at 40 °C, samples were washed twice in distilled water and finally the apex of the root was dyed with acetic orcein at 1% and then squashed. The account and interpretation of the chromosomes (10 metaphasic plates, five individuals) were carried out using an optical microscope (Axioskop Zeiss, Jena, Germany) with a digital camera (Canon PowerShot G6). The chromosomes were measured with the MicroMeasure 3.3 program (Reeves, 2001) and were classified according to arm radius (Levan *et al.*, 1964). The asymmetry of the karyotype (AsI %) was determined for each species analyzed using the formula of Arano and Saito (1980). The length of each chromosome was calculated as the percentage of the total genomic length of the corresponding set of haploid chromosomes. Corel Draw 8.0 was used to prepare the ideograms and the micro-photographs (1160x) were analyzed and contrasted with the Paint Shop Pro 7 Program.

RESULTS AND DISCUSSION

The two populations of *A. presliana* studied have an asymmetric karyotype with $2n = 2x = 16$ chromosomes (Table 1). *A. presliana* subsp. *presliana* has a haploid formula with $4m + 1sm\text{-sat} + 1st\text{-sat} + 2t$, that is, four

Table 1. Comparison of karyotype characteristics of *Alstroemeria presliana* subsp. *presliana* and *A. presliana* subsp. *australis*.

Species	2n	Chromosomal Set	AsI %	R
<i>A. presliana</i> subsp. <i>presliana</i>	16	4m + 1sm-sat + 1st-sat + 2t	64.5	3.2
<i>A. presliana</i> subsp. <i>australis</i>	16	2m + 1m-sat + 1sm + 4t	70.2	2.6

AsI %: karyotype asymmetry index according to Arano and Saito (1986); R: brachial index.

pairs of metacentric chromosomes, one submetacentric pair with a satellite, one subtelocentric pair with satellite and two telocentric pairs (Figure 1A, Table 1). *A. presliana* subsp. *australis* has a $2m + 1m\text{-sat} + 1sm + 4t$ haploid formula, which is three pairs of metacentric chromosomes, pair 2 with a satellite, one submetacentric pair and four pairs of telocentric chromosomes (Figure 1B, Table 1). The values of the asymmetry index of the karyotype (AsI %) and brachial index (R, coefficient between the greater and lesser chromosome pair) (Baeza *et al.*, 2007a) for each subspecies is summarized in Table 1. Graphic representation (ideogram) of the subspecies can be seen in Figure 2. The chromosome

sizes of *A. presliana* subsp. *presliana* and *A. presliana* subsp. *australis* are summarized in Tables 2 and 3, respectively.

Alstroemeria is a genus with great diversity in the Mediterranean region of central Chile. A high number of species and the highest percentage of endemism are concentrated in this region (Muñoz and Moreira, 2003). Both subspecies of *A. presliana* are found in this area. Based on collected material in the Nahuelbuta, Bayer (1987) described the subspecies *australis* in the Nahuelbuta mountain range, in the Araucanía Region of Chile. In addition to their distinct distribution, the

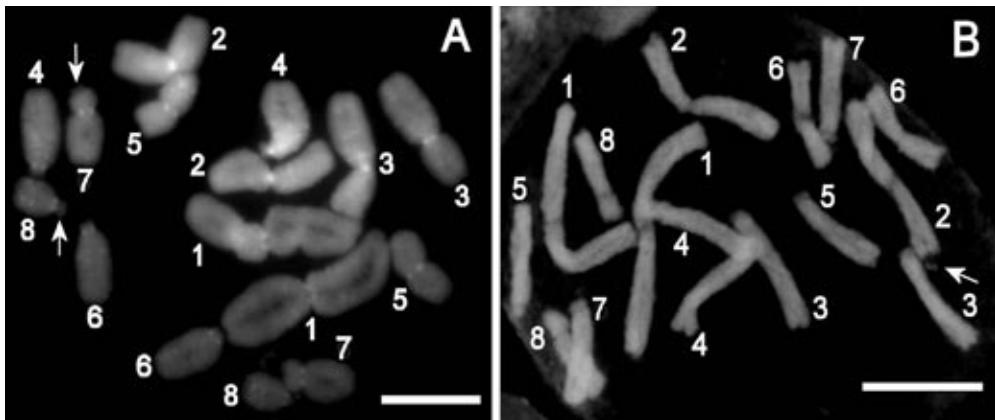


Figure 1. Metaphase plate of (A) *Alstroemeria presliana* subsp. *presliana*, Nevados de Chillán (Baeza 4192), (B) *A. presliana* subsp. *australis*, Parque Nacional Nahuelbuta (Baeza 4250 c).

Arrows indicate the positions of satellites. Scale bar = 10 μ m.

