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Cytological Studies on *Banisteriopsis* C.B. Robinson *ex* Small and *Heteropterys* Kunth (Malpighiaceae)

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Summary Cytological studies were carried out on 6 species of *Banisteriopsis* and 4 species of *Heteropterys* (Malpighiaceae, Malpighioideae), collected in forest and cerrado areas of southeastern Brazil, and a cultivated one. No previous chromosome counts are known for 3 species of *Banisteriopsis* and 3 species of *Heteropterys.* Chromosome numbers were obtained through mitotic (7 counts) and meiotic (5 counts) analysis. No meiotic abnormalities were encountered in the species studied, which presented high viable pollen rates. For the first time, karyomorphological characters were found for both genera, with a predominance of metacentric chromosomes. The chromosome number variation, based on $x=5$ and 10, with cases of polyploidy, and the similarity between the 2 related genera are discussed.

Key words Malpighiaceae, *Banisteriopsis*, *Heteropterys*, Cytogenetics, Karyomorphology.

The genera *Banisteriopsis* C.B. Robson *ex* Small and *Heteropterys* Kunth, belong to family Malpighiaceae, sub-family Malpighioideae, tribe Banisterieae, subtribe Banisteriinae (Anderson 1977). These genera were considered very related each other by Niedenzu (1928). This author differentiated the genera based on their marginal wing morphology. Vogel (1990) analyzed the pollen characters, and the morphology, anatomy and physiology of calyx glands in species from various genera of Malpighiaceae. Some features, as the absence of calyx glands in some species, led Vogel (1990) to the conclusion that *Banisteriopsis* and *Heteropterys* are 2 of the most derived genera in the family Malpighiaceae. Anderson (1979) also cited this absence of glands in floral calyx as a derived character for this family.

Banisteriopsis is represented by 92 species, distributed exclusively in America, mostly in the tropics. About 69 species of *Banisteriopsis* were found in Brazil, 2/3 of which are mainly distributed in the cerrado area, a savanna-like vegetation, of central Brazil (Gates 1982). The genus is divided in 3 sub-genera: *Banisteriopsis* (8 species), *Hemiramma* (58 species) and *Pleiopterys* (26 species). Although they are usually shrubby plants, some lianas and small trees also occur (Gates 1982). The taxonomy of the genus is one of the most problematic of Malpighiaceae (Makino-Watanabe *et al.* 1993).

Literature brings forward 17 cytological data for *Banisteriopsis* species, one of which remains non-identified (Forni-Martins *et al.* 1995). The chromosome number of the studied species seems relatively stable ($n=10$), and no karyomorphological data have ever been presented for this genus.

The genus *Heteropterys* Kunth comprises about 81 species of lianas, few erect shrubs, with yellow flowers and samaroid fruits (Niedenzu 1928). Although this is one of the largest genera in this family, only 9 of its species have undergone chromosome counts. They show a numeric sequence based on $x=5$. Two of them present divergent chromosome numbers: ($2n=34$) and *H. leona* ($2n=42$, 56, 58). There are no karyomorphological data for the genus *Heteropterys.*

This paper aims to contribute to the cytological knowledge of both *Banisteriopsis* and *Het-*

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eropterys, presenting new chromosome numbers and karyomorphological data for some species. Moreover, based on the chromosome numbers and observed karyotypes, it is intended to cytogenetically compare both genera, which are considered closely related and the 2 most derived in the family Malpighiaceae.

Materials and methods

We studied 6 species of *Banisteriopsis* and 4 of *Heteropterys* collected in areas of cerrado (Ititapina-SP, Carrancas-MG, Mogi-Mirim-SP), semi deciduous forest (Santa Genebra, Campinas-SP) and cultivates (UNICAMP campus). Vouchers are deposited at Unicamp herbarium UEC (Table 1).

For meiotic analysis and pollen viability we squashed anthers with 1.2% acetic carmine solution. For mitotic analysis root tips were pre-treated with PDB saturated solution, fixed in Carnoy's solution (alcohol 3 : 1 acetic acid, v/v) and hydrolyzed with 5N HCl. For the ideogram confection we analyzed 10 mitotic cells per species. The slide staining was made with 2% Giemsa solution. The karyotype indexes TCL (total chromatin length) and TF% (Huziwara 1962) were calculated. The chromosome nomenclature utilized is that proposed by Guerra (1986a).

