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Cytogenetic analysis in the terrestrial orchid *Sacoila argentina* (Griseb.) Garay from Paraguay

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ABSTRACT. *Sacoila argentina* (Griseb.) Garay is a terrestrial orchid with ornamental value found in northern Argentina, Bolivia, and Paraguay and is considered an object of conservation within the Paraguayan Chaco region, only found at Boquerón Department and chromosomally unknown until this study. Here, we present another natural population of *Sacoila argentina* for Paraguay (Paraguarí Department, Villa Florida, 57º07'48.71" W - 26º23'32.16" S) so extending its area of distribution. Conventional cytogenetic techniques were applied in order to analyze its genetic system. *Sacoila argentina* is a diploid species with $2n = 2x = 46$ small size chromosomes (1.21 - 4.45 µm) and 40.63 µm per haploid genome. Its karyotype, composed of 38*m* + 8*sm*, is bimodal (A_2=0.36/R=3.69) due to a pair (# 20, *sm*) that is more than two times larger than the mean length of the chromosome (1.77 µm), and symmetric (A_1=0.18/i=44.58/r>2=0.13) belonging to Stebbins category 2B. The chromosome pair # 20 also carries a terminal macrosatellite on its short arm with an active NOR. Sporogenesis is normal and pollen grain viability is high (80%). Meiotic behaviour is regular and chromosomes pair up as 23 bivalents in the male (pollen mother cells, PMC) as they do in the female meiosis (megaspore mother cells, MMCs), confirming the basic number $x = 23$ for *Sacoila*. In PMCs and MMCs at diakinesis/metaphase I, ring bivalents (80%) with distal chiasmata (85%) are common, and the mean of chiasmata per bivalent is high (1.80) in both germinal lines. The recombination index, affected by the meiotic number ($n = 23$) and the mean of chiasmata/cell (41.5) is high (RI=64.5). Natural populations of *Sacoila argentina* possess low density, are scarce, interspersed and just found at flowering time. For these reasons, it is a rarely collected species.

Keywords: Chromosomes; Genetic system; Orchidaceae; *Sacoila argentina*.

INTRODUCTION

Sacoila Raf. is a small genus of orchids with ca. ten species native to the tropics and subtropics of America (Garay, 1980) and three of them are found in Paraguay: *S. argentina* Griseb. (Garay), *S. lanceolata* (Aubl.) Garay, and *S. pedicellata* (Cogn.) Garay. *Sacoila argentina* is a terrestrial and annual orchid with yellow-orange flowers and basal leaves, distributed from Bolivia to Paraguay and northern Argentina (Garay, 1980; Correa, 1996). Its habitat comprises open and low forests, the borders of high forests where the light is greater, and dunes and loose sandy grounds. In Paraguay, the orchid is found only in the Boquerón Department and is considered a conservation object within the Paraguayan Chaco Region. Natural populations of *S. argentina* possess a low density, are scarce and interspersed, and are found only at flowering time. For these reasons, the species is rarely collected and chromosomally unknown.

This work reports the first cytogenetic analysis of *S. argentina* and extends its area of distribution in Paraguay.

MATERIALS AND METHODS

Plant material was collected from Paraguarí Department, Villa Florida, at 57º07'48.71" W-26º23'32.16" S, Paraguay. A voucher specimen, Daviña & Honfi 612, was deposited at the Herbarium of the Universidad Nacional de Misiones, Argentina (MNES).

