Botanical Journal of the Linnean Society, 2007, **154**, 313–320. With 21 figures

Cytogenetic studies of four South American species of *Paullinia* L. (Sapindaceae)

JUAN D. URDAMPILLETA[1], MARÍA S. FERRUCCI[2] and ANDRÉ L. L. VANZELA[1]*

[1]*Laboratório de Biodiversidade e Restauração de Ecossistemas, Departamento de Biologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Campus Universitário, 86051-990, Londrina, Paraná, Brazil*
[2]*Facultad de Ciencias Agrarias, Instituto de Botánica del Nordeste, Universidad Nacional del Nordeste, CC 209, 3400-Corrientes, Argentina*

Received May 2006; accepted for publication March 2007

Four South American species of *Paullinia* (*P. elegans*, *P. meliaefolia*, *P. pinnata*, and *P. rhomboidea*) were compared using conventional chromosome staining, C-Giemsa and C-chromomycin A_3/4′,6-diamidino-2-phenylindole (C-CMA_3/DAPI) banding, and fluorescence *in situ* hybridization (FISH) with a 45S ribosomal DNA (rDNA) probe. All species showed a somatic complement of $2n = 24$ chromosomes, agreeing with earlier records in some cases, and showing a tendency for the chromosome number to be conserved in this genus. The chromosome number of *P. rhomboidea* is a new report. The karyotypes differed in chromosome size and degree of karyotype asymmetry. The chromosomal band patterns and location of the 45S rDNA sites are reported for the first time in the genus. Terminal C-CMA_3 bands were associated with the 45S rDNA sites, but varied in number and size between the species. The occurrence of other C-Giemsa bands that were not revealed by CMA_3 suggests that more than one family of repetitive DNA may be involved in karyotype differentiation. The systematic implications of these results on the infrageneric relationships are discussed. *Botanical Journal of the Linnean Society*, 2007, **154**, 313–320.

ADDITIONAL KEYWORDS: 45S rDNA – C-chromomycin A_3/ 4′, 6-diamidino-2-phenylindole (C-CMA_3/DAPI) – chromosomes – fluorescence *in situ* hybridization (FISH) – heterochromatin – karyotype symmetry.

INTRODUCTION

Paullinia L. is one of the larger genera of the family Sapindaceae, comprising 180–200 species (Ferrucci, 1998; Weckerle & Rutishauser, 2005). It is distributed from the northern states of Mexico to northeast Argentina, but one representative, *P. pinnata*, can also be found in tropical Africa and Madagascar. Radlkofer (1895–96) divided the genus into 13 sections, with the infrageneric classification relying heavily on the anatomy and morphology of the capsules and on sepal connation. The tribe Paullinieae is recognized as a monophyletic group (Acevedo-Rodríguez, 1993), consisting of the genera *Paullinia*, *Cardiospermum* L., *Urvillea* Kunth, *Houssayanthus* Hunz., *Lophostigma* Radlk., and *Serjania* Mill. Of these, *Paullinia*, *Cardiospermum*, and *Urvillea* possess the most derived features, in particular septifragal capsules. *Paullinia* is easily recognized within the tribe by its fleshy capsules, generally reddish or white fleshy arillate seeds, and triporate pollen (Muller & Leenhouts, 1976; Ferrucci & Anzótegui, 1993). *Thinouia* Triana & Planch., placed by Radlkofer in the Paullinieae, should be placed in Cupanieae (Acevedo-Rodríguez, 1993).

Cytogenetic studies in *Paullinia* are scarce, with chromosome numbers reported for only seven species, all with $2n = 2x = 24$, the karyotypes of which have been described for only three (Mangenot & Mangenot, 1958; Semple, 1974; Ferrucci, 1981, 2000; Guerra, 1986; Ferrucci & Solís Neffa, 1997; Solís Neffa & Ferrucci, 1998, 2001). All information on karyotypes in

*Corresponding author. E-mail: andrevanzela@uel.br

Paullinia has been based on the use of conventional staining techniques; banding patterns and the location of ribosomal DNA (rDNA) sites are not known in this group. Staining techniques, such as C-Giemsa and C-chromomycin A_3/4′,6-diamidino-2-phenylindole (C-CMA_3/DAPI) banding and fluorescence *in situ* hybridization (FISH) using rDNA probes, are effective tools for the study of the longitudinal differentiation of chromosomes, and are useful in chromosome recognition, karyotype differentiation, and evolutionary studies in the genus.

The aim of the present study was to differentiate between four South American species of *Paullinia* on the basis of the following karyotype features: (1) prophase condensation pattern; (2) chromosome measurements; (3) occurrence and distribution of heterochromatin; and (4) location of 45S rDNA sites. The findings are discussed in order to better understand and evaluate the relationship between karyotype evolution and infrageneric systematics proposed for the genus.

