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Karyotypes, heterochromatin distribution and rDNA patterns in South American *Grindelia* (Asteraceae)

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Abstract

Grindelia is a genus with a complex evolutionary history with reticulate evolution. We studied the karyotype, fluorescent banding, and fluorescence *in situ* hybridization (FISH) using 18–5.8–26 S and 5 S ribosomal DNA probes to survey karyotypic diversity of South American *Grindelia* species. Chromosome basic numbers were $x = 6$ (with several ploidy levels: $2x$, $4x$, and $6x$). All the *Grindelia* studied conserved the patterns of CG-rich heterochromatin and 18-5.8-26 S rDNA. The third m sat-chromosome pair was homeologous in *Grindelia*. Chromosome variation, although not always large, accompanied the evolutionary divergence of the taxa studied. The *Grindelia* studied formed two species groups: (1) *G. globularifolia* and *G. pulchella* var. *pulchella,* (2) *G. buphthalmoides, G. cabrerae* var. *alatocarpa* and var. *cabrerae, G. chiloensis, G. orientalis*, and *G. prostrata.* These groups do not show any morphological affinities and their phylogenetic relationships are not clearly resolved, suggesting that these groups have recently diverged.

Keywords: Grindelia*, heterochromatin, karyotype, polyploidy, ribosomal genes*

Introduction

The cosmopolitan Astereae is the second largest tribe of the family, with ca. 220 genera and more than 3000 spp. (Funk et al. 2009). *Grindelia* Willd., in the Machaerantherinae subtribe (Nesom 2000; Morgan 2003), and *Haplopappus* are the only members that inhabit South America. Morgan et al. (2009) suggested that this subtribe had a complex evolutionary history with reticulate evolution.

Grindelia is a New World genus with amphitropical disjunction and ca. 67 spp.; approximately 26 of them are South American and the remainder are from North America and Mexico (Bartoli & Tortosa 2012), both groups being sister clades (Moore et al. 2012). South American taxa are more diverse in habit and have their centers of diversity in Argentina, including some perennial species with varying degrees of woodiness, sometimes suffruticose or herbaceous, often resinous with glandular trichomes (Bartoli & Tortosa 2014). Their yellow heads are solitary, radiate or discoid, with perfect disk florets that have tubular corollas, often with an abruptly expanded throat, and when present, pistillate ray florets; pappuses are deciduous, having 2–80 bristles with smooth to barbellulate margins. Their glabrous achenes are flattened or prismatic, sometimes winged.

Cytological data contribute to clarifying the origin, speciation, and phylogenetic relationships of plants (e.g. Weiss-Schneeweiss & Schneeweiss 2013). In the Astereae tribe, the chromosome number and morphology provided important insights into its systematics and evolution (e.g. Nesom 1994; Torrell et al. 2003). Jones (1985) suggested that chromosome features are basic to the better understanding of the relationships of the tribe, being more useful at the infrageneric than at the generic level. There are ca. 40 chromosome counts available in *Grindelia* (e.g. Whitaker & Steyermark 1935; Semple et al. 1992; Lane & Li 1993; Bartoli & Tortosa 1998a; Baeza & Schrader 2005; Moreno et al. 2012), indicating its base number is $x = 6$. Some species showed different ploidy levels (Dunford 1986; Semple et al. 1989; Moreno et al. 2012) and particularly in South America, ploidy is more variable, with diploids, tetraploids, and hexaploids that have already been

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Table I. Collection data (country, province, department, collector, and number, herbarium) and chromosome numbers of *Grindelia* species, indicating previous reports when available. Underlined references reported karyotypes.

Taxon	Voucher information	2*n*	Previous reports
Grindelia buphthalmoides DC.	Argentina, Buenos Aires, Tornquist, Ratto & Marzaro s/n, BAA 26016.	12	Wulff et al. (1996); Bartoli and Tortosa (1998a)
	Argentina, Buenos Aires, Tornquist, Barboza *et al.* 2314, CORD		
G. cabrerae var. *alatocarpa* Ariza	Argentina, Córdoba, San Justo, Chiarini 1049, CORD	12	
G. cabrerae var. *cabrerae* Ariza	Argentina, Córdoba, Colón, Chiarini *et al.* 922, CORD	12	Di Fulvio 1977
	Argentina, Córdoba, Colón, Chiarini *et al.* 923, CORD	12	
G. chiloensis (Cornel.) Cabrera	Argentina, Chubut, Futaleufu, Barboza *et al.* 3767, CORD	12	Covas and Schnack (1946); Schnack and Covas (1947); Rahn 1960; Hunziker et al. (1989); Bartoli et al. (1990), (1993); Bartoli and Tortosa (1994), 1998b; Wulff et al. (1996)
	Argentina, Chubut, F. Ameghino, Barboza *et al.* 3675, CORD		
	Argentina, La Pampa, Toay, Barboza et al. 3670, CORD		
	Argentina, Mendoza, Luján de Cuyo, Rato *et al.* 32, BAA		
	Argentina, Río Negro, Pilcanieyu, Bach 596, BAB		
G. globularifolia Griseb.	Argentina, Córdoba, San Alberto, Chiarini *et al.* 930, CORD	12	
G. orientalis A. Bartoli, Tortosa & G.H. Rua	Uruguay, Maldonado, Maldonado, Bartoli *et al.* s/n, BAA 22840.	12	Bartoli and Tortosa (1998a)
G. prostrata A. Bartoli & Tortosa	Argentina, La Rioja, Famatina, Chiarini 1143, CORD	12	
G. pulchella var. *discoidea* (Hook. & Arn.) A. Bartoli & Tortosa	Argentina, Córdoba, Punilla, Chiarini et al. 924, CORD	24	Solbrig et al. (1964), (1969)
	Argentina, Córdoba, Pocho, Chiarini *et al.* 927, CORD		
G. pulchella var. *pulchella* Dunal	Argentina, Córdoba, Cruz del Eje, Chiarini *et al.* 926, CORD	12	Covas and Schnack (1946); Solbrig et al. (1969); Turner et al. (1979); Hunziker et al. (1990); Bartoli and Tortosa (1998a)
	Argentina, Buenos Aires, San Antonio de Areco, F. Ratto s/n, BAA 26018		
G. ventanensis A. Bartoli & Tortosa	Argentina, Buenos Aires, Sierra de La Ventana. Parque Ernesto Tornquist, Ratto & Marzaro s.n., (BAA).	36	Bartoli and Tortosa (1994)
G. anethifolia (Phil.) A. Bartoli & Tortosa x *G. chiloensis*	Argentina, Neuquén, Ñorquin, Moore *et al.* 62, BAA 26038	24	

reported (Bartoli & Tortosa 1998a). Moore et al. (2012) hypothesized that polyploidy arose several times in both *Grindelia* clades. On the contrary, karyotypic data are scarce: only nine South American and seven North American *Grindelia* (Bartoli & Tortosa 1998a; Baeza & Schrader 2005; Moreno et al. 2012) have been studied to date.

