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ORIGINAL ARTICLE

Cytotaxonomical study in Brazilian species of *Solanum*, *Lycianthes* and *Vassobia* (Solanaceae)

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Abstract *Solanum* comprises about 1,400 species of shrubs, trees and vines. This group is cytogenetically interesting because it possesses karyotypes apparently conserved in chromosome number and shape, but with diversity in the repetitive DNA. The objective of this study is to characterize 16 species of *Solanum* and two species of closely related genera (*Lycianthes australe* and *Vassobia breviflora*) using cytogenetic parameters. All the species presented $2n = 24$, confirming previous chromosome counting. Additionally, nonreticulated nuclei, proximal condensation in prophase-metaphase and little variation in the karyotype symmetry were observed. *Solanum corymbiflorum* exhibited chromosomes approximately three times bigger in relation to the other species. GC-rich heterochromatin was preferentially located at terminal regions and AT-rich blocks always appear in the centromeric regions. The 45S rDNA sites were coincident with C/CMA_3^+ regions (satellites) and found in just one pair, except in *S. corymbiflorum* which presented two pairs. FISH with 5S rDNA showed signals in the paracentromeric region of one chromosome pair, except in *S. trachytrichium* and *S. gemellum* which showed two hybridization signals. The results point out to different ways of karyotype differentiation in *Solanum* and closely related genera and bring important issues on the value of the cytogenetical information for taxonomic studies.

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Introduction

Solanaceae posses about 90 genera and 3,000 species, which have great economical importance, as *Solanum tuberosum* L. (potato), *Solanum melogena* L (eggplant) and several peppers (*Capsicum* L.), used as food in several cultures (D'Arcy 1991). Members of the family Solanaceae show basic chromosome number varying from $x = 7$ to $x = 13$ and occurrence of symmetrical karyotypes with predominance of meta- and submetacentric chromosomes. These features are quite evident in *Capsicum* L. (Moscone 1993; Moscone et al. 1993) and *Nicotiana* L. (Nakamura et al. 2001; Kitamura et al. 2001). The genus *Solanum* L., with about 1,400 species of herbs, subtrees and trees (Nee 1999; Child and Lester 2001; Bohs 2005), possess species with $2n = 2x = 24$, besides polyploids with $2n = 4x = 48$, $2n = 6x = 72$ and $2n = 8x = 96$ (Hunziker 2001).

Chromosome banding and FISH studies show that the species of Solanaceae have a wide variation of repetitive DNA occurrence and distribution. We can mention the occurrence of AT-rich heterochromatin in the largest chromosomes of *Nicotiana kawakamii* Y. Ohashi (Nakamura et al. 2001), the predominance of centromeric C-bands in some species of *Capsicum* in relation to other that are more terminal (Moscone et al. 1993), and different heterochromatin types in *Cestrum* L. (Fregonezi et al. 2006). The positioning of the rDNA segments has been described in *S. bulbocastanum* Dunal (Dong et al. 2000) and *S. tuberosum* L. (Komarova et al. 2004), but scarcely documented in other native species of *Solanum*.

Molecular phylogeny studies have changed substantially the taxonomic organization in groups close to *Solanum*, e.g., through the inclusion of *Cyphomandra* Mart. ex Sendtn. as a section of *Solanum* L., at the same time that genera which are morphologically closer [such as *Lycianthes* (Dunal) Hassl.] have been maintained out of *Solanum* (Olmstead and Palmer 1997; Bohs and Olmstead 1997, 1999; Olmstead et al. 1999; Bohs 2004, 2005). The cytogenetic data do not support the above mentioned taxonomic arrangements, which are mainly based in sequences of few genes. In order to contribute to a better understanding of the taxonomic relationships among *Solanum* and related genera, this study aimed the karyotype characterization of some species of *Solanum,* as compared to *Lycianthes australe* and *Vassobia breviflora* (Sendtn.) Hunz.

Materials and methods

Plant material

Seeds and samples of *Solanum*, *Lycianthes* and *Vassobia* were collected in different localities of Brazil: Mato Grosso do Sul, Paraná and Rio Grande do Sul states (Table 1), and cultivated in pots at Laboratório de Biodiversidade e Restauração de Ecossistemas—LABRE, CCB, UEL, Londrina, Brazil. At least three individuals from each species were used for the karyotype analyses. Vouchers are kept in the herbarium of the Universidade Estadual de Londrina (FUEL). The species were organized in sections according to Nee (1999).

Conventional staining

The method described by Guerra (1983) with modifications was used for the study of somatic chromosomes. Roots were pretreated with 2 mM 8-hydroxyquinoline for 24 h and fixed in absolute ethanol: glacial acetic acid (3:1, v:v) for 12 h and kept at −20°C until used. Root tips were digested for 1 h in a mixture of 4% (w/v) cellulase and 40% pectinase (v/v), further hydrolyzed in 1 M HCl at 60°C for 11 min, dissected in a drop of 45% acetic acid and squashed. The cover slips were removed after freezing in liquid nitrogen. The material was stained with 2% Giemsa and permanent slides mounted in Entellan. The chromosome measurements performed using the MicroMeasure 3.3 software (http://www.biology.colostate.edu), allowed to calculate the: (1) haploid set length and (2) centromere position and (3) karyotype formulae. Chromosome types were classified according to Guerra (1986).

