Pl. Syst. Evol. 204: 257–273 (1997)

Plant Systematics and Evolution

Cytogenetics and cytotaxonomy of *Velloziaceae*

NATONIEL FRANKLIN DE MELO, MARCELO GUERRA, ANA MARIA BENKO-ISEPPON, and NANUZA LUIZA DE MENEZES

Received December 12, 1995; in revised version, February 5, 1996

Key words: *Velloziaceae*. – Cytotaxonomy, mitotic chromosomes, interphase nuclei, heterochromatin, condensed euchromatin, C-banding, fluorochrome staining.

Abstract: Chromosome number and other cytological features are reported from 35 species of *Velloziaceae*, including several African and Brazilian populations. All analyzed species show areticulate interphase nuclei and prophase/prometaphase chromosomes with proximal early condensation. Most heteropycnotic blocks do not seem to correspond to heterochromatin since, at least in *Vellozia patens*, they do not stain differentially after C-banding procedures. Regarding the chromosome number, three main groups could be identified. The first comprised diploid species of the genera *Nanuza*, *Vellozia* and the Brazilian species of *Xerophyta* with $2n = 14$ or 16; the second comprised tetraploid species with $2n = 34$, and included all Brazilian species of subfam. *Barbacenioideae*; the third group, of hexaploid species, comprised the African representatives of the genus *Xerophyta*. A single population of *Vellozia*, possibly of hybrid origin, had $2n \cong 32$. A basic number of $x = 8$ is proposed for the family. The karyological information supports the hypothesis that the *Velloziaceae* originated on the South American, rather than on the African continent.

The *Velloziaceae* constitute a tropical monocot family composed of ca. seven genera, six of them endemic to South America and one (*Xerophyta*) occurring also in Africa. In spite of the small size of the family (ca. 250 spp.), there is no agreement about the delimitation of its genera and subfamilies. As reviewed by MELLO-SILVA (1991), two main taxonomic systems remain under discussion. MENEZES (1971, 1980, 1988) and MENEZES & SEMIR (1991) divided the *Velloziaceae* into the subfam. *Barbacenioideae* (*Aylthonia* N. MENEZES, *Barbacenia* VAND., *Burlemarxia* MENEZES & SEMIR and *Pleurostima* RAF.) and *Vellozioideae* (*Vellozia* VAND. and *Xerophyta* JUSS.). SMITH & AYENSU (1976), while accepting these subfamilies, proposed a different composition (*Barbacenioideae* = *Barbacenia*, *Barbaceniopsis* L. B. SMITH, *Talbotiopsis* L. B. SMITH & AYENSU and *Xerophyta*, while *Velloziodeae* = *Nanuza* L. B. SMITH & AYENSU and *Vellozia*).

In the system of SMITH & AYENSU (1976), all four genera of MENEZES's subfam. *Barbacenioideae* are included in the genus *Barbacenia*. On the other hand, in the system of MENEZES (1971, 1980, 1988), the three genera *Barbaceniopsis*, *Talbotia* and *Nanuza* are included in the genus *Xerophyta*. Recently, SMITH agreed that

Talbotiopsis is an earlier valid name for the monospecific *Talbotia* and MENEZES accepted the monospecific genus *Nanuza* (see MELLO-SILVA 1991).

Previous observations on chromosome numbers in this family were reported by STENAR (1925), GOLDBLATT (1980) and GOLDBLATT & POSTON (1988). These latter authors analyzed the chromosome number of 16 species (6% of the total of *Velloziaceae*) belonging to four genera. The results suggested that a cytotaxonomic analysis would be very promising to clarify the suprageneric taxonomy of the family.

In the present work, we report chromosome counts for 33 other species and two recounts, including representatives of six genera. Further cytogenetic features are described as a contribution to understand the cytotaxonomic relationships of the family. The taxonomic treatment followed here is that of MENEZES (MENEZES 1971, 1980, 1988; MENEZES & SEMIR 1991, MENEZES & al. 1994) and the results will be compared later in both taxonomic systems.

Material and methods

Root tips of *Xerophyta elegans* (BALF.) N. MENEZES [= *Talbotia elegans* (BALF.) BAKER] were collected from a cultivated specimen growing in a greenhouse of the Botanical Garden of the University of Vienna, Austria. All remaining specimens were collected in the field. Some are cultivated at the Botanical Department of the Biosciences Institute of the University of São Paulo, Brazil. The voucher herbarium specimens are deposited at the herbarium of the University of São Paulo (SPF). The localities of collection, herbarium voucher numbers and chromosome numbers are listed in Table 1.

Young root tips from seedlings or from adventitious roots growing under the sheaths and stem tips or apical meristems from young leaf buds were pretreated with 8-hydroxyquinoline at 12 °C for 5–24 h and fixed in Carnoy 3:1. Several other anti-mitotic pre-treatments (colchicine, a-bromonaphtalene, para-diclorobenzene) were tested without giving better results. Young flower buds were collected inside and outside the sheaths and fixed in Carnoy.

Conventional chromosome staining with Giemsa and C-banding proceeded as described by GUERRA (1983) and SCHWARZACHER & al. (1980), respectively. Chromosome double-staining with the fluorochromes chromomycin-A (CMA) and 4′–6-diamidino-2- phenylindole (DAPI) was carried out as described by DEUMLING & GREILHUBER (1982). In some species the chromosome sizes were estimated from the negative films of the best five metaphases using a micrometric scale of the same enlargement. Chromocentre size and number were also estimated using a sample of 25 to 50 cells at typical interphase stage. Photomicrographs were taken with Agfa Copex Pan film for visible light and Kodak Tri-X Pan film for ultraviolet light.

Results

During the first two years of this work, we analyzed only root tips from young seedlings – traditionally the best material for mitotic chromosomal analysis. However, in the *Velloziaceae* the root meristem in young seedlings is very small and hence presents only a few cells suitable for chromosome counts. More recently, we have analyzed young tips of adventitious roots and young leaf buds with better results. It was noteworthy that nuclei and chromosome size seemed to be larger in the meristem of adventitious root tips than in the young seedlings. This was more evident when we compared slides from both meristems of a single species.

The nuclear chromatin structure was areticulate in all analyzed species (see, e.g., Figs. 1c, 2a–b and 3c), although slightly different within each ploidy level. The chromatin reticulum was slightly more stainable in some diploid and tetraploid species than in the hexaploid ones. In the meristematic nuclei of some tetraploid species, like *Burlemarxia pungens*, the chromocentres were sometimes irregularly shaped, with a slightly stained chromatin reticulum, trending to a semi-reticulate nuclear structure (Fig. 2a).

