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ORIGINAL ARTICLE

Diversification and geographical distribution of *Psidium* (Myrtaceae) species with distinct ploidy levels

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Abstract

Key message **Polyploidy (diploid to octoploid) was evidenced from seven *Psidium* species, besides the outcomes of the whole-genome duplication about the nuclear DNA content, DNA sequence, and distribution.**

Abstract The previous studies have reported the occurrence of polyploid species in *Psidium*, all deriving from the basic chromosome number $x=11$, which is conserved in Myrtaceae. Here, we aimed to assess the ploidy levels of seven *Psidium* species and to investigate the genomic outcomes of this karyotype change. Data on chromosome number, ploidy level, nuclear DNA content, and DNA sequence (SSR markers) were sought, quantified, and compared to geographical distribution of the studied *Psidium* species. A euploid series based on $x=11$ was evidenced, with diploid, tetraploid, hexaploid, and octoploid species. These species also differed regarding at least one of the other analyzed traits, especially the hexaploids and the octoploid in relation to the others. Diploid species show restricted geographical distribution in the Atlantic Forest, differently from the polyploid species, which occur in several biomes in Brazil. Ploidy level of the *Psidium* species is related with the nuclear genome size and both seems to be related with species' geographical distribution. Besides polyploidy, the genetic changes associated with numerical chromosome shift shown in this study, which increases the knowledge about the diversification and distribution of *Psidium* species.

Keywords Myrteae · Euploidy · Karyotype · Nuclear DNA content · SSR markers · Guava

Introduction

Polyploidy, euploidy—a numerical chromosome rearrangement characterized by whole-genome duplication (Stebbins 1950; Edger and Pires 2009; Marchant et al. 2016; Spoelhof et al. 2017)—is arguably the most important karyotype change that increases the diversification and drive speciation in plants (Edger and Pires 2009; Madlung 2013; Alix et al. 2017; Slijepcevic 2018). Polyploidy directly leads to extensive genomic (of the chromosome number to the DNA sequence), epigenetic, and transcriptomic changes (Dhooghe

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et al. 2011; Marchant et al. 2016; Segraves 2017; Spoelhof et al. 2017). Due to these changes, polyploid taxa can exhibit new phenotypes or even attributes (morphologic, phenologic, physiologic, and reproductive) in relation to their counterparts (Levin 2002; Dhooghe et al. 2011; Segraves 2017; Spoelhof et al. 2017; Shu et al. 2018), within only one or few generations (Otto and Whitton 2000; Beest et al. 2012).

These novelties potentially influence the ecology (Otto and Whitton 2000; Soltis and Soltis 2000; Segraves 2017), as the increased ecological tolerance, allowing the polyploids overlap the niche of their ancestors (Marchant et al. 2016), as well as to colonize new habitats (Stebbins 1985; Soltis and Soltis 2000; Segraves 2017; Spoelhof et al. 2017). This hypothesis is supported, among others, by several studies on polyploid cytotypes of the genera *Fragaria* (Hancock and Bringhurst 1981), *Eupatorium* (Watanabe 1986), *Plantago* (Van Dijk and Bakx-Schotman 1997), and *Aster* (Münzbergová 2007). In these studies, diploids are found to have restricted spatial distributions, contrary to the widely dispersed polyploids. Cytogeographical studies have shown that diploids and polyploids often occupy different regions of the landscape along ecological gradients, such as moisture, whereby polyploids are generally capable of occurring in drier habitats when compared to diploids (Kay 1969; Watanabe 1986; Maherali et al. 2009; Treier et al. 2009).

The polyploidy was estimated to account for the speciation of 2–4% of today's flowering plant species, with woody plants representing a lower fraction (Otto and Whitton 2000). After a century of study (Barker et al. 2016), the polyploidy has been identified in several taxa, mainly crops (Alix et al. 2017), being currently considered that the whole-genome duplication probably occurred in the ancestor of all angiosperm plants (Alix et al. 2017; Spoelhof et al. 2017). Nevertheless, the knowledge about the role of this genomic change in the diversification, speciation, and ecology in tropical lineages is still scarce (Husband et al. 2013; Spoelhof et al. 2017), especially for trees.

One of the polyploidy outcomes is the nuclear DNA content change (Kron et al. 2007; Slijepcevic 2018), which can promote modifications in the size and/or number of vegetative and/or reproductive structures of a new plant when compared to its ancestors (Stebbins 1950). These changes can affect fitness, and include alterations in growth rates, seed production, the so-called hybrid vigor or heterosis, coupled with effective dispersal, and higher germination rates (Baker 1965; Bretagnolle and Lumaret 1995; Otto and Whitton 2000; Soltis and Soltis 2000; Comai 2005; Sattler et al. 2016).