Chromosome banding

The banding was performed as described by Vanzela et al. (2002), based in the procedure of Schwarzacher et al. (1980). Root tips were digested in an enzyme solution composed of 4% cellulase (w/v) and 40% pectinase (v/v) at 37°C and dissected in a drop of 45% acetic acid. After removal of the cover slips, the slides were aged for 3 days, and then incubated in 45% acetic acid for 10 min at 60°C, 5% barium hydroxide for 10 min at room temperature and 2 × SSC, pH 7.0, for 10 min at 60°C. The samples were washed in distilled water, air dried and stained with the fluorochromes: 0.5 mg/mL CMA_3 for 1.5 h and 2 μg/mL DAPI for 30 min. Slides were mounted with a medium composed of glycerol/McIlvaine buffer (pH 7.0) 1:1, plus 2.5 mM $MgCl_2$.

FISH

The in situ hibridization was performed according Heslop-Harrison et al. (1991) and Cuadrado and Jouve (1994), with modifications. Slides were prepared as described for banding and immediately used for FISH. The p*Ta*71 probe containing the 45S rDNA sequence (Gerlach and Bedbrook 1979) was labeled with biotin-14-dATP by nick translation. The p*Ta*794 probe containing the 5S rDNA sequence (Gerlach and Dyer 1980) was labeled with digoxigenin-11-dUTP by nick translation. Each slide was treated with 34 μL of hybridization mixture containing 100 ng of labeled probe (2 μL of each probe), 100% formamide (15 μL), 50% polyethylene glycol (6 μL), 20 × SSC (3 μL), 100 ng of calf thymus DNA (1 μL) and 10% SDS (1 μL). The material was denatured at 90°C for 10 min and hybridization was performed overnight at 37°C in a humidified chamber. Post-hybridization washes were carried out in 2 × SSC, 20% formamide in 0.1 × SSC, 0.1 × SSC and 4 × SSC/0.2% Tween 20, all at 42°C. The probes were simultaneously detected with a solution composed of 5% BSA, avidin-FITC conjugated and anti-dig-rhodamine conjugated (100:1:1, v:v:v), and the post-detection baths were conducted in 4 × SSC/0.2% Tween 20 at room temperature. Slides were mounted with 25 μL of a medium composed of 23 μL of DABCO solution (1,4-diaza- bicyclo (2.2.2)-octane (2,3%), 20 mM Tris HCl, pH 8.0, (2%) and glycerol (90%), in distilled water), 1 μL of 2 μg/mL DAPI and 1 μL of 50 mM $MgCl_2$.

All the images were acquired with a Leica DM 4500 B microscope equipped with a DFC 300FX camera and the Leica IM50 4.0 software. All the images were optimized for best constrast and brightness with iGrafx Image software.

Table 1 Karyotype features of *Solanum species* (according Nee 1999), *Lycianthes australe* and *Vassobia breviflora*

Species	2*n*	KF	HSS (μm)	*R*	KA	Municipium, state and voucher
Subgenus *Leptostemonum* Dunal (Bitter)						
Section *Acanthophora* Dunal						
Solanum viarum Dunal	24	11 m + 1 sm	18.14	1.62	1A	Ibiporã, Paraná, 41933
S. palinacanthum Dunal	24	3 m + 9 sm	27.90	1.45	3A	Jataizinho, Paraná, 41938
S. artropurpureum Schrank	24	6 m + 6 sm	23.04	1.49	2A	Tibagi, Paraná, 41937
Section *Cryptocarpum* Dunal G. Don						
S. sisymbriifolium Lam.	24	6 m + 6 sm	27.41	1.53	2A	Londrina, Paraná, 41949
Section *Torva* Nees						
S. paniculatum L.	24	7 m + 5 sm	19.32	1.55	1A	Londrina, Paraná, 41950
S. guaraniticum A. St.-Hil.	24	8 m + 4 sm	19.90	1.37	2A	Faxinal, Paraná, 41935; Londrina, Paraná, 41936
S. scuticum M. Nee	24	8 m + 4 sm	25.87	1.69	2A	Londrina, Paraná, 41934
S. variabile Mart.	24	12 m	20.50	1.51	1A	Londrina, Paraná, 41084
Subgenus *Solanum* L.						
Section *Solanum* L.						
S. americanum Mill.	24	10 m + 2 sm	22.39	1.67	2A	Londrina, Paraná, 41939; Ibiporã, Paraná, 41940
Section *Geminata* (G. Don) Walp.						
S. pseudocapsicum L.	24	7 m + 5 sm	20.99	1.72	2A	Londrina, Paraná, 41941
S. trachytrichium Bitter	24	6 m + 6 sm	15.47	1.62	1A	Londrina, Paraná, 41942
Section *Brevantherum* (Seithe) D'Arcy						
S. granuloso-leprosum Dunal	24	6 m + 6 sm	13.50	1.60	1A	Sapopema, Paraná, 41943
S. mauritianum Scop.	24	7 m + 5 sm	21.04	1.46	2A	Porto Alegre, Rio Grande do Sul, 41952
S. gemellum Sendtn.	24	5 m + 7 sm	25.74	1.47	2A	Londrina, Paraná, 41953
Section *Dulcamara* (Moench) Dumort.						
S. amygdalifolium Steud.	24	8 m + 4 sm	13.08	1.62	2A	Aquidauana, Mato Grosso do Sul, 42116
Subgenus *Bassovia* (Aubl.) Bitter						
Section *Pachyphylla* Dunal						
S. corymbiflorum (Sendtn.) Bohs	24	6 m + 6 sm	77.88	1.43	2A	Sapopema, Paraná, 41947
Lycianthes australe Barboza et Hunz.	24	6 m + 6 sm	20.02	1.81	1A	Londrina, Paraná, 41951
Vassobia breviflora (Sendtn.) Hunz.	24	9 m + 3 sm	19.63	1.63	1A	Londrina, Paraná, 42117

KF karyotype formula, *HSS* haploid set size, *R* ratio between major and minor chromosome pair, *KA* karyotype asymmetry according to Stebbins (1971)

Results

The conventional analysis showed that the species of *Solanum*, *Lycianthes australe* and *Vassobia breviflora* presented nonreticulated interphase nuclei, proximal condensation at prophase (Fig. 1e, q), $2n = 2x = 24$, chromosomes measuring around 2 μm and predominance of meta- and submetacentrics (Figs. 1, 4; Table 1), with the exception of *Solanum corymbiflorum* (Sendtn.) Bohs which exhibited chromosomes with up to 6 μm (Figs. 1r, 4i; Table 1).

