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KARYOLOGICAL STUDIES OF SOME ALLIUM L. (AMARYLLIDACEAE) SPECIES IN IRAN

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Four species belonging to two subgenera of Iranian *Allium* species were chromosomally and karyotypically assessed, using squash technique and feulgen stain. Ploidy level of *A. longivaginatum*, *A. hooshidaryae* and *A. remediorum* (2n = 2x = 16) are reported for the first time. Results indicated that *A. rotundum* is tetraploid (2n=4x= 32). Two chromosome types (''m'', ''sm'') formed different karyotypic formulas. Mean chromosome length varied from 7.8-13.56 µm. The results show that *Allium longivaginatum* has the most asymmetrical karyotype.

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Key words: *Allium* species; chromosome number; cytogenetic; karyotype; Iran

مطالعات کروموزومی بر روی برخی از گونه های سرده پیاز (تیره نرگسیان) در ایران

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چهار گونه از دو زیر جنس پیازهای ایران، با استفاده از تکنیک اسکواش و رنگ آمیزی فولگن، مورد ارزیابی کروموزومی و کاریوتیپی قرار گرفتند. سطح پلوییدی گونه‌های A. Longivaginatum، A. hooshidaryae و A. remediorum (2n = 2x = 16) برای نخستین بار گزارش می شود. نتایج نشان داد که A. rotundum تتراپلویید (2n=4x= 32) است. دو تیپ کروموزومی (''m''، ''sm'') فرمول های کاریوتیپی مختلفی را تشکیل دادند. میانگین طول کروموزوم بین ۷/۸ –۱۳/۵۶ میکرومتر، متغیر بود. نتایج نشان می دهد که گونه A. longivaginatum دارای نامتقارن ترین کاریوتیپ است.

INTRODUCTION

Allium is one of the largest genera of monocots comprising currently more than 900 accepted species (World Checklist 2014) naturally occurring on the northern hemisphere. There is a main center of diversity in the eastern Mediterranean area, Southwest and Central Asia where *Allium* species constitute an important part of different plant societies (Fritsch & Abbasi 2013). The most common basic chromosome number in *Allium* is x = 8, but other numbers (x=7, 9, 10, 11) and variation in ploidy also occurs (2n=14–68) (Fritsch & Astanova 1998; Zhou & al. 2007). The majority of the Old World species have the basic chromosome number of x = 8 (Choi & Cota-Sanchez 2010). Among the 121 Iranian species of *Allium*, 76 species and subspecies are assigned to subgen. *Melanocrommyum* (Fritsch & Abbasi 2013) that is very diverse in Southwest Asia, particularly in Iran and Turkey. They are mostly diploid (x=8) with nearly uniform karyotypes (Fritsch & al. 2010; Fritsch 2012). In a karyological study on 23 species of this subgenus (Fritsch and Astanova 1998), all species were reported as diploid with 2n=16. Hosseini and Go (2010) and Akhavan & al. (2015), in cytological study of different species of *Allium*, reported diploid (2n=16) or tetraploid (2n=32) for the studied species.

Subgenus *Allium* is the largest subgenus of *Allium* comprising by far the largest number of species and section *Allium* is the most species-rich in the genus. In the subgenu *Allium*, often more chromosomes have

exceptionally long satellites comparing to other subgenera (Hanelt & al. 1992), which characterizes this group karyologically. Wendelbo (1971) assigned *A. longivaginatum* and *A. rubellum* under sect. *Scorodon* in subg. *Allium* but they were transferred by Khassanov (1998) and Friesen & al. (2006) into sect. *Longivaginata* and sect. *Avulsea* respectively. Other 9 Iranian species of section *Scorodon* were transferred to different sections as well. *Allium hooshidaryae* Mashayekhi, Zarre and R.M. Fritsch (Mashayekhi & al. 2005) was assigned to sect. *Compactoprason* but this species has been transferred to section *Pseudoprason*. Section *Pseudoprason* (Wendelbo) K. Perss. & Wendelbo includs three species (Fritsch & Abbasi 2013). *Allium hooshidaryae* and *A. sanandajense* known only from Kurdistan province up till now and *A. koelzii* mostly distributed in north east of Iran. *Allium remediorum* R.M. Fritsch was assigned to section *Procerallium* by Fritsch & al. (2010) and it shows only gradual morphological differences with typical *A. jesdianum*. Molecular markers (Gurushidze & al. 2010; Fritsch & al. 2010) presented evidence for a remarkable genetic distance, strongly supporting its recognition as separate species. According to the IPCN (Index to Plant Chromosome Numbers, www.tropicos.org/ Project/IPCN), information on the karyology of *A. hooshidaryae*, *A. longivaginatum* and *A. remediorum* are not documented.