In the Angiosperm family Myrtaceae, polyploidy has mainly been evidenced by chromosome counting in fleshy-fruited species of the clade Myrtoideae (Andrade and Forni-Martins 1998; Costa and Forni-Martins 2006a, b, 2007). This includes the Neotropical *Psidium*, a monophyletic group (Lucas et al. 2007; Rivero et al. 2012; Murillo et al. 2012) with rapid diversification rates (Vasconcelos et al. 2017). The genus comprises at least 100 species, distributed from Mexico and the Caribbean to Argentina and Uruguay (WCSP 2017). Sixty percent of the *Psidium* species occur in Brazil, being found in different biomes, such as evergreen tropical rain forests (Amazon and Atlantic Forest), savannas (Cerrado), and semi-arid forests (Caatinga) (BFG 2015). The large geographical distribution of many *Psidium* species is suggested to result from their superior competitive ability (Soares-Silva and Proença 2006). Staggemeier et al. (2016) have demonstrated that species of Myrtaceae exhibit a wide variety of fruit morphology and phenological strategies that support a variety of frugivorous sizes while retaining overall ecosystem functionality. Polyploidy can also explain this success. *Psidium* species have chromosome numbers of $2n=22$, 33, 44, 55, 66, 77, and 88, deriving from the basic chromosome number $x=11$ (Atchison 1947; Andrade and Forni-Martins 1998; Bolkhovskikh et al. 1969; Goldblatt 1981; Goldblatt and Johnson 1996; Moore 1977; Costa et al. 2008; Marques et al. 2016). Despite the existence of many polyploidy events in *Psidium*, the origin of polyploidy and its effects on the species' diversification and geographical distribution have not been investigated so far. Besides, the possible relationships among ploidy and geographical ranges in *Psidium* may offer a better understanding about how speciation affects the dispersal and establishment abilities of tropical species.

The previous studies have suggested the relationships between polyploidy and different measures of ecological 'success' (Stebbins 1947, 1950; Ehrendorfer 1980; Lewis 1980; Thompson and Lumaret 1992; Soltis and Soltis 2000; Alix et al. 2017; Segraves 2017). However, these hypotheses have rarely been tested (Segraves 2017) in the tropics, and the factors that contribute to the success of polyploids have seldom been identified. The consequences of whole-genome duplication on species' geographical distribution remain marginally explored. Therefore, the main goal of this study was to expand the knowledge about the *Psidium* genome, including the chromosome number, nuclear DNA content, DNA sequence, and the geographic distribution of six species indigenous to Brazil.

Materials and methods

Sampling

This study included six *Psidium* species indigenous to Brazil, as well as the naturalized *P. guajava* L. The selection was based on differences in the geographical distribution of the species using the BFG (2015) database. Five of the

indigenous species occur in two or more Brazilian biomes, and two are restricted to the Brazilian Atlantic Forest: *P. guineense* Sw. is widely distributed across the different Brazilian biomes (except the Pampas); *P. myrtoides* O. Berg and *P. cattleyanum* Sabine occur in the Cerrado, Caatinga and Atlantic Forest; *P. longipetiolatum* D. Legrand is found in the Cerrado and Atlantic Forest; and *P. oblongatum* O. Berg and *P. cauliflorum* Landrum & Sobral are restricted to the Atlantic Forest. Young and healthy leaves, from five individuals for each species, were collected in field expeditions and stored in silica gel for molecular analysis. As the number of fruits varied between the *Psidium* individuals, all fruits were collected for flow cytometry and cytogenetic analyses, being: 29 for *P. guajava*, nine for *P. oblongatum*, six for *P. cauliflorum*, 55 for *P. guineense*, 50 for *P. cattleyanum*, 38 for *P. myrtoides*, and 18 for *P. longipetiolatum*. One voucher per population was collected, dried (Peixoto and Maia 2013), and deposited at the RB herbarium of the Botanical Garden of Rio de Janeiro: *P. guajava* (Tuler, A 445), *P. oblongatum* (Carrijo, T 2105), *P. cauliflorum* (Tuler, A 511), *P. guineense* (Tuler, A 487), *P. cattleyanum* (Tuler, A 427), *P. myrtoides* (Tuler, A 451), and *P. longipetiolatum* (Tuler, A 450).

In vitro establishment, nuclear 2*C* value measurement, and chromosome number determination

Seeds of the seven *Psidium* species and *Solanum lycopersicum* Mill 'Stupické' (reference standard, $2C=2.00$ pg; Praça-Fontes et al. 2011) were disinfested under laminar flow hood (Oliveira et al. 2013) and inoculated into flasks containing MS medium (Murashige and Skoog 1962) supplemented with 3.0% (w/v) sucrose and 0.7% (w/v) type A agar, pH 5.7. The flasks were maintained at 25 °C under a 16/8 h light/dark regimen, with 36 μmol m^{-2} s^{-1} light radiation provided by two fluorescent lamps (20 W, Osram®). As performed by Marques et al. (2016), from the in vitro plantlets, leaves were collected for 2*C* value measurement, and roots were collected for 2*n* chromosome number determination. The use of in vitro plantlets was important owing to the unavailability of fruits during all months of the year in which the study was executed.

