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Karyological study of Balkan endemics *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka (Caryophyllaceae) in Bulgaria

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Abstract

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The study presents the somatic chromosome number of *Moehringia jankae* 2*n* = 24, and confirms the one for *Moehringia grisebachii* 2*n* = 24. The karyotype morphology of ten populations of the two species were described in detail. Karyotype variability was registered for *M. jankae* (Hcl = 17.99 - 37.58) and *M. grisebachii* (Hcl = 15.30 - 36.63), while the chromosome analysis showed mainly metacentric and submetacentric chromosomes for both species. Five different karyotype formulae were registered for all studied populations. Based on the calculated values of Mca (0.46 - 1.34 and 0.55 - 1.21), A1 (0.117 - 0.141 and 0.117 - 0.217), TF% (41.97 - 47.26 and 42.76 - 46.73) and Ask% (52.74 - 58.03 and 53.27 - 57.24), it was determined that the karyotype of *M. jankae* and *M.grisebachii* is symmetric. The chromosome number of *M. jankae* is announced for the first time.

Keywords: *Moehringia jankae*; *Moehringia grisebachii;* chromosome; karyotype; endemic plant species

Introduction

The genus *Moehringia* L. is composed of approximately 35 species (IPNI, 2019), distributed mainly on the Balkan Peninsula and the Central European Alps (Hind, 1988; Minuto et al., 2006; Fior & Karis, 2007). Most of species in Europe are considered local endemic. In Bulgaria are spread the endemic species *Moehringia jankae* and *Moehringia grisebachii*. Both species are included in the category "endangered" in the Red Book of Bulgaria (Stoeva, 2015; Stoyanov, 2015). Moreover, *M. jankae* is a protected species (Biological Diversity Act, 2002), classified as "Data Deficient" (DD) on a global scale (Bern Convention, 1979; Directive 92/43/EEC, 1992).

According to recent studies (Zhelyazkova et al., 2018) the populations of *M. jankae* in Bulgaria are found on the territory of Eastern Balkan Range (Sliven, Sinite Kamani Natural Park), while only some populations of *M. grisebachii* are found in those territories. Both species prefer rock crevices mainly in quartz porphyry, conglomerates and granite acid rocks. Similar to other species from the genus *Moehringia* L., *M. jankae* and *M. grisebachii* have limited distribution, often small populations, most of them topographically isolated from each other (Akeroyd, 1981; Minuto et al., 2006; Fior & Karis, 2007; Grozeva et. al., 2016; Lorite et al., 2018; Zhelyazkova et al., 2018). This particular characteristic determines their vulnerability in regards to genetic diversity and the need for their in depth recognition, study and conservation.

The development and progress of the molecular cytogenetics contributes for a better karyotype characterization of the endemic species, as well as for determining the bonds between similar species, when the ploidy level and chromosome number are the same. Determining the chromosome number, ploidy level, size of genome, karyotype asymmetry and other parameters helps reveal the direction of chromosome evolution. Usually in contemporary literature the data are often combined with independent one from molecular phylogenetics (Yakovlev & Peruzzi, 2012; Manhaes et al., 2019).

Cytological studies of genus *Moehringia* L. establish their chromosome number as x = 12, 13, 18, 24, 25, 26 (Rohweder, 1939; Litardiere, 1948; Sokolovskaya, 1960; Zhukova, 1967; Gadella & Kliphuis, 1971; Findley & McNeill, 1974; Hindakova, 1974; Májovský et al., 1976; Kieft & Van Loon, 1978; Strid, 1980; Kirschner et al., 1982; Goldblatt, 1985; Luque & Lifante, 1991; Gurzenkov, 1995; Probatova et al., 2006; Probatova, 2014; Probatova et al., 2018; Zhelyazkova et al, 2020). The aim of the study is to establish the somatic chromosome number and karyotype of *M. jankae* and *M. grisebachii* from their populations in Eastern Balkan Range.

Material and Methods

Mature seeds were collected during the vegetation period 2017 – 2019 from 10 natural populations of *M. jankae* and 10 natural populations of *M. grisebachii* on the territory of Eastern Balkan Range (Table 1; Fig.1 and Fig. 2).

Table 1. Natural populations of species *Moehringia jankae* and *Moehringia grisebachii* and their location in Eastern Balkan Range

Pop №	Species *Moehringia jankae*, location	Pop №	Species *Moehringia grisebachii*, location
Mj1	Kaloyanovi kuli area N 42° 42.755′ E 26° 23.015′, 756 m	Mg1	The east of Haiduschka pateka N 42° 42.785′ E 26° 21.349′, 921 m
Mj2	Haiduschka pateka area east of Karandila hotel N 42° 42.704′ E 26° 22.261′, 889 m	Mg2	The south-east of Karandila hotel N 42° 42.851′ E 26° 22.447′, 971 m
Mj3	Micro dam area N 42° 42.790′ E 26° 22.612′, 972 m	Mg3	Kaloyanovi kuli area N 42°42.833′ E 26° 23.169′, 685 m
Mj4	350 m south of Karandila hotel N 42° 42.709′ E 26° 22.355′, 933 m	Mg4	The west of Karandilska polyana N 42° 42.818′ E 26° 22.482′, 965 m
Mj5	450 m southwest of Karandila hotel N 42° 42.712′ E 26° 22.252′, 908 m	Mg5	Gornaka area N 42° 42.828′ E 26° 23.735′, 920 m
Mj6	The south-east of Kamilata area N 42° 42.593′ E 26° 22.196′, 851 m	Mg6	Haiduschka polyana N 42° 42.290′ E 26° 21.655′, 641 m
Mj7	The rocks between Karandila hotel and Kamilata N 42° 42.726′ E 26° 22.349′, 913-952 m	Mg7	Micro dam area N 42° 42.815′ E 26° 22.647′, 951 m
Mj8	The north of Kamilata area N 42° 42.673′ E 26° 22.217′, 866 m	Mg8	The east of Micro dam area N 42° 42.818′ E 26° 22.482′, 975 m
Mj9	The rock formations near Haiduschka pateka N 42° 42.603′ E 26° 22.180′, 857 m	Mg9	The south of Karandilska polyana N 42° 42.828′ E 26° 22.530′, 956 m
Mj10	The east of Kamilata area N 42° 42.647′ E 26° 22.198′, 863-869 m	Mg10	Kamilata area N 42° 42.595′ E 26° 22.181′, 838 m

