Pl. Syst. Evol. 258: 85–95 (2006)
DOI 10.1007/s00606-005-0393-6

Plant Systematics and Evolution
Printed in Austria

Karyotype relationships among four South American species of *Urvillea* (Sapindaceae: Paullinieae)

J. D. Urdampilleta[1], **M. S. Ferrucci**[2], **J. M. D. Torezan**[1], and **A. L. L. Vanzela**[1]

[1]Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de Biologia Geral, CCB, Universidade Estadual de Londrina, Paraná, Brazil
[2]Instituto de Botánica del Nordeste, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina

Received July 25, 2005; accepted October 3, 2005
Published online: February 17, 2006

Abstract. A cytogenetic study was conducted on four species of the genus *Urvillea* (Sapindaceae, Paullinieae): *U. chacoensis* Hunz., *U. filipes* Radlk. and *U. ulmacea* Kunth of the *Urvillea* section and *U. laevis* Radlk. of the *Stenelytron* section. The chromosome numbers in *U. chacoensis* (2n = 22) and *U. laevis* (2n = 24) were confirmed, and new chromosome numbers are reported for *U. filipes* with 2n = 22 and *U. ulmacea* with 2n = 88. Additionally, data on interphase nuclear structure, chromosome banding patterns (C-Giemsa and C-CMA_3/DAPI) and FISH with rDNA probes are also presented. The distribution of AT- and GC-rich regions and the physical mapping of ribosomal genes (45S and 5S rDNA sites) were established for the first time in these *Urvillea* species. Sections of *Urvillea* are cytogenetically differentiated according to basic chromosome number, where x = 11 in the section *Urvillea* and x = 12 in the section *Stenelytron*. This first section displayed an important karyotypic feature, the occurrence of large AT- and GC-rich bands at terminal chromosomal regions. The *Urvillea* section showed polyploidy and its species were differentiated by their banding patterns. *Urvillea chacoensis* showed several terminal AT-rich bands, while terminal AT- and GC-rich bands were both found in *U. ulmacea*. However, the section *Stenelytron* did not exhibit this banding pattern. The 45S rDNA sites appeared always associated with GC-rich regions and they were numerically variable among species, being located or not the same chromosome 5S rDNA sites. Variation in the repetitive DNA distribution and their role in karyotype differentiation among these *Urvillea* species are discussed.

Key words: Banding pattern, FISH, heterochromatin, Paullinieae, rDNA, *Urvillea*.

Introduction

Urvillea Kunth belongs to the largely neotropical tribe Paullinieae subtribe Paulliniinae (Sapindaceae), together with the genera *Cardiospermum* L., *Houssayanthus* Hunz., *Lophostigma* Radlk., *Paullinia* L. and *Serjania* Mill. The tribe comprises a number of woody vines, but also herbaceous annual vines such as *C. halicacabum* L. Among these genera, *Cardiospermum* is most closely related to *Urvillea*, because its members are characterized by papery, inflated capsules and seeds that usually

have a dry aril. However, *Urvillea* has capsules that are slightly inflated or flattened with marginal wings, while *Cardiospermum* capsules are completely inflated and apterous or have narrow marginal wings (Radlkofer 1932, Ferrucci 2000b).

Urvillea includes 17 species, where some have a wide distribution and others are endemic. This genus comprises two sections, *Urvillea* Kunth and *Stenelytron* Radlk., which are differentiated by morphological characteristics of fruits and seeds (Radlkofer 1932, Ferrucci 2000b). Section *Urvillea* exhibits 3-seeded capsules with inflated locules, and the section *Stenelytron* is characterized by 1-seeded capsules with flattened locules (Ferrucci 2000b). Five species have been studied in reference to cytogenetic characteristics, including chromosome number and karyotype (Ferrucci 1981, 1997a, 1997b; Nogueira et al. 1995; Lombello and Forni Martins 1998). Variation in basic chromosome number has been observed between the two sections, shown to be x = 11 for section *Urvillea* and x = 12 for section *Stenelytron* (Ferrucci 2000a). Approximately 70 to 90% of Angiosperm species may be considered to be polyploid (Morawetz 1986, Wendel 2000), although polyploid series are not frequent in Sapindaceae. In Paullinieae, only *Urvillea* shows variation in ploidy, for example, 2n = 44 in *U. uniloba* Radlk. and 2n = 22 and 86 in *U. ulmacea* Kunth (Ferrucci 1981, Nogueira et al. 1995).

Other cytogenetic data, such as banding patterns and rDNA site localization in species of *Urvillea* were not previously available. In Paullinieae, two species of *Cardiospermum* and two of *Serjania* were characterized with regard to C-Giemsa, CMA_3 and DAPI banding patterns, and found to have remarkable amounts of AT-rich terminal bands. According to Hemmer and Morawetz (1990), the presence of AT-rich blocks could represent a derived character in Sapindaceae. Thus, the present study was carried out to obtain new information about the distribution of repetitive DNA segments in *Urvillea*. *Urvillea chacoensis* Hunz., *U. filipes* Radlk. and *U. ulmacea* Kunth, all from the *Urvillea* section, and *U. laevis* Radlk. from the *Stenelytron* section, were karyotyped using conventional staining and C-Giemsa and C-CMA_3/DAPI banding procedures, and FISH with 45S and 5S rDNA probes for physical localization of ribosomal genes. Our results are discussed in light of karyotype evolution in the genus *Urvillea* to interpret relationships among the species studied.

