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Research Article

Chromosome characterization and variability in some Iridaceae from Northeastern Brazil

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Abstract

The chromosomes of 15 species of Iridaceae of the genera *Alophia*, *Cipura*, *Eleutherine*, *Neomarica* and *Trimezia* (subfamily Iridoideae) were examined after conventional Giemsa staining**.** The karyotypes of *Alophia drummondii* (2*n* = 14+1B, 28, 42 and 56), *Cipura paludosa* (2*n* = 14), *C. xanthomelas* (2*n* = 28) and *Eleutherine bulbosa* (2*n* = 12) were asymmetric; *Neomarica candida*, *N. caerulea*, *N. humilis*, *N. glauca*, *N. gracilis*, *N. northiana* and *Neomarica* sp. (2*n* = 18); *N.* cf. *paradoxa* (2*n* = 28), *Trimezia fosteriana* (2*n* = 52), *T. martinicensis* (2*n* = 54) and *T. connata* (2*n* = 82) were all generally symmetric. New diploid numbers of 2*n* = 56 for *Alophia drummondii*, 2*n* = 18 for *N. candida*, *N. humilis*, *N. glauca*, and *N. gracilis*, 2*n* = 28 for *N.* cf. *paradoxa*, and 2*n* = 82 for *T. connata* are reported. The karyotypic evolution of the studied species is discussed.

Key words: Iridaceae, disploidy, karyotypic evolution, polyploidy, asymmetrical karyotype.

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Introduction

The monocot family Iridaceae comprises approximately 2050 species distributed among 67 genera, with a major center of radiation in the southern African Sahara (Goldblatt *et al.*, 2008). The Neotropics are considered the second most important center of diversity for this family, with 250 known species and 30 genera; from which 18 genera and 160 species occur in Brazil (Eggers *et al.*, 2010; Judd *et al.*, 2008). Iridaceae is divided into seven subfamilies (Goldblatt *et al.*, 2008); the subfamily Iridoideae, the subject of the present work, is composed of four tribes and includes many exotic species that are cultivated as ornamental plants or to harvest saffron, especially those from the genera *Gladiolus*, *Neomarica*, *Crocus* and *Trimezia* (Lorenzi and Souza, 2001).

This family is relatively well studied in terms of its cytology, especially the Old World taxa, with diploid numbers reported for 1330 species (almost 65% of the total in the family). Proportionally fewer New World species have been studied (Goldblatt and Takei, 1997; Goldblatt, 1982).

Karyological data are available for only nine species from northeastern Brazil. This shortage of data represents an important gap in our knowledge of chromosome variation among the Brazilian representatives of this family and in our understanding of the mechanisms of karyotype evolution in this plant group.

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In the present study we investigated the mechanisms of chromosome evolution in different groups of Iridaceae from northeastern Brazil by examining the chromosome morphologies of 15 species from five genera of the tribes Trimezieae and Tigrideae (Iridoideae). Karyograms were prepared for 10 species and their karyotypic asymmetry indices were calculated.

Materials and Methods

Chromosome numbers, previously published and new reports of diploid numbers, plant origins and voucher numbers of all of the populations and taxa examined are presented in Table 1. The plants were grown in plastic containers in a 1:1 mixture of washed sand and agricultural soil. The species were collected in the field in six states in northeastern Brazil, except for *Neomarica glauca* (collected in Minas Gerais State, southeastern Brazil) and one population of *Trimezia connata* (collected in Pará State, northern Brazil). All the specimens collected were deposited in the herbarium Jayme Coelho de Morais of UFPB (EAN) (Table 1).

Chromosome analyses were performed using root tips pretreated with 0.002 M 8-hydroxyquinoline at 4 °C for 24 h. The material was then mixed in absolute ethanol/glacial acetic acid (3:1 v/v) for 3-24 h at room temperature (25 °C) and stored at -20 °C. To prepare slides for analysis, root tips were hydrolyzed in 5 N HCl at room temperature, frozen in liquid nitrogen to remove the coverslip, stained with Giemsa 2% for 15 min, and mounted in Entellan (Fukui, 1996). Chromosome measurements were made in

Table 1 - Diploid numbers of some Iridaceae species from the Brazilian Northeast. Diploid numbers indicated in bold were reported herein.

