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Karyotypes of some species of *Cestrum*, *Sessea*, and *Vestia* (tribe Cestreae, Solanaceae)

Las Penas[1] M. Laura, Franco E. Chiarini[1], Gabriel Bernardello[1*] and Carmen Benítez de Rojas[2]

[1] Instituto Multidisciplinario de Biología Vegetal (CONICET-UNC), C.C. 495, 5000 Córdoba, Argentina.
[2] Facultad de Agronomía, Universidad Central de Venezuela, Maracay, Apartado Correos 4579, Aragua, Venezuela.

Abstract — The somatic chromosomes of *Cestrum bigibbosum* Pittier, *C. buxifolium* Kunth, *Sessea corymbosa* Goudot ex Taylor and Phillips and *Vestia foetida* (Ruiz et Pav.) Hoffmanns. were examined by classical staining. The number $2n = 2x = 16$ was found for all species. The total genome length ranged from 57.70 to 92.09 μm and the average chromosome length from 7.21 to 11.51 μm. Karyotypes were notably symmetrical. Haploid karyotype formulae were 8 m pairs (*C. bigibbosum*, *V. foetida*) or 7 m + 1 sm pairs (*S. corymbiflora*, *C. buxifolium*). Except *Sessea corymbiflora* in which satellites were not visualized, the remaining species showed one satellited pair. The species can be distinguished by a combination of karyotype formula, karyotype length, and the position of satellites in a particular chromosome pair. Karyotype diversification in tribe Cestreae is suggested to have been accompanied by a tendency towards slight asymmetry and increased genome length.

Key words: *Cestrum*, karyotype, *Sessea*, somatic chromosome number, *Vestia*.

INTRODUCTION

Solanaceae is a family of flowering plants that has a great economic, floristic, and ethnobotanic importance (D'Arcy 1991; Hunziker 2001). According to the classification system, it is composed by a different number of subfamilies and tribes (D'Arcy 1991; Olmstead *et al.* 1999; Hunziker 2001); however, all of them agree in including subfam. Cestroideae Schltdl. and tribe Cestreae G. Don.

Within this tribe, and regardless of the system, the following three genera, that inhabit the American continent are included: *Cestrum* L., *Sessea* Ruiz et Pav., and *Vestia* Willd. The affinity among them is well supported by morphological, chemical, and molecular data (Faini *et al.* 1984; Olmstead and Palmer 1992; Fay *et al.* 1998; Olmstead *et al.* 1999; Hunziker 2001; Santiago-Valentin and Olmstead 2003). Fruit and seed traits are key to recognize them: *Cestrum* with berries containing 2-18 polyedric seeds, *Sessea* having capsules with 4-8 winged seeds, and *Vestia* showing larger capsules with ca. 50 polyedric seeds; in addition, the latter has exserted anthers, whereas the other two genera included anthers (Benítez de Rojas and D'Arcy 1998; Benítez de Rojas and Nee 2001; Hunziker 2001).

Cestrum is considered to have ca. 150 spp. (Benítez de Rojas and D'Arcy 1998) that range from Southern Florida (U.S.A.) to Southern Chile and Argentina, but are absent from the central Amazonian basin (Benítez de Rojas and D'Arcy 1998; Hunziker 2001). Some species are used as ornamentals (e.g., *C. diurnum* L., *C. nocturnum* L., *C. elegans* (Brongn.) Schltdl.) or as medicinal plants (e.g., *C. sendtnerianum* Mart., *C. laevigatum* Schltdl., *C. mathewsii* Dunal), or are toxic (*C. nocturnum*, *C. diurnum*, *C. laevigatum*, *C. parqui* L'Herit.) because of the presence of alkaloids (Halim *et al.* 1971; McLennan and Kelly 1984; Peixoto *et al.* 2000). *Sessea* has 15 spp. mainly distributed in cloud forests and paramos of the Andes of South America (Benítez de Rojas and Nee 2001), while *Vestia* is monotypic and consists of *V. foetida* (Ruiz et Pav.) Hoffmanns., an endemic species to temperate Chile well-known as medicinal (Hunziker 2001).

