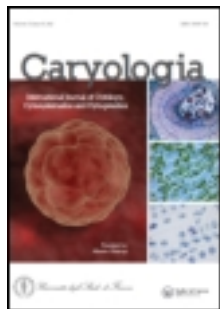


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Karyotypes of South American species of the Morelloid and Dulcamaroid clades (*Solanum*, Solanaceae)

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A karyotypic study on South American representatives of the Morelloid and Dulcamaroid clades of *Solanum* was conducted to contribute to a better understanding of their relationships. Mitotic chromosomes of 26 species were examined (13 of which were previously unknown). All taxa presented $2n = 2x = 24$ and had small chromosomes (less than 4 μm long) with the exception of *S. crispum*. Most species displayed symmetrical karyotypes, being 78% and 69% m, 19% and 25% sm and 3% and 6% st for the Morelloid and Dulcamaroid clades, respectively. *Solanum crispum* (Dulcamaroid clade) was unique by having mostly sm chromosomes and *S. sinuatirecurvum* (Morelloid clade) stood out with four st pairs and an exclusive pair of satellites in long arms. *Solanum tripartitum* was the sole entity exhibiting an sm pair with satellites. Most examined species of these clades resulted karyologically indistinguishable, based on conventionally stained mitotic chromosomes. Molecular analyses are needed to gain a better knowledge of the possible karyoevolutionary trends of both clades.

Keywords: Dulcamaroid; karyotypes; Morelloid; *Solanum*; South America

Introduction

Solanum L. is the largest genus of Solanaceae, with around 1400 species (Bohs 2005). It includes plants of economic significance: for their edible tubers, fruits or leaves, for their pharmaceutical compounds or for their ornamental value (Hunziker 2001; Weese and Bohs 2007); in addition, some are weeds of importance (Hunziker 2001).

Systematically, it is a complex genus. The classification of D'Arcy (1991) recognized seven subgenera, whereas other botanists made other infrageneric schemes based on different morphological characters (Nee 1999; Child and Lester 2001; Hunziker 2001). More recently, phylogenies have been produced and a natural infrageneric arrangement has been proposed (Olmstead et al. 1999, 2008; Bohs 2005; Weese and Bohs 2007), with 13 main clades including Morelloid and Dulcamaroid. These clades are closely related and are considered a sister group of ancient origin (Bohs 2005; Weese and Bohs 2007). Their monophyly was proposed according to sequences of ndhF chloroplast genes, DNA restriction sites, and nuclear ITS DNA sequence analyses (Olmstead and Palmer 1997; Bohs and Olmstead 1999; Bohs 2005; Weese and Bohs 2007).

The Morelloid clade has a worldwide distribution but predominates in the New World (Weese and Bohs 2007; Barboza et al. 2013), with South America being a conspicuous region due to the number of species. This clade mostly agrees with *Solanum section Morella* (Dunal) Bitter, which includes weeds and food plants. After Bohs (2005), its morphological synapomorphies include:

vining habit in many taxa, presence of unbranched, dendritic, or echinoid hairs, 3- to many-foliolate sympodial units, and fruits without stone cell aggregates. The Dulcamaroid clade is also distributed worldwide (Eurasia and the Americas) with its centre of diversity in the Andes and south-eastern Brazil (Knapp 2013); it includes entities from D'Arcy's three subgenera (1991). Some morphological features of this clade are: herbaceous or weakly woody habit, 2- to 3-foliolate sympodial units, pubescent filaments and styles in many taxa, and small stone cell aggregates in the fruits (Bohs 2005).

The knowledge of the structural and quantitative characteristics of the karyotype has proven to be important in evolutionary and taxonomic studies in several angiosperm groups (Shan et al. 2003; Weiss-Schneeweiss et al. 2003; Urdampilleta et al. 2005) and also in Solanaceae (e.g. Fregonezi et al. 2006; Moscone et al. 2007). There are numerous cytogenetic analyses dealing with *Solanum* species of economic importance performed with classical and molecular techniques (e.g. Tanskley et al. 1992; Dong et al. 2000; Cheema and Pant 2013). However, the karyology of less than half of *Solanum* species has been studied (Hunziker 2001). Karyotypes have been reported for several American species of *Solanum* and have proved to be useful in differentiating taxa and evolutionary trends; for instance, *Basarthurum* (Bernardello and Anderson 1990), *Lasiocarpa* (Bernardello et al. 1994), *Leptostemonum* (Chiarini and Bernardello 2006), and *Solanum* as a whole (e.g. Acosta et al. 2005, 2012; Rego et al. 2009; Chiarini et al. 2010; Melo et al. 2011).

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Table 1. Collection data of material studied of the Morelloid and Dulcamaroid clades, indicating previous reports when available. Asterisks indicate the first count and karyotype for the species.

Species	Voucher information	Figure	Previous number reports
Morelloid clade			
<i>S. aloysiifolium</i> Dunal	Argentina. Salta prov., Barboza et al. 2210	5A	
	Argentina. Tucumán prov., Barboza et al. 2152 *	5B	
<i>S. americanum</i> Mill.	Argentina. Tucumán prov., Barboza et al. 2139	5C	<i>n</i> = 12 (Ganapathi and Rao 1982)
<i>S. chenopodioides</i> Lam.	Argentina. Buenos Aires prov., Barboza et al. 2292	5D	<i>n</i> = 12 (Moscone 1992)
<i>S. concarense</i> Hunz.	Argentina. San Luis prov., Chiarini et al. 821 *	5E	
<i>S. hastatilobum</i> Bitter	Argentina. Catamarca prov., Chiapella et al. 2630 *	5F	
<i>S. echegarayi</i> Hieron.	Argentina. La Rioja prov., Barboza et al. 3253 *	1A	
<i>S. palitans</i> C.V. Morton	Argentina. Tucumán prov., Barboza et al. 2178	1B	<i>n</i> = 12 (Moscone 1992)
<i>S. pilcomayense</i> Morong	Argentina. Corrientes prov., Barboza et al. 2279	1C	
	Argentina. Entre Ríos prov., Barboza et al. 2287 *	1D	
<i>S. reductum</i> C.V. Morton	Argentina. Salta prov., Barboza et al. 3516 *	1E	
<i>S. sarrachoides</i> Sendtn.	Argentina. Neuquén prov., Chiapella et al. 1840	1F	
<i>S. sinuatirecurvum</i> Bitter	Argentina. Jujuy prov., Barboza et al. 3551 *	6A	
<i>S. triflorum</i> Nutt.	Argentina. Neuquén prov., Chiapella et al., 1839	6B	<i>n</i> = 12 (Moscone 1992)
<i>S. tripartitum</i> Dunal	Argentina. Jujuy prov., Barboza et al. 2228	6C	<i>n</i> = 12 (Acosta et al. 2005)
<i>S. zuloagae</i> Cabrera	Argentina. Salta prov., Barboza et al. 3569 *	6D	
Dulcamaroid clade			
<i>S. aligerum</i> Schldtl.	Argentina. Tucumán prov., Barboza et al. 2155	3A	<i>n</i> = 12 (Moscone 1992)
<i>S. amygdalifolium</i> Steud.	Argentina. Entre Ríos prov., Barboza et al. 1566 *	3B	
<i>S. angustifidum</i> Bitter	Argentina. Catamarca prov., Barboza et al. 3489 *	3C	
<i>S. crispum</i> Ruiz & Pav.	Chile VIII Región del Biobío, Chiapella et al. 1764 *	3D	
<i>S. dulcamara</i> L.	s/n cultivated	3E	<i>n</i> = 12 (Bir et al. 1978)
<i>S. endoadenium</i> Bitter	Argentina. Catamarca prov., Barboza et al., 3476	7A	<i>n</i> = 12 (Moscone 1992)
<i>S. salicifolium</i> Phil.	Argentina. Catamarca prov., Barboza et al. 3488	7B	<i>n</i> = 12 (Moscone 1992)
	Argentina. Córdoba prov., Chiarini et al. 818 *	7C	
	Argentina. La Rioja prov., Barboza et al. 3158	7D	
	Argentina. Córdoba prov., Chiarini et al. 794 *	7F	