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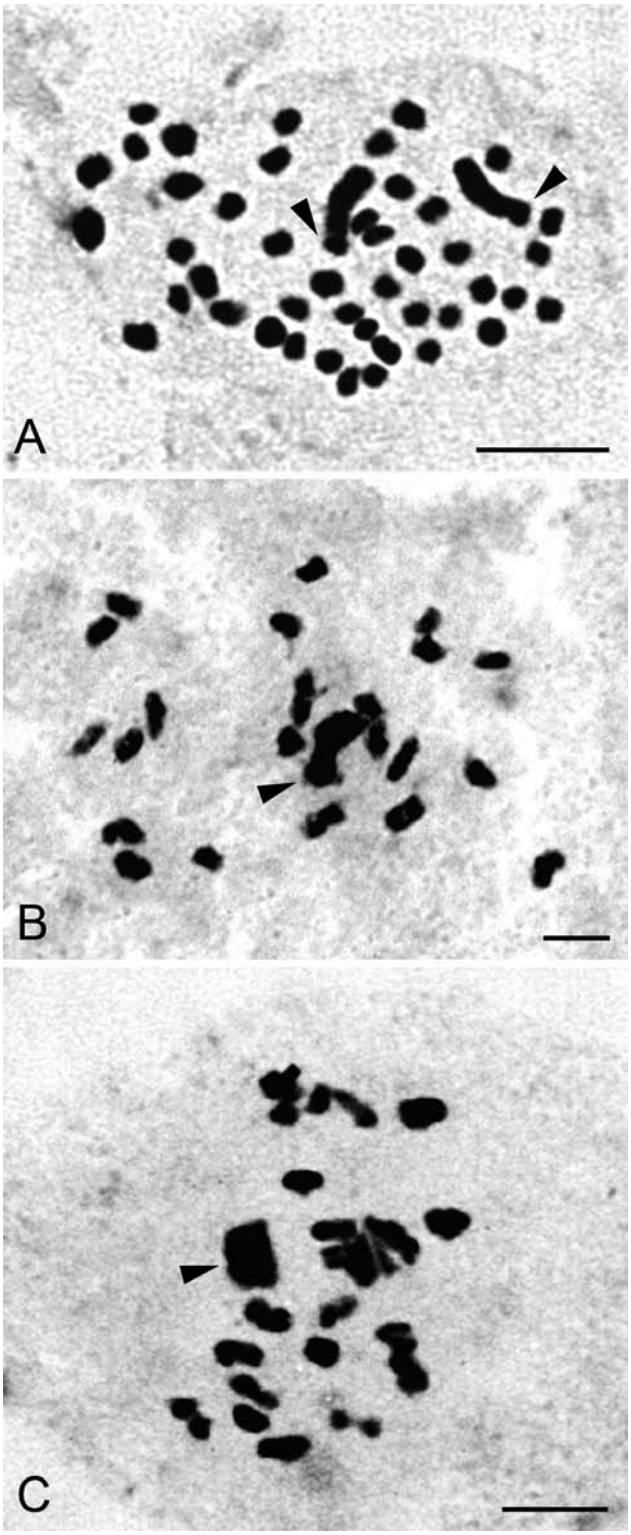

Figure 1. A-C, Somatic and meiotic chromosomes of *Sacoila argentina*. A, Mitotic metaphase with 2*n* = 46; B, MMC at metaphase I with 23 II; C, PMC at metaphase I with 23 II. Arrowheads in A show satellites on pair # 20 *sm*, and in B, C show the largest ring bivalent formed by pair # 20. Scale bars = 5 μm.

Meiotic studies were carried out in young floral buds and ovules both fresh and fixed in absolute ethanol: glacial acetic acid (3:1) and stained with 2% aceto-carmine according to Daviña (2001). Bivalent and chiasmata frequencies were estimated by analysis of 30 cells at diakinesis or metaphase I. Pollen stainability was estimated in 1000 grains stained with carmine-glycerine.

Mitotic studies were performed in young ovules pretreated with saturated 1-bromonaphthalene for 3 h at room temperature and hydrolyzed in HCl 1N for 2 min at 60ºC according to Daviña (2001). Ovaries were macerated in a drop of 2% aceto-orcein and then squashed.

For karyotype description the chromosomes were arranged in groups according to the position of the centromere (median, *m*; submedian, *sm*) and in order of decreasing size in each class. Chromosome nomenclature followed Levan et al. (1964). Satellites were classified according to Battaglia (1955). At least ten best metaphases were selected for making idiograms. The length of chromosome arms and satellites were measured on drawings made with a camera lucida (×2600). Karyotype asymmetry was estimated using the intrachromosome (A_1) and interchromosome (A_2) asymmetry indices of Romero Zarco (1986), as well as the categories of Stebbins (1971).

RESULTS

Sacoila argentina has been chromosomally studied for the first time showing 2*n* = 46 (Figure 1A). Its karyotype is composed of 38 metacentric and 8 submetacentric chromosomes (38 *m* + 8 *sm*), most of which can not be identified, but they were organized in pairs in order to present individual measurements (Tables 1, 2; Figure 2). A terminal macrosatellite in the short arm of the largest pair (# 20, *sm*) carrying the active NOR was observed (Figures 1A, 2). Total chromosome length is 81.25 μm, and mean chromosome length is 1.77 μm, ranging from 1.21 (*m*) to 4.45 μm (*sm*) (Table 2), a small size. Despite the uniformity in chromosome size, the karyotype of *S. argentina* is bimodal due to a pair (# 20, *sm*) more than two times larger than the mean chromosome length (Table 2). This is also evidenced by the interchromosome asymmetry index A_2 value (0.36) and by the largest/smallest chromosome ratio R value (3.69). Most *S. argentina* chromosomes are metacentrics, and the rest

