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Chromosome numbers in Bromeliaceae

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Abstract

The present study reports chromosome numbers of 17 species of Bromeliaceae, belonging to the genera *Encholirium, Bromelia, Orthophytum, Hohenbergia, Billbergia, Neoglaziovia, Aechmea, Cryptanthus* and *Ananas*. Most species present 2n = 50, however, *Bromelia laciniosa, Orthophytum burle-marxii* and *O. maracasense* are polyploids with 2n = 150, 2n = 100 and 2n = 150, respectively, while for *Cryptanthus bahianu*s, 2n = 34 + 1-4B. B chromosomes were observed in *Bromelia plumieri* and *Hohenbergia* aff. *utriculosa*. The chromosome number of all species was determined for the first time, except for *Billbergia chlorosticta* and *Cryptanthus bahianus*. Our data supports the hypothesis of a basic number of x = 25 for the Bromeliaceae family and decreasing aneuploidy in the genus *Cryptanthus*.

INTRODUCTION

Bromeliaceae is a plant family from tropical and subtropical America, with about 3,000 species in 54 genera (Leme, 1998). It is widely distributed on the American continent, from the States of Virginia and Texas in the southern United States to central Argentina and Chile (Smith, 1934). *Pitcairnia feliciana*, the only exception, is found in Guine, Africa (Smith and Downs, 1974). Brazil, one of the largest centers of diversity, has approximately 40% of known species, with the more evolved genera and species of the Bromelioideae subfamily and some more advanced taxa of the Pitcairnioideae and Tillandsioideae subfamilies found in the eastern region (Leme and Marigo, 1993). Although numerous botanical, ecological and evolutionary studies exist, cytogenetical analyses are limited to about 9% of the species, most of these cultivated and ornamental (Marchant, 1967; Sharma and Ghosh, 1971; McWilliams, 1974; Brown and Gilmartin, 1986, 1989; Lin *et al*., 1987). There is a predominance of 2n = 50 in these studies and aneuploidy appears to decrease to 2n = 34 in the genus *Cryptanthus*. However, no cytological data appears for 22 genera, and for the majority of those already studied only a small number of species have been investigated. Besides scarcity of cytological data, recordings of different numbers of chromosomes for the same species justify new counts. This study is the first report of 15 Bromeliaceae species, belonging to nine different genera, and presents new counts for *Billbergia chlorosticta* and *Cryptanthus bahianus*. Determination of chromosome number for species of *Encholirium, Orthophytum, Hohenbergia* and *Neoglaziovia* represents the first record for these genera.

MATERIAL AND METHODS

Plants were collected from their natural habitat, except for *Ananas lucidus, Aechmea blanchetiana* and *Billbergia morelii* which, although native to Bahia, were obtained from specimens in cultivation (Table I). At least three plants per species were analyzed. Voucher specimens were deposited at the ALCB herbarium, Instituto de Biologia, Universidade Federal da Bahia, Brazil. The plants were kept in containers with water up to the point of leaf insertion to encourage rooting. Root tips were pretreated with 0.002 M 8-hydroxyquinoline for 4 h at 18ºC and fixed in 3:1 ethanol-acetic acid for 18-24 h. Root tips were transferred to 70% alcohol and stored at 4ºC, then hydrolyzed in 1 N HCl for 8 min at 60ºC and stained following the Feulgen method (Sharma and Sharma, 1980). Squash preparations were made in a 1% acetic-carmine solution. Coverslips were removed in 45% acetic acid and slides and coverslips mounted in Canada balsam. Chromosome counts and measurements were made in 5-20 metaphases of each species.

RESULTS AND DISCUSSION

The majority of the species analyzed had 2n = 50, but 2n = 34 and 2n = 100 to about 150 were also observed (Table I). The chromosome number of all species was determined for the first time, except for *B. chlorosticta* and *C. bahianus* (Figures 1 and 2). Extremely small chromosome size (0.23-1.08 µm) hindered a comparative morphological analysis, but small differences suffice for characterization. While some species such as *B. chlorosticta, H. utriculosa* and *H. stellata* had chromosomes varying from 0.41-1.21 µm, those of *H. littoralis, B. morelii* and

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Table I - Origin, voucher number and chromosome number of species of the Bromeliaceae studied.