Fig. 1. Habitat and populations of species *Moehringia jankae* Griseb. ex. Janka

Fig. 2. Habitat and populations of species *Moehringia grisebachii* Janka

Seeds were placed to germinate on damp filter paper in petri dishes at room temperature in laboratory conditions. After growing to 1 - 1.5 cm the root tips were cut and treated with a drop of Colchicine for 3.5 h at room temperature, then fixed in Clark's fixation agent for 10 - 16 h at 4°C. After that the root tips were stained in 1% natural aceto-orcein solution for 24 h at room temperature. The aceto-orcein squash method was applied (Tanaka, 1959). Observation and pictures were made with Olympus U-TVO.5XC-3 camera and Olympus BX51 microscope, Japan. Karyomorphological measurements and building of idiograms were done using Drawid software (Kirov et al., 2017). From each population with at least five metaphase plates were measured the chromosomes in them and was determined the arm length (short arm S, long arm L), arm ratio AR, mean length of chromosomes CL, relative length percentage RL and centromere index CI, centromere position and karyotype formula.

Herbarium specimen of the karyologically studied plants were deposited in the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences. For determination and comparison of the established karyotypes were used and calculated different parameters, including: chromosome type (Grif & Agapova, 1986); the sum of the length of the haploid chromosome number Hcl; mean centromeric asymmetry Mca (Peruzzi & Eroğlu, 2013), where A is degree of asymmetry of karyotype (Watanabe et al., 1999); coefficient of variation of chromosome length CV_{CL} (Paszko, 2006); intrachromosomal asymmetry A_1 and interchromosomal asymmetry A_2 (Romero Zarko, 1986); total form percentage TF% (Huziwara, 1962); karyotype asymmetry index percentage Ask% (Arano, 1963); symmetric index Syi (Greilhuber & Speta, 1976).

Results and Discussion

Moehringia jankae

Based on the obtained results we could conclude that the detected chromosome number of this species is 2n = 24 and it is reported for the first time. Dominant type chromosomes are the metacentric chromosomes in all populations. In the populations south-east of Kamilata (Mj6), north of Kamilata (Mj8) and Haiduschka pateka (Mj9) was established karyotype of 24 metacentric chromosomes - $2n$ = 24m. Whereas in the populations from Haiduschka pateka east of Karandila hotel (Mj2), Micro dam (Mj3) and between Karandila hotel and Kamilata area (Mj7) the established karyotype is $2n$ = 22m + 2sm (22 metacentric chromosomes and 2 submetacentric). The populations from Kaloyanovi kuli (Mj1) and 350 m south of Karandila hotel (Mj4) have the karyotype of 20 metacentric and 4 submetacentric chromosomes - $2n$ = 20m + 4sm. The karyotype of 18 metacentric and 6 submetacentric chromosomes were discovered in the population 450 m southwest of Karandila hotel (Mj5) $2n$ = 18m + 6sm. For the population east of Kamilata area (Mj10) were established karyotype of 20 metacentric, 2 submetacentric and 2 intercentric chromosomes - 2n = 20m + 2sm + 2i. The data for the karyotype of the studied ten populations are presented in Tables 2 and 3. In Fig. 3 and 4 are shown respectively the chromosomes of these populations and their ideograms.

Table 2. Diploid chromosome number (2n), karyotype formula, chromosome length range (µm), total sum of the haploid chromosome length (Hcl, µm) of studied populations of species *Moehringia jankae* Griseb. ex Janka

Population	2n	Karyotype formula	Chromosome length range (µm)	Hcl
Mj1	24	2n = 20m + 4sm	0.81 – 4.11	25.60
Mj2	24	2n = 22m + 2sm	1.26 – 3.87	28.39
Mj3	24	2n = 22m + 2sm	1.37 – 4.56	31.71
Mj4	24	2n = 20m + 4sm	1.29 – 5.90	37.13
Mj5	24	2n = 18m + 6sm	0.80 – 2.79	18.96
Mj6	24	2n = 24m	0.88 – 2.38	17.99
Mj7	24	2n = 22m + 2sm	1.74 – 7.16	37.58
Mj8	24	2n = 24m	1.14 – 3.59	24.17
Mj9	24	2n = 24m	1.01 – 4.25	27.45
Mj10	24	2n = 20m + 2sm + 2i	0.81 – 4.41	24.50

For all studied populations of *M. jankae,* the longest chromosome is 7.16 ± 0.18 µm, whereas the shortest one is 0.80 ± 0.02 µm. The total sum of haploid chromosome length varied from 17.99 - 37.58 for Mj6 (south-east of Kamilata) and Mj7 (between Karandila hotel and Kamilata) (Table 2). The mean values for centromere index and arm ratio ranges from 43.19 - 47.39 and 0.77 - 0.90 respectively for populations Mj1 (Kaloyanovi kuli) and Mj8 (north of Kamilata). Relative length was similar in the most of studied populations – 8.36 for Mj3, Mj4, Mj7 and Mj8; 8.38 for Mj1, Mj2 and Mj9; 8.39 for Mj5 and Mj10 (Table 3).

Similarity was registered in karyotype characteristics in the some populations, which are closer to each other. For example, both population Mj2 and Mj9 (around Haiduschka pateka) and their characteristics such as: mean S (1.05 and 1.06), mean L (1.31 and 1.23), CL (2.36 and 2.28). Also populations Mj5 and Mj6 have similar karyotypes, where mean S - 0.67 and 0.69, mean L - 0.91 and 0.81, mean CL - 1.58 and 1.50. On the other hand these 4 populations have a different karyotype formula. Mean length of the chromosome in our study assort the populations into two main groups: in the first they are Mj5 and Mj6 (1.58 - 1.50), in the second they are Mj1, Mj2, Mj3, Mj8, Mj9 and Mj10 (2.01 - 2.64). The population Mj4 (CL - 3.09, Hcl - 37.13) and Mj7 (CL - 3.96, Hcl - 37.58) were different more than the other studied population of species *M. jankae* (Table 2, 3).