MATERIAL AND METHODS

Plant material

Four *Paullinia* species were studied: *P. elegans* Cambess., *P. meliaefolia* Juss., *P. pinnata* L., and *P. rhomboidea* Radlk. Each was represented by three populations, except *P. pinnata*, for which only one was analysed. The species and populations are listed in Table 1. Vouchers were deposited at FUEL (Herbarium of the Universidade Estadual de Londrina, Paraná, Brazil).

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 or followed by the HCl/Giemsa staining procedure (Guerra, 1983). Meristems were isolated and squashed in a drop of 45% acetic acid. After coverslip removal for freezing, the material was

Table 1. Karyotype features of the *Paullinia* species, all showing $2n = 24$

Species	Karyotype formula	DSL (σ)	Chromosome size (μm)		A1	A2	45S sites	Locality
			Range	Average				
P. elegans	4m + 4sm + 4st	43.7 (4.35)	2.57–1.18	1.82	0.48	0.24	6	ARGENTINA. Misiones. Urdampilleta *et al*. 147, Posadas (FUEL 34728). BRAZIL. Paraná. Urdampilleta *et al*. 128, Fz. Doralice (FUEL 34789); Urdampilleta *et al*. 131, Primeiro Maio (FUEL 34715)
P. pinnata	4m + 4sm + 4st	60.78 (3.85)	3.43–1.78	2.53	0.47	0.20	6	BRAZIL. Mato Groso do Sul. Urdampilleta *et al*. 231, Pantanal, Passo da Lontra (FUEL)
P. meliaefolia	6m + 1sm + 5st	69.3 (4.71)	3.76–2.07	2.88	0.51	0.20	4	BRAZIL. Paraná. Urdampilleta *et al*. 134, Arapongas (FUEL 34732); Urdampilleta *et al*. 178, Assai, Café Forte (FUEL); Urdampilleta *et al*. 186, São Luís (FUEL)
P. rhomboidea	6m + 3sm + 3st	34.92 (2.45)	2.14–0.89	1.45	0.46	0.29	2	BRAZIL. Paraná. Urdampilleta *et al*. 158, Jataizinho (FUEL 34726); Urdampilleta *et al*. 185, São Luís (FUEL), Urdampilleta *et al*. 233, Parque Arthur Thomas (FUEL)

A1 and A2, asymmetry indices; DSL, diploid set length (μm); σ, standard deviation; 45S sites, 45S rDNA site number.

stained with 2% Giemsa. Idiograms were prepared on the basis of measurements of five metaphases with similar condensation levels. The chromosome shape was defined according to Levan, Fredga & Sandberg (1964), and the asymmetry indices (A1 and A2) were determined according to Romero Zarco (1986).

Chromosome banding

Chromosome banding techniques were carried out on root tips softened in 4% cellulase plus 40% pectinase at 37 °C for 2 h and squashed in a drop of 45% acetic acid. After freezing and coverslip removal, the preparations were treated for C-banding according to Schwarzacher, Ambros & Schweizer (1980), with minor modifications. After hydrolysis in 45% acetic acid at 60 °C for 10 min, the slides were incubated in 5% $Ba(OH)_2$ at room temperature for 10 min and in 2 × SSC, pH 7.0, at 60 °C for 80 min. Samples were stained in 2% Giemsa, or with a drop of 0.5 mg ml^{-1} CMA_3 in McIlvaine buffer, pH 7.0, and distilled water (1 : 1) containing 2.5 mM $MgCl_2$ for 90 min, washed in distilled water, and subsequently stained with 2 μg ml^{-1} DAPI for 30 min. Slides were mounted with 50% glycerol in McIlvaine buffer containing 2.5 mM $MgCl_2$.

Fluorescence *in situ* hybridization

FISH was carried out following the method of Heslop-Harrison *et al*. (1991) and Cuadrado & Jouve (1994), with modifications (see Vanzela *et al*., 2002). The p*Ta*71 probe containing the 18S–5.8S–26S rDNA sequence isolated from wheat (Gerlach & Bedbrook, 1979) was used to locate the 45S rDNA chromosome sites. The probe was labelled with biotin-14–dATP by nick translation. Preparations were incubated in 100 μg ml^{-1} RNase, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series, and air-dried. A hybridization mix (30 μl per slide), containing 50–100 ng of labelled probe (4 μl), 100% formamide (15 μl), 50% polyethylene glycol (6 μl), 10% sodium dodecylsulphate (SDS) (1 μl), 100 ng of calf thymus DNA (1 μl), and 20 × SSC (3 μl), was denatured at 70 °C for 10 min and immediately chilled on ice. Chromosome denaturation/hybridization was performed at 90 °C for 10 min, 48 °C for 10 min, and 38 °C for 5 min using a thermal cycler (MJ Research), and placed in a humid chamber at 37 °C overnight. Post-hybridization washes were carried out in 2 × SSC, 20% formamide in 0.1 × SSC, 0.1 × SSC, 2 × SSC, and 4 × SSC/0.2% Tween 20, all at 42 °C. The hybridization sites were detected with avidin–fluorescein isothiocyanate (FITC) conjugate, and post-detection washes were carried out in 4 × SSC/0.2% Tween 20 at room temperature. Next, the samples were counter-stained with 2.5 μg ml^{-1} propidium iodide, and the slides were mounted in a solution of 50% antifade and 50% glycerol in McIlvaine buffer containing 2.5 mM $MgCl_2$.