Material and methods

Plant material. The species and populations studied (*U. chacoensis*, *U. filipes*, *U. ulmacea* and *U. laevis*), locations and collectors are included in Table 1. Samples were collected and cultivated in pots under greenhouse conditions. Vouchers were deposited at FUEL (Herbarium of the Universidade Estadual de Londrina, Paraná, Brazil) and at CTES (Instituto de Botánica del Nordeste, Corrientes, Argentina).

Conventional staining. Chromosome preparations were made from root tips pretreated with 2 mM 8-hydroxyquinoline for 4–5 h at 15 °C, fixed in ethanol: acetic acid (3:1, v:v) for 12 h and stored at −20 °C until use. Conventional analysis of chromosomes was performed using the HCl/Giemsa technique of Guerra (1983). Chromosome measurements were obtained from at least five metaphases to determine karyotype formula (KF), diploid set length (DSL) and heterochromatin percentage.

Chromosome banding. For chromosome banding, root tips were digested at 37 °C in a solution composed of 4% cellulase and 40% pectinase, and the chromosome squashed was made in a drop of 45% acetic acid. Slides were frozen in liquid nitrogen and then the cover slips removed. Slides were aged for three days at room temperature, treated with 45% acetic acid (10 min at 60 °C), 5% barium hydroxide (10 min at 25 °C), and 2xSSC, pH 7.0 (1 h 20 min at 60 °C), and then stained with 2% Giemsa (Schwarzacher et al. 1980). Alternatively, the slides were aged for three more days at room temperature and sequentially stained with 0.5 mg/ml CMA_3 for 1.5 h and 2 μg/ml DAPI for 30 min (see Vanzela et al. 2002). Samples stained with Giemsa were mounted with Entellan (Merck), but those stained with the fluorochromes were mounted in a medium composed of glycerol/McIlvaine buffer pH 7.0, 1:1 (v:v), plus 2.5 mM

$MgCl_2$. The cells were photographed with Imagelink HQ ASA 25 or T-max ASA 100, both from Kodak. Analyses were based on five well-spread metaphases for each treatment.

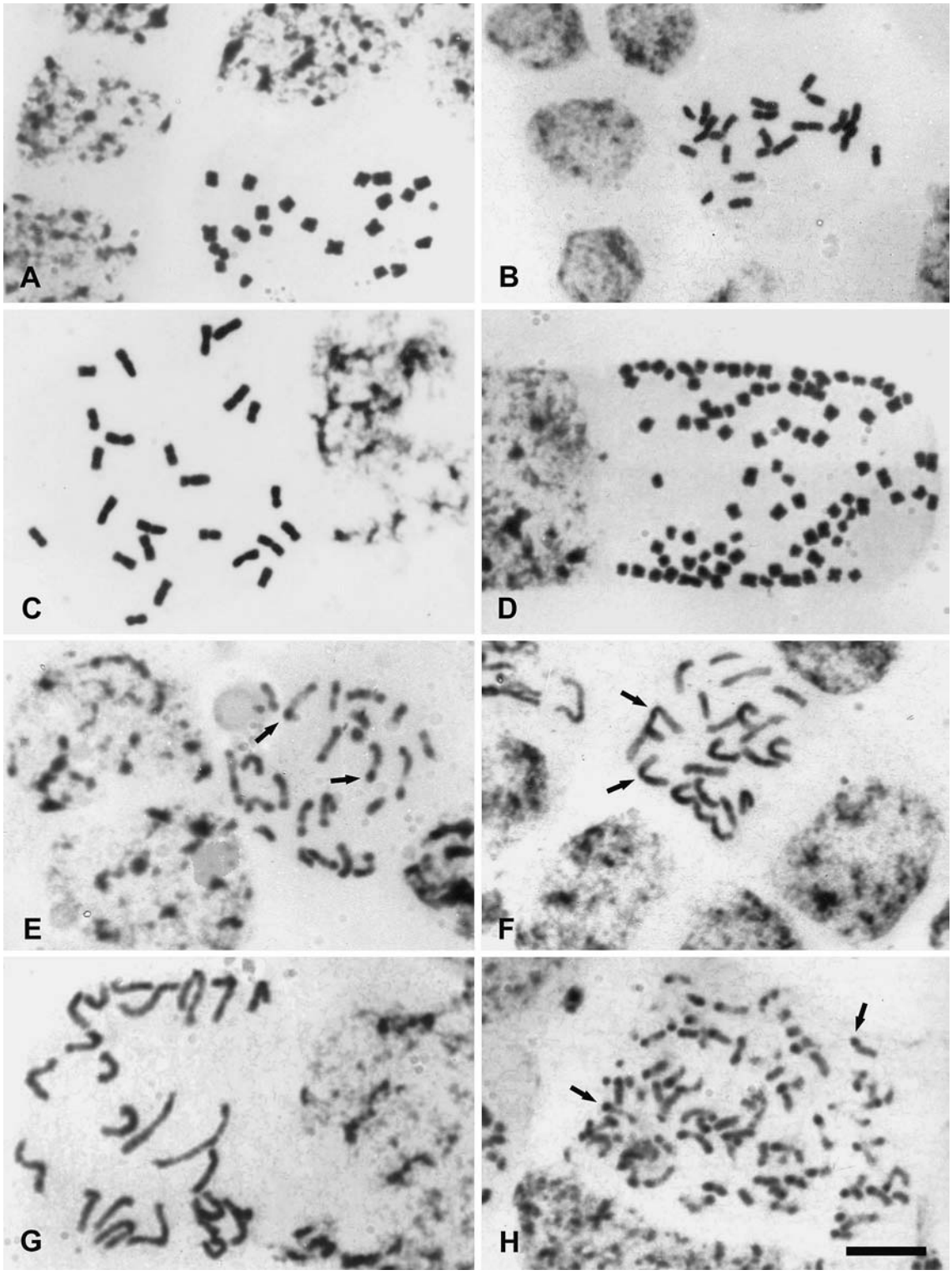
FISH. The location and number of rDNA sites were determined by fluorescence *in situ* hybridization using two plasmids: p*Ta*71 containing the 18S-5.8S-26S rDNA (Gerlach and Bedbrook 1979) and p*Ta*794 containing the 5S rDNA-gene repeat unit (Gerlach and Dyer 1980), according to Heslop-Harrison et al. (1991) and Cuadrado and Jouve (1994), both with minor modifications. Probes were labeled with biotin-14-dATP by nick translation. Preparations were incubated in 100 μg/ml RNase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series and air-dried. Each slide was treated with 30 μL of hybridization mixture containing 100–200 ng of labeled probe, 50% formamide (15 μL), 50% polyethylene glycol (6 μL), 20 × SSC (3 μL), 100 ng of calf thymus DNA (1 μL), and 10% SDS (1 μL). The probe mixture was denatured at 70 °C for 10 min and immediately chilled on ice. Chromosome denaturation/hybridization was done at 90 °C for 10 min, 48°C for 10 min, and 38 °C for 5 min, using a thermal cycler (MJ Research), and slides were placed in a humid chamber at 37 °C overnight. The hybridization sites were detected with avidin-FITC conjugate. The slides were counterstained and mounted in a solution composed of 25 μL of 50% antifade: 50% glycerol/ McIlvaine buffer, pH 7.0/ 2.5 mM $MgCl_2$ solution (1:1, v:v), plus 1 μL of 2.5 μg/ml propidium iodide. Metaphases were photographed with Fuji Color 200 ISO film.

