

## Chromosome studies of some *Thinouia* species (Sapindaceae) and the taxonomic implications

Juan D. Urdampilleta<sup>1,\*</sup>, María S. Ferrucci<sup>2</sup> & Eliana R. Forni-Martins<sup>1</sup>

<sup>1</sup> Programa de Pós-Graduação em Biologia Vegetal, Departamento de Botânica, Instituto de Biologia, Universidade Estadual de Campinas, Caixa Postal 6109, CEP 13083-970, Campinas, SP, Brasil (\*e-mail: juanurdampilleta@hotmail.com)

<sup>2</sup> Instituto de Botánica del Nordeste, Universidad Nacional del Nordeste, C.C. 209, Corrientes, Argentina

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The karyotypes of three species of the genus *Thinouia* (Sapindaceae, Paullinieae) from southern Brazil and Bolivia are analyzed. New counts and karyotypes are reported for the first time for *T. ventricosa* and *T. paraguayensis*, both with  $2n = 28$ . The karyotype formulae of *T. mucronata*, *T. paraguayensis* and *T. ventricosa* are  $8m + 14sm + 6st$ ,  $10m + 8sm + 10st$ , and  $14m + 4sm + 10st$ , respectively. In all the species investigated, the prophase chromosome condensation was always proximal and the pattern of the interphase nuclei was non-reticulate. These results are discussed in relation to previous chromosome studies for Paullinieae in order to infer the phylogenetic relationships within the tribe.

Key words: cytotaxonomy, interphase nuclei, karyotype, Sapindaceae, *Thinouia*

### Introduction

*Thinouia* (Sapindaceae, Paullinieae) is a small genus of woody vines with a Neotropical distribution ranging from Mexico to northern Argentina (Radlkofer 1932, Ferrucci 1991). The current tribe classification of the family recognizes two subtribes in Paullinieae: Thinouiinae with the genus *Thinouia* and Paulliniinae with *Cardiospermum*, *Houssayanthus*, *Lophostigma*, *Paullinia*, *Serjania* and *Urvillea*. The monotypic Thinouiinae includes species with actinomorphic flowers with an annular nectary disk, while in the other subtribe the flowers are zygomorphic with a unilateral nectary disk modified into two or four prominent glands.

As treated by Radlkofer (1878), *Thinouia* consists of two sections differing in petal morphology. The section *Thinouia* (as sect. *Lepidodine*), with a single species, *T. myriantha*, is characterized by petals equal to or longer than the petaloid appendage, and in section *Petalodine*, which has seven species (Radlkofer 1932, Ferrucci 1991), the petaloid appendage is longer than the petals. A further species, *T. obliqua*, has an uncertain position. The genus is easily recognized by its schizocarpic fruits and mericarps with a proximal locule and a distal wing. In addition, the fused sepals form a cupular 5-lobed calyx, and another remarkable feature is the precocious aperture of the calyx, which is shared with other American genera such as

### *Lophostigma* and *Matayba*.

As for the karyotypic studies of *Thinouia*, only *T. mucronata*, with  $2n = 28$ , has been cytogenetically studied, suggesting that the basic chromosome number of the genus could be  $x = 14$  (Ferrucci & Solís Neffa 1997, Solís Neffa & Ferrucci 1998). Those studies reported small chromosomes and a relatively symmetric karyotype, which is in contrast to other species of Paullinieae that frequently have larger chromosomes and lower chromosome numbers with moderately asymmetric karyotypes. (Hemmer & Morawetz 1990, Lombello & Forni-Martins 1998). However, within Paullinieae, *Thinouia* shares its chromosome number and size with *Lophostigma plumosum* (Ferrucci & Solís Neffa 1997).

This paper, which reports the karyomorphological results of *T. mucronata*, *T. paraguayensis* and *T. ventricosa*, aims at contributing to the knowledge on the karyotypic characters of Paullinieae. It comparatively presents their chromosome numbers and karyotypic structures and analyzes their interphase nuclei type and condensation patterns. The results are discussed in relation to previous studies in the tribe in order to improve our understanding of the Paullinieae evolution.