Taxon	Locality[1]	Voucher number[2]	2*n*	Figures	Previously reported 2*n*	Source[3]
Tribe Tigrideae Baker						
***Alophia* Herb.**						
A. drummondii (Graham) R.C. Foster	Esperança, PB	LPFelix, 11186	28, 56	1a, b	28	
	Pariconha, AL	LPFelix, 12169	42	1c, 3b	-	GT97
	Itapiúna, CE	LPFelix, 10004	14+1B	1d, 3a	-	-
***Cipura* Aubl.**						
C. paludosa Aubl.	Areia, PB	LPFelix, 10739				KH84; G82;
	Itapororoca, PB	LPFelix, 10732	14	1e, 3c	14	BG90; GT97
	Campo Maior, PI	LPFelix, 11032				
C. xanthomelas Maxim. ex Klatt	Castelo, PI	LPFelix, 11059				
			28	1f, 3d	28	GH87
	Campo Maior, PI	LPFelix, 11025				
***Eleutherine* Herb.**						
E. bulbosa (Mill) Urb.	Areia, PB	LPFelix, 12000				G82; KH84;
			12	1g, 3e	12, 14	G91; BG90;
	Taquaritinga do Norte, PE	LPFelix, 10654				GT97
Tribe Trimezieae						
***Neomarica* Sprague**						
N. candida (Hassl.) Sprague	Ilha Comprida, SP	LPFelix, 12802				
			18	2a, 4a	-	-
	Instituto Agronômico de Campinas, SP	IAC, 82221				
N. caerulea (Ker Gawl.) Sprague	Areia, PB	LPFelix, 12002	18	2b	24, 32	G82; KH84; GT97
N. glauca (Seub. ex Klatt) Sprague	Salto da Divisa, MG	NAPorto, S/N	18	2c	-	-
N. gracilis (Herb.) Sprague	Serra de Itabaina, SE	LPFelix, 12743	18	2d, 4b	-	-
N. humilis (Klatt) Capell.	Guaramiranga, CE	LPFelix, 10740				
			18	2e, 4c	-	-
	Maranguape, CE	LPFelix, 10055				
N. northiana (Schneev.) Sprague	Ibateguara, AL	LPFelix, 12003				
	Campina Grande, PB	LIFAlves, 02	18	2f, 4d	-	-
	Recife, PE	LPFelix, S/N				
	Belém, PA	LPFelix, 12671				
N. cf. *paradoxa* (Ravenna) Chukr	Morro do Chapéu, BA	LPFelix,11691	28	2g, 4f	-	-
Neomarica sp.	Caruaru, PE	LPFelix, S/N	18	2h, 4e	-	-
***Trimezia* Salisb. ex Herb.**						
T. connata Ravenna	Areia, PB	LPFelix, 11966				
			82	2i	-	-
	Belém, PA	LPFelix, 12762				
T. fosteriana Steyerm.	Recife, PE	LPFelix, 10805				
			52	2j	26	G82; GT97
	João Pessoa, PB	LIFAlves, 01				
T. martinicensis (Jacq.) Herb.	Brejo da Madre de Deus, PE	LPFelix, 11185	54	2l	40, 54, 80	G82; KH84; GT97

[1]The Brazilian states are abbreviated as: PB = Paraíba, CE = Ceará, MG = Minas Gerais, AL = Alagoas, PE = Pernambuco, BA = Bahia, PI = Piauí. [2]Voucher deposited in herbarium Jayme Coelho de Morais (EAN). [3]Sources abbreviations: GT97 = Goldblatt and Takei (1997); KH84 = Kenton and Heywood (1984); G82 = Goldblatt (1982); GB90 = Beltrão and Guerra (1990); GH87 = Goldblatt and Henrich (1987); G91 = Guerra (1991).

the best spreads. The terminologies for interphase nuclei and prophase condensation patterns were adapted from Benko-Iseppon and Morawetz (2000) and Yokota (1990), respectively. The material was photographed using an Olympus BX41 microscope equipped with an Olympus D-54 digital camera.

At least five metaphases were analyzed per population to determine the chromosome numbers and the three best cells were used for measuring chromosome lengths and for making the karyograms. The karyograms were organized in order of chromosome size. All measurements were performed using the Image Tool software program. The lengths of the short (s) and long arms (*l*) as well as the centromeric indices (CI = 100s/s + *l*) were determined, and the data were organized in decreasing size order. The chromosome nomenclature used was M for metacentrics (CI = 50.0-40.1), SM for submetacentrics (CI = 40.0-25.1), A for acrocentrics (CI = 25.1-0.01) and T for telocentrics (CI = 0.00). The following karyological parameters were determined: total chromosome length (TCL), mean chromosome length (CL), and the ratio between the largest and the smallest chromosomes (L/s). Karyotypic asymmetry was evaluated using the interchromosomal asymmetry index (A_2) proposed by Romero Zarco (1986) and the classification followed Stebbins (1971). The intrachromosomal asymmetry index (A_1) of Romero Zarco (1986) was not used because it was incompatible with the karyotypes studied.

Results

All species had semi-reticulated interphase nuclei (Figures 1b, c and 2c) with few interspecific differences in chromatin density or chromocenter sizes. The chromosome numbers varied from $2n = 12$ in *Eleutherine bulbosa* to $2n = 82$ in *Trimezia connata*, and the mean chromosome sizes varied from 1.03 (*Neomarica* cf. *paradoxa* and *T. martinicensis*) to 5.89 μm (*Neomarica northiana*), with a predominance of meta- and submetacentric chromosomes. The genus *Neomarica* had the highest number of chromosomes with satellites, varying from two to six among the different species. In relation to the interchromosomal

