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ORIGINAL ARTICLE

Molecular cytogenetic studies of the "*Xanthocephalum* group" (Asteraceae)

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Abstract Fourteen North American members of the "*Xanthocephalum* group" were studied by classical and molecular cytogenetics. Location and number of rDNA sites were determined by FISH. For the 5S rDNA, a probe was obtained from *Prionopsis ciliata*. Most species were diploid ($2n = 12$), although *Isocoma menziesii*, *Grindelia hirsutula, G. robusta*, both varieties of *G. stricta*, and one population of *G. camporum* were tetraploid ($2n = 24$). Diploid *Grindelia* and *Prionopsis ciliata* were $5m + 1sm$, tetraploids $10m + 2sm$, except *G. hirsutula* ($8m + 4sm$), and *Isocoma* and *Olivaea* $6m + 2sm$ and $3m + 3sm$, respectively. Most species had satellites on the short arms of *m* pairs: two in tetraploids and *P. ciliata* and one in diploids. Satellites were associated with two $CMA^+/DAPI^-$ bands in diploid species and four bands in tetraploids and in *P. ciliata*. rDNA loci (two in diploids to four in tetraploids) may be indicative of ploidy level. *Grindelia* tetraploids could have originated recently by autopolyploidy. Chromosome duplication was followed by modifications in the genome structure, resulting in higher heterochromatin amounts not associated with NORs. There is only one 5S site per basic genome in para or pericentromeric regions. Although not always large, chromosome variation has accompanied the evolutionary divergence of the taxa studied.

Keywords 18-5.8-26S and 5S rDNA · Asteraceae · Fluorescent banding · Karyotypes · North America · "*Xanthocephalum* group"

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Introduction

Chromosome banding and fluorescence in-situ hybridization (FISH) are valuable techniques for studies on genome organization and evolution in plants (Sumner 1990; Jiang and Gill 1994, 2006). CMA/DAPI banding has been widely used to characterize heterochromatin bands on the basis of their highly repeated DNA composition. The distribution pattern of constitutive heterochromatin in chromosomes with fluorescent banding can be used to detect chromosomal rearrangements that act in genome organization (Guerra 2000; Raskina et al. 2008). These banding patterns are also useful markers for identifying homologous chromosome pairs.

The physical mapping of tandem repeat genes (rDNA), and other genomic landmarks, with FISH resulted in useful markers to identify chromosome and fine karyotype comparisons for cytoevolution and cytotaxonomy (Cheng et al. 2001; Kato et al. 2004). Use of these data in studies of systematics and evolution contributes to evaluation of relationships among species and populations, and to better understanding of their divergence (Greilhuber and Ehrendorfer 1988; Jiang and Gill 1994).

Asteraceae comprise the largest family of vascular plants with ca. 23,000 species. The "*Xanthocephalum*

group" is included in the subtribe Machaerantherinae Nesom of tribe Astereae (Nesom 1991, 1994). This subtribe is mainly North American, the exceptions being *Grindelia* (with a disjunct distribution in North and South America) and the exclusively South American *Haploppapus* (Lane and Hartman 1996; Nesom 2000; Moore et al. 2012). It was divided by Nesom (1991) into five groups. Among these, the "*Xanthocephalum* group" was characterized by yellow ray flowers (if present), disk flowers with the corolla abruptly ampliated, deltate to ovate-elliptic style appendages, and basic chromosome number $x = 6$ (Nesom 1994). In this group, Nesom (1994) included the genera *Grindelia* Willd. (70 spp.), *Isocoma* Nutt. (16 spp.), *Olivaea* Sch. Bip. ex Benth. (2 spp.), *Stephanodoria* Greene (monotypic), and *Xanthocephalum* Willd. (5 spp.). Later, Lane and Hartman (1996) described *Rayjacksonia* (three North American spp.), which, on the basis of chloroplast DNA restriction site data, was included it in the "*Xanthocephalum* group". They also suggested the inclusion of *Stephanodoria* within the genus *Xanthocephalum* and resurrected *Prionopsis* Nutt. (monotypic), that had been included in *Grindelia* by Nesom et al. (1993).

Monophyly of the "*Xanthocephalum* group" is currently well supported by cpDNA restriction data and combined ITS and ETS sequence data (Morgan and Simpson 1992; Morgan 1997; Lane and Hartman 1996; Lane et al. 1996; Morgan 2003; Moore et al. 2012). Furthermore, the monophyletic condition of some genera included in the "*Xanthocephalum* group" is uncertain. For instance, in the restriction site cpDNA analysis performed by Lane et al. (1996), *Grindelia, Xanthocephalum, Isocoma*, and *Rayjacksonia* were either paraphyletic or polyphyletic (cf. their Fig. 9). Nevertheless, on the basis of the same cpDNA data plus morphology, Lane and Hartman (1996) argued that the different genera placed in the group should be maintained as such. Nesom (1997) pointed out that the monophyly of the genera could not be assessed with high certainty because Lane and Hartman (1996) sampled only one species per genus.

