ORIGINAL ARTICLE

A new insight into *Serjania* Mill. (Sapindaceae, Paullinieae) infrageneric classification: a cytogenetic approach

J. P. Coulleri · M. Dematteis · M. S. Ferrucci

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Abstract Serjania (Sapindaceae, Paullinieae) comprises about 230 species, and currently two infrageneric classifications have been proposed but both are difficult to apply. This work tested which infrageneric classification fitted better in relation to cytogenetic traits added to the main morphological features used by the authors of the subgenus arrangements to gain an insight into the evolutionary karyotype relationships. In order to test the relationship between karyotypes and the systematics of this genus, the karyotypes of five species of Serjania belonging to five different sections (sensu Radlkofer) were described. Known karyological information on 26 species was used to complement the results. With these data, a cluster analysis was set up to test which infrageneric classification fitted better. In addition, a principal component analysis (PCA) was performed to examine the relevance of the traits in the subgenus classification. All the karyotypes analyzed (including new as well as previous records) had 2n = 24chromosomes, and the karyotypes were asymmetrical: submetacentric and metacentric chromosomes were common, whereas telocentric chromosomes were rare. The PCA revealed seven principal components, the first two explained 52 % of the total variation, and the last ones were related to all the karyotypic features studied. The phenogram obtained reflected a scarce fitting into both infrageneric classifications, with only 3 sections of the 12 proposed by Radlkofer and two of the six sections proposed by Acevedo-Rodríguez being represented. Finally, regarding karyotype evolution, the constancy of chromosome number and the variation in the length of the complement suggest that structural chromosome changes would have played a leading role.

Keywords Cluster analysis · Karyotypes · Sapindaceae · Somatic chromosomes · Cytotaxonomy

Introduction

Sapindaceae s.str. is a moderately large family of trees, shrubs, and lianas that comprises about 140 genera and about 1,800 species, most of them with a tropical to subtropical distribution. However, some genera extend into temperate regions in Asia and North America, with the greatest diversity found in tropical Southeast Asia. For this paper we accepted the delimitation of the family sensu Radlkofer (1931–1934), recently reinforced by Buerki et al. (2010). Radlkofer (1931–1934) structured the family into 14 tribes, of which Paullinieae is the unique tribe with species that present a climbing habit. This tribe is a monophyletic group (Harrington et al. 2005; Buerki et al. 2009) comprising the genera Balsas Jiménez Ram. & Vega, Cardiospermum L., Houssayanthus Hunz., Lophostigma Radlk., Paullinia L., Serjania Mill. and Urvillea Kunth. Paullinieae is also the largest tribe, comprising almost a quarter of all Sapindaceae species. Serjania is a genus of monoecious climber shrubs, recognized by their squizocarpic fruits with three samaralike mericarps, with the locule in the distal portion. The genus is widely distributed in the American continent, from south of the United States to central Argentina, and comprises about 230 species (Ferrucci and Acevedo-Rodríguez 2005; Ferrucci and Somner 2010).

The most important contributions to the taxonomy of *Serjania* were made by Radlkofer (1874, 1875, 1931) who published the first synopsis for the genus (Radlkofer 1874),

J. P. Coulleri (⊠) · M. Dematteis · M. S. Ferrucci Instituto de Botánica del Nordeste (UNNE-CONICET), C.C. 209, 3400 Corrientes, Argentina e-mail: juancoulleri@gmail.com

and later the monograph (Radlkofer 1875) in which were recognized about 200 species grouped into 12 sections in accordance with reproductive and vegetative features. However, this infrageneric classification is difficult to use because the character states used are not exclusive to the sections. In addition, the locations of some species in certain sections are questionable because the author based the taxonomic position only on floriferous material. Acevedo-Rodríguez (1993) reduces the 12 sections to five and establishes a new one, the sect. *Confertiflora* based primarily on fruit and seed characters (Table 1). However, this classification presents some difficulties, of which the main one is the lack of characters used, and the characters used are mostly fruit related. Moreover, both classifications only consider similarities without an evolutionary perspective.

