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Cytogenetic studies in South American species of *Serjania* (Sapindaceae: Paullinieae)

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Abstract

Serjania Mill. (Paullinieae) is considered the most important neotropical genus of Sapindaceae due to species number and its widespread distribution. In this study, 14 species belonging to three sections were analyzed using conventional staining, C/CMA/DAPI banding, and fluorescence *in situ* hybridization (FISH) with a 18S-5.8S-26S rDNA probe. New chromosome counts are reported for *Serjania crassifolia*, *Serjania platycarpa*, and *Serjania regnellii*, all with $2n = 24$, which is remarkably constant for *Serjania*. The karyotypes are moderately asymmetric, and variations observed in A1 and A2 indices show resemblances between *S. platycarpa*, *Serjania hebecarpa*, and *S. crassifolia*, and between *Serjania communis*, *Serjania gracilis*, and *S. regnellii*. The banding pattern was homogeneous in *Serjania*. C/DAPI bands (AT-rich sites) were not clearly evidenced, but changes in the number and position of GC-rich sites (CMA bands) were observed. These segments were associated with 18S-5.8S-26S rDNA sites. The significance of the results is discussed in relation to chromosomal data available for the genus and in regard to the infrageneric treatment of *Serjania*.

Keywords: *18S-5.8S-26S rDNA, Giemsa C-banding, fluorochrome banding, FISH, cytotaxonomy, karyotype symmetry, Serjania*

Introduction

Serjania Mill. (Paullinieae) is an American genus with about 230 species, vines, or lianas, distributed in tropical and subtropical areas of the New World (Ferrucci & Acevedo-Rodríguez 2005), ranging from the southern United States to Uruguay and central Argentina (Acevedo-Rodríguez 1993). Within the Paullinieae, *Serjania* can be identified by its schizocarpic fruits, separated into three samaroid mericarps with a distal locule, which distinguishes it from other genera of *Paullinieae*, such as *Cardiospermum* L., *Houssayanthus* Hunz., *Paullinia* L., and *Urvillea* Kunth. A comprehensive revision of the genus was done by Radlkofer (1875), subdividing *Serjania* into 12 sections based mainly on fruit morphology. The proposed system is rather difficult to use because of the intergradations of characters used to define some of the sections. Acevedo-Rodríguez (1993), in revising the section *Platycoccus*, combined eight of Radlkofer's sections and created a new section, thus recognizing a total of six sections in *Serjania*. However, he pointed out that, considering the still rather chaotic systematics of *Serjania*, a complete revision of the genus is needed. A survey of pollen morphology in 31 species of *Serjania*, covering all six recognized sections, showed that the pollen morphology does not contribute much to the infrageneric classification of the genus (Van der Ham & Tomlik 1994).

Previous cytogenetic studies were realized in 35 species of *Serjania* belonging to five sections, according to Acevedo-Rodríguez (1993). The results suggested a conservation of the chromosome number $2n = 24$ (Fernández Casas & Fernández Piqueras 1981; Ferrucci 1981, 1985; Nogueira et al. 1995;

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Ferrucci & Solís Neffa 1997; Solís Neffa & Ferrucci 1997, 1998). Solís Neffa and Ferrucci (1997) described the karyotypes of 15 species and found intra- and interspecific changes in both chromosomal size and morphology; nevertheless, these changes did not present a direct relationship with the infrageneric classification proposed by Radlkofer (1875).

Studies of chromosome banding and location of repetitive DNA are limited in *Sapindaceae*. Hemmer and Morawetz (1990) analyzed the patterns of Giemsa C-bands in species of *Serjania* and *Cardiospermum*, and proposed that *Serjania* is characterized by the absence of bands, except in nucleolar organizer regions (NORs), whereas AT-rich heterochromatin blocks were found on the terminal regions of several chromosomes of *Cardiospermum*. Studies in some species of *Urvillea* and *Paullinia* showed interspecific variations in the occurrence of AT- and GC-rich bands and 18S-5.8S-26S rDNA site numbers (Urdampilleta et al. 2006, 2007). Variation in 18S-5.8S-26S rDNA site number is often related to the differences in ploidy level, however, some of these sites are often deleted in polyploids (Pellicer et al. 2010).

The aim of this study was to analyze the karyotype of 14 *Serjania* species from southern South America by conventional staining, in an attempt to characterize karyotypic diversity, as well as to find diagnostic characters that can contribute to the proposed infrageneric classification. This karyotype characterization was complemented, in some species, with studies on chromosome-banding pattern and 18S-5.8S-26S rDNA distribution.

Materials and methods

Table I shows the 14 taxa studied and for which vouchers were deposited at FUEL (Herbarium of the Universidade Estadual de Londrina, Paraná, Brazil) and at CTES (Instituto de Botánica del Nordeste, Corrientes, Argentina).

The chromosome preparations were obtained 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 12–24 h and stored at −20°C until the use. A conventional analysis was performed using the HCl/Giemsa technique proposed by Guerra (1983). The chromosome number was determined in all accessions of each species. Idiograms and karyotype analyses were conducted from chromosome measurements of at least five metaphases of each accession using MicroMeasure v3.3 (Colorado State University). The chromosome classification was done according to Levan et al. (1964). Karyotypes were compared using: average chromosome length (ACL), total chromosome length (TCL) of diploid complement, ratio between longest and shortest chromosome pairs (RI), average centromeric index (ACI), A_1/A_2 (Romero Zarco 1986), and CVci (Paszko 2006) asymmetry indices. In order to evaluate the differences among species, the mean values of TCL, ACL, RI, and ACI were compared by variance analysis (ANOVA). The differences between each pair of means were estimated using the test of Tukey ($\alpha = 0.01$). The data were processed with the statistical software Statistica v.6 (StatSoft, Inc.).

For chromosome banding, pretreated root tips were digested with 4% cellulase and 40% pectinase at 37°C for 2 h and dissected in a drop of 45% acetic acid. The coverslips were removed with liquid nitrogen. The C banding procedure was performed according to Schwarzacher et al. (1980) with some modifications. Samples were treated with 45% acetic acid (10 min at 60°C), 5% barium hydroxide (10 min at 25°C), and saline sodium citrate (SSC2×), pH 7.0 (1 h and 20 min at 60°C). The samples were then stained with 2% Giemsa and

Table I. Species and accessions of the examined individuals from the genus *Serjania*.

