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ORIGINAL ARTICLE

Karyological circumscription of *Ipheion* Rafinesque (Gilliesioideae, Alliaceae)

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Abstract *Ipheion* Rafinesque is a small genus formed by *I. uniflorum* ($2n = 12$, 2SM + 10A), *I. tweedieanum* ($2n = 14$A), and *I. recurvifolium* ($2n = 20$, 4SM + 16A). Three species of *Nothoscordum*, *N. felipponei*, *N. hirtellum*, and *N. vittatum* ($2n = 10$, 6M + 4A), were also later transferred to *Ipheion* based on the common presence of unifloral inflorescence. Karyotype analysis of the five former species was performed in this work, aiming to evaluate the circumscription of the genus. This analysis was based on chromosome size and morphology, asymmetry index, staining with chromomycin A3 (CMA) and 4′,6-diamidino-2-phenylindole (DAPI), and in situ hybridization with 5S and 45S rDNA probes. Tetraploid populations of *I. uniflorum*, probably autopolyploids of recent origin, with karyotype similar to the diploids, are described herein for the first time. Grouping analyses of the several sets of characters analyzed show the former three *Ipheion* species clearly separated from the *Nothoscordum* ones, which were more proximally related to other *Nothoscordum* species. Chromosome size, asymmetry indices, and number and position of 5S and 45S rDNA sites were the most important karyotype characters to define the genus *Ipheion*. These data indicate that the unifloral species of *Nothoscordum* belong to *Nothoscordum* and not to *Ipheion*, and the "unifloral inflorescence" should be a homoplasy common to both genera.

Keywords Chromosomes · rDNA sites · CMA^+ bands · *Ipheion* · *Nothoscordum* · Tribe Ipheieae

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Introduction

Ipheion Rafinesque is a small genus of the tribe Ipheieae (Gilliesioideae-Alliaceae) whose distribution is restricted to Argentina, Uruguay, and southern Brazil (Crosa and Marchesi 2002). The tribe Ipheieae also includes the genera *Leucocoryne* Lindl., *Nothoscordum* Kunth, *Tristagma* Poeppig (Fay and Chase 1996), and *Zoellnerallium* Crosa, with which *Ipheion* has a close relationship and shares many morphological characteristics (Guaglianone 1972; Crosa 1975; Crosa and Marchesi 2002). However, the circumscription of *Ipheion* is controversial.

The species traditionally included in *Ipheion* [*I. uniflorum* (Raf.) Traub., *I. tweedieanum* (Griseb.) Traub., and *I. recurvifolium* (Wright) Traub.], as well as some others from *Nothoscordum*, have unifloral inflorescences (Guaglianone 1972). Based on the similarities in the form of the scapes and the unifloral inflorescences, Guaglianone (1972) transferred *N. hirtellum* (Kunth) Traub and *N. vittatum* (Griseb.) Ravenna to *Ipheion*, creating the section *Hirtellum* Guagl., and included a new unifloral species, *I. dialystemon* Guagl., later considered a synonym for *Nothoscordum felipponei* Beauverd (Crosa 1975). However, karyological (chromosome number and morphology) and morphological (histology of the testa and morphology of the flowers, bracts, and seeds) data strongly indicated

that the species of the section *Hirtellum* should return to *Nothoscordum*, as the character "unifloral inflorescence" seems to represent a homoplasy common to both genera (Crosa 1975). Fay et al. (2006), based on the plastid sequences intron *trn*L, intron *rps*l6, intergenic spacer *trn*L-F and *rbc*L, as well as the internal transcribed spacer (ITS) of the nuclear ribosomal DNA, proposed that *Ipheion* is paraphyletic, with *I. uniflorum* and *I. sessile* (Phil.) Traub (=*I. recurvifolium*) being a sister group of *Tristagma*, while *I. dialystemon* and *I. hirtellum* (Guaglianone 1972) were included into the *Nothoscordum* clade.

Karyologically, the unifloral species of *Nothoscordum* are distinct from those of *Ipheion*. All unifloral species of *Nothoscordum* have karyotype $2n = 10$ (6M + 4A), with the exception of *N. izaguirreae*, with $2n = 24$ (24M) (Crosa 1975, 2006), and all of them have large chromosomes (up to 20 μm). In contrast, the karyotypes observed in the species of *Ipheion* are formed by smaller chromosomes (up to 8.5 μm) which are predominantly acrocentric (Crosa 1975). The chromosome numbers and karyotype formulae previously described for species of *Ipheion* are: $2n = 12$ (2SM + 10A) for *I. uniflorum*, $2n = 20$ (4SM + 16A) for *I. recurvifolium*, and $2n = 14$ (14A) for *I. tweedieanum* (Crosa 1975; Crosa and Marchesi 2002; Meric and Dane 2005).

