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Karyotype analysis in South American species of Myrtaceae

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In Myrtaceae (Myrteae), the diploid chromosome number $2n = 2x = 22$ is the most common, although variations of ploidy level occur, with some triploid ($2n = 3x = 33$) and tetraploid ($2n = 4x = 44$) records. Karyotype details in this group are scarce because the chromosomes are small (< 2 μm). In this work, we carried out a karyotypic analysis of 15 species of Myrtaceae grouped in different subtribes and genera. Measurements of chromosome length (long arm, *L*; short arm, *S*) were taken and several karyotypic parameters were calculated for each species. The karyotypes in fleshy-fruited taxa (Myrteae) were more varied than in the other previously analysed dry-fruited group (*Eucalyptus*, Eucalypteae), in which the chromosomes were exclusively metacentric. *Botanical Journal of the Linnean* Society, 2007, **155**, 571–580.

ADDITIONAL KEYWORDS: chromosome number – cytotaxonomy – Myrteae – Myrtoideae – polyploidy.

INTRODUCTION

The Myrtaceae is one of the most important families of Myrtales (*sensu* Angiosperm Phylogeny Group (APG), 2003), with 130 genera and *c*. 3800 species (Wilson *et al*., 2001). It was traditionally divided into two subfamilies – Myrtoideae with one tribe and Leptospermoideae with two tribes – according to Niedenzu (1893). Myrtoideae, with only the tribe Myrteae (Niedenzu, 1893), circumscribes three subtribes based mainly on embryo morphology: Eugeniinae, Myrciinae, and Myrtinae (Berg, 1855–1856, 1857–1859). Recently, Myrtaceae has had its intrafamilial circumscription re-evaluated by Wilson *et al*. (2005), who described 15 tribes in Myrtoideae (grouping all traditional genera with dry and baccoid fruits) and suggested another subfamily, Psyloxiloideae, with two monogeneric tribes, Psiloxyleae and Heteropyxideae. Following Berg (1855–1856, 1857–1859), all Brazilian species of Myrtaceae were allocated to Myrtoideae, tribe Myrteae.

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Chromosome studies in Brazilian and Neotropical species of Myrtaceae (Myrteae) are still scarce. Most of the studies have been carried out in Australasian species – traditional Leptospermoideae (Atchison, 1947; Brighton & Ferguson, 1976; Rye, 1979; Tyagi, McComb & Considine, 1991; Matsumoto *et al*., 2000; Lange & Murray, 2004). Forni-Martins, Pinto-Maglio & Cruz (1995), Andrade & Forni-Martins (1998), Pedrosa *et al*. (1999), and Forni-Martins & Martins (2000) performed the first studies in Brazilian species, in which they analysed chromosome numbers of ten species, including species of *Campomanesia* Ruiz & Pávon and *Myrcia* DC., and recorded previously unpublished counts.

Recently, Costa (2004) and Costa & Forni-Martins (2006a, b, 2007) reported chromosome counts in *c*. 50 species of Myrteae belonging to different subtribes (Eugeniinae, Myrciinae, and Myrtinae), finding a predominance of $2n = 22$. These were new for the majority of species and even for some genera, such as *Gomidesia* O. Berg, *Marlierea* Cambess., and *Plinia* L., and also enhanced the knowledge of chromosome numbers for the two most diverse genera of Myrteae, *Eugenia* L. and *Myrcia*. The authors confirmed the

basic chromosome number $x = 11$ for Myrtaceae, previously proposed by Atchison (1947) and Raven (1975). However, several other Neotropical genera, such as *Accara* Landrum, *Blepharocalyx* O. Berg, *Calycolpus* O. Berg, *Mosiera* Small, *Ugni* Turcz (Myrtinae), *Calyptranthes* Sw. (Myrciinae), *Calycorectes* O. Berg, *Hexaclamys* O. Berg, *Neomitranthes* D. Legrand, and *Siphoneugena* O. Berg (Eugeniinae), are still in need of chromosome studies.

According to Costa (2004), the identification of Brazilian species of Myrtaceae is difficult, because of hybridization and polyploidy, giving rise to types with morphological characters intermediate between those of the original taxa. Chromosome differentiation, especially chromosome number duplication, interrupted gene flow between them.

Chromosome numbers and karyotypic parameters, such as the form and size of chromosomes, amongst others, are of great importance, supplying characters for taxonomic studies (Jackson, 1971; Raven, 1975; Stace, 1991). Information on the morphology of the chromosomes in Myrteae species is practically nonexistent, probably because they are small, not exceeding 2 μm (Costa, 2004). Karyotype details have been described only for some species of *Eucalyptus* L'Hér. (Eucalypteae), revealing highly symmetrical karyotypes with exclusively metacentric chromosomes (Matsumoto *et al*., 2000; Matsumoto & Marin-Morales, 2001). In Myrteae, Vijayakumar & Subramanian (1985) described the karyotypic variation in different cultivated varieties of *Psidium guajava*. The species presented moderately symmetrical karyotypes [average total form percentage (TF%) = 33.9; $TF\% = 100\ \Sigma S\ \Sigma L^{-1}$, where S is the total sum of short arms and L is the total sum of chromosome lengths; Huziwara, 1962] and small chromosomes, varying from 1.8 to 0.8 μm. The study of *P. guajava* is the most detailed register of karyotype in this group, with three to six pairs of chromosomes being subtelocentric in this species, whereas, in *P. acutangulum*, a predominance of metacentric chromosomes has been observed (Forni-Martins & Martins, 2000).

These contrasting results strongly suggest that a karyotype survey of Myrtaceae could provide data that may be valuable for species characterization in this group, and the present work aims to evaluate this potential in some Brazilian species.