Species	Locality
S. communis Cambess.	BRAZIL. Paraná. Londrina, Urdampilleta *et al.* 214 (FUEL)
S. crassifolia Radlk.	BOLIVIA. Depto. Santa Cruz. Ferrucci *et al.* 1923 (CTES)
S. fuscifolia Radlk.	BRAZIL. Paraná. Londrina, Urdampilleta *et al.* 174 (FUEL)
S. glabrata Kunth	ARGENTINA. Misiones. Puerto Iguazú, Urdampilleta *et al.* 141 (FUEL)
S. glutinosa Radlk.	BRAZIL. Paraná. Londrina, Urdampilleta *et al.* 129 (FUEL)
S. gracilis Radlk.	BRAZIL. Paraná. Guartela, Urdampilleta *et al.* 217 (FUEL)
S. hebecarpa Benth.	BOLIVIA. Dpto. Santa Cruz. Prov. Chiquitos, Ferrucci *et al.* 1918 (CTES)
S. laruotteana Cambess.	BRAZIL. Paraná. Londrina, Urdampilleta *et al.* 173 (FUEL)
S. meridionalis Cambess.	ARGENTINA. Misiones. Garupá, Urdampilleta *et al.* 198 (FUEL)
S. multiflora Cambess.	BRAZIL. Paraná. Arapongas, Urdampilleta *et al.* 133 (FUEL)
S. perulacea Radlk.	BRAZIL. Paraná. Assaí, Urdampilleta *et al.* 179 (FUEL)
S. platycarpa Benth.	BOLIVIA. Depto. Santa Cruz. Prov. Ñuflo de Chavez, Ferrucci *et al.* 1836 (CTES)
S. regnellii Schltdl.	BRAZIL. São Paulo. Aguas de Lindóia, Urdampilleta *et al.* 240 (FUEL)
S. tripleuria Ferrucci	BOLIVIA. Depto. Chuquisaca. Prov. Calvo, Ferrucci *et al.* 1767 (CTES)

Table II. Karyotype parameters of *Serjania* species and sections studied.

Species	Section Radlkofer[1]	Section Acev.-Rodríguez[2]	Karyotype formulae	ACL	CV	TCL	RI	ACI	A_1	A_2	CVci
S. glutinosa	*Eurycoccus*	*Eurycoccus*	2 m + 8 sm + 2 st	2.20^{abc}	3.12–1.60	52.8^{abc}	1.94^{abc}	31.10^{bcd}	0.525	0.220	29.1
S. gracilis	*Eurycoccus*	*Eurycoccus*	3 m + 7 sm + 2 st	1.77^{a}	2.37–1.46	42.4^{a}	1.62^{a}	32.31^{bcd}	0.497	0.196	28.3
S. platycarpa⋆	*Eurycoccus*	*Eurycoccus*	9 m + 3 sm	1.85^{ab}	2.72–1.16	44.5^{ab}	2.32^{c}	40.56^{e}	0.303	0.257	14.4
S. laruotteana	*Eucoccus*	*Platycoccus*	2 m + 6 sm + 4 st	2.49^{abc}	3.95–1.77	59.6^{abc}	2.24^{bc}	30.12^{abc}	0.533	0.287	30.3
S. glabrata	*Holcococcus*	*Serjania*	3 m + 6 sm + 3 st	2.39^{abc}	3.33–1.75	57.6^{abc}	1.92^{abc}	32.00^{bcd}	0.499	0.233	32.7
S. tripleuria	*Holcococcus*	*Serjania*	2 m + 8 sm + 2 st	2.64^{bc}	3.67–1.95	63.3^{bc}	1.88^{ab}	33.15^{cd}	0.484	0.224	28.0
S. multiflora	*Oococcus*	*Serjania*	3 m + 8 sm + 1 st	2.65^{bc}	3.78–1.81	63.6^{bc}	2.10^{bc}	34.18^{d}	0.464	0.255	20.4
S. perulacea	*Oococcus*	*Serjania*	3 m + 6 sm + 2 st + 1 t	2.12^{abc}	2.99–1.59	50.8^{abc}	1.87^{ab}	30.76^{bc}	0.520	0.251	38.4
S. crassifolia⋆	*Simococcus*	*Serjania*	9 m + 3 st	2.18^{abc}	2.95–1.35	52.3^{abc}	2.18^{bc}	37.88^{e}	0.350	0.232	28.7
S. fuscifolia	*Simococcus*	*Serjania*	3 m + 7 sm + 2 st	2.25^{abc}	3.13–1.51	54.1^{abc}	2.07^{bc}	33.13^{cd}	0.484	0.239	24.6
S. hebecarpa	*Syncoccus*	*Serjania*	9 m + 1 sm + 2 st	2.36^{abc}	3.17–1.56	56.7^{abc}	2.03^{abc}	39.04^{e}	0.323	0.229	26.1
S. meridionalis	*Syncoccus*	*Serjania*	5 m + 4 sm + 3t	2.81^{c}	3.65–1.91	67.5^{c}	1.90^{abc}	32.98^{cd}	0.488	0.229	25.7
S. communis	*Platycoccus*	*Platycoccus*	2 m + 5 sm + 4 st + 1 t	2.33^{abc}	3.29–1.68	55.9^{abc}	1.98^{abc}	26.94^{a}	0.595	0.203	45.8
S. regnellii⋆	*Platycoccus*	*Platycoccus*	3 m + 6 sm + 2 st + 1 t	2.45^{abc}	3.38–1.77	58.7^{abc}	1.92^{abc}	29.29^{bc}	0.555	0.198	37.5

⋆New count in *Serjania*. [1]Classification by Radlkofer (1931–1934), [2]classification by Acevedo-Rodríguez (1993). [a,b,c,d,e]Tukey test, $\alpha = 0.01$. TCL, total chromosome length of diploid complement; ACL, average chromosome length; CV, chromosome variation (max.–min.); RI (ratio index), ratio of longest/shortest chromosome; ACI, average centromeric index; A_1 and A_2, intra- and interchromosomal asymmetry indices, respectively; CVci, coefficient of variation for the centromeric index.

mounted with Entellan (Merck). Some slides were sequentially stained with 0.5 mg/mL chromomycin A_3 (CMA_3) for 1.5 h and with 2 mg/mL 4′,6-diamidino-2-phenylindole (DAPI) for 30 min, and mounted in glycerol/McIlvaine buffer pH 7.0, 1:1 (v:v) plus 2.5 mM $MgCl_2$, after the C-banding treatment (see Vanzela et al. 2002).