Table 2. The *Solanum* taxa studied of the Morelloid and Dulcamaroid clades, with karyotype formulae, total haploid genome length (tl), mean chromosome length (C), mean arm ratio (r), mean asymmetry indices (intrachromosomal: A₁, interchromosomal: A₂), category of asymmetry of Stebbins (St), and standard deviation (SD).

Species	Karyotype formula	tl (SD)	C (SD)	r (SD)	A ₁	A ₂	St
Morelloid clade							
<i>S. aloysiifolium</i> 2210	10m* + 2sm	24.79 (0.41)	2.07 (0.41)	2.42 (0.30)	0.33	0.20	2 B
<i>S. aloysiifolium</i> 2152	7m* + 4sm + 1st	15.74 (0.30)	1.31 (0.20)	1.83 (0.19)	0.45	0.15	1 B
<i>S. americanum</i>	10m + 2sm	17.80 (0.23)	1.48 (0.22)	1.69 (0.21)	0.31	0.15	1 A
<i>S. chenopodioides</i>	10m + 2sm	13.55 (0.19)	1.13 (0.18)	1.85 (0.22)	0.29	0.16	1 A
<i>S. concarense</i>	11m + 1sm	19.22 (0.23)	1.60 (0.22)	1.74 (0.08)	0.32	0.14	1 A
<i>S. echegarayi</i>	9m* + 3sm	20.94 (0.23)	1.75 (0.24)	1.94 (0.34)	0.40	0.13	1 B
<i>S. hastatilobum</i>	9m* + 3sm	20.69 (0.28)	1.72 (0.27)	1.74 (0.18)	0.37	0.16	1 B
<i>S. palitans</i>	10m + 2sm	11.68 (0.17)	0.97 (0.16)	1.84 (0.17)	0.33	0.16	1 A
<i>S. pilcomayense</i> 2287	6m** + 5sm + 1st	17.07 (0.26)	1.42 (1.23)	1.35 (0.57)	0.40	0.09	1 B
<i>S. pilcomayense</i> 2279	11m + 1sm	16.17 (0.15)	1.35 (0.14)	1.50 (0.04)	0.25	0.10	1 A
<i>S. reductum</i>	11m + 1sm	20.69 (0.27)	1.72 (0.25)	1.72 (0.15)	0.33	0.15	1 A
<i>S. sarrachoides</i>	10m* + 2sm	20.72 (0.24)	1.72 (0.22)	1.61 (0.07)	0.35	0.13	1 A
<i>S. sinuatirecurvum</i>	5m* + 3sm + 4st	15.68 (0.24)	1.31 (0.31)	2.13 (0.21)	0.43	0.24	2 C
<i>S. triflorum</i>	10m + 2sm*	21.41 (0.29)	1.78 (0.32)	2.38 (0.30)	0.37	0.18	2 B
<i>S. tripartitum</i>	10m* + 2sm	12.65 (0.13)	1.05 (0.14)	1.67 (0.14)	0.32	0.13	1 A
<i>S. zuloagae</i>	11m* + 1sm	22.24 (0.34)	1.85 (0.30)	1.90 (0.15)	0.29	0.16	1 A
Dulcamaroid clade							
<i>S. aligerum</i>	10m* + 2sm	18.53 (0.21)	1.54 (0.19)	1.60 (0.14)	0.29	0.12	1 A
<i>S. amygdalifolium</i>	9m + 2sm + 1st	22.06 (0.14)	1.85 (0.96)	1.28 (0.70)	0.22	0.08	2 B
<i>S. angustifidum</i>	11m* + 1sm	26.13 (0.46)	2.18 (0.44)	2.15 (0.08)	0.33	0.20	2 A
<i>S. crispum</i>	2m* + 9sm + 1st	49.15 (0.60)	4.09 (3.83)	1.63 (0.84)	0.50	0.14	1 C
<i>S. dulcamara</i>	11m* + 1sm	22.13 (0.21)	1.84 (1.33)	1.54 (0.20)	0.22	0.11	1 A
<i>S. endoadenium</i>	8m + 3sm + 1st	18.65 (0.14)	1.55 (0.13)	1.29 (0.54)	0.41	0.08	1 B
<i>S. salicifolium</i> 3488	9m + 2sm + 1st	19.61 (0.13)	1.63 (0.87)	1.32 (0.81)	0.25	0.08	1 B
<i>S. salicifolium</i> 3158	7m* + 4sm + 1st	22.78 (0.16)	1.23 (1.08)	1.37 (0.70)	0.33	0.09	1 B
<i>S. salicifolium</i> 818	10m* + 2sm	22.55 (0.29)	1.88 (0.28)	2.02 (0.09)	0.37	0.15	2 A
<i>S. salicifolium</i> 794	10m + 2sm	17.16 (0.22)	1.43 (0.20)	1.99 (0.01)	0.37	0.14	1 A

Notes: Lengths are in μm . An asterisk indicates one chromosome pair with satellites in the short arm and two asterisks indicates one satellite chromosome pair in the long arm.

In the Morelloid and Dulcamaroid clades, few karyotype studies have been conducted (Moscone 1992; Acosta et al. 2005, 2012). Thus, in the present work, we provide a detailed morphometric karyotype analysis for 26 species, 13 of which are here reported for the first time. Our aims are: (1) to describe the chromosome number and to determine the karyotypes; and (2) to contribute to the improvement of the classification system and karyotype evolutionary trends in the Morelloid and Dulcamaroid clades.

Materials and methods

The species studied, locations, and collectors are listed in Table 1. Vouchers are kept at the herbarium of the Museo Botánico de Córdoba (CORD).