The main aim of this study was to determine chromosome number, ploidy level and general information on karyotype characteristics of *A. hooshidaryae* from sect. *Pseudoprason*, *A. longivaginatum* from sect. *longivaginata*, *A. remediorum* from sect. *Procerallium*, *A. rotundum* from sect. *Allium* and to examine chromosome and karyotype parameters associated with interspecific variation.

MATERIALS AND METHODS

The bulbs of four species, namely, *A. longivaginatum* Wendelbo, *A. rotundum* L., *A. hooshidaryae* Mashayekhi, Zarre and R.M. Fritsch and *A. remediorum* R.M. Fritsch were collected from natural habitats during 2016. Details regarding the studied materials are presented in table 1. After collecting samples, cold treatment was applied to break the dormancy of bulbs. For the analysis of somatic chromosomes, 1–1.2 cm long fresh root tips were collected from the cultivated bulbs. Roots were pretreated in a- bromo naphthalene for 3 hours at 4 °C. Sample roots were subsequently washed three times with distilled water (each 5 min) at room temperature. They were subsequently fixed in Carnoy's fixative (glacial acetic acid: ethanol; 3:1) overnight at 4 °C. After through washing with distilled water, excised roots were transferred to 70 % (v/v) aqueous ethanol and stored in a refrigerator until required. Hydrolysis was carried out with 1 M HCl for 15 min at 60 °C. Afterwards, root tips were stained in feulgen solution for 1 hour. The stained root tips were afterwards squashed in a driblet of 45 % (v/v) acetic acid. At least five metaphase plates were analyzed. The best metaphase plates were photographed, using a DP72 digital camera attached to the BX51 Olympus microscope. The morphology of chromosome is explained using nomenclatures proposed by (Levan & al. 1964). Eight chromosomal parameters were either measured or calculated, including long (L) and short (S) arms, chromosome length (CL) and arm ratio (AR), r value, relative length of chromosome (RL), chromosome form percentage (F %) and centromeric index (CI %). Moreover,12 different methods were used to assess the degree of karyotype asymmetry, comprising total form percentage (TF %; Huziwara 1962), total chromosome length of the haploid complement (HCL) and coefficient of variation of chromosome length (CV_{CL}; Paszko 2006), mean centromeric asymmetry (X_{CA}; Peruzzi and Eroglu 2013), mean centromeric index (X_{CI}) coefficient of variation of centromeric index (CV_{CI}; Paszko 2006), asymmetry index (AI; Paszko 2006), degree of karyotype asymmetry (A; Watanabe & al. 1999), percentage of karyotype symmetry (S%), Intra chromosomal assmetry Index (A_1 and A_2; Romero-Zarco 1986), percentage karyotype asymmetry index (AsK %; Arano 1963), Stebbins' classification (1971) and Romero-Zarco (1986) method.

RESULTS

Representative somatic metaphase plates and idiograms of haploid complement of studied *Allium* species are demonstrated in figs. 1 & 2, respectively. Comparison of length of chromosome arms indicated that subgenus *Melanocrommyum* had remarkable longer chromosomes (11.2 and 13.5µm) than subgenus *Allium* (7.8 and 8.1).

The mean value of total chromosome length (TL) was determined as 10.14 µm varying from 7.8 µm (*A. longivaginatum*) to 13.56 µm (*A. remediorum*). The mean CI of the complement varied from 41 % (*A. remediorum*) to 45 % (*A. rotundum*). Using Levan & al. (1964) chromosome nomenclature. Two chromosome types of ''m'' (centromere at median region) and ''sm'' (centromere at sub medium region), formed 4 different karyotypic formula. The karyotypic formula of species are shown in table 2. In addition, there is a pairs of satellites with size 2-3.84 µm, in *A. hooshidaryae* (S3-E1) locating on short arm. Karyotypes of all 4 species

were classified in the 1A, 1B, 2A class of Stebbins classification (Stebbins 1971).