Chromosomically, Solanaceae exhibits a dysploid series from $x = 7$ to $x = 14$, although other derived basic numbers such as $x = 17$, $x = 19$ and $x = 23$ have been recorded (Bolkhovskikh *et al.*

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[*] Corresponding author: phone (fax) ++54 351 4331056; e-mail: bernarde@imbiv.unc.edu.ar

1969; Moscone 1992; Hunziker 2001). Even though subfam. Cestroideae has several base numbers (x = 7 to x = 12), in tribe Cestreae only x = 8 was reported so far. In *Cestrum*, chromosome numbers are known for as few as 15 spp. (cf. Bolkhovskikh *et al.* 1969; Goldblatt 1984; Goldblatt and Johnson 1991, 1996) whereas in the genus *Sessea*, there are no chromosome counts available, and for *Vestia* there are only two reports (Goodspeed 1954; Tschischow 1956).

Regarding the chromosome morphology in the tribe Cestreae, our knowledge is even scarce: only the karyotypes of ten *Cestrum* species were studied (Gottschalk 1954; Sharma and Sharma 1957; Berg and Greilhuber 1992; 1993a; b; Badr *et al.* 1997); in addition, the presence of B chromosomes in *C. strigillatum* and *C. intermedium* by banding techniques (Fregonezi *et al.* 2004) and the absence of *Arabidopsis*-type telomeres in *Cestrum*, *Sessea*, and *Vestia* (Sykorova *et al.* 2003) were recently reported. No data are available for *Sessea* and *Vestia*.

Thus, karyological information is badly needed to improve our understanding of the systematic and evolutionary relationships among *Cestrum, Sessea*, and *Vestia*. The purpose of this paper is to analyze the karyotype of selected representatives of these Cestreae genera by means of a classical staining, trying to understand the taxonomic and evolutionary relationships of species and genera investigated.

The examined species are: *Cestrum bigibbosum* Pittier, a slender treelet with reclining branches that occurs in Colombia and Venezuela in evergreen cloud forests; *C. buxifolium* Kunth, a shrub or a dwarfed shrub irregularly branched that grows on paramos of Andean regions (2650-4000 m a.s.l.) of Colombia and Venezuela; *Sessea corymbiflora* Goudot ex Taylor and Phillips, a tree of ca. 6 m high ranging in subparamos at 2200-2900 m a.s.l. from Ecuador to Venezuela; *Vestia foetida* (Ruiz et Pav.) Hoffmanns., a 1-2 m tall plant species that inhabits wet shrublands of Chile, from Valdivia to Chiloé.

MATERIALS AND METHODS

Details of the studied material and voucher specimens are listed below.

C. bigibbosum. VENEZUELA. Mérida state, mun. Cardenal Quintero, 1600 m.s.m., 8° 29' N/ 71° 36' O, Benítez de Rojas and Rojas 6478 (MY, VIA).

Cestrum buxifolium. VENEZUELA. Mérida state, mun. Rangel, 3090 m.s.m., 8° 42' N/ 71° 47' O, Benítez de Rojas 6542 (MY).

Sessea corymbiflora. VENEZUELA. Mérida state, mun. Cardenal Quintero, 2650 m.s.m., 8° 50' N/ 70° 56' O, Benítez de Rojas 6437(MY).

Vestia foetida. SPAIN. Cultivated in the Real Jardín Botánico de Madrid, from seeds collected in Chile (CORD 923).