Phylogenetic analyses of closely related genera of the tribe Ipheieae, based on ITS and *rbc*L gene sequences, suggest that *Tristagma* is the sister group of *Ipheion* (Fay and Chase 1996). Actually, the genus *Tristagma* shares with the species *Ipheion uniflorum* and *I. tweedieanum* the same fundamental number (number of major chromosome arms in a diploid cell), FN = 14 or exact multiples of 14, but there are significant differences in their karyotype formulae, and chromosome numbers and sizes between the karyotypes of the two genera (Crosa 1975, 1981; Crosa and Marchesi 2002).

In the present study, detailed karyotype analysis was performed on five of the six species of the genus *Ipheion*, as recognized by Guaglianone (1972), aiming to evaluate the circumscription of the genus. The analysis was based on chromosome size and morphology, heterochromatin patterns revealed by the fluorochromes chromomycin A3 (CMA) and 4′,6-diamidino-2-phenylindole (DAPI), and distribution of 5S and 45S rDNA sites detected by fluorescent in situ hybridization (FISH). The results were compared with the available data for other *Nothoscordum* species.

Materials and methods

Plant material

Samples of *Ipheion uniflorum*, *I. tweedieanum*, and *I. recurvifolium* and two species included by Guaglianone (1972) into *Ipheion*, *I. dialystemon* and *I. hirtellum*, were analyzed. The two latter species will be here treated as *N. felipponei* and *N. hirtellum*, respectively. The collection sites and the number of individuals examined are presented in Table 1. The vouchers were deposited in the herbarium of the Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay (MUFA).

Chromosome banding

Root tips obtained from bulbs were pretreated with 0.05% colchicine during 24 h at 12°C, fixed in ethanol:acetic acid (3:1, v/v) for 2–24 h at room temperature, and stored at −20°C. Afterwards, fixed root tips were washed in distilled water and digested in a 2% (w/v) celullase (Onozuka)–20% (v/v) pectinase (Sigma) solution at 37°C for 90 min. The meristem was macerated in a drop of 45% acetic acid, and the coverslip removed in liquid nitrogen.

The C-banding technique was based on Schwarzacher et al. (1980). After 2 days aging, the preparations were hydrolyzed in 45% acetic acid for 10 min at 60°C, denatured in a saturated solution of barium hydroxide for 10 min at room temperature, renatured in 2× SSC for 80 min at 60°C, and stained with 2% Giemsa for 30 s. For CMA/DAPI double staining, the slides were aged for 3 days, stained with 10 μl 0.1 mg/ml CMA for 30 min, and restained with 10 μl 2 μg/ml DAPI for 60 min (Barros e Silva and Guerra 2010). The slides were mounted in glycerol:McIlvaine buffer pH 7.0 (1:1) and aged for 3 days before analysis using an epifluorescence Leica DMLB microscope. Images were captured using a Cohu charge coupled device (CCD) video camera using Leica QFISH software and later edited using Adobe Photoshop CS3 version 10.0.

Fluorescent in situ hybridization (FISH)

To localize the rDNA sites, a 500-bp 5S rDNA clone (D2) of *Lotus japonicus*, labeled with Cy3-dUTP (Amersham), and a 6.5-kb 18S-5.8S-25S clone (R2) of *Arabidopsis thaliana*, labeled with digoxigenin-11-dUTP, were used as probes (Pedrosa et al. 2002). Both labelings were done by nick translation. The 45S rDNA probe was detected with sheep anti-digoxigenin fluorescein isothiocyanate (FITC) conjugate (Roche) and amplified with rabbit anti-sheep FITC conjugate (Dako). FISH was performed as described by Pedrosa et al. (2002) with small modifications. The hybridization mix contained formamide 50% (v/v), dextran sulfate 10% (w/v), 2× SSC, and 5 ng/μl of each probe. The slides were denaturated at 75°C for 3 min, and the final stringency of hybridization was ca. 76%. Images of the best cells were captured as described before.