## Material and methods

The voucher specimens are deposited at FUEL (Herbarium of the Universidade Estadual de Londrina, Paraná, Brazil) and CTES (Instituto de Botánica del Nordeste, Corrientes, Argentina). The voucher specimens examined are the following:

*T. mucronata*. Brazil. Paraná. Londrina, São Luis, *J. D. Urdampilleta* 153 (CTES, FUEL); Londrina, Fz. Doralice, *J. D. Urdampilleta* 230 (CTES, FUEL).

*T. paraguayensis*. Bolivia. Dpto. Santa Cruz. Prov. Chiquitos, *M. S. Ferrucci, M. Dematteis & A. Schinini* 1915 (CTES, LPB, SI, USZ).

*T. ventricosa*. Brazil. Paraná. Londrina, *J. D. Urdampilleta* 123 (FUEL); Londrina, Fz. Doralice, *J. D. Urdampilleta* 125 (FUEL); Londrina, São Luis, *J. D. Urdampilleta* 189 (FUEL).

Chromosome preparations were obtained from root tips proceeding of germinating seeds. After a pretreatment with 2 mM 8-hydroxyquinoline for 4–5 h at 15 °C, they were fixed in ethanol:acetic acid (3:1, v:v) for 12 hours and

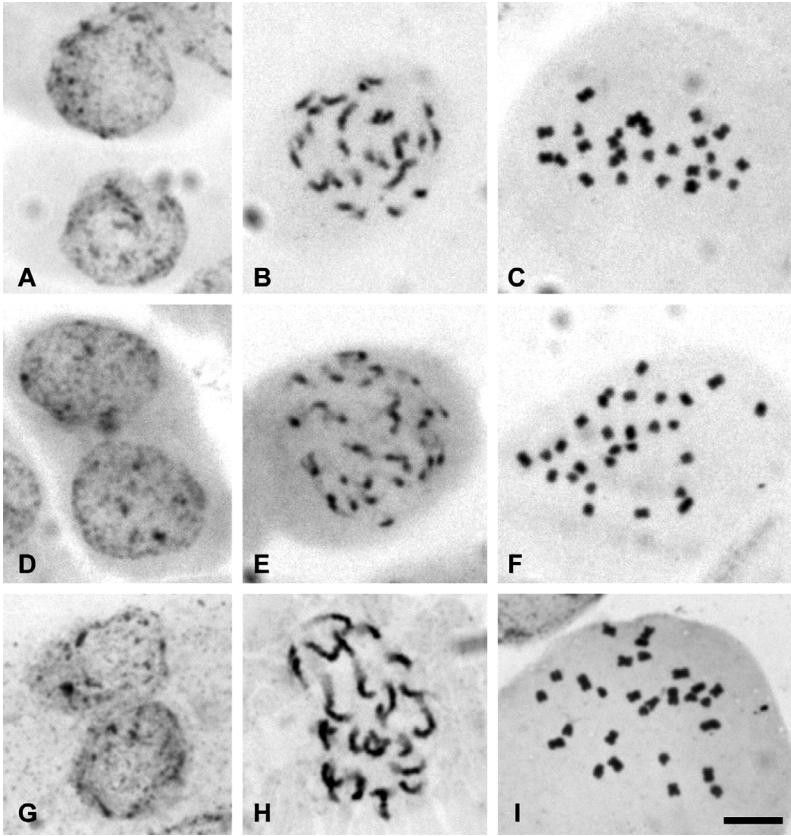
stored at –20 °C until use. For conventional chromosome analysis, the HCl/Giemsa technique (Guerra 1983) was used.

The five best metaphase plates, with similar condensations, were selected for making idiograms. Chromosome nomenclature follows that of Levan *et al.* (1964). The symbols m, sm, and st design metacentric, submetacentric and subtelocentric chromosomes, respectively. The karyotypes were described according to the chromosome morphology determined by the centromeric index (CI = short arm  $\times$  100/total chromosome length) as suggested by Levan *et al.* (1964) and the total chromosome length (TCL) and mean of chromosome length (CL) were calculated. Karyotype asymmetry was evaluated using the average centromeric index (TF%) (Huziwara 1962), and the intra- and interchromosomal asymmetry indexes  $A_1$  and  $A_2$ , respectively (Romero-Zarco 1986).