Subfamily/Species	Localities of collection	Voucher number	2n
Pitcairnioideae			
Encholirium spectabile Mart. ex Schult.f.	Milagres, BA, Brazil	ALCB-028635	50
Bromelioideae			
Aechmea aquilega (Salisbury) Grisebach	Baixa Grande, BA, Brazil	ALCB-028395	50
Aechmea blanchetiana (Baker) L.B. Smith	Salvador, BA, Brazil (cultivated)	ALCB-028638	50
Aechmea conifera L.B. Smith	Baixa Grande, BA, Brazil	ALCB-029469	50
Ananas lucidus Miller	Salvador, BA, Brazil (cultivated)	ALCB-029633	50
Billbergia chlorosticta Saunders Hortus	Amargosa, BA, Brazil	ALCB-029638	50
Billbergia morelii Brongniart	Salvador, BA, Brazil (cultivated)	ALCB-038104	50
Bromelia laciniosa Mart. ex Schultes	Baixa Grande, BA, Brazil	ALCB-029470	ca. 150
Bromelia plumieri (E. Morren) L.B. Smith	Baixa Grande, BA, Brazil	ALCB-029046	50 + 1-2B
Cryptanthus bahianus L.B. Smith	Baixa Grande, BA, Brazil	ALCB-028397	34 + 1-4B
Hohenbergia catingae Ule var. *catingae*	Milagres, BA, Brazil	ALCB-029258	50
Hohenbergia littoralis L.B. Smith	Salvador, BA, Brazil	ALCB-029259	50
Hohenbergia stellata Schultes	Baixa Grande, BA, Brazil	ALCB-029258	50
Hohenbergia aff. *utriculosa* Ule	Baixa Grande, BA, Brazil	ALCB-028394	50 + 2B
Neoglaziovia variegata (Arruda Câmara) Mez	Baixa Grande, BA, Brazil	ALCB-028634	100
Orthophytum maracasense L.B. Smith	Baixa Grande, BA, Brazil	ALCB-029635	150
Orthophytum burle-marxii L.B. Smith & Rangel	Lençóis, BA, Brazil	ALCB-036881	100

the *Bromelia* species did not reach 0.3 µm. Variation in chromosome size appeared within the same karyotype. *Bromelia* and *Billbergia* species were more uniform, while *Encholirium spectabile*, *Neoglaziovia variegata* and the *Aechmea* species presented continuous variation from largest to smallest chromosome, but without the clear expression of bimodality, observed by Marchant (1967) in the more advanced Bromelioideae and Tillandsioideae species.

Chromosome counts in the subfamily Pitcairnioideae have been published for *Brocchinia* species (in Goldblat and Johnson, 1993), *as well as Deuterocohnia, Dyckia, Fosterella, Hechtia, Pitcairnia, Lindmania* and *Puya* (Marchant, 1967; Sharma and Ghosh, 1971; Brown *et al.*, 1984; Varadarajan and Brown, 1985; Brown and Gilmartin, 1986, 1989). These counts showed n = 25, 2n = 50 in about 60 species analyzed and in only two, 2n = 100 (McWilliams, 1974; Brown *et al.*, 1984; Brown and Gilmartin, 1989; Marchant, 1967; Sharma and Ghosh, 1971). *Encholirium spectabile* with 2n = 50 represents the first count for the genus. The chromosomes varying from 0.53-1.07 µm are among the largest observed in this study (Figure 1a).

