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Chromosome Number for Bromeliaceae Species Occurring in Brazil

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Summary The chromosome number of 25 Bromeliaceae species from the genera *Dyckia*, *Vriesea*, *Aechmea*, *Ananas*, *Billbergia*, *Nidularium*, *Neoregelia*, *Neoglaziovia*, *Orthophytum*, *Portea*, *Quesnelia* and *Wittrockia* were assessed. All are diploid $2n=50$, except for *Orthophytum albopictum* and *Neoglaziovia variegata*, both tetraploids, $2n=100$. The chromosome counts are the first report for 19 of the 25 species evaluated. All chromosome counts reinforce $x=25$ as the basic number for the family.

Key words Chromosome number, Bromeliaceae.

Bromeliaceae is one of the largest families from tropical plants that have an exclusive origin in the American continents, except *Pitcairnia feliciana*, which is from the Gulf of Guinea, Africa. This family is distributed in the tropical and sub-tropical latitudes, from the Southern United States, in the states of Virginia and Texas, to the central regions of Chile and Argentine. The largest centers of origin and diversity are in South America. The most primitive members of the Pitcairnioideae and Tillandsioideae subfamilies are found in the northern region of Andean Mountains to Mexico and the Antilles, while the eastern part of Brazil has the most advanced species of the Bromelioideae subfamily, and the evolved species from the genera *Dyckia* from Pitcairnoideae and *Vriesea* from Tillandsioideae (Smith 1934, Leme and Marigo 1993). The cytogenetic analyses of the Bromeliaceae comprise approximately 12% of known species, with the majority being cultivated ornamental (Lindschau 1933, Weiss 1965, Gauthé 1965, Marchant 1967, Sharma and Ghosh 1971, McWilliams 1974, Brown *et al.* 1984, Varadarajan and Brown 1985, Brown and Gilmartin 1986, 1989, Lin *et al.* 1987, Brown *et al.* 1997, Baracho and Guerra 2000, Gitaí *et al.* 2000, Cotias de Oliveira *et al.* 2000, Palma-Silva 2003). The first chromosome counts revealed great variation with $2n=48$, 50, 56, 64, 72, 94, 96, 100 and 126, and contradictory interpretations on its basic number. On the hand, the precise counts realized by Marchant (1967) revealed a great uniformity in the chromosome number, which was $2n=50$, 100 and 34, based on basic number $x=25$ and a derived haploid number $n=17$. Brown and Gilmartin (1989) mainly counted the chromosome numbers from the members of the Tillandsioideae subfamily, and they confirmed a predominance of $2n=50$ and a basic number $x=25$. Even though almost 50% of the known species are found in Brazil, their cytogenetic analysis is scarce and for that reason, it represents a great source of subjects to study chromosome evolution in Bromeliaceae. In this report, we assess the chromosome numbers of 25 Bromeliaceae species, belonging to the genera *Dyckia*, *Vriesea*, *Aechmea*, *Ananas*, *Billbergia*, *Nidularium*, *Neoregelia*, *Neoglaziovia*, *Orthophytum*, *Portea*, *Quesnelia* and *Wittrockia*. This study is the first determination of 19 species and presents new counts for *Aechmea fulgens*, *Aechmea miniata* var. *discolor*, *Billbergia euphemiae* var. *euphemiae*, *Neoglaziovia variegata*,

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Nidularium rutilans and *Orthophytum saxicola*.

Material and methods

The most material in the studies were collected from natural habitats and others were obtained from specimens in cultivation (Table 1). The plants were kept in *xaxim* to encourage rooting. Root tips were pretreated with 0.002 M 8-hydroxyquinoline at 18°C for 4 h and fixed in Carnoy 3 : 1 overnight, transferred to 70% alcohol and stored in the refrigerator until used. They were then hydrolyzed in 1 N HCl for 8 min at 60°C and stained following the Feulgen method (Sharma and Sharma 1980). Squashes preparations were made in a 1% acetic-carmine solution. The slides were mounted in Entellan. Chromosome counts were made in 5–20 metaphases of 1–4 plants of each species. Chromosome size were estimated from the metaphases using a micrometric scale of the same enlargement.