Results

Conventional analysis showed for first time chromosome numbers for *U. filipes* (2n = 22) and for *U. ulmacea* (2n = 88), and confirmed the counts for *U. chacoensis* with 2n = 22 and *U. laevis* with 2n = 24 (see Table 1 and Fig. 1). Chromosome measurements showed that in *U. chacoensis* chromosome size varied from 2.13 μm to 1.15 μm, with a diploid set length (DSL) equal to 35.68 μm, and with a karyotype consisting of one metacentric (m) and 10 submetacentric (sm) chromosome pairs. *Urvillea filipes* exhibited chromosomes varying from 2.58 μm to 1.32 μm (DSL = 45.04 μm) and a karyotype composed of 4 m, 2 sm, 4 st (subtelocentric) and 1 t (telocentric) pairs. The chromosomes of *U. ulmacea* varied from 2.07 μm to 0.71 μm (DSL = 122.92), with a karyotype comprising 1 m, 23 sm and 20 st chromosome pairs. Finally, the chromosomes of *U. laevis* varied from 3.56 μm to 1.99 μm (DSL = 65.82 μm), and the karyotype included 4 m and 8 sm pairs (see Table 1 and Fig. 1).

The structure of interphase nuclei was semi-reticulate in all four species, but varied in other features. *Urvillea chacoensis* showed larger chromocenters and a terminal pattern of prophasic condensation (Fig. 1E). *Urvillea filipes* exhibited few and tenuous chromocenters and a proximal prophasic condensation (Fig. 1F). *Urvillea ulmacea* was very similar to *U. chacoensis* with regard to nuclear shape and prophasic condensation pattern, but was different due to the occurrence of larger nuclei and higher chromocenter numbers (Fig. 1H). *Urvillea laevis* showed nuclei with small chromocenters and heterogeneous prophasic condensation (Fig. 1G).

Chromosome banding showed that *Urvillea chacoensis* has several heterochromatic blocks, which agrees with the occurrence of large chromocenters observed. C-Giemsa bands were present in both terminal regions in the six major chromosome pairs, and also in the terminal region of the long arm of four chromosome pairs of medium and small size (Fig. 2A and 4). Interestingly, C-DAPI banding showed positive signals coincident with those visualized after C-Giemsa banding, which occupied 43.8% of the diploid set length, demonstrating that AT-rich heterochromatin is an important component of the *Urvillea chacoensis* genome (Fig. 2C and 4). C-CMA_3 banding showed terminal GC-rich regions in the short arms of three medium and small chromosome pairs, where some chromosomes also possessed AT-rich terminal bands (Fig. 2B, 2C and 4). In *U. filipes*, C-Giemsa and C-DAPI bands were not visualized, but terminal C-CMA_3^+ bands were present in the short arms of two pairs of


A
B
C
D
E
F
G
H

medium and small chromosomes, occupying less than 1% of the genome size (Figs. 2E, 4 and Table 1). *Urvillea ulmacea* had several terminal heterochromatic blocks, showing a complex band patterns. AT-rich segments appeared as numerous terminal and interstitial bands. The C-CMA_3^+ bands appeared as terminal blocks in several chromosomes, and in some cases these heterochromatic blocks were associated to AT-rich bands. Approximately 12.9% of the diploid set length was occupied by GC-rich blocks and 10.8% by AT-rich blocks (Fig. 2I, J and Table 1). In *U. laevis,* C-Giemsa banding revealed small bands present in the terminal region of some chromosomes (Fig. 2F). However, the C-CMA_3/DAPI banding showed tenuous terminal and intercalary $DAPI^+$ bands (Figs. 2H and 4) and terminal CMA_3^+ bands in the short arms of three chromosome pairs, occupying also less than 1% of the genome size. These GC-rich regions were heteromorphic in both homologous and heterologous chromosomes (Fig. 2G).