Karyological studies in *Serjania* have shown karyotypes of 29 species, all of them with 2n = 2x = 24 (Ferrucci 1981, 1985, 2000; Ferrucci and Solís Neffa 1997; Hemmer and Morawetz 1990; Nogueira Zampieri et al. 1995; Solís Neffa and Ferrucci 1997; Urdampilleta 2005, 2009). However, these authors did not propose any relationship between the karyological data and the infrageneric classifications, even though karyotypic features can be as systematically informative as morphological ones (e.g.

Table 1 Equivalence of the infrageneric classifications of Radlkofer(1875) and Acevedo-Rodríguez (1993)

Radlkofer	Acevedo-Rodríguez
S. sect. Platycoccus	S. sect. Platycoccus
	S. sect. Confertiflora
S. sect. Ceratococcus	S. sect. Ceratococcus
S. sect. Eurycoccus	S. sect. Eurycoccus
S. sect. Eucoccus	S. sect. Serjania
S. sect. Pachycoccus	
S. sect. Holcococcus	
S. sect. Dyctyococcus	
S. sect. Simococcus	
S. sect. Oococcus	
S. sect. Phacococcus	
S. sect. Syncoccus	
S. sect. Physococcus	S. sect. Physococcus

Stebbins 1971; Kenton et al. 1986; Chiarini and Bernardello 2006).

In this paper, we report five new karyotypes belonging to five species of *Serjania* of five different sections (sensu Radlkofer 1931). As well as these novel cytogenetic findings, we also consider the already known karyological information of 26 species belonging to ten sections sensu Radlkofer in order to: (1) examine the patterns of chromosome variation in relation to both infrageneric classifications; (2) test the infrageneric classification proposals in relation to the karyological data, plus a combination of those morphological characters used by the two authors for the subgenus arrangement; and (3) gain an insight into the evolutionary karyotype relationships in *Serjania*.

Materials and methods

Plant material

The species and collection data on which this karyological study was based are listed in Table 2. Species were arranged in sections according to the system proposed by Radlkofer (1931). All the species studied are also listed in Table 3, together with the bibliographic references of the karyotypic data used for the statistical analysis.

Karyological features

Primary roots were obtained from germinating seeds to study somatic chromosomes. The root tips were pretreated with 8-hydroxyquinoline for 3 h at room temperature, fixed in a 5:1 ethanol/lactic acid mixture for 24 h (Fernández 1973), and stained using the Feulgen technique. Meristems were macerated in a drop of lacto-propionic-orcein and squashing, and slides were permanently prepared using Euparal mounting medium (Asco Laboratories, Manchester, UK).

Ten metaphases were drawn for each species using a Zeiss camera lucida, and the five best were selected for the following measurements: short arm (s), long arm (l), mean chromosome length (MCL) and the centromeric index (CI). The nomenclature used for the description of the

Table 2 Species studied, and sources and figures (photomicrographs and idiograms) cited in the text

Section	Species	Source	Figures
Eurycoccus	S. altissima (Poepp.) Radlk.	Bolivia, dpt. La Paz. Coulleri et al. 72 (CTES)	1a, 2a
Syncoccus	S. orbicularis Radlk.	Brazil, state Bahia. Urdampilleta et al. 2641 (UEC, CTES)	1b, 2b
Simococcus	S. pannifolia Radlk.	Bolivia, dpt. La Paz. Coulleri et al. 82 (CTES)	2c
Pachycoccus	S. reticulata Cambess.	Brazil, state Bahia. Urdampilleta et al. 2624 (UEC, CTES)	2d
Eucoccus	S. sphaerococca Radlk.	Bolivia, dpt. Santa Cruz. Coulleri et al. 21 (CTES)	1c, 2e

