Heterochromatin and numeric chromosome evolution in Bignoniaceae, with emphasis on the Neotropical clade *Tabebuia* alliance

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Abstract

Bignoniaceae is a diverse family composed of 840 species with Pantropical distribution. The chromosome number \(2n = 40\) is predominant in most species of the family, with \(n = 20\) formerly being considered the haploid base number. We discuss here the haploid base number of Bignoniaceae and examine heterochromatin distributions revealed by CMA/DAPI fluorochromes in the *Tabebuia* alliance, as well as in some species of the Bignonieae, Tecomeae, and Jacarandeae tribes. When comparing the chromosome records and the phylogenies of Bignoniaceae it can be deduced that the base number of Bignoniaceae is probably \(n = 18\), followed by an ascendant dysploidy \((n = 18 \rightarrow n = 20)\) in the most derived and diverse clades. The predominant heterochromatin banding patterns in the *Tabebuia* alliance were found to be two terminal CMA\(^+\) bands or two terminal and two proximal CMA\(^+\) bands. The banding pattern in the *Tabebuia* alliance clade was more variable than seen in Jacarandeae, but less variable than Bignonieae. Despite the intermediate level of variation observed, heterochromatin banding patterns offer a promising tool for distinguishing species, especially in the morphologically complex genus *Handroanthus*.

Keywords: Chromosome number, CMA/DAPI, *Handroanthus*, polyploidy.

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Introduction

Bignoniaceae is a Pantropical family composed mostly of trees and lianas, and includes 82 genera and 840 species (Fischer et al., 2004; Lohmann and Ulloa, 2016). Eight tribes are nested within the family: Bignonieae, Catalpeae, Coleeae, Crescentieae, Jacarandeae, Oroxyleae, Tecomeae, and Tourrettieae, plus the informal Crescentiina clade, that comprises the Neotropical and Palaeotropical subclades (Olmstead et al., 2009). While the morphological features of most tribes of Bignoniaceae are well-characterized, the Crescentiina clade and its subclades are well-sustained lineages, although without clear morphological synapomorphies (Grose and Olmstead, 2007; Olmstead et al., 2009). The Crescentiina clade comprises two informal lineages: the exclusively Neotropical *Tabebuia* alliance and the Paleotropical clade with Asian and African genera (Olmstead et al., 2009). The *Tabebuia* alliance has 14 genera and 147 species of trees and shrubs that have composite and palmate leaves (Grose and Olmstead, 2007). Most species within that clade belong to *Tabebuia* Gomes ex DC. and *Handroanthus* Mattos, while the remaining genera are smaller but widely-distributed in the Americas (Gentry, 1992; Grose and Olmstead, 2007). There is great morphological variability within the *Tabebuia* alliance, so that the delimitation of its species is often difficult.

From a cytogenetic point of view, the Bignoniaceae family comprises two groups with distinct karyotypes. The first group has a wide range of chromosome numbers \((2n = 22, 28, 30, 36, 38, 40 \text{ and } 42)\) and includes the tribes Jacarandeae, Tecomeae, Oroxyleae, and the two genera *Argyilia* D.Don and *Delostoma* D.Don (Moore, 1974; Goldblatt and Gentry, 1979; Pizzano, 1998; Pizzano et al., 2015). The second group has the prevailing chromosome number \(2n = 40\), and includes Bignonieae, Catalpeae, and the Crescentiina clade (Goldblatt and Gentry, 1979; Pizzano, 1998; Alcorcés de Guerra, 2002; Ortolani et al., 2008; Firetti-Leggieri et al., 2011; Pizzano et al., 2015; Cordeiro et al., 2016a, 2017). Ploidy variations \((2n = 60, 80 \text{ and } 120)\) were found for a few species of the tribe Bignonieae and the clade *Tabebuia* alliance from the second group (Pizzano, 1998; Alves et al., 2013; Pizzano et al., 2015; Cordeiro et al., 2017).
Most species of Bignoniaceae show \( n = 20 \), and it has been proposed that \( x = 20 \) is the haploid base number for the family (Goldblatt and Gentry, 1979; Piazzano, 1998; Piazzano et al., 2015). However, when confronting the known chromosome numbers of Bignoniaceae and the phylogenetic analyses of Olmstead et al. (2009), it became evident that the most primitive clades (such as Jacarandaceae) are \( x = 18 \), suggesting that a different number from 20 could be the haploid base number of the family.