FISH using the p*Ta*71 probe (45S rDNA) located six terminal signals in *U. chacoensis* and *U. laevis*, four in *U. filipes* and approximately 24 sites in *U. ulmacea* (Fig. 3A, 3C, 3D and 3E). In *U. laevis* and *U. filipes*, the size of 45S rDNA sites varied among different chromosome pairs, but these sites were similar in size in *U. chacoensis*. FISH with p*Ta*794 probe in *U. laevis* revealed four 5S rDNA sites located adjacent to 45S rDNA sites (Fig. 3B). On the other hand, two 5S rDNA sites were detected in *U. chacoensis* and *U. filipes*, but not in those that possessed 45S rDNA segments (Fig. 3C and 3D).

Discussion

The genus *Urvillea* comprises 17 species, but the cytogenetical features of only five of them had been described previously. In this study, chromosome counts in four species were carried out, and for two of these species the numbers are described for the first time: *Urvillea filipes* with 2n = 22 and *U. ulmacea* with 2n = 88. The chromosome number reported here for *U. filipes* could confirm the systematic relationship of this species with other species from the section *Urvillea*, such as *U. chacoensis*, *U. peruviana* and the diploid cytotype of *U. ulmacea* (Ferrucci 1981, 1997a, 1997b). *U. ulmacea* is widely dispersed in the Americas, from Texas (United States) to Jujuy, Salta and Misiones Provinces (North Argentina). Due to its great geographic extension, several infraspecific taxa have been accepted (Radlkofer 1932, Barkley 1957). Previous chromosome counts in *U. ulmacea* showed 2n = 86 in material collected in Londrina, Paraná, Brazil (Nogueira et al. 1995). The chromosome number reported here (2n = 88) was obtained from three populations of Londrina and other locations in the northern region of Paraná State. Thus, we believe that 2n = 88 is the correct chromosome number for this cytotype, and that the number 2n = 86 could have been a miscount due to decrease in chromosome size. It is worth pointing out that 2n = 88 is a multiple of the cytotype 2n = 22, which was recently described for populations from Amambay - Paraguay, Jujuy - Argentina and Mato Grosso do Sul - Brazil (Ferrucci and Solís Neffa 1997, Ferrucci 2000a, Solís Neffa and Ferrucci 2001). Both cytotypes (2n = 22 and 2n = 88) point out that polyploidy is an

Fig. 1. Conventional staining in *Urvillea* species. (**A**) Semi-reticulate interphase nuclei and mitotic metaphase in *U. chacoensis* (2n = 22). (**B**) Semi-reticulate interphase nuclei and mitotic metaphase in *U. filipes* (2n = 22). (**C**) Mitotic metaphase in *U. laevis* (2n = 24). (**D**) Semi-reticulate interphase nuclei and mitotic metaphase in *U. ulmacea* (2n = 88). The Figures E to H show different prophasic condensation patterns. (**E**) Terminal condensation in *U. chacoensis*. Arrows indicate two terminal regions. (**F**) Proximal condensation in *U. filipes* (see arrows). (**G**) Heterogeneous condensation in *U. laevis*. (H) Terminal condensation in *U. ulmacea*. Arrows indicate two terminal regions. Bar = 10 μm

