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Chromosome number and genome size diversity in five Solanaceae genera

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Abstract. Sixteen species of Solanaceae, belonging to five genera, were studied karyologically through chromosome counting, chromosomal measurement, and karyotype symmetry. Genome size (GS) estimation was performed on fifteen species using flow cytometry. The chromosome number 2n=24 was found in all *Solanum* species and *Acnistus arborescens*, 2n=22 was found in *Brunfelsia uniflora,* and 2n=16 in *Cestrum* representatives. *Physalis pubescens* was the only specie with evidence of polyploidy, showing 2n=4x=48 chromosomes. The chromosome numbers of *S. adspersum*, *S. inodorum*, *S. flaccidum, S. sanctae-catharinae, and B. uniflora* were reported for the first time. Haploid karyotype length (HKL) was statistically different between the studied species. The polyploid *P. pubescens* showed the largest HKL value, 93.10 μm. In general, karyotypes were symmetrical with predominance of metacentric chromosomes. Chromosome size was small in most species (<4 μm), while *S. diploconos*, *C. laevigatum,* and *C. mariquitense*, species with high HKL values, exhibited larger chromosomes. Genome size estimation were unpublished for ten studied species and were the first estimation for the genera *Acnistus*, *Brunfelsia* and *Physalis*. Were observed about eight-fold differences between species with averages varying from 2C=2.57 pg to 2C=20.27 pg. As both HKL and GS showed a continuous variation. We observed partial similarity in the species ordered according to HKL and GS. The Solanaceae genera showed a constant chromosome number and a tendency to posse symmetrical karyotypes. The genome size also showed differences, which suggests that chromosome evolution in the group could be driven by alterations in the repetitive fractions of the genome.

Keywords. *Acnistus*, *Brunfelsia*, *Cestrum*, *Physalis*, *Solanum*, karyotype evolution.

INTRODUCTION

The Solanaceae family comprises about 2,500 species and 100 genera and have cosmopolitan distribution. The greatest diversity of the family is found in Neotropical regions (D'Arcy 1991; Hunziker 2001). Members of Solanaceae have great ecological and morphological diversity, characteristics which favoured the occupation of diverse habitats, such as desert regions, tropi-

cal rainforests, and even disturbed areas (D'Arcy 1991; Knapp 2002).

The family includes several species of important global food crops with high economic value, such as tomatoes (*Solanum lycopersicum*), potatoes (*Solanum tuberosum*), eggplants (*Solanum melongena*), and chilli peppers (*Capsicum* spp.), widely used drug plants, such as tobacco (*Nicotiana tabacum*), "datura" (*Datura stramonium*), and "angel's tears" *Brugmansia suaveolens*, as well as many ornamental plants, such as species of the genus *Brunfelsia*, *Cestrum* and *Petunia*. Many Solanaceae species, including tomatoes, potatoes, and tobacco, are model organisms for various biological studies, and their genomes are some of the most well studied among angiosperms (Knapp et al. 2004).

Karyotype information about species and groups are important for taxonomic and evolutionary studies, whereas karyological changes accompany speciation and, consequently, the diversification of the groups (Guerra et al. 2008, 2012, Chiarini et al. 2018). The chromosome number, nuclear DNA content, total length of the chromosome complement, asymmetry indices, and number and location of the rDNA sites and heterochromatic bands are the main data used in cytotaxonomic studies. Chromosome number data is the most available information and is not influenced by external agents, such as age of individuals, environmental conditions, and gene expression, providing accurate data about species evolution (Dobginy et al. 2004, Guerra et al. 2008, 2012). Cytogenetic characterization, accompanied by a genome size (GS) study, can offer important information about genome organization, phylogenetic relationships, and evolutionary trends. This approach has been successful used in some Solanaceae (Mishiba et al. 2000, Moscone 2003, Chiarini et al. 2014).

Chromosome data is available for some genera of Solanaceae, while for other genera there is not enough data or information about their chromosomes. *Lycium* and *Solanum* present constant chromosome number (2n=24 and polyploids) (Bernadello and Anderson 1990; Bernadello et al. 1994; Chiarini and Bernadello 2006; Rego et al. 2009; Stiefkens et al. 2010; Melo et al. 2011; Chiarini et al. 2014), while *Capsicum* shows 2n=24 and 2n=26 (Moscone 1993; Moscone et al. 2007; Aguilera et al. 2014; Grabiele et al. 2014; Romero-da Cruz and Forni-Martins 2015; Romero-da Cruz et al. 2017). For the Cestreae tribe, composed of *Cestrum*, *Sessea,* and *Vestia*, the only chromosome number reported to date is 2n=16 (Fregonezi et al. 2006; Las Peñas et al. 2006; Fernandes et al. 2009; Urdampilleta et al. 2015). The greatest range in chromosome number is found in *Nicotiana* (n=12 to n=32, and polyploids, Chase et al. 2003).