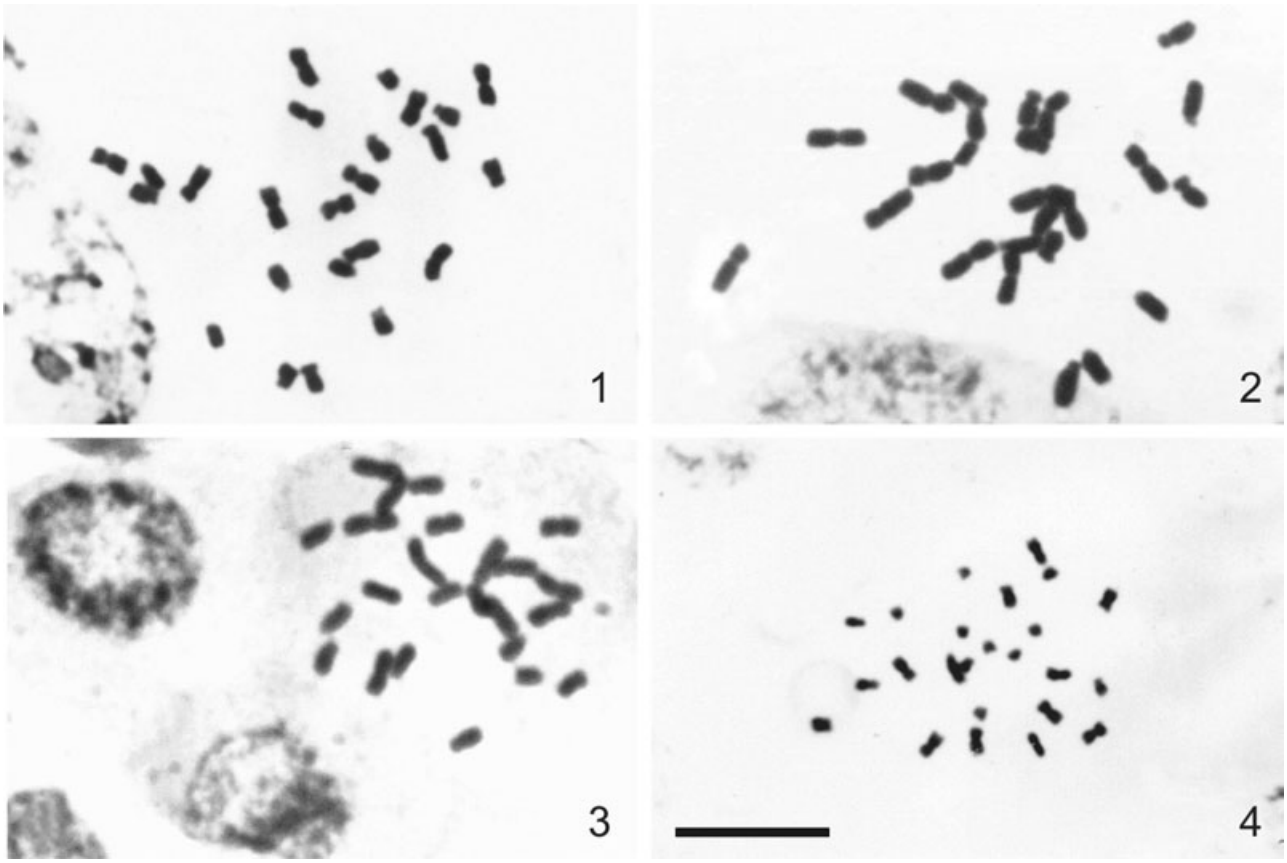
Photographs were taken using Kodak Imagelink HQ 25 ISO film for conventional staining and C-Giemsa banding, Kodak T-Max 100 ISO for C-CMA_3/DAPI banding, and Fuji Color 100 ISO for FISH.

RESULTS AND DISCUSSION

The chromosome number $2n = 2x = 24$ was confirmed for *P. elegans*, *P. meliaefolia*, and *P. pinnata*, and reported for *P. rhomboidea* for the first time (Table 1, Figs 1–4). The karyotypes are described in Table 1 and the idiograms are shown in Figure 5. The two species belonging to the section *Paullinia* (*P. elegans* and *P. pinnata*) have the same karyotype formulae composed by 4m + 4sm + 4st chromosomes. In *P. meliaefolia* (section *Caloptilon*) the karyotype consists of 6m + 1sm + 5st chromosomes, a more asymmetrical karyotype as a result of the presence of more subteliocentric chromosomes then in the other three species. The karyotype of *P. rhomboidea* (section *Phygoptilon*) is more symmetrical, comprising 6m + 3sm + 3st chromosomes. This is an important feature because it is shared with other species of the section *Phygoptilon*, such as *P. racemosa* Wawra and *P. coriacea* Casar. (Solís Neffa & Ferrucci, 1998, 2001).