MATERIAL AND METHODS

Material collection

The 15 analysed species, comprising eight genera in three subtribes of Myrteae (Eugeniinae, Myrciinae, and Myrtinae), had chromosome numbers determined previously by Costa & Forni-Martins (2006a, b, 2007). They were collected in different savannic (cerrado *s.s.*, campos rupestres) and forest (Atlantic Tropical Rain Forest) vegetations in south-eastern Brazil. Voucher details are recorded by Costa (2004) and Costa & Forni-Martins (2006a, b, 2007). The species were identified using specialized bibliography and by comparison with specimens in herbaria, and were confirmed by specialists (Marcos Sobral, UFMG; Eve Lucas, RBG Kew; Carolyn Proença, UnB). Voucher materials were deposited in the UEC Herbarium (Universidade Estadual de Campinas) (Table 1).

Karyotype analysis

To obtain mitotic metaphases, seeds were germinated at temperatures of 28–30 °C. The root tips were pretreated with 2 mM 8-hydroxyquinoline for 24 h at 8 °C, and fixed in Farmer's solution (ethanol–acetic acid, 3 : 1 v/v). For slide preparation, they were frozen (– 20 °C), stained using the Giemsa technique (Guerra, 1983), and squashes were made.

Measurements (in five to ten metaphases) of chromosome length (long arm, l; short arm, s) were made using the MicroMeasure program version 3.2 (Reeves & Tear, 2000). The nomenclature of the chromosome types followed Guerra (1986), with calculations of the centromeric index ($CI = s/l + s$) and the ratio between the arms ($R = l/s$). Ideograms were prepared for each species based on the average measurements of each chromosome pair. The total chromosome length (TCL; the sum of the length of all metaphase chromosomes), index of karyotypic symmetry ($TF\% = 100\ \Sigma S\ \Sigma L^{-1}$) (Huziwara, 1962), and the symmetry indices of Romero Zarco (1986), A_1 and A_2, often used for comparison of species with few differences in karyotypic symmetry, were also calculated. The intrachromosome asymmetry (A_1) is calculated from the ratio between the average values of the chromosome arms following the formula $A_1 = 1 - [\Sigma(s_i/l_i)/n]$, where s_i is the average length of short arms in every homologous chromosome pair, l_i is the average length of long arms in every homologous pair, and n is the number of homologous chromosome pairs. The interchromosome asymmetry (A_2) is given by the variation in length of the chromosomes, independent of the chromosome size, calculated following the formula $A_2 = SD/x$, where SD is the standard deviation and x is the mean of the chromosome lengths.

RESULTS AND DISCUSSION

The chromosome numbers of $2n = 22$ and 44 are distributed between the different species, genera, and subtribes, confirming the previous records of Costa & Forni-Martins (2006a, b, 2007). Of the 15 species (Figs 1–12), only four presented $2n = 4x = 44$: *Eugenia*

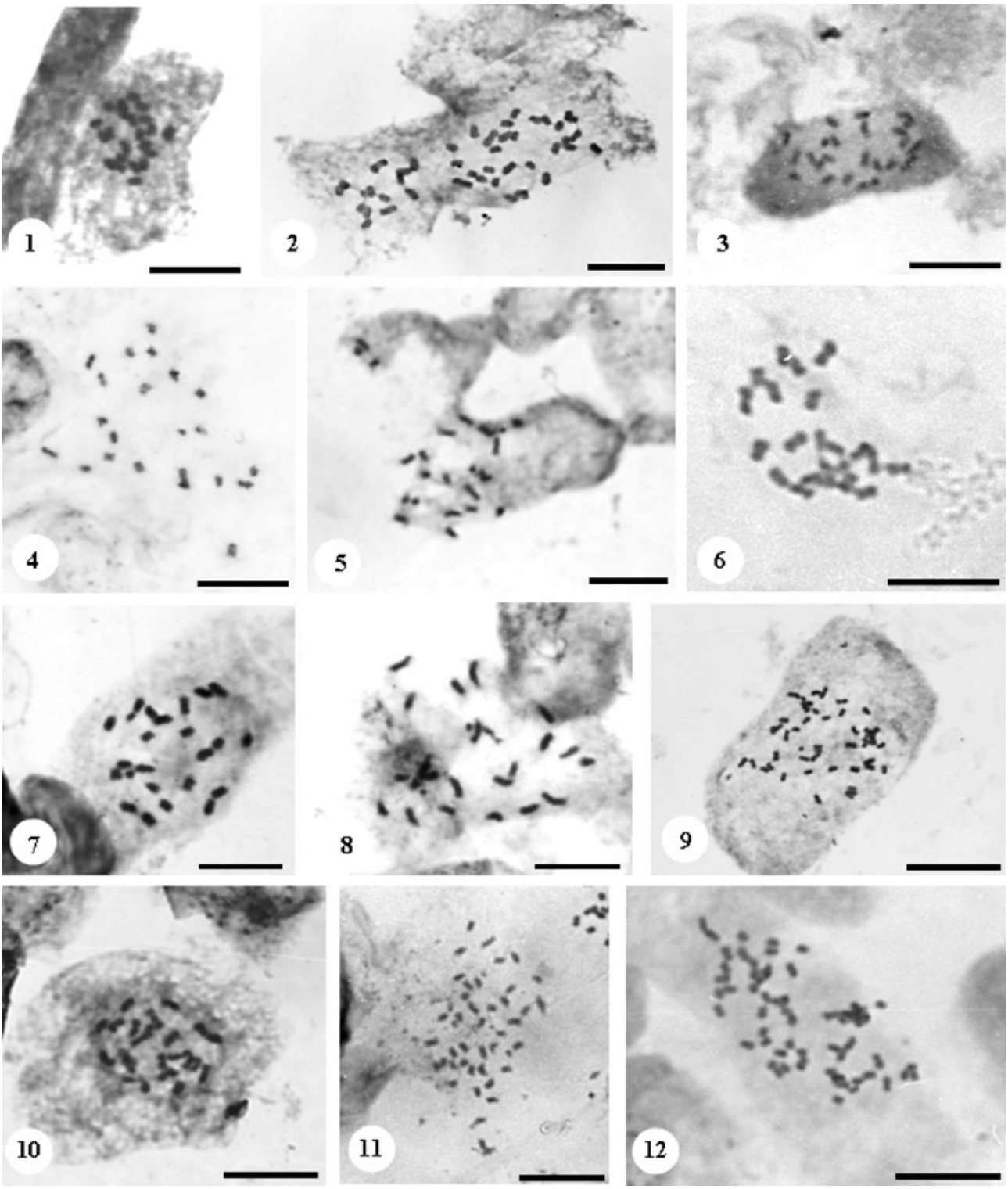
Table 1. Karyotypic parameters of Myrtaceae

