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

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Received 6 Feb. 2007, revised version received 14 Feb. 2007, accepted 21 Feb. 2007


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: Thinouiniae with the genus *Thinouia* and Paulliniiniae with *Cardiospermum*, *Houssayanthus*, *Lophostigma*, *Paullinia*, *Serjania* and *Urvillea*. The monotypic Thinouiniae 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 plamosum (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. 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 × 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 A1 and A2, 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 (α = 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 µm, and the average total chromosome length (TCL) varies between 26 and 30.5 µm (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 µm (0.69–1.28 µm). In T. paraguayensis, the karyotype is 10m + 8sm + 10st and the average chromosome length is 1.09 µm (0.85–1.46 µm). In T. ventricosa, the karyotype is 14m + 4sm + 10st, and the average chromosome length is 0.96 µm (0.64–1.41 µm). Although the chromosome size is not useful to distinguish the species, the
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 intrachromosomal 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
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The 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).

Table 1. Karyotype formula (KF), variation of length between the longest and the shortest chromosome (V, µm), mean chromosome length (CL, µm) and standard deviation (SD). With each column, means followed by different superscript letters differ significant (p ≤ 0.01).

<table>
<thead>
<tr>
<th>Species</th>
<th>2n</th>
<th>KF</th>
<th>V</th>
<th>CL (SD)</th>
<th>TCL (SD)</th>
<th>L (SD)</th>
<th>S (SD)</th>
<th>TF%</th>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. mucronata</td>
<td>28</td>
<td>8m + 14sm + 6st</td>
<td>1.28–0.69</td>
<td>0.93 ± 0.10</td>
<td>26.0 ± 0.83</td>
<td>0.62 ± 0.07</td>
<td>33.5 ± 0.12</td>
<td>3.04 ± 0.06</td>
<td>0.498 ± 0.14</td>
<td>0.125 ± 0.07</td>
</tr>
<tr>
<td>T. paraguayensis</td>
<td>28</td>
<td>10m + 8sm + 10st</td>
<td>1.46–0.85</td>
<td>1.09 ± 0.15</td>
<td>30.9 ± 0.69</td>
<td>0.75 ± 0.06</td>
<td>31.3 ± 0.14</td>
<td>0.34 ± 0.18</td>
<td>0.448 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>T. ventricosa</td>
<td>28</td>
<td>14m + 4sm + 10st</td>
<td>1.41–0.64</td>
<td>0.96 ± 0.22</td>
<td>27.0 ± 1.31</td>
<td>0.62 ± 0.08</td>
<td>35.7 ± 0.26</td>
<td>0.45 ± 0.05</td>
<td>0.31 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Asymmetry indexes A1 and A2 of the Thinouia species analyzed.

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

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 micro-morphological 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 A1. However, T. ventricosa is the species with the more asymmetric karyotype, i.e. the size variation among chromosomes (A1 = 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 Cardioespernum, Paullinia, Serjania and Urvilea 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, an 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 Urvilea and Cardioespernum, 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 dispoloay/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, macro-morphological evidence and the pollen type (Ferrucci & Anzótegui 1993) support its basal position within Paullinieae.

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

Acknowledgments

The authors thank Ing. Agr. Mercedes Avanza for help with statistic analyses, and the Brazilian agencies CAPES for financial support.
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