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Morphological variability of endemic *Sempervivum ciliosum* subsp. *ciliosum* (Crassulaceae) from the Balkan Peninsula

Original Article

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Abstract:

Sempervivum ciliosum belongs to the group of yellow-flowered houseleek species (*S. ciliosum* complex) endemic for the territory of the Balkan Peninsula. Concerning the traditional taxonomy of genus *Sempervivum*, there are several taxa included in the above-mentioned group (*S. ciliosum, S. jakucsii, S. klepa, S. octopodes* and *S. galicicum*). However, due to highly expressed phenotypic variability, it isn't possible to certainly establish whether all taxa from that group, including *S. ciliosum* as a type species, are morphologically clearly defined. The aim of this study was to analyze the level of variability of morphological traits within the populations of *S. ciliosum*, taken in a narrow sense. Measurements of 36 quantitative traits of the vegetative and the flowering region of *S. ciliosum* subsp. *ciliosum*, including populations from Bulgaria, Greece and North Macedonia, were processed in STATISTICA 8.0. Results of Descriptive statistics, Correlation analysis and Univariate variance analysis (ANOVA) indicate that most of the analyzed traits express moderate to high variability within all examined populations.

Key words:

morphological traits, phenotypic plasticity, variability, Balkans

Apstract:

Morfološka varijabilnost endemične vrste *Sempervivum ciliosum* subsp. *ciliosum* (Carassulaceae) sa Balkanskog poluostrva

Sempervivum ciliosum pripada grupi žutocvetnih vrsta čuvarkuća (*S. ciliosum* complex) endemičnih za teritoriju Balkanskog poluostrva. U odnosu na tradicionalno shvatanje taksonomije roda *Sempervivum*, nekoliko taksona je uključeno u gore pomenutu grupu (*S. ciliosum*, *S. jakucsii, S. klepa, S. octopodes* i *S. galicicum*). Međutim, zbog veoma izražene fenotipske varijabilnosti, nije moguće sa sigurnošću utvrditi da li su svi taksoni ove grupe, uključujući *S. ciliosum* kao tipsku vrstu, morfološki jasno definisani. Cilj ove studije bila je analiza stepena varijabilnosti morfoloških karaktera u okviru populacija *S. ciliosum* s.s. Merenja 36 kvantitativnih karaktera vegetativnog i cvetnog regiona *S. ciliosum* subsp. *ciliosum*, kod jedinki populacija iz Bugarske, Grčke i Severne Makedonije, obrađena su u programu STATISTICA 8.0. Rezultati deskriptivne statistike, korelacione analize i univarijantne analize varijanse (ANOVA) ukazuju da većina analiziranih karaktera ispoljava umerenu do visoku varijabilnost u okviru analiziranih populacija.

Ključne reči:

morfološki karakteri, fenotipska plastičnost, varijabilnost, Balkansko poluostrvo

Introduction

Genus *Sempervivum* L. belongs to a relatively large family Crassulaceae J.St.-Hil. The family includes 1300-1500, mostly leaf and stem succulent species divided into 33-35 genera (Berger, 1930; Thiede & Eggli, 2007; Mort et al., 2010). Although some of the species of this family are extensively studied, highly expressed phenotypic variability as well as the ability of hybridization obscures the already vague picture of their morphological variability, taxonomy, as well as the number of taxa within the family.

Sempervivum L. is represented by perennial, herbaceous, rosulate, hardy leaf and partially stem succulent plants. According to an earlier, more widely accepted understanding, the genus *Sempervivum* encompasses 40 to 60 species ('t Hart et al., 2005; Thiede & Eggli, 2007). However, the latest studies indicate that there are 46 species within the genus (Karaer et al., 2011; Klein & Kadereit, 2015; Klein

& Kadereit, 2016) as well as 16 heterotypic infraspecific taxa and 16 described natural hybrids sensu 't Hart et al. (2003). Blurred picture of the total number of species within this genus is a consequence of the difficult identification of the representatives as well as the fact that currently there is an extremely high number of the names pertaining to species rank (Karaer et al., 2011). The natural habitat of *Sempervivum* species includes rocky and dry terrains of the high mountains of Europe and Asia ('t Hart et al., 2003). The only outlier of this genus, located outside the main area in Europe and Asia, is *S. atlanticum* (Hook.f.) Ball, endemic of northern Africa (Karaer et al., 2011). The taxonomic problems within this genus primarily arise from pronounced phenotypic plasticity, reflected through the manifestation of morphological similarity, which is often neglected or overemphasized, when describing species.