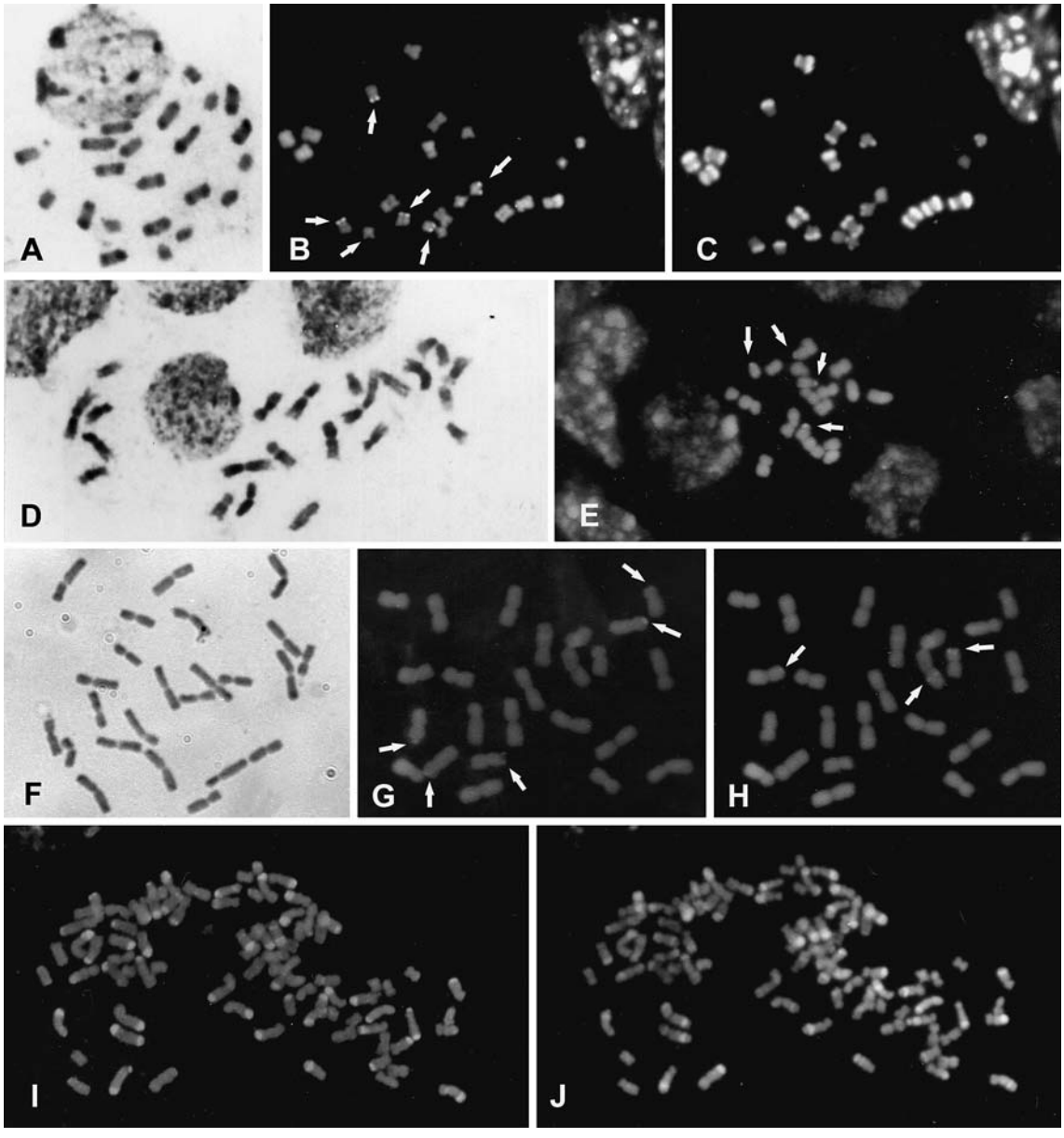


Fig. 2. Chromosome banding in *Urvillea*. (**A**) C-Giemsa, (**B**) C-CMA_3 and (**C**) C-DAPI banding in *U. chacoensis*. Arrows in the Figure B indicate small and terminal C-CMA_3 bands. (**D** and **E**) C-Giemsa and C-CMA_3 banding in *U. filipes*. Arrows indicate very small and terminal C-CMA_3 bands. (**F**, **G** and **H**) C-Giemsa, C-CMA_3 and C-DAPI banding in *U. laevis*. Arrows in Figure G indicate very small and terminal C-CMA_3 bands and in Figure H indicate some terminal and interstitial C-DAPI bands. (**I** and **J**) C-CMA_3 and C-DAPI banding, respectively, in *U. ulmacea*. Bar = 10 μm

important mechanism of karyotype differentiation among different populations of *Urvillea ulmacea*.

The basic chromosome number x = 8 has been suggested to be characteristic of primitive Angiosperms, such as the Magnoliales and Laurales (see Morawetz 1986), and it is therefore possible that the basic numbers of most of the extant Angiosperms originated through polyploidy and/or aneuploidy/disp-

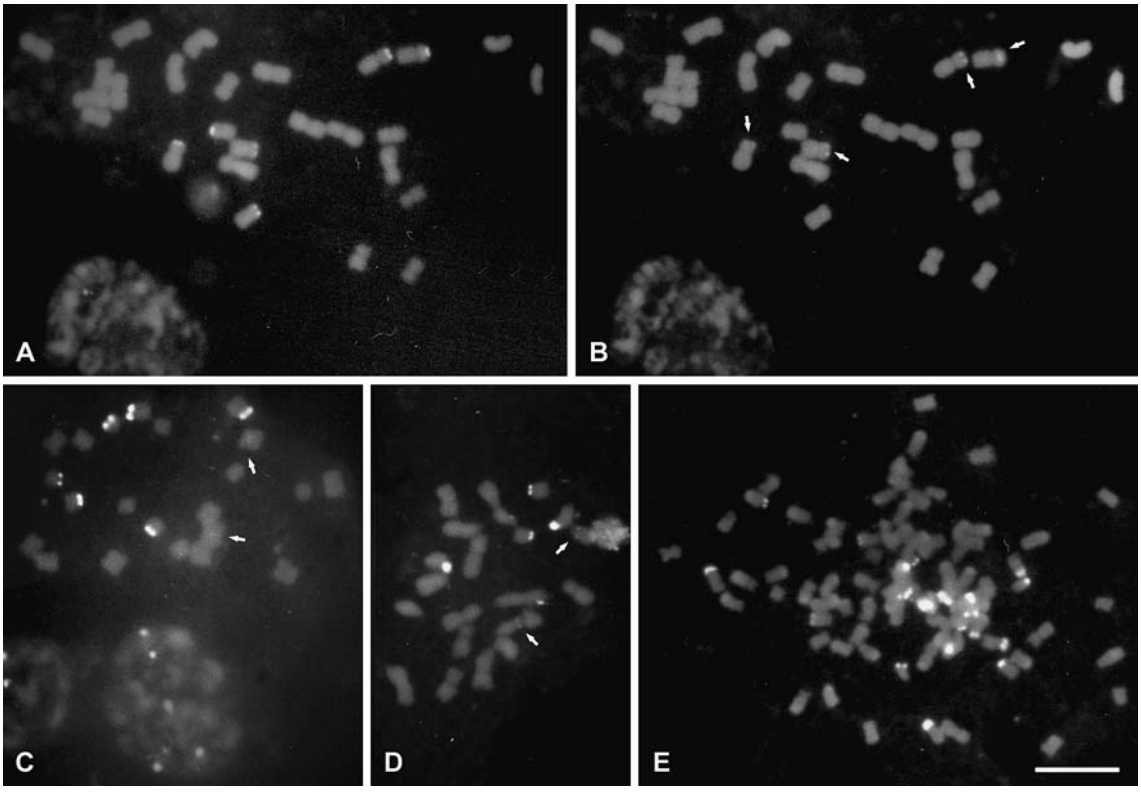


Fig. 3. FISH with rDNA probes in *Urvillea* species. (**A** and **B**) Hybridization with 45S and 5S rDNA probes (respectively) in *U. laevis*. Arrows indicate terminal 5S rDNA sites. (**C**) Simultaneous hybridization with 45S and 5S rDNA probes in *U. chacoensis*. Arrows indicate small and proximal 5S sites. (**D**) Simultaneous hybridization with 45S and 5S rDNA probes in *U. filipes*. Arrows indicate also small and proximal 5S sites. FISH with 45S and 5S rDNA probes were performed separately in both *U. chacoensis* and *U. filipes*, and after the location of each region, a simultaneous hybridization with the two probes was made for each species. (**E**) FISH with 45S rDNA probe in *Urvillea ulmacea*. Note the occurrence of only terminal hybridization signals. Bar = 10 μm

