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Original Paper
Karyotypic analyses and genomic affinity among Argentinean species of *Passiflora*

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Abstract

The genus *Passiflora* (*Passifloraceae*) has more than 500 species, nineteen are native to Argentina. By their austral distribution, these species were included in breeding programs to obtain ornamental varieties tolerant to low temperatures. The aim of this work was to know the cytogenetic characteristics of *Passiflora* genotypes present in a working collection, as an indispensable knowledge for the development of a breeding plan. Chromosomal preparations were performed and karyotypic characteristics, rDNA sites by FISH and affinity among subgenera by GISH were studied. Chromosome counts in fourteen Argentinean species confirmed the basic chromosome numbers previously published: x = 6 for subgenus *Decaloba*, x = 9 for subgenus *Passiflora* and x = 10 for *Dysosmia*. The karyotypic parameters (karyotype, haploid chromosome length and asymmetry indices) and genomic affinities among the subgenera, clarify most of the chromosomal evolution of the genus. The results obtained strongly suggest that the basic number x = 6 would be the original one and that x = 9 was originated by processes of polyploidy and descendent dysploidy. Since subgenus *Passiflora* possesses the largest genomes, it is postulated that evolutionary process leading to x = 9 was accompanied by unequal distribution of non-coding repetitive DNA, mainly transposable elements. These processes could explain the asymmetrical karyotypes of species of subgenus *Passiflora*.

Key words: chromosome numbers, *in situ* hybridization, karyotypes, native *Passiflora* to Argentina, ornamental plant breeding.

Resumen

El género *Passiflora* (*Passifloraceae*) cuenta con más de 500 especies, diecinueve de ellas son nativas de Argentina. Por su distribución austral, estas especies fueron incluidas en un plan de mejoramiento para obtener variedades ornamentales tolerantes a bajas temperaturas. El objetivo de este trabajo fue conocer las características citogenéticas de los genotipos de *Passiflora* en la colección de trabajo como conocimiento indispensable para el desarrollo de un plan de mejoramiento. Se realizaron preparados cromosómicos y se estudiaron las características cariotípicas, regiones ADNr por FISH y afinidad genómica entre subgéneros por GISH. Los recuentos cromosómicos realizados en catorce especies argentinas confirmaron los previamente publicados: x = 6 para el subgénero *Decaloba*, x = 9 para el subgénero *Passiflora* y x = 10 para el subgénero *Dysosmia*. Los parámetros cariotípicos (cariotipos, longitud del genoma haploide e índices de asimetría) y las afinidades genómicas analizadas por GISH entre las especies de los tres subgéneros, clarifican la mayor parte de la evolución cromosómica del género. Los resultados obtenidos sugieren que el número básico x = 6 sería el original y que x = 9 se habría originado por procesos de poliploidía y disploidía descendente. Debido a que las especies del subgénero *Passiflora* poseen los genomas más largos, se postula que el proceso evolutivo que llevó de x = 10 a x = 9 fue acompañado por una distribución desigual en los brazos cromosómicos de ADN repetitivo no codificante, principalmente elementos transponibles. Estos procesos explicarían la asimetría cariotípica del subgénero *Passiflora*.

Palabras clave: números cromosómicos, hibridación *in situ*, cariotipos, especies de *Passiflora* nativas de Argentina, mejoramiento genético de ornamentales.

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Introduction

The genus *Passiflora* L. (*Passifloraceae*) has more than 500 species; most of this diversity is found in South America. Fruits of some *Passiflora* species are edible, such as passion fruit (*P. edulis f. flavicarpa* O. Deg.), others species have medicinal properties (*P. incarnata* L.) and many of them are cultivated for their ornamental characteristics (*P. alata* Curtis). The versatility of the species of this genus highlights their potential as source of traits for plant breeding.

There are nineteen species of *Passiflora* reported as native to Argentina (Deginani 2001). Due to their taxonomic characteristics, the Argentinean species were divided in four subgenera: *Passiflora* (11 species), *Tacsonioides* (1), *Dysosmia* (2) and *Decaloba* (5) (Deginani 2001). The austral distribution of the Argentinean taxa, postulates them as tolerant to temperate environments, unlike most species of the genus that prefer tropical climates. For this reason, fourteen of these species present in the collection of the Institute of Floriculture (INTA, Buenos Aires) were included in a breeding program that aim to obtain ornamental varieties tolerant to low temperatures by means of interspecific hybridization (Bugallo *et al*. 2011). So far, hybrids were only obtained among some of the *Passiflora* subgenera species while none of the inter-subgenera crossings tested thrived (unpublished data).

The basic chromosome numbers reported for the subgenera were x = 9 for *Passiflora* and *Tacsonioides*, x = 10 for *Dysosmia* and x = 6 for *Decaloba* (Souza *et al*. 2008; Melo *et al*. 2001). The study of the variability of the DNAr sites that more quantity of Argentine species approached was the one of Melo & Guerra (2003), where ten of the species were included. According to phylogenetic studies, the basic numbers would support the monophyly of each subgenus (Muschner *et al*. 2003). Despite the morphological and cytogenetic differences, the molecular studies indicate that subgenera *Passiflora* and *Dysosmia* would be more related with each other and less with subgenus *Decaloba* (Sader *et al*. 2019; Muschner *et al*. 2003).

The aim of this work was to know the cytogenetic characteristics of the *Passiflora* species present in the working collection of the Institute of Floriculture (INTA - National Institute of Agricultural Technology, Argentina), as an indispensable knowledge for the development of a breeding plan to obtain tolerant to low temperatures plants. The breeding plan in *Passiflora* is included in a bigger project that aim to obtain ornamental varieties from native species to Argentina. For this reason, cytogenetic characterization of the 49 genotypes belonging to fourteen Argentinean *Passiflora* species, including fluorescent chromosome staining (DAPI) and *in situ* hybridization (FISH), using rDNA 18S and 5S as probes were performed. The chromosome counts and karyotypes provided new data and corroborated existing information. Furthermore, we explore the genomic affinity among the three subgenera by means of GISH assays, unsuccessfully inter-hybridized until now.

Materials and Methods

Plant material

For the cytological studies, the 49 genotypes of fourteen species of *Passiflora* present in the Institute of Floriculture (INTA - National Institute of Agricultural Technology, Argentina). These plants were the same used in the breeding program to obtain ornamentals from native species. The genotypes and their origin sites were listed in Table 1.

Chromosomal preparations

Mitotic metaphase preparations were performed in the fourteen species (Tab. 1). Fixed root tips were treated with an enzymatic solution (2% cellulase Onozuka R10 and 20% Pectinase) for 1 h at 37 °C. Slides with well-spread metaphases were selected by contrast-phase microscopy. The coverslips were removed by freezing on dry ice and the slides were air-dried and stored at 4 °C until use.

DAPI staining (4′,6-Diamidino-2-phenylindole)

The DAPI staining technique was applied, according to Sumner (1990). Slides were washed in McIlvaine buffer (citric acid-NaHPO buffer, pH 7), and then stained with 2 µg/ml DAPI. Slides were incubated in a moist box at 20 °C in the dark for 25 min. After staining, preparations were briefly washed, and slides were mounted in McIlvaine buffer pH 7 and sealed with rubber solution.

Karyotype analysis

For the chromosome characterization, the measurement on the microphotographs of the DAPI stained chromosome preparations were performed by means of MicroMeasure sofware

Table 1 – *Passiflora* species of the Institute of Floriculture collection, voucher numbers and collection sites.