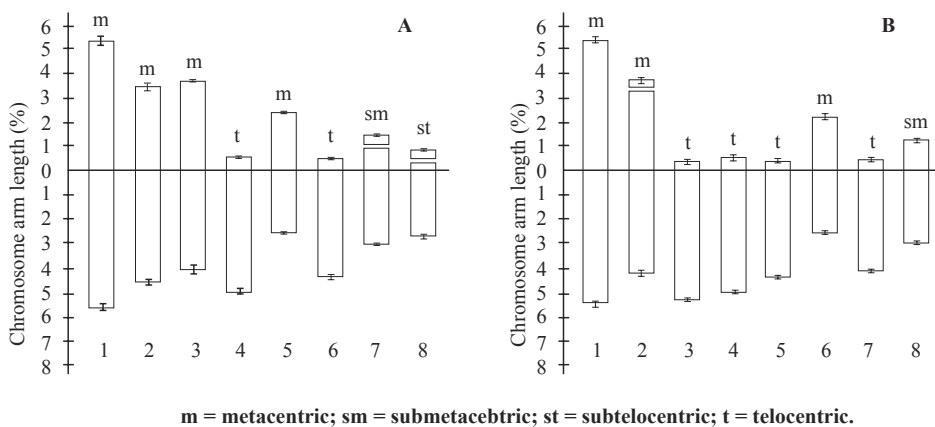


Figure 2. Haploid ideogram of (A) *Alstroemeria presliana* subsp. *presliana*, Nevados de Chillán (Baeza 4192), (B) *A. presliana* subsp. *australis*, Parque Nacional Nahuelbuta (Baeza 4250 c).

Chromosomes have been ordered in decreasing size.

size and the more intense pink coloring of the flowers distinguishes subsp. *australis* from the typical subspecies (Bayer 1987; Muñoz and Moreira, 2003), which have a broader distribution in Chile and Argentina (Neuquén).

There are many works related to chromosome studies in *Alstroemeria* species (De Jeu *et al.*, 1997; Kamstra *et al.*, 1997; Kuipers *et al.*, 1997; Buitendijk *et al.*, 1998; Kuipers *et al.*, 1998; Sanso and Hunziker, 1998; Kuipers *et al.*, 2002; Sanso, 2002; Zhou *et al.*, 2003; Jara *et al.*, 2004; Baeza *et al.*, 2006; 2007a; 2007b). Based on this evidence it is possible to establish that

there is a high degree of stability in terms of the number of diploid chromosomes ($2n = 16$), the absence of natural polyploids and the presence of groups in accordance to the morphology of the first three pairs of chromosomes (the larger ones). So, it is possible to recognize the following species groups: *A. graminea* Phil., *A. magnifica* Herb. (one pair of metacentric chromosomes), *A. andina* Phil. var. *venustula* (Phil.) M. Muñoz, *A. aurea* Graham, *A. angustifolia* Herb., *A. peregrina* L., *A. philippii* Baker (two pairs of metacentric chromosomes) and *A. ligtu* L. subsp. *ligtu* and *A. ligtu* L. subsp. *simsii* (Sprengel) Bayer (three pairs of metacentric chromosomes).

Table 2. Average length of chromosomes of *Alstroemeria presliana* subsp. *presliana* (Baeza 4192). Mean length calculated as percentage of haploid genome length of 10 metaphases.

Chromosome pair	Long arm length	Short arm length	Total relative length	Total absolute length	R	Chromosome type
	(%) ± SD		%	µm		
1	5.64 ± 0.22	5.31 ± 0.32	10.95	21.72	1.06	m
2	4.63 ± 0.20	3.41 ± 0.26	8.04	15.95	1.36	m
3	4.11 ± 0.35	3.60 ± 0.10	7.71	15.29	1.14	m
4	5.00 ± 0.17	0.56 ± 0.10	5.56	11.03	8.93	t
5	2.61 ± 0.10	2.35 ± 0.10	4.96	9.84	1.11	m
6	4.41 ± 0.20	0.49 ± 0.10	4.90	9.72	9.00	t
7	3.08 ± 0.14	1.37 ± 0.22	4.45	8.83	2.25	sm-sat
8	2.76 ± 0.17	0.68 ± 0.17	3.44	6.82	4.06	st-sat

SD: standard deviation; R: brachial index; m: metacentric; sm-sat: submetacentric with satellite; st-sat: subtelocentric with satellite; t: telocentric.

Table 3. Average length of chromosomes of *Alstroemeria presliana* subsp. *australis* (Baeza 4250 c). Calculated as a percentage of mean haploid genome length of 10 metaphases.

