



# Karyotype evolution in the genus *Jacaranda* Juss. (Jacarandaeae, Bignoniaceae): chromosome numbers and heterochromatin

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**ABSTRACT.** Most taxa in the Bignoniaceae have  $2n = 40$ , but the basal clade Jacarandaeae has  $2n = 36$ , suggesting that  $x = 18$  is the ancestral basic number for the family. Variations in heterochromatin band patterns in genera that are numerically stable, such as *Jacaranda*, could facilitate our understanding of the chromosomal and karyotypic evolution of the family. We characterized heterochromatin distributions in six *Jacaranda* species using chromomycin A3 (CMA) and 4'6-diamidino-2-phenylindole (DAPI). All of them had  $2n = 36$ , including first counts for *Jacaranda bracteata* Bureau & K. Schum., *Jacaranda irwinii* A.H. Gentry, *Jacaranda jasminoides* (Thunb.) Sandwith, and *Jacaranda rugosa* A.H. Gentry. Their karyotypes had four to eight terminal CMA<sup>+</sup>/DAPI<sup>-</sup> bands per monoploid set. In the section *Monolobos*, *Jacaranda brasiliiana* (Lam.) Pers. had eight terminal bands and *Jacaranda mimosifolia* D. Don had four; in the section *Dilobos*, *J. bracteata* had six bands per monoploid set, with the

other species having five. While three species in the section *Dilobos* had the same number of terminal bands, *J. irwinii* had two additional pericentromeric bands and a proximal heterozygotic band, and *J. bracteata* had two distended CMA bands. The consistent records of  $2n = 36$  in *Jacaranda* may represent a plesiomorphic condition for the Bignoniaceae; therefore, the family originated from an ancestor with  $x = 18$ . However,  $2n = 36$  may represent a derived condition, and the family could have had an ancestral basic number of  $x = 20$  that is still conserved in most representatives of the family.

**Key words:** *Jacaranda*; Cytotaxonomy; CMA/DAPI; Terminal heterochromatin; Karyotypic variation

## INTRODUCTION

The tribe Jacarandae comprises 55 species of trees and shrubs belonging to the genera *Jacaranda* and *Digomphia* Benth., and has a strictly Neotropical distribution (Olmstead et al., 2009). *Jacaranda* has the greatest number of species in the tribe, and is one of the largest genera of the family Bignoniaceae, with 49 species (Gentry and Morawetz, 1992; Lohmann and Ulloa, 2016). The taxon is characterized by bi-pinnate or pinnate leaves, flowers with corollas blue or blue-purple to magenta, staminode elongate, exceeding the stamens, a calyx short and broadly campanulate to cupular, and fruit oblong, flattened, perpendicular to the septum (Morawetz, 1982; Gentry and Morawetz, 1992).

The genus is subdivided into two sections: *Monolobos* DC. and *Dilobos* Endl. The section *Monolobos* has 1-thecate anthers, and comprises 18 species that are found in the Antilles, Central America, and the western part of South America; the section *Dilobos*, which is considered the most primitive, has 2-thecate anthers and comprises 31 species that are mainly found in Brazil (Morawetz, 1982; Gentry and Morawetz, 1992; Dos Santos and Miller, 1997). Molecular phylogenetic analyses have indicated that the tribe Jacarandae is the most basal in the family, and is a sister group to the other Bignoniaceae (Spangler and Olmstead, 1999; Olmstead et al., 2009). These analyses, however, only included four species, with two from the section *Monolobos* and two from the section *Dilobos*, and their relationships are unresolved (Olmstead et al., 2009).

The Bignoniaceae exhibits notable numerical karyotypic stability, with most species having  $2n = 40$  (Goldblatt and Gentry, 1979; Piazzano, 1998; Piazzano et al., 2015); the Jacarandae, however, have  $2n = 36$  in all of the 16 species of *Jacaranda* the karyotypes of which have been analyzed (Goldblatt and Gentry, 1979; Morawetz, 1982; Costa, 2006; Sampaio, 2010). There are no known chromosome records for the genus *Digomphia*.