Conventional cytological research has provided important insight in the systematics and evolution of tribe Astereae (Jones 1985; Nesom 1994; Torrell et al. 2003). There are approximately 40 chromosomically counted species of *Grindelia* (Whitaker and Steyermark 1935; Semple et al. 1992; Lane and Li 1993; Bartoli and Tortosa 1998; Baeza and Schrader 2005), 50 species in *Haplopappus* (Grau 1976; Semple et al. 1989; Baeza and Schrader 2005), 10 species in *Isocoma* (Lane and Li 1993), the three *Rayjacksonia* species, and the monotypic *Stephanodoria* and *Prionopsis* (Semple 1980; Jackson and Dimas 1981; Lane and Li 1993). These data indicate that the base number for the "*Xanthocephalum* group" is $x = 6$, with different ploidy levels: $2n$, $3n$, and $4n$ (Dunford 1986; Semple et al. 1989). On the other hand, no karyotypic data are available for all these taxa except for nine *Grindelia* and two *Haplopappus* species from South American (Bartoli and Tortosa 1998; Baeza and Schrader 2005).

With this background, North American members of the "*Xanthocephalum* group" (seven *Grindelia* and three *Isocoma* taxa, *Olivaea tricuspis*, *Prionopsis ciliata* and *Stephanodoria tomentella*) have been studied to determine their chromosome numbers, karyotypes, heterochromatin distribution, and 18-5.8-26S and 5S rDNA loci, to elucidate their importance in its systematics and evolution.

Materials and methods

Seeds were bulked from plants in natural populations. Details of the studied material and voucher specimens are included in Table 1.

For conventional staining, mitotic chromosomes in somatic cells of root-tips were analyzed from squashes of primary roots from germinated seeds. Seeds were soaked in tap water for 24 h and placed in Petri dishes lined with filter paper moistened with gibberellic acid (GA_3, 1,000 ppm); seeds were regularly watered with the same solution. Petri dishes were kept in the dark in a warming oven at 30 °C. Root tips were cut when the primary roots were 2–10 mm long and were pretreated in 8-hydroxyquinoline for 24 h at 4 °C. Root-tips were rinsed in distilled water and fixed in freshly prepared ethanol–glacial acetic acid (3:1) for 24 h at room temperature. After fixation, root tips were hydrolyzed with 5 M HCl for 40 min at room temperature and placed in Feulgen solution for 2 h at room temperature in the dark (Jong 1997). Root-tip meristem cells were isolated on a slide and squashed. Slides were made permanent in Euparal after removing the cover slips with liquid nitrogen-induced freezing.

Cells selected for measurements were photographed with phase contrast optics on a Zeiss Axiophot microscope equipped with a Leica DFC300FX camera. Ten cells, each from a different individual, were photographed, and the lengths of the short arm (s) and long arm (l), and total chromosome length (c), were measured for each chromosome pair. The centromeric index ($I = 100s/c$) and the arm ratio ($r = l/s$) were calculated and used to classify the chromosomes and determine homologues (in accordance with Levan et al. 1964), and satellites were classified in accordance with Battaglia (1955). Karyograms were constructed by organizing the chromosomes into groups according to their arm ratio (from *m* to *sm*), ordering them by decreasing length within each category. The resulting idiograms were based on the mean values obtained for measurements of all individuals for each species. Karyotype asymmetry was estimated by use of the indices of Romero Zarco (1986).

Table 1 Collection data and chromosome numbers of the members of the "*Xanthocephalum* group" studied, indicating previous reports when available

Species	Voucher information	2*n*	Previous reports
Grindelia camporum	USA, California, Los Angeles Co., RSABG seed collection 21950 (RSA)	12	Whitaker and Steyermark (1935); Semple et al. (1992)
	USA, California, Santa Clara Co., RSABG seed collection 18107 (RSA)	12	
	USA, California, Contra Costa Co., Moore 862 (JEPS)	12	
	USA, California, Tehama Co., Moore, Silveira and Anderson 551 (JEPS)	24	
G. hirsutula	USA, California, Santa Clara Co., RSABG seed collection 16825 (RSA)	24	Whitaker and Steyermark (1935)
	USA, California, Marin Co., Moore 818 (JEPS)	24	
	USA, California, Contra Costa Co., Moore 861 (JEPS)	24	
G. robusta	USA, California, Santa Barbara Co., RSABG seed collection 15596 (RSA)	24*	
G. procera	USA, California, Tulare Co., Moore 947-3 (JEPS)	12	Dunford (1986)
	USA, California, Kern Co., Moore 952-5 (JEPS)	12	
G. squarrosa	USA, Index Seminum Praga botanical Garden LD 799 (BAA)	12	Freeman and Brooks (1988)
G. stricta var. *angustifolia*	USA, California, Alameda Co., Moore and Park 870 (JEPS)	24	Lane and Li (1993)
G. stricta var. *platyphylla*	USA, California, Santa Barbara Co., RSABG seed collection 18555 (RSA)	24	Lane and Li (1993)
	USA, California, San Mateo Co., Moore 863 (JEPS)	24	
Isocoma menziesii var. *menziessi*	USA, California, San Luis Obispo Co., Moore 946 (JEPS)	24	Lane and Li (1993)
	Mexico, Baja California, RSABG seed collection 22108 (RSA)	24	
	Mexico, Baja California, RSABG seed collection 19212 (RSA)	12	
I. menziesii var. *vernonioides*	USA, California, Los Angeles Co., RSABG seed collection 21951 (RSA)	12	
I. menziesii var. *sedoides*	USA, California, Santa Barbara Co., RSABG seed collection 15601 (RSA)	12	Lane and Li (1993)
Olivaea tricuspis	Mexico, Aguascalientes, El Llano, Martínez R. 1281 (MEXU)	12	
Prionopsis ciliata	USA, Kansas, Wabausse Co. (BAA 26854)	12	Semple et al. (1992)
Stephanodoria tomentella	México, San Luis de Potosí, Calzada J. 25377 (MEXU)	12	Semple et al. (1989)

An *asterisk* means a new number for the taxon

To make chromosome preparations for fluorescent banding and FISH, root tips were washed twice in distilled water (10 min each), digested with a 2 % cellulase (Sigma-Aldrich, Vienna, Austria) and 20 % pectinase (from *Aspergillus niger*; Sigma–Aldrich) solution (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.