The ratio of the longest to shortest chromosome for the studied populations of *M. jankae* ranges from 2.7:1 to 5.07:1.

Table 3. Karyotype characteristics of studied populations of species *M. jankae*: S - length of the short arm, L - length of the long arm, AR –arm ratio CL –length of the chromosome, RL - relative length percentage, CI - centromere index

Population	S, µm mean±SD	L, µm mean±SD	AR mean	CL, µm mean±SD	RL mean	CI mean±SD
Mj1	0.89±0.04	1.23±0.09	0.77	2.13±0.09	8.38	43.19±1.93
Mj2	1.05±0.05	1.31±0.03	0.83	2.36±0.05	8.38	45.12±1.56
Mj3	1.20±0.06	1.45±0.09	0.85	2.64±0.09	8.36	45.77±1.55
Mj4	1.35±0.10	1.73±0.10	0.82	3.09±0.11	8.36	44.98±2.18
Mj5	0.67±0.02	0.91±0.03	0.80	1.58±0.03	8.39	44.09±1.41
Mj6	0.69±0.02	0.81±0.02	0.86	1.50±0.02	8.41	46.26±1.26
Mj7	1.81±0.11	2.15±0.09	0.85	3.96±0.13	8.36	45.68±1.71
Mj8	0.95±0.02	1.06±0.04	0.90	2.01±0.05	8.36	47.39±1.21
Mj9	1.06±0.05	1.23±0.06	0.87	2.28±0.06	8.38	46.58±1.91
Mj10	0.89±0.09	1.15±0.10	0.78	2.04±0.10	8.39	43.23±3.88

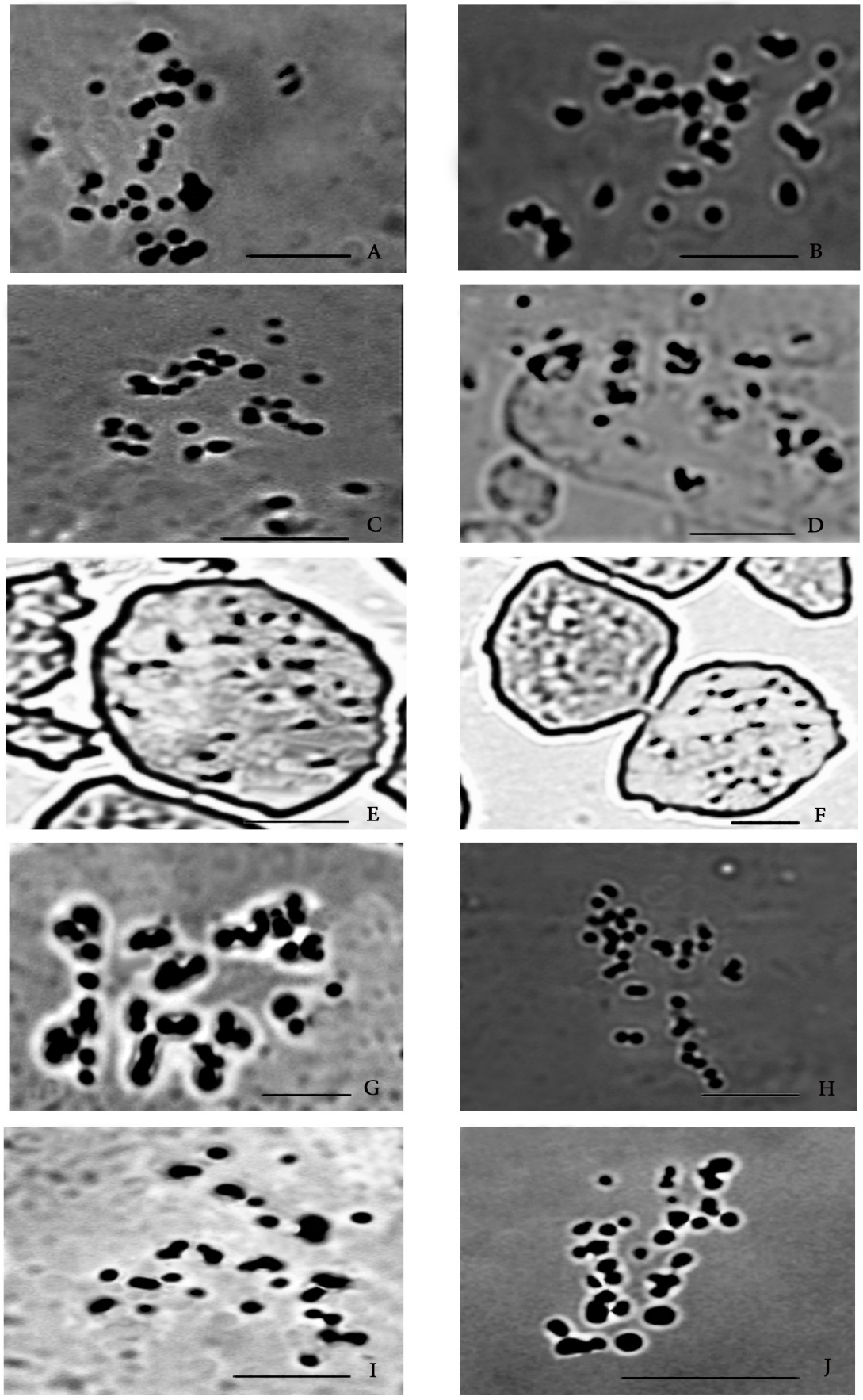

Fig. 3. Somatic chromosomes of *Moehringia jankae* in 10 populations: (A) Mj1; (B) Mj2; (C) Mj3; (D) Mj4; (E) Mj5; (F) Mj6; (G) Mj7; (H) Mj8; (I) Mj9 and (J) Mj10. Scale bar: 10 µm