The location and number of 18S-5.8S-26S rDNA sites were determined by FISH (Heslop-Harrison 1991; Cuadrado & Jouve 1994) using p*Ta*71, a probe containing *Triticum aestivum* 18S-5.8S-26S rDNA (Gerlach & Bedbrook 1979), labeled with biotin-14-dUTP by nick translation (BioNick, Invitrogen). Hybridization sites were detected with avidin-fluorescein isothiocyanate (FITC) conjugate and slides were counterstained and mounted with 25 μL of 50% antifade solution: 50% glycerol/McIlvaine buffer, pH 7.0 with 2.5 mM $MgCl_2$, plus 1 μL of 2.5 mg/mL propidium iodide.

Photographs were taken using Kodak Imagelink HQ 25 ISO for conventional staining, Kodak T-Max 100 ISO for C/CMA/DAPI, and Fuji Color 100 ISO for fluorescence *in situ* hybridization (FISH). Idiograms were conducted from chromosomal measurements and the localization of NORs (CMA/18S-5.8S-26S rDNA) was estimated from relative size and form of chromosomes.

Results

A summary of cytogenetic results, including karyotype formulas, measures, and asymmetry indexes are presented in Table II; the section to which the species belong takes into account both infrageneric treatments. All the chromosome counts in *Serjania* were based on $x = 12$ and the 14 species studied here share the diploid chromosome number $2n = 2x = 24$ (Figures 1 and 2).

Chromosome measurements revealed a relatively variable size for all species analyzed: TCL varied from 42.4 to 67.5 μm and chromosome sizes from 3.95 to 1.16 μm (Table II). *Serjania gracilis* was the species with the smallest chromosomes, whereas *Serjania meridionalis* showed the longest ones. The species showed significant differences ($p < 0.01$) in TCL, ACL, RI, and ACI parameters (Figure 3A–D). The RI allowed us to distinguish *S. gracilis* from the other studied species, due to the relatively homogeneous chromosome size. According to the ACI, *Serjania crassifolia*, *Serjania hebecarpa*, and *Serjania platycarpa* exhibit the most symmetrical karyotypes among the 14 species analyzed here (Figure 3D).

The karyomorphological data of the studied taxa (Table II) showed that the chromosome complement showed differences in karyotype formulae and asymmetry degree. The karyotypes are composed mainly of chromosomes with a median (m) and submedian (sm) centromere. However, in *S. crassifolia*, *S. hebecarpa*, and *S. platycarpa* most chromosomes were median (m) (Table II). Only three species, *Serjania communis*, *Serjania perulacea*, and *Serjania regnellii* exhibited chromosomes with terminal (t) centromeres (Figures 1, 2, and 6). Several parameters, such as A_1, A_2, CVci, and AI, revealed differences in karyotype asymmetry among species. The lowest asymmetry index, AI, was observed in *S. platycarpa*, *S. hebecarpa*, and *S. crassifolia*, however, this difference was not reflected in the CVci (Figure 3E and F) of *S. crassifolia* and *S. hebecarpa*. The parameter A_2 presented lower values in *S. communis*, *S. gracilis*, and *S. regnellii*, while *Serjania laruotteana* showed a high level of asymmetry for parameters A_1 and A_2.

In general, the species of *Serjania* were characterized as presenting semi-reticulate interphase nuclei (Figure 4A) and heterogeneous condensation at prophase (Figure 4B). Evident heterochromatic blocks were not observed after Giemsa C-banding, but only small and dispersed regions which were observed both in the more decondensed chromosomes and in the interphase nuclei of *S. gracilis* (Figure 4C).

After the CMA/DAPI banding in nine species of *Serjania*, *S. communis* (Figure 4D), *S. crassifolia* (Figure 4E), *Serjania fuscifolia* (Figure 4F), *Serjania glabrata* (Figure 4G), *S. laruotteana* (Figure 4H), *S. meridionalis* (Figure 4I), *Serjania multiflora* (Figure 4J), *S. perulacea* (Figure 4K), and *S. platycarpa* (Figure 4L), GC-rich sites (CMA^+) were located at terminal regions of short arms of different chromosome pairs. In general, medium and small chromosomes are bearers of these sites, with the exception of *S. laruotteana*, which exhibited GC-rich sites at the terminal region of the short arm of the first chromosomal pair (Figure 4H). The band numbers varied among species, for example, *S. glabrata* and *S. gracilis* showed just one chromosome pair with terminal GC-rich regions, while three pairs were observed in *S. laruotteana* and four pairs in *S. meridionalis*. However, in the majority of species, two chromosome pairs with GC-rich terminal regions were observed (Figure 4D–L). AT-rich regions ($DAPI^+$) were not observed in any of the 14 species studied.

By FISH with a 18S-5.8S-26S rDNA probe (p*Ta*71), it was possible to confirm a hybridization signal in seven species of *Serjania*: *S. communis* (Figure 5A), *S. crassifolia* (Figure 5B), *S. gracilis* (Figure 5C), *S. laruotteana* (Figure 5D), *S. perulacea* (Figure 5E), *S. platycarpa* (Figure 5F) and *S. regnellii* (Figure 5G). The hybridization sites were always terminal, but the number of sites observed varied from two (one pair) in *S. gracilis* (Figure 5C) to six in *S. laruotteana* (five sites were observed in Figure 5D). In the majority of species analyzed (*S. communis*,