Mitotic chromosomes were examined in squashes of root tips that were obtained from germinating seeds. Seeds were soaked for 24 hr in running water and then put in Petri dishes on moist filter paper and stored at 30°C. Root tips were fixed in a 3:1 ethanol:acetic acid mixture, after pretreatment in a saturated solution of p-dichlorobenzene in water for 2 hr, and were stained with Feulgen solution (basic fuchsine). Permanent mounts were made following the method of Bowen (1956). At least ten metaphases per species were photographed with a phase contrast optic Axiophot microscope. The photomicrographs were used to take measurements for each chromosome pair: s (short arm), l (long arm), and c (mean total chromosome length). The arm ratio (r=l/s) was then calculated and used to classify the chromosomes as recognized by Levan *et al.* (1964). In addition, total haploid chromosome length of the karyotype, based on the mean chromosome length (tl), average chromosome length (C), and average arm ratio (R) were calculated. Idiograms were based on the mean values for each species. The chromosomes were arranged first into groups according to their increasing arm ratio and then according to the decreasing length within each group. Karyotype asymmetry was estimated using the intrachromosomal (A_1) and the interchromosomal (A_2) indices of Romero Zarco (1986). Satellites were designated according to Battaglia (1955) and their lengths were added to those of the corresponding arms.

A phenetic analysis was performed by using six variables per genome: tl, R, C, A_1, A_2, and the presence of satellite. The program SYSTAT (version 7.0, SPSS Inc., 1997) was used to standardize a data matrix, to calculate the average Euclidean distance, and to generate an UPGMA (unweigthed pair-group method using arithmetical averages) phenogram.

RESULTS

The diploid chromosome number for all the studied species was 2n = 16. Figure 1 illustrates