Results and discussion

All the species presented a diploid number $2n=50$, except for *Orthophytum albopictum* and *Neoglaziovia variegata*, which are tetraploids, $2n=100$ (Table 1). First counts are reported for 19 species and five additional counts are for taxa reported previously. The size of chromosomes varied

Table 1. Localities of collection and chromosome number of species of the Bromeliaceae

Subfamily/species	Provenance	2*n*
Pitcairnioideae		
Dyckia platyphylla L. B. Smith	Rio de Janeiro, RJ** (Cultivated)	50
Tillandsioideae		
**Vriesea fosteriana* L. B. Smith	Rio de Janeiro, RJ (Cultivated)	50
**Vriesea picta* (Mez et Wercklé) L. B. Smith et Pittendr.	Rio de Janeiro, RJ (Cultivated)	50
**Vriesea saundersii* (Carrière) E. Morren ex Mez	Rio de Janeiro, RJ (Cultivated)	50
**Vriesea botafogensis* Mez	Rio de Janeiro, RJ (Cultivated)	50
Bromelioideae		
**Neoregelia carcharodon* (Baker) L. B. Smith	Rio de Janeiro, RJ (Cultivated)	50
**Neoregelia hoehneana* L. B. Smith	Rio de Janeiro, RJ (Cultivated)	50
**Neoregelia laevis* (Mez) L. B. Smith var. *albomarginata*	Rio de Janeiro, RJ (Cultivated)	50
**Neoregelia johannis* (Carrière) L. B. Smith	Rio de Janeiro, RJ (Cultivated)	50
**Neoregelia wilsoniana* M. B. Foster	Jaguaripe, BA	50
Nidularium rutilans E. Morren	Sertão das cobras, SP (Leme 1423)	50
**N. longiflorum* Ule	Rio de Janeiro, RJ (Leme 771)	50
**N. procerum* Lindman	Nova Friburgo, RJ (Leme 990)	50
**Quesnelia arvenis* (Vellozo) Mez	Rio de Janeiro, RJ (Cultivated)	50
**Quesnelia edmundoi* L. B. Smith var. *rubrobracteata* Pereira	Rio de Janeiro, RJ (Cultivated)	50
Aechmea fulgens (Brongn.) var. *fulgens*	Amargosa, BA	50
Aechmea miniata Beer hortus ex Baker var. *discolor* Beer	Valença, BA	50
Billbergia. euphemiae E. Morren var. *euphemiae*	Jaguaripe, BA	50
**Portea silveirae* Mez	Jaguaripe, BA	50
**Portea grandiflora* Philcox	Santa Teresinha, BA	50
**Ananas nanus* (L. B. Smith) L. B. Smith	Salvador, BA (Cultivated)	50
**Wittrockia gigantea* (Baker) Leme	Fervedouro, MG (Leme 2166)	50
Orthophytum saxicola (Ule) L. B. Smith	Ipirá, BA	50
**Orthophytum albopictum* Philcox	Mucugê, BA	100
Neoglaziovia variegata (Arruda Câmara) Mez	Morro do Chapéu, BA	100

* First chromosome number reported for species.

** The abbreviations of the localities correspond to Brazilian states: RJ, Rio de Janeiro; SP, São Paulo; BA, Bahia; MG, Minas Gerais.

from 0.36–1.2 μm. In addition to reduced chromosome size, we observed larger chromosomes in *Vriesea picta* and *V. saundersii* (0.53–1.21 μm) and small chromosomes in *Nidularium rutilans* (0.35–0.56 μm). Due to the small size, no further, more detailed karyotype analysis can be carried out.

Chromosome in the subfamily Pitcairnioideae have been registered for the genus *Brocchinia*, *Deuterocohnia*, *Dyckia*, *Fosterella*, *Hechtia*, *Pitcairnia*, *Lindmania* and *Puya* (Lindschau 1933, Sharma and Ghosh 1971, Brown *et al.* 1984, Varadarajan and Brown 1985, Brown and Gilmartin 1986, 1989, Marchant 1967, Brown *et al.* 1997, Baracho and Guerra 2000, Gitaí *et al.* 2000, Cotias de Oliveira *et al.* 2000). These counts showed $n=25$, $2n=50$ in about 68 species analyzed and only five polyploids with $2n=100$, 150. *Dyckia platyphylla* with $2n=50$ chromosomes varying from 0.53–1.1 μm, are among the largest observed in this study. Eleven of the approximately 120 species of *Dyckia* have been counted chromosomally showed $2n=50$, except for *D. argentea*, *D. lorentziana* and *D. remotiflora* with $2n=100$ (Sharma and Ghosh 1971, Baracho and Guerra 2000).