The cytological analysis permitted the recognition of three groups of species: diploids, tetraploids and hexaploids (Table 1).

The diploid group comprised 19 species belonging to the genera *Vellozia*, the monospecific *Nanuza* and the South American species of *Xerophyta* (See Table 1). Representative prophases and metaphases of some diploid species are shown in Fig. 1. Most species of this group presented $2n = 14$ or 16. Despite the low chromosome number of the diploids, it was sometimes very difficult to establish, if the correct number was $2n = 14$ or $2n = 16$. Most diploid species presented one or two pairs of relatively large satellites which at metaphase might easily be misinterpreted as being small chromosomes (Fig. 1a, e, f). On the other hand, during early prophase, most of the chromosomes showed large heteropycnotic proximal blocks whereas the "smallest chromosomes" or satellites were equally very decondensed, becoming sometimes almost unstainable (Fig. 1g). However, at early and late metaphase the satellites were highly condensed and often not contiguous with the remainder of the chromosome arms (Fig. 1a). In *Vellozia variegata* it was not possible to determine, if the correct number was $2n = 16$ with one satellited chromosome pair or $2n = 14$ with two pairs of satellited chromosomes. In *Vellozia glabra* we counted $2n = 16–18$ in most metaphases and $2n = 14$ plus four very weakly stained small chromosomes or satellites in most prophases (Fig. 1f–g). Since we could not with certainty assert that the four smallest structures in $2n = 18$ cells were satellites, we prefer to report this count as $2n = 14–18$. When only very small satellites were present, e.g., in *X. minima* ($2n = 14$), there was no doubt about the chromosome number (Fig.1c).

In the diploid species the morphology of the chromosomes showed a median to submedian position of the centromere in most of them (Fig.1d–f). They clearly showed conspicuously the largest chromosome size and the largest karyotype asymmetry in the family. The largest chromosome complement of all species was observed in *Nanuza plicata* (Fig. 1d–e). Chromosome measurements in *Xerophyta minima* and *Vellozia* aff. *patens* showed similar sizes although slightly larger in the former. In most interphase cells of both species there were 11 to 13 chromocentres with an average size of 1.04μm (Table 2).

The prophase chromosomes of all *Velloziaceae* species showed deeply stained proximal blocks, although they were especially large in the diploid ones (Fig. 1c, d, g). In *Nanuza plicata*, the condensing pattern of the prophase chromosomes was highly differentiated, allowing the identification of almost all chromosome pairs of the complement (Fig. 1d). The only species analyzed with C-banding, the diploid *Vellozia patens*, showed only dot-like proximal C-bands and some sub-terminal larger, but weaker bands (Fig. 1h). The latter seemed to represent the NOR-associated heterochromatin as judging from their number and chromosomal location. The CMA/DAPI staining of the chromosomes of *V. nivea* revealed the absence

Table 1. List of the taxa analyzed with respective localities, herbarium vouchers, chromosome numbers and previous counts. *GP* GOLDBLATT & POSTON (1988) *ST* STENAR (1925)

Taxon	Provenance, voucher number, collector	Chromosome number		Previous counts
		2n	n	
Aylthonia graminifolia (L. B. SMITH) N. MENEZES, comb. nov. ined.	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, CFSC 11167, PRADO & al.	34	–	–
A. pulverulenta (L. B. SMITH & AYENSU) N. MENEZES	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, CFSC 11234A, MENEZES & al.	34	–	–
Aylthonia sp. nova	Brazil, Minas Gerais, Itambé do Mato Dentro, CFCR 2832, MENEZES & al.	34	–	–
Barbacenia aff. *albiflora* L. B. SMITH	Brazil, Minas Gerais, Biri-Biri, Diamantina.	–	17	GP
B. coronata P. RAVENNA	Brazil, Minas Gerais, Pico Itambé.	–	17	GP
B. globata GOETHARD & RENHARD	Brazil, Minas Gerais, Conselheiro Mata.	–	17	GP
B. paranaensis L. B. SMITH [= *Aylthonia paranaensis* (L. B. SMITH) N. MENEZES]	Brazil, Minas Gerais, Diamantina, Serra do Espinhaço.	–	17	GP
Burlemarxia pungens N. MENEZES & SEMIR	Brazil, Minas Gerais, main road from Diamantina to Conselheiro Mata, CFCR 12323, PIRANI & al.	34	–	–
B. spiralis (L. B. SMITH & AYENSU) N. MENEZES & SEMIR	Brazil, Minas Gerais, Extração, CFCR 12295, MENEZES & CASTRO–SOUZA.	34	–	–
Nanuza plicata (MART.) L. B. SMITH & AYENSU	Brazil, 2 populations: 1. Rio de Janeiro, Niterói, 13466, MENEZES; 2. Minas Gerais, Diamantina, 237, BENKO–ISEPPON.	16	–	–
Pleurostima fanniei N. MENEZES	Brazil, Rio de Janeiro, Santa Maria Madalena, SPF 33.990, MENEZES.	34	–	–
P. inclinata (GOETHARD & HENRARD) N. MENEZES	Brazil, Minas Gerais, Conselheiro Mata, CFCR	34	–	–

	12322, MENEZES & CASTRO–SOUZA.			
P. longiscapa (GOETHARD & HENRARD) N. MENEZES	Brazil, Minas Gerais, main road from Diamantina to Conselheiro Mata, CFCR 11910, MENEZES & al.	34	–	–
P. nuda (L. B. SMITH & AYENSU) N. MENEZES, comb. nov. ined.	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, CFSC 11614, MENEZES & CASTRO–SOUZA.	34	–	–
P. purpurea (HOOKER) RAF.	Brazil, Rio de Janeiro, Pedra da Gávea, SPF 635, MENEZES.	34	–	–
P. riparia N. MENEZES & MELLO-SILVA	Brazil, Minas Gerais, Grao Mogol, CFCR 8400, ZAPPI & al.	34	–	–
P. rogieri (MOORE & AYRES) N. MENEZES, comb. nov. ined.	Brazil, Rio de Janeiro, Mangaratiba, near the main road Rio-Santos, 227, MENEZES.	34	–	–
Vellozia alata L. B. SMITH	Brazil, Minas Gerais, 3 km N of Chapeado Sol, Serra do Cipó.	–	7	GP
V. bahiana L. B. SMITH & AYENSU	Brazil, Bahia, Medina, SP-110129, W. MAIA.	14	–	–
	Brazil, Bahia, exact locality unknown.	–	8	GP
V. aff. *candida* MIKAN	Brazil, Rio de Janeiro, Niterói, 1230, MENEZES.	14	–	–
V. candida MIKAN	Brazil, Rio de Janeiro, Morro da Urca, Pedra da Gávea, 1232, MENEZES.	14–16	–	–
V. caruncularis MART. ex SEUBERT	Brazil, Minas Gerais, 9 km W of Serro Cerrado.		7	GP
V. compacta MART. ex SEUBERT	Brazil, Minas Gerais, 27 km W of Serro.		8	GP
V. crassicaulis MART. ex J. A. & J. H. SCHULT. (= *V. albiflora* POHL)	Brazil, Minas Gerais, 2 populations: 1. Road from Araçuaí to Itaobim, 1022, MENEZES. 2. Caraça, SPF-34233, 527, MENEZES.	14–16	–	–
V. aff. *declinans* GOETHARD & HENRARD	Brazil, Minas Gerais, Road from Diamantina to Milho Verde, 1304, MENEZES.	14	–	–
V. epidendroides MART. ex SCHULT. × aff. *pusilla* L. B. SMITH & AYENSU	Brazil, Minas Gerais, Diamantina, 202, BENKO–ISEPPON.	32	–	–
V. flavicans MART. ex SCHULT.	Brazil, Minas Gerais, Road from Belo Horizonte to Ouro Preto, 1345, MENEZES.	16	–	–