Mitotic chromosomes were examined in squashes of root tips obtained from germinating seeds. Seeds were soaked for 24 h in running water and then put in Petri dishes on moist filter paper at 25 °C. Root tips were pre-treated with 2 mM 8-hydroxyquinoline for 6 h at 14 °C,

fixed in ethanol : acetic acid (3:1, v/v) for 24 hours and stored at -20 °C until use for conventional chromosome analysis according to the HCl/Giemsa technique (Guerra 1983). At least five metaphases of each species were analysed with a Zeiss Axiophot phase contrast microscope (Jena, Germany) and photographed with a Leica DFC300FX camera (Wetzlar, Germany). Photographs were used to take the following measurements for each chromosome pair: s (short arm), l (long arm) and c (total chromosome length), using the freeware computer application MicroMeasure version 3.2 available on the internet at <http://www.colostate.edu/Depts/Biology/Micro-Measure>. The arm ratio ($r = l/s$) was then calculated and used to classify the chromosomes as defined by Levan et al. (1964). Karyotype asymmetry was obtained using the intrachromosomal (A_1) and the interchromosomal (A_2) asymmetry index described by Romero Zarco (1986). Chromosome types were classified first into groups according to their increasing arm ratio and then according to the decreasing length within each group. To look for associations between pairs of the mentioned

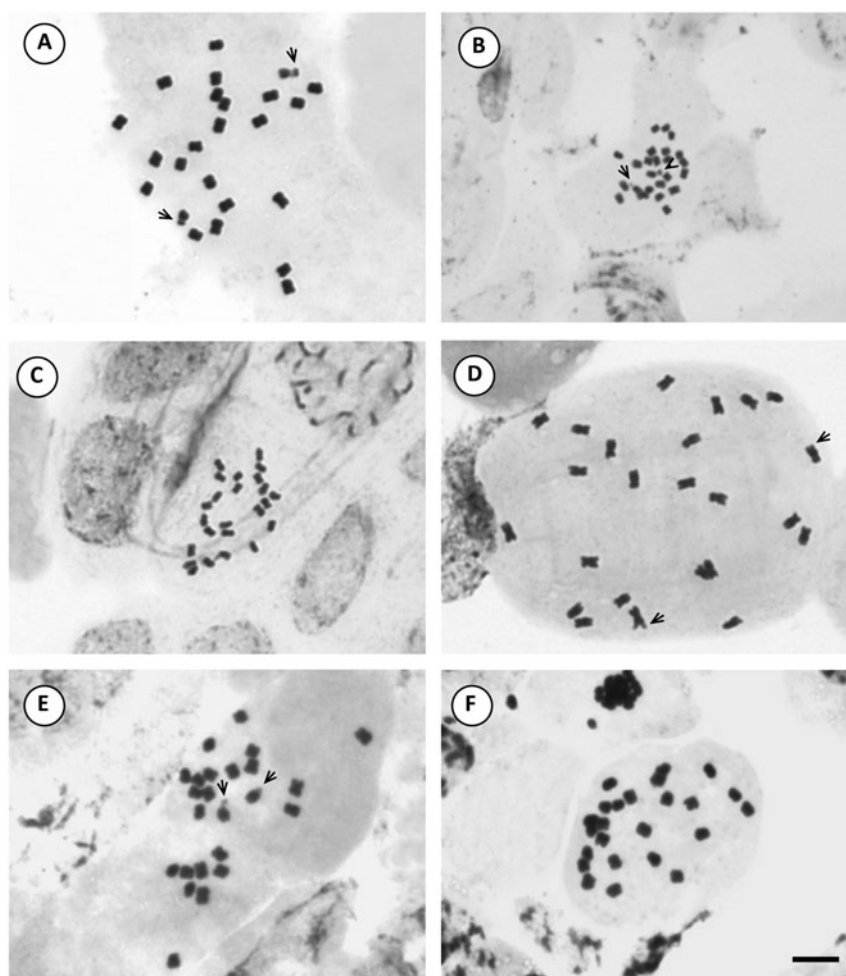


Figure 1. Photomicrographs of mitotic metaphases in *Solanum* species of Morelloid clade with $2n = 24$. (A) *S. echegarayi*; (B) *S. palitans*; (C) *S. pilcomayense* 2279; (D) *S. pilcomayense* 2287; (E) *S. reductum*; (F) *S. sarrachoides*. Scale bar = 6 μ m, all photomicrographs at the same scale. Arrows indicate satellites.

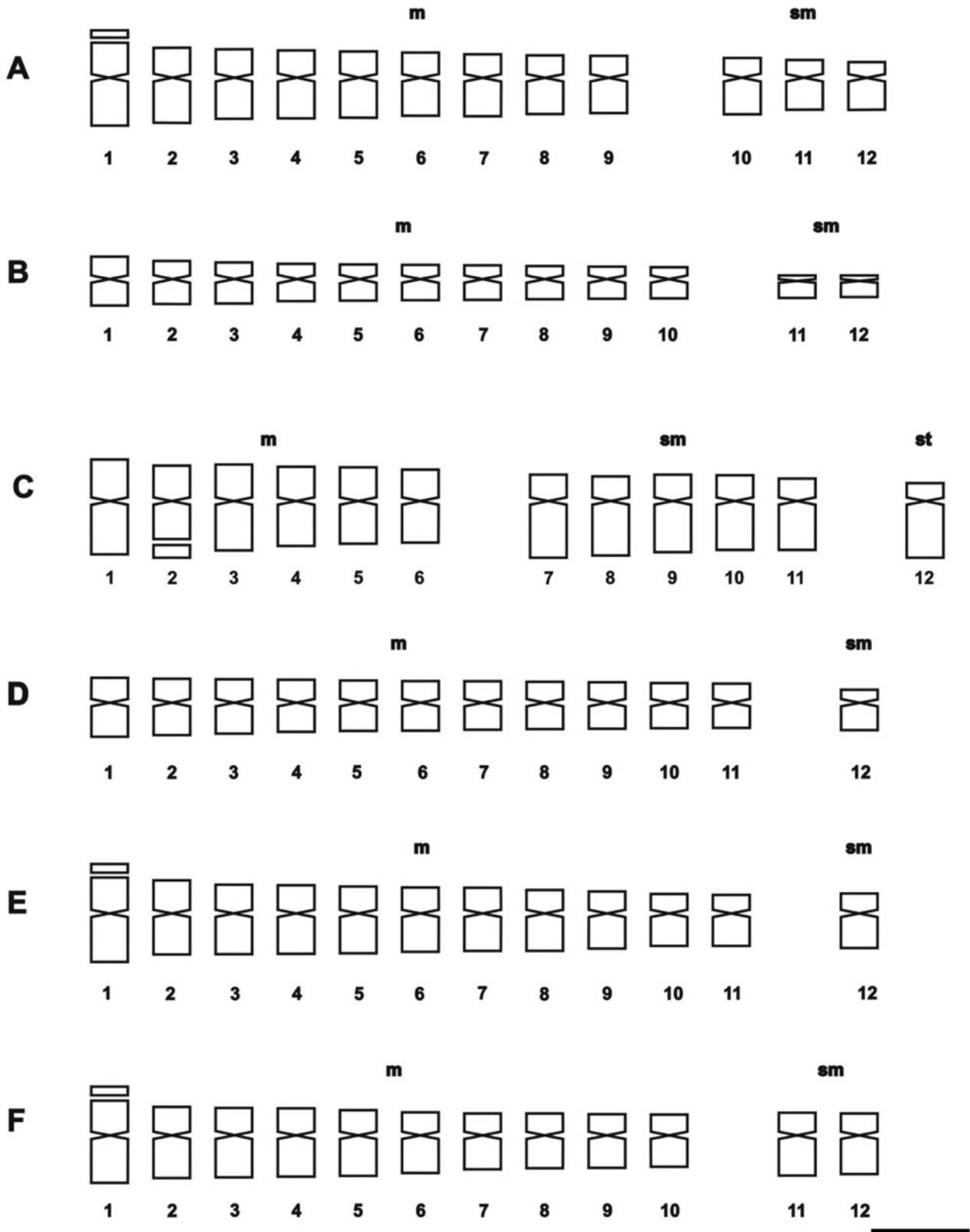


Figure 2. Idiograms of *Solanum* species of Morelloid clade. (A) *S. hastatilibum*; (B) *S. palitans*; (C) *S. pilcomayense* 2287; (D) *S. pilcomayense* 2279; (E) *S. reductum*; (F) *S. sarrachoides*. Scale bar = 3 μ m.

karyotype variables, linear regression tests were performed using Infostat (Infostat Group 2002).