The distribution pattern of constitutive heterochromatin with fluorescent banding detects chromosomal rearrangements in the genome organization in Angiosperms (Guerra 2000; Raskina et al. 2008). In addition, mobility and distribution patterns of rDNA genes serve as species-specific karyotype markers (Hasterok et al. 2006; Chacón et al. 2012). In *Grindelia*, there are few articles dealing with these aspects: in South America rDNA genes in two species and in North America rDNA genes and banding in five taxa (Baeza & Schrader 2005; Moreno et al. 2012).

Upon this background, we studied the chromosome numbers, karyotypes, heterochromatin distribution, and 18-5.8-26S and 5S rDNA loci of 11 South American *Grindelia* taxa in order to elucidate their importance in its systematics and evolution.

Materials and methods

Plant material and chromosome preparations

Details of the material studied from natural populations and voucher specimens are shown in Table I.

For conventional staining, mitotic chromosomes of somatic cells from root tips were analyzed from squashes of 2–10 mm long primary roots from germinated seeds. Root tips were pretreated with 2 mM 8-hydroxyquinoline for 24 h at 4°C temperature, rinsed in distilled water, and fixed in freshly made ethanol:glacial acetic acid (3:1) for 24 h at room temperature. Then, they were hydrolyzed with 5N HCl for 40 min at room temperature and put in Feulgen solution for 2 hours in the dark (Jong 1997).

Meristem cells were isolated on a slide and squashed. Slides were made permanent in Entellan© after removing the cover slips with liquid nitrogen-induced freezing.

Root tips for banding and FISH preparations were washed twice in distilled water (10 min each), digested with a 2% cellulase (Sigma-Aldrich, Austria) and 20% pectinase solution (from *Aspergillus niger*; Sigma-Aldrich, Austria) for 45 min at 37°C, and squashed in a drop of 45% acetic acid (Schwarzacher et al. 1980). After coverslip removal in liquid nitrogen, the slides were stored at −20°C.

Karyotype analysis

Ten metaphases, each from a different individual, were photographed with phase contrast optics on a Zeiss Axiophot microscope equipped with a Leica DFC300FX camera. Measurements were made for the lengths of the short arm (*s*), long arm (*l*), and total chromosome length (*c*) for each pair. The centromeric index ($I = 100s/c$) and the arm ratio ($r = l/s$) were calculated and used to classify chromosomes and determine homologs according to Levan et al. (1964). Karyograms were constructed by organizing the chromosomes into groups according to their arm ratio and ordering them by decreasing length within each category. The resulting idiograms were based on the mean values obtained from the measurements of all individuals of each species. Karyotype asymmetry was estimated using the coefficient of variation of chromosome length (CV_{CL}) and mean centromeric asymmetry (MCA) (Peruzzi & Eroğlu 2013).

Fluorescent banding

Slides for fluorescent banding were stained with a drop of 0.5 mg/ml Chromomycin A_3 (CMA) in McIlvaine buffer, pH 7.0 and distilled water (1:1) containing 2.5 mM MgCl for 90 min, subsequently stained with 2 µg/ml 4′-6-diamidino-2-phenylindole (DAPI) (both Sigma-Aldrich, Austria) for 30 min, and finally mounted in McIlvaine's buffer–glycerol v/v 1:1 (Schweizer 1976; Schweizer & Ambros 1994). Photomicrography was performed with a Zeiss Axiophot (Jena, German) microscope coupled with a Leica DFC300FX camera a BX51 (Wetzlar, Germany).

The relative lengths of short and long chromosome arms (data not shown) and bands were calculated (considering haploid karyotype length = 100%) in five metaphases per species, each from a different individual. The amount of heterochromatin was expressed as a percentage of the total length of the haploid karyotype.

Fluorescent in situ hybridization

The location and number of rDNA sites were determined using as probe the p*Ta*71 containing the 18-5.8-26S rDNA (Gerlach & Bedbrook 1979) labeled with biotin-14-dATP (BioNick, Invitrogen Carlsbad, USA). For the 5S rDNA, a probe was obtained from the genome of *Prionopsis ciliata* (Nutt.) Nutt. (Moreno et al. 2012) and then labeled with digoxigenin-11-dUTP (Roche Diagnostics, USA).

The FISH protocol was according to Schwarzacher and Heslop-Harrison (2000), with minor modifications. Preparations were incubated in 100 µg/ml RNAse, post-fixed in 4% (w/v) paraformaldehyde, dehydrated in a 70–100% graded ethanol series, and air-dried. On each slide 15 µ1 of hybridization mixture was added (3 ng/µl of probe, 100% formamide, 50% dextran sulfate, 20 x SSC, and 10% SDS), previously denatured at 70°C for 10 min. Chromosome denaturation/hybridization was carried out at 90°C for 10 min, 48°C for 10 min, and 38°C for 5 min using a thermal cycler (Mastercycler, Eppendorf, Hamburg, Germany). Slides were placed in a humid chamber at 37°C overnight. The probes were detected with avidin–FITC and antidigoxigenin–rodamine conjugates and counterstained and mounted with 25-µl antifade (Vectashield Vector Lab., USA), containing 2 ng/µl of DAPI. Photomicrography was performed with a Zeiss Axiophot (Jena, German) microscope coupled with a Leica DFC300FX camera a BX51 (Wetzlar, Germany)

Results

Representative metaphases of each species are shown in Figure 1. The results obtained for the karyotypes analyzed are in Table II. Most taxa were diploid with $2n = 12$. The exceptions were the tetraploids *G. pulchella* var. *discoidea* and *G. anethifolia* × *G. chiloensis* with $2n = 24$ and the hexaploid *G. ventanensis* with $2n = 36$. Some individuals of *G. cabrerae* var. *cabrerae* had 1–2 accessory chromosomes (Figure 1(c)).

