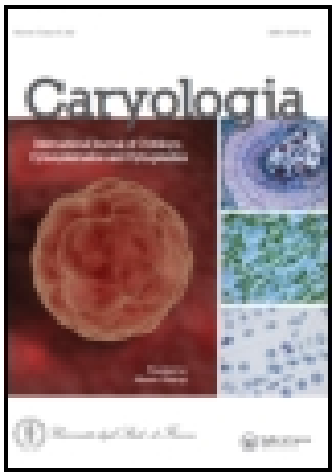

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Karyotypes and DNA content in Bignoniaceae

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Karyotypes and DNA content in Bignoniaceae

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Cytogenetic studies in 22 Bignoniaceae species were performed. Most taxa are from Argentina, one from Brazil, and two are cultivated (from South Africa and USA). All data are new, including first counts for *Bignonia binata*, *Handroanthus ochraceus*, *Tabebuia aurea* and the genus *Podranea*. Most taxa are diploid ($2n = 40$): members of tribes Bignonieae (*Adenocalymma*, *Amphilophium*, *Bignonia*, *Cuspidaria*, *Dolichandra*, *Fridericia*, and *Tynanthus*), Catalpeae (*Catalpa*) and the *Tabebuia* alliance (*Handroanthus* and *Tabebuia*). *Dolichandra unguis-cati* and *Handroanthus chrysotrichus* were polyploid ($2n = 80$). Tribes Jacarandeae (*Jacaranda*) and Tecomeae (*Tecoma*) were unusual (with $2n = 36$), whereas *Podranea ricasoliana* (Tecomeae) had $2n = 38$. The basic number $x = 20$ is proposed as the base number for the family. Chromosomes are small. The average length was 1.21 µm. Average haploid karyotype length was 28.13 µm, ranging from 18.63 in *Dolichandra cynanchoides* to 37.63 in *D. unguis-cati*. Type m chromosomes were the most common. One to five sm pairs were found in 16 species and one st pair in *Cuspidaria convoluta* and *Podranea ricasoliana*. One to four microsatellites, in long or short arms, were detected in nine species. Karyotypes are symmetrical. Asymmetry indices ranges were: $A_1 = 0.11–0.23$, $A_2 = 0.14–0.22$. The karyotypes of *P. ricasoliana* and *C. convoluta* were the most asymmetrical. Most species were karyologically indistinguishable based on conventional staining, but some could be distinguished by a combination of traits. 1C nuclear DNA content for 12 species were within the range 0.64–2.02 pg. In Bignoniaceae there is a common karyotypical pattern of mostly small m chromosomes with few cryptic chromosomal rearrangements.

Keywords: Argentina; Bignoniaceae; chromosome numbers; DNA content; karyotypes; polyploidy

Introduction

Although Pantropical, Bignoniaceae is one of the most diverse plant families in South America and its members are important components of Neotropical forests (Gentry 1974; Fischer et al. 2004; Lohman 2006). It has a central position within the Asteridae and includes *c*.80 genera and 840 species of shrubs, trees, and climbers (Fischer et al. 2004; Lohmann and Ulloa 2013). Several of the basally branching lineages, e.g. Jacarandeae, Tourrettieae, and *Argylia* are strictly New World, as also are tribe Bignonieae and the *Tabebuia* alliance (Spangler and Olmstead 1999; Grose and Olmstead 2007a, 2007b; Olmstead et al. 2009). Comparatively few taxa have economic significance outside horticulture, but numerous species are used for food, timber, containers, medicinal, and ritual purposes (Gentry 1992).

Despite the great potential of chromosome information for taxonomy (Stebbins 1958; Jones 1970), already proved in many plant families (e.g. Amaryllidaceae, Ran et al. 2001; Sapindaceae, Urdampilleta et al. 2013; Solanaceae, Tate et al. 2009; Chiarini et al. 2014), Bignoniaceae cytological studies are scarce and fragmentary (cf. Goldblatt and Gentry 1979; Piazzano 1998; Firetti-Leggieri et al. 2011). Around 15% of its species have their chromosome numbers counted (e.g. Smith 1941; Venkatasubban 1944, 1945; Covas and Snack 1946; Goldblatt and Gentry 1979; Gentry 1980; Goldblatt 1989; Piazzano 1998; Alcorcés de Guerra 2002; Chen et al. 2004; Alcorcés de Guerra and Méndez Natera 2007; Kumar et al. 2008; Firetti-Leggieri et al. 2011, 2013). In addition, karyotypic analyses are even more infrequent (e.g. Alcorcés de Guerra 2002; Chen et al. 2004), probably due to their small chromosome size. This is unfortunate, because the knowledge of the structural and quantitative characteristics of karyotypes have been significant in evolutionary and taxonomic studies in many angiosperm groups (e.g. Shan et al. 2003; Weiss-Schneeweiss et al. 2003; Moscone et al. 2007).