Results

Banisteriopsis Four different chromosome numbers were obtained: $n=10$, $n=20$, $n=40$ and $2n=30$ (Table 1). Karyomorphological data (Table 1, Fig. 1) were used to construct the chromosome ideogram of *B. stellaris*, *B. pubipetala* and *Banisteriopsis* sp. (Fig. 2). The chromosomes observed are relatively short. Only *B. pubipetala* presented 3.85 μm-long chromosomes, relatively long when compared with other species of Malpighiaceae. The chromosomes are mostly metacentric (Fig. 2) and their length variation is gradual within the karyotypes.

No abnormalities were observed in the meiotic process, which resulted in high viable pollen indexes: *B. laevifolia*, 90.3%, *B. variabilis*, 92.7% and *B. campestris*, 95.7%. Only *B. stellaris* presented a minor index, 65.9%, although no meiotic problems were encountered.

Heteropterys Two different chromosome numbers were obtained: $n=10$ and $2n=30$ (Table 1, Fig. 1). The chromosomes are longer than those observed in *Banisteriopsis. H. chrysophylla* presented the longest chromosome, with 3.8 μm, and *H. pteropetala* the shortest one, 1.6 μm (Table 1). The chromosome length variation within the karyotype is gradual. No meiotic abnormality was observed in *H. chrysophylla*, whose viable pollen index is 96.5%.

Discussion

The high index of viable pollen observed in the studied species indicates a meiotic process without abnormality for *Banisteriopsis* and *Heteropterys.* Singhal *et al.* (1985) indicated that the meiotic process used to be problematic in some genera. They studied the species: *Stigmaphyllon ciliatum*, *Hiptage benghalensis* and *Banisteriopsis laevifolia*, which presented 59, 56 and 53% of viable pollen, respectively. Forni-Martins *et al.* (1992) presented data on meiotic abnormalities, as trivalent formation and irregular chromosome disjunction, in *Peixotoa.* For *Stigmaphyllon lalandianum*, Lombello and Forni-Martins (1998) found 91.1% of viable pollen and 100% normal tetrads, indicating meiosis without any problem, corroborated by the data presented here.

The chromosome numbers of *Banisteriopsis* are $n=10$ stable, except for *B. pubipetala* (Tables 1, 2). Three cases of polyploidy were observed: *B. variabilis* ($n=20$), *B. pubipetala* ($2n=30$) and *B. stellaris* ($n=40$, $2n=80$). Anderson (1993) related a case of polyploidy in *B. Muricata* ($n=20$). The literature data (Table 2) show only 2 divergent count: *B. laevifolia* and *B. muricata.* For *B.*

Table 1. Species of *Banisteriopsis* and *Heteropterys* with cytogenetic studies

Species	*n*	2*n*	Chromosome length (μm)	TCL	TF%	KF	Col.	Locality
B. campestris	10	—	—	—	—	—	L.36	Carrancas-MG
B. laevifolia	10	—	—	—	—	—	FM	Itirapina-SP
*B. pubipetala**	—	30	2.0±0.29–3.9±0.48	91.3±10.27	42.9	12 m+3 sm	L.47	Campinas-SP
*B. stellaris**	40	80	1.1±0.10–2.1±0.16	118.0±14.63	44.3	30 m+10 sm	L.61	Itirapina-SP
*B. variabilis**	20	—	—	—	—	—	FM	Itirapina-SP
Banisteriopsis sp.	—	20	1.4±0.11–2.4±0.13	38.2±2.21	45.0	10 m	L.34	Carrancas-MG
*H. anoptera**	—	20	—	—	—	—	L.43	Carrancas-MG
*H. chrysophylla**	10	20	2.2±0.25–3.8±0.33	59.6±5.05	42.6	8 m+2 sm	L.52	UNICAMP
*H. pteropetala**	—	20	1.6±0.30–2.7±0.33	41.8±5.75	45.9	9 m+1 sm	L.51	Itirapina-SP
Heteropterys sp.	—	30	1.7±0.18–3.4±0.37	77.7±7.27	44.3	13 m+2 sm	L.30	Mogi-Mirim-SP

Total chromatin length (TCL), TF% index, karyotype formula (KF) (m, metacentrics; sm, sub-metacentrics), collector number (Col.) (L, Lombello; FM, Forni-Martins) and locality of collection.