Table 3 Species analyzed inthe cluster analysis andbibliographic references for thekaryological data used in thispaper

Section	Species	Reference
Platycoccus	S. communis Cambess.	Nogueira Zampieri et al. (1995)
	S. cuspidata Cambess.	Ferrucci and Solís Neffa (1997)
	S. paludosa Cambess.	Ferrucci and Solís Neffa (1997)
	S. regnellii Schltdl.	Urdampilleta (2005)
Ceratococcus	S. setigera Radlk.	Ferrucci and Solís Neffa (1997)
Serjania (Dictyococcus)	S. subdentata Juss. ex Poir.	Hemmer and Morawetz (1990)
	S. atrolineata C. Wright	Without karyological data
	S. exarata Radlk.	Without karyological data
Eurycoccus	S. gracilis Radlk.	Nogueira Zampieri et al. (1995)
	S. altissima (Poepp.) Radlk.	This paper
	S. glutinosa Radlk.	Ferrucci and Solís Neffa (1997)
	S. subimpunctata Radlk.	Ferrucci and Solís Neffa (1997)
	S. platycarpa Benth.	Urdampilleta (2005)
Eucoccus	S. caracasana (Jacq.) Willd.	Ferrucci and Solís Neffa (1997)
	S. sphaerococca Radlk.	This paper
	S. laruotteana Cambess.	Nogueira Zampieri et al. (1995)
Pachycoccus	S. erecta Radlk.	Ferrucci and Solís Neffa (1997)
	S. marginata Casar.	Ferrucci and Solís Neffa (1997)
	S. reticulata Cambess.	This paper
Holcococcus	S. exarata Radlk.	Guervin (1961)
	S. glabrata Benth.	Ferrucci and Solís Neffa (1997)
	S. tripleuria Ferrucci	Ferrucci (1985)
Simococcus	S. clematidifolia Cambess.	Ferrucci and Solís Neffa (1997)
	S. pannifolia Radlk.	This paper
	S. fuscifolia Radlk.	Nogueira Zampieri et al. (1995)
	S. crassifolia Radlk.	Urdampilleta (2005)
Oococcus	S. multiflora Cambess.	Nogueira Zampieri et al. (1995)
	S. perulacea Radlk.	Ferrucci and Solís Neffa (1997)
Phacococcus	S. brachycarpa A. Gray ex Radlk.	Without karyological data
	S. mexicana (L.) Willd.	Without karyological data
	S. grammatophora Radlk.	Without karyological data
	S. rubicaulis Benth. ex Radlk.	Without karyological data
Physococcus	S. inflata Poepp.	Without karyological data
-	S. sufferruginea Radlk.	Without karyological data
	S. longistipula Radlk.	Without karyological data
	S. grosii Schltdl.	Without karyological data
Syncoccus	S. foveata Griseb.	Ferrucci and Solís Neffa (1997)
	S. hebecarpa Benth.	Ferrucci and Solís Neffa (1997)
	S. meridionalis Cambess.	Ferrucci and Solís Neffa (1997)
	S. orbicularis Radlk.	This paper

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chromosome morphology was that proposed by Levan et al. (1964). In addition karyotype asymmetry was estimated using the indices proposed by Romero Zarco (1986) (A₁ = intrachromosomal asymmetry index, and A₂ = interchromosomal asymmetry index) which indicate the size variation among chromosomes. Idiograms were drawn based on the mean CI and arranged according to increases in this value (from metacentric, m, to telocentric, t), and then according to decreases in length within each group. Means were compared by one-way ANOVA after Bartlett's test for homogeneity. In addition, Tukey's test was applied to evaluate the differences between each pair of means. A scatter plot of the asymmetry indices is also presented.