Sempervivum ciliosum taken in a broader sense represents a group of taxa, described from the territory of the central and southern parts of the Balkan Peninsula. Although intriguing taxonomical complex, *S. ciliosum* is still an under-researched group that includes *S. ciliosum* Craib as a type species, *S. jakucsii* Pénzes, *S. klepa* Micevski, *S. octopodes* Turrill and *S. galicicum* (A. C. Sm.) Micevski. *Sempervivum ciliosum* s.s. is a perennial, leafy succulent plant with closed or semi-open rosettes, 2-4 cm in diameter. Rosette leaves are oblong-oblanceolate to oblong-lanceolate, acute, characterized by the presence of long trichomes, both on the abaxial and adaxial surface as well as on the margin of leaves which can often be interwoven with each other and with trichomes of adjoining leaves. The flowering stem is erect, 4-10 cm long, cauline leaves are slightly wider than those in the rosette. Inflorescence is compact, composed of 9- to 14-merous flowers with acute, reddish-brown sepals, lemon yellow to yellow petals (8-12 × 1.5-2 mm), pale green carpels and pale yellow stamens. Within *S. ciliosum* two subspecies have been described ('t Hart, 2002; 't Hart et al., 2003): *Sempervivum ciliosum* subsp. *ciliosum* characterized by lemon yellow, 10-12 × 1.5 mm, petals, and *S. ciliosum* subsp. *octopodes* recognizable by pale yellow at base purple or lilac petals and purplish filaments. According to 't Hart et al. (2003) the first subspecies also includes *S. ciliosum* var. *galicicum* A. C. Sm., described from Mt. Galičica in North Macedonia. However, taxonomical position of this taxon is still not completely resolved since Micevski (1998) considers it as a separate taxon (*S. galicicum*) at the species rank. Due to highly expressed phenotypic plasticity and scarce literature data available of the morphological features, it is not possible to certainly establish whether all described species of this group are, so-called, valid or well-defined taxa. Therefore, the possibility of having more or fewer taxa, than the number already reported in the literature is present. Furthermore, taxonomic relations between taxa, nomenclature, and status of species within the complex are extremely vague.

Studies of the morphological variability of vegetative traits and traits from flowering region, despite the need for more detailed studies, have not been conducted for the taxa within *S. ciliosum* s.s. In accordance with current taxonomic concept ('t Hart et al., 2003), the species *S. ciliosum* here was considered in the narrow sense, as *S. ciliosum* subsp. *ciliosum* (incl. *S. ciliosum* var. *galicicum* A. C. Smith). Aim of this study was to examine the degree of variability of selected quantitative morphological traits from both previously mentioned regions of individuals from *S. ciliosum* subsp. *ciliosum* populations originated from different parts of the Balkan Peninsula. Therefore, the purpose of this study in theoretical terms was to determine the level of morphological variability of *S. ciliosum* and accordingly to clarify the taxonomic relationships both in the *S. ciliosum* complex and throughout the genus *Sempervivum*. The practical purpose was to provide additional information for a valid description of the taxa analyzed and to improve the keys for identifying species, and subspecies of this genus.

Material and methods

Plant material

Morphological traits from the vegetative and the flowering region were analyzed on 45 individuals from three populations collected from the territory of central and southern Balkan Peninsula. From each population, individuals were deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš (HMN) and Herbarium of the University of Belgrade (BEOU). Detailed information of habitat characteristics, legators and voucher numbers, as well as localities from where populations were collected, are given in **Tab. 1** and **Fig. 1**. During the field research, *S. ciliosum* was collected at a much smaller number of localities than expected because many of the reported distribution data for this species refer to other, morphologically similar species from the genera *Jovibarba* and *Sempervivum*. Finally, this species spontaneously hybridizes with other species of the genus *Sempervivum*, resulting in the large presence of hybrid individuals in most of the studied populations, also excluded from the study. In order to eliminate the influence of ecological factors on the variability of morphological traits of selected populations in their natural environment, all individuals, prior to morphometric analysis, were subjected to a period of growing, for a minimum

Table 1. Localities, habitat characteristics, legators name and voucher numbers of individuals from analyzed populations of *Sempervivum ciliosum* subsp. *ciliosum.*

Locality	Altitude (m)	Habitat	Subtratum	Legator	Voucher No.
Bulgaria, Mt. Rila, Rila	580	cliffs	silicate	Zlatković, B., Gussev, Ch.	HMN 13899
Greece, Mt. Orvilos	1737	subalpine rocky grasslands (*Daphno-Festucetea*)	limestone	Lakušić, D., Kuzmanović, N., Janković, I.	BEOU 46354
North Macedonia, Mt. Galičica, Trpejca	1570	rocks	limestone	Zlatković, B., Lazarević, P.	HMN 13991

of one year, in a greenhouse. Collected individuals from each population were planted in pots of the same diameter, on Floradur® substrate (FloraGard, Vertriebs GmbH für Gartenbau, Germany). After a growing period, 15 individuals from each population were morphometrically processed.

