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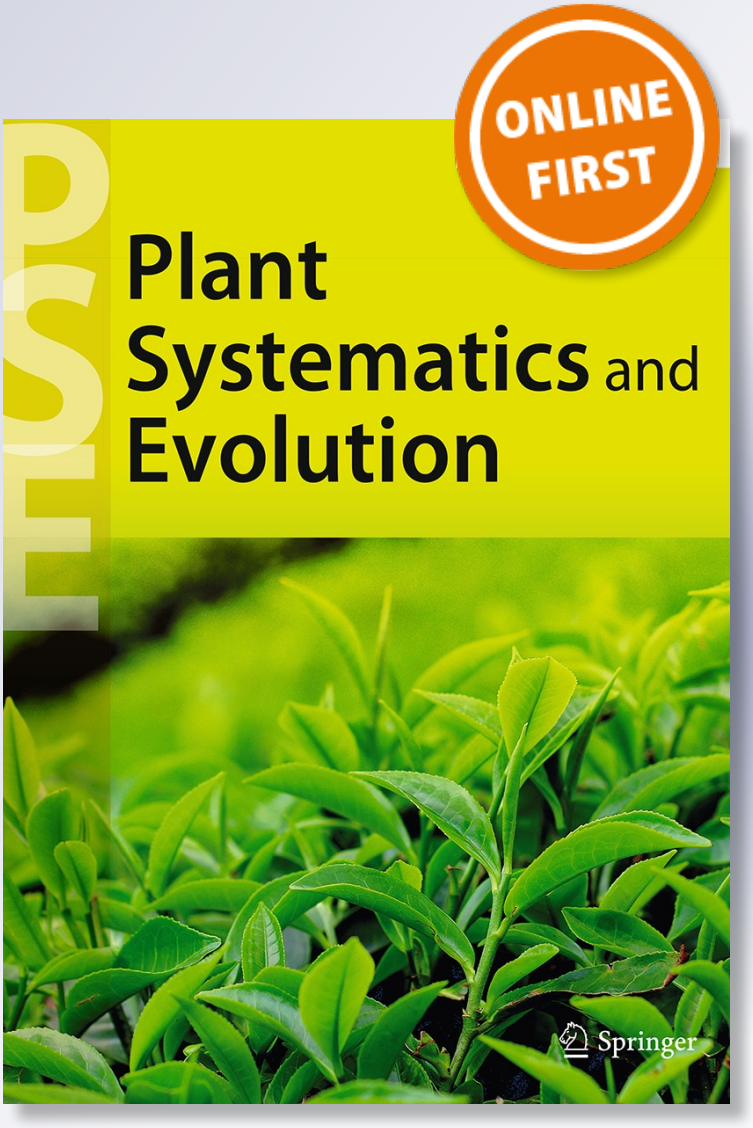
Plant Systematics and Evolution

ISSN 0378-2697

Plant Syst Evol
DOI 10.1007/s00606-014-1158-x



Springer

Plant Syst Evol
DOI 10.1007/s00606-014-1158-x

ORIGINAL ARTICLE

Chromosomal differentiation of Tribe Cestreae (Solanaceae) by analyses of 18-5.8-26S and 5S rDNA distribution

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Received: 13 September 2013 / Accepted: 28 April 2014

Abstract Tribe Cestreae is monophyletic with three genera: *Cestrum*, *Sessea*, and *Vestia*. Karyotypically, it is outstanding within Solanaceae by several features: (1) basic number $x = 8$, (2) large chromosome sizes, (3) complex heterochromatin patterns, (4) occurrence of B-chromosomes (Bs) in *Cestrum* with particular banding patterns and rDNA sites distribution, and (5) absence of *Arabidopsis*-type telomeres. Seventeen South American Cestreae species from the three genera were studied using fluorescence in situ hybridization (FISH) with ribosomal DNA regions (5S and 18-5.8-26S) as probes, with the aim of recognizing specific or group-specific chromosomal markers and analyzing karyotype diversity in a systematic and evolutionary context. The first chromosome number report for *Cestrum euanthes*, *C. kunthii*, *C. lorentzianum*, and *C. tomentosum* is included. Variation in number and distribution of rDNA loci was observed among the species, concerning both As and Bs chromosomes. Despite the constancy of the karyotype and numbers of rDNA loci, the mapping of 18-5.8-26S and 5S rDNA loci allowed to differentiate Cestreae genera and species groups within *Cestrum*, highlighting the importance of these markers as cytotaxonomic character in this tribe.

Keywords B-chromosomes · *Cestrum* · Chromosome numbers · Karyotype evolution · Ribosomal DNA · *Sessea* · *Vestia*

Handling editor: Eric Schranz.

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Introduction

Solanaceae is a plant family of economic, floristic, and ethnobotanic importance, including species such as potato, tomato, chili peppers, and tobacco (Hunziker 2001). Molecular studies showed that it is composed of several clades (Olmstead et al. 2008). This phylogeny recognized four subfamilies (Goetzeoideae, Cestroideae, Nicotianoideae, Solanoideae), two unranked informal clades within Solanoideae (Atropina and Salpichroina), and several genera and tribes unassigned to a subfamily or within a more inclusive clade.

Among the Cestroideae, tribe Cestreae is monophyletic and consists of three American genera: *Cestrum* L., *Sessea* Ruiz et Pav., and *Vestia* Willd. (Hunziker 2001). Their affinity 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; Särkinen et al. 2013). 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.