Table 4 Matrix sh	owing	the cyt	ogeneti	c and	morphoio	gıcal leat	ures of the	e species s	studied										
Species	ш	шs	51	t	CI	MCL	\mathbf{A}_1	A_2	NPVBFB	PVBC	CALYX	COL	SOW	CBLW	DC	HLLP	SS	CS	SI
S. altissima ^a	10	9	8	0	34.36	2.01	0.63	0.27	0	0	5	1	0	1	0	0	0	0	0
S. atrolineata	I	I	I	I	I	I	I	Ι	.0	1	5	0	0	0	1	0	1	0	1
S. brachycarpa	I	I	I	I	I	I	I	I	0	0	4	1	0	0	0	0	7	0	0
S. caracasana	8	10	4	7	32.22	2.6	0.52	0.22	8	0	5	0	2	0	1	0	0	0	0
S. clematidifolia	8	12	7	7	34.16	1.77	0.48	0.19	3	0	5	1	0	1	1	0	1	0	0
S. communis	4	10	8	7	26.94	2.33	0.595	0.203	3	0	4	1	0	1	0	0	1	1	0
S. crassifolia	16	7	7	4	40.1	2.18	0.35	0.23	10	0	5	0	2	0	0	0	ю	0	0
S. cuspidata	9	9	10	7	27.86	2.6	0.61	0.23	3	0	4	1	0	1	0	0	7	0	0
S. erecta	8	8	4	4	30.83	2.6	0.55	0.23	4	0	5	1	1	1	0	0	-	0	0
S. exarata	8	8	9	7	31.03	2.6	I	Ι	0	0	5	1	0	1	0	0	-	0	1
S. foveata	8	12	7	7	33.92	2.53	0.48	0.19	0	0	5	0	0	0	0	0	7	0	0
S. fuscifolia	4	8	10	7	33.1	2.25	0.48	0.24	9	1	5	0	0	1	0	0	-	1	0
S. glabrata	8	10	4	7	32.1	3.15	0.52	0.63	5	0	5	0	2	0	1	0	0	0	1
S. glutinosa	10	9	9	7	34.04	1.97	0.48	0.22	0	0	5	1	1	1	0	0	0	0	0
S. gracilis	4	8	8	4	30.33	1.77	0.5	0.196	0	0	5	1	0	1	0	0	2	0	0
S. gramatophora	I	I	I	I	I	I	I	I	0	0	4	1	0	1	1	0	2	0	0
S. grosii	I	I	I	I	I	I	I	I	0	0	5	0	0	1	0	0	2	0	1
S. hebecarpa	16	7	9	0	38.25	2.47	0.37	0.27	0	0	5	0	0	0	0	0	0	0	0
S. inflata	I	I	I	I	Ι	I	I	Ι	3	0	5	0	0	1	1	0	0	0	1
S. laruotteana	10	8	4	2	34.5	2.64	0.46	0.2	8	0	4	-	0	1	0	0	0	0	1
S. longistipula	Ι	I	I	Ι	Ι	Ι	I	Ι	33	0	5	0	0	0	1	0	1	0	0
S. marginata	8	8	9	7	30.52	3.09	0.56	0.21	3	0	5	1	1	1	0	0	1	0	0
S. meridionalis	8	12	7	7	33.89	2.1	0.48	0.22	0	0	5	1	0	1	0	0	7	1	1
S. mexicana	I	I	I	I	I	I	I	I	0	0	4	0	0	0	0	0	1	1	1
S. multiflora	4	10	10	0	31.01	2.65	0.46	0.25	8	0	5	0	0	0	1	0	1	0	0
S. orbicularis ^a	10	14	0	0	37.13	1.27	0.25	0.336	0	0	5	0	0	1	0	0	1	0	0
S. paludosa	10	10	2	5	32.81	2.63	0.55	0.22	33	0	5	0	2	1	0	0	1	0	0
S. pannifolia ^a	4	8	12	0	35.33	2.75	0.51	0.3	8	0	5	0	0	1	1	0	-	1	-
S. perulacea	8	8	9	7	31.25	2.28	0.54	0.22	5	1	5	0	0	1	0	0	7	0	0
S. platycarpa	12	10	7	0	37.4	1.85	0.3	0.25	0	0	5	0	0	1	1	0	7	0	0
S. polyphylla	I	I	I	I	I	I	I	I	9	0	5	0	0	1	0	0	-	0	-
S. regnellii	9	12	4	7	29.29	2.45	0.555	0.198	3	0	4	1	0	1	0	0	7	0	0
S. reticulata ^a	12	8	4	0	34.38	1.31	0.22	0.305	0	0	5	0	1	1	1	0	-	0	0
S. rubicaulis	I	I	I	Ι	I	I	I	I	0	0	4	0	0	1	0	0	З	0	-
S. setigera	8	8	9	2	31.22	3.24	0.54	0.25	3	0	5	0	2	1	0	1	1	0	0

