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Morphological and karyological variability of the Balkan endemics *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka (Caryophyllaceae) from Eastern Balkan Range (Bulgaria)

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Abstract

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Seven populations of *Moehringia jankae* and eleven populations of *M. grisebachii* at Eastern Balkan Range – Sinite Kamani Natural Park were morphologically and karyologically tested. The chromosome numbers, the karyotype characteristics and the stomata type of *M. grisebachii* and *M. jankae* were described. Intrapopulation, interpopulation and interspecies variabilities were established. The chromosome number $2n = 2x = 24$ have been found in all studied populations. The karyotypes of *M. jankae* and *M. grisebachii* consist of metacentric and submetacentric chromosomes, differences in morphology and size of chromosomes have been identified and in the studied populations of *M. grisebachii* were established one pair of chromosomes with satellites. The main source of phenotype variation was intrapopulation variability mainly due to characteristics of habitats of both species and their biological type. More variable in all populations of *M. jankae* and *M. grisebachii* were vegetative traits and the most variable was height of stem. The registered interpopulation variability was affected by the differences in the karyotype, the altitude, the exposure and the type of rock, number and area of population. Indumentum, dimentions of leaves and flowers and morphological features of pollen and seeds had taxonomic significance for distinguishing *M. jankae* from *M. grisebachii*.

Keywords: karyology; *Moehringia grisebachii*; *Moehringia jankae*; morphology; population variations

Introduction

Genus *Moehringia* L. comprises 25 species occurring in north-temperate North America, Europe and Asia (Hind, 1993; Rabeler & Hartman, 2005; Dequan & Rabeler, 2008). Most of the species only occur in Europe (Minuto, 2006). Here, three main centers of speciation have been recognized, i.e. the Balkan Peninsula, the Iberian Peninsula and the Central European Alpine system (Hind, 1988).

The genus is represented in Bulgarian flora by 5 species (Kuzmanov & Kozhuharov, 1966; Assyov & Petrova, 2012), two of which *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka are Balkan endemics, included in the Red Data Book of Bulgaria under category Endangered (Stoeva, 2015; Stoyanov, 2015). *M. jankae* is protected by the Biological Diversity Act of Bulgaria (2002) and it is included in Appendix I of Bern Convention (1979) and in IUCN Red Lists of Threatened Plants (Bilz, 2011) as „Data Deficient". Populations of the species have been registered in Bulgaria – Eastern Balkan Range (Sinite Kamani Nature Park) and Shumen Heights (Grozeva et al., 2004; Zahariev & Radoslavova, 2010; Zahariev, 2014; Stoeva, 2015; Zhelyazkova et al., 2018) and Romania – Dobrudzha (Oprea & Sârbu, 2009; Petrescu, 2007; Gavrilă & Anghel, 2012). In the above areas *M. grisebachii* also forms its populations and the species is reported for Sredna Gora Mts as well (NW of Pesnopoi village; Eastern – between Rozovets village and Bratan summit), Thracian Lowland (northwards from Matochina village) and Tundzha Hilly Country (Sakar Mt) in Bulgaria (Assyov & Petrova, 2012; Stoyanov, 2015; Zhelyazkova et al., 2018) and for the European part of Turkey – from Elmacık to Kırklareli (Dőnmez et al., 2013). Until now *M. jankae* and *M. grisebachii* have not been the object of a comprehensive study.

The morphology of their seeds has been studied (Minuto et al., 2006; 2011; Dönmez et al., 2013) as well as the blastogenesis of *M. grisebachii* (Gherghişan, 2013). Both species have been a subject of karyological study (Zhelyazkova et al., 2020a; b).

Our previous research (Grozeva et al., 2016) established the present-day distribution of *M. jankae* and *M. grisebachii* on the territory of Sinite Kamani Nature Park and the state of their populations in the park. The objective of the present study was to trace the intrapopulation, interpopulation and interspecies' morphological and karyological variability of the registered populations of *M. jankae* and *M. grisebachii* and to identify the traits that have the greatest taxonomic value.

Materials and Methods

The object of study were 7 populations of *M. jankae* and 11 populations of *M. grisebachii* on the territory of Eastern Balkan Range - Sinite Kamani Nature Park (Table 1). In the nature park the two species formed their populations in rock crevices (Fig. 1, 2), sometimes occurring jointly, and the dominant species in the population was *M. grisebachii.*

Table 1. Studied populations of *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka

Locality	Ecological conditions	Population
Moehringia jankae (Mj)		
Mj1. South-west of Karandila Hotel complex, 850 m a.s.l. N 42°42.704′, E 26°22.254′	The population is located on the southern side of vertical conglomerates. The plant community is dominated by lichens and mosses.	Area - 55 m^2, numbers 56 specimens.
Mj2. The rocks around Karandilska polyana, 955 m a.s.l. N 42°42.873′, E 26°22.452′	The population is located on the southern side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses. Accompanying species is *M. grisebachii*.	Area - 796 m^2, numbers 61 specimens.
Mj3. The high eastern rocks around the Microyazovir area, 972 m a.s.l. N 42°42.790′, E 26°22.612′	The population is located on the western and north-western side on vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses. Accompanying species is *M. grisebachii*.	Area - 25.5 m^2, numbers 19 specimens.
Mj4. The rocks around Haidushka pateka, 590-621 m a.s.l. N 42°42.873′, E 26°22.452′	The population is located on the eastern, western and southern sides of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 20.3 m^2, 72 specimens.
Mj5. Haidushka pateka to the south of Karandila Hotel complex, 879 m a.s.l. N 42°42.652′, E 26°22.158′	The population is located on the western side of vertical conglomerates. The plant community is dominated by lichens and mosses.	Area - 0.5 m^2, numbers 17 specimens.
Mj6. Kaloyanovi kuli area, 756 m a.s.l. N 42°42.755′, E 26°23.015′	The population is located on the western side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 19 m^2, numbers 48 specimens.
Mj7. Kamilata area, 857 m a.s.l. N 42°42.601′, E 26°22.183′	The population is located on the eastern and southern sides of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses. Accompanying species is *M. grisebachii*.	Area - 4.3 m^2, numbers 31 specimens.
Moehringia grisebachii (Mg)		
Mg1. The rock formations around Haidushka polyana, 641 m a.s.l. N 42°42.290′, E 26°21.655′	The population is located on the eastern side of vertical conglomerates. The plant community is dominated by lichens and mosses.	Area - 14 m^2, numbers 26 specimens.
Mg2. The south-east of Haidushka pateka, 951 m a.s.l. N 42°42.828′, E 26°22.432′	The population is located on the southern side of vertical conglomerates. The plant community is dominated by lichens and mosses.	Area - 12 m^2, numbers 22 specimens.

Mg3. The east of Haidushka pateka, 921 m a.s.l. N 42°42.782′, E 26°22.348′	The population is located on the south-eastern side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses. Accompanying species is *M. jankae*.	Area - 25 m^2, numbers 26 specimens.
Mg4. The rock formations at the bend of the trail from Gornaka area, 803 m a.s.l. N 42°42.664′, E 26°22.869′	The population is located on the eastern side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 15.3 m^2, numbers 24 specimens.
Mg5. The rock formations around the junction to Kaloyanovi kuli area above the panel, 685 m a.s.l. N 42°42.833′, E 26°23.169′	The population is located on the southern side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 1.7 m^2, numbers 11 specimens.
Mg6. Karandila area – around Karandilska polyana, 994-998 m a.s.l. N 42°42.833′, E 26°22.525′	The population is located on the northern and north-eastern side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses. Accompanying species is *M. jankae*.	Area - 1720 m^2, numbers 347 specimens.
Mg7. The high eastern rocks near the Microyazovir area, 975 m a.s.l. N 42°42.480′, E 26°22.423′	The population is located on the western and north-western side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 25.5 m^2, numbers 45 specimens.
Mg8. Gornaka area, 920 m a.s.l. N 42°42.828′, E 26°23.735′	The population is located on the western side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 60 m^2, numbers 17 specimens.
Mg9. Karakyutyuk area, 851-854 m a.s.l. N 42°43.861′, E 26°18.662′	The population is located on the western side of vertical limestone. The plant community is dominated by lichens and mosses.	Area - 4.7 m^2, numbers 22 specimens.
Mg10. Kamilata area, 838 m a.s.l. N 42°42.595′, E 26°22.179′	The population is located on the western side of vertical conglomerates. The plant community is dominated by lichens and mosses. Accompanying species is *M. jankae*.	Area - 17.5 m^2, numbers 28 specimens.
Mg11. Golyama Chatalka area, 1022-1049 m a.s.l. N 42°43.362′, E 26°21.093′	The population is located on the western side of vertical quartz porphyry rocks. The plant community is dominated by lichens and mosses.	Area - 29.5 m^2, numbers 25 specimens.