Morphometric procedures

A total of 36 quantitative morphological traits were analyzed for this study i.e., 18 morphological traits each from the vegetative and the flowering region. All morphological traits of the vegetative part (**Tab. 2**), except the height of the plant (PH), which was determined using the ruler, were measured using high precision digital caliper (Mahr Federal 4107107 16U, Esslingen, Germany) during the flowering period. Measurements of morphological traits from the inflorescence and flower (**Tab. 3**) were performed in several ways. Quantitative traits such as the number of flowers in the main inflorescence (NF), as well as number of sepals, petals, stamens and carpels (NS, NP, SN, NC), as meristic characters, are counted within all examined individuals during the flowering period. The angle of branching of the main inflorescence (ABI) was determined using a protractor. Measurements of length and width of the main inflorescence (LI, WI), length of the pedicel (LP) and diameter of flower (DF) are conducted by using a digital caliper. Length and width of parts of different segments of the flower are measured on scanned pictures of appropriate permanent slides, using Digimizer Image Analysis software (MedCalc Software©, Belgium). From all examined individuals, to prepare permanent slides, one flower was selected from a different region of the same inflorescence (central, middle and outer). Each permanent slide was prepared after floral structures of each flower were carefully dissected and placed on slides. After preparation, slides were scanned on a resolution of 600 dpi on HP Scanjet 200. As background, during the process of scanning, blue paper was used in order to provide high-contrast with yellowish floral parts. Obtained values of measured floral parts for a flower, from each region of the inflorescence, were statistically processed to obtain mean values of analyzed traits. This way of analyzing floral parts allowed us to reduce differences in dimensions of floral parts between flowers from a different region of an inflorescence. It should be also emphasized that, except for height of the plant (PH), diameter of the stem (DS), length of internodes in the lower and upper part of the stem (LIL, LIU), the number of flowers in the main inflorescence (NF) and angle of branching of the main inflorescence (ABI), measurements of all selected quantitative traits were performed in triplicate.

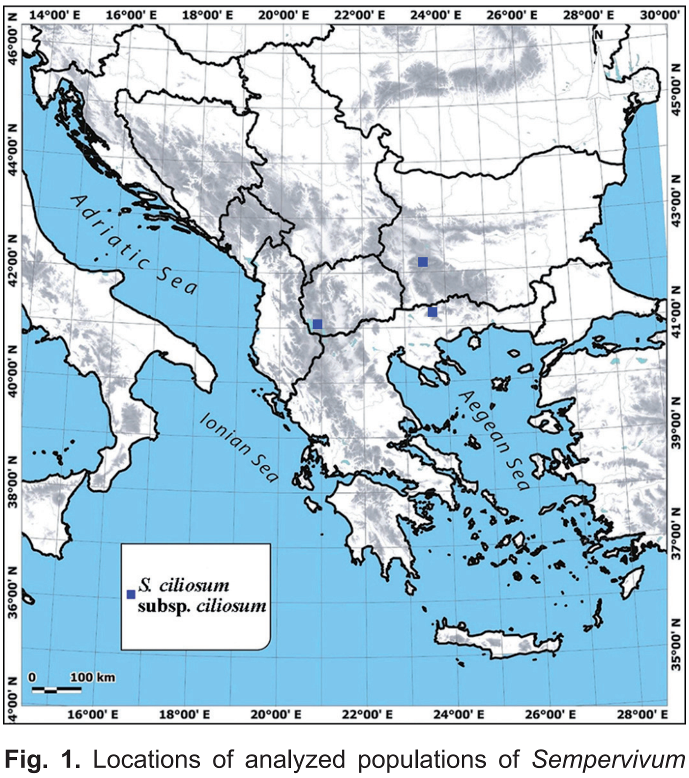

Fig. 1. Locations of analyzed populations of *Sempervivum ciliosum* subsp. *ciliosum.*

Table 2. Results of descriptive statistics and ANOVA of morphological traits from the vegetative region of analyzed individuals of *Sempervivum ciliosum* subsp *ciliosum*.

