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Chromosomal features and evolution of Bromeliaceae

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Abstract. New cytological information and chromosome counts are presented for 19 taxa of 15 genera of the Bromeliaceae, among them, data for 15 taxa and five genera are reported for the first time. The basic number x = 25 is confirmed and polyploidy seems to be the main evolutionary mechanism in Bromeliaceae. Most of the analyzed species presented 2n = 50. Polyploids have been detected in *Deinacanthon urbanianum* with 2n = ca.160 and *Bromelia laciniosa* with 2n = ca.150. In *Deuterocohnia lorentziana* we observed individuals with two different ploidy levels (2n = 50 and 2n = 100) growing together in the same pot. *Ayensua uaipanensis* showed the uncommon number 2n = 46. After triple staining with CMA_3/Actinomycin/DAPI one or two CMA^+/$DAPI^-$ bands could be observed in the studied species (*Aechmea bromeliifolia*, *Greigia sphacelata* and *Ochagavia litoralis*). The role of these features in the evolution of the family is discussed, revealing new aspects of the evolution of the Bromeliaceae.

Key words: Chromosomes, polyploidy, interphase nuclei, bimodal karyotypes, fluorochrome staining, silver staining, C banding, heterochromatin.

The Bromeliaceae comprise 58 genera with app. 2700 species and is almost exclusively confined to the neotropics. Their representatives are distributed in a wide range of habitats, from rain forests to dry savannas, campos rupestres and semi-arid regions (Smith and Till 1998). Even though they include a significant number of terrestrial species, they represent the second largest angiosperm family in number of epiphytic species (Gentry 1993, Benzing 2000).

In spite of their remarkable diversity and adaptation to different ecosystems, they show a relative conservation regarding the chromosome numbers, with a predominance of 2n = 50, representing the diploid level, with many authors suggesting x = 25 as the main base number (Marchant 1967; McWilliams 1974; Brown et al. 1984, 1997; Brown and Gilmartin 1983, 1989).

Many of the chromosome numbers initially reported by some authors (e.g. Lindschau 1933, Gauthé 1965, Weiss 1965) were carried out with the classical microtome section technique generating a variety of basic chromosome numbers which putatively supported the separation of the subfamilies Pitcairnioideae and Tillandsioideae. Many of these numbers that deviated from the base x = 25, have not been confirmed by later studies (Marchant 1967, McWilliams 1974)

and their value should be considered carefully (Brown and Gilmartin 1986).

Most previous works have been restricted to chromosome counts from meiotic cells, with only few details on karyotype architecture. Considering the relative conservation of chromosome numbers it is interesting to aggregate further cytological information as interphase nuclei structure, condensing behavior in prophase to prometaphase, chromosome size and morphology as well as banding patterns in order to assess a possible role of the heterochromatin in the karyotype evolution. The present study aims to screen different genera of Bromeliaceae regarding these cytological features in order to evaluate their usefulness for phylogenetic purposes.

Materials and methods

All species studied are listed in Table 1 with source, voucher numbers and previous reports of chromosome numbers, if any is available. Cytological observations were carried out from root tips, flower bud primordia or leaf meristems. For mitotic arrest, meristematic tissues were pre-treated with 8-hydroxiquinoline (2 mM) for 24 h and fixed in Carnoy (Ethanol:Acetic acid, 3:1). The standard chromosome preparations (HCl/Giemsa) followed the technique described by Benko-Iseppon and Morawetz (2000). C-banding was performed after Schwarzacher et al. (1980) with minor modifications and the fluorochrome staining with CMA/Actinomycin/DAPI followed the procedures described by Deumling and Greilhuber (1982) after the method developed by Schweizer (1976). Silver staining of interphase nuclei followed Hizume et al. (1980) with minor modifications.

Measurements of chromosome maximal and minimal sizes were based on drawings of 2–4 well-contracted metaphase plates of each species presented as compared with a micrometric scale. Since all species presented chromosomes of small size, a minimum of 10 good metaphase or prometaphase spreads were checked in order to identify the correct chromosome number.

Photomicrographs were taken with Kodak Technical Pan for conventional staining and with Kodak T-Max 400 for fluorochrome staining.

Results

Chromosome numbers and cytological features of the 19 analyzed taxa are presented in Table 1 and 2 and illustrated in Figs. 1 and 2. First cytological analyzes are presented for 15 taxa assigned to the three subfamilies as follows: (a) Bromelioideae (10 taxa): *Ananas nanus*, *Deinacanthon urbanianum, Fascicularia bicolor* ssp. *bicolor*, *F. bicolor* ssp. *canaliculata*, *Greigia* aff. *mulfordii* var. *micrantha*, *G. sphacelata*, *Ochagavia elegans*, *O. litoralis; Orthophytum disjunctum* (b) Pitcairnioideae (4 taxa): *Ayensua uaipanensis; Deuterocohnia lorentziana; Dyckia saxatilis, Pitcairnia atrorubens*, and *Puya mirabilis* (c) Tillandsioideae (1 taxon): *Catopsis floribunda*. For the genera *Ayensua*, *Deinacanthon*, *Fascicularia*, *Greigia* and *Ochagavia* these are the first karyological data.

Additional features as interphase nuclei structure, chromosome condensing behavior, and banding patterns (C-banding, fluorochrome staining with CMA/Actinomycin/DAPI and silver staining with $AgNO_3$) were carried out for the first time in Bromeliaceae.