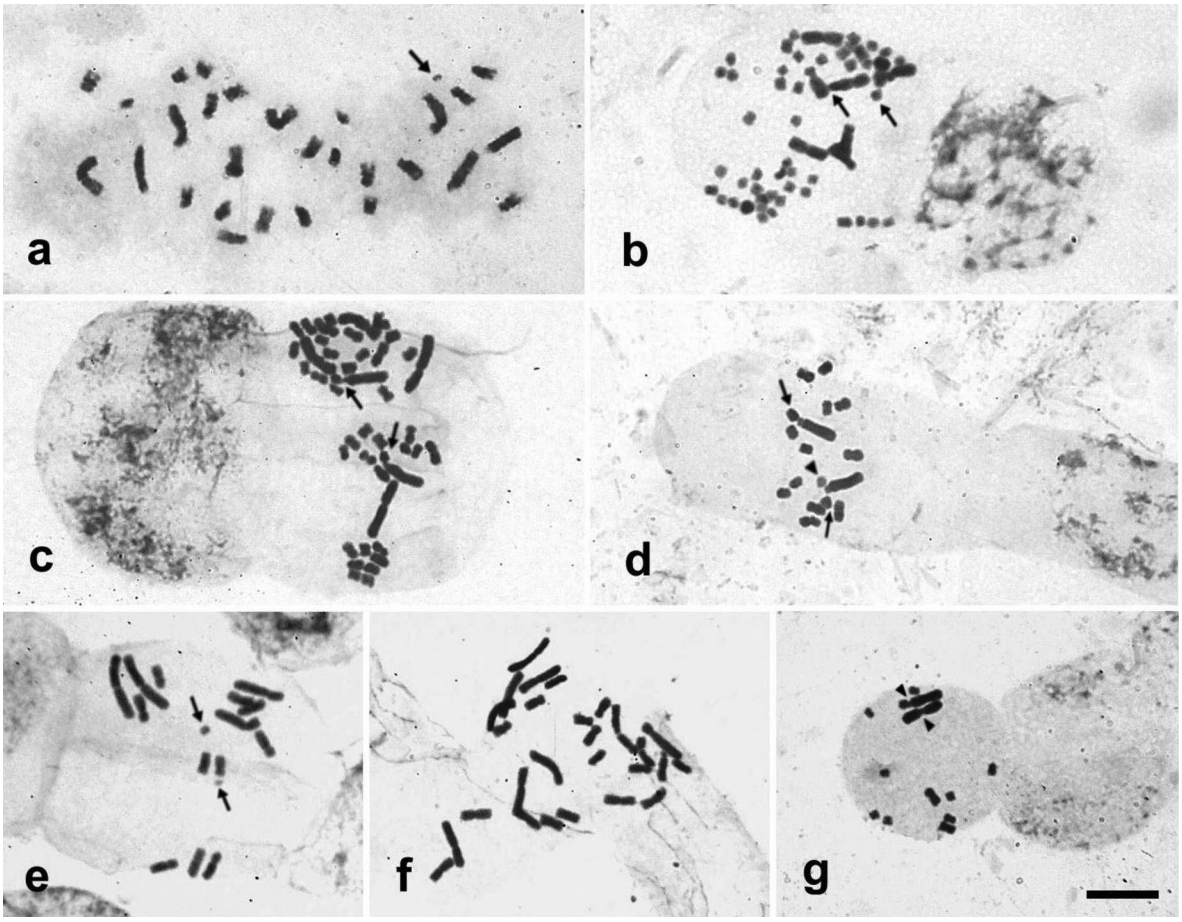

Figure 1 - Mitotic metaphase and interphase nuclei of species of the Tribe Tigrideae from northeastern Brazil**:** (a-d) *Alophia drummondii* with (a) $2n = 28$ and one visible satellite; (b) $2n = 56$ and two satellites and (c) $2n = 42$ and one satellited pair; (d) $2n = 14+1B$ with a pair of distended satellites and a heteropicnotic B chromosome (arrowhead); (e) *Cipura paludosa* with $2n = 14$ and a pair of distended satellites; (f) *Cipura xanthomelas* with $2n = 28$; (g) *Eleutherine bulbosa* ($2n = 12$) with a distended secondary constriction (arrowhead). Arrows indicate satellites in (a), (b), (c), (d) and (e). The bar corresponds to 10 μm.