Chromosome data are available for the subfamily Bromelioideae for species from the genera *Acanthostachys, Aechmea, Ananas, Araeococcus, Billbergia, Bromelia, Canistrum, Cryptanthus, Neoregelia, Nidularium, Portea, Pseudananas, Quesnelia, Streptocalyx* and *Wittrockia*. The most common number is 2n = 50, although some variations, e.g., 2n = 54, 48, 96, have been found (McWilliams, 1974; Brown *et al.*, 1984; Brown and Gilmartin, 1989; Marchant, 1967; Sharma and Ghosh, 1971).

The *Aechmea* species had 2n = 50 and chromosomes varying from 0.36-0.86 µm in *A. aquilega*, to 0.46-0.80 µm in *A. blanchetiana* and 0.46-1.03 µm in *A. conifera*. The other species in the genus show a certain uniformity in the number n = 25 (Marchant, 1967; Brown and Gilmartin, 1989) and 2n = 50 (Lindschau, 1933, in McWilliams, 1974). The only exception reported is 2n = 54 for *A. ornata* (Lindschau, 1933, in McWilliams, 1974) and n = 21 for *A. tillandsioides* (Marchant, 1967).

Ananas lucidus with 2n = 50 had more uniform chromosomes, varying from 0.67-0.89 µm (Figure 1e). *Ananas* also had a certain uniformity of 2n = 50 in the five species already analyzed, but triploids have been previously recorded in *A. comosus* and tetraploids in *A. ananassoides* (Lin *et al.*, 1987).

The two *Billbergia* species analyzed showed 2n = 50. *B. morelii* had small chromosomes, 0.23-0.30 µm, while *B. chlorosticta* had large ones, 0.86-1.07 µm (Figure 1f, g). The count of 2n = 50 for *B. chlorosticta* differs from the first report of 2n = 54 by Brown and Gilmartin (1986).

Neoglaziovia is a genus with only three species, of which *N. variegata* had 2n = 100 with chromosome size varying from 0.40-0.93 µm, (Figure 1h). No chromosome data exist for the remaining species of this genus.

The two species of *Bromelia* examined differed in polyploid level and in the presence of B chromosomes: *B. plumieri* had 2n = 50 + 1-2B and *B. laciniosa*, 2n = ca. 150, but both had very small chromosomes, in the same range: 0.23-0.26 µm (Figure 1i, j). In *B. plumieri* B chromosomes had a size similar to others of the same set. Only four species have been analyzed of the *Bromelia* genus, showing atypical numbers of 2n = 48 for *B. alta*, 2n = 94 for *B. goeldiana* (cf. McWilliams, 1974) and 2n = 96 for *B. pinguim* and *B. antiacantha* (Lin *et al.*, 1987).

