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CENTRO DE CIÊNCIAS AGRÁRIAS E ENGENHARIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E MELHORAMENTO

# Estudos citogenéticos em *Dorstenia* L. (Moraceae)

LUCAS MESQUITA BARRETO

Alegre - ES
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Dissertação apresentada ao Programa de Pós-Graduação em Genética e Melhoramento do Centro de Ciências Agrárias e Engenharias da Universidade Federal do Espírito Santo, como parte das exigências para obtenção do título de Mestre em Genética e Melhoramento.

Orientadora: Profa Dra Tatiana Tavares Carrijo.

Coorientadora: Profa Dra Milene Miranda Praça Fontes.

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# Sumário

## Contextualização teórica

*Dorstenia* é um gênero de Angiospermas que pertence à família Moraceae, que se destaca morfologicamente dos demais por apresentar representantes herbáceos (Figuras 1a-d) e arbustivos (Carauta, 1978). Várias espécies possuem rizomas (Figura 1e), outra característica pouco usual em Moraceae, o que facilita a propagação vegetativa. As espécies podem ser dioicas ou monoicas, e as inflorescências estão representadas por cenantos de formas variadas (Berg, 2001), nos quais flores unissexuais femininas e masculinas encontram-se dispostas em um eixo plano (Figura 1f). Os frutos drupáceos são dispersos pela própria planta, caracterizando um modo de dispersão de diásporos autocórico (Berg, 2001).

As espécies de *Dorstenia* ocorrem predominantemente nas Américas Central e do Sul e África, e em uma pequena parte da Ásia (Figura 2), o que suscitou perguntas no meio científico sobre qual seria o centro de origem do gênero. A carência de evidências ecológicas para justificar dispersão a longa distância de um continente para o outro (dado que as espécies são autocóricas), e a elevada riqueza de espécies encontradas tanto na África (61 spp.) quanto no Neotrópico (47 spp.), foram os alicerces para a estruturação da hipótese de que o gênero teria se originado há 105 milhões de anos, antes da separação da América do Sul e da África (Berg e Hijman, 1999; McLoughlin, 2001). Entretanto, estudos filogenéticos (Figura 3) embasados em dados moleculares (regiões ITS) apontam para a origem da família Moraceae no Cretáceo médio, há aproximadamente 89,1 milhões de anos, e diversificação após a separação da África e da América do Sul. Neste cenário, o gênero *Dorstenia* teria se originado

há aproximadamente 20 milhões anos no continente Africano, e migrado para o continente americano (Zerega et al. 2005). Porém, estes resultados foram inconclusivos quanto à distribuição das espécies em ambos os continentes ter ocorrido por dispersão a longa distância ou vicariância.

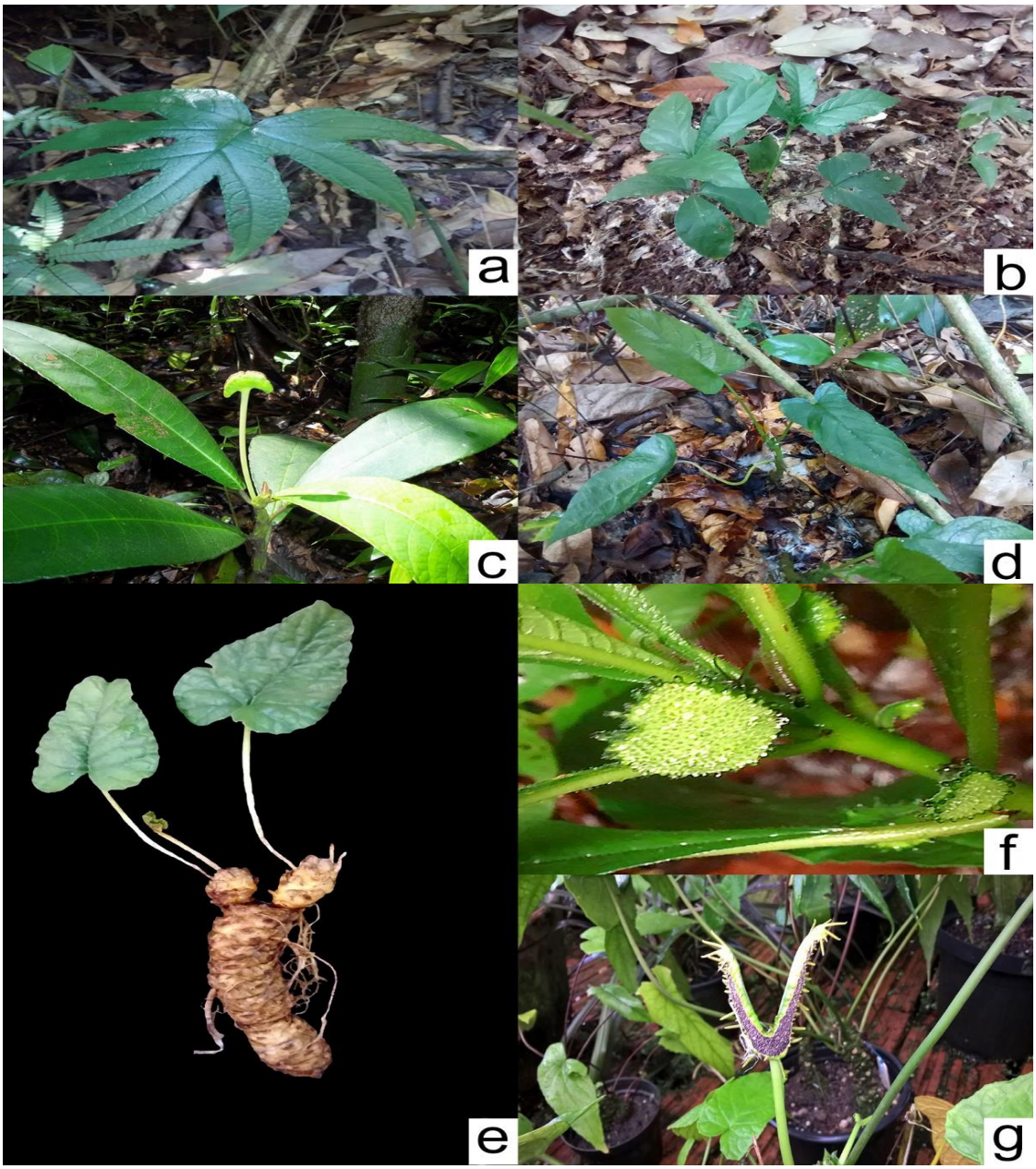


**Figura 1.** Representantes herbáceos das espécies Neotropicais de *Dorstenia* (a) *D. arifolia*, (b) *D. bonijesu*, (c) *D. elata*, (d) *D. grazielae*, (e) representação do rizoma em *D. cayapia*, (f) inflorescência do tipo cenanto em *D. hirta* e (g) cenanto bifurcado em *D. dolichocaula* representante da seção *Sychinia.*

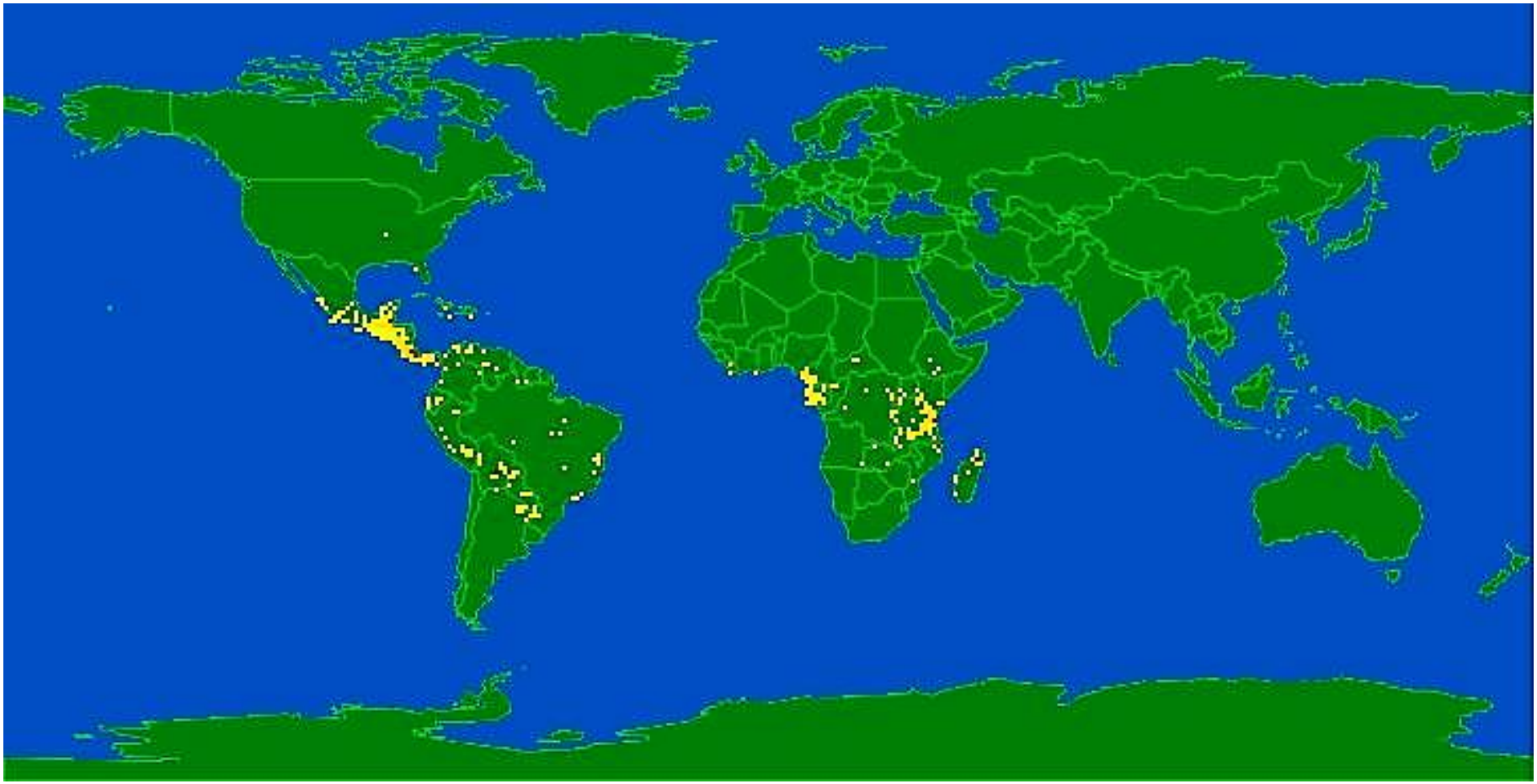

**Figura 9.** Mapa de distribuição das espécies do gênero *Dorstenia*. Os pontos em amarelo representam as localizações de coleta (Fonte: Tropicos).