Character abbreviation, name and unit		N	Mean	Min.	Max.	Std. Dev.	CV %	F	p
PH	Height of the plant (cm)	45	23.97	16.50	36.30	4.99	21	6.33	0.0040**
DR	Diameter of the rosette (cm)	45	3.33	2.15	4.68	0.56	17	4.08	0.0240*
LLR	Length of the lower part of the rosettes leaf (mm)	45	21.62	9.05	40.99	7.17	33		0.0001***
LUR	Length of the upper part of the rosettes leaf (mm)	45	3.88	2.41	5.49	0.71	18	4.67	0.0137*
WLR	Width of leaves from the middle part of the rosette (mm)	45	6.48	4.68	9.17	1.06	16	0.15	0.8575 n.s.
TLR	Thickness of leaves from the middle part of the rosette (mm)	45	2.55	1.68	3.58	0.55	22		0.0002***
DS	Diameter of the stem (mm)	45	4.09	2.05	6.72	1.15	28		0.0000***
LIL	Length of internodes in the lower part of the stem (mm)	45	12.19	7.89	17.35	2.22	18	1.44	0.2475 n.s.
LIU	Length of internodes in the upper part of the stem	45	8.24	4.77	13.68	2.31	28	2.12	0.1327 n.s.
LLL	Length of the lower leaf of the stem (mm)	45	27.27	11.14	40.76	6.08	22	7.33	0.0019**
WLL	Width of the lower leaf of the stem (mm)	45	6.88	4.07	9.05	1.11	16	2.25	0.1181 n.s.
TLL	Thickness of the lower leaf of the stem (mm)	45	2.92	1.18	4.05	0.57	19	2.54	0.0912 n.s.
LML	Length of the middle leaf of the stem (mm)	45	25.23	15.37	36.88	4.70	19	3.19	0.0512 n.s.
WML	Width of the middle leaf of the stem (mm)	45	6.51	3.40	8.93	1.26	19	1.58	0.2181 n.s.
TML	Thickness of the middle leaf of the stem (mm)	45	2.51	1.23	3.59	0.52	21	4.55	0.0162*
LUL	Length of the upper leaf of the stem (mm)	45	19.83	12.69	28.77	3.52	18	5.76	0.0061**
WUL	Width of the upper leaf of the stem (mm)	45	4.66	3.01	6.72	0.92	20	2.94	0.0640 n.s.
TUL	Thickness of the upper leaf of the stem (mm)	45	1.61	1.03	2.10	0.27	17	0.16	0.8513 n.s.

(N – number of individuals, Mean – Mean value, Min. – Minimum value, Max. – Maximum value, Std. Dev. – Standard Deviation, CV% – Coefficient of Variation, F – Fisher's coefficient, p – level of significance, ***- extremely high statistical significance, **- high statistical significance, *- low statistical significance; n.s.- not significant)

Statistical analysis

For the purposes of this study, variability of selected quantitative morphological traits was analyzed using basic statistical analysis (Descriptive statistics), Correlation analysis and Univariate analysis of variance (ANOVA). All values of measured traits were statistically processed using STATISTICA 8.0 (StatSoft, Inc., Tulsa, USA).

Results

Descriptive statistics

Based on obtained results of descriptive statistics, quantitative morphological traits from the vegetative region manifest a low to moderate degree of variability (**Tab. 2, Fig. 2**). Morphological traits which stand out by a higher degree of variability, based on highest values of standard deviation, which is the

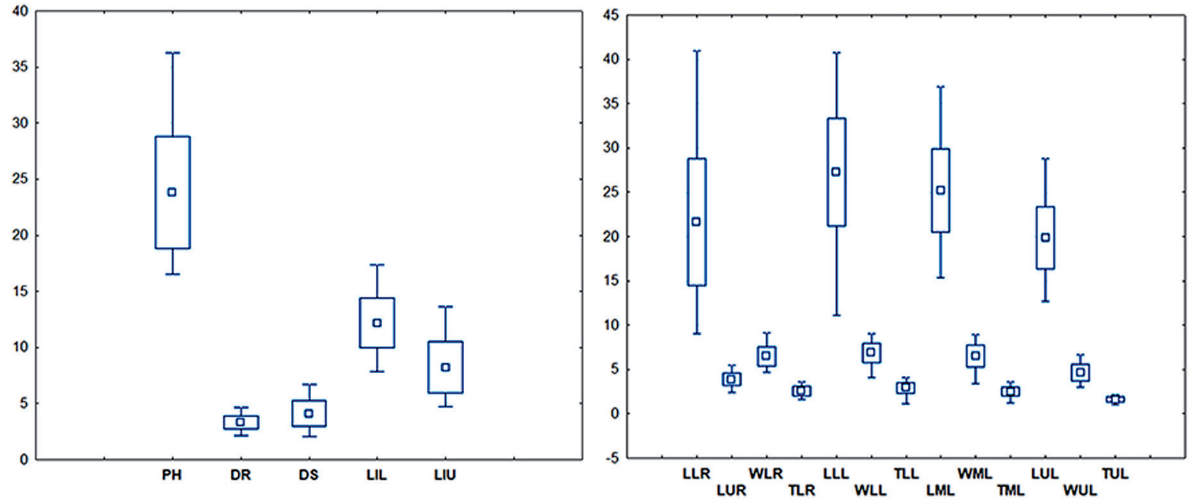

Fig. 2. Box and Whisker plots of basic statistic parameters of analyzed vegetative morphological traits of *Sempervivum ciliosum* subsp. *ciliosum*: height of the plant (PH); DR-diameter of the rosette; DS- diameter of the stem; LIL- length of internodes in the lower part of the stem; LIU- length of internodes in the upper part of the stem; LLR- length of the lower part of the rosettes leaf; LUR-length of the upper part of the rosettes leaf; WLR- width of leaves from the middle part of the rosette; TLR- thickness of leaves from the middle part of the rosette; LLL- length of the lower leaf of the stem; WLL- width of the lower leaf of the stem; TLL- thickness of the lower leaf of the stem; LML- length of the middle leaf of the stem; WML- width of the middle leaf of the stem; TML- thickness of the middle leaf of the stem; LUL- length of the upper leaf of the stem; WUL- width of the upper leaf of the stem; TUL- thickness of the upper leaf of the stem (middle point – Mean value; box – Mean ± SD; whisker – Minimum value - Maximum value).