Table 1 Samples analyzed of *Ipheion* and *Nothoscordum* species with voucher number, provenance, number of individuals analyzed, diploid number ($2n$), karyotype formula, fundamental number (FN), chromosome size range, average chromosome size, haploid complement size, asymmetry index (A_1 and A_2), and number of rDNA sites

Species	Voucher	Provenance	Number of individuals	$2n$	Karyotype formula	FN	Chromosome size range (μm)	Average chromosome size (μm)	Haploid complement size (μm)	A_1	A_2	rDNA sites	
												5S	45S
Ipheion recurvifolium (Wright) Traub.	MVFA 33781	Paso Roldán, Depto. Florida, Uruguay	4	20	4SM + 16A	24	5.8–12.1	4.48	89.5	0.76	0.21	4	4
I. tweedieanum (Baker) Traub.	MVFA 21953	Ruta 24, km. 45.5, Depto. Río Negro, Uruguay	2	14	14A	14	5.5–10.2	4.26	59.6	0.86	0.18	2	14
I. uniflorum (Lindl.) Raf.	MVFA 33773	Ruta 26, km. 191, Depto. Tacuarembó, Uruguay	6	12	2SM + 10A	14	7.1–10.0	4.38	52.5	0.78	0.11	2	10
	MVFA 33774	Abra de Perdomo, Depto. Maldonado, Uruguay	1	24	4SM + 20A	28	6.3–9.3	4.07	97.7	0.76	0.07	4	20
	MVFA 33775	Laguna del Sauce, Depto. Maldonado, Uruguay	5	24	4SM + 20A	28	–	–	–	–	–	4	20
	MVFA 33776	Fuerte San Miguel, Depto. Rocha, Uruguay	1	24	4SM + 20A	28	–	–	–	–	–	4	20
Nothoscordum felipponei Beauverd	MVFA 33777	Minas, Depto. Lavalleja, Uruguay	2	10	6M + 4A	16	9.2–15.3	12.14	60.7	0.43	0.18	8	8
	MVFA 33778	Cerro Verdún, Depto. Lavalleja, Uruguay	1	10	6M + 4A	16	–	–	–	–	–	8	8
	MVFA 33779	Ruta 6, km. 152, Depto. Florida, Uruguay	1	10	6M + 4A	16	–	–	–	–	–	8	8
	MVFA 33780	Minas, Depto. Lavalleja, Uruguay	1	10	6M + 4A	16	–	–	–	–	–	8	8
N. hirtellum (Kunth) Herter	MVFA 33782	Sierra de las Ánimas, Depto. Maldonado, Uruguay	3	10	6M + 4A	16	9.9–17.4	14.24	71.2	0.45	0.21	2	14

Chromosome measures and cluster analysis

For each species, 5–7 metaphases with clear chromosome morphology were measured using software Adobe Photoshop CS3 version 10.0. Chromosome arm ratio (AR = length of the long arm/length of the short arm) was used to classify chromosomes as metacentric (AR = 1–1.4), submetacentric (AR = 1.5–2.9), or acrocentric (AR ≥ 3.0), according to Guerra (1986). Mean lengths of the whole chromosome complement, shortest and longest chromosome of the complement, and chromosome pairs bearing CMA bands and rDNA sites were compared. The karyotype symmetry was calculated according to the formula proposed by Romero Zarco (1986), to estimate the intrachromosomal asymmetry [$A_1 = 1 - (\sum b/B)/n$; b = average length for short arms in every chromosome pair, B = average length for long arms in every chromosome pair, n = chromosome number] and the interchromosomal asymmetry ($A_2 = S/X$; S = standard deviation, X = mean chromosome length).

A phenetic analysis was performed based on the following characters: (a) chromosome number, (b) fundamental number, (c) karyotype asymmetry indices A_1 and A_2, (d) average chromosome size, (e) percentage of acrocentric chromosomes in the karyotype, (f) number of 5S rDNA sites in the proximal, interstitial, and terminal position, (g) number of 45S rDNA sites on metacentric chromosomes, (h) number of 45S rDNA sites on the short arms of acrocentric chromosomes, and (i) number and position of CMA^+ bands (except the bands colocalized with 45S rDNA sites). *Allium cepa* was used to compare the grouping of *Ipheion* and *Nothoscordum* species in relation to another species of the family that has already been investigated for the same set of cytological parameters. Karyotype parameters of *Allium cepa* were based on Do et al. (2001) for the cultivar "Cheonjudaego." CMA banding for *A. cepa* was based on Kim et al. (2002) and on our own results using a commercial onion (unpublished results). For *N. arenarium* and *N. pulchellum*, the data were based on Souza et al. (2009) and Guerra and Felix (2000), respectively. The data were analyzed using Multi-Variate Statistical Package-MVSP version 3.13p (http://www.kovcomp.com/). Clustering was performed using the unweighted pair-group method (UPGMA).