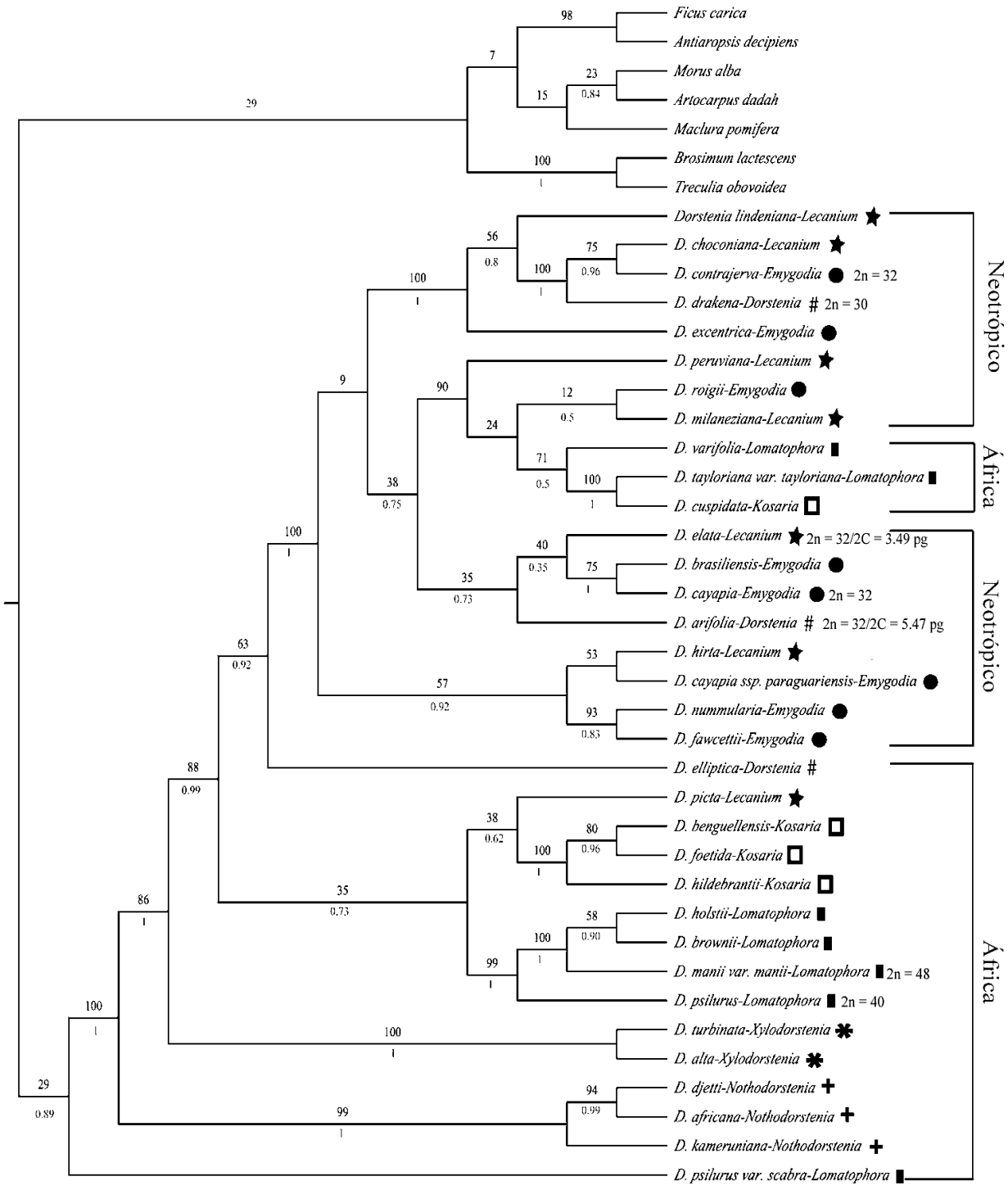


**Figura 10.** Filogenia proposta para o gênero *Dorstenia*, 2n representa o número cromossômico diploide, enquanto o valor 2C mostra o conteúdo de DNA nuclear em picogramas (pg) (Fonte: Misiewicz e Zerega 2012).

Do ponto de vista taxonômico e filogenético, *Dorstenia* é um gênero bem delimitado e claramente monofilético (Misiewicz e Zerega, 2012), o qual foi estabelecido por Linnaeus (1753) com base em *D. contrajerva*, espécie nativa do México. As duas primeiras espécies brasileiras descritas, *D. brasiliensis* e *D. arifolia*, são de autoria de Lamarck (1786), seguindo-se todas as demais. Aspectos da distribuição geográfica e caracteres como hábito e morfologia do cenanto foram utilizados por autores que trabalharam a sistemática de *Dorstenia* em nível infragenérico, estabelecendo seções para agrupar as espécies. Historicamente, a divisão em seções ou diferenciação em grupos de espécies levou em consideração os seguintes aspectos: distribuição geográfica (Sprengel, 1826); forma da inflorescência e aspectos do cenanto (Endlicher, 1842; Lemaire, 1863); hábito e/ou forma de vida (Fisher e Meyer 1846; Walpers, 1948-1849; Miquel 1853; Carauta, 1976-1978), a combinação de aspectos morfológicos e de distribuição geográfica (Bureau, 1873), hábito, forma de vida e caracteres florais (Engler, 1898); hábito, suculência e distribuição geográfica (Berg e Hijman, 1999). Estes últimos autores, propuseram a sistematização de *Dorstenia* em 9 seções, amplamente aceita atualmente: *Nothodorstenia*, *Xylodorstenia*, *Acauloma*, *Bazzemia*, *Lomatophora*, *Kosaria*, *Emygdioa*, *Lecania* e *Dorstenia*.

Uma seção denominada *Sychinia* foi estabelecida por Fisher e Meyer (1846) com base no gênero monotípico *Sychinium ramosum*, descrito por Desvaux (1826) como gênero relacionado a *Dorstenia*, porém distinto em virtude do peculiar cenanto bifurcado (Figura 1g), desconhecido para as demais espécies de *Dorstenia* até então. Posteriormente, Pilger (1937) descreveu *D. dolichocaula*, espécie ocorrente no estado do Rio de Janeiro, que juntamente com *D. capricorniana*, descrita por Carauta, Valente e Sucre (1974), ampliou a

riqueza de espécies com cenanto bifurcado. Berg (2001), no entanto, sinonimizou *Sychinia* (espécies de cananto bífido) à *Lecania* (espécies de cenanto inteiro). Esta sinonímia não foi aceita por Carauta (1978) que manteve a seção *Sychinia*, permanecendo esta questão também em aberto. Considerando as divergências em relação a escolha dos caracteres morfológicos adequados para classificar as espécies de *Dorstenia* em nível infragenérico, torna-se fundamental o uso de outros marcadores, como moleculares e citogenéticos, para esclarecimento desta questão.

O entendimento dos processos relacionados à diversificação cromossômica em *Dorstenia* podem trazer luz a questões evolutivas e taxonômicas no gênero. No entanto, estudos desta natureza são escassos na literatura frente à riqueza de espécies existentes, sobretudo no Neotrópico. Os estudos citogenéticos do gênero se resumem principalmente ao uso da citogenética clássica, sendo conhecidos número (Krause, 1931; Le Coq, 1964; Hoen, 1983; Oginuma e Tobe, 1995), morfologia cromossômica e conteúdo de DNA de três espécies (Amaral-Silva et al. 2016). Na obra Flora Neotropica, Berg (2001) apresenta um resumo dos aspectos citogenéticos para o gênero (Tabela 1). Basicamente, o número cromossômico em *Dorstenia* varia de 2n=24 a 2n=72. As espécies africanas apresentam número básico de x = 12 e 13 com número 2n = 24, 26, 28, 36, 40, 42, 48, 52, 64, 72, incluindo diploides, triploides, tetraploides, pentaploides, hexaploides e aneuploides, enquanto as Neotropicais apresentam x = 14, 15 e 16 com número 2n = 28, 30 e 32 (Tabela 1). Esses estudos têm revelado uma variabilidade cariotípica no gênero, que podem ter ocorrido por meio de rearranjos cromossômicos.

