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Karyomorphology and karyotype asymmetry in the South American *Caesalpinia* species (Leguminosae and Caesalpinioideae)

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ABSTRACT. With the purpose of addressing the pattern of karyotype evolution in *Caesalpinia* species, chromosome morphology was characterized in five species from Brazil, and karyotypic asymmetry was analyzed in 14 species from South America. All accessions had the chromosome number 2n = 24, which was first described here for *Caesalpinia laxiflora* Tul. and *Cenostigma macrophyllum* Tul. The karyotype formula of *C. laxiflora*, *Caesalpinia pyramidalis* Tul., and *C. macrophyllum* was 12 m. The formula varies amongst the populations of *Caesalpinia bracteosa* Tul. (11 m + 1 sm) and *Caesalpinia echinata* Lam. (10 m + 2 sm and 9 m + 3 sm). The intra- and interspecific variations in chromosome length were significant (analysis of variance, $P < 0.05$). Analyzing the asymmetry index (AI), revealed that *Caesalpinia calycina* Benth. had the most asymmetrical karyotype (AI = 10.52), whereas *Caesalpinia paraguarienses* (D. Parodi) Burkat. and *Caesalpinia gilliesii* (Hook.) Benth. had the most symmetrical karyotypes (AI = 0.91 and 1.10, respectively). There has been a trend to lower AI values for the *Caesalpinia* s.l. species assigned in *Libidibia* and intermediate values for those combined into *Poincianella*. On the

other hand, the karyotypes of *Erythrostemon* species had extremely different AI values. This study confirms the existence of karyotype variability in *Caesalpinia* s.l. while revealing a possible uniformity of this trait in some of the new genera that are being divided from *Caesalpinia* s.l. More broadly, the 2n = 24 chromosome number is conserved. Metacentric chromosomes and low AI values predominate among *Caesalpinia* s.l. and *Cenostigma*.

Key words: Brazilwood; Caesalpinieae; Cytogenetics; Evolution

INTRODUCTION

Caesalpinia L. (Leguminosae - Caesalpinioideae - Caesalpinieae), analyzed as *Caesalpinia* L. s.l., comprises approximately 140 species with morphological characteristics that are very similar to each other (Lewis, 1998; Gasson et al., 2009). Caesalpinioideae has great interspecific and intraspecific variability in the number of chromosomes, and many species have diploid or an unknown chromosome number (Beltrão and Guerra, 1990; Jena et al., 2004; Biondo et al., 2005, Rodrigues et al., 2012). Taxonomic studies support the hypothesis that Caesalpinioideae is a non-natural group comprising paraphyletic tribes (Tucker and Douglas, 1994; Käss and Wink, 1996; Doyle et al., 1997, 2000; Herendeen, 2000; Bruneau et al., 2001; Kajita et al., 2001). Thus, phylogenetic analysis with molecular markers aided in the understanding of this subfamily (Haston et al., 2005; Bruneau et al., 2001, 2008).

Cytogenetic studies are important to Caesalpinioideae studies, but they are still scarce in this subfamily (Biondo et al., 2005). Cytogenetic analysis has been used in cytotaxonomic analyses, particularly by comparing the number and morphology of chromosomes of species at different taxonomic levels. Variations in the number and position of satellites in karyotypes allow inferences about the relationship between karyotype evolution and related taxa.

In the Caatinga biome of Brazil, Caesalpinieae is represented by nine genera, four of which belongs to Caesalpinia clade: *Cenostigma* Tul., *Erythrostemon* Cav., *Libidibia* (DC.) Schltdl., and *Poincianella* Britton & Rose, with these last three genera segregated from *Caesalpinia* s.l. (Gasson et al., 2009; Queiroz, 2009, 2010; Warwick and Lewis, 2009; Manzanilla and Bruneau, 2012). The *Caesalpinia* s.l. species that are reported to be native to Brazil occur in natural populations from Bahia and other states in northeastern Brazil, mainly in semiarid region. Most of these species belong to the *Poincianella*, *Erythrostemon* and *Libidibia* genera (Lewis, 1998; Queiroz, 2009, 2010). Only five Brazilian *Caesalpinia* s.l. species have their karyomorphological data published (Rodrigues et al., 2012). Of the *Caesalpinia* s.l. species that are native to other countries, only three species have been thoroughly examined in terms of karyomorphology (Cangiano and Bernardello, 2005). The literature reporting a higher number of *Caesalpinia* s.l. species only refers to the chromosome number 2n = 24, the identification of some polyploid populations, and partial karyomorphological variations.

Species of great economic value for the local populations from some areas of the Cerrado and Caatinga Biomes only have their chromosome number known, such as *Caesalpinia bracteosa* Tul. and *Caesalpinia pyramidalis* Tul. As for *Caesalpinia laxiflora* Tul. and *Cenostigma macrophyllum* Tul., which are also native to the Caatinga, not even the chromosome number is known. The chromosome number 2n = 24 is found in *C. echinata* Lam. (Beltrão

and Guerra, 1990). However, its karyomorphology remains unreported in the literature. This species has a high economic value and, because of that, it has been overexploited; its natural habitat (Atlantic Forest) is also very devastated (Rocha, 2004; Rondon et al., 2006). Today, *C. echinata* is endangered (Varty, 1998). This species has a genetic diversity compatible with the existence of botanical varieties, subspecies, or complex of species (Juchum et al., 2008), but the few remaining natural populations are structured (Cardoso et al., 1998; Melo et al., 2007). Furthermore, there is still devastation of forest fragments where some of these populations live.

Despite 2n = 24 being a common chromosome number in *Caesalpinia*, important data have been obtained from the karyomorphological analysis via classical cytogenetic techniques, which allow for species characterization and can contribute to the understanding of this clade. Thus, the different karyomorphological parameters and the comparative analysis of karyotype asymmetry have that potential. It is known that more symmetrical karyotypes show a high total form percent (TF%), a chromosomal asymmetry parameter (Huziwara, 1962), or low karyotype asymmetry index (AI) (Paszko, 2006). These two asymmetry parameters have been adopted to analyze different taxonomic levels. AI was seen to be quite informative in studies of infra-generic karyotypic heterogeneity, as demonstrated for eight *Calamagrostis* accessions (Poaceae) (Paszko, 2006) and six *Coffea* species (Pierozzi et al., 2012). As demonstrated in the analysis of 217 species representing different Liliaceae genera (Peruzzi et al., 2009), AI is perceived as a suitable parameter for suprageneric evolutionary studies.