Code	Subtribe/species	Collector number	Habitat	2*n*	KF	TCL	RMCL	TF%	A_1	A_2
	Eugeniinae									
1	*Eugenia bracteata* Vell.	*I. R. Costa* 434	CLT	22	8m + 3sm	17.03	1.02–0.57	40.91	0.295	0.167
2	*E. hyemalis* Cambess.	*I. R. Costa et al.* 442	CRP	44	16m + 6sm	34.14	1.36–0.43	20.83	0.266	0.298
3	*E. punicifolia* (Kunth) DC.	*I. R. Costa et al.* 492	CRP	22	2m + 9sm	14.75	1.13–0.49	35.84	0.424	0.255
4	*E. uniflora* L.	*I. R. Costa* 420	CLT	22	7m + 4sm	15.20	1.28–0.40	40.70	0.302	0.354
5	*Myrciaria delicatula* (DC.) O. Berg	*I. R. Costa et al.* 425	ROC	22	10m + 1sm	16.40	1.18–0.48	43.81	0.212	0.254
6	*M. tenella* (DC.) O. Berg	*I. R. Costa et al.* 494	CER	22	6m + 5sm	21.90	1.34–0.63	38.92	0.321	0.359
7	*Plinia cauliflora* (Mart.) Kausel	*I. R. Costa* 421	CLT	22	11m	12.84	0.82–0.44	44.23	0.198	0.187
	Myrciinae									
8	*Gomidesia* sp.	*I. R. Costa et al.* 481	TRF	22	2m + 9sm	18.02	1.35–0.52	37.50	0.385	0.285
9	*Marlierea tomentosa* Cambess.	*K. Matsumoto* 800	TRF	22	4m + 7sm	13.50	0.77–0.46	37.62	0.388	0.142
10	*Ma. warmingiana* Kiaersk.	*K. Matsumoto* 836	TRF	22	9m + 2sm	18.70	1.09–0.76	42.70	0.238	0.228
11	*Myrcia lingua* (O. Berg) Mattos	*I. R. Costa et al.* 430	CER	22	8m + 3sm	19.60	1.23–0.50	45.37	0.204	0.251
12	*Myrcia* sp.	*K. Matsumoto* 833	TRF	44	16m + 6sm	32.57	1.00–0.50	42.39	0.372	0.165
	Myrtinae									
13	*Campomanesia pubescens* (Mart. ex DC.) O. Berg	*I. R. Costa et al.* 428	CER	22	8m + 3sm	22.25	1.61–0.56	42.68	0.198	0.187
14	*Psidium cattleianum* Afzel. ex Sabine	*I. R. Costa* 486	CER	44	19m + 3sm	31.90	1.09–0.45	43.18	0.248	0.336
15	*P. cinereum* Mart. ex DC.	*I. R. Costa et al.* 509	CLT	44	12m + 10sm	28.25	0.91–0.38	41.53	0.285	0.246

A_1 and A_2, asymmetry indices (Romero Zarco, 1986); KF, karyotypic formula (m, metacentric; sm, submetacentric); RMCL, range in mean chromosome length (μm); TCL, total chromosome length (μm); TF%, symmetry index (Huziwara, 1962); 2*n*, diploid chromosome number.

Habitat: CER, cerrado vegetation; CLT, cultivated at UNICAMP; CRP, campos rupestres vegetation; ROC, rocky outcrop; TRF, Atlantic Tropical Rain Forest vegetation.

Code numbers indicate species in Figure 16.



Figures 1–12. Mitotic metaphases in species of Myrteae. Figs 1–6. Subtribe Eugeniinae. Fig. 1. *Eugenia bracteata* ($2n = 22$). Fig. 2. *E. hyemalis* ($2n = 44$). Fig. 3. *E. uniflora* ($2n = 22$). Fig. 4. *Myrciaria delicatula* ($2n = 22$). Fig. 5. *M. tenella* ($2n = 22$). Fig. 6. *Plinia cauliflora* ($2n = 22$). Figs 7–9. Subtribe Myrciinae. Fig. 7. *Gomidesia* sp. ($2n = 22$). Fig. 8. *Marlierea tomentosa* ($2n = 22$). Fig. 9. *Myrcia* sp. ($2n = 44$). Figs 10–12. Subtribe Myrtinae. Fig. 10. *Campomanesia pubescens* ($2n = 22$). Fig. 11. *Psidium cattleianum* ($2n = 44$). Fig. 12. *P. cinereum* ($2n = 44$). Scale bars, 5 μm.

hyemalis (Fig. 2), *Myrcia* sp. (Fig. 9), *Psidium cattleianum* (Fig. 11), and *P. cinereum* (Fig. 12). The basic chromosome number of $x = 11$ in Myrtaceae (Myrteae) is constant, with polyploids occurring in several species (Atchison, 1947; Andrade & Forni-Martins, 1998; Costa, 2004; Costa & Forni-Martins, 2006a, b, 2007).