Results

Karyotype analysis of the 26 *Solanum* species here studied showed a constant chromosome number: $2n =$

$2x = 24$. The chromosome numbers of 13 species, indicated with an asterisk in Table 1, are reported for the first time.

Table 2 summarizes the morphometric characteristics of each taxa. The Morelloid clade has small chromosomes. The largest chromosome length and haploid genome length values were observed in *S. aloysiifolium* 2210 (2.07 μm and 24.79 μm , respectively), whereas the lowest ones were detected in *S. palitans* (0.97 μm and 11.68 μm , respectively). *Solanum crispum* from the Dulcamaroid clade was outstanding, not only between both clades but also in the genus, for having the largest chromosome length and haploid genome length (4.09 μm and 49.15 μm , respectively). On the other hand, also within the Dulcamaroid clade, *S. salicifolium* 3158 presented the lowest chromosome length (1.23 μm), whereas the lowest haploid genome lengths corresponded to *S. aligerum* (18.53 μm).

The presence of satellites was inconsistent in the examined species, varying according to the sample. The number of cells in which they could be visualized was variable and satellites could be detected only in one homologue. The occurrence of one satellited pair is common in the Morelloid clade: seven species displayed it in the short arms, and *S. pilcomayense* 2287 in the long arms (Figures 1D, 2C). Six analysed species of the Dulcamaroid clade exhibited one pair of satellites in the short arms. *Solanum crispum* was also outstanding because its satellites were larger than the short arms in which they were attached (Figures 3D, 4D).

The more frequent karyotype formula of the Morelloid clade was $10m + 2sm$ (in *S. aloysiifolium* 2210, *S. americanum*, *S. chenopodioides*, *S. palitans*,

S. sarrachoides, *S. triflorum*, *S. tripartitum*), followed by $11m + 1sm$ (*S. concarense*, *S. pilcomayense* 2279, *S. reductum*, *S. zuloagae*), and $9m + 3sm$ (*S. echeagarayi*, *S. hastatilobum*) (Figures 1, 5, 6, 9, 10). Three species displayed a unique formula showing st pairs: *S. pilcomayense* 2287 with $6m + 5sm + 1st$, *S. aloysiifolium* 2152 with $7m + 4sm + 1st$, and *S. sinuatirecurvum* with $5m + 3sm + 4st$ (Figures 1D, 5B, 6A).

In the Dulcamaroid clade, the majority of species exhibit m chromosomes (Figures 3, 4, 7, 8), except in *S. crispum* with nearly all sm chromosomes (Figure 3D). *S. amygdalifolium* showed st chromosomes, along with *S. salicifolium* 3488 ($9m + 2sm + 1st$) and *S. salicifolium* 3158 ($7m + 4sm + 1st$). In contrast, *S. aligerum*, *S. salicifolium* 818, *S. salicifolium* 794 ($10m + 2sm$), *S. angustifidum* and *S. dulcamara* ($11m + 1sm$) (Figures 4A, C, E, 7C, E) did not show st chromosomes. Neither in this clade nor in the Morelloid clade were t chromosomes detected.

The karyotypes of the studied taxa were mainly symmetrical; *S. pilcomayense* 2287 with $A_1 + A_2 = 0.35$ (Morelloid clade) and *S. amygdalifolium* with $A_1 + A_2 = 0.30$ (Dulcamaroid clade) were the most symmetrical (Table 2). *S. sinuatirecurvum* with $A_1 + A_2 = 0.67$ (Morelloid clade) and *S. crispum* with $A_1 + A_2 = 0.64$ (Dulcamaroid clade) were the most asymmetrical. After the classification of Stebbins (1971), the species of both clades showed different categories. Nine Morelloid clade species fell into the 1A category, four into 1B, two into 2B and just *S. sinuatirecurvum* into 2C. In the Dulcamaroid clade, three species fell into the 1A category, three into 1B, two into 2B, and one into each of 2A and 1C (*S. angustifidum* and *S. crispum*, respectively).

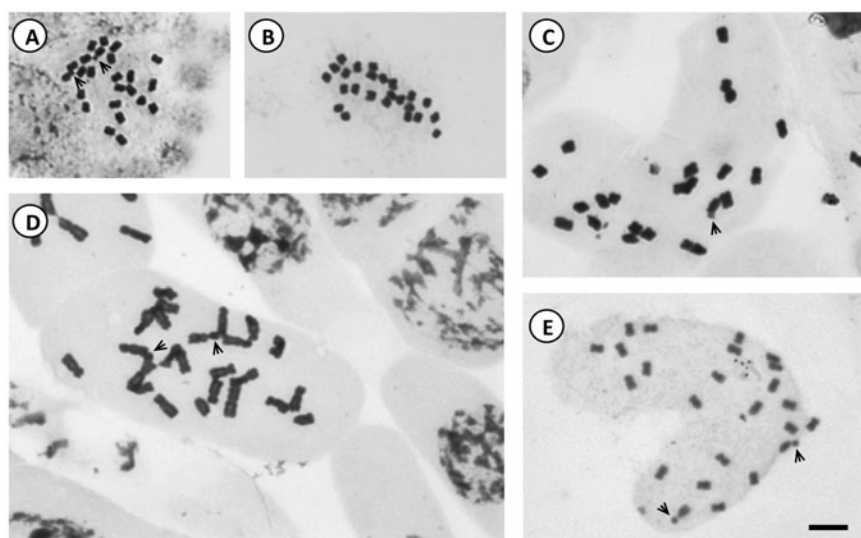


Figure 3. Photomicrographs of mitotic metaphases in *Solanum* species of Dulcamaroid clade with $2n = 24$. (A) *S. aligerum*; (B) *S. amygdalifolium*; (C) *S. angustifidum*; (D) *S. crispum*; (E) *S. dulcamara*. Scale bar = 6 μm , all photomicrographs at the same scale. Arrows indicate satellites.