Alterações cromossômicas numéricas e estruturais têm sido relatadas como precursoras de mudanças em vários táxons de espécies vegetais. Por consequência, as variações no tamanho do genoma nuclear entre espécies filogeneticamente relacionadas (Bonifácio et al. 2012; Raskina et al. 2008). As relações evolutivas do gênero *Dorstenia* ainda são imprecisas principalmente pelo número reduzido de estudos. Análises cariotípicas e o mensuramento do conteúdo de DNA nuclear se mostraram informativas quanto à evolução do cariótipo e sistemática de *Dorstenia* (Amaral-Silva et al. 2016). No entanto, falta na literatura uma investigação direcionada a utilizar os caracteres do cariótipo para tratar de questões relacionadas a sistemática de *Dorstenia*, sendo este o principal objetivo deste trabalho.

**Tabela 1.** Resumo dos estudos citogenéticos referentes ao número cromossômico no gênero *Dorstenia*, destacando em sombreado as espécies do Neotrópico

| Espécies de *Dorstenia* | Número cromossômico | Autores |
|---|---|---|
| *D. arifolia* Lam. | 2n = 32 | Hoen (1983); Amaral-Silva (2016) |
| *D. bahiensis* Klotzsch. ex Fisch. | 2n = 32 | Hoen (1983) |
| *D. barteri* Bur | 2n = 24 | Krause (1931) |
| *D. bonijesu* Carauta e Valente | 2n = 32 | Amaral-Silva (2016) |
| *D. cayapia* subsp. *cayapia* Vell. | 2n = 32 | Hoen (1983) |
| *D. cayapia* subsp. *asaroides* Vell. | 2n = 32 | Hoen (1983) |
| *D. contrajerva* L. | 2n = 32 | Oginuma (1995) |
| *D. convexa* Wild. | 2n = 24 | Krause (1931) |
| *D.drakena* L. | 2n = 30 | Hoen (1983) |
| *D. elata* Hook. | 2n = 26* | Krause (1930)* |
| | 2n = 32** | Krause (1931)**; Amaral-Silva (2016)** |
| *D. frutescens* Engl. | 2n = 26 | Le Coq (1964) |
| *D. hirta* Desv. | 2n = 28 | Krause (1931); Hoen (1983) |
| *D. mannii* Hook. | 2n = 48 | Krause (1931) |
| *D. massoni* Bur. | 2n = 40 | Krause (1931) |
| *D. psilurus* Welw. | 2n = 40 | Krause (1931) |
| *D. scabra* (Bureau) Engl. | 2n = 40 | Krause (1931) |
| *D. ramosa* Desv. | 2n = 32 | Krause (1930) |
| *D. tenuis* Bonpl. ex Bareau. | 2n = 32 | Hoen (1983) |
| *D. turnerifolia* Fischer e Meyer | 2n = 32 | Krause (1931) |
| *Inc. D. argentata* Hook. f | 2n = 28 | Le Coq (1964) |
| *D. urceolata* Schott | 2n = 32 | Krause (1931); |
| *D. volkensii* Engl. | 2n = 24 | Le Coq (1964) |
| *D. yambuyaensis* Wild. | 2n = 24 | Krause (1931) |

* Primeiro número cromossômico descrito; ** Correção do número cromossômico;

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**Chapter**

# The role of cytogenetic data to the taxonomy in Neotropical species of *Dorstenia* L. (Moraceae)

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**The role of cytogenetic data to the taxonomy in Neotropical species of *Dorstenia* L. (Moraceae)**

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## Abstract

Previous cytogenetic studies in *Dorstenia* mention that the species may have 24 to 72 chromosomes, and suggested a conserved chromosome number 2n = 32 for the Neotropic species. However, some information reported in the literature are dubious or insufficient to assess the potential of cytogenetic data to the better understand of systematics and evolution issues within this genus. Here, eight species of Neotropical *Dorstenia* had their karyotypes characterized, and the nuclear DNA content measured. *Dorstenia bahiensis*, *D. cayapia*, *D. grazielae*, *D. hirta* and *D. turnerifolia* had their karyotypes characterized and the DNA nuclear content measured for the first time. Morphological plant characters and morphometric data were submitted to cluster analysis, followed by a test of group sharpness, and ordination analysis, aiming to support the discussion about the potential of cytogenetic data to infrageneric systematic of *Dorstenia*. The species showed chromosome number of 2n = 32, varying in chromosomes morphology. The karyotypes least asymmetric were observed in *Dorstenia elata*, and the more asymmetric were registered in *D. bahiensis* and *D. bonijesu*. The 2C value ranged from 3.21 picograms (pg) *D. bahiensis* to 5.47 pg in *D. arifolia*. Morphologically similar species, like *D. hirta* and *D. turnerifolia*, grouped together based on morphometric data. The sharp groups based on morphometric data correspond to species circumscribed under the sections *Dorstenia, Lecania* and *Emygodia*, previously established based on the plant morphology. Our results supports that the chromosome number 2n = 32 is possible conserved in the Neotropical species of *Dorstenia*, and indicate the potential of cytogenetic data to the systematics of this genus.

**Key-words:** Chromosome structure, flow cytometry, cytotaxonomy, cytogenetics.

## Introduction

Events that lead in karyotype changes may be due to structural and numerical rearrangements in the chromosome, occurred during the evolutionary processes (Lysak et al. 2006), resulting in the increase or decrease of the genome portions (Leitch et al. 2008). These processes can generate phenotypic differences between taxa, partially explaining the morphological differences among species within a genus (Kron et al. 2007). A recent study has shown the variability in chromosome morphology in three Neotropical species *Dorstenia* L. (Moraceae), despite the constant number of 2n = 32 (Amaral-Silva et al. 2016), indicating that this variability is given by structural chromosome rearrangements. At this point knowledge, it is essential to confirm if the chromosomal number of 2n = 32 is constant in the other Neotropical species of this genus, as well as check some dubious results in the literature due to some species synonymization.

The classical cytogenetics has contributed to evolutionary and taxonomic inferences in related species of the same genus (Morales et al. 2013; Prančl et al. 2014). Identical chromosome number may indicate a close phylogenetic relationship between species, while different suggests reproductive isolation (Fishman et al. 2014), and a further phylogenetic divergence. Considering that karyotype differences can be observed even among species that have no variations in chromosome number (Nani et al. 2015), the observation of chromosomal class, number and the position of secondary constrictions (Guerra 2012) are important karyotype features for interspecific distinction. This information may be useful to the understanding of the boundaries between related or highly variable species, assisting in taking taxonomic decisions.

Nuclear DNA content quantification has increasingly been used in taxonomic studies for its feasibility and reproducibility (Bennet and Leitch 2005a, 2005b; Leitch et al. 2008; Bennet and Leitch 2011; Chumová et al. 2015). Flow cytometry (FCM) use to measure the nuclear and chromosomal DNA content. These aspects make the FCM useful to reveal differences between taxa, mostly in groups of species that have chromosome number conserved (Mabuchi et al. 2005). Variations in the size of interspecific nuclear genome reported in the literature for this kind indicates that the karyotypes differ between species by changes in chromosome structure (Amaral-Silva et al. 2016). These modifications can be detected by changes in chromosome classes, chromosomes total length, and the length of long and short arms of chromosomes. Thus, to identify these changes make possible to infer about homologies and differences in karyotype within related taxa (Guerra 2008; Acosta et al. 2015).

Pantropical genus *Dorstenia* is a monophyletic genus (Misiewicz and Zerega 2012), well defined morphologically. The species are characterized as herbs or subshrubs with the type coenanthium inflorescence. Aspects of geographical distribution, habit and morphology of flowers and inflorescences were used to propose an infrageneric classification for *Dorstenia*, establishing sections to organize morphological variation patterns showed by species (e.g., Carauta 1976-1978; Berg and Hijman 1999). Based on these characteristics, Berg and Hijman (1999) proposed the systematization of *Dorstenia* currently accepted in nine sections: *Nothodorstenia, Xylodorstenia, Acauloma, Bazzemia, Lomatophora, Kosaria, Emygdioa, Lecania* and *Dorstenia*. *Sychinia* section was established by Fisher and Meyer (1846) based on the monotypic genus

*Sychinium ramosum*, described by Desvaux (1826) as a genus related to *Dorstenia*, but distinct due to the peculiar coenanthium bifurcated, which was unknown until that moment. Berg (2001) synonymized *Sychinia* to *Lecania*, but this synonymous was not accepted by Carauta (1978), remaining this open question.

Previous contributions applying cytogenetics analysis were effect to delimit genera and infrageneric taxa within Asteraceae (Via Do Pico and Dematteis 2014) and groups of species in Alliaceae (Souza et al. 2012). However, species showing the same chromosome number, ploidy level or structural changes, cannot be necessarily related (Guerra 2008). In this scenario, a detailed comparative karyotype analyses proved to be an important tool for plant taxonomy (Guerra 2012), given basis to the improve classifications (Stace 2000).

Here we intend to investigate if the cytogenetic data is useful for a better understanding of the infrageneric classification of *Dorstenia* (Berg and Hijman 1999). For this purpose, were outlined the following goals: 1. To increase the knowledge about *Dorstenia* species karyotype, from the study of a larger number of Neotropical taxa applying classical cytogenetic techniques; 2.To verify the occurrence of interspecific variation in nuclear DNA content in studied species, applying FCM; 3. To evaluate the potential of cytogenetics data to provide useful information to infrageneric *Dorstenia* classification.