Fig. 1. *Moehringia jankae* Griseb. ex Janka, population from Haidushka pateka – Mj4 (photo N. Grozeva)

Fig. 2. ***Moehringia grisebachii*** **Janka, population around Karandilska polyana – Mg6 (photo N. Grozeva)**

Karyological analyses were conducted during the vegetation periods of 2017 - 2018. Chromosome numbers and karyotypes have been reported on lasting preparations of metaphase root apex plates of seeds germinated in laboratory conditions collected in the natural habitats of the species. The roots were treated and squashed according to the accepted methods (Zhelyazkova et al., 2020a; b). The voucher specimens are kept in the herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM).

The chromosomal type was determined by the centromere index I = s/s+l, according to the classification proposed by Grif & Agapova (1986). Three metaphase plates were measured from each population. The idiograms were obtained with the help of the Adobe Photoshop CS6.0 program. The karyotype asymmetries were estimated with coefficient variation of chromosome length (CV_{cl}) based on Paszko (2006) and mean centromeric asymmetry (M_{ca}) proposed by Peruzzi & Eroğlu (2013).

Observations and morphometric studies were conducted during the vegetation periods of 2014 - 2017. The morphological analysis comprised 24 quantitative traits: 1. Height of stem, mm; 2. Basal leaves length (mm); 3. Basal leaves width (mm); 4. Basal leaves length/width ratio; 5. Basal leaves petiole length (mm); 6. Upper leaves length (mm); 7. Upper leaves width (mm); 8. Upper leaves length/ width ratio; 9. Upper leaves petiole length (mm); 10. Flower diameter (mm); 11. Sepals length (mm); 12. Sepals width (mm); 13. Sepals length/width ratio; 14. Petals length (mm); 15. Petals width (mm); 16. Petals length/width ratio; 17. Flower petiole length (mm); 18. Capsule (fruit) length (mm); 19. Capsule width (mm); 20. Capsule length/width ratio; 21. Seed length (mm); 22. Seed width (mm); 23. Seed thickness (mm); 24. Seed length/width ratio. The following qualitative traits have also been analysed: shape, colour and hairing of stem, leaf lamina, leaf petiole, sepals and petals; shape and colour of capsule and seed. Due to lack of interpopulation differences the results have been presented as a summary for each species.

The dimensions of flower, seed and capsule, the specifications of epidermis of the leaf lamina and the morphology of pollen have been recorded in laboratory conditions from flowers, fruits and seeds gathered in advance from each population. All other traits have been recorded *in situ*.

To study the morphology of stomata, flower, pollen, seed and for a more detailed research of the type of indumentum the Scanning Electron Microscopy (SEM) method has been used. The research has been performed on herbarized plant parts in the laboratory of the Faculty of Chemistry and Pharmacy at Sofia University „St. Kliment Ohridski“. At least 15 pollens, 10 seeds and plant parts have been researched from each population. Morphological specifications of pollen were evaluated according to Moore et al. (1997) and Punt et al. (2007). The data from the scanning electron microscope studies have been presented as a summary for each species due to lack of significant variability in and among their populations.

The results obtained for the traits related to morphological specifications have been processed statistically by Statistica for Windows 10, Statsoft. The statistical significance of differences among various traits, interpopulation and intrapopulation variation of traits has been established by ANOVA. To group populations on the basis of similarity of 24 quantitative morphological traits Cluster analysis (CA) has been applied. To allocate populations in groups according to the strength and direction of effect of the main traits (from the included 24 morphological traits) Principal Component Analysis (PCA) has been applied.

Results

Intrapopulation and interpopulation karyological and morphological variability of M. jankae. As a result of the karyological study diploid chromosome number $2n = 2x = 24$ were found in all studied populations of *M. jankae* (Fig. 3). Two types of chromosomes: metacentric and submetacentric were established in the karyotypes. Karyomorphometric data about the populations are shown in Table 2. The total sum of haploid chromosome length (hcl) ranged from 53.06 to 62.91 µm. In the studied populations some differences have been found in the morphology and the size of chromosomes (Table 2). For both populations from the high eastern rocks around the Microyazovir area (Mj3) and from Kamilata area (Mj7) a karyotype of 9 pairs of metacentric chromosomes and 3 pairs of submetacentric chromosomes have been established, while for the other 5 populations – from south-west of Karandila hotel complex (Mj1), the rocks around Karandilska polyana (Mj2), the rocks around Haidushka pateka (Mj4), Haidushka pateka to the south of Karandila hotel complex (Mj5), Kaloyanovi kuli area (Mj6) 11 pairs of metacentric chromosomes and 1 pair of submetacentric chromosomes have been registered (Table 2). The average length of chromosomes in the karyotypes varied from 4.42 µm in the population from south-west of Karandila hotel complex (Mj1) to 5.24 µm in that from Microyazovir area (Mj3). The analysis of the interchromosomal and intrachromosomal karyotype asymmetry indices showed values from 1.29 to 1.44 for CV_{cl} and from 1.27 to 2.44 for M_{ca}. The highest value of CV_{cl} was registered for the population from the rocks around Karandilska polyana (Mj2) and the lowest for that from Kamilata area (Mj7). The highest and the lowest values of M_{ca} were established for the population from Kaloyanovi kuli area (Mj6) and for the one from south-west of Karandila hotel complex (Mj1), respectively.

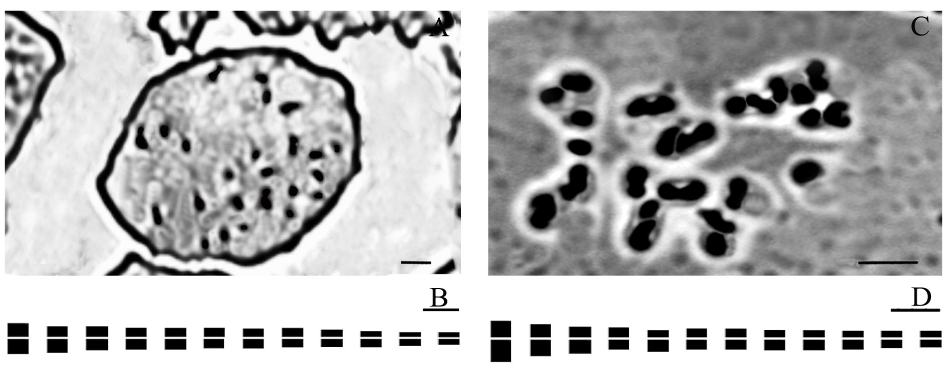

Fig. 3. ***Moehringia jankae*** **Griseb.: A, C – microphotograph of root tip mitosis and idiogram of the population from the high eastern rocks around the Microyazovir area (Mj3); B, D – microphotograph of root tip mitosis and idiogram of the population from south-west of Karandila hotel complex (Mj1), scale bar 10 µm**

Table 2. Karyomorphometric data for the srudied populations of *M. jankae* Griseb. *ex Janka*, chromosome size variation (µm) - short (S) and long (L); total sum of the haploid chromosome lenght (hcl, µm), coefficient variation of chromosome length (CV_{cl}), mean centromeric asymmetry (M_{ca})

Population	Karyotype	S	L	Hcl	CV_{cl}	M_{ca}
Mj1	*2n = 22m + 2sm*	3.06	8.31	53.06	1.37	1.27
Mj2	*2n = 22m + 2sm*	3.28	8.35	59.75	1.29	2.42
Mj3	*2n = 18m + 6sm*	2.91	8.6	62.91	1.44	1.41
Mj4	*2n = 22m + 2sm*	3.32	8.18	59.54	1.38	1.29
Mj5	*2n = 22m + 2sm*	3.27	8.03	60.89	1.41	2.36
Mj6	*2n = 22m + 2sm*	3.24	8.55	59.7	1.33	2.44
Mj7	*2n = 18m + 6sm*	3.34	8.25	59.35	1.42	1.35

The data about the mean arithmetic of the studied quantitative traits of each trait are presented in Table 3. Intrapopulation variability has been traced on the basis of variance values. Their values were different both for the specific traits of each population and between populations. More variable in all populations were the vegetative traits, the most variable being the height of stem. Greater variability has been established for length of basal leaves lamina, lamina length/width ratio and length of its petiole and length of upper leaves lamina and length/width ratio. In two of the populations from the south-west of Karandila hotel complex (Mj1) and from the rocks around Karandilska polyana (Mj2), the length of basal leaves lamina had considerably lower level of variability compared to the other 5 populations.