All karyotype data referring to the chromosome size and morphology are previously unknown (Figs 13–15, Table 1). No secondary constrictions were observed, probably because of the small size of the chromosomes and the non-specific staining. The length of the chromosomes varied from 0.38 μm in *P. cinereum* to 1.61 μm in *Campomanesia pubescens* (Table 1). Of the species with $2n = 22$, TCL varied from 12.84 μm in *Plinia cauliflora* to 22.25 μm in *C. pubescens*.

A higher value of TCL (34.14 μm) was observed in *E. hyemalis* as a result of its polyploid chromosome number ($2n = 44$).

In all species, the variation of size between the chromosomes was gradual, and it was not possible to recognize groups of long, intermediate, or small chromosomes.

In Myrtaceae, karyomorphological analysis does not support the subtribal classification in Myrteae. In other groups, such as Malpighiaceae, it supports the intrafamilial classification into two large subfamilies: Malpighioideae, with $x = 5$ small chromosomes, lianoid species, and winged fruits; and Byrsonimoideae, with $x = 6$ large chromosomes, shrub or tree species, with non-winged fruits (Lombello & Forni-Martins, 2002). In Sapindaceae, cytotaxonomy has contributed to the knowledge of taxonomic and evolutionary relationships. Variations in the karyotypes, as a result of a decrease in the chromosome number associated with an increase in the absolute length of the chromosomes, have supplied indications of the change from the tree to the lianoid pattern, characteristic of the Paullineae (Lombello & Forni-Martins, 1998).

In this study, *Plinia cauliflora* was the only species to present exclusively metacentric chromosomes (Figs 6, 13). In general, a predominance of metacentric chromosomes was observed, with the exception of some species with a larger proportion of submetacentric chromosomes, such as *E. bracteata* and *E. punicifolia* (in Eugeniinae) and *Marlierea tomentosa* (Myrciinae), all with $2n = 22$. These three species, plus *Myrciaria tenella* (Eugeniinae) and *Gomidesia* sp. (Myrciinae), were the only ones to present TF% lower than 40.00 (Table 1). TF% indicates a moderate degree of karyotype symmetry in the majority of species, reaching a maximum value in *Myrcia lingua* (45.37). Vijayakumar & Subramanian (1985) also observed karyotypes with a moderate degree of symmetry in Myrtaceae in different cultivated varieties of

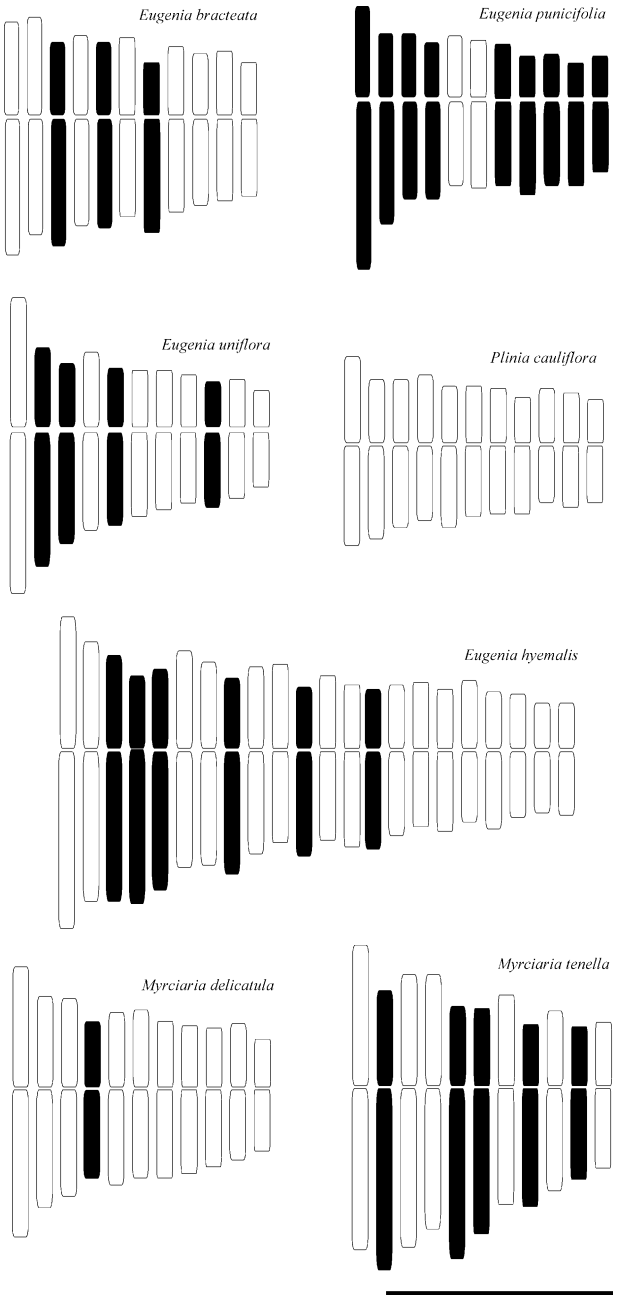


Figure 13. Idiograms of Eugeniinae species. Metacentric chromosomes in white and submetacentric chromosomes in black. Scale bar, 1 μm.