The karyotype size and symmetry analysis of these four *Paullinia* species show an evident interspecific variation. *P. meliaefolia* exhibits the largest chromosomes (Fig. 2), with a diploid combined chromosome set length (DSL) of 69.30 μm and an individual chromosome length variation from 3.76 to 2.07 μm. Symmetry analysis shows that *P. meliaefolia* possesses the largest value of A1, as a result of its large number of telocentric chromosomes, and the smallest value of A2, which it shares with *P. pinnata* (Table 1, Fig. 6), as described previously by Solís Neffa & Ferrucci (1998). *P. meliaefolia* exhibits the most asymmetrical karyotype of the four species analysed here and may be associated with other morphological features, such as three-winged capsules, persistent wings, non-bipartible endocarp, and five free sepals, which characterize the section *Caloptilon* (Radlkofer, 1931–32).

Paullinia rhomboidea shows the karyotype with the smallest chromosomes, with a DSL of 34.9 μm, and an individual chromosome length ranging from 2.14 to 0.89 μm. Karyotype symmetry analysis indicates that *P. rhomboidea* has the smallest A1 value (0.46), because of its lack of telocentric chromosomes, and the largest A2 value (0.29), because of the larger difference in size between the largest and smallest chromosomes (Table 1, Fig. 4). This karyotype type has also been described for *P. racemosa* (Solís Neffa & Ferrucci,



Figures 1–4. Mitotic metaphases conventionally stained showing $2n = 24$. Fig 1. *Paullinia elegans*. Fig. 2. *P. meliaefolia*. Fig. 3. *P. pinnata*. Fig. 4. *P. rhomboidea*. Scale bar, 10 μm.

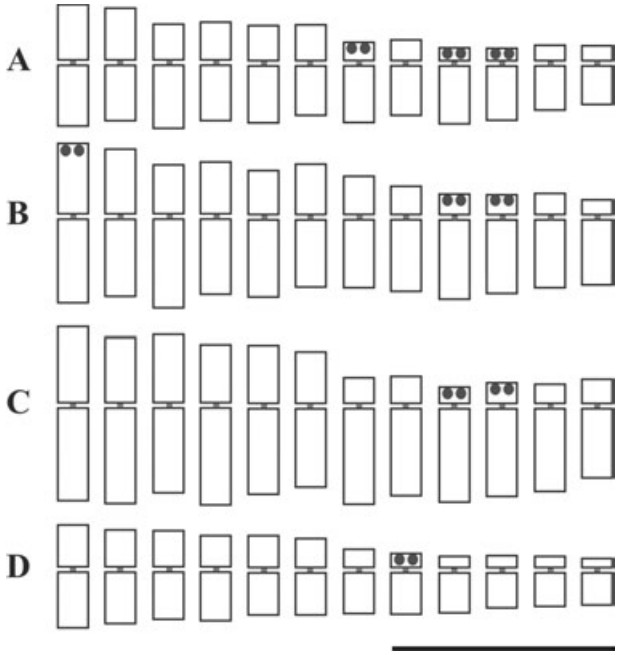


Figure 5. Idiograms of the *Paullinia* species studied. A, *P. elegans*. B, *P. pinnata*. C, *P. meliaefolia*. D, *P. rhomboidea*. Scale bar, 5 μm. ●, CMA_3^+/rDNA 45S sites.

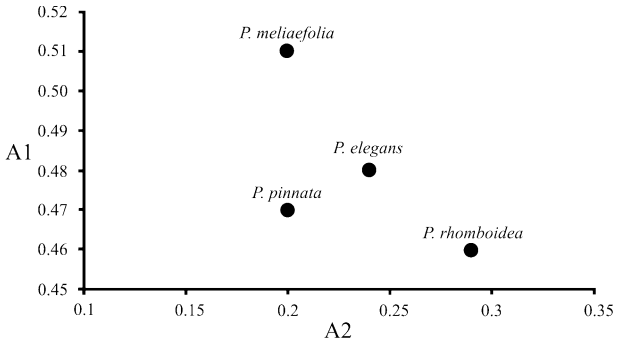


Figure 6. Dispersion diagram based on the asymmetry coefficients A1 and A2 obtained for the *Paullinia* species.