In the subgenera *Leptostemonum* Dunal (Bitter) and *Solanum* there was a predominance of metacentrics in relation to submetacentrics (Fig. 4; Table 1). However, the more frequent karyotype formulae was 6 m + 6 sm, observed in *S. atropurpureum* Schrank, *S. sisymbriifolium* Lam., *S. trachytrichium* Bitter, *S. granuloso-leprosum* Dunal, *S. corymbiflorum* and *Lycianthes australe* Barboza et Hunz. (Fig. 4c, d, l, m, i, q; Table 1). In spite of similar chromosome types in these species, the haploid set size was variable, from 13.08 to 27.9 μm, except to *S. corymbiflorum* that showed 77.88 μm (Table 1).

Karyotypes containing a larger number of metacentrics were observed in *S. paniculatum* L., *S. pseudocapsicum* L. and *S. mauritianum* Scop., with 7 m + 5 sm (Fig. 4e, k, n), *S. guaraniticum* St.-Hil., *S. scuticum* M. Nee and

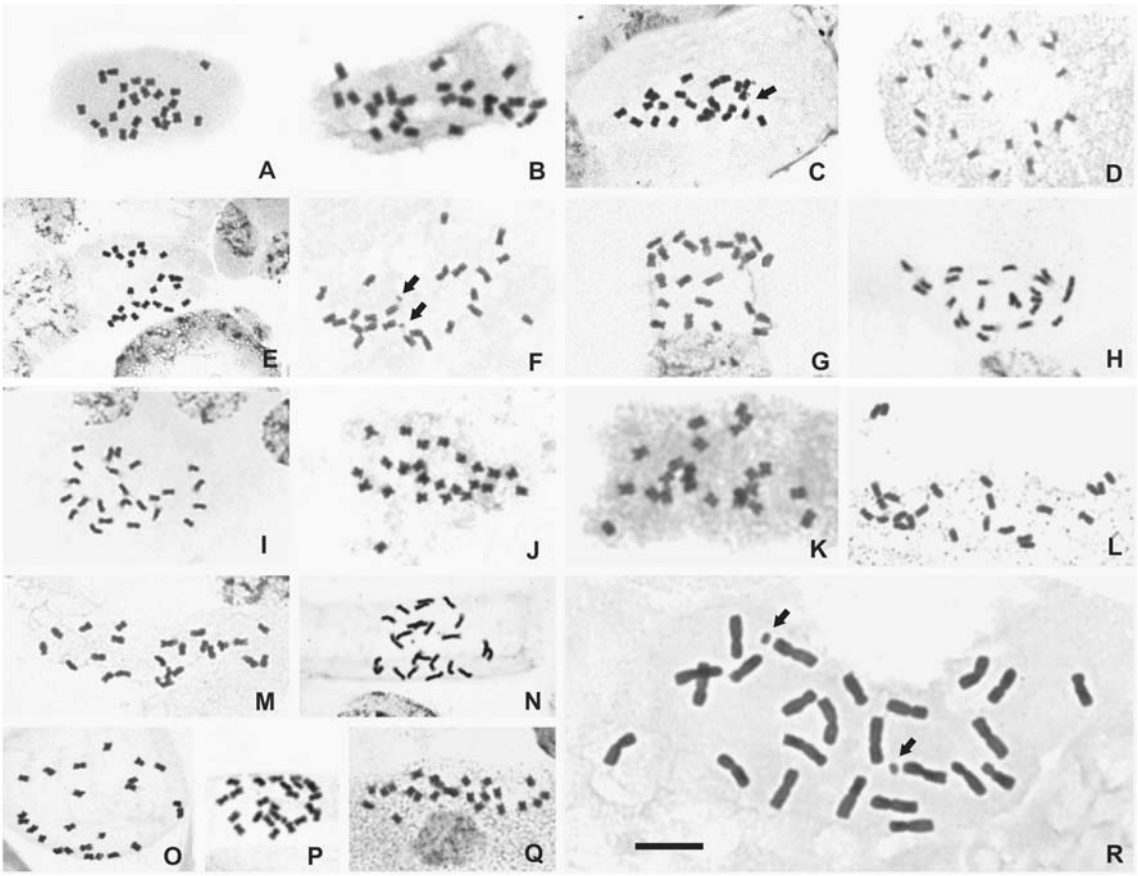

Fig. 1 Conventional staining in **a** *Solanum americanum*, **b** *S. gemellum*, **c** *S. atropurpureum*, **d** prometaphase of *S. viarum*, **e** *S. granuloso-leprosum*, **f** *S. palinacanthum*, **g** *S. pseudocapsicum*, **h** prometaphase of *S. scuticum*, **i** prometaphase of *S. variabile*, **j** *S. trachytrichium*, **k** *S. paniculatum*, **l** *S. mauritianum*, **m** *S. sisymbriifolium*, **n** prometaphase of *Vassobia breviflora*, **o** *S. guaraniticum*, **p** *S. amygdalifolium*, **q** *Lycianthes australe* and **r** *S. corymbiflorum*. *Arrows* indicate satellites. *Bar* = 10 µm