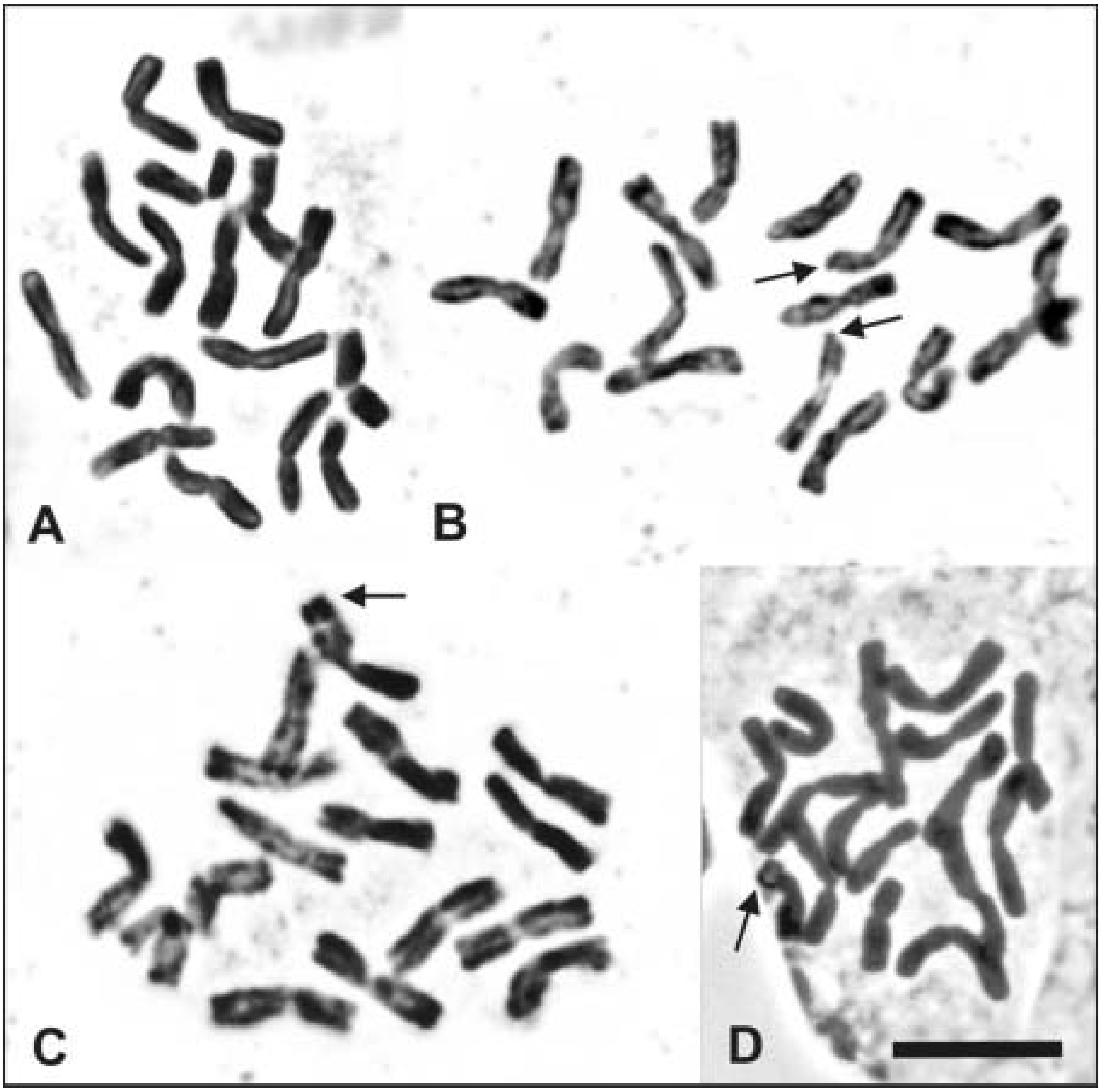

Fig. 1 — Mitotic metaphases of Cestreae species. **A.** *Sessea corymbiflora*. **B.** *Vestia foetida*. **C.** *Cestrum bigibbosum*. **D.** *Cestrum buxifolium*. Arrows point to satellites. Bar represents 10 μm. All pictures at the same scale.

Table 1 — *Cestreae* taxa studied, haploid karyotype formulae, ordering number of the satellited pair (SAT), mean chromosome length (C) ± s.d.; mean arm ratio (R) ± s.d.; total haploid chromosome length (tl) ± s.d.; intrachromosomal asymmetry index (A_1); interchromosomal asymmetry index (A_2)

Species	Haploid karyotype formula	SAT	C (μm)	R	tl (μm)	A_1	A_2
C. bigibbosum	8 m*	5	8.73 ± 0.36	1.24 ± 0.20	69.91 ± 1.32	0.17	0.26
C. buxifolium	7 m* + 1 sm	6	11.51 ± 1.54	1.37 ± 0.31	92.09 ± 1.34	0.24	0.28
S. corymbiflora	7 m + 1 sm	-	10.52 ± 061	1.26 ± 0.22	84.20 ± 1.12	0.19	0.24
V. foetida	8 m*	6	7.21 ± 0.63	1.16 ± 0.10	57.70 ± 0.95	0.13	0.08

the range of metaphases encountered. Precise idiograms calculated from means are given for these species in Fig. 2.

The average chromosome length varied from 7.21 to 11.51 µm (Table 1). The shortest measured chromosome pair was n° 8 in one cell of *V. foetida* (6 µm), whereas the longest was pair n° 2 in a cell of *C. buxifolium* (14 µm).

The karyotypes of the species studied are notably symmetrical (Fig. 1, 2). Effectively, they are composed exclusively of *m* chromosomes or just have one *sm* pair (Fig. 2, Table 1). The karyotype of *V. foetida* is the most symmetrical and, comparatively, *C. buxifolium* shows some differences in the chromosome sizes of the different pairs (Fig. 2, Table 1).