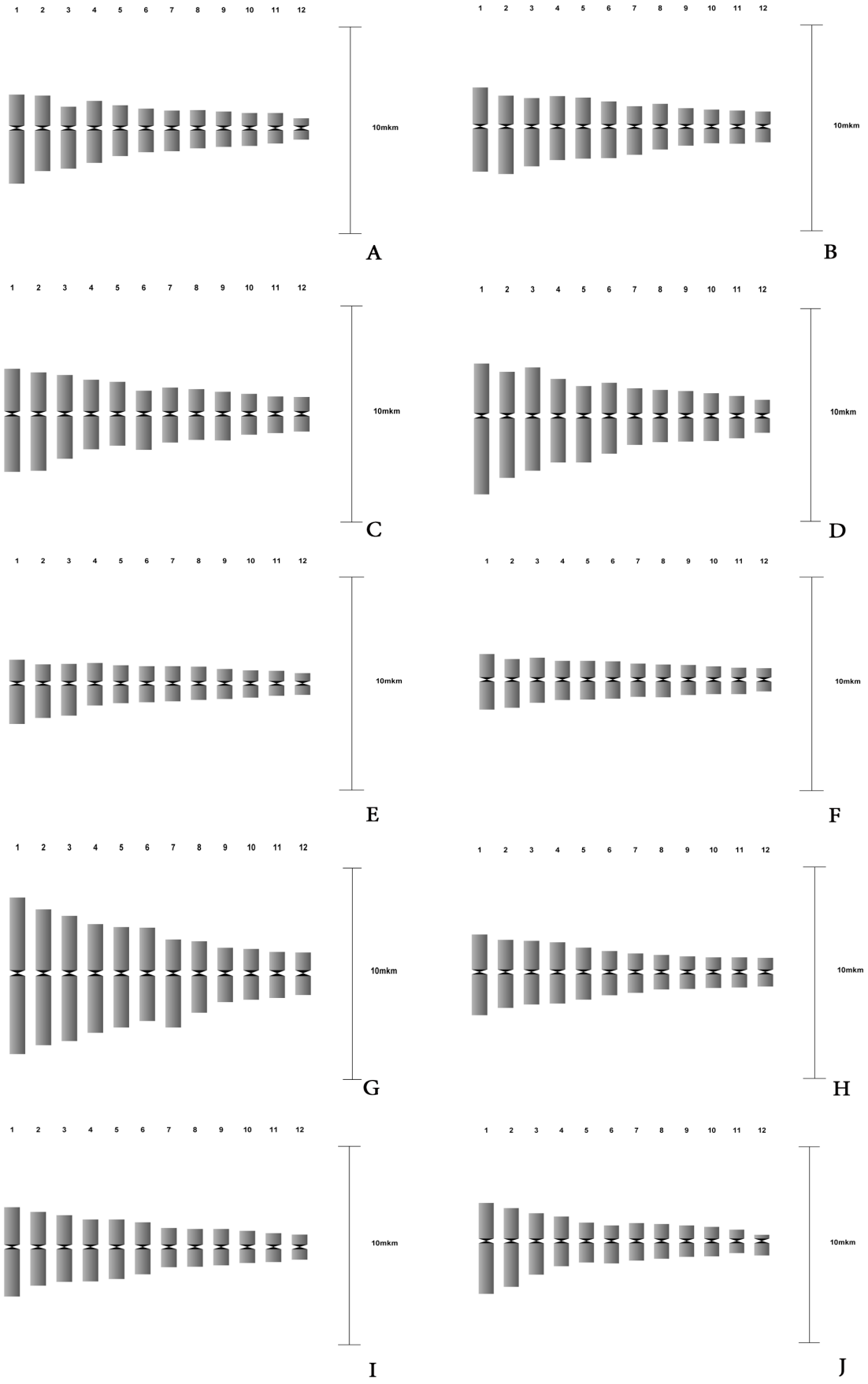

Fig. 4. Haploid idiograms of *Moehringia grisebachii* in 10 populations: (A) Mj1; (B) Mj2; (C) Mj3; (D) Mj4; (E) Mj5; (F) Mj6; (G) Mj7; (H) Mj8; (I) Mj9 and (J) Mj10

For all studied populations were calculated values of the asymmetric indices (Table 4). The TF% index for the species *M. jankae* ranged from 41.97 in population Mj1 (Kaloyanovi kuli) to 47.26 in Mj8 (north of Kamilata) and showed that the karyotype of the species is closer to symmetric. The values for intrachromosomal asymmetry A1 were lowest in Mj8 (0.005) and highest in Mj1 and Mj5 (450 m southwest of Karandila hotel) (0.013), respectively for CV_{cl} the lowest were in Mj6 (south-east of Kamilata) (1.62) and highest in Mj1 (4.64). The values for the interchromosomal asymmetry A2 varied from 0.016 to 0.046 in the populations Mj8 (north of Kamilata) and Mj1 (Kaloyanovi kuli), respectively the M_{ca} varied from 0.46 to 1.34 in the same populations. The AsK% index also showed lowest and highest value in Mj8 and Mj1, from 52.74 - 58.03. The values of Syi vary from 72.31 to 89.63 for these two populations (Mj1 and Mj8).

Table 4. Asymmetry indices of studied populations of species *Moehringia jankae* Griseb. ex Janka. Total form percentage (TF%), karyotype asymmetry percentage (AsK%), Watanabe index (A), symmetric index (Syi), intrachromosomal asymmetry (A_1), interchromosomal asymmetry (A_2), coefficient of variation of chromosome length (CV_{cl}) and mean centromeric asymmetry (M_{ca})

Population	TF%	AsK%	Syi	A	A1	A2	CV_{cl}	M_{ca}
Mj1	41.97	58.03	72.31	0.013	0.231	0.046	4.64	1.34
Mj2	44.41	55.59	79.88	0.009	0.171	0.022	2.25	0.93
Mj3	45.24	54.76	82.60	0.008	0.149	0.038	3.78	0.79
Mj4	43.93	56.07	78.33	0.010	0.175	0.032	3.23	1.01
Mj5	42.49	57.51	73.90	0.013	0.196	0.020	2.00	1.25
Mj6	46.11	53.89	85.56	0.006	0.136	0.016	1.62	0.65
Mj7	45.76	54.24	84.38	0.007	0.155	0.032	3.20	0.71
Mj8	47.26	52.74	89.63	0.005	0.099	0.025	2.49	0.46
Mj9	46.14	53.86	85.67	0.006	0.126	0.025	2.48	0.64
Mj10	43.49	56.51	76.96	0.011	0.217	0.049	4.90	1.09

