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DARLEY APARECIDO TAVARES FERREIRA

CARIÓTIPO E CONTEÚDO DE DNA NUCLEAR DE *Passiflora* L.: UMA CONTRIBUIÇÃO PARA SISTEMÁTICA E EVOLUÇÃO DO GÊNERO

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Tese apresentada à Universidade Federal do Espírito Santo, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento, para obtenção do título de "***Doctor Scientiae***"

Orientadora: Profa. Dra. Milene Miranda Praça-Fontes

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Comissão Examinadora:

Dra. Milene Miranda Praça Fontes
Universidade Federal do Espírito Santo
(Orientadora)

Dra. Isane Vera Karsburg
Universidade Estadual do Mato Grosso
(Membro externo)

Dra. Tatiana Tavares Carrijo
Universidade Federal do Espírito Santo
(Membro interno)

Dra. Stefanie Cristina de Oliveira
(Membro externo)

Dr. Fábio Demolinari de Miranda
Universidade Federal do Espírito Santo
(Membro interno)

DEDICO

Aos meus pais Divina e Antônio,

A minha esposa Jéssica,

A minha filha Mariana.

OFEREÇO

A minha orientadora Milene

"Pra quem tem pensamento forte, o impossível é só questão de opinião".

Charlie Brown Jr.

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BIOGRAFIA

Darley Aparecido Tavares Ferreira, filho de Antônio Ferreira e Divina Tavares Ferreira e nascido em Alta Floresta, Mato Grosso em 18 de setembro de 1984. Ingressou no ano de 2008 no curso de Licenciatura Plena em Ciências Biológicas na Universidade Estadual do Mato Grosso – UNEMAT campus Alta Floresta – MT, obtendo em 2012 o título de licenciado em Ciências Biológicas. No primeiro semestre de 2013, ingressou no Programa de Pós-Graduação em Produção Vegetal do Centro de Ciências Agrárias da Universidade Federal do Espírito Santo, em nível de Mestrado atuando na área de Biotecnologia e Ecofisiologia do Desenvolvimento de Plantas, mais especificamente, na área de Cultura de Tecidos e Citogenética de Plantas, sob a orientação do Prof. Dr. Wellington Ronildo Clarindo, submetendo-se à defesa de dissertação em agosto de 2014. Ainda no segundo semestre de 2014 ingressou no doutoramento pelo Programa de Pós-Graduação em Genética e Melhoramento da Universidade Federal do Espírito Santo, atuando na linha de pesquisa Citogenética e Biologia Evolutiva, sob a orientação inicial do Prof. Dr. Wellington Ronildo Clarindo e final da Profa Dra. Milene Miranda Praça-Fontes, submetendo-se à defesa da Tese em 27/08/2018.

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RESUMO

Passiflora L. compreende cinco subgêneros subdivididos em 16 superseções, 31 seções e 13 séries. O gênero têm sido estudado sob diversos aspectos, botânicos, sistemáticos e evolutivos. Entretanto, sua delimitação em nível infragenérico ainda é passível de discussão. Dados clássicos do cariótipo e o tamanho do genoma nuclear têm sido considerados informativos para o refinamento desse conhecimento. Com base nesse cenário, o presente estudo teve dois focos: a) revisar, ampliar e atualizar as informações sobre o cariótipo e o valor nuclear 2C em espécies pertencentes aos subgêneros *Astrophea*, *Decaloba*, *Deidamioides* e *Passiflora*. b) analisar os dados no ponto de vista da sistemática e evolução. Como resultado, o conhecimento do número cromossômico foi expandido para nove espécies e confirmado para 19. A classe dos cromossomos foi conhecida para 19 espécies e reavaliada para nove. O cariograma foi estabelecido para todas as espécies analisadas, sendo em 24 delas pela primeira vez e em quatro atualizados. As contagens do número de cromossomos revelaram 2n = 12 para o subgênero *Decaloba*, 2n = 18 e 2n = 20 para o subgênero *Passiflora*, 2n = 24 nos subgêneros *Astrophea* e *Deidamioides* e o inédito 2n = 48 cromossomos para *P. contracta* (subgênero *Deidamioides*). A classe dos cromossomos variou entre pares metacêntricos e submetacêntricos com exceção de alguns acrocêntricos em *P. lindeniana* (dois pares) e *P. arborea* (três pares), ambas do subgênero *Astrophea*. A presença desses cromossomos acrocêntricos evidênciou a provável ocorrência de disploidia dentro do

gênero. O conhecimento do tamanho do genoma no gênero *Passiflora* também foi ampliado para 19 espécies e atualizados para 22. Os valores 2C apresentaram diferenças de até 925% entre algumas espécies, onde o menor valor 2C encontrado foi 0,59 pg (*P. capsularis* - *Decaloba*) e o maior 5,46 pg (*P. quadrangularis* - *Passiflora*). Para algumas espécies o aumento no tamanho do genoma está correlacionado com o aumento no número de cromossomos, uma consequência da poliploidia. Já em outras, esta relação não foi observada sugerindo a ocorrência de rearranjos estruturais. Em conclusão, o cariótipo e o tamanho do genoma nuclear do gênero *Passiflora* sugerem diversificação por poliploidia e disploidia ao longo da evolução. O número x = 6 é provavelmente o número cromossômico ancestral do gênero. Além disso, as análises de dados foram complementares às abordagens sistemáticas do gênero e forneceram suporte para a atual classificação subgenérica deste táxon.

Palavras chave: citogenética, citometria de fluxo, cariograma, tamanho do genoma nuclear, passiflora

FERREIRA, Darley Aparecido Tavares Ferreira, D.Sc., Universidade Federal Do Espírito Santo, Centro de Ciências Agrárias e Engenharias, agosto de 2018. **Karyotype and nuclear DNA content of *Passiflora* L.: a contribution to, systematics and evolution of the genus.** Orientadora: Milene Miranda Praça-Fontes.

ABSTRACT

Passiflora L. comprises five subgenera subdivided into subdivided into 16 supersections, 31 sections and 13 series. The genus has been studied in several aspects, botanical, systematic and evolutionary. However, its delimitation at the infrageneric level is still subject to discussion. Classical karyotype data and the size of the nuclear genome have been considered informative for the refinement of this knowledge. Based on this premise, the present study had two focus: a) revisiting, expanding and updating the information on karyotype and nuclear value 2C in species belonging to the subgenus *Astrophea*, *Decaloba*, *Deidamioides* and *Passiflora*. b) Analyze the data in the viewpoint of the systematic and evolution. As result, chromosome number knowledge was expanded for nine species and confirmed for 19. Chromosomes class was determinated for 19 species and reevaluated for nine. In all species analyzed the karyogram was assembled, being in 24 taxa for the first time and in four updated. Chromosome number counts revealed 2n = 12 for the subgenus *Decaloba*, 2n = 18 and 2n = 20 for the subgenus *Passiflora* and 2n = 24 on the subgenera *Astrophea* and *Deidamioides* and the unpublished 2n = 48 chromosomes in *P. contracta* (subgenus *Deidamioides*). Chromosome class ranged between metacentrics and submetacentrics pairs with exception of some acrocentrics in *P. lindeniana* (two pairs) and *P. arborea* (three pairs), both of the subgenus *Astrophea*. This presence of acrocentrics chromosomes evidenced

probable occurrence of dysploidy within the genus. The knowledge of genome size in the genus *Passiflora* was also extended to 19 species and updated to 22. Nuclear 2C value exhibited differences up to 925% betweem species, where the lowest value found was 0.59 pg (*P. capsularis* - *Decaloba*) and the greater 5.46 pg for (*P. quadrangularis* - *Passiflora*). For some species the increase in genome size is correlated with the increase of the chromosome number, a consequence of the polyploidy. Already in others, this relation was not observed suggesting the occurrence of structural rearrangements. In conclusion, the karyotype and genome size of the genus *Passiflora* suggest diversification by polyploidy and disploidia. The number x = 6 is probably the ancestral chromosome number of the genus. Moreover, the data analyzes were complementary with systematic approaches of the genus and provided support for the current subgeneric classification of this taxon.

Key words: cytogenetics, flow cytometry, kayograms, nuclear genome size, passionflowers.

I. INTRODUÇÃO

Passiflora L. é o maior gênero da família Passifloraceae com cerca de 577 espécies distribuídas no Neotrópico (PÉRES et al., 2018). Representado por trepadeiras herbáceas e lenhosas, arbustos e árvores, o gênero apresenta ampla variabilidade de caracteres vegetativos e florais. O histórico taxonômico do gênero está representado por diferentes propostas de classificações que divergem em número de subgêneros, incluindo outras características infragenéricas (KILLIP 1938; FEUILLET; MACDOUGAL 2003; MUSCHNER et al., 2003; YOCKTENG; NADOT, 2004; HANSEN et al., 2006; MUSCHNER et al., 2012). Apesar das várias contribuições disponíveis, o tema parece estar longe de uma definição.

Killip (1938) propôs a divisão em 22 subgêneros 13 seções e 23 séries. Posteriormente, Escobar (1989) adicionou mais um subgênero a esta conformação. Já Feuillet; MacDougal (2003), propuseram a redução desta classificação para quatro subgêneros (*Astrophea*, *Decaloba*, *Deidamioides* e *Passiflora*) e estes subdivididos em 16 superseções, 31 seções e 13 séries. Análises da sistemática filogenética com uso de diferentes marcadores moleculares forneceram suporte parcial ou total a este rearranjo (MUSCHNER et al., 2003; YOCKTENG; NADOT, 2004; HANSEN et al., 2006; MUSCHNER et al., 2012). Entretanto, subgêneros adicionais vêm sendo sugeridos tornando as delimitações referidas ainda não satisfatórias (YOCKTENG; NADOT, 2004; MUSCHNER et al., 2012; KROSNICK et al., 2009). Além disso, diferentes autores apontam inconsistências em outras categorias infra-subgenéricas sugerindo revisões (MEZZONATO-PIRES et al., 2017a; ROME; D'EECKENBRUGGE, 2017; MEZZONATO-PIRES et al., 2017b; OCAMPO; COPPENS D´EECKENBRUGGE; 2017).

O status dos dados citogenéticos clássicos e do tamanho do genoma nuclear é considerado informativo para tomadas de decisões no âmbito da taxonomia, sistemática e

evolução (STACE, 2000). Em *Passiflora*, grande parte dos estudos investiram na contagem do número cromossômico que se encontra disponível para 26% das espécies do gênero. Entretanto, outros aspectos importantes como a morfometria e classificação dos cromossomos abrangem 5,2% e o estabelecimento do cariograma 1% de todas espécies. Quanto ao tamanho do genoma nuclear, embora a citometria de fluxo seja um método consolidado para o mensuramento, somente 12% das espécies do gênero possuem os valores do conteúdo de DNA determinados. Além disso, a maioria das espécies analisadas por estas ferramentas representaram somente os subgêneros *Decaloba* e *Passiflora*.

O gênero *Passiflora* também apresenta ampla variabilidade para o número de cromossomos. Considerando as espécies com contagens reportadas na literatura, seis grupos cariológicos podem ser definidos: a) 2n = 12, 24, 36; b) 2n = 14; c) 2n =18, 36; d) 2n = 20; e) 2n = 22; e f) 2n = 24 cromossomos (STOREY, 1950; SNOW; MACDOUGAL, 1993; DE MELO et al., 2001; e DE MELO; GUERRA, 2003; BELO et al., 2015). Entretanto, a definição do número de cromossomos ancestral para o gênero ainda é conflituosa e possui diferentes proposições: x = 3 (STOREY, 1950), x = 9 (SNOW; MACDOLGAL, 1993), x = 6 (DE MELO et al., 2001) e x = 12 (HANSEN et al., 2006). A proposta de x = 6 têm sido a mais aceita, sendo corroborada por análises da citogenética molecular com base no número e posição dos sítios rDNA 5S e 45S (DE MELO; GUERRA, 2003). Em contrapartida, Hansen et al. (2006) consideraram esta hipótese convincente mais não conclusiva. Então, apoiado em árvores filogenéticas moleculares, estes autores sugeriram que considerar x = 12 como ancestral é mais parcimonioso. De fato, esta é uma questão que ainda permanece em aberto para o gênero *Passiflora.*

Assim como o número cromossômico, alguns estudos têm evidenciado que o tamanho do genoma nuclear em espécies do gênero *Passiflora* também varia substancialmente. Souza et al. (2004) empregando a citometria de fluxo em oito espécies,

reportaram valores 2C entre 1,83 pg (*Passiflora suberosa* L.) a 5,36 pg (*Passiflora quandrangularis* L.). Usando a mesma ferramenta, Yotoko et al., (2011) investigaram 50 espécies e os valores 2C encontrados variaram de 0,52 pg para *Passiflora palmeri* Killip a 4,41 pg para *Passiflora alata* Curtis. Estas análises têm contribuído em estudos de caracterização de genótipos (SOUZA et al., 2004), evolutivos (YOTOKO et al., 2011) e como caracter taxonômico (AMORIM et al., 2014).

Dentro deste contexto, há necessidade de revisar, expandir e atualizar as informações clássicas do cariótipo e do tamanho do genoma de *Passiflora* visando construir uma matriz de dados que abarque além do número cromossômico, outras informações, como a classificação dos cromossomos, montagem do cariograma e conteúdo de DNA nuclear. Estes dados podem ser úteis para melhorar a compreensão taxonômica, sistemática e evolutiva do gênero *Passiflora.*

II. JUSTIFICATIVA DO TRABALHO

A construção de uma matriz de dados abrangendo informações sobre número cromossômico, classe dos cromossomos, cariograma e conteúdo de DNA nuclear podem revelar informações valiosas para melhorar a compreenção destas lacunas. Apesar dos avanços da biologia molecular e citogenética molecular, que tem produzido uma enorme riqueza de dados, as informações tradicionais continuam sendo de grande relevância para que eles possam atingir seu pleno impacto.

III. OBJETIVOS

3. 1. Objetivo geral

O objetivo do trabalho foi revisar, expandir e atualizar a caracterização do genoma do gênero *Passiflora* quanto ao cariótipo e tamanho do genoma nuclear e avaliar o potencial dos dados sob a luz da sistemática e evolução do cariótipo.

3. 2. Objetivos específicos

Determinar o número de cromossomos, caracterizar morfométricamente os cromossomos e estabelecer o cariograma para espécies pertencentes aos subgêneros *Astrophea*, *Decaloba*, *Deidamioides* e *Passiflora*;

Estimar por meio da citometria de fluxo o conteúdo de DNA nuclear valor 2C em espécies dos subgêneros *Astrophea*, *Decaloba*, *Deidamioides* e *Passiflora*;

Identificar evidências do cariótipo e do tamanho do genoma nuclear que possam contribuir com a compreensão sistemática e evolutiva do gênero *Passiflora*.

IV. REVISÃO BIBLIOGRÁFICA

4.1. O gênero Passiflora

Passiflora L. é o gênero mais representativo dos 16 gêneros que compõem a família Passifloraceae abarcando cerca de 577 espécies (PÉRES et al., 2018). Seus representantes podem ser encontrados desde o sul da Argentina estendendo-se até a América Central, México e sul dos Estados Unidos. Além disso, algumas espécies também habitam a Ásia e Ilhas do Pacífico Sul (MUSCHNER et al., 2003; HANSEN et al., 2006).

Economicamente o gênero *Passiflora* é reconhecido pelo cultivo de algumas espécies para produção de frutas consumidas *in atura* ou em forma de sucos (SANTOS et al., 2014). Apresenta também potencial ornamental e medicinal, sendo fonte de matéria prima na produção de diversos fármacos (YOCTENG; NADOT, 2004). Além dos aspectos econômicos, as passifloras assumem papel importante nas interações ecológicas proporcionando néctar a diferentes organismos polinizadores como abelhas, vespas, aves e morcegos, sendo assim, consideradas potenciadoras da biodiversidade (FARIA; STEHMANN, 2010).

As espécies que representam o gênero *Passiflora* são caracterizadas morfologicamente por plantas trepadeiras, arbustivas e árvores. Suas folhas são simples, inteiras ou lobadas, sempre alternas, com margem inteira ou serrilhada, podendo possuir ou não glândulas nectarianas (FEUILLET; MACDOUGAL, 2007; YOCKTENG; NADOT, 2004). As flores de *Passiflora* são hermafroditas, compostas por cinco pétalas e cinco sépalas, cinco estames e três estigmas, e apresentam uma ampla gama de formas e cores. Outra característica floral marcante é a presença da corona de filamentos na maioria das espécies exibindo formas, tamanho e cores variadas. Além da diversidade floral, os frutos das

passifloras também apresentam grande heterogeneidade, com tamanhos pequenos, médios e grandes, nas formas ovoides, globosas e fusiformes (ULMER; MACDOUGAL, 2004).

4.2. Classificação taxonômica do gênero Passiflora

O gênero *Passiflora* foi descrito por Linnaeus (1753) durante o período colonial espanhol na América do Sul. Inicialmente, De Candolle (1828) reconheceu oito seções para o gênero *Passiflora* as quais foram denominadas de *Astrophea*, *Polyanthea, Tetrapathea*, *Cieca, Decaloba, Granadilla, Tacsonioides e Dysosmia.* Quase um século depois, Harms (1925) organizou o gênero em três seções: *Decaloba, Tryphostemmatoides* e *Tetrastylis*, sendo a primeira subdividida nas subseções *Polyanthea*, *Cirrhiflora* e *Deidamioides*.