Species	ш	шs	st	t	CI	MCL	\mathbf{A}_{1}	\mathbf{A}_2	NPVBFB	PVBC	CALYX	COL	SOW	CBLW	DC	HLLP	SS	CS	SI
S. sphaerococca ^a	8	10	9	0	36.92	3.9	0.24	0.33	8	0	4	0	2	0	0	0	1	0	0
S. subdentata	9	16	0	0	37.1	1.7	I	I	5	0	5	1	0	1	0	0	0	0	1
S.	12	×	0	0	35.3	1.84	0.45	0.21	0	0	5	0	2	1	0	0	0	0	0
subimpunctata																			
S. sufferuginea	I	I	I	I	I	I	I	I	3	1	5	0	0	0	0	0	1	0	1
S. tripleuria	4	8	10	7	30.1	2.64	0.48	0.22	б	0	5	0	0	0	1	0	4	0	1
<i>m, sm, st, t</i> number <i>A</i> ₂) mean interchro	of eacl mosom	h type (al asyn	of chro. 1metry	moson index	ne; <i>CI</i> mea ; <i>NPVBFB</i>	n centron number e	of periphe	x; MCL m ral vascul	ean chromosc ar bundles in	me length the florifer	with a stands ous branch; J	PVBC pre	ion of 0.3 sence of 6	S; A _I mean complanate	intrachi periphe	omosomal ral vascula	asymm ur bundl	etry ind es; CAI	lex, NX

The numbers of each type of chromosome (m, sm, st) and t, MCL, CI and asymmetry indices are listed in

Morphological traits

Table 4.

We studied a combination of the independent characters used by Radlkofer (1931) and Acevedo-Rodríguez (1993). These traits were reproductive features including floral characteristics, and were as follows:

(1) Sepal number (CALYX) Fruit features:

(2) Locule flattened versus inflated (COL)

(3) Width of the septum (WOS)

(4) Presence of a constriction between the locule and the wing (CBLW)

(5) Presence of dorsal crest in the seminiferous portion (DC)

(6) Presence of a horn-like projection in the seminiferous portion (HLLP);

Seed traits:

(7) Seed shape (SS)

- (8) Cotyledon shape (CS)
- (9) Seed insertion (SI)

The following vegetative features were also taken into account:

(10) Number of peripheral vascular bundles in the floriferous branch (NPVBFB)

(11) Presence of complanate peripheral vascular bundles (PVBC)

All these traits were codified (Appendix) for the construction of a matrix (Table 4).