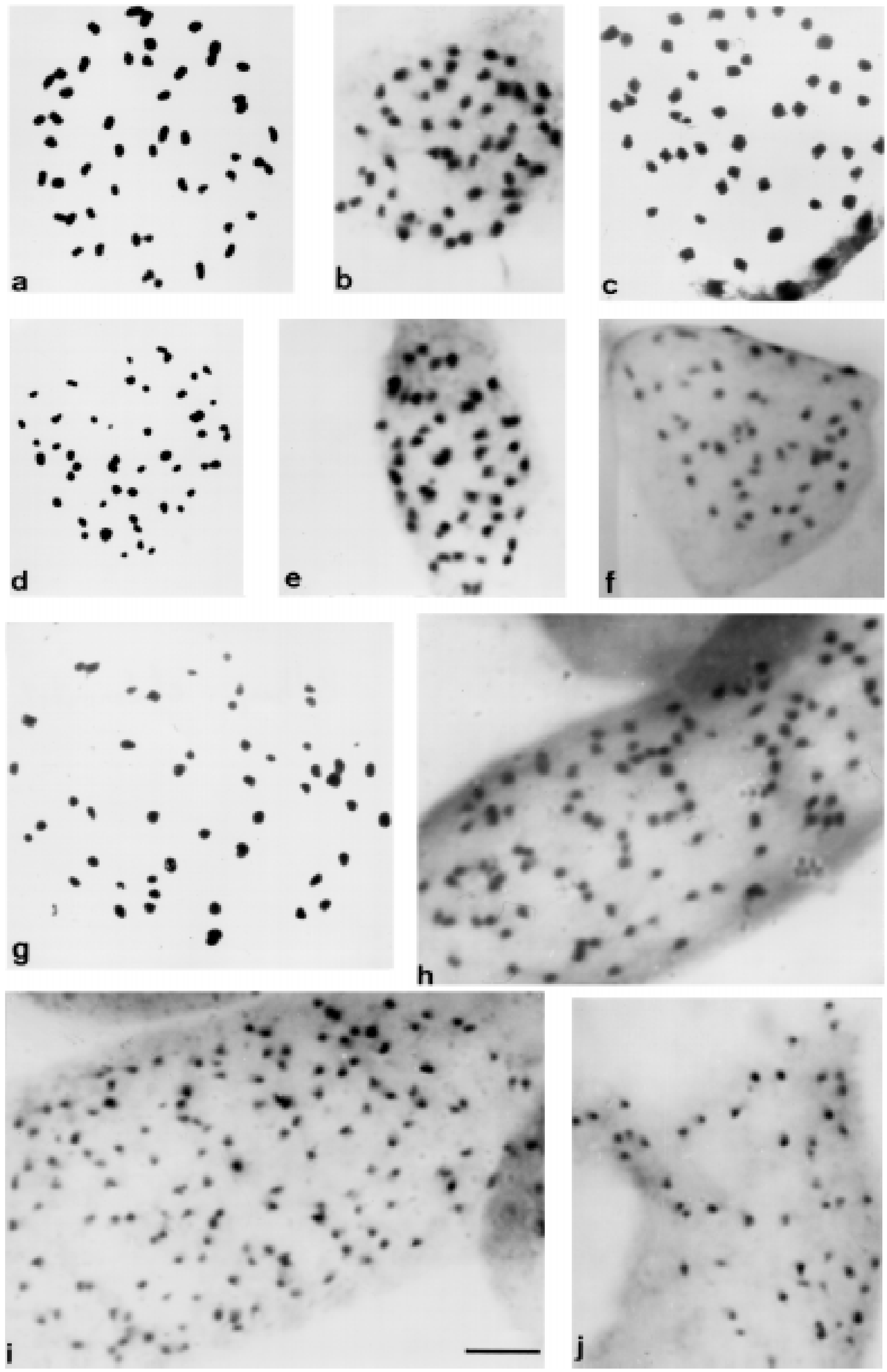

Figure 1 - Mitotic metaphases in species of the Bromeliaceae. **a.** *Encholirium spectabile*, 2n = 50; **b.** *Aechmea blanchetiana*, 2n = 50; **c.** *A. conifera*, 2n = 50; **d.** *A. aquilega*, 2n = 50; **e.** *Ananas lucidus*, 2n = 50; **f.** *Billbergia morelii*, 2n = 50; **g.** *B. chlorosticta*, 2n = 50; **h.** *Neoglaziovia variegata*, 2n = 100; **i.** *Bromelia laciniosa*, 2n = 150; **j.** *B. plumieri*, 2n = 50. Bar in **i** represents 5 µm for all figures.