Subgenus	Species	Code	Site of colection
Passiflora	*Passiflora alata* Curtis	20041119A1	Donation
Passiflora	*Passiflora alata* Curtis	20050814B1	San Justo, Buenos Aires
Passiflora	*Passiflora alata* Curtis	20100131A1	Puerto Madero, Ciudad de Buenos Aires
Passiflora	*Passiflora alata* Curtis	20140606A1	Famaillá, Tucumán
Passiflora	*Passiflora amethystina* JC Mikan	20050707A7	Donation
Passiflora	*Passiflora caerulea* L.	20050318A1	Guayquiraró, Corrientes
Passiflora	*Passiflora caerulea* L.	20051202C1	Cerro Azul, Misiones
Passiflora	*Passiflora caerulea* L.	20051202G1	Cerro Corá, Misiones
Passiflora	*Passiflora caerulea* L.	20071124A1	Chañar Ladeado, Santa Fe
Passiflora	*Passiflora caerulea* L.	20071124A2	Chañar Ladeado, Santa Fe
Passiflora	*Passiflora caerulea* L.	20071124A4	Chañar Ladeado, Santa Fe
Passiflora	*Passiflora caerulea* L.	20071124A9	Chañar Ladeado, Santa Fe
Passiflora	*Passiflora caerulea* L.	20071124A8	Chañar Ladeado, Santa Fe
Passiflora	*Passiflora caerulea* L.	20091012J4	San Martín, Corrientes
Passiflora	*Passiflora caerulea* L.	05-101	Valle de Lerma, Salta
Passiflora	*Passiflora caerulea* L.	05-102	Salta capital, Salta
Passiflora	*Passiflora caerulea* L.	05-43	Castelar, Buenos Aires
Passiflora	*Passiflora caerulea* L.	20050608A2	INTA Castelar, Buenos Aires
Passiflora	*Passiflora caerulea* L.	20050608A1	INTA Castelar, Buenos Aires
Passiflora	*Passiflora capsularis* L.	20081101A2	San Pedro, Misiones
Passiflora	*Passiflora cincinnata* Mast.	05-46#1	Donation
Passiflora	*Passiflora cincinnata* Mast.	05-46#2	Donation
Passiflora	*Passiflora edulis f. flavicarpa* O. Deg.	20050707A3	Donation
Passiflora	*Passiflora edulis f. flavicarpa* O. Deg.	12-169	Donation
Passiflora	*Passiflora edulis f. flavicarpa* O. Deg.	12-170	Donation
Passiflora	*Passiflora edulis f. flavicarpa* O. Deg.	07-247	Donation
Passiflora	*Passiflora edulis f. flavicarpa* O. Deg.	12-357	Donation
Passiflora	*Passiflora edulis f. flavicarpa* O. Deg.	12-391	Donation
Passiflora	*Passiflora elegans* Mast.	06-618	Santo Tomé, Corrientes
Passiflora	*Passiflora elegans* Mast.	06-619#1	Santo Tomé, Corrientes
Passiflora	*Passiflora elegans* Mast.	06-619#2	Santo Tomé, Corrientes
Passiflora	*Passiflora elegans* Mast.	06-619#3	Santo Tomé, Corrientes
Passiflora	*Passiflora elegans* Mast.	10-461	IF-INTA (06-619#3 open pollination)
Dysosmia	*Passiflora foetida* L.	20070126A6	Matacos, Formosa
Dysosmia	*Passiflora foetida* L.	20070126B4	Bermejo, Formosa
Decaloba	*Passiflora misera* Kunth.	06-83	Federación, Entre Ríos
Decaloba	*Passiflora misera* Kunth.	20061129B1	Santo Tomé, Corrientes
Decaloba	*Passiflora misera* Kunth.	20061129E2	Santo Tomé, Corrientes

Subgenus	Species	Code	Site of colection
Passiflora	*Passiflora mooreana* Hook.	20061201A1	Mercedes, Corrientes
Passiflora	*Passiflora mooreana* Hook.	20070125B1	Matacos, Formosa
Decaloba	*Passiflora morifolia* Mast.	20080411E1	Ledesma, Jujuy
Decaloba	*Passiflora suberosa* L.	06-477#1	IF-INTA
Decaloba	*Passiflora suberosa* L.	06-477#2	IF-INTA
Decaloba	*Passiflora suberosa* L.	20050707A4	Donation
Decaloba	*Passiflora suberosa* L.	20091209C1	San Javier, Santa Fe
Passiflora	*Passiflora tenuifila* Killip	20140423H1	Donation
Passiflora	*Passiflora tucumanensis* Hook.	20090922A1	Pucará, Jujuy
Passiflora	*Passiflora tucumanensis* Hook.	20110203B1	Belén, Catamarca
Passiflora	*Passiflora tucumanensis* Hook.	20110204A1	Andalgalá, Catamarca

version 3.3 (<www.colostate.edu/Depts/Biology/MicroMeasure>). The haploid chromosome length (HCL) for each studied species was calculated as the mean length of the chromosomes in a cell of at least five cells and then was divided by the ploidy level. The nomenclature used for chromosome morphology is that of Levan *et al.* (1964). For the analyses, we used two intrachromosomal asymmetry indices: A_1 [A1 = 1-(short arm/long arm)/n] (Romero Zarco 1986) and MCA [MCA = (long arm - short arm)/(long arm + short arm)*100] (Peruzzi & Eroğlu 2013). Also, two coefficients of variation were studied: CV of centromeric index (CVci) [CVci = standard deviation of the centromeric index/mean centromeric index x 100] and CV of the chromosome length (CVcl) [CVcl = standard deviation of the chromosome length/mean chromosome length x 100] (Paszco 2006). Mean values of the karyotypes were calculated from measurements of a minimum of five scattered metaphase plates in each accession. The idiograms for each species were constructed using Microsoft Power Point 2016. The relationships between HCL and asymmetry indices were analyzed by means of linear regressions and ANOVA tests with BSS comparisons.

FISH DNA probes

For FISH analysis, rDNA 5S and 18S sequences were isolated and amplified from total genomic DNA of *P. edulis f. flavicarpa* by polymerase chain reaction (PCR). The 5s primer sequences were designed according to Coelho *et al.* (2016)

S: 3' GTGCGATCATACCAGCAGCTTAA TGCACCGG 5'-

A: 5' GAGGTGCAACACGAGGACTTCC CAGGAGG 3'.

The 18S primers were designed according to the maize sequence of 18S rDNA (Realini 2017), S: 3' GAACCTACACCATCGGCAAA 5'- A: 5' TCATTACTCCGATCCCGAAG 3'.

The probes were biotin and digoxigenin-labelled by PCR, using fluorescently conjugated nucleotide 16-biotin dUTP (Sigma) and 11-digoxigenin-dUTP (Roche).

GISH DNA probes

To test the genomic affinity among the plants in the collection GISH assays were performed among species of the three subgenera available for breeding of genus *Passiflora*. To improve resolution of GISH technique, genomic DNA probe from species with the smaller chromosomes (subgenus *Decaloba* and *Dysosmia*) were hybridized on mitotic preparations of species with larger chromosomes (subgenus *Passiflora*). For the GISH trials, *P. suberosa* was selected for having an intermediate amount of DNA for the group according to Sader *et al*. (2019). To represent subgenus *Dysosmia*, DNA of the only available species of that group in the collection, *P. foetida*, was used as probe. These probes were tested on mitotic preparations of *P. amethystina*, *P. caerulea* and *P. edulis* which have a mean DNA size for subgenus *Passiflora*. Total genomic DNA from young leaves from species was isolated using a Wizard Genomic DNA Purification kit (Promega) and biotin labelled using the BioNick DNA Labeling System, (Thermo Fisher Scientific) according to manufacturer instructions.

In situ hybridization

For the *in situ* hybridization procedure, slide preparations were incubated in 100 µg mL-1 of RNAse in 2× saline sodium citrate (2 × SSC) for 1 h at 37 ºC and washed in 2 × SSC at room temperature. The slides were post-fixed in 4 % (w/v) paraformaldehyde for 10 min and washed in 2 × SSC. The preparations were dehydrated in a graded ethanol series and air-dried. For FISH assays, each slide was subjected to 30 µL of the hybridization mixture (15 µl of formamide, 6 µl of dextran sulfate, 3 µl of 2XSSC buffer, Sodium Dodecyl Sulfate-SDS 10% and 1 ul of salmon sperm DNA) with 50 ng of each labelled probe.

For GISH experiments, 45 ng of labeled genomic DNA (probe) and 450 ng of blocking DNA were dissolving them together in hybridization mixture according to Melo *et al*. (2015).

The hybridization mixtures were denatured for 15 min at 75 ºC. The slides were placed on a thermocycler at 75 ºC for 7 min, 45 ºC for 10 min and 38 ºC for 10 min, and then incubated overnight at 37 ºC. Following hybridization, the slides were incubated in the detection buffer containing a solution of 2.5 % bovine serum albumin and the corresponding detection antibody Cy3 conjugate or antidigoxigenin- fluorescein isothiocyanate (FITC) for 1 h at 37 ºC and washed in 4× SSC/Tween buffer. Slides were counterstained with 1 µg mL-1 of DAPI in 4× SSC/Tween buffer for 40 min at room temperature and then mounted in VectaShield solution (Vector Lab). Slides were examined with a Carl Zeiss Axiophot epifluorescence microscope with appropriate Carl Zeiss filters coupled with a Leica DC 250 digital camera and with an image analyzer Leica IM 1000. The location of hybridization signals were based on the observation of at least 5 complete metaphases for each analyzed species.