The same situation applies to nuclear DNA content (C-value for unreplicated haploid nuclei) in Bignoniaceae. At present, the DNA nuclear content of only nine species has been reported (Bennett and Leitch 2010), although it is an important source of information (e.g. Bennett and Leitch 2005; Gregory 2005). Comparative C-values have helped in understanding genome size evolution (Bennett and Leitch 2005) and have been correlated with minimum generation time, life history, phenology, and significant parameters for plant breeders, including frost resistance, biomass production, and ecological adaptations (e.g. Ohri 1998). Moreover, nuclear DNA amounts are a useful tool in the study of phylogenetic relationships between taxonomically related groups (e.g. Ohri 1998; Zonneveld 2001).

Karyotype studies and nuclear DNA content measurements were performed in 22 species from 13 genera of Bignoniaceae to fill the gaps in cytogenetic

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knowledge. These data are significant for understanding their systematic relationships and to discuss karyotypic evolution in the light of the available the molecular phylogenies. In our study, representatives of five of the eight main clades of Bignoniaceae (Olmstead et al. 2009) are included: Jacarandeae, Tecomeae, the *Tabebuia* alliance, Catalpeae, and Bignonieae. Most taxa (19) are native of Argentina, where 22 genera and 57 species are registered (Arbo and Lohman 2008).

Materials and methods

Table 1 lists the materials studied and their provenance. Most are native from Argentina and neighboring countries, except the cultivated *Podranea ricasoliana* from South Africa and *Catalpa bignonioides* from the USA.

Mitotic chromosomes were studied in root tips of germinating seeds, which were first soaked and then put in Petri dishes at 30°C in the dark. Roots were pretreated in a 8-hydroxiquinolein 0.002 M water solution, 3 h at room temperature, and later, they were fixed in a 3:1 (ethanol:acetic acid) mix for 24 h, hydrolyzed for 1 min in HCl 1 N at 60°C, and dyed with 2% lactopropionic orcein. Squashes were made in a drop of 45% acetic acid and were made permanent according to Bradley's method (1948). Ten metaphase plates from 10 individuals of each species were examined under a Zeiss Axiophot microscope (Oberkochen) and were photographed. The photomicrographs were used to take measurements of the following features for each chromosome pair: s (short arm length), l (long arm length), and c (total chromosome length). The arm ratio (r = l/s) was calculated and utilized to classify the chromosomes as recognized by Levan et al. (1964) as: m – metacentric (r = 1.00–1.69), sm – submetacentric (r = 1.70–2.99), or st – subtelocentric (r = 3.00–6.99). Battaglia's (1955) terminology for satellites was used. The satellite lengths were added to the lengths of the corresponding arms. In addition,

Table 1. Bignoniaceae species studied and collection data (all from Argentina). For cultivated specimens the origin is provided. Herbarium specimens are deposited at Museo Botánico de Córdoba (CORD).