Psidium, with TF% varying from 31.1 to 38.4 and with three to six chromosomes being subtelocentric. The extremes in the family are an asymmetrical karyotype record in *E. caryophyllata* (TF% = 23.70; Vijayakumar & Subramanian, 1985) and the high degree of symmetry in species of *Eucalyptus*, in which most chromosomes are metacentric, with values of TF% varying from 45.90 to 48.60 (Matsumoto *et al*.,

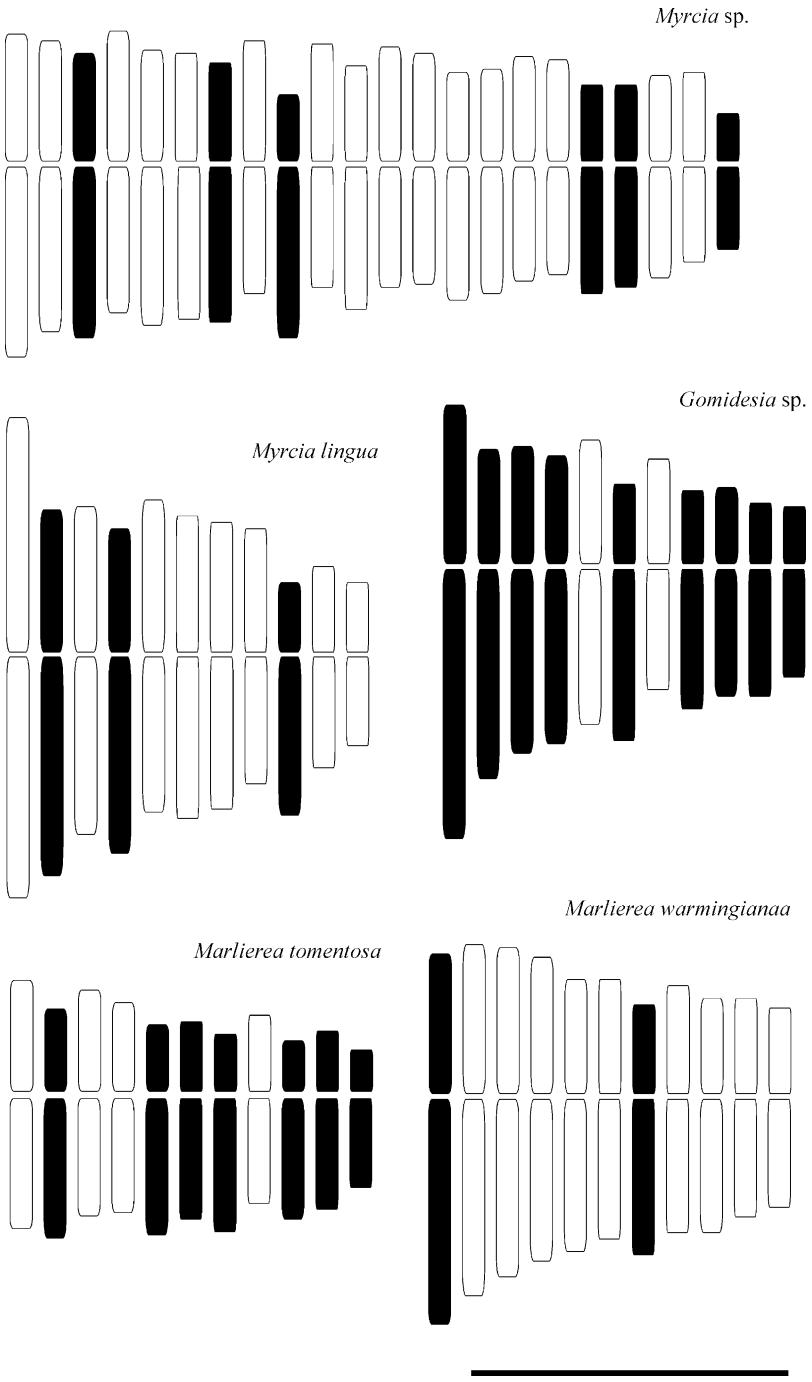


Figure 14. Idiograms of Myrciinae species. Metacentric chromosomes in white and submetacentric chromosomes in black. Scale bar, 1 μm.

2000; Matsumoto & Marin-Morales, 2001). In *Eucalyptus*, only two species present a pair of submetacentric chromosomes (Mora, Palma-Rojas & Jara-Seguel, 2005).

The estimates of TF% (Huziwara, 1962) are based only on differences in centromere position and do not evaluate the relative size of the chromosomes, the original parameter considered by Lewitsky (in Stebbins, 1971). The asymmetry index of Romero Zarco (1986) combines centromeric position and relative size of the chromosomes, leading to results different from those obtained with the TF% index. Species with