Killip (1938), analisando caracteres morfológicos (principalmente florais) em mais de 350 espécies, propôs uma complexa divisão do gênero *Passiflora* em 22 subgêneros 13 seções e 23 séries. Posteriormente, Escobar (1989) adicionou mais um subgênero a essa conformação totalizando 23 taxa. Diversos pesquisadores ainda utilizam esta classificação que pode ser conhecida na Figura 1:

Gênero *Passiflora* segundo Killip (1938) e Escobar (1989)*.

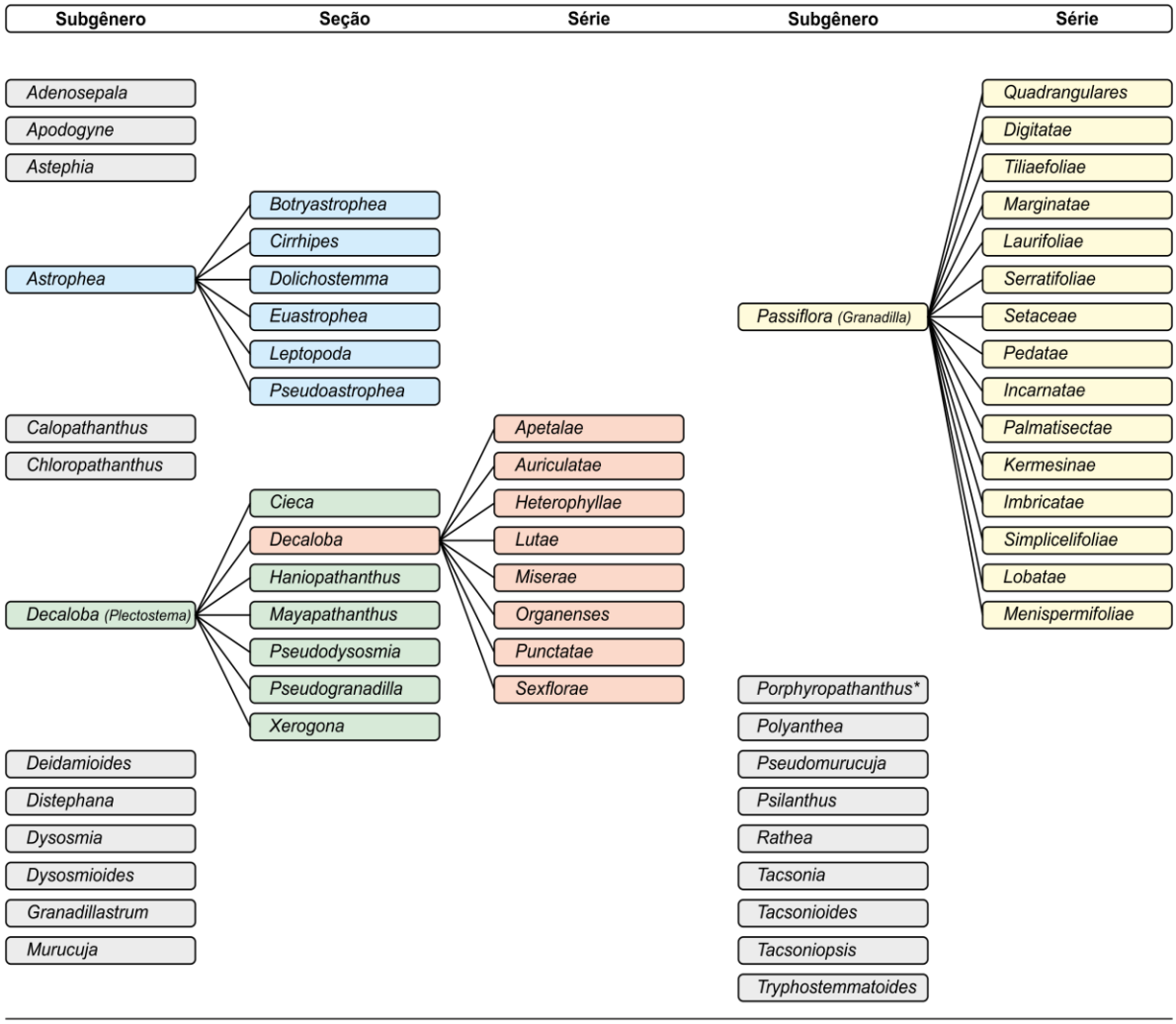

Figura 1. Esquema representativo da classificação infragenérica do gênero *Passiflora* em 23 subgêneros, 13 seções e 23 séries.

Também apoiado em caracteres morfológicos e ecológicos, Feuillet; MacDougal (2003), propuseram uma nova classificação taxonômica para o gênero. Para estes autores, *Passiflora* se divide em somente quatro subgêneros os quais foram chamados de *Astrophea*, *Decaloba*, *Deidamioides* e *Passiflora*. Estes subgêneros foram subdivididos em 16 superseções, 31 seções e 13 séries conforme demonstrado na Figura 2. Apesar de alguns autores optarem pela classificação anterior, esta têm sido a mais aceita.

Gênero *Passiflora* segundo Feuillet; MacDougal (2003)

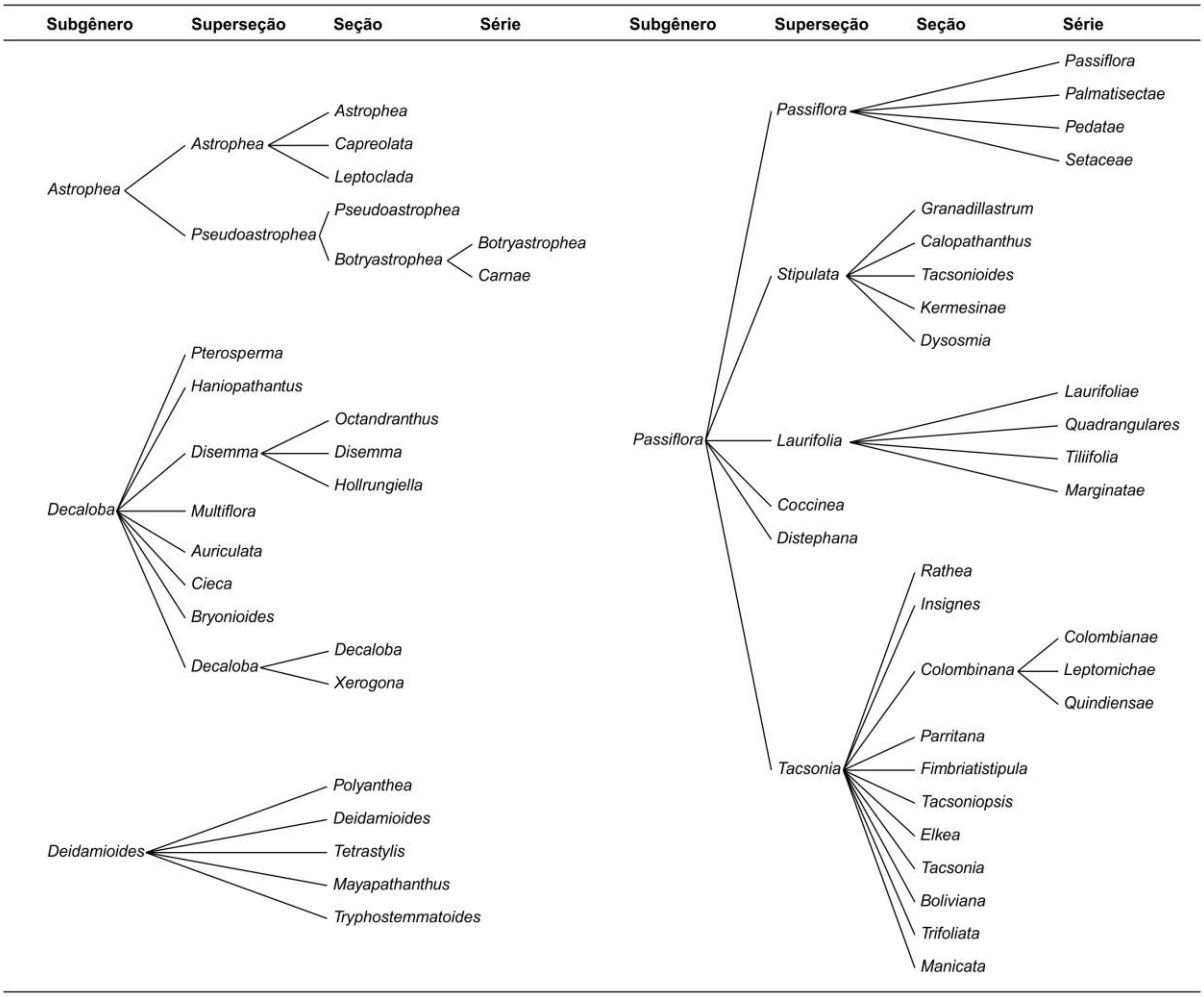

Figura 2. Esquema representativo da classificação infragenérica do gênero *Passiflora* em quatro subgêneros, 16 superseções, 31 seções e 13 séries.

A redução do número de subgêneros dentro do gênero *Passiflora* também têm sido sustentada por diferentes análises filogenéticas moleculares. A primeira evidência foi reportada por Downie et al. (1996) que avaliou a ausência ou presença do intron *rpoCl* (gene do cloroplasto) em 10 espécies do gênero. Neste estudo, espécies pertencentes ao atual subgênero *Decaloba* tiveram a presença do intron *rpoCL* retida enquanto em espécies do subgênero *Passiflora* o gene estava presente. Entretanto, o tamanho da amostra foi pequeno para inferências mais conclusivas.

Posteriormente, Muschner et al. (2003) apresentaram a primeira filogenia molecular do gênero *Passiflora* para 61 espécies representando 11 subgêneros dos 23 propostos por

Killip (1938) e Escobar (1989). Neste trabalho foram utilizados os espaçadores internos transcritos do DNA ribossômico e nuclear (nrITS), as regiões espaçadoras plastidais *trnL-trnF* e o gene plastidial *rps4*. Como resultado, três clados foram fortemente apoiados (bootstrap > 99) a qual foram chamados *Astrophea*, *Decaloba* e *Passiflora*, corroborando com a nova classificação proposta por Feuilet; MacDougal (2003).

Em seguida, Yockteng; Nadot (2004) avaliaram as relações filogenéticas de 90 espécies do gênero *Passiflora* a partir do gene nuclear da glutamina sintetase expresso em cloroplasto (ncpGS). Estes autores também corroboraram a classificação de Feuilet e MacDougal (2003), mas sugeriram a manutenção de três subgêneros de Killip (1938): *Polyanthea*, *Dysosmia* e *Tetrapathea*.

Inspirados na investigação de Downie et al (1996), Hansen et al. (2006) expandiram a investigação das relações filogenéticas a partir do intron *rpoCL* para 136 espécies representando 17 subgêneros de Killip (1938). Além disso utilizaram também as regiões espaçadoras do cloroplasto *trnL/trnT* em 61 espécies. Os resultados destas análises também suportaram a redução do número de subgêneros como propostos por Feuilet; MacDougal (2003).

Krosnick; Freudenstein (2005) analisaram por meio das sequências de DNA plastidial (*trnL-F* intron e espaçador) e nuclear (ITS) as relações filogenéticas da superseção *Disemma*, taxon do subgênero *Decaloba* que habita o velho mundo. Nesta investigação, os pesquisadores incluíram os gêneros monotípicos *Hulungria* e *Tetrapathea* que foram fortemente apoiados como membros do gênero *Passiflora*. Ancorado nesta informação, Krosnick et al., (2009) descreveram um novo subgênero para o gênero *Passiflora* a qual denominaram de subgênero *Tetrapathea*.

Muschner et al., (2012) analisaram 106 espécies representando os quatro subgêneros de Feuilet; MacDougal (2003). Sete regiões de DNA foram estudadas, compreendendo

genomas plastidial, mitocondrial e nuclear. As análises reconheceram os subgêneros *Astrophea*, *Decaloba* e *Passiflora*. No entanto, para o subgênero *Deidamioides* os autores sugeriram uma revisão taxonômica alegando que a seção *Tryphostematoides* poderia ser considerada como um novo subgênero.

4.3. Número cromossômico e evolução do cariótipo em Passiflora

Estudos baseados na citogenética clássica determinaram o número cromossômico para diferentes espécies do gênero *Passiflora* a qual podemos destacar os mais representativos os de STOREY, 1950; SNOW & MACDOUGAL 1993; DE MELO et al. 2001, DE MELO E GUERRA 2003; HANSEN et al. 2006; DE MELO et al. 2014. Storey (1950) reportou a ocorrência de 2n = 12, 2n = 18, 2n = 20, 2n = 24 e 2n = 36 cromossomos. Diante desses dados, foi sugerido pela primeira vez que x = 3 ou x = 6 poderiam ser os números de cromossomos ancestral do gênero. Para assumir x = 6 como número básico, o autor menciona que as espécies com 2n = 18 cromossomos deveriam ser consideradas triploides. Geralmente, a triploidia é considerada um obstáculo para geração de espécies férteis devido a ocorrência substancial de irregularidades meióticas. Como essa situação não foi observada nas espécies investigadas, Storey considerou a hipótese pouco provável e sugeriu que x = 3 forneceria explicações mais plausíveis para a origem das espécies com 2n = 18 cromossomos

Subsequente, Raven (1975) reportou que x = 9 poderia ser o número de cromossomos ancestral do gênero *Passiflora* e que as espécies com n = 6 seriam taxa derivadas. Em contrapartida, Morawetz (1986) apoiou x = 6 resgatando novamente a possibilidade de espécies com 2n = 18 cromossomos terem uma origem triploide. Para tanto, ou autor apoiou-se na ausência de uma série disploide entre 2n = 12 e 2n = 18 cromossomos

e, também, em uma espécie triploide x = 9 (*Passiflora caponii* 2n = 27) documentada por Fedorov (1969).

Posteriormente, o número básico x = 9 foi novamente proposto para o gênero *Passiflora* no trabalho de Snow e MacDougal (1993). Como justificativa, foi considerado a simetria do cariótipo onde espécies com 2n = 18 exibiram cariotipos mais simétricos em relação as espécies com 2n = 12 cromossomos. De acordo com as considerações de Stebbins (1971), esta característica seria uma condição de ancestralidade.

Para De Melo et al. (2001) e De Melo et al., (2003), as espécies do gênero *Passiflora* estão divididas cariologicamente em quatro grupos: x = 6 (2n = 12, 24, 36), x = 9 (2n = 18), x = 10 (2n = 20) e x = 12 (2n = 24). Embora o número de cromossomos tenha sido diferente entre as espécies, x = 6 foi proposto como número ancestral do gênero. Para tanto, foi sugerido que os demais números cromossômicos teriam surgido como consequência de alterações cromossômicas envolvendo a poliploidia (x = 6 para x = 12), seguido por disploidia descendente (x = 12 para x = 10 e x = 9). Esta hipótese foi fortemente corroborada pela citogenética molecular quanto ao número e a posição dos sítios rDNA 5S e 45S.

Não obstante, Hansen et al. (2006) consideraram a proposta de x = 6 convincente, mas não conclusiva. Então, ancorados em arvores filogenéticas moleculares, propuseram que x = 12 seria mais parcimonioso como número básico de cromossomos do gênero *Passiflora*. Para os autores, x = 12 requer um passo evolutivo a menos comparado x = 6. Além disso, é representado em espécies com características morfológicas consideradas ancestrais e em outros gêneros da família Passifloraceae. No entanto, reconhecem que um único passo evolutivo é pouco para designar o número básico de cromossomos e sugeriram a expansão dos dados citogenéticos em *Passiflora*, especialmente a contagem de cromossomos, envolvendo táxons pouco conhecidos, como o subgênero *Astrophea* e *Deidamioides*.

4.4. Conteúdo de DNA nuclear de Passiflora

Empregando a citometria de fluxo, Souza et al. (2004) mensuraram o conteúdo de DNA nuclear (em picogramas, pg) de sete espécies de *Passiflora* com a finalidade de caracterizar genótipos de interesse. Esses autores reportaram diferenças significativas para o tamanho do genoma nuclear dentro do gênero. Os valores obtidos variaram entre 1,83 pg (*Passiflora suberosa* L., 4x = 24 cromossomos) a 5,36 pg (*Passiflora quandrangularis* L., 2x = 18 cromossomos).

Em uma abordagem evolutiva, Yotoko et al., (2011) também empregaram esta ferramenta e avaliaram 50 espécies pertencentes aos subgêneros *Decaloba* e *Passiflora*. Neste trabalho a variação no tamanho do genoma também foi significativa. O menor tamanho de genoma encontrado foi 2C = 0,52 pg para *Passiflora palmeri* Killip e o maior 2C = 4,41 pg para *Passiflora alata* Curtis. Estes valores foram comparados com diâmetro da flor para avaliar se as diferenças no tamanho do genoma refletem processos adaptativos ou neutros (ganho ou perda de DNA ao longo da evolução). As análises evidenciaram correlações positivas entre os caracteres quando se considerou os dois subgêneros juntos ou só o subgênero *Passiflora*. O mesmo não foi constatado para as espécies do subgênero *Decaloba*. Diante disso, os autores discutiram que as variações no tamanho do genoma de *Passiflora* refletem um processo adaptativo, e que as espécies com genomas menores podem ter surgido por perda de DNA das espécies com genomas maiores. Para chegar a esta inferência, os autores se apoiaram no modelo proporcional de evolução, em que taxas mais rápidas ocorrem em genomas maiores. Assim, seria mais difícil genomas pequenos se tornarem e permanecerem maiores.