Only about 8% of Solanaceae taxa have available GS data. This character has more variability than chromosome number (Soltis et al. 2003). In *Solanum*, the GS ranges of from forty-fold in species with 2n=24 chromosomes. The smallest reported C-value is in *S. chacoense*, 1C=0.63 pg (Bennett and Smith 1976), while the largest value is 1C= 24.80 pg, found in *S. hartwegii* (Pringle and Murray 1991).

Nevertheless, there are still many gaps in karyotypic knowledge for the Solanaceae family and such information (i.e. genome size, chromosome number, and karyotype variables) is important to complete current data and to better understand the systematic relationships and chromosome evolution of the family. Therefore, the objectives of this study were: (1) to report original chromosome numbers and describe the karyotype variables in distinct genera of the Solanaceae family, (2) to determine the genome size (GS) using flow cytometry for the first time for many species.

MATERIAL AND METHODS

Plant material

Sixteen species from the genera *Acnistus*, *Brunfelsia*, *Cestrum*, *Solanum,* and *Physalis* were collected in Southeastern Brazil. Voucher specimens were deposited into the Herbarium at the University of Campinas (UEC). Data collection is detailed in Table 1.

Chromosome preparations

Seeds of at least three individuals per species were germinated in Petri dishes. In some cases, 1 ml gibberellic acid (GA_3) was applied to break seed dormancy (Ellis et al. 1985). According to previous tests, root meristems were pre-treated with different solutions to block the cell cycle to obtain good chromosome spread and condensation (Table 2). The root apices were fixed in 3:1 ethanol: acetic acid (v:v) mixture that was stirred for a minimum of 12 h at room temperature (RT) and stored at -6ºC until slide preparation. Slides were made using root meristems that were previously digested in a solution of 1% macerozima, 2% cellulase, and 20% pectinase for 10-15 minutes at 37ºC and squashed in a drop of 45% acetic acid. Coverslips were removed after freezing in liquid nitrogen for 15 minutes. The cells were photographed under a microscope Olympus BX51 with a DP72 camera attached and images were captured using Olympus DP2 BSW program (Olympus Corporation).

Table 1. Cytogenetics data of Solanaceae species: Species and voucher specimen; provenance of materials; chromosome number, haploid karyotype formula (HKF), median haploid karyotype length (HKL), variation in chromosome length (VCL); symmetry indices A1 and A2; median DNA content (2C values).

Species (Voucher specimen)	Provenance	2n	HKF	HKL – µm (CI)	VCL – µm	A1	A2	2C values – pg (CI)
Acnistus arborescens Schltdl. (Monge 2787)	Brazil: Rio Grande do Sul; Aratinga	24	12m	45.93 (2.78)	3.17-4.38	0.17	0.10	6.56 (0.06)
Brunfelsia. uniflora D. Don (Mesquita 15)	Brazil; São Paulo; Campinas	22	7m+4sm	50.51 (0.50)	3.89-5.37	0.32	0.10	6.58 (0.13)
Cestrum laevigatum Schltdl. (Mesquita 12)	Brazil; São Paulo; Campinas	16	6m+2sm	78.72 (2.96)	7.92-10.88	0.23	0.10	20.27 (0.43)
C. mariquitense Kunth (Mesquita 14)	Brazil; São Paulo; Campinas	16	7m+1sm	73.91 (6.38)	7.35-11.39	0.21	0.12	-
Physalis pubescens L. (Vasconcellos Neto 00-068)	Brazil; São Paulo; Jundiaí	48	19m+5sm	93.10 (2.87)	1.43-2.80	0.32	0.18	12.98 (0.09)
Solanum								
Cyphomandra clade								
Solanum diploconos (Mart.) Bohs (Mesquita 23)	Brazil; São Paulo; Jundiaí	24	8m+4sm	74.72 (1.86)	4.63-7.49	0.32	0.14	19.22 (0.43)
Dulcamaroid clade								
S. flaccidum Vell. (Mesquita 07)	Brazil; São Paulo; Campinas	24	9m+2sm+1st	26.73 (1.14)	1.83-2.50	0.27	0.10	2.57 (0.25)
S. inodorum Vell. (Vasconcellos Neto 20401)	Brazil; São Paulo; Jundiaí	24	5m+7sm	38.33 (3.90)	2.83-3.86	0.39	0.09	4.63 (0.06)
Geminata clade								
S. pseudocapsicum L. (Mesquita 24)	Brazil; São Paulo; Jundiaí	24	9m+3sm	28.61 (7.70)	1.76-2.72	0.28	0.13	2.94 (0.11)
Leptostemonum clade								
***Acanthophora* section**								
S. acerifolium Sendt. (Mesquita 02)	Brazil; São Paulo; Campinas	24	10m+2sm	36.17 (1.09)	1.71-3.87	0.26	0.23	5.69 (0.15)
S. palinacanthum Dunal (Mesquita 20)	Brazil; São Paulo; Ubatuba	24	5m+7sm	37.86 (0.72)	2.51-3.86	0.41	0.13	5.00 (0.10)
***Torva* section**								
S. adspersum Witasek (Monge 2748 c 240)	Brazil; Rio de Janeiro; Arraial do Cabo	24	9m+3sm	25.09 (1.62)	1.77-2.44	0.25	0.09	3.19 (0.04)
S. scuticum M. Nee (Vasconcellos Neto 8503)	Brazil; São Paulo; Jundiaí	24	9m+3sm	27.66 (2.10)	1.95-2.79	0.31	0.04	3.42 (0.06)
S. variabile Mart (Monge 2324)	Brazil; São Paulo; Itacaré	24	9m+3sm	33.45 (0.51)	2.15-3.26	0.24	0.11	3.54 (0.09)
Uncertain position								
S. concinnum Schott ex Sendtn. (Mesquita 08)	Brazil; São Paulo; Campinas	24	6m+6sm	31.82 (0.37)	2.26-2.86	0.39	0.09	3.65 (0.25)
S. sanctae-catharinae Dunal (Vasconcellos Neto 20873)	Brazil; São Paulo; Jundiaí	24	10m+2sm	23.15(2.56)	1.68-2.35	0.26	0.09	3.79 (0.07)