Slides for CMA/DAPI 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 and subsequently stained with 2 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) (both Sigma–Aldrich) for 30 min, and finally mounted in McIlvaine's buffer–glycerol 1:1 (*v*/*v*) (Schweizer 1976; Schweizer and Ambros 1994). The amount of heterochromatin was expressed as a percentage of the total length of the haploid karyotype.

The location and number of rDNA sites were determined by FISH using as probe the p*Ta*71 containing the 18-5.8-26S rDNA (Gerlach and 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* by PCR, by using the primers UP46 (5′-GTGCGATCATACCAGC(A/G)(G/T)TAATG CACCGG-3′) and UP47 (5′-GAGGTGCAACACGAGGA CTTCCCAGGAGG-3′) (Gottlob-McHugh et al. 1990) labeled with digoxigenin-11-dUTP (Roche Diagnostics). The FISH procedure was in accordance with Schwarzacher and Heslop-Harrison (2000), with minor modifications. The 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 µl hybridization mixture was added (3 ng/µl of probe, 100 % formamide, 50 % dextran sulfate, 20× SSC, and 10 % SDS), previously denatured at 70 °C for 10 min. Chromosome denaturation/hybridization was conducted 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), and slides were placed in a humid chamber at 37 °C overnight. The probes were detected with avidin-FITC conjugate and antidigoxigenin-rodamine conjugate and counterstained and mounted with 25 µl antifade (Vectashield Vector Laboratories, Burlingame, USA), containing 2 ng/µl DAPI.

Results

Representative metaphases of each species are presented in Fig. 1. Results obtained for the karyotypes analysed are listed in Table 2. Both diploids and tetraploids were found. For *Grindelia camporum*, *G. procera*, *G. squarrosa*, *Isocoma menziessi* var. *vernonioides* and var. *sedoides*, *Olivaea tricuspis*, *Prionopsis ciliata*, and *Sthephanodoria tomentella* $2n = 2x = 12$, whereas for *I. menziesii* var. *menziesii*, *G. hirsutula, G. robusta*, both varieties of *G. stricta*, and one population of *G. camporum* $2n = 4x = 24$. Some individuals of *G. procera* had 1 or 2 accessory chromosomes, and some individuals of *G. robusta* were aneuploid with $2n = 25$.

Karyotypes were exclusively composed of *m* and *sm* chromosomes (Table 2; Fig. 2). Diploid *Grindelia* had the formula 5 $m + 1$ *sm*; on the other hand, tetraploids duplicated it: 10 $m + 2$ *sm* (Table 2; Fig. 2) with the exception of *G. hirsutula* with 8 $m + 4$ *sm*. *Prionopsis ciliata* had a comparable formula with diploid *Grindelia* species, whereas *Isocoma* and *Olivaea* species studied had distinctive formulas: 6 $m + 2$ *sm* and 3 $m + 3$ *sm*, respectively (Table 2; Fig. 2).

There are variations in total genomic length among the diploid species, from 14.31 µm in *G. squarrosa* to 26.10 µm in *I. menziessi* var. *sedoides*. *Olivaea* and *Prionopsis* had closer values to *G. squarrosa* whereas *G. camporum* and *G. procera* had intermediate values (Table 2). Tetraploid *Grindelia* species had 2.8 to 3.2-fold amounts (Table 2).

Most species had satellites on the short arms of *m* pairs: two satellited pairs in the polyploids *G. robusta* and both varieties of *G. stricta* and one pair in the diploids *G. camporum*, *G. procera*, *G. squarrosa, I. menziessi* var. *sedoides*, and *O. tricuspis* (Fig. 2). Two taxa were outstanding: *Prionopsis* because, although diploid, it had two satellited pairs (Fig. 2) and *G. hirsutula* with no satellites detected with this technique.

Fig. 1 Mitotic metaphases of "*Xanthocephalum* group" species with Feulgen staining. **a** *Grindelia camporum* ($2n = 12$). **b** *G. squarrosa* ($2n = 12$). **c** *Isocoma menziessi* var. *sedoides* ($2n = 12$). **d** *Olivaea tricupis* ($2n = 12$). **e** *Prionopsis ciliata* ($2n = 12$). **f** *Stephanodoria tomentella* ($2n = 12$). **g** *G. hirsutula* ($2n = 24$). **h** *G. robusta* ($2n = 24$). **i** *G. stricta* var. *angustifolia* ($2n = 24$). **j** *G. stricta* var. *platyphylla* ($2n = 24$). *Arrows* point to satellites. *Bars* represent 5 µm

CG-rich heterochromatin was always detected. In all cases, satellites were associated with two $CMA^+/DAPI^-$ bands in diploid species and four bands in all tetraploids and *P. ciliata* (Fig. 3). Specifically, there were additional bands characterizing some species. In *I. menziessi* var. *sedoides*, the satellited chromosome pair was heteromorphic, with one $CMA^+/DAPI^-$ interstitial band in a long arm (Figs. 2, 3). *G. stricta* var. *platyphylla* had in pair #1 two terminal bands in the short arms, whereas pair #2 had four interstitial bands (two in short arms and two in long arms) and pair #10 had two terminal bands in long arms (Figs. 2, 3). In *P. ciliata*, pairs #1, 2, 4, and 6 had pericentromeric bands (Figs. 2, 3). Additionally, prometaphases of the tetraploid *I. menziessi* had terminal bands in two pairs in both arms and three pairs in short arms (Fig. 3).