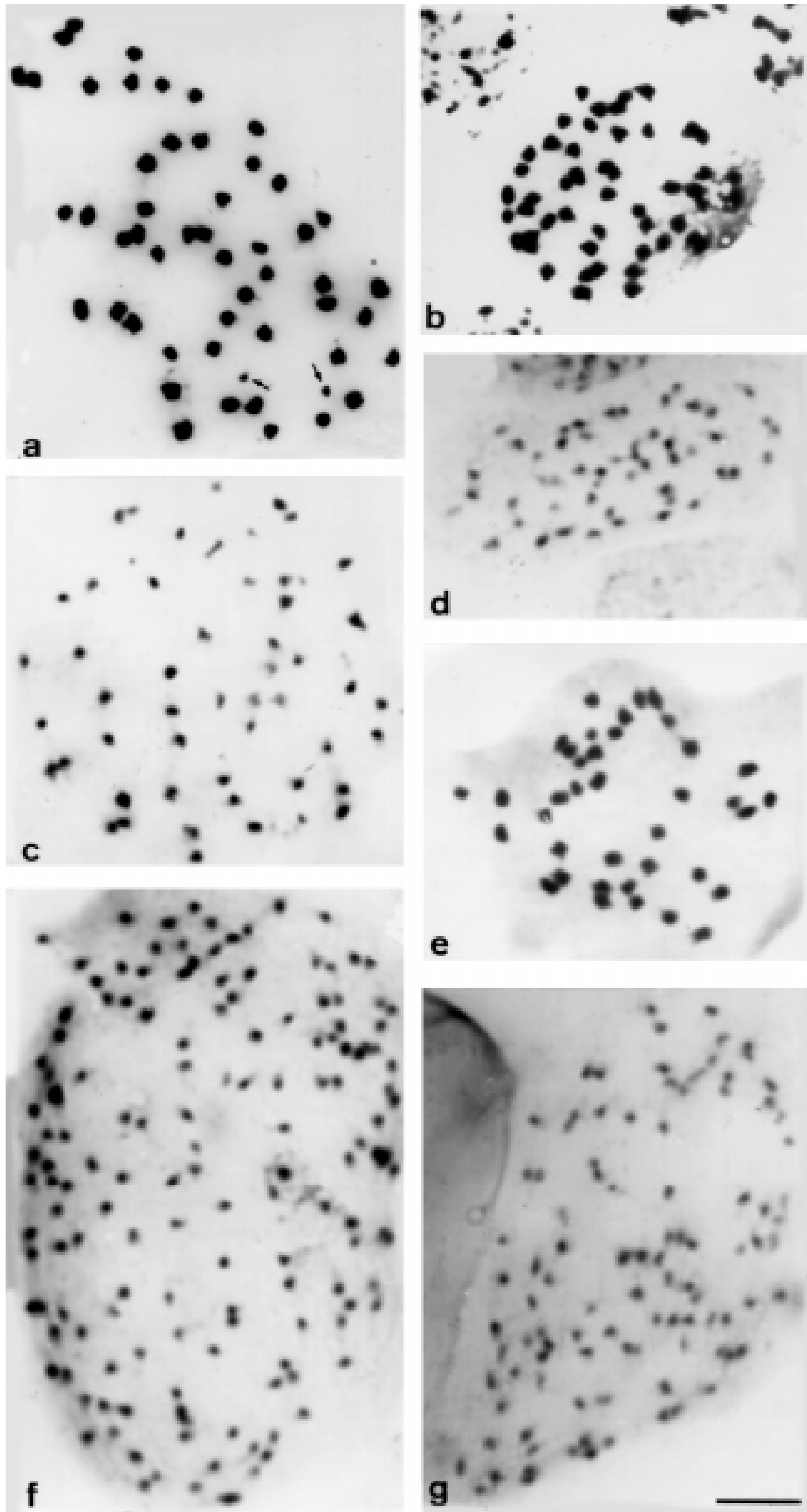

Figure 2 - Mitotic metaphases in species of the Bromeliaceae. **a.** *Hohenbergia utriculosa*, 2n = 50 + 2B; **b.** *H. stellata*, 2n = 50; **c.** *H. catingae* var. *catingae*, 2n = 50; **d.** *H. littoralis*, 2n = 50; **e.** *Cryptanthus bahianus*, 2n = 34 + 4B; **f.** *Orthophytum maracasense*, 2n = 150; **g.** *O. burle-marxii*, 2n = 100. Bar in **g** represents 5 µm for all figures. Arrows indicate B-chromosomes.