All analyzed species presented semi-reticulated interphase nuclei and proximal-anterior chromosome condensing behavior during prophase to prometaphase (Table 2; Fig. 1b, c, e, m; Fig. 2b, e, g) mostly with small spheroid or rod shaped chromocenters (e.g. Fig. 1b, e) with exception of *Deuterocohnia lorentziana* that presented filamentous chromocenters (Fig. 2b).

The number $2n = 50$ was observed in 16 species (Table 1), ten belonging to subfamily Bromelioideae, four to Pitcairnioideae and two to Tillandsioideae, covering a total of 12 of the 15 genera analyzed. Higher ploidy levels could be observed in two species of Bromelioideae (*Bromelia laciniosa*, with $2n = $ ca. 150; Fig. 1d) and *Deinacanthon urbanianum* ($2n = $ ca.160; Fig. 1e). Since this last number deviates from the $x = 25$ base number, we carried out additional counts confirming this number.

Table 1. List of analyzed species including provenance, collector, voucher number and chromosome numbers (present data and previous reports). For species with more than a sigle previous chromosome count, respective authors are indicated by letters in upper case

Taxon	Source, collector and voucher number	[Chromosome numbers]		Author
		Present study	Previous reports	
Bromelioideae				
Aechmea bromeliifolia (Rudge) Baker	Wild, Brazil, Bahia, Rio de Contas, leg. Benko-Iseppon BRO-1220, det. Wanderley	2n = 50	2n = 50	Brown et al. (1997)
Ananas comosus (L.) Merrel	Cultivated, Brazil, Pernambuco, Recife, local market, leg. et det. Benko-Iseppon BRO-1246	2n = 50	2n = 48[d], 50[a,b,c,d,f], 75[b,d], 100[a], n = 25[a]	[a]Collins and Kerns (1931, 1936, 1938), [b]Bhowmik (1977), [c]Chen et al. (1985), [d]Lin et al. (1987), [e]Brown and Gilmartin (1989), [f]Cotias-de-Oliveira et al. (2000)
A. nanus (L.B. Sm.) L.B. Sm.	Cultivated, Brazil, Pernambuco, Recife, Campus UFPE; leg. et det. Gitaí BRO-001	2n = 50	–	
Bromelia laciniosa Mart. ex Schultes	Wild, Brazil, Bahia; Paulo Afonso, leg. Benko-Iseppon 21/040398, det. Wanderley	2n = ca.150	2n = ca.150	Cotias-de-Oliveira et al. (2000)
Deinacanthon urbanianum (Mez.) Mez.	Cultivated, Germany, Palmengarten of the City of Frankfurt, 98-17786-0, leg. Horst 08.09.1972, H018, det. Horres	2n = ca.160	– –	–
Fascicularia bicolor (Ruiz & Pavon) Mez. ssp. *bicolor*. E.C. Nelson & Zizka	Cultivated, Germany, Palmengarten of the City of Frankfurt, 98-16846-3, seeds from RBG Kew, FR 6a, det. Horres	2n = 50	–	–

Table 1. (Continued)

Taxon	Source, collector and voucher number	[Chromosome numbers]		Author
		Present study	Previous reports	
F. bicolor (Ruiz & Pavon) Mez. ssp. *canaliculata* E.C. Nelson & Zizka	Cultivated, Germany, Palmengarten of the City of Frankfurt, 90-17118-3, seeds from RBG Kew, FR 16a, det. Horres	2n = 50	–	–
Greigia spec. nov. (aff. *G. mulfordii* var. *micrantha*)	Cultivated, Germany, Palmengarten of the City of Frankfurt, collected in Colombia, Cundina marca, Monserrate, 04°36′035″N, 074°03'238"W, leg. Grant 30.04.1999, 99-19040-0, H157, det. Horres	2n = 50	–	–
G. sphacelata (Ruiz & Pavon) Regel	Cultivated, Germany, Palmengarten of the City of Frankfurt, collected in Chile, Province Concepción, Forest at Hualqui, 98-16855-1, H27a, det. Horres	2n = 50	–	–
Ochagavia elegans R. Philippi	Cultivated, Germany, Palmengarten of the City of Frankfurt, 98-16852-3a, II 93, seeds from RBG Kew 1987-2763, FR23a, det. Horres	2n = 50	–	–
O. litoralis (Phil.) Zizka, Trumpler & Zoellner	Cultivated, Germany, Palmengarten of the City of Frankfurt, 94-14614-3, H115, det. Horres	2n = 50	–	–
Orthophytum disjunctum L.B. Sm.	Wild, Brazil, Pernambuco, Camocin de São Félix, leg. Sales de Melo BRO-009, det. Wanderley	2n = 50	–	–

Table 1. (Continued)