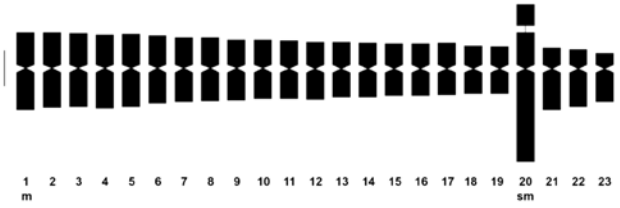

Figure 2. Idiogram of *Sacoila argentina* (38 *m* + 8 *sm*). Scale bar = 1 μm.

Table 1. Quantitative parameters of chromosomes of *Sacoila argentina*.

Pair	s (μm) ±SE[a]	l (μm) ±SE[a]	c (μm) ±SE[a]	I[a]	RL±SE[a]	Type
1	1.00±0.01	1.17±0.05	2.17±0.01	46.13	5.35±0.03	m
2	1.01±0.02	1.10±0.01	2.11±0.02	47.96	5.20±0.02	m
3	0.99±0.01	1.08±0.02	2.07±0.01	47.92	5.09±0.02	m
4	0.93±0.05	1.12±0.01	2.05±0.01	45.26	5.04±0.03	m
5	0.95±0.03	1.08±0.06	2.03±0.03	46.81	4.99±0.04	m
6	0.91±0.03	0.97±0.03	1.88±0.03	48.28	4.61±0.03	m
7	0.85±0.01	0.92±0.02	1.77±0.01	48.17	4.35±0.02	m
8	0.82±0.01	0.91±0.02	1.72±0.02	47.50	4.24±0.02	m
9	0.78±0.01	0.86±0.01	1.64±0.01	47.37	4.03±0.01	m
10	0.78±0.01	0.82±0.01	1.60±0.01	48.65	3.93±0.01	m
11	0.75±0.02	0.82±0.01	1.57±0.02	47.95	3.87±0.02	m
12	0.71±0.02	0.84±0.01	1.55±0.03	45.83	3.82±0.02	m
13	0.71±0.02	0.78±0.02	1.49±0.01	47.83	3.66±0.02	m
14	0.69±0.01	0.78±0.01	1.47±0.01	47.06	3.61±0.01	m
15	0.66±0.01	0.72±0.01	1.38±0.01	47.82	3.39±0.01	m
16	0.66±0.01	0.72±0.01	1.38±0.03	47.82	3.39±0.02	m
17	0.66±0.01	0.72±0.01	1.38±0.03	47.82	3.39±0.02	m
18	0.58±0.02	0.67±0.01	1.25±0.01	46.55	3.08±0.02	m
19	0.56±0.01	0.65±0.02	1.21±0.01	46.43	2.97±0.02	m
20	1.65±0.01	2.80±0.01	4.45±0.01	37.05	10.95±0.01	sm
21	0.52±0.01	1.16±0.01	1.68±0.02	30.77	4.14±0.02	sm
22	0.43±0.06	1.03±0.03	1.46±0.03	29.41	3.61±0.04	sm
23	0.39±0.03	0.95±0.06	1.34±0.04	29.03	3.29±0.04	sm

[a]I=mean centromeric index; c=mean chromosome length; l=mean long arm length; s=mean short arm length; RL=relative chromosome length expressed as percentages of total complement length; SE=standard error.

are submetacentrics, so its karyotype is symmetrical in respect to the centromere position on chromosomes as evidenced by the mean centromeric index (i) value (44.58), intrachromosome asymmetry index A_1 value (0.18) and proportion of chromosome pairs with arm ratios higher than 2 (0.13) (Table 2). Thus, the karyotype of *S. argentina*, although bimodal, is rather symmetrical and belongs to category 2B of Stebbins (1971) (Table 2).

The sporogenesis of *S. argentina* has been normal in all its stages, not only in the female but also in the male germline. Since meiotic behaviour is identical in megaspore mother cells (MMCs) and pollen mother cells (PMCs) the meiotic chromosome associations at diakinesis and metaphase I for both lines are jointly presented (Table 3). *Sacoila argentina's* gametic number is $n = 23$ and its chromosomes are arranged as 23 bivalents (Table 3; Figures 1B, C). Most bivalents are rings (80%) with distal chiasmata (85%), and the mean of chiasmata per bivalent is high (1.80) (Table 3). Also, the recombination index, affected by the meiotic number ($n = 23$) and the mean of chiasmata/cell (41.5) is high (RI = 23 + 41.5 = 64.5) (Table 3). Pollen grain viability is elevated (80%) (Table 3).