loidy. According to Wendel (2000), approximately 70% of Angiosperm species could be considered polyploids. Thus, the basic numbers x = 14, 15 and 16 present in 90% of the genera of Sapindaceae can be palaeotetraploids (Morawetz 1986). Only three genera, *Allophylus* L. (Thouinieae), *Melicoccus* P. Br. (Melicocceae) and *Urvillea*, exhibit a polyploid series (Ferrucci and Solís Neffa 1997). The number 2n = 4x = 44 found in *U. uniloba* was the first case of polyploidy within *Urvillea* (Ferrucci 1981). This species is closely associated with *U. ulmacea* (2n = 8x = 88), and both point out the important role of polyploidy in the karyotype differentiation in Sapindaceae. Additionally, the basic chromosome number x = 11 is shared by *Cardiospermum* and *Urvillea*, which exhibit other basic numbers, that is, x = 7, 9, 10 and 11 in *Cardiospermum* and x = 11 and 12 in *Urvillea* (Ferrucci 2000a). The relationship between *Cardiospermum* and *Urvillea* is also evidenced by morphological characteristics such as pericarp with papery locules that are inflated and inconspicuous and a dry aril (Weckerle and Rutishauser 2005).

Two sections are recognized in the genus *Urvillea* (*Urvillea* and *Stenelytron*), based on

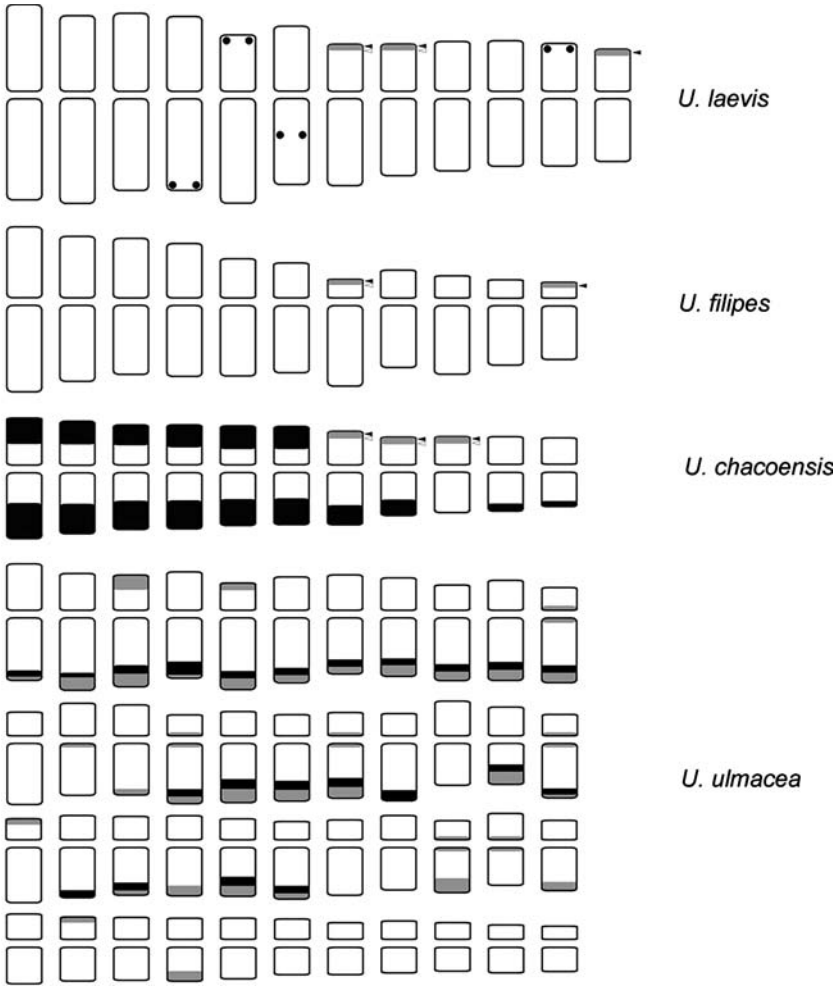


Fig. 4. Idiograms of the species of *Urvillea* studied: ■ = AT rich regions; ■ = GC rich regions; ▲ = 45S rDNA sites; ▲ = 5S rDNA sites. Bar = 5 μm

morphological features, which are also differentiated by the basic chromosome number: x = 11 in Sect. *Urvillea* and x = 12 in Sect. *Stenelytron* (Ferrucci 1981, 1997b; Nogueira et al. 1995; Ferrucci and Solís Neffa 1997; Lombello and Forni Martins 1998; Solís Neffa and Ferrucci 2001). According to Hemmer and Morawetz (1990) and Acevedo-Rodríguez (1993), the lower number (x = 11) could have originated by a reduction in the basic number, characterizing a derivative feature in the tribe Paullinieae. This feature also is related to other apomorphies, such as climbing habit, tendrils and type of pollen, making Paullinieae a derivate among other tribes (Acevedo-Rodríguez 1993). However, the karyotype evolution of the tribe mediated by descending disploidy is a hypothesis that requires further testing, mainly through new counts and recounts of different genera of the tribe and the family.