Table 1 (continued)

Taxon	Provenance, voucher number, collector	Chromosome number		Previous counts
		2n	n	
V. glabra MIKAN	Brazil, Minas Gerais, Serra do Cipó, SPF 61.879, RÉ.	14–18	–	–
V. grao-mogolensis L. B. SMITH	Brazil, Minas Gerais, Serra do Grão-Mogol, CFCR 9687, MELLO–SILVA & al.	16	–	–
V. hirsuta GOETHARD & HENHARD	Brazil, Minas Gerais, Diamantina, Serra do Espinhaço.		7	GP
V. nanuzae L. B. SMITH & AYENSU	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, CFSC 11.598, MENEZES & CASTRO-SOUZA.	16	–	–
V. nivea L. B. SMITH & AYENSU	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, CFSC 11.236, MENEZES & al.	16	–	–
V. aff. *patens* L. B. SMITH & AYENSU	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, 191, BENKO–ISEPPON.	14	–	–
V. patens L. B. SMITH & AYENSU	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, 186, BENKO–ISEPPON.	16	–	–
V. pterocarpa L. B. SMITH & AYENSU	Brazil, Minas Gerais, Diamantina.	–	8	GP
V. pusilla L. B. SMITH & AYENSU	Brazil, Minas Gerais, Serra do Cipó, Santana do Riacho, CFSC-9896, SPF-44450, MENEZES & al.	16	–	–
V. riedeliana GOETHARD & HENRARD	Brazil, Minas Gerais, 27 km W of Serro.	–	7	GP
V. tubiflora (A. RICH) KUNTH	Brazil, Minas Gerais, Rio Itacambicuru, Grão Mogol.	–	7	GP
V. variegata GOETHARD & HENRARD	Brazil, Rio de Janeiro, Rio de Janeiro, Santa Maria Madalena, 1229, MENEZES.	14–16	–	–

Vellozia sp. nova	Brazil, Minas Gerais, Diamantina, 253, BENKO-ISEPPON.	16	–	–
Xerophyta abietina (MART.) SPRENGEL	Brazil, Minas Gerais, Diamantina, Extração, 1046, MENEZES.	14	–	–
Xerophyta dasylirioides BAKER var. *pectinata* (BAKER) H. PERR	Africa, Madagascar, 103 km N of Ihosy, MB 176, D. J. DUPUY & MENEZES.	48	–	–
Xerophyta elegans (BALF.) BAKER [= *Talbotiopsis elegans* (HOOK f.) L. B. SMITH]	Africa, Madagascar, cultivated, Botanical Garden, Vienna University, Austria.	48	–	–
	South Africa, exact locality unknown.	–	24	GP
	South Africa, exact locality unknown.	–	24–26	ST
X. giuliettiae N. MENEZES & SEMIR, sp. nov. ined.	Brazil, Minas Gerais, Diamantina, 261, BENKO–ISEPPON.	14	–	–
X. humilis (BAKER) DUR. & SCHINZ	South Africa, without precise locality.		24	GP
X. minima (POHL) BAKER	Brazil, Minas Gerais, 2 populations: 1. Serra do Cipó, CFSC 11208, MENEZES & al.; 2. Diamantina, 196, BENKO-ISEPPON.	14	–	–
X. retinervis BAKER	South Africa, Pretoria, hills at Bot. Res. Inst.	–	24	GP
X. sellovyi (SEUBERT) BAKER	Brazil, Minas Gerais, Piedade, CFCR 2807, SPF 22287, MENEZES & al.	14	–	–