Vestia is a monotypic genus (*V. foetida* Hoffmanns.) endemic to Chile, while *Sessea* has ca. 15 species mainly distributed in Andean regions. Concerning macroscopic features, some *Sessea* species might be confused with *Cestrum* species, although the two genera can be clearly differentiated by the fruit type (Benítez de Rojas and Nee 2001; Francey 1935, 1936; Benítez de Rojas and D'Arcy 1998) and they are recognized as separated clades in a molecular phylogenetic study (Montero-Castro et al. 2006).

Cestrum embraces ca. 150 species and is distributed throughout the tropical regions of the New World, from southern Florida and northern Mexico to Argentina. Its

Published online: 09 October 2014

major diversity center is located in Brazil, Bolivia, Peru, and Northern Argentina, with ca. 100 species (Romanutti and Hunziker 1998; Hunziker 2001). Some of them (e.g., *C. nocturnum* L., *C. elegans* (Brongn.) Schltdl., and *C. parqui* L'Hér.) were introduced in other regions of the world. The monophyly of *Cestrum* was confirmed (Montero-Castro et al. 2006), but the inter-relationships of its species are still unclear. Molecular analyses are inconsistent with former infrageneric classifications (Dunal 1852; Francey, 1935, 1936), and according to Montero-Castro et al. (2006), the geographical proximity predicts phylogenetic relationships in *Cestrum* and could be explained by vicariance events.

Karyotypically, Cestreae is outstanding within Solanaceae because of the following features, which support its monophyly and make it of interest to study karyotype evolution and diversity of distribution of repetitive DNA sequences: (1) basic number $x = 8$ (Tschischow 1956; Bolkhovskikh et al. 1969; Goldblatt 1984; Goldblatt and Johnson 1991, 1996; Las Peñas et al. 2006); (2) largest chromosome sizes among the family (Fernandes et al. 2009; Fregonezi et al. 2006; Las Peñas et al. 2006); (3) complex patterns of heterochromatin in *Cestrum*, like the cold-sensitive regions (Berg and Greilhuber 1992, 1993; Fregonezi et al. 2006); (4) occurrence of B-chromosomes in several *Cestrum* species with particular banding patterns (Fregonezi et al. 2004) and distribution of 45S and 5S rDNA (Sykorova et al. 2003b; Fernandes et al. 2009); and (5) absence of *Arabidopsis*-type (TTTAGGG)n telomeres, which seem to be replaced by an A/T-rich minisatellite (Sykorova et al. 2003a, b).

Thus, species of *Cestrum*, *Sessea*, and *Vestia* were studied by fluorescence in situ hybridization (FISH) using rDNA regions (5S and 18-5.8-26S) as probes, with the aim of recognizing specific or group-specific chromosomal markers and analyzing karyotype diversity in a systematic and evolutionary context.

Materials and methods

Plant material

The studied species of *Cestrum, Sessea*, and *Vestia* and their collection data are detailed in Table 1.

Chromosome preparations

Chromosome preparations were obtained from root meristems pretreated with 2 mM 8-hydroxyquinoline at 15 °C for 4–5 h, fixed in ethanol: acetic acid (3:1, v:v) for 24 h at room temperature and stored at −20 °C until use. Slides were made using root meristems previously digested with a solution of 4 % cellulase and 40 % pectinase to 37 °C for 2 h and chromosome squashes were made in 45 % acetic acid. Coverslips were removed after freezing in liquid nitrogen and the slides were air dried.

Fluorescence in situ hybridization (FISH)

The FISH protocol was according to Schwarzacher and Heslop-Harrison (2000), with minor modifications. The location and number of rDNA sites were determined using the following probes: the p*Ta*71 containing the 18-5.8-26S gene of wheat (Gerlach and Bedbrook 1979) labeled with biotin-14-dATP by nick translation (BioNick, Invitrogen Carlsbad); a 5S rDNA fragment obtained by PCR using the primers 5L1 and 5L2 (Shibata and Hizume 2002) and genomic DNA of *Cestrum parqui* as template, labeled with digoxigenin-11-dUTP (DIG Nick translation mix, Roche). The 18-5.8-26S probe was detected with avidin-FITC conjugate (Sigma-Aldrich), the 5S probe was detected with antidigoxigenin-rhodamine (Roche), and then counterstained and mounted with 25 µl antifadeVectashield® (Vector Lab.), containing 1.5 µg/ml of DAPI. Photomicrographs were obtained with a Zeiss Axiophot microscope (Jena, Germany) coupled to a Leica DFC300FX camera (Wetzlar, Germany). Combination of channels into RGB color images were made in Adobe Photoshop CS4 (Adobe Systems Incorporated) software.

The nomenclature used for the description of mitotic chromosome morphology follows Levan et al. (1964).

Results

Chromosome number and karyotype structure

The chromosome number $2n = 16$ was found in all the species studied, and supernumerary chromosomes (B-chromosomes) were found in *Cestrum euanthes*, *C. parqui*, and *C. nocturnum* (Table 1; Figs. 1, 2). Karyotypes were highly symmetrical: *Cestrum* and *Sessea* showed seven metacentric (*m*) pairs (#1–7) plus one submetacentric (*sm*) (#8) (Table 1). *Vestia foetida* exclusively showed eight *m* pairs (Table 1). For all the species, A-chromosome size ranged from 7 to 13.5 µm.

