

Morphometric analysis of vegetative and reproductive organs of the *Fragaria* species

Original Article

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Abstract:

This paper presents results of a study on morphological variability of *Fragaria vesca*, *F. moschata* and *F. viridis* populations collected in their natural habitats in Serbia, Macedonia and Montenegro. The goals of the study were to determine the degree of morphological variability as well as the morphological characters with the most significant impact on differentiation of these species. The morphometric study was carried out on 7 populations (142 individuals x 19 morphological characters). Analyses included standard univariate (Descriptive, ANOVA) and multivariate (Principal Component Analysis, Canonical Discriminant Analysis, UPGMA classification) analyses. The obtained results indicate moderate to high variability in most of the analysed characters. *Fragaria moschata* has shown the highest level of variability for almost all characters in comparison to *F. vesca* and *F. viridis*. The most significant differentiation in *Fragaria* species was present in following characters: Leaf-L, Lam-cent-L, Lam-cent-W, Lam-lat-L, Ped-L and Sep-int-L.

Key words:

Fragaria, morphological characters, variability pattern

Apstract:

Morfometrijska analiza vegetativnih i reproduktivnih organa vrsta roda *Fragaria*

U ovoj studiji istraživana je morfološka varijabilnost populacija vrsta *Fragaria vesca*, *F. moschata* i *F. viridis* sakupljenih na njihovim prirodnim staništima u Srbiji, Makedoniji i Crnoj Gori. Ciljevi ovog rada su bili da se ustanovi nivo morfološke varijabilnosti kao i da se utvrde morfološki karakteri koji najviše doprinose u diferencijaciji ovih vrsta. Morfometrijska studija je sprovedena na 7 populacija (142 individua x 19 morfoloških karakterata). Uradene su standardne statističke metode univarijantne (Deskriptivna, ANOVA) i multivarijantne (Analiza glavnih komponenti (PCA), Kanonijska diskriminantna analiza (CDA), UPGMA klasifikacija) analize. Dobijeni rezultati ukazuju na umerenu do visoku varijabilnost većine analiziranih karakterata. *Fragaria moschata* je pokazala najveći stepen varijabilnosti za skoro sve karaktere u poređenju sa *F. vesca* i *F. viridis*. Karakteri koji su najviše doprineli u diferencijaciji istraživanih *Fragaria* vrsta su: Leaf-L, Lam-cent-L, Lam-cent-W, Lam-lat-L, Ped-L and Sep-int-L.

Ključne reči:

Fragaria, morfološki karakteri, varijabilnost

Introduction

Genus *Fragaria* L. includes 24 species (DiMeglio et al., 2014) distributed in Eurasia, North America and South America. Flora Europea (Tutin et al., 1968) lists 5 species: *Fragaria vesca* L., *F. moschata* Weston, *F. viridis* Weston, *F. virginiana* Mill. and *F. × ananassa* (Weston) Rozier, while Euro+Med Plantbase also includes *F. chiloensis* (L.) Weston.

In Serbia, this genus is represented with three wild

species: *F. vesca*, *F. moschata* and *F. viridis* (Gajić, 1972). These species are also present in Macedonia and Montenegro (Micevski, 1998; Euro+Med Plantbase). *Fragaria vesca* has the Eurasian origin and the most extensive native range among all *Fragaria* species, as it is distributed from the west of the Urals throughout Northern Europe and across the North American continent (Hummer et al., 2011). In Serbia it has a wide distribution (Gajić, 1972) and may be found in meadows and forests in following vegeta-



tion associations: *Quercetum frainetto-cerris*, *Querceto-Carpinetum*, *Quercetum montanum*, *Fagetum montanum*.

Fragaria moschata is distributed in Central and Southern Europe and the central part of Russia and Ukraine. In Serbia this species grows in forests (*Querceto-Fraxinetum*, *Quercetum farnetto-cerris* and *Querceto-Carpinetum*), at forest edges, burned areas, open grassy hillsides and meadows, usually in shadowy places and tall grass, preferring saturated fertile soils.

Fragaria viridis is distributed almost throughout Europe, as well as in Siberia and at the Canary Islands. In Serbia it is recorded at meadows (*Galieta-Festucetum vallesiaca*, *Chrysopogonetum pannonicum*), forests and burned areas (Gajić, 1972).

Morphological recognition among species is complicated within the genus *Fragaria*, due to high levels of morphological variations and similarities (Nosrati et al., 2015).