## Material and Methods

**Sample —** Eight species of *Dorstenia* were included in this study: *D. arifolia* Lam., *D. bahiensis* Klotzsch ex Fisch*, D. bonijesu* Carauta and Valente*, D. cayapia* Velloso, *D. elata* Hook*, D. grazielae* Carauta, Valente and Sucre*, D.*

*hirta* Desv*, D. turnerifolia* Fischer and Meyer. The samples were collected in localities of the Espírito Santo state, defined from the data query registered at the site Species Link (2015). One individual per species (within about 5 collected) were dried and prepared to be included in the VIES herbarium collection, representing the voucher material (*D. arifolia* – T.T Carrijo 1516; *D. bahiensis* – J. Luber 241; *D. bonijesu* – T.T Carrijo 1939; *D. cayapia* – J. Luber 239; *D. elata* – T.T Carrijo 1618; *D. grazielae* – J. Luber 240; *D. hirta* – T.T Carrijo 1556; *D. turnerifolia* – J. Luber 171). The other individuals were grown in water (B.O.D. incubator maintained at 28°C), to obtain roots for cytogenetics studies.

**Cytogenetic analysis —** Roots were treated with 4 µM amiprophos-methyl (APM) for 14 – 15 h at 4°C, washed with distilled water, fixed in methanol: acetic acid (3: 1 v/v) and stored at -20 °C. After 24 h, the roots were washed in distilled water and macerated in pectinase solution 1:45 (enzyme: distilled water) for 1 h 45 min or 2 h at 34 °C. Slides were prepared (Carvalho et al. 2007), and prometaphases/metaphase images were captured with a video camera Nikon DS - Fi1c engaged on a Nikon 80i microscope (Nikon, Japan). Karyotype of *Dorstenia* species were characterized according to Levan et al. (1964), reviewed by Guerra (1986). The interchromosomic index asymmetry ($A_2$) of the karyotype was evaluated using the method proposed by Zarco (1986). Five high quality metaphases from the best treatment were used for this analysis.

**FCM —** Nuclear genome size of the *Dorstenia* species was estimated in accordance (Galbraith et al. 1983; Otto 1990; Praça-Fontes et al. 2011). Nuclei suspensions were analyzed in a flow cytometer Partec PAS II/III (Partec GmbH, Germany). Histograms were analyzed with the Partec Flow Max software tools to measure nuclear DNA content. *Solanum lycopersicum* Linnaeus, 1753 “Stupické”

(internal standard, 2C = 2.00 pg, Praça-Fontes et al. (2011) for FCM was grown in the field.

**Cytogenetic and Morphological multivariate analysis —** Two matrices, one based on cytogenetics data and other based on morphological data, were built to perform a multivariate analysis (cluster and ordination analysis). For cytogenetic data, a binary matrix was assembled including the eight studied species x the centromere position in the 16 pairs of chromosomes (Appendix 1). For morphological data, a binary matrix of eight studied species x 20 morphological features, including qualitative and quantitative traits, was build (Appendix 2). Morphological features were obtained from the taxonomic species description of Flora Neotropica (Berg 2001), and floras made to *Dorstenia* in Brazil (Carauta 1974, 1976). Vegetative and reproductive characters, which were chosen to compose the matrix, are informative for interspecies distinction, avoiding those one that have more than two states for the same species, as leaf form, for example. Cluster analysis was performed to reveal groups, followed by ordination analysis, which was useful to reveal patterns of association between species and characters. Gower index of similarity was used to perform both analysis, as the original matrix included qualitative and quantitative characters. The unweight arithmetic average clustering (UPGMA) algorithm carried out clustering. Considering that cluster analysis will always reveal groups, a test for the significance of partition levels (Pillar 1999) were applied. Cluster analysis and the test for fuzziness of the partitions in cluster analysis were performed using Multiv v.2.4 software (Pillar 2006); PCoA was carried out in the R software (R Development Core Team, 2014) using the VEGAN package (Oksanen et al.

2013). Cluster dendrograms were performed in R software using hclust function and the VEGAN function vegdist.

## Results

### Cytogenetic analysis

*Dorstenia* species studied here showed consistent chromosome number 2n = 32 (Fig. 1), but exhibited different morphology karyotype with each other. *Dorstenia arifolia* show two pairs of metacentric chromosomes (1 and 4), eight pairs of submetacentrics (2, 3, 7, 10, 13, 14, 15 and 16), and six of acrocentrics (5, 6, 8, 9, 11 and 12). *Dorstenia bahiensis* show one pair of submetacentric chromosomes (14), fourteen acrocentrics (1, 2 ,3 4, 5, 6, 7, 8, 8, 10, 11, 12, 13 and 15), and one telocentric (16). *Dorstenia bonijesu* show seven pairs of submetacentric chromosomes (1, 2 ,3 ,4, 5, 8 and 9), and nine acrocentrics (6, 7, 10, 11, 12, 13, 14, 15 and 16). *Dorstenia cayapia* show four pairs of metacentric chromosomes (1, 2, 3 and 12), five submetacentrics (5, 11, 14, 15 and 16), and seven acrocentrics (4, 6, 7, 8, 9, 10 and 13). *Dorstenia elata* show two pairs of metacentric chromosomes (1 and 14), eleven submetacentrics (2, 3, 5, 6, 7, 8, 9, 11, 12, 13 and 15), and three acrocentrics (4, 10 and 16). *Dorstenia grazielae* show one pair of metacentric chromossomes (3), four submetacentrics (2, 4, 10 and 16), and eleven acrocentrics (1, 5, 6, 7, 8, 9, 11, 12, 13, 14 and 15). *Dorstenia hirta* show two pairs of metacentric chromosomes (6 and 9), tem submetacentrics (1, 2, 3, 4, 5, 7, 8, 12, 13 and 15), and four acrocentrics (10, 11, 14 and 16). Finally, *D. turnerifolia* show eleven pairs of submetacentric chromosomes (1, 2, 3, 4, 5, 7, 8, 10, 12, 13 and 16), and five acrocentrics (6, 9, 11, 14 and 15). (Table 1).

**Table 1.** Morphometry of the metaphasic chromosomes of Neotropical *Dorstenia* species, interchromosomic asymmetry index ($A_2$) and DNA content (2C)

| Nº Chrom. | ***Dorstenia arifolia*** (2C = 5.47 pg) ($A_2$ = 0.16) | | ***Dorstenia bahiensis*** (2C = 3.21 pg) ($A_2$ = 0.21) | | ***Dorstenia bonijesu*** (2C = 4.05 pg) ($A_2$ = 0.21) | | ***Dorstenia cayapia*** (2C = 4.34 pg) ($A_2$ = 0.16) | | ***Dorstenia elata*** (2C = 3.49 pg) ($A_2$ = 0.11) | | ***Dorstenia grazielae*** (2C = 5.37 pg) ($A_2$ = 0.20) | | ***Dorstenia hirta*** (2C = 4.83 pg) ($A_2$ = 0.15) | | ***Dorstenia turnerifolia*** (2C = 4.24 pg) ($A_2$ =0.19 ) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** | **Total (µm)** | **Clas. r** |
| 1 | 3.34 | M | 3.34 | A | 3.61 | SM | 2.54 | M | 2.32 | M | 3.12 | A | 2.36 | SM | 2.99 | SM |
| 2 | 3.30 | SM | 2.99 | A | 3.39 | SM | 2.45 | M | 2.23 | SM | 2.94 | SM | 2.27 | SM | 2.63 | SM |
| 3 | 3.30 | SM | 2.90 | A | 3.25 | SM | 2.27 | M | 2.09 | SM | 2.72 | M | 2.23 | SM | 2.63 | SM |
| 4 | 3.03 | M | 2.58 | A | 2.94 | SM | 2.27 | A | 2.05 | A | 2.54 | SM | 2.09 | SM | 2.58 | SM |
| 5 | 3.85 | A | 2.54 | A | 2.85 | SM | 2.23 | SM | 2.05 | SM | 2.45 | A | 2.00 | SM | 2.45 | SM |
| 6 | 2.76 | A | 2.50 | A | 2.76 | A | 2.23 | A | 2.05 | SM | 2.45 | A | 1.96 | M | 2.27 | A |
| 7 | 2.72 | SM | 2.41 | A | 2.67 | A | 2.18 | A | 1.96 | SM | 2.41 | A | 1.96 | SM | 2.23 | SM |
| 8 | 2.67 | A | 2.32 | A | 2.58 | SM | 1.96 | A | 1.96 | SM | 2.27 | A | 1.91 | SM | 2.14 | SM |
| 9 | 2.67 | A | 2.23 | A | 2.54 | SM | 1.96 | A | 1.91 | SM | 2.23 | A | 1.87 | M | 2.14 | A |
| 10 | 2.63 | SM | 2.14 | A | 2.50 | A | 1.91 | A | 1.87 | A | 2.23 | SM | 1.83 | A | 2.09 | SM |
| 11 | 2.54 | A | 2.14 | A | 2.32 | A | 1.87 | SM | 1.87 | SM | 2.18 | A | 1.78 | A | 1.96 | A |
| 12 | 2.54 | A | 2.09 | A | 2.27 | A | 1.87 | M | 1.69 | SM | 2.14 | A | 1.74 | SM | 1.87 | SM |
| 13 | 2.45 | SM | 1.87 | A | 2.00 | A | 1.69 | A | 1.69 | SM | 2.05 | A | 1.56 | SM | 1.87 | SM |
| 14 | 2.41 | SM | 1.83 | SM | 1.96 | A | 1.69 | SM | 1.65 | M | 1.87 | A | 1.56 | A | 1.69 | A |
| 15 | 2.27 | SM | 1.69 | A | 1.87 | A | 1.51 | SM | 1.65 | SM | 1.74 | A | 1.47 | SM | 1.60 | A |
| 16 | 2.23 | SM | 1.47 | T | 1.69 | A | 1.42 | SM | 1.56 | A | 1.20 | SM | 1.42 | A | 1.47 | SM |
| Total | 44.71 | - | 37.04 | - | 41.20 | - | 32.05 | - | 30.60 | - | 36.54 | - | 30.01 | - | 34.61 | - |

M – metacentric; SM – submetacentric; A – acrocentric; T – telocentric.