Species	Voucher information	Figure
Tribe Bignonieae		
Adenocalymma marginatum (Cham.) DC.	Prov. Misiones, Dept. Iguazú, Iguazú, G. Rivera 34.	1A
Amphilophium crucigerum (L.) L.G. Lohmann	Prov. Misiones, Dept. Iguazú, camino Garganta del Diablo, G. Rivera 71.	1B
Amphilophium cynanchoides (DC.) L.G. Lohmann	Prov. Córdoba, Dept. Colón, Villa Warcalde, G. Rivera 26.	1C
Amphilophium paniculatum DC (L.) Kunth	Prov. Tucumán, Dept. Burruyacú, El Cajón, G. Rivera 68.	1D
Bignonia binata Silva Manso	Prov. Misiones, Dept. Iguazú, Iguazú, G. Rivera 86.	1E
Cuspidaria convoluta (Vell.) H.A. Gentry	Prov. Córdoba, Dept. Colón, cultivated, G. Rivera 79. Origin Corrientes province.	1F
Dolichandra cynanchoides Cham.	Prov. Córdoba, Dept. Colón, El Diquecito, G. Rivera 40.	1G
Dolichandra unguis-cati (L.) L.G. Lohmann	Prov. Jujuy, Dept. Ledesma, Serranía de Calilegua, G. Rivera 17.	1H
Dolichandra dentata (K. Schum.) L.G. Lohmann	Prov. Córdoba, Dept. Colón, cultivated, G. Rivera 23. Origin Corrientes province.	1I
Fridericia dichotoma (Jacq.) L.G. Lohmann	Prov. Jujuy, Dept. Ledesma, Serranía de Calilegua, G. Rivera 16.	1J
Tynanthus micranthus Corr. Méllo ex K. Schum.	Prov. Misiones, Dept. Iguazú, route 101, G. Rivera 80.	1K
Tribe Jacarandeae		
Jacaranda mimosifolia D. Don	Prov. Córdoba, Dept. Colón, El Diquecito, G. Rivera 39.	1L
***Tabebuia* alliance**		
Handroanthus chrysotrichus (Mart. ex DC.) Mattos	Prov. Misiones, Dept. Capital, cultivated, A. Cardozo 117. Origin Rio Grande do Sul state (Brazil).	1M
Handroanthus heptaphyllus (Vell.) Mattos	Prov. Córdoba, Dept. Capital, cultivated, G. Rivera 5. Origin Corrientes province.	1N
Handroanthus impetiginosus (Mart. ex DC.) Mattos	Prov. Córdoba, Dept. Capital, cultivated, G. Rivera 4. Origin Catamarca province.	1O
Handroanthus ochraceus (Cham.) Mattos	Prov. Córdoba, Dept. Capital, cultivated, G. Rivera 6. Origin Jujuy province.	1P
Handroanthus pulcherrimus (Sandwith) S. Grose	Prov. Córdoba, Dept. Capital, cultivated, G. Rivera 101. Origin Corrientes province.	–
Tabebuia aurea (Silva Manso) Benth. & Hook. f. ex S. Moore	USA, Hawaii, Dept. Maui, cultivated. Origin Corrientes province.	1Q
Tribe Tecomeae		
Podranea ricasoliana (Tanfani) Sprague	Prov. Córdoba, Dept. Capital, cultivated, G. Rivera 49. Origin South Africa.	1S
Tecoma garrocha Hieron.	Prov. La Rioja, Dept. Capital, dique Los Sauces, G. Rivera 50.	–
Tecoma stans (L.) Kunth in H.B.K.	Prov. Tucuman, Dept. Tafi del valle, San Javier, G. Rivera 12.	1R
Tribe Catalpeae		
Catalpa bignonioides Walter	Prov. Córdoba, Dept. Capital, cultivated, G. Rivera 11. Origin USA.	1T

haploid karyotype length (HKL) based on the mean chromosome lengths for each species, average chromosome length, and average arm ratio were calculated. Idiograms were based on the mean values for each species. The chromosomes were arranged into groups according to increasing arm ratio. As chromosomes were very small and quite similar in length, only groups of chromosome pairs were drawn, except for some pairs that could be recognized due to having satellites. Karyotype asymmetry was estimated using Stebbins' (1971) categories and Romero Zarco's indices (1986): A_1 = intrachromosomal asymmetry index, which indicates the length difference among the chromosome arms, and A_2 = interchromosomal asymmetry index, which indicates the size variation among the chromosomes. No karyotype data were obtained from *Tecoma garrocha* because we had scarce material.

DNA content was measured in telophase nuclei (2C) at the root apex of germinating seeds (Tito et al. 1991). Seeds were germinated and fixed as for the previous method but without pretreatment. *Amaranthus cruentus* var. Don Guiem was used as standard to calculate genome size in picograms; its genome size (2C = 1.26 pg) was calibrated according to Bennett and Smith (1976). After fixation, roots were rinsed for 30 min in distilled water. Hydrolysis was carried out with 5 N HCl at 20°C. Different times of hydrolysis were tested and the optimum period determined was 40 min. After hydrolysis, the roots were rinsed three times with distilled water for 15 min. Staining was done with Feulgen at pH 2.2 for 2 h in the dark. Then, material was rinsed three times in SO_2 water for 10 min each rinse, then rinsed again with distilled water (10 min) and squashed in 45% acetic acid. The cover slip was removed after freezing with CO_2 and the material was dehydrated in absolute alcohol, mounted in Euparal, and maintained in the dark until measurements were made. The amount of Feulgen staining per nucleus was measured at a wavelength of 570 nm using the scanning method in a Cytoscan Zeiss microdensitometer in the Instituto Fitotécnico Santa Catalina (Llavallol, Buenos Aires). Each measurement considered is the average of two readings. Differences in DNA content between taxa were tested through an ANOVA and comparisons between means using Scheffe's method using MINITAB (version 7).

Results

Figure 1 illustrates the range of chromosomes encountered. Most taxa are diploid with $2n = 40$ (Table 2, Figure 1): members of tribes Bignonieae *(Adenocalymma*, *Amphilophium*, *Bignonia*, *Cuspidaria*, *Dolichandra*, *Fridericia*, and *Tynanthus* species), Catalpeae (*Catalpa*), and the *Tabebuia* alliance (*Handroanthus* and *Tabebuia* species). Only two species were tetraploid with $2n = 80$: *Dolichandra unguis-cati* and *Handroanthus chrysotrichus* (Table 1, Figure 1H, 1M). Tribes Jacarandeae (*Jacaranda mimosifolia*) and Tecomeae (*Tecoma* species) were unusual in having $2n = 36$ (Table 2, Figure 1L, 1R) and *Podranea ricasoliana*, also in Tecomeae, had $2n = 38$ (Table 2, Figure 1S).