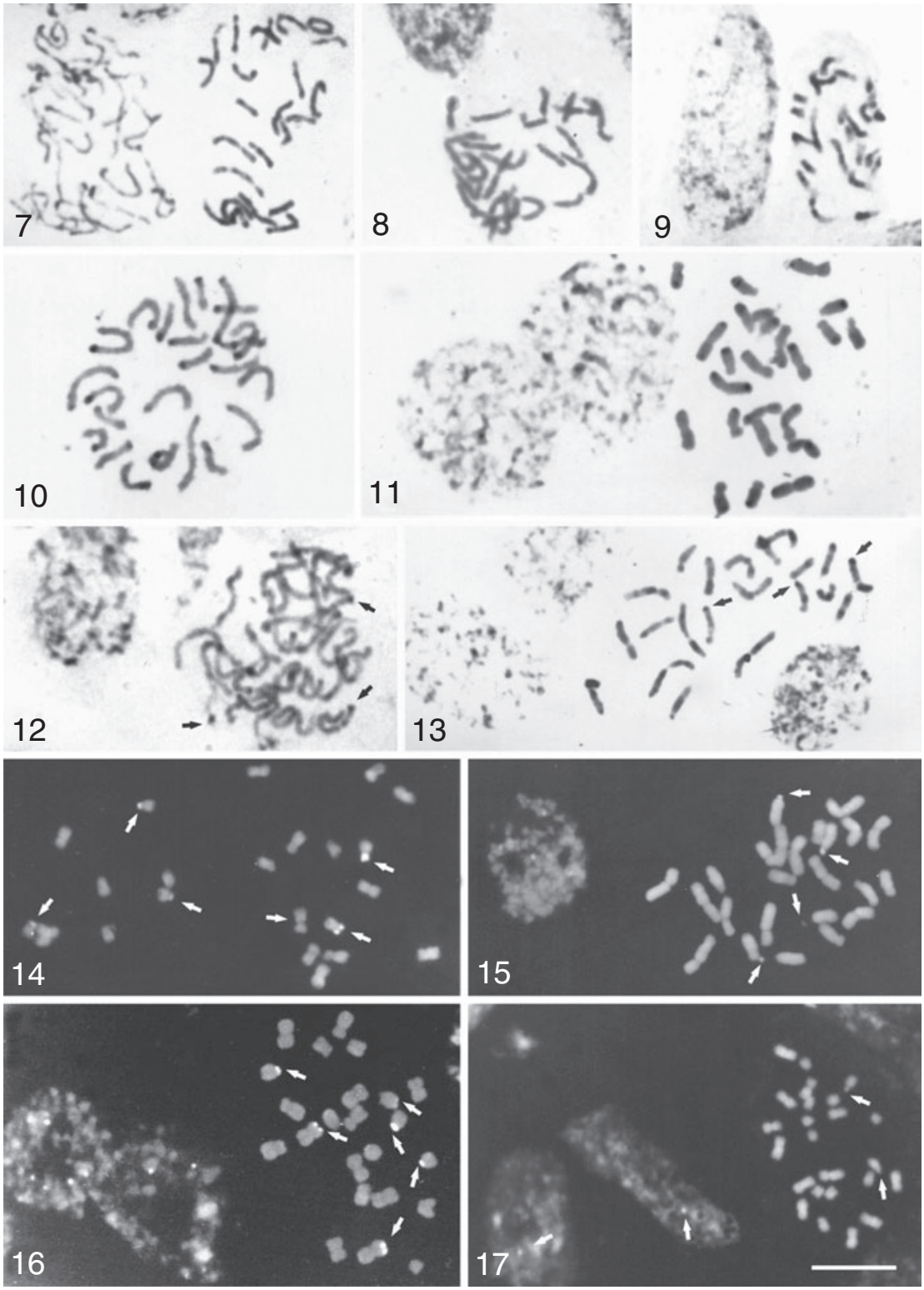
1998), both species belonging to the section *Phygoptilon*. Within this section, *P. rhomboidea* is closely related to *P. racemosa*, although they are easily distinguished, as the former species has bicompound leaves and the latter has compound leaves. The other two species in the section, known cytologically only by their chromosome number, are *P. coriacea* Casar. and *P. weinmanniifolia* Mart. (Ferrucci, 2000), which have small-sized chromosomes in common with *P. racemosa* and *P. rhomboidea*. Although there is little cytogenetic information about this section, it is possible that karyotypes with small chromosomes are associated with other features that are common to the section *Phygoptilon*, such as capsules that are three-winged or, less often, with three pronounced dorsal longitudinal ribs, endocarp in the wings finally divided into two parts, and four sepals, the third and the fourth being fused (Radlkofer, 1931–32).

Paullinia elegans (Fig. 1) and *P. pinnata* (Fig. 3) show chromosomes of intermediate size, with a diploid set length of 43.7 and 60.8 μm, respectively, and a chromosome size variation from 2.57 to 1.18 μm and from 3.43 to 1.78 μm, respectively (Table 1). The karyotype organization of *P. elegans* found here is similar to that described for this species by Solís Neffa & Ferrucci (1998), with minor differences in the chromosome types. *P. elegans* and *P. pinnata* belong to the section *Paullinia*, which is characterized by five free sepals, wingless capsules, and mesocarp strongly nerved. When the mesocarp is dry, it appears slightly ligneous and delicately oblique-striated. The karyotype similarities observed between these two species tend to support their placement in this section (Radlkofer, 1931–32).