Diploids, tetraploids, hexaploids, octaploids and decaploids have been reported, as well as several intermediate ploidy levels of interspecific hybrids of *Fragaria* species (Nosrati et al., 2015). *Fragaria vesca* and *F. viridis* are diploids, while *F. moschata* is the only hexaploid species (DiMeglio et al., 2014). The best-studied form is *F. vesca*, included in many studies dealing with morphological variability caused by ecological conditions (Hancock & Bringhurst, 1978; Jenson & Hancock, 1981; Huseinović & Osmanović, 2010; Malnikova et al., 2013; Huseinović et al., 2014) or by genetic diversity

(Labokas & Bagdonaitė, 2005; Zhang et al., 2014). The quantification of variation at morphological and molecular levels within and between populations of *F. vesca* and the American octoploid species *F. chiloensis* and *F. virginiana* have been described by Harisson et al. (1997, 2000). Phenotypic variation within the diploid section of genus *Fragaria* was also a subject of several studies (Sargent et al., 2004; Labokas & Bagdonaitė, 2005). However, no quantitative studies of morphological variability have been carried out to measure the phenotypic variation between diploid and hexaploid *Fragaria* species.

This study aimed to research the morphological variability of vegetative and reproductive organs of two diploid *Fragaria* species (*F. vesca* and *F. viridis*) and a hexaploid species *F. moschata* and to determine the morphological characters with the most significant impact on differentiation between these species.

Materials and methods

Sample collections

The seven populations of *Fragaria* species (*F. vesca*, *F. viridis* and *F. moschata*) were collected for morphological analysis. The samples were taken from Serbia, Montenegro and Macedonia (Tab. 1) during two vegetation seasons (2017 and 2018). The voucher specimens were deposited in the Herbarium Moesiacum Niš (HMN) at Department of Biology and Ecology, University of Niš. Identification of collected material was made by using literature sources