Other important and little used cytogenetic features are interphase nuclei type and prophasic condensation pattern. *Urvillea chacoensis* and *U. ulmacea* showed a terminal condensation pattern, probably related to C-Giemsa and C-CMA_3/DAPI banding, and the interphase nuclei in these species were semi-reticulate with several chromocenters. On the other hand, *U. filipes* showed a proximal condensation pattern and *U. laevis* a heterogeneous condensation

Table 1. Karyotype formula (KF), range of chromosome length (Range), diploid set length (DSL), percentage of heterochromatin and presumptive DNA composition inferred from fluorescence patterns

Species	2n	KF	Range (μm)	DSL (μm)	Interphase nuclei, Condensation pattern	AT (%) bands	GC (%) bands	Location
Sect. *Urvillea*								
U. chacoensis	22	1m + 10sm	2.13–1.15	35.7	Semireticulate, Terminal	43.8	4.8	**Bolivia:** Dpto. Chuquisaca. Ferrucci et al. 1763, Prov. Calvo (CTES); Dpto. Santa Cruz. Ferrucci et al. 1912, Prov. Chiquitos (CTES).
U. filipes	22	4m + 2sm + 4st + 1t	2.58–1.32	45.0	Semireticulate, Proximal			**Bolivia:** Dpto. Santa Cruz. Ferrucci et al. 1783, Prov. Cordillera (CTES); Ferrucci et al. 1789, Prov. Ichilo (CTES); Ferrucci et al. 1906, Prov. Velasco (CTES).
U. ulmacea	88	1m + 23 sm + 20st	2.07–0.71	122.9	Semireticulate, Terminal	10.8	12.9	**Brazil:** Paraná. Urdampilleta 192, Londrina (FUEL); Urdampilleta et al. 246, Paiquerê (FUEL); Urdampilleta et al. 265, Imbaú (FUEL).
Sect. *Stenelytron*								
U. laevis	24	4m + 8sm	3.56–1.99	65.8	Semireticulate, Heterogeneous			**Brazil:** Paraná. Urdampilleta 146, Londrina (FUEL 34738); Urdampilleta et al. 149, Ibi porã (FUEL 34745); Urdampilleta et al. 247, Paiquerê, (FUEL). **Bolivia:** Dpto. Santa Cruz. Prov. Chiquitos. Ferrucci et al. 1911 (CTES); Ferrucci et al. 1920 (CTES).

pattern, which were not correlated with banding pattern, and these species exhibited semi-reticulate interphase nuclei that were finely granulated. Nonetheless, these features can constitute an important tool in karyosystematics studies of *Urvillea*.

According to Hemmer and Morawetz (1990), the tribe Paullinieae not only shows a larger size and smaller number of chromosomes, but also differs from other Sapindaceae groups by the presence of several terminal $DAPI^+$ bands on the chromosomes. These authors studied the banding patterns of some species of *Cardiospermum* and *Serjania*, and reported the occurrence of terminal $DAPI^+$ and $CMA_3{}^+$ bands in at least four species. The C-Giemsa and C-CMA_3/DAPI banding patterns were not previously studied in species of *Urvillea*, and the results obtained here point out the importance of this heterochromatic segment in karyotype differentiation in the *Urvillea* section. These results indicate that C-CMA_3/DAPI banding can be useful in the distinction of *Urvillea* species studied here, as well as other species of the genus, in addition to morphological features. *Urvillea filipes* showed no $DAPI^+$ bands, while the *U. chacoensis* genome consisted of 43% AT-rich heterochromatin. Conversely, *U. ulmacea* had 12.9% $CMA_3{}^+$ bands and 10.8% $DAPI^+$ bands. *Urvillea chacoensis* possesses the smallest chromosome number and the smallest DSL, but presented the largest percentage of heterochromatin among the four species studied here. Besides, the physical positioning of bands, either GC- or AT-rich, appeared mainly in the terminal regions, corresponding to an equilocal pattern distribution (see Fig. 4). This situation agrees with the heterochromatin dispersion model proposed by Schweizer and Loidl (1987), in which chromosome positioning can favor Rabl's orientation, and in this way, facilitate the amplification and translocation of repetitive sequences among some heterologous chromosomes at interphase.

Ribosomal genes of *Urvillea* species were located for first time in this study and, as commonly found in plants, the 45S rDNA sites appear to be associated with GC-rich regions, located preferentially in the terminal regions of the short arms of different chromosomal types (see Guerra 2000). However, several GC-rich heterochromatic blocks that were visualized in *U. ulmacea* were not associated with 45S rDNA sites, but were adjacent to AT-rich heterochromatin. FISH with 45S and 5S rDNA probes employed in *Urvillea* showed an important interspecific variation in number and position of these segments. The number of 45S rDNA sites did not correlate with the systematics proposed for the genus. That is, *U. chacoensis* (section *Urvillea*) and *U. laevis* (section *Stenelytron*) showed six 45S rDNA sites, while *U. filipes* from the *Urvillea* section showed four of them. On the other hand, the physical localization of 5S rDNA sites exhibited, in relation to 45S rDNA sites, a good relationship with the systematics of *Urvillea*. The 45S and 5S rDNA sites in *U. laevis* appeared located in the same chromosome, while in *U. chacoensis* and *U. filipes* these sites were located in different chromosomes. Additionally, the number of 5S rDNA sites seems to vary between the two sections, since two chromosome pairs were visualized in section *Stenelytron* and only one in section *Urvillea*.