The chromosomes of all taxa are small (Table 2; Figure 1). The average chromosome length varied from 0.90 µm (*H. chrysotrichus*) to 1.50 µm (*A. paniculatum*), with a general mean of 1.15 µm. The average HKL was 28.13 µm for all species, ranging from 18.63 in *Dolichandra cynanchoides* to 37.63 µm in the tetraploid *D. unguis-cati*.

Idiograms calculated from means are given in Figure 2. Because of their small size and comparable shape, it was difficult to match all homologs. In all species, m chromosomes were the most common (88% of them). In addition, one to five sm chromosome pairs were found in 16 species. On the other hand, st chromosomes were rare, with one pair present in two species: *Cuspidaria convoluta* and *Podranea ricasoliana*.

From one to four microsatellites, located either in the long or in the short arms, were detected in nine species (Table 2, Figure 2). Their presence was variable within each species; usually, satellites were observed in both members of the respective chromosome pair, although sometimes only in one homolog. Satellites were more commonly located on the short arms; only *Amphilophium paniculatum*, *Cuspidaria convoluta*, *Catalpa bignonioides*, and *Dolichandra dentata* had one or two pairs on the long arms (Figure 2).

Overall, karyotypes were symmetrical: most species fell into 2A or 1A Stebbins' (1971) categories (Table 2). Asymmetry indices of Romero Zarco (1986) were as follows: A_1 = 0.11–0.23 and A_2 = 0.14–0.22 (Table 2). The karyotypes of *P. ricasoliana* and *C. convoluta* were the most asymmetrical because of the presence of st chromosomes.

Some species could be distinguished by a combination of karyotype formulae, haploid karyotype length, and position of satellites on a particular chromosome pairs, e.g. *Amphilophium cynanchoides*, *Catalpa bignonioides*, *Cuspidaria convoluta*, *Dolichandra dentata*, and *Tecoma stans* (Table 2, Figure 2).

1C nuclear DNA content was obtained for 12 diploid species, from 0.64 pg in *Tecoma stans* to 2.02 pg in *Amphilophium paniculatum* (Table 2). The nuclear DNA content was correlated with total length of the haploid complement. The average amount was 1.49 pg.

Discussion

This is the first chromosome number report for the species *Bignonia binata*, *Handroanthus ochraceus*, and *Tabebuia aurea* and for the genus *Podranea* Sprague. In addition, the first sporophytic number for *Catalpa bignoniodes* is reported, which coincides with preceding gametic data ($n = 20$; Mehra 1976). For the remaining species, we confirmed previous number reports (Piazzano 1998).

Tribe Jacarandeae showed $x = 18$ (Goldblatt and Gentry 1979; Piazzano 1998), as we registered in

