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## Chromosome studies in Brazilian species of *Campomanesia* Ruiz & Pávon and *Psidium* L. (Myrtaceae Juss.)

RIBEIRO DA COSTA ITAYGUARA<sup>1\*,2</sup> and ELIANA REGINA FORNI-MARTINS<sup>2</sup>

<sup>1</sup> Programa de Pós-graduação em Biologia Vegetal, Universidade Estadual de Campinas (UNICAMP), Instituto de Biologia (IB), CP 6109, CEP 13083-970, Campinas, SP, Brazil. Fax: ++19 37886168.

<sup>2</sup> Departamento de Botânica, IB, UNICAMP, CP 6109, CEP 13083-970, Campinas, SP, Brazil. Fax: ++19 37886168

**Abstract** — In this work, we carried out chromosome counts of four species of *Campomanesia* (*C. adamantium*, *C. guaviroba*, *C. phaea* and *C. pubescens*) and four of *Psidium* (*P. acutangulum*, *P. cattleianum*, *P. cinereum* and *P. guajava*) from different populations. In *Campomanesia*,  $2n=22$  was found in all of the species analyzed, and no polyploidy was seen. For *Psidium*, only *P. guajava* had  $2n=22$  and another three species had  $2n=44$ . No abnormalities in meiosis were observed. High levels of pollen stainability (> 80%) was seen in all species. About 50% of Myrtinae species were polyploid, indicating the importance of polyploidy in the evolution of this group.

**Key words:** Chromosomes, Myrtaceae, Myrtinae, Myrtoideae and Polyploidy.

### INTRODUCTION

With about 133 genera and > 3800 species (WILSON *et al.* 2001), Myrtaceae has a wide distribution in tropical and subtropical regions, and is well represented in temperate regions of Australia (CRONQUIST 1981). Traditionally (NIEDENZU 1893), this family is divided into two subfamilies: Myrtoideae with bacoid fruits, opposite leaves and a pantropical distribution; and Leptospermoideae with dry and capsular fruits and alternate leaves, essentially australasian. All Neotropical Myrtaceae are included in one tribe, the fleshy-fruited Myrteae (BERG 1855-56; BERG 1857-59), with about 70 genera and 2400 species (SCHMID 1980), of which two thirds are distributed in the New World (LANDRUM 1986). BERG (1855-56; 1857-59) divided Myrteae into three subtribes: Eugeniinae (globose embryos with a short distinct radicle), Myrciinae (embryos with foliar cotyledons and a long radicle) and Myrtinae (originally named *Pimentinae*, with reduced cotyledons and a long radicle) (LANDRUM and KAWASAKI 1997). Following a recent phylogenetic analysis based on *ITS* and *psbA-trnH* sequence data, LUCAS *et al.* (2005) found Eugeniinae and Myrtinae (mainly paleotropical distribution, with some species found in the Mediterranean) as paraphyletic groups and

Myrciinae (exclusively neotropical) to be monophyletic. According to the latter authors, *Psidium* was also monophyletic in the subtribe Myrtinae.

MCVAUGH (1956) considered the American Myrtaceae a complex group that needed extensive systematic studies and BARROSO (1991) also suggested combined biosystematics studies with regional surveys in order to defining the taxa.

There have been few chromosome studies of Neotropical species of Myrtaceae. Most studies carried out with Australian species and only about eight Neotropical species (Myrteae) (ATCHINSON 1947; RYE 1979). The Neotropical genera of fleshy-fruited Myrteae studied so far include *Campomanesia*, *Eugenia*, *Myrceugenia*, *Myrcia*, *Myrcianthes*, *Luma* and *Psidium* (ANDRADE and FORNI-MARTINS 1998; ATCHINSON 1947; COSTA 2004; COSTA and FORNI-MARTINS 2005; 2006; FORNI-MARTINS *et al.* 1995; FORNI-MARTINS and MARTINS 2000; SANDERS *et al.* 1983; LANDRUM 1981).