Results

The three *Ipheion* species displayed karyotypes formed by submetacentric and acrocentric chromosomes, whose sizes varied from 5.5 to 12.1 µm. The chromosome numbers were identical to those previously reported: $2n = 12$ for *I. uniflorum*, $2n = 14$ for *I. tweedieanum*, and $2n = 20$ for *I. recurvifolium*. However, most populations of *I. uniflorum* analyzed were tetraploid with $2n = 24$, whereas all other samples previously counted were diploid. C-banding analysis of *I. uniflorum* revealed heterochromatin on the short arms of all acrocentric chromosomes, and on the proximal region of the submetacentric pair (Fig. 1a). Double staining with CMA and DAPI fluorochromes revealed CMA^+ bands in the same position as the C-banding technique (Fig. 1b). Because CMA/DAPI staining produces results very similar to C-banding and has the advantage of being a more simple and rapid technique, for the remaining species only this technique was performed.

The diploid sample of *I. uniflorum* ($2n = 12$, 2SM + 10A) had chromosome sizes varying from 7.1 to 10.0 µm and a haploid chromosome complement length of 52.5 µm. The arm ratio (AR) for the submetacentric pair was 1.5, whereas for the acrocentric pairs it varied from 6.2 to 8.5. The 45S rDNA sites were colocalized with the CMA^+ bands of the short arms of the acrocentric chromosomes, while the 5S rDNA sites were located in the interstitial region of a pair of acrocentric chromosomes (Fig. 1c). The tetraploid samples of *I. uniflorum* had $2n = 24$ (4SM + 20A) and an exact duplication of the number of CMA^+ bands and rDNA sites in relation to the diploid form (Fig. 1d). The length of the haploid chromosome complement was 97.7 µm, which was only slightly smaller than that expected based on the diploid cytotype. Arm ratios and chromosome sizes were also only slightly different from those of the diploid cytotype (Fig. 3a, b). After FISH, the proximal bands of the submetacentric chromosomes of both cytotypes were observed as $DAPI^+$ bands (Fig. 1d).

Ipheion tweedieanum had $2n = 14$ (14A), with similarly sized short arms of the acrocentric chromosomes (ca. 1 µm), and gradual size variation in the long arms (4.5–9.4 µm). The total chromosome complement length was 59.6 µm. CMA^+ bands were observed on the short arms of all acrocentric chromosomes and were always colocalized with 45S rDNA sites (Fig. 1f, g). During interphase, all the CMA^+ bands of the acrocentric chromosomes were associated with the nucleolus (Fig. 1e), suggesting that they were active nucleolar organizing regions (NORs). At prophase, the CMA bands were often oriented towards a single large area, corresponding to the nucleolus (Fig. 1e). Sometimes, fine secondary constrictions were observed at the end of these bands. The only 5S rDNA site observed was localized in the interstitial region of the long arm of the smallest chromosome pair (Figs. 1g, 3c).

Ipheion recurviflorum had $2n = 20$ (4SM + 16A), with FN = 24 (considering only chromosome arms clearly larger than the short arms containing rDNA sites), and a chromosome complement length of 89.5 µm, with chromosome sizes varying from 5.8 to 12.1 µm. Two chromosome pairs were conspicuously smaller than the others,