consequence of large scale differences of minimum and maximum values from mean values, are: length of the lower part of the rosettes leaf (LLR, 21.62 ± 7.17), length of the lower leaf of the stem (LLL, 27.27 ± 6.08), height of the plant (PH, 23.97 ± 4.99) and length of the middle leaf of the stem (LML, 25.23 ± 4.70). Values of the most variable trait of the vegetative region, length of the lower part of the rosettes leaf (LLR), ranges from 9.05 mm, as a minimum, up to 40.99 mm, as a maximum value. Less variable trait, the height of the plant (PH), range from 16.50 cm to 36.30 cm. Furthermore, lengths of the leaves of the stem are distributed in the range of minimal and maximal values of 11.14-40.76 mm for the lower leaves (LLL) and 15.37-36.88 mm for the middle leaves (LML). Morphological traits from the vegetative region exhibiting the lowest degree of variability are the following: thickness of the upper leaf of the stem (TUL, 1.61 ± 0.27) with a range of minimal and maximal values of 1.03-2.10 mm, thickness of the middle leaf of the stem (TML, 2.51 ± 0.52) with values in range of 1.23-3.59 mm, thickness of the leaves from the middle part of rosette (TRL, 2.55 ± 0.55) which is within a range of 1.68-3.58 mm, diameter of the rosette (DR, 3.33 ± 0.56) characterized by a minimum value of 2.15 cm and maximal value of 4.68 cm, and thickness of the lower leaf of the stem (TLL, 2.92 ± 0.57) with values within a range of 1.18-4.05 mm. Obtained values of the coefficient of variation indicate that only length of the lower part of the rosettes leaf is highly variable (LLR, CV=33%) while remaining of analyzed morphological traits from vegetative region show moderate degree of variability (CV=10-30%).

Quantitative morphological traits from flowering region, generally, and according to obtained results, are less variable regarding to traits of the vegetative region. Although traits of the inflorescence and flower show a low to a high degree of variability, the number of traits with low variability is significantly higher in regards to highly variable ones. As the most variable traits stand out: length of the main inflorescence (LI, 65.92 ± 25.66), width of the main inflorescence (WI, 70.38 ± 24.95), number of flowers in the main inflorescence (NF, 37.75 ± 18.37) and angle of branching of the main inflorescence (ABI, 46.20 ± 14.94). The most variable trait, length of the main inflorescence (LI) has a range of minimal and maximal values from 20.41 mm to 121.49 mm. Thus, the width of the main inflorescence (WI) is within the range of 29.43-137.35 mm, number of flowers in the main inflorescence (NF) varies from 7 to even 103 flowers per inflorescence and minimal value of angle of branching of the main inflorescence (ABI) is 24.00° while the maximal value is 90.00°. The morphological traits of lowest variability are as follows: width of carpels (CW, 2.08 ± 0.25), width of petals (WP, 2.11 ± 0.31) width of sepals (WS, 1.58 ± 0.33), length of longer filaments of the flower (LLF, 5.13 ± 0.48) and length of shorter filaments of the flower (LSF, 4.63 ± 0.49). Based on the values of the coefficient of variation most of the analyzed traits from the flowering region are in the extent of moderate variability, while only four of analyzed traits show a high degree of variability: number of flower in the main inflorescence (NF, CV=49%), length of the main inflorescence (LI, CV=39%), width of the main inflorescence (WI, CV=35%) and angle of branching of the main inflorescence (ABI, CV=32%). These same traits, as already explained, have been characterized as the most variable, based on the values of standard deviation. Thus, length of longer filaments of the flower (LLF) and length of petals (LP) with the values of coefficient of variation of 9%, number of carpels (NC) with coefficient of correlation of 8%, number of sepals (NS), as well as number of petals (NP) and number of stamens (SN), all characterized with the value of 7% of the same coefficient, are presented as the least variable. Results for minimum and maximum values, as well

Table 3. Results of descriptive statistics and ANOVA of morphological traits from the inflorescence and flower of analyzed individuals of *Sempervivum ciliosum* subsp. *ciliosum*.