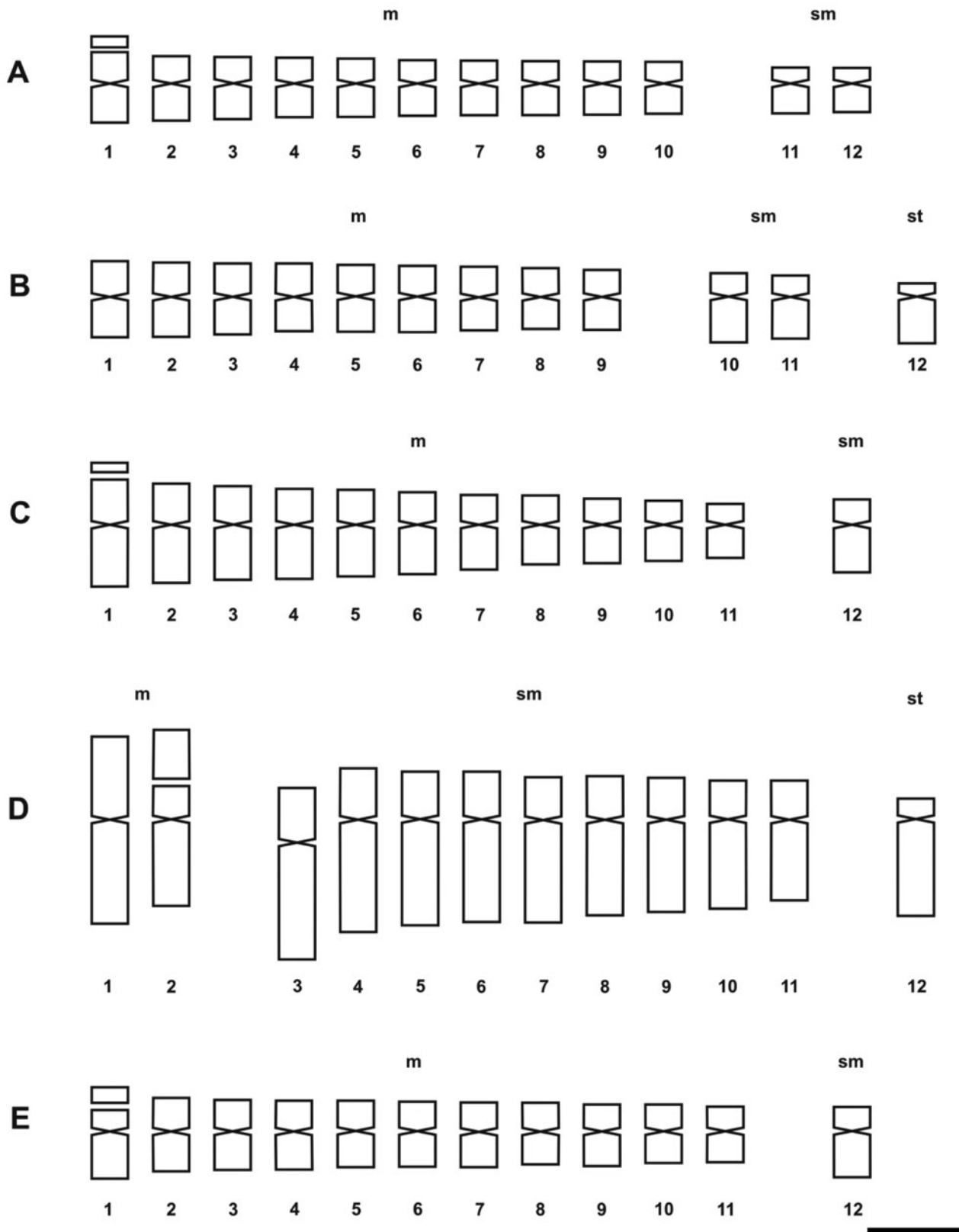


Figure 4. Idiograms of *Solanum* species of Dulcamaroid clade. (A) *S. aligerum*; (B) *S. amygdalifolium*; (C) *S. angustifidum*; (D) *S. crispum*; (E) *S. dulcamara*. Scale bar = 3 μ m.

Discussion

Solanum species here examined are diploid with $x = 12$. This is the most frequent number for the genus and for

Solanaceae as well, being present in more than 50% of the known species (cf. Acosta and Moscone 2000; Hunziker 2001; Acosta et al. 2005, 2012). A few exceptions

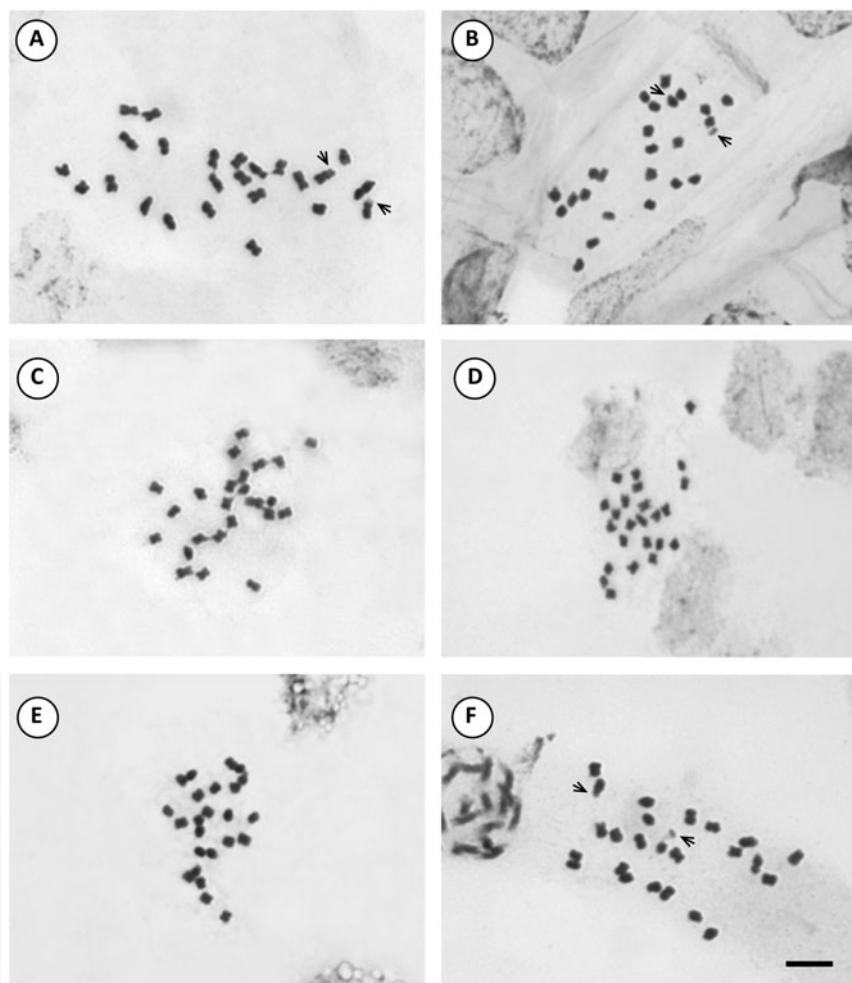


Figure 5. Photomicrographs of mitotic metaphases in *Solanum* species of Morelloid clade with $2n = 24$. (A) *S. aloysiifolium* 2210; (B) *S. aloysiifolium* 2152; (C) *S. americanum*; (D) *S. chenopodioides*; (E) *S. concarensis*; (F) *S. hastatilibum*. Scale bar = 6 μm , all photomicrographs at the same scale. Arrows indicate satellites.

have been detected in *Solanum* within the *Leptostemonum* clade (*S. mamosum* and *S. platense* with $n = 22$; Chiarini and Bernardello 2006) and the *Archaeosolanum* clade (seven species with $n = 23$; Pinto Maggio et al. 1997).

Solanum as a whole shows small chromosomes of less than 4 μm (e.g. Bernardello and Anderson 1990; Bernardello et al. 1994; Acosta et al. 2005; Chiarini and Bernardello 2006; Rego et al. 2009; Melo et al. 2011), as mostly found here. The *Cyphomandra* clade is an exception with chromosome sizes ranging from 3 to 14 μm (e.g. Pringle and Murray 1991; Moscone 1992; Miguel et al. 2012), as found here in *S. crispum* with chromosomes longer than 4 μm .