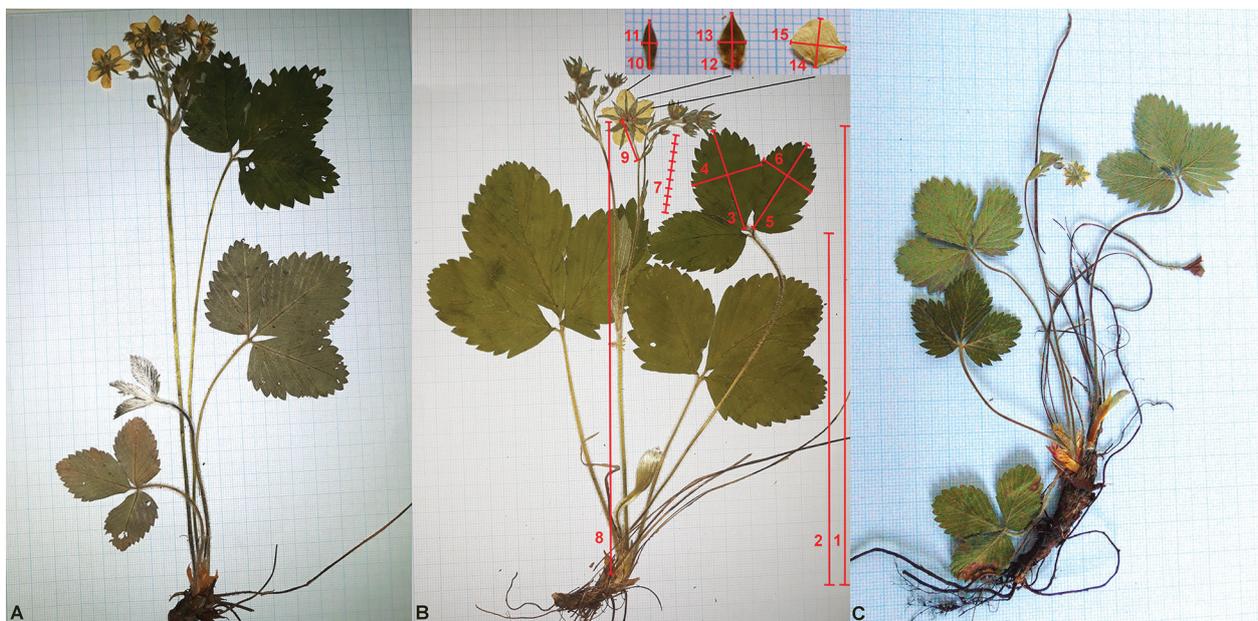


Fig 1. Some morphological characters of the vegetative and reproductive organs of the analysed species: A-*Fragaria moschata*, B-*F. vesca*, C-*F. viridis* (1-Leaf-L, 2-Peti-L, 3-Lam-cent-L, 4-Lam-cent-W, 5-Lam-lat-L, 6-Lam-lat-W, 7-Lob-lam-cent-N, 8-Scap-L, 9-Ped-L, 10-Sep-ext-L, 11-Sep-ext-W, 12-Sep-int-L, 13-Sep-int-W, 14-Pet-L, 15-Pet-W).

Table 1. The list of analysed populations of the *Fragaria* species

No.	Taxon	Locality	Number of individuals	Voucher number (HMN)	Legators
1.	<i>Fragaria viridis</i>	Stara planina Mt. (Serbia)	20	13697	Veličković, M. Nikolić, D.
2.	<i>Fragaria moschata</i>	Rtanj Mt. (Serbia)	20	13703	Veličković, M. Nikolić, D.
4.	<i>Fragaria moschata</i>	Vlasina (Serbia)	20	13701	Veličković, M. Nikolić, D.
3.	<i>Fragaria vesca</i>	Stol Mt. (Serbia)	20	13707	Veličković, M. Nikolić, D.
5.	<i>Fragaria vesca</i>	Kraljevica (Serbia)	12	13710	Veličković, M. Nikolić, D.
6.	<i>Fragaria vesca</i>	Jablanica (Macedonia)	20	13958	Raca I. Randelović, V.
7.	<i>Fragaria vesca</i>	Prokletije (Montenegro)	30	13959	Raca, I., Ljubisavljević, I. Jenačković, D. Randelović, V.

(Tutin et al., 1968; Gajić, 1972; Markova, 1973).

Morphometric analyses

The 7 morphological characters of vegetative organs and 12 morphological characters of reproductive organs were selected for analyses. Vegetative morphological characters included: length of leaf (Leaf-L), length of petioles (Peti-L), length of central lamina (Lam-cent-L), width of central lamina (Lam-cent-W), number of lobes on central lamina (Lob-lam-cent-N), length of lateral lamina (Lam-lat-L) and width of lateral lamina (Lam-lat-W). The reproductive morphological characters included: length of scape (Scap-L), length of peduncles (Ped-L), length of external sepal (Sep-ext-L), width of external sepal (Sep-ext-W), number of lobes on external sepal (Lob-sep-ext-N), number of external sepals with lobes (Sep-ext-Lob-N), length of internal sepal (Sep-int-L), width of internal sepal (Sep-int-W), length of petal (Pet-L), width of petal (Pet-W), number of flowers (Flow-N), number of flower branches (Flow-branch-N) (**Fig. 1**).

The plant material was first digitised by the camera (Sony 20 megapixels) and then measured using ImageJ software.

Statistical analysis

Descriptive statistics (mean, max, min, standard deviation and coefficient of variation) were computed to evaluate the range of variation for morphological traits.

The coefficient of variability was used to describe

the morphological variability of characters. Moderately variable characters are those with CV=20-40%, highly morphologically variable had CV higher than 40%, while stable characters are those with a coefficient of variation below 10%.

Analysis of variance (ANOVA) was performed to identify levels of significant variation for each character. Multivariate analyses (Principal component analysis (PCA), Canonical discriminant analysis (CDA) and Cluster analyses (UPGMA) based on Mahalanobis distances) were performed to examine relationships between the studied species and to find the best characters that facilitate discrimination between the species. Statistical analyses were performed using the package Statistika 8.0 (Statsoft 2007).

Results and discussion

Morphological variability of vegetative and reproductive characters - descriptive analysis

The results of the descriptive statistics have shown significant variability for almost all morphological characters (**Tab. 2**)

Generally, characters of vegetative and reproductive organs of *Fragaria* species have shown moderate to high degrees of variability. Following characters may be considered most variable within *F. moschata* populations, characterised by large-scale deviation of maximum and minimum values from the mean values, and with highest standard deviation values: Ped-L (23.40 ± 9.01), Leaf-L ($22.55 \pm$

Table 2. Results of descriptive statistics for the morphological characters of the *Fragaria* species.