Chromosome data are available for the subfamily Tillandsioideae for species from the genera *Catopsis*, *Glomeropitcairnia*, *Guzmania*, *Tillandsia* and *Vriesea* (Lindschau 1933, Weiss 1965, Gauthé 1965, Brown *et. al.* 1984, Brown and Gilmartin 1989, Brown *et al.* 1997, Baracho and Guerra 2000, Gitaí *et al.* 2000, Cotias de Oliveira *et al.* 2000, Palma-Silva 2003). The majority of the 101 species counts have been $n=25$. The report of $n=22$, 21, 20, 19 and 18 reveal the presence of aneuploidy in this subfamily. The four *Vriesea* species analyzed here, had $2n=50$, but clear differences appear in chromosome size. While *V. fosteriana* and *V. botagensis* had chromosome varying from 0.53–0.64 μm and 0.50–0.89 μm, respectively, those of *V. picta* and *V. saundersii* varied from 0.53–1.21 μm, showing the clear expression of bimodality. In this karyotypes 10–12 chromosomes presented weak coloration (Fig. 1a). The chromosomal counts have already been carried out in 29 of the 227 species of the genus, all of them with $n=25$, except for *V. hieroglyphica* with the somatic number $2n=56$ (Gauthé 1965).

Chromosome counts in the subfamily Bromelioideae are available for species from the genera *Acanthostachys*, *Aechmea*, *Ananas*, *Araeococcus*, *Billbergia*, *Bromelia*, *Canistrum*, *Cryptanthus*, *Cottendorfia*, *Deinacanthon*, *Greigia*, *Fascicularia*, *Hohenbergia*, *Lymania*, *Neoglaziovia*, *Neoregelia*, *Nidularium*, *Orthophytum*, *Portea*, *Pseudananas*, *Quesnelia*, *Streptocalyx* and *Wittrockia* (Lindschau 1933, Weiss 1965, Marchant 1967, Sharma and Ghosh 1971, McWilliams 1974, Brown *et al.* 1984, Varadarajan and Brown 1985, Brown and Gilmartin 1986, 1989, Lin *et al.* 1987, Brown *et al.* 1997, Cotias de Oliveira *et al.* 2000, Baracho and Guerra 2000, Gitaí *et al.* 2000, Palma-Silva 2003). A majority of such species are $2n=50$, with some $2n=100$, 150, although variations this numbers, *e.g.* $2n=54$, 48, 96, 160 have been found. *Portea*, a genus of only nine species, is endemic to Brazil and only one chromosome count has been reported for *P. karmesina* with $2n=50$ (Weiss 1965). *Portea grandiflora* and *P. silveirae*, both with $2n=50$ chromosomes varying from 0.53–1.1 μm, are among the largest observed in this study (Fig. 1b).

Eleven of the ~95 species of *Neoregelia* have been counted chromosomally, and they showed $2n=50$ (Marchant 1967) or $2n=54$ (Lindschau 1933, Weiss 1965). The five species here analyzed, were all $2n=50$ chromosomes varying in the range 0.36–0.82 μm.

Wittrockia is a small genus with 12 species, endemic to Brazil. There is a previous register only for *W. amazonica* (as *Canistrum amazonicum*, Weiss 1965) with $2n=50$. The same chromosome number was observed for *W. gigantea* in this paper. Their chromosomes vary from 0.45–0.64 μm.

The count $2n=100$ for *Orthophytum albopictum* is the third known polyploid in the genus (Fig. 1c). The chromosomes are small, 0.46–0.68 μm. The count $2n=50$ for *O. saxicola*, confirm the first report (Ramirez-Morillo and Brown 2001). *Orthophytum* is an endemic genus in Brazil, with 26 species, from which there are chromosomal counts for four, two of them being polyploids, $2n=100$ and 150 (Cotias de Oliveira *et al.* 2000) and two diploids, $2n=50$ (Baracho and Guerra