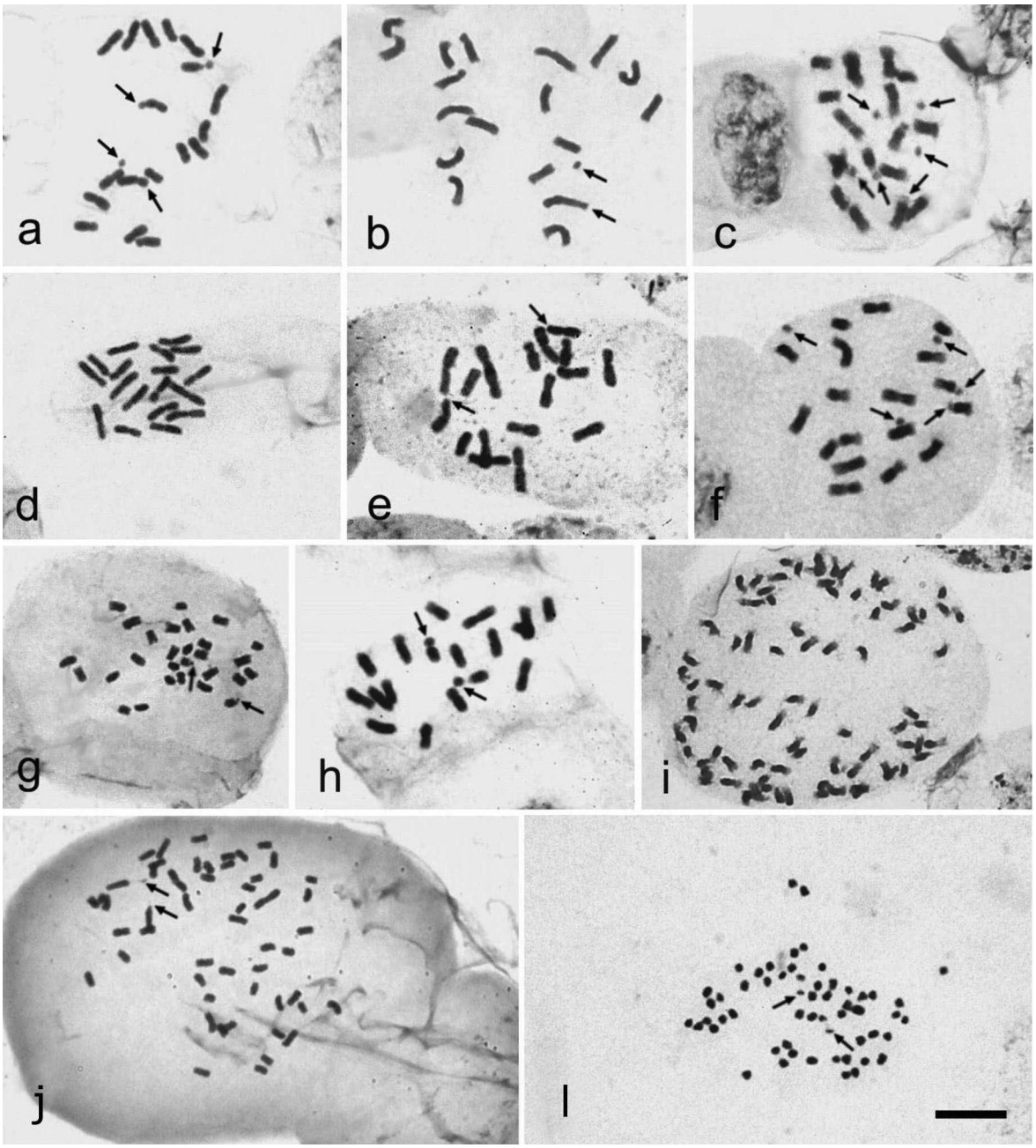

Figure 2 - Mitotic metaphase and interphase nuclei of species of the Tribe Trimezieae from northeastern Brazil: (a) *Neomarica candida* with 2*n* = 18 and four satellites; (b) *N. caerulea* with 2*n* = 18 and two satellites; (c) *N. glauca* with 2*n* = 18 and six satellites; (d) *N. gracilis* (2*n* = 18); (e) *N. humilis* (2*n* = 18) with one satellited pair; (f) *N. northiana* with 2*n* = 18 and five satellites; (g) *N.* cf. *paradoxa* with 2*n* = 28 and two satellites; (h) *Neomarica* sp. with 2*n* = 18 and two satellites; (i) *Trimezia connata* with 2*n* = 82; (j) *T. fosteriana* with 2*n* = 52 and a pair of terminal satellites; (l) *T. martinicensis* with 2*n* = 54 and a distended pair of satellites. Arrows indicate satellites. The bar corresponds to 10 μm.

asymmetry index (A_2), *Alophia drummondii*, *Cipura paludosa*, *C. xanthomelas* and *Eleutherine bulbosa* had asymmetric karyotypes, while *Neomarica candida*, *N. caerulea*, *N. humilis*, *N. glauca*, *N. gracilis*, *N. northiana*, *Neomarica* sp., *N.* cf. *paradoxa*, *Trimezia connata*, *T. fosteriana*, and *T. martinicensis* had symmetric karyotypes.

The diploid numbers of all of the species examined are summarized in Table 1. The karyotypic formula and other morphometric parameters of the 15 species examined are presented in Table 2. Figures 1 and 2 show the mitotic metaphases of the species analyzed, and the karyograms of ten species are illustrated in Figures 3 and 4. Analyses of the main karyotypic features of the species studied are detailed below:

Tribe Tigrideae

All the species examined from this tribe had bimodal and asymmetric karyotypes, with one or more chromosome

Table 2 - List of analyzed species and their karyotypic formula, total chromosome length (TCL), mean chromosome length (CL), mean centromere index (CI), interchromosomal asymmetry index (A2), proportion between the largest and smallest chromosomes (L/s), Stebbins asymmetry category (Steb.).

Taxon	Karyotypic formula	TCL (μm)	CL (μm)	CI (%)	A_2	L/s	Steb.
Tribe Tigrideae							
Alophia							
Alophia drummondii	38M+2SM+2A	98.76 ± 1.66	2.35	42.30	0.70	6.67	3c
	(10M+4SM)+1B	20.28 ± 0.91	1.45	43.10	0.63	2.84	3c
Cipura							
Cipura paludosa	4M+2SM+8A	67,81 ± 2,41	4.84	39.01	0.50	4.28	3c
C. xanthomelas	10M+8SM+10A	117.68 ± 2.32	4.10	38.70	0.55	2.28	3c
Eleutherine							
E. bulbosa	11M+1A	31.34 ± 2.02	2.62	41.92	0.77	5.00	3c
Tribe Trimezieae							
Neomarica							
N. candida	4M+8SM+6A	72.29 ± 0.68	4.02	36.93	0.16	1.86	3a
N. caerulea	10M+6SM+2A	80.34 ± 0.71	4.46	38.98	0.16	1.78	3a
N. glauca	8M+6SM+4A	66.11 ± 0.58	3.67	-	0.13	1.43	-
N. gracilis	6M+8SM+4A	83.44 ± 0.53	4.64	41.12	0.11	1.41	
N. humilis	14M+2SM+2A	98.71 ± 0.97	5.48	40.30	0.18	1.82	3a
N. northiana	16M+2SM	106.13 ± 0.87	5.89	44.29	0.15	1.61	3a
N. cf. *paradoxa*	20M+8SM	28.94 ± 0.13	1.03	-	0.12	1.78	-
Neomarica sp.	8M+6SM+4A	86.25 ± 0.58	4.79	41.98	0.18	1.54	3a
Trimezia						2.06	
T. connata	-	182.86 ± 0.65	2.23	-	0.29	2.83	-
T. fosteriana	-	104.61 ± 0.52	2.02	-	0.25	2.55	-
T. martinicensis	-	55.66 ± 0.17	1.03	-	0.16	1.83	-