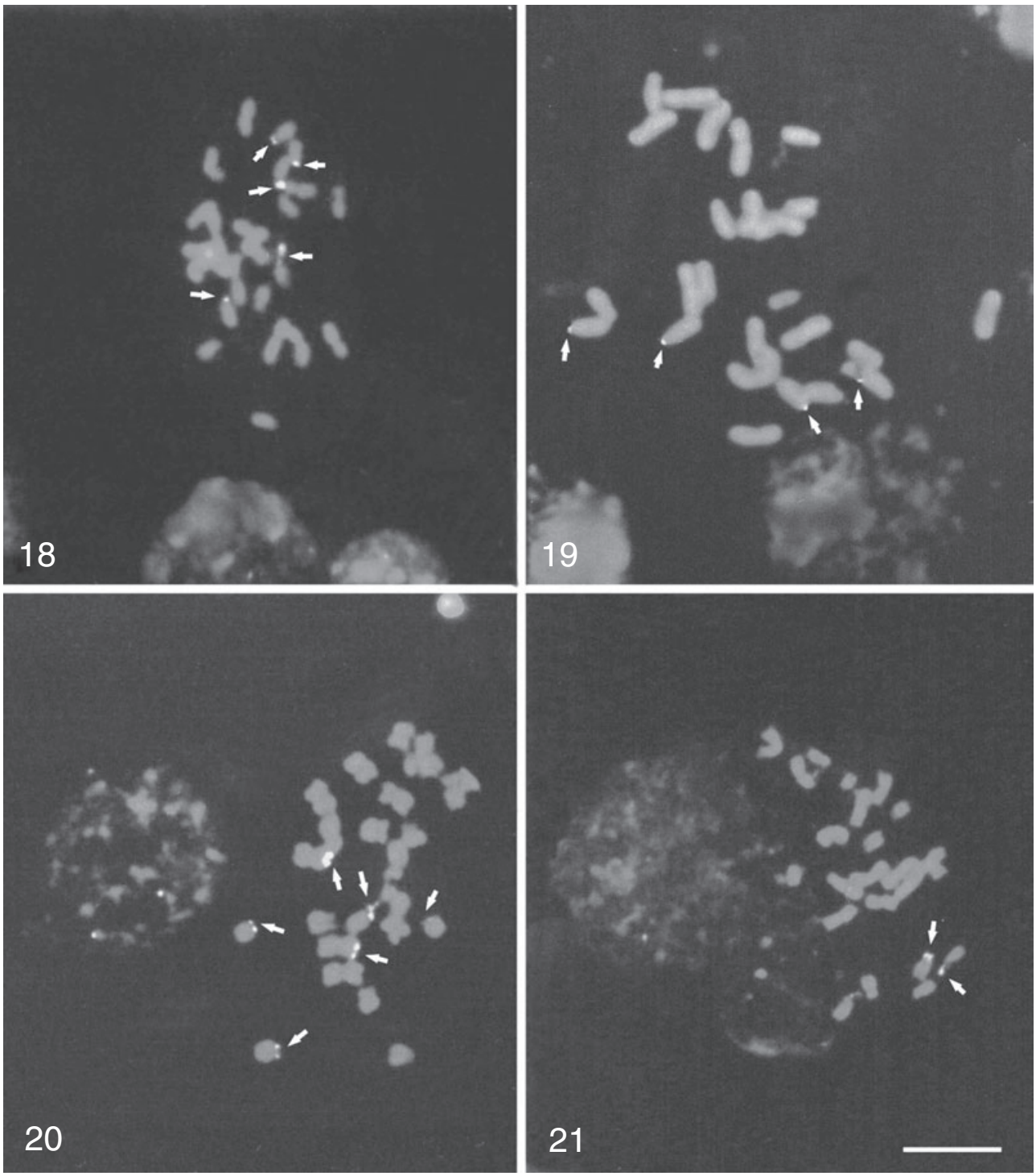
Other cytogenetic parameters compared between these species were the interphase nuclear morphology and prophase condensation pattern. The semi-reticulate nuclei were constant between the four species, but the prophase condensation showed a distinction of *P. rhomboidea* from the rest of the species. *P. elegans*, *P. meliaefolia*, and *P. pinnata* showed a heterogeneous condensation pattern, with condensation in the proximal, interstitial, and terminal regions (Figs 7, 8), whereas *P. rhomboidea* showed a proximal condensation pattern, suggesting a differentiated genome organization (Fig. 9). These data permit the separation of the section *Phygoptilon* from the sections *Paullinia* and *Caloptilon*. Unfortunately, there are no other comparative studies regarding prophase condensation in Sapindaceae to support a discussion about this matter.