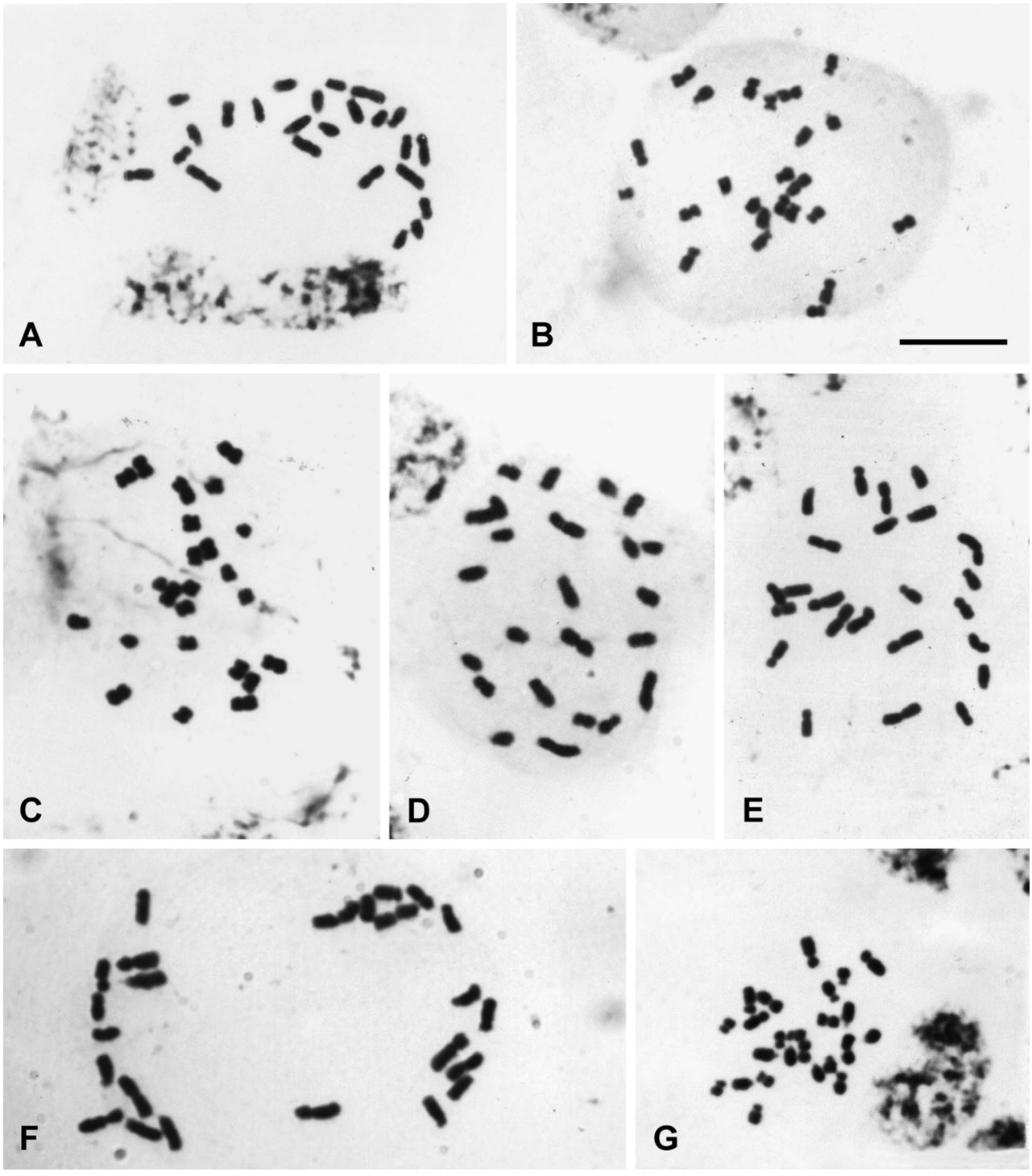

Figure 1. Conventional staining (HCl/Giemsa) in *Serjania* species. (A) *S. glutinosa*; (B) S. *gracilis*; (C) *S. platycarpa*; (D) *S. laruotteana*; (E) *S. glabrata*; (F) *S. tripleuria*; (G) *S. perulacea*. Bar = 10 μm.

S. crassifolia, *S. perulacea*, *S. platycarpa* and *S. regnellii*), two chromosome pairs (Figure 5A–G) with rDNA signals were observed. In general, the 18S-5.8S-26S rDNA/CMA^{+} sites were observed in terminal regions of the short arms of chromosomes with intermediate and minor size, with the exception of *S. laruotteana* that showed sites in the major chromosome.

Discussion

Chromosome number in Serjania *and its taxonomic implications in Paullinieae*

Within the Sapindaceae family, the tribe *Paullinieae* is cytogenetically the best known, with the genus *Serjania* being the most studied. Here, the chromosome numbers of *S. crassifolia*, *S. platycarpa*, and