Chromosome numbers and morphologies are the features most used in karyotype analyses and ground cytotoraxonomy studies (Guerra, 2008), although those characteristics can be uninformative in groups where chromosome numbers are stable and the chromosomes are small (< 3 \( \mu m \)) (Guerra, 2000, 2012). Bignoniaceae have chromosome sizes of \( \sim 2 \mu m \), meta- submetacentric morphology, and \( 2n = 36 \) or 40 is predominant in the majority of species (Goldblatt and Gentry, 1979; Piazzano et al., 2015; Cordeiro et al., 2016b, 2017). Banding pattern characterizations can therefore often help discriminate between cytotypes with stable chromosome numbers, sizes and morphologies. The fluorochromes Chromomycin A3 (CMA) and \( 4',6'-\text{diamidino-2-phenylindole (DAPI)} \) are specific for GC-rich (CMA) or AT-rich (DAPI) regions respectively, and usually stain regions with tandem repeats of non-coding DNA (Schweizer, 1976; Guerra, 2000). They have been used mainly to characterize karyotypes with chromosomes that have the same size and morphology, and to differentiate the karyotypes of species with identical chromosome numbers (see Almeida et al., 2007; Barros e Silva et al., 2010; Cordeiro et al., 2016b; Almeida et al., 2016). The different patterns found can help determine taxonomic distinctions and clarify relationships among species (Carvalho et al., 2005; Almeida et al., 2007; Oliveira et al., 2015), as well as contribute to the description of new taxa, such as Epidendrum sanchezii E.Pessoa & L.P.Felix (Pessoa et al., 2014), Ameroglossum manalo-felizii L.P.Felix & E.M.Almeida (Almeida et al., 2016), and Spondias bahiensis P.Carvalho, Van den Berg and M.Machado (Almeida et al., 2007; Machado et al., 2015). Preliminary studies in the tribe Jacarandaceae (Cordeiro et al., 2016b) indicated that heterochromatin distribution appeared to follow a specific pattern (8-16 CMA\(^-\) terminal bands), while in the tribe Bignonieae (Cordeiro et al., 2017) heterochromatin distribution is quite variable among the species. That result demonstrates that regions rich in GC base pairs (CMA\(^-\)) can be variable even among closely related species of Bignoniaceae, and that a specific pattern for each group or tribe may not exist.

The main objective of this work was to describe the cytotoraxonomic differences between related species of Bignoniaceae (mainly in the Neotropical lineage of the Tabebutia alliance clade) by examining their heterochromatin distributions, and discuss the haploid base number of the Bignoniaceae based on compilations of the chromosome numbers known for all lineages of the family.

Materials and Methods

Taxon sampling

Heterochromatin banding patterns of 12 species of the Tabebuia alliance clade were analyzed (Figure 1), as well as those of three species of Jacarandaceae, two species of Tecomeae, and two species of Bignonieae tribes. The species, vouchers, and primarily karyological information are presented in Table 1. The vouchers were deposited in the EAN herbarium. An average of three specimens of each species were grown in plastic pots in the experimental garden of the Centro de Ciências Agrárias of the Universidade Federal da Paraíba. When the roots reached 2 cm in length, 15 root tips per specimen were excised and analyzed.

Cytogenetic analyses

Mitosis was examined in root tips that had been pretreated with 0.002 M 8-hydroxyquinoline (8-HQ) for 24 h at 4 \( ^\circ \)C, fixed in 3:1 (v/v) absolute ethanol/glacial acetic acid for 30 min, and then stored in a freezer at -20 \( ^\circ \)C. The roots were digested with an enzymatic solution (2% cellulase and 20% pectinase) for one hour at 37 \( ^\circ \)C. Root tips were squashed in 45% acetic acid and coverslips were removed by freezing in liquid nitrogen. The samples were aged for three days at room temperature and stained with 10 \( \mu L \) of CMA (0.1 mg/mL) for 1 h, and then with 10 \( \mu L \) of DAPI (1 \( \mu g/mL \)) for 30 min. The samples were mounted in glycerol/McIlvaine’s buffer at pH 7.0 (1:1, v/v) and kept in the dark for three days (Cordeiro et al., 2017).

The best metaphases were photographed using an AxioCam MRC5 digital camera and AxioVision 4.8 software (Carl Zeiss Microscopy GmbH, Jena Germany). Measurements were made using Uthscsa Image Tool (IT) v 3.0 software. The final images were prepared using Adobe Photoshop CS3 v 10.0 (Adobe Systems Incorporated, San Jose, USA). Chromosome morphology was determined using the centromeric index, following Guerra (1986).