The chromosomes of the studied species also differ by the presence of secondary constrictions in the short and long arms (Fig. 2 and 3). *Dorstenia arifolia* presented secondary constriction in the short arm of chromosome pairs 1 and 3 (Fig. 2a). *Dorstenia bahiensis* presented secondary constriction only in the long arm of the chromosome 4 (Fig. 2b). *Dorstenia grazielae* presented secondary constriction in the short arms of chromosomes 3 and the long arm of chromosome 7 (Fig. 3b). Chromosomes with secondary constrictions were not evidenced in *D. bonijesu* and *D. cayapia* (Fig. 2c and d), *D. elata D. hirta* and *D. turnerifolia* (Fig. 3a, c and d). The highest chromosome complement was measured in *D. arifolia* (44.71 µm), followed by *D. bonijesu* (41.20 µm), *D. bahiensis* (37.04 µm), *D. grazielae* (36.54 µm), *D. turnerifolia* (34.61 µm), *D. cayapia* (32.05 µm), *D. elata* (30.60 µm) and *D. hirta* (30.01 µm) (Table 1). The interchromosomic asymmetry index $A_2$ differs among species, as shown in Table 1.

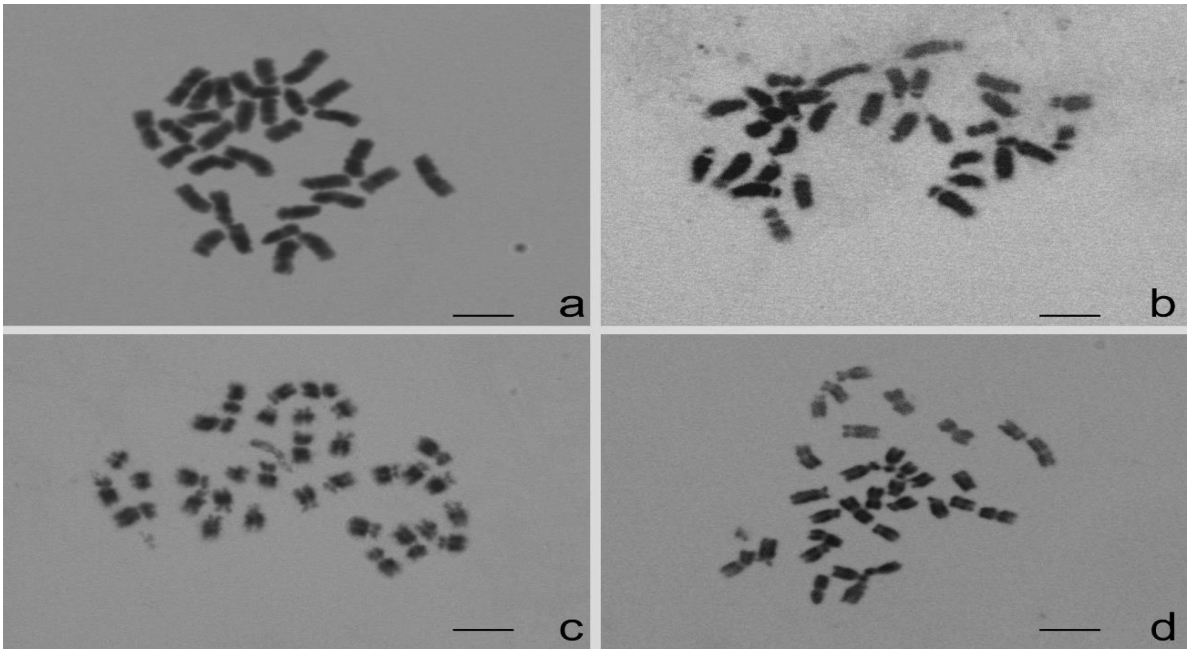


**Figure 1.** Representative karyotypes of *Dorstenia* species (2n = 32 chromosomes) obtained from roots treated with APM 4 µM during 15h, (a) *D. arifolia*, (b) *D. bahiensis*, (c) *D. bonijesu*, (d) *D. cayapia*. Chromosomes were stained with Giemsa 5%. Bar = 5 µm.

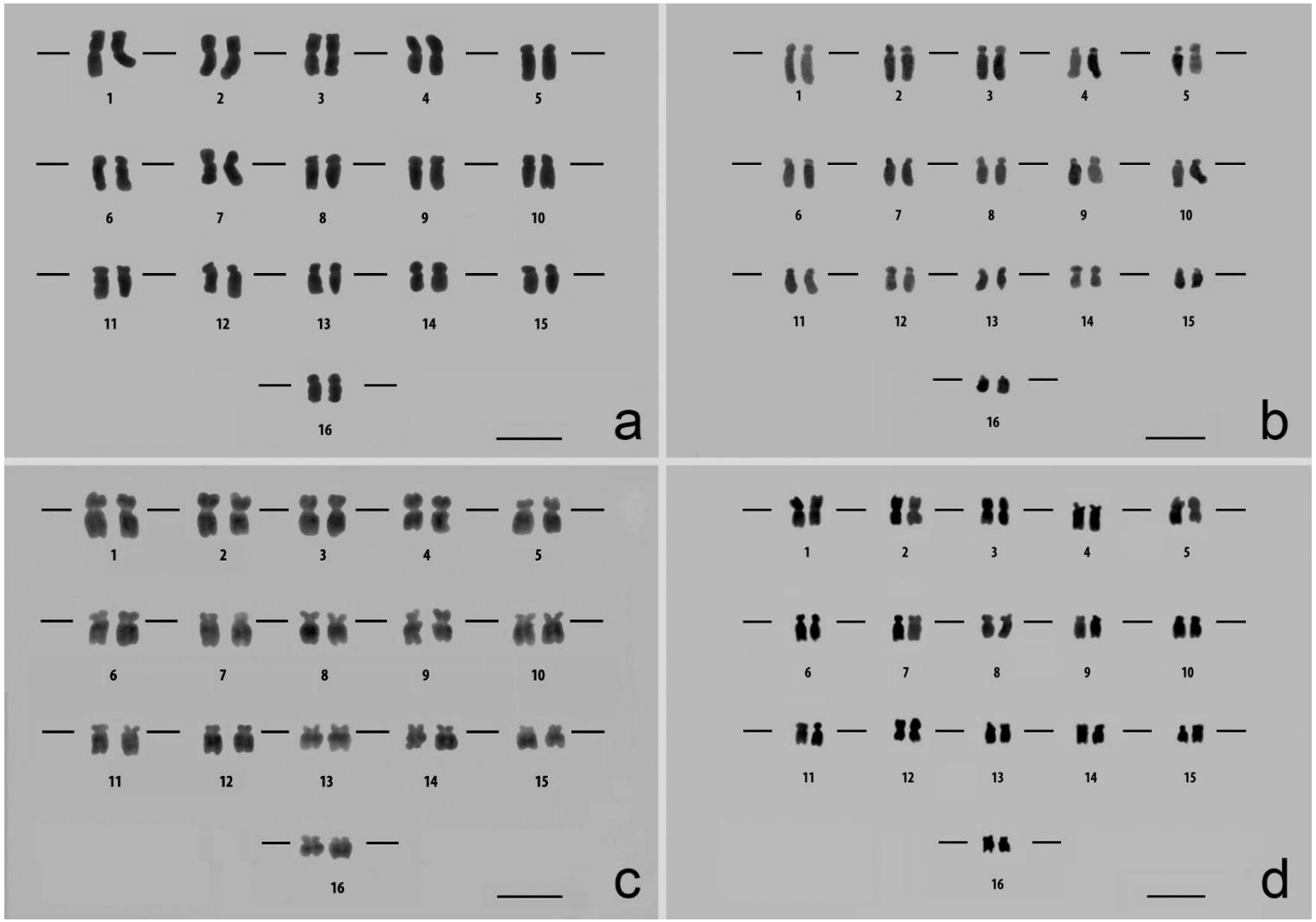


**Figure 2.** Karyograms of *Dorstenia* species (2n = 32 chromosomes), built from metaphase chromosomes obtained from roots treated with 4 APM µM, during 15 h, (a) *D. arifolia*, (b) *D. bahiensis*, (c) *D. bonijesu*, (d) *D. cayapia*. Chromosomes were stained with Giemsa 5%. Bar = 5 µm.