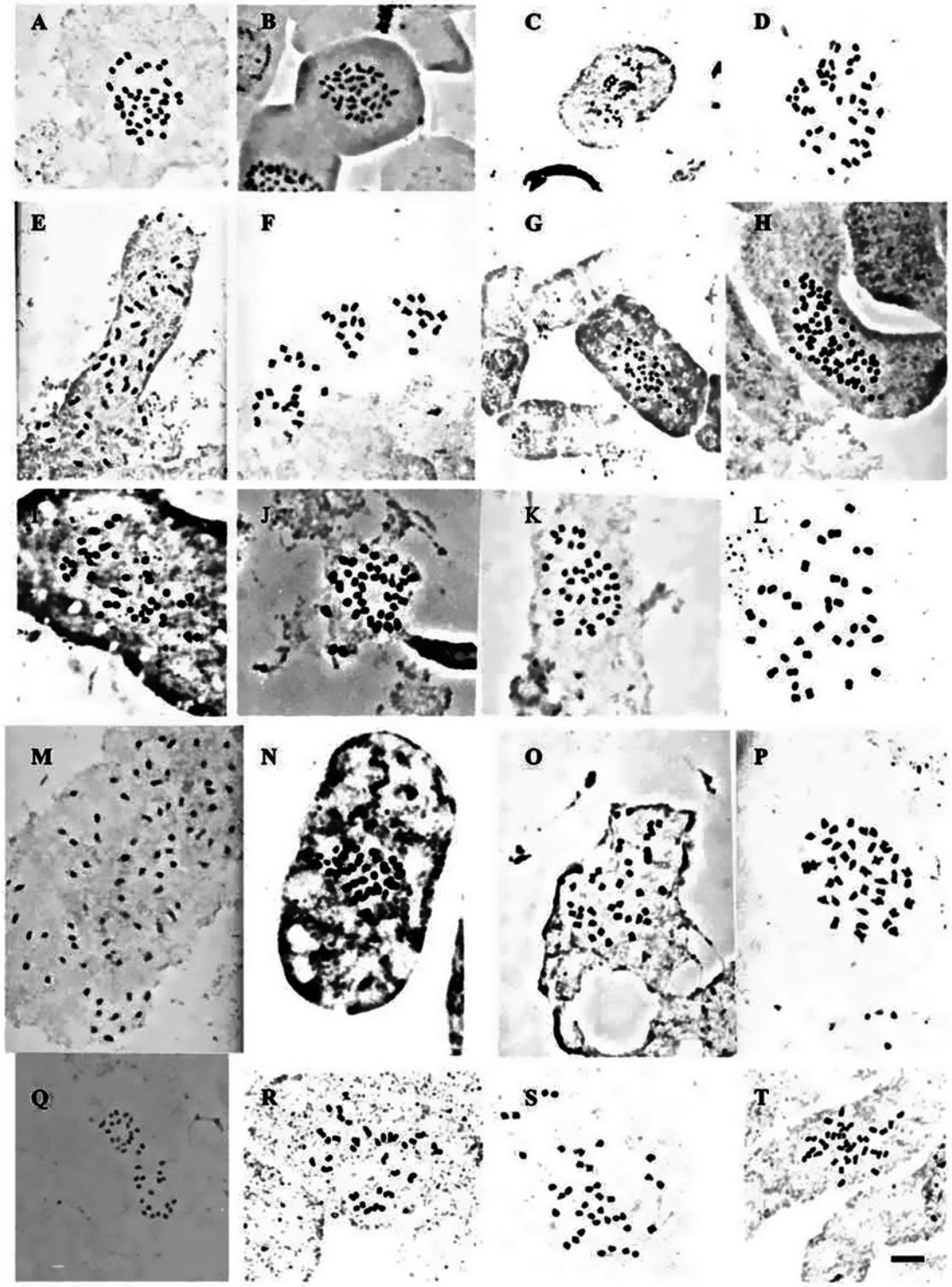

Figure 1. Photomicrographs of Bignoniaceae mitotic chromosomes. (A) *Adenocalymma marginatum*; (B) *Amphilophium crucigerum*; (C) *Amphilophium cynanchoides*; (D) *Amphilophium paniculatum*; (E) *Bignonia binata*; (F) *Cuspidaria convoluta*; (G) *Dolichandra cynanchoides*; (H) *Dolichandra unguis-cati*; (I) *Dolichandra dentata*; (J) *Fridericia dichotoma*; (K) *Tynanthus micranthus*; (L) *Jacaranda mimosifolia*; (M) *Handroanthus chrysotrichus*; (N) *H. heptaphyllus*; (O) *H. impetiginosus*; (P) *H. ochraceus*; (Q) *Tabebuia aurea*; (R) *Tecoma stans*; (S) *Podranea ricasoliana*; (T) *Catalpa bignonioides*. Scale = 5 μm.

J. mimosifolia. In Tecomeae, there are different chromosome numbers reported: $x = 11$ (*Incarvillea*; Chen et al. 2004), $x = 15$ in *Argylia* (Covas and Schnack 1946), $x = 17$ and 18 in *Tecoma* (Nakajima 1936; Goldblatt and Gentry 1979; Piazzano 1998; our data), $x = 18$ in *Tecomaria* (Goldblatt and Gentry 1979), and

Table 2. Chromosome features and 1C nuclear DNA content in Bignoniaceae species.