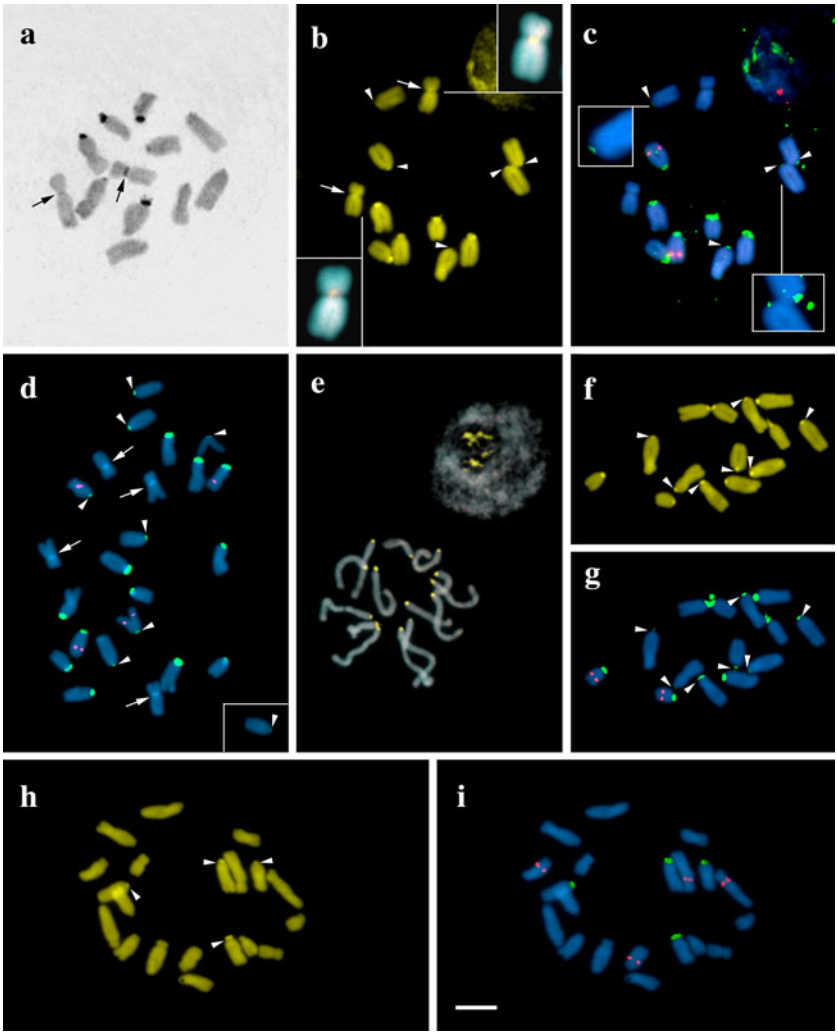

Fig. 1 Distribution of heterochromatin and 5S (*red*) and 45S (*green*) rDNA sites in the chromosome complement of *Ipheion* species. **a–c** *I. uniflorum* 2*x* showing C-bands (**a**), CMA^+ bands (**b**) and rDNA sites (**c**). **d** *I. uniflorum* 4*x* showing rDNA sites. **e–g**, *I. tweedieanum* interphase and prophase nuclei stained with CMA/DAPI (**e**), and metaphase displaying CMA^+ bands (**f**) and rDNA sites (**g**). **h–i** *I. recurvifolium* with CMA^+ bands (**h**) and rDNA sites (**i**). *Inserts* in **b** show the submetacentric pair with a proximal CMA^+ band, in **c** show details of some small rDNA sites, at both higher magnification and contrast, and in **d** it shows a single chromosome of the same metaphase that was separated from the others. *Arrowheads* point to the smaller CMA^+ bands colocalized with 45S rDNA sites. *Arrows* in **a**, **b**, and **d** point to the proximal heterochromatin of the submetacentric pair. *Bar* in **i** represents 10 μm

one being submetacentric (pair IX) and the other acrocentric (pair X) (Fig. 3d). CMA^+ bands were observed only on the short arms of the acrocentric pairs IV and V (Fig. 1h), being colocalized with 45S rDNA sites. The 5S rDNA sites were localized on the proximal regions of two acrocentric pairs (Fig. 1i).

Nothoscordum felipponei and *N. hirtellum* showed $2n = 10$ (6M + 4A), haploid chromosome length of 60.7 and 71.2 μm, respectively, and individual chromosome sizes varying between 9.2 and 15.3 μm and between 9.9 and 17.4 μm, respectively. The heterochromatin was $CMA^+/DAPI^-$and colocalized with 45S rDNA sites, although some CMA^+ bands were very small and not always visible (Fig. 2a–b). Small 45S rDNA sites were found on the telomeric region of most arms of the metacentric chromosomes, mainly in *N. hirtellum*.

Nothoscordum felipponei had 45S rDNA sites on the long arms of two pairs of metacentric chromosomes (pairs I and II) and on the chromosome termini of two acrocentric pairs (Fig. 2b). The 5S rDNA sites were located in the proximal and terminal region of the short arm of pair I, and in the proximal region of both the short and the long arms of pair III (Fig. 2b). *Nothoscordum hirtellum* had 45S rDNA sites on the terminal regions of the long and short arms of pairs I and II, on the terminal region of the short arm of pair III, and probably on the short arms of the two pairs of acrocentric chromosomes (pairs IV and V). On the other hand, 5S rDNA sites were located only in the proximal region of pair II (Figs. 2c, 3e, f).

Cluster analysis using the indicated karyotype features as variables separated the *Ipheion* and *Nothoscordum* species into two groups (Fig. 4), whereas *A. cepa* was separated to another cluster. *Nothoscordum hirtellum* and *N. felipponei* were grouped together with the other two species of *Nothoscordum* for which similar karyotype parameters are known (Guerra and Felix 2000; Souza et al. 2009). Within the clade of *Ipheion* species, *I. uniflorum* 2*x* and 4*x* showed reduced percentage similarity because the haploid chromosome number and the fundamental number were used to calculate the distance between these samples.