Table 2. Karyotype parameters of *Sacoila argentina.*

2*n*	46
x	23
Karyotype formula	38 *m* + 8 *sm*
TCL	81.25 μm
c	1.77 μm
c max	4.45 μm (*sm*)
c min	1.21 μm (*m*)
i	44.58
A_1	0.18
A_2	0.36
R	3.69
r>2	0.13
Stebbins category	2B

A_1, A_2=intrachromosomal and interchromosomal asymmetry indices; c=mean chromosome length; c max, c min=maximum and minimum chromosome length; i=mean centromeric index; r>2=proportion of chromosome pairs with arm ratio>2; R=largest/smallest chromosome ratio; TCL=total complement length.

Table 3. Meiotic chromosome associations at diakinesis and metaphase I of *Sacoila argentina*.

n	23
Bivalents per cell ±SE	
Rings	18.40±0.22
Rods	4.60±0.23
Total	23
Chiasmata per cell ±SE	
Distals	35.30±0.21
Interstitials	6.20±0.45
Total	41.50±0.22
Chiasmata per bivalent ±SE	1.80±0.02
Pollen stainability	80 %

SE = standard error.

DISCUSSION

Sacoila argentina presented $2n = 46$ and the same number was found in *S. lanceolata* by Cocucci (1956) (sub *Stenorrynchos australis* Lindl., $n = 23$), Grabiele et al. (2005) from natural populations of Argentina and Felix & Guerra (2005) from Brazil. At present, these are the only two species of *Sacoila* that have been cytogenetically analyzed. The chromosome number $2n = 46$ was also found in species of the tribe Spirantheae Endl. other than *Sacoila* (*Eltroplectris* Raf., *Mesadenella* Pabst & Garay*, Pelexia* Poit. ex Rich., *Sarcoglottis* C. Presl, and *Skeptrostachys* Garay) by Martinez (1985), Dematteis and Daviña (1999), Felix and Guerra (2005) and Grabiele et al. (2005).

The karyotype formula and morphometric parameters of chromosomes of *S. argentina* (Tables 1, 2) are similar to those found in *S. lanceolata* by Grabiele et al. (2005) despite the fact that ovules were used in the former and root meristems in the latter species analysis. The general features of karyotype described for *S. argentina* are also shared by the other analyzed genera of subtribe Stenorrynchidinae Szlach. (Spirantheae), like *Eltroplectris*, *Mesadenella* and *Skeptrostachys* (Martinez, 1985; Dematteis and Daviña, 1999; Grabiele et al., 2005), and this is despite the uncommon $2n = 26$ from *E. schlechteriana* (Porto *et* Brade) Pabst (Martínez, 1985; Dematteis and Daviña, 1999).

The presence of 23 bivalents confirms the basic chromosome number $x = 23$ for *Sacoila* proposed by Felix and Guerra (2005) and Grabiele et al. (2005), despite the fact that it may be a derived number.

The female meiosis behaviour found in *S. argentina* indicates reduced megaspore (*n*) production. However, it does not specify a particular mode of reproduction, if taking into account the results on the breeding system of *S. lanceolata* from Catling (1987). This author describes sexual reproduction for *S. lanceolata* var. *lanceolata* and var. *paludicola* from Guyana and Florida respectively, in which a normal sequence of pollination, pollen tube growth, fertilization, and embryo seed development occurs; however, the variety *lanceolata* from Florida reproduces by agamospermy, in which the female gametophytes developed from reduced megaspores (*n*) degenerate, and integumental cells became embryos (Catling, 1987). Reduced megaspores (*n*) are *pre* conditions to expecting normal and reduced gametophythes, but for reasons described above, our findings of female meiotic behaviour do not definitively suggest a sexual mode of reproduction for *S. argentina*, which should be confirmed by cytoembryological studies. *Sacoila lanceolata* and *S. argentina* also possess differences in their range of distribution and population size. The former species is widespread in the Americas from Florida to northern and central Argentina, forming large populations while the latter species is restricted to Bolivia, Paraguay, and northern Argentina (Garay, 1980; Catling, 1987) with small and interspersed populations.

The high recombination index of *S. argentina* suggests that, if the species reproduces by sexual means, the genetic variability can be distributed throughout the population. However in *S. argentina*, there is an elevated chance that the consequences will occur, mainly because the species has small populations. Efforts to increase the number of individuals by biotechnological means and to look for new natural populations could be a good strategy for overcoming the severe breeding system barriers to natural conservation of the species.