Based on 12 different methods, the karyotype asymmetry was assessed (table 2). Most of the methods showed different symmetric species but the same asymmetric species. For example, the highest value TF % was detected in *A. longivaginatum* (44.12; table 2; the most symmetric) and the lowest value was observed in *A. rotundum* (39.37; the most asymmetric). The highest and the lowest values of coefficient of variation (CV %) were identified on *A. rotundum* (21.07 %; the most asymmetric) and *A. remediorum* (7.74 %; the most symmetric), respectively. The highest value of X_{CA} was identified in *A. rotundum* (19.13 %) while *A. longivaginatum* demonstrated the lowest value (12.33 %). The mean CV_{CI} was determined as 11.09µm, varying from 7.7 µm (*A. hooshidaryae*) to 16.23 µm (*A. rotundum*). The highest value of A was identified in *A. rotundum* (0.19) while *A. longivaginatum* demonstrated the lowest value (0.12). The highest and the lowest values of S% were distinguished in *A. remediorum* (76.5) and *A. rotundum* (53.2), respectively. The highest value of AsK % was identified in *A. rotundum* (60.6) while *A. longivaginatum* demonstrated the lowest value (55.8). Therefore as we can see in table 2, all karyotype asymmetry method recognized, *A. longivaginatum* as a most asymmetric species.

DISCUSSION

Three *Allium* species (*A. longivaginatum, A. Hooshidaryae* and *A. remediorum*) were diploid (2n=2x=16), which was reported for the first time. *Allium rotundum* was tetraploid (2n=4x=32) and this is in agreement with previous studies (Ruiz Rejon & Sanudo 1976; Jacobsen & Ownbey 1977; Özhatay 1993). However, 2n=16 and 2n=48 have been reported for this species as well (Pogosian 1983; Murín & al. 2000). A pair of satellite were observed in *A. hooshidaryae*, which were located on the short arm of the chromosomes.

In the present study, two chromosome types of ‘‘m’’ and ‘‘sm’’ were identified in four different species of *Allium*. The results of this study and previously published data (Akhavan & al. 2015; Genç & al. 2013; Fritsch & Astanova 1998) indicate the symmetric karyotype comprising of 5-8 metacentric and 0-4 submetacentric chromosomes as a common karyological feature of A. subgen. *Melanocrommyum*. Hosseini and Go (2010) have reported ‘‘st’’ chromosome type in subgen. *Allium* (*A. iranicum* Wendelbo, in different populations), but we observed almost symmetric karyotype in *A. rotundum* and *A. longivaginatum*.

The karyotype asymmetry was assessed, based on either qualitative classification method or quantitative indices. Hence, according to Stebbins (1971), karyotypes of different species of studied Iranian *Allium* in the present report were located in the groups of 1A, 2A and 2 B which are classified as relatively symmetric. Paszko (2006) discussed that Stebbins' (1971) classification is a qualitative method, hence is less powerful and flexible in terms of the types of conclusions it can provide. Thus, more quantitative indices need to be considered to achieve greater measurement accuracy. For example, Akhavan & al. (2015) reported the average of CV_{CL} as 13.38 while this parameter in our study was 9.7 for subgen. *Melanocrommyum*. The mean value of CV_{CI} and AI parameters were 9.82 and 1.63 respectively. In this study, it was 8.28 and 1.29 for species of subgen. *Melanocrommyum* and symmetric species of this subgenus were confirmed in both study. In case of TF %, CVCL, A1, A2, AsK %, S%, A, XCA, XCI and CVCI all of the species were symmetric, but the most asymmetric species was *A. longivaginatum*. Based on the theory that more symmetric karyotypes are more primitive (Stebbins 1971), *A. rotundum* might represent the most primitive karyotype among studied specie. Variation between indices value were observed in karyology of studied species and these differences were significant enough to provide species discrimination at subgeneric level and this was confirmed by cluster analysis based on TF%, AsK%, A and XC_I. However, the karyotype homogeneity and similar chromosome numbers are not valuable characters in distinguishing some closely related taxa at section level. In this study a few number of species belong to four different section were examined and we cannot generalize this issue for the genus *Allium*. Future investigation of chromosomal karyotype with enhancement of species number from different sections may be useful in clarifying the sectional delimitation.