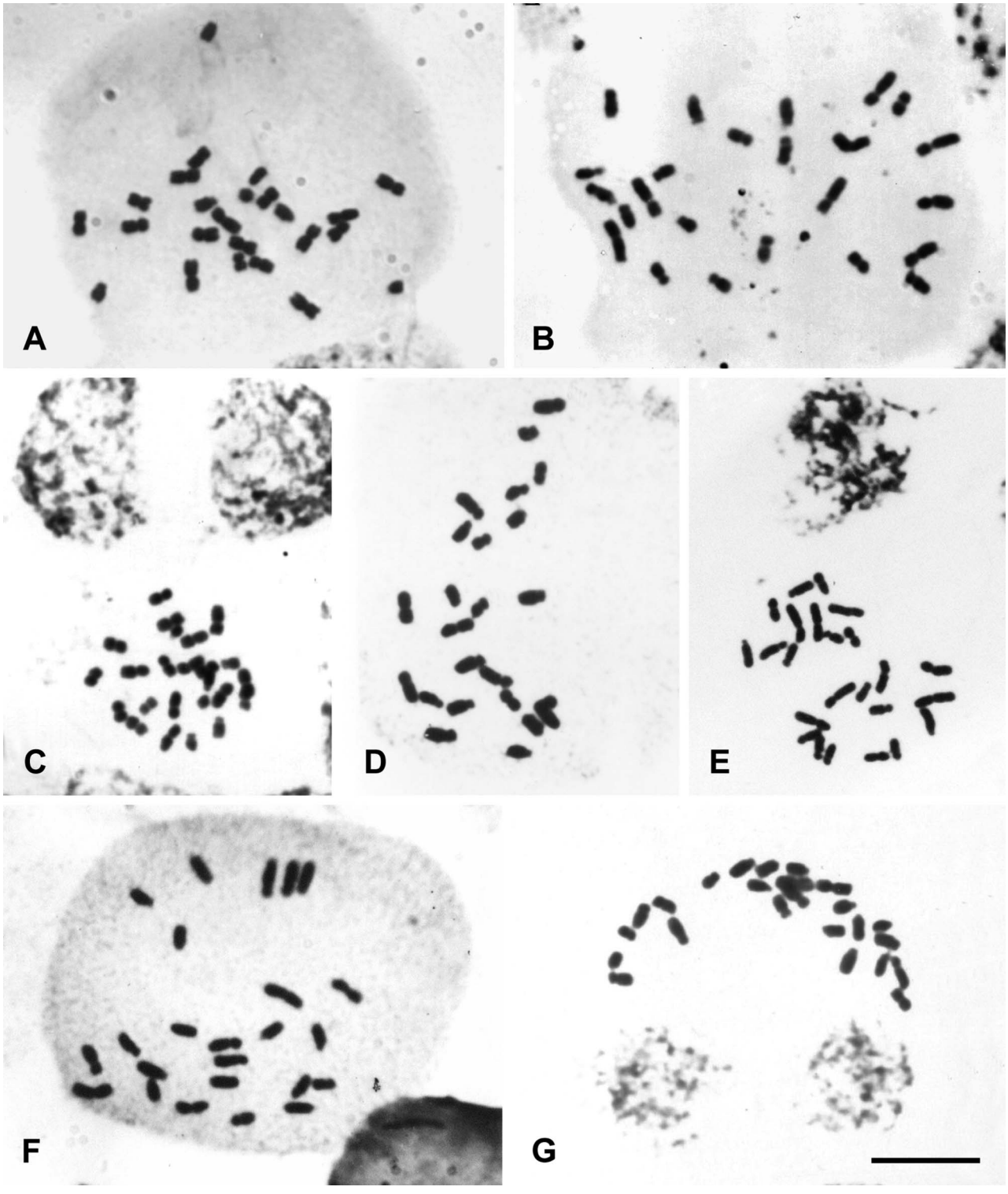

Figure 2. Conventional staining (HCl/Giemsa) in *Serjania* species. (A) *S. crassifolia*; (B) *S. fuscifolia*; (C) *S. hebecarpa*; (D) *S. meridionalis*; (E) *S. multiflora*; (F) *S. communis*; (G) *S. regnellii*. Bar = 10 μm.

S. regnellii are reported for the first time. Considering the new chromosome counts presented in this study, the 38 species of *Serjania* studied represent 17% of this genus, confirming $2n = 2x = 24$ as a conserved character for the genus. It is important to point out that *Houssayanthus* and *Paullinia* also share this character (Ferrucci 1981; Ferrucci & Solís Neffa 1997; Solís Neffa & Ferrucci 1998). Within the tribe, variations in chromosome number were found for *Urvillea* with $x = 11$ and 12, and also for *Cardiospermum* with $x = 7$, 9, 10, and 11 (Ferrucci 2000). Among the genera of the subtribe Paullinia, *Serjania* is closely related to

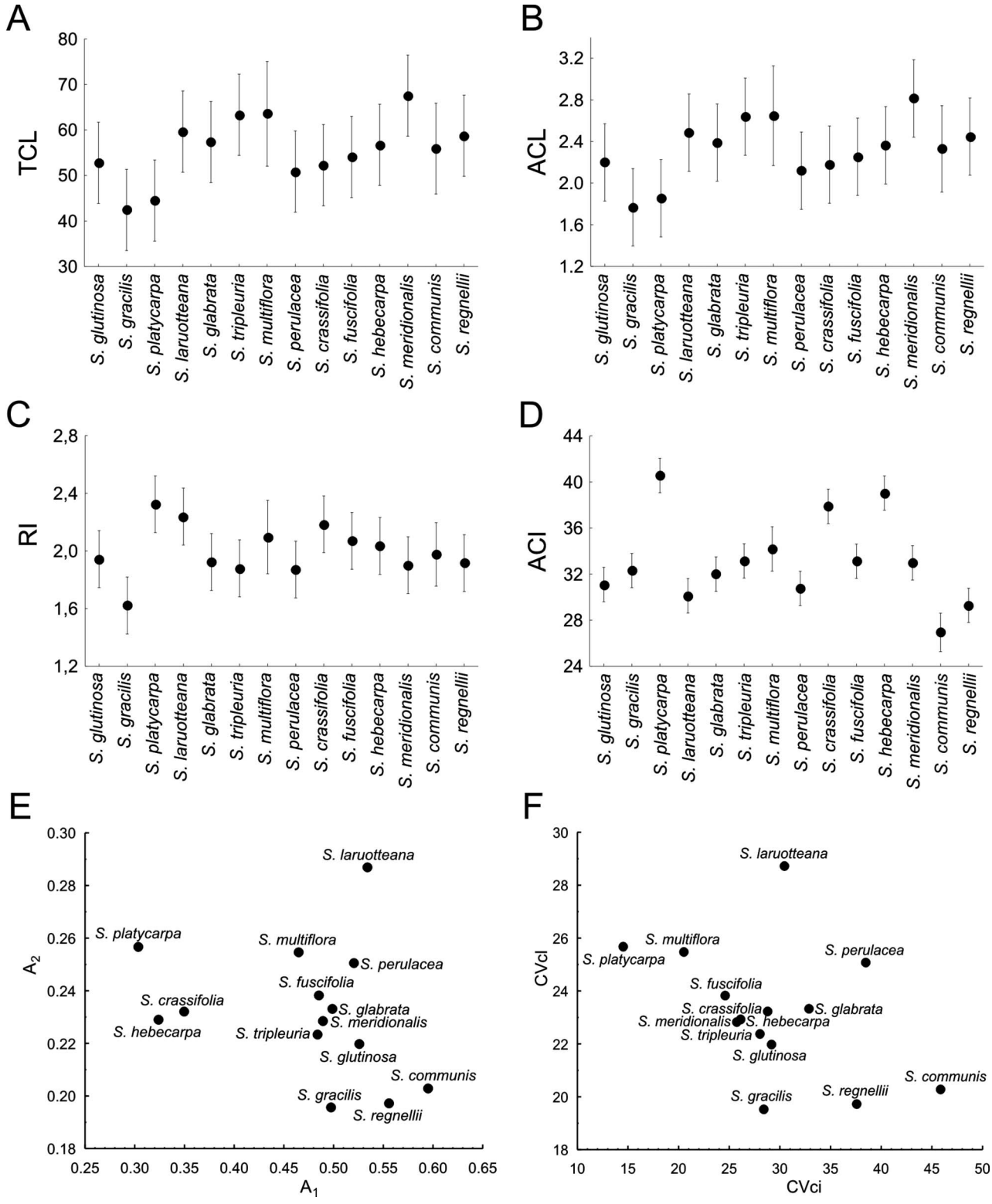

Figure 3. Variation of four chromosome characters, TCL, ACL, RI, and ACI, in *Serjania* species studied (A–D). Vertical bars denote 0.99 confidence intervals. Scatter diagrams for *Serjania* accessions with A_1 and A_2 (Romero Zarco 1986), and CVcl (A_2 x100) and CVci (Paszko 2006) parameters (E and F).