CI – Confidence interval at 95% of semi range.

Karyotype analysis

Five metaphases of each species, with the same degree of chromosome condensation, were used to determine the chromosome number. The measurements were taken using the MicroMeasure© software (3.3). Ideograms were made using measurements of the following means for each chromosome pair: S (short arm length), L (long arm length) and C (total chromosome length) using the formula C= S+L. In addition, haploid karyotype length (HKL) was calculated by the sum of the haploid chromosome lengths. The arm ratio (r) was calculated using the formula r= L/S and was used to classify chromosomes according to Levan et al. (1964). For ideograms, chromosomes were first grouped by morphology (r=1.00-1.69 metacentric-m; r=1.70-2.99 submetacentric-sm; r=3.00-6.99 subtelocentric-sm) and then by decreasing size order within each group.

The karyotype symmetry was described using the indices A1= 1-[(Σbi/Bi)/n] (bi = mean of the short arm

of each chromosome pair, Bi = average of the long arm of each chromosome pair, n = number of chromosome pairs) and A2=x/s (s = standard deviation; x = average chromosome complement length) (Zarco 1986). A1 index measures intrachromosomal asymmetry which indicates differences in the size of chromosome arms. A2 index measures the interchromosomal asymmetry and indicates the variation in chromosome lengths. In terms of length, chromosomes were classified according to Lima de Faria (1980) as very small (≤1 μm), small (>1 μm and ≤4 μm), intermediate (>4 and ≤12) and big (>12 and ≤60).

Flow cytometry

The same species that were cytogenetically analysed (except for *Cestrum mariquitense*) were cultivated in a greenhouse and used for GS measurements. For each species, three individuals were measured in three repetitions, for a total of nine samples. Approximately 1 cm^2 of young leaf tissue was used to prepare the nuclear suspensions, according to Dolezel et al. (2007). The material of each species of interest and a piece of internal leaf standard (*Pisum sativum* "Ctirad" 2C=9.09 pg (Dolezel et al. 1998) were sliced with a razor blade and placed into a Petri dish on ice. About 1 ml of LB01 buffer (Dolezel et al. 1989) was used to extract the nuclei. A nylon mesh with 40 microns was used to filter the sample (CellTrics, PARTEC), then, 25 μL 1 mg/mL propidium iodide and 25 μL 1 mg/mL RNAse were added to the nuclear suspension. The measurement was performed on a BD FACS Calibur flow cytometer, for each sample an average of 10,000 nuclei were analysed. The 2C value was calculated using the linear relationship between fluorescence signals from stained nuclei of the unknown sample and the reference standard. The nomenclature for genome size classification followed Leitch et al. (1998) with modification by Soltis et al. (2003): values <1.4 pg and between 1.4 to 3.5 pg correspond to "very small" and "small" genomes, respectively. On the other hand, values between 3.51-13.99 pg, >14 pg and >35 pg are considered "intermediate," "large", and "very large" genomes, respectively.

Statistical analyses

The HKL values, as well of GS values of each species, were compared using Past 3.18 ® (Øyvind Hammer, Natural History Museum, University of Oslo). The Kruskal-Wallis nonparametric test was performed to compare the averages among the species and Dunn's post-hoc test (Dunn 1954) was carried out after significant Kruskal-Wallis test.

RESULTS

Karyotype analysis

The somatic chromosome numbers were 2n=2x=24 (*Acnistus* and *Solanum*), 2n=2x=22 (*Brunfelsia*), 2n=2x=16 (*Cestrum*) and 2n=4x=48 (*Physalis*) (Table 1; Fig. 1).