Pitcairnioideae				
Ayensua uaipanensis (Maguire) L.B. Sm.	Cultivated, Germany, Palmengarten of the City of Frankfurt, collected in Venezuela, Auyantepui, 2000 m N.N.; leg. Bütschi (Bern) 1992, 92-9510-2, H011, det. Horres	2n = 46	–	–
Deuterocohnia lorentziana (Mez) Spencer & L.B. Sm.	Cultivated, Germany, Palmengarten of the City of Frankfurt, s.no., ex B.G. Heidelberg (130007), det. Horres	2n = 50, ca. 100	–	–
Dyckia saxatilis Mez.	Wild, Brazil, Bahia; Rio de Contas, leg. Benko-Iseppon BRO-1228, det. Wanderley	2n = 50	–	–
Pitcairnia atrorubens (Beer) Baker	Cultivated, Germany, Palmengarten of the City of Frankfurt, collected in Costa Rica, 89-16095-2, 20.07.1988 grown from seeds by Billensteiner, det. Horres	2n = 50	–	–
Puya mirabilis (Mez.) L.B. Sm.	Cultivated, Germany, Palmengarten of the City of Frankfurt, 86-418-1-3, det. Horres	2n = 50	–	–
Tillandsioidae				
Catopsis floribunda L.B. Sm.	Cultivated, Germany, Palmengarten of the City of Frankfurt, s.no., det. Horres	2n = 50	–	–
Tillandsia dodsonii L.B. Sm.	Cultivated, Germany, Palmengarten of the City of Frankfurt, 90-9649-4-2, det. Horres	2n = 50	n = 25, 2n = 50	Brown and Gilmartin (1989), Brown et al. (1984, 1997)

Table 2. Cytogenetic features of the Bromeliaceae analyzed as evaluated from conventional, fluorochrome, and $AgNO_3$ staining of mitotic cells in different stages. Legend: – = not studied; ca. = approximate number

Species	2n	Chromos. maximal sizes (μm)	Chomocenters of Interphase nuclei		Number of chromosome pairs with		Maximal number of nuclei with $AgNO_3$ staining	Karyotype Architecture
			Form	Distribution	CMA^+ /$DAPI^-$ terminal bands	Satellites		
Bromelioideae								
Aechmea bromeliifolia	50	2.72–1.36	Small, spheroid or rod shapped	Irregular	2	–	4	decreasing size
Ananas comosus	50	1.38–0.83	Small, mostly spheroid	Regular	–	–	–	± homogeneous size
Ananas nanus	50	1.6–1.1	Small, spheroid to dot-like	Regular	–	–	–	± homogeneous size
Bromelia laciniosa	ca.150	1.25–0.25	Small, mostly spheroid	Irregular	–	–	–	± homogeneous size
Deinacanthon urbanianum	ca.160	1.6–0.5	Spheroid to rod shapped	Regular	–	–	–	± homogeneous size
Fascicularia bicolor ssp. *bicolor*	50	1.47–0.59	Small, mostly spheroid	Regular	–	–	–	decreasing size
Fascicularia bicolor ssp. *canaliculata*	50	2.06–0.88	Small, mostly spheroid to dot-like	Regular	–	–	–	± homogeneous size
Greigia spec. nov. (aff. *G. mulfordii* var. *micrantha*)	50	1.14–0.57	Small, spheroid or rod shapped	Irregular	–	2	–	–
Greigia sphacelata	50	1.71–0.86	Small, spheroid or rod shapped	Irregular	1	–	–	decreasing size
Ochagavia litoralis	50	2.61–1.18	Small, spheroid or rod shapped	Regular to polarized	1	–	–	decreasing size
Ochagavia elegans	50	1.38-0.83	Small, mostly spheroid	Regular	–	–	–	± homogeneous size

Table 2. (Continued)

Orthophytum disjunctum	50	1.61–0.97	Small, mostly spheroid	Regular	–	–	–	–
Pitcairnioideae								
Ayensua uaipainensis	46	1.62–0.81	Spheroid or rod shapped	Regular to polarized	–	–	–	–
Deuterocohnia lorentziana (= *Abromeitiella lorentziana*)	5	2.29–1.14	Large, filamentous, cloudy-like	Irregular	–	–	–	Bimodal, with 6 small and 19 larger pairs, mainly submeta- to metacentric
	ca.100	1.94–0.5	Small, spheroid or rod shapped	Irregular	–	2	–	Bimodal, with 1 larger pair
Dyckia saxatilis	50	2.25–1.25	Small, spheroid to threaded	Irregular	–	–	2	± homogeneous size
Pitcairnia atrorubens	50	1.43–1.14	Small, spheroid or rod shapped	Regular	–	–	–	–
Puya mirabilis	50	1.71–0.86	Mostly dot-like	Irregular	–	–	–	Bimodal, with 2 pairs of larger chromosomes
Tillandsioideae								
Catopsis floribunda	50	1.43–0.86	Small, spheroid to threaded	Regular	–	–	–	–
Tillandsia dodsonii	50	1.71–0.86	Small, rod shapped to threaded	Regular	–	–	–	± homogeneous size