The number and morphology of chromosomes are the distinctions that are more frequently used in cytogenetics (Biondo et al., 2005). A direct comparative analysis of cytogenetic data in closely related species allows pinpointing the unique features of each species, as well as those that are common to all or to most of them (Guerra, 1990). In recent studies, AI and its components were observed to be suitable for comparative analysis at different taxa, encouraging us to use it in analysis of different *Caesalpinia* s.l. and *Cenostigma* species. Accordingly, the chromosome number and morphometric characteristics of five species were determined in an attempt to provide input to cytotaxonomic studies on Caesalpinieae from Caatinga. Moreover, Paszko's AI and its components were used to investigate the karyotypic heterogeneity of 14 *Caesalpinia* s.l. and *Cenostigma* species.

MATERIAL AND METHODS

Sample collection and preparation of slides

The original cytogenetic data of five Brazilian endemic tree species were obtained as a complement to our previous karyomorphological studies (Rodrigues et al., 2012). Samples of each species were collected at different localities of Bahia State: *C. bracteosa* in Oliveira dos Brejinhos (13°36'07.6"S, 41°46'33.8"W), *C. echinata* in Ilhéus (14°47'20"S, 39°2'56"W) and Feira de Santana (12°16'01"S, 38°58'1"W), *C. laxiflora* and *C. pyramidalis* in Bom Jesus da Lapa (13°19'0.9"S, 43°20'14.8"W). As for *C. echinata*, a third sample was collected in Recife, Pernambuco (8°2'32.82"S, 34°53'55.9"W). *C. echinata* was a rare species in nature, and it was collected from urban parks, whereas all the others species were collected from their natural occurrence sites. In turn, *C. macrophyllum* Tul. was obtained in an area of urban expansion located 5 km from Ibotirama (12°09'19.5"S, 43°10'03.9"W).

The identification of samples was confirmed by comparing the materials available at the herbarium of UESC, and a copy of each species was kept in the herbarium (voucher: *C. bracteosa*

16043, *C. echinata* 2929, *C. laxiflora* 16038, *C. pyramidalis* 16039, and *C. macrophyllum* 16041). Botanical samples were likewise kept in the Herbarium of UEFS (Feira de Santana, BA).

Apical samples of roots were used to prepare the slides as described before (Rodrigues et al., 2012).

Analysis of cytogenetic data

Short arm (SA), long arm (LA), and satellites (SAT) were measured using the Image Tool 3.0 software. From this data, we calculated the ratio between chromosome arms (r = LA / SA), total chromosome length (TCL = SA + LA + SAT), the relative length of each chromosome, the average chromosome length ($\chi = \Sigma TCL$ / number of chromosomes), the haploid lot length (HLL), the AI for TF% (Huziwara, 1962), and the AI for A_1 of Romero Zarco (1986). The karyotypic formula was established, and the satellites were classified according to the pattern used before (Rodrigues et al., 2012). The karyograms and ideograms were obtained with the help of the Adobe Photoshop CS4.0 program.

Morphometric data were statistically analyzed in a completely randomized design with five replicates. Statistical analyses were performed using the Sisvar software (Ferreira, 2003). Analysis of variance (ANOVA) was applied to assess differences in the mean chromosome length of species and between accessions of the same species, as well as variations in chromosome length from the first to the twelfth chromosome pair of each species. Means were compared by applying the Scott-Knott test at 5% probability.

Analysis of karyotypic heterogeneity in *Caesalpinia*

The 14 *Caesalpinia* s.l. and *Cenostigma* species were tested in terms of karyotype heterogeneity according to the Paszko's AI estimates and based on scatter plots of the variation sources comprising this parameter (Paszko, 2006). Most of the data are derived from cytogenetic studies carried out following the same methodological procedures (five species described in the previous section and five species analyzed by Rodrigues et al., 2012). As for *Caesalpinia crista* L., measurements were collected from karyograms available in the literature, whereas the values were standardized based on the HLL calculated by Jena et al. (2004). With regard to the other three species, measurements were directly taken from the respective ideograms in previous results (Cangiano and Bernardello, 2005).

Most of the species analyzed in this study were previously placed in new combinations of genera segregated from *Caesalpinia* s.l. (Lewis, 1998; Gasson et al., 2009; Queiroz, 2009, 2010). Despite the high taxonomic variation of *Caesalpinia* clade, sampling was restricted to those species whose detailed karyomorphological data have been obtained so far.

RESULTS

Morphometric analyses of five Brazilian *Caesalpinia* species

In this study, the observed chromosome number in *C. bracteosa*, *C. echinata*, *C. laxiflora*, *C. pyramidalis*, and *C. macrophyllum* was 2n = 24 (Figures 1 and 2). This chromosome number is commonly found in the *Caesalpinia* species.

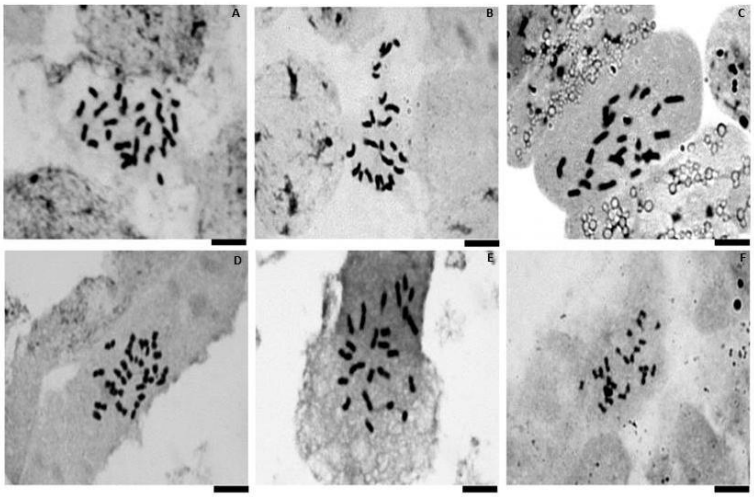

Figure 1. Conventional staining in the mitotic metaphases of *Caesalpinia* L. s.l. species. **A.** *C. bracteosa* Tul.; **B.** *C. echinata* Lam. (Ilhéus); **C.** *C. echinata* Lam. (Recife); **D.** *C. laxiflora* Tul.; **E.** *C. pyramidalis* Tul.; **F.** *Cenostigma macrophyllum* Tul. Bar = 10 µm.