Character abbreviation, name and unit		N	Mean	Min.	Max.	Std. Dev.	CV %	F	p
LI	Length of the main inflorescence (mm)	45	65.92	20.41	121.49	25.66	39	6.01	0.0050**
WI	Width of the main inflorescence (mm)	45	70.38	29.43	137.35	24.95	35	2.78	0.0738*
NF	Number of flowers in the main inflorescence	45	37.75	7.00	103.00	18.37	49	13.15	0.0000***
ABI	Angle of branching of the main inflorescence (˚)	45	46.20	24.00	90.00	14.94	32	12.48	0.0001***
PL	Length of the pedicel (mm)	45	1.82	0.92	2.83	0.51	28	13.41	0.0000***
DF	Diameter of the flower (mm)	45	21.53	16.65	27.12	1.85	9	9.26	0.0005***
NS	Number of sepals of the flower (mm)	45	11.58	10.00	13.67	0.82	7	16.55	0.0000***
LS	Length of sepals of the flower (mm)	45	4.66	3.45	7.37	0.68	15	18.29	0.0000***
WS	Width of sepals of the flower (mm)	45	1.58	1.10	3.12	0.33	21	15.86	0.0000***
NP	Number of petals of the flower	45	11.59	10.00	13.67	0.84	7	17.63	0.0000***
LP	Length of petals of the flower (mm)	45	9.87	8.27	12.11	0.91	9	31.91	0.0000***
WP	Width of petals of the flower (mm)	45	2.11	1.49	3.10	0.31	15	15.36	0.0000***
SN	Number of stamens of the flower	45	23.09	20.33	27.33	1.71	7	14.06	0.0000***
LSF	Length of shorter filaments of the flower	45	4.63	3.40	6.14	0.49	11	0.72	0.4936n.s.
LLF	Length of longer filaments of the flower	45	5.13	4.09	6.47	0.48	9	2.42	0.1011n.s.
NC	Number of carpels of the flower	45	11.47	10.00	13.67	0.86	8	20.66	0.0000***
LC	Length of carpels of the flower (mm)	45	5.70	4.12	6.93	0.72	13	46.39	0.0000***
CW	Width of carpels of the flower (mm)	45	2.08	1.53	2.67	0.25	12	15.66	0.0000***

(N – number of individuals, Mean – Mean value, Min. – Minimum value, Max. – Maximum value, Std. Dev. – Standard Deviation, CV% – Coefficient of Variation, F – Fisher's coefficient, p – level of significance, ***- extremely high statistical significance, **- high statistical significance, *- low statistical significance)

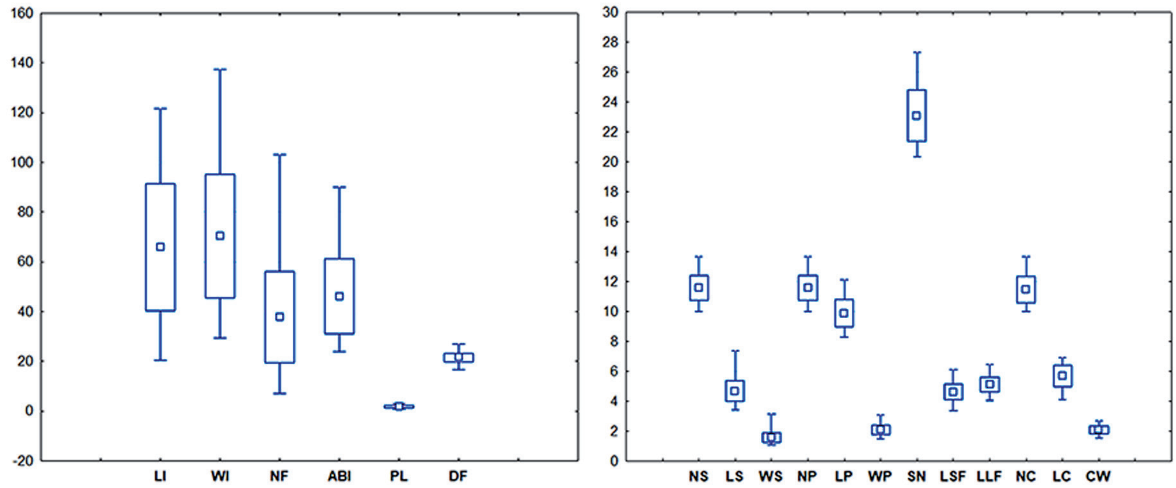

Fig. 3. Box and Whisker plots of basic statistic parameters of analyzed morphological traits from flowering region of *Sempervivum ciliosum* subsp. *ciliosum*: LI- length of the main inflorescence; WI- width of the main inflorescence; NF- number of flowers in the main inflorescence; ABI- angle of branching of the main inflorescence; PL- length of the pedicel; DF-diameter of the flower; NS- number of sepals; LS- length of sepals; WS- width of sepals; NP- number of petals; LP- length of petals; WP- width of petals; SN- number of stamens; LSF- length of shorter filaments; LLF- length of longer filaments; NC- number of carpels; LC- length of carpels; CW- width of carpels (middle point – Mean value; box – Mean ± SD; whisker – Minimum value - Maximum value).

as mean values, standard deviation and coefficient of variation, of all analyzed morphological traits of flowering region, are given in **Tab. 3** and **Fig. 3**.