Karyotypes of all species showed m and sm chromosomes (Table II; Figure 2). Diploid *Grindelia* had the formula 5 m + 1 sm, except for *G. globularifolia* and *G. pulchella* var. *pulchella* with 4 m + 2 sm (Table II; Figure 2). All diploid species had one satellited m pair on the short arms. In the *Grindelia* polyploids, there are 2–3 satellited pairs according to their ploidy level at the same localization (Figure 2).

All species had symmetrical karyotypes with slight size variations between the chromosomes and chromosome arms, with scarce differences between species (Table II).

There were variations in the total karyotype length between the diploid species, from 13.14 µm in *G. orientalis* to 22.41 µm in *G. cabrerae* var. *cabrerae*

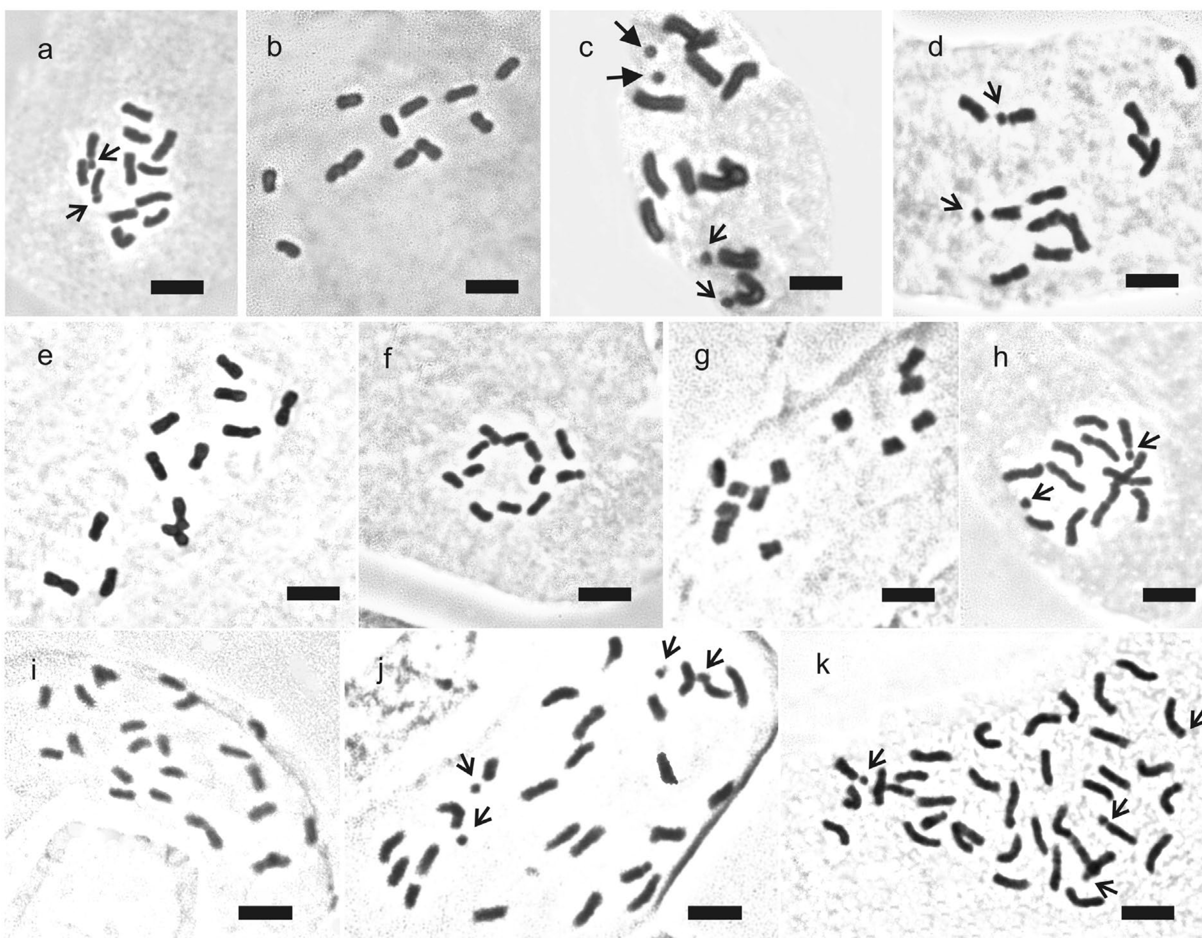

Figure 1. Mitotic metaphases of *Grindelia* species with Feulgen staining. (a) *G. buphthalmoides*. (b) *G. cabrerae* var. *alatocarpa*. (c) *G. cabrerae* var. *cabrerae* ($2n$ = 12). (d) *G. chiloensis*. (e) *G. globularifolia*. (f) *G. orientalis*. (g) *G. prostrata*. (h) *G. pulchella* var. *pulchella*. (i) *G. pulchella* var. *discoidea* $2n$ = 24. (j) *G. ventanensis* $2n$ = 36. (k) *G. anethifolia* x *G. chiloensis* $2n$ = 24. Arrows point to satellites. Bold arrows point to B chromosomes. Bars represent 5 µm.

Table II. Karyotype data of diploid taxa studied. Mean total haploid chromosome length (tl), mean chromosome length (C), mean arm ratio (r), coefficient of variation of chromosome length (CVCL), mean centromeric asymmetry (MCA), ratio between the largest and smallest chromosomes in the complement (R), and heterochromatin amount expressed as percentage of the karyotype length (% Ht). Asterisks indicate one chromosome pair with satellites on the short arms.