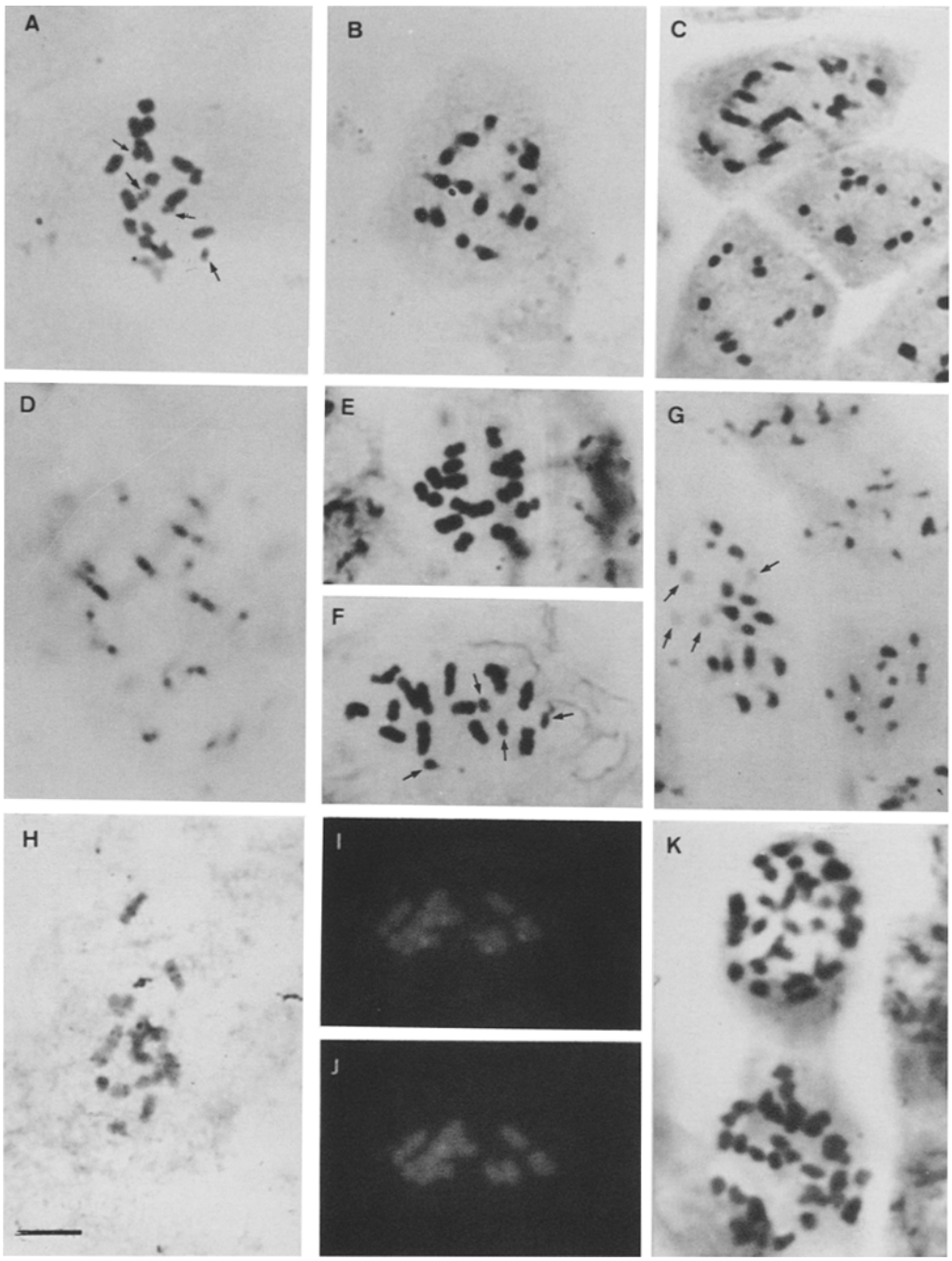

Fig. 1. Mitotic chromosomes and interphase nuclei of American *Vellozioideae*. *A* Metaphase and *B* prometaphase chromosomes of *V. crassicaulis* (2n = 16) with large heteropycnotic blocks. *C* Prophase and interphase nuclei of *Xerophyta minima* (2n = 14). *D* Prophase and *E* metaphase of *Nanuza plicata* (2n = 16). Observe the characteristic prophase condensing pattern. *F* Metaphase and *G* prometaphase of *V. glabra* (2n = 14–18). *H* C-banded metaphase of *V. patens* (2n = 16). *I*, *J* CMA/DAPI double stained metaphase of *V. nivea* (2n = 16) showing four CMA + blocks (I) and DAPI homogeneous stained chromosomes (J). *K* Two prometaphases of the putative hybrid *V. epidendroides* × *V.* aff. *pusilla* (2n ≅ 32). Arrows point out putative satellites. Bar: 5 μm

Table 2. Variation in chromosome size, chromocentre size and chromosome number in ten species with different ploidy level

Taxon	2n	Haploid complement size (μm)	Chromosome size, smallest–largest (μm)	Chromocentre number per nucleus	Average size of chromocentres (μm)
Xerophyta minima	14	13.72	2.44–1.49	11–13	1.04
Vellozia aff. *patens*	16	12.04	2.09–1.33	11–13	1.04
Aylthonia graminifolia	34	20.74	1.68–0.92	17–20	0.81
Aylthonia pulverulenta	34	21.08	1.55–0.95	17–20	0.77
Burlemarxia pungens	34	28.39	2.15–1.33	18–22	1.10
Pleurostima longiscapa	34	18.87	1.33–0.82	22–25	0.76
Pleurostima nuda	34	14.96	1.04–0.69	18–21	0.54
Pleurostima purpurea	34	22.95	2.05–0.98	21–24	0.68
Pleurostima rogieri	34	18.36	1.37–0.79	21–25	0.67
Xerophyta dasylirioides var. *pectinata*	48	20.16	1.26–0.63	30–40	0.52

of DAPI bands and the occurrence of four terminal large blocks of CMA + chromatin (Fig. 1i–j). At interphase such blocks were often associated with the nucleolus.

A single sample of *Vellozia*, possibly of hybrid origin (*V. epidendroides* × *V.* aff. *pusilla*, MELLO-SILVA, pers. comm.), presented $2n \cong 32$. Its chromosomes and chromocentres seemed to be smaller than those generally present in *Vellozia* species (Fig. 1k). Also, its prophase chromosomes did not show large proximal heteropycnotic blocks as observed in the diploids. However, the quality of the cytological preparations was not good enough to permit a more detailed investigation.

The tetraploid group included only representatives of subfam. *Barbacenioideae: Aylthonia* (3 species), *Burlemarxia* (2 species) and *Pleurostima* (7 species). They presented a stable chromosome number ($2n = 34$), but were conspicuously variable in chromosome complement size. Figure 2 illustrates the variation in chromosome size and nuclear structure in this group. In our sample of 12 species, the largest chromosome complement size was observed in *Burlemarxia pungens* (Fig. 2a) – about twice the size of the smallest one found in *Pleurostima nuda* (Fig. 2d, Table 2). The variation in chromosome size as well as in chromocentre size and number was similar in the two *Aylthonia* species, but much higher among *Pleurostima* species. The haploid chromosome complement size of *P. purpurea* (Fig. 2c) was 50% larger than that of *P. nuda* (Table 2).

Chromosome morphology, observed in many partially spread metaphases, varied between the metacentric and the submetacentric type. The satellites, one or two pairs, were always small and caused no problems in counting (Fig. 2e).

All meiotic stages were observed in young flower buds of *P. rogieri* collected outside the sheaths. Pairing was normal with 17 bivalents at metaphase I and a normal segregation at anaphases I and II (Fig. 3a). In very few nuclei at anaphase I,