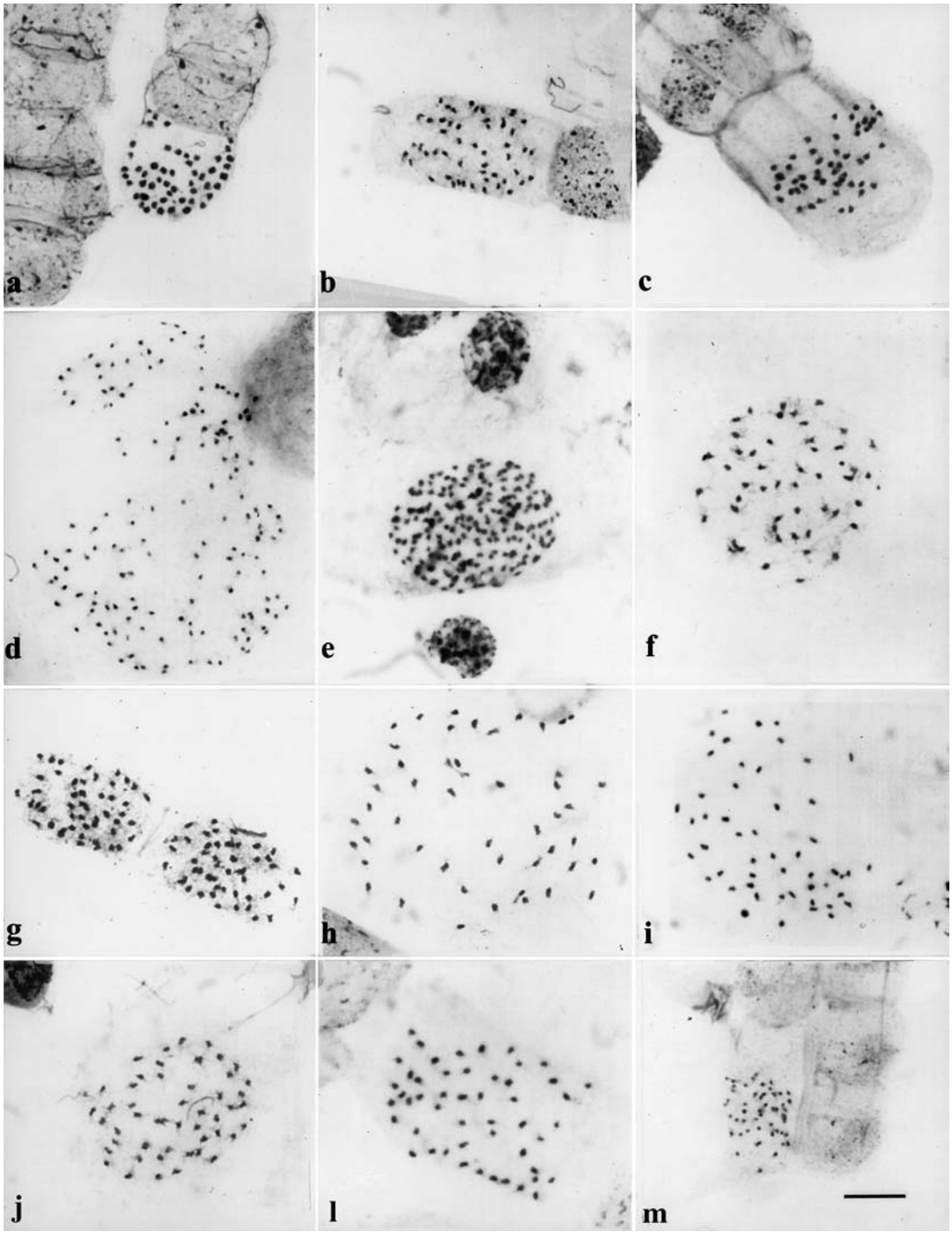

Fig. 1. Mitotic chromosomes and interphase nuclei in Bromeliaceae. **a** *Aechmea bromeliifolia* (2n = 50); **b–c** *Ananas comosus* and *A. nanus* (both 2n = 50); **d** *Bromelia laciniosa* (2n = 150); **e** *Deinacanthon urbanianum* (2n = ca.160); **f–g** *Fascicularia bicolor* ssp. *bicolor* and *F. bicolor* ssp. *canaliculata* (both 2n = 50); **h–i** *Greigia* spec. nov. and *G. sphacelata* (both 2n = 50); **j–l** *Ochagavia elegans* and *O. litoralis* (both 2n = 50); **m** *Orthophytum disjunctum* (2n = 50). Bar (in **m**) corresponds to 10 μm