Taxon	Karyotype formulae	tl (µm)	C (µm)	*r*	CV_{CL}	MCA	R	%Ht
Grindelia buphthalmoides	5 *m** + 1 *sm*	16.54	2.76	1.27	12.33	9.65	1.39	2.41
G. cabrerae var. *alatocarpa*	5 *m** + 1 *sm*	13.59	2.26	1.26	8.47	10.42	1.30	3.93
G. cabrerae var. *cabrerae*	5 *m** + 1 *sm*	22.41	3.73	1.38	19.80	14.56	1.75	2.01
G. chiloensis	5 *m** + 1 *sm*	17.89	2.98	1.27	9.40	8.77	1.29	2.22
G. globularifolia	4 *m** + 2 *sm*	19.09	3.18	1.42	10.79	15.18	1.49	1.77
G. orientalis	5 *m** + 1 *sm*	13.14	2.18	1.29	11.95	10.78	1.39	1.70
G. prostrata	5 *m** + 1 *sm*	13.73	2.29	1.23	12.84	9.02	1.52	2.08
G. pulchella var. *pulchella*	4 *m** + 2 *sm*	20.45	3.41	1.46	10.61	16.68	1.41	1.46

(Table II). The polyploids showed comparatively lower lengths: 4*x* *G. pulchella* var. *discoidea* 17.6 µm, 4*x* *G. anethifolia* × *G. chiloensis* 17.02 µm and 6*x* *G. ventanensis* 19.45 µm.

Fluorescent banding patterns only showed CMA^+/$DAPI^-$ NOR-associated heterochromatin bands in the satellites (Figure 4). The mean total amount of this heterochromatin was 2.4% of the haploid genome length (Table II), regardless of the ploidy level.

The number of 18-5.8-26S rDNA sites coincided with the satellite number: two in diploids, four in tetraploids, and six in the hexaploid (Figures 2 and 4). These sites were associated with CMA^+/$DAPI^-$ bands in all cases. The 5S rDNA sites were two, four,

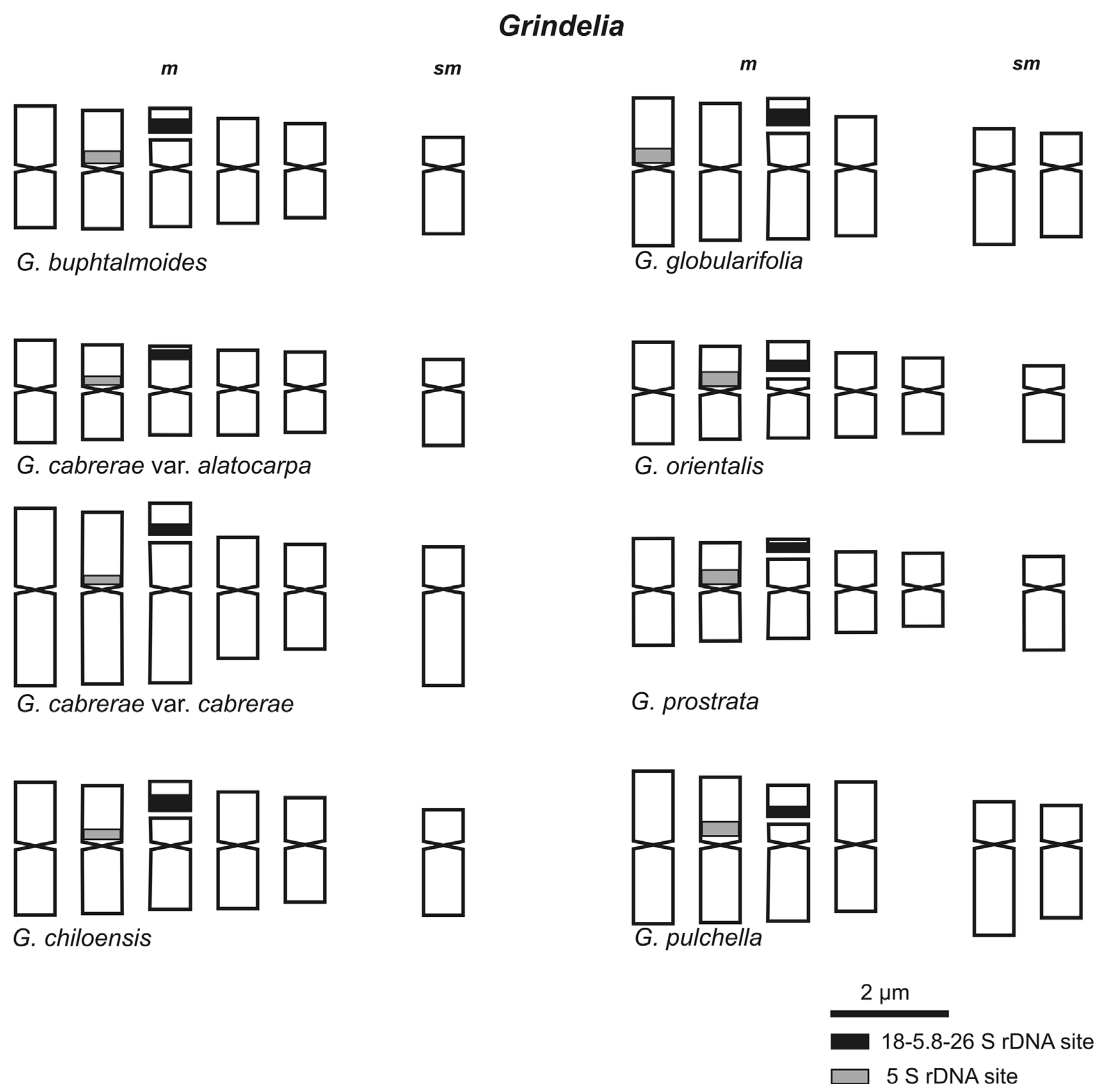

Figure 2. Idiograms of *Grindelia* species based on mean chromosome values. Bar represents 2 µm. All at the same scale.

or six sites according to the ploidy level of the taxon, and were located in the pericentromeric region of a short arm of an m pair. In all the species studied, both ribosomal genes were always asynthenic.

Discussion

In Astereae, a range from 4 to ca. 432 chromosomal numbers occurs (Goldblatt & Johnson 2015; Watanabe 2015). Semple and Watanabe (2009) proposed that the base number $x = 9$ is ancestral for the tribe. In particular, *Grindelia* has the base number $x = 6$ (e.g. Grau 1976; Brown & Clark 1981; Dunford 1986, Baeza & Schrader 2005; Moreno et al. 2012), which originated via downward dysploidy during the tribe diversification, as suggested for Cichorieae (*Hypochaeris*, Weiss et al. 2003; *Crepis*, Enke & Gemeinholzer 2008) and Gnaphalieae (*Leontopodium*, Russell et al. 2013).