The B-chromosomes were variable in number within and among individuals: 1–3 in *C. euanthes* and 1–10 in *C. nocturnum* (Table 1). In the case of *C. parqui*, some populations showed a constant absence of Bs, while some other populations always presented 1–3 Bs (Fig. 2d). Regarding the size, all Bs were 5–6 times smaller than A-chromosomes, ranging from 1.7 to 2.8 µm (Figs. 1, 2). The Bs of the different species were similar in size and morphology, being in all cases submetacentric/subtelocentric (Figs. 1, 2).

Table 1 Distribution of 18-5.8-26S and 5S rDNA in the studied species of the tribe Cestreae and collection data of the populations analyzed

Species	KF	B-chrom.	rDNA				Synteny	Collection data and voucher	References
			18-5.8-26SS		5S				
			N	*P*	*N*	*P*			
Cestrum									
C. amictum Schlcht.	7*m* + 1*sm*	–	4	t	2	c	+		Fregonezi et al. (2006)
C. corymbosum Schltdl.	7*m* + 1*sm*	–	4	t	2	c	+		Fernandes et al. (2009)
C. elegans (Brongn.)Schltdl.	7*m* + 1*sm*	–	4	t	2*	c (c, i, st, t)	+	Cultivated, NLD020, 804750025	In this work
C. euanthes Schltdl.	7*m* + 1*sm*	1–3	4	t	2*	c (c)	–	Argentina, Prov. Corrientes, *Barboza 2127* (CORD)	In this work
C. fasciculatum (Schltdl.) Miers	7*m* + 1*sm*	–	4	t	2	c	+	Cultivated, NLD020, 944750044	In this work
C. intermedium Sendtn.	7*m* + 1*sm*	1–2	4	t, st	2	c	+		Fregonezi et al. (2004, 2006)
C. kunthii Francey	7*m* + 1*sm*	–	4	t	2*	c (c)	–	Argentina, Prov. Tucumán, *Urdampilleta 749* (CORD)	In this work
C. laevigatum Schltdl.	7*m* + 1*sm*	–	4	t	2	c	–		Fernandes et al. (2009)
C. lorentzianum Griseb.	7*m* + 1*sm*	–	4	t, st	2*	c (c)	–	Argentina, Prov. Tucumán, *Barboza 2141 and 2144* (CORD)	In this work
C. megalophylum Dunal	7*m* + 1*sm*	–	5	t	2	c	–		Fernandes et al. (2009)
C. nocturnum L.	7*m* + 1*sm*	1–10	4	t, st	2*	c (c, i, st, t)	+	Cultivated, *Chiarini 701* (CORD)	In this work
C. parqui Benth.	7*m* + 1*sm*	–	4	t, st	2*	c (c)	–	Argentina, Prov. Córdoba, *Urdampilleta 553–554* (CORD)	In this work
C. parqui Benth.	7*m* + 1*sm*	1–2	4	t, st	2	c	–	Argentina, Prov. Corrientes, *Barboza 2284* (CORD)	In this work
C. sendtnerianum Mart.	7*m* + 1*sm*	–	4	t	2	c	–		Fregonezi et al. (2006)
C. strigilatum Ruiz & Pav	7m + 1*sm*	1	4	t, st	2	c	–		Fregonezi et al. (2004, 2006)
C. strigilatum Ruiz and Pav	7*m* + 1*sm*	–	4	t, st	2	c	+	Cultivated, NLD020, 814750087	In this work
C. tomentosum L.	7*m* + 1*sm*	–	4	t, st	2	c	–	Cultivated, NLD020, 934750170	In this work
Sessea									
S. regnellii Taub.	7*m* + 1*sm*	–	4	t	2	c	–	Argentina, Prov. Misiones, *Urdampilleta 573* (CORD)	In this work
Vestia									
V. foetida Hoffmanns.	8*m*	–	4	t	2*	c (c, i, st, t)	+	Cultivated, NLD020 814750079	In this work

* Dispersion of rDNA in A-chromosomes

N number of rDNA loci, *P* position of rDNA loci, *c* centromeric, *i* intercalary, *st* subterminal, *t* terminal

KF Karyotype formulae, *NLD020* Experimental Garden of the Radboud University of Nijmegen, *CORD* Herbarium of the Museo Botánico de Córdoba