Analyses that involve staining with the fluorochromes chromomycin A3 (CMA) and 4'6-diamidino-2-phenylindole (DAPI) have proven to be very useful in taxonomic studies, and in examinations of karyotypic evolution in plant groups with stable chromosome numbers. Numerically stable species of *Citrus* L. (Guerra, 1993; Carvalho et al., 2005), *Spondias* L. (Almeida et al., 2007), and *Acianthera* Scheidw. (Oliveira et al., 2015), for example, have been karyotypically characterized based on their patterns of CMA/DAPI bands. Although analyses of CMA/DAPI banding patterns have been important in defining species and investigating karyotypic evolution, this technique has not been widely used in the Bignoniaceae.

A number of *Jacaranda* species have restricted distributions (Gentry and Morawetz, 1992), or are considered rare or threatened with extinction (Lohmann et al., 2013). Species such as *Jacaranda mimosifolia* D. Don are widely cultivated as ornamentals (Gentry, 1992). However, most species of the genus are difficult to cultivate, and produce recalcitrant seeds that can only be stored for short periods of time before losing their germinative ability (Sangalli et al., 2012), often making it difficult to obtain material appropriate for cytological analyses of its species.

Approximately 30% of *Jacaranda* species have been karyotypically analyzed, with all of them having  $2n = 36$  and symmetrical karyotypes, making them difficult to characterize using conventional staining techniques (Goldblatt and Gentry, 1979; Morawetz, 1982; Forni-Martins and Martins, 2000; Costa, 2006; Sampaio, 2010). The use of different staining techniques in *Jacaranda* could differentiate the chromosomes of its species, including the karyotypic characterization of the subsections *Monolobos* and *Dilobos*, and morphologically similar species. Therefore, this study aimed to characterize the karyotypes of six species of *Jacaranda* belonging to the sections *Monolobos* (two species) and *Dilobos* (four species) using CMA and DAPI.

## MATERIAL AND METHODS

Almost all of the plant material used was collected in the field (Caatinga and Cerrado vegetation), with only one cultivated species taken from an urban garden (*J. mimosifolia* D. Don). Specimens of all of the material analyzed were deposited in the Prof. Jayme Coelho de Moraes Herbarium at Universidade Federal da Paraíba, Brazil.

Cytogenetic analyses were performed on root tips treated with 0.002 M 8-hydroxyquinoline at 4°C for 24 h, fixed in Carnoy's solution (absolute ethanol:glacial acetic acid; 3:1, v/v) for 3 h, and subsequently stored at -20°C. To prepare the slides, the root tips were digested in an enzymatic solution (2% cellulase + 20% pectinase) and maintained under humid conditions at 37°C for 1 h. The material was then squashed in 45% acetic acid and the slides were frozen in liquid nitrogen to remove their cover slips. The slides were then stained with a solution of DAPI (2 µg/mL):glycerol (1:1, v/v). The best slides were selected and cleared in ethanol:acetic acid (3:1) for 30 min, and subsequently were maintained in absolute alcohol for 2 h. The slides were cured for three days and then stained for 1 h with 10 µL CMA (0.1 mg/mL), and then with 10 µL DAPI (2 µg/mL) for 30 min. They were then mounted in glycerol:McIlvaine buffer, pH 7.0 (1:1, v/v), and stored for three days in the dark to stabilize the fluorochromes (Guerra and Souza, 2002).

At least three slides were analyzed for each species, with the best metaphases being photographed using an AxioCam MRc 5 video camera (Carl Zeiss Vision GmbH, München-Hallbergmoos, Germany) coupled to a Zeiss Scope.A1 microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) using the AxioVision v.4.8 software. Chromosomal measurements were made using the ImageTool v.3.0 software (<http://compdent.uthscsa.edu/dig/itdesc.html>). The images were edited and the karyograms mounted using Adobe Photoshop CS3 v.10.0.

## RESULTS

All of the species exhibited  $2n = 36$ , and had symmetrical karyotypes with medium-sized metacentric and submetacentric chromosomes that varied in median length from 1.83

$\pm 0.34 \mu\text{m}$  in *J. mimosifolia* to  $2.82 \pm 0.54 \mu\text{m}$  in *Jacaranda rugosa* A.H. Gentry (Table 1). Previously reported chromosome counts and the new counts recorded here for *Jacaranda* represent a total of 20 species analyzed (41% of the genus), and all of them have  $x = 18$  (Table 2).