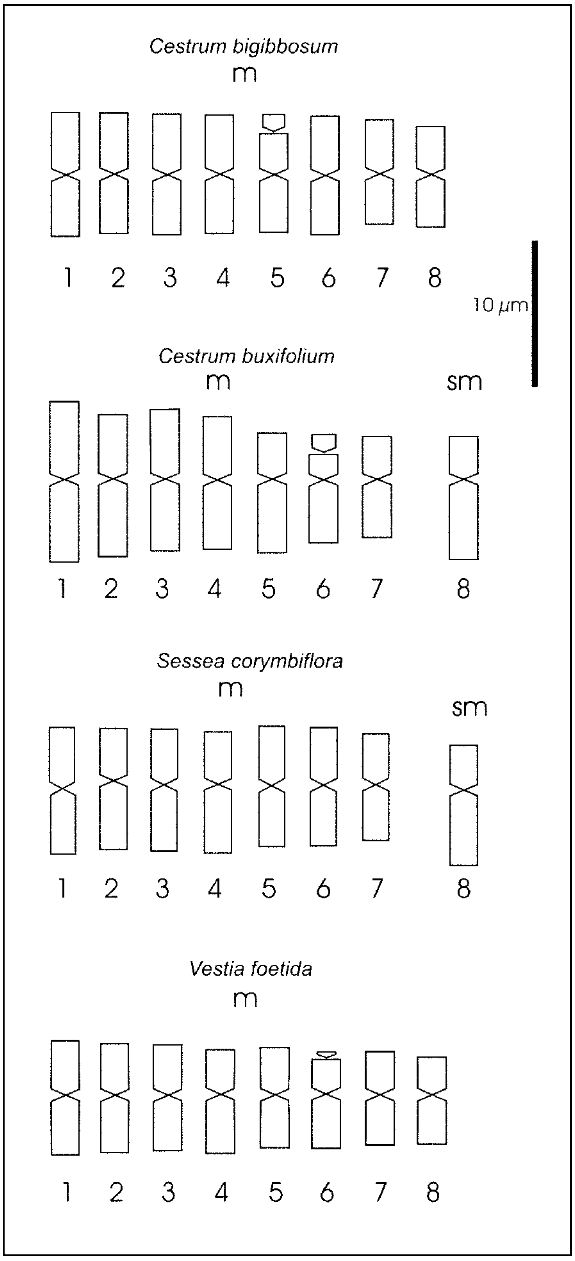

Fig. 2 — Idiograms of *Cestreae* species based on mean chromosome values. All at the same scale.

Except *Sessea corymbiflora* in which satellites were not visualized, the remaining species shows one satellited pair. According to its shape and position, they are always terminal microsatellites. They have been observed in the 80-100 % of the examined cells of each species.

Although the karyotypes are comparable, the examined species can be distinguished by a combination of karyotype formula, karyotype length, and the position of satellites in a particular chromosome pair (Table 1), as shown in the phenogram obtained based on these features (Fig. 3). *Vestia* is the most different because of its shorter chromosomes and extremely symmetrical karyotype. From the remaining cluster, *C. buxifolium* separates because of its higher total haploid karyotype length, and S. *corymbiflora* and *C. bigibbosum* are closer with comparable R, A_1, and A_2 values.

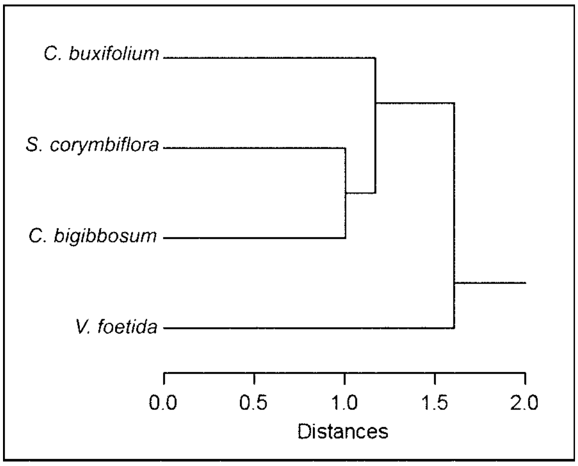

Fig. 3 — UPGMA phenogram derived from average taxonomic distance for the *Cestreae* species studied, based on karyological data.