pairs being larger than the rest. *Alophia drummondii* had $2n = 14+1B$, 28, 42 and 56, with one-two satellites located on the short arm of one of the larger metacentric pairs (Figures 1a, b, c, d). One population of *A. drummondii* from Esperança, Paraíba State, had individuals with $2n = 28$ and 56 (Figure 1a, b) with four large chromosome pairs. We were not able to obtain good chromosome condensation, which made centromere visualization difficult, particularly for the smaller chromosomes. The individuals with $2n = 56$ (Figure 1b) showed four large chromosome pairs, two acrocentric and two metacentric, as well as other smaller pairs, varying from meta- to submetacentric, with two satellites located on the larger metacentric pairs. The population of *Alophia drummondii* from Pariconha, Alagoas State, had $2n = 42$ (Figures 1c, 3b), with three large chromosome pairs and additional smaller pairs, the karyotypic formula was 38M+2SM+2A, the chromosomes measured 1.29-6.51 μm, and the interchromosomal asymmetry was $A_2 = 0.70$ (Table 2, Figure 3b). The individuals from this population were larger and had larger fruits when compared to the ones with $2n = 28$ and 56, but showed no noticeable differences in their floral morphologies.

One population of *Alophia drummondii* from Itapiúna, Ceará State, had a strongly asymmetric karyotype, with $2n = 14+1B$ (Figures 1d, 3a), chromosomes measuring 1.97-5.61 μm (karyotypic formula 10M+4SM+1B), and interchromosomal asymmetry $A_2 = 0.63$. It was included in the category 3c from Stebbins. This population had lighter colored flowers, but the floral morphologies did not noticeably differ from the other population of the species. Two distended satellites with proximal secondary constrictions were observed on the large acrocentric pair (Figure 3a). The supernumerary chromosome was smaller and heteropycnotic when compared to the other chromosomes in the complement and it was not observed in the other *A. drummondii* cytotypes.

Cipura paludosa has $2n = 14$, chromosomes measuring 2.21 to 9.48 μm (Figures 1e, 3c), and the karyotypic formula 4M+2SM+8A, with two satellites located on the smaller submetacentric pair. Heteromorphisms in submetacentric pairs I and II due to differences in the sizes of the long arms was observed in all populations (Figure 3c). This species showed interchromosomal asymmetry $A_2 = 0.50$ and was thus classified into category 3c from Stebbins (Table 2). In contrast, *C. xanthomelas* was tetraploid with

$2n = 28$ (Figures 1f, 3d) and had 2.64 to 6.02 μm long meta- and submetacentric chromosomes, with a karyotypic formula 10M+8SM+10A, interchromosomal asymmetry $A_2 = 0.55$ and no visible satellites (Table 2, Figure 3d). *Eleutherine bulbosa* had $2n = 12$ (Figures 1g, 3e) and a karyotype composed of 11M+1A. The largest chromosome pair was heteromorphic, with one metacentric and one acrocentric chromosome. This species had a strongly asymmetric karyotype, chromosomes measuring 1.36 to 6.80 μm, an interchromosomal asymmetry index $A_2 = 0.77$ and was included in category 3c from Stebbins (Table 2, Figure 3e).

Tribe Trimezieae

Neomarica candida, *N. caerulea*, *N. humilis*, *N. glauca*, *N. gracilis*, *N. northiana*, and *Neomarica* sp. had $2n = 18$, whereas *N.* cf. *paradoxa* had $2n = 28$ (Table 1). Although *N.* cf. *paradoxa* differed in chromosome numbers and sizes, the other species of *Neomarica* had small differences in their karyotypic formula and chromosome sizes, but always had a predominance of meta- and submetacentric chromosomes (Table 2). The mean chromosome lengths varied from 1.03 μm in *Neomarica* cf. *paradoxa* to 5.89 μm in *N. northiana* with two, four, or six satellites. *N. candida* had the karyotypic formula 4M+8SM+6A, chromosomes measuring 3.66-5.69 μm, and four to six satellites located on meta- and submetacentric chromosomes (Figures 2a and 4a). *N. caerulea* had 10M+6SM+2A, chromosomes 3.35 to 5.64 μm long and two satellites located on the short arms of metacentric chromosomes (Figure 2b). *N. glauca* (8M+6SM+4A) had chromosomes measuring 2.51 to 4.58 μm and two to four satellited pairs (Figure 2c); *N. gracilis* (6M+8SM+4A) had chromosomes measuring 3.87 to 4.76 μm and no satellites were observed (Figures 3d and 4b); and in *N. humilis* (14M+2SM+2A) chromosome sizes varied from 3.72 to 6.76 μm, with one satellited pair (Figures 2e and 4c). *N. northiana* had 16M+2SM, chromosomes measuring 4.57 to 7.33 μm, and two to six satellites were observed on the short arms of the meta- and acrocentric chromosomes (Figures 2f and 4d). In *Neomarica* sp., chromosome sizes varied from 5.81 to 6.45 μm, with one pair of satellites and the karyotypic formula was 8M+6SM+4A (Figures 2h and 4e). *N.* cf. *paradoxa* had the karyotypic formula 20M+8SM, chromosomes measuring 0.98 to 2.77 μm, with one or two satellites. This species was different from the others in diploid number and in the smaller size of its chromosomes (Figures 2g and 4f). Interchromosomal asymmetry (A_2) varied from 0.11 in *N. gracilis* to 0.18 μm in *N. northiana* and *Neomarica* sp., all included in the 3a asymmetry category from Stebbins (1971), which corresponds to relatively symmetric karyotypes (Table 2).