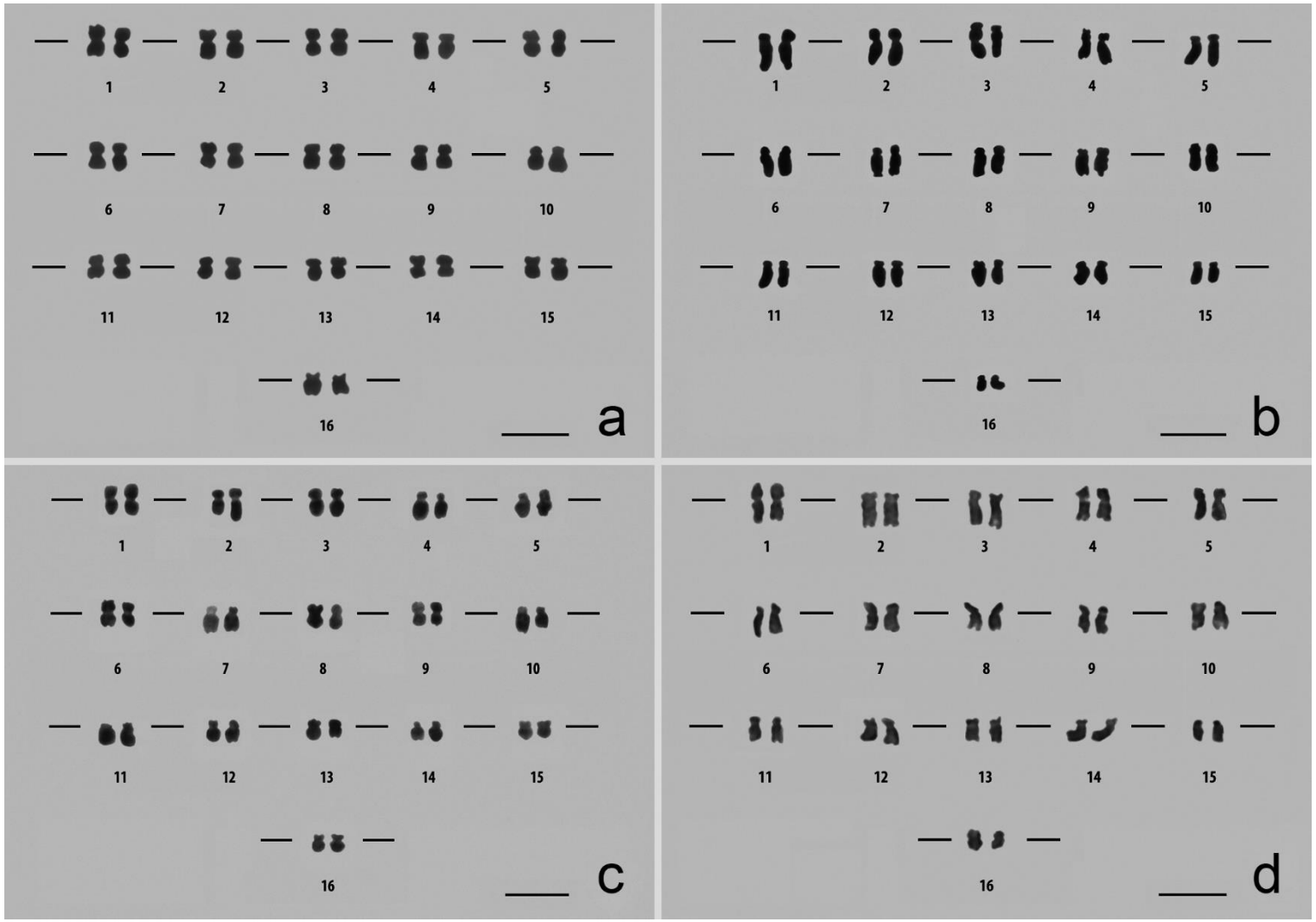


**Figure 3.** Karyograms of *Dorstenia* species (2n = 32 chromosomes), built from metaphase chromosomes obtained from roots treated with 4 APM µM during 15h, (a) *D. elata*, (b) *D. grazielae*, (c) *D. hirta*, (d) *D. turnerifolia*. Chromosomes were stained with Giemsa 5%. Bar = 5 µm

**FCM**

Nuclear suspensions generate histograms showing $G_0/G_1$ peaks with coefficient of variation below than 5%. The values in picograms (pg) and base pair (bp) are shown in Table 2. These results show that the value of the mean nuclear DNA content vary among species. *Dorstenia arifolia* show the highest 2C value, which is 20,65% higher than the 2C intermediary value of *D. cayapia*, and 41,31% higher than *D. bahiensis*, species that show the lower 2C value.

**Table 2.** Mean nuclear 2C values of diploid (in pg) and 1C haploid (in bp) complement, in descending order

| Species | 2C | 1C |
|---|---|---|
| *Dorstenia arifolia* | 5.47 pg ± 0.002 | 2.67 x $10^9$ bp |
| *D. grazielae* | 5.37 pg ± 0.080 | 2.62 x $10^9$ bp |
| *D. hirta* | 4.83 pg ± 0.004 | 2.36 x $10^9$ bp |
| *D. cayapia* | 4.34 pg ± 0.035 | 2.13 x $10^9$ bp |
| *D. turnerifolia* | 4.24 pg ± 0.042 | 2.07 x $10^9$ bp |
| *D. bonijesu* | 4.05 pg ± 0.0035 | 1.98 x $10^9$ bp |
| *D. elata* | 3.49 pg ± 0.014 | 1.70 x $10^9$ bp |
| *D. bahiensis* | 3.21 pg ± 0.016 | 1.57 x $10^9$ bp |

**Multivariate analyses based on cytogenetics and morphological data**

Cluster analysis based on cytogenetic data followed by the partition test revealed three sharp groups (Fig. 4) as follows: *Dorstenia elata*, *D. bonijesu*, *D. hirta* and *D. turnerifolia* (G1), *D. cayapia* (G2), *D. arifolia*, *D. bahiensis* and *D. grazielae* (G3).

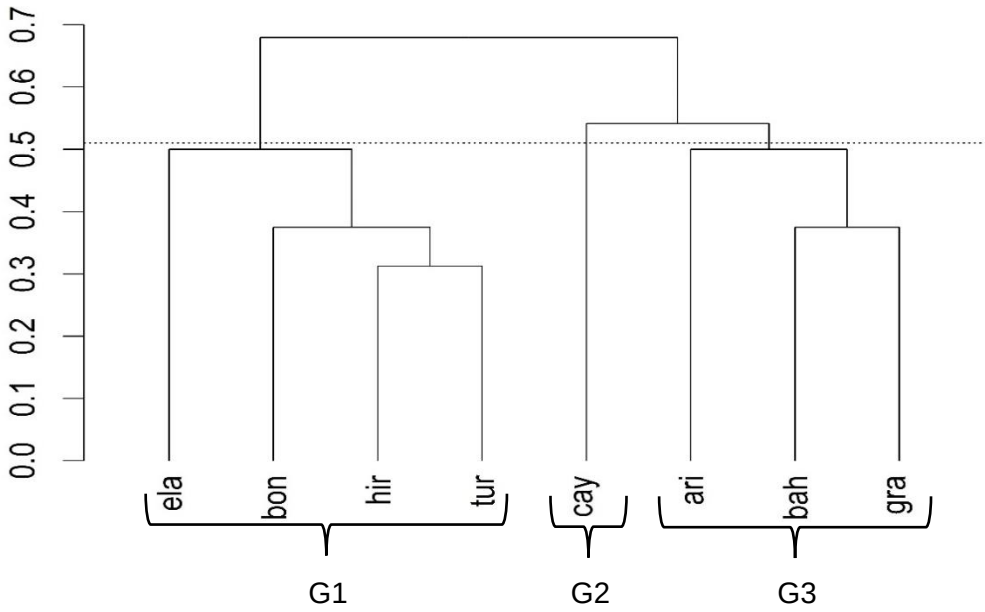


**Figure 4.** Cluster analysis and test of groups partition based on karyotype characteristics of *Dorstenia* species studied (Appendix 1). The dotted line indicates the partition of the groups considering all species analyzed. ari: *D. arifolia*; bah: *D. bahiensis*; bon: *D. bonijesu*; cay: *D. cayapia*; her: *D. elata*; gra: *D. grazielae*; hir: *D. hirta*; tur: *D. turnerifolia.*

Ordination analysis of cytogenetic data (Fig. 5) showed the chromosome pairs that grouped the species in their groups. The chromosome pairs 3, 4, 8 and 12 (submetacentric), as well as the pair 14 (acrocentric), except *D. elata* which features 4 pairs acrocentric and 14 metacentric, explains the grouping *D. elata, D. bonijesu, hirta D.* and *D. turnerifolia* (G1). The chromosome pairs 4 (acrocentric) and 11 (submetacentric) explain *D. cayapia* as monospecific group (G2). The chromosome pairs 8 and 11 (both acrocentric), explain *D. arifolia, D. bahiensis* and *D. grazielae* (G3).

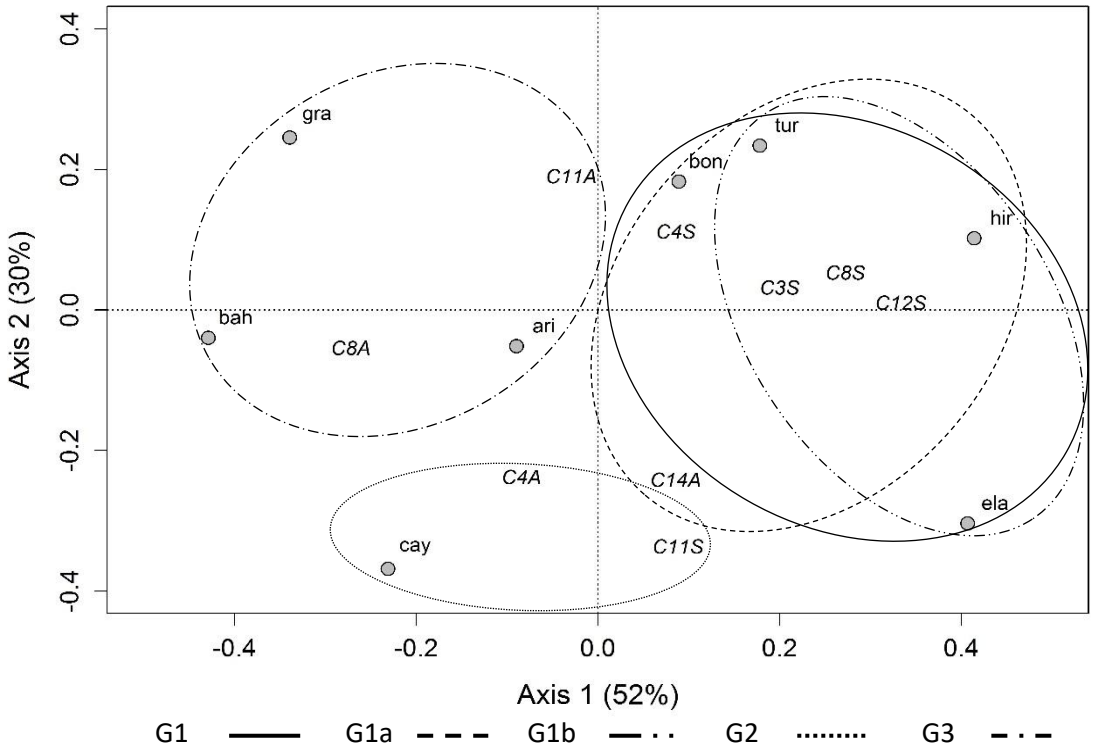


**Figure 5.** Ordination analysis (PCoA) of Neotropical *Dorstenia* species. The dotted lines indicate the groups formed by the species. The acronyms in tiny indicate the species, and the uppercase symbols followed by number indicate the number of chromosomes and their respective classes.