Although the differences in HKL between some species were significant ($p<0.05$) according to statistical analysis (Table 1; Fig. 2), this variation was gradual, and no groups were formed. *Solanum sanctae-catharinae* showed the lowest median value (23.15 μm) with a variation of 1.68-2.35 μm from the smallest to largest chromosome pair. Other *Solanum* species also presented low HKL (except for *S. diploconos*), with values reaching 38.33 μm in *S. inodorum* (2.83 to 3.86 μm). Species with intermediate HKL values were *A. arborescens* (45.93 μm, 3.17 to 4.38 μm) and *B. uniflora* (50.51 μm, 3.89 to 5.38 μm). High HKL values were found in *C. mariquitense* with 73.91 μm (7.35 to 11.39 μm), *S. diploconos* with 74.72 μm (4.63 to 7.49 μm), and *C. laevigatum* with 78.72 μm (7.92 to 10.88 μm). *Physalis pubescens* showed

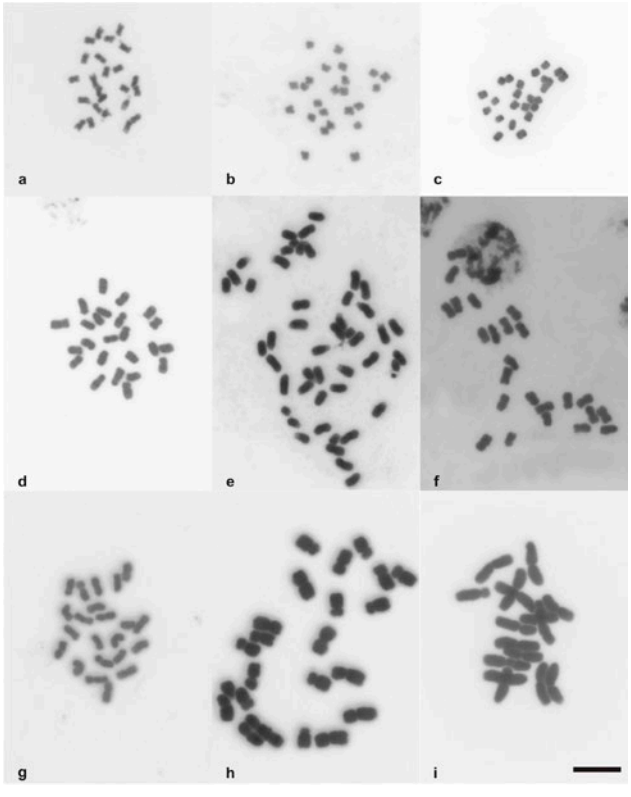

Fig. 1. Somatic metaphases of five genera of Solanaceae. **a** *Solanum flaccidum*. **b** *S. adspersum*. **c** *S. sanctae-catharinae*. **d** *S. inodorum*. **e** *Physalis pubescens*. **f** *Acnistus arborescens*. **g** *Brunfelsia uniflora*. **h** *S. diploconos*. **i** *Cestrum laevigatum*. Bar=10 μm.

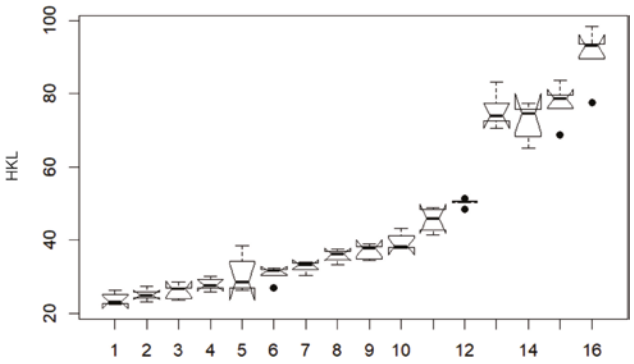

Fig. 2. Boxplots illustrating the continuous variability of HKL (Haploid Karyotype Length), as inferred from de Kruskal Wallis analysis. The numbers on the x axis represent the species ordered by crescent HKL values (in µm): *S. sanctae-catharinae* (1), *S. adspersum* (2), *S. flaccidum* (3), *S. scuticum* (4), *S. pseudocapsicum* (5), *S. concinnum* (6), *S. variabile* (7) *S. acerifoium* (8) *S. palinacanthum* (9) *S. inodorum* (10), *A. arborescens* (11) *B. uniflora* (12) *C. mariquitense* (13) *S. diploconos* (14) *C. laevigatum* (15) *P. pubescens* (16). The central box represents 50% of the data from de upper to lower quartile. The horizontal bar expresses the median position. The extremity of the vertical lines indicates minimum and maximum values of HKL, if they are no outliners. When outliners are present, they are represented by circles.

the highest HKL value (93.10 µm), even though it is a polyploid species with chromosomes ranging from 1.43 to 2.8 µm.