Amorim et al., (2014) mensuraram o tamanho do genoma nuclear de *Passiflora capsularis* L. e *Passiflora rubra* L. que apresentaram 2C = 057 pg e 2C = 062 pg

respectivamente. Estes dados foram utilizados como um caracter taxonômico na avaliação dessas espécies que eram confundidas. Com o tamanho de genoma semelhante associado a informações morfológicas, citogenéticas e moleculares, os dois taxa foram consideradas variedades de uma mesma espécie.

Considerando estes estudos, o tamanho do genoma nuclear é conhecido para 70 espécies, o que corresponde a somente 12 % das espécies do gênero. Além disso, as espécies amostradas representam somente os subgêneros *Decaloba* (14 espécies) *Passiflora* (56 species) e *Deidamioides* (1 espécie) sumarizados na Tabela de Dados Suplementares S1. Isso reforça a necessidade de realização de novas análises para expandir o conhecimento acerca do tamanho do genoma nuclear deste taxon tão importante em *Passiflora.*

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VI. ARTIGO

Observação: manuscrito formatado de acordo com as normativas da revista **Annals of Botany**

RESEARCH IN CONTEXT

Cytogenetics and flow cytometry in *Passiflora* L: Systematics and evolution

Darley Aparecido Tavares Ferreira[1], Milene Miranda Praça-Fontes[1*], Ariane Tonetto Vieira[1], Andrei Caique Pires Nunes[2], Adésio Ferreira[1], Wellington Ronildo Clarindo[3]

[1]Laboratório de Citogenética e Cultura de Tecidos, Centro de Ciências Agrárias e Engenharias, Universidade Federal do Espírito Santo. ZIP: 29.500-000 Alegre – ES, Brazil.

[2]Universidade Federal do Sul da Bahia, Campus Jorge Amado. Centro de Formação em Ciências e Tecnologias Agroflorestais. ZIP: 45.613-204 Itabuna-BA, Brazil.

[3]Laboratório de Citogenética e Citometria, Departamento de Biologia Geral, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Viçosa. ZIP: 36.570-000 Viçosa – MG, Brazil.

**For correspondence: E-mail milenemiranda@yahoo.com*

ABSTRACT

• **Background and aims** The systematics and evolutionary history of the genus *Passiflora* is controversial, and various taxonomic classifications and ancestral chromosome numbers have been proposed. Here we examined whether classical karyotype data and nuclear genome size can be informative and contribute to a better understanding of these gaps.

• **Methods** We applied classical cytogenetics to revise the chromosome number of 19 species, and define it for further nine. The classification of chromosome pairs was re-evaluated for nine species and established for further 19. The karyogram was assembled for the first time for 24 of the species, and updated for the other four. Using flow cytometry, the nuclear DNA content measurement was revisited for 22 species and expanded for 19. The data were analyzed in light of the evolutionary and systematics knowledge about the genus *Passiflora*.

• **Key results** The chromosome number 2n = 12 was found for the subgenus *Decaloba*, as well as 2n = 18 and 2n = 20 for *Passiflora*, 2n = 24 for *Astrophea* and *Deidamioides*, and the unpublished 2n = 48 for *Passiflora contracta* (subgenus *Deidamioides*). Metacentric and submetacentric chromosomes predominated in all karyotypes, except *P. lindeniana* and *P. arborea* (subgenus *Astrophea*), which presented some acrocentric pairs. The nuclear 2C value ranged from 0.59 pg (*P. capsularis*) to 5.46 pg (*P. quadrangularis*), with the differences thus exceeding 800%.

• **Conclusions** The karyotypes and nuclear genome size of the genus *Passiflora* suggest diversification by polyploidy and dysploidy. The number x = 6 is probably the ancestral chromosome number of the genus. Furthermore, data analyses were complementary to systematics approaches of the genus, and provided support to the current classification of this taxon.

Key words: Cytogenetics, karyogram, flow cytometry, nuclear genome size, systematic karyotype evolution, *Passiflora* L.

INTRODUCTION

Passiflora L. is the most representative genus within the family Passifloraceae, comprising about 577 species found in the neotropical flora (Pérez *et al*., 2018). Composed of herbaceous plants, woody vines, shrubs and small trees, the genus presents wide variability of vegetative and floral characters. The systematics history of *Passiflora* is marked by various proposed classifications that differ in number of subgenera, including other infrageneric characteristics (Killip, 1938; Feuillet and MacDougal, 2003; Muschner *et al*., 2003; Yockteng and Nadot, 2004; Hansen *et al*., 2006; Muschner *et al*., 2012). Despite the various available contributions, this issue seems to still be far from a definition.

The genus *Passiflora* also exhibits karyotype diversity. Species with known chromosome number present different karyological groups: x = 6 (2n = 12, 24 and 36) in the subgenus *Decaloba*, x = 9 (2n = 18, 36 and 72) and x = 10 (2n = 20) in the subgenus *Passiflora*, and x = 12 (2n = 24) in the subgenera *Astrophea*, *Deidamioides* and *Tetrapathea* (De Melo and Guerra, 2003; Hansen *et al*., 2006). Other chromosome counts have been less frequently reported, such as for *Passiflora holosericea* and *P. lobata*, subgenus *Decaloba*, with 2n = 14 (Snow and MacDougal, 1993), and for *P. sublanceolata*, subgenus *Passiflora* (Belo *et al*., 2015), with 2n = 22. Considering this diversity in chromosome number, two hypotheses have been proposed based on the basic number of the genus *Passiflora*. First, De Melo *et al*. (2001) suggested x = 6 as ancestral condition, from which other numbers would have arisen by polyploidy (x = 6 → x = 12) with subsequent dysploidy (x = 10 and x = 9), being then considered basic secondary numbers. Differences in the number and chromosome position of CMA+ bands (De Melo *et al*., 2001) and of 5S and 45S rDNA sites (De Melo and Guerra, 2003) corroborate this hypothesis. In contrast, optimizing different chromosome numbers in parsimonious phylogenetic trees, Hansen *et al*. (2006) suggested x = 12 as the

basic chromosome number of the genus *Passiflora*. For these authors, x = 12 requires one evolutionary step less in comparison to x = 6 to explain the other chromosome numbers (x = 10, x = 9 and x = 6). Furthermore, x = 12 is present in another Passifloraceae genus, *Adenia* Forssk, a taxon phylogenetically related to *Passiflora.* Thus, certainty about the ancestral chromosome number of the genus *Passiflora* still lacks.

Karyotype and nuclear DNA content data are considered of great importance for systematics and evolutionary approaches (Stace, 2000). Furthermore, they constitute valuable information for genetic breeding strategies (Souza *et al*., 2004; Amorim *et al*., 2014). Accordingly, the chromosome number of approximately 150 species from the genus *Passiflora* L. has been determined (Storey, 1950; Snow and MacDougal, 1993; De Melo *et al*., 2001; De Melo and Guerra, 2003; Souza *et al*., 2003; Hansen *et al*., 2006; De Melo *et al*., 2014), as well as the nuclear DNA content of 70 species (Souza *et al*., 2004; Yotoko *et al*., 2011). Nevertheless, chromosome images are only available for about 80 species (Snow and MacDougal, 1993; Olaya Arias *et al*., 2002; De Melo and Guerra, 2003; Souza *et al*., 2003; Cuco *et al*., 2005; De Melo *et al*., 2014), chromosome morphometry for 30 species (Souza *et al*., 2003; Cuco *et al*., 2005; Amorim *et al*., 2014; De Melo *et al*., 2014; Belo *et al*., 2015), and karyograms for only six species (Cuco *et al*., 2005; Praça *et al*., 2008; Viana and Souza, 2012; Amorim *et al*., 2014). Moreover, most of this information is available only for species of the subgenera *Decaloba* and *Passiflora.* Therefore, it is imperative to revisit, expand and update the karyotype data of the genus *Passiflora* in order to increase the knowledge on the systematics and evolutionary history of this taxon.

The nuclear 2C value of species from the genus *Passiflora* has been measured in pre-breeding studies (Souza *et al*., 2004; Amorim *et al*., 2014) and evolutionary approaches (Yotoko *et al*., 2011). These works revealed nuclear genome size variations that exceeded 800% in species with divergent chromosome numbers. Among these, the largest genome size

was found for *P. quadrangularis* (2n = 18) with 2C = 5.36 pg (Souza *et al*., 2004), and the lowest for *P. organensis* (2n = 12) with 2C = 0.42 pg (Yotoko *et al*., 2011). Futhermore, differences between species with same chromosome number were also observed. In the subgenus *Passiflora* (2n = 18), Souza *et al*. (2004) identified genome sizes ranging from 2C = 3.16 pg in *P. edulis* to 2C = 5.36 in *P. quadrangularis*. Yotoko *et al*. (2011) reported 2C = 0.52 pg for *P. palmeri* and 2C = 4.42 pg for *P. alata*. For the subgenus *Decaloba* (2n = 12), the latter study reported differences ranging from 2C = 0.42 pg in *P. organensis* to 2C = 1.98 pg in *P. auriculata*. Despite these studies, little is known so far about the genome size in the genus *Passiflora*, with information available for less than 12% of the species.

Considering the above, the main aim of this study was to investigate whether the variations in chromosome number, chromosome class and nuclear genome size can be informative towards better understanding the systematics and evolutionary history of the genus *Passiflora*. In particular, we aimed to revise available data as well as provide new information about chromosome number and class, karyogram assembly and nuclear genome size for different species of four subgenera, considering intra-subgeneric levels. In addition, these genome features were reviewed for *Passiflora*.

MATERIAL AND METHODS

Collected material

Seeds of different species from the genus *Passiflora* were obtained from Atlantic Rainforest fragments in the state of Espírito Santo, Brazil; Amazon Rainforest fragments in the state of Mato Grosso, Brazil; forest fragments from the Atlantic Coast in Limón Province, Costa Rica; forest fragments in Oaxaca and Jalisco, Mexico; Brazil Plants Collection, Mogi

das Cruzes, São Paulo, Brazil; Collection Nationale Italie Greenhouse, Ripalta Cremasca, Italy; and personal collections of Harry Geilen, Amstenrade, and Arjen Lommen, Ede, the Netherlands.

In vitro plantlet recovery

Seeds of each *Passiflora* species were scarified; disinfested in laminar flow chamber by immersion in 70% ethanol for 1 min, followed by 0.1% Tween 20 supplemented with 2.5% sodium hypochlorite for 20 min; and washed four times in sterile distilled water. The seeds were inoculated into medium containing ½MS salts, 10 mL L^{-1} MS vitamins (Murashige and Skoog, 1962), 30 g L^{-1} sucrose and 7g L^{-1} agar. The seeds and resulting plantlets were cultivated under photoperiod with 16 h of light at 25 ± 2°C. The plantlets provided roots for cytogenetic analyses and young leaves for flow cytometry.

Chromosome number and karyogram

Karyotype characterization was performed in 28 species of the genus *Passiflora* (Supplementary Data Table S1). The analysis included the subgenera *Astrophea* (three species), *Decaloba* (eight species), *Deidamioides* (two species) and *Passiflora* (15 species). Roots were treated with 4 μM amiprophos-methyl (APM, Sigma®) for 16 h at 4°C. Subsequently, apical root meristems were washed with distilled water, fixed in solution of ethanol and acetic acid (3:1, Merck®), and stored at -20°C. After 24 h, the root meristems were macerated in enzymatic pool containing 4% cellulase (w/v), 0.4% hemicellulase (w/v), 1% macerozyme (w/v), 100% pectinase (v/v), at the concentrations ranging of 1:50 to 1:90 (pool : distilled water), for 2 h – 2 h:15 min at 34°C; fixed in ethanol : acetic acid (3:1,

Merck®); and stored at -20°C. The slides were prepared by root meristem dissociation and air-drying techniques and stained in 5% Giemsa (Merck®) for 5 min (Praça *et al*., 2008). Images of prometaphases and metaphases were captured using a Nikon 80i microscope (Nikon, Japan) equipped with a 100x Nikon Plan Fluor oil immersion objective of 1.30 numerical aperture and aplanat achromat condenser of 0.7 aperture, and coupled to a monochromatic CCD digital video camera DS-Fi1c of 8-bit gray (Nikon, Japan), in turn coupled to a Pentium Intel Core i5 computer (Thermaltake, Asus) featuring the NIS-Elements 3.0 imaging software (Nikon, Japan). The karyotype of each *Passiflora* species was characterized according to Levan *et al*. (1964), reviewed by Guerra (1986). Ten to 20 prometaphases/metaphases of each species were characterized for karyogram assembly.

Nuclear 2C value

The nuclear genome size was measured for species representative of the four *Passiflora* subgenera: *Astrophea* (four species), *Decaloba* (11 species), *Deidamioides* (two species) and *Passiflora* (24 species) (Supplementary Data Table S1). Flow cytometry was performed associating the procedures proposed by Galbraith *et al*. (1983), Otto (1990) and Praça-Fontes *et al*. (2011). For nuclei extraction, fragments of young leaves from the standard (*Solanum lycopersicum* L. ´Stupické´, 2C = 2.00 pg) and from each *Passiflora* species were simultaneously chopped (Galbraith *et al*., 1983) in Petri dishes containing 0.5 mL OTTO-I lysis buffer (Otto, 1990) supplemented with 2.0 mM dithiothreitol and 50 μg mL^{-1} of RNAse. Subsequently, 0.5 mL of the same buffer was added, the suspension was filtered through a 30-μm nylon mesh and centrifuged at a rate of 100 ×*g* for 5 min. The supernatant was discarded, and the pellet resuspended and incubated in 100 μL of OTTO-I lysis buffer for 10 min. The nuclei suspension was stained in 1.5 mL of OTTO-I : OTTO-II buffer (Otto, 1990)

supplemented with 2.0 mM dithiothreitol, 50 g mL^{-1} RNAse and 75 μM propidium iodide for 30 min in the dark, followed by filtration through 20-μm nylon mesh (Praça-Fontes *et al*., 2011). The nuclear suspensions were analyzed in flow cytometer equipped with a laser source (488 nm). The nuclear 2C value was derived from the ratio between fluorescence intensity of G_0/G_1 peaks of each sample and that of the standard. From the obtained histograms, the coefficients of variation were calculated using the FloMax software (Partec, Germany). For each *Passiflora* species, at least six repetitions were performed at three distinct days.

Statistical analysis

The mean 2C values of 41 *Passiflora* species were used for standardized Euclidean Distance calculations and subjected to UPGMA (*Unweighted Pair Group Method with Arithmetic Mean*) clustering, using Mojena's (1977) criteria. The same were used to karyological data (chromosome number and class) and mean 2C values of 27 *Passiflora* species. All analyses were carried out using the software GENES (Cruz, 2013).

RESULTS

Chromosome number and karyogram

Roots meristems treated with 4 μM APM for 16 h at 4ºC provided on average ten prometaphases/metaphases per slide. The use of the enzymatic pool, combined with the meristematic dissociation and air-drying techniques, provided prometaphases/metaphases free of cytoplasmic traces and without chromosome overlapping. Prometaphases with chromosomes at different compaction levels as well as metaphases were obtained (Figs. 1, 2),

exhibiting chromosomes with well-defined primary constriction. These cytological aspects were important to determine the chromosome number, characterize the chromosome morphometry and assemble at least ten karyograms for each species. Accordingly, such cytogenetic data was obtained for 28 *Passiflora* species, 13 of which were characterized here for the first time.

The chromosome number 2n = 12 was found for the species of the subgenus *Decaloba* (Figs. 1 and 3); 2n = 18 for the subgenus *Passiflora* (Figs. 2 and 4), except *P. foetida* with 2n = 20 (Fig. 4A); and 2n = 24 for the subgenus *Astrophea* (Fig. 5). In the subgenus *Deidamioides*, 2n = 24 was determined for *P. arbelaezii* (Fig. 6A) and 2n = 48 for *P. contracta* (Fig. 6B), the latter being a chromosome number previously unreported for the genus *Passiflora*.