The numbers of 18-5.8-26S rDNA sites were coincident with the number of satellites: two in diploids and four in tetraploids and *P. ciliata* (Figs. 2, 4). These were associated with $CMA^+/DAPI^-$ bands in all cases.

For the 5S rDNA probe an amplicon of approximately 500 bp was obtained from genomic DNA of *P. ciliata* using specific primers (not shown). For the species studied here there were two or four 5S rDNA sites according to ploidy level, always attached to short arms. In most species, these loci were located in *m* chromosomes, except for *O. tricuspis*. Both ribosomal genes were always asynthenic.

All the species studied had symmetrical karyotypes according to the indices calculated, these had slight size variations among chromosomes (Table 2). *I. menziessi* var. *sedoides* and *Olivaea tricuspis* had the most asymmetrical karyotypes in respect of length differences among the chromosome arms (A_1), but the remaining taxa had few differences among them (Table 2).

Discussion

Several genera of the Cosmopolitan tribe Astereae have been chromosomically examined mainly as counts (e.g. *Aster*, Huziwara 1967; Mandákova and Münzbergová 2006; *Brachyscome*, Watanabe et al. 1999; *Conyza*, Urdampilleta et al. 2005; *Hieracium*, Coşkuncelebi and Hayirlioğlu-Ayaz 2006). Worldwide, most genera have the basic number $x = 9$ (Solbrig 1964; Nesom 1978; Jones 1985; Torrell et al. 2003; Semple 2008). However, taxa

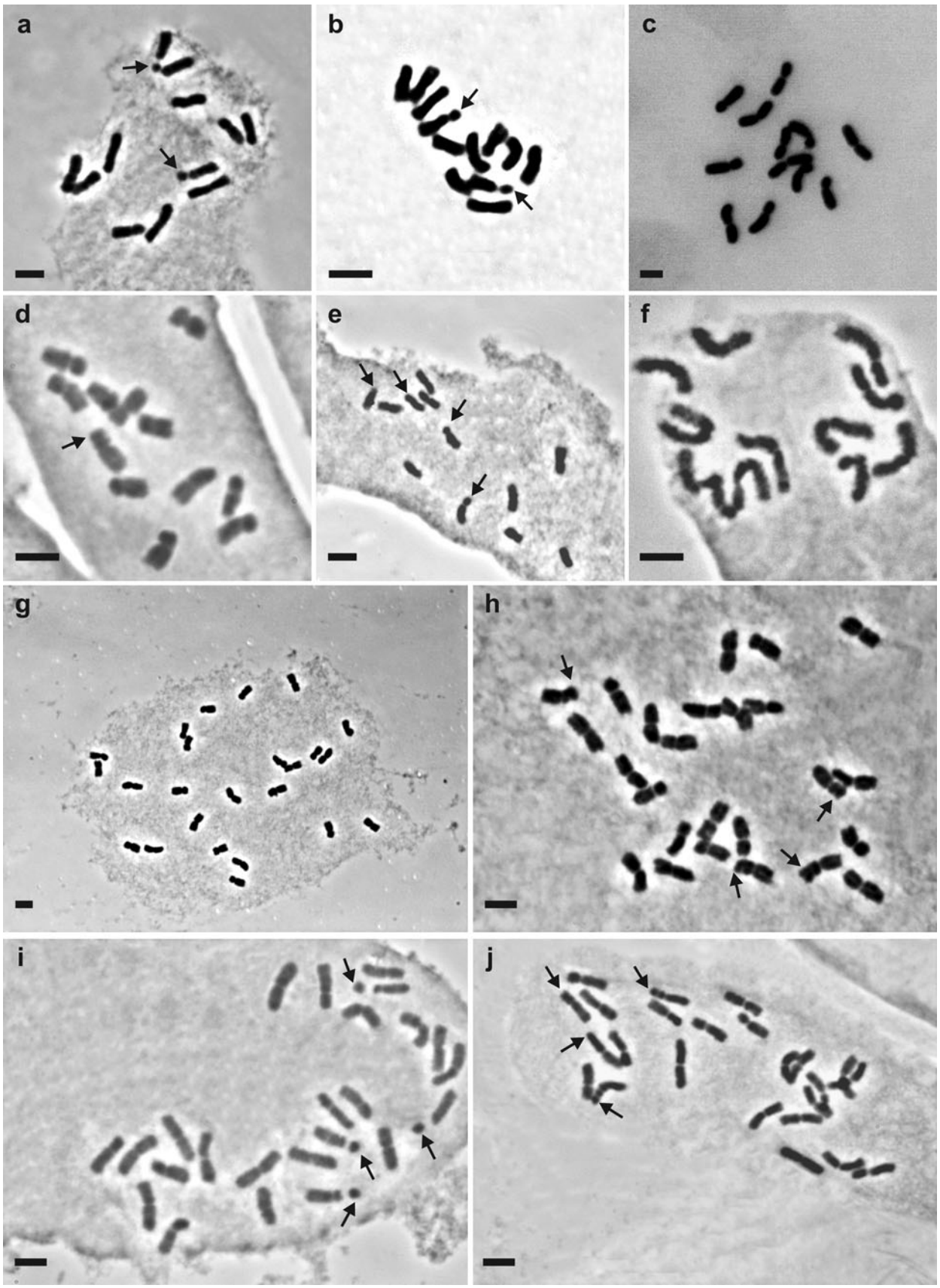
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Table 2 Taxa of the "*Xanthocephalum* group" studied