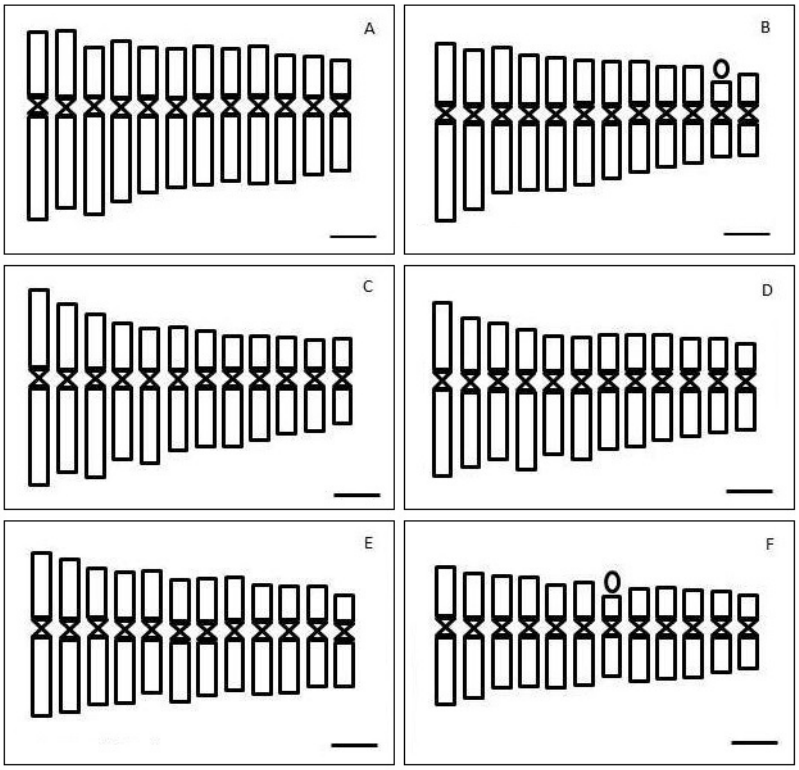

Figure 2. Ideograms of *Caesalpinia* L. s.l. species. **A.** *C. bracteosa* Tul.; **B.** C. *laxiflora* Tul.; **C.** *C. echinata* Lam. (Ilhéus/ Feira de Santana); **D.** *C. echinata* Lam. (Recife); **E.** *C. pyramidalis* Tul.; **F.** *Cenostigma macrophyllum* Tul. Bar = 1 cm.

The values of χ reveals little variation among the five *Caesalpinia* species examined (Tables 1 and 2); χ ranged from 3.12 µm in *C. bracteosa* to 2.03 in *Caesalpinia* sp, but it remained nearly constant between *C. laxiflora* and *C. pyramidalis* (2.52 and 2.51 µm, respectively) and between the accessions of *C. echinata* from different locations (2.40 µm in Ilhéus, 2.41 µm in Feira de Santana, and 2.43 µm in Recife).

Table 1. Karyomorphological data regarding the metaphases of *Caesalpinia* s.l. species.

Species*	Data	Chromosome pair											
		1	2	3	4	5	6	7	8	9	10	11	12
bra	SA	1.56	1.65	1.23	1.40	1.23	1.21	1.23	1.24	1.16	1.00	0.99	0.88
	LA	2.58	2.28	2.47	2.12	1.92	1.76	1.72	1.70	1.63	1.66	1.46	1.35
	TCL	4.14	3.93	3.70	3.52	3.15	2.98	2.96	2.94	2.79	2.66	2.45	2.23
	SD (±)	0.95	0.92	0.91	0.40	0.56	0.74	0.70	0.67	0.58	0.72	0.36	0.57
	S-K	a	a	a	a	b	b	b	b	b	b	b	b
	r	1.65	1.38	2.00	1.51	1.56	1.45	1.40	1.37	1.40	1.66	1.47	1.53
	CM	m	m	sm	m	m	m	m	m	m	m	m	m
echI	SA	1.70	1.25	1.14	0.92	1.06	0.83	0.86	0.87	0.89	0.70	0.75	0.60
	LA	1.91	1.72	1.78	1.82	1.46	1.61	1.39	1.36	1.13	1.11	0.96	0.90
	TCL	3.61	3.06	2.92	2.75	2.52	2.43	1.25	1.22	2.02	1.81	1.71	1.50
	SD (±)	0.66	0.32	0.32	0.28	0.40	0.39	0.32	0.27	0.22	0.20	0.17	0.24
	S-K	a	b	b	b	c	c	c	c	d	d	d	d
	r	1.12	1.27	1.56	1.98	1.38	1.94	1.61	1.57	1.27	1.59	1.27	1.49
	CM	m	m	m	sm	m	sm	m	m	m	m	m	m
echF	SA	1.71	1.54	1.21	1.14	0.80	0.85	0.82	0.76	0.81	0.68	0.70	0.66
	LA	2.14	1.94	1.74	1.58	1.70	1.45	1.35	1.32	1.17	1.11	0.93	0.82
	TCL	3.85	3.48	2.95	2.72	2.50	2.31	2.17	2.08	1.98	1.78	1.63	1.48
	SD (±)	0.58	0.53	0.40	0.25	0.17	0.21	0.15	0.14	0.12	0.12	0.15	0.09
	S-K	a	a	b	b	c	c	c	c	c	d	d	d
	r	1.24	1.25	1.43	1.38	2.13	1.69	1.63	1.73	1.44	1.64	1.32	1.23
	CM	m	m	m	m	sm	m	m	sm	m	m	m	m
echR	SA	1.72	1.54	1.13	0.95	0.87	0.98	0.92	0.72	0.75	0.75	0.65	0.67
	LA	2.20	2.03	2.13	1.57	1.64	1.35	1.27	1.39	1.19	1.04	0.94	0.79
	TCL	3.92	3.57	2.27	2.53	2.51	2.34	2.20	2.11	1.95	1.79	1.59	1.46
	SD (±)	0.63	0.80	0.88	0.32	0.50	0.38	0.26	0.30	0.24	0.26	0.36	0.33
	S-K	a	a	a	b	b	b	b	b	c	c	c	c
	r	1.28	1.31	1.87	1.65	1.88	1.37	1.38	1.92	1.57	1.38	1.43	1.17
	CM	m	m	sm	m	sm	m	m	sm	m	m	m	m
lax	SA	1.47	1.34	1.41	1.23	1.15	1.07	1.04	1.01	0.91	0.82	0.85	0.69
	LA	2.45	2.11	1.70	1.64	1.63	1.50	1.33	1.22	1.09	0.99	0.95	0.78
	TCL	3.92	3.45	3.11	2.87	2.78	2.57	2.37	2.23	2.00	1.81	1.80	1.47
	SD (±)	0.14	0.24	0.21	0.24	0.25	0.17	0.25	0.20	0.18	0.23	0.18	0.12
	S-K	a	b	c	d	d	e	e	e	f	f	g	g
	r	1.66	1.57	1.20	1.33	1.41	1.40	1.27	1.20	1.19	1.20	1.11	1.13
	CM	m	m	m	m	m	m	m	m	m	m	m	m
pyr	SA	1.61	1.49	1.23	1.16	1.20	1.04	1.06	1.05	0.87	0.83	0.84	0.66
	LA	1.94	1.79	1.67	1.58	1.31	1.47	1.31	1.25	1.32	1.32	1.16	1.09
	TCL	3.55	3.28	2.90	2.74	2.51	2.51	2.37	2.30	2.19	2.15	2.00	1.75
	SD (±)	0.59	0.58	0.54	0.66	0.67	0.53	0.47	0.44	0.35	0.34	0.34	0.38
	S-K	a	a	b	b	c	c	c	c	c	c	c	c
	r	1.20	1.20	1.36	1.36	1.10	1.41	1.24	1.19	1.52	1.59	1.38	1.65
	CM	m	m	m	m	m	m	m	m	m	m	m	m
mac	SA	1.24	1.10	1.04	1.01	0.83	0.86	0.92	0.72	0.68	0.65	0.63	0.55
	LA	1.70	1.50	1.26	1.23	1.28	1.19	1.10	1.10	1.01	0.89	0.84	0.78
	TCL	2.94	2.60	2.30	2.24	2.11	2.05	2.02	1.82	1.69	1.54	1.47	1.33
	SD (±)	0.75	0.66	0.54	0.52	0.57	0.58	0.62	0.47	0.47	0.50	0.32	0.19
	S-K	a	a	a	a	a	a	b	b	b	b	b	b
	r	1.37	1.36	1.21	1.21	1.54	1.38	1.20	1.52	1.48	1.32	1.33	1.41
	CM	M	m	m	m	m	m	m	m	m	m	m	m