In all subgenera, most of the species revealed karyotypes composed by metacentric and submetacentric chromosomes. Exceptions were *P. lindeniana* and *P. arborea* of the subgenus *Astrophea*, which presented two and three acrocentric pairs, respectively. Variations in the number of metacentric and submetacentric chromosome pairs were observed among the species. Within the subgenus *Decaloba* (Fig. 3), *P. micropetala* (Fig. 3D) presented five metacentric pairs (1, 2, 3, 5 and 6) and a submetacentric one (4). In contrast, *P. coriacea* (Fig. 3F) exhibited one metacentric (2) and five submetacentric pairs (1, 3, 4, 5 and 6). In turn, in *P. auriculata* (Fig. 3H) half of the chromosome pairs were metacentric (1, 2 and 6) and the other half submetacentric (3, 4 and 5). In the subgenus *Passiflora, P. foetida*, with 2n = 20 (Fig. 4A), displayed four metacentric pairs (4, 5, 6 and 8) and six submetacentric ones (1, 2, 3, 7, 9 and 10). Among the species with 2n = 18 chromosomes, *P. actinia* (Fig. 4B) showed six metacentric (3, 4, 5, 7, 8, and 9) and three submetacentric pairs (1, 2 and 6), while *P. speciosa* (Fig. 4F) exhibited two metacentric pairs

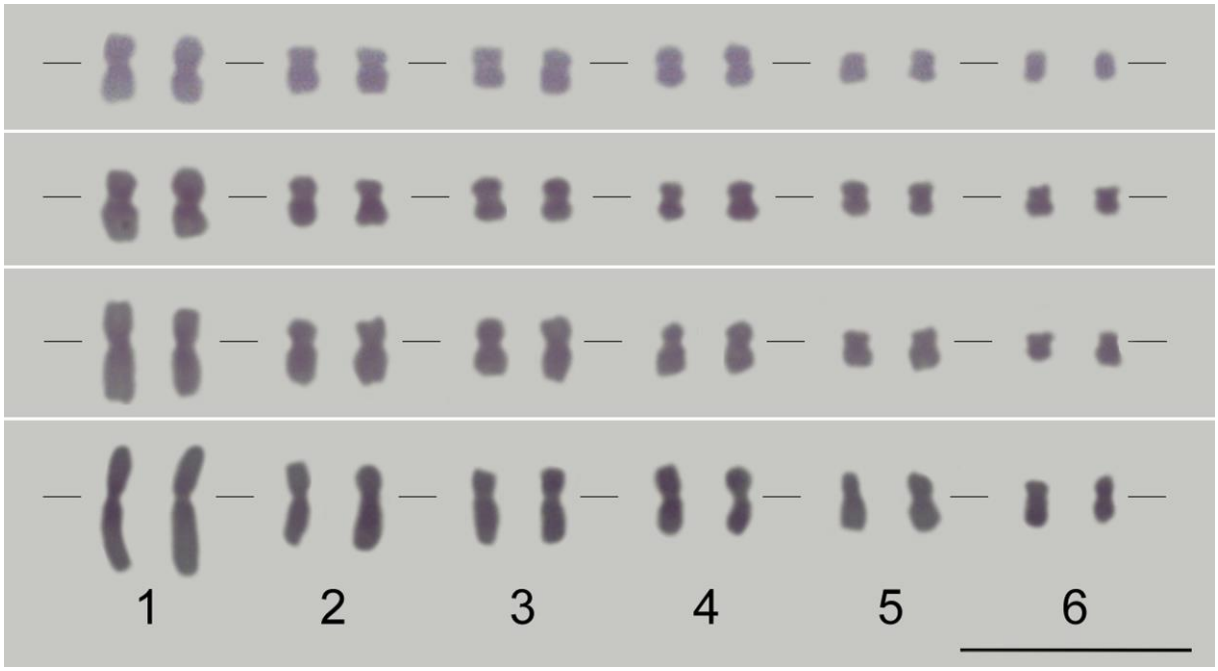

Fig. 1. Four representative karyograms of *P. megacoriacea* (2n = 12), supersection *Cieca*, subgenus *Decaloba*, showing higher (a) to lower chromatin compaction level (d). This difference is notable in the chromosome pair 1 of each karyogram. Chromosome pair identification was facilitated by karyograms assembled from chromosomes with well-defined telomeres and primary constrictions, as well as relatively lower compaction levels (d). Bar = 5 μm. Note: All karyograms shown in this study were stained with 5% Giemsa for 5 min.

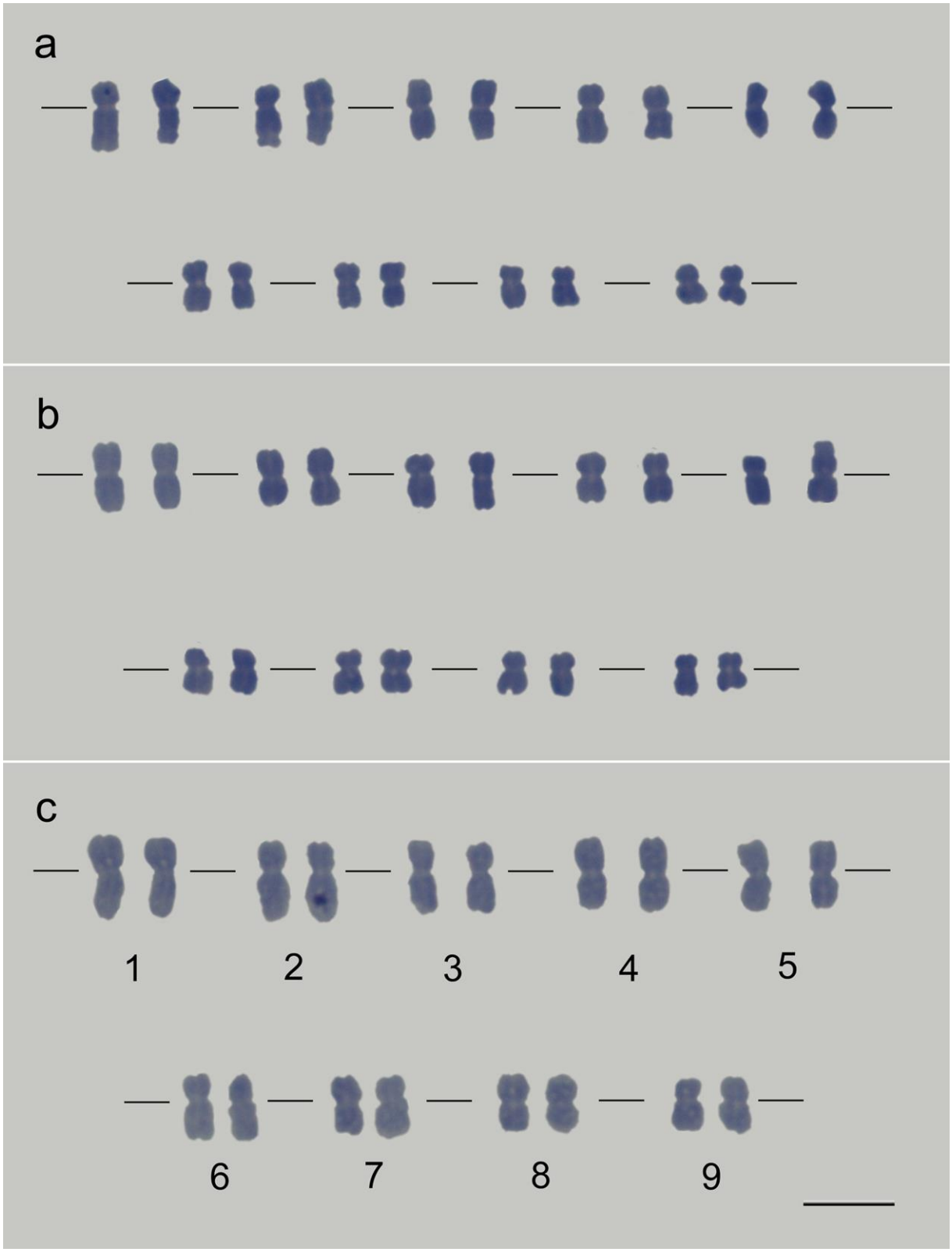

Fig. 2. Karyograms of *P. nigradenia* (2n = 18), supersection *Laurifolia*, subgenus *Passiflora*, presenting chromosomes with different chromatin compaction levels (a–c). Also note the distinct compaction levels in the homologous chromosome pair 5 of karyogram b. Bar = 5 μm.

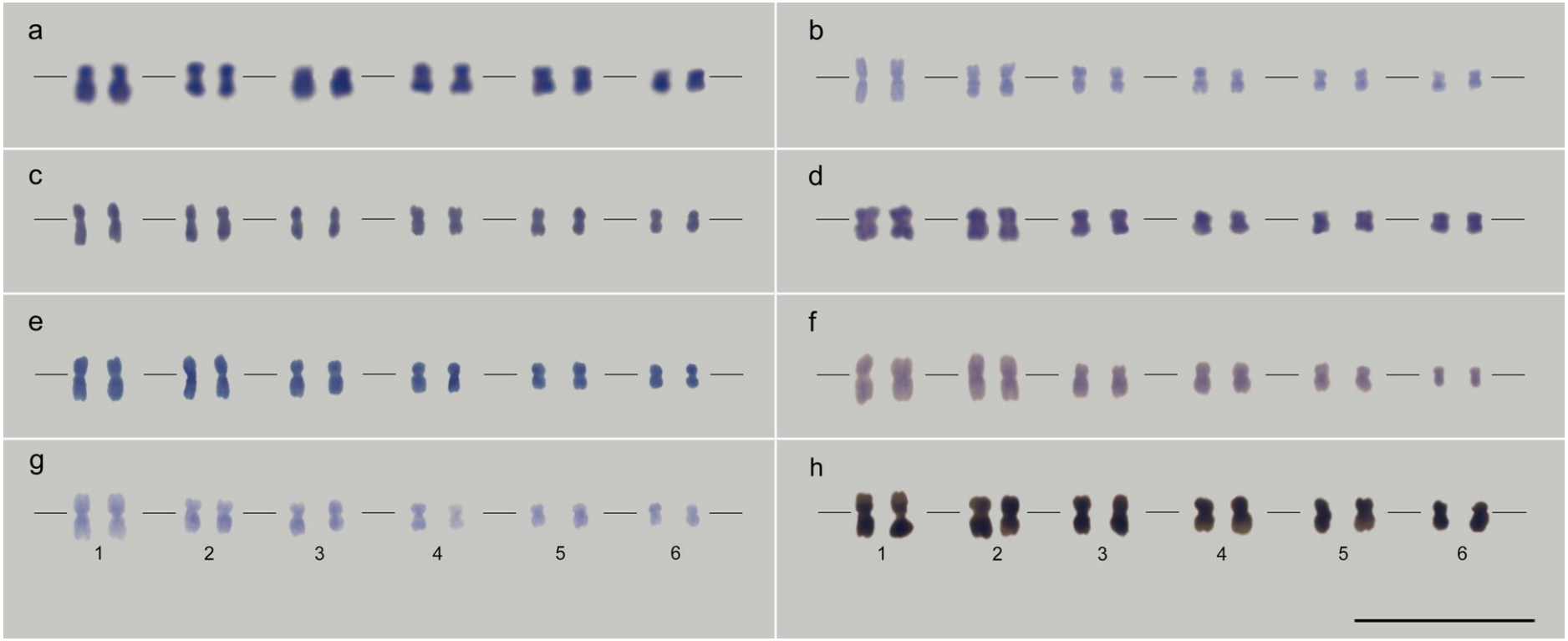

Fig. 3. Karyograms of eight species from the subgenus *Decaloba.* All species exhibited 2n = 12 chromosomes. a) *P. capsularis*, b) *P. porophylla*, c) *P. trifasciata*, d) *P. micropetala*, and e) *P. mexicana* representing the supersection *Decaloba*; f) *P. coriacea* and g) *P. megacoriacea* of the supersection *Cieca*; and h) *P. auriculata* of the supersection *Auriculata*. Chromosome classes ranging between metacentric and submetacentric. Bar = 5 µm.

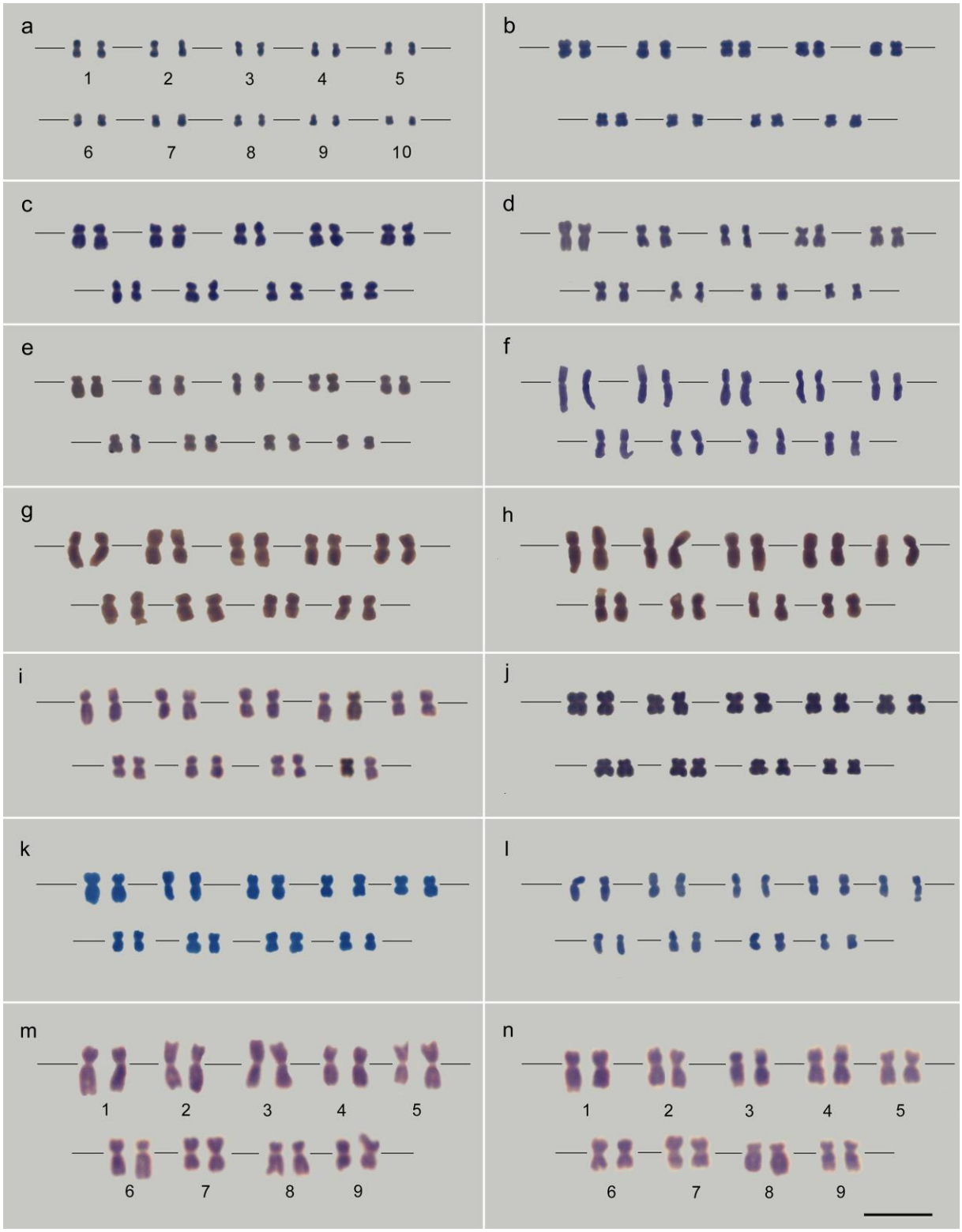

Fig. 4. Karyograms of 14 species from the subgenus *Passiflora*. *P. foetida* (a) was the only species with 2n = 20 chromosomes. All other species presented 2n = 18: b) *P. actinia*, c) *P. setacea*, d) *P. cincinnata*, e) *P. subpeltata*, f) *P. speciosa*, g) *P. edulis*, h) *P. miniata*, i) *P.*

seemannii, j) *P. laurifolia*, k) *P. amethystina*, l) *P. alata*, m) *P. nitida*, and n) *P. quadrangularis*. Chromosome classes ranged between metacentric and submetacentric. Note the occurrence of different chromatin compaction levels in the chromosomes of same homologous pairs, such as pair 6 of *P. miniata* (h) and pair 5 of *P. alata* (l), with visible secondary constriction in only one of the chromosomes. These karyograms represented the supersections *Stipulata* (a, e and k), *Laurifolia* (b, i, j, l, m and n), *Passiflora* (c, d and g), *Coccinea* (f), and *Distephana* (h). Bar = 5 μm.