Species	Karyotype formulae	2*n*	Tl	*C*	*r*	A_1	A_2	*R*	% Ht	
									CMA–NORs	CMA
Grindelia camporum	5 *m** + 1 *sm*	12	22.93	3.82	1.29	0.19	0.10	1.41	2.10	–
G. hirsutula	8 *m* + 4 *sm*	24	41.91	3.49	1.09	0.25	0.09	1.37	1.75	–
G. procera	5 *m** + 1 *sm*	12	20.58	3.43	1.34	0.21	0.10	1.37	1.57	–
G. robusta	10 *m*** + 2 *sm*	24	48.33	4.02	1.31	0.20	0.09	1.44	2.17	–
G. squarrosa	5 *m** + 1 *sm*	12	14.31	2.38	1.26	0.17	0.10	1.37	1.45	–
G. stricta var. *angustifolia*	10 *m*** + 2 *sm*	24	49.46	4.12	1.34	0.23	0.09	1.36	1.61	–
G. stricta var. *platyphylla*	10 *m*** + 2 *sm*	24	42.05	3.50	1.35	0.23	0.10	1.45	1.60	1.54
Isocoma menziessi var. *sedoides*	4 *m** + 2 *sm*	12	26.10	4.35	1.505	0.28	0.12	1.39	1.43	0.60
Olivaea tricuspis	3 *m** + 3 *sm*	12	16.79	2.79	1.60	0.35	0.09	1.31	1.97	–
Prionopsis ciliata	5 *m*** + 1 *sm*	12	15.54	2.59	1.24	0.15	0.11	1.43	5.61	10.11

Karyotype formulae: mean total haploid chromosome length (tl); mean chromosome length (*C*); mean arm ratio (*r*); intrachromosomal asymmetry index (A_1); interchromosomal asymmetry index (A_2); ratio between the largest and the smallest chromosomes in the complement (*R*) and heterochromatin amount expressed as percentage of the karyotype length (% Ht). The number of asterisks indicate the number of chromosome pairs with satellites on the short arms

from the American continent had great diversity with many base numbers: $x = 2, 3, 4, 5, 6, 7, 8, 9, 11$, and 13; of these, members of the "*Xanthocephalum* group" had $x = 6$ (Whitaker and Steyermark 1935; Raven et al. 1960; De Jong and Beaman 1963; Nesom 1991, 1993, 1997; Lane and Li 1993; Lane and Hartman 1996).

The numbers obtained here agreed with previous reports (cf. Table 1), with the exception of *G. robusta*, never reported before. Populations examined were either diploid or tetraploid. In addition, there are reports of several ploidy levels for some *Isocoma* and *Grindelia* species (Dunford 1964, 1969, 1986; Morton 1981; McLaughlin 1986; Semple et al. 1989, 1992; Lane 1993; Lane and Li 1993; Carr et al. 1999), as we found in *G. camporum* only.

Grindelia polyploids found here could have originated recently by autopolyploidy, as suggested by the exact duplication of the diploid karyotype formula and the number of chromosomes with NORs. Natural hybrids are rare or unknown suggesting that isolating mechanisms could be external (e.g., geographic range), and supporting the autopolyploid hypothesis. On the other hand, hybrids within the "*Xanthocephalum* group" had been artificially synthesized among closely related genera (Nesom 1994 and papers cited therein).

Diploid *Grindelia* presented either 6 *m* (Baeza and Schrader 2005) or 4 *m* + 2 *sm*—formula after Bartoli and Tortosa (1998) corrected by Baeza and Schrader (2005) in South America or 5 *m* + 1 *sm* in North America (this work). The taxa studied displayed symmetrical karyotypes and different ploidy levels, which are typical of this genus (cf. literature in Table 1; Bartoli and Tortosa 1998; Baeza and Schrader 2005; this work).

CMA/DAPI banding has been widely used for angiosperms to identify heterochromatic bands with regard to their highly repeated DNA composition. The general patterns of band numbers and heterochromatin amounts can be conserved at the generic level (Galasso et al. 1997; Moscone et al. 1996; Marcon and Guerra 2005) or can be variable (Miranda et al. 1997; Guerra 2000; Fregonezi et al. 2006), as found here in the "*Xanthocephalum* group" and in other Asteraceae (Kamari 1992; Cerbah et al. 1995; D'Amato 2000; Dimitrova and. Greilhuber 2000; Ruas et al. 2000; Fregonezi et al. 2004; Garcia et al. 2009).