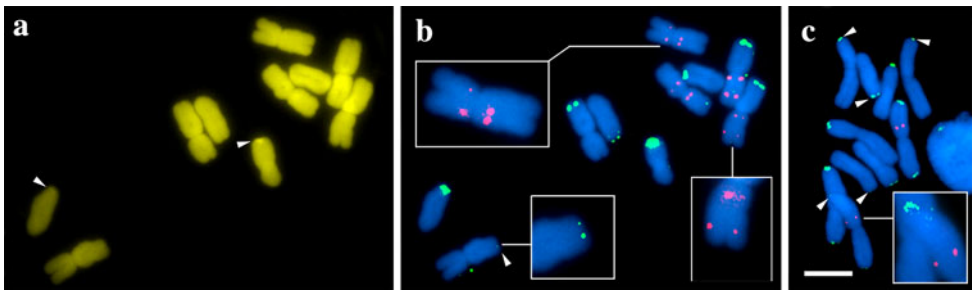

Fig. 2 Distribution of CMA^+ bands and rDNA sites in the chromosome complements of *Nothoscordum felipponei* (**a**, **b**), and rDNA sites in *N. hirtellum* (**c**). **a** CMA/DAPI-stained metaphase showing CMA^+ band. **b**, **c** In situ hybridization with 5S (*red*) and 45S (*green*) rDNA probes. *Inserts* in **b** and **c** show some rDNA sites at higher magnification and contrast. *Arrowheads* point to the smaller CMA^+ bands/45S rDNA sites. *Bar* in **c** represents 10 µm