S. amygdalifolium Steud., with 8 m + 4 sm (Fig. 4f, g, p) and in *Vassobia breviflora* (Sendtn.) Hunz., with 9 m + 3 sm (Fig. 4r). In those species, the haploid set size varied from 13.08 to 25.87 µm (Table 1). Only three species were not grouped: *Solanum viarum* with 11 m + 1 sm and 18.14 µm of haploid set size, *S. americanum* Mill. with 10 m + 2 sm and 22.39 µm and *S. variabile* Mart. with only metacentrics and 20.50 µm (Fig. 4a, j, h, respectively; Table 1). *Solanum palinacanthum* Dunal and *S. gemellum* Sendtn. also stood out because they exhibited more submetacentrics (3 m + 9 sm, 5 m + 7 sm) and higher haploid set sizes with 27.90 and 25.74 µm (Fig. 4b, o, respectively; Table 1).

The chromosome banding showed four heterochromatin distribution types. In *Vassobia breviflora* (Figs. 2q, 4r) and in the subgenera *Leptostemonum* and *Solanum* (*S. pseudocapsicum*, *S. sisymbriifolium*, *S. americanum, S. guaraniticum* and *S. variabile*), terminal C-CMA_3^+ bands in the largest chromosomes and major blocks associated to satellites were found (Figs. 2e, f, i, l, m, 4k, d, j, f, g, h). In other species, as *S. atropurpureum* (Figs. 2a, 4c), *S. viarum* Dunal (Figs. 2b, 4a), *S. mauritianum* (Figs. 2c, 4n), *S. trachytrichium* (Figs. 2d, 4l), *S. granuloso-leprosum* (Figs. 2h, 4m), *S. amygdalifolium* (Figs. 2k, 4p), *S. gemellum* (Figs. 2p, 4o) and *Lycianthes australe* (Figs. 2r, 4q), only C-CMA_3^+ bands associated with satellites were observed. *Solanum paniculatum* and *S. palinacanthum,* besides of C-CMA_3^+ associated with satellites, exhibited proximal, terminal and interstitial dots (Figs. 2n, o, 4b, e). *Solanum corymbiflorum*, of the section *Pachyphylla* Dunal (subgenus *Bassovia* (Aubl.) Bitter), with the largest chromosomes, showed only two pairs with terminal C-CMA_3^+ bands (Figs. 2t, 4i). Except *Lycianthes australe* (Figs. 2s, 4q) which showed centromeric C-$DAPI^+$ bands, the remaining species did not present this heterochromatin type.

The double FISH with 45S and 5S rDNA probes always showed two terminal sites of 45S rDNA and two terminal or pericentromeric hybridization sites of 5S rDNA (Fig. 3). The exceptions were *Vassobia breviflora* (Figs. 3p, 4r) and *S. corymbiflorum* (Figs. 3r, 4i), which exhibited two pairs with terminal 45S rDNA sites and two signals of 5S rDNA.