In the tetraploids *G. stricta* var. *platyphylla* and *I. menziessi* var. *menziessi*, chromosome duplication was followed by modifications in the genome structure, resulting in higher heterochromatin amounts not associated with NORs, as reported for other groups (Fregonezi et al. 2004; Garcia et al. 2009). As a whole, genome restructuring in polyploids is more rapid and extensive than in diploids (Adams and Wendel 2005; Clarkson et al. 2005; Comai 2005; Parisod et al. 2010). This could be because of unequal recombination, transposable element proliferation, or a combination of both factors.

In plant chromosomes, localization of CG-rich heterochromatin in proximal positions is less frequent that AT-rich heterochromatin (Schweizer 1976; Deumling and Greilhuber 1982; Galasso et al. 1997; Moscone et al. 1996), although it has been detected in *Areca* (Arecaceae, Röser 1994), *Feroniella* (Rutaceae, Guerra 2000), *Phyrrocactus* (Cactaceae, Las Peñas et al. 2008), *Artemisia* (Asteraceae, Vallès and Mc Arthur 2001), and *Prionopsis* (this work). On the other hand, the tetraploids *G. stricta* var. *platyphylla* and *I. menziessi* did not have a defined band pattern.

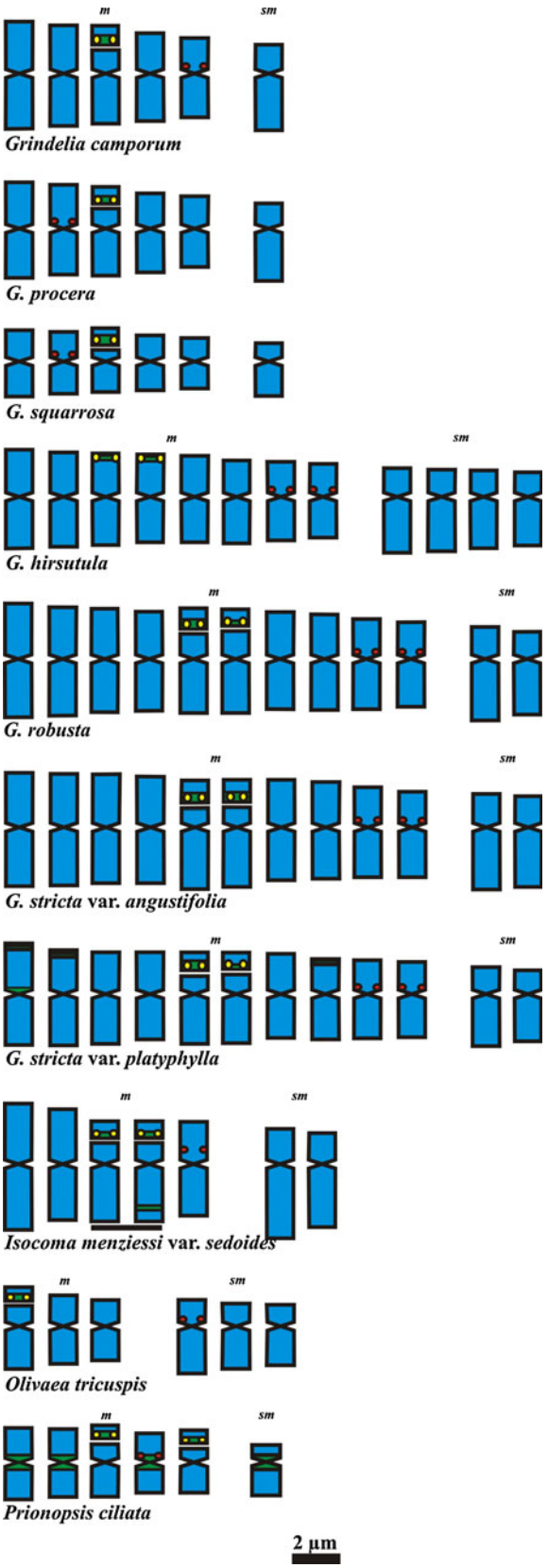

Fig. 2 Idiograms of "*Xanthocephalum* group" species based on mean chromosome values. The *bar* represents 2 µm. All at the same scale

The FISH technique enables physical mapping of sequences to their location within the genome, in particular repetitive sequences that cannot be easily mapped by any other method (Schwarzacher 2003). Both 5S and 18-5.8-26S rDNA genes have been extensively used to establish chromosomal homeologies, because they are abundant and highly conserved in higher plants (Moscone et al. 1999; Taketa et al. 1999; Adams et al. 2000; Cai et al. 2006; Hasterok et al. 2006; Vanzela et al. 2003). The number of 18S-26S rDNA sites in the species studied varied from two in diploids to four in tetraploids; thus, they may be indicative of the ploidy level. Besides, available data indicate these signals are highly conserved in Asteraceae (Fregonezi et al. 2004; Ruas et al. 2005; Baeza and Schrader 2005). In the "*Xanthocephalum* group", NOR-bearing pair #3 may be homeologous, excepting in *O. tricuspis* and two South American *Grindelia* species previously studied (Baeza and Schrader 2005). Generally, 5S sites are more numerous than 18-5.8-26S sites (Hemleben and Werts 1988; Sastri et al. 1992; Moscone et al. 1999), but in the taxa studied here there is only one per basic genome. In addition, its location in para or pericentromeric regions, as reported here, is frequent in gymnosperms and angiosperms (Kulak et al. 2002; Besendorfer et al. 2002). In the "*Xanthocephalum* group" polyploids, and in other plants (Liu et al. 2001; Lim et al. 2004; Pires et al. 2004), diplodization processes (inactivation or amplification of loci, or gene silencing) have not occurred, probably because of their recent origin.