Results

Karyotypes and idiograms

Chromosomal counts were performed for 49 genotypes corresponding to 14 Argentinean *Passiflora* species (Tab. 1). The mitotic chromosomes morphology, rDNA chromosome position and representative idiograms for each species of *Passiflora* are presented in Figures 1 and 2. All the studied species showed intra-karyotypic variation in the chromosome length (Tab. 2).

Chromosome counts in subgenus *Decaloba* species were 2n = 12 for *P. capsularis*, *P. misera* and *P. morifolia*, and 2n = 24 in *P. suberosa*. In the species of subgenus *Passiflora*, the somatic numbers were 2n = 18 for *P. alata*, *P. amethystina*, *P. caerulea*, *P. cincinnata*, *P. edulis*, *P. elegans*, *P. tenuifila* and *P. tucumanensis*, 2n =18 and 2n = 36 for *P. mooreana*, while *P. foetida* from subgenus *Dysosmia* presented 2n = 20 (Fig. 1).

The joint analysis of the species of genus *Passiflora* showed a range of chromosome sizes (Fig. 2), from a maximum values of 8.9 ± 1.1 µm (chromosome 1 of *P. alata*) to a minimum value of 1.0 ± 0.2 µm (chromosome 10 of *P. foetida*) (Tab. 2). The haploid chromosome length (HCL) values ranged from 57.95 ± 3.68 µm in *P. alata* to 10.61 ± 1.24 µm in *P. capsularis* (Tab. 3).

In subgenus *Passiflora*, all of the studied species exhibited diploid levels, with somatic numbers 2n = 2x = 18 while in *P. mooreana*, diploid and tetraploid individuals (2n = 4x = 36) were detected. Total haploid chromosome lengths (HCL) in this subgenus varies between 57.95 ± 3.68 µm and 25.32 ± 3.10 µm, in *P. alata* and *P. tucumanensis*, respectively. All chromosomes were metacentric, except for submetacentric chromosome 1 of *P. mooreana* and chromosome 6 of *P. amethystine* (Fig. 2). The mean values of intrachromosomal asymmetry indexes A1 calculated for the subgenus were between 0.30 ± 0.04 for *P. mooreana*, and 0.19 ± 0.03 for *P. caerulea*. Likewise, the mean values of the centromeric asymmetry indexes M_{CA}, were between 0.19 ± 0.03 and 0.11 ± 0.02, respectively. The coefficient of variation of chromosome length CVcl was between 13.77 in *P. caerulea* to 20.44 in *P. alata*, while the coefficient of variation of centromeric index CVci was between 3.88 in *P. tucumanensis* and 6.64 in *P. mooreana* (Tab. 3).

In species of the subgenus *Decaloba,* haploid genome length ranged from 17.67 ± 3.09 µm for *P. morifolia* to 10.61 ± 1.24 µm for *P. capsularis*. In this subgenus, the mean values of A1 indexes were between 0.39 ± 0.05 for *P. suberosa* and 0.32 ± 0.07 for *P. morifolia*. M_{CA} indexes ranged from 0.27 ± 0.04 for *P. suberosa*, and 0.20 ± 0.04 for *P. capsularis* and *P. morifolia*. For subgenus *Decaloba*, values of CVcl were between 25.48 and 40.58 while those of CVci were between 12.80 and 5.78 (Tab. 3).

The only studied species of the subgenus *Dysosmia*, *P. foetida* (2n = 2x = 20) showed a length of the basic chromosomal complement of 14.71 ± 3.08 µm. All chromosomes were metacentric except for the submetacentric chromosome 4. A1 index was 0.29 ± 0.04, MCA was 0.18 ± 0.03, CVcl was 26.03 and CVci 6.75 (Tab. 3).

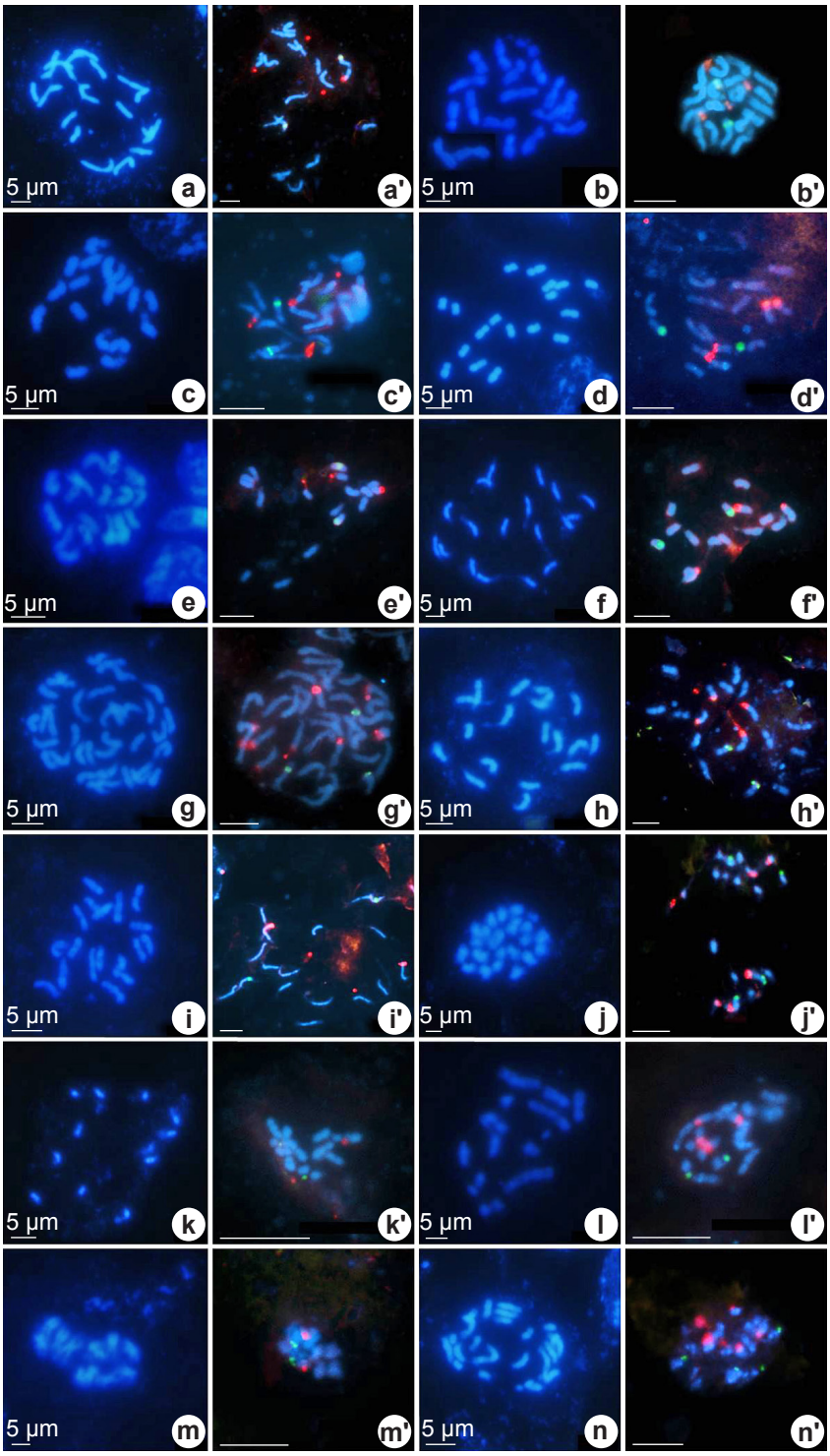

Figure 1 – a-n. Mitotic chromosomes of species of *Passiflora*. Dapi counterstain (blue), 18s sites biotin-Cy3 labelled (red) and 5s sites digoxigenin-antidigoxigenin-FITC labelled (green) – a. *P. alata*, b. *P. amethystina*, c. *P. caerulea*, d. *P. cincinnata*, e. *P. edulis f. flavicarpa* and f. *P. elegans* with 2n = 2x = 18; g. *P. mooreana* with 2n = 4x = 36; h. *P. tenuifila*, i. *P. tucumanensis* with 2n = 2x = 18; j. *P. foetida* 2n = 2x = 20; k. *P. capsularis*, l. *P. misera* and m. *P. morifolia* with 2n = 2x = 12; n. *P. suberosa* with 2n = 4x = 24. Letters without apostrophe indicates DAPI stain, with apostrophe indicates FISH. Scale bars: µm.