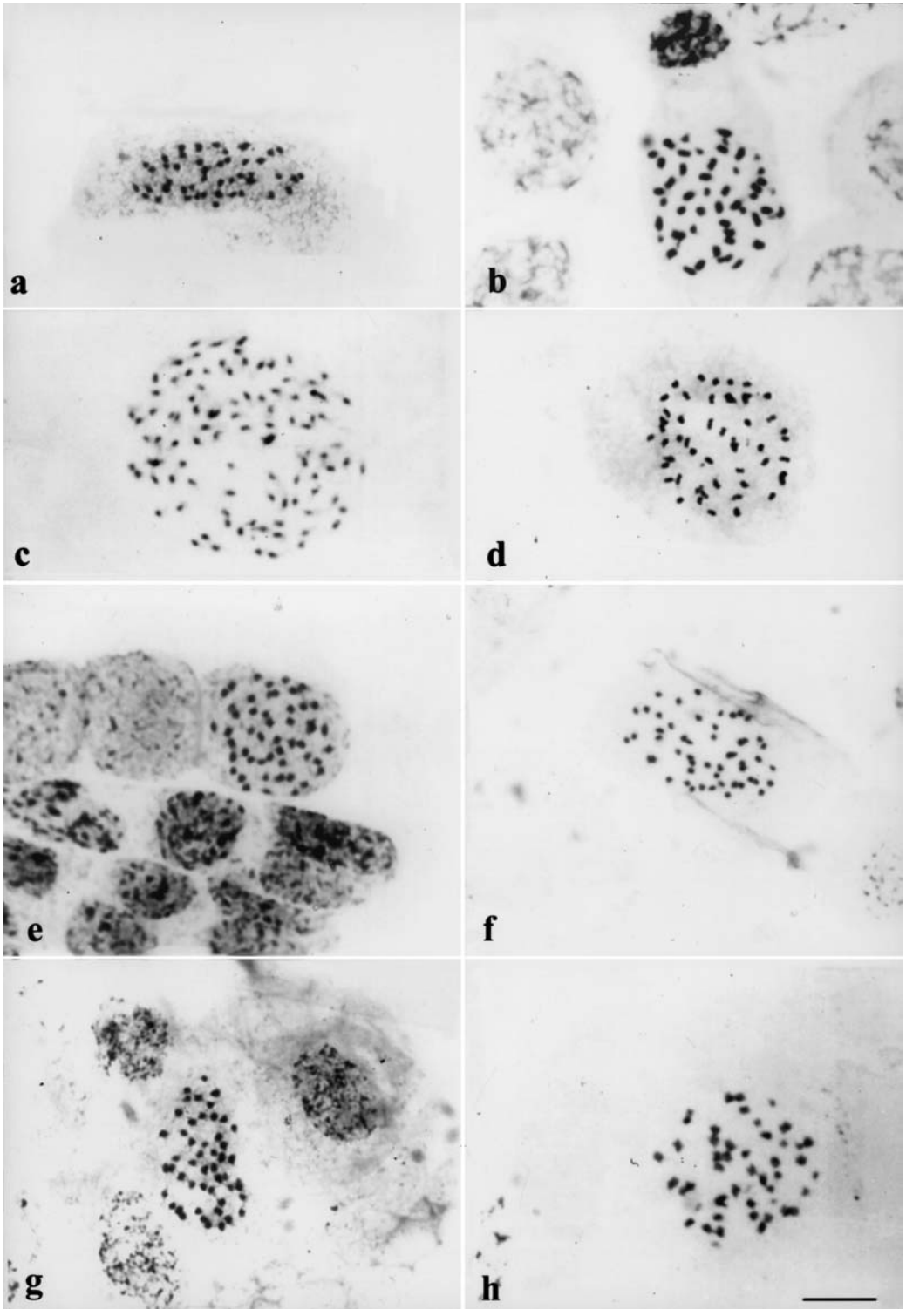

Fig. 2. Mitotic chromosomes and interphase nuclei in Bromeliaceae. **a** *Ayensua uaipansensis* (2n = 46); **b–c** *Deuterocohnia lorentziana* (2n = 50 and 2n = 100 respectively); **d** *Dyckia saxatilis* (2n = 50); **e** *Pitcairnia atrorubens* (2n = 50); **f** *Puya mirabilis* (2n = 50); **g** *Catopsis floribunda* (2n = 50); **h** *Tillandsia dodsonii* (2n = 50). Bar (in **h**) corresponds to 10 μm

A single case of polyploidy was found for the subfamily Pitcairnioideae in some individuals of *Deuterocohnia lorentziana*. It is remarkable that in different individuals of this species growing side by side in pods two different chromosome numbers ($2n = 50$ or $2n = 100$; Fig. 2b-c) could be observed. Still more surprising both genomes had quite different chromosome sizes and morphology, with the diploids bearing larger chromosomes (between 2.29 and 1.14 μm) and a tendency to bimodality, with 19 larger and 6 smaller chromosome pairs (Fig. 2b) while the tetraploids presented smaller chromosomes (1.94–0.5 μm) with only two pairs of larger chromosomes (Fig. 2c). We carried out additional collection and obtained the same results. The individuals belong to the same species and could not be distinguished morphologically.

For *Ayensua uaipanensis* we found $2n = 46$ (Fig. 2a), but the remaining cytological features are similar to that of the other Bromeliaceae, with small chromosomes (1.62–0.81 μm) and similar interphase nuclei structure and chromosome condensing behavior.

All studied species had chromosomes of small size, with lengths varying between 2.72 and 0.5 μm. The larger chromosomes were observed in *Aechmea bromeliifolia* (2.72–1.36 μm; Fig. 1a) and the smaller in *Greigia* aff. *mulfordii* var. *micrantha* (1.14–0.57 μm; Fig. 1h), both with $2n = 50$, followed by *Bromelia laciniosa* ($2n = ca.150$) with sizes between 1.25 and 0.25 μm (Fig. 1d).

Most species presented chromosomes of similar sizes (i.e. regular karyotypes), as shown in *Ananas comosus* (Fig. 1b), *A. nanus* (Fig. 1c), *Fascicularia bicolor* ssp. *bicolor* (Fig. 1f) and *O. litoralis* (Fig. 1l).

After C banding in two species (*Deinacanthon urbanianum* and *Dyckia saxatilis*) no heterochromatic bands could be observed (data not shown). On the other hand, the triple staining with CMA/Actinomycin/DAPI revealed one to two pairs with $CMA^+/DAPI^-$ terminal (or subterminal) bands in three members of the Bromelioideae analyzed: *Aechmea bromeliifolia* (2 pairs; Fig. 3e–f); *Greigia sphacelata* (1 pair; Fig. 3a–b) and in *Ochagavia litoralis* (1 pair; Fig. 3c–d). Considering the small chromosome sizes and in order to increase C band resolution, a combination of C banding and CMA/DAPI staining was performed in *Aechmea bromeliifolia* (in this study the species with larger chromosomes) but only faint DAPI bands could be observed in few cells (data not shown), maybe due to differential condensing chromosome regions in late prometaphase.