Correlative variability

Results from correlation analysis of analyzed morphometric traits have shown that all three degrees of linear correlation were represented, both as negative and positive form of correlations. Majority of analyzed traits, 54.60%, show a weak positive linear correlation with values of correlative coefficient (r) between 0.00 and 0.50. A weak negative correlation ($-0.50 < r < 0.00$) is represented in 32.06% pairs of morphological traits. Moderate, positive ($0.50 < r < 0.80$) or negative ($-0.50 < r < -0.80$), correlation is less represented, i.e. in 10.63% as positive correlation form, respectively, 0.79% as negative correlation form. Thus, strong linear connectivity was established only as a positive form of correlation ($0.80 < r < 1.00$), in three pairs of traits from vegetative and nine pairs of traits from the flowering region (1.90% in total). The pairs of traits with highest degree of correlation ($r > 0.90$) are: length of shorter filaments of the flower/length of longer filaments of the flower (LSF/LLF), number of sepals/number of petals (NS/NP), length of sepals/width of sepals (LS/WS), number of petals/number of carpels (NP/NC), number of petals/number of stamens (NP/SN), number of sepals/number of carpels (NS/NC), number of stamens/number of carpels (SN/NC), number of sepals/number of stamens (NS/SN) and thickness of the middle leaf of the stem/thickness of the lower leaf of the stem (TML/TLL) (**Tab. 4**). The correlation among pairs of traits from the vegetative region, as well as pairs of traits from the vegetative and the flowering region is mostly represented by weak negative and moderate positive linear correlation, while a correlation between pairs of traits from the flowering region is mostly represented by weak negative correlation.

Analysis of variance (ANOVA)

The significance of mean values differences were tested using Analysis of variance (ANOVA) for all selected morphological traits. Based on p values (*** $p < 0.001$), most of the analyzed traits are extremely statistically significant, and as such may be considered as the most significant for general morphological variability of analyzed populations. As the most variable morphological traits, from the vegetative region, stand out: diameter of the stem (DS) with p=0.0000, length of the lower part of the rosettes leaf (LLR) with p=0.0001 and thickness of leaves from the middle part of the rosette (TLR) with p=0.0002 (**Tab. 2**). Statistically significant traits at extremely high level, from the flowering region, are: diameter of the flower (DF, p=0.0005), the angle of branching of the main inflorescence (ABI, p=0.0001), as well as all morphological traits of flower parts, all characterized by p=0.0000, except for the length of shorter filaments of the flower (LSF) and length of longer filaments of the flower (LLF), which proves to be not significant (**Tab. 2**). Among previously mentioned morphological traits, according to the highest obtained values of Fisher's coefficient (F), the

Table 4. Values of correlation coefficient (*r*) for pairs of morphological traits with strong positive correlation (bold values) within analyzed populations of *Sempervivum ciliosum* subsp. *ciliosum*.

	LLL	WLL	TLL	LML	TML	LUL	NS	LS	WS	NP	LP	SN	LSF	LLF	NC	LC
LLL	1.00	0.81	0.37	0.76	0.28	0.65	-0.17	-0.01	0.00	-0.19	0.39	-0.12	-0.26	-0.15	-0.30	0.55
WLL	**0.81**	1.00	0.63	0.74	0.56	0.62	-0.19	0.14	0.21	-0.18	0.27	-0.15	-0.17	-0.16	-0.25	0.41
TLL	0.37	0.63	1.00	0.66	0.91	0.57	-0.20	0.15	0.17	-0.17	-0.21	-0.18	-0.12	-0.21	-0.15	-0.13
LML	0.76	0.74	0.66	1.00	0.67	0.85	-0.38	-0.05	-0.04	-0.38	0.07	-0.31	-0.13	-0.13	-0.40	0.22
TML	0.28	0.56	**0.91**	0.67	1.00	0.61	-0.30	0.01	0.04	-0.25	-0.28	-0.26	-0.17	-0.26	-0.19	-0.22
LUL	0.65	0.62	0.57	**0.85**	0.61	1.00	-0.40	-0.17	-0.18	-0.40	0.04	-0.35	-0.16	-0.15	-0.44	0.23
NS	-0.17	-0.19	-0.20	-0.38	-0.30	-0.40	1.00	0.49	0.39	0.96	0.23	0.92	0.09	0.10	0.94	-0.17
LS	-0.01	0.14	0.15	-0.05	0.01	-0.17	0.49	1.00	0.95	0.48	0.28	0.50	0.12	0.07	0.51	0.01
WS	0.00	0.21	0.17	-0.04	0.04	-0.18	0.39	**0.95**	1.00	0.38	0.27	0.43	0.14	0.07	0.42	0.08
NP	-0.19	-0.18	-0.17	-0.38	-0.25	-0.40	**0.96**	0.48	0.38	1.00	0.20	0.93	0.08	0.09	0.93	-0.19
LP	0.39	0.27	-0.21	0.07	-0.28	0.04	0.23	0.28	0.27	0.20	1.00	0.22	0.44	0.57	0.08	0.84
SN	-0.12	-0.15	-0.18	-0.31	-0.26	-0.35	**0.92**	0.50	0.43	**0.93**	0.22	1.00	0.08	0.08	0.90	-0.18
LSF	-0.26	-0.17	-0.12	-0.13	-0.17	-0.16	0.09	0.12	0.14	0.08	0.44	0.08	1.00	0.96	0.07	0.33
LLF	-0.15	-0.16	-0.21	-0.13	-0.26	-0.15	0.10	0.07	0.07	0.09	0.57	0.08	**0.96**	1.00	0.05	0.48
NC	-0.30	-0.25	-0.15	-0.40	-0.19	-0.44	**0.94**	0.51	0.42	**0.93**	0.08	**0.90**	0.07	0.05	1.00	-0.34
LC	0.55	0.41	-0.13	0.22	-0.22	0.23	-0.17	0.01	0.08	-0.19	**0.84**	-0.18	0.33	0.48	-0.34	1.00