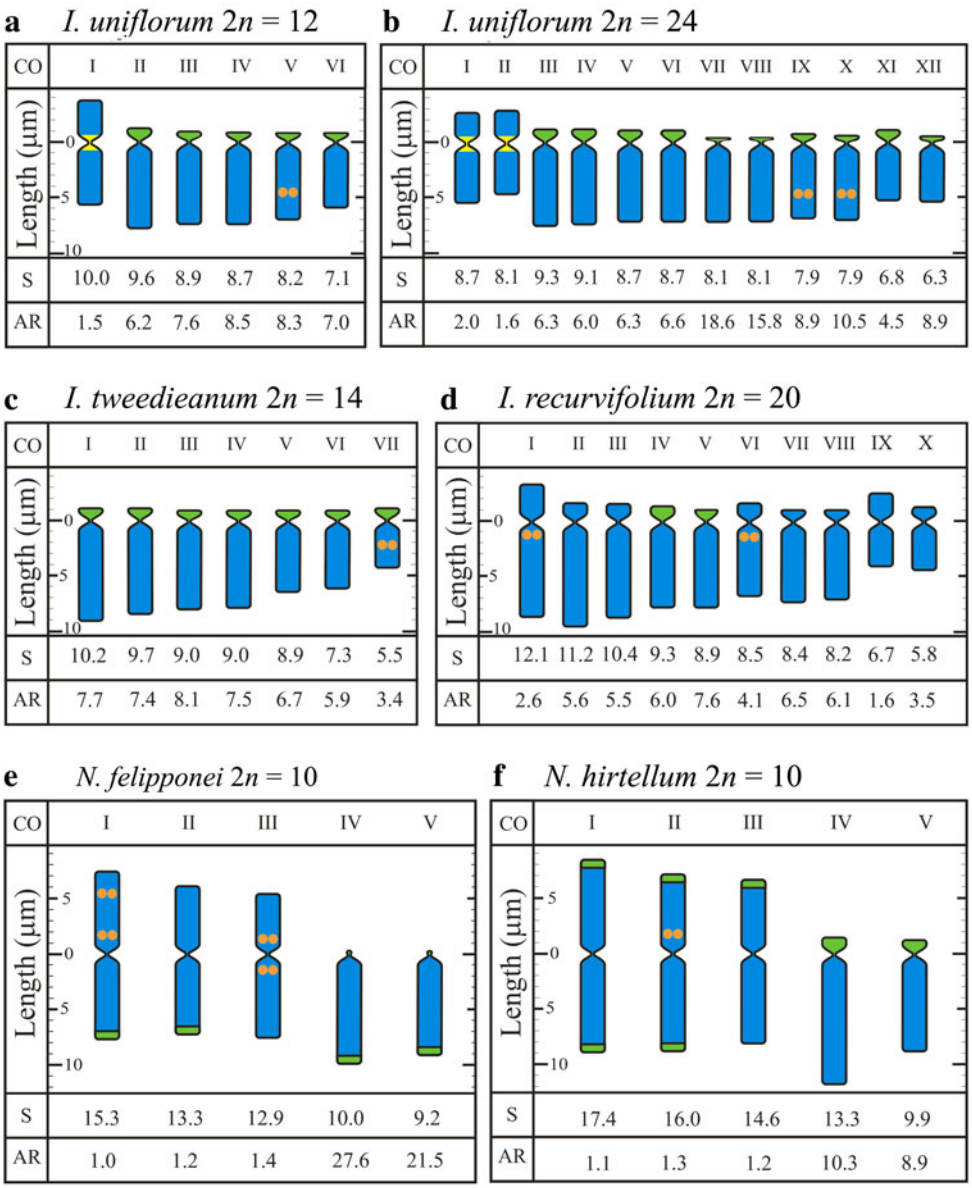

Fig. 3 Idiograms of *Ipheion* and *Nothoscordum* species showing chromosome size (*S*), arm ratio (*AR*), CMA^+ bands (*yellow*), 5S (*red*), and CMA^+ bands colocalized with 45S rDNA sites (*green*). Chromosomes were ordered (CO) by decreasing size, except for *I. uniflorum* 4*x*, where the metacentric chromosomes were conserved in the initial positions

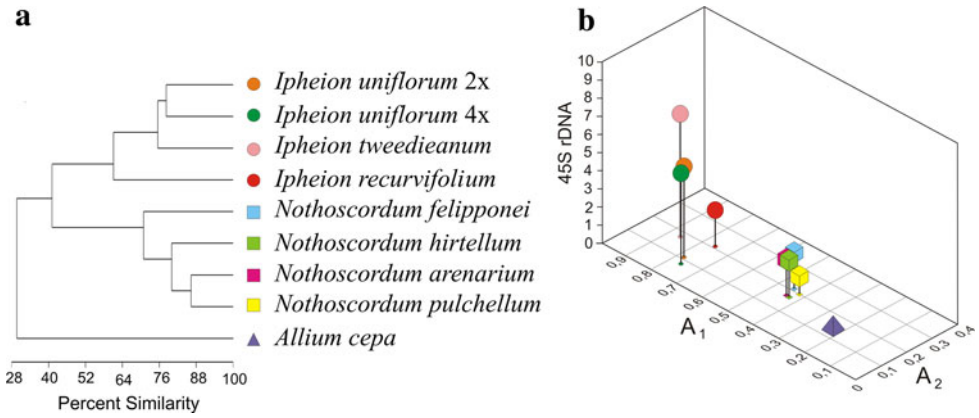

Fig. 4 Relationship among karyotypes of *Ipheion* and *Nothoscordum* species. **a** Dendrogram using the unweighted pair-group method and *Allium cepa* as outgroup. **b** Ratio to asymmetry index (A_1 and A_2) and number of 45S rDNA sites on the short arms of acrocentrics. *Symbols* represent the species indicated in **a**

Discussion

The karyotype formulae, chromosome numbers, and chromosome sizes of the species of *Ipheion* and *Nothoscordum* examined here were similar to those described earlier (Crosa 1975; Crosa and Marchesi 2002; Meric and Dane 2005), while the analysis of heterochromatic bands, asymmetry index, and rDNA sites furnished new information about the karyotypes of these species. The tetraploid samples of *I. uniflorum*, with $2n = 24$, described here for the first time, showed an almost exact duplication of chromosome morphology, CMA^+ bands, and rDNA sites, in relation to the diploid cytotype, probably representing an autopolyploidy of recent origin. Recent autopolyploids frequently conserve most karyotype features, such as the chromosome morphology, heterochromatic bands, DNA amount, and number and position of rDNA sites, while older polyploids tend to lose some of these sequences and become less similar to their diploid relatives (Weiss and Maluszynska 2000; Bennett and Leitch 2005; Kovarik et al. 2008).

The distribution of the 45S rDNA sites, restricted to the short arms of the acrocentric chromosomes, is a common characteristic of the three species traditionally included in *Ipheion*. The two other species analyzed here, *N. felipponei* and *N. hirtellum*, had 45S rDNA sites on most chromosome termini of the acrocentrics and on terminal region of some metacentric chromosomes. The meaning of the diffusion of 45S rRNA genes throughout all or almost all acrocentric chromosomes, observed in *I. uniflorum* and *I. tweedieanum*, is unknown, but it has also been found in some other genera with similar karyotype, as for example, *Zamia* and *Alstroemeria* (Tagashira and Kondo 2001; Baeza et al. 2007). In another species of the tribe Ipheieae, *Zoellnerallium andinum* ($2n = 24$, 8M + 16A), the short arms of all acrocentric chromosomes reacted positively with silver nitrate and hybridized with the 45S rDNA probe (unpublished data). The localization of nucleolar organizer regions (NORs) on the short arms of the acrocentric chromosomes, identified either by silver nitrate impregnation or by FISH with 45S rDNA probe, was also observed in the three other species of *Nothoscordum* analyzed previously (Sato et al. 1979; Guerra and Felix 2000; Souza et al. 2009).