Moehringia grisebachii

The chromosome number was reported as $2n = 24$ (Zhelyazkova et al., 2020) in previous studies. In this study was determined that the chromosome number of the species was 2n = 24, as well. Dominant type chromosomes are the metacentric chromosomes in all populations. The populations east of Haiduschka pateka (Mg1), south-east of Karandila hotel (Mg2), Kaloyanovi kuli (Mg3), west of Karandilska polyana (Mg4) and Karandilska polyana (Mg10) have a karyotype formula 2n = 24m (24 metacentric chromosomes). Meanwhile the populations from the areas Gornaka (Mg5) and east of Micro dam (Mg8) have a different one – 2n = 22m + 2sm (22 metacentric chromosomes and 2 submetacentric). For the population from Micro dam (Mg7) the established karyotype formula is 2n = 20m + 4sm (20 metacentric and 4 submetacentric chromosomes). The populations south of Karandilska polyana (Mg9) and Haiduschka polyana (Mg6) also have a different karyotype formula 2n = 20m + 2sm + 2i (20 metacentric, 2 submetacentric and 2 intercentric chromosomes) and 2n = 18m + 4sm + 2i (18 metacentric, 4 submetacentric and 2 intercentric chromosomes). Data for the karyotype of the studied 10 populations are shown in Table 5 and 6. In Fig. 5 and 6 are illustrated respectively the chromosomes of these populations and their idiograms.

Table 5. Diploid chromosome number (2n), karyotype formula, chromosome length range (µm), total sum of the haploid chromosome length (hcl, µm) of studied populations of species *Moehringia grisebachii* Janka

Population	2n	Karyotype formula	Chromosome length range (µm)	Hcl
Mg1	24	2n = 24m	0.74 – 2.77	17.78
Mg2	24	2n = 24m	0.86 – 1.89	15.30
Mg3	24	2n = 24m	1.29 – 4.16	31.59
Mg4	24	2n = 24m	0.66 – 3.24	20.18
Mg5	24	2n = 22m + 2sm	0.83 – 2.73	20.18
Mg6	24	2n = 18m + 4sm + 2i	1.15 – 4.33	30.12
Mg7	24	2n = 20m + 4sm	1.32 – 4.51	35.83
Mg8	24	2n = 22m + 2sm	1.05 – 3.84	26.64
Mg9	24	2n = 20m + 2sm + 2i	1.39 – 4.20	36.63
Mg10	24	2n = 24m	1.05 – 2.66	19.78

For all studied populations of *M. grisebachii*, the longest chromosome is 4.51 ± 0.06 µm, whereas the shortest one chromosome is 0.66 ± 0.09 µm. The total sum of haploid chromosome length varied from 15.30 - 36.63 for Mg2 (south-east of Karandila hotel) and Mg9 (south of Karandilska polyana) (Table 4). The mean value for centromere index ranged from 43.46 for population Mj9 to 46.82 for population Mj10 (Karandilska polyana). Arm ratio varied from 0.80 for population Mj6 (Haiduschka polyana) to 0.88 for three populations - Mj1 (east of Haiduschka pateka), Mj5 (Gornaka) and Mj10 (Karandilska polyana). Relative length ranged from 8.37 (Mj3, Mj6, Mj7, Mj9) to 8.41 (Mg2) (Table 5).

In the observed karyotype characteristics the different populations were more similar to mean S, L, CL as follow: Mj4 (0.76, 0.92, 1.68), Mj5 (0.78, 0.90, 1.68) and Mj10 (0.77, 0.88, 1.65); Mj7 (1.32, 1.67, 2.99) and Mj9 (1.32, 1.74, 3.05). Of them only populations Mg4 and Mg10 have same karyotype formulas. Although these populations were not situated closer to each other, they have a similar karyotype characteristic relative to the calculated averages. The populations Mg7 (CL - 2.99, Hcl - 35.83) and Mg9 (CL - 3.05, Hcl - 36.63) are differ more than the other studied population of species *M. grisebachii* (Table 5, 6).

The ratio of the longest to shortest chromosome for the studied populations of *M. grisebachii* ranged from 2.2:1 to 4.9:1.

Table 6. Karyotype characteristics of studied populations of species *M. grisebachii*: S - mean length of the short arm, L - mean length of the long arm, AR - arm ratio CL - mean length of the chromosome, RL - relative length percentage, CI - centromere index

PopN	S, µm mean±SD	L, µm mean±SD	AR mean	CL, µm mean±SD	RL mean	CI mean±SD
Mg1	0.69±0.03	0.80±0.02	0.88	1.48±0.05	8.40	46.63±0.71
Mg2	0.58±0.02	0.69±0.02	0.86	1.27±0.03	8.41	46.12±1.39
Mg3	1.20±0.05	1.43±0.06	0.86	2.63±0.05	8.37	46.19±1.64
Mg4	0.76±0.05	0.92±0.03	0.84	1.68±0.06	8.38	45.46±1.65
Mg5	0.78±0.03	0.90±0.03	0.88	1.68±0.05	8.40	46.73±1.20
Mg6	1.07±0.06	1.44±0.08	0.80	2.51±0.07	8.37	43.89±1.96
Mg7	1.32±0.09	1.67±0.08	0.82	2.99±0.08	8.37	44.93±2.17
Mg8	1.01±0.06	1.21±0.05	0.87	2.22±0.08	8.38	46.20±1.43
Mg9	1.32±0.06	1.74±0.09	0.78	3.05±0.07	8.37	43.46±2.07
Mg10	0.77±0.03	0.88±0.030	0.88	1.65±0.01	8.39	46.82±1.68