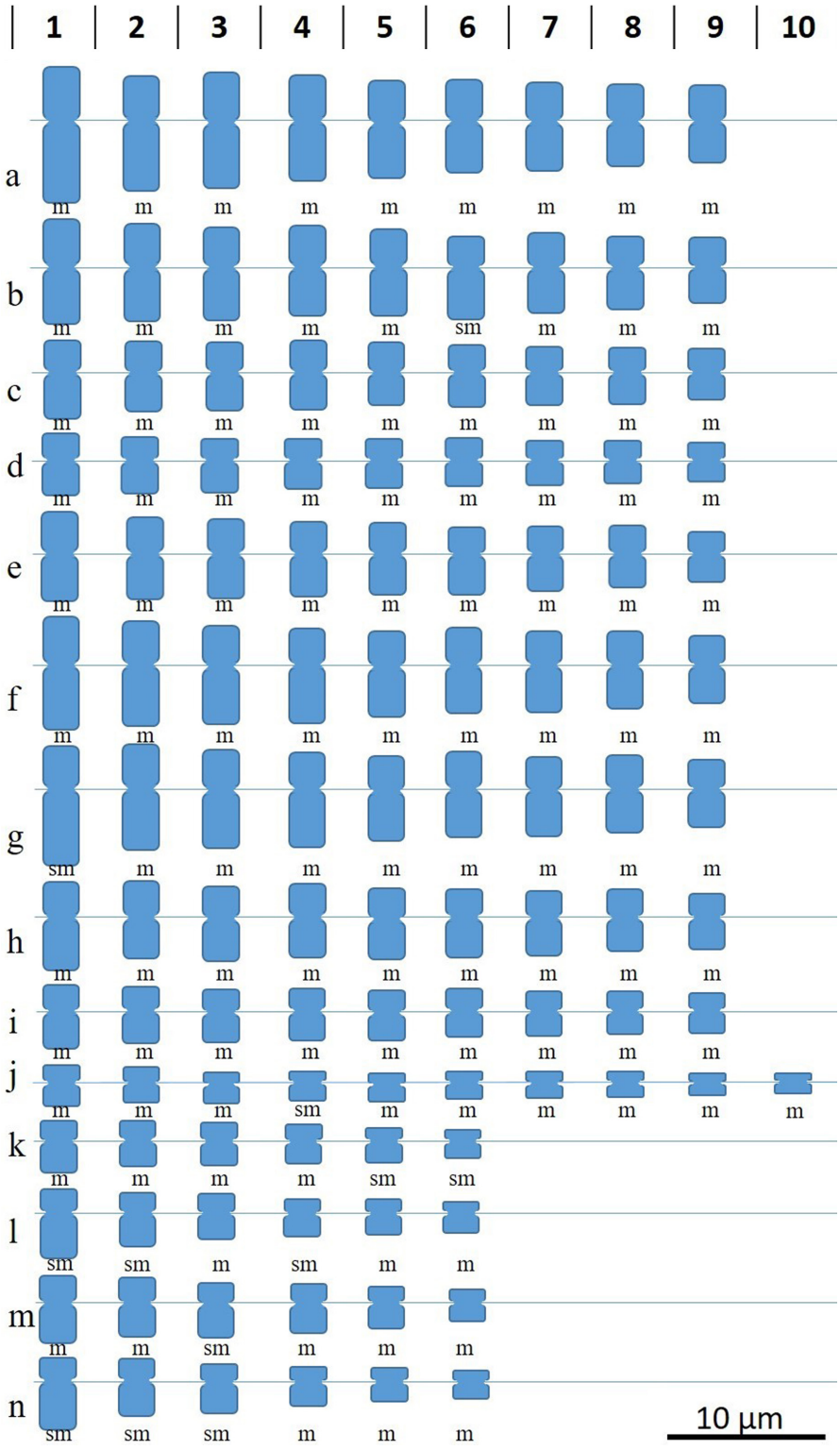

Figure 2 – a-n. Karyotypes and centromeric position of species of *Passiflora* – a. *P. alata*; b. *P. amethystina*, c. *P. caerulea*, d. *P. cincinnata*, e. *P. edulis f. flavicarpa* and f. *P. elegans* with 2n = 2x = 18; g. *P. mooreana* with 2n = 4x = 36; h. *P. tenuifila*, i. *P. tucumanensis* with 2n = 2x = 18; j. *P. foetida* 2n = 2x = 20; k. *P. capsularis*, l. *P. misera* and m. *P. morifolia* with 2n = 2x = 12; n. *P. suberosa* with 2n = 4x = 24. Scale bar: 10 µm.

Table 2 – Morphometric characteristics of the chromosomes of species of *Passiflora*. Mean measurements and standard deviation of CL = Chromosome length (μm). Different letter indicates significative differences among the length of the chromosomes of the set (p =< 0.05). L = long arm (μm); S = short arm (μm); CI = centromeric index (%).

Chromosome		1	2	3	4	5	6	7	8	9	10
P.alata	CL	8.9±1.1[a]	7.7±0.9[b]	7.2±0.7[b]	6.8±0.4[c]	6.4±0.4[c]	5.9±0.4[d]	5.7±0.3[d]	5.0±0.3[e]	4.4±0.2[f]	-
	L	5.1±0.9	4.3±0.9	4.1±0.4	3.7±0.5	3.4±0.5	3.1±0.6	3.0±0.4	2.7±0.3	2.5±0.2	-
	S	3.3±0.8	3.2±0.9	3.0±0.9	2.7±0.7	2.6±0.4	2.5±0.3	2.3±0.4	2.2±0.2	2.2±0.3	-
	CI	39.8	42.9	42.7	42.7	41.9	43.9	44.2	45.3	46.5	-
P. amethystina	CL	7.2±0.8[a]	6.4±0.5[b]	6.1±0.5[b]	5.8±0.5[c]	5.5±0.5[c]	5.2±0.6[d]	5.0±0.6[d]	4.6±0.6[e]	4.2±0.6[e]	-
	L	3.5±0.6	3.4±0.6	3.3±0.2	3.0±0.3	3.0±0.4	3.2±0.4	2.8±0.4	2.6±0.1	2.2±0.2	-
	S	3.0±0.5	2.7±0.2	2.4±0.5	2.5±0.3	2.3±0.4	1.9±0.2	2.0±0.2	1.8±0.3	1.8±0.3	-
	CI	45.6	44.2	41.8	45.8	42.7	36.9	42.0	41.8	45.2	-
P. caerulea	CL	4.9±0.9[a]	4.4±0.7[b]	4.2±0.7[b]	4.0±0.6[b]	3.9±0.5[b]	3.8±0.6[c]	3.6±0.5[c]	3.4±0.5[c]	3.1±0.5[c]	-
	L	2.9±0.8	2.4±0.5	2.4±0.4	2.3±0.3	2.0±0.3	2.1±0.3	2.0±0.3	1.9±0.4	1.6±0.3	-
	S	2.0±0.4	1.9±0.3	1.8±0.3	1.8±0.3	1.8±0.3	1.7±0.3	1.5±0.2	1.5±0.2	1.4±0.2	-
	CI	40.4	44.1	43.6	48.8	47.6	44.1	42.8	43.2	46.2	-
P. cincinnata	CL	4.2±0.9[a]	3.8±0.7[a]	3.5±0.8[b]	3.3±0.7[b]	3.1±0.7[b]	3.0±0.6[b]	2.8±0.5[c]	2.6±0.3[c]	2.4±0.3[c]	-
	L	2.2±0.2	2.1±0.2	2.0±0.2	1.8±0.2	1.7±0.2	1.6±0.2	1.5±0.2	1.4±0.2	1.3±0.1	-
	S	1.6±0.2	1.4±0.2	1.3±0.2	1.2±0.2	1.2±0,1	1.3±0.1	1.1±0.2	1.1±0.1	0.9±0.1	-
	CI	42.2	40.2	38.6	41.5	42.1	45.0	43.3	44.4	43.6	-
P. edulis	CL	6.4±0.5[a]	5.5±0.5[b]	5.1±0.6[c]	5.0±0.6[c]	4.7±0.7[c]	4.5±0.7[c]	4.2±0.6[d]	3.9±0.5[d]	3.4±0.5[e]	-
	L	3.0±0.4	2.8±0.4	2.8±0.4	2.7±0.6	2.5±0.4	2.6±0.6	2.3±0.5	2.0±0.3	1.7±0.4	-
	S	2.6±0.4	2.2±0.3	2.1±0.4	2.0±0.3	1.9±0.3	1.6±0.1	1.6±0.2	1.7±0.2	1.3±0.5	-
	CI	46.8	44.1	43.3	42.1	42.7	39.0	41.7	45.4	43.4	-
P. elegans	CL	4.3±0.6[a]	3.9±0.4[b]	3.7±0.3[c]	3.5±0.3[c]	3.3±0.3[d]	3.2±0.3[d]	3.1±0.3[e]	2.9±0.2[e]	2.7±0.3[f]	-
	L	2.5±0.3	2.4±0.3	2.1±0.2	2.0±0.4	2.0±0.2	1.8±0.2	1.8±0.2	1.6±0.3	1.5±0.2	-
	S	1.7±0.4	1.5±0.3	1.5±0.2	1.4±0.2	1.3±0.3	1.3±0.3	1.3±0.2	1.3±0.2	1.2±0.1	-
	CI	39.8	38.8	42.3	41.7	38.4	41.9	42.2	44.3	43.4	-
P. mooreana	CL	6.2±0.9[a]	5.7±0.9[b]	5.3±0.9[b]	5.0±0.8[b]	4.7±0.9[c]	4.5±0.9[c]	4.3±0.9[c]	4.1±0.9[c]	3.7±0.9[d]	-
	L	4.6±1.5	3.7±0.7	3.5±0.7	3.5±0.9	2.9±0.7	2.9±0.7	2.8±0.8	2.6±0.6	2.2±0.6	-
	S	2.5±0.8	2.6±0.7	2.4±0.5	2.1±0.6	2.1±0.4	2.2±0.4	1.9±0.5	1.9±0.5	1.6±0.4	-
	CI	35.3	41.0	40.1	38.4	39.9	44.0	41.1	43.7	42.4	-
P. tenuifila	CL	4.9±1.1[a]	4.5±0.9[b]	4.3±0.8[b]	4.0±0.7[c]	3.9±0.7[c]	3.7±0.6[c]	3.6±0.6[c]	3.3±0.6[d]	3.1±0.6[d]	-
	L	3.1±0.8	2.4±0..3	2.5±0.4	2.3±0.4	2.3±0.5	2.4±0.5	2.2±0.5	1.9±0.2	1.8±0.2	-
	S	2.1±0.5	2.1±0.4	1.9±0.5	1.9±0.3	1.8±0.3	1.6±0.3	1.5±0.3	1.6±0.2	1.3±0.3	-
	CI	40.0	46.4	42.6	45.2	43.1	39.6	41.0	44.5	41.8	-
P. tucumanensis	CL	3.6±0.6[a]	3.2±0.4[b]	3.0±0.4[c]	2.9±0.3[c]	2.8±0.3[d]	2.7±0.3[d]	2.5±0.3[e]	2.4±0.4[e]	2.2±0.4[f]	-
	L	2.2±0.5	1.9±0.3	1.7±0.2	1.7±0.3	1.6±0.2	1.5±0.2	1.4±0.1	1.3±0.1	1.2±0.2	-
	S	1.5±0.2	1.3±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.1±0.2	1.1±0.2	0.9±0.2	-
	CI	40.8	40.7	42.0	42.3	43.8	44.4	44.8	45.0	44.0	-