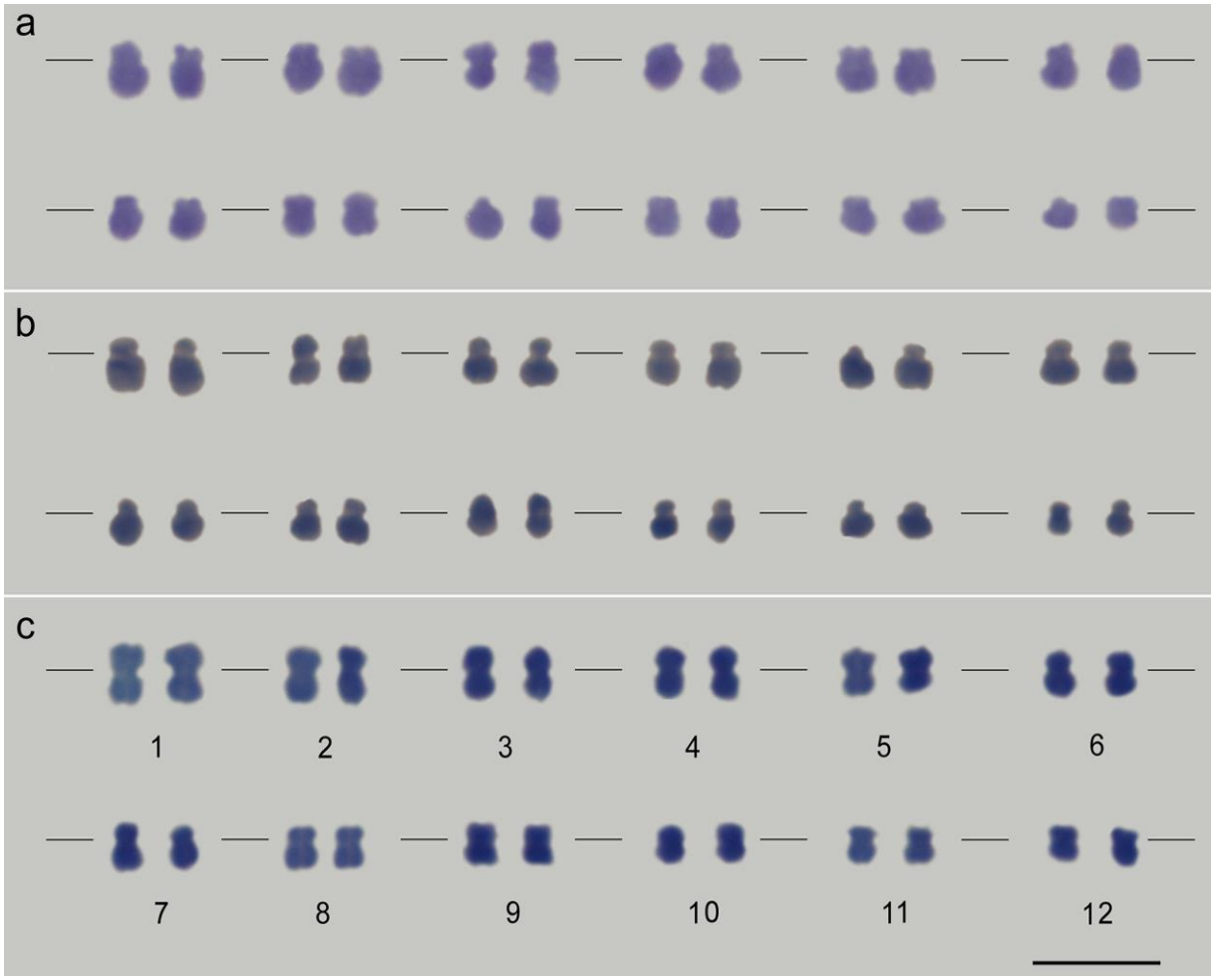

Fig. 5. Karyograms of three species from the subgenus *Astrophea*, supersection *Astrophea*, section *Astrophea*. All species exhibited 2n = 24 chromosomes: a) *P. lindeniana*, b) *P. arborea*, and c) *P. macrophylla*. Chromosome classes were predominantly metacentric and

submetacentric, besides acrocentric pairs found in *P. lindeniana* (chromosomes 4 and 10) and *P. arborea* (chromosomes 5, 7 and 11), which were hitherto unpublished. Didactically, the three chromosome classes can be sequentially observed in the *P. arborea* karyogram (b): chromosome 2 = metacentric, chromosomes 3 and 4 = submetacentric, and chromosome 5 = acrocentric. Bar = 5 μm.

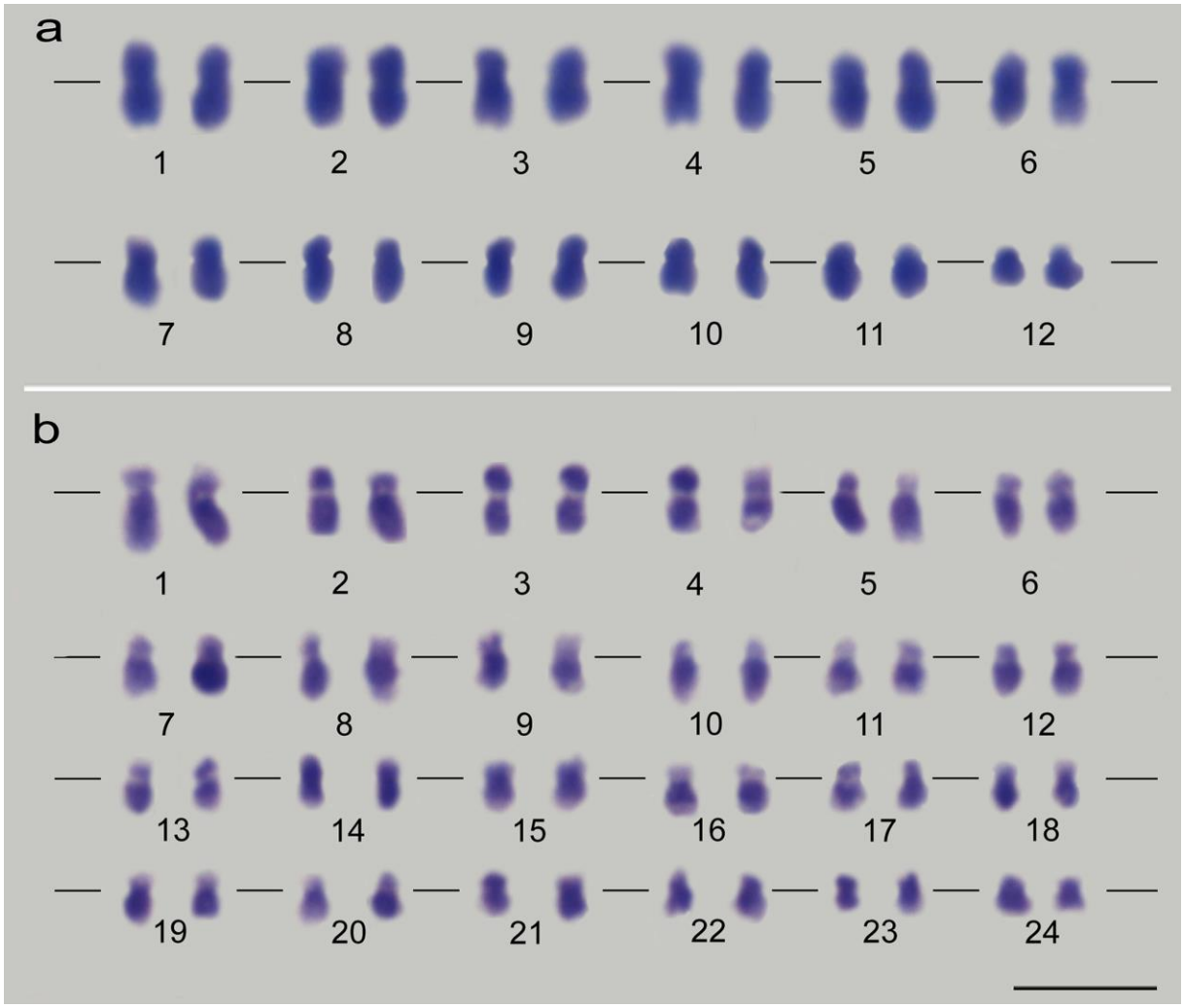

Fig. 6. Karyograms of the two analyzed species from the subgenus *Deidamioides*: a) *P. arbelaezii*, section *Tryphostemmatoides*, 2n = 24 chromosomes, exhibiting metacentric and submetacentric pairs; b) *P. contracta*, section *Tetrastylis*, 2n = 48 chromosomes, all submetacentric. Bar = 5 μm.

(7 and 8) and seven submetacentric ones (1, 2, 3, 4, 5, 6 and 9). Species of the subgenus *Astrophea* showed the most varied chromosome classes. Of the twelve chromosome pairs seen in *P. lindeniana* (Fig. 5A), three were metacentric (5, 8 and 9), seven were submetacentric (1, 2, 3, 6, 7, 11 and 12) and two were acrocentric (4 and 10). *P. arborea* (Fig. 5B) exhibited two metacentric (2 and 8), seven submetacentric (1, 3, 4, 6, 9, 10 and 12) and three acrocentric pairs (5, 7 and 11). In *P. macrophylla* (Fig. 5C), ten metacentric (1, 2, 3, 4, 5, 6, 7, 8, 10 and 12) and two submetacentric pairs (9 and 11) were evidenced. In the subgenus *Deidamioides*, *P. arbelaezii* (Fig. 6A) showed five metacentric pairs (1, 2, 5, 6 and 10) and seven submetacentric ones (3, 4, 7, 8, 9, 11 and 12). Finally, all 24 chromosomes pairs of *P. contracta* (Fig. 6B) were found to be submetacentric.

Nuclear 2C value

All flow cytometry histograms had coefficients of variation below 5% for the G_0/G_1 peaks of *Passiflora* species (samples) and *S. lycopersicum* (standard). This value evidenced that the suspensions contained satisfactory amounts of intact, isolated and stoichiometrically stained nuclei. Thus, the mean nuclear 2C value could be determined for the 41 analyzed *Passiflora* species, having been previously reported for 22 of them (Souza *et al*., 2004, Yotoko *et al*., 2011) and being measured here for the first time in 19. The mean values obtained in this study, as well as those previously reported, are shown in the Supplementary Data Table S1.

Considering all *Passiflora* species, the mean 2C value revealed a large interspecific variation of up to 925%, with the lowest value being found for *P. capsularis* (2C = 0.59 pg) and the highest for *P. quadrangularis* (2C = 5.46 pg). Mean 2C value variations were also observed at the subgeneric level: In *Decaloba*, 2C = 0.59 pg for *P. capsularis* and 2C = 2.00

pg for *P. auriculata* (variation range: 339%); in *Passiflora*, 2C = 0.77 pg for *P. arida* and 2C = 5.46 pg for *P. quadrangularis* (variation range: 709%); in *Astrophea*, 2C = 2.24 pg for *P. lindeniana* and 2C = 4.36 pg for *P. pittieri* (variation range: 180%); and in *Deidamioides*, 2C = 2.24 for *P. arbelaezii* and 2C = 4.78 pg for *P. contracta* (variation range: 213%).

As expected, differences in genome size were found for some species with increasing numbers of chromosome sets as represented in Figure 7 and Supplementary Data Table S1. *P. coriacea* exhibited 2n = 12 chromosomes and mean 2C = 1.00 pg (Fig. 7A). In *P. lindeniana*, with 2n = 24 chromosomes, the mean genome size was 2C = 2.42 pg (Fig. 7C). *P. contracta*, with 2n = 48, presented mean 2C = 4.78 pg (Fig. 7E). In contrast, some species with marked chromosome number divergences exhibited similar mean 2C values. For instance, *P. foetida* (2n = 20, subgenus *Passiflora*) had 2C = 1.04 pg, a value similar to that of *P. coriacea* (2n = 12, subgenus *Decaloba*), with 2C = 1.00 pg (Fig. 7A, B). *P. coccinea* (2n = 18, subgenus *Passiflora*) and *P. auriculata* (2n = 12, subgenus *Decaloba*) showed the same nuclear genome size of 2C = 2.00 pg. Similar observations were also made for *P. arbelaezii* (2n = 24, subgenus *Deidamioides*) and *P. tripartita* (2n = 18, subgenus *Passiflora*), both with 2C = 2.53 pg (Supplementary Data Table S1). On the other hand, some species, such as *P. miniata* (2n = 18 and 2C = 3.40 pg) (Fig. 7D), had a greater genome size than species with 2n = 20 and 2n = 24 (Fig. 7B and D). Surprisingly, the species *P. alata* (2C = 5.06 pg), *P. nitida* (2C = 5.35 pg) and *P. quadrangularis* (2C = 5.46 pg), all with 2n = 18 chromosomes, had nuclear genome sizes larger than 2C = 4.78 pg, which was found for *P. contracta* with 2n = 48.

Nuclear 2C value and karyological data clustering

UPGMA clustering based on the mean 2C values of the investigated *Passiflora* species revealed three groups (Fig. 8). Cluster I was composed of twelve species, ten from the

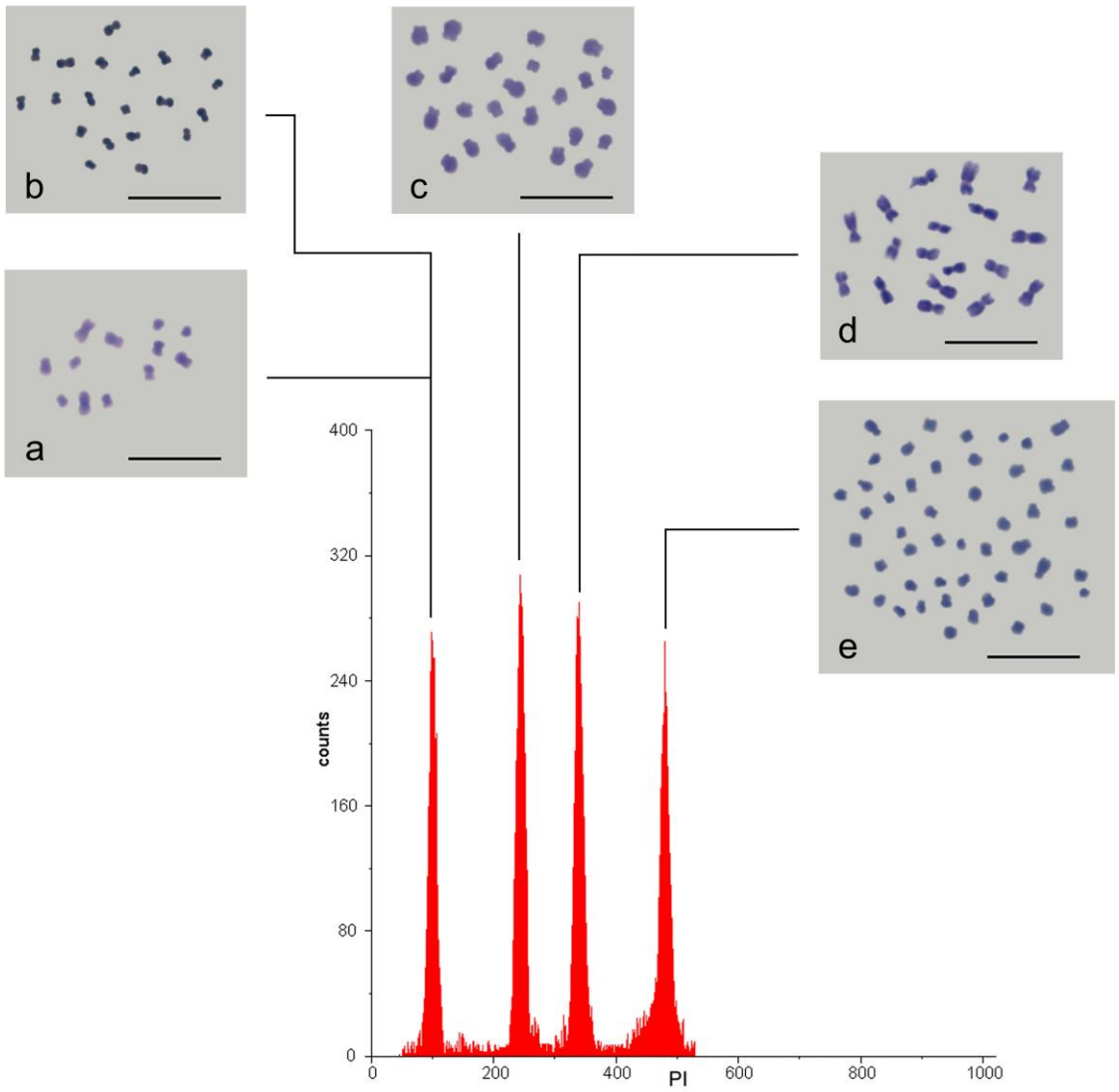

Fig. 7. Karyotype of five species from the genus *Passiflora* with respective G_0/G_1 nuclei peaks in one representative histogram. (a) *P. coriacea*, subgenus *Decaloba*, 2n = 12 chromosomes, 2C = 1.00 pg, and (b) *P. foetida*, subgenus *Passiflora*, 2n = 20 chromosomes, 2C = 1.04 pg, G_0/G_1 nuclei peak in channel 100. (c) *P. lindeniana*, subgenus *Astrophea*, 2n = 24, 2C = 2.42 pg, G_0/G_1 nuclei peak in channel 242. (d) *P. miniata*, subgenus *Passiflora*, 2n = 18 chromosomes, 2C = 3.40 pg, G_0/G_1 nuclei peak in channel 340. (e) *P. contracta*, subgenus *Deidamioides*, 2n = 48 chromosomes, 2C = 4.78 pg, G_0/G_1 nuclei peak in channel 478. In the histogram, the *y*-axis represents the number of G_0/G_1 nuclei. Bar = 5 μm.

subgenus *Decaloba* and two from the subgenus *Passiflora*. The nuclear genome size ranged from 2C = 0.59 pg (*P. capsularis*) to 2C = 1.58 pg (*P. suberosa*). Cluster II consisted of 19 species from four subgenera: One from *Decaloba*, 14 from *Passiflora*, three from *Astrophea*, and one from *Deidamioides*. This group included species with mean 2C values between 2.00 pg (*P. auriculata* and *P. coccinea*) and 3.40 pg (*P. miniata*). In turn, cluster III grouped ten species from three subgenera: Eight from *Passiflora*, one from *Astrophea*, and one from *Deidamioides*. In this group, the mean 2C values oscillated from 3.77 pg (*Passiflora seemannii* Griseb.) to 5.46 pg (*P. quadrangularis*). Each subgenus was also represented by different sections or supersections (discriminated by colors on Fig. 8).