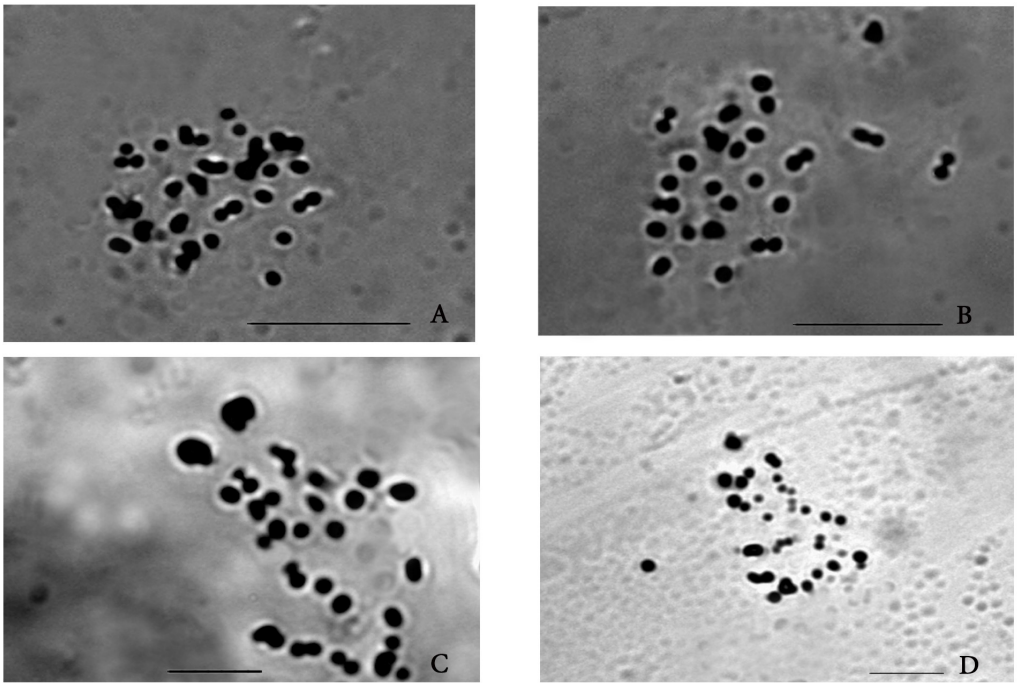

Fig. 5. Somatic chromosomes of *Moehringia grisebachii* in 10 populations: (A) Mg1; (B) Mg2; (C) Mg3; (D) Mg4; Scale bar: 10 µm

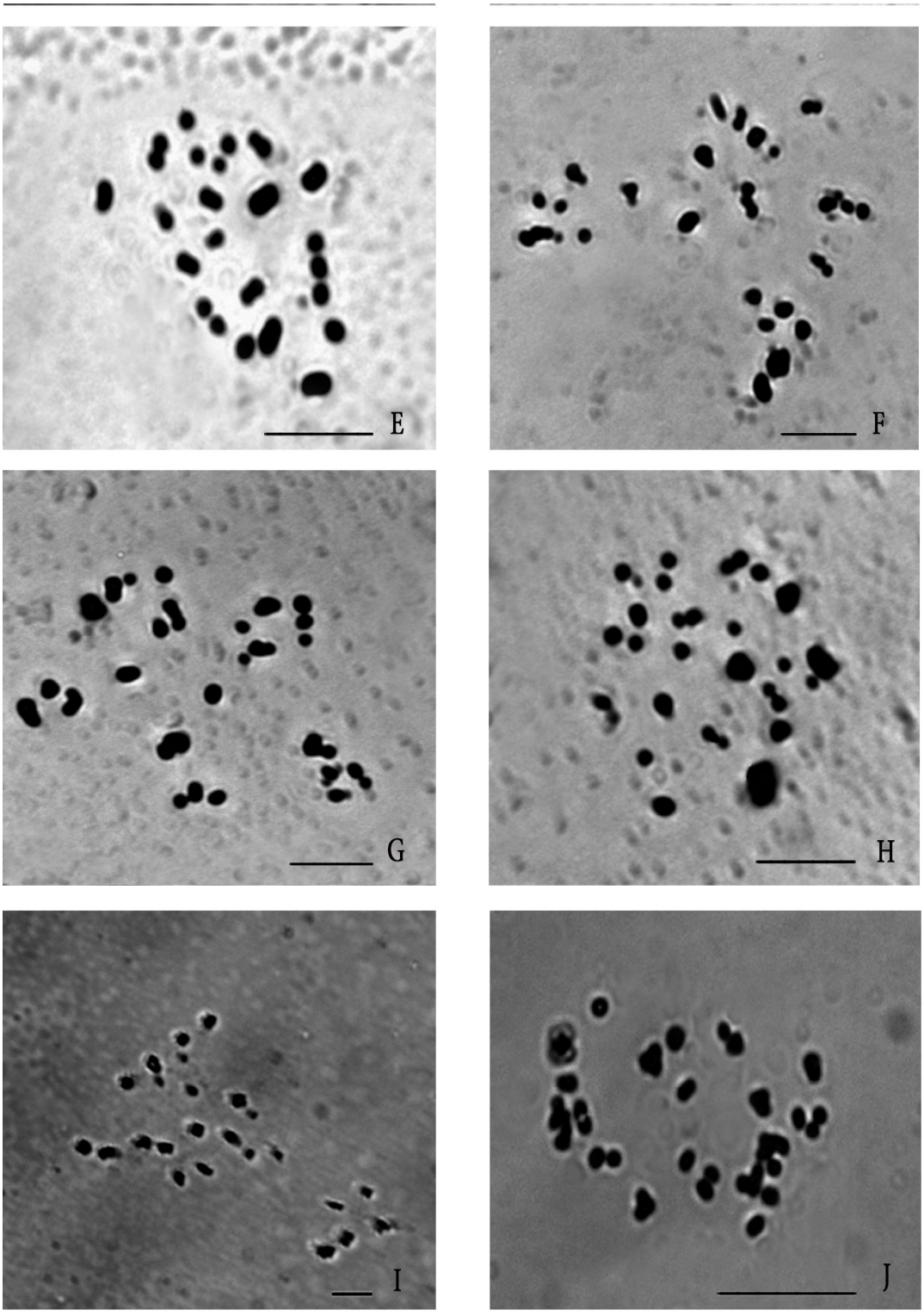

Fig. 5. /continued/ Somatic chromosomes of *Moehringia grisebachii* in 10 populations: (E) Mg5; (F) Mg6; (G) Mg7; (H) Mg8; (I) Mg9 and (J) Mg10. Scale bar: 10 µm