Table 3. Mean (upper numbers in each row), variance value (lower numbers in each row, V%) and percentage of intrapopulation variation (SSv, %) in the overall morphological variation of the seven studied populations of *Moehringia jankae* Griseb. ex Janka for each of the 24 observed quantitative traits

	Mj1*	Mj2	Mj3	Mj4	Mj5	Mj6	Mj7	SSv %
1	27.33	38.47	49.87b	70.73a	50.27b	69.53a	52.83b	38.91
V%	48.81	159.98	104.98	179.50	182.21	168.27	148.85	
2	9.15a**	6.56a	10.44acd	15.43b	12.61bcd	13.77bc	11.28acd	71.21
V%	3.96	3.72	29.60	28.01	24.61	38.24	11.58	
3	2.10acf	1.97acdfg	2.57be	2.45abef	1.94acfg	2.11abcdf	1.59cdg	79.56
V%	0.14	0.70	0.29	0.38	0.83	0.29	0.07	
4	4.54ab	6.54ab	4.18a	6.42ab	8.14ab	6.92ab	7.39ab	93.75
V%	12.19	13.79	15.63	13.98	29.15	12.36	9.43	
5	6.87ae	5.65acde	6.80ade	10.85b	9.47be	9.33bce	7.20acde	75.79
V%	2.70	1.98	18.89	5.02	17.27	15.10	9.17	
6	2.72	4.86bc	5.96ac	7.75a	5.52bd	9.67ad	8.03ad	60.71
V%	8.16	5.33	10.85	8.39	3.03	8.04	9.28	
7	1.89ac	1.77abcd	1.49abcd	1.42bd	1.86acd	1.45bcd	1.45bcd	89.11
V%	0.14	0.13	0.37	0.14	1.18	0.21	0.15	
8	1.64ad	2.73ac	4.78bcd	5.67b	3.73c	7.07b	5.86bd	65.06
V%	4.38	1.11	12.59	5.91	3.55	6.95	9.05	
9	1.80a	2.13ad	3.07bcd	3.51b	2.21ac	2.89abcd	2.80abcd	88.92
V%	1.60	2.41	4.92	3.07	2.06	2.53	2.03	
10	2.34abc	2.36abc	2.23abc	2.46ab	2.13abc	2.46ab	2.07ac	92.12
V%	0.19	0.22	0.10	0.37	0.32	0.34	0.21	
11	1.86a	1.87a	1.85a	1.84a	1.90a	1.85a	1.87a	97.63
V%	0.008	0.018	0.021	0.021	0.013	0.010	0.010	
12	0.87ab	0.89ab	0.87ab	0.86ab	0.91ab	0.84a	0.87ab	94.81
V%	0.010	0.008	0.008	0.011	0.010	0.008	0.007	
13	2.17a	2.13a	2.14a	2.16a	2.10a	2.22a	2.18a	97.86
V%	0.052	0.068	0.075	0.057	0.055	0.061	0.071	
14	1.88	1.76abc	1.69ab	1.75abc	1.70abc	1.75abc	1.78ac	77.94
V%	0.013	0.017	0.023	0.014	0.007	0.007	0.010	
15	0.85a	1.11cd	1.07cd	0.93ab	1.09c	0.95ab	1.00bc	72.76
V%	0.010	0.107	0.007	0.008	0.009	0.008	0.006	
16	2.23	1.67bc	1.59b	1.89a	1.57b	1.84a	1.79ac	53.93
V%	0.073	0.140	0.024	0.050	0.023	0.037	0.043	
17	10.40ac	9.60abc	9.07c	10.93adf	8.73bc	11.27adf	11.93adef	72.06
V%	2.83	5.54	1.50	3.64	1.64	3.21	5.07	
18	1.61a	1.63a	1.65a	1.74b	1.61a	1.88	1.74b	52.34
V%	0.004	0.011	0.017	0.008	0.008	0.012	0.007	
19	1.72a	1.19b	1.81a	1.79a	1.23b	1.74a	1.79a	25.03
V%	0.01	0.03	0.04	0.01	0.05	0.01	0.02	
20	0.94a	1.40b	0.92a	0.98a	1.36b	1.08	0.98a	35.02
V%	0.005	0.039	0.016	0.008	0.064	0.003	0.006	
21	0.90a	0.91ab	0.87a	0.95ab	0.93ab	0.88ab	0.89ab	92.25
V%	0.006	0.014	0.007	0.008	0.011	0.010	0.006	
22	0.79a	0.80ab	0.79ab	0.75a	0.81ab	0.73a	0.79ab	91.75
V%	0.004	0.011	0.010	0.007	0.009	0.008	0.004	
23	0.37ab	0.35abc	0.31bc	0.39ab	0.37ab	0.38ab	0.39ab	84.50
V%	0.004	0.004	0.001	0.004	0.005	0.003	0.004	
24	1.18ac	1.15ac	1.11bc	1.27	1.15abc	1.20ac	1.14a	74.06
V%	0.007	0.008	0.005	0.011	0.007	0.009	0.006	

The least variable in all populations were the traits characterizing sepals, petals, capsules and seeds.

The results from ANOVA (Table 2) showed that dominant in the overall variability in 21 of the studied 24 traits was the intrapopulation one.

The data from CA based on morphological relatedness (Euclidean distances between population centroids), showed some grouping of 7 populations by degree of similarity (Fig. 4). In the first group, which forms cluster A, the greatest similarity along the entire set of quantitative traits among specimens had two of the populations from the rocks around Haidushka pateka (Mj4) and from Kaloyanovi kuli area (Mj6), located at an altitude between 590 and 756 m. The second group (cluster B) comprised the other 5 populations – south-west of Karandila hotel complex (Mj1), the high eastern rocks around the Microyazovir area (Mj3), from the rocks around Karandilska polyana (Mj2), Haidushka pateka to the south of Karandila hotel complex (Mj5), Kamilata area (Mj7), located at an altitude between 850 and 972 m.

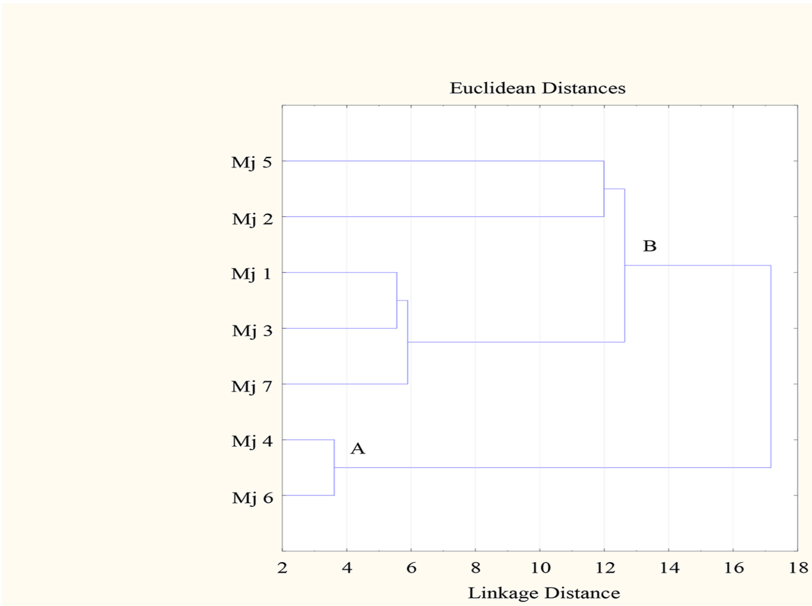

Fig. 4. Dendrogram of CA of *Moehringia jankae* Griseb. ex Janka populations based on 24 traits of vegetative and generative morphological variability

The PCA for the distribution of populations according to the studied 24 morphological traits confirms the similarity shown in the CA. Three of the studied populations – south-west of the Karandila hotel complex (Mj1), the high eastern rocks around the Microyazovir area (Mj3) and Kamilata area (Mj7) had positive values both on factor 1, describing 44.21%, and on factor 2, describing 21.19% (Table 4). The population from Haidushka pateka to the south of Karandila hotel complex (Mj5) was positive for factor 1 and negative for factor 2. The other three populations from Kaloyanovi kuli area (Mj6), the rocks around Karandilska polyana (Mj2) and Haidushka pateka (Mj4) had positive values for factor 2, but negative for factor 1.