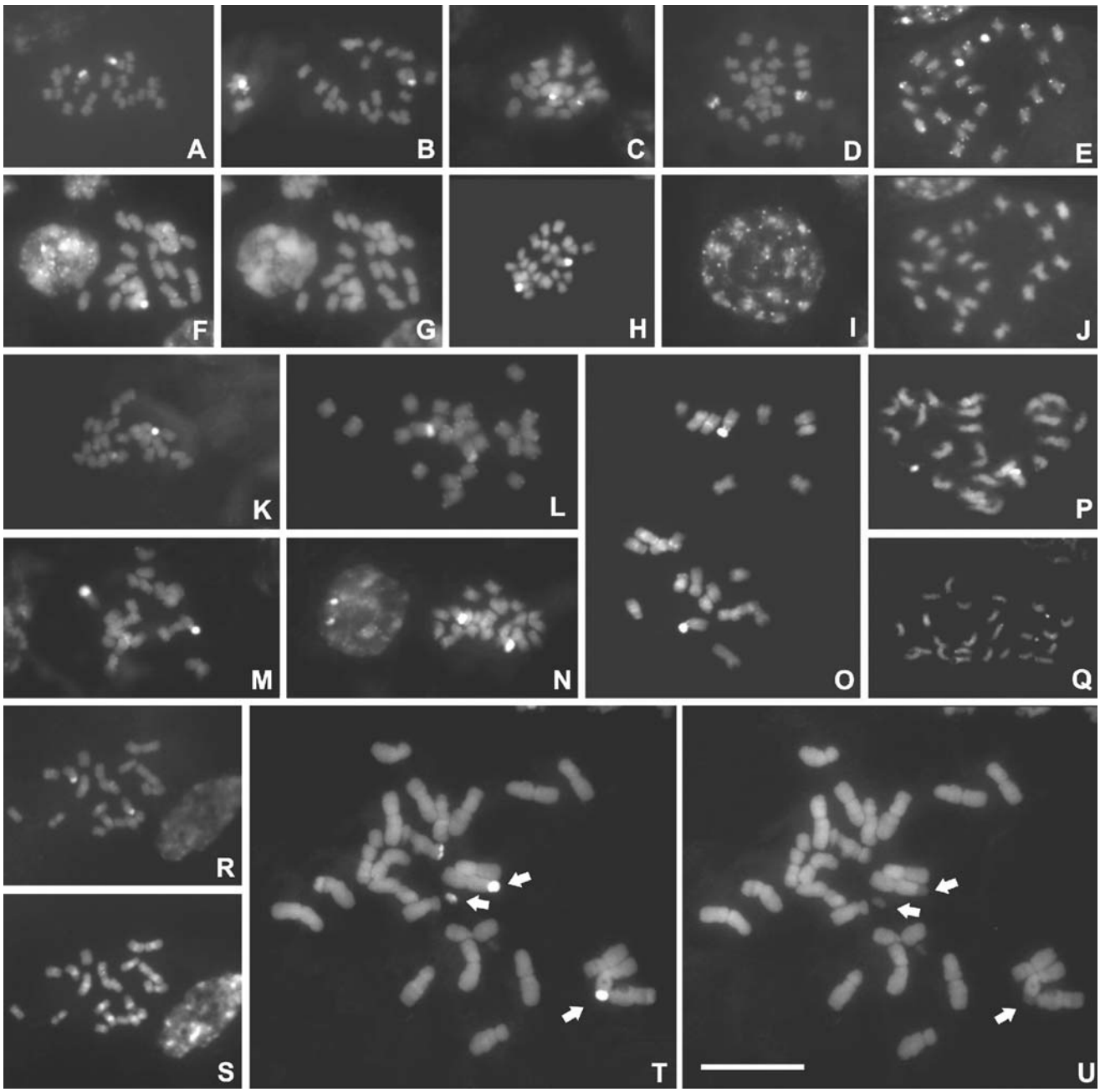

Fig. 2 Chromosome banding in species of *Solanum*, *Vassobia breviflora* and *Lycianthes australe*. C-CMA$_3$ banding in *Solanum artropurpureum* (**a**), *S. viarum* (**b**), *S. mauritianum* (**c**), *S. trachytrichium* (**d**), *S. pseudocapsicum* (**e**), *S. sisymbriifolium* (**f**), *S. granuloso-leprosum* (**h**), *S. americanum* (**i**), *S. amygdalifolium* (**k**), *S. guaraniticum* (**l**), *S. variabile* (**m**), *S. paniculatum* (**n**), *S. palinacanthum* (**o**), *S. gemellum* (**p**), *Vassobia breviflora* (**q**), *Lycianthes australe* (**r**), *S. corymbiflorum* (**t**). C-DAPI banding in *S. sisymbriifolium* (**g**), *S. pseudocapsicum* (**j**), *S. corymbiflorum* (**u**), see negative bands (*arrows*), *Lycianthes australe* (**s**), with centromeric C-DAPI positive bands. *Bar* = 10 µm

In all cases, the hybridization sites with 45S rDNA probes were coincident with satellites detected by C-CMA$_3^+$ banding (Fig. 4). However, the pair bearer of 45S rDNA varied among species. These sites were located in the pair ten in *Solanum* sections *Acanthophora*, *Pachyphylla* and *Brevantherum*, in the pair 11 at section *Torva* and *Vassobia breviflora* and in different pairs at the remaining groups (Fig. 4). The hybridization signals obtained with 5S rDNA probe were always located in the pair nine in *Solanum viarum, S. atropurpureum, S. palinacanthum*, *S. scuticum, S. guaraniticum, S. americanum* and *S. pseudocapsicum*. However, the pair nine was not constant in shape, being meta or submetacentric, and the 5S rDNA location was terminal or interstitial, independent of the chromosome

shape (Figs. 3a–e, g, i, 4a–c, f–g, j–k). The remaining species showed hybridization signals in different chromosome pairs, e.g., *Vassobia breviflora* in the pair six (Figs. 3p, 4r), *Solanum amygdalifolium* and *Lycianthes australe* in the pair seven (Figs. 3j, q, 4p, q), *S. paniculatum* and *S. corymbiflorum* in the pair eight (Figs. 3f, r, 4e, i), *S. sisymbriifolium* and *S. variabile* in the pair ten (Figs. 3h, k, 4d, h), *S. granuloso-leprosum* and *S. mauritianum* in the pair 12 (Figs. 3n, o, 4m, n). On the other hand, *Solanum trachytrichium* and *S. gemellum* exhibited four 5S rDNA hybridization signals. In the first one, in the interstitial position of the pair five and in the terminal position of the pair eight (Figs. 3l, 4l). In *Solanum gemellum,* in the interstitial position of the pair eight and in the terminal position of the pair ten (Figs. 3m, 4o).

Discussion

The chromosome number $2n = 24$ was found in all the studied species and is in accordance with those available at *Index to Plant Chromosome Numbers* (http://mobot.mobot.org/W3T/Search/ipcn.html), except to *Solanum scuticum*, *S. gemellum*, *S. amygdalifolium* and *Lycianthes*