Within the genus *Ipheion* there is great karyotypic similarity between *I. uniflorum* and *I. tweedieanum*, as they share the same fundamental number (FN = 14), similar chromosome morphology, and high number of 45S rDNA sites. The haploid karyotype formulae of *I. uniflorum* (1SM + 5A) and *I. tweedieanum* (7A) suggest that the submetacentric chromosome of *I. uniflorum* corresponds to two acrocentric chromosomes of *I. tweedieanum*. Therefore, Robertsonian translocation may have played an important role in the karyotype evolution of *Ipheion*, as has been suggested for *Nothoscordum* (Crosa 1972; Jones 1998; Guerra 2008).

Among the three *Ipheion* species, *I. recurvifolium*, is the only one that does not show a clear relationship with the fundamental number, diploid numbers, or karyotype formulae observed in the others. Its relatively high chromosome number and duplicate number of 5S rDNA sites suggest that it is tetraploid (Crosa 1975; Crosa 2004). In this case, different types of chromosomal rearrangements should have contributed to modifying the karyotype of this species after the polyploidization event, as observed in other polyploid genera (see, e.g., Dierschke et al. 2009). Nevertheless, the three species of *Ipheion* share several karyotype similarities in the chromosome size, high percentage of acrocentric chromosomes, 45S rDNA sites restricted to the short arms of acrocentrics, and absence of duplicated sites of 5S rDNA. Multiple sites of 5S rDNA were also observed in *N. felipponei*, *Allium cepa*, and several other species of *Allium* (Lee et al. 1999). Besides, they also share other morphological characteristics such as the spathe formed by single bracts, and the complex structure of its flower and of the testa (Crosa 1975).

Differently from *Ipheion*, the phylogenetically related genera *Nothoscordum*, *Leucocoryne*, and *Tristagma* have chromosomes that are predominantly metacentric and larger (Crosa 1972, 1981, 1988), and a fundamental number of

FN = 16 for the diploid species of the former two genera and FN = 14 for the diploid species of the latter. The closely related genus *Zoellnerallium*, with only two species known (Crosa 2004), has a karyotype more similar to *Ipheion*, with predominance of relatively small acrocentric chromosomes ($2n = 24$; 8M + 16A). However, *Zoellnerallium* species have the same fundamental number (FN = 32) found in tetraploid species of *Nothoscordum* and *Leucocoryne* (Crosa 1972, 1988). Assuming that increasing number of acrocentric chromosomes is a derived characteristic (Schubert 2007), the evolution of *Ipheion* may have been accompanied by successive centric fissions followed by formation of rDNA sites on the short arms of the acrocentric chromosomes. Hall and Parker (1995) found that, in *Hypochaeris radicata*, the formation of acrocentric chromosomes with 45S rDNA sites on the short arms was associated with centric fission. A similar mechanism may have been involved in the origin of *I. uniflorum* and *I. tweedieanum* karyotypes.

Nothoscordum felipponei and *N. hirtellum*, included into *Ipheion* by Guaglianone (1972), were karyotypically very distinct from the other three *Ipheion* species. On the other hand, these two species were quite similar to the species with $x = 5$ of *Nothoscordum* section *Nothoscordum*, both in chromosome number, size, and morphology, as in their patterns of CMA^+ bands and 5S and 45S rDNA distribution (unpublished data). Additionally, uniflorum and plurifloral species of *Nothoscordum* share the presence of spathes with two bracts, tepals free at their base, stamen filaments adnate to perigonium basis, black and smooth seeds, and undifferentiated parenchymatic tissue in the testa (Crosa 1975). Phenetic distance analysis showed that the karyotypes of *Ipheion* and *Nothoscordum* species investigated here can be grouped into two main clusters, separating the three *Ipheion* species from the *Nothoscordum* ones and also from the outgroup (*Allium cepa*). The main karyotype parameters that circumscribe the genus *Ipheion* are the asymmetry indices A_1 and A_2 and the number of 45S rDNA sites on short arms of acrocentric chromosomes. Therefore, the unifloral species of *Nothoscordum* should be excluded from *Ipheion*, which is a monophyletic group.

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