Karyotypes are symmetrical with A1 and A2 values for each species ranging from 0.17 to 0.41 and from 0.04 to 0.23, respectively. Most species presented a predominance of metacentric chromosomes (Table 1, Fig. 3) that characterized most intrachromosomal symmetry shown in the A1 index. *Acnistus arborescens* had the most symmetrical karyotype, composed of only metacentric chromosomes and A1=0.17. Three species had less symmetrical karyotypes: *Solanum inodorum* and *S. palinacanthum* showed predominance of submetacentric chromosomes (5m+7sm) and A1 value of 0.39 and 0.41, respectively. *Solanum concinnum* also presented A1=0.39, but karyotype formulae 6m+6sm.

Interchromosomal index A2 showed that all species have few variations in chromosome size of the karyotypes. *Solanum scuticum* showed the small A2 value (0.04) and *Solanum acerifolium* presented the highest A2 value (0.23), characterizing the most interchromosomal asymmetry among studied species (Table 1).

C-value

Genome size estimates of all the studied species are shown in Table 1 and histograms for selected species are shown in Fig. 4. According to statistical analysis, GS showed significant differences among some of the studied species (Fig. 5). A variation of about eight-fold was observed, ranging from 2C=2.57 pg (*S. flaccidum*, Fig. 4a) to 2C=20.27 pg (*C. laevigatum*, Fig. 4d). The GS presented continuous variation, so distinct groups were not characterized (Fig. 5). Most species had small (2C=2.57 pg in *S. flacidum* to 2n=3.79 pg in *S. sanctae-catharinae*) and intermediate genomes (2C=4.63 pg in *S. inodorum* to 6.56 pg in *A. arborescens* and 6.58 pg in *B. uniflora*). The species with larger genomes were *P. pubescens* (2n=12.98 pg), *S. diploconos* (2C=19.22 pg), and *C. laevigatum* (2C=20.27 pg).

DISCUSSION

Chromosome number

The chromosome number data found here are new for *S. adspersum*, *S. inodorum*, *S. flaccidum,* and *S. sanctae-catharinae*, with 2n=24 chromosomes, as well as for *B. uniflora*, with 2n=22. For the remaining species, the chromosome number obtained corroborated with data found in the literature for *Acnistus* (2n=24), *Cestrum* (2n=16), *Solanum* (2n=24), and *Physalis* (2n=48) (Heiser 1963; Pedrosa et al. 1999; Fernandes et al. 2009; Rego et al. 2009; Urdampilleta et al. 2015).

All the species in this study, except for *P. pubescens*, which is a tetraploid, are diploid. Although diploid is the most frequent ploidy level (including other species of *Physalis*), polyploidization has played an important role in the evolution of some Solanaceae genera (e.g., *Nicotiana*, Chase et al. 2003; *S. elaeagnifolium*, Scaldaferro et al. 2012). The chromosome number most frequent in the family is 2n=24, found in more than 85% of the previously studied Solanaceae species (Olmstead et al. 2008) though a diploid series from 2n=14 to 2n=26 is present in some genera (eg. *Petunia* and *Calibrachoa*, Mishiba et al. 2000, *Cestrum, Sessea and Vestia,* Las Peñas et al. 2006, *Capsicum*, Moscone et al. 2007).

Many authors have postulated hypotheses for the ancestral chromosome base number in the family. Raven (1975) proposed x=7 and 12 for the order Solanales and Solanaceae family, respectively, while Badr et al. (1997) suggested the hypothesis of x=7 or x=8. Moscone (1992) corroborate with the proposition of Badr et al. (1997), suggested x=7 as the basic chromosome number for Solanaceae. Olmstead and Palmer (1992) and Olmstead et al (2008) based in phylogenetic studies, suggests an ancestral position of subfam. Cestroideae (x=8), and x=12 as a derivate basic chromosome number the family.