DISCUSSION

The chromosome number for *Vestia foetida* confirms previous data on other accessions (Goodspeed 1954; Tschischow 1956), whereas the numbers for the other three species are new, including the first count for the genus *Sessea*. In all cases, the basic chromosome number x = 8 is verified for the tribe Cestreae (Sharma and Sharma 1957; Bolkhovskikh *et al.* 1969; Moscone 1992). Outside tribe Cestreae, this base number is only known in some Australian *Nicotiana* species (Hunziker, 2001), a genus included in tribe Nicotianeae. Some authors (Moscone 1992; Badr *et al.* 1997) suggested that x = 8 together with x = 7 are the base numbers for Solanaceae, a hypothesis supported by molecular studies that place subfam. Cestroideae as ancestral (Olmstead and Palmer 1992; Olmstead *et al.* 1999). More advanced taxa, such as Solanoideae, increased their basic number to x = 12 (Olmstead and Palmer 1992; Olmstead *et al.* 1999).

Concerning to the size, chromosomes of the studied species are comparatively large, since most Solanaceae have chromosomes of 1-3 µm long (e.g. Moscone 1992; Badr *et al. 1997;* Chiarini 2003; Moscone *et al.* 2005). Species of *Solanum* sect. *Cyphomandropsis* (Moscone 1992) and sect. *Cyphomandra* (Pringle and Murray 1991; Bohs 1994) have also chromosome sizes comparable to the Cestreae species analyzed up-to-now (Sharma and Sharma 1957; Berg and Greilhuber 1993a, b; our results). Taking into account the DNA amount, Sykorova *et al.* (2003), based in their own data and in Bennett and Leitch (2001), calculated that *Cestrum parqui* has the largest mean chromosome size of any Solanaceae species reported. In this sense, an analysis of the absence of *Arabidopsis*-type telomeres in *Cestrum*, *Sessea*, and *Vestia* has suggested the hypothesis that an over-active replacement of that region would lead to chromosome enlargement and genome size gain (Sykorova *et al.* 2003).

The presence of macro-, micro-, or linear satellites has been noted in *Cestrum*. Most species present three satellited pairs, although some have either two or four (Gottschalk 1954; Sharma and Sharma 1957; Berg and Greilhuber 1992, 1993 a, b). The species here examined have only one satellited pair, suggesting that it is a variable trait for the genus. The position in the chromosomes of these secondary constrictions also varies: generally they are located in the short arm of the most asymmetrical pair (either *sm* or *st*) and/or in the short arm of an *m* pair (Sharma and Sharma, 1957; Berg and Greilhuber, 1992, 1993, a, b; our results).

The karyotype formulae obtained here are comparable and related to the karyotypes known for *Cestrum*: most have seven *m* chromosomes pairs together with one pair of *sm* or *st* chromosomes (Gottschalk 1954; Sharma and Sharma 1957; Badr *et al.* 1997; Berg and Greilhuber 1992, 1993 a, b), or exclusively *m* pairs (our data). Thus, the three genera cannot be characterized by a determined karyotype formula.

Overall, the karyotypes of Solanaceae are mostly symmetrical, with a predominance of *m* chromosomes of rather similar size (e.g., Moscone 1989, 1990, 1999; Sheidai *et al.* 1999; Acosta and Moscone 2000; Stiefkens and Bernardello 2002; Acosta *et al.* 2005; Moscone *et al.* 2005), a trend supported by our data. Exceptionally, complements mostly composed by *sm* and *st* chromosomes are found in some *Nicotiana* (Burns 1982; Villa 1984) and some *Solanum* species (Bernardello and Anderson 1990; Acosta *et al.* 2005). The fact that many genera (including the studied here) display a constancy in chromosome shape and size between species, was proposed as an evidence of karyotypic orthoselection in Solanaceae, which preserves rather similar complements because they seem to be more stable (Moscone *et al.* 2003; Acosta *et al.* 2005).

However, a general trend in the tribe can be suggested. *Vestia foetida* has a comparatively more symmetrical karyotype and the shortest genome length, showing a basal position in the phylogenetic trees (Olmstead *et al.* 1999; Sykorova *et al.* 2003). Thus, karyotype diversification in Cestreae may have been accompanied by a tendency towards slight asymmetry and increased genome length (Sykorova *et al.* 2003). Nevertheless, more species of *Cestrum* and *Sessea* should be analyzed to confirm this trend.

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