Nuclear 2*C* value measurement by flow cytometry is relevant to screen the polyploid taxa and record ploidy changes, as well as the increase and decrease in genome size that occurs after this event (Bennetzen and Kellogg 1997; Petrov 2002; Soltis et al. 2003). Therefore, leaf fragments of *S. lycopersicum* 'Stupické' plantlets (reference standard) grown in vitro and of each *Psidium* species (samples) were chopped together, and the nuclei were extracted and isolated (Otto 1990; Coser et al. 2012; Marques et al. 2016). The resulting suspensions were stained with buffer containing propidium iodide (Praça-Fontes et al. 2011; Coser et al. 2012) and analyzed in a Partec PAS® flow cytometer (Partec® GmbH, Munster, Germany) (Coser et al. 2012; Marques et al. 2016). The FlowMax® software (Partec®) was used to analyze the histograms. The mean nuclear genome size (2*C*) was measured by dividing the mean channel of the fluorescence peak corresponding to the standard's G_0/G_1 nuclei by that of each sample. At least 20 in vitro plantlets were used for each species.

Just as for flow cytometry, in vitro plantlets were fundamental to accomplish the cytogenetic evaluation. From these plantlets, roots were removed and immediately treated with 4 μM amiprophos-methyl (APM, Nihon Bayer Agrochem K. K.®) for 5 h at 30 °C. The roots were washed with distilled water (dH_2O) for 20 min, fixed in fresh methanol:acetic acid (Merck®) solution (3:1), stored at -20 °C for at least 24 h, washed again with dH_2O for 20 min, and macerated with enzymatic solution for 2 h at 34 °C (Coser et al. 2012; Marques et al. 2016). Root meristem dissociation and air-drying (Carvalho et al. 2007) procedures were used to prepare the slides, which were analyzed using a Nikon 80i microscope (Nikon, Japan). Metaphase images were captured with a Media Cybernetics® Evolution™ charge-coupled device (CCD) video camera (Nikon, Japan) coupled to this microscope.

Molecular analysis

The transferability rates of 141 SSR (simple sequence repeat) markers were obtained from a previous study (Tuler et al. 2015). Of these, 32 SSR were selected which amplified for the seven species analyzed in this study (Table 1). Details of DNA extraction and SSR amplification are available in Tuler et al. (2015). The number of alleles per locus and observed heterozygosis per primer was estimated. Data obtained for alleles of each individual were subjected to dissimilarity index analysis using the weighted index.

Results

In vitro establishment, nuclear 2*C* value measurement, and chromosome number determination

All seeds of *Psidium* and *S. lycopersicum* germinated after 30 days, providing morphologically normal plantlets, which were maintained under controlled environmental in vitro conditions. From the in vitro plantlets, nuclear genome size analysis evidenced high intrageneric variation of mean 2*C* values among the seven *Psidium* species. *Psidium cauliflorum* showed the lowest nuclear genome size, $2C=0.93\pm0.002$ pg ($1C=0.465$ pg), followed by *P.*

Table 1 SSR primers used in the detection of polymorphism among the seven species of *Psidium*

SSR primer	Forward	Reverse	FR	Number of alleles
mPgCIR 2	AGTGAACGACTGAAGACC	TTACACATTCAGCCACTT	0	5
mPgCIR 16	AATACCAGCAACACCAA	CATCCGTCTCTAAACCTC	0	4
mPgCIR 19	AAAATCCTGAAGACGAAC	TATCAGAGGCTTGCATTA	0	4
mPgCIR 21	TGCCCTTCTAAGTATAACAG	AGCTACAAACCTTCCTAAA	0	3
mPgCIR 26	CTACCAAGGAGATAGCAAG	GAAATGGAGACTTTGGAG	0	8
mPgCIR 91	GCGGTGGATTTGAATTTAG	CCAAGTAACCCACAACAATA	1	6
mPgCIR 94	CAACCTTCCCGTGATTATT	CTAGCTTCTTCAGTGGGAAC	1	9
mPgCIR 97	GACCTCAGTAGTTCAGCATGT	TAGAGTGGACGGGAGGAG	1	4
mPgCIR 98	CATCAACTTTCCAGGCATA	CCATTCAGTCGGTTTGAC	1	5
mPgCIR 99	TCAAAGTCCAAAACTCATGC	GGGATGGAGTAAAGATGAAA	1	8
mPgCIR 104	ATTCCCGTGGATTATGTATC	ACAACCATTTTCTCCTCATC	1	5
mPgCIR 108	AGGACCTCACAGAAGTTCAC	CGCTGTTTACACTGTCGTT	0	5
mPgCIR 137	GGGGAATGCAGAGATTGT	AGATGATGGTCTCGCTTTT	1	5
mPgCIR 148	CATACAGAGTCGGATGGTTT	GCTGCTGGTCTTAAAGCTAA	1	9
mPgCIR 158	ATCACCACTACTCCACTCGT	TAGAAGGTGCTCTAGGCTCA	0	1
mPgCIR 163	TCTTTGCACATCAAACTCG	CATGGTATCAATAGGTCAAGC	1	3
mPgCIR 188	TGGATGAATCAGGAGGATTA	TTGTGGGGAAGAAACTACTG	1	2
mPgCIR 192	ACGCTAACTATCGAAATGCT	ACTACGCACTTGATGGAGAT	1	6
mPgCIR 198	CTCGATCAGAAGAACAACATC	ACTGTTCCTGATGGCTCTC	1	7
mPgCIR 209	CTAAAGCCACATCCAGCA	CTAACATTTGCCTTCTACAGC	1	4
mPgCIR 231	CTCCAAGAAAATGGAAAGG	TGAAAACACCAAACAGCAC	1	3
mPgCIR 233	GACTGAAGACCCAAATACCA	TTAGGCTGAAATGCTCCTTA	1	2
mPgCIR 242	TTAAGGTGGGACCAAGAAG	GACGTATCGGATCAAGTTTC	1	4
mPgCIR 256	AGGTGCATGATTACGATTCT	CGAGGTTCTTGATGTTGTCT	1	6
mPgCIR 277	AGCCGATTATGATTACCTGA	CGATTCACTCCCTCATTACT	–	6
mPgCIR 287	GCTGGTGCAAAAGTAGTCA	GCAGTTCTTTTCCTTCTAACC	–	4
mPgCIR 345	CTGGGAGACTTTTCAAGG	GAGTCCGATGTTGATGAAG	–	5
mPgCIR 347	CTCTGAAAGGGAGAGGACTT	AGAATCTTCGCCTATTGCTT	–	4
mPgCIR 414	AACAACACGCTTTGAAGTTT	CCCAGAAAGATGAGACAAAG	–	4
mPgCIR 420	CAACTTTGCTAGAGATGAAGC	ATGTAGTAATCGAAGAAATGGTT	–	5
mPgCIR 437	ACAACAGTTCTGATCCCAAA	CTCGGAGACACAGAGGTCTA	–	3
mPgCIR 439	GCATCTTGCTTCTGTCACTT	GGAGATGTGCAACGTATTTT	–	3