Lophostigma Radlk. but differs from it in terms of chromosome number. *Lophostigma* is a basal genus, when considering floral characters, such as the calyx with five free sepals and the semi-annular disk, and chromosome number ($2n = 2x = 28$). The $2n = 28$ is a plesiomorphic character shared with *Thinouia* and species of other related tribes, such as *Thouinieae* and *Cupanieae* (Ferrucci 2000).

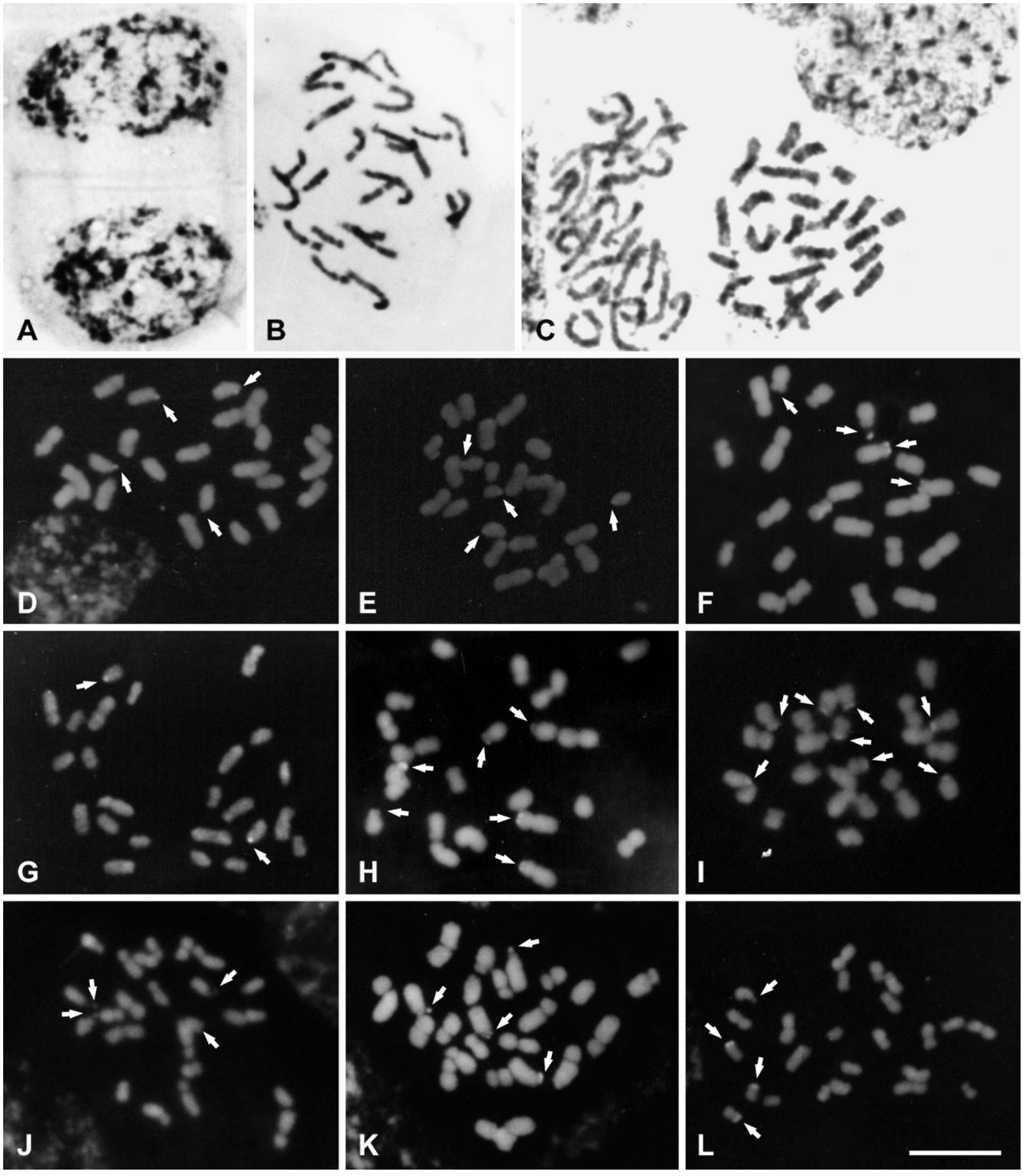

Figure 4. Conventional staining (HCl/Giemsa) in *Serjania* species: semi-reticulate interphase nuclei (A), prophasic condensation patterns (B), and C-banding in *S. gracilis* (C). CMA banding patterns in *S. communis* (D), *S. crassifolia* (E), *S. fuscifolia* (F), *S. glabrata* (G), *S. laruotteana* (H), *S. meridionalis* (I), *S. multiflora* (J), *S. perulacea* (K), and *S. platycarpa* (L). The arrows indicate CG-rich regions. Bar = 10 μm.