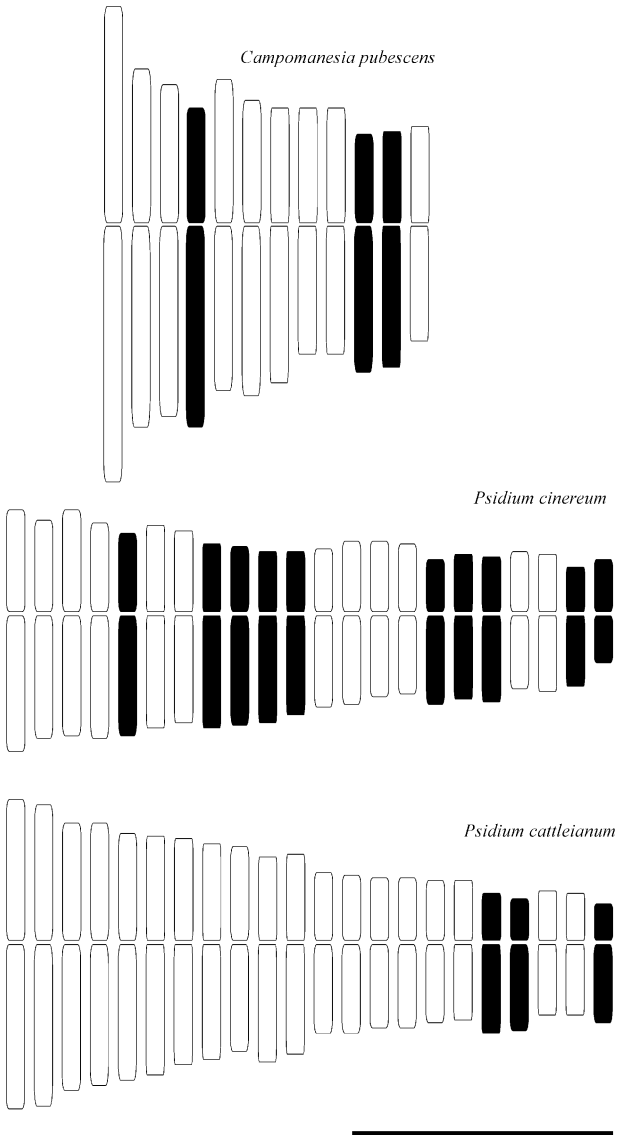


Figure 15. Idiograms of Myrtinae species. Metacentric chromosomes in white and submetacentric chromosomes in black. Scale bar, 1 μm.

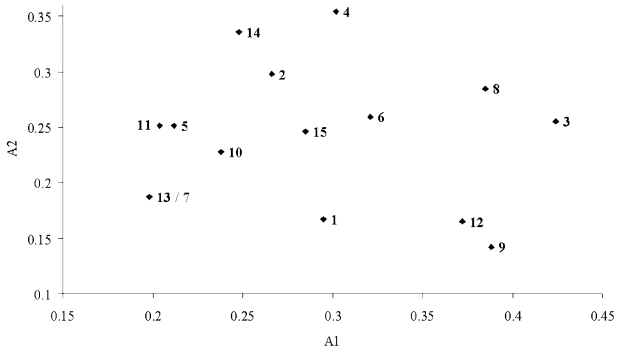


Figure 16. Dispersion of symmetry indices. Intra-chromosome (A_1) and inter-chromosome (A_2) symmetry (Romero Zarco, 1986). Code numbers for the species are identified in Table 1.

higher values of A_1 and A_2 have more karyotype asymmetry through the accumulation of major differences in centromere position and chromosome size. Of the five species with TF% lower than 40.00, *Gomidesia* sp. and *E. punicifolia* present more asymmetrical karyotypes, according to Romero Zarco (1986). *Marlierea tomentosa* has the highest values of A_1 (differences in centromeric position), but has chromosomes with similar lengths (low values of A_2). A similar situation occurs in *Myrcia* sp., which presents TF% = 42.39 (Table 1). To facilitate a comparative discussion of the karyotypes, TF% is used alone, because of the availability of this information in the literature.

Below, we discuss the results for each subtribe of Myrteae (*sensu* Berg, 1855–1856, 1857–1859).

Subtribe Eugeniinae O. Berg

In *Eugenia*, TCL varied from 14.75 μm in *E. punicifolia* to 34.14 μm in *E. hyemalis*, which is the only tetraploid species ($2n = 4x = 44$). A predominance of metacentric chromosomes was observed in two species, *E. hyemalis* and *E. uniflora*, whereas, in *E. bracteata* and *E. punicifolia*, the majority of the chromosomes were submetacentric. *Eugenia punicifolia* showed the most asymmetrical karyotype (TF% = 35.84) of all the analysed species, because of its high proportion of submetacentric chromosomes (Table 1, Figs 13, 16). The only previous study in species of Eugenia was presented by Vijayakumar & Subramanian (1985) for *E. caryophyllata*, which had $2n = 22$, with a high degree of asymmetry (TF% = 23.70), and chromosomes slightly larger than those found here, with variation from 2 to 1 μm, resulting in a TCL value of 29.60 μm. However, this species is now included in *Syzygium*, as *S. aromaticum* (L.) Merr & L. M. Perry, considered phylogenetically distant from *Eugenia* (Lucas *et al*., 2005; Wilson *et al*., 2005). In accordance with M. Dornelas (UNICAMP, Brazil, pers. comm.), *Syzygium* species present a genome size two to four times larger than that of *Eugenia* species.

The *Myrciaria* species, both with $2n = 22$, displayed considerable differences in karyotypic parameters (Table 1, Figs 13, 16). *Myrciaria tenella* has a more asymmetrical karyotype (TF% = 38.92), with chromosomes varying between 1.38 and 0.63 μm and TCL = 21.90 μm, by contrast with *M. delicatula*, which has a more symmetrical karyotype (TF% = 43.81) and slightly smaller chromosomes (1.18–0.48 μm; TCL = 16.40 μm).

In *Plinia*, the only species so far analysed, *Plinia cauliflora* ('jaboticaba'), presented the most symmetrical karyotype in Eugeniinae, with TF% = 44.23, and the smallest Romero Zarco symmetry indices of the subtribe, the chromosomes varying from 0.82 to 0.44 μm and TCL = 12.84 μm (Table 1, Figs 13, 16).

The taxonomic delimitation between *Myrciaria* and *Plinia* is complex, with a persistent calyx in *Plinia* species and a deciduous calyx in *Myrciaria* species (Sobral, 1993). Unfortunately, because of limited sampling (two species of *Myrciaria* and one of *Plinia*), the comparison of karyotype data did not help us to solve the taxonomic problems related to these genera. The karyological data of chromosome morphology, TCL and TF%, were more similar between *M. delicatula* and *Plinia cauliflora* than between *Myrciaria* species and *Plinia*.

Subtribe Myrciinae O. Berg

For *Gomidesia*, the only analysed species (still unidentified) had chromosomes varying between 1.35 and 0.52 μm and TCL = 18.02 μm. The karyotype was more asymmetrical than those of other species and genera of Myrciinae, with TF% = 37.50 (Table 1, Figs 14, 16).