Cluster analysis was also performed using the variables nuclear 2C DNA content, chromosome number and chromosome class, resulting in five clusters (Fig. 9). Cluster I grouped all species from the subgenus *Decaloba*. Cluster II was composed of all species from the subgenus *Passiflora* as well as *P. arbelaezii*, belonging to the subgenus *Deidamioides*. The clusters III and IV grouped species from the subgenus *Astrophea*, and cluster V comprised one species from the subgenus *Deidamioides*. Therefore, this analysis was able to discriminate the highest number of species by subgenus, represented by different sections or supersections.

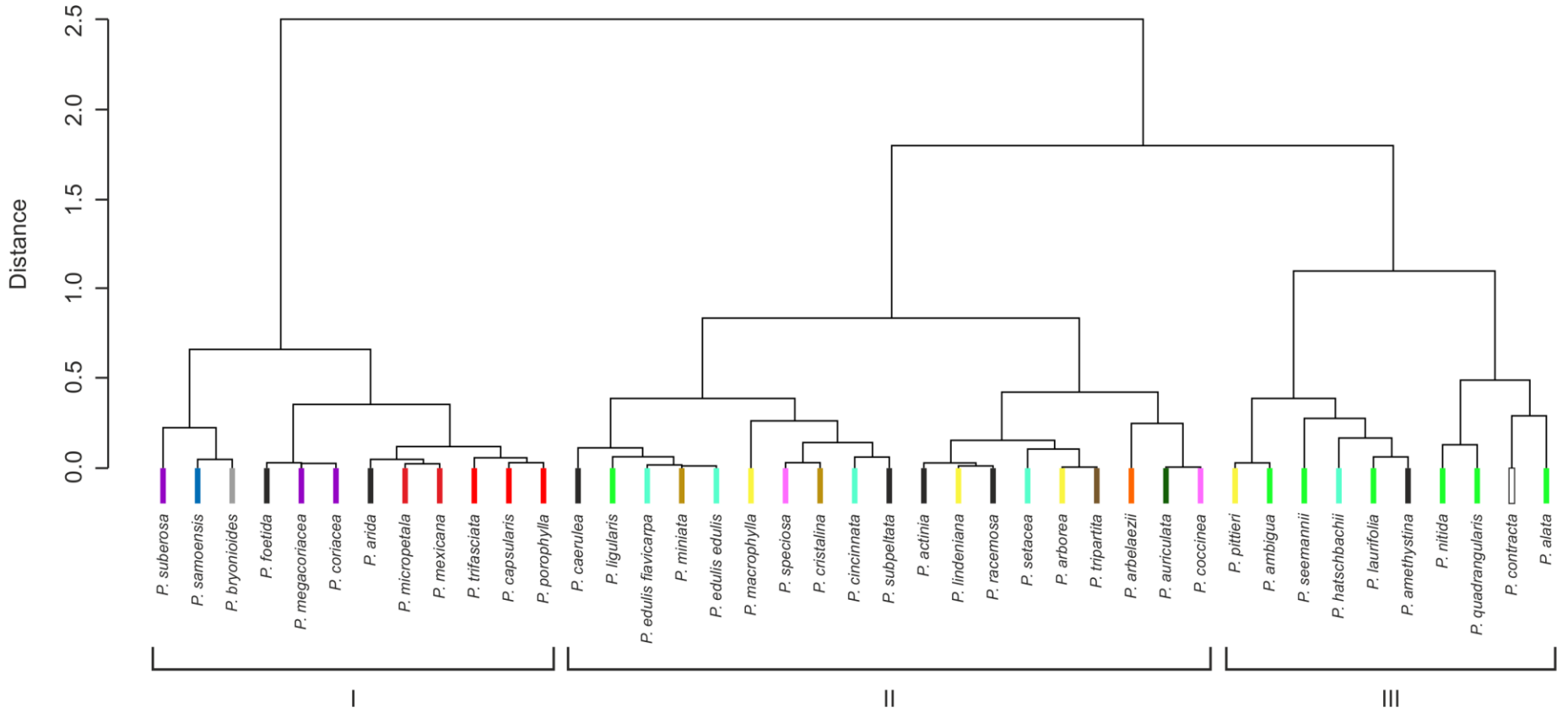

Fig. 8. Dendrogram based on the nuclear 2C values of 41 species from the genus *Passiflora*, with color markings representing supersection or section. Mojena's criteria indicate three clusters composed of different taxa. Cluster I was represented by subgenus *Decaloba* – supersections *Cieca* (purple), *Disemma* (dark blue), *Bryonioides* (gray) and *Decaloba* (red); and subgenus *Passiflora* – supersection *Stipulata* (black). Cluster

II was composed of subgenus *Passiflora* – supersections *Stipulata* (black), *Laurifolia* (light green), *Passiflora* (light blue), *Distephana* (golden), *Coccinea* (pink) and *Tacsonia* (brown); subgenus *Astrophea* – supersection *Astrophea* (yellow); subgenus *Deidamioides* – section *Tryphostemmatoides*; and subgenus *Decaloba* – supersection *Auriculata*. Cluster III grouped the subgenus *Astrophea* – supersection *Astrophea* (yellow); subgenus *Passiflora* – supersection *Laurifolia* (light green) and *Stipulata* (black); and subgenus *Deidamioides* – section *Tetrastylis* (no color). Note the similarity in nuclear genome size among the species of the supersection *Decaloba* (red) in cluster I and species of the supersection *Laurifolia* (light green), mostly grouped in cluster III. Also note the divergence in nuclear genome size in species of the supersection *Stipulata* (black), with representatives in the three clusters.

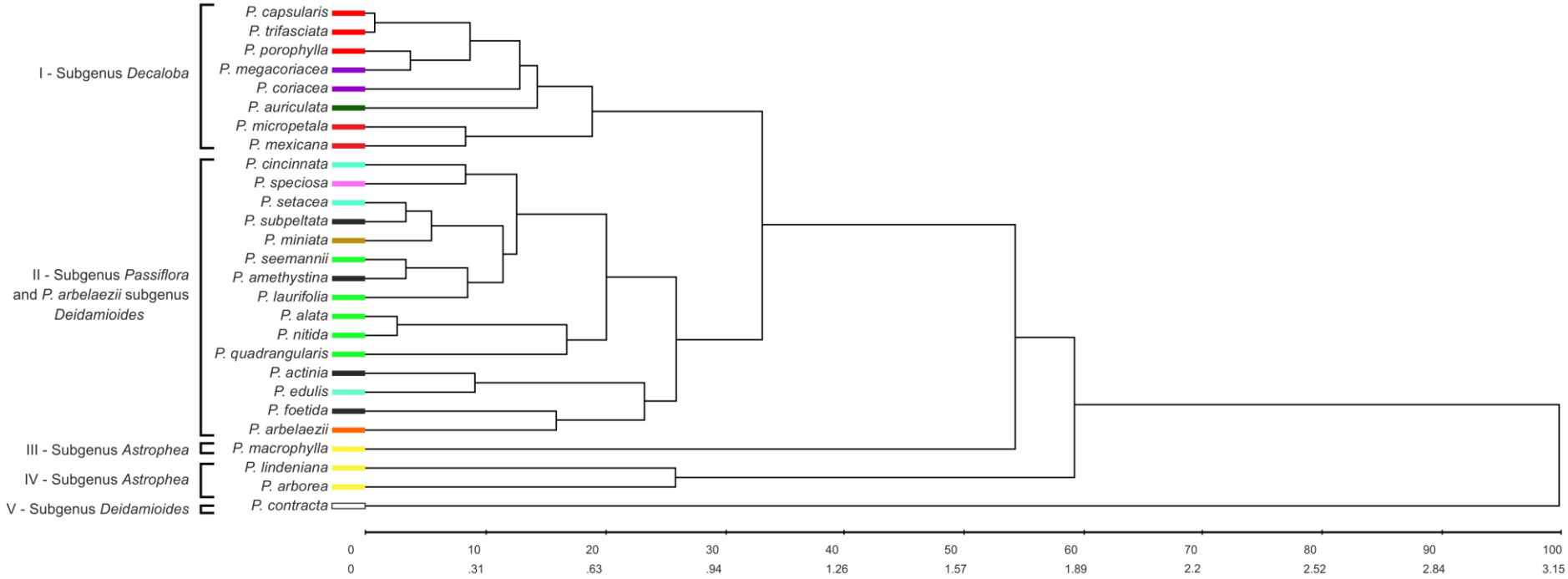

Fig. 9. Dendrogram based on nuclear 2C values, chromosome number and chromosome class of 28 species from the genus *Passiflora*, with clustering of the subgenera in five groups. Color markings represent the supersection or section. Cluster I comprised species from the subgenus *Decaloba* – supersections *Decaloba* (red), *Cieca* (purple) and *Auriculata* (dark green). Cluster II included the subgenus *Passiflora* – supersections *Passiflora* (light blue), *Coccinea* (pink), *Stipulata* (black), *Distephana* (golden) and *Laurifolia* (light green); and one species from the subgenus *Deidamioides* – section *Tryphostemmatoides* (orange). Clusters III and IV comprised species of the subgenus *Astrophea* – supersection *Astrophea* (yellow). Cluster V consisted of one species from the subgenus *Deidamioides* – section *Tetrastylis* (no color).

DISCUSSION

Chromosome number diversity

The present study evidenced species with chromosome numbers 2n = 12, 2n = 18, 2n = 20, 2n = 24 and 2n= 48. In the subgenus *Decaloba*, the species of the supersections *Auriculata*, *Cieca* and *Decaloba* included here exhibited 2n = 12 chromosomes (Fig. 3). This number has also been reported for species from the supersections *Bryonioides* and *Disemma* (Snow and MacDougal, 1993; De Melo *et al.*, 2001; De Melo and Guerra, 2003) and *Multiflora* (Hansen *et al*., 2006; Yotoko *et al*., 2011) (Supplementary Data Table S1). Despite 2n = 12 being common in the subgenus *Decaloba*, some variations have been described in other studies. Snow and MacDougal (1993) reported 2n = 14 chromosomes for *P. holosericea* L. (supersection *Multiflora*) and for *P. lobata* (supersection *Bryonioides*). Hansen *et al*. (2006) documented 2n = 18 chromosomes for *P. lancetilensis* and *P. microstipula* (supersection *Pterosperma*), as well as 2n = 22 or 2n = 24 (J. MacDougal, personal comm.) in *P. guatemalensis* (supersection *Haniopathantus*). Further, polyploidy was observed in *P. suberosa* (a polyploid series – 2n = 12, 24, 36 chromosomes) and *P. tenuiloba* (2n = 24), of the supersection *Cieca*); in *P. exsudans* (2n = 24), of the supersection *Bryonioides*; and *P. lutea* (2n = 24, 84) and *P. misera* (2n = 12, 36), of the supersection *Decaloba* (Snow and MacDougal, 1993; De Melo *et al*., 2001). For *P. suberosa* and *P. misera*, the accessions with different chromosome numbers (polyploid condition) have been found in geographically distant regions, suggesting speciation (Souza *et al*., 2004; De Melo *et al*., 2001). Considering the present results as well as previous studies, the data on chromosome numbers for the subgenus *Decaloba* was updated, covering 60 species (Supplementary Data Table S1), corresponding to 25% of this taxon.

For the subgenus *Passiflora*, five of its six supersections were investigated (*Coccinea*, *Distephana*, *Laurifolia*, *Passiflora* and *Stipulata*). Nearly all these taxa presented 2n = 18 chromosomes, except *P. foetida* (supersection *Stipulata*, section *Dysosmia*), which showed 2n = 20. These results support previously reported counts (Storey, 1950; Snow and MacDougal, 1993; De Melo *et al*., 2001). The occurrence of 2n = 20 chromosomes in *P. foetida* is not common in the section *Dysosmia*. Some studies have reported 2n = 18 (Hansen *et al*., 2006) and 2n = 22 (Santos *et al*., 2012; Belo *et al*., 2015) for species of this section. This indicates that the diversification of the species in this taxon involved events of dysploidy, as suggested by De Melo *et al*. (2001) and De Melo and Guerra (2003). Apart from the section *Dysosmia*, chromosome number variations have been recorded for other taxa of the subgenus *Passiflora*, such as 2n = 36 in *P. mooreana* (supersection *Stipulata*, section *Granadillastrum*) (Chiapero *et al*., 2013). For *P. incarnata* (supersection *Passiflora*, series *Passiflora*), cytotypes with 2n = 18 and 2n = 36 chromosomes were reported (Snow and MacDougal, 1993). The most isolated case was 2n = 12 chromosomes for *P. coactilis* (Rice *et al*., 2014), which is the dominant number in the subgenus *Decaloba*. *P. coactilis* belongs to the supersection *Tacsonia*, section *Colombiana* and series *Lepitomichae*, the same taxa as for *P. antioquiensis* (Snow and MacDougal, 1993) and *P. ampullaceal* (Hansen *et al*., 2006), which showed 2n = 18. Considering all the data, the chromosome number is now available for 85 species of the subgenus *Passiflora* (Supplementary Data Table S1), corresponding to approximately 35% of its representatives.

The chromosome number 2n = 24 has been reported for species of the subgenus *Astrophea* (Berry, 1987; De Melo *et al*., 2001; De Melo and Guerra, 2003; Hansen *et al*., 2006). In the present study, the chromosome number was revisited in *P. lindeniana* and expanded for *P. arborea* and *P. macrophylla*, all belonging to the supersection *Astrophea*, section *Astrophea*. For the three species, the number 2n = 24 chromosomes was verified (Fig.

5A–C). These results corroborate those of Berry (1987) for *P. lindeniana* and the chromosome number described previously for *P. pittieri*, section *Capreolata* (Hansen *et al*., 2006). In the supersection *Pseudoastrophea*, 2n = 24 chromosomes were also reported for *P. pentagona* (De Melo *et al*., 2001), *P. haematostigma* (De Melo and Guerra, 2003) and *P. candida* (Hansen *et al*., 2006), all belonging to the section *Pseudoastrophea*. Considering these studies, the chromosome number is available for seven of the 60 species of the subgenus *Astrophea*. For the sections *Leptoclada* and *Botryastrophea*, the chromosome number remains unknown.

In the subgenus *Deidamioides*, 2n = 24 chromosomes were also found for *P. arbelaezii*, the first count within the section *Tryphostemmatoides* (Fig. 6A). This number is the same as for *P. deidamioides* Harms (section *Deidamioides*), *P. cirrhiflora* (section *Polyanthea*) and *P. ovalis* (section *Tetrastylis*) (Hansen *et al*., 2006). Surprisingly, *P. contracta*, which also belongs to the section *Tetrastylis*, exhibited 2n = 48 chromosomes (Fig. 6B). Initially, *P. contracta* and *P. ovalis* were considered to be the same species. However, Vitta and Bernarcci (2004) reexamined *P. ovalis* collections and recognized two geographically isolated taxa exhibiting some distinct morphological features. Probably, the differentiation and speciation of *P. contracta* involved polyploidization events in *P. ovalis*, which presents 2n = 24 chromosomes (Hansen *et al.*, 2006), resulting in 2n = 48 chromosomes. Considering the evolutionary karyotype hypothesis of De Melo *et al*. (2001) and De Melo and Guerra (2003), *P. contracta* has an octaploid status. Chromosome counts in *Deidamioides* have now been carried out for five of the 15 species of the subgenus. Among the subdivisions of the taxon, a chromosome number has only not yet been described for species of the section *Mayapathanthus*.

Chromosome classification

Chromosomes at different compaction levels, especially in prometaphase, are a common feature in cytogenetic procedures without a synchronization process (Carvalho and Carvalho, 2016). This step contributes to the morphometric characterization of the chromosomes, and consequently to karyogram assembly. Moreover, it is recommended to assemble several karyograms in order to generate more accurate data on the chromosome class. A large number of prometaphase/metaphases exhibiting different compaction levels was obtained in this study, especially for some species, for instance *P. megacoriacea* (Fig. 1) and *P. nigradenia* (Fig. 2). Around ten karyograms were assembled for each of the 28 investigated species, ensuring the reliability of the karyotype characterization. These results represent a great expansion of the available chromosome knowledge, which had been previously limited to only two species of the subgenus *Decaloba* (Amorim *et al*., 2014) and four of the subgenus *Passiflora* (Cuco *et al*., 2005; Viana and Souza 2012).

Morphometry, chromosome class and karyogram were presented for 28 *Passiflora* species, being 24 karyogram published for the first time (Supplementary Data Table S1, Figs. 1–6). The data corroborate previous suggestions that the karyotypes in the genus *Passiflora* predominantly have metacentric and submetacentric chromosomes (Snow and MacDougal, 1993; De Melo *et al*., 2001; De Melo and Guerra, 2003; De Melo *et al*., 2014). In addition, acrocentric chromosomes were observed in the subgenus *Astrophea* for *P. lindeniana*, pairs 4 and 10 (Fig. 5A), and *P. arborea*, pairs 5, 7 and 11 (Fig. 5B). The presence of acrocentric chromosome pairs in species with 2n = 24 is an evidence that supports the hypothesis of descending dysploidy occurring within the genus *Passiflora* (De Melo *et al*., 2001; De Melo and Guerra, 2003). The fusion of acrocentric chromosomes (Robertsonian translocation) is a

dysploidy mechanism that leads to the reduction of the chromosome number, and has been reported in plants (Mas de Xaxars *et al*., 2015).