**Table 1.** Characteristics of the *Jacaranda* species analyzed.

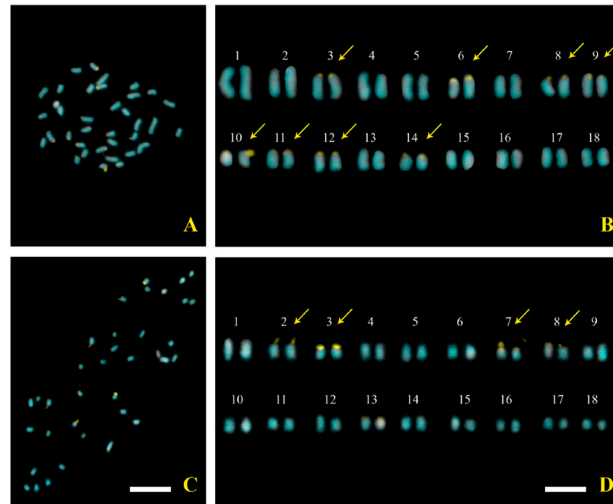
Section/species	Habit	Biome	2n	Median size
<i>Monolobos</i>				
<i>Jacaranda brasiliiana</i> (Lam.) Pers.	Tree	Cerrado	36	2.64 $\mu\text{m}$
<i>J. mimosifolia</i> D. Don	Tree	Cultivate	36	1.83 $\mu\text{m}$
<i>Dilobos</i>				
<i>J. bracteata</i> Bureau & K. Schum.*	Shrub	Cerrado	36	2.18 $\mu\text{m}$
<i>J. irwinii</i> A.H. Gentry*	Shrub	Cerrado	36	2.10 $\mu\text{m}$
<i>J. jasminoides</i> (Thunb.) Sandwith*	Shrub	Caatinga	36	2.17 $\mu\text{m}$
<i>J. rugosa</i> A.H. Gentry*	Shrub	Caatinga	36	2.82 $\mu\text{m}$

\*First chromosome count for the species.

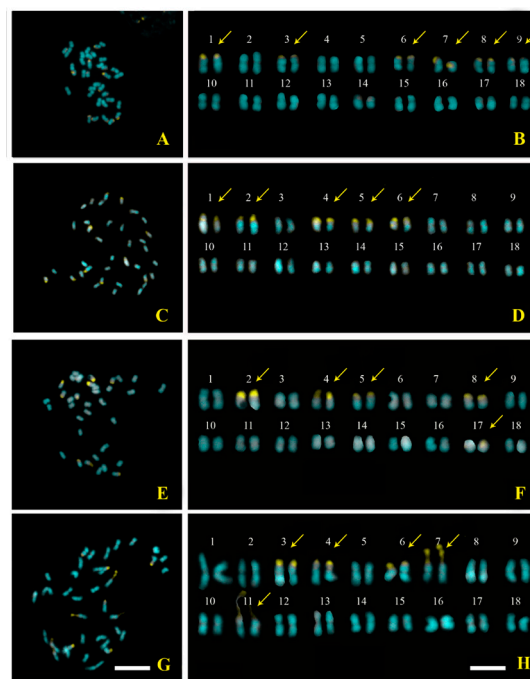
**Table 2.** Chromosome numbers recorded for *Jacaranda* species and their respective bibliographic references.