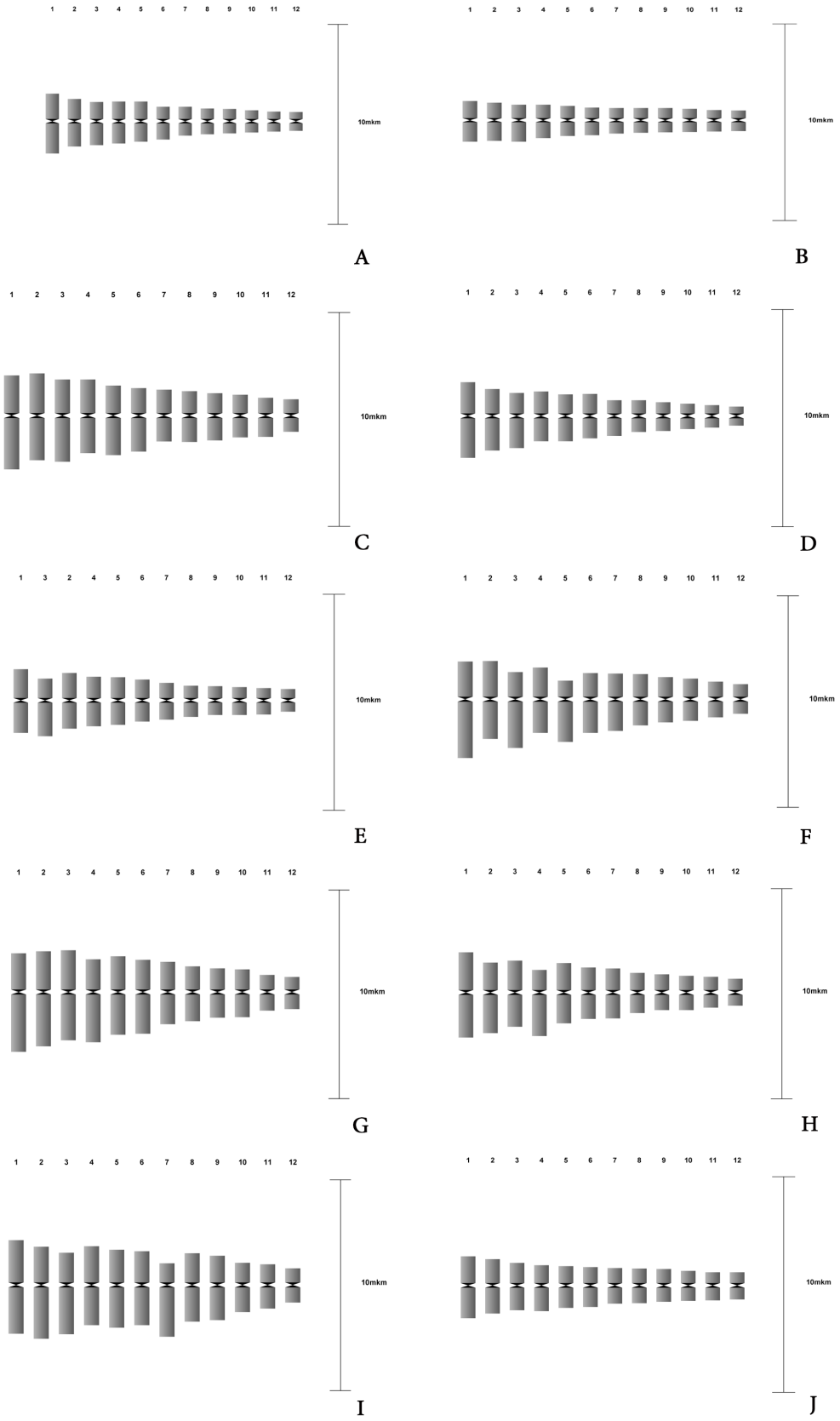

Fig. 6. Haploid idiograms of *Moehringia grisebachii* in 10 populations: (A) Mg1; (B) Mg2; (C) Mg3; (D) Mg4; (E) Mg5; (F) Mg6; (G) Mg7; (H) Mg8; (I) Mg9 and (J) Mg10

For all studied populations were calculated the values of asymmetric indices (Table 7). TF% of the species *M. grisebachii* varies from 42.76 in Mg6 (Haiduschka polyana) to 46.73 in Mg10 (Karandilska polyana), proving that the karyotype of the species is closer to symmetric. The indices for intrachromosomal asymmetry A_1 and CV_{cl} vary respectively from 0.117 in Mg5 (Gornaka) to 0.217 in Mg9 (south of Karandilska polyana) and from 1.06 in Mg10 (Karandilska polyana) to 3.94 in Mg1 (east of Haiduschka pateka). The indices for interchromosomal asymmetry A_2 and M_{ca} vary from 0.011 in Mg10 to 0.039 in Mg1 and from 0.55 in Mg10 to 1.21 in Mg6. AsK% varies from 53.27 to 57.24 in Mg10 and Mg6 (Haiduschka polyana). The values of Syi are from 74.71 in Mg6 (Haiduschka polyana) to 87.71 in Mg10 (Karandilska polyana).