In general, Myrtaceae shows little variation in chromosome number, with  $2n=22$  in most genera (RYE 1979). Some species of *Eugenia* are dispolids, with  $2n=24$ , 42, 45, 46 and 54 (BOLKHOVSKIKH *et al.* 1969; DARLINGTON and WYLIE 1961). Polyploid species are frequent in fleshy-fruited taxa (ANDRADE and FORNI-MARTINS 1998; COSTA 2004; COSTA and FORNI-MARTINS 2005; 2006; FORNI-MARTINS and MARTINS 2000), but are rare in dry and capsular-fruited groups, in which dispolid species predominate (RYE 1979).

\* Corresponding author: e-mail: itayguara@yahoo.com

ANDRADE and FORNI-MARTINS (1998) showed the importance of polyploidy in the evolution of this family, with *Eugenia pluriflora* having  $n=33$  and *Psidium* sp. with  $2n=66$ . Polyploidy is frequent in Myrtinae, mostly in *Psidium*, with  $2n=33$ , 44, 55, 66, 77 and 88 (ATCHINSON 1947; ANDRADE and FORNI-MARTINS 2000; BOLKHOVSKIKH *et al.* 1969; GOLDBLATT 1981; GOLDBLATT and JOHNSON 1990; MOORE 1977). Polyploidy is frequently associated with inter-specific hybridization and restores the hybrid fertility (GUERRA 1988). COSTA (2004) and COSTA and FORNI-MARTINS (2006) attributed the difficulty in identifying Neotropical Myrtaceae to possible events of hybridization associated with polyploidy, seen in several species of *Eugenia* (subtribe Eugeniinae). Hybridization could explain the existence of intermediate characters between closely related species, with the gene flow among them being interrupted by chromosome differentiation, especially by polyploidy.

The aim of this work was to determine the chromosome numbers of some species of *Campomanesia* and *Psidium*, and to access the importance of polyploidy in the evolution of these groups.

## MATERIAL AND METHODS

**Material collecting** - Four species of *Campomanesia* and four species of *Psidium* were collected in different habitats (cerrado *s.s.*, "campos rupestres", tropical rain forest) in southeastern and

midwestern Brazil (Table 1). The species and populations were selected according to the availability of material for chromosome studies (floral buds and mature fruits with seeds). The species were initially identified based on literature reports and by comparison with herbaria collections and, were subsequently confirmed by specialist (Dr. Carolyn Proença — Departamento de Botânica, Universidade de Brasília). Vouchers specimens were deposited in the UEC herbarium at Universidade Estadual de Campinas (UNICAMP).

**Chromosome studies** - For meiotic studies, floral buds were fixed in Farmer solution (ethanol:acetic acid, 3:1, v/v) for 24h and stored in 70% alcohol at freezer. The cytological preparations were obtained by squashing the anthers in acetocarmine 1.2% (MEDINA & CONAGIN 1964). To obtain mitotic metaphases, seeds were germinated at temperature of 28°-30°C. The radicular meristems were pre-treated with 2mM 8-hydroxyquinoline for 24h, at 8°C. The roots were fixed in Farmer solution and stored in 70% alcohol and frozen until slide preparation and staining with Giemsa (GUERRA 1983). The slides were examined by light microscopy and meiotic and mitotic cells with a good chromosome condensation and spreading were photographed with a photomicroscope.

**Pollen stainability** - Was accessed using a slightly modified technique of MEDINA and CONAGIN (1964), using acetocarmine 1,2%. About 1000

Table 1 — Species of *Campomanesia* and *Psidium* studied, showing the gametic ( $n$ ) and somatic ( $2n$ ) chromosome number and details of the voucher specimens. (\*) first records for the species, (-) not analyzed. Habitat: CRUP — Campo Rupestre vegetation, CERR — Cerrado vegetation, RFOR — Tropical Rain Forest, CULT — em cultivo. States: DF — Distrito Federal, MG — Minas Gerais, SP — São Paulo.