Silver staining in interphase nuclei was carried out for two species (*Aechmea bromeliifolia* and *Pitcairnia atrorubens*) revealing a maximal number of four nucleoli in the first and two in the second species. Two pairs of satellited chromosomes could be observed in *Greigia* aff. *mulfordii* var. *micrantha* and also in *Dyckia saxatilis*.

This is the first report presenting photomicrographs of mitotic chromosomes in Bromeliaceae. Due to the small size, the karyotype architecture was described only for the species with good spreads of condensed metaphase chromosomes. General features observed are presented in Table 2. All members of subfamily Bromelioideae analyzed showed symmetric karyotypes with chromosomes of regular size, similar to the only Tillandsioideae studied (*Tillandsia dodsonii*). It is noteworthy that of four Pitcairnioideae studied, only a single species presented regular karyotypes (chromosomes with similar sizes), with the remaining three presenting a tendency to bimodality (Table 2).

Discussion

Considering the similarities regarding the interphase nuclei structure, the condensing behavior and the chromosome sizes, Bromeliaceae can be considered a natural group.

As shown in Table 1, there were previous counts for only four of the 19 taxa analyzed in the present work. Our results confirmed previous reports of Brown et al. (1997) for *Aechmea bromeliifolia* ($2n = 50$) and of Brown and Gilmartin (1989) and Brown et al. (1997) for *Tillandsia dodsonii* ($n = 25$; $2n = 50$). Our

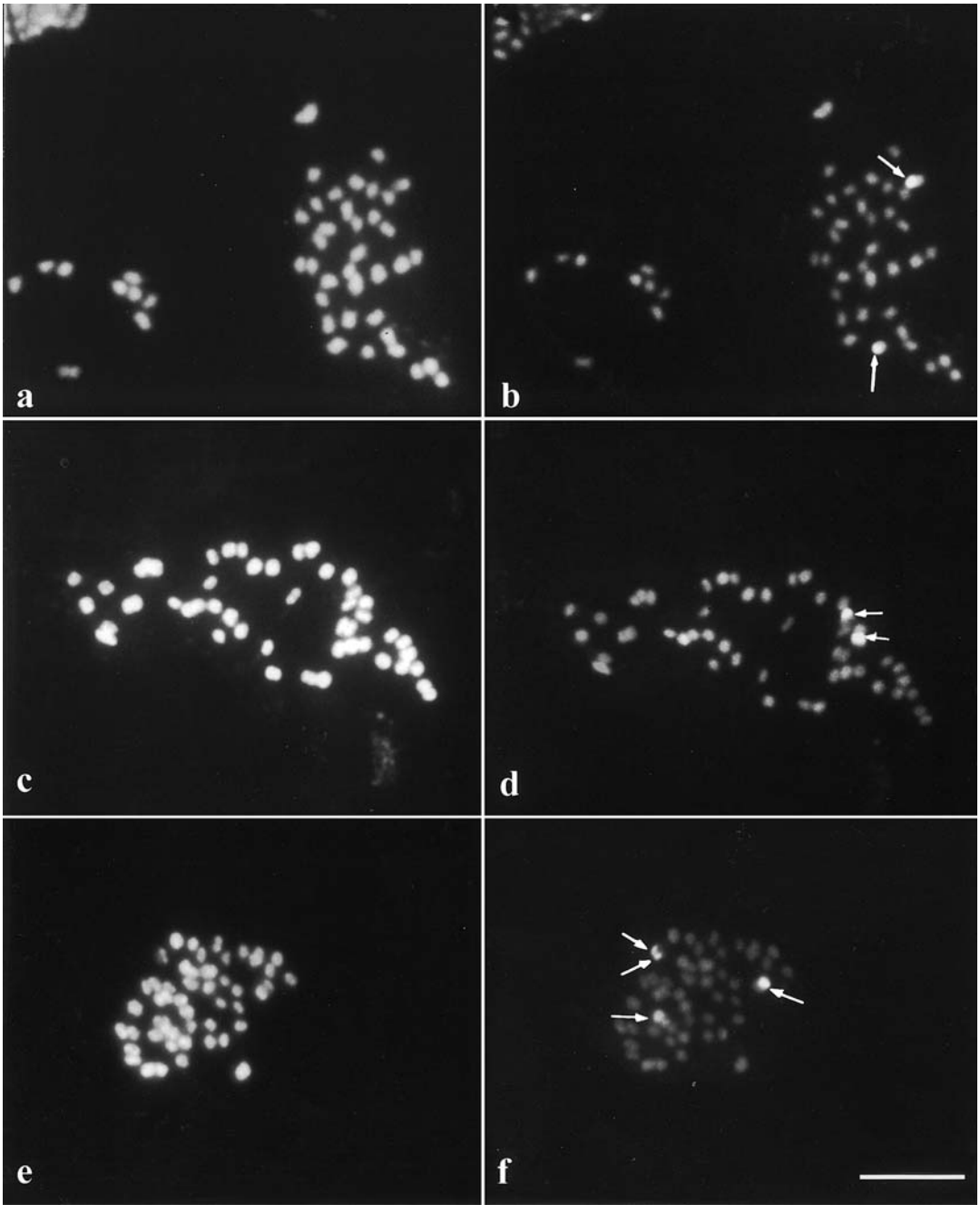

Fig. 3. Metaphase chromosomes of Bromeliaceae after sequential staining with the fluorochromes DAPI/AMD/CMA_3. **a–b** *Greigia sphacelata*; **c–d** *Ochagavia litoralis*; **e–f** *Aechmea bromeliifolia*. Arrows indicate CMA^+ bands. Bar (in **f**) corresponds to 10 μm

observation of small chromosomes of similar sizes in *Bromelia laciniosa* with 2n = ca.150 also confirms the single counting for this species carried out in another Brazilian population collected in Bahia (Cotias-de-Oliveira et al. 2000).