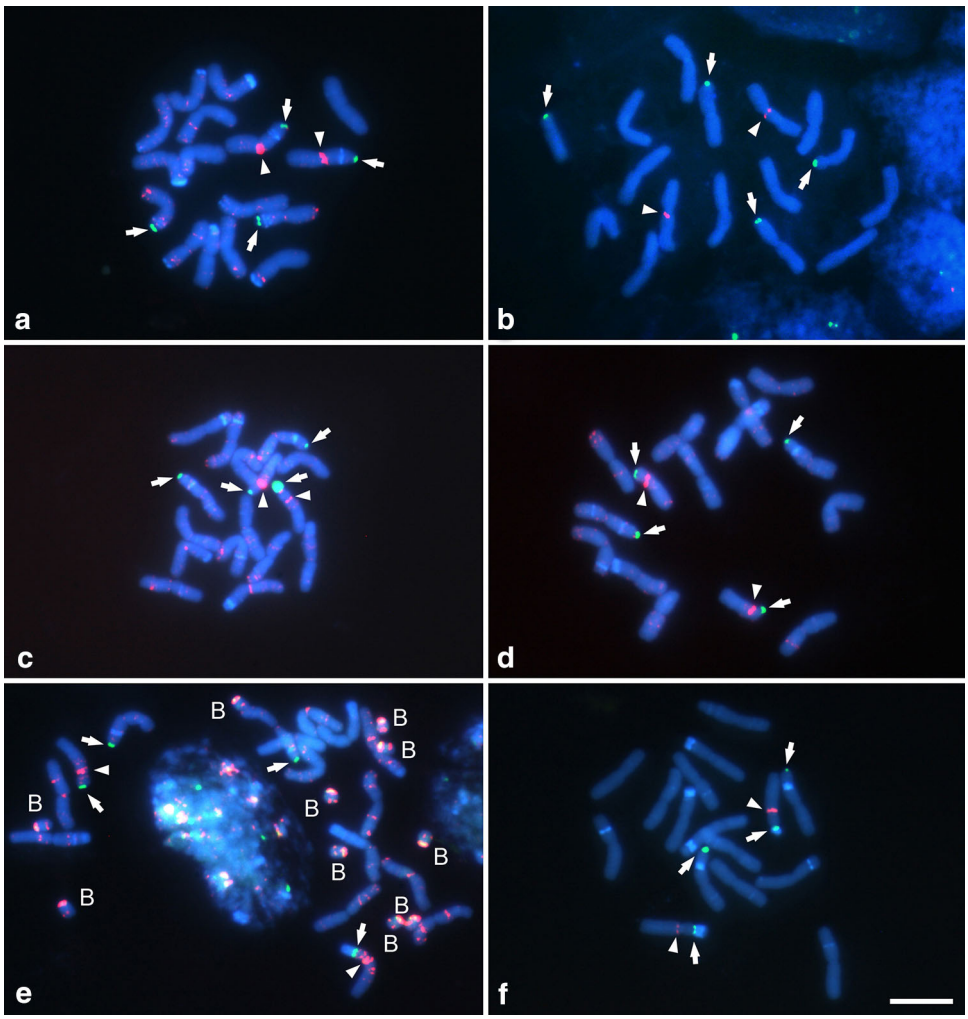

Fig. 1 Distribution of rDNA loci (18-5.8-26S, *arrow*, *green*, and 5S, *arrowhead*, *red*) in Cestreae. **a** *Vestia foetida*, **b** *Sessea regnellii*, **c** *Cestrum elegans*, **d** *C. fasciculatum*, **e** *C. nocturnum*, **f** *C. strigilatum*. In **a**, **c**, **d**, and **e** the dispersion of 5S rDNA in other chromosomes besides the pair #8 is observed. In **e**, ten B-chromosomes (marked with *B*) are visualized. *Bar* = 10 μm

Fluorescence in situ hybridization with rDNA probes

The number of major 18-5.8-26S and 5S rDNA loci was identical for all species studied: four hybridization signals for the 18-5.8-26S and two for the 5S site. However, their position varied among the species (Table 1; Figs. 1, 2). In addition, minor hybridization signals of 5S rDNA were detected in several species in variable number (Figs. 1a, c–e, 2a–c, e). These results are presented schematically in Fig. 3 where only chromosomes bearing rDNA sites are shown.

As a by-product of the FISH technique, a variable number of bands strongly dyed with DAPI were seen in most species, placed in chromosomes with rDNA sites as well as in chromosomes without rDNA sites. This pattern of DAPI bands, allowed distinguishing species groups, especially those with DAPI bands in chromosomes with rDNA sites (see "Discussion" below).

Vestia foetida showed four pairs of terminal DAPI bands and two pairs of intercalary DAPI bands (Fig. 1a). The 18-5.8-26S rDNA probe revealed two chromosome pairs with signals in terminal regions, which also presented intercalary DAPI bands. At the same time, one of these pairs presented a strong 5S rDNA signal in pericentromeric position. In addition, minor 5S rDNA hybridization signals were detected in most chromosomes (Fig. 1a).

Sessea regnellii (Fig. 1b) showed no DAPI bands in terminal or in intercalary regions. The 18-5.8-26S rDNA probe revealed four hybridization signals in terminal regions: two in an *m* chromosome pair and two in an *sm* pair. The 5S rDNA probe showed two strong hybridization signals in pericentromeric regions in an *m* chromosome pair (Fig. 1b).

Cestrum elegans, *C. fasciculatum*, *C. nocturnum*, and *C. strigilatum* (Fig. 1c–f) presented terminal, subterminal, and intercalary DAPI$^+$ bands. In these species, FISH results showed two pairs of strong 18-5.8-26S rDNA signals: one pair in terminal regions of an *m* pair and another pair in terminal or subterminal regions of short arms of pair #8 (*sm*). The 18-5.8-26S rDNA sites were observed in