* First report.

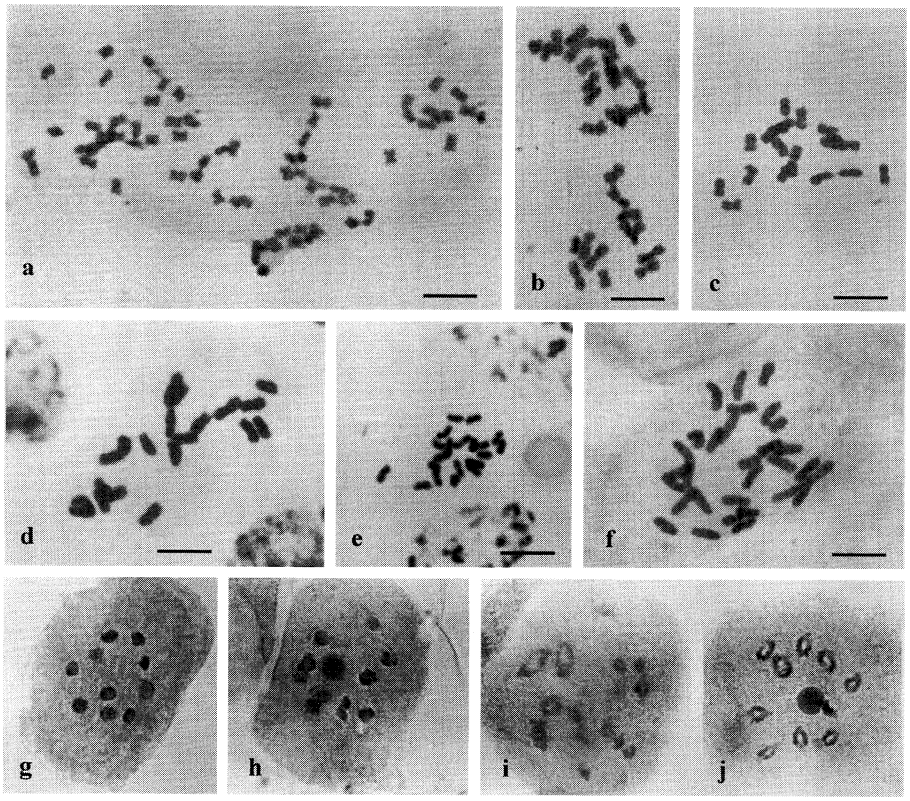

Fig. 1. Metaphase configurations in (a) *Banisteriopsis stellaris* ($2n=80$), (b) *B. pubipetala* ($2n=30$), (c) *Banisteriopsis* sp. ($2n=20$), (d) *Heteropterys chrysophylla* ($2n=20$), (e) *H. pteropetala* ($2n=20$), (f) *Heteropterys* sp. ($2n=30$), (g) *B. campestris* ($n=10$), (h) *B. laevifolia* ($n=10$), (i) *B. variabilis* ($n=20$) and (j) *H. chrysophylla* ($n=10$). Bar=5 μm.

laevifolia, the haploid numbers are $n=20$ (Pal 1964) and $n=10$ (Gates 1982, Singhal *et al.* 1985). For *B. muricata* Anderson (1993) presented $n=20$, different from $n=10$ (Gates 1982) and $2n=20$ (Fouët 1966). Gates (1982) questioned the taxonomic identification of *B. laevifolia* by Pal (1964), because there was no voucher of the studied material.

If these are really divergent counts we can consider them cases of chromosome races, as in *Duguetia furfuracea* (Annonaceae) that presents 3 karyological races in different areas of cerrado, with $2n=16$, 24 and 32 (Morawetz 1984), or as *Mikania micrantha* (Compositae) that presents $2n=36$, 42 and 72 in 12 studied populations (Maffei *et al.* 1999). These intra-specific numeric alterations may indicate a speciation process.