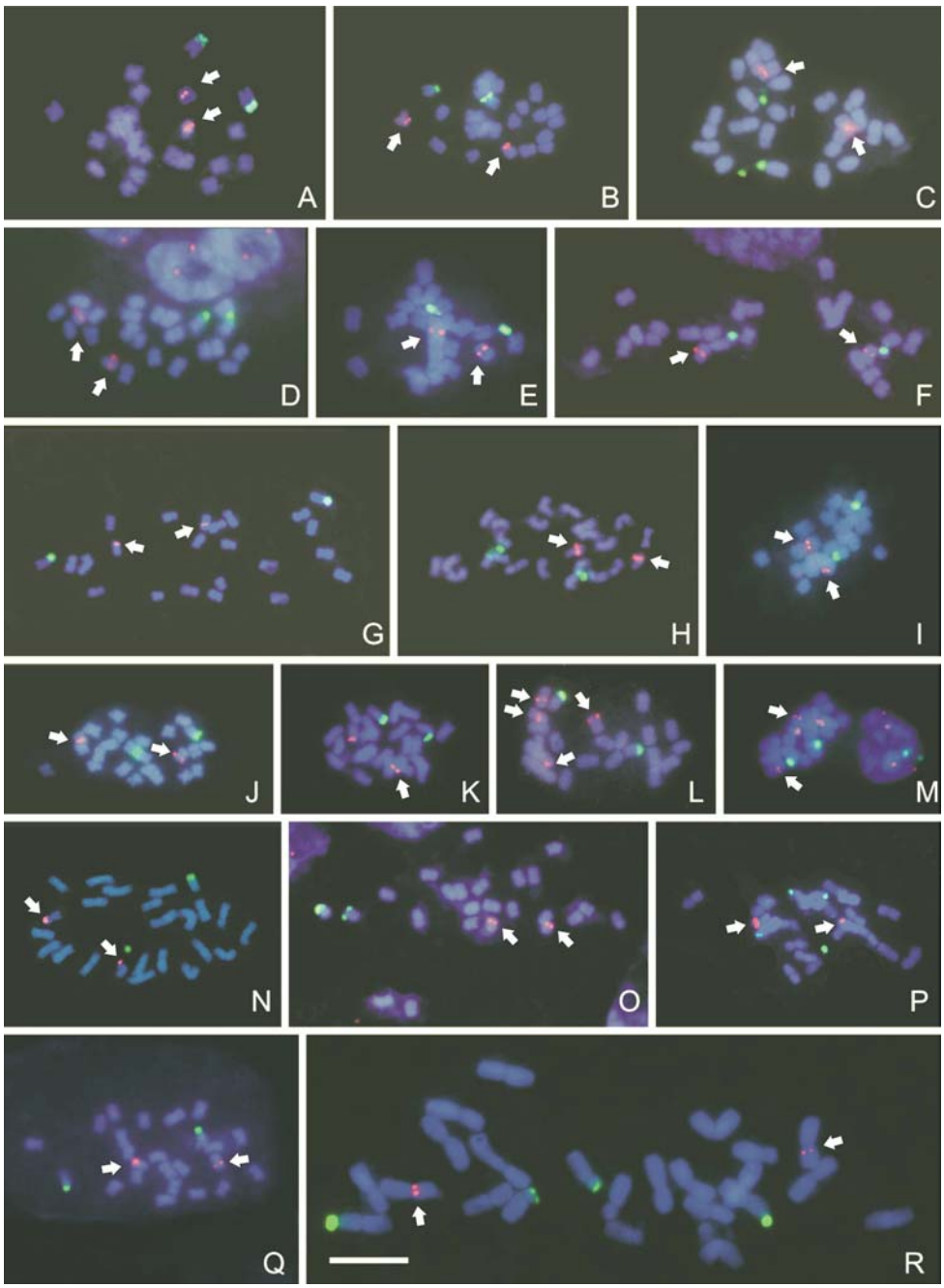

Fig. 3 Double FISH with rDNA probes in species of *Solanum*, *Vassobia breviflora* and *Lycianthes australe*. Note two and four 45S rDNA sites (the largest signals) always located in terminal regions of the chromosomes. *Arrows* indicate always two 5S rDNA hybridization signals in almost species, excepted for *S. trachytrichium* (l) and *S. gemellum* (m). In *S. gemellum* only two sites of 5S rDNA are indicate. FISH in *Solanum viarum* (**a**), *S. artropurpureum* (**b**), *S. palinacanthum* (**c**), *S. scuticum* (**d**), *S. guaraniticum* (**e**), *S. paniculatum* (**f**), *S. americanum* (**g**), *S. sisymbriifolium* (**h**), *S. pseudocapsicum* (**i**), *S. amygdalifolium* (**j**), *S. variabile* (**k**), *S. trachytrichium* (**l**), *S. gemellum* (**m**), *S. granuloso-leprosum* (**n**), *S. mauritianum* (**o**), *Vassobia breviflora* (**p**), *Lycianthes australe* (**q**) and *S. corymbiflorum* (**r**). *Bar* = 10 µm