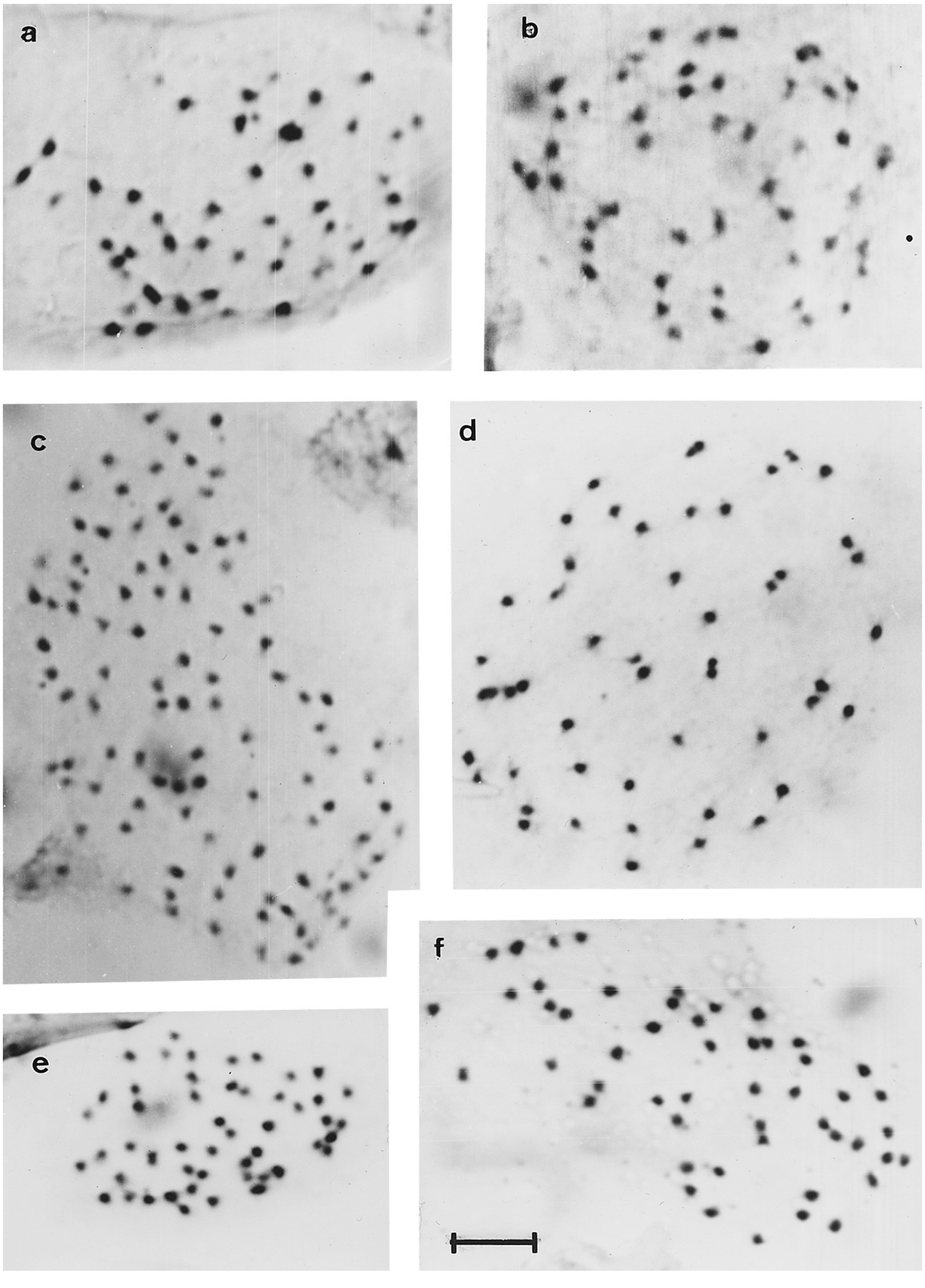

Fig. 1. Mitotic chromosomes of some species of Bromeliaceae. a) *Vriesea fosteriana*, $2n=50$. b) *Portea silveirae*, $2n=50$. c) *Orthophytum albopictum*, $2n=100$. d) *Aechmea miniata* var. *discolor*, $2n=50$. e) *Nidularium longiflorum*, $2n=50$. f) *Nidularium procerum*, $2n=50$. Bar: 5 μm.

2000, Ramirez-Morillo and Brown 2001).

Quesnelia is a small genus, endemic in Brazil, with 15 species and one chromosome count $2n=50$, 54 for *Q. liboniana* (Matsuura and Suto 1935, McWilliams 1974, Brown *et al.* 1997). *Quesnelia arvensis* and *Q. edmundoi* var. *rubrobracteata* showed $2n=50$, but clear differences appear in chromosomes size. While *Quesnelia arvensis* has the smallest chromosome varying from 0.46–0.89 μm, *Q. edmundoi*, varies from 0.32–0.75 μm.