The number of metacentric and submetacentric chromosomes varied among species (Supplementary Data Table S1). In *Decaloba*, metacentric or submetacentric chromosomes predominated. Previously, Amorim *et al*. (2014) and De Melo *et al*. (2014) showed chromosomes to be mostly metacentric (*P. capsularis, P. rubra* and *P. coriacea*) or only metacentric (*P. ferruginea* and *P. micropetala*). In the subgenus *Passiflora*, of 15 species characterized in this study, 13 presented mostly submetacentric chromosomes. This class was shared by the chromosome pair 1 of all species analyzed in this taxon (Figs. 2, 4 and Supplementary Data Table S1). These results diverged from previous reports where chromosomes were mostly or only metacentric (Souza *et al*., 2003; Cuco *et al*., 2005; De Melo *et al*., 2014). Regarding the two investigated species of the subgenus *Deidamioides*, *P. arbelaezii* (Fig. 6A) presented predominance of submetacentric chromosomes and *P. contracta* (Fig. 6B) exhibited only submetacentric chromosomes pairs. Thus far, no karyotype characterization had been reported for this taxon.

Divergences in karyotype formula (Supplementary Data Table S1) were detected in species revisited in the present study. In *Decaloba* (2n = 12), *P. capsularis* (Fig. 1A) revealed the formula 2n = 4m + 8sm, whereas Amorim *et al*. (2014) characterized this species as 2n = 8m + 4sm. The karyotype formulae for *P. micropetala* (Fig. 3D) and *P. coriacea* (Fig. 3F) were 2n = 4m + 8m and 2n = 2m + 10sm, respectively. In contrast, De Melo *et al*. (2014) reported 2n = 12m for *P. micropetala* and 2n = 10m + 2sm for *P. coriacea*. Species of the subgenus *Passiflora* presented the karyotype formulae 2n = 10m + 6sm for *P. actinia* (Fig. 4A), 2n = 6m + 10sm for *P. setacea* (Fig. 4C), 2n = 6m + 12sm for *P. cincinnata* (Fig. 4D), 2n = 8m + 10sm for *P. alata* (Fig. 4L), and 2n = 4m + 14sm for *P. quadrangularis* (Fig. 4N). Differently, Souza *et al*. (2003) reported 2n = 14m + 4m for *P. alata* and 2n = 10m + 8sm for

P. quadrangularis. Cuco *et al*. (2005) described the species *P. cincinnata*, *Passiflora amethystina* and *P. edulis* with the same chromosome classification, 2n = 14m + 4sm. In turn, Praça *et al*. (2008) reported 2n = 12m + 6sm for *P. edulis*. For the karyotypes of *P. actinia* and *P. setacea*, De Melo *et al*. (2014) did not detect variations in chromosome class, suggesting 2n = 18m. These differences probably arose due to varying chromatin compaction levels, as well as the analyzed number of prometaphases/metaphases.

Nuclear 2C value and its relationship with karyotype evolution in Passiflora

This work enhanced the knowledge on nuclear genome size of the genus *Passiflora*, now expanded to 81 species (Supplementary Data Table S1). Here, 2C values ranged from 2C = 0.59 pg (*P. capsularis*) to 2C = 5.46 pg (*P. quadrangularis*). Large 2C value variations in this genus were also observed in the only two previousl enhanced y available studies (Souza *et al*., 2004; Yotoko *et al*., 2011). Considering all 2C values, the genus *Passiflora* can be defined as having "very small" (2C value ≤ 2.8 pg) and "small" (2.8 pg < 2C value ≤ 7.0 pg) nuclear genome size, as proposed for angiosperms (Leitch *et al*., 1998; Bennett and Leitch, 2011).

The variation in genome size of the genus *Passiflora* is largely due to the increase in chromosome number, which is the outcome of polyploidy, as summarized in Fig. 7 for *P. coriacea* (2n = 12, 2C = 1.00 pg), *P. lindeniana* (2n = 24, 2C = 2.42 pg) and *P. contracta* (2n = 48, 2C = 4.78 pg). However, this correlation is not observed in other species, such as *P. coriacea* (2n = 12, 2C = 1.00 pg) and *P. foetida* (2n = 20, 1.04 pg), suggesting the occurrence of rearrangements. These results, associated with the presence of acrocentric chromosomes in species with 2n = 24 chromosomes (as discussed in the previous topic), suggests x = 6 as the ancestral chromosome number of the genus *Passiflora*. These results corroborate the studies

by De Melo et al. (2001) and De Melo and Guerra (2003). For didactic purposes, we adapted the data of the present study to the schematic representation of these authors (Fig. 10), simplifying the probable evolutionary route of the genus.

Nuclear genome size and systematics implications

UPGMA clustering (Fig. 8) showed that nuclear genome size data can provide support for the systematics of the genus *Passiflora*. Cluster I, composed of smaller genome sizes, grouped ten of the eleven investigated species of the subgenus *Decaloba.* Moreover, the proximity to the genome size in some artificial groups (supersections) was remarkable. Species of the supersection *Decaloba* (red color) were clustered in the same subgroup, with mean 2C value below 0.75 pg. Sorted into other subgroups, the supersections *Cieca* (purple color), *Bryonioides* (gray color) and *Disemma* (dark blue color) exhibited mean nuclear genome size above 2C = 1.00 pg. Phylogenetic analyses by Krosnick *et al*. (2013) showed that the supersections of the subgenus *Decaloba* are closely related, except *Auriculata* and *Multiflora*. In the present study, *P. auriculata* of the supersection *Auriculata* (dark green color) presented mean 2C = 2.00 pg, being assigned to Cluster II. Therefore, the nuclear genome size corroborated the distance of this taxon within the subgenus *Decaloba*.

The genome size in species of the subgenus *Astrophea* circumscribed to the supersection *Astrophea* (yellow color) generated two clusters and supported the classification by Feuillet and MacDougal (2003) for section level. The species of the section *Astrophea* were grouped in Cluster II, although *P. macrophylla* occupied a subgroup different from that of the others species. In turn, *P. pittieri*, section *Capreolata*, was assigned to Cluster III. In contrast, systematics approaches performed on pollen morphology by Mezzonato-Pires *et al*. (2017b) revealed the proximity between *P. pittieri*, *P. arborea* and *P. lindeniana*, while *P.*

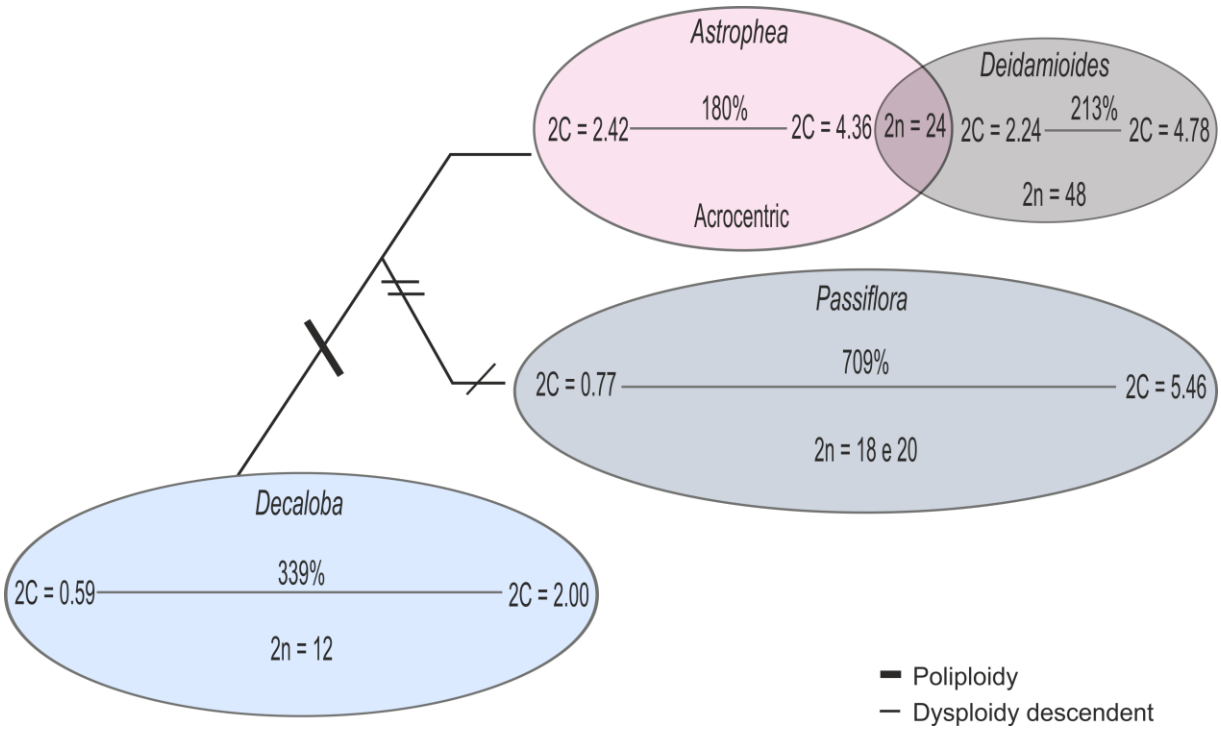

Fig. 10 (adapted from De Melo *et al.*, 2001). Changes in chromosome number and, consequently, nuclear 2C value in the genus *Passiflora* due to the occurrence of polyploidization events followed by reductional dysploidy. Chromosome set duplication events occurred in the subgenus *Decaloba* (2n = 12, nuclear genome size ranging from 2C = 0.59 to 2.00 pg), resulting in species with 2n = 24 chromosomes, for instance in the subgenera *Astrophea* and *Deidamioides* (2n = 24 and 2n = 48 in *Deidamioides*, 2C = 2.24 to 4.78 pg). Dysploidy events promoted reduction of the chromosome number, originating the species of the subgenus *Passiflora* (2n = 18 and 2n = 20, 2C = 0.77 to 5.46 pg). Based on the hypothesis proposed by De Melo et al. (2001) about the *Passiflora* karyotype evolution, polyploidy and dysploidy had a significant role in the diversification of this genus. The chromosome characterization revealed predominantly metacentric and submetacentric pairs in all subgenera. Only in species of the subgenus *Astrophea* were acrocentric chromosome pairs observed. The 2C values reached differences of about 925% in the genus *Passiflora*: 339%

among the species of the subgenus *Decaloba*, 709% in the subgenus *Passiflora*, 180% in the subgenus *Astrophea*, and 213% in the subgenus *Deidamioides*.

macrophylla was more distant. Nevertheless, these variations are expected in artificial groups, created from the analysis of different characters.

In the subgenus *Deidamioides*, the nuclear genome size was established for *P. arbelaezii* (2C = 2.24 pg) of the section *Tryphostemmatoides* (orange color), Cluster II, and *P. contracta* (2C = 4.78 pg) of the section *Tetrastylis* (no color), Cluster III. The difference between the genome sizes of these species is a direct consequence of their chromosome numbers, respectively 2n = 24 and 2n = 48. This data provided support to the molecular systematics analysis performed by Cazé *et al*. (2013) in this subgenus, where a large genetic distance was described for these taxa.

Within the subgenus *Passiflora*, similarities in nuclear genome size were observed for most species of the supersection *Laurifolia* (light green color). Presenting higher 2C values, these species were grouped in Cluster III, with the exception of *P. ligularis*, which has a medium nuclear 2C value and was assigned to Cluster II. The nuclear genome size may be important for taxonomic reviews in the supersection *Laurifolia*, whereas within this taxon the delimitations have been considered difficult (Rome and d'Eeckenbrugge, 2017). The species *P. amethystina*, supersection *Stipulata* (black color), and *P. hatschbachii*, supersection *Passiflora* (light blue color), had mean 2C values similar to those of the supersection *Laurifolia* in Cluster III. Recently, based on qualitative characters, Ocampo and Coppens d´Eeckenbrugge (2017) related that some species of the supersection *Stipulata* are close to the supersection *Laurifolia*. These authors also reported that, within the supersections *Stipulata* and *Passiflora*, some species are not closely related. Regarding nuclear genome size, this fact was observed particularly in the supersection *Stipulata*, which had representatives in the three

clusters. Other species of the supersection *Passiflora* were grouped in Cluster II, but in different subgroups. The same was observed for species of the supersection *Coccinea* (pink color) and supersection *Distephana* (golden color), for which reported classifications have been inconsistent (Vanderplank, 2006).

Nuclear genome size and karyological data in Passiflora systematics

Analyzed together, nuclear genome size, chromosome number and chromosome class data discriminated most of the species by subgenus (Fig. 9). This data supported the morphological analyses by Feuillet and MacDougal (2003) and molecular analyses based on different markers (Muschner *et al*., 2003; Yockteng and Nadot, 2004; Hansen *et al*., 2006; Muschner *et al*., 2012). Species of the subgenus Decaloba constituted Cluster I, and represented the supersections *Decaloba* (red color), *Cieca* (purple color) and *Auriculata* (light green color). In the work of Krosnick *et al*. (2013), phylogenetic relationships of the subgenus *Decaloba* were examined and revealed proximity between the supersections *Decaloba* and *Cieca*. However, species of the supersection *Auriculata* had more distant positions. In the present investigation, *P. auriculata* (supersection *Auriculata*) exhibited nuclear genome size twice as high as that found in representatives of the supersections *Decaloba* and *Cieca*. Despite this difference, similar aspects of the karyotype favored the grouping of these taxa, such as a chromosome number of 2n=12 and chromosome class ranging between metacentric and submetacentric.

Representing the supersections *Passiflora* (light blue color), *Coccinea* (pink color), *Distephana* (golden color) and *Stipulata* (black color), species of the subgenus *Passiflora* formed the Cluster II together with *P. arbelaezii* of the subgenus *Deidamioides*, section *Tryphostemmatoides*. Despite evidenced variations in the nuclear genome size of the

subgenus *Passiflora*, karyotype aspects favored its discrimination. Species of this subgenus exhibited 2n = 18 chromosomes (except *P. foetida* with 2n = 20) and chromosome class ranging between metacentric and submetacentric, with predominance of submetacentric chromosomes, including the chromosome pair 1 of all species. Predominance of submetacentric chromosome pairs was also observed in *P. arbelaezii* (2n = 24) of the subgenus *Deidamioides*, a characteristic that may explain its presence in this cluster. This result corroborates molecular phylogenetic studies reported by Yockteng and Nadot (2004) and Krosnick *et al*. (2013), where relationships between species of the subgenera *Deidamioides* and *Passiflora* were described. Nevertheless, species of the section *Tryphostemmatoides* (to which *P. arbelaezii* belongs) were related with species of the subgenus *Astrophea* (Muchner *et al*., 2012; Krosnick *et al*., 2013). In addition, the present analysis supported *P. foetida* as part of the subgenus *Passiflora*, despite its small genome size and different chromosome number. These results corroborate previous molecular cytogenetic analyses (De Melo *et al*., 2001; De Melo and Guerra, 2003) and molecular studies (Yockteng and Nadot, 2004) fueling this longstanding discussion.

The subgenus *Astrophea* was also discriminated by cluster analysis. However, although the three analyzed species belong to the supersection and section *Astrophea* (yellow color) and presented chromosome number 2n = 24, *P. macrophylla* was assigned to Cluster III, and *P. arborea* and *P. lindeniana* to Cluster IV. This delimitation is related to peculiar characteristics of the karyotype of these species. In *P. macrophylla*, most of the chromosome pairs were metacentric, whereas *P. arborea* and *P. lindeniana* had a predominance of submetacentric chromosome pairs, besides some acrocentric ones. Therefore, karyological data and nuclear genome size did not support these species within the same supersection or section as proposed by Feuillet and MacDougal (2003). On the other hand, this data is in accordance with the analysis performed on metrical variables of pollen by Mezzonato-Pires *et*

al. (2017a), where *P. macrophylla* was also segregated from *P. arborea* and *P. lindeniana*. In addition, it corroborates the suggestion of Mezzonato-Pires *et al*. (2017b), based on analysis of seed morphology, of an infra-subgeneric taxonomic revision of the subgenus *Astrophea*.

Cluster V was the most isolated in this analysis, and only included *P. contracta* (subgenus *Deidamioides*, section *Tetrastylis*) (no color). This segregation was favored by the karyotype showing 2n = 48 chromosomes, a number very distant from that of all other species. Furthermore, the chromosome classification presented no range, with all chromosome pairs being submetacentric. Submetacentric chromosomes were predominant in most analyzed species of the subgenus *Passiflora*. Molecular phylogenies reported by Muchner *et al*. (2003) and Krosnick *et al*. (2013) revealed that *P. ovalis*, a species also circumscribed to the section *Tetrastylis*, is closely related to species of the subgenus *Passiflora*.

CONCLUSION

In conclusion, karyotype data and nuclear 2C value evidenced the probable occurrence of polyploidy and dysploidy during the diversification of the genus *Passiflora*. Our results suggest x = 6 as the basic chromosome number of the genus, providing support to the molecular cytogenetic study. Furthermore, data analyses complemented the phylogenies of the genus and corroborated the current subgeneric classification. In sum, this study contributes to the knowledge about the genus *Passiflora* and its diversity, and provides valuable information for future investigations.