Character	<i>Fragaria vesca</i>					<i>Fragaria moschata</i>					<i>Fragaria viridis</i>									
	N	Mean	Min.	Max.	Std. Dev.	N	Mean	Min.	Max.	Std. Dev.	N	Mean	Min.	Max.	Std. Dev.	N	Mean	Min.	Max.	Std. Dev.
Leaf-L (cm)	82	12.02	4.83	22.69	3.91	32.53	35	22.55	8.67	39.96	7.06	31.29	20	11.50	6.09	16.73	2.54	22.04		
Peti-L (cm)	82	8.39	2.60	16.77	3.22	38.42	35	16.49	5.40	28.83	5.72	34.70	20	8.29	3.06	12.71	2.24	27.01		
Lam-cent-L (cm)	82	3.34	1.99	5.06	0.76	22.67	35	5.32	2.68	10.49	1.62	30.55	20	2.90	2.02	4.02	0.66	22.90		
Lam-cent-W (cm)	82	2.47	1.31	3.81	0.53	21.43	35	3.44	1.74	6.52	0.93	26.94	20	2.06	1.19	2.86	0.47	22.92		
Lob-lam-cent-N	82	7.44	5.00	11.00	1.32	17.69	35	7.86	6.00	10.00	1.06	13.51	20	7.60	6.00	10.00	1.19	15.63		
Lam-lat-L (cm)	82	2.74	1.37	4.91	0.86	31.55	35	4.48	2.42	8.00	1.16	25.85	20	2.74	1.81	3.76	0.54	19.79		
Lam-lat-W (cm)	82	2.19	1.06	3.90	0.63	30.57	35	3.03	1.58	5.06	0.81	26.76	20	1.92	1.37	2.70	0.40	20.61		
Scap-L (cm)	81	2.34	0.70	10.11	1.70	72.76	35	4.25	1.31	8.60	2.09	49.22	20	3.00	1.35	6.00	1.13	37.59		
Ped-L (cm)	81	11.97	4.06	20.55	3.72	31.10	35	23.40	9.16	41.06	9.01	38.52	20	9.22	6.16	13.21	1.79	19.45		
Sep-ext- L (cm)	82	0.40	0.23	0.80	0.10	24.95	34	0.49	0.25	0.91	0.15	31.37	20	0.28	0.21	0.42	0.06	21.54		
Sep-ext-W (cm)	82	0.13	0.06	0.23	0.04	29.45	34	0.11	0.04	0.19	0.04	39.67	20	0.08	0.06	0.13	0.02	21.14		
Lob-sep-ext-N	73	2.00	2.00	2.00	0.00	0.00	22	2.00	2.00	2.00	0.00	0.00	20	2.00	2.00	2.00	0.00	0.00		
Sep-ext-lob-N	43	1.37	1.00	3.00	0.62	45.05	22	1.45	1.00	5.00	0.91	62.68	20	1.55	1.00	2.00	0.51	32.93		
Sep-int-L (cm)	82	0.42	0.26	0.71	0.08	20.35	35	0.59	0.26	0.97	0.17	27.96	20	0.33	0.26	0.45	0.06	18.74		
Sep-int-W (cm)	82	0.19	0.09	0.40	0.05	24.99	35	0.19	0.11	0.38	0.05	26.66	20	0.14	0.10	0.21	0.03	22.86		
Pet-L (cm)	71	0.49	0.28	0.88	0.14	28.88	34	0.68	0.29	0.94	0.17	25.10	20	0.38	0.25	0.66	0.12	32.00		
Pet-W (cm)	71	0.48	0.21	0.90	0.16	32.82	34	0.58	0.24	1.00	0.17	29.98	20	0.32	0.18	0.50	0.08	25.76		
Flow-N	82	3.15	1.00	6.00	1.33	42.12	35	4.09	1.00	8.00	1.67	40.86	20	2.50	1.00	5.00	1.05	42.05		
Flow-branch-N	82	1.40	1.00	4.00	0.66	47.35	35	1.00	1.00	1.00	0.00	0.00	20	1.50	1.00	3.00	0.61	40.47		

Table 3. Results of ANOVA and PCA analysis for quantitative characters of individuals of the *Fragaria* species.