Genera / Species (and populations)	$n$	$2n$	Habitat, Municipality, State and collector
<b>Campomanesia</b>			
<i>C. adamantium</i> (Camb.) O. Berg* (pop1)	11	-	CRUP, Serra do Cipó, MG, I. R. Costa 437
(pop2)	11	-	CRUP, São Thomé das Letras, MG, C. F. Verola 35
(pop3)	-	22	CERR, Brasília, DF, I. R. Costa 621
<i>C. guaviroba</i> (DC.) Kiaersk.*	-	22	RFOR, Sete Barras, SP, M. A. R. Andrade**
<i>C. phaea</i> (O. Berg) Landrum*	-	22	RFOR, Sete Barras, SP, M. A. R. Andrade**
<i>C. pubescens</i> (DC) O. Berg. (pop1)	11	-	CERR, Mogi Guaçu, SP, I. R. Costa 428
(pop2)	-	22	CERR, Campinas, SP, I. R. Costa**
(pop3)	-	22	CERR, Assis, SP, I. R. Costa 501
<b>Psidium</b>			
<i>P. acutangulum</i> DC.	-	44	CULT, Campinas, SP, I. R. Costa 568
<i>P. cattleianum</i> Sabine (pop1)	-	44	CULT, Campinas, SP, I. R. Costa 486
(pop2)	22	-	RFOR, Cananéia, SP, C. Urbanetz 153
(pop3)	22	-	RFOR, Sete Barras, SP, I. R. Costa 523
<i>P. cinereum</i> Mart. ex DC.* (pop1)	-	44	CERR, Itirapina, SP, I. R. Costa 509
(pop2)	22	-	CERR, Assis, SP, I. R. Costa 496
<i>P. guajava</i> L.	-	22	CULT, Campinas, SP, I. R. Costa 476

\*\* no number availability.

pollen grains from at least five floral buds of each species were analyzed.

## RESULTS

In *Campomanesia*, the chromosome number was  $2n=22$  in all species analyzed: *C. adaman-*

*tium*, *C. guaviroba*, *C. phaea* and *C. pubescens* (Figure 1, Table 1). Three populations of *C. pubescens* and three of *C. adamantium* presented diploid state ( $n=11$  or  $2n=22$ ) (Table 2). For *Psidium*, the diploid state ( $2n=22$ ) was found only in *P. guajava*, whereas the others species (*P. acutangulum*, *P. cattleianum* — three populations and *P. cinereum*) were tetraploid ( $2n=4x=44$ ).