FR: functional region = yes (1), no (0), absent (–)

guajava, $2C = 0.95 \pm 0.021$ pg ($1C = 0.475$ pg) and *P. oblongatum*, $2C = 0.98 \pm 0.004$ pg ($1C = 0.490$ pg). In comparison, the other species presented significantly greater mean values: *P. guineense* exhibited $2C = 1.86 \pm 0.003$ pg ($1C = 0.930$ pg), a nuclear genome size approximately twofold higher than in *P. cauliflorum, P. guajava*, and *P. oblongatum. P. myrtoides* showed $2C = 3.07 \pm 0.0045$ pg ($1C = 1.535$ pg), or 3.30 times higher; *P. cattleyanum* had $2C = 3.57 \pm 0.00$ pg ($1C = 1.785$ pg), or 3.84 times higher; and *P. longipetiolatum* displayed $2C = 5.12 \pm 0.002$ pg ($1C = 2.560$ pg), or 5.51 times higher than in the three above-mentioned species. Thus, mean $2C$ value data suggest that karyotype alterations occurred during evolution, promoting a strong variation in nuclear genome size (Fig. 1; Table 2).

Based on this hypothesis, a cytogenetic approach was performed from the same in vitro plantlets to assess the chromosome number of each species, as well as polyploidy in *P. guineense, P. myrtoides, P. cattleyanum*, and *P. longipetiolatum. Psidium cauliflorum, P. guajava* and *P. oblongatum* presented $2n = 22$ chromosomes; *P. guineense* $2n = 44$; *P. myrtoides* and *P. cattleyanum* $2n = 66$; and *P. longipetiolatum* $2n = 88$. Therefore, cytogenetics revealed a euploid series comprising diploid (*P. cauliflorum, P. guajava*, and *P. oblongatum*), tetraploid (*P. guineense*), hexaploid (*P. myrtoides* and *P. cattleyanum*), and octoploid species (*P. longipetiolatum*) (Fig. 1; Table 2).

Considering the non-replicated monoploid genome $x = 11$ for the *Psidium* species sampled here, the $1Cx$ DNA

