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KARYOTYPES OF *MACROSIPHONIA PETRAEA* AND *M. VIRESCENS* (APOCYNACEAE)*

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Summary: The mitotic chromosomes of *Macrosiphonia petraea* (A. St.-Hil.) K. Schum. and *M. virescens* (A. St.-Hil.) Müll. Arg., 2n = 16, two suffrutescent herbaceous species of South America, have been studied for the first time. The karyotypes, which are also the first records for the genus, are fairly symmetric with small chromosomes, their length ranging from 1.21 to 2.36 µm in *M. petraea* and from 1.32 to 2.82 µm in *M. virescens*. Total chromosome length (TCL) calculated for *M. petraea* is 27.06 ± 1.38 µm and for *M. virescens*, 31.85 ± 0.32 µm. The basic chromosome number in relation to the evolution of the family is discussed.

Key words: Apocynaceae, *Macrosiphonia petraea, Macrosiphonia virescens*, karyotype.

Resumen: Cariotipos de *Macrosiphonia petraea* y *M. virescens* (Apocynaceae). Se estudian por primera vez los cromosomas mitóticos de *Macrosiphonia petraea* (A. St.-Hil.) K. Schum. y *M. virescens* (A. St.-Hil.) Müll. Arg., 2n = 16, dos sufrútices herbáceos de Sud América. Los cariotipos, que son también los primeros estudiados en el género, son bastante simétricos con cromosomas pequeños, siendo su longitud de 1,21 a 2,36 µm en *M. petraea* y de 1,32 a 2,82 µm en *M. virescens*. La longitud total cromosómica (TCL) calculada para *M. petraea* es de 27,06 ± 1,38 µm y para *M. virescens*, de 31,85 ± 0,32 µm. Se discute el número básico cromosómico en relación a la evolución de la familia.

Palabras clave: Apocynaceae, *Macrosiphonia petraea, Macrosiphonia virescens*, cariotipo.

INTRODUCTION

The genus *Macrosiphonia* Müll. Arg. comprises nowadays near six species distributed in the plains or "campos" of South America southeastern (Woodson, 1933; Xifreda, in prep.). The other several species from SE of the United States and Mexico, currently classified as congeneric, were distinctively recognized and recently transferred to *Telosiphonia* (Woodson) Henrickson (Henrickson, 1996).

Macrosiphonia petraea (A. St.-Hil.) K. Schum. and *M. virescens* (A. St.-Hil.) Müll. Arg. are two of the four species of *Macrosiphonia* that grow in Argentina (Ezcurra, 1999). Both are suffrutescent herbs. *Macrosiphonia petraea* occurs in Bolivia, Brazil, Paraguay, Uruguay and extends down in Argentina as far as the Córdoba, Entre Ríos, and Buenos Aires provinces. The distribution of *M. virescens* is restricted only to NE of Argentina, at Misiones Province, SE of Brazil and Paraguay.

The possibility to obtain seeds and live plants during a research trip has originated this contribution, with the first cytological information on both species and the apocynaceous genus. Previous knowledge on chromosome data of native argentine representatives of Apocynaceae was scanty and attested by chromosome counts of only three species discussed below (Coleman & Smith, 1969; Piovano, 1987; Martínez, 1987).

MATERIAL AND METHOD

Seeds were obtained from naturally growing populations. The origin of the accessions studied are:

Macrosiphonia petraea

ARGENTINA. *Prov. Entre Ríos. Dpto. Colón*, Parque Nac. El Palmar, La Glorieta, 25-III-1996, Xifreda 1509.

Macrosiphonia virescens

ARGENTINA. *Prov. Misiones, Dpto. San Ignacio*, road to Teyucuaré, ca. 5 km from San Ignacio, 2-IV-1996, Xifreda 1664.

* Dedicated to Prof. Dr. Juan H. Hunziker on the ocassion of his 75th anniversary.