Mean values in µm of the short arm (SA), long arm (LA) and total chromosome length (TCL). Standard deviation of TCL (SD), Scott-Knott test regarding the average length of each chromosome (S-K), arm ratio (r) and classification of chromosome morphology (CM) (m = metacentric, sm = submetacentric). *Name abbreviations of *Caesalpinia* L s.l. species: *bra* = *C. bracteosa* Tul.; *echI* = *C. echinata* Lam. from Ilhéus; *echF* = *C. echinata* from Feira de Santana; *echR* = *C. echinata* from Recife; *lax* = *C. laxiflora Tul.*; *pyr* = *C. pyramidalis* Tul.; *mac* = *Cenostigma macrophyllum* Tul.

Table 2. Karyotype parameters in *Caesalpinia* L. s.l.

Species name (sampling locations)	HLL (µm)	χ (µm)	TF%	A_1	KF
C. bracteosa Tul.	37.47	3.12 ± 0.49	37.47	0.34	11 m + 1 sm
C. echinata Lam. (Ilhéus)	28.83	2.40 ± 0.29	40.45	0.32	10 m + 2 sm
C. echinata Lam. (Feira de Santana)	28.97	2.41 ± 0.19	40.42	0.32	10 m + 2 sm
C. echinata Lam. (Recife)	29.26	2.43 ± 0.42	39.95	0.32	9 m + 3 sm
C. laxiflora Tul.	30.26	2.52 ± 0.14	42.52	0.23	12 m
C. pyramidalis Tul.	30.20	2.51 ± 0.46	43.04	0.25	12 m
Cenostigma macrophyllum Tul.	24.36	2.03 ± 0.30	40.20	0.27	12 m

Haploid lot length (HLL), average length of chromosomes (χ), asymmetry index of total form percent (TF%), intrachromosomal asymmetry indexes (A_1), and karyotype formula (KF), where m is metacentric and sm is submetacentric.

The variation in chromosome size reached 53.8% in *C. bracteosa* Tul., 37.5% in *C. laxiflora*, 49.2% in *C. pyramidalis*, 45.2% in *C. macrophyllum*, 41.55% in the *C. echinata* from Ilhéus, 38.44% in the *C. echinata* Lam. from Feira de Santana, and 37.25% in the *C. echinata* from Recife. *C. bracteosa* has the largest HLL as compared to *C. macrophyllum*.

A pair of chromosomes in satellite form was only observed in *C. laxiflora* and *C. macrophyllum* (Table 2). There was also the minisatellite-type, sized 0.43 and 0.46 µm, in these species, respectively. Nevertheless, the satellites were located on the short arms of separate chromosome pairs. In *C. laxiflora*, the satellite is found at the chromosome pair number 10, whereas in *C. macrophyllum*, it is located at the pair number 7.

For the seven populations analyzed, the three different karyotypic formulas determined and the TF% values showed that these populations had quite symmetrical karyotypes (Tables 1 and 2). Exclusively metacentric chromosomes were observed in *C. laxiflora*, *C. pyramidalis*, and *C. macrophyllum* (formula 12 m). Only the third chromosome pair was seen to be submetacentric in *C. bracteosa* (11 m + 1 sm).

Depending on the locality examined, two or three pairs of submetacentric chromosomes were observed in *C. echinata*. In addition, there were variations in the pair where they were found (10 m + 2 sm in Ilhéus and Feira de Santana; 9 m + 3 sm in Recife). The highest TF% was 43.04% in *C. pyramidalis*, whereas the lowest value was 37.47% in *C. bracteosa*. The variation in TF% between the species was 13%.

ANOVA revealed a significant difference ($P < 0.05$) relative to the average chromosome size between the species (Table 3). Yet, there were no significant differences regarding this trait in *C. echinata* between the sampling localities (Table 4). ANOVA also revealed a significant difference relative to the χ within each species (Table 5). The coefficient of variation (CV) in all of the analyses ranged from 8.33% in *C. laxiflora* to 26.59% in *C. bracteosa*.