Concerning the taxonomic value of our data, species can be distinguished by a combination of karyotype formula, position of NORs in a particular chromosome pair, and percentage of heterochromatin (Table 2; Fig. 3). Thus, chromosome variation, although not always large, has accompanied evolutionary divergence of the taxa studied, a general phenomenon observed in plants and animals (Goodspeed 1954; Riesemberg 2001). The finding of B-chromosomes and aneuploid individuals are an indication that chromosome variation in the group is dynamic. In spite of the amount of data on the distribution of B-chromosomes, there is lack of understanding of their significance in plant systematics; the fact remains that where B-chromosomes do occur they influence nuclear DNA values (Jones et al. 2008). In addition, their adaptive significance in natural populations showed little if any substantial evidence to support such a role (Jones and Houben 2003).

Jones (1985) suggested that chromosome features are basic to an understanding of the relationships of the Astereae, but they are more useful at the infrageneric than the generic level. However, we found that the genera examined here had significant differences. The heterochromatin distribution pattern in *Stephanodoria* and *Prionopsis* is unique. *Olivaea* and *Isocoma* had different formulae; in

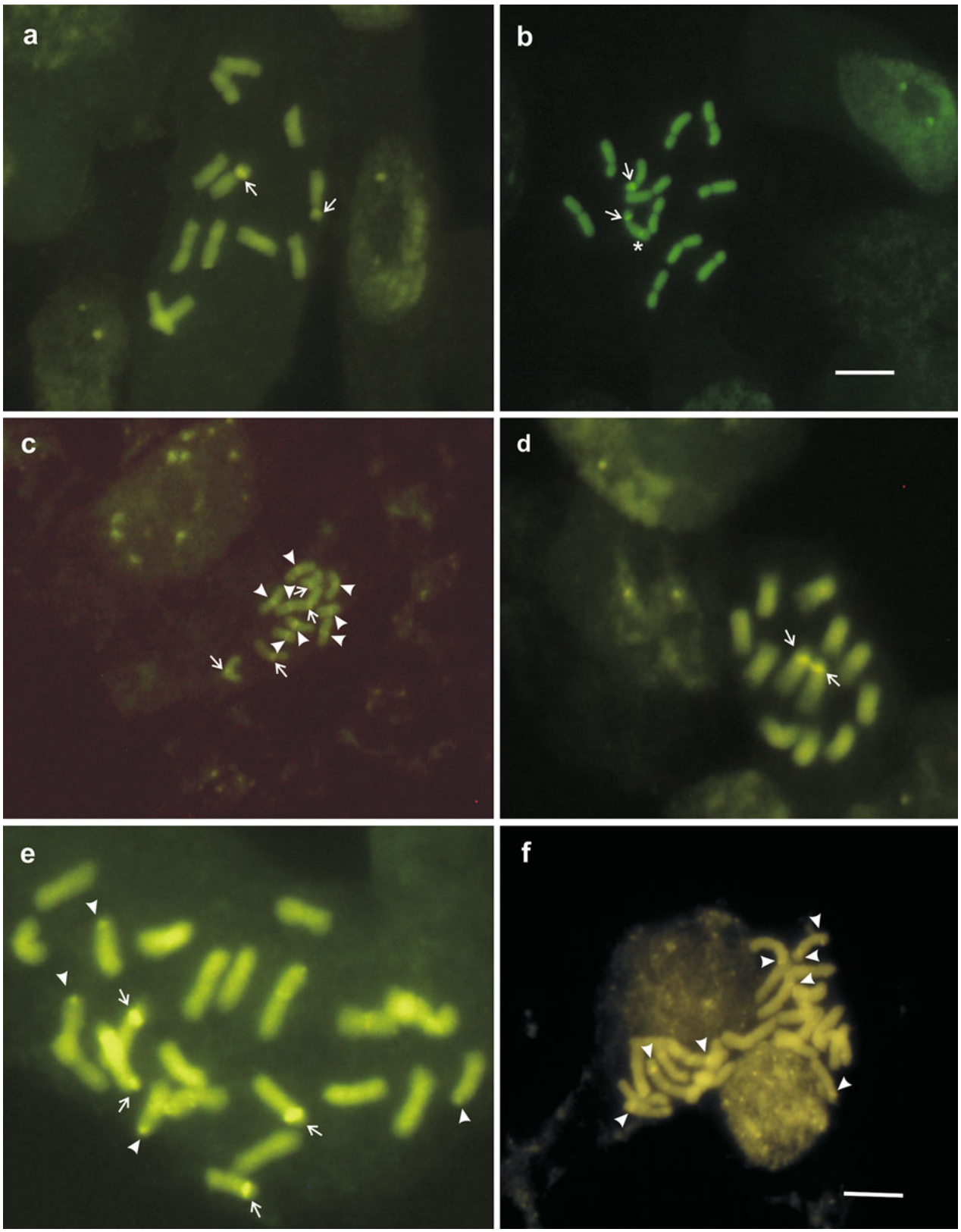

Fig. 3 Fluorescent chromosome banding in "*Xanthocephalum* group". **a** *Grindelia camporum.* **b** *Isocoma menziessi* var. *sedoides.* **c** *Prionopsis ciliata.* **d** *Olivaea tricupis*. **e** *G. stricta* var. *platyphylla*. **f** *I. menziessi* var. *menziessi* ($2n = 24$). *Arrows* show $CMA^{+}/DAPI^{-}$ NOR-associated bands. *Arrow heads* show $CMA^{+}/DAPI^{-}$ bands. The *asterisk* indicates a $CMA^{+}/DAPI^{-}$ heteromorphic band. The *bar* represents 5 μm