We report the chromosome numbers of *G. cabrerae* var. *alatocarpa*, *G. globularifolia*, and *G. prostrata* for the first time, whereas counts for the remaining taxa had been confirmed in previous reports in individuals from other populations (Table I). We also found supernumerary chromosomes in *G. cabrerae* var. *cabrerae* for the first time, as had been previously reported for *G. procera* (Moreno et al. 2012) and *G. pulchella* (Hunziker et al. 1990).

Most South American diploid *Grindelia* showed 4 m + 2 sm or 6 m (Bartoli & Tortosa 1998a; Baeza & Schrader 2005; this work). Moreover, we found 5 m + 1 sm in several species (*G. buphthalmoides*, *G. cabrerae* var. *cabrerae* and var. *alatocarpa*, *G. chiloensis*, and *G. orientalis*, Figure 4), as formerly detected in several North American species (Moreno et al. 2012).

In most angiosperms, GC-rich heterochromatin is more frequent in the paracentromeric

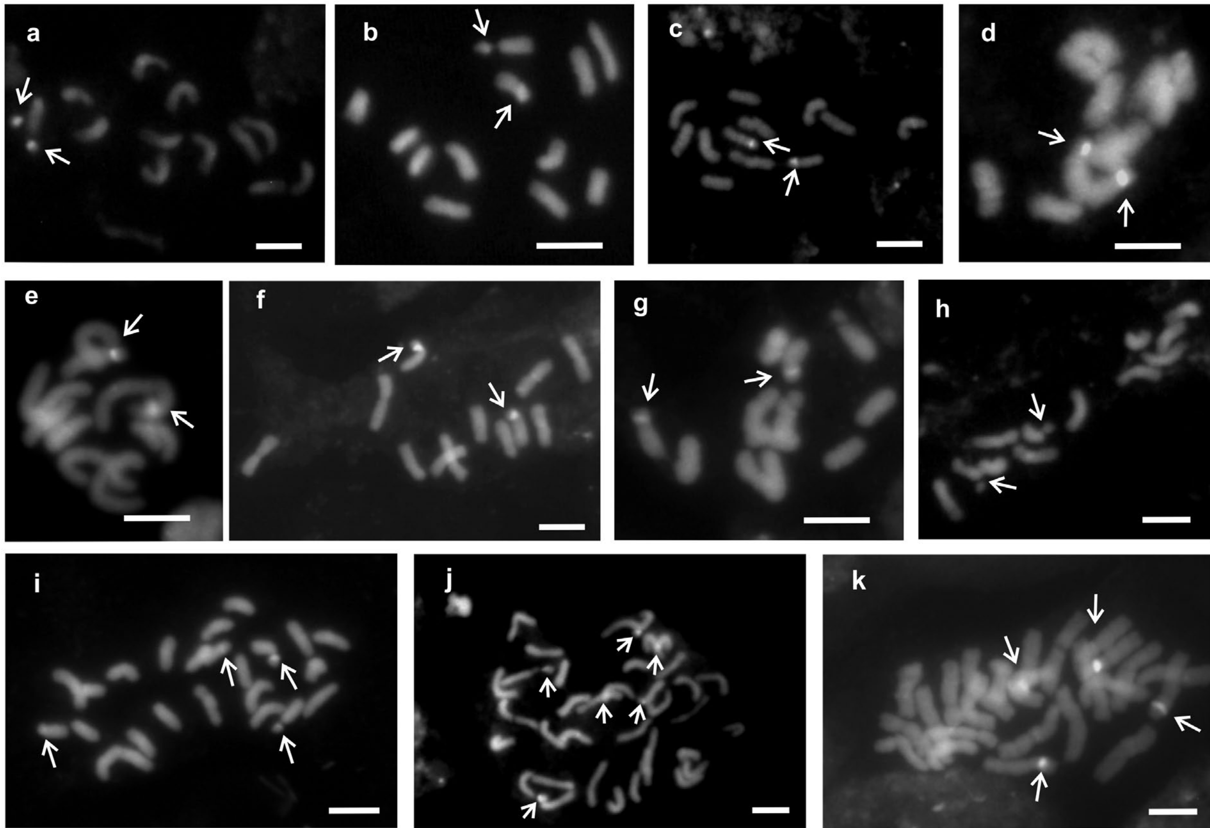

Figure 3. Fluorescent chromosome banding in *Grindelia* species. (a) *G. buphthalmoides.* (b) *G. cabrerae* var. *alatocarpa.* (c) *G. cabrerae* var. *cabrerae.* (d) *G. chiloensis.* (e) *G. globularifolia.* (f) *G. orientalis.* (g) *G. prostrata.* (h) *G. pulchella* var. *pulchella.* (i) *G. pulchella* var. *discoidea.* (j) *G. ventanensis.* (k) *G. anethifolia* x *G. chiloensis.* Arrows show $CMA^+/DAPI^-$ NOR-associated bands. Bar represents 5 μm.

and/or terminal regions, especially associated with rDNA (Guerra 2000), as recorded in all the species studied. Different genera conserved the heterochromatin pattern (Galasso et al. 1997; Moscone et al. 1996; Weiss-Schneeweiss et al. 2003), as occurs in *Grindelia* with only CMA^+ bands associated with NORs (Moreno et al. 2012; this work). Related genera in the Astereae had comparatively more CG-rich heterochromatin with different localizations, conserved in the different species as well (Moreno et al. 2012).