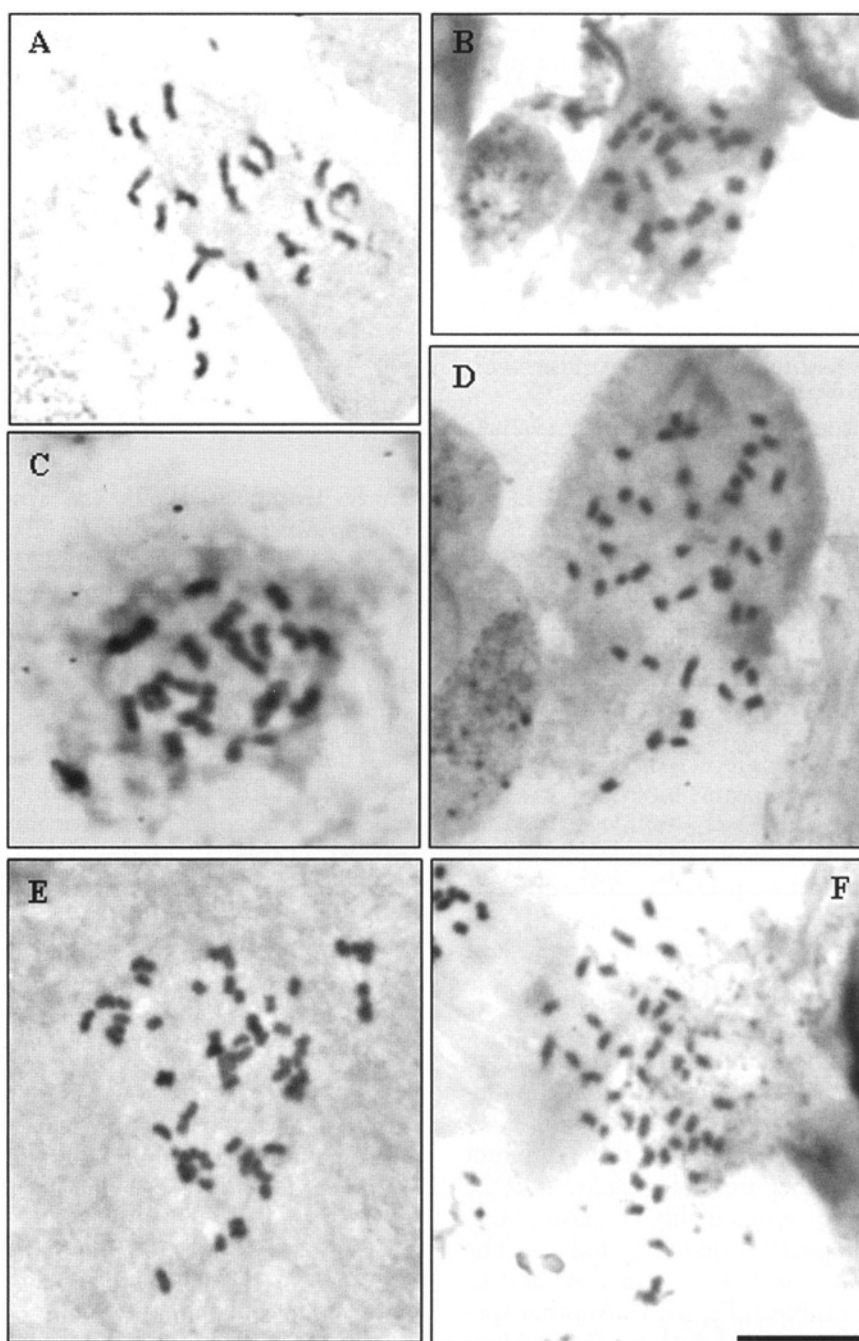


Fig. 1 — Chromosomes of *Campomanesia* and *Psidium* species. (A) *Campomanesia adamantium* ( $2n=22$ ), (B) *C. phaea* ( $2n=22$ ), (C) *C. pubescens* ( $2n=22$ ), (D) *Psidium acutangulum* ( $2n=44$ ), (E) *P. cattleianum* ( $2n=44$ ) and (F) *P. cinereum* ( $2n=44$ ).

Table 2 — Chromosome numbers in other species of *Campomanesia* and *Psidium*. Species currently considered synonyms are listed in parentheses. (-) not analyzed

Species	<i>n</i>	<i>2n</i>	Reference
<i>Campomanesia cambessedeanae</i> O. Berg	—	22	FORNI-MARTINS <i>et al.</i> 1989
<i>Campomanesia pubescens</i> (DC.) O. Berg	11	—	FORNI-MARTINS and MARTINS 2000
<i>Psidium acutangulum</i> DC.	—	44	FORNI-MARTINS and MARTINS 2000
<i>Psidium cattleianum</i> Sabine	—	88	ATCHISON 1947
<i>Psidium cerasoides</i> Cambess. (= <i>C. guaviroba</i> (A. P. de Candolle) Kiaerskou)	—	88	MOUSSEL 1965
<i>Psidium chinense</i> Sw.	—	22	NAITHANI and SRIVASTAVA 1965
<i>Psidium coriaceum</i> O. Berg (= <i>P. cattleianum</i> Sabine)	—	77	SINGHAL <i>et al.</i> 1980
<i>Psidium friedrichsthalianum</i> Niedenzu	11	22	SRIVASTAVA 1977a
<i>Psidium guajava</i> L.	—	22	VIJAYAKUMAR and SUBRAMANIAN 1985
		21, 22, 30, 33	D'CRUZ and RAO 1962
		22-28, 32-34	MAJUNDER and MUKKERJEE 1972
		33	KUMAR and RANADE 1952
		44	SRIVASTAVA 1977b
<i>Psidium molle</i> Bertol. (= <i>P. guineense</i> Sw.)	—	55	SRIVASTAVA 1970
<i>Psidium montanum</i> Sw.	—	33	MOUSSEL 1965
<i>Psidium pumilum</i> Vahl (= <i>P. guajava</i> L.)	11	—	SINGHAL <i>et al.</i> 1980
<i>Psidium variabile</i> O. Berg (= <i>P. cattleianum</i> Sabine)	—	88	ATCHINSON 1947
<i>Psidium</i> sp (araçá)	—	66	ANDRADE and FORNI-MARTINS 2000