Table 3. Summary of ANOVA regarding the average length of chromosomes between *Caesalpinia* L. s.l. species: *C. bracteosa* Tul., *C. echinata* Lam. (Ilhéus, Feira de Santana and Recife), *C. laxiflora* Tul., *C. pyramidalis* Tul. and *Caesalpinia* sp (2n = 24).

Variation sources	d.f.	MS
Taxa	6	0.5203
Error	28	0.1466
CV (%)		15.38

d.f. = degrees of freedom; MS = mean square; CV = coefficient of variation. Significant at 1% probability by F test.

Table 4. Summary of ANOVA for the average chromosome length (χ) of *Caesalpinia echinata* Lam. regarding the three sampling places.

Variation sources	d.f.	MS	F
Taxa	2	0.0017	0.0170^{ns}
Error	12	0.0999	
CV (%)		13.09	

d.f. = degrees of freedom; MS = mean square; CV = coefficient of variation. ^{ns}Not significant at 1% probability by the F test.

Table 5. Summary of ANOVA regarding the length of chromosomes in *Caesalpinia* L. s.l. species.

Variation sources	d.f.	MS						
		bra	*ech*I	*ech*F	*ech*R	*lax*	*pyr*	*sp1*
Taxa	11	1.0000	1.8911	2.6573	3.0364	2.7597	1.3693	0.9070
Error	48	0.2921	0.1174	0.0873	0.2418	0.0441	0.2575	0.2629
CV (%)		26.59	14.25	12.23	20.15	8.33	20.14	25.59

d.f. = degrees of freedom; MS = mean square; CV = coefficient of variation. Species name abbreviations are indicated in Table 1. Significant at 1% probability by the F test.

Chromosomal heterogeneity in *Caesalpinia* group

Interchromosomal variations regarding the position of the centromere (CV_{CI}) and chromosome size (CV_{CL}), as well as the estimated AI values, revealed that there is chromosomal diversity among *Caesalpinia* and *Cenostigma* species (Figure 3A). Among the 14 species analyzed for karyotypic heterogeneity, only 8 had published karyomorphological data. The scatter plot of CV_{CI} x CV_{CL} shows that the AI values were equally influenced by these two variation components in most species except *C. echinata*, *C. laxiflora*, and *C. macrophyllum*. In the ranking of species based on AI, three groups are observed (Figure 3B): a group of species with symmetrical karyotypes [*C. paraguariensis* (D. Parodi) Burkart and *C. gilliesii* (Wall. ex Hook.) Benth.], a group with an asymmetrical karyotype (*Caesalpinia calycina* Benth.), and a group with all of the other species, which display intermediate and gradual AI values.

The components of variation that integrate AI (CV_{CI} and CV_{CL}) showed no significant correlation related to the HLL (R^2 = 36.3 and 12.4, respectively). Furthermore, there was no significant correlation between the parameters of chromosome asymmetry AI and TF% (R^2 = 31.9) relative to the 14 species analyzed. Nine of the 14 species analyzed in this study are part of re-established genera from *Caesalpinia* s.l. (Table 6).

DISCUSSION

Chromosome diversity

The *Caesalpinia* s.l. and *Cenostigma* species that were karyomorphometrically evalu-