As obsaerved in *Banisteriopsis*, *Heteropterys* also showed a sequence based on multiples of 5, with cases of polyploidy and inter- and intra-specific numeric variation. As for the unidentified species, it presented the same diploid number as that found by Chen and Huang (1989) for *H. salicidolia,* a hexaploid with $2n=30$. In addition to these cases of polyploidy, Semple (1970) presented the diploid number of *H. angustifolia* ($2n=34$), which is probably due to polyploidy followed by aneuploidy. Intra-specific numeric variations are observed in *H. leona* (Table 2). Roy and Mishra (1962) presented intra-specific variation for this species ($2n=42$, 56).

Although the group of species studied within the genera *Banisteriopsis* and *Heteropterys* is still small and the literature does not mention any diploid number $2n=10$ for Malpighiaceae, the basic chromosome number for both genera is probably $x=5$, rectifying that ($x=10$) proposed by Gates (1982) for *Banisteriopsis*. We present this hypothesis based on species with $2n=30$ in both

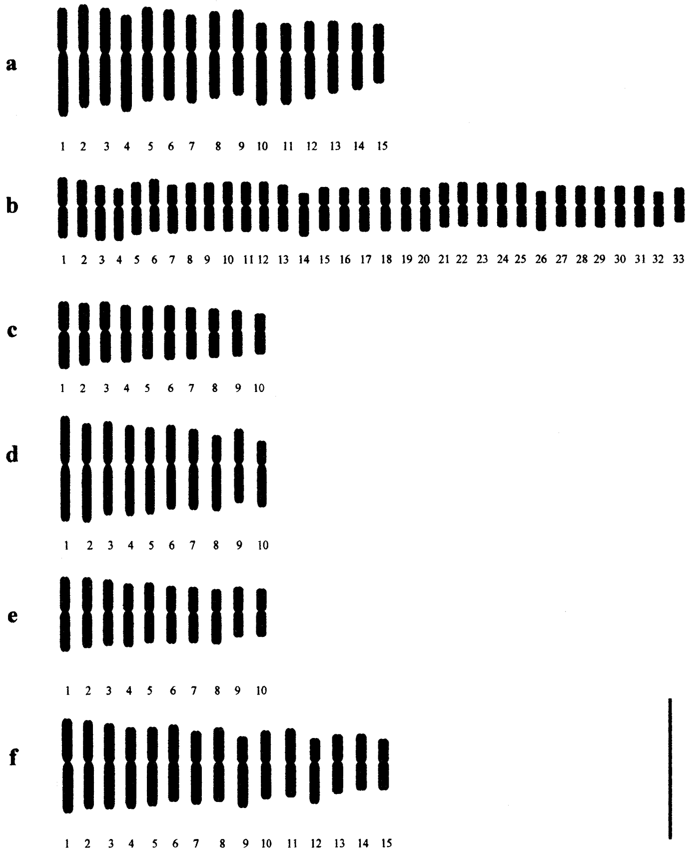

Fig. 2. Ideograms of (a) *Banisteriopsis pubipetala*, (b) *B. stellaris*, (c) *Banisteriopsis* sp., (d) *Heteropterys chrysophylla*, (e) *H. pteropetala* and (f) *Heteropterys* sp. Bar=5 μm.

genera like *B. pubipetala*, *H. salicifolia* and *Heteropterys* sp. (Table 2).

This basic number $x=5$ is coherent with the basic number $x=10$ proposed by Anderson (1977) for the entire family Malpighiaceae. Even considering that some tribes present divergent basic numbers, as Galphimieae and Byrsonimeae ($x=6$) of sub-family Byrsonimoideae, and the sub-tribe Sphedamnocarpinae ($x=9$), of sub-family Malpighioideae (Bawa 1973, Forni-Martins *et al.* 1995), $x=10$ is certainly the most widespread in the family.

We may thus consider the existence of several basic numbers in Malpighiaceae, as $x=5$, 6, 9, 10 and 12, reinforcing the hypothesis of DiFulvio (1979) and Forni-Martins *et al.* (1995).

The karyotypes presented are 1A type, as described by Stebbins (1971). Following this author, symmetrical karyotype indicates a primitive chromosome group. Since this pattern is not valid for all groups, it is not a model for karyotype evolution (Guerra 1986b). Based on palynological and morphological characters, Vogel (1990) showed solid data on the derived position of these genera in the family.