The location of 18-5.8-26S rDNA sites did not vary randomly, occurring preferentially on the short arms and in terminal chromosome regions in angiosperms (Roa & Guerra 2012); *Grindelia* was no exception to this trend (Baeza & Schrader 2005; Moreno et al. 2012). Thus, North and South American *Grindelia* species exhibited the same 18-5.8-26S gene position in the third m pair (Moreno et al. 2012). This chromosome pair is homeologous among *Grindelia* and *Isocoma* species (Baeza & Schrader 2005; Moreno et al. 2012). In the *Grindelia* species examined so far, there is only one 5S locus per basic genome (Baeza & Schrader 2005; Moreno et al. 2012; this work), whereas in the related genus *Haplopappus* there are two loci (Baeza & Schrader 2005). In addition, a pericentromeric 5S rDNA location is frequent in species of *Grindelia* and the "Xanthocephalum group" (Moreno et al. 2012), but not in *Haplopappus* (Baeza & Schrader 2005). In *Grindelia*, both ribosomal genes indicate the ploidy level.

Different ploidy levels, either intraspecific or interspecific, are typical of *Grindelia* and they appear to have an important role in its evolution (Bartoli & Tortosa 1998a; Baeza & Schrader 2005; Moreno et al. 2012; this work). There are diploid species throughout its range, in addition to hexaploid species and tetra- and hexaploid cytotypes in South America and tetraploid species and cytotypes in North America (e.g. Dunford 1986; Bartoli & Tortosa 1998a, 1998b; Moreno et al. 2012). Thus, the wide distribution range of polyploids in *Grindelia* suggests they increased their dispersal capability or ecological tolerance, a phenomenon reported in other genera (e.g. *Draba*, Brochmann et al. 2004; *Polystachya*, Russell et al. 2010; *Leontopodium*, Russell et al. 2013).

Genome downsizing after polyploidy is a general response in angiosperms (e.g. Leitch & Bennett 2004; Tayalé & Parisod 2013). In South American *Grindelia*, the genomic length (per basic genome)

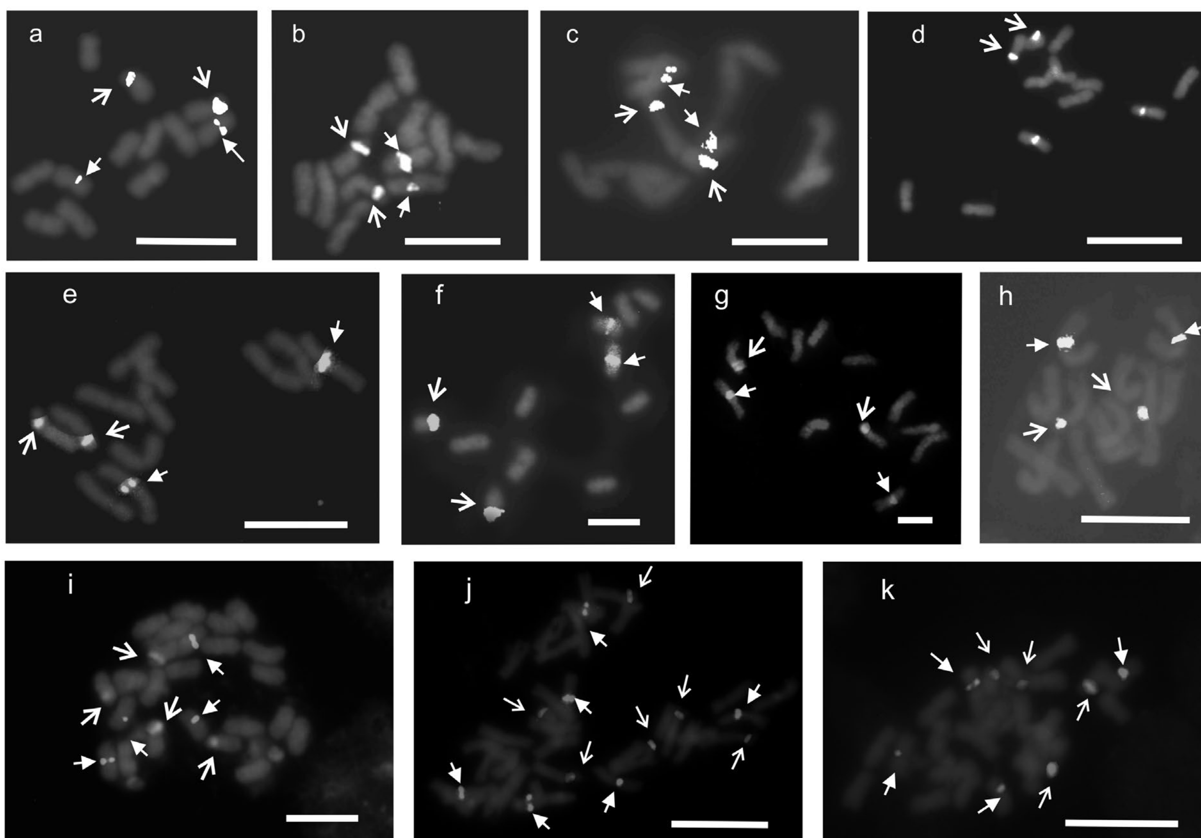

Figure 4. Somatic chromosomes detected by FISH using 18–5.8–26S and 5S rDNA probes in *Grindelia* species. (a) *Grindelia buphthalmoides.* (b) *G. cabrerae* var. *alatocarpa*. (c) *G. cabrerae* var. *cabrerae*. (d) *G. chiloensis.* (e) *G. globularifolia.* (f) *G. orientalis*. (g) *G. prostrata*. (h) *G. pulchella* var. *pulchella*. (i) *G. pulchella* var. *discoidea*. (j) *G. ventanensis*. (k) *G. anethifolia* x *G. chiloensis.* Arrows show 18-5.8-26S rDNA sites and bold arrows show 5S rDNA hybridization sites. Bar represents 5 μm.

almost always decreased with higher ploidy levels, whereas this fact was not observed in North American taxa (Moreno et al. 2012).

Natural hybrids are rare in *Grindelia*, suggesting that isolating mechanisms are external (e.g. geographic range). On the other hand, hybrids in the "Xanthocephalum group" were artificially synthesized between closely related genera (Nesom 1994 and papers cited therein). Several authors (Bartoli & Tortosa 1998b; this work) reported populations of the natural hybrid *G. anethifolia* × *G. chiloensis* particularly in South America, suggesting that their genomes are not differentiated and they do not have any geographic barriers.