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Suplementary Data Table S1 *2C nuclear value (pg), 2n chromosome number and chromosome class pairs of the genus Passiflora (present study PS, previous reports PR, metacentric M, submetacentric SM, acrocentric A, numbers in [ ] indicate the references*)

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Genus *Passiflora* Subgenus *Decaloba* Supersection *Decaloba* Section *Decaloba*	*P. affinis*	-	-	-	12 [15]	-	-
	P. aurantia	-	-	-	12 [6]	-	-
	P. bicornis	-	-	-	12 [1]	-	-
	P. biflora	-	-	-	12 [6]	-	-
	P. candollei	-	-	-	12 [15]	-	-
	P. cubensis	-	-	-	12 [6]	-	-
	P. gilbertiana	-	-	-	12 [6]	-	-
	P. leptoclada	-	0.52 [21]	-	12 [21]	-	-
	P. lutea	-	-	-	24 [30] 84 [30]	-	-
	P. mexicana Robert Frías S. 03	0.73	-	12	-	M (1, 2, 5, 6) SM (3, 4)	-
	P. micropetala M. Peixoto 05	0.71	0.50 [21]	12	12 [25]	M (1-3, 5,6) SM (4)	M (1-6) [25]
	P. misera	-	0.51 [21]	-	12 -36 [8]	-	-
	P. murucuja	-	-	-	12 [15]	-	-
	P. nubicola	-	-	-	12 [7]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Section *Decaloba*	*P. oblongata*	-	-	-	12 [15]	-	-
	P. organensis	-	0.42 [21]	-	12 [21]	-	-
	P. perfoliata	-	-	-	12 [15]	-	-
	P. pohlii	-	0.59 [21]	-	12 [21]	-	-
	P. porophylla Vell., K.F. Borges & H.G. Castro 07	0.62	-	12	-	M (1, 3, 5) SM (2, 4, 6)	-
	P. porphyretica	-	-	-	12 [6]	-	-
	P. pulchella	-	-	-	12 [6]	-	-
	P. standleyi	-	-	-	12 [6]	-	-
	P. tricuspis	-	0.57 [21]	-	12 [6, 8, 11]	-	-
	P. trifasciata Ferreira, D.A.T. 12	0.66	-	12	-	M (5, 6) SM (1-4)	-
	P. tulae	-	0.55 [21]	-	12 [21]	-	-
	P. vespertilio	-	0.65 [21]	-	12 [21]	-	-
Section *Xerogona*	*P. capsularis* K.F. Borges 04 & H.G. Castro	0.59	0.63 [24]	12	12 [6,8,11 24]	M (2, 6) SM (1, 3-5)	M (1,3,5,6) SM (2,4) [24]
	P. citrina	-	-	-	12 [6]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Section *Xerogona*	*P. cobanensis*	-	-	-	12 [6]	-	-
	P. conzattiana	-	-	-	12 [6]	-	-
	P. costaricensis	-	-	-	12 [6]	-	-
	P. escobariana	-	-	-	12 [6]	-	-
	P. quinquangularis	-	-	-	12 [6]	-	-
	P. rovirosae	-	-	-	12 [6]	-	-
	P. rubra L.	-	0.62 [24]	-	12 [6, 8, 11]	-	M (1,3,5,6) SM (2,4) [24]
	P. sanguinolenta	-	-	-	12 [6]	-	-
Supersection *Cieca*	*P. coriacea* Ferreira, D.A.T. 33	1.00	-	12	12 [25]	M (2) SM (1, 3-6)	M (2,3,5,6) SM (1,4) [25]
	P. juliana	-	-	-	12 [6]	-	-
	P. megacoriacea Vassalli, P. 01	1.02	-	12	-	M (1, 4, 6) SM (2, 3, 5)	-
	P. obtusifolia	-	-	-	12 [6]	-	-
	P. suberosa Ferreira, D.A.T. 10	1.58	1.83 [13]	-	24 [6, 8, 11]	-	-
	P. tenuiloba	-	-	-	24 [32]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Supersection *Disemma* Section *Disemma*	*P. aurantia*	-	-	-	12 [6]	-	-
	P. cinnabarina	-	-	-	12 [6]	-	-
	P. herbertiana	-	-	-	12 [8]	-	-
	P. samoensis Geilen, H. 01	1.34	-	-	-	-	-
Supersection *Bryonioides*	*P. bryonioides* Robert Frías S. 03	1.39	-	-	12 [6]	-	-
	P. morifolia	-	1,01 [21] 2.80 [19]	-	12 [11]	-	-
	P. adenopoda	-	-	-	12 [7]	-	-
	P. oaxacensis	-	-	-	12 [6]	-	-
	P. karwinskii	-	-	-	12 [6]	-	-
	P. dioscoreifolia	-	-	-	12 [7]	-	-
	P. gracilis	-	-	-	12-18-20 [17]	-	-
	P. lobata	-	-	-	14 [6]	-	-
	P. exsudans	-	-	-	24 [6]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Supersection *Auriculata*	*P. auriculata* M Peixoto 02	2.00	1.98 [21]	12	12 [15, 21]	M(1,2,3) SM (3,4,5)	-
	P. ferrugínea	-	-	-	12 [25]	-	M (1-6) [25]
Supersection *Multiflora*	*P. holosericea*	-	-	-	14 [6]	-	-
	P. multiflora	-	-	-	12 [15]	-	-
	P. truncata	-	1.40 [21]	-	12 [21]	-	-
	P. microstipula	-	-	-	18 [15]	-	-
Supersection *Haniopathantus*	*P. guatemalensis*	-	-	-	22 or 24 [15]	-	-
Subgenus *Astrophea* Supersection *Astrophea* Section *Astrophea*	*P. arborea* Vecchia, M. 02	2.53	-	24	-	M (2,8) SM (1,3, 4, 6, 9, 10, 12) A (5,7,11)	-
	P. lindeniana Vecchia, M. 01	2.42	-	24	24 [03]	M (5, 8, 9, 12) SM (1,3,6, 7, 12) A (4,10)	-
	P. macrophylla Vecchia M. 03	2.77	-	24	-	M (1-8, 10, 12) SM (9, 11)	-
Section *Capreolata*	*P. pittieri* Vecchia M. 04	4.36	-	-	24 [15]	-	-
Supersection *Pseudoastrophea*	*P. pentagona*	-	3.70 [19]	-	24 [6]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Supersection *Pseudoastrophea*	*P. candida*	-	-	-	24 [15]	-	-
	P. haematostigma	-	-	-	24 [6]	-	-
Subgenus *Deidamioides* Section *Tryphostemmatoide*	*P. arbelaezii* Vassalli, P. 02	2.24	-	24	-	M (1, 2, 5, 6, 10) SM (3, 4, 7-9, 11, 12)	-
Section *Tetrastylis*	*P. contracta* Ferreira, D.A.T 20	4.78	-	48	-	SM (1-24)	-
	P. ovalis	-	-	-	24 [15]	-	-
Section *Polyanthea*	*P. cirrhiflora*	-	-	-	24 [15]	-	-
Section *Deidamioides*	*P. deidamioides*	-	1.63 [21]	-	24 [15]	-	-
Subgenus *Tetrapathea*	*P. tetrandra*	-	-	-	24 [2]	-	-
Subgenero *Passiflora* Supersection *Stipulata* Section *Dysosmia*	*P. arida* Ferreira, D.A.T. 35	0.77	-	-	-	-	-
	P. campanulata	-	-	-	18 [15]	-	-
	P. ciliata	-	-	-	18 [15]	-	-
	P. foetida Ferreira, D.A.T. 09	1.04	0.96 [21] 2.80 [19]	20	20 [1, 5, 6]	M (4-6, 8) SM (1-3, 7, 9, 10)	-
	P. palmeri	-	0.53 [21]	-	18 [21]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Section *Dysosmia*	*P. sublanceolata*	-	-	-	18 [15] 22 [27]	-	-
	P. villosa	-	-	-	18 [15]	-	-
Section *Granadillastrum*	*P. actinea* M. Peixoto 09	2.40	2.11 [21]	18	18 [25]	M (3-5, 7-9) SM (1, 2, 6)	M (1-9) [25]
	P. amethystina K.F. Borges 01 & H.G. Castro	4.12	3.40 [19]	18	18 [10]	M (4, 7, 8) SM (1-3, 5, 6, 9)	M (2-6,8,9) SM (1,7) [10]
	P. caerulea Ferreira, D.A.T. 06	3.27	2.77 [21] 3.20 [19]	18	18 [21, 23]		8M + 1SM [23]
	P. eichleriana	-	2.42 [21]	-	18 [21]	-	-
	P. elegans	-	-	-	18 [11]	-	-
	P. galbana	-	3.52 [13]	-	18 [12]	-	18 M [12]
	P. garckei	-	-	-	18 [15]	-	-
	P. gardinerii	-	3.84 [21]	-	18 [21]	-	-
	P. gibertii	-	3.92 [21]	-	18 [12]	-	-
	P. jilekii	-	1.87 [21]	-	18 [8]	-	-
	P. menispermifolia	-	4.55 [5]	-	18 [15]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
	P. mooreana	-	-	-	36 [23]	-	16M + 2SM [23]
	P. mucronata	-	3.40 [13]	-	18 [8]	-	14M + 4SM [12]
	P. picturata	-	4.34 (21)	-	18 [21]	-	-
	P. sidiifolia	-	1.86 [21]	-	18 [21]	-	-
Section *Granadillastrum*	*P. subpeltata* M. Peixoto 04	2.99	-	18	18 [8]	M (3, 4, 7, 8) SM (1, 2, 5, 6, 9)	-
	P. subrotunda.	-	2.64 [21]	-	18 [25]	-	M (1-9) [25]
	P. tenuifolia	-	-	-	18 [9]	-	-
	P. trisulca	-	-	-	18 [6]	-	
	P. tucumanensis.	-	-	-	18 [23]	-	8M + 1SM [23]
	P. urubiciensis	-	3.16 [21]	-	18 [21]	-	-
Section Kermesiane	*P. edmundoi*	-	3.43 [13]	-	18 [11]	-	16M + 2SM [12]
	P. kermesina	-	2.47 [21]	-	18 [8]	-	-
	P. loefgrenii	-	2.62 [21]	-	18 [21]	-	-
	P. miersii	-	2.80 [13]	-	18 [21, 25]	-	M (1-9) [25]
	P. watsoniana	-	2.61 [21]	-	18 [21]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Section *Calopathanthus*	*P. racemosa* Ferreira, D.A.T. 34	2.43	2.15 [21]	-	18 [21]	-	-
Section *Tacsonioides*	*P. reflexiflora*	-	-	-	18 [15]	-	-
	P. umbilicata	-	-	-	18 [6]	-	-
Supersection *Laurifolia* Series *Laurifoliae*	*P. acuminata*	-	-	-	18 [15]	-	-
	P. ambigua M. Peixoto 08	4.33	-	-	-	-	-
	P. capparidifolia	-	4.10 [21]	-	18 [21]	-	-
	P. cerasina		2.64 [21]	-	18 [21]	-	-
	P. crenata	-	-	-	18 [15]		-
Series *Laurifoliae*	*P. ischnoclada*	-	1.80 [21]	-	18 [21]	-	-
	P. laurifolia M. Peixoto 01	4.06	3.90 [19]	18	-	M (2, 6-8) SM (1, 3-5, 9)	-
	P. nigradenia Geilen, H. 02	-	-	18	18 [12]	M (4, 5, 8) SM (1-3, 6, 7, 9)	-
	P. nitida Ferreira, D.A.T. 08	5.33	3.70 [21] 4.80 [19]	18	18 [8]	M (3, 5, 6, 8) SM (1, 2, 4, 7, 9)	-
Series *Tiliifolia*	*P. ligularis* M. Peixoto 07	3.33	2.82 [21]	18	18 [21]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Series *Tiliifolia*	*P. magnifica*	-	-	-	18 [6]	-	-
	P. maliformis	-	3.78 [13]	-	18 [12]	-	-
	P. Platyloba	-	3.29 [21]	-	18 [21]	-	-
	P. seemannii Vassalli, P. 03	3.77	-	18	18 [15]	M (3, 4, 8) SM (1, 2, 5-7, 9)	-
	P. serratodigitata	-	3.71 [13]	-	18 [4, 25]	-	M (1-9) [25]
Series *Quadrangulares*	*P. alata* Ferreira, D.A.T. 19	5.06	4.41 [21]	18	18 [12]	M (4, 5, 7, 9) SM (1-3, 6, 8)	14M + 4SM [12]
	P. quadrangularis Ferreira, D.A.T. 01	5.46	5.36 [13]	18	18 [12]	M (6, 7) SM (1-5, 8, 9)	10M + 8SM [12]
	P. trialata Feuillet & J. MacDougal	-	-	-	18 [15]	-	-
Supersection *Passiflora* Series *Passiflora*	*P. bahiensis*	-	-	-	18 [25]	-	M (1-5, 7,9) SM (6,8) [25]
	P. cacaoensis	-	-	-	18 [22]	-	M (1-9) [22]

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Series *Passiflora*	*P. cincinnata* Ferreira, D.A.T. 07	2.93	3.01 [18] 2.80 [19]	18	18 [10, 23]	M (6, 7, 9) SM (1-5, 8)	SM (1 e 7) M (2-6, 8-9) [10]
	P. edulis edulis Ferreira, D.A.T. 13	3.39	3.19 [13]	18	18 [1, 6]	M (2-7) SM (1, 8, 9)	-
	P. edulis f. *flavicarpa* K.F. Borges 05	3.38	2.50 [21] 3.20 [13]	18	18 [1, 6, 14, 16, 22]	M (2-7) SM (1, 8, 9)	M (1,4) SM (2,3,5-9) [14] M (2-7) SM (1,8, 9) [16] M (1-9) [22]
	P. filamentosa	-	-	-	18 [25]	-	M (1-9) [25]
	P. incarnata	-	1.32 [21]	-	18- 36 [6]	-	-
	P. iodocarpa		2.60 [21]		18 [21]	-	-
	P. malacophylla	-	-	-	18 [12]	-	14SM + 4SM [12]
Series *Setaceae*	*P. hatschbachii* Ferreira, D.A.T. 27	3.98	1.76 [21]	-	18 [21, 25]	-	M (1-9) [25]

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Series *Setaceae*	*P. setacea* M. Peixoto 03	2.63	2.60 [26]	18	18 [25]	M (3, 6, 8, 9), SM (1, 2, 4, 5, 7)	M (1-9) [25]
Supersection *Coccinea*	*P. coccinea* M. Peixoto 06	2.00	2.67 [21] 3.20 [19]	18	18 [21, 25]	-	M (1,2,5,6,8,9) SM (3,4,7) [25]
	P. speciosa K.F. Borges 09	3.08	3.08 [28]	18	18 [28]	M (7, 8) SM (1-5, 9)	-
	P. vitifolia	-	2.83 [21]	-	18 [6, 25]	-	M (1-9) [25]
Supersection *Distephana*	*P. cristalina* Ferreira, D.A.T. 11	3.11	-	-	-	-	-
	P. glandulosa	-	-	-	18 [11]	-	-
	P. miniata Ferreira, D.A.T. 14	3.40	3.40 [28]	18	18 [28]	M (4, 6, 7, 9) SM (1-3, 5, 8)	-
Supersection *Tacsonia* Section *Tacsonia*	*P. mixta*	-	-	-	18 [8]	-	-
Section *Rathea*	*P. andina*	-	-	-	18 [15]	-	-
Section *Insignes*	*P. pilosicorona*	-	2.80 [21]	-	18 [21]	-	-
Section *Elkea*	*P. cumbalensis*	-	-	-	18 [4]	-	-

Continued

Suplementary Data Table S1 (*continued, present study PS, previous reports PR, numbers in [ ] indicate the references)*

Taxon*	Species (Voucher)	2C nuclear value		Chromosome number		Chromosome class pairs[#]	
		PS	PR	PS	PR	PS	PR
Section *Insignes*	*P. tarminiana*	-	-	-	18 [10]	-	-
	P. tripartita Lommen, A. 01	2.53	-	-	18 [16]	-	-
Section *Parritana*	*P. parritae*	-	-	-	18 [15]	-	-
Section *Colombiana* Series *Leptomichae*	*P. ampullacea*	-	-	-	18 [15]	-	-
	P. antioquiensis	-	3.08 [20]	-	18 [6]	-	-
	P. coactilis	-	-	-	12 [30]	-	-
Section *Trifoliata*	*P. trifoliata*	-	-	-	18 [15]	-	-
Series *Manicata*	*P. manicata*	-	-	-	18 [1]	-	-
	P. trisecta	-	-	-	18 [15]	-	-

*Classification according Feuillet and MacDougal (2003).

M, metacentric; SM, submetacentric; A, acrocentric.

Previous data based on information in [1] Storey (1950); [2] Fedorov (1974); [3] Berry (1987); [4] Escobar (1987); [5] Arumuganathan and Earle (1991); [6] Snow and MacDougal (1993); [7] MacDougal (1994); [8] De Melo et al. (2001); [9] Deginani and Escobar (2002); [10] Olaya Arias et al (2002); [11] De Melo and Guerra (2003); [12] Souza et al. (2003); [13] Sousa et al. (2004); [14] Cuco et al. (2005); [15] Hansen et al. (2006); [16] Praça et al. (2008); [17] Souza et al. (2008); [18] Pain Pinto et al. (2010); [19] Bennett and Leitch (2011); [20] Yockteng et al. (2011); [21] Yotoko et al. (2011); [22] Viana and Souza (2012); [23] Chiapero et al. (2013); [24] Amorim et al. (2014); [25] De Melo et al. (2014); [26] Vieira et al. (2014); [27] Belo et al. (2015); [28] Ferreira et al. (2015); [29] Bennett and Leitch (2012); [30] Rice et al. (2014).