Even presenting similar karyotypes, with predominance of metacentric and absence of acrocentric chromosomes, the TF% index indicates a small variance of chromosome symmetry among the studied species. The most asymmetric karyotype belongs to *H. chrysophylla*. *Heteroperys* sp. shows the greatest intra-specific chromosome length variation (1.7 to 3.4 μm). *B. stellaris* presents the highest TCL (Table 1), due to its chromosome number (Table 1, Figs. 1, 2).

There is probably no correlation between the chromosome number variation and the distribution of the species of *Banisteriopsis* in its sub-genera. There are counts $n=10$ in the 3 sub-genera,

Table 2. Species of *Banisteriopsis* and *Heteropterys* with chromosome number report, distributed in subgenera following Gates (1982) (*Banisteriopsis*) and Niedenzu (1928) (*Heteropterys*), with respective chromosome numbers (*n* and 2*n*) and references

Genus	Subgenus	Species	*n*	2*n*	References
Banisteriopsis	*Banisteriopsis*	*B. acapulcensis* var. *llanensis*	10	—	Gates 1982
	Hemiramma	*B. acerosa*	10	—	Gates 1982
		B. andersonii	10	—	Anderson 1993
		B. angustifolia	10	—	Anderson 1993
		B. argyrophylla	10	—	Gates 1982
		B. caapi	—	20	Baldwin 1946
		B. campestris	10	—	Anderson 1993
					Present paper
		B. cipoensis	10	—	Anderson 1993
		B. laevifolia	10	—	Gates 1982
					Singhal *et al.* 1985
					Present paper
			20	—	Pal 1964
		B. muricata	10	—	Gates 1982
			20	—	Anderson 1993
			—	20	Foüet 1966
		B. oxyclada	10	—	Gates 1982
		B. pulchra	10	—	Gates 1982
		B. stellaris	40	80	Present paper
		B. variabilis	20	—	Present paper
		B. vernoniifolia	10	—	Gates 1982
	Pleiopterys	*B. hypericifolia*	10	—	Gates 1982
		B. pubipetala	—	30	Present paper
		B. valvata	10	—	Anderson 1993
	Indeterminate	*Banisteriopsis* sp.	—	20	Forni-Martins *et al.* 1995
		Banisteriopsis sp.	—	20	Present paper
Heteropterys		*Heteropterys angustifolia*	—	34	Semple 1970
		H. campestris	10	—	Anderson 1993
		H. chrysophylla	10	20	Present paper
		H. coleoptera	10	—	Ormond *et al.* 1981
		H. hypercifolia	10	—	Difulvio 1979
			—	20	Mangenot and Mangenot 1962
		H. leona	—	42, 56	Roy and Mishra 1962
			—	58	Pal 1964
		H. pteropetala	—	20	Present paper
		H. salicifolia	—	30	Chen and Huang 1989
		H. sericea	10	—	Anderson 1993
		H. escallonifolia	10	—	Anderson 1993
		H. byrsonimifolia	10	—	Anderson 1993
		H. anoptera	—	20	Present paper
		Heteropterys sp.	—	30	Present paper

although data only exist for one species of sub-genus *Banisteriopsis*, and 3 species of *Pleyopterys* (Table 2). *Banisteriopsis pubipetala* is the only hexaploid presented for the genus, but there are 3 more counts $2n=30$ for sub-tribe Banisteriinae: *Heteropterys salicifolia* (Chen and Huang 1989), *Peixotoa reticulata* (Anderson 1993) and *Peixotoa* sp. (Forni-Martins *et al.* 1989).

The occurrence of polyploid series in cerrado plants of both genera, as *B. laevifolia* with $n=10$, *B. variabilis* with $n=20$, *B. stellaris* with $n=40$, not to mention *H. anoptera* with $n=20$ and *Heteropterys* sp. with $2n=30$, indicates the development of a reproductive barrier between sympatric species, based on the ploidy levels of these species.

These genera need more attention, because more than taxonomic tools, karyomorphological data may give important arguments for the evolutional analysis of the group.

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