Finally, all *Grindelia* studied displayed symmetrical karyotypes with two karyotype formulae: 5 m + 1 sm for the North and South American taxa, and 4 m + 2 sm exclusively for the South American taxa (Bartoli & Tortosa 1998a; Moreno et al. 2012; this work). In addition, the shared homeology of the 18-5.8-26S gene supports a North American origin of the genus with subsequent migration to South America, as suggested by Moore et al. (2012). Chromosome variation, although not always large, accompanied the evolutionary divergence of the taxa studied. Our cytological data recognized two species groups among the taxa studied: one) *G. globularifolia* and *G. pulchella* var. *pulchella,* and two) the six remaining species. However, these groups do not show morphological affinities and their phylogenetic relationships are not clearly resolved (Moore et al. 2012), which indicates that these taxa have recently diverged.

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References

Baeza C, Schrader O. 2005. Comparative karyotype analysis in *Haplopappus* Cass. and *Grindelia* Willd. (Asteraceae) by double FISH with rRNA specific genes. Plant Syst Evol 251: 161–172.

Bartoli A, Roitman GG, Frayssinet N. 1990. Estudio comparativo de poblaciones diploides y tetraploides de *Grindelia chiloensis* (Asteraceae) [Comparative study of diploid and tetraploid populations of Grindelia chiloensis (Asteraceae)]. Darwiniana 30: 171–177.

Bartoli A, Roitman GG, Frayssinet N. 1993. Presencia de hexaploides (2*n* = 36) en *Grindelia chiloensis* (Asteraceae). Bol Soc Argent Bot 29: 139–142.
Bartoli A, Tortosa RD. 1994. Tres nuevas especies de *Grindelia* (Asteraceae), afines a *G. chiloensis* (Corn.) Cabr [Three new species of Grindelia (Asteraceae), akin to G. chiloensis (Corn.) Cabr]. Kurtziana 23: 131–150.
Bartoli A, Tortosa RD. 1998a. Estudios cromosómicos en especies sudamericanas de *Grindelia* (Astereae, Asteraceae) [Chromosomic studies in South American species of Grindelia (Astereae, Asteraceae)]. Kurtziana 26: 165–171.
Bartoli A, Tortosa RD. 1998b. Intermediacy between *Grindelia chiloensis* and *Haplopappus pectinatus* (Asteraceae). Taxon 47: 337–345.
Bartoli A, Tortosa RD. 2012. Revision of the North American species of Grindelia (Asteraceae) 1 Ann Missouri Bot Gard 98: 447–513.
Bartoli A, Tortosa RD. 2014. Grindelia. In: Anton AM, Zuloaga F, editors. Flora Argentina Vol 7 Tomo 1. San Isidro: Instituto de Botánica Darwinion. pp. 163–181.
Brown GK, Clark WD. 1981. Chromosome numbers in South American Haplopappus Cass. (Compositae). Amer J Bot 68: 1218–1221.
Brochmann C, Brysting AK, Alsos IG, Borgen L, Grundt HH, Scheen AC, et al. 2004. Polyploidy in arctic plants. Biol J Linn Soc 82: 521–536.
Chacon J, Sousa A, Baeza CM, Renner SS. 2012. Ribosomal DNA distribution and a genus-wide phylogeny reveal patterns of chromosomal evolution in *Alstroemeria* (Alstroemeriaceae). Amer J Bot 99: 1501–1512.
Covas G, Schnack B. 1946. Número de cromosomas en Antófitas de la región de Cuyo (República Argentina). Revista Argent Agron 13: 153–166.
Di Fulvio TE. 1977. Recuentos cromosómicos en Angiospermas Argentinas III [Chromosomic counts in Argentinian Angiosperms III]. Kurtziana 10: 249.
Dunford MP. 1986. Chromosome relationships of diploid species of Grindelia (Compositae) from Colorado, New Mexico, and adjacent areas Amer J Bot 73: 297–303.
Enke N, Gemeinholzer B. 2008. Babcock revisited: new insights into generic delimitation and character evolution in *Crepis* L. (Compositae: Cichorieae) from ITS and matK sequence data. Taxon 57: 756–768.
Funk VA, Susanna A, Stuessy TF, Robinson H. 2009. Systematics, evolution, and biogeography of compositae. Vienna: International Association for Plant Taxonomy.
Galasso I, Sublimi Saponetti L, Pignone D. 1997. Cytotaxonomic studies in *Vigna*: 3. Chromosomal distribution and reacting properties of the heterochromatin in five wild species of the section *Vigna*. Caryologia 49: 311–319.
Gerlach WL, Bedbrook JL. 1979. Cloning and characterization of ribosomal RNA genes from wheat and barley. Nucl Acids Res 7: 1869–1885.
Goldblatt P, Johnson DE. 2015. Index to plant chromosome numbers. St. Louis: Missouri Botanical Garden.
Grau J. 1976. Chromosomenzahlen von sussamerikanischen Haplopappus Arten. Mitteil Bot Staats München 12: 403–410.
Guerra M. 2000. Patterns of heterochromatin distribution in plant chromosomes. Genet Molec Biol 23: 1029–1041.
Hasterok R, Wolny E, Hosiawa M, Kowalczyk M, Kulak-Ksiazczyk S, Ksiazczyk T. 2006. Comparative analysis of rDNA distribution in chromosomes of various species of Brassicaceae. Ann Bot 97: 205–216.
Hunziker JH, Escobar A, Xifreda CC, Gamerro JC. 1990. Estudios cariológicos en Compositae VI [Karyologic studies in Compositae VI]. Darwiniana 30: 115–121.
Hunziker JH, Wulff A, Xifreda CC, Escobar A. 1989. Estudios cariológicos en Compositae V [Karyologic studies in Compositae V]. Darwiniana 29: 25–39.
Jones AG. 1985. Chromosomal features as generic criteria in the Astereae. Taxon 34: 44–54.
Jong J. 1997. Laboratory manual of plant cytological. Edinburgh: Royal Botanical Garden.
Lane MA, Li J. 1993. Documented chromosome numbers 1993: 1. Chromosome number reports in Compositae with emphasis on Tribe Astereae of the Southwestern United States and Mexico. SIDA 15: 539–546.
Leitch IJ, Bennett MD. 2004. Genome downsizing in polyploid plants. Biol J Linn Soc 82: 651–663.
Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.
Moore AJ, Bartoli A, Tortosa RD, Baldwin BG. 2012. Phylogeny, biogeography, and chromosome evolution of the amphitropical genus *Grindelia* (Asteraceae) inferred from nuclear ribosomal and chloroplast sequence data. Taxon 61: 211–230.
Moreno NC, Stiefkens L, Las Peñas ML, Bartoli A, Tortosa R, Bernardello G. 2012. Molecular cytogenetic studies of the "Xanthocephalum group" (Asteraceae). Plant Syst Evol 298: 1503–1514.
Morgan DR. 2003. nrDNA external transcribed spacer (ETS) sequence data, reticulate evolution, and the systematics of *Machaeranthera* (Asteraceae). Syst Bot 28: 179–190.
Morgan DR, Korn RL, Mugleston SL. 2009. Insights into reticulate evolution in Machaerantherinae (Asteraceae: Astereae). 5S ribosomal RNA spacer variation, estimating support for incongruence, and constructing reticulate phylogenies. Amer J Bot 96: 920–932.
Moscone EA, Lambrou M, Ehrendorfer F. 1996. Fluorescent chromosome banding in the cultivated species of *Capsicum* (Solanaceae). Plant Syst Evol 202: 37–63.
Nesom GL. 1994. Subtribal classification of the Astereae (Asteraceae). Phytologia 76: 193–274.
Nesom GL. 2000. New subtribes for North American Astereae (Asteraceae). SIDA 19: 263–268.
Peruzzi L, Eroglu H. 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp Cytogenet 7: 1–9.
Rahn K. 1960. Chromosome numbers in some South American angiosperms. Bot Tidssk 56: 117–127.
Raskina O, Barber JC, Nevo E, Belyayev A. 2008. Repetitive DNA and chromosomal rearrangements: speciation-related events in plant genomes. Cytog Genome Res 120: 351–357.
Roa F, Guerra M. 2012. Distribution of 45S rDNA sites in chromosomes of plants: Structural and evolutionary implications. BMC Evol Biol 12: 225.
Russell A, Safer S, Weiss-Schneeweiss H, Temsch E, Stuppner H, Stuessy TF, et al. 2013. Chromosome counts and genome size of *Leontopodium* species (Asteraceae: Gnaphalieae) from South-western China. Bot J Linn Soc 171: 627–636.
Russell A, Samuel R, Klejna V, Barfuss MH, Rupp B, Chase MW. 2010. Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) as shown by plastid DNA sequences and low-copy nuclear genes. Ann Bot 106: 37–56.
Schwarzacher T, Ambros P, Schweizer D. 1980. Application of Giemsa banding to orchid karyotype analysis. Plant Syst Evol 134: 293–297.
Schwarzacher T, Heslop-Harrison P. 2000. Practical *in situ* hybridization. Oxford: Bios Scientific Publishers Limited.
Schweizer D. 1976. Reverse fluorescent chromosome banding with chromomycin and DAPI. Chromosoma 58: 307–324.
Schweizer D, Ambros P. 1994. Chromosome banding. In: Gosden JR, editor. Methods in molecular biology. Totowa: Humana Press. pp. 97–112.