The *Trimezia* species studied herein had symmetric karyotypes composed of small chromosomes, with mean

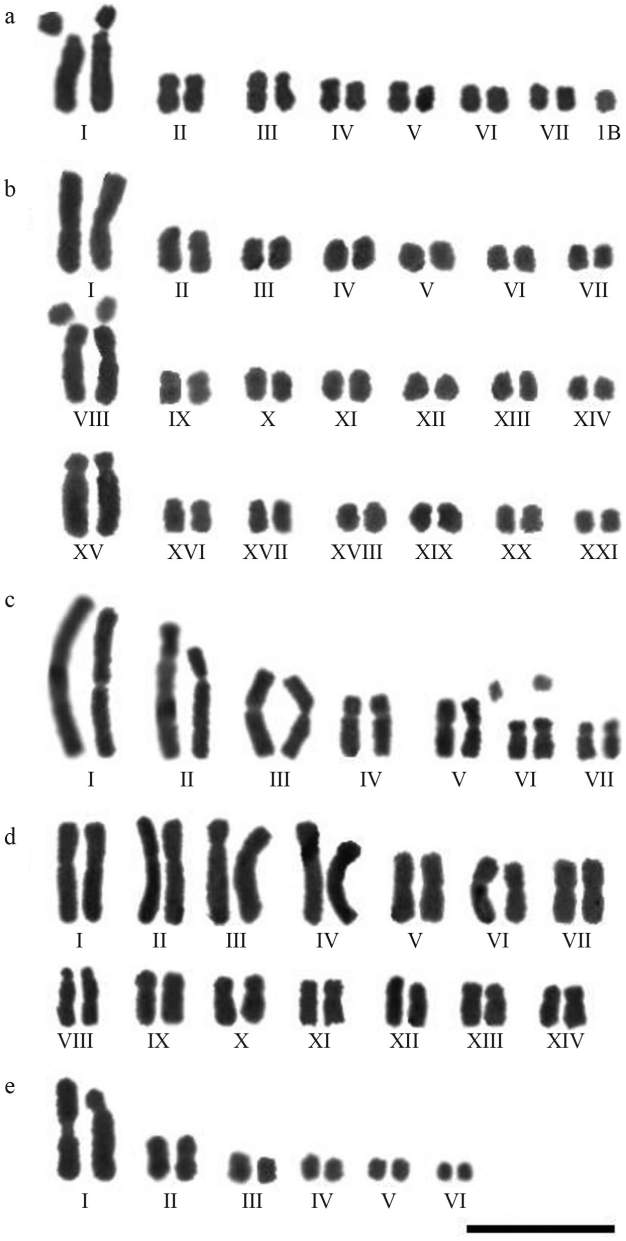

Figure 3 - Karyograms of some species of the Tribe Tigrideae from northeastern Brazil. (a-b) *Alophia drummondii*: (a) $2n = 14+1B$ and (b) $2n = 42$; (c) *Cipura paludosa* ($2n = 14$); (d) *C. xanthomelas* ($2n = 28$); (e) *Eleutherine bulbosa* ($2n = 12$). The bar corresponds to 10 μm.

chromosome lengths varying from 1.03 μm (*T. martinicensis*) to 2.23 μm (*T. connata*), with distended or terminal satellited pairs. Populations of *Trimezia connata* from Areia (Paraíba State) and Belém (Pará State) had $2n = 82$ (Table 1, Figure 2i), small chromosomes without visible secondary constrictions and no satellites were observed. *Trimezia fosteriana* had $2n = 52$, with a pair of satellites and terminal secondary constrictions in the plants from Recife (Pernambuco State) and João Pessoa (Paraíba State) (Table 1, Figure 2j). *Trimezia martinicensis* had $2n = 54$ in all individuals from a single wild population at