Chromosome		1	2	3	4	5	6	7	8	9	10
P. foetida	HCL	2.3±0.6[a]	2.0±0.5[a]	1.6±0.3[b]	1.5±0.3[b]	1.4±0.3[b]	1.3±0.2[b]	1.2±0.2[c]	1.2±0.2[c]	1.1±0.2[c]	1.0±0.2[c]
	L	1.2±0.3	1.1±0.3	1.1±0.25	1.0±0.3	0.9±0.2	0.8±0.2	0.7±0.2	0.7±0.1	0.6±0.2	0.6±0.1
	S	0.9±0.4	0.9±0.2	0.7±0.1	0.5±0.2	0.6±0.2	0.6±0.2	0.5±0.1	0.5±0.1	0.4±0.1	0.4±0.1
	CI	43.1	44.7	38.0	35.0	39.5	41.5	42.4	40.9	41.0	42.1
P. capsularis	HCL	2.4±0.3[a]	2.0±0.2[b]	1.8±0.2[c]	1.7±0.2[c]	1.5±0.2[d]	1.3±0.2[d]	-	-	-	-
	L	1.8±0.4	1.4±0.4	1.3±0.3	1.2±0.4	1.1±0.2	0.9±0.2	-	-	-	-
	S	1.1±0.4	1.0±0.3	1.0±0.3	0.9±0.2	0.7±0.2	0.5±0.1	-	-	-	-
	CI	38.5	41.8	43.7	41.9	36.8	37.4	-	-	-	-
P. misera	HCL	3.7±1.0[a]	3.1±0.4[b]	2.6±0.5[b]	2.5±0.6[b]	2.1±0.7[c]	1.7±0.7[c]	-	-	-	-
	L	2.7±1.3	1.9±0.6	1.5±0.3	1.3±0.5	1.1±0.3	1.1±0.3	-	-	-	-
	S	1.3±0.3	1.1±0.4	1.1±0.2	0.7±0.3	0.7±0.2	0.5±0.1	-	-	-	-
	CI	32.2	35.8	43.8	37.3	44.8	42.5	-	-	-	-
P. morifolia	HCL	3.2±0.7[a]	2.6±0.6[b]	2.3±0.5[b]	2.1±0.4[b]	1.8±0.4[c]	1.5±0.4[c]	-	-	-	-
	L	2.4±0.5	1.9±0.3	2.0±0.6	1.7±0.3	1.4±0.2	0.9±0.1	-	-	-	-
	S	1.5±0.4	1.4±0.4	1.1±0.1	1.0±0.2	0.9±0.1	0.7±0.1	-	-	-	-
	CI	39.3	42.3	36.8	38.2	38.5	42.4	-	-	-	-
P. suberosa	HCL	4.5±1.0[a]	3.6±0.5[b]	3.0±0.4[c]	2.5±0.3[d]	2.1±0.3[e]	1.6±0.3[f]	-	-	-	-
	L	2.9±0.3	2.1±0.4	1.8±0.3	1.3±0.2	1.1±0.1	0.9±0.2	-	-	-	-
	S	1.3±0.5	1.2±0.3	1.0±0.3	0.8±0.2	0.7±0.1	0.6±0.1	-	-	-	-
	CI	30.4	36.8	36.0	38.0	39.1	41.2	-	-	-	-

The size of the basic chromosomal complement was larger in the species of the subgenus *Passiflora* than in the others. Comparison of haploid genome length shows that *P. alata* value is significantly larger than the rest of the studied species (57.95 µm), followed by *P. amethystina* (49.91 µm). No significant differences were found between the sizes of the chromosomal complements of the species *P. mooreana*, *P. caerulea* and *P. edulis*, neither between *P. elegans*, *P. cincinnata* and *P. tucumanensis*, as well as between the species of the subgenus *Decaloba* (*P. misera*, *P. morifolia*, *P. suberosa* and *P. capsularis*) and *P. foetida* (Tab. 3).

Close relationships were found between haploid chromosome length and asymmetry indices A1 ($R^2 = 0.65$), CVcl ($R^2 = 0.56$) and MCA ($R^2 = 0.50$), while the regression was weaker with CVci ($R^2 = 0.30$) (Fig. 3).

FISH rDNA sequences mapping

Fluorescent *in situ* hybridization (FISH) assays on mitotic metaphases of native *Passiflora* species, using 5S and 18S sequences as probe showed variations in the number of hybridization signals among the species. Two 5S hybridization signals were detected per somatic cell in species of subgenus *Passiflora* (*P. alata*, *P. amethystina*, *P. caerulea*, *P. cincinnata*, *P. edulis f. flavicarpa*, *P. elegans*, *P. tucumanensis*), and subgenus *Decaloba* (*P. capsularis*, *P. misera* and *P. morifolia*) (Fig. 1). On the other hand, four 5S hybridization signals were found in the tetraploids *P. mooreana* form subgenus *Passiflora* and *P. suberosa* from *Decaloba*, as well as in the diploid *P. foetida* from *Dysosmia* (Fig. 1g,j,n). Regarded to the 18S hybridization signals, *P. capsularis* and *P. morifolia* from subgenus *Decaloba* presented two signals; while *P. misera* from the same subgenus and several species from subgenus *Passiflora* (*P. alata*, *P. cincinnata* and *P. edulis f. flavicarpa*) exhibited four. *Passiflora amethystina*, *P. caerulea*, *P. elegans* and *P. tucumanensis* showed six 18S hybridization signals and *P. suberosa* (subgenus *Decaloba*) showed eight (Fig. 1). The tetraploid cytotype of *P. mooreana* (subgenus *Passiflora*) showed ten 18s signals (Fig. 1g').