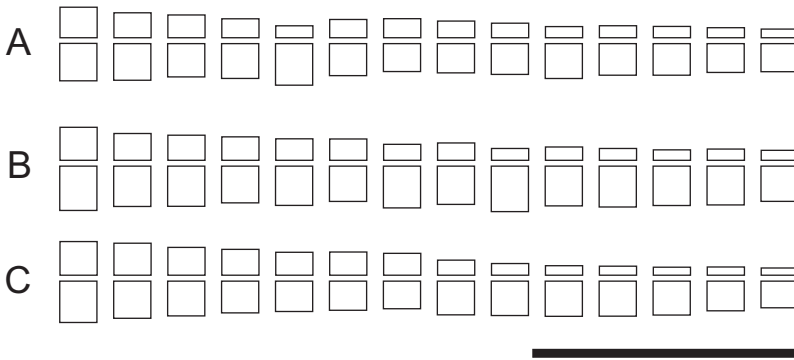
In order to evaluate the differences between species according to the TCL, CL, L (length of long arm) and S (length of short arm) variables, the mean values of the calculated variables were compared, for each species, with ANOVA. The differences between each pair of mean were estimated using the Tukey test ( $\alpha = 0.01$ ). The data were processed with the statistical software InfoStat (2006).

## Results

All the three studied species have  $2n = 28$  (Fig. 1). The chromosome size of the species belongs to the category of very small to small as defined by Lima de Faría (1980), in which chromosome lengths range between 0.64 and 1.46  $\mu\text{m}$ , and the average total chromosome length (TCL) varies between 26 and 30.5  $\mu\text{m}$  (Table 1). The karyotype of *T. mucronata* consists of  $8m + 14sm + 6st$ , differing slightly from the other species by its smaller chromosome length of 0.93  $\mu\text{m}$  (0.69–1.28  $\mu\text{m}$ ). In *T. paraguayensis*, the karyotype is  $10m + 8sm + 10st$  and the average chromosome length is 1.09  $\mu\text{m}$  (0.85–1.46  $\mu\text{m}$ ). In *T. ventricosa*, the karyotype is  $14m + 4sm + 10st$ , and the average chromosome length is 0.96  $\mu\text{m}$  (0.64–1.41  $\mu\text{m}$ ). Although the chromosome size is not useful to distinguish the species, the



**Fig. 1.** HCl/Giemsa stained chromosomes of *Thinouia*. — **A–C:** *T. mucronata*. — **D–F:** *T. paraguayensis*. — **G–I:** *T. ventricosa* **A, D, G:** interphase nuclei; **B, E, H:** prophase nuclei; **C, F, I:** metaphase chromosomes. Scale bar = 5  $\mu$ m.



**Fig. 2.** Idiograms of the haploid complement of the three species of *Thinouia*. — **A:** *T. mucronata*. — **B:** *T. paraguayensis*. — **C:** *T. ventricosa*. Scale bar = 5  $\mu$ m.

number of m, sm and st chromosomes might be an important character of diagnostic value (Table 1 and Fig. 2).

The intrachromosomal asymmetry index ( $A_1$ ) indicates little variation among the length of the chromosome arms of the three species (Table 1). On the other hand, the interchromosomal asymmetry index ( $A_2$ ) shows somewhat more variation among them (Table 1). The dispersion diagram of asymmetry indices points out that *T. paraguayensis* has the greatest intrachromo-

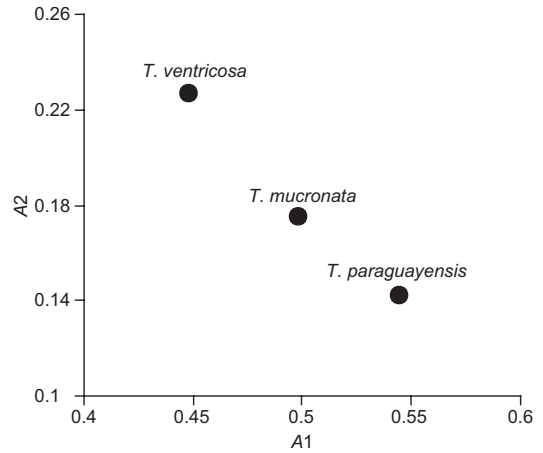
somic asymmetry index while *T. ventricosa* has the greatest interchromosomal value (Fig. 3).