Within the Sapindaceae, *Paullinieae* is characterized by a reduction in basic chromosome number and by an increase in chromosome size (Lombello & Forni Martins 1998; Solís Neffa & Ferrucci 2001). This trend to reduction in chromosome number is supported by cytogenetic, molecular, and morphological studies (Acevedo-Rodríguez 1993; Ferrucci 2000; Harrington et al. 2005). These are apomorphic characters expressed in most genera of this tribe (Hemmer & Morawetz 1990). It is possible that chromosome number reduction in *Paullinieae* occurred in some common ancestor to *Lophostigma* and

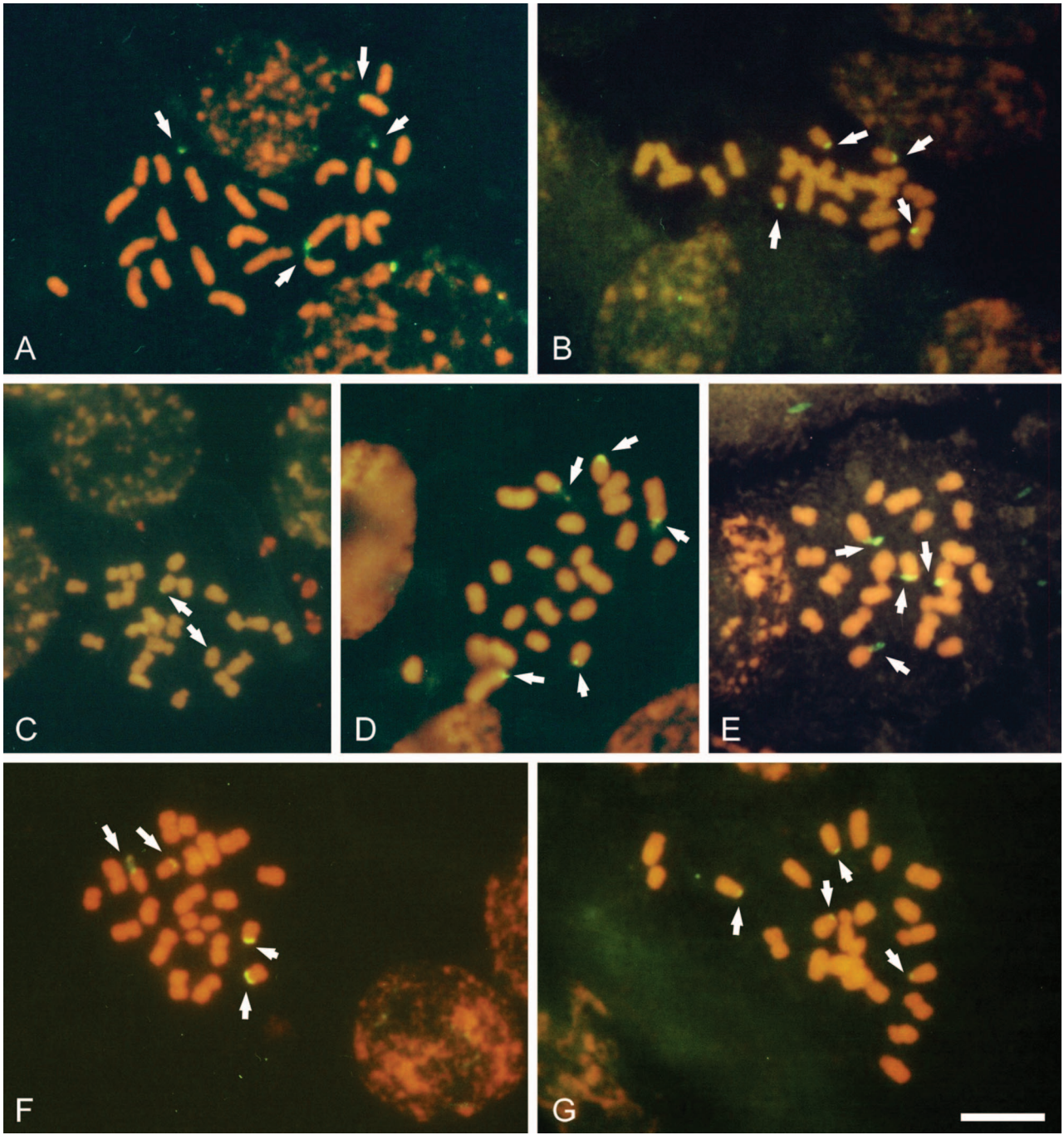

Figure 5. Fluorescence *in situ* hybridization (FISH) with probe p*Ta*71 (18S-5.8S-26S rDNA) (arrows) in *S. communis* (A), *S. crassifolia* (B), *S. gracilis* (C), *S. laruotteana* (D), *S. perulacea* (E), *S. platycarpa* (F), and *S. regnellii* (G). Bar = 10 μm.

other genera of the tribe. However, new counts are necessary as well as the development of chromosome markers enabling a more secure comparison among karyotypes of these two genera.

Karyotype symmetry in Serjania

Two methods were used to estimate the karyotype asymmetry and the A_1/A_2 index system (Romero Zarco 1986), which allowed a better observation of karyotype variation in *Serjania*. The parameter asymmetry intrachromosome index, A_1, let us clearly recognize two groups: one composed by species with a higher number of chromosomes with median centromeres (m), observed in *S. crassifolia*, *S. hebecarpa*, and *S. platycarpa*, and another group with more submedian (sm), subterminal (st), and terminal (t) pairs, which includes the remaining species studied here.

Previous studies recognized that karyotypes of *Serjania* species are moderately asymmetric, with gradual changes in chromosome size and an increase in asymmetry in species with large chromosomes