Table 3 – Number of rDNA sites and asymmetry indexes in the karyotypes of *Passiflora* species. HCL = haploid length of the chromosome set; CVcl = coefficient of variation of chromosome length; CVci = coefficient of variation of centromeric index (Paszko 2006); A1 = intrachromosomal asymmetry index (Romero Zarco 1986); MCA = mean centromeric asymmetry (Peruzzi & Eroğlu 2013); SD = standard deviation; * values of 1C genome size according to Sader *et al.* (2019). Different letters in HCL indicates statistical differences among species.

	Somatic number	Karyotype formula	5s sites	18s sites	HCL (µm)-SD	CVcl	CVci	A1-SD	MCA-SD	1C genome size *
P. alata	2n = 2x = 18	9m	2	4	57.95±3.68[a]	20.44	4.50	0.22±0.02	0.13±0.01	2.208
P. amethystina	2n = 2x = 18	9m	2	6	49.91±5.08[b]	14.79	6.48	0.24±0.05	0.14±0.03	-
P. caerulea	2n = 2x = 18	8m+1sm	2	6	40.96±10.29[c]	13.77	5.80	0.19±0.03	0.11±0.02	1.386
P. cincinnata	2n = 2x = 18	9m	2	4	28.82±5.99[e]	17.10	4.81	0.26±0.02	0.16±0.01	-
P. edulis	2n = 2x = 18	9m	2	4	42.39±4.18[c]	17.97	5.18	0.22±0.04	0.15±0.03	1.258
P. elegans	2n = 2x = 18	9m	2	6	30.80±2.62[e]	14.10	4.86	0.27±0.04	0.17±0.03	-
P. mooreana	2n = 4x = 36	8m+1sm	4	10	43.41±8.44[c]	18.51	6.64	0.30±0.04	0.19±0.03	-
P. tenuifila	2n = 2x = 18	9m	2	6	35.23±6.39[d]	14.96	5.49	0.24±0.02	0.15±0.01	-
P. tucumanensis	2n = 2x = 18	9m	2	6	25.32±3.10[e]	16.24	3.88	0.23±0.04	0.14±0.03	-
P. foetida	2n = 2x = 20	9m+1sm	4	6	14.71±3.08[f]	26.03	6.75	0.29±0.04	0.18±0.03	0.481
P. capsularis	2n = 2x = 12	4m+2sm	2	2	10.61±1.24[f]	25.48	7.04	0.37±0.05	0.20±0.04	0.319
P. misera	2n = 2x = 12	3m+3sm	2	4	15.70±3.25[f]	40.58	12.80	0.37±0.02	0.26±0.03	0.253
P. morifolia	2n = 2x = 12	5m+1sm	2	2	17.67±3.09[f]	27.69	5.78	0.32±0.07	0.20±0.05	0.505
P. suberosa	2n = 4x = 24	3m+3sm	4	8	17.31±2.51[f]	38.11	9.96	0.39±0.05	0.27±0.04	0.684

Genomic affinity among subgenera

In situ genomic hybridizations were performed on the mitotic chromosomes of species from subgenus *Passiflora* (*P. amethystina*, *P. caerulea* and *P. edulis flavicarpa*) with probes of genomic DNA from *P. suberosa* (subgenus *Decaloba*) and *P. foetida* (subgenus *Dysosmia*) (Fig. 4). The last mentioned probe was also applied on *P. suberosa* chromosomes (*Decaloba* subgenus). When the DNA probe from *P. foetida* (subgenus *Dysosmia*) was used on mitotic preparations of subgenus *Passiflora* species, uniform hybridization along entire chromosomes was found. On the other hand, the same probe applied on *P. suberosa* (subgenus *Decaloba*) chromosomes showed weak hybridization signals focused in some centromeres and secondary constrictions. Hybridization of *P. suberosa* genomic DNA on mitotic preparations of *P. amethystina*, *P. caerulea* and *P. edulis*, from subgenus *Passiflora*, showed weaker dispersed hybridization signals over some chromosomes, on centromeres and secondary constrictions (Fig. 4).

Discussion

Karyotypic parameters

This work provides a classical and molecular cytogenetic characterization of fourteen species of *Passiflora*, the largest number of Argentinean species studied together to this day.

The chromosomal counts performed in the present work were in agreement with previous reports, confirming the presence of three basic chromosome number: x = 6 for subgenus *Decaloba*, x = 9 for subgenus *Passiflora* and x = 10 for subgenus *Dysosmia* (Sader *et al*. 2019; Coelho *et al*. 2016; Sühsner *et al*. 2016; Melo *et al*. 2015; Bugallo *et al*. 2013; Chiapero *et al*. 2013; Souza *et al*. 2008; Hansen *et al*. 2006; Soares-Scott *et al*. 2005; Muschner *et al*. 2003; Melo & Guerra 2003; Souza *et al*. 2004; Deginani & Escobar 2002; Melo *et al*. 2001; Deginani 2001; Storey 1950).

In this work, the first karyotype measurements for genotypes belonging to *P. caerulea*, *P. elegans*, *P. mooreana*, *P. tenuifila*, *P. tucumanensis*, *P. misera*, *P. morifolia* and *P. suberosa* were provided. Only

metacentric chromosomes were observed in the karyotypes of *P. alata*, *P. edulis* and *P. cincinnata*, while previous publications on Brazilian genotypes recorded two submetacentric pairs for these species (Souza *et al*. 2003; Coelho *et al*. 2016). On the other hand, Praça *et al.* (2008) report only three pairs of metacentric chromosomes for *P. edulis* from other Brazilian collection sites, suggesting intraspecific polymorphisms in chromosome morphology. Although the karyotypes of the studied species of *Passiflora* are very symmetrical, this parameter was higher in the subgenus *Passiflora*, with almost exclusively metacentric chromosomes, than in the subgenera *Dysosmia* and *Decaloba*, which possess metacentric and submetacentric chromosomes.

The comparison of the haploid genome length measurements in the present work with that obtained in Brazilian genotypes, resulted in higher values than those of Souza *et al.* (2003) for *P. alata*; than those of Beena & Beevy (2015), Cuco *et al.* (2003) and Coelho *et al*. (2016) for *P. edulis f. flavicarpa*; than those of Cuco *et al*. (2003) and Coelho *et al*. (2016) for *P. cincinnata* and that of Cuco *et al.* (2003) for *P. amethystina*. On the other hand, our measurement of the haploid genome of *P. capsularis* resulted in values lower than those found by Amorim *et al.* (2014). The variation founded in the lengths of haploid genomes between different authors could arise from differences in chromosome condensation or could represent