Aechmea is a large genus of approximately 172 species. The available chromosomal data for 29 species reveal $2n=50$ and some $2n=54$. Atypical counts with $n=21$ were registered for *A. tillandsioides* (Marchant 1967) and *A. exudans* (Baracho and Guerra 2000). Our count $2n=50$ for *Aechmea miniata* var. *discolor* (Fig. 1d) disagrees with the first determination $2n=54$ (Lindschau 1933) and confirms $n=25$ (Marchant 1967). On the one hand, the count $2n=50$ for *Aechmea fulgens* confirms previous reports (Lindschau 1933, Marchant 1967, Brown *et al.* 1997).

Thirteen of the ~62 species of *Billbergia* have been counted chromosomally. The majority presented $2n=50$, but variations of $2n=48$, 52 and 54 were registered (Sharma and Ghosh 1971, Weiss 1965, Lindschau 1933). The new count $2n=50$ for *Billbergia euphemiae* var. *euphemiae* disagrees with previous report $2n=52$ (Sharma and Ghosh 1971). Their chromosomes varying from 0.50–0.86 μm.

Nidularium, a genus of approximately 54 species, is endemic to Brazil, having a previous chromosomal register for only 10 out of them. All the counts have been $2n=50$, except three them with $2n=54$, analyzed by Lindschau (1933) and Weiss (1965). Our count from *N. procerum* disagrees with previous count $2n=54$ (Weiss 1965). The count for *N. rutilans* confirmed earlier determination (Marchant 1967). This species had smallest chromosomes, varying 0.36–0.56 μm, while *N. procerum* and *N. longiflorum*, 0.43–0.75 μm (Fig. 1e).

Ananas contains 8 species and there are chromosomal counts for seven, including the first report for *A. nanus*, in this paper. Although most species studied present $2n=50$ (Brown and Gilmartin 1989, Sharma and Ghosh 1971, Cotias de Oliveira *et al.* 2000), there were observed variations in polyploid level in *A. comosus* (pineapple), with $2n=50$, 75 and *Ananas ananassoides*, with $2n=50$, 75, 94 (Lin *et al.* 1987, Brown *et al.* 1997).

Neoglaziovia, an endemic genus from Brazil, with 3 species and chromosomal count only for *N. variegata* (Cotias de Oliveira *et al.* 2000). This new count for populations from other locality, confirms previous report $2n=100$.

All chromosomal counts in this paper reflect the basic $x=25$ number. In spite of $2n=50$ being constant, some species, such as *Nidularium rutilans*, *N. procerum*, *N. longiflorum*, *Aechmea fulgens* var. *fulgens*, *Neoregelia wilsoniana*, *Quesnelia edmundoi* and *Ananas nanus*, presented until four supernumerary chromosomes in different cells, from the same root tip (Fig. 1f). The occurrence of these chromosomes is very common in mitotic analyses of Bromeliaceae and generate records with $2n=52$, 54 and 56 (Lindschau 1933, Weiss 1965, Gauthé 1965, Sharma and Ghosh 1971). This may explain the disagreement between new chromosome counts and that from Lindschau (1933), Weiss (1965) and other authors. On the other hand, several somatic records with $2n=48$, 72, 94, 96 (Lindschau 1933, Sharma and Ghosh 1971, Lin *et al.* 1987) have also not been confirmed in new counts and may have resulted from the observation of incomplete metaphases, or with chromosome superposition, in diploid, triploid and tetraploid species, respectively.

The extremely small Bromeliaceae chromosomes do not permit carrying out a detailed morphological analysis, although chromosomal bimodality in the *Vriesea* species was observed. Marchant (1967) registered a bimodal karyotype in some *Vriesea*, as well as in other Tillandsioideae, correlating bimodality with advanced characteristics, such as epiphytism. Nevertheless, more recent analyses have also presented bimodality in *Deuterocohnia lorentziana*, from the Pitcairnioideae, the most primitive subfamily among the Bromeliaceae (Gitaí *et al.* 2000) contradicting the correlation between bimodality and evolutionary advance, admitted by Marchant (1967).

Interestingly, the polyploids here analyzed grow in semi-arid regions. Even though many diploid species of Bromeliaceae are found in this ecosystem, it is possible that polyploidy in *Orthophytum* and *Neoglaziovia* have been an important role for their adaptation in xeric habitats. This association between polyploidy and extreme environmental conditions, has also been observed by Forni-Martins *et al.* (1995) in species from the Brazilian savanna. This reinforces the idea that polyploidy can also favor the adaptation in semi-arids regions in the tropics.

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