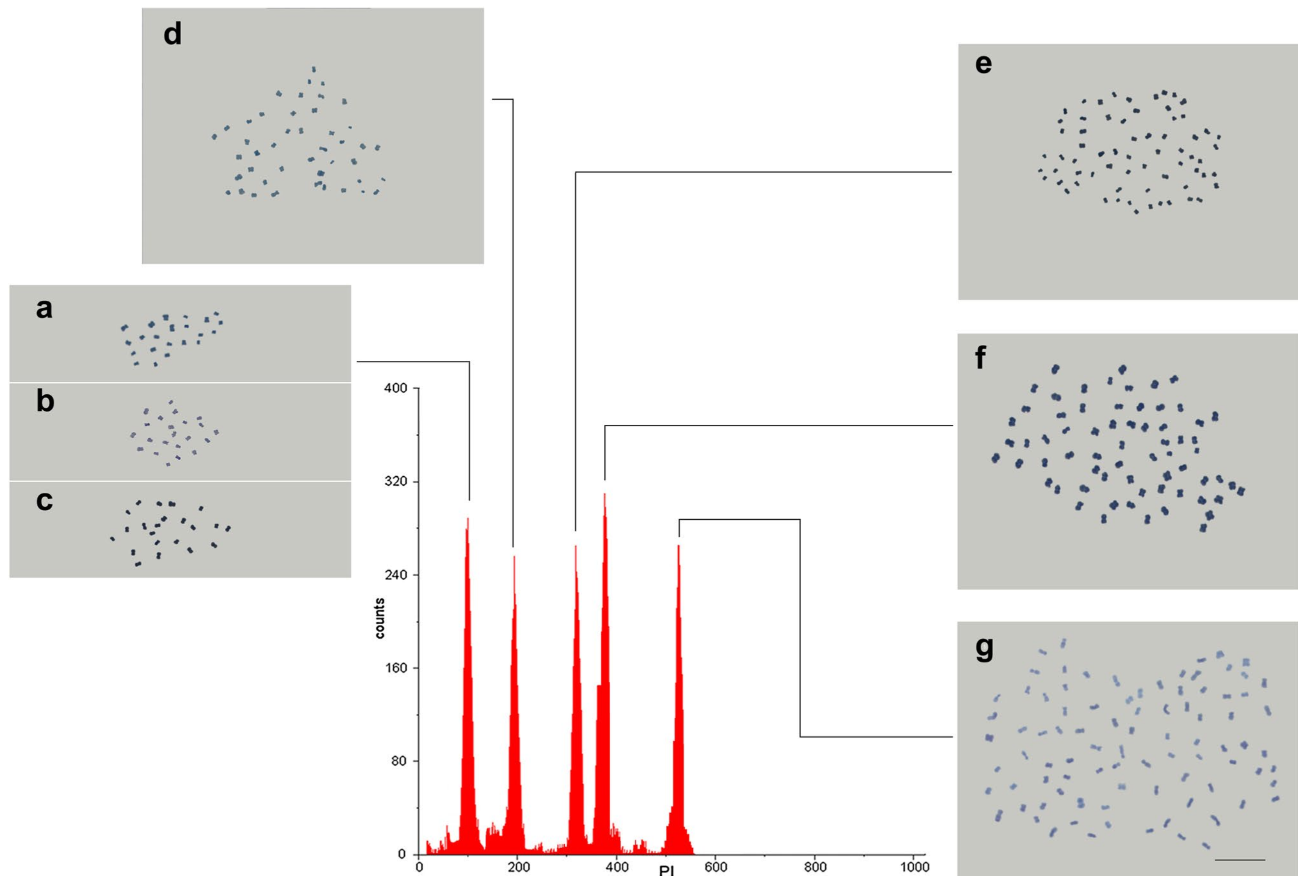

Fig. 1 Schematic histogram and karyotype of the seven *Psidium* species. Flow cytometry was executed separately for each species using the internal standard *S. lycopersicum* (2*C* = 2.00 pg; Praça-Fontes et al. 2011). G_0/G_1 nuclei peaks of each *Psidium* species are represented in the same histogram, as follows: *P. cauliflorum* (2*C* = 0.93 pg), *P. guajava* (2*C* = 0.95 pg) and *P. oblongatum* (2*C* = 0.98 pg) in channel 100; *P. guineense* (2*C* = 1.86 pg) in channel 200; *P. myrtoides* (2*C* = 3.07 pg) in channel 323; *P. cattleyanum* (2*C* = 3.57 pg) in channel 376; and *P. longipetiolatum* (2*C* = 5.12 pg) in channel 539. Following the lines from each G_0/G_1 peak, the karyotype of each species is shown: **a** *P. cauliflorum*, **b** *P. guajava* and **c** *P. oblongatum* with $2n = 2x = 22$ chromosomes; **d** *P. guineense* with $2n = 4x = 44$ chromosomes; **e** *P. myrtoides* and **f** *P. cattleyanum* with $2n = 6x = 66$ chromosomes; and **g** *P. longipetiolatum* with $2n = 8x = 88$ chromosomes. Note the euploid series in these *Psidium* species based on $x = 11$, the similar nuclear genome size of the diploid species, and the clear nuclear DNA content difference between hexaploid species. Bars = 5 µm

Table 2 C: 2*n* chromosome number (ploidy level), D: mean 2*C* nuclear DNA content (pg), T: transferability (data from Tuler et al. 2015), H: percentage of heterozygous loci for 32 SSR markers, A: mean of the alleles in seven species of *Psidium*

Species	C	D	T	H (%)	A
Psidium guajava	22 (2*X*)	0.95	100	34.3	1.40
Psidium oblongatum	22 (2*X*)	0.98	80.9	37.5	1.59
Psidium cauliflorum	22 (2*X*)	0.93	53.9	0.00	1.00
Psidium guineense	44 (4*X*)	1.86	97.8	40.0	1.68
Psidium cattleyanum	66 (6*X*)	3.57	74.4	34.3	1.43
Psidium myrtoides	66 (6*X*)	3.07	80.9	37.5	1.50
Psidium longipetiolatum	88 (8*X*)	5.12	65.2	50.0	1.81

value (DNA content of basic chromosome number *x*; Greilhuber et al. 2005) was: 0.465 pg for *P. cauliflorum* and *P. guineense*; 0.475 pg for *P. guajava*; 0.490 pg for *P. oblongatum*; 0.512 pg for *P. myrtoides*; 0.595 pg for *P. cattleyanum*; and 0.640 pg for *P. longipetiolatum*.