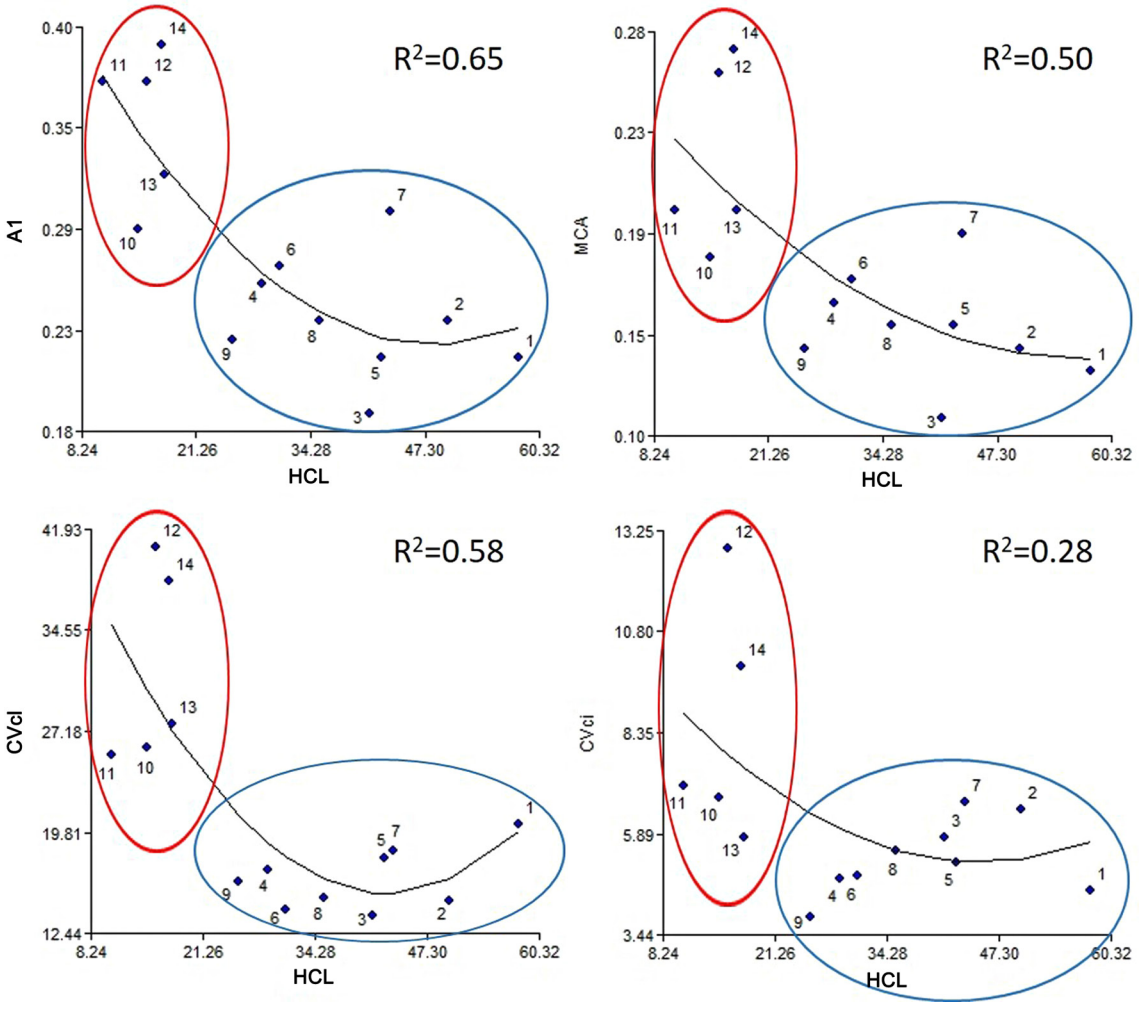

Figure 3 – Regression between asymmetry indices and the length of the haploid genome in genus *Passiflora*. 1 = *P. alata*; 2 = *P. amethystina*; 3 = *P. caerulea*; 4 = *P. cincinnata*; 5 = *P. edulis f. flavicarpa*; 6 = *P. elegans*; 7 = *P. mooreana*; 8 = *P. tenuifila*; 9 = *P. tucumanensis*; 10 = *P. foetida*; 11 = *P. capsularis*; 12 = *P. misera*; 13 = *P. morifolia*; 14 = *P. suberosa*. Red circle: subgenera Decaloba and Dysosmia (x = 6 and x = 10); blue circle: subgenus Passiflora (x = 9).

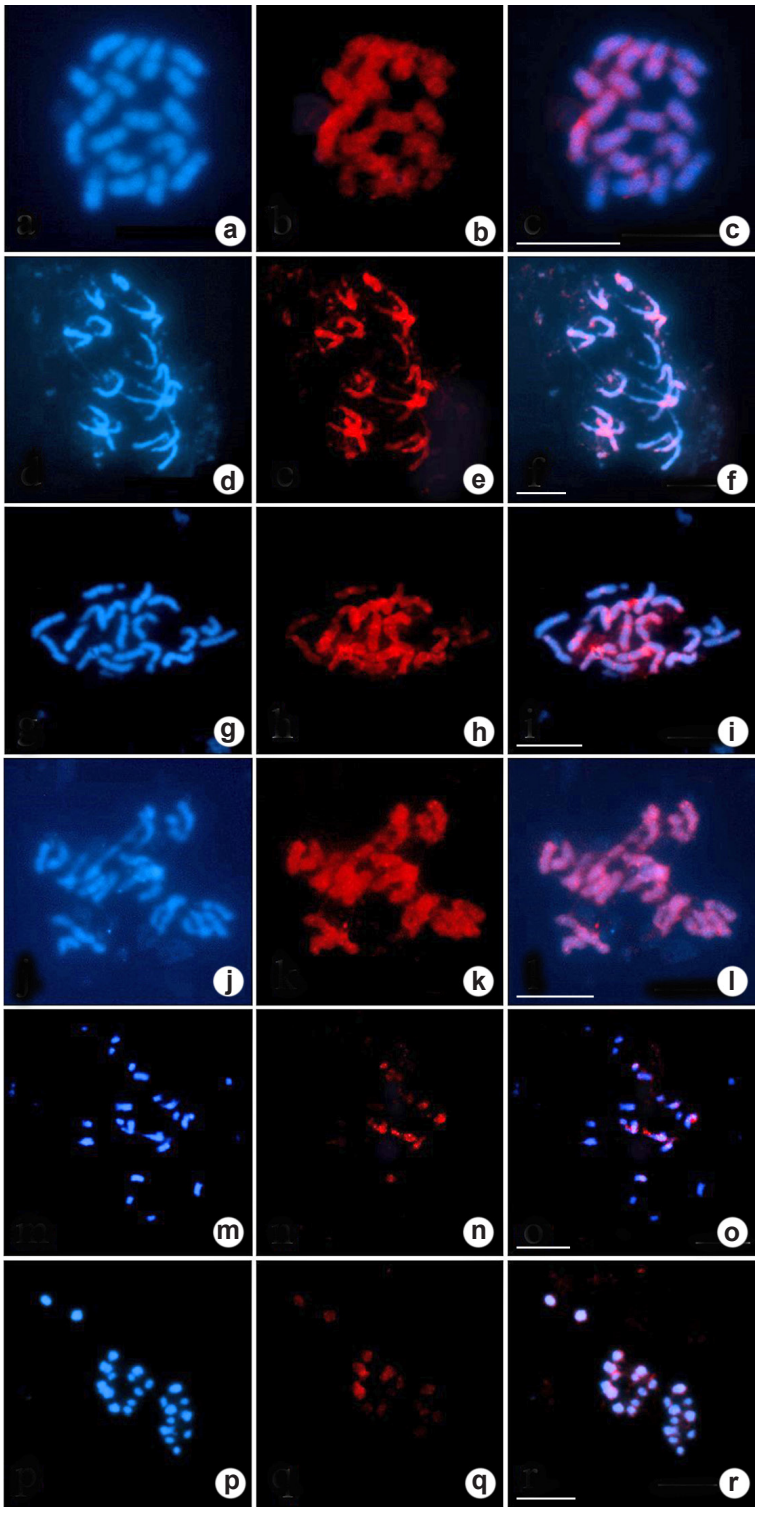

Figure 4 – a-c. *Passiflora suberosa* prove (subgenus *Decaloba*) on mitotic chromosomes of *P. caerulea* (subgenus *Passiflora*); d-f. *Passiflora suberosa* prove on mitotic chromosomes of *P. edulis f. flavicarpa* (subgenus *Passiflora*); g-i. *Passiflora suberosa* prove on mitotic chromosomes of *P. amethystina* (subgenus *Passiflora*); j-l. *Passiflora foetida* (subgenus *Dysosmia*) prove on mitotic chromosomes of *P. amethystina* (subgenus *Passiflora*); m-r. *Passiflora foetida* prove on mitotic chromosomes of *P. suberosa* (subgenus *Decaloba*).

variability in the genome size among populations of the same species. A regression test among haploid chromosome lengths and published values of 1C genome size for eight of the studied species (Sader *et al*. 2019) showed a close correlation ($R^2 = 0.96$). In addition, Bugallo *et al.* (unpublished data) found a close relationship between the length of the haploid genome and the amount of DNA in some genotypes studied in the present work.

The regression tests between the haploid chromosome length (HCL) and the asymmetry indices A1, CVcl and MCA not only show that species with longest genomes have more symmetrical karyotypes but also exhibit similarity among the karyotype of *P. foetida* (subgenus *Dysosmia*) and the species of the subgenus *Decaloba*, both in haploid chromosome length and in karyotypic symmetry (Fig. 3).

Ribosomal DNA sites

The number of sites 5s coincided with the ploidy level in *Decaloba* and *Passiflora* subgenus, showing two signals in somatic cells of the genotypes recognized as diploids and four of them in the tetraploids. On the other side, *P. foetida* is a diploid that showed four marks. These results could suggest the occurrence of polyploidy with the possibility of an ancestor x = 5, but no species related to this number is known to date, although it could also arise from an amplification of this sequence.