Species	2*n*	Karyotype formula	Satellited pairs number	HKL (μm)	c(μm)	r	A_1	A_2	St	1C(pg)
Tribe Bignonieae										
Adenocalymma marginatum	40	20 m	—	19.72	0.99	1.10	0.01	0.16	1A	—
Amphilophium crucigerum	40	18 m + 2 sm	—	21.55	1.10	1.19	0.14	0.17	2A	0.78 ± 0.23
Amphilophium cynanchoides	40	17 m + 3 sm	3	20.52	1.03	1.23	0.14	0.18	2A	1.84 ± 0.30
Amphilophium paniculatum	40	15 m + 5 sm	1	30.05	1.50	1.50	0.23	0.22	3A	2.02 ± 0.23
Bignonia binata	40*	18 m + 2 sm	—	20.21	1.01	1.20	0.13	0.18	2A	—
Cuspidaria convoluta	40	17 m + 2 sm + 1 st	1	29.55	1.31	1.47	0.16	0.18	2A	1.69 ± 0.11
Dolichandra cynanchoides	40	18 m + 2 sm	3	18.63	0.93	1.21	0.12	0.18	2A	1.59 ± 0.18
Dolichandra unguis-cati	80	40 m	2	37.63	0.94	1.06	0.12	0.19	2A	1.34 ± 0.18
Dolichandra dentata	40	20 m	4	20.60	1.03	1.07	0.06	0.14	1A	—
Fridericia dichotoma	40	20 m	1	26.20	1.15	1.31	0.11	0.20	1A	—
Tynanthus micranthus	40	20 m	—	27.22	1.36	1.07	0.10	0.12	1A	—
Tribe Jacarandeae										
Jacaranda mimosifolia	36	14 m + 4 sm	—	21.10	1.17	1.26	0.14	0.16	2A	—
***Tabebuia* alliance**										
Handroanthus heptaphyllus	40	18 m + 2 sm	—	22.75	1.18	1.19	0.15	0.15	2A	1.82 ± 0.20
Handroanthus impetiginosus	40	19 m + 1 sm	2	23.83	1.19	1.22	0.16	0.15	2A	1.61 ± 0.24
Handroanthus ochraceus	40*	18 m + 2 sm	—	28.80	1.44	1.35	0.15	0.14	2A	1.30 ± 0.14
Handroanthus pulcherrimus	40	17 m + 3 sm	—	24.05	1.19	1.18	0.16	0.14	2A	—
Handroanthus chrysotrichus	80	40 m	—	35.70	0.90	1.17	—	—	1A	1.88 ± 0.18
Tabebuia aurea	40*	18 m + 2 sm	—	26.21	1.31	1.05	0.13	0.15	2A	
Tribe Tecomeae										
Tecoma stans	36	14 m + 4 sm	—	21.35	1.18	1.40	0.15	0.16	2B	0.64 ± 0.02
Tecoma garrocha	36	—	—	—	—	—	—	—	—	1.07 ± 0.07
Podranea ricasoliana	38**	17 m + 1 sm+ 1 st	—	19.10	1.01	1.28	0.15	0.16	2A	—
Tribe Catalpeae										
Catalpa bignonioides	40	18 m + 2 sm	3	26.47	1.32	1.27	0.19	0.20	2A	—

Abbreviations: m = metacentric, sm = submetacentric, st = subtelocentric, HKL = haploid karyotypes length, c = mean chromosome length, r = mean arm ratio, A_1 = mean intrachromosomal asymmetry index, A_2 = mean interchromosomal asymmetry index, St = karyotype asymmetry category (Stebbins 1971), pg = picograms, mean ± SD.
*New count for the species.
**New count for the genus.

x = 19 in *Campsis* (Venkatasubban 1944), *Pandorea* (Nakajima 1936) and *Podranea* (our data). In the *Tabebuia* alliance, *Handroanthus* presented *x* = 20 (Piazzano 1998; Alcorcés de Guerra 2002; Alcorcés de Guerra and Méndez Natera 2007; our data). In tribe Catalpeae, *x* = 20 is known (Suessenguth 1942; Mehra 1976; our data). Tribe Bignonieae embraces the most derived taxa (Spangler and Olmstead 1999; Olmstead et al. 2009) and chromosomically most taxa showed *x* = 20, as we also found here, with a doubtful report of 2*n* = 36 (38) for *Mansoa difficilis* (Goldblatt and Gentry 1979).

According to Raven (1975), *x* = 20 is the most frequent base number of the family. However, he proposed that the ancestral base number would be *x* = 7, from which *x* = 20 would have arisen by a six-fold polyploidization process with a subsequent loss of a chromosome pair. The remaining numbers would have arisen in a similar way. This hypothesis was based on the assumption that *Oroxylum*, considered then as one of the most primitive genera, had *n* = 14 and 15 (Goldblatt 1976). Later authors on the same grounds supported this explanation (Goldblatt 1976; Goldblatt and Gentry 1979; Gentry 1980; Piazzano 1998; Chen et al. 2004; Fischer et al. 2004). However, recent molecular phylogenetic studies (Spangler and Olmstead 1999; Olmstead et al. 2009) clearly showed that tribe Oroxyleae is not basal; thus, the previous justification presently does not hold. There is no doubt that paleopolyploidy was a significant mechanism of chromosomal evolution of Bignoniaceae and it may have already been in the origin of the basic chromosome number. It is clear that *x* = 20 is the most frequent number for the family and this was also reported for the sister clades Paulowniaceae and Schlegeliaceae, with high chromosome numbers and 2*n* = 40 (e.g. Liang and Chen 1997; Goldblatt and Gentry 1979). Thus, a most parsimonious explanation would be to consider *x* = 20 as basic for Bignoniaceae with the recurrent loss of chromosomes by disploidy.

Presently, polyploidy is relatively rare in Bignoniaceae. Only three species are currently known as polyploid, two from tribe Bignonieae: *D. unguis cati* (2*n* = 80; Goldblatt and Gentry 1979; Jullier 1989; our data) and *Pyrostegia venusta* (Ker-Gawl.) Miers (2*n* = 60; Joshi and Hardas 1956), and *H. chrysotrichus* from the *Tabebuia* alliance (2*n* = 80; Piazzano 1998; our data). These polyploids were probably originated from meiosis alterations that produced unreduced gametes.