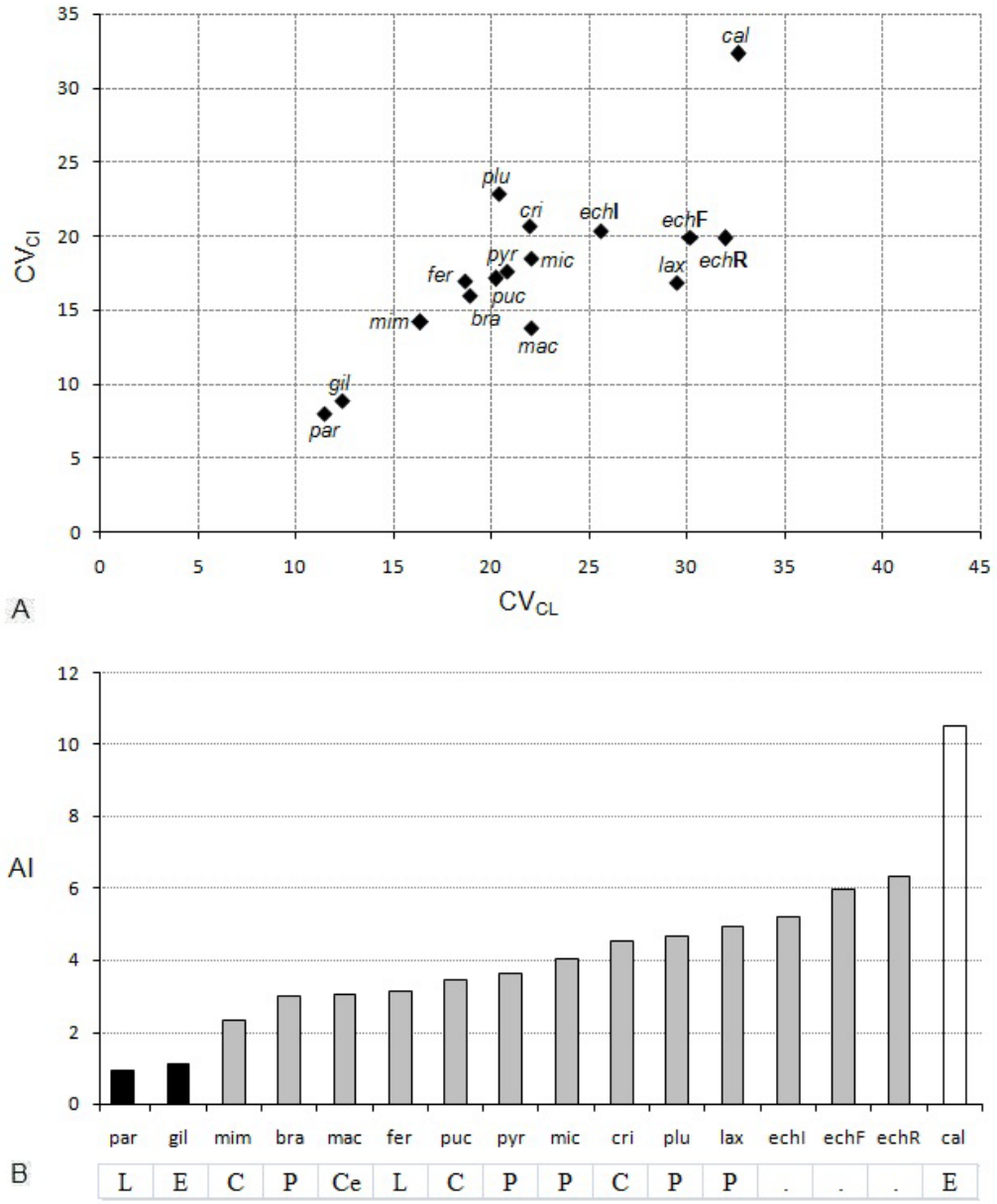

Figure 3. Scatter diagram of chromosome characteristics for *Caesalpinia* L. s.l. and *Cenostigma* species. Abbreviations of species names: bra = *C. bracteosa*; cal = *C. calycina*; echI = *C. echinata* from Ilhéus; echF = *C. echinata* from Feira de Santana; echR = *C. echinata* from Recife; fer = *C. ferrea*; gil = *C. gilliesii*; lax = *C. laxiflora*; mic = *C. microphylla*; min = *C. mimosifolia*; par = *C. paraguariensis*; pul = *C. pulcherrima*; plu = *C. pluviosa*; pyr = *C. pyramidalis*; mac = *Cenostigma macrophyllum*. **A.** Coefficient of variation (CV) of the chromosome length (CV_{CL}) against the centromeric index (CV_{CI}) parameters. **B.** *Caesalpinia* s.l. species asymmetry index as for Paszko (2006) where L = *Libidibia*; E = *Erythrostemon*; PE = *Poincianella-Erythrostemon* group; C = *Caesalpinia* s.s.; P = *Poincianella*; Ce = *Cenostigma*.

Table 6. Coding adopted for the different species in the present study, species names and their new combinations of genera in *Caesalpinia* s.l.

Code	Species of *Caesalpinia* s.l. and *Cenostigma*	New combinations of species names	Ref
par	*Caesalpinia paraguarienses* (D.Parodi) Burkat.	*Libidibia paraguariensis* (D. Parodi) G.P. Lewis	1
gil	*Caesalpinia gilliesii* (Hook.) Benth.	*Erythrostemon gilliesii* (Hook.) D. Dietr.	2
mim	*Caesalpinia mimosifolia* Griseb.	-	1
bra	*Caesalpinia bracteosa* Tul.	*Poincianella bracteosa* (Tul.) L.P. Queiroz	3
mac	*Cenostigma macrophylum* Tul.	-	1
fer	*Caesalpinia ferrea* var. *leiostachya* Benth.	*Libidibia ferrea* var. *leiostachya* (Benth.) L.P. Queiroz	4
puc	*Caesalpinia pulcherrima* Sw.	-	1
pyr	*Caesalpinia pyramidalis* Tul.	*Poincianella pyramidalis* var. *diversifolia* (Benth.) L.P. Queiroz	4
mic	*Caesalpinia microphylla* Mart.	*Poincianella microphylla* (Mart. ex G. Don) L.P. Queiroz	3
cri	*Caesalpinia crista* L.	-	1
plu	*Caesalpinia pluviosa* var. *phetophoroides* Bent.	*Poincianella pluviosa* var. *peltophoroides* (Benth.) L.P. Queiroz	4
lax	*Caesalpinia laxiflora* Tul.	*Poincianella laxiflora* (Tul.) L.P. Queiroz	3
*ech*I	*Caesalpinia echinata* Lam.	-	1
*ech*F	*Caesalpinia echinata* Lam.	-	1
*ech*R	*Caesalpinia echinata* Lam.	-	1
cal	*Caesalpinia calycina* Benth.	*Erythrostemon calycina* (Benth.) L.P. Queiroz	3

Ref = References: 1 = IPNI (2013); 2 = Manzanilla e Bruneau (2012); 3 = Queiroz (2009); 4 = Queiroz (2010).

ated in this study showed the diploid chromosome number $2n = 24$. The chromosome number of *C. laxiflora* and *C. macrophyllum* was cytogenetically described for the first time; the chromosome number of *C. pyramidalis*, *C. echinata*, and *C. bracteosa* was confirmed; and the chromosome morphometric data for these five species were described in this research for the first time. Therefore, the data obtained in this research confirmed 2n = 24 as being common to the *Caesalpinia* species. Our data was obtained from three different populations of *C. echinata* and only one population of each of the other species.

In spite of previous reports on tetraploid populations of *C. bracteosa* with 2n = 48 (Alves and Custódio, 1989), this variation was not found in this study, probably because the only population samples were made up of diploid individuals. Among Caesalpinioideae, the diploid chromosome number is variable, and species with 2n = 20, 24, 26, and 28 were already described (Jena et al., 2004; Souza and Benko-Iseppon, 2004; Biondo et al., 2005). However, in spite of the taxonomic diversity of *Caesalpinia*, the variations in regard to 2n = 24 only refer to euploids, suggesting a value of n = 12. The analysis of meiotic chromosomes should be performed in order to better characterize the peculiarities of the polyploidy that was found in some species. Among the 140 species of *Caesalpinia* L., the chromosome number is known for only 22 species (14.6%) (Atchison, 1951; Goldblatt, 1981; Alves and Custódio, 1989; Kumari and Bir, 1989; Beltrão and Guerra, 1990; Lewis, 1998; Jena et al., 2004; Cangiano and Bernardello, 2005; Rodrigues et al., 2012), making the need for more studies on this group clear in order to better distinguish this trait in the diverse genera of Caesalpinioideae.

The analysis of various populations of the same species can reveal cytological stability of the species and even the existence of cryptic species or varieties (Guerra, 1990). Although the analysis of the chromosome number continues to be the principal instrument of cytotaxonomy, the use of more informative techniques such as chromosome morphometry and *in situ* hybridization can show differences in the structures of the chromosomes among closely related species, revealing more details about the evolution and karyotypic diversity among the *Caesalpinia* species.

Complete morphometric data were obtained for five more species of *Caesalpinia* (Ta-

bles 1 and 2). Previously, this level of detail about the chromosomes of *Caesalpinia* referred to nine species, only 6.4% of the known species (Jena et al., 2004; Cangiano and Bernardello, 2005; Rodrigues et al., 2012). By combining the data from these studies and the present study, the morphometric detailing of the chromosomes of 14 species (10%) was made available.