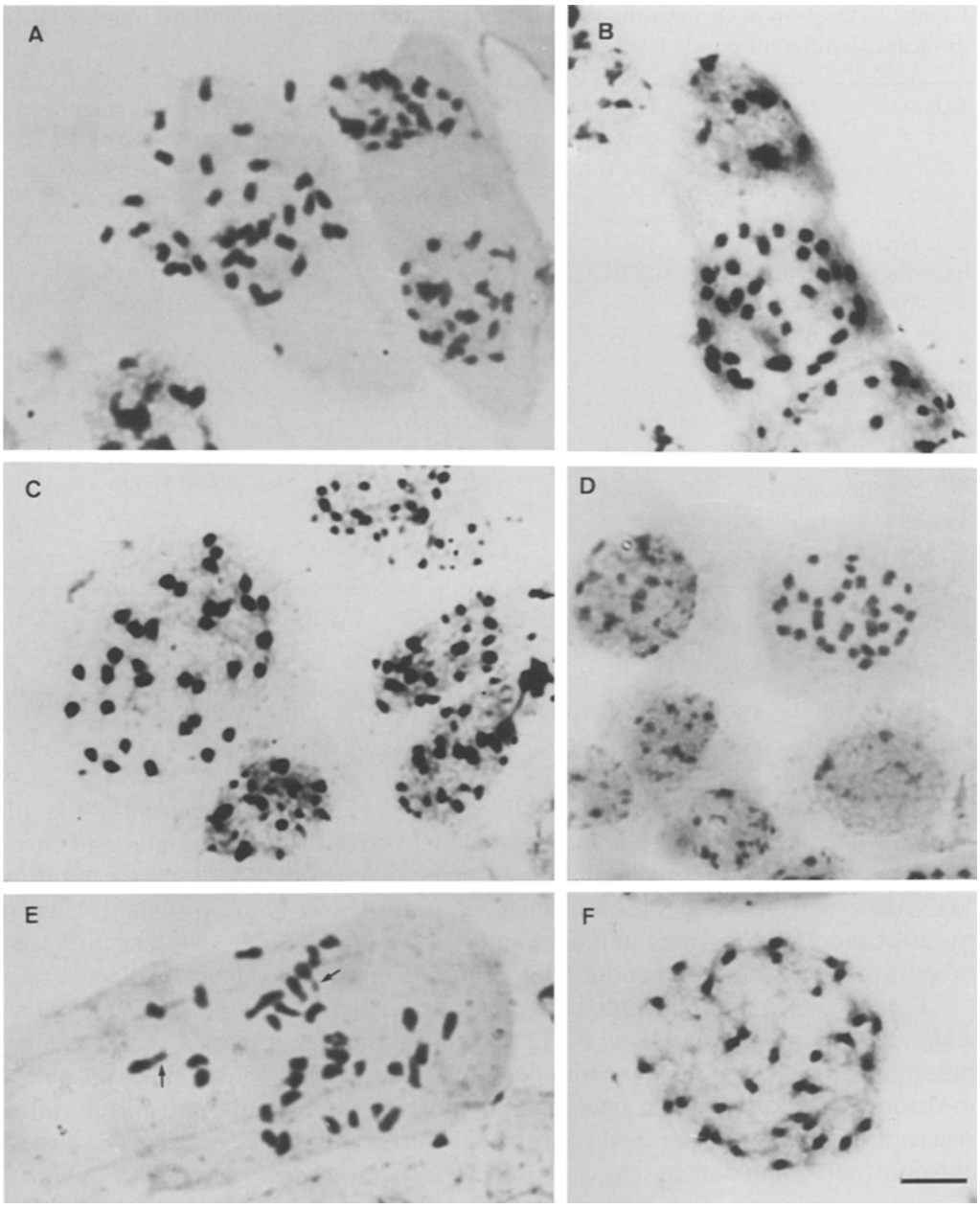

Fig. 2. Mitotic chromosomes and interphase nuclei of some tetraploid species of subfam. *Barbacenioideae*. *A Burlemarxia pungens* (2n = 34). *B Aylthonia graminifolia* (2n = 34). *C Pleurostima purpurea* (2n = 34). *D P. nuda* (2n = 34). *E P. riparia* (2n = 34). *F P. rogieri* (2n = 34). Arrows in *E* point out satellites. Bar: 5 μm

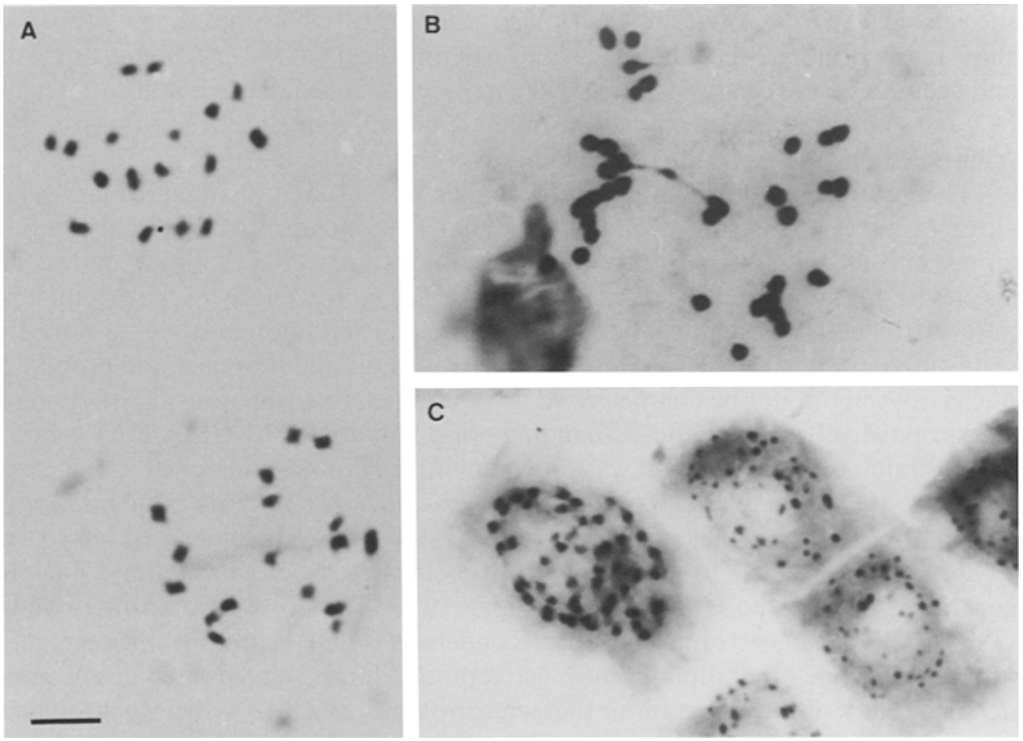

Fig. 3. Meiotic configurations of *Pleurostima rogieri* (*A, B*) and mitotic nuclei of *Xerophyta elegans* (*C*). *A* metaphase II (n = 17). *B* Anaphase I with bridge. *C* Mitotic prometaphase (2n = 48) and interphase nuclei. Bar: 5 μm

bridges and fragments were observed, suggesting the occurrence of small paracentric inversions (Fig. 3b).

Xerophyta dasylirioides var. *pectinata* and *X. elegans* (Fig. 3c) were the only hexaploid species (2n = 48) analyzed. The chromosome size was amongst the smallest ones in the family, whereas the number of chromocentres per interphase nucleus (30–40) was the highest among the species of our sample (Table 2). Details of chromosome morphology and satellites could not be observed.