Table 4. Factor scores based on PCA and correlation of the seven studied populations of *Moehringia jankae* Griseb. ex Janka

	Factor 1	Factor 2
Mj1*	3.65243	2.63262
Mj2	-1.00196	0.43033
Mj3	3.53706	0.11925
Mj4	-3.88573	0.15826
Mj5	1.46771	-4.72917
Mj6	-4.37992	0.58067
Mj7	0.61041	0.80804

*numbering of populations follows the indications in Table 1

In the conducted SEM studies, hairing on the plant parts of *M. jankae* has not been registered (Fig. 5, A-F). Three types of stomata: paracytic, anomocytic and diacytic have been found on the stem, leaf and flower petiole of the studied specimens in all populations (Fig. 5, A-E). Stomata of the anomocytic type prevailed on the adaxial (upper) leaf surface (Fig. 5, D), while diacytic type prevailed on the abaxial (lower) leaf surface (Fig. 5, E). Sepals were always without a convex middle vein (Fig. 5, F). The pollen was pantoporate, with radial symmetry, medium size, with 12 - 14 pores (Fig. 5, G). Pollen diameter was from 24.8 to 27.3, pore diameter from 3.6 to 4.7 µm and interpore distance 6.2 - 7.5 µm. Surface was uniformly granulose and with small verrucae, on the tectum, single minute perforations were noted. Seeds were subcircular with convex lateral faces and absence of dorsal ridge (Fig. 5, H-J). The strophilae consisted of short cells of equal length (Fig. 5, K). Seed coat was formed by round cells having a weak secondary sculpturing with 3-5 teeth on each side with irregular contours (Fig. 5, L).

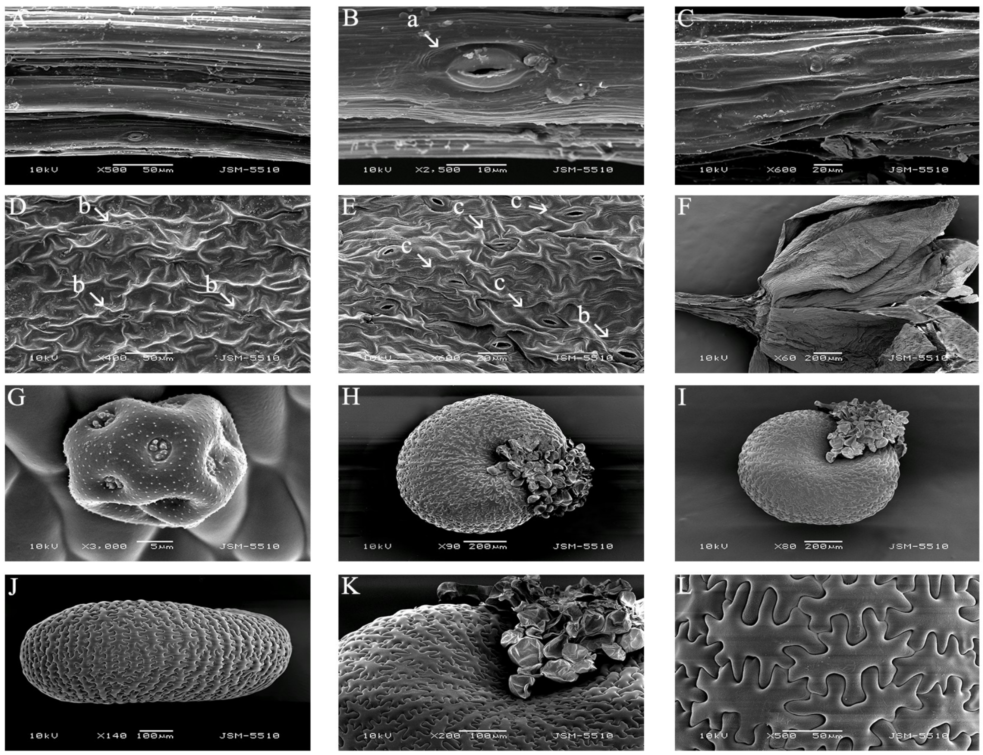

Fig. 5. SEM micrographs of *Moehringia jankae* Griseb. ex Janka: A-B) stem surface; C) flower petiole surface; D) adaxial (upper) leaf surface; E) abaxial (lower) leaf surface; F) sepals with a part of flower petiole surface; G) pollen grain; H-I) seed surface from both sides; J) seed edge; K) details of seed surface; L) strophilae and seed surface; a) paracytic; b) anomocytic; c) diacytic

Intrapopulation and interpopulation karyological and morphological variability of M. grisebachii. As a result of the karyological study, for the eleven studied populations of *M. grisebachii* diploid chromosome number $2n = 2x = 24$ has been established (Fig. 6). Two types of chromosomes: metacentric and submetacentric have been established in the karyotypes. Karyomorphometric data about the studied populations is shown in Table 5. Some differences in the size and the morphology of chromosomes have been registered.

The total sum of haploid chromosome length (hcl) ranged from 38.13 to 42.10 µm. In 6 of the studied populations - the south-east of Haidushka pateka (Mg2), the rock formations around the junction to Kaloyanovi kuli area above the panel (Mg5), Gornaka area (Mg8), Karakyutyuk area (Mg9), Kamilata area (Mg10), Golyama Chatalka area (Mg11) a karyotype of 9 pairs of metacentric chromosomes and 3 pairs of submetacentric chromosomes have been established and in one of the pairs of submetacentric chromosomes satellites have been registered. In the remaining 5 populations – the rock formations around Haidushka polyana (Mg1), the east of Haidushka pateka (Mg3), the rock formations at the bend of the trail from Gornaka area (Mg4), Karandila area – around Karandilska polyana (Mg6), the high eastern rocks near the Microyazovir area (Mg7) a karyotype of 4 metacentric pairs and 8 pairs of submetacentric chromosomes have been registered and again in one of the pairs of submetacentric chromosomes satellites have been registered.

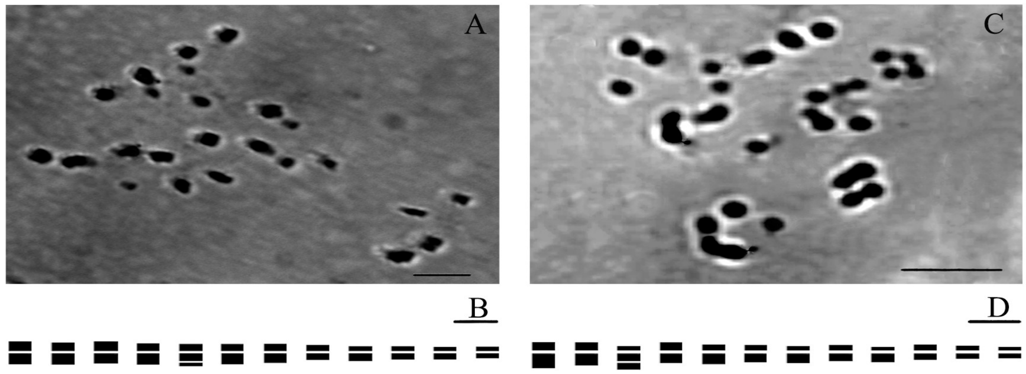

Fig. 6. ***Moehringia grisebachii*** **Janka.: A, C – microphotograph of root tip mitosis and idiogram of the population from the south-east of Haidushka pateka (Mg2); B, D – microphotograph of root tip mitosis and idiogram of the population from the high eastern rocks near the Microyazovir area (Mg7); scale bar 10 µm**