In Solanaceae and in *Solanum* there is a prevalence of m and sm chromosomes (e.g. Trivedi and Sinha 1986; Bernardello and Anderson 1990; Bernardello et al. 1994; Moscone 1990, 1999; Acosta and Moscone 2000; Acosta et al. 2005; Chiarini and Bernardello 2006). On the other hand, st chromosomes in *Solanum* are rare, either as one

pair (e.g. Trivedi and Sinha 1986; Bernardello and Anderson 1990; Acosta et al. 2005; Chiarini and Bernardello 2006) or two pairs (e.g. Bernardello et al. 1994; Chiarini and Bernardello 2006). In the Morelloid clade, we detected an outstanding species with four st pairs: *S. sinuatirecurvum*. This is the first report in this clade, and even in the genus *Solanum*, with this many st pairs.

The number of satellited pairs is variable within Solanaceae; for instance, *Capsicum* species displayed one to four satellited pairs (Moscone 1999), *Lycium* always showed one (Stiefkens and Bernardello 2002) and species of *Hyoscyamus* may have one to three pairs (Sheidai et al. 1999). Many *Solanum* showed satellites in the short arms: one pair (as here found in both clades), two pairs (in *S. pseudolulo*, Bernardello et al. 1994), three pairs (in species of the *S. indicum* complex, Krishnappa and Chennaveeraiah 1975). There are relatively few reported cases in which satellites were located on the long arms, namely *Nicotiana* (Moscone 1989), *Datura* (Palomino and Bye 1988; Moscone 1989), *Ce-*

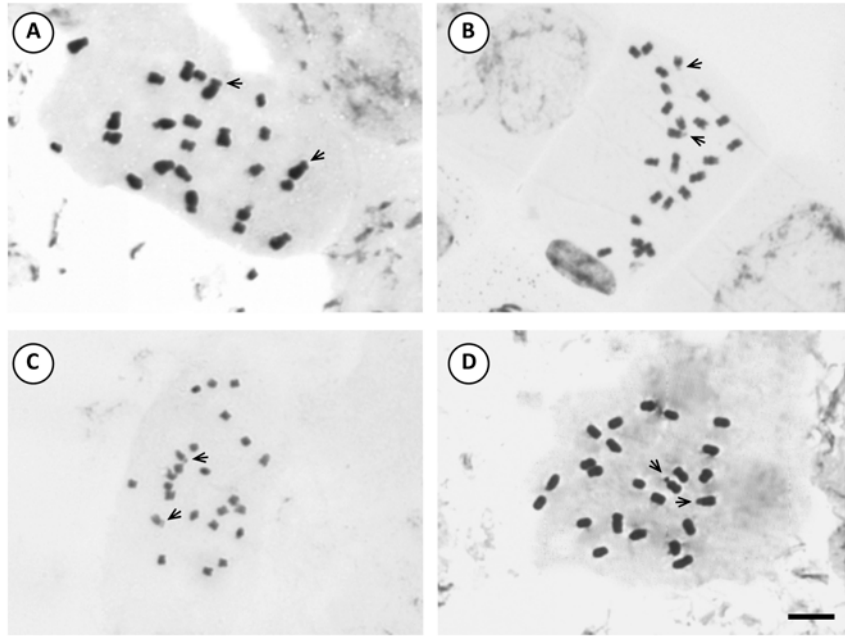


Figure 6. Photomicrographs of mitotic metaphases in *Solanum* species of Morelloid clade with $2n = 24$. (A) *S. simuatirecurvum*; (B) *S. triflorum*; (C) *S. tripartitum*; (D) *S. zuloagae*. Scale bar = 6 μm , all photomicrographs at the same scale. Arrows indicate satellites.

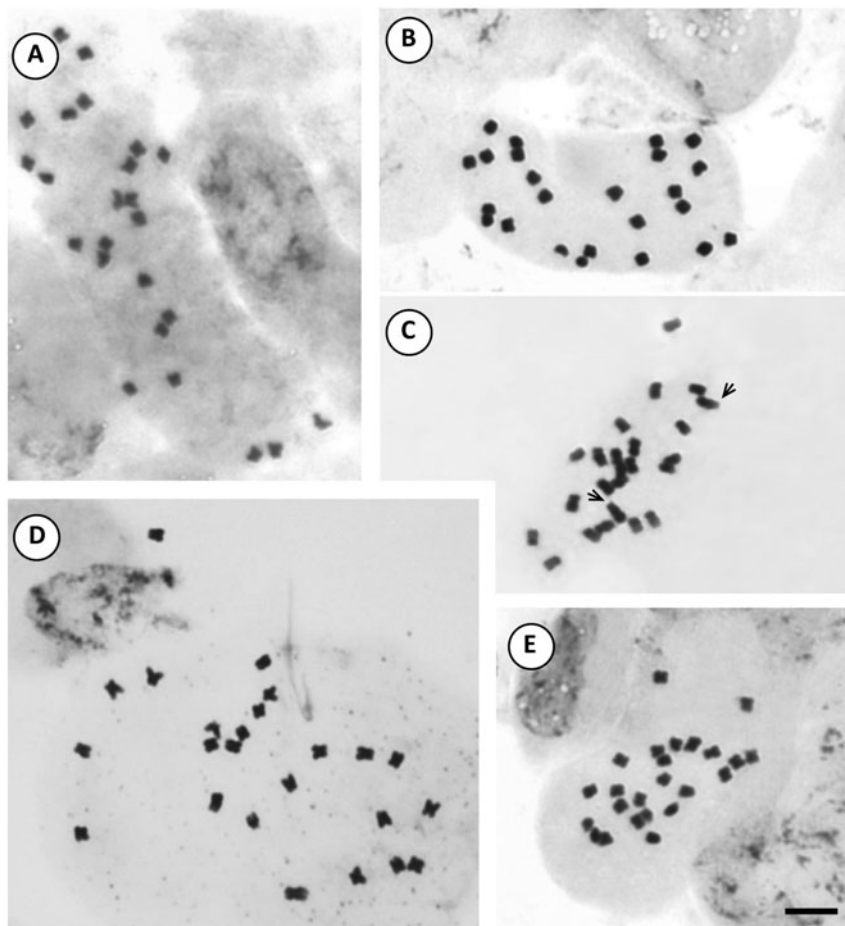


Figure 7. Photomicrographs of mitotic metaphases in *Solanum* species of Dulcamaroid clade with $2n = 24$. (A) *S. endoadenium*; (B) *S. salicifolium* 3488; (C) *S. salicifolium* 818; (D) *S. salicifolium* 3158; (E) *S. salicifolium* 794. Scale bar = 6 μm , all photomicrographs at the same scale. Arrows indicate satellites.