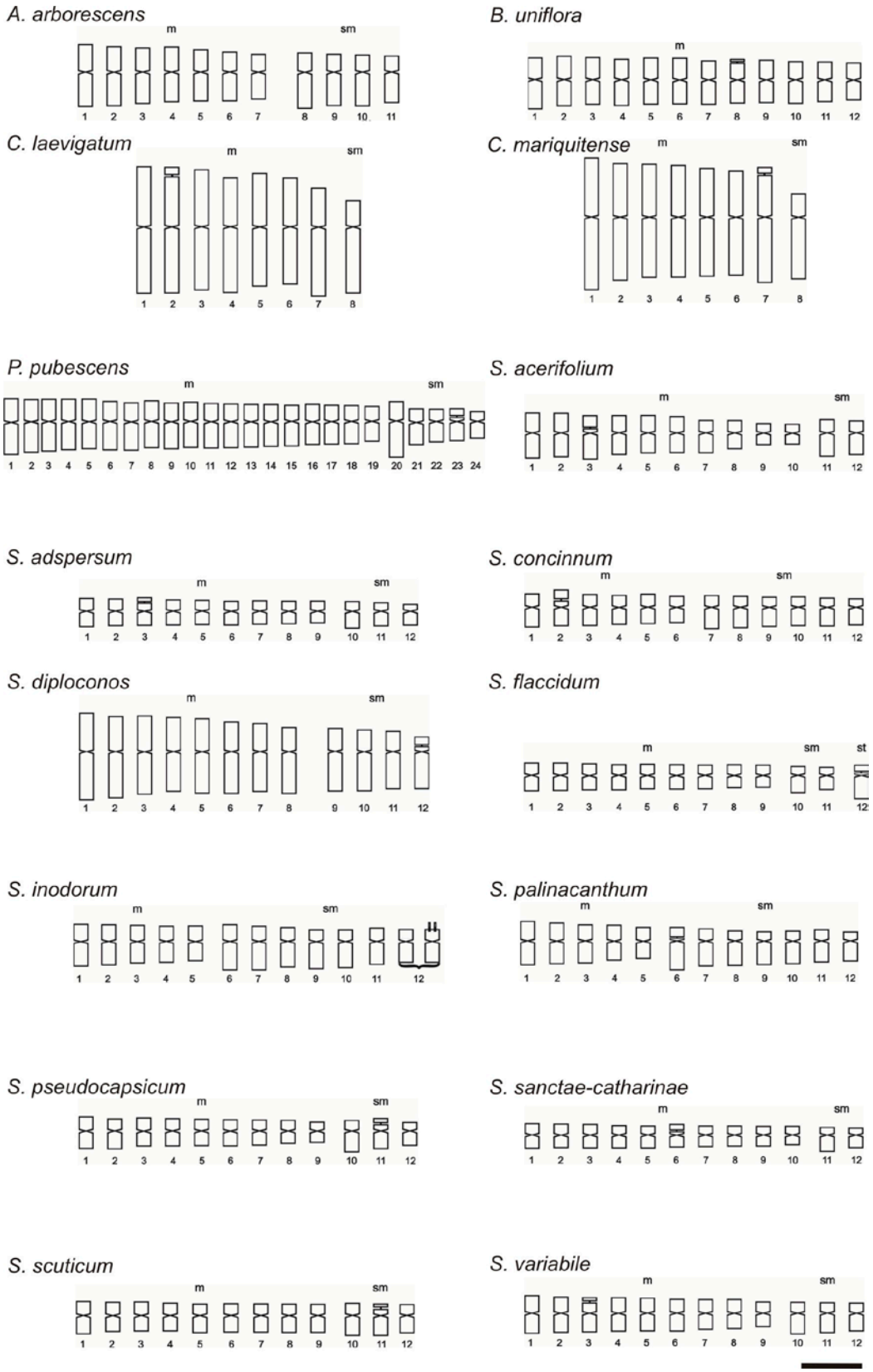

Fig. 3. Ideograms of the investigated Solanaceae species based on median chromosome values. Bar=5 μm.

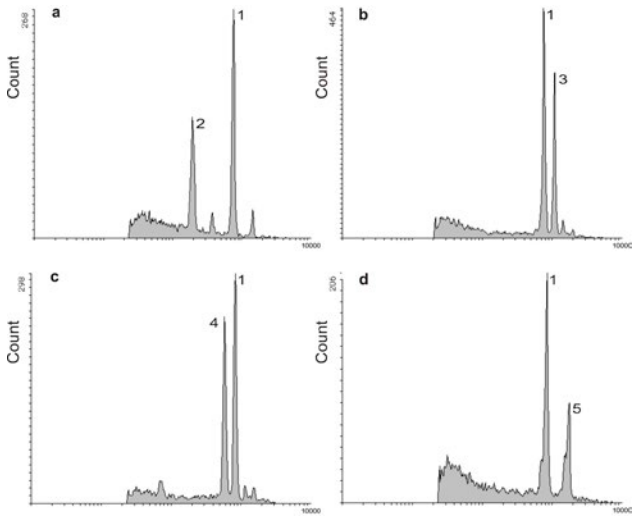

Fig. 4. Flow cytometry histograms (iodide propidium fluorescence intensity of nuclei) showing DNA amounts from leaf tissues of some Solanaceae species. **1** *Pisum sativum* "Ctirad" (standard). 2 *S. flaccidum*. **3** *P. pubescens*. **4** *A. arborescens*. **5** *C. laevigatum*.