For almost all ten genera with previous reports we found similar chromosome num-

bers as reported for related species, with prevalence of $n = 25/2n = 50$. Deviating numbers have been all generated by the classical microtome section technique (e.g. Billings 1904, Lindschau 1933, Matsuura and Suto 1935, Gauthé 1965, Weiss 1965) used by most of the researchers up to 1965. After review of all chromosome numbers reported in Bromeliaceae we observed that most of the uncommon numbers reported by these authors were not confirmed in later studies, suggesting that they should be considered carefully. This is serious when considering that of the about 220 species previously studied, around 80 are referred only in studies published before 1965, revealing that recounts for this family are as important as new chromosome counts.

The genus *Ananas* showed a uniform karyotype with regular chromosomes in both species analyzed (*A. comosus* and *A. nanus*, both with $2n = 50$). Tetraploid specimens ($2n = 100$) have been reported in *A. macrodontes* (= *Pseudananas sagenarius* (Arruda) Camargo), while triploids ($2n = 75$) and tetraploids ($2n = 100$) have also been found in *A. comosus* (Collins and Kerns 1931, 1936, 1938; Lin et al. 1987). A single case of aneuploidy in *A. ananassoide*s was published with $2n = 48$ (Lin et al. 1987), maybe due to crossing experiments for breeding purposes.

Ayensua uaipanensis, a monotypic genus currently placed in Pitcairnioideae (Smith and Till 1998) presented $2n = 46$ chromosomes, that disagrees with the basic number $x = 25$ found in most Bromeliaceae. This species is native to two Tepuis of the Guayana Highlands and is also characterized by particular leaf anatomical features (Horres and Zizka 1995). It is notable that *Ayensua* was first placed in Velloziaceae (Maguire and Wurdack 1957) before being identified to be a bromeliad (Ayensu 1969, Smith 1969). Recently molecular analyses of *trn*L intron sequences of the chloroplast genome (Horres et al. 2000) positioned *Ayensua* together with *Brocchinia* as a basal clade with sister group relationship to the remainder of the family. The subfamilial position of the genus *Brocchinia* has also been discussed. *Brocchinia* is the only genus of the monophyletic tribe Brocchinieae (Varadarajan and Gilmartin 1988), and is currently placed in the Pitcairnioideae (Smith and Till 1998), even though this species display some characters typical for the subfamily Tillandsioideae (Benzing 2000). The single count reported for this genus was carried out by Oberprieler and Vogt (1993) that reported $2n = 18$ for *B. cinerea* (Delile) Vis. Unfortunately we were not able to analyze cytogenetic features of *Brocchinia* up to now. Our results for *Ayensua* reveal that despite the contrasting chromosome numbers remaining features as chromosome size, condensing behavior and the interphase nuclei features are in accordance with those observed in other Bromeliaceae. On the other hand, these features are also present in other monocot families like Velloziaceae (Franklin-de-Melo et al. 1997) and Xyridaceae (Benko-Iseppon and Wanderley 2002). Molecular data clearly place *Ayensua* within *Brocchinia* (Horres et al. 2000, Horres et al. in print, Givnish et al. in print) so that the study of cytological features within *Ayensua*/*Brocchinia* is a very interesting case study for evolutionary trends within Bromeliaceae.

The observation of two different ploidy levels in morphologically undistinguishable individuals of *Deuterocohnia lorentziana* ($2n = 50, 150$; growing side by side in the same pot) is surprising, especially considering the differences in chromosome sizes and morphology (Table 2). While the diploids presented larger chromosomes (between 2.29 and 1.14 μm) and a tendency to bimodality (19 larger and 6 smaller chromosome pairs) the tetraploids presented smaller chromosomes (1.94 – 0.5 μm) with only two pairs of larger chromosomes. These observations suggest that some sequences were eliminated from the genome during the process of polyploidization, a phenomenon previously observed in other families of Angiosperms (e.g. Souza and Benko-Iseppon 2004). Evidences of sequence elimination during polyploidization processes have been shown before, suggesting that polyploidy-induced sequence elimination is a directed, non

random process (Feldman et al. 1997, Liu et al. 1998). Since the plants analyzed in this study have been grown from seeds, one cannot exclude the possibility of interspecific out crossing in the genesis of the polyploidization event, reinforcing the importance of hybridization in the evolution of Bromeliaceae.