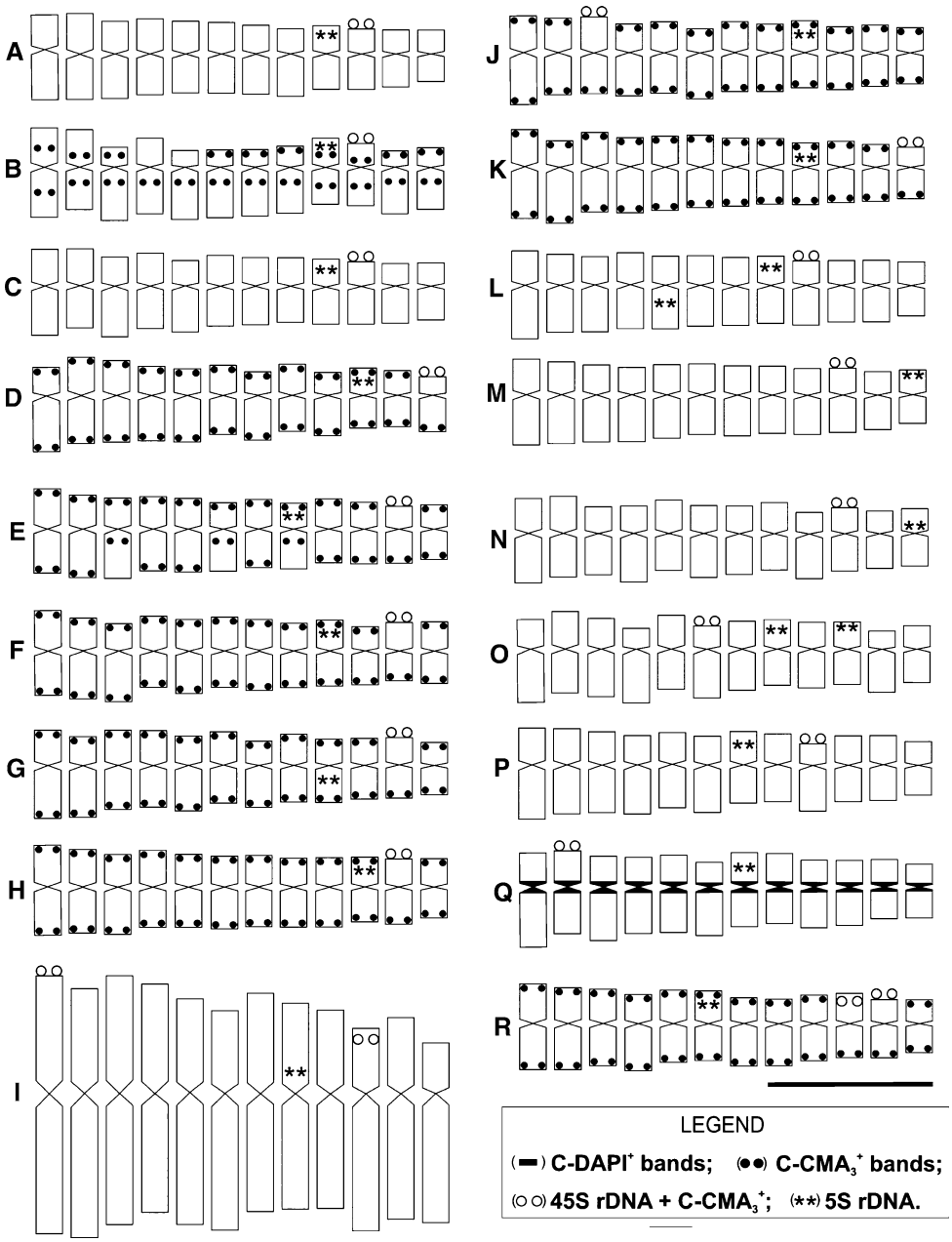

Fig. 4 Idiograms with the physical location of repetitive DNA segments in *Solanum* species, *Lycianthes australe* and *Vassobia breviflora*. The species of *Solanum* are organized according to Nee (1999). Subgenus *Leptostemonum* section *Acanthophora*: *Solanum viarum* (**a**), *S. palinacanthum* (**b**), *S. artropurpureum* (**c**). Section *Melongena*: *S. sisymbriifolium* (**d**). Section *Torva*: *S. paniculatum* (**e**), *S. guaraniticum* (**f**), *S. scuticum* (**g**), *S. variabile* (**h**). Subgenus *Bassovia* section *Pachyphylla*: *S. corymbiflorum* (**i**). Subgenus *Solanum* section *Solanum*: *S. americanum* (**j**). Section *Geminata*: *S. pseudocapsicum* (**k**), *S. trachytrichium* (**l**): Section *Brevantherum*: *S. granuloso-leprosum* (**m**), *S. mauritianum* (**n**). Section *Extensum*: *S. gemellum* (**o**). Section *Dulcamara*: *S. amygdalifolium* (**p**). *Lycianthes australe* (**q**) and *Vassobia breviflora* (**r**). *Bar* = 10 μm

australe, which are new records. About 50% of the species of Solanaceae present chromosome numbers multiple of $x = 12$, which is considered the basic chromosome number (Hunziker 2001). This number is predominant in the subfamily Solanoideae, in the genera *Triguera*, *Lycopersicon*, *Lycianthes* and *Vassobia* (Hunziker 2001), as well as in *Solanum* (Olmstead et al. 1999; Hunziker 2001; Acosta et al. 2005). Only two species of the section *Acanthophora* showed $2n = 22$, *Solanum mammosum* L. and *S. platense* Dieckman (Chiarini and Bernardello 2006). Numbers different from $x = 12$ have been reported in other groups, as in the subfamily Cestroideae (cf. Hunziker 2001).

The conventional analysis also showed invariably non-reticulated nuclei and proximal condensation at prophase. This is in accordance with Guerra (2000) that suggests that small chromosomes (<3 μm) are associated with these features. In relation to chromosome sizes, our results showed that except *S. corymbiflorum*, whose haploid set size was 77.8 μm, the remaining species presented small chromosomes with haploid set size varying from 13.08 to 27.9 μm, about three times smaller than in *S. corymbiflorum*. A wide variation in the haploid complement size of *Solanum* and *Lycianthes* (from 16.30 to 38.75 μm) has been also reported by Bernardello and Anderson (1990), Bernardello et al. (1994), Acosta et al. (2005) and Chiarini

and Bernardello (2006). All the species studied here exhibited predominance of meta and submetacentric chromosomes, with the smallest being always submetacentric, except *Solanum variabile* and *S. americanum* that showed the smallest metacentric. The tendency of chromosome size reduction accompanied by a change in the centromere position was also observed in the idiograms reported by Bernardello and Anderson (1990), Pringle and Murray (1991), Bernardello et al. (1994), Acosta et al. (2005) and Chiarini and Bernardello (2006). However, our results showed that there is no correlation between karyotype features and groupings and section of Nee (1999).