Discussion

The most characteristic karyological feature of the *Velloziaceae* was the interphase nuclear structure, which showed an areticulate type well conserved in all the species analyzed here. The variation in chromocentre number was directly related to ploidy level whereas chromocentre size showed an inverse relationship with ploidy level. Such a conservation of the nuclear structure is not common at the family level among the angiosperms (Delay 1949), pointing to the monophyletic origin of this family. Areticulate nuclei are related mainly to karyotypes with small chromosomes and low DNA content (Delay 1949, Barlow 1977), and are commonly associated with phylogenetically more advanced (Guerra 1987, Morawetz 1988) or more

specialized taxa (BARLOW 1977, LEVIN & FUNDERBURG 1979). Since the *Velloziaceae* show many primitive morphological and palynological characters (MENEZES 1980) and occur in very specialized habitats, the well-defined chromatin organization may be a consequence of their adaptation to extreme environmental conditions in the "campos rupestres" and other xeric habitats.

The presence of high amounts of condensed chromatin, observed as large chromocentres at interphase and as proximal blocks at prophase, mainly in the largest chromosomes, seems to be an important feature in the evolution of diploid species in this family. Beside its importance to the cytotaxonomy of the group, it is noteworthy in its unusual reaction to the C-banding and fluorochrome staining. In spite of the fact that the blocks were observed in almost all interphase and prophase nuclei, they could not be characterized as typical heterochromatin since they were neither positively stained after C-banding nor differentiated by CMA/DAPI staining. A similar staining reaction was observed in the chromosomes of *Costus* species (GUERRA 1988) where the blocks were characterized mainly as condensed euchromatin. On the other hand, the four CMA + /DAPI − blocks observed in *Vellozia nivea* seemed to represent NOR-associated heterochromatin which is generally GC-rich and CMA + (SCHWEIZER 1976). The observation of these four CMA + blocks suggests that the four decondensed isolated chromosome segments observed in some other diploids may also represent large isolated satellites. A more detailed study of the variation in the heteropycnotic or CMA + heterochromatic blocks would be very helpful to clarify the infrageneric cytotaxonomy of *Vellozia* and may furnish further information on the structural karyotypic differentiation and evolution in the diploids.

The present study represents a significant increase in the percentage of cytologically analyzed *Velloziaceae* species. Together with the previous data from GOLDBLATT & POSTON (1988) there is now chromosome number information from 49 species (ca. 20% of the family) covering all recognized genera.

Only three ploidy levels and four different chromosome numbers were found in the family: diploids with n = 7, 8; tetraploids with n = 17 and hexaploids with n = 24. The diploid chromosome complements were not easy to count exactly, mainly due to the difficulty in distinguishing satellites from small chromosomes during metaphase. This may explain the disagreement between our chromosome count and that from GOLDBLATT & POSTON (1988) for *V. bahiana* (Table 2). At prophase and prometaphase the satellites were often weakly stained. Such behavior of satellite chromatin is rather uncommon since it is generally more condensed during prophase (FAVARGER 1978). We have counted 2n = 14–18 in *Vellozia glabra*, but this was probably due to the presence of large satellites (Fig. 1f, g). A meiotic analysis would be very helpful in this case. The number n = 9 was previously attributed to *Vellozia* by GOLDBLATT (apud RAVEN 1975). However, he later recognized it as a miscount and reported n = 8 as the correct number (GOLDBLATT 1980).

No clear-cut chromosomal differences were observed between species of *Vellozia* and South American species of *Xerophyta* (recognized as *Vellozia* subg. *Xerophytoides*, according to SMITH & AYENSU 1976). On the contrary, they showed a very similar variation in chromosome number, chromosome size and prophase condensing behavior. Similarly, SALATINO & al. (1989), comparing the distribution of foliar epicuticular alkanes of 110 species of *Velloziaceae* found no substantial differences

between profiles of Brazilian *Xerophyta* and those of *Vellozia* species. HARBORNE & al. (1994) found the same complex lipophilic flavonoids and mono-C-glycoflavones in both groups. The inclusion of Brazilian *Xerophyta* within the genus *Vellozia* sensu SMITH & AYENSU (1976) was also supported by a weighted cladistic analysis based on morphological and anatomical characters (MENEZES & al. 1994).

The former *Xerophyta plicata*, separated by SMITH & AYENSU (1976) as the monospecific genus *Nanuza*, also showed $2n = 16$ and the same cytological characteristics as other diploid species of *Xerophyta*. However, it had the largest chromosome size and the biggest heteropycnotic blocks in the family. This may support its distinction from the remainder of the *Xerophyta* species (see MENEZES & al. 1994). Furthermore, the flavonoid profile of the *Vellozioideae* clearly suggests an isolated position for *Nanuza plicata* within the subfamily (WILLIAMS & al. 1994). The discovery of the uncommon biflavonoid amentoflavona unique for this species suggests that it may be among the more "primitive" members of the family (WILLIAMS & al. 1987).

The tetraploid group included representatives of all four genera of *Barbacenioideae*. They were very stable in chromosome number although variable in chromosome size. In *Pleurostima* the variation in chromosome size between species was almost as large as in the whole tetraploid group. The early karyological evolution of the tetraploids probably involved diploidization (meiotic behavior like a diploid), restriction of chromosome size and enlargement and/or reduction of the chromosome complement size by heterochromatin (or condensed euchromatin) elimination. This could explain the observed meiotic stability, the reduced amount of heteropycnotic chromatin and the higher karyotype symmetry in tetraploids in relation to diploids.

The number $n = 17$ in the tetraploids seemed to be common and exclusive to the whole subfam. *Barbacenioideae*. Its origin is still unclear. GOLDBLATT & POSTON (1988) suggested that it would represent an aneuploid derived from a hypothetical paleotetraploid with $n = 16$ or $n = 18$. They did not consider the possibility of a hybrid origin from two diploid species with $n = 8$ and $n = 9$, although they admitted that the hypothetical $x = 9$ may be the original base number for the family. Since natural polyploids are often of hybrid constitution (GREILHUBER & EHRENDORFER 1988), e.g., the tetraploid found in *Vellozia*, an allopolyploid origin of subfam. *Barbacenioideae* from a hybrid between species with $n = 8$ and the hypothetical $n = 9$ has also to be considered. However, $n = 17$ arising by increasing dysploidy of a tetraploid with $n = 16$ seems to be the more parsimonious explanation.