The results found within the *Passiflora* subgenus indicate that the number of 18s signals would match the phylogeny based on internal transcribed spacers and plastid spacers trnL-trnF elaborated by Muschner *et al.* (2003). These authors placed in the same clade four species with six 18s signals, *P. caerulea*, *P. amethystina*, *P. elegans* and *P. tenuifila*. However, Sader *et al*. (2019) did not include *P. elegans* in this group. Muschner *et al.* (2003) grouped in a clade the three species showing four 18s signals: *P. alata*, *P. cincinnata* and *P. edulis*. Sader *et al*. (2019) also include *P. foetida,* which presented 6 marks.

While Coelho *et al*. (2016) coincided with us in the number of 5s signals for *P. edulis* and *P. cincinnata* and Praça *et al*. (2008) does it in the number of 18s signals for *P. edulis*. The first authors found an extra pair of 45s signals for both of these species (unit 45s includes 18s sequence). The variation in the number of rDNA zones could be due, among others, to the amplification and jumping to different chromosomal sites (Schubert & Wobus 1985).

Genomic affinity among subgenera

The GISH technique allows comparing the genomic affinity between two species. Applying GISH with the use of probes of species belonging to one subgenus on the chromosomes of species from another subgenus, the zones with shared repetitive sequences could be exhibited. In mitotic preparations of species of the subgenus *Passiflora*, the genomic probe of *P. foetida* (subgenus *Dysosmia*) showed complete homology in all the chromosomes while probes of *P. suberosa* (subgenus *Decaloba*) showed scattered signals along the chromosomes. This would support the results of the molecular phylogenies of Muschner *et al.* (2003) and Sader *et al*. (2019) in which they affirm that there would be more phylogenetic proximity between the subgenus *Dysosmia* (*P. foetida*) and the subgenus *Passiflora* than between the latter subgenera with respect to the subgenus *Decaloba*, to which *P. suberosa* belongs.

The genomic probe of *P. foetida* from subgenus *Dysosmia*, with x = 10, only partially marked six to eight chromosomes in the mitotic preparations of *P. suberosa* (2n = 24; x = 6) from subgenus *Decaloba*. Melo & Guerra (2003) proposed that *P. foetida* has a later origin than the species of x = 6, the hybridization signals in only six to eight chromosomes of *P. suberosa* suggest a possible affinity with a shared ancestral genome of x = 6. One of the difficulties of working with these subgenera is the small size of their chromosomes that makes it difficult to visualize the hybridization signals.

Concluding remarks

The data obtained in this work support the hypotheses formulated by Melo *et al*. (2001) and Melo & Guerra (2003). In their works, the authors propose that x = 6 would be the original basic chromosomal number of the *Passifloraceae* family and that the basic numbers derived from it would have occurred due to processes of dysploidy and polyploidy. According to the results obtained in the present work regarding the number of rDNA sites, karyotypes and size of basic chromosomal complements, we agree with these authors hypothesis on the evolutionary history of the genus *Passiflora.* They state that x = 6 is the basic genome because they have only one 5s rDNA site per genome, which is supported by our FISH results. The original basic number (present in this work in diploid species as *P. morifolia*, *P. misera* and *P. capsularis*) would derived by polyploidy in another

x = 6 + 6, n = 12, presenting four 5s DNAr marks (like *P. suberosa*). Then, by descending dysploidy, 2n = 24 could generate 2n = 20, x = 10, keeping the four DNAr signals (*P. foetida*) and, by another event of descending dysploidy, x = 9 (*P. alata*, *P. amethystina*, *P. caerulea*, among others) (x = 6 a x = 12 a x = 10 a x = 9) (Fig. 5). We find that this hypothesis explain in a more parsimonious way the chromosomal evolutionary process than that exposed by Sader *et al*. (2019). They affirm that the genome evolved by ascending dysploidy (x = 6 a x = 7–8 a x = 9 a x = 10), generating in the last instance, the basic number x = 10. Although this option is possible, the fact of not knowing any *Passiflora* species with intermediate basic numbers x = 7 or x = 8 make it less parsimonious. These authors also discuss prediction models, questioning the validity of some of the algorithms applied for evolutionary studies. In addition, it is necessary to take into account that *P. foetida* (x = 10) is the most variable species of the whole genus with almost 50 varieties dispersed not only in America but also in Africa, Southeast Asia and Australia (Ulmer & MacDougal 2004).

The work of "molecular clock" by Muschner *et al*. (2012) shows that the diversification of the subgenus *Decaloba*, with x = 6, would have occurred 29 million years ago while that of the subgenus *Passiflora*, with x = 9, would have done it almost 17 million years ago. Unfortunately, neither Muschner *et al.* (2012) nor the Sader *et al*. (2019) studies included other representatives of basic number x = 10, such as *P. chrysophylla*, *P. arida*, *P. pectinata*, *P. sublanceolata* and *P. urbaniana*, among others. In the present work, we found *P. foetida* (subgenus *Dysosmia*) more related to the species of the subgenus *Passiflora* than to those of the subgenus *Decaloba*. On the other hand, the karyotype characteristics and the length of the haploid genome were strikingly similar between the subgenera *Dysosmia* and *Decaloba*. For this reason, we support the hypothesis that *P. foetida* (x = 10) would be an actual species related to the one that link the original subgenera *Decaloba* (x = 6) and *Passiflora* (x = 9), as formulated by Melo *et al*. (2001).

In general terms, the growth in size of a genome could become from both polyploidy events and amplification of transposable elements (Bennetzen *et al*. 2005). According to these authors, there would be the possibility that some species show greater activity of one of the mechanisms of genomic size variation, being able to grow more than decrease their genome. The species evaluated in this work with chromosome basic number x = 9 possess the longest genomes of the genus, suggesting that the process of descending dysploidy (which reduced the basic number x = 10 to x = 9) was accompanied by an increase in the DNA content that is reflected in the chromosomal length of the subgenus *Passiflora*. In the case of this subgenus, the responsible for the amplification of the genome would be the activation of transposable elements.

The distribution of DNA among chromosome arms could derive in changes in the karyotype intrachromosomal and interchromosomal asymmetry (Peruzzi *et al*. 2009; Peruzzi & Eroglu 2013). Sometimes, the same amount of DNA is added to each chromosome arm (equal increase),

Figure 5 – a-b. Hypothesized chromosomic evolutionary process of genus *Passiflora* – a: hypothesis proposed by Melo *et al.* (2001) and Melo & Guerra (2003) supported by this work: original basic number x = 6 undergoes a polyploidy event that generates an x = 6 + 6, 2n = 24, by descending dysploidy it would produce 2n = 20, x = 10 and by another event of descending dysploidy x = 9; b. hypothesis proposed by Sader *et al.* (2019): basic number x = 6 would suffer several events of ascending dysploidy originating basic number x = 7-8, 9 and 10.

resulting in an increase in the intrachromosomal karyotype symmetry. In other cases, the amount of DNA added is different between chromosome arms (unequal increase), resulting in a decrease of the intrachromosomal symmetry (Poggio *et al*. 2014). The highly symmetrical karyotypes in the subgenus *Passiflora* associated with the longest genomes of the genus suggest that the pattern of DNA accumulation caused an unequal increase in both chromosomal arms. The karyotypes of x = 9 were the most symmetrical, presented more DNA and longest genomes suggesting that, if they derive from x = 6, the rearrangements involved in the establishment of x = 9 caused genomic obesity and that the distribution of repetitive DNA was unequal being greater in the short arms and leading to more symmetrical karyotypes.

In summary, the karyotypic parameters studied in 14 species as well as the genomic affinities among different species belonging to subgenus *Decaloba*, *Dysosmia* and *Passiflora* clarify most of the chromosomal evolution of the genus and provides information to be applied in the breeding program.

The data obtained lead to confirm previous data and strongly suggest that the basic number x = 6 would be the original one, and that x = 9 was originated by processes of polyploidy and descendent dysploidy. The high molecular affinity between subgenus *Dysosmia* (*P. foetida*) and species of the subgenus *Passiflora* supports their closeness but it does not clarify which of the two originated the other. Since the genus *Passiflora* possesses the longest genomes, it is postulated that evolutionary process leading x = 10 to x = 9 was accompanied by unequal distribution of non-coding repetitive DNA, mainly transposable elements. These processes could explain the assymetrical karyotypes of species of subgenus *Passiflora*.

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