Statistical analysis

Principal component analysis (PCA) was carried out to examine the importance of each trait in the infrageneric classification. Also, a cluster analysis of the karyotypic and morphological data was carried out to examine the similarities among species and sections. A data matrix of 40 operational taxonomic units \times 18 variables (Table 4) was constructed. The program INFOSTAT (version 2011; Di Rienzo et al. 2008) was used to standardize the data matrix, to estimate the PCA and the cophenetic correlation in this analysis, and to calculate the average Euclidean distance and generate a dendrogram using the unweighted pair-group method with arithmetical average (UPGMA). The dendrogram distortion was measured by computing the cophenetic correlation coefficient (*r*).

seed insertion

seed shape; CS cotyledon shape; SI

in the seminiferous portion; HLLP presence of a horn-like lateral projection in the seminiferous portion; SS i

first time

for the

is reported

number

in which chromosome

Species

Results

The new accounts of the five species of *Serjania (S. altissima* (Poepp.) Radlk., *S. orbicularis* Radlk., *S. pannifolia* Radlk., *S. reticulata* Cambess., and *S. sphaerococca* Radlk.)



reveal a diploid chromosome number of 2n = 24, as in all the previous records for the genus. Figure 1 illustrates the mitotic metaphases of three of the new accounts, and Fig. 2 shows the idiograms of the five new records. ANOVA applied to the species with the new chromosome counts showed a wide variability in the mean values (MCL F = 15.31, P < 0.01; CI F = 8.01, P < 0.01).

Among the 31 karyotypes analyzed (5 new accounts and 26 extracted from previous reports) the *sm* chromosome was predominant. Four species (*S. setigera* Radlk., *S. marginata* Casar., *S. exarata* Radlk., and *S. perulacea* Radlk.) exhibited the most common karyotype formula (8m + 8sm + 6st + 2t), the longest and the shortest pairs belonging to the *sm* and *st* chromosomes, respectively. According to the classification of chromosomes according to length proposed by Lima de Faría (1980), this genus shows small chromosomes. Whereas the MCL ranges from



Fig. 1 Photomicrographs of mitotic metaphases of the *Serjania* species studied. **a** *S. altissima*, **b** *S. orbicularis*, **c** *S. sphaerococca*. *Scale bar* 3 μm

Fig. 2 Idiograms of *Serjania* species for which the karyotypes are presented for first time based on mean values. **a** *S. altissima*, **b** *S. orbicularis*, **c** *S. pannifolia*, **d** *S. reticulata*, **e** *S. sphaerococca*



1.27 to 3.9 μ m, and the mean CI of the complement varies between 26.94 and 40.1 (Table 4).

In general the karyotypes are quite asymmetrical, as shown by the Romero Zarco indices whose ranges are $A_1 = 0.22-0.63$ and $A_2 = 0.19-0.63$. The karyotypes of *S. reticulata* Cambess., *S. confertiflora* Radlk., *S. communis* Cambess., *S. sphaerococca* Radlk., and *S. orbicularis* Radlk. are the most symmetrical, while the karyotypes of *S. cuspidata* Cambess., *S. paludosa* Cambess., *S. caracasana* (Jacq.) Willd., *S. erecta* Radlk., *S. glabrata* Benth., and *S. regnellii* Schldtl. are comparatively asymmetrical. The Romero Zarco indices are plotted in Fig. 3.

In the dendrogram obtained (Fig. 4) nine clusters (A–I) can be recognized determined by a combination of similarities in the following character states: pentamerous calyx, locule inflated and seed trigonous-obovoid insert at base of the locule (cluster A); A₁ asymmetry index 0.52–0.54, locule inflated, septum dilated and constriction between the locule and the wing (cluster B); 10-12 sm chromosomes, septum narrow, constriction between the locule and the wing absent, dorsal crest in the locule and substraight cotyledons (cluster C); 6 *m* chromosomes, tetramerous calyx, locule flattened, without constriction between the locule and the wings, and an ellipsoid seed basally inserted (cluster D); tetramerous calyx, fruit locule inflated, without dorsal crest and seed inserted at the middle of the locule (cluster E); CI values 30.52-38.25, pentamerous calyx and seed trigonous-obovoid (cluster F); three peripheral vascular bundles and a trigonous-obovoid seed, the external cotyledon curved and the inner biplicated (cluster G); narrow fruit septum, without constriction between the locule and wings and seed basally inserted, with the external cotyledon curved and the inner biplicated (cluster H); $10-12 \ m$ chromosomes and $6-8 \ st$ chromosomes, peripheral vascular bundles absent, pentamerous calyx, fruit without constriction between the locule and the wing, and seed basally (cluster I).