The diploid species, *P. cauliflorum* and *P. oblongatum*, are endemic to the Atlantic Forest, restricted to a few locations, mainly in rainforest regions. The octoploid *P. longipetiolatum* is also restricted to the Atlantic Forest, occurring in Ombrophilous Forest, Semideciduous forest in the states of southeastern (Espírito Santo, Minas Gerais, Rio de Janeiro, and São Paulo states) and Mixed Ombrophilous Forest in south (Paraná, Rio Grande do Sul, and Santa Catarina states). The tetraploid (*P. guineense*) and hexaploid (*P. myrtoides* and *P. cattleyanum*) are widely distributed in Brazil, occurring under different environmental conditions in the Atlantic Forest, Caatinga, Cerrado, and Amazon Rainforest (Fig. 2).

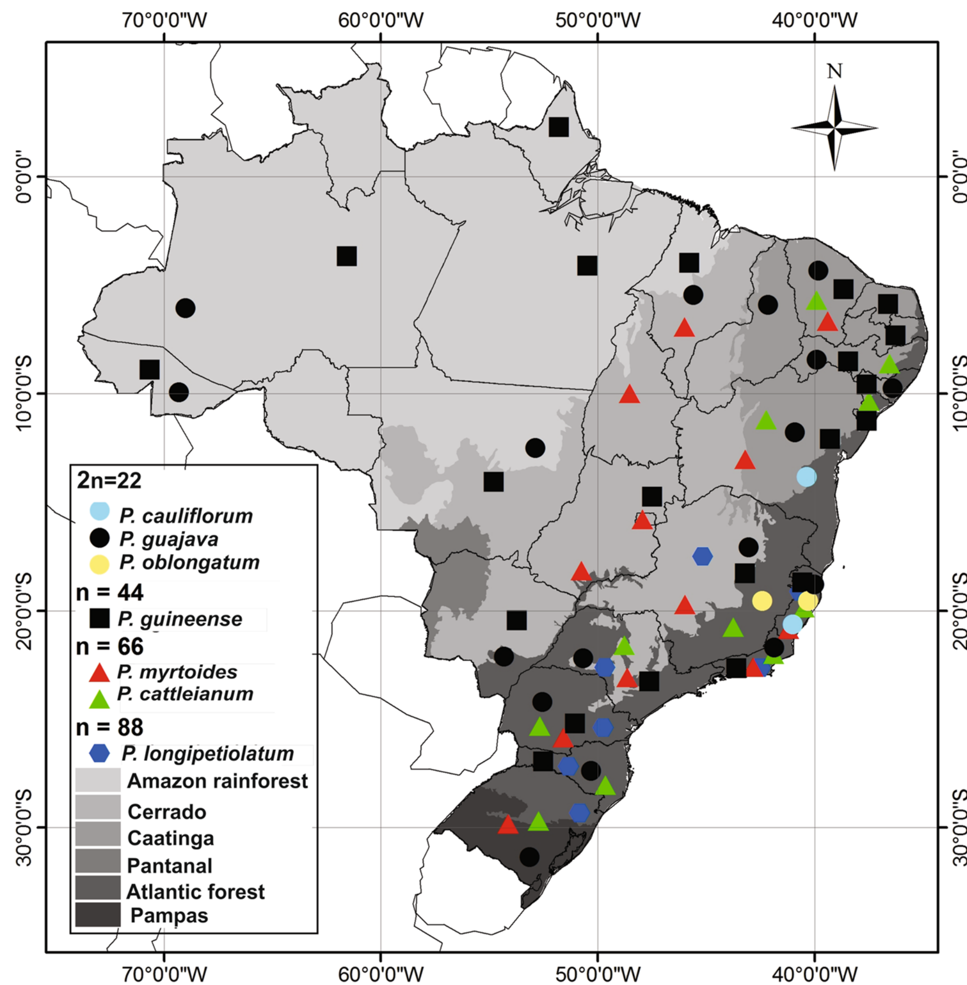

Fig. 2 Distribution of the seven *Psidium* species in Brazilian biomes based on data from the literature, herbaria, and the present study. Distribution of the diploid species ($2n=2x=22$ chromosomes): *P. cauliflorum* (blue circle), *P. guajava* (black circle), and *P. oblongatum* (yellow circle). Note that *P. cauliflorum* and *P. oblongatum* (yellow circle) only occur in the Atlantic Forest. Tetraploid species ($2n=4x=44$ chromosomes): distribution of *P. guineense* (black square) was not registered in Pantanal and the Pampas. Hexaploid species ($2n=6x=66$ chromosomes): *P. cattleyanum* (green triangle) and *P. myrtoides* (red triangle) occur in the Atlantic Forest, Cerrado, Caatinga, and the Pampas. Octoploid species ($2n=8x=88$ chromosomes): *P. longipetiolatum* (blue hexagon) is found distributed in the Atlantic Forest from Minas Gerais to Rio Grande do Sul. The coordinates used to define the species' geographical distribution were obtained with the application Google Earth, using the locations indicated on the labels of the herbarium specimens. The map of geographical distribution was made using the program DIVA GIS 5.4