Table 5. Karyomorphometric data for the srudied populations of *M. grisebachii* Griseb. *ex Janka*, chromosome size variation (µm) - short (S) and long (L); total sum of the haploid chromosome lenght (hcl, µm), coefficient variation of chromosome length (CV_{cl}), mean centromeric asymmetry (M_{ca})

Population	Karyotype formula	S	L	Hcl	CV_{cl}	M_{ca}
Mg1	2n = 8m + 14sm + 2sm^{sat}	1.95	4.25	39.84	2.32	2.28
Mg2	2*n* = 18m + 4sm + 2sm^{sat}	2.05	4.75	42.10	2.42	2.36
Mg3	2*n* = 8m + 14sm + 2sm^{sat}	1.95	4.25	39.68	1.33	2.18
Mg4	2*n* = 8m + 14sm + 2sm^{sat}	1.95	4.34	41.76	2.02	2.44
Mg5	2*n* = 18m + 4sm + 2sm^{sat}	2.00	4.30	39.85	1.35	2.07
Mg6	2*n* = 8m + 14sm + 2sm^{sat}	2.02	4.26	38.75	2.41	1.37
Mg7	2*n* = 8m + 14sm + 2sm^{sat}	1.68	5.05	38.13	2.42	2.35
Mg8	2*n* = 18m + 4sm + 2sm^{sat}	1.95	4.40	40.28	1.34	2.05
Mg9	2*n* = 18m + 4sm + 2sm^{sat}	2.00	4.32	39.24	1.21	2.12
Mg10	2*n* = 18m + 4sm + 2sm^{sat}	1.95	4.28	41.18	1.41	1.26
Mg11	2*n* = 18m + 8sm + 2sm^{sat}	1.85	4.33	41.54	2.17	2.03

The average length of chromosomes in the karyotypes varies from 3.18 µm in the population from the high eastern rocks near the Microyazovir area (Mg7) to 3.51 µm in that from the south-east of Haidushka pateka (Mg2). The values for interchromosomal and intrachromosomal karyotype asymmetry indices were from 1.21 to 2.42 for CV_{cl} and from 1.26 to 2.44 for M_{ca}. The population from the south-east of Haidushka pateka (Mg2) and from the high eastern rocks near the Microyazovir area (Mg7) was characterized by the highest value of CV_{cl} and the population from Karakyutyuk area (Mg9) by the lowest value of this parameter (Table 5). The population from the rock formations at the bend of the trail from Gornaka area (Mg4) and that from Kamilata area (Mg10) showed the highest and the lowest values of M_{ca}, respectively.

Table 6. Mean (upper numbers in each row), variance value (lower numbers in each row, V%) and percentage of intrapopulation variation (SSv, %) in the overall morphological variation of the eleven studied populations of *Moehringia grisebachii* Janka for each of the 24 observed quantitative traits

	Mg1*	Mg2	Mg3	Mg4	Mg5	Mg6	Mg7	Mg8	Mg9	Mg10	Mg11	SSv %
1	43.47a**	23.73d	47.47ab	46.13ac	56.6bc	40.4a	39.6a	32.13ad	56.6bc	34.07ad	25.13d	70.22
V%	720.84	59.92	177.98	242.98	468.54	139.40	245.97	163.98	468.54	310.92	125.55	
2	11.97a	10.49ab	10.49ab	9.55b	9.8b	9.6b	11.45ab	10.81ab	9.8b	10.5ab	9.9b	92.59
V%	9.64	2.42	7.87	6.93	7.75	6.97	16.71	4.11	7.75	6.29	5.26	
3	1.63ac	1.64ac	1.15b	1.73c	1.54ad	1.48ae	1.71ac	1.53a	1.49a	1.33d	1.62ac	62.95
V%	0.03	0.04	0.03	0.02	0.09	0.07	0.02	0.03	0.05	0.09	0.05	
4	7.58a	6.54ac	9.25b	5.49c	6.66acd	6.83acd	6.80acd	7.06acd	6.79acd	8.36bd	6.28ac	83.97
V%	7.11	2.26	8.39	3.73	5.78	6.70	7.35	4.22	6.34	8.09	3.55	
5	9.2a	8.41ab	8.33ab	7.33bc	7.27b	9.08abd	10.41ad	8.77ab	7.67ab	8.07ab	7.74ab	84.34
V%	10.17	12.05	7.10	9.10	8.21	9.44	10.44	12.60	9.52	13.07	9.10	
6	8.72abce	8.76abce	8.13abc	9.26abcde	10.16cde	9.17abcde	9.03abcde	9.45acde	9.49acdee	9.73acde	8.75abce	89.60
V%	1.13	2.43	2.77	2.95	4.01	3.08	1.61	1.54	3.58	3.20	2.57	
7	1.61acd	1.63ad	1.71acd	1.40bc	1.41bc	1.63acd	1.63ad	1.57abcd	1.64ad	1.23b	1.55abcd	75.00
V%	0.03	0.04	0.13	0.09	0.06	0.06	0.03	0.05	0.05	0.06	0.04	
8	5.48ab	5.49ab	5.02ab	6.80cd	7.50cd	5.68ab	5.64ab	6.11ac	5.95abc	8.18d	5.66ab	70.73
V%	0.43	1.68	2.74	2.32	5.49	1.33	1.41	1.19	2.87	3.44	0.18	
9	4.47a	3.67ab	3.13b	3.13b	2.67b	2.93b	3.20	3.53ab	3.67ab	3.10b	3.00b	92.41
V%	4.55	2.38	3.12	2.55	1.81	2.35	3.46	2.55	2.10	3.01	3.57	
10	1.70abcf	1.93abcdf	1.77abcdf	2.01abcdef	2.15bcdef	2.01abcdef	2.39cde	2.07abcdf	2.15bcdef	2.39cde	1.83abcdf	85.16
V%	0.17	0.49	0.28	0.03	0.26	0.05	0.49	0.12	0.26	0.72	0.20	
11	1.85a	1.91a	1.88a	1.87a	1.89a	1.89a	1.87a	1.89a	1.90a	1.90a	1.87a	97.71
V%	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	
12	0.91a	0.95a	0.94a	0.93a	0.91a	0.90a	0.90a	0.91a	0.89a	0.92a	0.93a	95.88
V%	0.011	0.008	0.005	0.008	0.007	0.006	0.007	0.010	0.011	0.012	0.009	
13	2.06ab	2.01ab	2.01ab	2.04ab	2.08ab	2.11ab	2.09ab	2.08ab	2.17a	2.08ab	2.04ab	95.88
V%	0.09	0.03	0.03	0.07	0.04	0.04	0.02	0.04	0.08	0.03	0.07	
14	1.99a	2.03a	2.02a	2.01a	2.05a	2.01a	2.04a	2.01a	1.97a	2.01a	2.04a	96.17
V%	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	
15	1.08a	0.98d	1.07ab	1.07ac	0.96d	0.97d	0.99bcd	1.09a	1.01ad	1.01ad	0.97d	85.82
V%	0.006	0.009	0.058	0.005	0.005	0.018	0.017	0.007	0.006	0.008	0.011	
16	1.85a	2.09bcef	1.94ab	1.89a	2.15cf	2.10bcd	2.10bcef	1.86a	1.95ade	2.00adfg	2.12cg	80.24
V%	0.03	0.04	0.06	0.02	0.05	0.08	0.07	0.03	0.02	0.04	0.09	
17	10.80ab	11.41ab	10.80ab	10.20ab	11.33ab	11.27ab	11.40ab	11.05ab	11.53a	11.20ab	11.11ab	95.52
V%	2.31	2.35	3.31	4.03	3.52	3.50	2.03	2.35	4.12	3.03	2.72	
18	1.75abc	1.80ab	1.80ab	1.76abc	1.77abc	1.73ac	1.80ab	1.79ab	1.96	1.79abc	1.79ab	68.50
V%	0.008	0.006	0.010	0.007	0.011	0.006	0.007	0.006	0.008	0.007	0.006	
19	1.79abcde	1.70ae	1.79abcd	1.83abcd	1.81abcd	1.77abcde	1.77abcde	1.73abde	1.77abcde	1.75abcde	1.73abde	91.76
V%	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.01	
20	0.98abcd	1.07be	1.01abcd	0.97abcd	0.98abcd	0.98abcd	1.02abcd	1.05ab	1.11e	1.03abcd	1.04abd	80.92
V%	0.006	0.011	0.009	0.007	0.008	0.005	0.010	0.011	0.006	0.004	0.006	
21	0.99a	1.01a	0.96a	0.95a	0.97a	0.98a	0.99a	0.98a	0.95a	0.99a	0.99a	95.43
V%	0.005	0.012	0.011	0.004	0.005	0.003	0.011	0.012	0.007	0.006	0.006	
22	0.84a	0.85a	0.81a	0.83a	0.84a	0.83a	0.83a	0.86a	0.81a	0.85a	0.83a	96.41
V%	0.005	0.011	0.010	0.005	0.011	0.004	0.004	0.008	0.005	0.006	0.011	
23	0.43	0.39a	0.35	0.37	0.40a	0.39a	0.38a	0.39a	0.40a	0.39a	0.38a	93.11
V%	0.004	0.006	0.004	0.005	0.003	0.005	0.006	0.005	0.004	0.004	0.005	
24	1.19a	1.18a	1.19a	1.14a	1.17a	1.19a	1.21a	1.14a	1.18a	1.17a	1.21a	96.09
V%	0.015	0.010	0.013	0.005	0.013	0.010	0.020	0.006	0.007	0.011	0.022	