Most of the genera show clearly a basic number $x = 8$. It is the only basic number of *Nanuza, Talbotia* and the African *Xerophyta*, and it is at least well-represented in *Vellozia*. The haploid number $n = 7$ was found only in some *Vellozia* and Brazilian *Xerophyta*, and seems to be a derivate number of $x = 8$. Therefore, $n = 8$ represents more probably the basic number of the family, which agrees with the phylogeny proposed by MENEZES & al. (1994). GOLDBLATT & POSTON (1988) proposed $x = 9$ as the basic number of the family, based mainly on a supposed origin of $n = 17$ from descending aneuploidy of a tetraploid with $n = 18$. However, $n = 17$ may also have resulted from amphiploidy or from an increasing dysploidy of a tetraploid with

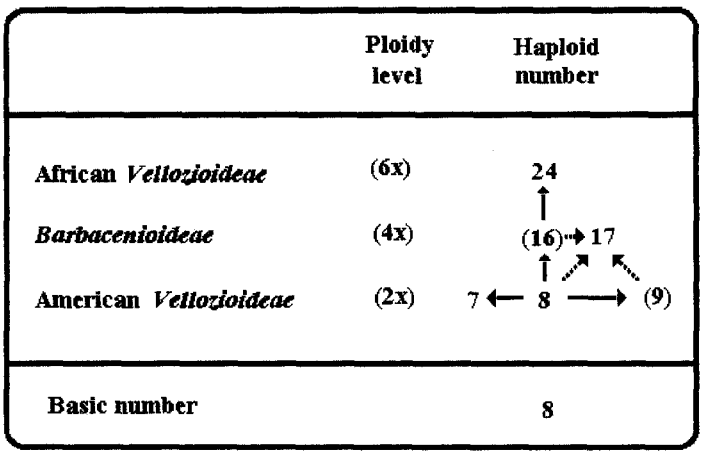

Fig. 4. Diagrammatic scheme of the most likely phylogenetic relationships among all haploid numbers in *Velloziaceae* from the basic number x = 8. Hypothetical numbers are given in parenthesis. Doubtful connections are represented by dotted arrows. Continuous arrows indicate very probable connections

n = 16 (x = 8). A scheme of the most likely evolution of haploid numbers from the base number x = 8 is presented in Fig. 4.

Based on morphological and anatomical reasons AYENSU (1973) presented a considerable mass of evidence for the origin of the *Velloziaceae* in the African continent and its later migration to South America. MENEZES (1980) discussed extensively the arguments of AYENSU (1973). Aided by further morpho-anatomical, embryological and taxonomic data, she sustained the opposite hypothesis of a South American origin for the family. However, as noted by AYENSU (1973), no final conclusion should be undertaken until comprehensive analyses from other approaches, such as pollen and chromosome studies, are considered. Nevertheless, our existing information about the cytogenetics of this family may shed some light on this problem. First, the primitive morphological and anatomical characters of the African *Xerophyta* may be a consequence of its ancient hexaploid condition. Plant taxa with a high ploidy level tend to be "slow" in terms of evolutionary changes, conserving most of their phenotypic characteristics. On the other hand, diploids like the American *Vellozia* and some allotetraploids, like subfam. *Barbacenioideae*, tend to be more diversified and derived (see STEBBINS 1971, GRANT 1981). Secondly, old polyploid genera often tend to occupy a marginal position in the general world distribution of the family (EHRENDORFER 1980, LEWIS 1980). If this is true for the *Velloziaceae*, the occurrence of old polyploids in Africa may indicate the limit of the family distribution in the past, rather than its centre of origin. Third, the hypothesis of an African origin for the *Velloziaceae* presupposes the existence of diploids in this continent, at least in the past, but until now their presence has not been demonstrated. It can be argued that they could be extinct in their original home (Africa), being represented now only in their secondary centre of distribution (South America). However, the apparent absence of diploid species in Africa can hardly be

explained on the base of extinction, since the characteristic xeric habitat of the *Velloziaceae* is very similar in both continents and seems to have remained relatively unchanged since the separation of the continents (AYENSU 1973).

Bearing in mind the above evidence, we believe that the most reasonable hypothesis to explain the actual distribution of the *Velloziaceae* has to consider that: (i) its original stock of x = 8 diploids arose in South America; (ii) the limit of distribution of the family was extended to Africa where the new habitats were more successfully explored by species of a higher ploidy level (2n = 48 = 6x); (iii) dysploidy followed or preceded by amphiploidy may have taken place among South American *Vellozioideae*, giving rise to a new clade with n = 17.

A detailed discussion on the chromosomal relationships of the *Velloziaceae* with possible related families would be still too speculative, mainly because there is no agreement about which family is closest. The *Bromeliaceae* as suggested by DAHLGREN (1977) also present x = 8, although this number occurs mainly in the advanced tribe *Tillandsieae* (SHARMA & GHOSH 1971). The areticulate nuclear structure, observed in all *Velloziaceae*, has also been reported for some *Bromeliaceae* (DELAY 1949). Therefore, from a cytological point of view this family could be considered very close to the *Velloziaceae*. The *Haemodoraceae*, as suggested by HUTCHINSON (1934) and CRONQUIST (1988:555) to be the closest related family, have x = 8 and the *Hypoxidaceae* (TAKHTAJAN 1969, AYENSU 1973) have x = 6, 9 and 11 (see SHARMA 1984). This latter author considered that the *Hypoxidaceae* may have evolved from the *Milliganieae*, which have x = 8, so that both families could also be directly related to the *Velloziaceae*. Unfortunately we are not aware of any descriptions of the structure of the interphase nuclei of these last two families and chromosome number records for both are still very scanty.

We thank Prof. RENATO MELLO-SILVA (Universidade de São Paulo) for the identification of many species and interesting discussions, Prof. Dr SIMON J. MAYO (Royal Botanic Gardens, Kew) for corrections to the English manuscript and other valuable suggestions, and ANA EMILIA B. E SILVA for much technical help. We also thank the Botanical Garden of the University of Vienna, Austria, for the permission to collect root tips of *Xerophyta elegans* growing in their greenhouse. This study was partially supported by CNPq, FACEPE and FAPESP.

References

AYENSU, E. S., 1973: Phytogeography and evolution of the *Velloziaceae*. – In MEGGERS, B. J., AYENSU, E. S., DUCKWORTH, W. D., (Eds): Tropical forest ecosystems in Africa and South America: comparative review, pp. 105–119. – Washington: Smithsonian Institution Press.

BARLOW, P., 1977: Determinants of nuclear chromatin structure in angiosperms. – Ann. Sci. Nat. Bot., 12e Sér., **18**: 193–206.

CRONQUIST, A., 1988: The evolution and classification of flowering plants. – New York: New York Botanical Garden.

DAHLGREN, R., 1977: A commentary on a diagrammatic presentation of the angiosperms in relation to the distribution of character states. – Pl. Syst. Evol., Suppl. **1**: 253–283.