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Voucher specimens are deposited in the Herbarium of Instituto Darwinion (SI).

Cytological examination of somatic chromosomes was performed on young root tips of germinating seeds that were pretreated in an aqueous solution of 2 mM 8-hydroxiquinoline, for 4 h at room temperature (20 ± 3° C). They were then fixed in 1 acetic acid- 3 absolute ethanol for at least 24 h, placed in 70% alcohol, and stored at 4-5° C until required. Following a 15 min. hydrolysis in 5 N HCl at 20 ± 3° C, the roots were washed in distilled water, stained and squashed using 2 % propionic acid haematoxylin adding ferric citrate as a mordant (Sáez, 1960).

The determination of karyotype parameters was carried out working with enlarged photographs of selected cells. Measurements were made on four cells but karyotype of each species was described from ten scattered mid metaphases belonged to ten individuals. The mean chromosome length (CL) and centromeric index (CI) were calculated for each chromosome pair. The nomenclature used for the description of the chromosome morphology is that proposed by Levan *et al.* (1964). In most m pairs homologies are just tentative. Chromosome pairs were aligned and numbered in order of their decreasing value of CL. For each cell, values of CL were added to give the total chromosome length (TCL). The chromosome length percentage relative to total chromosome length for each chromosome type (RL) was then calculated.

Results

Macrosiphonia petraea and *M. virescens* had not been studied previously cytologically. The somatic chromosome numbers were found to be 2n = 16 and they constitute the first counts for the genus as well. The karyotypes, which are also the first records for *Macrosiphonia*, are fairly symmetric. Mitotic chromosomes and karyograms are shown in Fig. 1.

The karyotype formula of *Macrosiphonia petraea* included 6 pairs of chromosomes with their centromeres located in the median region (m), one with centromeres probably in the submedian region (sm), and one pair of chromosomes with centromeres in the terminal region (t) (Fig. 1B). The chromosomes were small, the lengths ranged from 1.21 to 2.36 µm (Table 1).

The karyotype formula of *M. virescens* consisted of 6 pairs of chromosomes with their centromeres located in the median region (m), one with centromeres in the submedian region (sm), and one pair of chromosomes with centromeres in the terminal region (t) (Fig. 1D). Microsatellites were located on the long arm of chromosome pair n° 3 and on the short arm of chromosome pair n° 7. The chromosomes were short, the lengths ranged from 1.32 to 2.82 µm. Chromosome pairs n° 7 and 8 turned out to be very similar in length. The telocentric chromosome pairs are the shortest ones in both complements (Table 1).

Total chromosome length (TLC) of the studied species measured were 27.06 ±1.38 µm for *M. petraea* and 31.85 ± 0.32 µm for *M. virescens*, and the average chromosome lengths about 1.7 µm and 2 µm respectively (Table 2). The chromosome length percentages relative to TCL for each chromosome type (RL) are similar for both complements (Table 1).

The quotient (ratio) longest/ shortest chromosome was 1.95 ± 0.17 in M. *petraea* and 2.14 ± 0.15 in *M. virescens* (Table 2). Therefore, it is possible to accept that the size difference between the extreme chromosome pairs is about twofold.

Discussion

Like other large and predominately tropical families of flowering plants, the Apocynaceae remains poorly known cytologically. Small chromosomes and difficulties in cytological preparations have prevented for cytotaxonomical studies.

Previous cytological information of the thirty nine native argentine species has been restricted only to three taxa: *Prestonia acutifolia* (Müll. Arg.) K. Schum., n = 16-17 (Coleman & Smith, 1969), 2n = 18 (van der Laan & Arends, 1985), *Aspidosperma quebracho-blanco* Schltdl., 2n = 36, x = 9 (Piovano, 1987) and *Mandevilla pentlandiana* (A. DC.) Woodson, 2n = 16 (Martínez, 1987).