Base chromosome number and karyotype evolution

The base chromosome number analysis is based on 179 species of Bignoniaceae, distributed in all of the clades retrieved by Olmstead et al. (2009) for the family. The list of samples, and their chromosome numbers and respective references are presented in Table S1 (Supplementary Material). Karyotype and molecular phylogenetic data were compiled for representatives of the Bignoniaceae. The numbers of species analyzed in each Bignoniaceae clade and their chromosome numbers and frequencies are presented in a phylogeny adapted from Olmstead et al. (2009) to demonstrate their putative chromosome number evolution. Information concerning heterochromatin patterns is presented for Bignoniaceae, Tabebuia alliance, Tecomeae,
and Jacarandae. The chromosomes types (A, B, C, D, E and F) follow Cordeiro et al. (2017).

Results

Chromosome numbers

The chromosome number of 12 species of the Tabebuia alliance clade was analyzed, as well as those of three species of Jacarandae, two species of Tecomeae, and two species of Bignonieae tribes. The karyotypes of the 19 species analyzed were predominantly symmetrical, principally with metacentric or sub-metacentric chromosomes. Their sizes ranged from 1.02 μm ± 0.13 in Tabebuia aurea (Silva Manso) Benth. & Hook. f. ex S. Moore to 2.19 μm ± 0.3 in J. praetermissa. The chromosome number of most of the

Figure 1 - Some of the species of the Neotropical lineage Tabebuia alliance clade sampled. A. Crescentia cujete, B. Handroanthus chrysotrichus, C. H. impetiginosus, D. H. ochraceus, E. H. serratifolius, F. H. umbellatus, G. Sparattosperma leucanthum, H. Tabebuia aurea, I. T. elliptica, J. T. rosea, K. T. roseoalba, L. Zeyheria tuberculosa.
Tabebuia alliance was $2n = 40$ (Crescentia L., Sparattosperma Mart. ex Meisner, Tabebuia Gomez, and Zeyheria Mart.). However, Handroanthus Mattos showed $2n = 40$ [H. impetiginosus (Mart. ex DC.) Mattos and H. umbellatus] as well as $2n = 80$ [H. chrysotrichus (Mart. ex DC.) Mattos and H. ochraceus (Cham.) Mattos], and $2n = 120$ [H. serratifolius (Vahl.) S.O. Grose]. The remaining species showed $2n = 36$ [Jacaranda mimosifolia D.Don., J. jasminoides (Thunb.) Sandwith., J. praetermissa, and Tecoma stans (L.) Juss. ex Kunth], $2n = 38$ [Podranea ricasoliana (Tanfani) Sprague], or $2n = 40$ (A. citrinum and F. chica) (Table 1).

New chromosome records are described for Handroanthus umbellatus (Sond.) Mattos, Sparattosperma leucanthum (Vell.) K.Schum, Tabebuia elliptica (DC.) Sandwith, T. roseoalba (Bertol.) Bertero ex A. DC. and Z. tuberculosa (Vell.) Bureau ex Verl. (2n = 40; Tabebuia alliance), as well as for Fridericia chica (Bonpl.) L.G.Lohmann (2n = 40; Bignoniae tribe) and Jacaranda praetermissa Sandwith (2n = 36; Jacarandaceae tribe). Additionally, a new cytotype is de-

**Table 1** - Species of Bignoniaceae analyzed and their main karyological parameters. Heterochromatin patterns: A - large telomeric CMA$^+$/bands, B - small telomeric CMA$^+$ bands, C - proximal CMA$^+$ bands, F - lack of heterochromatic bands. Abbreviations of the Voucher: JMPC - Joel Maciel Pereira Cordeiro, LPF - Leonardo Pessoa Felix, EMA - Eron Mendonça de Almeida, SAAL - Saulo Antonio Alves de Lima. Abbreviations in the Origin: PB - Paraíba State, BA - Bahia State, PI - Piauí State, and MG - Minas Gerais State, Brazil.