Serjania setigera is not included in any group, being the only one with a horn-like lateral projection in the locule, and *S. tripleuria* is also not clustered in any group because of the presence of the unique feature of a subtransverse, trigonous-obovoid seed, although it is near cluster B, with which shares the fruit character states of an inflated fruit locule and a constriction between the locule and the wing. *Serjania laurotteana* is not included in cluster D because of differences in the seed, primarily the shape (trigonousovoid) and the seed insertion (in the middle), but shares with cluster D other characteristics which define it.

The PCA indicated seven principal components, the first three accounting for 70 % of the total variation, and the first two (plotted on a two-dimensional graph, Fig. 5) accounting for 52 %. Component one (31 % of the variation) emphasizes the position of the centromere (number of *m* and *st* chromosomes, and the CI), the A₁ asymmetry index, sepal number (CALYX), fruit characteristics including locule flattened versus inflated (COL), width of the septum (WOS) and cotyledon shape (CS). Component two (21 % of the variation) is related to the number of *st* chromosomes, the MCL, A₂ asymmetry index, the number Fig. 4 UPGMA phenogram derived from average Euclidean distance among species of Serjania. The Eurycoccus, Simococcus and Physococcus sections (sensu Radlkofer 1931) are recognized. Cophenetic correlation r = 0.966. Roman numerals represent the Radlkofer classification (I Platycoccus, II Ceratococcus, III Eurycoccus, IV Eucoccus, V Pachycoccus, VI Holcococcus, VII Dictyococcus, VIII Simococcus, IX Oococcus, X Phacococcus, XI Physococcus, XII Syncoccus). and Arabic numerals represent the Acevedo-Rodríguez classification (1 Platycoccus, 2 Confertiflora, 3 Ceratococcus, 4 Serjania, 5 Eurycoccus, 6 Physococcus)



of peripheral vascular bundles in the floriferous branch (NPVBFB), the presence of a constriction between the locule and the wing and seed insertion (CBLW).

In summary, on the basis of chromosomal and morphological traits both sectional arrangements are slightly represented in the phenogram.

Discussion

General karyotype characteristics

The results of this study reveal a detailed picture of *Serjania* chromosomes and their pattern of variation in

relation to their infrageneric position. The genus shows a diploid chromosome number equal to 2n = 24, the basic number being x = 12. This last feature is common to 75 % of the species of the Paullinieae tribe (Ferrucci 2000). The karyotypes recorded (both new and previously reported) show a common pattern, with the majority having metacentric (*m*) and submetacentric (*sm*) chromosomes, and lacking telocentric (*t*) chromosomes (Hemmer and Morawetz 1990; Nogueira Zampieri et al. 1995; Ferrucci and Solís Neffa 1997; Solís Neffa and Ferrucci 1997; Ferrucci 2000; Urdampilleta 2005, 2009).

The karyotype formulae and the quantitative analysis show a great variability among the species, although it is not possible to differentiate the species of *Serjania* using



only the chromosome data. However, the variability of both cytogenetic and morphological traits allows clustering of the species. The numbers of each type of chromosome and their length were the most variable characters. The MCL allowed indirect estimation of the variation in the length of the complement. The maximum ratio between the lengths of the shortest and the longest complement in *Serjania* species was 47.67 μ m, from 29.51 μ m in *S. orbicularis* to 77.18 μ m in *S. setigera*. The variation in complement length among the species is, perhaps, one of the more striking changes that have occurred during the divergence and evolution of the chromosome complement.