Table 1. Sampling locations and chromosome numbers of *Allium* Species.

Subgenus	Section	Species Code	Species	Location	Origin	2n
Allium	*Allium*	S1	*A. rotundum*	Prov. East Azerbaijan, Tabriz, Misho mountain	non-Endemic	32
Melanocrommyum	*Longivaginata*	S2	*A. longivaginatum*	Prov. East Azerbaijan, Ahar, Goicha bel Village	Endemic	16
	Pseudoprason	S3-E1	*A. hooshidaryae* (E1)	Prov. West Azerbaijan, Bookan, The fields of near the road	Endemic	16
		S3-E2	*A. hooshidaryae* (E2)	Prov. Kurdistan, Divandarreh, Doozakhdarreh village	Endemic	16
	Procerallium	S4	*A. remediorum*	Prov. Kurdistan, Sanandaj, Shian Village	Endemic	16

Table 2. Mean chromosomal and karyotypic parameters of *Allium* spp. S1: *A. longivaginatum*, S2: *A. rotundum*, S3: *A. hooshidaryae*, S4: *A. remediorum*. S: short arm, L: long arm, TL: total chromosome length, AR: arm ratio, RL: relative length of chromosome, F%: chromosome form percentage, CI: centromeric index, HCL: total chromosome length of the haploid complement, TF%: total form percentage, CVCL: coefficient of variation of total chromosome length, A1: intra chromosomal asymmetry index, A2: inter chromosomal asymmetry index, AI: asymmetry index, AsK%: arano index of karyotype asymmetry, S%: symmetry index, A: degree of karyotype asymmetry, XCA: mean centromeric asymmetry, XCI: mean centromeric index, CVCI: coefficient of variation of centromeric index.

Parameters	Species				Mean	Range	Species of range	
	S1	S2	S3	S4			Min	Max
S (µm)	3.15	3.44	5.71	4.70	4.25	3.15-5.71	S1	S3
L (µm)	4.85	4.35	7.85	6.51	5.89	4.35-7.85	S2	S3
TL (µm)	8.009	7.801	13.56	11.21	10.14	7.8-13.56	S2	S3
AR	1.54	1.31	1.38	1.40	1.40	1.31-1.54	S2	S1
r value	0.69	0.79	0.73	0.72	0.73	0.69-0.73	S1	S3
RL%	12.50	6.25	12.52	12.51	10.93	6.25-12.52	S2	S3
F %	4.92	2.75	5.27	5.23	4.54	2.75-5.27	S2	S3
CI	0.45	0.43	0.42	0.41	0.42	0.41-0.45	S4	S1
HCL	64.07	124.81	108.55	89.71	96.78	64.07-124.81	S1	S2
TF %	39.37	44.12	42.19	41.91	41.89	39.37-44.12	S1	S2
CV_{CL}	21.07	11.94	11.66	7.74	13.10	7.74-21.07	S4	S1
A_1	0.303	0.206	0.26	0.27	0.25	0.2-0.3	S2	S1
A_2	0.21	0.11	0.11	0.07	0.12	0.07-0.21	S4	S1
AI	3.41	1.38	0.89	0.68	1.59	0.68-3.41	S4	S1
AsK %	60.62	55.87	57.80	58.08	58.09	55.87-60.62	S2	S1
S%	53.24	69.27	71.63	76.52	67.66	53.24-76.52	S1	S4
A	0.19	0.12	0.15	0.16	0.15	0.12-0.19	S2	S1
X_{CA}	19.13	12.33	15.60	16.11	15.79	12.33-19.13	S2	S1
X_{CI}	0.405	0.43	0.42	0.41	0.41	0.40-0.43	S1	S2
CV_{CI}	16.23	11.57	7.70	8.87	11.09	7.7-16.23	S3	S1

	Species				
	S1	S2	S3-E1	S3-E2	S4
ST[a]	1B	2A	2A	2A	1A
KF[b]	12m+4sm	28m+4sm	14m+2sm	16m	12m+4sm

