NEW CHROMOSOME NUMBERS IN *PILOCARPUS* VAHL (RUTACEAE)[1]

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RESUMO – (Novos números cromossômicos em *Pilocarpus* Vahl (Rutaceae). Contagens cromossômicas em oito espécies de *Pilocarpus* Vahl (Rutaceae) do Brasil são relatadas pela primeira vez. Os números cromossômicos foram determinados a partir de células de ponta de raiz de mudas mantidas em casa-de-vegetação e obtidas em expedições de coleta. O método Feulgen foi empregado para a coloração dos cromossomos. O pré-tratamento das pontas de raiz foi feito em solução saturada de alfa-bromonaftaleno por duas horas à temperatura ambiente (20-25°C). Foram identificados os números diplóides 2n=44 e 2n=88. Os resultados sugerem a ocorrência de tetraploidia em *P. spicatus* St.-Hil. e em *P. carajaensis* Skorupa, e um possível número básico x=22 para o gênero *Pilocarpus*.

Palavras-chave – *Pilocarpus*, Rutaceae, Cusparieae, citogenética, poliploidia

ABSTRACT – (New Chromosome numbers in *Pilocarpus* Vahl (Rutaceae). Chromosome counts for eight species of *Pilocarpus* Vahl (Rutaceae) a native of Brazil are reported for the first time. Chromosome numbers were determined from mitotic root tip cells of seedlings derived from field collections and grown in the greenhouse. Feulgen staining was used. Initial pre-treatment of root tips was done by using a saturated aqueous solution of alpha-bromonapthalene for two hours at room temperature (20-25°C). Chromosome numbers of 2n=44 and 2n=88 were determined for the examined taxa. The present results suggest the occurrence of tetraploidy in *P. spicatus* St.-Hil. and *P. carajaensis* Skorupa, and a possible basic number x=22 to the genus *Pilocarpus*.

Key words – *Pilocarpus*, Rutaceae, Cusparieae, cytology, polyploidy

Introduction

Pilocarpus (Rutaceae) is a neotropical genus of shrubs or small trees and comprises 16 species (Skorupa 1996). It occurs from Southern Mexico to Southern South America. The entire genus is of interest due to the occurrence of pilocarpine, an alkaloid widely used in ophthalmology, especially in the treatment of glaucoma (Lewis & Elvin-Lewis 1977). This alkaloid is found in the leaves of some species, the most important of which in terms of alkaloid production, being *P. microphyllus* Stapf ex Wardl. (Trease & Evans 1983).

Rutaceae chromosome numbers are relatively scarce and scattered in the literature.

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Stace *et al.* (1993) state that only 73 out of 150 genera of Rutaceae (ca. 49%) and approximately 250 out of ca. 1.800 species of the family (14%) have some information on their chromosome number. Specifically in Pilocarpinae, a subtribe of Cusparieae, with four genera (*Pilocarpus*, *Metrodorea*, *Esenbeckia* and *Raulinoa*), only *Pilocarpus* and *Esenbeckia* have one species in which the chromosome number was determined. Indeed, *Pilocarpus* chromosome numbers are found in Honsell (1954), Kaastra (1978), Guerra (1984) and Pagliarini & Pereira (1992), when dealing with *P. pennatifolius*.

The purpose of this paper is to present new chromosome numbers for *Pilocarpus* as a contribution towards a better knowledge of the genus.

Material and methods

The eight *Pilocarpus* species, including their diploid chromosome numbers and collecting sites are given in Tab. 1. Chromosome numbers were determined from mitotic root tips cells of seedlings derived from field collections and grown in the greenhouse at Cenargen/ Embrapa, Brasília, Brazil. Feulgen staining was used (Löve & Löve 1975). A pre-treatment of root tips was done by using a saturated aqueous solution of alpha-bromonapthalene for two hours at room temperature (20-25°C). Root tips were then transferred to ethanol : acetic acid solution (3:1) for 24h and rinsed three times in 95% ethanol. Root tips were hydrolyzed in 1N HCl solution for 12 min. at 60°C, followed by exposure to a 2% pectinase aqueous solution for 45 min.

Voucher herbarium specimens were deposited in the herbarium of Cenargen (CEN).