Of the two species of *Marlierea*, both with $2n = 22$, *Ma. tomentosa* had the more asymmetrical karyotype (TF% = 37.62), with little variation in size between the chromosomes (Fig. 14). By contrast, *Ma. warmingiana* showed greater karyotype symmetry (TF% = 42.70). These differences may be a result of the higher proportion of submetacentric chromosomes in *Ma. tomentosa* (Table 1, Figs 14, 16). The smallest karyotype was observed in *Ma. tomentosa* (Table 1, Fig. 14), with TCL = 13.50 μm.

In *Myrcia*, the two analysed species did not show much difference in the size of the chromosomes (Table 1, Fig. 3), but TCL varied from 19.60 μm in *My. lingua* to 32.57 μm for *Myrcia* sp., because of the duplicated number of chromosomes ($2n = 44$) in the latter. Both species presented TF% > 40.00 (Table 1), with *Myrcia* sp. presenting a greater difference between the chromosome arms.

Myrciinae is considered to be the most highly derived subtribe of Myrteae, and the delimitation of the three genera (*Gomidesia, Marlierea*, and *Myrcia*) is unclear, especially between *Myrcia* and *Marlierea*, which are separated by their differing hyphanthium development and modes of floral bud rupture (Landrum & Kawasaki, 1997). In a recent phylogenetic analysis (Lucas *et al*., 2005), the resolution between the genera has remained obscure, although *Gomidesia* appears as a monophyletic group, considering both morphological and molecular data. The karyotypic analysis also does not contribute towards the characterization of these genera, because there are similarities in the size and morphology of the chromosomes between the species.

Subtribe Myrtinae O. Berg

In this group, all analysed species had symmetrical karyotypes, with TF% values above 40.00 and a predominance of metacentric chromosomes (Table 1, Fig. 15).

Campomanesia pubescens had chromosomes larger than those of the two species of *Psidium*, *P. cattleianum* and *P. cinereum* (Table 1, Figs 15, 16). Although the species of *Psidium* each had $2n = 44$, their TCL values (31.90 and 28.25 μm) were only about 70% greater than that of *C. pubescens* (TCL = 22.25 μm), with $2n = 22$. *Campomanesia pubescens* also had a more symmetrical karyotype. The karyotypes of *Psidium* species had differences in the parameters TCL, TF%, and karyotypic formula (Table 1), with that of *P. cinereum* more asymmetrical than that of *P. cattleianum* (Figs 15, 16).

Vijayakumar & Subramanian (1985) provided karyotype details for different cultivated plants of *P. guajava*, all with $2n = 22$ and with chromosomes varying from 1.8 to 0.8 μm. Karyotypes revealed differences between cultivars in the relative numbers of metacentric and submetacentric chromosomes (TCL between 21.2 and 32.8 μm) and TF% values (between 31.1 and 38.4). In the same species, Kumar & Ranade (1952) found a triploid cytotype ($2n = 3x = 33$). According to L. Landrum (Arizona State University, pers. comm.), diverse Neotropical *Psidium* have a hybrid origin, with *P. guajava* and *P. guineense* as parents (both have $2n = 22$; I. R. Costa & E. R. Forni-Martins, unpubl. data).

Myrtinae is considered to be the most primitive subtribe of Myrteae and *Psidium* the most highly derived genus in this group. Lewitski (in Jackson, 1971) stated that asymmetrical karyotypes are indicative of highly derived taxa, but this relationship cannot be established in Myrteae as yet. Additional karyotype studies are required in a larger number of species to reach any conclusion.

Although karyotypic characterization in Myrteae is still not useful in the delimitation of the subtribes or genera, it seems promising for the characterization of the species in the genera. In contrast, in *Eucalyptus* (Eucalypteae), the similar and very symmetrical karyotypes were not useful indicators of species differentiation (Matsumoto *et al*., 2000; Matsumoto & Marin-Morales, 2001; Mora *et al*., 2005). The Eucalypteae is considered to be one of the most primitive in Myrtaceae (Wilson *et al*., 2001, 2005), and the high level of symmetry observed in the karyotypes of *Eucalyptus* agrees with Lewitski (in Jackson, 1971), who

anticipated the occurrence of symmetrical karyotypes in the most primitive groups. Karyotypes of fleshy-fruited Myrtaceae (Myrteae) were shown to be more derived by their moderate degree of karyotypic symmetry relative to that of dry-fruited taxa (Chamelauciae, Eucalyptieae, Melaleuceae, etc.).

Until now, karyotypic analysis in Myrtaceae has been useful only for the characterization of some species, and not for distinguishing genera or subtribes, as a result of the very similar karyotype characters throughout these groups, possession of small chromosomes, gradual variation of size, and the predominance of metacentric chromosomes. Additional karyotype analysis of a larger number of species may identify useful parameters for the delimitation of genera. In the future, the application of other techniques, such as nucleolar-organizing region (NOR) banding and *in situ* hybridization, will be necessary to identify the secondary constrictions and other indicators of longitudinal differentiation on the chromosomes of this group.

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REFERENCES

Andrade FG, Forni-Martins ER. 1998. Estudos cromossômicos em espécies de Myrtaceae. *Genetics and Molecular Biology* **21** (Suppl.)**:** 166.

Angiosperm Phylogeny Group (APG). 2003. An update of angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141:** 399–436.

Atchison E. 1947. Chromosome numbers in the Myrtaceae. *American Journal of Botany* **34:** 159–164.

Berg O. 1855–1856. Revisio Myrtacearum Americae. *Linnaea* **27:** 1–472.

Berg O. 1857–1859. *Myrtaceae*. In: von Martius CFP, ed. *Flora Brasiliensis* **14:** 1–655.