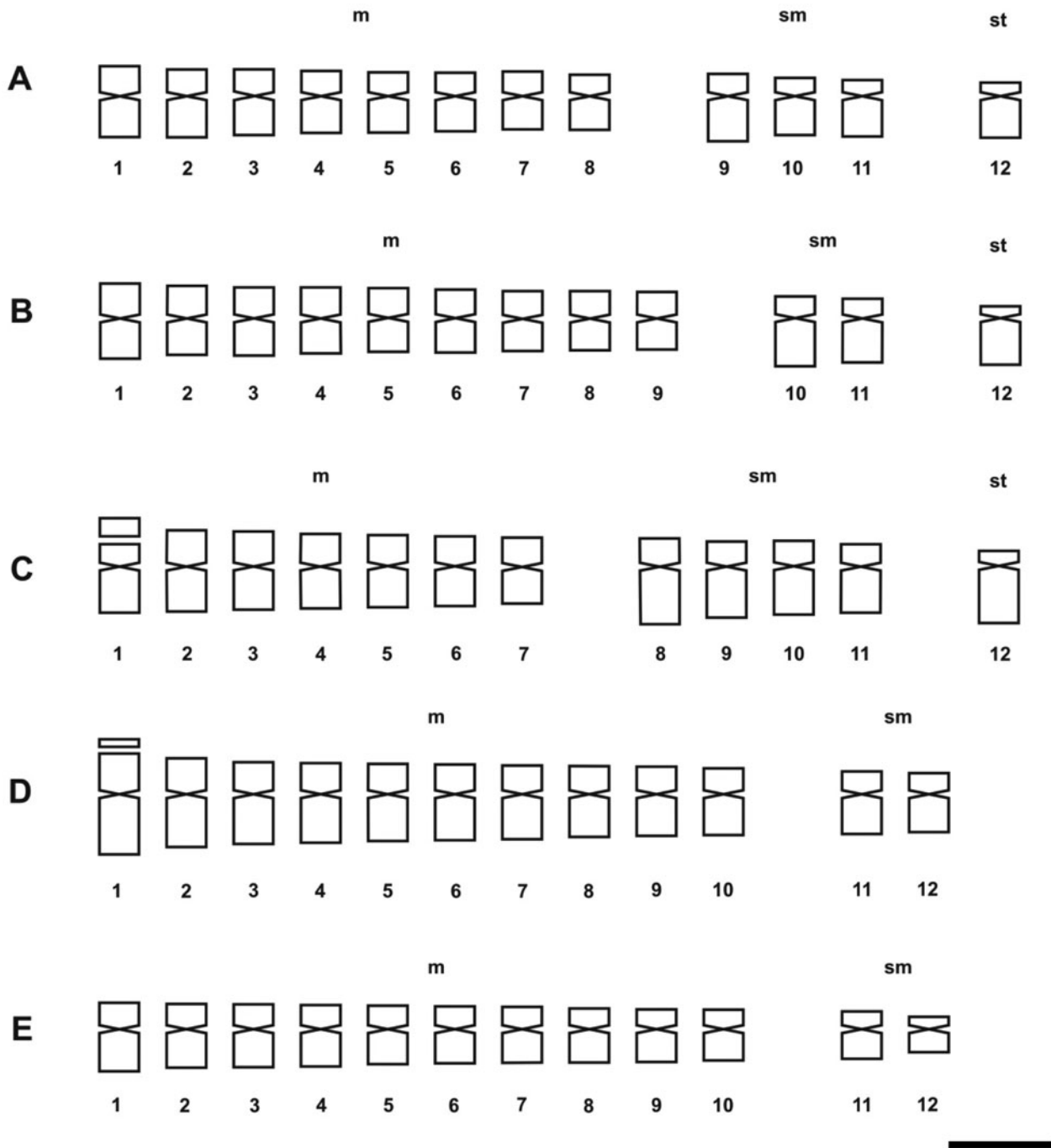


Figure 8. Idiograms of *Solanum* species of Dulcarnaroid clade. (A) *S. endoadenium*; (B) *S. salicifolium* 3488; (C) *S. salicifolium* 3158; (D) *S. salicifolium* 818; (E) *S. salicifolium* 794. Scale bar = 3 μm .

strum (Sharma and Sharma 1957), and *Solanum* (Bernardello and Anderson 1990). *Solanum pilcomayense* 2287 (Morelloid clade) was the only taxon studied with satellites in the long arms. Regarding the magnitude of the satellites, it can vary depending on the species, being of remarkable size in *S. crispum* (Dulcarnaroid clade), where they are even larger than the arms.

The genus *Solanum* is complex and there are many problems in delimiting its taxa and defining an acceptable

infrageneric circumscription (e.g. D'Arcy 1991; Nee 1999; Bohs 2005; Weese and Bohs 2007). Our karyotypic analysis of species of the Morelloid and Dulcarnaroid clades allowed the detection of differences between the examined taxa. Thus, chromosome variation, although not always high, accompanied evolutionary divergence of the species studied, a general phenomenon observed in both plant and animal kingdoms (e.g. Goodspeed 1954; Rieseberg 2001). At the same time, karyotype similarities may

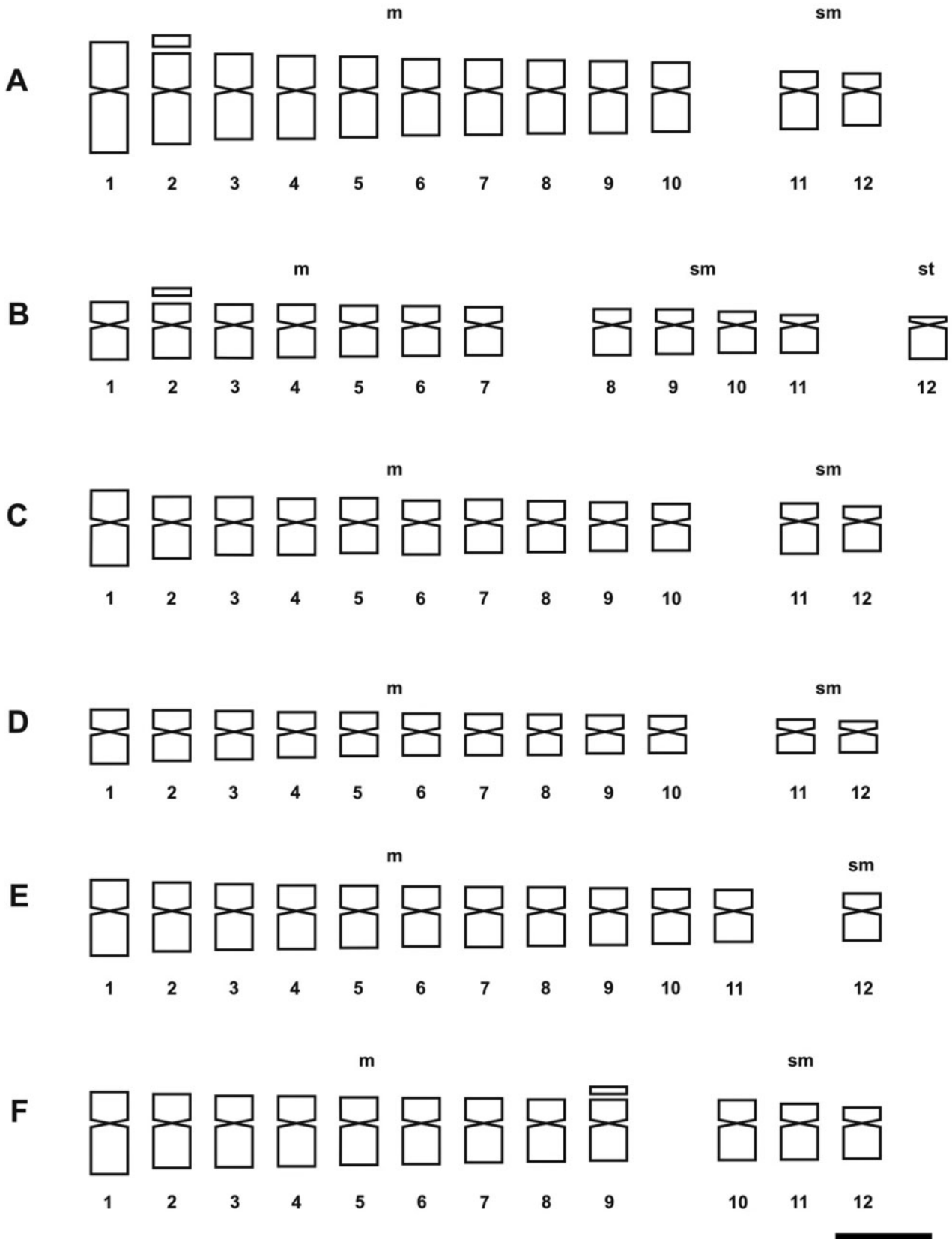


Figure 9. Idiograms of *Solanum* species of Morelloid clade. (A) *S. aloysiifolium* 2210; (B) *S. aloysiifolium* 2152; (C) *S. americanum*; (D) *S. chenopodioides*; (E) *S. concarense*; (F) *S. echegarayi*. Scale bar = 3 μ m.