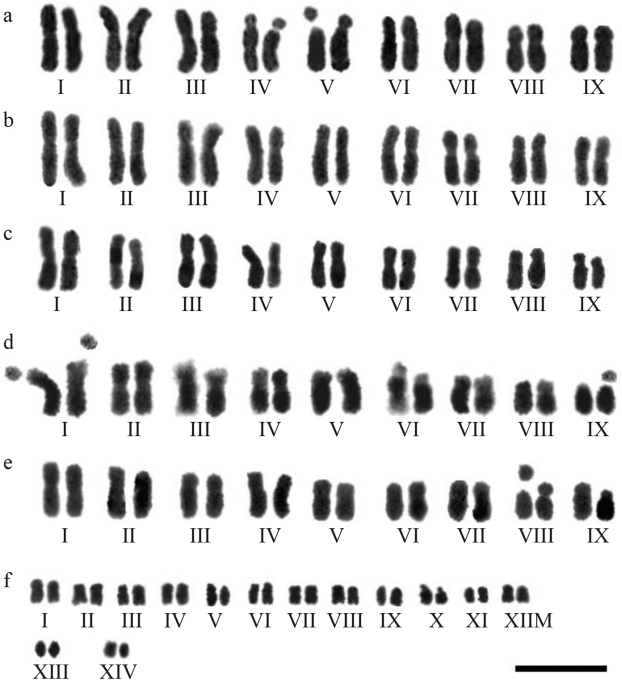

Figure 4 - Karyograms of some species of the Tribe Trimezieae from northeastern Brazil. (a) *Neomarica candida*; (b) *N. gracilis*; (c) *N. humilis*; (d) *N. northiana*; (e) *Neomarica* sp. (2*n* = 18); (f) *N.* cf. *paradoxa* (2*n* = 28). The bar corresponds to 10 μm.

Brejo da Madre de Deus (Table 1, Figure 2l), with a proximal secondary constriction on one chromosome pair.

Discussion

The Iridaceae are variable in chromosome numbers, sizes and morphologies, with numbers ranging from 2*n* = 6 in *Crocus candilus* (Brighton, 1976) to 2*n* = ca. 230 in *Libertia grandiflora* (Kenton and Heywood, 1984). Such variability may be related to cycles of polyploidy and disploidy reduction in both Neotropical (Goldblatt *et al.*, 1998) and Old World species (Goldblatt and Takei, 1993). Disploidy and aneuploidy appear to have had an important role in the chromosome evolution of this family, especially among the Tigridioideae. In our study, heteromorphisms of the long arms of the large chromosomes of *Cipura paludosa* (2*n* = 14) suggested the presence of reciprocal translocations. Sengupta and Sen (1988) reported heteromorphism in a single large chromosome pair in several specimens of *C. paludosa*, including hybrids and callus tissue cultivated *in vitro*, and a predominance of 2*n* = 12. Chromosome heteromorphisms have been occasionally reported in the Iridaceae, especially in the Tigrideae, with previous records for *Eleutherine bulbosa* in pair I due to an inversion followed by tandem duplications (Guerra, 1991), as well as for *Gelasine elongata* (R. Graham) Ravenna (formerly *Gelasine azurea*) as a result of a complex series of translocation events (Kenton and Rudall, 1987). The precise identification of the structural changes involved in these heteromorphisms will only be possible after meiotic analyses or after the use of differential staining techniques that reveal more detailed chromosome features (Alves, in preparation).

Inter- and intraspecific chromosome numerical variations

The finding of 2*n* = 28 for *Alophia drummondii* (Tigrideae) agrees with previous reports for this species in North America (Goldblatt and Takei, 1997; Kenton and Heywood, 1984), but diverges from the other diploid numbers found in this species, 2*n* = 14+1B, 2*n* = 42 and 2*n* = 56. The karyotypes of the North American populations have two large chromosome pairs (one meta- and one acrocentric), in contrast to the cytotypes with 2*n* = 28 and 56, that show four larger chromosome pairs (two metacentric and two acrocentric pairs) and of the cytotypes with 2*n* = 42 that have three larger chromosomes pairs (two metacentric and two acrocentric).

Our record of 2*n* = 14+1B for *Alophia drummondii* is new for this genus and confirms the basic number x = 7 for this taxon, as was also observed in other genera of Tigrideae (Goldblatt and Takei, 1997; Goldblatt and Snow, 1991). This data suggest that *A. drummondii* originated from a species with a low chromosome number that formed cytotypes with 2*n* = 28, 42 and 56 through successive polyploidy cycles. B chromosomes, which were probably suppressed in the polyploid cytotypes, were also reported in southern Brazilian forms of the genus *Herbertia* (Goldblatt, 1982).

The karyotypic differences between these disjunct populations of *A. drummondii* (as well as some of the individuals from the Esperança population in Paraíba State) suggest that structural karyotypic changes, in addition to polyploidy, would best explain the karyotypic evolution of this species and the maintenance of its bimodality. Other species of the family exhibit intraspecific polyploidy, such as *Gladiolus italicus* with 2*n* = 60, 90, 110-120 (Raamsdonk and De Vries, 1989) and *Iris japonica* with 2*n* = 18, 30, 32, 36, 60 (Zhou *et al.*, 2003). In *Lapeirousia* (an African genus that also shows high bimodality), karyotypic differences seem to have originated from chromosome fusions and from the loss of redundant centromeres, resulting in increases in chromosome size and reductions in diploid numbers without significant variation in genome size (Goldblatt and Takei, 1997, 1993). The differences in the numbers and morphologies of the large chromosome pairs observed in the population of *A. drummondii* examined herein indicate that structural changes occurred during the karyotypic evolution of this species. Robertsonian translocations in *Ornithogalum tenuifolium* (Stedje, 1987) may have resulted in the formation of a chromosome race with 2*n* = 4 (2*n* = 6 → 2*n* = 4).