Acronyms	F	p	PCA 1	PCA 2	PCA 3
Leaf-L	64.718	0.000	-0.932	-0.160	-0.080
Peti-L	56.213	0.000	-0.898	-0.129	-0.104
Lam-cent-L	52.904	0.000	-0.919	-0.173	0.066
Lam-cent-W	37.295	0.000	-0.862	-0.150	0.176
Lam-lat-L	47.633	0.000	-0.907	-0.257	-0.116
Lam-lat-W	31.539	0.000	-0.873	-0.230	-0.012
Lam-cent-lob-N	1.407	0.249	-0.398	-0.092	0.225
Scap-L	65.050	0.000	-0.898	-0.131	-0.028
Sep-Int-L	45.775	0.000	-0.672	0.477	-0.137
Sep-Int-W	8.180	0.000	-0.194	0.644	0.334
Pet-L	29.814	0.000	-0.616	0.450	-0.226
Pet-W	17.237	0.000	-0.499	0.605	-0.072
Sep-Ext-L	21.148	0.000	-0.578	0.552	-0.147
Sep-Ext-W	14.970	0.000	-0.079	0.701	0.378
Lob- Sep-Ext-N	11.130	0.000	0.398	0.413	-0.520
Flow-branch-N	7.503	0.001	0.407	0.050	0.328
Flow-N	9.115	0.000	-0.427	0.246	0.488
Ped-L	15.185	0.000	-0.573	-0.202	-0.301
Sep-Ext-Lob-N	3.837	0.025	0.234	0.263	-0.661

(F- Fisher’s coefficient, p- the level of significance ($p < 0.05$); PCA axes- Component with loadings > 0.700 is highlighted in bold)

7.06), Peti-L (16.49 ± 5.72). The number of lobes on central lamina (Lob-lam-cent-N) ranged from 6 to 10 lobes. The least variable character of the vegetative region within *F. moschata* populations was Lam-lat-W (3.03 ± 0.81). Of characters of the flowering parts, characters Sep-ext-W (0.11 ± 0.04) and Sep-int-W (0.19 ± 0.05) were distinguished as the least variable. The number of lobes on external sepal (Lob-sep-ext-N) was 2, while number of external sepals with lobes (Sep-ext-Lob-N) ranged from 1 to 5. The number of flowers (Flow-N) was represented by a range from 1 to 8, while number of flower branches (Flow-branch-N) was 1.

Slightly smaller variability of characters was recorded within *F. vesca* populations in comparison to *F. moschata* populations. The most variable characters of the vegetative region were Leaf-L (12.02 ± 3.91) and Peti-L (2.60 ± 16.77). The number of lobes on the central lamina (Lob-lam-cent-N) ranged from 5 to 11. The least variable characters of the vegetative region in *F. vesca* populations were Lam-cent-W (2.47 ± 0.53) and Lam-lat-W (2.19 ± 0.63). Among the characters of the flowering parts, characters Sep-ext-W (0.13 ± 0.04), Sep-int-W (0.19 ± 0.05) and

Sep-int- L (0.42 ± 0.08) were distinguished as the least variable.

Lobes on external sepals (Lob-sep-ext-N) was 2, while external sepals with lobes (Sep-ext-Lob-N) ranged from 1 to 3. Flowers (Flow-N) was from 1 to 6, while flower branches (Flow-branch-N) ranged from 1 to 4.

The morphological characters of vegetative and reproductive organs of *F. viridis* individuals have shown the least variability when compared with *F. moschata* and *F. vesca* populations. The most variable characters of the vegetative region were Leaf-L (11.5 ± 2.54) and Peti-L (8.29 ± 2.24). The number of lobes on central lamina (Lob-lam-cent-N) ranged from 6 to 10.

The least variable characters of the vegetative region within *F. viridis* populations were Lam-lat-W (1.92 ± 0.40) and Lam-cent-W (2.06 ± 0.47). Within the characters of the flowering parts, characters such as Sep-ext-W (0.08 ± 0.02) and Sep-int-W (0.14 ± 0.03) were determined to be the least variable.

Lobes on external sepals (Lob-sep-ext-N) was 2, while external sepals with lobes (Sep-ext-Lob-N) ranged from 1 to 2. Flowers (Flow-N) was repre-

sented by a range from 1 to 5, while flower branches (Flow-branch-N) ranged from 1 to 3.