Giemsa C-banding enabled *P. elegans* to be differentiated from the other three species. *P. elegans* showed terminal heterochromatic blocks in several chromosomes of the complement, in addition to some interstitial bands (Figs 10, 11). There were no visible heterochromatic blocks in the other species, except for *P. meliaefolia*, which showed some small, terminal Giemsa C-bands, best observed in pre-metaphase (Figs 12, 13). The terminal C-Giemsa blocks found in *P. elegans* and *P. meliaefolia* were observed, not as typical GC- or AT-rich segments after C-CMA_3/DAPI staining, but as a typical neutral heterochromatin, as, when stained with CMA_3 and DAPI, the fluorescence intensity was the same. Neutral bands have been described in some Arecaceae species belonging to the genera *Coccothrinax* Sarg. and *Schippia* Burret (Röser, 1994), as well as in other species of angiosperms (Guerra, 2000). In *Paullinia*, the C-CMA_3 bands indicating GC-rich sites were found in the short arms (Figs 14–17). However, interspecific variations in band numbers and chromosome types were found. *P. elegans* (Fig. 14) and *P. pinnata* (Fig. 16) showed six terminal C-CMA_3^+ bands, in addition to a tenuous and interstitial/proximal band in an sm pair in *P. elegans* (Figs 5, 14). *Paullinia meliaefolia* and *P. rhomboidea* exhibited two and one chromosome pairs, respectively, with small C-CMA_3^+ terminal bands. In *P. meliaefolia*, bands appeared on the short arm of telocentric pairs (Figs 5, 15). These data agree with those of Solís Neffa & Ferrucci (1998), who recognized satellites on the telocentric chromosomes (such as those shown in Fig. 5). In *P. rhomboidea*, bands were visible terminally on the short arm of a small subtelocentric pair (Fig. 5). The association of satellite positions, C-Giemsa and C-CMA_3 bands, and 45S rDNA sites has been approached previously in the literature (see Guerra, 2000). This situation is also evident in *Paullinia*. The segments detected after FISH with the p*Ta*71 probe coincided with the number and positions of C-CMA_3^+ bands (Table 1, Figs 18–21). Two sites of 45S rDNA were localized in *P. rhomboidea* (Fig. 21), four in *P. meliaefolia* (Fig. 19), and six in *P. elegans* and *P. pinnata* (Figs 18, 20, respectively), all being in the terminal positions on the short arms, but in different chromosome pairs. In *P. meliaefolia* the 45S rDNA sites were localized in acrocentric chromosomes, whereas, in *P. elegans*, *P. pinnata*, and *P. rhomboidea*, they appeared on the subtelocentric pairs. Especially in *P. pinnata*, the 45S rDNA hybridization site was located on the first metacentric chromosome pair, a finding which allowed this species to be differentiated from the other species studied (Figs 5, 20). This feature could represent a specific marker not explored until now, and this finding suggests the involvement of rDNA in karyotype differentiation in the group.

Our results allow us to propose the existence of at least two highly repetitive DNA types involved in karyotype differentiation in *Paullinia*, which interestingly follow a pattern of equilocal distribution. The first corresponds to GC-rich repetitive



Figures 7–17. Mitotic pre-metaphases conventionally stained and C-chromomycin A_3 (C-CMA_3) banding patterns in *Paullinia*. Figs 7,8. Heterogeneous condensation pattern in *P. elegans* and *P. meliaefolia*, respectively. Fig. 9. Proximal condensation pattern in *P. rhomboidea*. Figs 10, 11. C-Giemsa banding in *P. elegans*. Figs 12, 13. C-Giemsa banding in *P. meliaefolia*. Arrows show the terminal C-Giemsa blocks. Figs 14–17. C-CMA_3 banding in *P. elegans*, *P. meliaefolia*, *P. pinnata*, and *P. rhomboidea*, respectively. Arrows show C-CMA_3^+ blocks. Scale bar, 10 μm.



Figures 18–21. Fluorescence *in situ* hybridization (FISH) with the p*Ta*71 probe (rDNA 45S) in *Paullinia*. Fig. 18. *P. elegans*. Fig. 19. *P. meliaefolia*. Fig. 20. *P. pinnata*. Fig. 21. *P. rhomboidea*. Arrows show the terminal hybridization sites. Scale bar, 10 μm.

DNA, which is associated with the nucleolar organizing region (NOR), and the second corresponds to C-Giemsa heterochromatic segments, which are neutral after C-CMA_3/DAPI staining. Similarly, the occurrence of two or more DNA repetitive types has been observed in species of the genus *Urvillea*. AT-rich sequences were shown in *U. chacoensis* Hunz. and *U. ulmacea* Kunth, whereas, in *U. ulmacea*, the GC-

rich sequences were associated with AT sites forming complex blocks of heterochromatin (Urdampilleta *et al*., 2006).