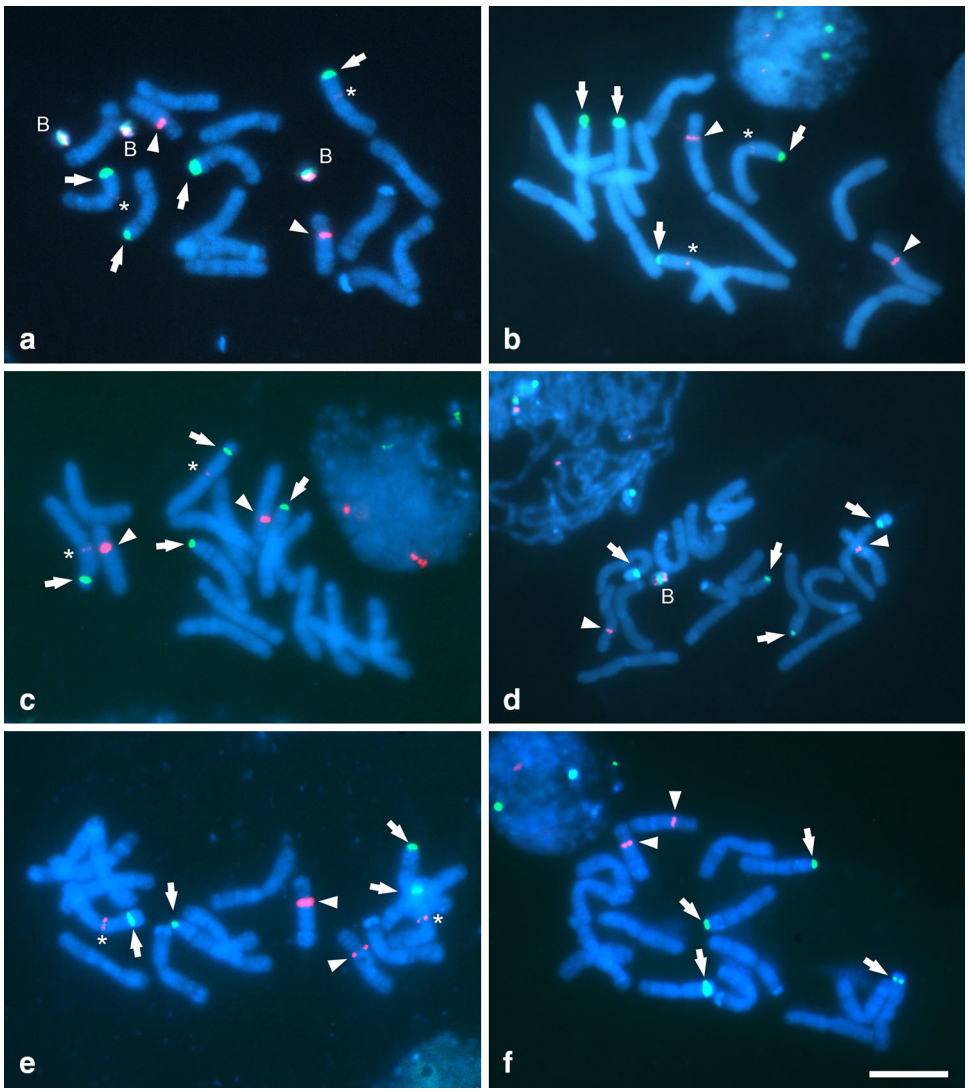

Fig. 2 Distribution of rDNA loci (18-5.8-26S, arrow, green, and 5S, arrowhead, red) in Cestreae. **a** *Cestrum euanthes*, **b** *C. kunthii*, **c** *C. lorentzianum*, **d** *C. parqui* (GB 2284), **e** *C. parqui* (JDU 553), **f** *C. tomentosum*. The *asterisk* indicates the dispersion of 5S rDNA in other chromosomes besides the pair #8. In **a** and **d**, B-chromosomes are visualized (marked with *B*). *Bar* = 10 μm

terminal regions of pair #8 in *C. elegans* and *C. fasciculatum* (Fig. 1c, d); however, in *C. nocturnum* and *C. strigilatum* these signals were subterminal with a terminal DAPI$^+$ band (heteromorphic in *C. nocturnum*) (Fig. 1e, f). Physical mapping revealed major 5S rDNA loci in the pericentromeric regions of pair #8 of four taxa. A remarkable number of dispersed minor 5S rDNA hybridization signals were detected in most chromosomes of *C. elegans*, *C. fasciculatum*, and *C. nocturnum*.

In *C. euanthes*, *C. lorentzianum*, *C. parqui*, and *C. tomentosum* (Fig. 2a–f), two pairs of intense 18-5.8-26S rDNA signals were identified in two *m* pairs, and one pair of 5S rDNA sites in pericentromeric regions of the only *sm* pair. A few dispersed 5S signals were observed in pericentromeric regions of an *m* chromosome in *C. euanthes*, *C. kunthii*, *C. lorentzianum*, and *C. parqui* (only in the sample *Barboza 2284*) (Fig. 2a–e). In *C. euanthes* and *C. kunthii*, four 18-5.8-26S rDNA sites were localized in terminal regions, whereas in *C. lorentzianum*, *C. parqui*, and *C. tomentosum*, two 18-5.8-26S rDNA sites were subterminal plus a terminal DAPI$^+$ band. In *C. euanthes*, *C. lorentzianum*, *C. parqui*, and *C. tomentosum*, minor intercalary DAPI bands were detected in a chromosome pair with the terminal 18-5.8-26S rDNA site.

Physical mapping of ribosomal RNA genes in the Bs

In *Cestrum euanthes*, *C. nocturnum*, and *C. parqui*, Bs showed hybridization signals for the 18-5.8-26S and 5S rDNA sites, although varying in number and position (Fig. 4). Two adjacent 18-5.8-26S and 5S signals were detected in *C. nocturnum* (Fig. 4a). *C. euanthes* presented two terminal 5S rDNA signals and one pericentromeric 18-5.8-26S rDNA site (Fig. 4b) whereas in *C. parqui* showed only one terminal 5S rDNA signal and one pericentromeric 18-5.8-26S rDNA (Fig. 4c).