indicate relationships between taxa. Based on morphological features, Nee (1999), does not consider *S. triflorum*, *S.*

tripartitum and *S. palitans* to be closely related species, placing *S. triflorum* in sect. *Solanum* (Morelloid clade)

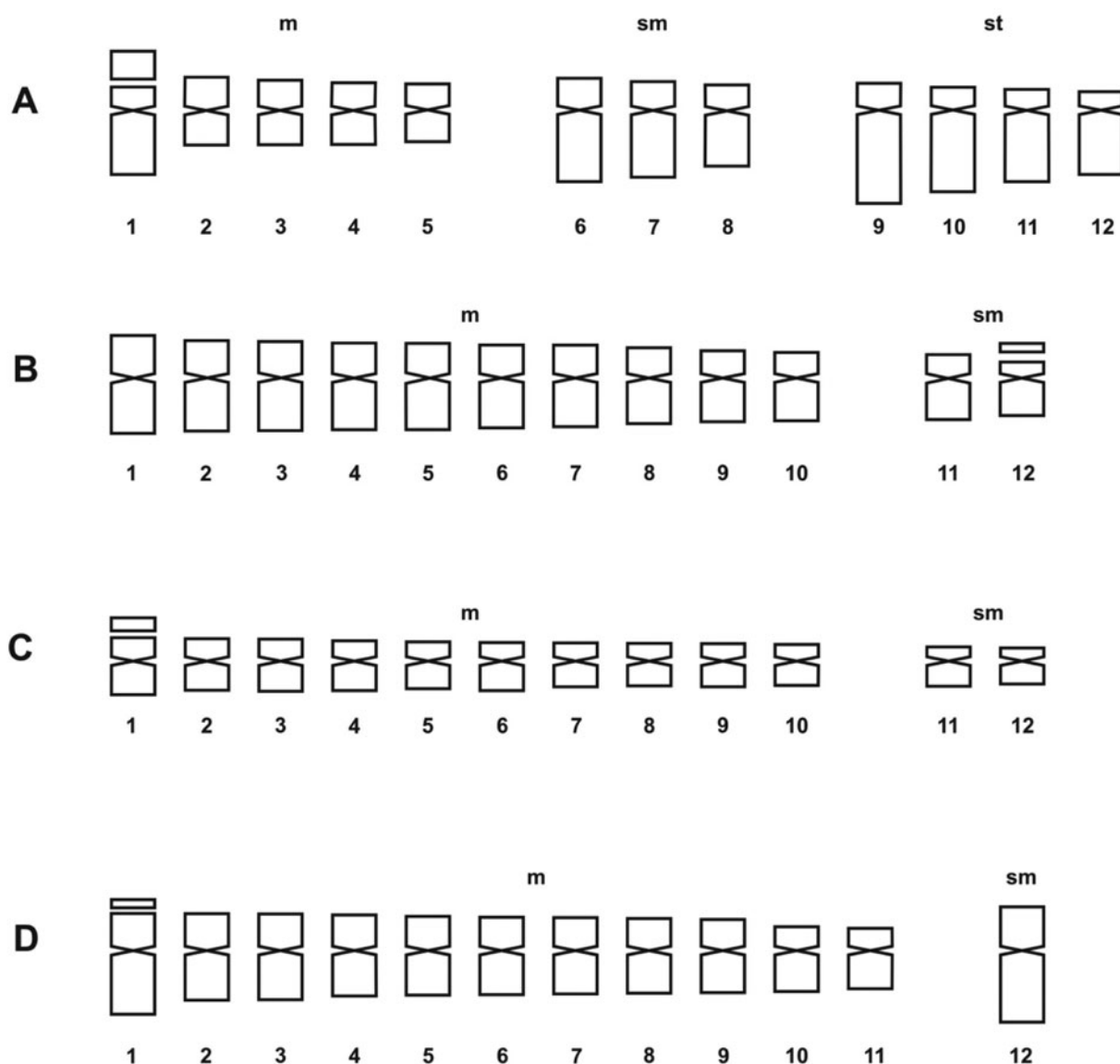


Figure 10. Idiograms of *Solanum* species of Morelloid clade. (A) *S. sinuatirecurvum*; (B) *S. triflorum*; (C) *S. tripartitum*; (D) *S. zuloagae*. Scale bar = 3 μ m.

and *S. tripartitum* and *S. palitans* in sect. *Dulcamara* (Dulcamaroid clade). By contrast, Bohs (2005) and Weese and Bohs (2007), analysing sequences of the *ndhF* chloroplast genes, and Barboza et al. (2013), analysing morphology, included them in the Morelloid clade. Our results agreed with the latter scheme, since these taxa displayed the same karyotypic formula. At the same time, *S. tripartitum* and *S. palitans* fell in the category 1A of Stebbins (1971) and showed similar values of *tl* and *C* (Table 2), whereas *S. triflorum* was included in the 2B category and its values of *tl* and *C* were higher. These facts would indicate that *S. tripartitum* and *S. palitans* are closely related

species, in agreement with Bohs (2005) and Weese and Bohs (2007). Moreover, these two species are morphologically very similar, to the point that they are often confused (Barboza et al. 2013).

Stebbins (1971) included *Solanum* in his 1A category due to the known presence of highly symmetrical karyotypes, as was later reported by several authors (e.g. Bernardello and Anderson 1990; Acosta et al. 2005; Chiarini and Bernardello 2006). However, our data indicated that the Morelloid and Dulcamaroid clades have a range of karyotypes, from symmetrical (most species) to less expectedly, more asymmetrical (e.g. *S. sinuatirecurvum*, *S. crispum*).

Recent data indicate that Solanaceae in general, and *Solanum* in particular, have a low rate of chromosomal changes and that many small rearrangements not observed at the cytological level have occurred (Wu and Tanskley 2010), as previously suggested (e.g. Stebbins 1971; Bernardello and Anderson 1990; Bernardello et al. 1994).

Most examined species of these clades were karyologically indistinguishable, based on conventionally stained mitotic chromosomes, although some species are clearly noticeable, such as *S. sinuatirecurvum* and *S. crispum*. Additional molecular karyotypic analyses with banding and FISH techniques are needed in the Morelloid and Dulcamaroid clades to gain a better knowledge of the possible karyoevolutionary trends.

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