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LITERATURE CITED

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

Catling, P.M. 1987. Notes on the breeding system of *Sacoila lanceolata* (Aublet) Garay (Orchidaceae). Ann. Missouri Bot. Gard. **74:** 58-68.

Cocucci, A. 1956. Cromosomas gaméticos de *Stenorrhynchos australis* Lindl. y *Tradescantia radiata* Clarke. Trabajos Mus. Bot. Univ. Nac. Córdoba **2:** 781-783.

Correa, M.N. 1996. Orchidaceae. *In* F.O. Zuloaga and O. Morrone (eds.), Catálogo de las Plantas Vasculares de la República Argentina I. Pteridophyta, Gymnospermae y Angiospermae Monocotyledoneae. Monogr. Missouri Bot. Gard. **60:** 242-271.

Daviña, J.R. 2001. Estudios citogenéticos en algunos géneros argentinos de Amaryllidaceae. Tesis Doctoral UNC, pp. 184.

Dematteis, M. and J.R. Daviña. 1999. Chromosome studies on some Orchids from South America. Selbyana **20:** 235-238.

Felix, L.P. and M. Guerra. 2005. Basic chromosome numbers in terrestrial orchids. Pl. Sys. Evol. **254:** 131-148.

Garay, L.A. 1980. A generic revision of the Spiranthinae. Bot. Mus. Leafl. **28:** 278-425.

Grabiele, M., J.C. Cerutti, D.H. Hojsgaard, A.I. Honfi, and J.R. Daviña. 2005. Caracterización cariotípica de especies de Spirantheae Endl. (Spiranthoideae-Orchidaceae) de Argentina. J. Basic Appl. Genet. **17:** 119.

Levan, A., K.L. Fredga, and A.A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52:** 201-220.

Martínez, A.J. 1985. The chromosomes of Orchids VIII. Spiranthinae and Cranichidinae. Kew Bull. **40:** 139-147.

Romero Zarco, C. 1986. A new method for estimating karyotype asymmetry. Taxon **3:** 531-536.

Stebbins, G.L. 1971. Chromosomal Evolution in Higher Plants. New York: Addison-Wesley Co.

產自巴拉圭之陸生蘭花 *Sacoila argentina* (Griseb.) Garay 之細胞遺傳學分析

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Sacoila argentina (Griseb.) Garay 是一種具觀賞價值之陸生蘭花，分佈於北阿根廷，玻利維亞，及巴拉圭。在巴拉圭之 Chaco 區域被定爲保育對象，只見於 Boquerón 省，其染色體之資料直到本文才揭曉。在此，我們介紹另一巴拉圭之自然族群 (取自 Paraguarí 省，Florida 村，西經 57º07’48.71” – 南緯 26º23’32.16”) 因此擴展它的分佈地區。傳統之細胞遺傳技術被用來分析其基因系統。*Sacoila argentina* 及二倍體 $2n = 2x = 46$ 小染色體 (1.21 - 4.45 µm)，單倍體基因組長 40.63 µm，它核型含 38*m* + 8*sm*，乃二型體 (A_2=0.36/R=3.69) ，此係因爲有一對染色體 (# 20, *sm*) 比染色體之長度平均 (1.77 µm) 超過 2 倍大。核型乃對稱的 (A_1=0.18/i=44.58/r>2=0.13) 屬於 Stebbins 分類 2B。染色體對 # 20 也在具活躍之 NOR 的短臂上帶一尾端大衛星 DNA，孢子形成正常，花粉粒之存活率高 (80%)。減數分裂行爲正常，而染色體對在雄 (花粉母細胞) 爲 23 二價體，如同雌（大孢子母細胞）性減數分裂時之數目，証實基本數目 $x = 23$，在花粉母細胞及大孢子母細胞之 diakinesis/metaphase I 期，環形二價體 (80%) 與遠方 chiasmata (85%) 乃常見者，而每一個二價體之平均 chiasmata 數是高的 (1.80)，無論是雄的或雌的部份。重組指標 [受減數分裂數 $n = 23$，及每一細胞之 chiasmata 平均數 (41.5) 所影響] 是高的 (RI=64.5)。*Sacoila argentina* 之自然族群稀少，密度低，分散，且只在開花期才看得到；基於上述理由，此乃很少被收集到之物種。

關鍵詞：染色體；基因系統；Orchidaceae；*Sacoila argentina*。