| Tribe/Alliance/species | Voucher | Origin | 2n  | Median size (µm) | Heterochromatin patterns | Figure |
|------------------------|---------|--------|-----|------------------|--------------------------|--------|
| Jacarandaceae          |         |        |     |                  |                          |        |
| *Jacaranda jasminoides* (Thunb.) Sandwith. | JMPC, 131 | Sertânia-PB | 36  | 2.09             | 6A + 4B + 26F               | 3A     |
| J. mimosifolia D.Don    | LPF, 14457 | Areia-PB | 36  | 1.84             | 6A + 2B + 28F               | 3B     |
| J. praetermissa Sandwith* | LPF, 17606 | Serra da Capivara-PB | 36  | 2.19             | 2A + 8B + 26F               | 3C     |
| Tecomaee               |         |        |     |                  |                          |        |
| Podranea ricasoliana (Tanfani) Sprague | JMPC, 135 | Areia-PB | 38  | 1.07             | 6B + 32F                   | 3D     |
| Tecoma stans (L.) Juss. ex Kunth | LPF, 14412 | Paulo Afonso-BA | 36  | 1.16             | 2A + 4C + 30F               | 3E     |
| Bignonieae             |         |        |     |                  |                          |        |
| Anemopaegma citrinum Mart. ex DC.** | JMPC, 1254 | Pico do Jabe-PB | 40  | 1.32             | 2A + 2B + 2D + 34F          | 3F     |
| Fridericia chica (Bonpl.) L.G.Lohmann* | JMPC, 1043 | Sertânia-PB | 40  | 1.66             | 2A + 2B + 4C + 32F          | 3G     |
| Tabebuia alliance       |         |        |     |                  |                          |        |
| Handroanthus chrysotrichus (Mart. ex DC.) | EMA, 814 | Campina Grande-PB | 80  | 1.44             | 4A + 4B + 4C + 68F          | 1B, 3H |
| H. impetiginosus (Mart. ex DC.) Mattos | SAAL, 86 | Areia-PB | 40  | 1.39             | 2A + 2C + 36F               | 1C, 3I |
| H. ochraceus (Cham.) Mattos | SAAL, 84 | João Pessoa-PB | 80  | 1.42             | 6B + 74F                   | 1D, 3J |
| H. serratifolius (Vahl.) S. O. Grose. Mattos | JMPC, 251 | Areia-PB | 120 | 1.63             | 4A + 6B + 4C + 106F         | 1E, 4A |
| H. umbellatus (Sond.) Mattos* | JMPC, 1043 | Sertânia-PB | 40  | 1.66             | 2A + 2B + 4C + 32F          | 1F, 4B |
| Sparattosperma leucanthum (Vell.) K.Schum.* | LPF, 15402 | Alvorada de Minas-MG | 40  | 1.55             | 2A + 38F                   | 1G, 4C |
| Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S. Moore | JMPC, 1078 | Piripiri-PB | 40  | 1.02             | 2A + 38F                   | 1H, 4D |
| T. elliptica (DC.) Sandwith* | SAAL, 81 | Santa Rita-PB | 40  | 1.86             | 2A + 38F                   | 1I, 4E |
| T. rosea (Bertol.) Bertero ex A. DC. | JMPC, 154 | Areia-PB | 40  | 1.51             | 2A + 2C + 36F               | 1J, 4F |
| T. roseoalba (Ridl.) Sandwith* | LPF, 14590 | Campina Grande-PB | 40  | 1.67             | 2A + 2C + 36F               | 1K, 4G |
| Zeyheria tuberculosa (Vell.) Bureau ex Verl.* | LPF, 14468 | Maracás-BA | 40  | 1.85             | 2A + 2C + 36F               | 1L, 4H |
| Crescentieae           |         |        |     |                  |                          |        |
| Crescentia cucute L.   | JMPC, 137 | Serra da Raiz-PB | 40  | 1.21             | 2A + 38F                   | 1A, 4I |

*First chromosome count for the species.
**New cytotype for the species.
scribed for *Anemopaegma citrinum* Mart. ex DC. (2n = 40; Bignoniaceae tribe).

**Base chromosome number and karyotype evolution**

The chromosome numbers of 179 species of Bignoniaceae (belonging to all of its clades) were compared (Table S1). Overall, most species showed 2n = 40 (67%) and 2n = 36 (19%). Chromosome numbers were compiled in a phylogeny adapted from Olmstead *et al.* (2009) to infer chromosome number evolution (Figure 2). The chromosome number 2n = 36 (n = 18) was principally distributed within the tribe Jacarandeae, while 2n = 40 (n = 20) appeared especially in the tribes Bignonieae and Catalpeae, in the clade Crescentiina, and in Tourrettieae. Other chromosome numbers occurred in *Argylia* (2n = 30) and *Delostoma* (2n = 42), and in the tribes Oroxyleae (2n = 28 and 30) and Tecomeae (2n = 22, 38 and 48).