Among the four parameters analyzed (CL, TCL, L and S), significant differences were observed among the species in TCL and L. The TCL and L are significantly greater in *T. paraguayensis* than in the other two species. No significant differences were found for the other variables ( $p > 0.01$ ), so the *Thinouia* species have similar karyotypes (Table 1 and Fig. 4).

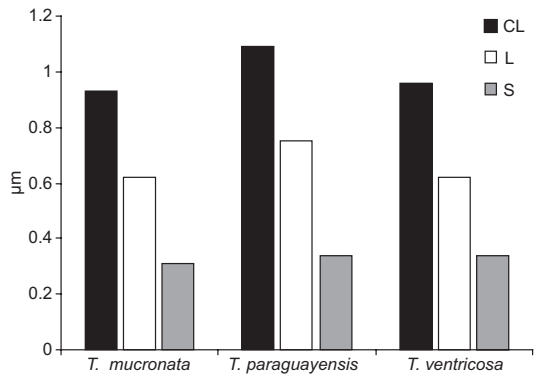
In all the studied species, the interphase

**Table 1.** Karyotype formula (KF), variation of length between the longest and the shortest chromosome ( $V$ ,  $\mu\text{m}$ ), mean chromosome length ( $CL$ ,  $\mu\text{m}$ ) and standard deviation, total chromosome length (TCL,  $\mu\text{m}$ ) and standard deviation, mean long-arm length ( $L$ ,  $\mu\text{m}$ ), mean short-arm length ( $S$ ,  $\mu\text{m}$ ), TF%, intrachromosomal asymmetry index ( $A_1$ ) and interchromosomal asymmetry index ( $A_2$ ). Within each column, means followed by different superscript letters differ significantly ( $p \leq 0.01$ ).

Species	2n	KF	V	CL (SD)	TCL (SD)	L (SD)	S (SD)	TF%	$A_1$	$A_2$
<i>T. mucronata</i>	28	8m + 14sm + 6st	1.28–0.69	0.93 <sup>a</sup> (0.16)	26.0 <sup>a</sup> (0.83)	0.62 <sup>ab</sup> (0.07)	0.31 <sup>a</sup> (0.12)	33.5	0.498	0.175
<i>T. paraguayensis</i>	28	10m + 8sm + 10st	1.46–0.85	1.09 <sup>a</sup> (0.15)	30.5 <sup>b</sup> (0.69)	0.75 <sup>b</sup> (0.06)	0.34 <sup>a</sup> (0.14)	31.3	0.544	0.142
<i>T. ventricosa</i>	28	14 m + 4sm + 10st	1.41–0.64	0.96 <sup>a</sup> (0.22)	27.0 <sup>a</sup> (1.31)	0.62 <sup>ab</sup> (0.08)	0.34 <sup>a</sup> (0.18)	35.7	0.448	0.227



**Fig. 3.** Asymmetry indexes  $A_1$  and  $A_2$  of the *Thinouia* species analyzed.



**Fig. 4.** Mean chromosome (CL), long arm (L) and short arm (S) lengths of the studied species of *Thinouia*.

nucleus structure is non-reticulate with a finely granulated reticulum, and few, small spheroid chromocenters evenly dispersed throughout the nuclei (Fig. 1). The chromosomal behavior during the prophasic condensation was constant in all the studied species. After Giemsa coloration, intensely colored proximal regions were visualized during prophase and prometaphase, while the distal regions of the chromosome arms were extended, showing a typical proximal condensation pattern (Fig. 1).