Pollen grains stainability was > 86% in all of the analyzed species (Table 3).

Table 3 — Values for pollen stainability (% , s.d.) in species of *Campomanesia* and *Psidium*. N=number of pollen grains analyzed.

Specie (population)	Ploidy	N	% (s.d.)
<i>Campomanesia adamantium</i> (pop 1)	2x	1378	88,4 (0,7)
<i>Campomanesia adamantium</i> (pop2)	2x	1434	90,5 (2,2)
<i>Psidium acutangulum</i>	4x	1635	93,8 (1,9)
<i>Psidium cattleianum</i> (pop2)	4x	1118	86,4 (2,6)
<i>Psidium cattleianum</i> (pop3)	4x	1325	96,1 (1,7)
<i>Psidium cinereum</i> (pop1)	4x	1487	96,7 (2,0)

## DISCUSSION

The chromosome number of  $n=11$  for *C. pubescens* from a *cerrado s.s.* population in the municipality of Itirapina (FORNI-MARTINS and MARTINS 2000) was confirmed here for three different populations from other areas of *cerrado s.s.* in the state of São Paulo (municipality of Assis, Mogi Guaçu and Campinas) (Tables 1 and 2). The counts for *C. adamantium*, *C. guaviroba* and *C. phaea* are inedit (Table 1, Figure 1). Another species, *C. cambessedeanae*, studied by FORNI-MARTINS *et al.* (1995), is also diploid with  $2n=22$  (Table 2). There are no records of polyploid species in this genus, although *Psidium cerasoides*, with

$2n=88$  (MOUSSEL 1965), was synonymized with *Campomanesia guaviroba* by LANDRUM (1986) (Table 2). A diploid state ( $2n=22$ ) has also been observed for species in the remaining genera of Myrtinae, including *Acca selloviana* (O. Berg) Burret (VIJAYAKUMAR and SUBRAMANIAN 1985), *Pimenta officinalis* Lindl. and *P. racemosa* (P. Miller) J. W. Moore (MOUSSEL 1965).

There are reports of chromosome counts for 12 species of *Psidium* (Table 2). Our results provide one additional record for this genus (*P. cinereum*) (Table 1, Figure 1). A diploid state ( $2n=22$ ) have been recorded in only four species (*P. chinense*, *P. friedrichsthalianum*, *P. guajava* and *P. pumilum*), which corresponds to 28.6% of the species with a known chromosome number (Tables 1 and 2). The remaining 10 species (71.4%) show variations in level of ploidy, with a variable somatic number of  $2n=33$ , 44, 55, 66, 77 and 88 (Table 2).

*Psidium guajava* containing many dispoloid individuals or populations ( $2n=21$ , 22, 28, 20, 32 and 34) (D'CRUZ and RAO 1962; MAJUNDER and MUKKERJEE 1972), as well as polyploids, with  $2n=33$  (KUMAR and RANADE 1962) and  $2n=44$  (SRIVASTAVA 1977b) (Table 2). Hence, this species has several cytotypes (chromosomic races), with the chromosome number varying in a dispoloid series (from  $2n=21-34$ ) or with different levels of ploidy ( $2n=22$ , 33 and 44).

In *P. cattleianum*, three cytotypes are known: tetraploid ( $2n=4x=44$ ) (this work), heptaploid ( $2n=7x=77$ ) and octaploid ( $2n=8x=88$ ). These polyploid cytotypes were studied when *P. cattleianum* was still considered as *P. coriaceum*, with  $2n=77$  (SINGHAL *et al.* 1980) and *P. variabile*, with  $2n=88$  (ATCHINSON 1947) (Table 2).