Tropical woody angiosperms are characterized by small chromosomes and high diploid numbers (Mehra and Bawa 1969; Raven 1975; Levin and Funderburg

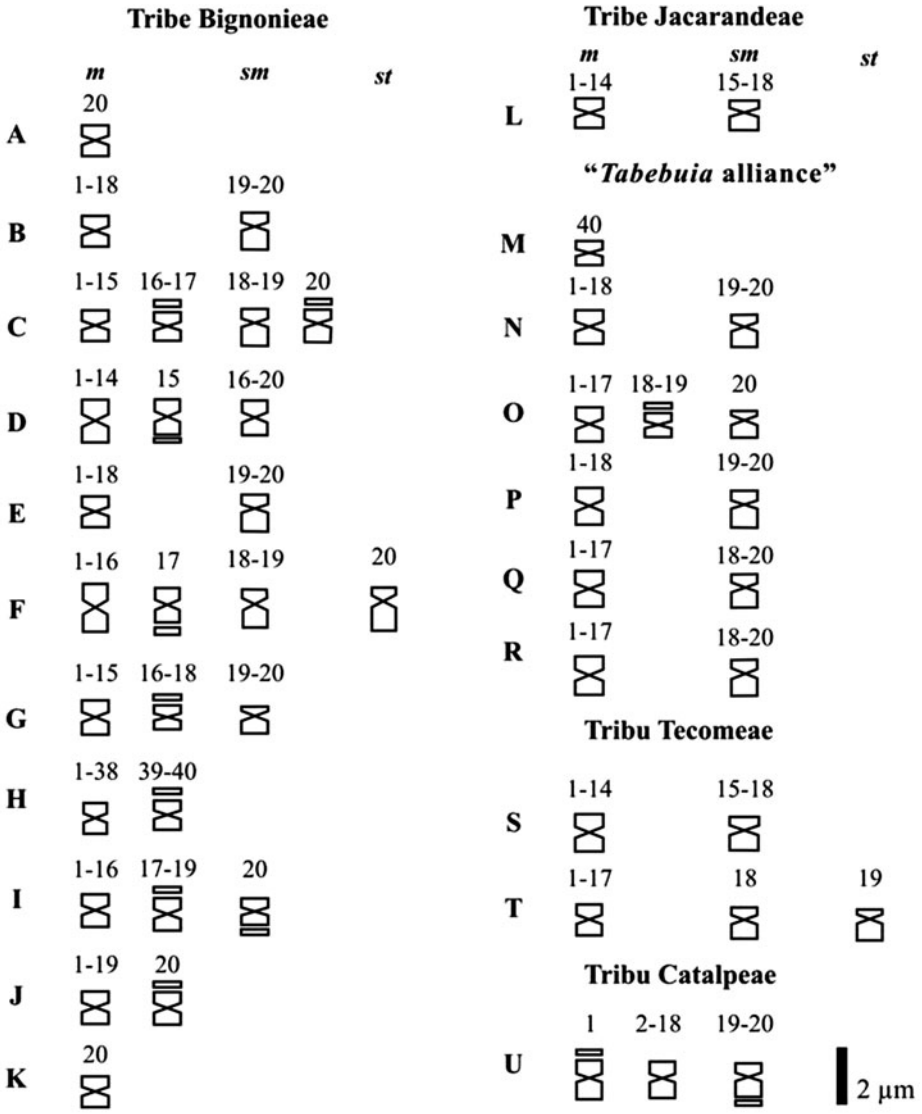

Figure 2. Idiograms of Bignoniaceae. (A) *Adenocalymma marginatum*; (B) *Amphilophium crucigerum*; (C) *Amphilophium cynanchoides*; (D) *Amphilophium paniculatum*; (E) *Bignonia binata*; (F) *Cuspidaria convoluta*; (G) *Dolichandra cynanchoides*; (H) *Dolichandra unguis-cati*; (I) *Dolichandra dentata*; (J) *Fridericia dichotoma*; (K) *Tynanthus micranthus*; (L) *Jacaranda mimosifolia*; (M) *Handroanthus chrysotrichus*; (N) *H. heptaphyllus*; (O) *H. impetiginosus*; (P) *H. ochraceus*; (Q) *H. pulcherrimus*; (R) *Tabebuia aurea;* (S) *Tecoma stans*; (T) *Podranea ricasoliana*; (U) *Catalpa bignonioides*. Scale = 2 µm.