[a] ST Stebbin's (1971) classification, [b] KF Karyotype formula

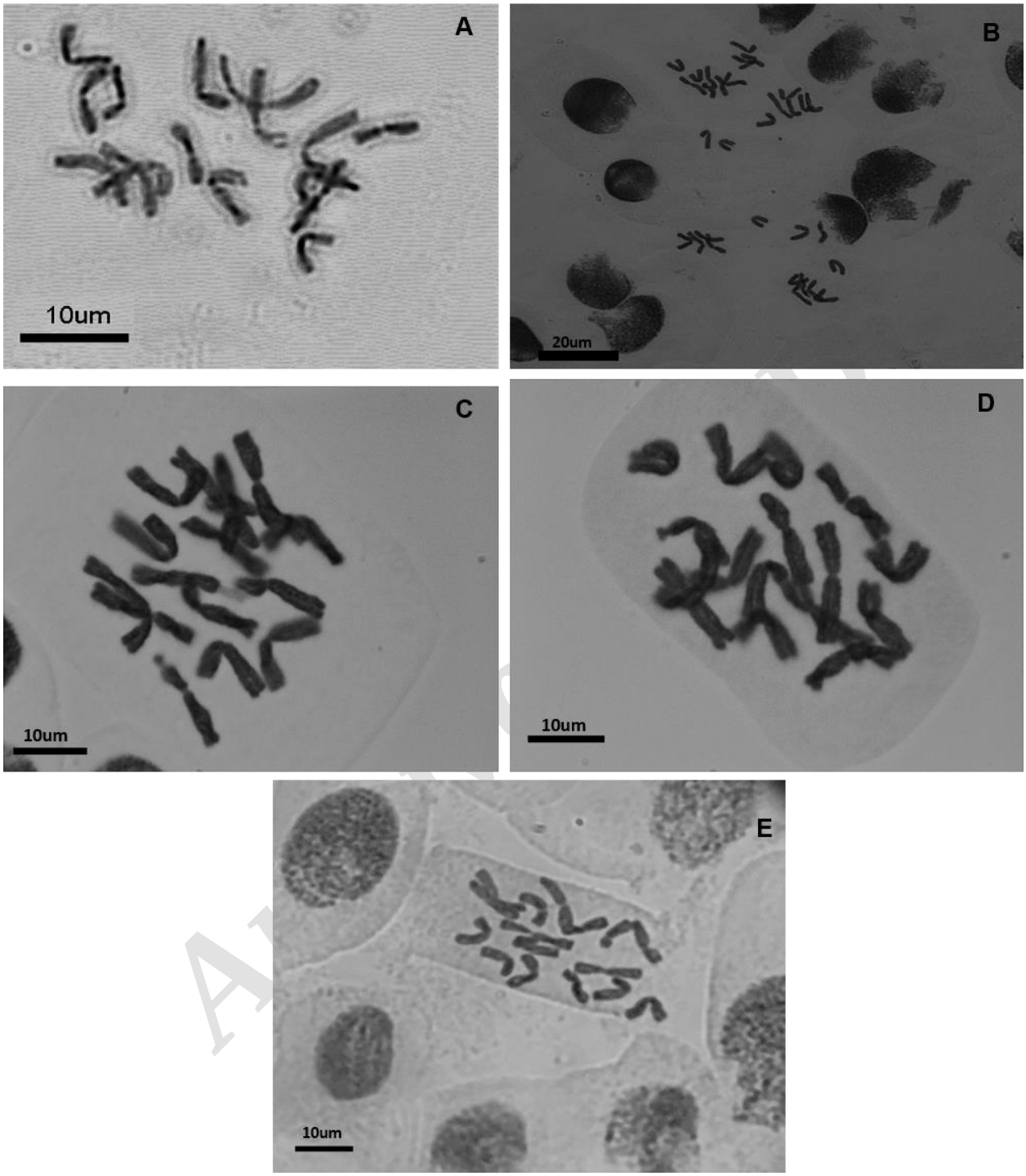

Fig. 1. Somatic chromosomes in *Allium*. A, *A. longivaginatum*; B, *A. rotundum*; C, *A. hooshidaryae* (E1); D, *A. hooshidaryae* (E2); E, *A. remediorum*.