Differences on the chromosome size have been useful to delimit some groups in Solanaceae. The section *Pachyphylla* of *Solanum* possess larger chromosomes in relation to other groups of the genus. Besides, some morphological features, such as anthers with thickened and glandular connectives, were also considered important to maintain this group as an independent genus, *Cyphomandra* (Bohs 1989, 1994; Hunziker 2001; Child and Lester 2001). However, phylogenetical analysis based in the sequence of conserved chloroplast genes, *ndh*F and *rbc*L, focusing *Solanum* and closely related genera, indicate that *Cyphomandra* can be included in *Solanum* (Bohs 1995; Olmstead and Palmer 1997; Bohs and Olmstead 1997, 1999; Olmstead et al. 1999; Bohs 2004, 2005). *Solanum corymbiflorum*, previously named *Cyphomandra corymbiflora* Sendtn. exhibits much bigger chromosomes and the largest haploid set size. The conventional cytogenetic analysis indicate that this species can be maintained in a genus separated of *Solanum* (*Cyphomandra*), as proposed by Child and Lester (2001), Moscone (1992) and Hunziker (2001). If *Cyphomandra* is included in *Solanum*, as pointed out by the above mentioned phylogenetic studies, therefore we will have to admit that the karyotypes of the section *Pachyphylla* become differentiated from the other species of *Solanum* by a substantial DNA accumulation, without important changes in the chromosome number and shape (see Fig. 4i).

The location of 45S ribosomal genes follows the common distribution pattern found in most plant species, always occurring in the terminal chromosome regions. This has been also reported in other genera of Solanaceae as *Solanum bulbocastanum* (Dong et al. 2000), *Capsicum* (Moscone et al. 1995), *Nicotiana* (Lim et al. 2000), and *Cestrum* (Fregonezi et al. 2006). In spite of the constancy of 45S rDNA location, the number of sites was variable, since in *S. corymbiflorum* and *Vassobia breviflora* four sites were observed. However, this is not an innovation in Solanaceae, as long as the number of hybridization sites in *Cestrum* using of the same probe also varied (Fregonezi et al. 2006). FISH with 5S rDNA probe showed that in almost all cases the signals appear in the short arm of the smallest chromosomes of the complement, except *S. trachytrichium* and *S. scuticum* where signals in the large arm were found. *Solanum trachytrichium* and *S. gemellum* exhibited four hybridization sites and the remaining species two signals of 5S rDNA. In all cases, the signals appeared in the smaller chromosomes of the set. On the other hand, Dong et al. (2000) showed paracentromeric 5S rDNA sites in the major chromosome of the set in *S. bulbocastanum*. In other genera, as *Nicotiana* (Lim et al. 2000; Kitamura et al. 2001), *Lycopersicon* (Lapitan et al. 1991) and *Cestrum* (Fregonezi et al. 2006) the 5S rDNA were also located in the paracentromeric region. These data suggest that the 45S and 5S rDNA segments do not present a conserved location and number of sites among species of *Solanum* and other Solanaceae. This feature seems to be common to other plant groups, e.g., Sapindaceae (Urdampilleta et al. 2006).

The C-banding followed by CMA_3/DAPI staining, as described by Vanzela and Guerra (2000), was useful to determinate different heterochromatin type distribution (see also Fregonezi et al. 2006). The different bands can be grouped in: (1) terminal C-CMA_3^+ bands in all the chromosomes, besides a block associated to satellites, (2) only C-CMA_3^+ bands associated to satellites, (3) proximal, terminal and interstitial C-CMA_3^+ dots, besides those C-CMA_3^+ associated to satellites, and (4) centromeric C-$DAPI^+$ bands. The presence of C-CMA_3^+ terminal dots can approximate species of distinct groups, as *S. sisymbriifolium, S. paniculatum, S. guaraniticum, S. scuticum, S. variabile* (sections *Melongena* and *Torva*), *S. americanum* (section *Solanum*), *S. pseudocapsicum* (section *Geminata*) and *Vassobia breviflora*. The occurrence of interstitial dots in *S. palinacanthum* indicates that the section *Acanthophora* is apparently more diverse. Fregonezi et al. (2006) studied species of *Cestrum* and detected different repetitive DNA families (dots), occupying different equilocal/equidistant regions, as well as observed in *Solanum* and *Vassobia* studied here. A similar behavior of bands distribution was reported to *Solanum* section *Petota* (Pijnaker and Ferwerda 1984), *Capsicum* (Moscone et al. 1993) and *Lycopersicon* (Peterson et al. 1996).

To conclude, the results of chromosome banding and FISH with rDNA probes show that they can be useful to characterize species and possibly populations and that the cytogenetical tools can complement the phylogenetical studies. We considered here the karyotypes of *Vassobia breviflora* and *Lycianthes australe* as external members for comparison with *Solanum*. *Vassobia breviflora* presents anthers with longitudinal dehiscence and ovary with a nectary, and possess karyotype features similar to species of subgenera *Melongena, Torva* and *Solanum*. *Lycianthes* and *Solanum* are considered separate genera, in spite of

possessing similar morphologic features as poricidal anthers and ovary without a nectary (Hunziker 2001). They also share similar cytogenetic features, as $2n = 24$, non-reticulated nuclei, proximal condensation at prophase, small chromosomes, similar karyotype structure and occurrence of two terminal 45S rDNA sites. If the same comparisons are made between *Cyphomandra* and *Solanum*, where there are more morphological differences and cytogenetical contrasts that in *Solanum* and *Lycianthes* (in spite of the occurrence of proximal C-DAPI bands in *Lycianthes*), therefore *Cyphomandra* should be also maintained separated of *Solanum*, as *Lycianthes* is. In spite of the great importance and contributions of the molecular phylogeny studies, we have doubts about the sustentation of the recent arrangement in *Solanum*, which is based on only a few chloroplast genes. We consider that molecular data should be applied along with other tools, including morphological, anatomical, chemical and cytogenetical information.

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