This comparative study of chromosome banding patterns and rDNA site distribution suggests that the karyotype of these species are involved in a dynamic process of amplification and dispersion of repeated DNA segments that can be used as karyotypical and micromorphological markers in the determination of species and populations. The banding patterns and rDNA distribution agree with the systematics of *U. chacoensis*, *U. filipes*, *U. ulmacea* and *U. laevis* based in morphological features. These results open new possibilities for the generation of information for future studies of the systematics and evolution of the genus *Urvillea*, as well as providing a better understanding of how the karyotypes of Paullinieae and other Sapindaceae groups are differentiated.

The authors are grateful to the Brazilian agencies Fundação Araucária, CAPES, IAP-SEMA and ProPPG for financial support and to Dr. Albert Leyva for reading the manuscript.

References

Acevedo Rodríguez P. (1993) Systematics of *Serjania* (Sapindaceae). Part I: A revision of *Serjania* Sect. *Platycoccus*. Mem. New York Bot. Gard. 67: 1–93.

Barkley F. A. (1957) Sapindaceae of southern South America. Lilloa 28: 111–179.

Cuadrado A., Jouve N. (1994) Mapping and organization of highly-repeated DNA sequences by means of simultaneous and sequential FISH and C-banding in 6x-*Triticale*. Chrom. Res. 2: 231–338.

Ferrucci M. S. (1981) Recuentos cromosómicos en Sapindáceas. Bonplandia 5: 73–81.

Ferrucci M. S. (1997a) *Urvillea peruviana* (Sapindaceae) una nueva especie andina. Bonplandia 9: 237–240.

Ferrucci M. S. (1997b) El número cromosómico de *Urvillea laevis* (Sapindaceae). Bonplandia 9: 305–306.

Ferrucci M. S. (2000a) Cytotaxonomy of *Sapindaceae* with special reference to the tribe Paullinieae. Genet. Mol. Biol. 23: 941–946.

Ferrucci M. S. (2000b) Revisión de los géneros de *Cardiospermum* y *Urvillea* para el neotrópico (Sapindaceae). Tesis Doctoral. Universidad Nacional de Córdoba, Córdoba, Argentina.

Ferrucci M. S., Solís Neffa V. G. (1997) Citotaxonomía de Sapindaceae sudamericanas. Bol. Soc. Argent. Bot. 33: 77–83.

Gerlach W. L., Bedbrook J. R. (1979) Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucleic Acids Res. 7: 1869–1885.

Gerlach W. L., Dyer T. A. (1980) Sequence organization of the repeating units in the nucleus of wheat which contain 5S rRNA genes. Nucleic Acids Res. 8: 4851–4865.

Guerra M. S. (1983) O uso do Giemsa em Citogenética Vegetal: comparação entre a coloração simples e o bandamento. Ciência e Cultura 35: 190–193.

Guerra M. S. (2000) Patterns of heterochromatin distribution in plant chromosomes. Genet. Mol. Biol. 23: 1029–1041.

Heslop-Harrison J. S., Schwarzacher T., Anamthewat-Jonsson K., Leitch A. R., Shi M., Leitch I. J. (1991) *In situ* hybridization with automated chromosome denaturation. Technique 3: 109–116.

Hemmer W., Morawetz W. (1990) Karyological differentiation in Sapindaceae with special reference to *Serjania* and *Cardiospermum*. Bot. Acta 103: 372–383.

Lombello A. R., Forni-Martins E. R. (1998) Chromosomal studies and evolution in Sapindaceae. Caryologia 51: 81–93.

Morawetz W. (1986) Remarks on karyological differentiation patterns in tropical woody plants. Pl. Syst. Evol. 152: 49–100.

Nogueira Zampieri C., Ruas P. M., Ruas C. F., Ferrucci M. S. (1995) Karyotypic study of some species of *Serjania* and *Urvillea* (Sapindaceae; Tribe Paullinieae). Amer. J. Bot. 82: 646–654.

Radlkofer L. (1932) Sapindaceae. In: Engler A. (ed.) Pflanzenr. 98 (IV, 165), Wilhelm Engelmann, Leipzig, pp. 352–370.

Schwarzacher T. P., Ambros P., Schweizer D. (1980) Application of Giemsa banding to orchid karyotype analysis. Pl. Syst. Evol. 134: 293–297.

Schweizer D., Loidl J. (1987) A model for heterochromatin dispersion and the evolution of C-band patterns. Chrom. Today 9: 61–74.

Solís Neffa V. G., Ferrucci M. S. (2001) Karyotype analyses of some Paullinieae species (Sapindaceae). Caryologia 54: 371–376.

Vanzela A. L. L., Ruas C. F., Oliveira M. F., Ruas P. M. (2002) Characterization of diploid, tetraploid and hexaploid *Helianthus* species by chromosome banding and FISH with 45S rDNA probe. Genetica 114: 105–111.

Weckerle C. S., Rutishauser R. (2005) Gynoecium, fruit and seed structure of Paullinieae (Sapindaceae). Bot. J. Linn. Soc. 147: 159–189.

Wendel J. F. (2000) Genome evolution in polyploids. Plant Molec. Biol. 42: 225–249.

Addresses of the authors: Juan D. Urdampilleta, José M. D. Torezan, André L. L. Vanzela (correspondence; e-mail: andrevanzela@uel.br), Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de Biologia Geral, CCB, Universidade Estadual de Londrina, 86051-990, Paraná, Brazil. María S. Ferrucci, Instituto de Botánica del Nordeste, Facultad de Ciencias Agrarias, Universidad Nacional del Nordeste, Corrientes, Argentina.