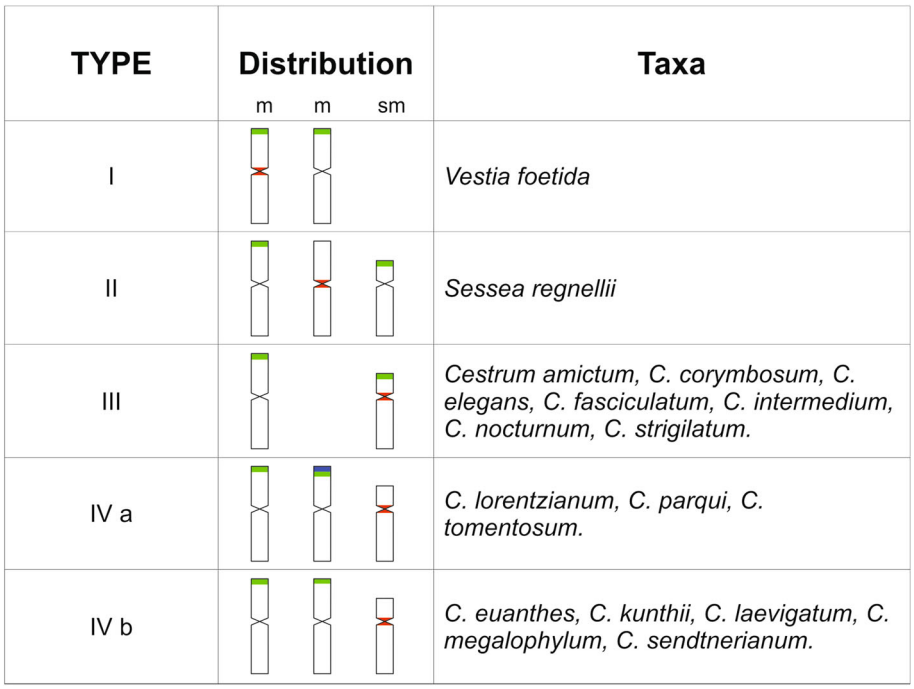

Fig. 3 Scheme showing types of chromosome organization of rDNA loci (18-5.8-26S, *green*, and 5S, *red*) observed in Cestreae, only chromosomes bearing rDNA sites are considered

Discussion

Chromosome numbers and karyotype structure

All species studied were diploid with $2n = 16$. The basic number $x = 8$ is conserved in Cestreae and differentiates it from other Solanaceae. Our results confirm previous numbers of several species from other localities (Tschischow 1956; Sharma and Sharma 1957; Bolkhovskikh et al. 1969; Moscone 1992; Las Peñas et al. 2006; Fregonezi et al. 2006; Fernandes et al. 2009), being the first chromosome number report for *Cestrum euanthes*, *C. kunthii*, *C. lorentzianum*, and *C. tomentosum*.

Tribe Cestreae is additionally characterized by large chromosomes and symmetrical karyotypes (Fregonezi et al. 2006; Las Peñas et al. 2006; Fernandes et al. 2009). Karyotypes of the studied species are relatively symmetrical, have similar chromosome sizes, and are composed exclusively of *m* chromosomes (*Vestia foetida*) or with one *sm* pair (the remaining species).

B-chromosomes had been previously reported in Solanaceae (Acosta and Moscone 2011) and in *Cestrum*. These supernumerary chromosomes had been observed in *C. intermedium* and *C. strigilatum* by Fregonezi et al. (2004), and in a hybrid *Cestrum* (*C. parqui* x *C. aurantiacum*) by Sykorova et al. (2003b). In *C. euanthes*, *C. nocturnum*, and *C. parqui*, Bs varied in number at both intra- and inter-individual level, which indicates possible mechanisms of mitotic and/or meiotic instability (Camacho et al. 2000). The size of Bs in *Cestrum* is 3-5 fold lower than A-chromosomes, as usually reported in plants (Jones and Houben 2003). The morphology of Bs in *Cestrum* is similar, but differences in composition and size of heterochromatin blocks suggest that they have been originated independently from each other (Fregonezi et al. 2004). This hypothesis needs to be verified, since the variation in heterochromatin observed in the Bs may be also explained by their rapid evolution (Houben et al. 2014), which make them subject of structural changes such as accumulation of repetitive DNAs (Lamb et al. 2007; Klemme et al. 2013).