Intrapopulation variability has been traced on the basis of variance values. Their values differ and, as in *M. jankae*, more variable in all populations were the vegetative traits, the most variable being the height of stem. Greater variability has been established for length of basal leaves lamina, length/width ratio of lamina and length of its petiole. In the population from Haidushka pateka (Mg2) the length/width ratio of basal leaves lamina and in the population from Golyama Chatalka (Mg11) the length/width ratio of upper leaves lamina have considerably lower level of variability compared to the other studied populations.

With the lowest variability in all populations were the traits characterizing the dimensions of sepals, petals, capsules and seeds, as well as width of basal and upper leaves lamina. In the populations from the rock formations at the bend on the trail from Gornaka area (Mg4) and around Karandilska polyana (Mg6) low level of variability has been registered for the flower diameter as well.

The results from ANOVA (Table 5) showed that in all populations dominant in the general variability was the intrapopulation one.

The data from the CA outline certain grouping among the 11 populations, as a result of which 4 clusters were formed (Fig. 7). In cluster A, by degree of morphological similarity, 5 populations were included for which a similarity in the karyotype is found. The remaining 3 clusters included 6 populations that again had similar karyotypes (Table 5).

The greatest morphological relatedness has been registered among the populations from Golyama chatalka area (Mg11) and the south-east of Haidushka pateka (Mg2), which were similar in number (Table 1) and formed cluster C. High similarity has been established between two other pairs of populations – Kaloyanovi kuli (Mg5) and Karakyutuk area (Mg9); Kamilata area (Mg10) and Gornaka area (Mg8), forming two other clusters D and B, respectively. In the second pair of populations (Mg8 and Mg10) the established morphological relatedness was probably influenced by the similarity of exposure (Table 1).

In cluster A, two subclusters were distinguished. In the first one there were 3 populations – around Karandilska polyana (Mg6), Mikroyazovir area (Mg7) and Haidushka polyana (Mg1). The registered greater similarity between the first two populations was probably influenced by the similarity of altitude (Table 1). The second subcluster included the other 2 populations – the east of Haidushka pateka (Mg3) and from the rock formations at the bend of the trail from Gornaka area (Mg4). The morphological relatedness between them was probably the result of similarity in number, exposure (Table 1).

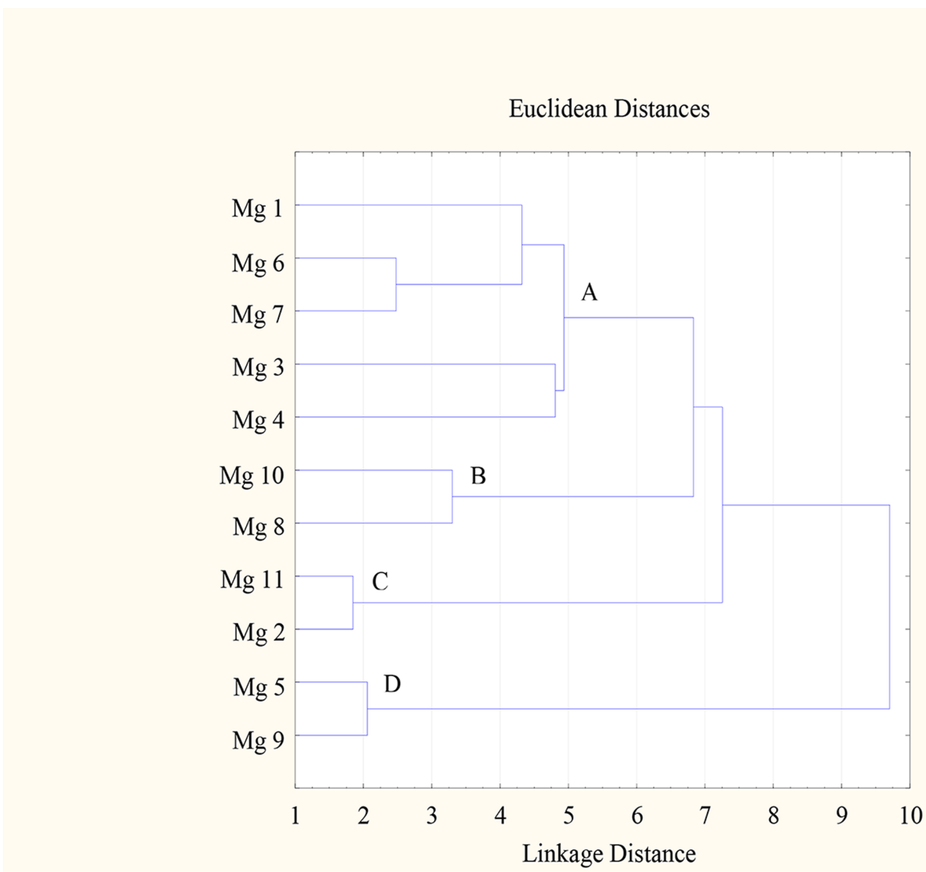

Fig. 7. Dendrogram of CA of *Moehringia grisebachii* Janka populations based on 24 traits of vegetative and generative morphological variability

Table 7. Factor scores based on PCA and correlation of the eleven studied populations of *Moehringia grisebachii* Janka

Populations	Factor 1	Factor 2
Mg1*	3.58073	0.12512
Mg2	-3.86055	-1.7905
Mg3	0.64062	-3.53904
Mg4	2.42064	-2.03224
Mg5	-2.8922	-1.57884
Mg6	0.66646	0.67274
Mg7	2.32707	0.71346
Mg8	-0.25219	1.93765
Mg9	-0.54165	-0.57601
Mg10	-1.32581	3.6285
Mg11	-0.76314	-1.43916

*numbering of populations follows the indications in Table 1

The populations east of Haidushka pateka (Mg3) and from the rock formations at the bend of the trail from Gornaka area (Mg4) had positive values on Factor 1 and negative on Factor 2. The populations from Gornaka area (Mg8) and from Kamilata area (Mg10) had positive values on factor 2, but negative on factor 1. The populations from Kaloyanovi kuli area (Mg5), Karakyutyuk area (Mg9), Golyama chatalka area (Mg11) and the south-east of Haidushka pateka (Mg2) were negative on both factors.

The conducted SEM studies found that the studied plant parts of *M. grisebachii* are covered by multicellular non-branched straight hairs (trichomes) with length from 18.80 to 83.33 µm with the strongest hairing on the stems, leaves and flowers petioles (Fig. 8, A-D).