**Heterochromatin patterns**

The heterochromatin banding patterns of the 19 species analyzed showed GC-rich (CMA+/DAPI) bands located on the telomeric or proximal regions of the chromosomes (Figures 3 and 4). The species belonging to Jacarandeae, Tecomeae, and Bignonieae tribes had distinct patterns of CMA+/DAPI bands. Jacarandeae had five pairs of telomeric bands in *J. jasminoides* (Figure 3A) and *J. praetermissa* (Figure 3C), and four telomeric pairs in *J. mimosifolia* (Figure 3B). Tecomeae had three pairs of inconspicuous telomeric bands in *P. ricasoliana* (Figure 3D), and one telomeric pair plus two proximal pairs in *T. stans* (Figure 3E). Bignonieae displayed two telomeric pairs as well as two telomeric and proximal pairs in *A. citrinum* (Figure 3F), and 16 telomeric pairs and three telomeric

![Figure 2 - Chromosome numbers of the Bignoniaceae clades. Values on the branches indicate bootstrap parsimony analysis and the posterior probability of Bayesian inference; Asterisks indicate 100% posterior probabilities (topology and support values following Olmstead *et al.*, 2009). Circle sizes correspond to the numbers of species with chromosome records in each clade. Chromosomes types A, B, C, D, E and F follow Cordeiro *et al.* (2017).](image-url)
pairs with bands on the short and long arm in *F. chica* (Figure 3G).

Most species in the *Tabebuia* alliance had karyotypes with a pair of chromosomes with large CMA+/DAPI+ telomeric bands, as seen in *Crescentia cujete* L. (Figure 4I), *S. leucanthum* (Figure 4C), *T. elliptica* (Figure 4E), and *T. aurea* (Figure 4D). Karyotypes with two telomeric and two proximal bands were observed in *H. impetiginosus* (Figure 3I), *Tabebuia rosea* (Bertol.) Bertero ex A.DC. (Figure 4F), *T. roseoalba* (Figure 4G), and *Z. tuberculosa* (Figure 4H). The remaining species of *Handroanthus* showed distinct heterochromatin patterns: four telomeric bands (two large

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**Figure 3** - Distribution of heterochromatic bands (CMA+, in yellow) of species of Jaracandeae, Tecomeae, Bignonieae and the *Tabebuia* alliance. A. *Jacaranda jasminoides* (2n = 36), B. *J. mimosa* (2n = 36), C. *J. praetemissa* (2n = 36), D. *Podranea ricasoliana* (2n = 38), E. *Teoma stans* (2n = 36), F. *Anemopaegma citrum* (2n = 40), G. *Fridericia chica* (2n = 40), H. *Handroanthus chrysotrichus* (2n = 80), I. *H. impetiginosus* (2n = 40), J. *H. ochraceus* (2n = 80). Scale bar in J corresponds to 10 μm. Arrow heads indicate minor CMA bands; inserts in D, H and J highlight chromosomes with inconspicuous CMA bands.
and two small) and four proximal bands in *H. umbellatus* (Figure 4B), four small telomeric bands in *H. ochraceus* (Figure 3J), eight telomeric bands (four large and four small) and four proximal bands in *H. chrysotrichus* (Figure 3H), and ten telomeric bands (four large and six small) and four proximal bands in *H. serratifolius* (Figure 4A).

**Discussion**

**Chromosome number evolution in Bignoniaceae**

Raven (1975) suggested $x = 7$ as the ancestral base number for Bignoniaceae, with the most common $n = 20$ being generated by a six-fold polyploidization followed by the loss of one pair of chromosomes; that base number was suggested because he considered Oroxylenae ($n = 14$ and 15) to be the most primitive tribe in Bignoniaceae. Several cytological studies in Bignoniaceae (Goldblatt and Gentry, 1979; Piazzano, 1998; Chen et al., 2004) agreed with the hypothesis of Raven (1975). More recent works, such as Piazzano et al. (2015), however, suggested $x = 20$ as the basic number of Bignoniaceae. The principal justification for that would be the large number of species with $2n = 40$, and groups considered correlated with Bignoniaceae, such as

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**Figure 4** - Distribution of heterochromatic bands (CMA+, in yellow) of species of the *Tabebuia* alliance (including Crescentiae). A. *Handroanthus serratifolius* ($2n = 120$), B. *H. umbellatus* ($2n = 40$), C. *Sparattosperma leucanthum* ($2n = 40$), D. *Tabebuia aurea* ($2n = 40$), E. *T. elliptica* ($2n = 40$), F. *T. rosea* ($2n = 40$), G. *T. roseoalba* ($2n = 40$), H. *Zeyheria tuberculosa* ($2n = 40$), I. *Crescentia cujete* ($2n = 40$). Scale bar in I corresponds to 10 μm. Arrow heads indicate minor CMA bands; inserts in A highlight chromosomes with inconspicuous CMA bands.
Paulowniaceae and Schlegeliiaceae, which also share the haploid number \( n = 20 \).