Distribution of rDNA and cytotaxonomy

rDNA distribution often allows grouping phylogenetically related species and can be used as a cytotaxonomic character (Tate et al. 2009; Lan and Albert 2011; Roa and Guerra 2012; Urdampilleta et al. 2013; Chiarini et al. 2014). Our results confirm previous works on *Cestrum strigilatum* and *Sessea regnellii* (Fregonezi et al. 2006; Fernandes et al. 2009) and report new data about distribution of 18-5.8-26S and 5S rDNA sites in *C. elegans*, *C. euanthes*, *C. fasciculatum*, *C. kunthii*, *C. lorentzianum*, *C. nocturnum*, *C. parqui*, *C. tomentosum*, and *Vestia foetida*. All species studied of Cestreae have conserved the terminal and pericentromeric positions for 18-5.8-26S and 5S rDNA, respectively. Despite the constancy of the karyotype features and numbers of rDNA loci, Cestreae genera and *Cestrum* groups could be differentiated by the relative distribution of 18-5.8-26S and 5S rDNA sites (Fig. 4). To verify this hypothesis and assess the value of the rDNA

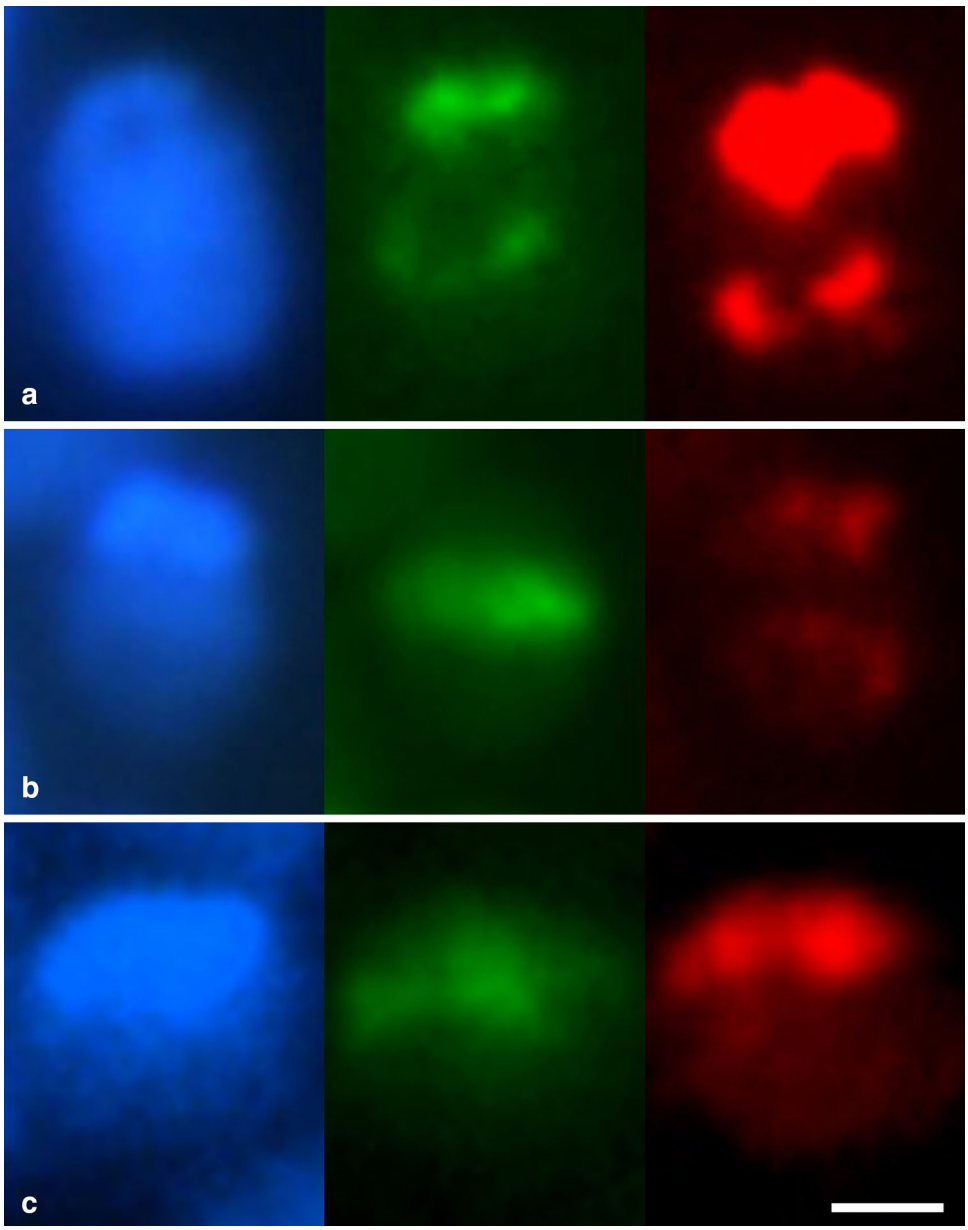

Fig. 4 Details of B-chromosomes by showing RGB channels after FISH. Distribution of rDNA loci (18-5.8-26S, *center*, *green*, and 5S, *right*, *red*) in B-chromosomes of Cestreae. **a** *Cestrum nocturnum*, **b** *C. parqui*, **c** *C. euanthes*. *Bar* = 1 μm

distribution as a cytotaxonomic character, it is necessary to include more species in phylogenetic studies.

Tribe Cestreae is a monophyletic group, with *Vestia* occupying a basal position (Olmstead et al. 1999; Montero-Castro et al. 2006). Compared to the other Cestreae, *Vestia foetida* has the most symmetrical karyotype and it has the shortest genome length (Las Peñas et al. 2006). Another chromosome feature that characterize *V. foetida* is the synteny of rRNA genes on an *m* pair. Thus, the chromosome pattern of *V. foetida* may be the ancestral state, and the diversification in Cestreae may have been accompanied by change in the karyotype formula from 8 *m* to 7 *m* +1 *sm*, together with chromosome rearrangements that led to the relocation of rRNA genes.