1979). Our findings as well as previous cytological studies (e.g. Venkatasubban 1944, 1945; Goldblatt and Gentry 1979; Gentry 1980; Goldblatt 1989; Piazzano 1998; Alcorcés de Guerra 2002; Chen et al. 2004; Alcorcés de Guerra and Méndez Natera 2007; Kumar et al. 2008; Firetti-Leggieri et al. 2011, 2013) pointed out that in all cases, chromosomes are small, with lengths mostly ranging from *c*.0.70 to 2.0 µm. Grant (1958) suggested that a high chromosome number can increase the potential for recombination. The decrease in chromosome size would be balanced by the increase in number, which would act as a driving force of evolutionary advance and would ensure an optimal recombination in tree species (Mehra and Bawa 1969). At the same time, herbaceous members of the family, like *Incarvillea*, presented larger chromosomes of *c*.4 µm long in some species (Chen et al. 2004).

At the interspecific level, a reason for the variation in satellite number may be that satellited chromosomes are composed of heterochromatin, which is highly variable. Moreover, translocations may be responsible for changing the satellite position, whereas duplications and deletions can cause differences in number (Moscone et al. 1995; Chiarini and Barboza 2008). Given the hypothesis that species of Bignoniaceae with higher numbers originated from a recurrent polyploidization process, it is expected that they present multiple satellites or nuclear organiser regions (NOR).

Unfortunately, few previous karyotypic papers are available with which to compare our results. *Handroanthus*

species previously studied (Alcorcés de Guerra 2002) had symmetrical karyotypes as well, mostly with m chromosomes. On the other hand, Asian *Incarvillea* species examined (Chen et al. 2004) showed more asymmetrical karyotypes. A widely accepted conception is that symmetrical karyotypes would be more primitive and would be associated with perennial, woody species, while in annuals and herbaceous species they would be more asymmetric (Stebbins 1950, 1971). This was confirmed for different families (e.g. Brandham 1983; Ehrendorfer 1983), but for Bignoniaceae more cytological data are needed.

The obtained DNA nuclear amount data are comparable to the few reported so far for Bignoniaceae. The known range for the family was 2C = 0.61–1.74 pg (Ohri and Kumar 1986; Ohri et al. 2004; Bennett and Leitch 2010) and we here detected slightly higher figures with a maximum of 2.02 pg for *Amphilophium paniculatum*. *Tecoma stans* was previously studied (Bennett and Leitch 2010) and our values are very close to the published ones. Also *H. impetiginosus* was previously analyzed under the name *Tabebuia palmeri* (Ohri et al. 2004), from which we obtained a slightly higher value.

Compared to closely related families for which DNA contents are available and concerning absolute values, Bignoniaceae measurements are, on average, similar to those of Verbenaceae, Pedaliaceae, and Acanthaceae, lower than Orobanchaceae, and higher than Lentibulariaceae (Hanson et al. 2005; Suda et al. 2005; Loureiro et al. 2007; Bennett and Leitch 2010; Vesely et al. 2011).

Within some genera, DNA contents are related to life form, with annuals having lower amounts than perennials (e.g. Bennett 1972; Albach and Greilhuber 2004; Price et al. 2005). In Bignoniaceae, the only species with scarce DNA content data registered are woody species.

Species of the same genus may vary in their DNA content. In *Bulnesia* (Zygophyllaceae), for example, Poggio and Hunziker (1986) reported a six-fold difference, while in *Solanum* (Solanaceae) there was up to a 24-fold variation (Bennett 1976; Pringle and Murray 1991). The available Bignoniaceae data showed that the differences were always less than single-fold: between *Amphilophium cynanchoides* and *A. paniculatum* it was lower than 9% (our data), between *Tecoma garrocha* and *T. stans* 70% (our data), between *Jacaranda mimosifolia* and *J. cuspidifolia* 10% (Ohri and Kumar 1986; Ohri et al. 2004), and between four *Handroanthus* species (our data) it was less than 6%. Thus, Bignoniaceae would be relatively conservative in terms of DNA contents, although more data are badly needed.

There are several examples of the relationship between DNA content and chromosome size (Nagl and Ehrendorfer 1974; Dimitrova and Greilhuber 2000; Garnatje et al. 2004). In general, the nuclear DNA content positively correlates with the total length of the haploid complement in each species, with some examples of the opposite pattern (Moscone et al. 2003). With our findings, no correlations can be drawn, and nor can they with chromosome numbers.

Although some examined species were karyologically indistinguishable, based on conventionally stained mitotic chromosomes, some species are clearly noticeable. On the other hand, our data did not provide useful information to characterize either genera or clades/tribes in Bignoniaceae. In spite of the remarkable morphological variation in Bignoniaceae, it seems that there is a common karyotypical pattern of mostly m chromosomes with few cryptic chromosomal rearrangements. Additional and extensive karyotypic analyses are badly needed, not only with classical but also with molecular techniques (e.g. banding and FISH).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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