Molecular phylogeny, however, suggests a different story. Paulowniaceae and Schlegeliiaceae are not closely related to Bignoniaceae (Olmstead et al., 2009; Refulio-Rodriguez and Olmstead, 2014). According to Olmstead et al. (2009), the first diverging lineage within Bignoniaceae was Jacarandaceae \((2n = 36)\), followed by a strongly supported clade (core Bignoniaceae) with Tourretteae \((2n = 40)\), and then Argylia \((2n = 30)\), Tecomeae \((2n = 18, 22, 34, 36, 38, \text{and} 40)\), and a large clade including Oroxyliaceae \((2n = 28, 30)\), Crescentiina (mostly \(2n = 40\), but also \(36, 38, 80 \text{and} 120)\), and Bignoniaceae (mostly \(2n = 40\), but also \(38, 60, \text{and} 80\)) \( (\text{Figure} 2) \). Among the most basal lineages \( \text{(Jacarandaceae, Tourretteae, Argylia, Tecomeae, and Delostoma)} \) only 8.7% of the species \( (\text{five species}) \) have \(2n = 40\), whereas 56.1% \( (\text{32 species}) \) show \(2n = 36\) \( (\text{Table} S1, \text{Figure} 2) \). Consequently, the haploid base number for the family is \( x \neq 20 \). Very likely, the haploid number is \( x = 18 \), which was followed by an ascendant dysploidy \((n = 18 \rightarrow n = 20)\) in the most derived and diversified clades of the family.

Jacarandaceae and Tourretteae are the most primitive group for Bignoniaceae. Jacarandaceae include two genera \( (\text{Jacaranda Juss. and Digomphia Benth.) and approximately 55 species that are widely distributed throughout the Neotropics (Gentry, 1980; Olmstead et al., 2009). The chromosome number in the Jacaranda is very well characterized by the \(2n = 36\) \( (\text{Cordeiro et al., 2016b}) \). Tourretteae include two small genera subwoody to herbaceous vines \( (\text{Eccremocarpus Ruiz} \& \text{Pav. and Tourretia DC}) \) and six species distributed in the Andes and north in the Central American Cordilleras to Mexico \( (\text{Gentry, 1980; Olmstead et al., 2009}) \). There are chromosomal records in this tribe only for \text{Touretia lappacea \( (\text{L’Hér.) Willd.} \) \( (2n = 40) \) \( \text{(Goldblatt and Gentry, 1979)} \). Although the chromosomal record for Tourretteae and Jacarandaceae are different, these two basal tribes share some traits, as the doubly compound leaves and pollen that is psilate and tricolpate \( \text{(Olmstead et al., 2009)} \). Further sampling in Tourretteae can confirm whether \(2n = 40\) is a typical chromosomal number for the tribe species or if there may be other chromosome numbers, as also observed in Tecomeae.

Tecomeae is placed between the basal \( \text{(Jacarandaceae and Tourretteae)} \) and most derived clades of the Bignoniaceae \( (\text{Crescentiina, Bignoniaceae, Catalpeae}) \). The tribe is characterized by wide variations in chromosome numbers \( (2n = 22, 36, 38, 40, \text{and} 48) \), unlike other tribes where \(2n = 36 \) \( \text{(Jacarandaceae)} \) or \(2n = 40 \) \( \text{(Bignoniaceae, Catalpeae, and Crescentiina clade)} \) predominate \( (\text{Table} S1, \text{Figure} 2) \). Variations in chromosome numbers in Tecomeae represent events of ascending and descending dysploidy resulting in different chromosome numbers. The presence of \( n = 20 \) in Tourretteae suggests that this number could have arisen at the Core Bignoniaceae by ascending dysploidy, while the other numbers could have arisen by ascending \((n = 21, 24)\) and descending \((n = 11, 14, 15, 19)\) dysploidy.

Most species of the derived clade comprising Catalpeae, Oroxyliaceae, Crescentiina, and Bignoniaceae \( \text{(Olmstead et al., 2009)} \) have the karyotype \(2n = 40\). Among the 122 species with known chromosome numbers within this clade, 92.6% \( (113 \text{ species}) \) show \(2n = 40\). Only six species show \(2n \neq 40 \) \( [\text{two species of Mansoa DC. in Bignoniaceae, two species of Oroxyliaceae, and Spathodea cAMPamulata P. Beauv. and Radermacheroxilocarpa \( \text{(Roxb.) Roxb. ex K. Schum.} \); \text{Table S1}] \). The remaining species are polyploids of the haplotype \( n = 20 \) \( (2n = 60, 80, 120) \). This large clade comprises around 80% of the species of Bignoniaceae \( \text{(Olmstead et al., 2009)} \), which makes \(2n = 40\) the most common karyotype in the family. The four tribes and informal groups in this derived clade show marked geographical patterns. The most species-rich tribe \( \text{(Bignoniaceae)} \) is Neotropical \( \text{(Fischer et al., 2004)} \) as is one lineage of the informal Crescentiina \( \text{(which also has one Paleotropical clade)} \) \( \text{(Grose and Olmstead, 2007)} \). Catalpeae is from temperate North America and China and the tropical Greater Antilles \( \text{(Gentry, 1980; Olsen and Kirkbride Jr, 2017)} \), while the smallest tribe, Oroxyliaceae, is from tropical southern and southeastern Asia and Malaysia \( \text{(Olmstead, 2013)} \). Their wide distribution around the world and the high numbers of species in those tribes make \( n = 20\) the most common haploid number in Bignoniaceae. The haploid number \( n = 20 \) could be related to actual diversity and the occupation of a wide variety of habitats.