Some controversy exists about the delimitation of the genus *Sessea*. Based on morphology, Carvalho and Schnoor (1997) transferred *S. regnellii* and *S. brasiliensis* to *Cestrum*, a point of view not accepted by other specialists (Benítez de Rojas and Nee 2001). Moreover, there is some molecular evidence suggesting that *Sessea* would be paraphyletic (Montero-Castro et al. 2006). Our results in *S. regnellii* showed differences in both the distribution of rDNA and karyotype formulae that distinguished it from *Cestrum* and *Vestia*. Chromosome studies in a larger number of *Sessea* species are necessary to clearly settle the generic boundaries.

The presence of one *sm* pair bearing a pericentromeric 5S rDNA locus is a conserved character shared by all

species of *Cestrum* studied to date (Fregonezi et al. 2006; Fernandes et al. 2009; this work). On the contrary, the 18-5.8-26S rDNA distribution allows to define two groups in *Cestrum*: one characterized by the synteny of the rDNA in the only *sm* pair (*C. amictum*, *C. corymbosum*, *C. elegans*, *C. fasciculatum*, *C. intermedium*, *C. nocturnum*, and *C. strigilatum*), and another one with a 18-5.8-26S rDNA distribution independent of the 5S rDNA loci (*C. euanthes*, *C. kunthii*, *C. laevigatum*, *C. lorentzianum*, *C. megalophylum*, *C. parqui*, *C. sendtnerianum*, and *C. tomentosum*). This separation into two groups coincides with molecular phylogenetic studies (Montero-Castro et al. 2006) which recognize two clades: one including *C. elegans*, *C. fasciculatum*, and *C. nocturnum*, and the other one including *C. tomentosum*. Within the latter, *C. lorentzianum*, *C. parqui*, and *C. tomentosum* were differentiated by distal heterochromatin blocks (DAPI bands after FISH) in a NOR-bearing pair.

Variation in number and distribution of rDNA loci is commonly observed in many different plant groups (Roa and Guerra 2012). In general, the number of 18-5.8-26S rDNA loci is reported to be more polymorphic than the 5S rDNA loci (Garcia et al. 2012). Different mechanisms have been postulated to explain the mobility of rDNA sites (Raskina et al. 2008; Lan and Albert 2011). Chromosome rearrangements, such translocations involving rDNA, could have occurred in the evolution of Cestreae. However, the dispersion of 5S rDNA in *C. elegans*, *C fasciculatum*, and *V. foetida* and the dispersion of 5S and 18-5.8-26S rDNA in Bs of *C. euanthes*, *C. nocturnum*, and *C. parqui* could be due to transposable elements (TEs), since it leads to an increased number of sites in the genome. The transposon-mediated mobility of rDNA, proposed for *Allium* (Schubert and Wobus 1985), was supported by permanent clustering of different TEs with 18-5.8-26S or 5S rDNA (Raskina et al. 2008). *Cestrum strigilatum* and *C. intermedium* exhibited a concentration of these elements in some terminal regions associated with NORs and hybridization signals on both arms of the Bs (Fregonezi et al. 2007). This fact confirms the hypotheses that dispersion of 5S and 18-5.8-26S rDNA in *Cestrum* could have occurred by transposon-mediated transpositional events, being Bs the preferred 'landing sites', due to their neutral character (Jones and Houben 2003). Further cytogenetic/molecular/bioinformatic studies are required for the elucidation of the relationship of TEs with the rDNA dispersion.

The distribution of rRNA genes (5S and 18-5.8-26S rDNA) in Cestreae species studied to date (Fregonezi et al. 2006; Fernandes et al. 2009; this work) permit to describe four types of genotype organization (Fig. 4). Type I is characteristic of *V. foetida*, where all chromosomes are *m*. Types II, III, and IV have the #8 *sm* pair and are distinguished by their rDNA distribution. In *Sessea*, type II was observed, whereas in *Cestrum* species are grouped in III or IV types with two subgroups, IVa and IVb, differentiated by the presence of heterochromatin blocks. Considering the species included in Cestreae phylogenetic and cytogenetic studies, the present rDNA distribution observed may have arisen from at least three significant chromosomal changes: (1) Modification of pair #8 that led to the transition between *m* and *sm* chromosomes or reverse; (2) chromosomal arrangement that led to the synteny of rRNA genes in pair #8; (3) chromosomal arrangement that led to the loss of synteny of rRNA genes in pair #8. Though it is possible to raise some hypotheses on the direction of changes, it is necessary to include more species to confirm these processes.

Our data, together with previous cytogenetic information, agree with the traditional circumscription of *Cestrum*, *Sessea*, and *Vestia* (Francey 1935, 1936) and support the monophyly of Cestreae. In the case of *Cestrum*, we support the existence of at least two of the clades proposed in the molecular phylogeny of Montero-Castro et al. (2006). In Cestreae, like in different plant groups (Ran et al. 2001; Liu et al. 2003; Kokubugata and Forster 2006; Urdampilleta et al. 2013; Chiarini et al. 2014), the use of molecular cytogenetics, specifically rDNA regions, analyzed together (5S and 18-5.8-26S rDNA), proved to be a useful tool in recognizing plant groups phylogenetically related.

Acknowledgments The authors are grateful to the Argentinean agencies: CONICET, ANPCyT—FONCyT, MINCyT—Córdoba, and SECyT—Universidad Nacional de Córdoba for financial support and Dr. Gloria Barboza for determination and collection of plant materials.

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