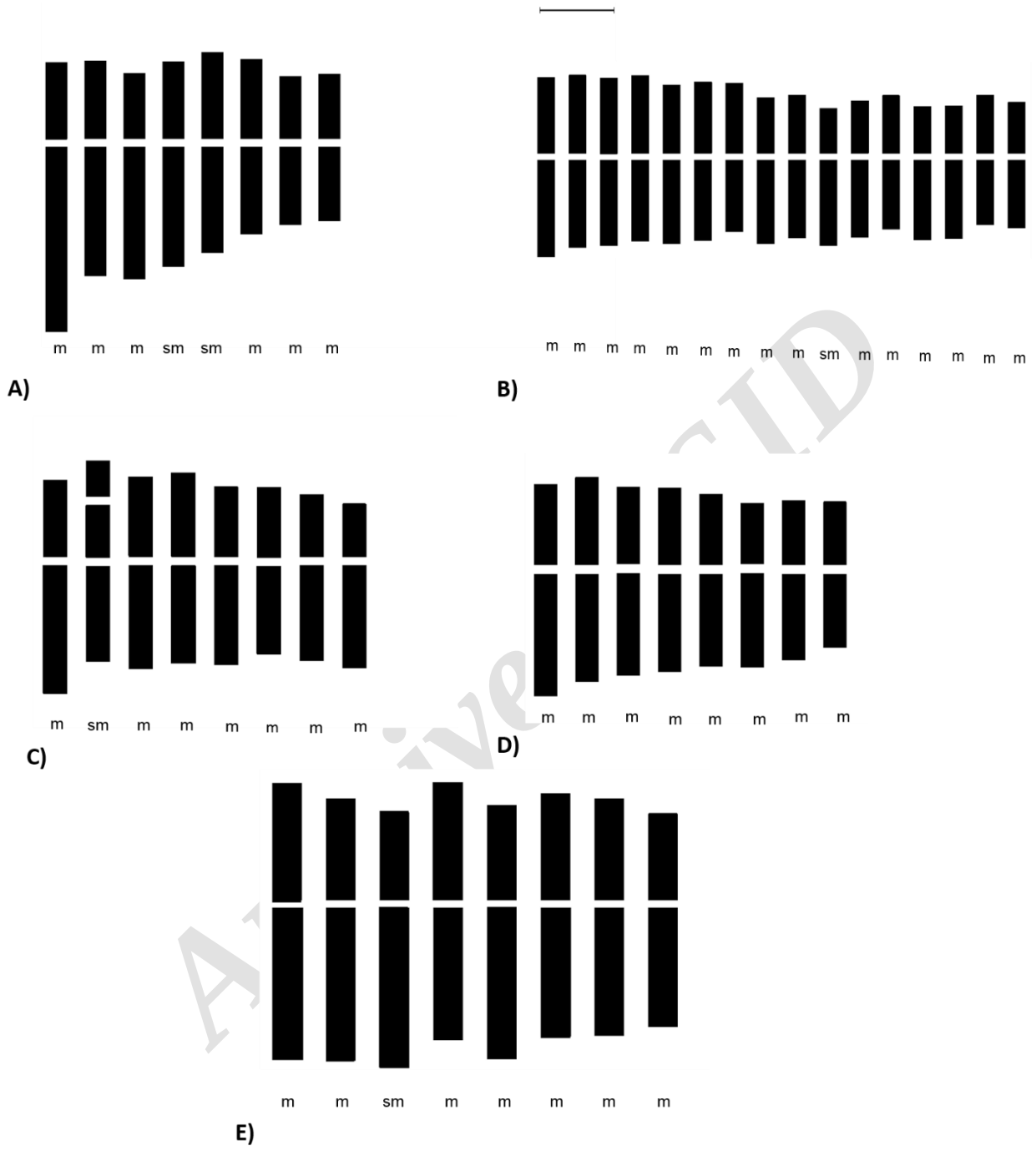

Fig. 2. Idiograms representing the mean karyotypes of the investigated species. A, *A. longivaginatum*; B, *A. rotundum*; C, *A. hooshidaryae* (E1); D, *A. hooshidaryae* (E2); E, *A. remediorum*. Bar 5 µm

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