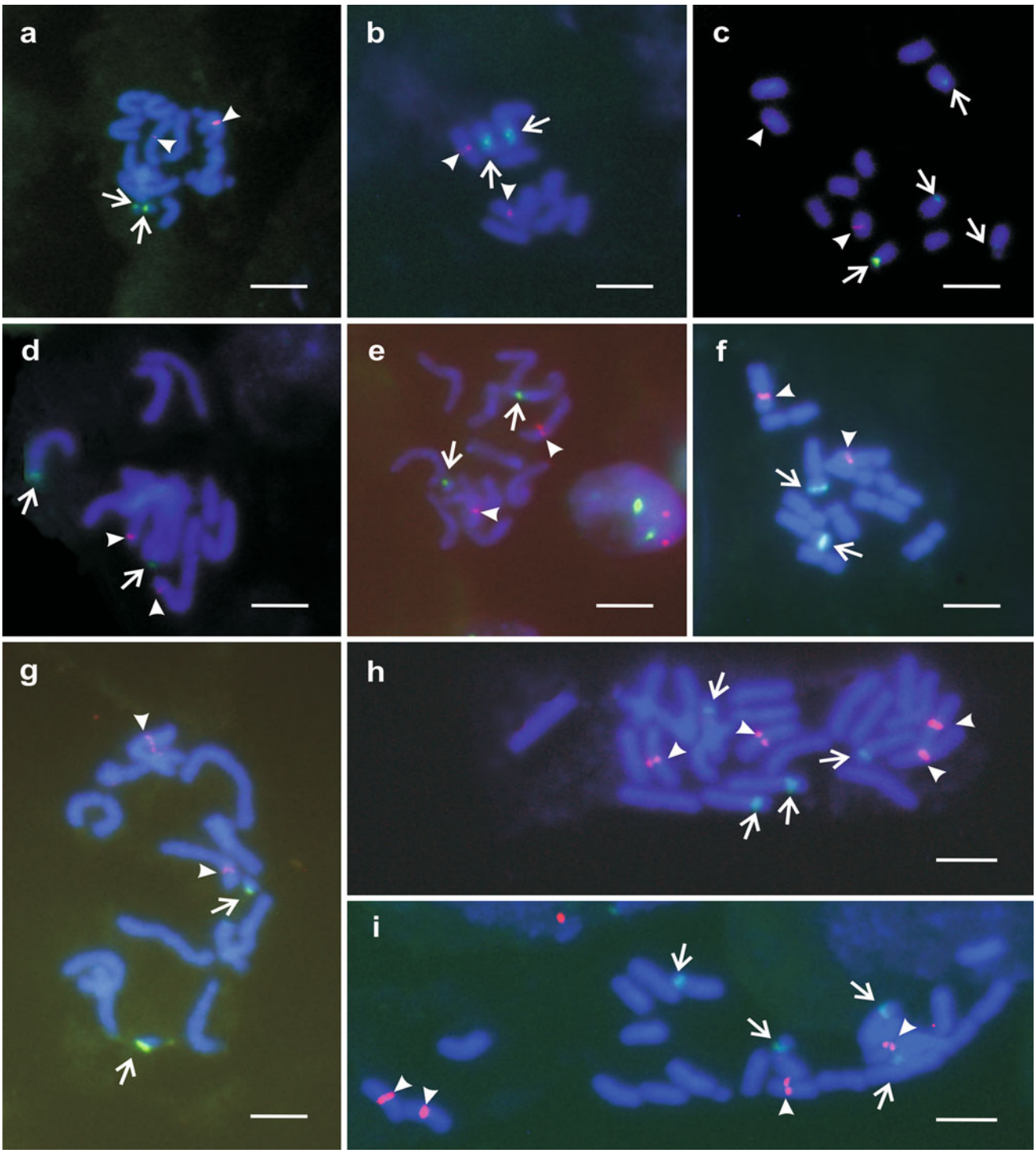

Fig. 4 Somatic chromosomes detected by FISH using 18-5.8-26S and 5S rDNA probes. **a** *G. procera.* **b** *G. squarrosa.* **c** *P. ciliata.* **d** *I. menziessi* var. *vernoides.* **e** *S. tomentella.* **f** *O. tricuspis.* **g** *I. menziessi* var. *sedoides.* **h** *G. stricta* var. *angustifolia.* **i** *G. hirsutula. Arrows* show 18-5.8-26S rDNA sites and *arrow heads* show 5S rDNA sites hybridization. The *bar* represent 5 μm

addition, *Olivaea* had distinctive localization of both rDNA genes. Finally, *Grindelia* species had common characteristics, sharing with *Isocoma* the homeologous position of the 18-5.8-26S gene. In summary, classic and molecular cytogenetic data in this group proved to be helpful to understanding its karyoevolutionary trends.

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Note added in proof After the following recent article (May 2012), *Grindelia robusta* is considered as a synonym of *G. humilis* var. *humilis*: Bartoli A, Tortosa RD (2012) Revision of the North American Species of *Grindelia* (Asteraceae). Ann Missouri Bot Gard 98:447–513.

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