Cluster analysis based on plant morphological data followed by the partition test revealed five sharp groups (Fig. 6) as follows: *Dorstenia grazielae*, *D. bahiensis* and *D. bonijesu* (G1), *D. arifolia* (G2), *D. cayapia* (G3), *D. elata* (G4), *D. hirta* and *D. turnerifolia* (G5). The ordination analysis revealed that morphological characters that better explains these groups are the plurinervate stipule, orbicular receptacle and petiole size (G1). The characteristic of being acaleuscent, show rosulate leaves and elliptical receptacle explain the monospecific *D. arifolia* (G2). The orbicular receptacle and the rosulate leaves explains the monoespecific group *D. cayapia* (G3). Finally, the last monospecific

group *D. elata* (G4) is explained by being caulescent and show distic leaves. Distic leaves and the uninervate stipule explain *D. hirta* and *D. turnerifolia* (G5).

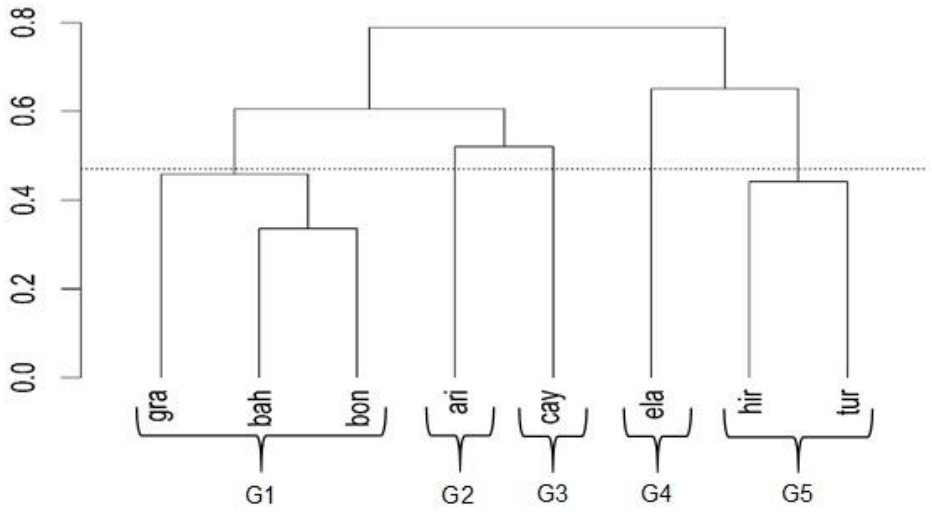


**Figure 6.** Cluster analysis showing the groups revealed by the test of group partition, based on morphological plant characters of *Dorstenia* species (Appendix 2). The dotted line indicates the partition of the groups considering all species analyzed. ari: *D. arifolia*; bah: *D. bahiensis*; bon: *D. boniiesu*; cav*: D. cavapia*; her: *D. elata*; gra: *D. grazielae*; hir*: D. hirta;* tur:

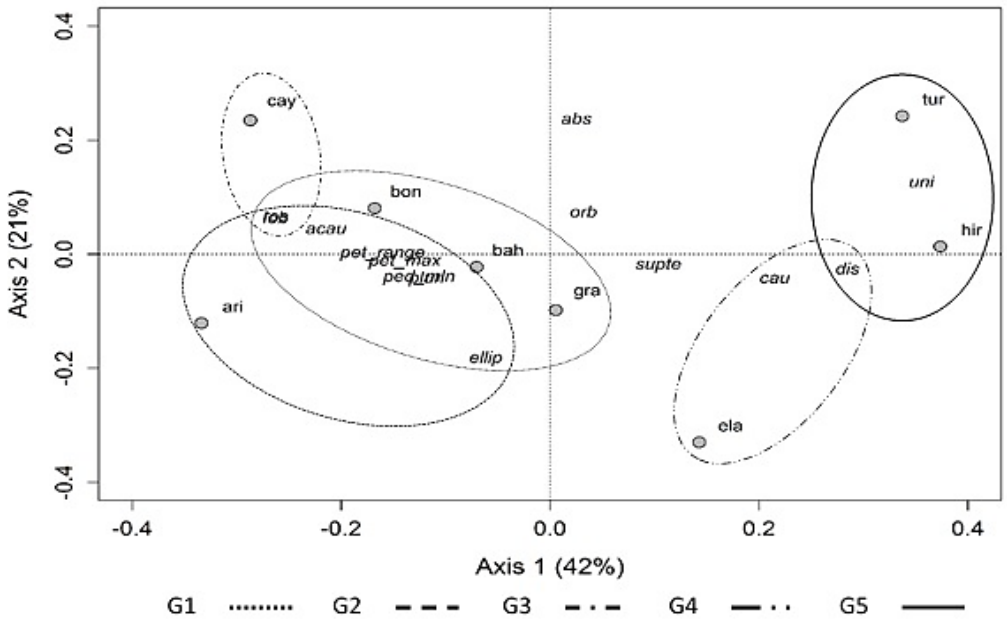


**Figure 7.** Ordination analysis (PCoA) of Neotropical *Dorstenia* species. The dotted lines indicate the groups formed by the species from morphological plant characters. The next acronyms the points indicate the species, and other morphological characters.

## Discussion

Chromosomes similar in number and morphology among species of *Dorstenia* would suggest common ancestry, despite the karyotype variation and nuclear DNA content. The karyotype characterization of *D. bahiensis*, *D. cayapia*, *D. grazielae*, and *D. hirta* and *D. turnerifolia*, including determination of the chromosomes number of *D. grazielae* is presented for the first time in this study. The chromosome number 2n = 32 reported to *D. arifolia* (Hoen 1983; Amaral-Silva et al. 2016)*, D. bahiensis* (Hoen 1983)*, D. bonijesu* (Amaral-Silva et al. 2016)*, D. cayapia* (Hoen 1983)*, D. elata* and *D. turnerifolia* (Krause 1931), was confirmed. However, the *Dorstenia hirta,* characterized by Krause (1931) e Hoen (1983) as possessing 2n = 28, was not confirmed. In fact, this species shows 2n = 32 chromosomes, as well as the other species. These results give more safety to state that the chromosome number of 2n = 32 seems to be conserved in Neotropical species of *Dorstenia*.

The basic chromosome number of x = 14, 15 and 16 suggested for Neotropical species *Dorstenia* by Berg (2001) were based on the first cytogenetic studies published to the genus during the 1930 and 1980 decades. It is possible that Berg (2001) has taken as a base chromosome numbers of 2n = 28, reported to *D. hirta* by Krause (1931) for setting x = 14 as a possible basic numbers of chromosomes in *Dorstenia*. This statement is justified by the results presented in this study, which strongly indicate the basic chromosome number x = 16 for this genus. It is possible that chromosomal determination methods used by Krause (1931) have led to mistakes in chromosomal count. Likewise, the basic number x = 15 (Berg 2001) was possibly based in the description of 2n = 30 for *D. drakena* reported by Hoen (1983). As if no other study was conducted for this species

later, this question remains open. Furthermore, the constant number of chromosomes 2n = 32 in Neotropical *Dorstenia* species indicates that the karyotype of the genus possibly involved by euploidy and aneuploidy events, since the most common basic chromosome numbers reported for Moraceae are x = 13 and 14.

The karyomorphological differences revealed by the morphometric analyzes indicate that structural chromosomal rearrangements occurred during the evolution of Neotropical *Dorstenia* species. Interchromosomal asymmetry index ($A_2$) supports this statement, since all species have the same chromosome number. Further asymmetry indexes indicate a more derived karyotype, while lower levels indicate less asymmetry derived karyotype (Stebbins 1971). In this sense, the karyotypes of *D. bahiensis* and *D. bonijesu* showed higher values of asymmetry among the studied species due to the predominance of acrocentric chromosomes. This data indicates that the karyotype of these species is more derived compared to *D. elata*, which is characterized by the presence of submetacentric chromosomes.

The $A_2$ reported in the literature for *D. bonijesu* and *D. elata* (Amaral-Silva et al. 2016) was confirmed here. The inclusion of *D. bahiensis* here has shown that this species has a pair of telocentric chromosomes, indicating higher derivation in relation to *D. bonijesu*, despite similar $A_2$ value of both species. Besides *D. bahiensis* and *D. bonijesu* share the most asymmetric karyotype, cluster analysis (Fig. 6) showed that these species share most morphological similarity to each other, forming a distinct group (G1) which also includes *D. grazielae*, classified under the section *Dorstenia* (Berg 2001).