The four *Hohenbergia* species analyzed here were all 2n = 50, but clear differences appear in chromosome size. *H. littoralis* has the smallest chromosomes (0.23-0.25 µm), followed by *H. catingae* var. *catingae* (0.86-0.89 µm), *H. stellata* (0.71-1.03 µm) and *H. utriculosa* (0.82-1.21 µm). *H. utriculosa* had two B chromosomes clearly smaller than the others of the same set (Figure 2a-d).

The *Cryptanthus* genus markedly deviates from 2n = 50, common in the Bromeliaceae family. In the single species examined, *C. bahianus* had 2n = 34 + 1-4 B chromosomes (Figure 2e). The number of B chromosomes varied from cell to cell of the same and different plants. Size ranged from 0.53-1.08 µm. Marchant (1967) recorded n = 17 for *C. bahianus, C. acaulis* and *C. zonatus* and 2n = 34 for *C. beuckeri*, while Lindschau, 1933 (in McWilliams, 1974) observed 2n = 54 for *C. beuckeri* and *C. bivittatus.* But Sharma and Ghosh (1971) found 2n = 36 for the latter species.

Orthophytum burle-marxii and *O. maracasense* had 2n = 100 and 2n = 150, respectively. The chromosomes in both species varied from 0.45-0.87 µm (Figure 2f, g).

Generally, a certain uniformity in the somatic number of 2n = 50 prevails in the Bromeliaceae. However, first observations on root tips showed some variation in the number for individual species, and discrepancies exist in numbers found by mitotic and meiotic analyses (cf. Brown and Gilmartin, 1986). The high number and small size of the chromosomes may have contributed to erroneous counts, based on pro-metaphases or metaphases with overlapping chromosomes. However, in some cases variation may be due to the presence of B chromosomes, as observed in *B. plumieri*, *H.* aff. *utriculosa* and *C. bahianus* (Table I) and 2n = 54 for *B. chlorosticta* and *Aechmea ornata* (Brown and Gilmartin, 1986; Lindschau, 1933, cited in McWilliams, 1974).

The karyotype 2n = 50, 100 and 150 observed here supports a hypothesized basic number of x = 25 for the family (Marchant, 1967), while the diploid numbers registered in species of the *Cryptanthus* and others such as n = 21 in *Aechmea tillandsioide*s, n = 19 in *Tillandsia leiboldiana* var. *leiboldiana*, n = 20 and 22 in *T. complanata* suggest aneuploidy decreasing from this basic number (Marchant, 1967; Brown and Gilmartin, 1989).

Polyploidy as well seems to play an important role in the chromosome evolution of the family, from the original basic number x = 25 (Brown and Gilmartin, 1989) to the diversification of some genera. Till (1984, cited in Brown and Gilmartin, 1986) observed a tendency to polyploidy in the subgenus *Diaphoranthema* (*Tillandsia*) recording 12 tetraploid species. The *Orthophytum* species showed a tetraploid karyotype, 2n = 100, and hexaploid, 2n = 150, and the only *Neoglaziovia* species analyzed had 2n = 100, while four of the six species of *Bromelia* analyzed were polyploid, with 2n = 100 and 150.

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RESUMO

Este trabalho apresenta o número de cromossomos de 17 espécies de Bromeliaceae, pertencentes aos gêneros *Encholirium, Bromelia, Orthophytum, Hohenbergia, Billbergia, Neoglaziovia, Aechmea, Cryptanthus* e *Ananas*. A maioria tem cariótipo 2n = 50, mas *Bromelia laciniosa*, *Orthophytum burle-marxii* e *O. maracasense* são poliplóides com 2n = 150, 100 e 150, respectivamente, enquanto *Cryptanthus bahianus* tem 2n = 34 + 1-4B. Cromossomos B foram observados também em *Bromelia plumieri* e *Hohenbergia* aff. *utriculosa*. O número de cromossomos de todas as espécies foi determinado pela primeira vez, exceto para *Billbergia chlorosticta* e *Cryptanthus bahianus*. Os dados obtidos reforçam a hipótese de um número básico x = 25 para a família Bromeliaceae e aneuploidia decrescente no gênero *Cryptanthus*.

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