According to values of the coefficient of variation, the most variable character ($CV > 70\%$) within the *F. vesca* populations was Scap-L. High level of variability was shown by characters Sep-ext-lob-N, Flow-N and Flow-branch-N. One character had no detected variability: Lob-sep-ext-N. All the other characters have shown moderate variability ($20\% < CV < 40\%$). Morphological characters with high variability within *F. moschata* populations were: Sep-ext-lob-N, Scap-L, and Flow-N. All the other characters have shown moderate variability, except character Lob-lam-cent-N with low variability and Lob-sep-ext-N and Flow-branch-N, which did not show any variability (Tab. 2). The characters

characters of flowering region, Scap-L ($F = 65.05$) and Sep-Int-L ($F = 45.78$) were the most significant. This set of characters with the highest F values is the most suitable for describing morphological differentiation between *Fragaria* species, based on the results of this analysis.

Morphological differentiation of analysed *Fragaria* species

The first three principal components accounted for 57.44% of the total observed variance (Tab. 2). The characters which contributed the most to variability along the first axis (36.19%) are the following: Leaf-L, Lam-cent-L, Lam-cent-W, Lam-lat-L, Ped-L and Sep-int-L. The second principal component

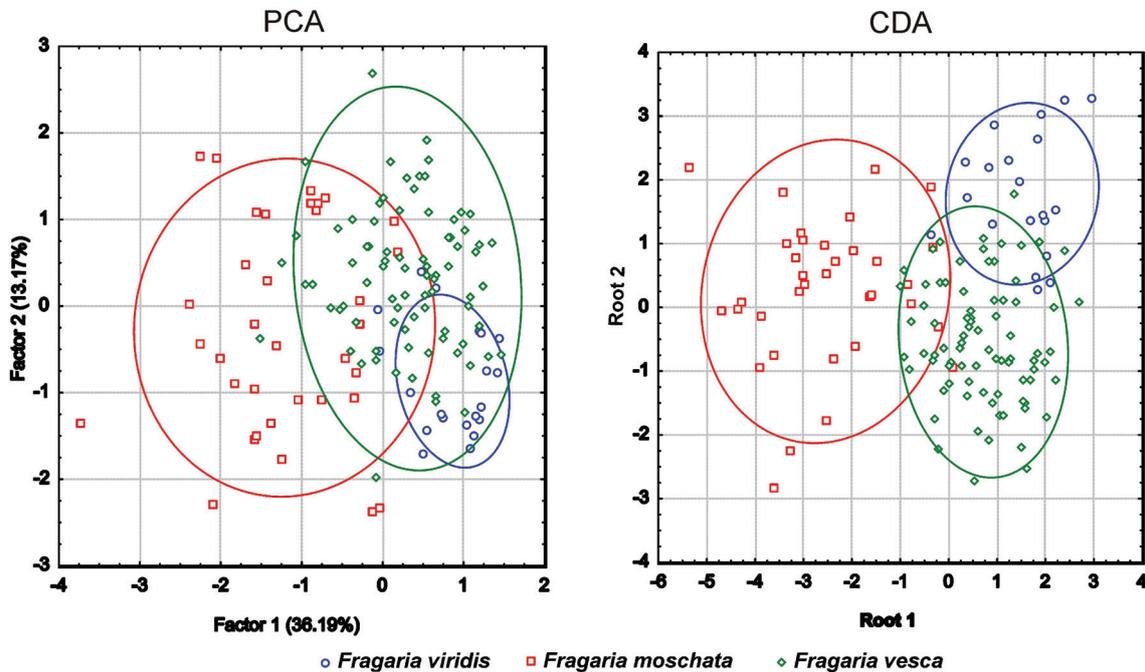


Fig 2. Results of the principal component analysis (PCA) and canonical discriminant analysis (CDA) based on morphometric characters of the *Fragaria* species

with low variability ($CV < 20\%$) within population of *F. viridis* were Lob-lam-cent-N, Lam-lat-L, Ped-L and Sep-int-L. Only Flow-N and Flow-branch-N have shown high variability ($CV > 40\%$), while all other investigated characters have shown a moderate degree of variability (Tab. 2).

Analysis of variance (ANOVA)

The ANOVA test has shown that all characters, except Lam-cent-lob-N, were statistically significant ($p < 0.005$) (Tab. 3). According to Fisher's coefficient (F), almost all vegetative characters may be treated as the most significant ($F > 30$). Among the

(13.17%) had high contributing factor loadings from Sep-ext-W. The third principal component accounted for 8.08% of the total variation.