Molecular analysis

Thirty-two SSR markers were chosen amongst the 132 SSR markers developed for *P. guajava*, according to Tuler et al. (2015), based on transferability in the six *Psidium* species (Table 2). Diploid species showed the lowest heterozygosity rates, with *P. cauliflorum* ($2x=22$) having all 32 *loci* in homozygosis (heterozygosity rate of 0.00%), corresponding to a mean allele number per *locus* equivalent to 1.00. In contrast, the octoploid *P. longipetiolatum* exhibited the highest heterozygosity rate (50.00%), as well as the highest mean number of alleles per *locus* among all species (1.81) (Table 2).

The 32 SSR loci differed among the species. A total of 149 alleles were amplified, with a mean of 4.6 alleles per locus. The SSR loci mPgCIR 26, mPgCIR 94, mPgCIR 99, and mPgCIR 148 produced the largest number of alleles (8 or 9), whereas mPgCIR 158, mPgCIR 188, and mPgCIR 233 generated the smallest (1 or 2). In general, SSR from transcribed regions showed more allelic forms (5.5 alleles per locus) than those from non-transcribed regions (4.8 alleles per locus) (Table 1).

Discussion

Nuclear 2*C* value, chromosome number, and molecular data evidenced the euploid, and dynamic and progressive genomic modifications in the seven *Psidium* species, expanding the data about tropical tree species (Husband et al. 2013; Spoelhof et al. 2017). The four polyploid species of *Psidium* in this study are an example of natural euploidy derived from whole-genome duplication. Therefore, these species were originated from intraspecific whole-genome duplication (autopolyploidy—endoreplication or endomitosis of the zygote or fusion of non-reduced reproductive cells), or from the interspecific crossing (allopolyploidy—hybridization) involving or not the whole-genome duplication (Stebbins 1950; Sattler et al. 2016; Shu et al. 2018). This is a dramatic quest that remains open, which must be look for each species. Due to the relatively recent (~ 9.9–20.8 Ma, Oligocene–Miocene) radiation of the tribe Myrteae (Thornhill et al. 2015; Berger et al. 2016), these *Psidium* species represent recent polyploids.

The progressive increase in nuclear DNA content, from the diploid species *P. cauliflorum, P. guajava*, and *P. oblongatum* to the polyploid species *P. guineense, P. cattleyanum, P. myrtoides*, and *P. longipetiolatum,* indicates strong karyotype differences related to numerical changes (euploidy). Therefore, there is a relation between the chromosome number and the nuclear genome size of the seven species. Interspecific and intraspecific variations in nuclear 2*C* value in *Psidium* have been reported for *P. guajava* 'White'—$2C = 0.507$ pg and *P. guajava* 'Red'—$2C = 0.551$ pg (Costa et al. 2008), *P. acutangulum*—$2C = 1.167$ pg (Costa et al. 2008), *P. australe*—$2C = 2.97$ pg (Souza et al. 2015), *P. guineense*—$2C = 1.85$ pg (Marques et al. 2016) and $2C = 2.02$ pg (Souza et al. 2015), and *P. cattleyanum*—$2C = 1.053$ pg (Costa et al. 2008) and $2C = 1.99–5.47$ pg (Souza et al. 2015).

Chromosome counting corroborated the obtained 2*C* values and shed light on the karyotype divergences (Fig. 1). Diploidy was confirmed for *P. guajava* (Costa et al. 2008; Souza et al. 2015; Marques et al. 2016), as well as tetraploidy for *P. guineense* (Souza et al. 2015; Marques et al. 2016) and hexaploidy for *P. cattleyanum* (Souza et al. 2015). Nuclear genome size and chromosome number were characterized for the first time in *P. cauliflorum* and *P. oblongatum*, which exhibited the same nuclear 2*C* value and chromosome number as *P. guajava*. The family Myrtaceae is basically diploid ($2n = 2x = 22$), as illustrated by Australasian species of the genera *Eucalyptus* and *Melaleuca* (Atchison 1947; Brighton and Ferguson 1976; Rye 1979). The tribe Myrteae also displays a predominance of $2n = 2x = 22$, except for *Eugenia, Myrcia*, and *Psidium*, in which polyploid species are also found (Costa and Forni-Martins 2006a, b, 2007; Silveira et al. 2017).