## Discussion

Chromosome numbers and karyotypes of *T. ventricosa* and *T. paraguayensis* are new to science, while our count on *T. mucronata* ( $2n = 28$ ) con-

firms the number previously reported for two populations of Argentina. The karyotype formula for the proportional number of the m- and sm-type chromosomes differs from what has been reported in earlier studies (Ferrucci & Solís Neffa 1997, Solís Neffa & Ferrucci 1998). Such differences could be due to the observed chromosome condensation.

In the *Thinouia* species analyzed, the very small to small chromosomes and symmetric karyotypes are features that this genus shares in the tribe with *Lophostigma*. It seems to be primitive in the Paullinieae. In addition, the species studied belong to the same section and these results support the infrageneric classification proposed by Radlkofer (1878). The karyotype variation is important not only as a genetic variability, but also because they represent an important micromorphological feature for this genus, and are frequently associated with species differentiation.

According to the results obtained, *T. paraguayensis* can be distinguished from *T. mucronata* and *T. ventricosa* by some karyotypical features such as the total chromosome length and long-arm lengths. *Thinouia paraguayensis* has the most asymmetric karyotype, i.e. the difference between short- and long-arm lengths, as indicated by a smaller TF% and a greater  $A_1$ . However, *T. ventricosa* is the species with the more asymmetric karyotype, i.e. the size variation among chromosomes ( $A_2 = 0.227$ ). The karyotype symmetry gives an idea of the karyotype evolution, the general tendency being that the species with more symmetric karyotypes are primitive (Stebbins 1971). In this study *T. paraguayensis* and *T. ventricosa* should thus be considered the most advanced species of the three studied taxa.

Results on the non-reticulate interphase nucleus structure and the proximal chromosomal behavior during the prophase condensation of *T. mucronata*, *T. ventricosa* and *T. paraguayensis* are here reported for the first time. Most of the analyzed species belonging to the genera *Cardiospermum*, *Paullinia*, *Serjania* and *Urvillea* have semi-reticulate interphase nuclei (Hemmer & Morawetz 1990, Urdampilleta et al. 2006), which suggests that *Thinouia* species might contain a smaller amount of DNA per nucleus than the other species in the tribe (Nagl & Fusening

1979). In Sapindaceae, a non-reticulate nuclear structure has also been recently recorded for two species of *Koelreuteria* (Urdampilleta et al. 2005). In both genera, the non-reticulate nuclei were associated with small chromosomes.

While the prophase condensation pattern in *Thinouia* species is always proximal, in the other Paullinieae genera it is variable. Proximal, heterogeneous and terminal condensation patterns are observed in some species of *Urvillea* and *Cardiospermum*, whereas in *Serjania*, the heterogeneous pattern is predominant (Hemmer & Morawetz 1990, Urdampilleta et al. 2006).

The basic numbers,  $x = 7, 9, 10, 11, 12, 14, 15$  and  $16$  have been reported for the family. Paullinieae thus shows an almost complete aneuploid series from  $x = 7$  to  $x = 14$ . In this tribe, the most advanced of the family, the highest basic chromosome numbers  $x = 15$  and  $x = 16$  are absent. Considering that  $x = 7$  is the primitive base number in Sapindaceae (Ferrucci 1989), the remaining chromosome numbers would be derived through polyploidy and disploidy/aneuploidy. *Thinouia* is considered the most primitive genus within the tribe (Radlkofer 1932, Ferrucci 2000). Despite the fact that, based on cladistic analyses, Acevedo-Rodríguez (1993) placed *Thinouia* in the tribe Cupanieae, macromorphological evidence and the pollen type (Ferrucci & Anzótégui 1993) support its basal position within Paullinieae.

*Thinouia* shares its basic chromosome number with *Lophostigma plumosum*, which supports the evolutionary tendency, based in macromorphology and pollen morphology, proposed for Paullinieae (Ferrucci & Anzótégui 1993). The basic number  $x = 14$  in this tribe would represent a plesiomorphic character shared with Thinouinieae, Sapindeae, Aphanieae, Cupanieae, Dodonaceae and Harpullieae.

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