The chromosome numbers of *Macrosiphonia petraea* and *M. virescens,* 2n = 16, x = 8, here presented are the first records obtained for the genus and have helped to confirm its previous suspected systematic affinities. *Mandevilla* is the most closely related genus to *Macrosiphonia*. Both were considered congeneric and pertaining to the subfamily Apocynoideae, tribe Ichnocarpeae Benth. & Hook. f. *emend.* Pichon, subtribe Mandevillinae Pichon (Pichon, 1951). On the other hand,

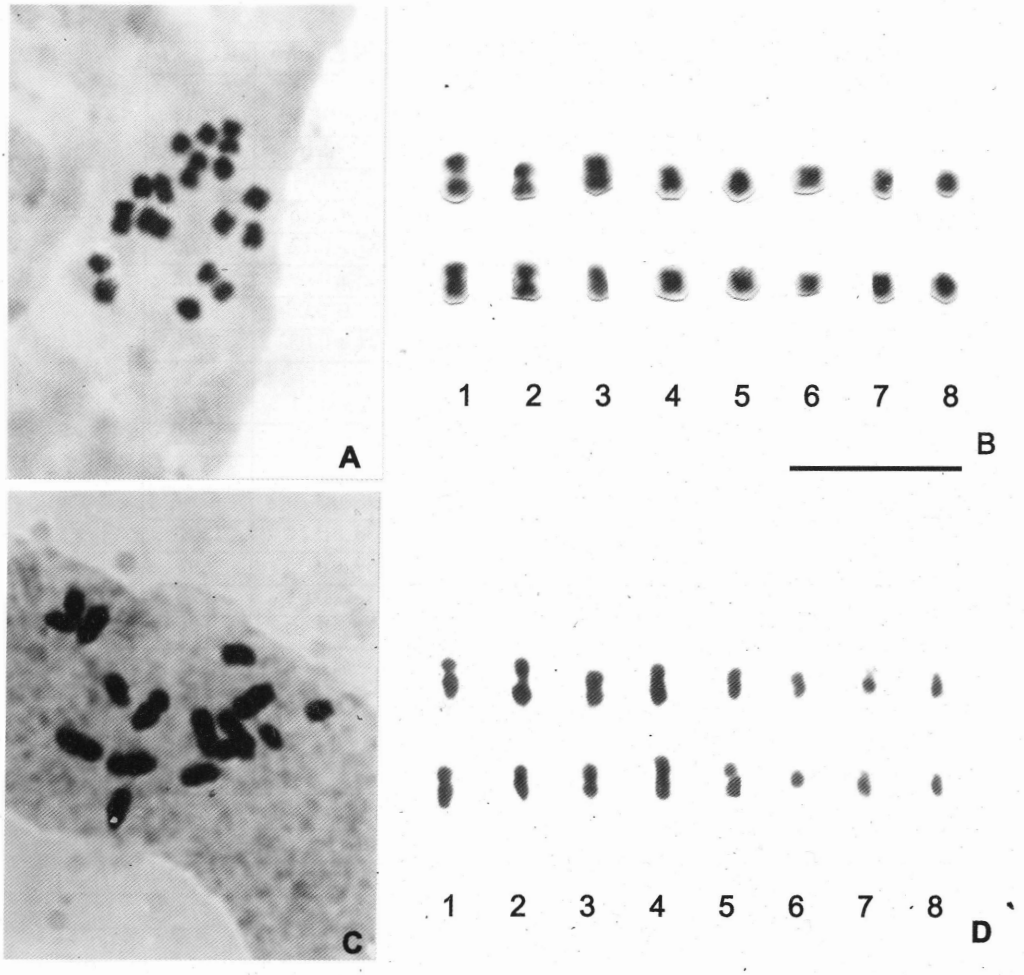

Fig. 1. Somatic metaphases and respective karyograms. A-B: *Macrosiphonia petraea*; C-D: *M. virescens*. Bar = 10 µm.