Species	2n	Source
<i>J. acutifolia</i> Bonpl.	36	Sampaio (2010)
<i>J. bracteata</i> Bureau & K. Schum.	36	Present study
<i>J. brasiliiana</i> (Lam.) Pers.	36	Morawetz (1982); Costa (2006); present study
<i>J. caerulea</i> (L.) J. St.-Hil.	36	Morawetz (1982)
<i>J. caroba</i> (Vell.) DC.	36	Forni-Martins and Martins (2000); Sampaio (2010)
<i>J. cuspidifolia</i> Mart.	36	Costa (2006)
<i>J. decurrens</i> Cham.	36	Sampaio (2010)
<i>J. hesperia</i> Dugand	36	Goldblatt and Gentry (1979); Morawetz (1982)
<i>J. irwinii</i> A.H. Gentry	36	Present study
<i>J. jasminoides</i> (Thunb.) Sandwith	36	Present study
<i>J. macrantha</i> Cham.	36	Morawetz (1982)
<i>J. micrantha</i> Cham.	36	Morawetz (1982)
<i>J. mimosifolia</i> D. Don	36	Goldblatt and Gentry (1979); Piazzano (1998); Costa (2006); present study
<i>J. paucifoliata</i> Mart. ex DC.	36	Sampaio (2010)
<i>J. puberula</i> Cham.	36	Morawetz (1982)
<i>J. pulcherrima</i> Morawetz	36	Morawetz (1982)
<i>J. rugosa</i> A.H. Gentry	36	Present study
<i>J. rufa</i> Silva Manso	36	Morawetz (1982); Sampaio (2010)
<i>J. subalpina</i> Morawetz	36	Morawetz (1982)
<i>J. ulei</i> Bureau & K. Schum.	36	Sampaio (2010)

The heterochromatic banding patterns observed in the *Jacaranda* species studied here were characterized by the presence of four to eight terminal bands rich in CG sequences (CMA<sup>+</sup>/DAPI<sup>-</sup>) per monoploid set. In the section *Monolobos*, *Jacaranda brasiliiana* (Lam.) Pers. had eight terminal CMA<sup>+</sup>/DAPI<sup>-</sup> bands per monoploid set (Figure 1A, B), while *J. mimosifolia* had four, two of them being distended (Figure 1C, D). In the section *Dilobos*, *Jacaranda bracteata* Bureau & K. Schum. had six terminal CMA<sup>+</sup>/DAPI<sup>-</sup> bands per monoploid set (Figure 2A, B), while *Jacaranda irwinii* A.H. Gentry (Figure 2C, D), *J. jasminoides* (Thunb.) Sandwith (Figure 2E, F), and *J. rugosa* (Figure 2G, H) had five, the latter with two chromosome pairs showing distended CMA<sup>+</sup> bands, which probably corresponded to nucleolar organizer regions (NORs). Of the species analyzed, only *J. irwinii* had a proximal heterozygotic CMA band at one of the homologs of chromosome pair 1 and two proximal bands on pair 6 (Figure 2D).



**Figure 1.** Mitotic metaphases and karyograms of *Jacaranda* species from the section *Monolobos*. **A. B.** *Jacaranda brasiliensis* ( $2n = 36$ ); **C. D.** *Jacaranda mimosifolia* ( $2n = 36$ ). Scale bar in **C** corresponds to 10  $\mu\text{m}$ ; scale bar in **D** corresponds to 5  $\mu\text{m}$ . Arrows indicate homologous chromosomes with terminal CMA<sup>+</sup> bands.



**Figure 2.** Mitotic metaphases and karyograms of *Jacaranda* species from the section *Dilobos*. **A. B.** *Jacaranda bracteata* ( $2n = 36$ ); **C. D.** *Jacaranda irwinii* ( $2n = 36$ ); **E. F.** *Jacaranda jasminoides* ( $2n = 36$ ); **G. H.** *Jacaranda rugosa* ( $2n = 36$ ). Scale bar in **G** corresponds to 10  $\mu\text{m}$ ; scale bar in **H** corresponds to 5  $\mu\text{m}$ . Arrows indicate homologous chromosomes with terminal CMA<sup>+</sup> bands.

## DISCUSSION

The present study confirmed previous counts of  $2n = 36$  for *J. mimosifolia* and *J. brasiliiana* (Goldblatt and Gentry, 1979; Morawetz, 1982; Piazzano, 1998; Costa, 2006), with the other counts being new for the genus. The numerical stability observed in *Jacaranda* has been reported in other genera of Bignoniaceae, particularly in the tribes Catalpeae, Bignonieae, and Crescentieae, and the Tabebuia alliance clade, although in this, and in most of the other genera in Bignoniaceae,  $2n = 40$  predominates (Goldblatt and Gentry, 1979; Piazzano, 1998; Piazzano et al., 2015). Difficulties have been encountered in identifying some species of *Jacaranda* due to morphological similarities in terms of their habits, leaf structures, the colors and morphologies of their flowers, and similarities in the shapes of their fruits (Gentry and Morawetz, 1992; Silva-Castro et al., 2007). Although chromosome numbers have been widely and traditionally used in plant cytotaxonomy (Guerra, 2008), this parameter is not informative in genera with similar chromosome numbers and morphologies, as *Jacaranda*. In these cases, the use of differential staining techniques, such as CMA/DAPI banding, has proven to be viable alternatives for cytotaxonomic studies, particularly when characterizing closely related species (Guerra, 2000).