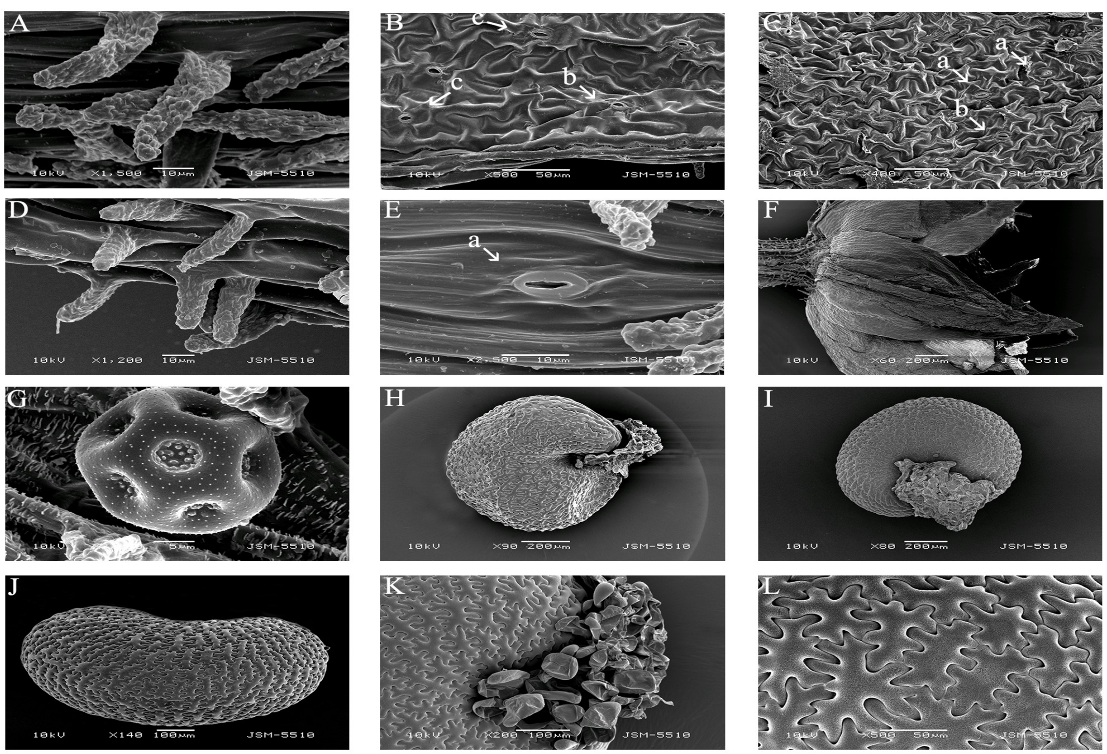

Fig. 8. SEM micrographs of *Moehringia grisebachii* Janka: A) stem surface; B-C) leaf surface from both sides; D) flower petiole surface; E) stem surface with stomata; F) adaxial (upper) leaf surface; G) abaxial (upper) leaf surface; H) sepals with a part of flower petiole; I) pollen grain; J-K) seed surface from both sides; L) seed edge; M) details of seed surface; N) strophilae and seed surface; a) paracytic; b) anomocytic; c) diacytic

Three types of stomata: paracytic, anomocytic and diacytic have been found on the stem, leaf and flower petiole of the studied specimens in all populations (Fig. 8, B, C, E). On the adaxial leaf surface stomata of the paracytic type prevailed, while on the adaxial leaf surface – diacytic type prevailed. Sepals were with convex middle vein (Fig. 8, F). The pollen was pantoporate, with radial symmetry, medium size, with 12 - 14 pores (Fig. 8, G). Pollen diameter was from 23.2 to 29.8, pore diameter from 3.4 to 5.5 µm and interpore distance 4.5 - 6.5 µm. Tectum was without minute perforations, surface was uniformly granulose and with small verrucae. Seeds were subcircular, one side being with convex face, the other – with typical concavity on the surface (Fig. 8, H-I). Absence of dorsal ridge (Fig. 8, J). The strophilae consisted of short cells of equal length (Fig. 8, K). Seed coat was formed by round cells having a weak secondary sculpturing with 3-5 teeth on each side with irregular contours (Fig. 8, L).

Interspecies karyological and morphological variability. The chromosome number established from Bulgarian population of *M. jankae* and *M. risebachii* was diploid $2n = 2x = 24$. The karyotypes consisted of metacentric and submetacentric chromosomes, the metacentric ones being prevalent in the *M. jankae* populations, as in 6 of the populations of *M. grisebachii* and in all studied populations of *M. grisebachii*, satellites of one of the pairs of submetacentric chromosomes have been established

The data about the mean arithmetic of the studied quantitative traits of each trait have been presented in Table 8.

Table 8. Mean (upper numbers in each row), variance value (lower numbers in each row, V%) and percentage of the interspecies variation (SSv, %) in the overall morphological variation of *Moehringia jankae* Griseb. ex Janka and *Moehringia grisebachii* Janka for each of the 24 observed quantitative traits

	Moehringia grisebachii (Mg)	*Moehringia jankae* (Mj)	SSv %
1	40.485***	51.696***	92.20
V%	379.9	357.9	
2	10.396a	11.154a	99.20
V%	7.5	30.2	
3	1.532***	2.239***	67.28
V%	0.071	0.483	
4	7.057**	5.986**	98.60
V%	5.95	37.47	
5	8.389a	8.029a	99.63
V%	5.14	13.02	
6	9.150***	6.116***	73.59
V%	5.14	13.02	
7	1.546a	1.617a	99.33
V%	2.75	10.85	
8	6.136***	4.311***	86.59
V%	2.91	8.36	
9	3.318**	2.702**	96.98
V%	2.91	3.19	
10	2.037***	2.338***	92.69
V%	0.319	0.245	
11	1.883a	1.858a	98.85
V%	0.010	0.016	
12	1.524a	0.873a	99.69
V%	60.714	0.009	
13	2.071**	2.149**	97.20
V%	0.048	0.060	
14	2.016**	1.746**	44.54
V%	0.013	0.017	
15	1.018a	1.014a	99.98
V%	0.015	0.037	
16	2.005****	1.771***	85.01
V%	0.058	0.100	
17	11.100***	9.956***	91.15
V%	2.98	3.83	

18	1.794***	1.684***	81.57
V%	0.010	0.017	
19	1.766***	1.584***	86.69
V%	0.016	0.099	
20	1.515**	1.109**	97.16
V%	2.500	0.062	
21	0.979***	0.909***	87.49
V%	0.007	0.010	
22	0.835***	0.777***	90.30
V%	0.007	0.009	
23	0.389***	0.358***	94.58
V%	0.005	0.004	
24	1.179a	1.177a	99.99
V%	0.012	0.011	

Differences for the specific parameters by the two species are statistical significant at *P<0.05; **P<0.01; ***P<0.001, if have not equal letters

Intrapopulation variability has been traced on the basis of variance values. Their values differed and for both species with the strongest variability was the height of the stem. Greater variability in *M. grisebachii* has been registered for the width of sepals and in *M. jankae* – for the length of basal leaves and the length/width ratio.

Statistically reliable differences for the studied 24 quantitative traits between *M. jankae* and *M. grisebachii* have been proven for 17 of them. Greater differences between the two species have been found for the following traits: height of stem; width of basal leaves lamina; length of upper leaves lamina and length/width ratio; flower diameter; length/width ratio of petals; length of flower petiole; length and width of capsule; length, width and thickness of seed.

Statistically reliable difference has not been proved between the two species for 7 of the studied traits: length of lamina and petiole of basal leaves; width of upper leaves lamina; length and width of sepals; width of petals; length/width ratio of seeds.

For the populations of *M. grisebachii* and *M. jankae* three types of stomata have been established: paracytic, anomocytic and diacytic (Fig. 5, A-E and 8, B-E). Anomocytic type stomata prevailed in adaxial leaf surface of *M. jankae* and paracytic type prevailed in adaxial leaf surface of *M. grisebachii.*

Differences between *M. grisebachii* and *M. jankae* have been registered in relation to the sepal morphology (Fig. 5, F and 8, F). In *M. grisebachii* unlike *M. jankae*, sepals had a convex middle vein.

Our results showed that the pollen of *M. jankae u M. grisebachii* had dimension between 22 and 30 µm, pantoporate, with radial symmetry, with 12 - 14 pores (Fig. 5, G and 8, G). In *M. grisebachii* the diameter of pollen and pores varied within wider boundaries, but statistically reliable differences between *M. jankae* and *M. grisebachii* along those parameters have not been found. Statistically reliable differences have been registered for the size of interpore distances and in *M. jankae* they were bigger.