The PCA ordination diagram of population samples (Fig. 2) indicated a slight differentiation of *F. moschata* and *F. viridis* populations along the first PC axis. The individuals of *F. vesca* are overlapping with individuals of *F. moschata* and *F. viridis*.

Canonical discriminant analyses have shown the separation of the three morphological groups of populations that match the analysed species (Fig. 2). Populations of *F. viridis* and *F. moschata* were differentiated along the first DA axis, while the popula-

tion of *F. viridis* was slightly differentiated along the second axis.

Cluster analysis of morphometric data has shown differentiation into two clusters, the first consisted of *F. vesca* and *F. viridis*, and the second included only *F. moschata* (Fig. 3).

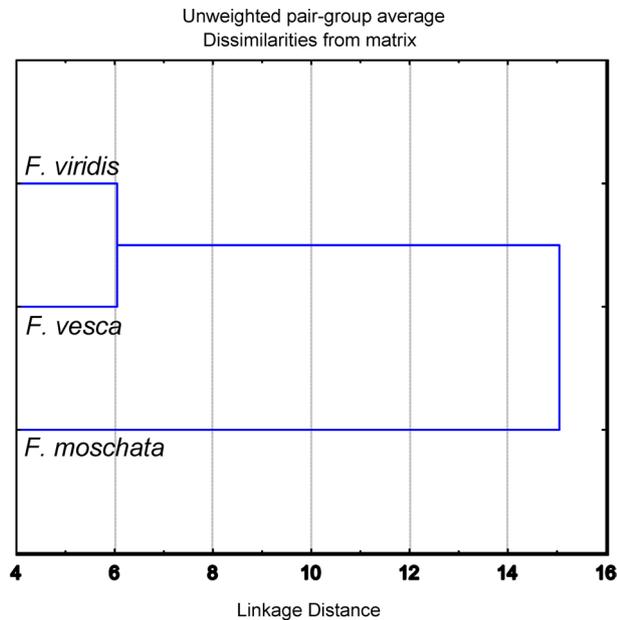


Fig 3. Results of cluster analysis (UPGMA) for morphological characters of the *Fragaria* species based on Mahalanobis distances

Our study confirmed the presence of a high degree of variability in vegetative and reproductive characters of *F. vesca* populations, which is in agreement with the previous studies on this species (Sargent et al., 2004; Labokas & Bagdonaitė, 2005; Huseinović & Osmanović, 2010; Maliníková et al., 2013). The study of phenotypic diversity of *F. vesca* and *F. viridis* by Labokas & Bagdonaitė (2005) recognised some differences in the number of flowers, length of petioles, length and width of leaf between *F. vesca* and *F. viridis*. In our sample, inflorescences of *F. vesca* populations were composed of 6-12 flowers while *F. viridis* produced a lower number of flowers (4-7). In comparison to populations of *F. viridis*, *F. vesca* was characterised by shorter petioles, longer inflorescences and a higher ratio of peduncle to rosette leaf length (Labokas & Bagdonaitė, 2005). Our study has shown different results regarding the range of variability of mentioned characters in *F. vesca* and *F. viridis* populations. Namely, the number of flowers in *F. vesca* populations ranged from 1 to 7 and in *F. viridis* populations from 1 to 5. Also *F. vesca* individuals had longer petioles than *F. viridis*. According to the available literature, morphological variability of *F. moschata* was never studied before.

This species has shown the highest level of variability for almost all characters. *F. moschata* individuals had the highest values for characters pertaining to leaves (Leaf-L, Leaf-W, Lam-cent-L, Lam-cent-W, Lam-lat-L, Lam-lat-W) and almost all morphological characters of the flowering part of the plant.

Conclusion

The morphological characters of vegetative and reproductive organs of *Fragaria* species have shown high to moderate levels of variability. The most variable morphological characters of vegetative organs were Peti-L and Leaf-L, while the most variable morphological characters of reproductive organs were Scap-L, Flow-branch-N, Flow-N, and Sep-ext-Lob-N. Analysis of variance has shown that all morphometric characters except Lam-cent-lob-N contributed significantly to differentiation of analysed *Fragaria* species. Morphological characters responsible for differentiation of analyzed *Fragaria* species included: Leaf-L, Lam-cent-L, Lam-cent-W, Lam-lat-L, Ped-L and Sep-int-L.

Acknowledgements. This investigation is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia grant number 173030.

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