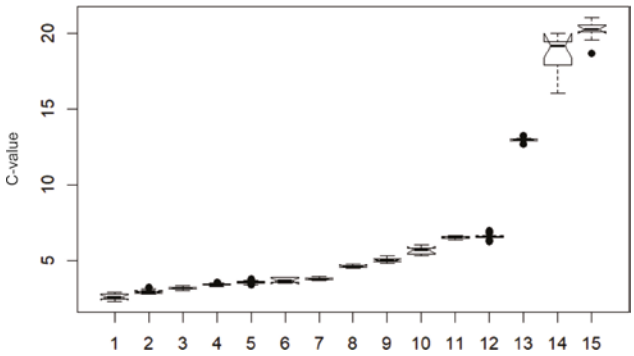

Fig. 5. Boxplots illustrating the continuous variability of GS (Genome Size), as inferred from de Kruskal Wallis analysis. The numbers on the x axis represent the species ordered by crescent C-values (in pg): *S. flaccidum* (1), *S. pseudocapsicum* (2), *S. adspersum* (3), *S. scuticum* (4), *S. variabile* (5), *S. concinnum* (6), *S. sanctae-catharinae* (7) *S. inodorum* (8) *S. palinacanthum* (9) *S. acerifolium* (10), *A. arborescens* (11) *B. uniflora* (12) *P. pubescens* (13) *S. diploconos* (14) *C. laevigatum* (15). The central box represents 50% of the data from de upper to lower quartile. The horizontal bar expresses the median position. The extremity of the vertical lines indicates minimum and maximum values of HKL, if they are no outliners. When outliners are present, they are represented by circles.

The lack of chromosomal data for several genera and for the Solanaceae sister group, the family Convolvulaceae, as well as the presence of distinct basic numbers in other Solanales families, as in Hydroleaceae, x=8 and 10 (Constance 1963) and Montiniaceae, x=12 (Goldblatt 1979), has hampered to establish a consensus about a basic chromosome number and understand the direction of chromosome number evolution for the family.

Karyotype structure

Differences in chromosome size were seen between the Solanaceae species here investigated.

The relatively small chromosome size and HKL observed in the species here of *Solanum*, except for *S. diploconos* (statistically distinct, and previously considered a species of the distinct genus *Cyphomandra*), and *P. pubescens*, have been reported in some studies for *Solanum* (Bernardello and Anderson 1990; Acosta et al. 2005; Chiarini et al. 2006; Rego et al. 2009; Melo et al. 2011; Moyetta et al. 2013), and another Solanaceae genera, as *Lycianthes* and *Vassobia* (Rego et al. 2009) and *Lycium*, (Stiefkens and Bernadello 2002,)

Acnistus arborescens and *B. uniflora* shows chromosomes and consequently, HKL values, with intermediate size, when compared to *Solanum* and *Physalis*. These karyotype characteristics are also present in *Capsicum* (Moscone 1996), *Sclerophylax* and *Nolana* (Lujea and Chiarini 2017), genera also belonging to Solanaceae. Although the intermediate size of the chromosomes, a constant chromosome number, karyotype symmetry and chromosomes majority metacentric appear to maintain in these groups.

The tribe Cestreae (subfam. Cestroideae) embraces the genera *Cestrum*, *Sessea* and *Vestia*, presents the largest chromosomal sizes of the family (Fregonezi et al. 2006, Peñas et al. 2006). *Cestrum laevigatum* and *C. mariquitense,* investigated here, showed the largest chromosome size high HKL values, confirming the trend for the tr. Cestreae (Fregonezi et al. 2006). Such increase in chromosome size for the tribe can be due to the absence of *Arabidopsis*-type telomeres $(TTTAGGG)_n$ for short interstitial telomeric sequences (SITS), leading to the lack of control of the telomerase-dependent replication. These sequences may associate with other DNA sequenc-

Table 2. Pretreatments used for each genus of Solanaceae studied.

Genus	Pretreatments
Acnistus and *Physalis*	8-hydroxyquinoline 0.002M + cycloheximide 25mg/L (1:1), 8 h, 4ºC
Brunfelsia	8-hydroxyquinoline 0.002M, 6 h, 14ºC
Cestrum	Colchicine 0.1% 6 h, RT*
Solanum	Saturated solution of r-dichlorobenzene 2 h, RT*
Solanum (*S. diploconos*)	Saturated solution of r-dichlorobenzene 5 h, RT*

*RT=room temperature.

es and assist in their dispersion leading to an increase in genome size (Sykorova et al. 2003a, b).

Besides chromosome number, other widely conserved karyotype characters in Solanaceae genera, are chromosome morphology and karyotype symmetry. Symmetrical karyotypes with a predominance of metacentric chromosome pairs are found in the five genera studied here. *Acnistus arborescens* was the unique specie that had only metacentric chromosomes, thus, had the most symmetrical karyotype and only *S. flaccidum* showed a subtelocentric chromosome pair. Other genera of the family Solanaceae in which it is possible to observe these characteristics are*Capsicum* (Pozzobon et al. 2006; Moscone et al. 1993, 2007), *Lycium* (Stiefkens and Bernadello 2012), *Lycianthes,* and *Vassobia* (Rego et al. 2009).

Among the angiosperms, karyotype asymmetry can be associated with derivate taxa (Stebbins 1971) In some groups of the Solanaceae family, intermediate asymmetry values, can be observed, such as the tr. Cestreae (Las Peñas et al. 2006), *Solanum* sect. *Acanthophora* (Chiarini et al. 2014) However, for species, the karytotype asymmetry was not associate with basal or derived position of the taxa in the phylogeny. Regarding karyotype asymmetry, no evolutive trend was found for the analysed genera or among the representatives of *Solanum*. Overall, karyotype asymmetry seems to occur randomly within some groups of the family.