Table 7. Asymmetry indices of studied populations of species *Moehringia grisebachii Janka*. Total form percentage (TF%), karyotype asymmetry percentage (AsK%), Watanabe index (A), symmetric index (Syi), intrachromosomal asymmetry (A_1), interchromosomal asymmetry (A_2), coefficient of variation of chromosome length (CV_{cl}) and mean centromeric asymmetry (M_{ca})

Population	TF%	AsK%	Syi	A	A1	A2	CV_{cl}	M_{ca}
Mg1	46.25	53.75	86.06	0.006	0.123	0.039	3.94	0.62
Mg2	45.83	54.17	84.59	0.007	0.141	0.023	2.32	0.70
Mg3	45.75	54.25	84.34	0.007	0.137	0.023	2.26	0.71
Mg4	44.99	55.01	81.77	0.008	0.162	0.035	3.47	0.84
Mg5	46.45	53.55	86.73	0.006	0.117	0.027	2.72	0.59
Mg6	42.76	57.24	74.71	0.012	0.196	0.028	2.79	1.21
Mg7	44.13	55.87	79.00	0.010	0.178	0.026	2.63	0.98
Mg8	45.36	54.64	83.01	0.008	0.129	0.037	3.67	0.77
Mg9	43.15	56.85	75.91	0.011	0.217	0.024	2.42	1.14
Mg10	46.73	53.27	87.71	0.005	0.118	0.011	1.06	0.55

The length of the chromosomes within a taxon's karyotype can have a rather wide range (Schubert, 2007; Hamouche et al., 2010). It's proven that the intrapopulation chromosome variety shows not only as a variation in the number of chromosomes but also in plants with the same chromosome number, sometimes in various karyotype structures. Usually the evolutionary change of a karyotype is complex and involves various mechanisms (Weiss-Schneeweiss & Schneeweiss, 2013). According to the theory for flowering plants the asymmetric karyotypes come from symmetric ones (Sharma & Sen, 2002; Medeiros-Neto, 2017). Ohri (1988) claims that a karyological study can show variations that are not shown in the morphology of the plant. In a study by Hamideh et al. (2009) this was confirmed in four populations of the endemic *Thymus daenensis*, as there are differences in the centromere position and the total sum of haploid chromosome length, and lack of morphological differences.

In previous studies of the species *Moehringia grisebachii* and *Moehringia jankae*, was reported that the species have more significant intrapopulation variability, at the cost of the interpopulation one. For *M. grisebachii* through morphometric measurements was reported a 63.87% intrapopulation variability, while for *M. jankae* it can be up to 72%, as established using DNA analysis with ISSR markers (Zhelyazkova et al., 2019a; 2019b). The present karyomorphological data show 5 different karyotype formulae, however they are found in the populations of the two species, which raises the question for a possible hybridization between them.

A study by Bhatnagar et al. (2018) shows that diversification of the species is related to structural changes in chromosomes. Moreover, the habitat conditions and anthropogenic activities can be responsible for the frequency of inversions, causing karyomorphological changes, and therefore variation in the karyotype formulae. The karyotype asymmetry (A1) is considered an indirect indicator for the number of chromosomal rearrangements occurring between the species (García-Barriuso et al., 2010; Bozkurt et al., 2017). In our results, the karyotype of all populations of *M. jankae* and *M. grisebachii* was mainly symmetric and the highest asymmetry indices belonged respectively to the population north of Kamilata (Mj8) and the population from Karandilska polyana (Mg10).

Data of the karyotype changes with the emergence of various karyotype formulae in populations of the same endemic species are not a novelty in literature. Konichenkoa et al. (2014) studied the karyotype morphology of six populations of the endemic *Astragalus sericeocanus* from different places along Baikal Lake. Despite each population having the same chromosome number (2n = 16), were determined three different karyotype formulae for them (2n = 16 = 8m + 8sm, 2n = 16 = 6m + 10sm, and 2n =16 = 4m + 12sm), and the plants from all studied populations had similar chromosome morphology.

The authors report for a variation in the sum of the length of the haploid chromosome number (Hcl) in the six populations of *Astragalus sericeocanus* from 25.61 µm to 30.55 µm. The wide variation in karyotype formulae and the variation in the size of their chromosomes found in our study can be explained with the large scale of our study on 20 different populations, as well as the habitat specifics of *M. jankae* and *M. grisebachii*.

Zhou et al. (2008) reports karyotype variation in the endemic *Orychophragmus violaceus* complex (Brassicaceae). Within the species *O. violaceus* they register two karyotype formulae for the nine studied populations where a metacentric chromosome pair is replaced with a submetacentric, and within the species *O. hupehensis* they also register two karyotype formulae for the two studied populations, with similar replacement of a metacentric pair with submetacentric and a satellite. In the discussion of the study's results on the possible reasons for karyotype variation the authors comment on the inbred depression of the low in numbers populations, the changeable conditions in rocky habitats, the influence of the altitude (around 1000 m), as well as the geographical isolation of the endemics.

Data similar to the present study is found in a study on *Psephellus aucherianus* complex, where the chromosome number of all population was the same (2n = 30), but the karyomorphological results showed that the populations of *Ps. aucherianus* complex had wide variation and differed among each other (Bozkurt et al, 2017). The authors determine that their karyotype formula is highly specific and different not only for all species but their populations too. On the other hand the chromosome indices showed that all populations had mostly symmetrical karyotypes, like in the determined by us karyotypes of the species *M. jankae* and *M. grisebachii.*

For ten population of *M. jankae* the calculated mean Hcl (28.34), was similar to the mean Hcl for ten population of *M. grisebachii* (25.40).

The observed changes in karyomorphology and the variations in the size of the genome, according to the contemporary literature can be explained with the various microclimate conditions, altitude, latitude, temperature, rains, natural selection and isolation of the populations, as well as the specific types of the habitats (Bozkurt et al, 2017). The habitats and extreme conditions where species *M. jankae* and *M. grisebachii* exist and the registered differences of the karyomorphometric data from this study support these theories.

Conclusion

In the present karyological study of a total of 20 populations of the species *M. jankae* and *M. grisebachii* were determined 5 different karyotype formulae. The karyotype formulae differed mainly by the presence or absence of one or two submetacentric pairs, while the karyotype of both species included mainly metacentric chromosomes. Both the established different karyotype formulae, repeating in the populations for both species, as well as the established variations in the general size of the karyotype, did not allow for a direct association with the karyotype changes on between species level in *M. jankae* and *M. grisebachii*. In relation with the repeating karyotype formulae in the different populations of both species and their considerable variation in chromosome size, the performed karyological analysis doesn't make a definite differentiation between the populations *M. jankae* and *M. grisebachii*.

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