Macrosiphonia was maintained as independent in the same subtribe by Allorge *et al.* (1981), on the base of morphological and chemotaxonomical characters. The Apocynoideae is the most advanced subfamily of Apocynaceae *s. str.*, with a complex floral structure recognized as a gynostegium, formed by the ventral side of anthers cemented to the style-head. This represents a high-level anther (Pichon, 1948; Fallen, 1986). Recently, Endress & Bruyns (2000) proposed a new classification in which both genera are considered into *Mesechiteae* Miers.

The karyotypes of both *Macrosiphonia* species were quite similar except for the presence of satellites and the chromosome sizes. Their chromosomes, as in other apocynaceous members, are small and mostly provided with median to submedian primary constrictions. Also the chromosome length percentage relative to total chromosome length for each chromosome type (RL) is similar. But meanwhile *M. virescens* exhibited two chromosome pairs with microsatellites, *M. petraea* did not show visible ones in the examined cells. At present, the absence of karyological information on other Apocynaceae taxa prevents further comments on taxonomic aspects.

The basic chromosome number x=8 occurs in the family with a frequency of five percent (*Catharanthus* G. Don, *Apocynum* L., *Trachomitum* Woodson in part, *Mandevilla* Lindl.), being the smallest number x = 6 (*Echites* P. Browne, *Urechites* Müll. Arg.; van der Laan & Arends, 1985).

Concerning the basic chromosome number in

Table 1. Centromeric position (according to Levan *et al.*, 1964), chromosome length (CL) and chromosome length percentage relative to TCL for each chromosome type (RL) of two *Macrosiphonia* species haploid genomes. Average values (CL) and standard error (S.E.) are presented.

Chromosome pair	Centromeric position	CL ± S.E. (µm)	RL (%)
M. petraea			
1	m	2.36 ± 0.18	17.44
2	m	2.13 ± 0.16	15.74
3	m	1.87 ± 0.08	13.82
4	m	1.66 ± 0.16	12.27
5	m	1.57 ± 0.12	11.60
6	m	1.45 ± 0.09	10.72
7	sm?	1.28 ± 0.05	9.46
8	t	1.21 ± 0.03	8.94
M. virescens			
1	m	2.82 ± 0.14	17.71
2	m	2.59 ± 0.18	16.27
3	m- microsatellite	2.34 ± 0.05	14.70
4	m	1.98 ± 0.20	12.44
5	m	1.88 ± 0.14	11.81
6	m	1.59 ± 0.06	9.99
7	sm- microsatellite	1.39 ± 0.01	8.73
8	t	1.32 ± 0.10	8.29

Table 2. Comparison of some karyotype characteristics of *Macrosiphonia petraea* and *M. virescens*. TCL = total chromosome length (2n); CL = average chromosome length. Averages and standard error are indicated.

Karyotype characteristic	*M. petraea*	*M. virescens*
TCL (µm)	27.06 ± 1.38	31.85 ± 0.32
CL (µm)	1.69 ± 0.09	1.99 ± 0.01
Length longest pair/ Length shortest pair	1.95 ± 0.17	2.14 ± 0.15
Length pair n° 1 – Length pair n° 8 (µm)	1.15 ± 0.19	1.50 ± 0.09

Apocynaceae, in general, there is possible to consider that 11 was the primary basic number in primitive species. The primitiveness of the basic number 11 which occurs in at least 15 different genera, distributed in both subfamilies is supported by their perennial woody habit (Roy Tapadar, 1964). Rosatti (1989) based on the van der Laan & Arends' review (1985) established that x = 11 has been the only chromosome number determined in about 60 percent of the 62 genera for which such data are available. Likewise x = 8 and x = 9 characterize taxa within advanced, although diverse, positions within the family. Van der Laan & Arends (1985) agree with Raven 's opinion (1975) in that x = 6, 8, 9 and 10 have evolved by descending aneuploidy from x =11, the rather widespread basic chromosome number, and consequently are derived numbers.

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