Semple JC, Chmielewski JG, Lane M. 1989. Chromosome number determinations in fam. Compositae, tribe Astereae. III. Additional counts and comments on generic limits and ancestral base numbers Rhodora. 91: 296–314.

Semple JC, Chmielewski JG, Xiang C. 1992. Chromosome number determinations in fam. Compositae, tribe Astereae. IV. Additional reports and comments on the cytogeography and status of some species of *Aster* and *Solidago*. Rhodora 94: 48–62.

Semple JC, Watanabe K. 2009. A review of chromosome numbers in Asteraceae with hypotheses on chromosomal base number evolution. In: Funk VA, Susanna A, Stuessy TF, Bayer RJ, editors. Systematics, evolution, and biography of Compositae. Vienna: International Association for Plant Taxonomy. pp. 61–72.

Schnack B, Covas G. 1947. Estudios cariológicos en Antófitas [Karyologic studies in Anthophytes]. Haumania 1: 32–41.

Solbrig OT, Anderson LC, Kyhos DW, Raven PH, Rudenberg L. 1964. Chromosome numbers in compositae V. Astereae II. Am J Bot 51: 513–519.

Solbrig OT, Anderson LC, Kyhos DW, Raven PH. 1969. Chromosome numbers in compositae VII: Astereae III Am J Bot 56: 348–353.

Tayalé A, Parisod C. 2013. Natural pathways to polyploidy in plants and consequences for genome reorganization Cytog Genome Res 140: 79–96.

Torrell M, Cerbah M, Siljak-Yakovlev S, Valles J. 2003. Molecular cytogenetics of the genus *Artemisia* (Asteraceae, Anthemideae): fluorochrome banding and fluorescence *in situ* hybridization.I. Subgenus *Seriphidium* and related taxa. Plant Syst Evol 239: 141–153.

Turner BL, Bacon J, Urbatsch L, Simpson B. 1979. Chromosome numbers in South American Compositae Am J Bot 66: 173–178.

Watanabe K. 2015. Index to chromosome numbers in Asteraceae. Kobe: Kobe University.

Weiss H, Stuessy TF, Grau J, Baeza CM. 2003. Chromosome reports from South American *Hypochaeris* (Asteraceae) Ann Missouri Bot Gard 90: 56–63.

Weiss-Schneeweiss H, Stuessy TF, Siljak-Yakovlev S, Baeza CM, Parker J. 2003. Karyotype evolution in South American species of *Hypochaeris* (Asteraceae, Lactuceae). Plant Syst Evol 241: 171–184.

Weiss-Schneeweiss H, Schneeweiss GM. 2013. Karyotype diversity and evolutionary trends in angiosperms. In: Greilhuber J, Dolezel J, Wendel JF, editors. Plant Genome Diversity Vol 2. Vienna: Springer. pp. 209–230.

Whitaker T, Steyermark J. 1935. Cytological aspects of *Grindelia* Species Bull Torrey Bot Club 62: 69–73.

Wulff AF, Hunziker JH, Escobar A. 1996. Estudios cariológicos en Compositae. VII. Darwiniana 34: 213–231.