Considering polyploid species, *P. myrtoides* shows the same $2n = 6x = 66$ chromosomes as *P. cattleyanum*, but its 2*C* value is 0.50 pg lower than in the latter. These results suggest the occurrence of structural chromosome changes during the karyotype evolution in *Psidium*. Besides euploidy, the karyotype evolution also involves aneuploidy, which did not observed in the *Psidium* species of this study, and structural chromosome rearrangements (Sattler et al. 2016; Slijepcevic 2018). Alternatively, *P. cattleyanum* and *P. myrtoides* may have originated from distinct progenitors (auto- or allopolyploids). The number of $2n = 8x = 88$ chromosomes reported here for *P. longipetiolatum* has been previously reported for *P. cattleyanum* (Atchison 1947), reinforcing that polyploidy occurs in this genus. Contrary to the previous studies (Atchison 1947; Costa and Forni-Martins 2006a, b; Costa et al. 2008; Souza et al. 2015), no variation in 2*C* value or chromosome number was found between the distinct individuals of *P. guajava* and *P. cattleyanum*.

The interspecific differences in the nuclear DNA content among the *Psidium* species (Fig. 1) represent outcomes of the polyploid origin due that the basic chromosome number $x = 11$, which is conserved in Myrtaceae. A polyploid series from $x = 11$ was confirmed for *Psidium*, as well as the mean nuclear 2*C* value was showed for each ploidy level (Fig. 1). The polyploidy has mainly been reported for the tribe Myrteae (Silveira et al. 2017; Costa and Forni-Martins 2006a, b, 2007), which includes fleshy-fruited species in South America. According to the phylogenetic relationships proposed for Myrtaceae (Vasconcelos et al. 2017), the tribe Eucalypteae is a basal clade in the subfamily Myrtoideae. The diploid species of the tribes Eucalypteae present $2C = 1.13$ pg (*Eucalyptus globulus*, Azmi et al. 1997). Differently, for the *Psidium* sampled here, the mean value for nuclear genome size ($2C = 1.85$ pg for *P. guineense* to 2*C* = to $2C = 5.75$ pg for *P. longipetiolatum*) increased through polyploidy events.

The SSR markers showed that polyploidy in *Psidium* also resulted in higher heterozygosity rate and mean number of alleles. This is a direct effect of this karyotype change. The high polymorphism of the primers mPgCIR 26, 94, 99, and 148 was a result of the higher number of alleles present in polyploid species (*P. cattleyanum*: 3 alleles for mPgCIR 94; *P. myrtoides*: 4 alleles for mPgCIR 26; *P. guineense*: 4 alleles for mPgCIR 148; and *P. longipetiolatum*: 4 alleles for mPgCIR 99 and 4 alleles for mPgCIR 94). The occurrence of more than two alleles per SSR *locus* has also been reported in accessions of *P. guajava* (mPgCIR 253, Aranguren et al. 2010), *P. guineense, P. cattleyanum*, and *P. friedrichsthalianum* (mPgCIR 255, Costa and Santos 2013).

Most of the polymorphic primers are derived from functional regions (Table 1; e.g., mPgCIR 94, 99, and 148).

Functional regions are associated with control and variation of adaptive characteristics and/or important traits for occupation of new habitats, thus affecting the species' distribution (Grattapaglia et al. 2012). Based on nuclear 2*C* value, chromosome number, and molecular data, we suggest that the large ecological and geographical amplitudes that the four polyploid *Psidium* species occupy can be linked to their polyploid condition.

The polyploid *Psidium* species (*P. guineense, P. cattleyanum, P. myrtoides*, and *P. longipetiolatum*) have large geographical distribution compared to the endemic and diploid species (*P. cauliflorum* and *P. oblongatum*). For instance, *P. guineense* occurs in all Brazilian biomes (Atlantic Forest, Caatinga, Cerrado, and Amazon Rainforest), and this broad geographical distribution comprises a wide range of environmental conditions. *Psidium guajava* is the only diploid species presenting large geographical distribution in Brazil. This can be explained by its cultivation for economic purposes. Considering that polyploids show broader ecological tolerances and higher colonization abilities in comparison to diploids (Stebbins 1950; Grant 1981), it is possible that the phenotypical diversity presented by the four evaluated polyploid *Psidium* species enables their exploration of new habitats.

Conclusion

Euploidy, based on the basic chromosome set $x=11$, was confirmed for four of the seven *Psidium* species studied here. The chromosome number explains the increase in nuclear genome size and genetic diversity, discriminating the tetraploid and octoploid species. Therefore, polyploidy contributed to the diversification in the studied *Psidium* species, representing an important mechanism of speciation. As a challenging field, further understanding of the evolutionary history and diversification of *Psidium* will probably require approaches including the understanding of the phenotypic variation associated with the species' geographic distributions, and the development of phylogenetic studies. Such studies, however, will be better understood in the light of the cytogenetic and molecular patterns revealed in this study.

Author contribution statement ACT, TTC, MLG, and WRC conceived, designed, and conducted the study. ACT, ALP, and TTC identified the species of *Psidium*. ACT, MSS, and WRC carried out the cytogenetic analyses. WRC and CRC performed the flow cytometry analysis. ACT and MFSF conceived and conducted the molecular marker analysis. All authors equally contributed to the writing, editing and revision of the manuscript, and approved the final version for submission.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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