The average length of the chromosomes in the species of *Caesalpinia* that were analyzed varied up to 53.8%, as in *C. macrophyllum* (2.03 µm) relative to *C. bracteosa* (3.12 µm). In the studies developed by Jena et al. (2004) and Cangiano and Bernardello (2005), the chromosome size varied from 1.90 µm in *C. gilliessi*, *C. paraguarienses*, and *Caesalpinia mimosifolia* Griseb. to 2.07 µm in *C. crista*, a variation of 9.1%. However, in the studies carried out by Rodrigues et al. (2012), this value was greater for the majority of the species that were studied, and chromosome sizes included 2.05 µm in *C. microphylla* Mart., 2.57 µm in *Caesalpinia pluviosa* var. *peltophoroides* Bent., 2.58 µm in *Caesalpinia pulcherrima* Sw., 2.87 µm in *Caesalpinia ferrea* var. *leiostachya* Benth., and 3.32 µm in *C. calycina*, indicating variation up to 62%. The average chromosome size that was found in studies carried out among the Caesalpinioideae (Auler and Battistin, 1999; Biondo et al., 2005) varies between 1.80 and 2.50 µm, giving 33.3% variation. Assuming greater variation among genera, our data indicate the need for a more significant sampling of species in this subfamily.

The HLL varied from 37.47 µm in *C. bracteosa* to 24.36 µm in *C. macrophyllum.* These values corroborate the results of Rodrigues (2012), who observed a variation of 39.86 µm in *C. calycina* to 24.63 µm in *C. microphylla*. The values found by Cangiano and Bernardello (2005) for the HLL varied from 20.67 to 24.74 µm; these values were slightly lower than our values. However, one should be cautious when making comparisons of HLL between samples that were analyzed by different laboratories that use different technical procedures, such as the type of antimitotic used and pretreatment time to which the roots are submitted before the preparation of the slides.

Among the species of *Caesalpinia* that were analyzed karyomorphologically, five species had the formula that was defined in this study, three were defined by Cangiano and Bernadello (2005), and the other five were defined by Rodrigues et al. (2012). The four species that showed a karyotype formula that was composed exclusively of metacentric chromosomes (12 m) showed the lowest values for the HLL, which varied from 24.36 µm in *C. macrophyllum* to 30.26 µm in *C. laxiflora*. Of the species with a formula that was not 12 m, only *C. echinata* had low values of HLL (approximately 29 µm). These species are grouped in *Poincianella* based on the anatomy of wood (Gasson et al., 2009). *C. pluviosa* was shown to be distinct among this new genus because its formula had a more derived karyotype characteristic compared to the other species in *Poincianella*. In fact, a more exhaustive analysis of the chromosome morphometry of *C. pluviosa* is suggested because it is a species with diverse varieties (Lewis, 1998).

The species with high rates of TF% asymmetry had all metacentric chromosomes except *C. echinata* from Ilhéus and Feira de Santana, which had two submetacentric chromosomes. Consequently, the species with lower rates of TF% asymmetry were those that showed a greater number of submetacentric chromosomes than metacentric chromosomes, with the exception of *C. bracteosa*, which had 11 m + 1 sm and a TF% of 37.47. These same standards were observed by Rodrigues et al. (2012), who also observed the presence of a subtelocentric chromosome in *C. calycina*, which had a karyotype formula of 8 m + 3 sm + 1st and a low rate of asymmetry. This shows that the species of *Caesalpinia*, which showed karyotypes with a low quantity of metacentric chromosomes or with a greater quantity of morphologically different chromosomes, are the species with more asymmetrical karyotypes.

The karyotypes of *Caesalpinia* are relatively symmetrical with a predominance of metacentric and submetacentric chromosomes (Kumari and Bir, 1989; Cangiano and Bernardello, 2005; Rodrigues et al., 2012). Nonetheless, morphological dissimilarities were observed between the karyotypes. This was verified in *C. laxiflora*, *C. pyramidalis*, and *C. macrophyllum*, which showed a karyotypic formula of 12 m as previously observed for *C. microphylla* (12 m) (Rodrigues et al., 2012). In turn, *C. bracteosa* showed a formula of 11 m + 1 sm, whereas *C. echinata* from the cities of Ilhéus and Feira de Santana presented a formula of 10 m + 2 sm and *C. echinata* from Recife showed a formula of 9 m + 3 sm. The species analyzed in previous studies showed a higher number of submetacentric chromosomes than in this study, reaching 4 m + 8 sm in *C. pluviosa* var. *peltophoroides*. Karyotype asymmetry may be associated with speciation, whereas the symmetrical parameters are the most primitive types (Stebbins, 1971). This can be confirmed here because *C. pyramidalis*, *C. laxiflora*, and *C. macrophyllum* had symmetrical karyotypes (12 m) and showed the highest AIs (TF% = 43.04, 42.52, and 40.20%, respectively). These species were therefore found to be more ancient than *C. echinata* from Ilhéus (10 m + 2 sm) with a TF% of 40.42%, *C. echinata* from Feira de Santana (10 m + 2 sm) with a TF% of 39.90%, *C. echinata* from Recife (9 m + 3 sm) with a TF% of 39.70%, and *C. bracteosa* (11 m + 1 sm) with a TF% of 37.47%. When comparing the TF% from the three sites that were evaluated for *C. echinata*, there were almost no differences, indicating some stability.

Of the five species whose morphometric data were obtained in this study, the ones with the lowest values of A_1 were seen to tend to have metacentric chromosomes (Tables 1 and 2). The karyotype formula of *C. laxiflora*, *C. pyramidalis*, and *C. macrophyllum* was 12 m, and their respective A_1 values were 0.23, 0.25, and 0.27 respectively. On the other hand, *C. pluviosa* was the species with the highest A_1 (0.40); yet, this species had the lowest number of metacentric chromosomes (4 m + 8 sm) among *Caesalpinia* species (Rodrigues et al., 2012).

Differences relative to the presence and location of satellites were only detectable in two of the five species analyzed. Besides being useful as genetic markers, variations in the number, position, and size of secondary constrictions and satellites are frequently observed in plants, and they can be incorporated or deleted during the evolutionary process. Minisatellites were found on the short arm of metacentric chromosomes in only two of the five species studied; these species only had one pair of chromosomes in satellite formation that was basically the same size: 0.43 µm in *C. laxiflora* and 0.46 µm in *C. macrophyllum*. Yet, the satellites are in different chromosome pairs: on the eleventh chromosome pair of *C. laxiflora* and on the seventh chromosome pair of *C. macrophyllum*. Chromosomes with a satellite form are not common in Caesalpinioideae (Kumari and Bir, 1989; Souza and Benko-Iseppon, 2004). In this study, two species had satellites, and all nine of the species previously characterized had satellites in one or more chromosome pairs (Jena et al., 2004; Cangiano and Bernardello, 2005; Rodrigues et al., 2012).