Considering the general features of the karyotypes that represent different ploidy levels in Bromeliaceae (in the present work the three levels $2n = 50$, $2n = 100$ and $2n = 150$–160 could be observed) regarding chromosome sizes two trends may be identified: (I) generally larger chromosomes in lower ploidy levels and smaller in higher ploidy levels and (II) lower contrast between maximal and minimal chromosome sizes in polyploids than in diploids. Taking these evidences into account one may suppose that some chromatin elimination occurred in the course of polyploidization events during the evolution of Bromeliaceae. This hypothesis should be evaluated with a larger number of species in the future.

No B chromosomes have been observed in our study, even though Cotias-de-Oliveira et al. (2000) have reported their observation, as in *Cryptanthus bahianus* L.B. Sm. ($2n = 34+1-4B$) and *Hohenbergia* aff. *ulticulosa* Ule ($2n = 50+2B$).

Marchant (1967) observed two groups of chromosomes of different sizes in some species, characterizing bimodal karyotypes. In our study some discrete bimodal karyotypes have been observed as in *Deuterocohnia lorentziana* ($2n = 50$). Studies of karyotype in Bromeliaceae revealed bimodality especially in Tillandsioideae (genera *Tillandsia* and *Vriesea*), and also as a tendency in Bromelioideae (Brown and Gilmartin 1986, 1989). In our study bimodal karyotypes were observed in two species of Pitcairnioideae (*Deuterocohnia lorentziana* and *Puya mirabilis*; Table 2), but not in the studied members of Bromelioideae and Tillandsioideae. An evaluation of a large number of species from all three subfamilies should be carried out in order to evaluate if this feature could represent an evolutionary trend specific to a given subfamily, as suggested before.

Since the Bromeliaceae are conservative regarding chromosome base numbers, an evaluation of heterochromatin evolution could be helpful to the understanding of infrafamilial relationships.

After the triple staining with CMA_3/Actinomycin/DAPI one to two $CMA^+/DAPI^-$ bands could be observed in the three studied species (see Table 2) probably corresponding to the NOR-associated heterochromatin. No additional C bands could be observed in *Deinacanthon urbanianum* and *Dyckia saxatilis*. Also the attempt to associate C banding with CMA/DAPI staining, performed in *Aechmea bromeliifolia* (the species with larger chromosomes in the present study) revealed no heterochromatic bands. These evidences suggest that Bromeliaceae are relatively poor in heterochromatin, a typical feature of plants with small chromosome sizes. This is also confirmed by the observations of interphase nuclei structure, with most species presenting quite small chromocenters.

A maximum number of two satellited chromosome pairs was observed in *Greigia* aff. *mulfordii* var. *micrantha* and in the diploid individuals of *Deuterocohnia lorentziana*. The silver ($AgNO_3$) staining also confirmed the assumption that $CMA^+/DAPI^-$ bands correspond to the NORs. This method stains the nucleoli of the NO-chromosomes that are active in the latest interphase, and revealed a maximum of four nuclei for *Dyckia saxatilis* (also with two pairs with $CMA^+/DAPI^-$ terminal bands). It is assumed that each chromosome set originally presented a single pair of NO-chromosomes (Stebbins 1971), but this number normally increases during polyploidization. The presence of two pairs of such chromosomes may confirm the theory that the Bromeliaceae are paleopolyploids with base chromosome number $x = 25$ that probably evolved from hybridization of species with lower ploidy levels not available anymore (Brown and Gilmartin 1989, Brown et al. 1997).

A very frequent cytological trend in angiosperms is dysploidy. It has been defined as a change in chromosome number due to structural rearrangements, with occurrence in many other monocotyledonous families (Franklin-de-Melo et al. 1997, Guerra 2000, Benko-Iseppon and Wanderley 2002). The available chromosome data for Bromeliaceae reveals a high importance of polyploidy in the evolution of the family with an almost complete lack of dysploid changes. Additionally the prevalence of the number $2n = 50$ (and its relative genome size) may represent the ideal (i.e. most successful) character combination for this family, indicating that hybridization events resulted mainly in individuals that maintained the same ploidy level as the parental species in the course of species diversification within this family.

Recently the reproductive biology of hybrid bromeliads was studied in natural populations of *Pitcairnia* species, with hybrids bearing intermediate features relatively to the parents (Wendt et al. 2001). The genetic variability was also investigated using isoenzymatic polymorphisms in genotypes of *Pitcairnia geykesii* revealing a high diversity level within species distributed in different populations of inselbergs (Sarthou et al. 2001). The karyological evaluation of such natural hybrids, including modern methods as GISH (genomic *in situ* hybridization) could bring interesting evidences for the understanding of chromosome speciation processes within this family.

In view of the presented features and up to date literature data, the karyological evolution in Bromeliaceae can be summarized by: (i) conservation regarding interphase nuclei structure and chromosome condensing behavior; (ii) limited chromosome number diversification with dominance of $2n = 50$, with few exceptions; (iii) minute chromosomes with low amount of heterochromatin; (iv) presence of one to two pairs of NOR-bearing chromosomes in species with $2n = 50$, suggesting that this ploidy level corresponds to the original tetraploid level; (v) presence of bimodal karyotypes in some diploid species ($2n = 50$) with polyploids ($2n = 100$, 150, 160) showing smaller chromosomes of similar sizes.

The present work shows that, despite the importance of chromosome numbers, the aggregation of additional karyological features in the evaluation of Bromeliaceae may bring very interesting evidences about the phylogenetic relationships of its members and should be considered as a useful tool for the understanding of evolutionary trends in the family.

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