The colour of seed of all studied populations of the two species was dark and dull, the shape was subcircular and the strophiole were formed by numerous short cells of approximately equal length. Differences in the seed morphology between *M. jankae* and *M. grisebachii* have been found in relation to lateral faces. In *M. jankae* seeds were with convex lateral faces, while in *M. grisebachii* one side was with convex face and the other – with typical concavity on the surface.

Discussion

The reported till now chromosome counts for *Moehringia* have been varied and too limited. According to Probatova et al. (2006) the highest polyploidy in the genus *Moehringia* was registered for *M. lateriflora*, the species was very polymorphic, and it had the largest area of distribution. The most frequent chromosome number was $2n = 2x = 24$ and it had been reported by different researchers for the populations of *Moehringia trinervia* (L.) CLAIRV. (Rohweder, 1939; Litardiere, 1948; Gadella & Kliphuis, 1971; Hindakova, 1974; Strid, 1980; Kirschner et al., 1982); *Moehringia muscosa* L. (Findley & McNeill, 1974; Májovský et al., 1976; Kieft & Van Loon, 1978), *Moehringia intricata* R. Roem. ex Willk. (Luque & Lifante, 1991), *Moehringia lateriflora* (L.) Fenzl, $2n = 24$ (Probatova et al., 2018). The diploid chromosome count of $2n = 26$ was reported for *Moehringia intricata* (Luque & Lifante, 1991). Tetraploid chromosome number $2n = 48$ was reported for *Moehringia lateriflora* (L.) Fenzl (Zhukova, 1967; Goldblatt, 1985; Probatova et al., 2006, Probatova, 2014), *Moehringia trinervia* (Litardiere, 1948). For *Moehringia lateriflora* (L.) Fenzl, $2n = 36$ (Gurzenkov, 1995) and $2n = 50$-52 (Sokolovskaya, 1960) were also reported.

The chromosome numbers and the karyotype characteristics established for the current populations of *M. jankae* and *M. grisebachii* are in accordance with those indicated in our previous studies for other Bulgarian populations of the two species (Zhelyazkova et al., 2020a; b).

The results of the present complete population research confirm the data from our previous research (Grozeva & Cvetanova, 2011; 2013) on the species from Bulgarian flora, namely that vegetative traits are more variable than generative ones.

The main source of phenotype variation in all studied populations is the intrapopulation one. This, according to our observations, is related to the characteristics of habitats of *M. jankae* and *M. grisebachii* – rock crevices, and the biological type of the species – perennial herbaceous plants. Within the boundaries of one and the same population moisture varied throughout the entire vegetation in very wide range and the development of individual specimens had different speed in the various periods.

Moreover, part of the specimens which developed from seeds did not always reach flowering and fruiting, others were from last year tufts and they had higher stems and larger leaf laminas and they always reached flowering and fruiting. This determined the dominant role of intrapopulation within total variability of these traits. Higher interpopulation variability has been established for the other 3 traits – height of stem, width and length/width ratio of capsule. Due to the different exposure of the slope, in the various populations moisture was distributed differently and respectively specimens had quite different length of stem; for example, the lowest average values for the height of stem was observed in the two populations located on a slope facing the south (Table 1) – south-west of Karandila Hotel complex (Mj1) and around Karandilska polyana (Mj2). They were the highest in the population from Haidushka pateka (Mj4), where the greater part of specimens were on rocks facing the east, and less – on rocks facing the south and the west.

The data from CA of the studied populations of *M. jankae* and *M. grisebachii* suggests that there was a correlation between the degree of morphological variability and the altitudes in the populations of *M. jankae* and between the degree of karyological and morphological variability in those of *M. grisebachii.* For some of the populations of both species relationships were established between morphological similarity and exposure, number and area of population. Stomatal features are taxonomically important for identification and delimitation of various taxa at different taxonomic levels (Dilcher, 1974). Stomata type of *M. grisebachii* and *M. jankae* are described here for the first time. When performing SEM studies of the populations of both species differences in the quantification of individual types of stomata were recorded only for the adaxial leaf surface – in *M. grisebachii* prevailed paracytic type and in *M. jankae* prevailed anomocytic type. The diacytic type was also recorded in both species and prevailed on abaxial leaf surface. The present study matched with the result of Grewal (2000) who reported diacytic type of stomata in Caryophyllaceae. Sahreen et al. (2010) found for 16 species of the genus *Silene* from Pakistan a variety of stomata, i.e. diacytic, anomocytic and anisocytic. Li et al. (2016) reported *Miosoton aquaticum* and *Stellaria media* from populations in China to have anomocytic type of stomata.

The pollen morphology was an important character used by systematists for the families of the Centrospermae. In studies on the pollen morphology of *Caryophyllaceae* Skvarla (1975), Skvarla & Nowicke (1976), Nowicke & Skvarla (1977), Ghazanfar (1984), Arkan & İnceoğlu (1992) and Yıldız (1996a, 1996b; 2001a, 2001b) demonsrated that the pollen was usually medium sized ranging from 25 to 50 µm. The characteristics of pollen of *M. jankae and M. grisebachii* from Bulgarian populations were related to the species *Arenaria serpillifolia* (Candau, 1987).

The importance of seed morphology in *Moehringia* for classification has long been recognized (Heywood, 1969; Barthlott, 1984). According to Minuto et al. (2011) seeds display constant characters that can be useful for classification. Seeds of 27 species of *Moehringia* and four related representatives of *Arenaria* (old M. sect. Pseudomoehringia) were examined by Minuto et al. (2006; 2011), using scanning electron microscopy. Among the studied species were *M. jankae* and *M. grisebachii* with populations from Northern Dobrudzha, Romania. Dönmez et al. (2013) studied 8 taxa of Caryophyllaceae, belonging to the genera *Minuartia* L.*, Moehringia* L.*, Petrorhagia* (Ser.) Link and *Silene* L., among which was *M. grisebachii* with population from Kofçaz, Turkey. The specifics pointed out by Minuto et al. (2006; 2011) and Dönmez et al. (2013) about the seeds of *M. jankae* and *M. grisebachii* in relation to their shape and colour, surface and the strophilae corresponded to the findings in the present study. Testa cells had the traits pointed by Minuto et al. (2006) for the „grisebachii species“ – round cells having a weak secondary sculpturing, showing 3-4 teeth on each side with irregular contours resembling a cogwheel, and with an outer surface formed by convex (mamillate) epidermal periclinal walls. The strophiole, which according to Minuto et al. (2011) is a key feature of *Moehringia,* in all studied populations of *M. jankae* and *M. grisebachii* is formed by numerous short cells of approximately equal length, which Minuto et al. (2006) determined as papillate strophiole. Minuto et al. (2006) and Dönmez et al. (2013) did not report a specificity of the lateral faces of seeds in the Romanian and Turkish populations, which is recorded for Bulgarian populations. According to Minuto et al. (2011) the morphological variation of the seeds in *Moehringia* seems to reflect the geographical distribution of the taxa, but ecological adaptation may have played an important evolutionary role.

Conclusion

The data in the present study gave us ground to conclude that *M. jankae* and *M. grisebachii* are distinguished both by the characteristics of the karyotype (morphology and size of the chromosomes) and by some morphological features.

With regard to quantitative traits, *M. jankae* differ from *M. grisebachii* by wider basal leaf laminas, shorter upper leaf laminas and petioles and slightly bigger flowers, where the length of petals did not usually exceed that of sepals. However, the differentiation of both species only on the basis of these traits was not always sure since the dimensions of leaf laminas and petioles varied within wide range in each population and depended on the ecological conditions. Most probably, as the results show, sometimes the altitude, the exposure and the type of rock, the area and the number of populations also played a role.

Concerning the qualitative traits, *M. jankae* and *M. grisebachii* can be distinguished on the basis of sepal and pollen morphology and the specifics of lateral faces of seeds. A sure trait for their distinction was the hairing of stems, leaves and flower petioles in *M. grisebachii* with multicellular non-branched straight hairs, which is not present in *M. jankae*.

For thorough tracing of the karyological and morphological variability of *M. jankae* and *M. grisebachii* and more precise outlining of the traits for their distinction, population studies are needed covering the greater part of their area of distribution.

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