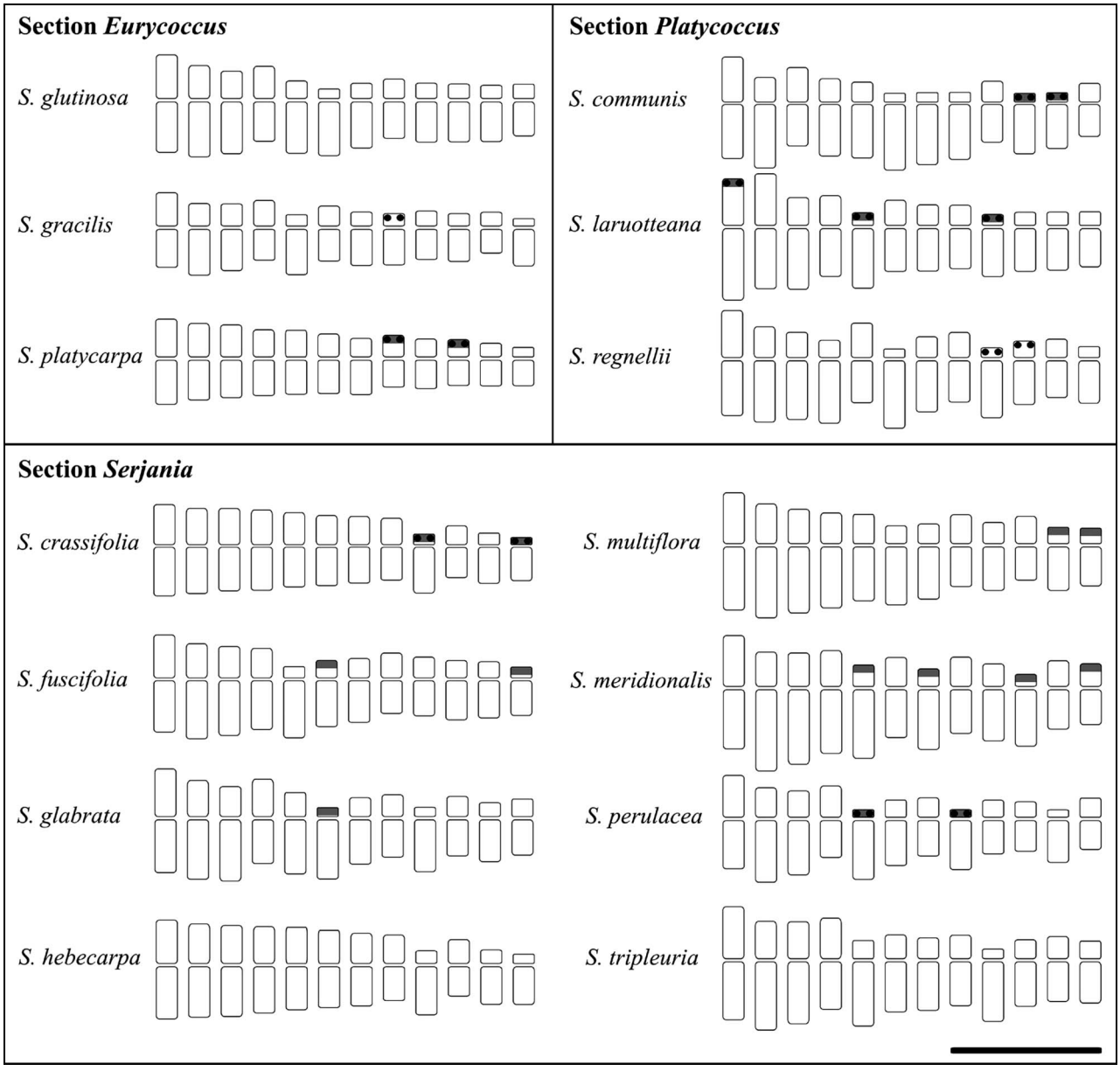

Figure 6. Idiograms of *Serjania* species studied, grouped in Acevedo-Rodríguez (1993). (▬) CMA bands, (●●) 18S-5.8S-26S rDNA. Bar = 5 μm.

(Solís Neffa & Ferrucci 1997). *Serjania crassifolia*, *S. hebecarpa*, and *S. platycarpa* are characterized by a high number of metacentric chromosomes and a major symmetry, with *S. crassifolia* and *S. hebecarpa* karyotypes being notably similar. These results agree with the infrageneric treatment proposed by Acevedo-Rodríguez (1993), which assembles *S. crassifolia* and *S. hebecarpa* in the section *Serjania*. Our results also agree with Solís Neffa and Ferrucci (1997, 2001), who identified *S. hebecarpa* and *Serjania cissoides* as the species with the most symmetrical karyotype. Thus, *S. crassifolia*, *S. hebecarpa*, and *S. cissoides* would form a group of closely related species. In this study, the most symmetrical karyotype was observed in *S. platycarpa*, belonging to the section *Eurycoccus*, which is clearly different from the other two species analyzed here.

The *S. laruotteana* karyotype shows a high degree of asymmetry. Radlkofer (1931) placed this species in the section *Eucoccus*, but Acevedo-Rodríguez (1993) included it in the section *Platycoccus*, together with *S. communis* and *S. regnellii.* These species share the lowest ACI among the species studied here; however, *S. laruotteana* differs notably from *S. communis* and *S. regnellii* when we observe the dispersion of A_1/A_2.

Banding pattern and rDNA in Serjania

The species of *Serjania* are notably homogeneous in terms of C/CMA/DAPI banding patterns, where a

lack of Giemsa C-bands is observed (Hemmer & Morawetz 1990; Nogueira et al. 1995). Another characteristic that attracts attention is the presence of semi-reticulate nuclei, due to a large amount of nuclear DNA (Nagl & Fusening 1979). In addition, it was suggested by Hemmer and Morawetz (1990) that repetitive DNA might be dispersed in the *Serjania* genome, without forming heterochromatin blocks.

In secondary constrictions, GC-rich repetitive DNA regions are frequently associated with rDNA genes, and so-called NOR-HC (heterochromatin associated with NORs), which habitually occupy terminal regions of short arms (Guerra 2000). Although sequential staining between CMA and FISH was not done in *Serjania*, it is possible to suppose an association between sequences of 18S-5.8S-26S rDNA and GC-rich sites (Figure 6), which would agree with the results obtained by Hemmer and Morawetz (1990). Interspecific differences in NOR number and position were suggested in previous studies on *Serjania* (Nogueira et al. 1995; Solís Neffa & Ferrucci 1997; Ferrucci 2000), which were confirmed by CMA banding and FISH. In general, NORs are present in terminal regions of short arms of medium and small chromosomes. The single exception is *S. laruotteana* in which the NOR is located in the terminal region of the short arm of the first chromosomal pair, which turned out to be a specific marker.

In conclusion, the karyological diversity observed in *Serjania* does not support the infrageneric classifications and sections proposed by Radlkofer (1931) or Acevedo-Rodríguez (1993). Similar results were obtained by a study on pollen morphology in ca. 30 species (Van der Ham & Tomlik 1994), indicating that pollen morphology does not exhibit differences useful for recognizing sections. However, karyotype analysis showed that it is possible to group some species, such as *S. glabrata* and *Serjania tripleuria*, or *S. communis* and *S. regnellii*, suggesting that the taxonomic treatment is not well defined. It will be interesting to obtain additional chromosomal information for *Serjania* species, as this information will allow us to relate *Serjania* to other genera of the tribe and contribute to the knowledge on the phylogenetic relations in Paullinieae. Considering the difficulties in interpreting the phylogenetic relationships in *Serjania*, it is imperative to perform molecular studies aimed at obtaining a natural intraspecific grouping for this genus.

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