Brighton CA, Ferguson IK. 1976. Chromosome counts in the genus *Melaleuca (Myrtaceae)*. *Kew Bulletin* **31:** 27–33.

Costa IR. 2004. Estudos cromossômicos em espécies de Myrtaceae Juss. no sudeste do Brasil. Unpublished Master's Dissertation, Universidade Estadual de Campinas, Brazil.

Costa IR, Forni-Martins ER. 2006a. Chromosome studies in Brazilian species of *Campomanesia* Ruiz & Pávon and *Psidium* L. (Myrtaceae Juss.). *Caryologia* **59:** 7–13.

Costa IR, Forni-Martins ER. 2006b. Chromosome studies in species of *Eugenia*, *Myrciaria* and *Plinia* (Myrtaceae) from south-eastern Brazil. *Australian Journal of Botany* **54:** 409–415.

Costa IR, Forni-Martins ER. 2007. Chromosome studies in *Gomidesia*, *Marlierea*, *Myrceugenia* and *Myrcia* (Myrtaceae, subtribe Myrciinae). *Kew Bulletin* **62:** 113–118.

Forni-Martins ER, Martins FR. 2000. Chromosome studies on Brazilian cerrado plants. *Genetics and Molecular Biology* **23:** 947–955.

Forni-Martins ER, Pinto-Maglio CAP, Cruz ND. 1995. Chromosome numbers in Brazilian cerrado plants. *Revista Brasileira de Genética* **18:** 281–288.

Guerra M. 1983. O uso do Giemsa em citogenética vegetal – comparação entre a coloração simples e o bandamento. *Ciência e Cultura* **35:** 190–193.

Guerra M. 1986. Reviewing the chromosome nomenclature of Levan *et al. Revista Brasileira de Genética* **9:** 741–743.

Huziwara Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. *American Journal of Botany* **49:** 116–119.

Jackson RC. 1971. The karyotype in systematics. *Annual Review of Ecology and Systematics* **2:** 327–368.

Kumar LSS, Ranade SG. 1952. Autotriploidy in Guava (*Psidium guajava* Linn.). *Current Science* **3:** 75–76.

Landrum LR, Kawasaki ML. 1997. The genera of Myrtaceae in Brazil: an illustrated synoptic treatment and identification keys. *Brittonia* **49:** 508–536.

Lange PJ, Murray BG. 2004. Chromosome numbers in *Kunzea* (Myrtaceae). *Australian Journal of Botany* **52:** 609–617.

Lombello RA, Forni-Martins FR. 1998. Chromosomal studies and evolution in Sapindaceae. *Caryologia* **51:** 81–93.

Lombello RA, Forni-Martins FR. 2002. Cytogenetics of twelve species of Malpighiaceae A. Juss. from Southeastern Brazil. *Caryologia* **55:** 241–250.

Lucas EJ, Belsham SR, NicLughada EM, Orlovich DA, Sakuragui CM, Chase MW, Wilson PG. 2005. Phylogenetic patterns in the fleshy-fruited Myrtaceae – preliminary molecular evidence. *Plant Systematics and Evolution* **251:** 35–51.

Matsumoto ST, Marin-Morales MA. 2001. Paternity study of an interspecific natural hybrid of the genus *Eucalyptus* L'Hér (Myrtaceae) based on cytogenetic data. *Cytologia* **66:** 437–443.

Matsumoto ST, Marin-Morales MA, Ruas CF, Ruas PM. 2000. Cytogenetic analysis of seven species of *Eucalyptus* L'Her. (Myrtaceae). *Caryologia* **53:** 205–212.

Mora F, Palma-Rojas C, Jara-Seguel P. 2005. Comparación del cariótipo de *Eucalyptus globulus y Eucalyptus*

cladocalix (Myrtaceae). *Agricultura Técnica (Chile)* **65:** 20–25.

Niedenzu F. 1893. Myrtaceae. *Die Natürlichen Pflanzenfamilien* **III:** 57–107.

Pedrosa A, Gitaí J, Barros e Sila AE, Felix LP, Guerra M. 1999. Citogenétca de Angiospermas coletadas em Pernambuco – V. *Acta Botanica Brasilica* **13:** 49–60.

Raven PH. 1975. The bases of angiosperm phylogeny: cytology. *Annals of the Missouri Botanical Garden* **62:** 724–764.

Reeves A, Tear J. 2000. *MicroMeasure for windows*, Version 3.3. Free program distributed by the authors over the Internet. URL http://www.colostate.edu/Depts/Biology/MicroMeasure [accessed in March 2006].

Romero Zarco C. 1986. A new method for estimating karyotype asymmetry. *Taxon* **35:** 526–530.

Rye BL. 1979. Chromosome number variation in the Myrtaceae and its taxonomic implications. *Australian Journal of Botany* **27:** 547–573.

Sobral M. 1993. Sinopse de *Myrciaria* (Myrtaceae). *Napaea* **9:** 13–41.

Stace CA. 1991. *Plant taxonomy and biosystematics*. Cambridge: Cambridge University Press.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. London: Edward Arnold.

Tyagi AP, McComb J, Considine J. 1991. Cytogenetic and pollination studies in the genus *Verticordia* DC. *Australian Journal of Botany* **39:** 261–272.

Vijayakumar N, Subramanian D. 1985. Cytotaxonomical studies in South Indian Myrtaceae. *Cytologia* **50:** 513–520.

Wilson PG, O'Brien MM, Gadek PA, Quinn CJ. 2001. Myrtaceae revisited: a reassessment of intrafamilial groups. *American Journal of Botany* **88:** 2013–2025.

Wilson PG, O'Brien MM, Heslewood MM, Quinn CJ. 2005. Relationships within Myrtaceae *sensu lato* based on a *mat*K phylogeny. *Plant Systematics and Evolution* **251:** 3–19.