Reported chromosome numbers suggest that polyploidy is restricted to the clades \text{Tabebuia alliance and Bignoniaceae} \( \text{(Goldblatt and Gentry, 1979; Piazzano, 1998; Firetti-Leggeri et al., 2011, 2013; Cordeiro et al., 2017)} \). Reproductive analyses of \text{Handroanthus and Anemopaegma} \text{Mart. ex Meisn. indicated self-pollination, sporophytic and pseudogamous apomixis, and polyembryonic seeds \text{(Piazzano, 1998; Bittencourt Jr and Moraes, 2010; Firetti-Leggeri et al., 2013) – which are common features in polyploid species \text{(Piazzano, 1998; Firetti-Leggeri et al., 2013)} \). Piazzano et al. (2015) suggested that the polyploidy observed in those species probably originated by meiotic alternation, leading to the production of non-reduced gametes. The absence of a morphological continuum between sympatric species of the same genera \text{(personal observations) suggests an autoploidy origin.}

**Heterochromatin patterns**

Heterochromatin in the basal lineage of Jacarandaceae \( \text{(Bignoniaceae)} \) is composed exclusively by 8-16 terminal CMA\(^{+}\) bands, while the following lineages \( \text{(Tecomeae, Bignoniaceae, Tabebuia alliance)} \) also demonstrate pericentric CMA\(^{+}\) bands, but with reductions in the numbers of terminal CMA\(^{+}\) blocks \( \text{(Cordeiro et al., 2016b, 2017; Figures 3 and 4)} \). In certain plant groups, such as the Caesalpinia group \( \text{(Van-Lume et al., 2017)} \), and sect. \text{Acana-}
of Solanum L. (Chiarini et al., 2014) and Nierembergia Ruiz & Pav. (Acosta et al., 2016), the heterochromatin patterns appear to follow a specific evolutionary pattern for the species in the different clades. In genera such as Lycium L. (Stiefkens et al., 2010), Pereskia Mill. (Castro et al., 2016), and Ceiba Mill. (Figueredo et al., 2016), however, the heterochromatin pattern appears to be quite conserved and demonstrate only small variability among the different species. In most plant groups, however, heterochromatin patterns tend to be random and quite distinct, even among closely related species (Berjano et al., 2009; Scaldaferrro et al., 2012; Grabiele et al., 2018; Van-Lume and Souza, 2018). For Bignoniaceae as a whole, three heterochromatin patterns can be seen, with the occurrence of a specific pattern for Jacarandaeae (terminal CMA+ blocks), conserved patterns for the diploid species of the Tabebuia alliance (two terminal CMA+ blocks and 0-2 pericentromeric CMA+ blocks), and a pattern of random attributions in relation to the numbers and positions of CMA+ blocks in the tribe Binonieae (Cordeiro et al., 2017).

The heterochromatin banding patterns of the Bignoniaceae tribe in Bignoniaceae (Cordeiro et al., 2017) are characterized by strong differences in the sizes and locations of the CMA+ blocks, and six chromosome types are recognized based on heterochromatic regions. The two species of Bignoniaceae analyzed here confirm the patterns described before for the tribe, with the occurrence of type A (large telomeric CMA+ bands), type B (small telomeric CMA+ bands), type D (telomeric and proximal CMA+ bands), and type F chromosomes (showing a lack of heterochromatic bands) in A. citrinum, and type A, B, E (two telomeric CMA+ bands) and F chromosomes in F. chica (Table 1, Figure 2). The species sampled in Tabebuia alliance, Jacarandaeae, and Tecomeae have four chromosome types (according to Cordeiro et al., 2017): type A, type B, type C (proximal CMA+ bands), and type F.