In contrast, in *P. cinereum* no cytotypes were seen. Polyploid cytotypes were also reported by COSTA (2004) and COSTA and FORNI-MARTINS (2005a) in *Eugenia* (subtribe Eugeniinae). The occurrence of cytotypes and polyploid species is a frequent event in fleshy-fruited Myrteae (ATCHINSON 1947; ANDRADE and FORNI-MARTINS 1998; COSTA 2004; COSTA and FORNI-MARTINS 2005; 2006; RYE 1979), reaching ca. 22.5% in *Eugenia*. The differentiation of chromosome races or cytotypes corresponds to one important intermediate stage because genetic isolation eventually creates a barrier to gene flow (STACE 1991) that can favor speciation (BRIGGS and WALTERS 1997). Probably, because of the large species diversity in this family, the number of polyploid species was probably underestimated.

Myrtinae is the most primitive subtribe in the Myrteae, with the genus *Psidium*, where about 75% of the species are polyploidy, being the most derived of this group (Table 2). Polyploidy is one of the most important adaptive mechanisms in plants and allows a wider geographical and ecological distribution compared to the parental diploids (BRIGGS and WALTERS 1997). STEBBINS (1950) considered that angiosperms with a chromosome number  $n > 11$  had originated by polyploidy. In evolutionary terms, polyploid species are considered derivatives, in contrast to species with a smaller chromosome number, although it is extremely difficult to reconstitute their origin (BRIGGS and WALTERS 1997, STACE 1991). Based on the high frequency of polyploid species in the *cerrado*, MORAWETZ (1990) affirmed that savanna-type vegetation (*cerrado s.s.*, including "*campos rupestres*") had a more recent origin than forest formations. According SARMIENTO (1983), a widespread gene flow was established from humid forests (Amazon and Atlantic) to the savanna formations (*cerrado*), implying extensive speciation in the *cerrado*.

FORNI-MARTINS and MARTINS (2000) contested the affirmations of MORAWETZ (1990) since chromosome counts are known for only about 3% of the flora of the *cerrado*. In addition, several forest species also have high chromosome numbers and some families contain both formations, with elevated chromosomal numbers. Based on

these arguments, FORNI-MARTINS and MARTINS (2000) suggested that there had been a bi-directional flow between savannas and forests.

The Myrtaceae is widely represented in a variety of habitats, including the *cerrado* (CASTRO *et al.* 1999), "*campos rupestres*" (KAWASAKI 1989) and forest formations (OLIVEIRA-FILHO and FONTES 2000). Comparison of the chromosome counts found in the present study showed that polyploid species in *Psidium* occurred in both habitats. COSTA and FORNI-MARTINS (2005; 2006) reported polyploidy in *Eugenia* and *Myrcia*, which are also very well represented in the *cerrado* and in forests, but with a greater concentration of polyploid species in *cerrado* and "*campos rupestres*". However, more studies are necessary to elucidate the origin of *cerrado* flora.

The high degree of pollen stainability ( $> 85\%$ ) agreed with the values registered for other species of Myrteae (COSTA and FORNI-MARTINS 2006). These authors documented no irregular association during meiosis in diploids species of Eugeniinae (*Eugenia*). Some reports have described sexual reproduction among species that are self-compatible and self-incompatible (NICLUGHADHA and PROENCA 1996). DAVIS (1966) stated that apomixy was widely distributed in Myrtoideae. Such species are generally incapable of sexual reproduction, but can reproduce by vegetative propagation or seeds with no previous fecundation (agamospermy) (STACE 1991). RYE (1979) related apomixy to the occurrence of polyploidy, but such a relation has not always been observed (COSTA 2004). The meiotic regularity seen here would be expected in diploids and in species with an even polyploid number, especially in species derived from inter-specific hybridization followed by chromosomal duplication (GUERRA 1988). In the present study, polyploid species with an odd level of ploidy ( $3n, 5n, \dots$ ), considered to be apomitic, were not studied.

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