Our study analysed five species from other sections (*Acantophora* and *Torva*) of the Leptostemonum clade. In sect. *Acantophora* (*S. acerifolium* and *S. palinacanthum*), we observed greater HKL and karyotype asymmetry than in sect. *Torva* (*S. adspersum*, *S. scuticum* and *S. variabile*). Karyotype asymmetry was previously reported for the Lepstotemomun clade (Chiarini et al. 2014). In other cases, asymmetry was random within a group, as in *Solanum* Morelloid and Dulcamaroid clades (Moyetta et al. 2013). Both species belonging to clade Dulcamaroid that were studied corroborated this data, while *S. inodorum* (HKL=38.33 µm) presented a more asymmetrical karyotype (5m + 7sm and A1=0.39, A2=0.09) than *S. flaccidum* (HKL=26.73 µm, 9m + 2sm + 1st and A1=0.27, A2=0.10).

The karyotype characteristics described for the studied species and genera as well as in other Solanaceae groups, a constancy in the chromosome number, karyotype symmetry and chromosome morphology, indicates karyotype orthoselection, which preserves similar chromosomal complements, regardless of chromosome size (Acosta et al. 2005; Moscone et al. 2003). According to Wu and Tanksley (2010), inversions have occurred at a much higher rate than translocations throughout the evolutionary history of Solanaceae, thereby preserving chromosome morphology favouring chromosomal uniformity

C-value/DNA content

Despite the great number of representatives in Solanaceae, the GS estimation is available for a small proportion of species and genera. Only 12 Solanaceae genera have data about GS, representing approximately 10% and 186 species that corresponding to 7% of Solanaceae representatives. Genome size data for *A. arborescens*, *B. uniflora* and *P. pubescens* are the first estimation for the relative genera. Some species of *Cestrum* and *Solanum* have their GS measured but the data here obtained are unpublished for *C. laevigatum*, *S. flaccidum*, *S. inodorum*, *S, adspersum*, *S, scuticum*, *S. variabile*, *S. concinnum* and *S. sanctae-catharinae*.

The GS variation observed in the species studied partially coincides with the variation observed in HKL. In general, species with small, intermediary, or high HKL presented the same GS classification. Among the five species with small HKL, four presented small values of DNA content (*S. adspersum*, *S. flaccidum, S. pseudocapsicum* and *S. scuticum*). Similarly, of the six species with high HKL, five showed high values of DNA content (*A. arborescens*, *B. uniflora*, *S. diploconos*, *C. laevigatum* and *P. pubescens*). The estimation of nuclear DNA using flow cytometry is more accurate than measuring chromosomes. This accuracy is supported by statistical tests and by species boxplots, where the dispersion of HKL data (Figure 2) was greater than GS data (Figure 5). Calculating HKL is more subject to external effects (Stace 2000). Methodological standardization, especially the degree of chromosomal condensation in the mitotic metaphase, is important for obtaining chromosomal sizes and comparing the results obtained between species (Stace 2000).

Although angiosperms have high diversity in their DNA content, the predominance of a small genome size causes a tendency with modal values equal to 1C = 0.7 pg (Leitch et al. 1998). This distribution, strongly skewed towards small genomes, is associated with the ancestral condition of the group and large genomes could have arose more than once during angiosperm evolution (Leitch et al. 1998; Soltis et al. 2003).

Among the species studied, and in the Solanaceae family in general, we observed a predominance of small genomes (see Bennett and Leitch 2012). The cosmopolitan distribution of the family and occurrence in a wide variety of habitats (D'Arcy 1991; Hunziker 2001; Knapp 2002) are related to some phenotypic characteristics that

are correlated to the low DNA content. Species with low DNA content tend to be found in varying habitats and those with very large genomes appear to be excluded from extreme habitats (Knight and Ackerly 2002).

Despite the predominance of small genomes in Solanaceae, there are some groups with intermediary or large genome sizes, such as species of the genera *Nicotiana*, *Cestrum*, *Capsicum,* and the Cyphomandra clade of *Solanum* (see Bennett and Leitch 2012). There are two main factors associated with increased genome size in plants, polyploidy events or whole genome duplication (Soltis et al. 2003; Leitch and Leitch 2013; Wendel et al. 2015) and an increase in the repetitive elements of DNA (mainly transposable elements) (Leitch and Leitch 2013; Bennetzen and Wang 2014). In Solanaceae, it is likely that the changes in genome size of these groups is related to repetitive elements, since there are few groups with polyploidy relatives causing an increase in DNA content.

CONCLUSIONS

We conclude that some karyotype characters are well conserved in the Solanaceae family at the generic level. Chromosome numbers are very constant, with few reports of polyploidy and aneuploidy and the predominance of chromosome morphology and karyotype symmetry. The family represents a model for karyotypic orthoselection and the karyotype evolution in Solanaceae may have been driven by repetitive DNA reorganization that led to GS diversification, but did not affect chromosome number and morphology.

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