The pattern of two CMA+ telomeric bands (chromosome type A) seen in most species of the Tabebuia alliance is very common among Angiosperms, and usually corresponds to a nucleolar organizer region (Guerra, 2000; Roa and Guerra, 2012). Telomeric CMA+ blocks are most likely related to rDNA sites as seen in most plant species (Barros e Silva et al., 2010; Castro et al., 2016; Marinho et al., 2018). Differences among species could be related to chromosome rearrangements and the amplification and reduction of rDNA sites caused by satellites or transposable sequences (Mehrotra and Goyal, 2014; Evtushenko et al., 2016; Saze, 2018).

The vegetative morphologies of Handroanthus species having yellow corollas are very similar (Gentry, 1992), with H. chrysotrichus and H. ochraceus being very close, even when comparing their flowers, leaves, and fruits. Those two species show a continuum of morphological variations, and hybridization or introgression has therefore been suggested (Gentry, 1992; Bittencourt Jr and Moraes, 2010). However these species have a distinctive heterochromatin banding pattern 4A + 4B + 4C in H. chrysotrichus and 4B in H. ochraceus. Similarly, T. roseoalba and T. elliptica have very similar flowers and fruits, although they can be differentiated by their 3- or 5-foliate leaves respectively (Gentry, 1992). The heterochromatin banding patterns of those two species are distinct, with the former having two proximal plus two telomeric bands (2A + 2C), while the latter has only two telomeric bands (2A). While banding patterns are still seldom-used in taxonomic studies, the results reported here support their utility in such analyses.

The chromosome numbers and heterochromatin banding patterns of J. jasminoides, J. praetermissa and J. mimostfolia support published data for the genus (Cordeiro et al., 2016b). Jacaranda is one of the largest genera of Bignoniaceae, with more than 50 species widely distributed in the Neotropics (Gentry and Morawetz, 1992). The genus is very well characterized by the chromosome number 2n = 36 (Morawetz, 1982; Cordeiro et al., 2016b) and by having 8 to 16 small and terminal CMA+ bands (Cordeiro et al., 2016b). In addition to its stable chromosome features, Jacaranda has a very consistent morphology, with all of its species having pinnate or bipinnate leaves, calyx lobes that are deeply divided, stamnodes longer than the stamens, and oblong and strongly flattened capsules opening through a rupture perpendicular to the septum (Morawetz, 1982; Gentry and Morawetz, 1992; Olmstead et al., 2009).

The heterochromatin banding patterns of Tecomeae have been poorly studied. The karyotypes of the two species of the tribe analyzed here, however, were relatively distinct from the species belonging to Jacarandaeae, the Tabebuia alliance clades, and Bignoniaceae species (Cordeiro et al., 2017). Although T. stans shows 2n = 36, its heterochromatin banding pattern (2A + 4C) is quite distinct from species with similar chromosome numbers, such as Jacaranda (8-16 A + B; Cordeiro et al., 2016b). Regarding P. ricasoliana, this species has an uncommon chromosome number for the Bignoniaceae (2n = 38) and six small terminal CMA+ bands – a unique pattern in the family (which usually has at least two large terminal CMA+ bands) (Cordeiro et al., 2016b, 2017). Although there is still little data available concerning banding patterns in Tecomeae, their lack of synapomorphies (Olmstead et al., 2009), wide distributions (Olmstead, 2013), high variability of life forms (including herbs, shrubs, trees, and lianas), environments occupied (from tropical to temperate forests, to both Andean and Himalayan mountains), and variations in chromosome numbers of the species within this clade, make this tribe one of the major challenges in Bignoniaceae.

Conclusion

The revision of the chromosome numbers previously reported for Bignoniaceae, allied to previous phylogenetic studies for the family, support a basic haploid chromosome
number different from 20 for the family. The most likely primary base number for the family is \( x = 18 \), which is the most common haploid number among its basal lineages. Ascending dispoloidy leading to \( x = 20 \) is consistent with the chromosome numbers found in the most derived and diversified lineages, where that number predominates. A broad study involving reconstructions of chromosome counts in families related to Bignoniaceae, as well as in all of its clades, would help clarify the evolution of the karyotype of the family.

The chromosomes of the *Tabebuia* alliance showed only GC-rich bands (CMA+/DAPI) located in telomeric or proximal regions. The banding pattern within that clade was more variable than seen in *Jacaranda*, but less variable than in Bignoniaceae. Despite the intermediate level of variation observed, heterochromatin banding patterns offer a promising tool for distinguishing species, especially in the morphologically complex genus *Handroanthus*.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

JMPC performed the research, MK contributed karyotypic evolution and taxonomic identifications, LGS contributed with cytogenetic analysis and karyotypic evolution, LPF advised the research. All authors contributed to the analysis of the results and to the writing of the manuscript.

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Supplementary material

The following online material is available for this article:

Table S1 - Chromosome numbers recorded for the Big-noniaceae family and their respective bibliographic references.

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