Results and discussion

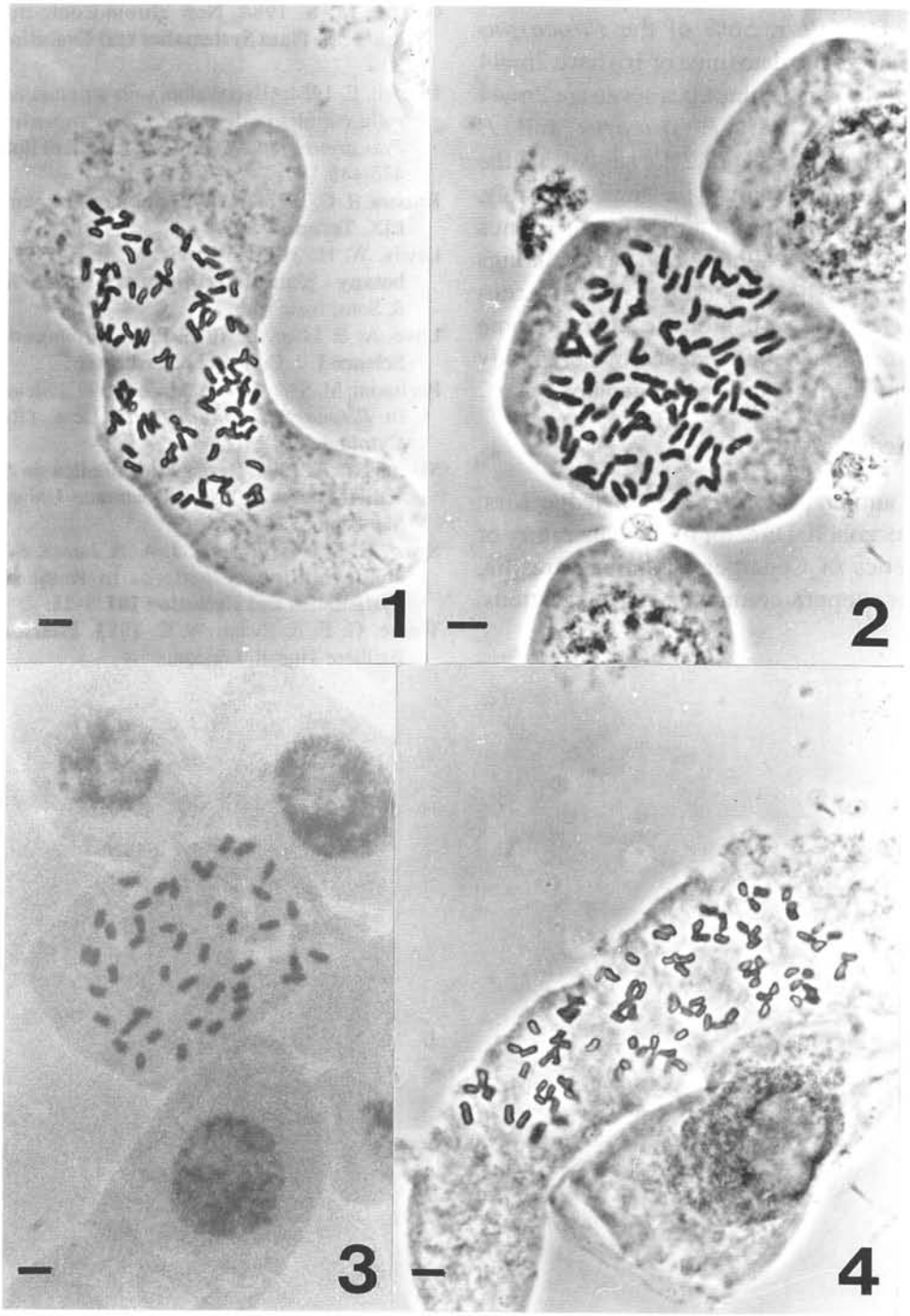
Two chromosome numbers were found among the taxa (Tab. 1). Of the eight species, six showed 2n=44, while two 2n=88. Figures 1-4 show mitotic cells in metaphase from three *Pilocarpus* species. Most of the chromosomes are meta- or submetacentric and have ca. 3µm.

Previous chromosome counts for *P. pennatifolius* are found in Honsell (1954), Kaastra (1978), Guerra (1984) and Pagliarini & Pereira (1992). All authors found 2n=44, except Honsell (1954) who reported 2n=36. Considering the results reported here and also the pertaining literature, it seems that Honsell's report (2n=36) is not correct, as already

Table 1. Taxa analyzed, chromosome numbers, collecting sites and herbarium vouchers of the *Pilocarpus* species examined.

Taxa	2n	Locality, State*	Voucher
P. carajaensis Skorupa	88	Parauapebas, PA	Skorupa & Elzamar 996
P. giganteus Engl.	44	Cubatão, SP	Skorupa & Pompéia 981
P. grandiflorus Engl.	44	Ilhéus, BA	Skorupa 1014
P. microphyllus Stapf ex Wardl.	44	Açailândia, MA	Vieira *et al.* 850
P. microphyllus Stapf ex Wardl.	44	Dr. Eliseu, PA	Vieira *et al.* 855
P. pauciflorus St.-Hil. ssp. *clavatus*	44	Itapetinga, BA	Skorupa *et al.* 991
P. spicatus St.-Hil. ssp. *spicatus* var. *spicatus*	88	Colatina, ES	Skorupa *et al.* 912
P. spicatus St.-Hil. ssp. *spicatus* var. *lealii* (Machado) Kaastra	88	Valença, BA	Skorupa *et al.* 987
P. spicatus St.-Hil. ssp. *aracatensis* Kaastra	88	Pacatuba, CE	Skorupa *et al.* 1003
P. spicatus St.-Hil. ssp. *longeracemosus* (Mart. ex Engl.) Kaastra	88	Seabra, BA	Skorupa 1008
P. sulcatus Skorupa	44	Tanque Novo, BA	Skorupa 1012
P. trachylophus Holmes	44	Tanque Novo, BA	Skorupa 1010

* Brazilian States: BA=Bahia, CE=Ceará, ES=Espírito Santo, MA=Maranhão, PA=Pará, SP=São Paulo



Figures 1-4. Chromosomes in ***Pilocarpus***. 1. ***P. spicatus*** subsp. ***aracatensis*** (Skorupa *et al.* 1003), 2n=88. 2. ***P. spicatus*** subsp. ***longeracemosus*** (Skorupa 1008), 2n=88. 3. ***P. microphyllus*** (Vieira *et al.* 850), 2n=44. 4. ***P. carajaensis*** (Skorupa & Elzamar 996), 2n=88. (bars=4μm).

suggested by Pagliarini & Pereira (1992).

Assuming that 2n=44 is the correct number in *P. pennatifolius*, 56% of the *Pilocarpus* species analyzed to date (nine of 16) have 2n=44 or 2n=88. Among the nine taxa, seven are 2n=44 (77.8%) while two (*P. spicatus* and *P. carajaensis*) are 2n=88 (22.2%), suggesting the occurrence of tetraploidy in the genus. Possibly, the basic chromosome number for the genus *Pilocarpus* is x=22, although further countings may indicate the occurrence of different numbers. Studies on meiotic chromosome behavior or on genetic evidence of polyploidy could support the results presented here.

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