*Jacaranda* had variable numbers of terminal CMA<sup>+</sup>/DAPI<sup>-</sup> bands, which allowed individual karyotypic characterizations of each of the six species analyzed here. The numbers of bands were variable among the arborescent species (section *Monolobos*), with four and eight pairs of bands in *J. mimosifolia* and *J. brasiliiana*, respectively. The shrub species (section *Dilobos*) exhibited greater karyotypic stability, with three of the four species analyzed having five pairs of terminal CMA<sup>+</sup> bands, with only *J. bracteata* having six bands. Of the species with five pairs of terminal CMA<sup>+</sup> bands, *J. rugosa* differed by having two pairs with notably distended NORs, while *J. irwinii* had proximal bands on two chromosome pairs.

Although variations in the numbers and sizes of the CMA/DAPI bands were observed among the *Jacaranda* species analyzed here, these differences may not be sufficient to karyotypically distinguish between the sections *Monolobos* and *Dilobos*, because other species of the genus will probably have terminal CMA<sup>+</sup> bands with sizes and numbers similar to those reported here, independent of the section to which they have been assigned. Such similarities between *Jacaranda* sections have been observed in phylogenetic analyses of base-pair sequences in the *ndhF*, *rbcL*, and *trnL-F* regions (Olmstead et al., 2009). Although 1-2-thecate anthers have been of practical use in separating these two sections (Gentry and Morawetz, 1992), this morphological characteristic does not appear to constitute a synapomorphy. It will therefore be necessary to undertake wider samplings of the species of both sections in order to support (or not) the current infrageneric classification of *Jacaranda*.

The patterns of CMA/DAPI bands observed in *Jacaranda* species diverge from those most frequently reported for angiosperms, with a single band corresponding to a heterochromatic NOR accompanied by pericentromeric bands and variable numbers of terminal bands (reviewed by Guerra, 2000). Although the numbers and sizes of the bands in *Jacaranda* are variable, they demonstrate equilocal distributions in the terminal regions of the chromosomes of most species, suggesting a consistent mechanism of chromosomal evolution. The presence of multiple copies of repeated DNA sequences in the terminal chromosome regions may have been caused in this case by pairing non-homologous chromosomes, thus amplifying recombination frequencies, and, consequently, the number of terminal heterochromatin loci in the genome (Bennetzen and Wang, 2013).

Regarding karyotypic numerical stability, two hypotheses may explain the constant occurrence of  $2n = 36$  in *Jacaranda*. The first hypothesis is based on the molecular phylogeny proposed by Olmstead et al. (2009), in which Jacarandae appears as the basal group of the family Bignoniaceae, which would support  $x = 18$  as the probable ancestral basic number of the family. The second hypothesis, which is supported by most of the authors that have discussed the karyotypic evolution of the Bignoniaceae (Goldblatt and Gentry, 1979; Piazzano, 1998; Piazzano et al., 2015), proposes  $x = 20$  as the basic chromosome number of the family, based on the consistent appearance of  $2n = 40$  in most of its genera (as well as closely related families, such as the Paulowniaceae and Schlegeliaceae). According to the latter hypothesis, the chromosome numbers reported for *Jacaranda* would be derived from  $2n = 40$ , which would have experienced chromosomal rearrangements of the centric fission type, with stabilization at  $2n = 36$ . However, whether by chromosomal rearrangement or because of the primitive characteristics of the Bignoniaceae, the establishment of a karyotype with  $2n = 36$  in *Jacaranda* was related to the evolution of one of the most diverse groups in the Bignoniaceae, with approximately 50 species that are adapted to a wide variety of Neotropical habitats (Gentry and Morawetz, 1992).

### Conflicts of interest

The authors declare no conflict of interest.

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