DELAY, C., 1949: Recherches sur la structure des noyaux quiescents chez les phanérogames. – Ph.D. Thesis. Paris.

DEUMLING, B., GREILHUBER, J., 1982: Characterization of heterochromatin in different species of the *Scilla siberica* group (*Liliaceae*) by in situ hybridization of satellite DNAs and fluorochrome banding. – Chromosoma **84**: 535–555.

EHRENDORFER, F., 1980: Polyploidy and distribution. – In LEWIS, W. H., (Ed.): Polyploidy – biological relevance, pp. 45–60. – New York, London: Plenum Press.

FAVARGER, C., 1978: Philosophie des comptages de chromosomes. – Taxon **27**: 441–448.

GOLDBLATT, P., 1980: Polyploidy in angiosperms: monocotyledons. – In LEWIS, W. H., (Ed.): Polyploidy – biological relevance, pp. 219-239. – New York, London: Plenum Press.

– POSTON, M. E., 1988: Observations on the chromosome cytology of *Velloziaceae*. – Ann. Missouri Bot. Gard. **75**: 192–195.

GRANT, V., 1981: Plant speciation, 2nd edn. – New York: Columbia University Press.

GREILHUBER, J., EHRENDORFER, F., 1988: Karyological approaches to plant taxonomy. – ISI Atlas of Science: Animal Pl. Sci. **1**: 289–297.

GUERRA, M., 1983: O uso do Giemsa na citogenética vegetal – comparação entre a coloração simples e o bandeamento. – Ci. & Cult. **35**: 190–193.

– 1987: Cytogenetics of *Rutaceae* IV. Structure and systematic significance of interphase nuclei. – Cytologia **52**: 213–222.

– 1988: Characterization of different types of condensed chromatin in *Costus* (*Zingiberaceae*). – Pl. Syst. Evol. **158**: 107–115.

HARBORNE, J. B., WILLIAMS, C. A., GREENHAM, J., EAGLES, J., 1994: Variations in the lipophilic and vacuolar flavonoids of the genus *Vellozia*. – Phytochemistry **35**: 1475.

HUTCHINSON, J., 1934: The families of flowering plants, 2. Monocotyledons. – London: Macmillan.

LEVIN, D. A., FUNDERBURG, S. W., 1979: Genome size in angiosperms: temperate versus tropical species. – Amer. Naturalist **114**: 784–795.

LEWIS, W. H., 1980: Polyploidy in plant evolution: summary. – In LEWIS, W. H., (Ed.): Polyploidy – biological relevance, pp. 241–268. – New York: Plenum Press.

MELLO-SILVA, R. DE, 1991: The infra-familial taxonomic circumscription of the *Velloziaceae*: a historical and critical analysis. – Taxon **40**: 45–51.

MENEZES, N. L., 1971: New taxa and new combinations in *Velloziaceae*. – Ci. & Cult. **23**: 421–422.

– 1980: Evolution in *Velloziaceae*, with special reference to androecial characters. – In BRICKELL, C. D., CUTLER, D. F., GREGORY, M., (Eds): Petaloid monocotyledons. – Linn. Soc. Symp. Ser. **8**: 117–138.

– 1988: Evolution of the anther in the family *Velloziaceae*. – Bol. Bot. Univ. São Paulo **10**: 33–41.

– SEMIR, J., 1991: *Burlemarxia*, a new genus of *Velloziaceae*.–Taxon **40**: 413–426.

– MELLO-SILVA, R. DE, MAYO, S. J., 1994: A cladistic analysis of the *Velloziaceae*. – Kew Bull. **49**: 71–92.

MORAWETZ, W., 1988: Karyosystematics and evolution of Australian *Annonaceae* as compared with *Eupomatiaceae, Himantandraceae* and *Austrobaileyaceae*. – Pl. Syst. Evol. **159**: 49–79.

RAVEN, P. H., 1975: The bases of angiosperm phylogeny: cytology. – Ann. Missouri Bot. Gard. **62**: 724–764.

SALATINO, M. L. F., SALATINO, A., MENEZES, N. L., MELLO-SILVA, R. DE, 1989: Alkanes of foliar epicuticular waxes of *Velloziaceae*. – Phytochemistry **28**: 1105–1114.

SCHWARZACHER, T., AMBROS, P., SCHWEIZER, D., 1980: Application of Giemsa banding to orchid karyotype analysis. – Pl. Syst. Evol. **134**: 293–297.

SCHWEIZER, D., 1976: Reverse fluorescent chromosome banding with chromomycin and DAPI. – Chromosoma **58**: 307–324.

SHARMA, A. K., 1984: Chromosome evolution in the monocotyledons – an overview. – In SHARMA, A. K., SHARMA, A., (Eds): Chromosome evolution in eucaryotic groups II: 169–187. – Boca Raton: CRC Press.

– GHOSH, I., 1971: I. Cytotaxonomy of the family *Bromeliaceae*. – Cytologia **36**: 237–247.

SMITH, L. B., AYENSU, E. S., 1976: A revision of American *Velloziaceae*. – Smithsonian Contrib. Bot. **30**: 1–172. – Washington: Smithsonian Institution Press.

STEBBINS, G. L., 1971: Chromosomal evolution in higher plants. – London: Arnold.

STENAR, A. H. S., 1925: Embryologische Studien II. Embryologie der Amaryllideen. – Ph.D. Thesis, Uppsala.

TAKHTAJAN, 1969: Flowering plants: origin and dispersal (translated from Russian by JEFREY, G. C.). – Washington, D. C.: Smithsonian Institution Press.

WILLIAMS, C. A., HARBORNE, J. B., TOMAS-BARBERAN, F. A., 1987: Biflavonoids in the primitive monocots *Isophysis tasmanica* and *Xerophyta plicata*. – Phytochemistry **26**: 2553–2555.

– GREENHAM, J., EAGLES, J., 1994: Differences in flavonoid patterns between genera within the *Velloziaceae*. – Phytochemistry **36**: 931–940.

Addresses of the authors: NATONIEL FRANKLIN DE MELO, EMBRAPA–CPATSA, 56.300 – 000, Petrolina – PE, Brazil. –Dr MARCELO GUERRA and Dr ANA MARIA BENKO-ISEPPON, Departamento de Genética, CCB, Universidade Federal de Pernambuco, 50.732–970 – Recife – PE, Brazil. – Dr NANUZA LUIZA DE MENEZES, Depto. de Botânica, Instituto de Biociências, Universidade de São Paulo, 05422–970, São Paulo – SP, Brazil.

Accepted February 9, 1996 by I. KRISAI–GREILHUBER