Despite the chromosome number being the same in all the species examined (2n = 24) and some *Caesalpinia* species having the same karyotype formula, combining different karyomorphological data allows species to be differentiated. The use of molecular cytogenetic techniques revealed a need to complement these classic analyses of chromosome morphology. Especially in specimens that have large intraspecific variations, such as *C. pluviosa* and *C. echinata*, more comprehensive studies should include the sampling of different populations.

Karyotypic heterogeneity in *Caesalpinia* s.l. and *Cenostigma*

In taxonomic terms, *Caesalpinia* is considered very variable. Therefore, different species in this group are being combined into new genera (Lewis, 1998; Gasson et al., 2009; Queiroz, 2009, 2010; IPNI, 2013) (Table 6). In this study, the amplitude of CV_{CL} and CV_{CI} values tended to be in the same range in some of the new genera. Species from the group *Poincianella* (*C. bracteosa*, *C. pyramidalis*, *C. microphylla*, *C. pluviosa*, *C. echinata*, and *C. laxiflora*) have intermediate symmetry values. *Libidibia* species, on the other hand, had karyotypes with low and intermediate AI values (*C. paraguariensis* and *C. ferrea*). Only two extreme karyotypes in terms of asymmetry refer to species assigned to the same genus (*C. gilliessi* and *C. calycina* in *Erythrostemon*). Hence, this characteristic is generally consistent with the newly proposed combinations of genera in *Caesalpinia*. In this research, however, the analysis only involves 14 of the 140 *Caesalpinia* species, revealing the need to further analyze these species to better delineate the karyomorphological types as a function of the newly instated genera.

The karyomorphological data generated in this study, along with those available in the literature, have allowed the parameters of karyotype asymmetry to be determined in 14 *Caesalpinia* species (Figure 3). CV_{CL} was the variation component that most influenced the karyotype asymmetry in *C. echinata*, *C. laxiflora*, and *C. macrophyllum*, which can be confirmed in the higher separation of these three species in relation to the scatter plot diagonal line of CV_{CL} x CV_{CI}. These two parameters influenced AI values from other species in a near-equal manner. In both cases, these two parameters clarified the heterogeneity of karyotypes from all of the species analyzed. The scatter plot based on the variation sources comprising the AI is suitable to compare karyotypes of species from different taxa, as demonstrated in the analysis of different plant families (Paszko, 2006; Peruzzi et al., 2009; Pierozzi et al., 2012).

By analyzing the scatter plot of CV_{CI} x CV_{CL}, *C. calycina* was found to have the greatest intrachromosomal variation regarding size and position, showing that this was the karyotype with the highest derivation in the chromosome morphology among the 14 species studied (Figure 3A). Likewise, *C. pluviosa* had a relatively high variation relative to the position of the centromere, as well as an intermediate level of variation in the chromosome size. Therefore, these two species have the highest karyotype asymmetry. Among the three species that were analyzed by Cangiano and Bernardello (2005), *C. paraguariensis* and *C. gilliessi* were the ones with the lowest variations in the centromere position and chromosome size, characteristics considered to be plesiomorphic. The largest variations in chromosome size were observed in *C. echinata* and *C. laxiflora*.

An overview of the species analyzed can be made based on the AI values; this parameter associates the contributions of the two variation sources with the variation in karyotype heterogeneity (CV_{CI} and CV_{CL}). In this study, the AI values revealed three groups (Figure 3B). Group 1 is formed by the species that showed the most asymmetrical karyotype (*C. calycina*). This characteristic is considered to be evolutionarily derived. Group 2, in turn, is formed by species with more symmetrical karyotypes (*C. paraguariensis* and *C. gilliesii*), a characteristic that is considered to be evolutionary basal. The other species examined are found in the intermediate group and showed values with minor differences between each other and great differences from the other two groups.

The two species of *Libidibia* had intermediate and small AI values (*C. ferrea* and

C. paraguariensis, respectively), and these values are close (Figure 3B). The two species of *Erythrostemon* (*C. calycina* and *C. gilliesii*) were divided into different groups according to their contrasting AI values. These AI values indicate an opposite evolutionary trend between their karyotypes. However, *C. calycina* and *C. gilliesii* were reassigned to the same genus, *Erythrostemon*, according to their wood morphology (Gasson et al., 2009) and other traits. Only a pair of species was examined cytogenetically from those genera. Each pair of species consisted of species from geographically distant locations.

In this study, 14 species were differentiated from each other based on AI (Figure 3B). Of these, five species were analyzed phylogenetically using the intron *trn*L sequences. Despite it is a relatively well-conserved sequence, *C. gilliesii*, *C. calycina*, *C. ferrea*, *C. echinata*, and *C. pluviosa* were found on well-defined branches (bootstrap >50%; 10,000 repetitions; Juchum et al., 2008). *C. ferrea*, *C. gilliessi* and *C. macrophylum* are among the six species with lower values of AI, among the 14 species (Figure 3). Those two species of *Caesalpinia* s.l are in the same branch of *Cenostigma gardneriana* in the tree based on molecular data (Manzanilla and Bruneau, 2012). Hence, as proposed by Paszko (2006), it is possible to show the presence of evolutionary differences or congruencies in the morphology of chromosomes and the karyotype asymmetry of *Caesalpinia* species because AI data were consistent with molecular phylogeny data for these species.

The taxonomic history of *Caesalpinia* clade revels that is composed of several genera to which *Caesalpinia* species have been taxonomically assigned (Lewis, 1998). Morphological data on wood anatomy (Gasson et al., 2009) and molecular analysis (Manzanilla and Bruneau, 2012) confirmed the above-mentioned points. Despite the identical chromosome number (2n = 24) for these species, morphometric chromosome changes based on classical cytogenetics revealed differences between the species and represent an important basis for future studies of molecular cytogenetics in this group.

Most Brazilian *Caesalpinia* species analyzed in this study showed intermediate asymmetry values and have been proposed for the genus *Poincianella* (Lewis, 1998; Gasson et al., 2009; Queiroz, 2009; 2010). Despite the limited number of *Caesalpinia* species whose karyomorphological data were known, chromosomal features can potentially be used as to support the allocation of *Caesalpinia* in the different genera, at least in the case of *Poincianella*.

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