Chromosome pair	Long arm length	Short arm length	Total relative length	Total absolute length	R	Chromosome type
	(%) ± SD		%	µm		
1	5.59 ± 0.20	5.53 ± 0.20	11.19	20.00	1.01	m
2	4.35 ± 0.25	3.83 ± 0.25	8.18	14.62	1.14	m-sat
3	5.44 ± 0.10	0.41 ± 0.15	5.85	10.46	13.27	t
4	5.18 ± 0.20	0.56 ± 0.15	5.74	10.26	9.25	t
5	4.53 ± 0.15	0.47 ± 0.15	5.00	8.94	9.63	t
6	2.64 ± 0.25	2.28 ± 0.25	4.92	8.79	1.16	m
7	4.23 ± 0.20	0.47 ± 0.15	4.70	8.40	9.00	t
8	3.11 ± 0.10	1.25 ± 0.10	4.36	7.79	2.45	sm

SD: standard deviation; R: indice braquial; m: metacentric; m-sat: metacentric with satellite; sm: submetacentric; t: telocentric.

A particular case is *A. hookeri* Lodd. subsp. *hookeri*, where populations-groups have two pairs of metacentric chromosomes and three pairs of metacentric chromosomes. But the groups are geographically separated by several kilometers (Baeza *et al.*, 2007b). The same situation was observed in the analyzed subspecies of *A. presliana*. Comparing the first three pairs of chromosomes, differences were noted in terms of morphology. The chromosome 3 in subspecies *presliana* is metacentric and in the subspecies *australis* it is telocentric. Therefore asymmetry index of the karyotype (AsI %) in the subspecies *presliana* is much lower than in the subspecies *australis*, indicating that the subspecies *australis* has a higher number of acrocentric chromosomes. In addition, chromosome 2 of the subspecies *australis* has a microsatellite in the short arm, which is not observed in subspecies *presliana*. The position of chromosome 5 (metacentric) in the subspecies *presliana* could also be located in position 6, because there is no significant difference between these two chromosomes, which means that the type and position of chromosomes 5 and 6 would be identical in both subspecies. Microsatellites are clearly present in chromosomes 7 and 8 in the subspecies *presliana* and are submetacentric and subtelo-centric, respectively, while in the subspecies *australis* they are telocentric and submetacentric without microsatellites. With regard to the chromosome size (Tables 2 and 3) it was observed that in *A. presliana* subsp. *presliana* the total value of the haploid length of the chromosomes is 99.2 μm , while in the subspecies *australis* it is 89.26 μm , which indicates a clear difference between them.

It is probable that a more detailed study of the full range of distribution of each subspecies, including material from Argentina, as well as using not only chromosomes studies, but also detailed isozymes, and floral morphological studies could help in defining whether the subspecies *australis* is a new species in Chile. This result is of great interest given that *Alstroemeria* represents a genetic resource for our country in genetic improvement programs in different parts of the world such as Holland, USA, Germany and Poland, among others.

CONCLUSIONS

On the basis of the results obtained from this research it can be concluded that there are clear karyotypic differences, in morphology, symmetry and size of chromosomes among the subspecies of *Alstroemeria presliana* in Chile.

RESUMEN

***Alstroemeria presliana* Herb. (Alstroemeriaceae) en Chile bajo una perspectiva citogenética.** Carlos M. Baeza^{1*}, Otto Schrader², Eduardo Ruiz¹, y M. Negritto¹. *Alstroemeria* (Alstroemeriaceae) es un género endémico de Sudamérica y presenta dos grandes centros de distribución: Chile y Brasil. En Chile se distribuye desde el norte, cerca de Iquique (20°13' S, 70°09' O) hasta la Patagonia (53°10' S, 70°54' O). La zona central del país presenta el mayor número de especies. *A. presliana* Herb. crece con una distribución que va desde Curicó (34°59' S, 71°14' O) a Cautín (38°45' S, 72°34' O) en Chile y en la Provincia de Neuquén (36°50' S, 71°05' O), Argentina. Se hizo un estudio comparativo del cariotipo de *A. presliana* subsp. *presliana* con *A. presliana* subsp. *australis* Ehr. Bayer. Las dos poblaciones presentaron un cariotipo asimétrico, con $2n = 2x = 16$ cromosomas, pero con cromosomas diferentes: *A. presliana* subsp. *presliana* presenta una fórmula haploide $4m + 1sm\text{-sat} + 1st\text{-sat} + 2t$, esto es, cuatro pares de cromosomas metacéntricos, un par submetacéntrico con satélite, un par subtelo-centric con satélite y dos pares telocéntricos. En cambio, *A. presliana* subsp. *australis* tiene una fórmula haploide constituida por $2m + 1m\text{-sat} + 1sm + 4t$, esto es, dos pares de cromosomas metacéntricos, un par metacéntrico con satélite, un par submetacéntrico y cuatro pares de cromosomas telocéntricos. Estos resultados evidencian que el cariotipo entre las subspecies es muy diferente, lo que podría estar indicando que la subspecie *australis* correspondería a una nueva especie dentro del género.

Palabras clave: Alstroemeriaceae, cariotipo, número cromosómico, Chile.

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