Interspecific variation in nuclear DNA content pointed to a loss of genome portions during the evolution of Neotropical *Dorstenia* species. *Dorstenia bahiensis* showed the lowest value 2C (3.21 pg), confirming the result that the species has the karyotype more derivate, found through the asymmetry index. On the other hand, *D. elata* presented a 2C value = 3.49 pg, next to *the D. bahiensis*, indicating that there was also loss of genome portions. Despite the low 2C value, the $A_2$ showed that the karyotype of *D. elata* has the lowest values compared to the other species. This indicates that the karyotype evolution of these species followed different paths, and the occurrence of structural rearrangements resulted more karyotype changes in *D. bahiensis* than *D. elata. Dorstenia arifolia* (2C = 5.47 pg) and *D. grazielae* (2C = 5.37 pg) have the highest 2C values. However, the asymmetry index showed that the karyotype of *D. grazielae* ($A_2$ = 0.20) suffered more changes regarding the karyotype of *D. arifolia* ($A_2$ = 0.16) and *D. elata* ($A_2$ = 0.11). The lack of relationship between the value 2C and $A_2$ can be explained by previous studies results, which mentioned that the increase and decrease of the genome may be associated with ecological and environmental aspects as geographic distribution and altitude (Cullis 2005; Nora et al. 2013). Therefore, the structural rearrangements that led to diversification of the karyotype and interspecific variability in 2C value of species may have occurred as biological responses.

Evolutionary studies comparing the genome size and other characters such as cytological, physiological, morphological, reproductive and ecological have been conducted based on phylogenetic aspects (Beaulieu et al. 2007; Whitney et al. 2010; Herben et al. 2012). The phylogenetic reconstruction proposed by Misiewicz and Zerega (2012) for *Dorstenia*, presents a clade

consisting of three species analyzed in this study (*D. arifolia, D. cayapia and D. elata*). In this phylogeny, *Dorstenia arifolia* emerge as a sister group of *D. cayapia* and *D. elata*. This is expected, considering the evolutionary theory of Stebbins (1971), which argues that less derived species would have higher DNA content compared to more derivative species. However, it is not observed for *D. elata*. Despite the lower DNA content, this species emerges as less derived in relation to D. *cayapia* in Misiewicz and Zerega (2012) phylogeny. These results may be signaling DNA losses and gains throughout the evolution of *Dorstenia* they may be associated with other causes such as environmental factors, for example, and not exhibit phylogenetic signal.

Furthermore, the cluster analysis (Fig. 6) revealed that *D. arifolia*, *D. cayapia* and *D. elata* are the most morphologically different species from each other, due this species formed monospecific groups (G2, G3 and G4). The size of the genome could not be correlated with phylogenetic aspects of the genus *Hordeum* (Jakob et al. 2004). These authors also mentioned that DNA content can change rapidly according to the environmental parameters, and that only monophyletic related groups that show similar DNA content were correlated with environmental parameters. The lack of relation between nuclear DNA content and phylogeny of *Dorstenia* may indicate that environmental factors may have influenced at the diversification of Neotropical species. However, the development of phylogenetic reconstructions including greater amount of Neotropical species is needed for further clarification on this issue.

A comparative karyotype analysis has proven to be useful for understanding the evolutionary direction among related taxa in previous studies (Schubert 2007; Peruzzi 2009). Here, the karyotype similarities between *D. hirta*

and *D. turnerifolia* (Fig. 3c and d) may explain, the morphological similarity observed between these species. The cluster and ordination analysis based on cytogenetic data (Fig. 4 and 5) revealed that the chromosome pairs 3, 4, 8, 12 and 14 are shared between *D. hirta* and *D. turnerifolia*, and both species do not share the pairs 12 with *D. bonijesu*, four and 14 to *D. elata*. These species also share morphological characters, revealed by cluster and ordination analysis (Fig. 6 and 7), which reinforces the hypothesis of common ancestry, and supports its circumscription under Section *Lecania* (Berg, 2001).

Similarly, the multivariate analysis based on cytogenetic data show that *D. cayapia* is the most different species among taxa studied here. This difference is explained by the chromosome pairs 4 and 11. Although not shown in the analyzes, *D. cayapia* is the only species with larger amount (four pairs) of metacentric chromosomes, a chromosome class considered less derived (Stebbins 1971). The group of *D. arifolia, D. bahiensis,* and *D. grazielae* (G3) revealed that these species share the chromosome pairs 8 and 11. Within this group, *D. bahiensis* and *D. grazielae* are more similar to each other, as both of these species have more acrocentric chromosomes. This data points to most frequent structural rearrangements during evolution, which could be confirmed by $A_2$. According to the "minimal interaction hypothesis" proposed by Imai et al. (1986), the evolution of the karyotype generally tends to increase the number of acrocentric chromosomes. In this sense, the evolutionary history of the karyotype of the Neotropical species of *Dorstenia* leads to the indication that structural rearrangements may have led to an increased number of acrocentric chromosomes, and consequent diversification of the genus karyotype.

## Conclusion

Nuclear DNA content show that deletions were responsible for chromosomes diversification, indicating loss of genome portions during *Dorstenia* evolution. The number of chromosomes 2n = 32 seems to be conserved in Neotropical species *Dorstenia* since remained the same in unpublished karyotype analysis of *D. grazielae*, as well as in the number of chromosomes reported to *D. hirta* fixed in this study. The multivariate analysis revealed groups of species based by both chromosomal and morphological characters similar to the sections established by Berg's last taxonomic revision, which may indicate the potential of cytogenetic to *Dorstenia* systematic.

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Appendix 1

Matrix of character chromosome

| Species | Chromosomes | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 | C13 | C14 | C15 | C16 |
| *D. arifolia* | 1 | 2 | 2 | 1 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 2 |
| *D. bahiensis* | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 4 |
| *D. bonijesu* | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| *D. cayapia* | 1 | 1 | 1 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 1 | 3 | 2 | 2 | 2 |
| *D. elata* | 1 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 1 | 2 | 3 |
| *D. grazielae* | 3 | 2 | 1 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 |
| *D. hirta* | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 3 | 3 | 2 | 2 | 3 | 2 | 3 |
| *D. turnerifolia* | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 3 | 2 |

Appendix 2

Matrix of character states

| Species/ Characters | Abbreviation | Habit | | Rizome | | Stem | | Leaf - phyllotaxy | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | herb | subshurbs | subterranean | supraterranean | acaulescent | caulescent | spiral | rosulate | distichous |
| *D. arifolia* | ari | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 |
| *D. bahiensis* | bah | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| *D. bonijesu* | bon | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 |
| *D. cayapia* | cay | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 |
| *D. elata* | ela | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| *D. grazielae* | gra | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| *D. hirta* | hir | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| *D. turnerifolia* | tur | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |

| Species/ Characters | Abbreviation | Leaf - consistence | | Leaf - variegation | | Leaf - form | | Petiole - < length (cm) | Petiole - > length (cm) | Petiole - Length (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | chartaceous | subcoriaceous | absent | present | lobate | entire | | | |
| *D. arifolia* | ari | 1 | 0 | 0 | 1 | 1 | 1 | 8 | 42 | 34 |
| *D. bahiensis* | bah | 1 | 0 | 0 | 1 | 0 | 1 | 10 | 21 | 11 |
| *D. bonijesu* | bon | 1 | 0 | 0 | 1 | 1 | 1 | 4.5 | 30 | 25.5 |
| *D. cayapia* | cay | 1 | 0 | 1 | 0 | 1 | 1 | 3 | 25 | 22 |
| *D. elata* | ela | 1 | 1 | 0 | 1 | 0 | 1 | 5 | 8 | 3 |
| *D. grazielae* | gra | 1 | 1 | 0 | 1 | 0 | 1 | 10 | 35 | 25 |
| *D. hirta* | hir | 1 | 0 | 0 | 1 | 0 | 1 | 0.5 | 3 | 2.5 |
| *D. turnerifolia* | tur | 1 | 0 | 1 | 1 | 0 | 1 | 2 | 5 | 3 |

| Species/ Characters | Abbreviation | Stipule - venation | | Inflorescence - position | | Inflorescence - receptacle shape | | Inflorescence -receptacle margin shape | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | uninervate | plurinervate | horizontal | erect | flat | concave | elliptic | oval | orbicular | subtriangular |
| *D. arifolia* | ari | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 |
| *D. bahiensis* | bah | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| *D. bonijesu* | bon | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| *D. cayapia* | cay | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| *D. elata* | ela | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 |
| *D. grazielae* | gra | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 0 |
| *D. hirta* | hir | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| *D. turnerifolia* | tur | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |

| Species/ Characters | Abbreviation | Inflorescence - peduncle insertion | | Peduncle size (cm) | Peduncle size (cm) | Peduncle size (cm) | Staminate flowers arrangement | | Endocarp - surface | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | centrally attached | excentrally attached | | | | not peripheral | peripheral | turberculate | smooth |
| *D. arifolia* | ari | 1 | 0 | 9 | 20 | 11 | 1 | 0 | 1 | 0 |
| *D. bahiensis* | bah | 1 | 0 | 5 | 15 | 10 | 1 | 0 | 1 | 0 |
| *D. bonijesu* | bon | 1 | 0 | 6 | 12 | 6 | 1 | 0 | 1 | 1 |
| *D. cayapia* | cay | 1 | 0 | 2 | 18 | 16 | 1 | 0 | 0 | 1 |
| *D. elata* | ela | 1 | 1 | 2 | 25 | 23 | 1 | 0 | 1 | 0 |
| *D. grazielae* | gra | 1 | 0 | 5 | 12 | 7 | 1 | 0 | 1 | 0 |
| *D. hirta* | hir | 1 | 0 | 1.5 | 7 | 5.5 | 1 | 0 | 1 | 0 |
| *D. turnerifolia* | tur | 1 | 0 | 0.5 | 2.5 | 2 | 0 | 1 | 1 | 0 |