Trimezia and *Neomarica* are exclusively New World genera, but their basic chromosome numbers are still uncer-

tain (Goldblatt *et al.*, 1998). The genus *Neomarica* exhibited a relatively constant $2n = 18$ in several species in this study, suggesting that this genus is more stable in diploid number than other genera of the tribe Trimezieae. However, *Neomarica* cf. *paradoxa* ($2n = 28$) is karyologically and morphologically distinct from the other species of the genus, and appears to be very similar to *Trimezia* both morphologically and cytologically.

The $2n = 82$ populations of *T. connata* from the states of Paraíba and Pará (from forested areas) are reported herein for the first time. This species is similar in size and morphology to *T. martinicensis* with $2n = 80$, as observed by Goldblatt and Takei (1997). These two species are very similar in terms of their vegetative and floral morphology, so that misidentifications are quite possible (see Goldblatt and Takei, 1997). However, the presence of *T. connata* and *T. martinicensis* in very distinct ecological environments, together with their karyotypic differences, suggest that these populations should be considered different species (perhaps cryptospecies). Differences in the location of the rDNA sites and in the satellites between diploid and tetraploid cytotypes of *Crocus vernus* suggest that their cytotypes are not directly related and that they could in fact represent different species (Frello and Heslop-Harrison, 2000).

Previous reports of $2n = 26$ for *T. fosteriana* (Goldblatt and Takei, 1997), a frequently cultivated species (Lorenzi and Souza, 2001), diverge from our result of $2n = 52$ for plants from Pernambuco and Paraíba. The $2n = 54$ in the population of *T. martinicensis* from Pernambuco State was similar to the result reported by Kenton and Heywood (1984) for a similar population from southern Brazil, but our results differed from those reported for populations from Jamaica and Martinique, with $2n = 40$ (Kenton and Heywood, 1984), and from Venezuela, with $2n = 80$ (Goldblatt and Takei, 1997). Further analyses are needed in *Trimezia* to assess its basic chromosome number.

Karyological features of the groups studied

The basic chromosome numbers of the New World Trimezieae (which include *Neomarica*, *Trimezia* and *Peseudotrimezia*) are still uncertain (Goldblatt and Takei, 1997). Five species of *Trimezia* reviewed by Goldblatt and Takei (1997) had variable diploid numbers ($2n = 26, 28, 40, 52, 54, 60, 76$ and 80) and variable chromosome sizes and morphologies (Kenton and Heywood, 1984). The occurrence of polyploidy cycles, followed by increasing or decreasing disploidy makes it difficult to determine the basic number in this group. *Neomarica* and *Trimezia* (Trimezieae) have symmetric karyotypes, but *Neomarica* is numerically more stable. The previous records of $2n = 18$ for *N. northiana* were confirmed in our *Neomarica* sample. However, the previous reports of $2n = 24$ and 32 for *N. caerulea* (Goldblatt and Takei, 1997; Kenton and Heywood, 1984; Goldblatt, 1982) did not agree with our results for any of the species studied, which had $2n = 18$ or $2n = 28$ in *N.* cf. *paradoxa* (and unusually small chromosomes). The diploid number variations previously reported, as well as our own data, support x = 9 or 8 as the probable basic number for *Neomarica* (Goldblatt *et al.*, 1998). Our data, as well as the presence of an erect rhizome in *N.*cf. *paradoxa*, suggest that its inclusion in *Neomarica* should be reconsidered (as well as the inclusion of other species with similar underground organs).

The karyotypes of Tigrideae reported herein, as well as those of other genera (Kenton and Heywood, 1984), are clearly asymmetric, bimodal, and show two to eight large meta-acrocentric chromosome pairs, while the remaining pairs are small and predominantly metacentric. Populations of *Eleutherine bulbosa* from Central America have karyotypes that are more symmetrical than those of the populations from northeastern Brazil (Guerra, 1991), which suggests that the latter may have originated from an ancestral stock with a symmetric karyotype (Chiarini, 2005; Stebbins, 1971). In the other species, as well as in *Lapeirousia* (Goldblatt and Takei, 1997), unequal translocations could have caused the bimodality seen and these may offer evolutionary advantages in cases of ecological alterations (Levin, 2002).

Karyotype asymmetry indices have been widely used to infer mechanisms of chromosomal evolution in plants (Paszko, 2006) (especially the interchromosomal asymmetry index [A_2]). This index provided a good measure of the general morphology of the chromosome sets of the species analyzed herein. The A_2 index suggests that the members of Tigrideae seem to have a more clearly derived karyotype than the individuals of Trimezieae, which is confirmed by important synapomorphies, such as plicate leaves, a distinctive type of bulb and the derived x = 7 (Goldblatt, 1990). This view is also supported by recent molecular phylogenetic studies of Iridaceae (Goldblatt *et al.*, 2008).

In summary, two karyological groups can be clearly identified in Iridoideae: the tribes Trimezieae, with more symmetric karyotypes and evolution through polyploidy, mainly in *Trimezia* (with or without disploid variants); and Tigrideae, with asymmetric karyotypes, with occasional heterozygous chromosome pairs.

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Internet Resources

Image Tool (IT) Version 3m0, http://ddsdx.uthscsa.edu/DIG/download.htm (July 24, 2007).

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