Because there is little cytological information available for *Paullinia*, these chromosome analyses provide valuable data to delineate the infrageneric systematics of the genus. Knowledge of chromosome morphology becomes more important in cases of closely related taxa with the same chromosome number. More sensitive techniques may be useful in identifying individual chromosomes and may reveal karyotype variations and possible phylogenetic relationships between species of *Paullinia*.

ACKNOWLEDGEMENTS

The authors thank the Brazilian agencies Fundação Araucária, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Instituto Ambiental do Paraná – Secretaria Estadual do Meio Ambiente (IAP-SEMA), and Conselho Nacional de Desenvolvimento Científico (CNPq) for financial support. Dr A. Leyva provided English language editing of the manuscript.

REFERENCES

Acevedo-Rodríguez P. 1993. Systematics of *Serjania* (Sapindaceae). Part I: a revision of *Serjania* Sect. *Platycoccus*. *Memoirs of the New York Botanical Garden* **67:** 1–93.

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

Ferrucci MS. 1981. Recuentos cromosómicos en Sapindáceas. *Bonplandia* **5:** 73–81.

Ferrucci MS. 1998. *Sapindaceae*. In: Hunziker AT, ed. *Flora Fanerogámica Argentina* 52: 1–44.

Ferrucci MS. 2000. Cytotaxonomy of *Sapindaceae* with special reference to the tribe Paullinieae. *Genetics and Molecular Biology* **23:** 941–946.

Ferrucci MS, Anzótegui LM. 1993. El polen de Paullinieae (Sapindaceae). *Bonplandia* **6:** 211–243.

Ferrucci MS, Solís Neffa VG. 1997. Citotaxonomía de Sapindaceae sudamericanas. *Boletín de la Sociedad Argentina de Botánica* **33:** 77–83.

Gerlach WL, Bedbrook JR. 1979. Cloning and characterization of ribosomal RNA genes from wheat and barley. *Nucleic Acids Research* **7:** 1869–1885.

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

Guerra MS. 1986. Citogenética de angiospermas coletadas em Pernambuco. I. *Revista Brasileira de Genética* **9:** 21–40.

Guerra MS. 2000. Patterns of heterochromatin distribution in plant chromosomes. *Genetics and Molecular Biology* **23:** 1029–1041.

Heslop-Harrison JS, Schwarzacher T, Anamthewat-Jonsson K, Leitch AR, Shi M, Leitch IJ. 1991. *In situ* hybridization with automated chromosome denaturation. *Technique* **3:** 106–109.

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

Mangenot S, Mangenot G. 1958. Deuxième liste de nombres chromosomiques nouveaux chez diverses dicotylédones et monocotylédones d'Afrique occidentale. *Bulletin du Jardin Botanique de l'État à Bruxelles, Bruxelles* **28:** 315–329.

Muller J, Leenhouts PW. 1976. A general survey of pollen types in Sapindaceae in relation to taxonomy. In: Ferguson IK, Muller J, eds. *The evolutionary significance of the exine. Linnean Society Symposium* **1:** 407–495.

Radlkofer L. 1895–96. *Monographie der Sapindaceen-Gattung Paullinia*. München: Verlag der K.B. Akademie, 67–381.

Radlkofer L. 1931–32. Sapindaceae. In: Engler A, ed. *Das Pflanzenreich*, Vol. 98. Leipzig: Wilhelm Engelmann, 219–352 (IV. 165).

Romero Zarco C. 1986. A new method for estimating karyotype asymmetry. *Taxon* **35:** 526–530.

Röser M. 1994. Pathways of karyological differentiation in palms (Arecaceae). *Plant Systematics and Evolution* **189:** 83–122.

Schwarzacher TP, Ambros P, Schweizer D. 1980. Application of Giemsa banding to orchid karyotype analysis. *Plant Systematics and Evolution* **134:** 293–297.

Semple JC. 1974. Chromosome numbers of phanerogams. 5. *Annals of the Missouri Botanical Garden* **61:** 902–903.

Solís Neffa VG, Ferrucci MS. 1998. Cariotipos de Sapindaceae sudamericanas. *Boletín de la Sociedad Argentina de Botánica* **33:** 185–190.

Solís Neffa VG, Ferrucci MS. 2001. Karyotype analyses of some Paullinieae species (Sapindaceae). *Caryologia* **54:** 371–376.

Urdampilleta JD, Ferrucci MS, Torezan JMD, Vanzela ALL. 2006. Karyotype relationships among four South American species of *Urvillea* (Sapindaceae: Paullinieae). *Plant Systematics and Evolution* **258:** 85–95.

Vanzela ALL, Ruas CF, Oliveira MF, Ruas PM. 2002. Characterization of diploid, tetraploid and hexaploid *Helianthus* species by chromosome banding and FISH with rDNA probe. *Genetica* **114:** 105–111.

Weckerle CS, Rutishauser R. 2005. Gynoecium, fruit and seed structure of Paullinieae (Sapindaceae). *Botanical Journal of the Linnean Society* **147:** 159–189.