Karyotypes and systematics

The chromosome data obtained in this study and previously reported allowed us to compare for the first time the two proposed *Serjania* infrageneric classification (Radlkofer 1931 versus Acevedo-Rodríguez 1993) in relation to cytogenetic and morphological traits. In the phenogram obtained, from the nine clusters observed, sensu Radlkofer (1931), the clusters A, E, I and the subclade F' represent the sections Simococcus, Physococcus, Eurycoccus and Pachycoccus, respectively. However, in relation to the

sectional arrangement proposed by Acevedo-Rodríguez (1993), only the sections Eurycoccus (cluster I) and Physococcus (cluster E) were detected.

The lack of adjustment of the species clustering in relation to both infrageneric classifications may be due to the particular difficulties in the application of each. The main difficulty with the classification of Radlkofer (1931) is the absence of specific traits of each section. On the other hand, the reductionist approach that relies heavily on fruit characters in the taxonomic classification of Acevedo-Rodríguez (1993) may have resulted in a sectional arrangement with unresolved groupings.

The PCA revealed that the karyological features play an important role in the characterization of the species, since their presence is significant in the first two components. However, only some clusters share karyotypic features between the species. Cluster A which represents the Simococcus section and cluster F shows the most variable karyotypic features among the species. Whereas, the species grouped in cluster I, section Eurycoccus, shares karyotypic features including *m* type chromosome number (10–12), with the exception of *S. gracilis* which has 4 *m* chromosomes, 6–10 *sm* chromosomes, a MCL in the range 1.77–2.01 and an A₂ asymmetry index in the range

0.196–0.27. The species in cluster B have similar karyotypic formulae [8m + (8 or 10)sm + (4 or 6)st + 2t] and also have the same CI values (31.25-36.92).

Chromosomes and evolution

The constancy in chromosome number observed among the species studied here and reported in the literature (Ferrucci 1981, 1985, 2000; Ferrucci and Solís Neffa 1997; Hemmer and Morawetz 1990; Nogueira Zampieri et al. 1995; Solís Neffa and Ferrucci 1997; Urdampilleta 2005, 2009) indicates that numerical changes have not been important in the chromosomal evolution of the Serjania species. Instead, differences in karyotype formula and asymmetry indices among the species suggest that the main cause of chromosome evolution is structural rearrangements (e.g. duplications, pericentric inversions, and translocations). Stability of chromosome complement in a group of species, in this case the genus as a whole, may be explained by nonrandom chromosomal mutations which are constrained by the chromosome structure and the type of change that can be produced (Shaw et al. 1983; King 1970).

Finally, variability in the MCL and indirectly the variation in the length of the complement indicate that cyclic changes in complement length have occurred. Also, variation in the asymmetry indices used for establishment of the evolutionary relationship of *Serjania* may not be the correct approach, since this may not have been unidirectional, and both increases and decreases in complement length may have participated in the evolution and diversification of the genus, even within the related group of species.

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Appendix

Character and trait states used in the cluster analysis: **1.** Peripheral vascular bundles in the floripherous branch: 0, 3, 4, 5, 6, 8, 10 (according to the number of bundles present); **2.** Presence of peripheral bundles complanated: (0) absent, (1) present; **3.** Sepal number: (4) tetramerous calyx, (5) pentamerous calyx; **4.** Locule: (1) flattened, (0) inflated; **5.** Width of the septum: (0) narrow, (1) subnarrow, (2) dilated; **6.** Presence of a constriction between the locule and the wing and seed insertion: (0) present, (1) absent; **7.** Presence of dorsal crest in the seminiferous portion: (0) present, (1) absent; **8.** Presence of a horn-like lateral projection in the seminiferous portion: (0) absent, (1) present; **9.** Seed shape: (0) trigonous-ovoid, (1) trigonous-obovoid, (2) ellipsoid, (3) subspheric; **10.** Cotyledon shape: (0) the external curved and the inner biplicated, (1) substraight; **11.** Seed insertion (0) base, (1) middle.

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