following traits can be considered as the most significant: length of carpels (LC, F=46.39), length of petals (LP, F=31.91) number of carpels (NC, F=20.66), length of sepals (LS, F=18.29), and number of petals (NP, F=17.63). This morphological traits may be considered as those that best indicate morphological differences between analyzed populations of *S. ciliosum*.

Furthermore, traits statistically not significant for delamination of analyzed populations from vegetative region (**Tab. 2**), based on high p values (and extremely low F values), are as follows: width of leaves from the middle part of the rosette (WLR, p=0.8575), width of the middle leaf of the stem (WML, p=0.2181), length of internodes in the lower part of the stem (LIL, p=0.2475), length of internodes in the upper part of the stem (LIU, p=0.1327), width of the lower leaf of the stem (WLL, p=0.1181), thickness of the lower leaf of the stem (TLL, p=0.0912), width of the upper leaf of the stem (WUL, p=0.0640) and length of the middle leaf of the stem (LML, p=0.0512). Above-mentioned set of morphological traits should not be considered as relevant for describing main morphological differences between the analyzed populations of *S. ciliosum* subsp. *ciliosum*.

Discussion

Morphological variability of any taxa from *S. ciliosum* complex, including *S. ciliosum* s.s has not been extensively researched. Relatively limited amounts of scientific data, which indicate the degree of variability of morphological traits of previously mentioned taxon, especially those from the flowering region, are available in the current floristic literature (Hagemann, 1986; Parnell & Favarger, 1993; 't Hart, 2002; 't Hart et al., 2003). From the other side, understanding of degree and pattern of the variability of morphological traits is the first step leading to an understanding of taxonomical relation between all related yellow-flowered taxa from Balkan Peninsula belonging to *S. ciliosum* group.

Obtained results in this study indicate that morphological traits from the vegetative region are characterized by a greater degree of variability than those from the flowering region. A high degree of variability, pronounced within the vegetative morphological traits, especially the leaf traits, may be, to some extent, justified by the possibility of these plants to store water in leaves. But, regardless of the growing

Table 5. A comparative review of values of morphological traits of *S. ciliosum* subsp. *ciliosum* from this study and corresponding traits of *S. ciliosum* s.s. represented in literature (Hagemann, 1986; Parnell & Favarger, 1993; 't Hart, 2002, 't Hart et al., 2003).

Character name and abbreviation	This study	Hagemann, 1986	Parnell & Favarger, 1993	't Hart, 2002	't Hart et al., 2003
Height of the plant (**PH**)	23.97-36.30 cm	12 cm	4-10 cm	–	13 cm
Diameter of the rosette (**DR**)	2.15-4.68 cm	2-3.5 cm	2-3(-5) cm	2-5 cm	2-5 cm
Length of rosette leaves	11.46-46.48 mm*	15-20 mm	10 mm	7-10(-25) mm	7-10(-25) mm
Width of leaves from the middle part of the rosette (**WRL**)	4.68-9.17 mm	3-4(-6) mm	4 mm	3-4(-6) mm	3-4(-6) mm
Diameter of the flower (**DF**)	16.65-27.12 mm	16-25 mm	–	–	16-25 mm
Length of the sepals (**LS**)	3.45-7.37 mm	3-4 mm	–	5-7 mm	5-7 mm
Length of petals (**LP**)	8.27-12.11 mm	8-10 mm	10-12(-15) mm	8-12 mm	–
Width of the petals (**WP**)	1.49-3.10 mm	1.5-2 mm	–	1.5-2 mm	–
Length of shorter filaments (**LSF**) Length of longer filaments (**LLF**)	4.63-6.14 mm 5.13-6.93 mm	–	–	~ 6 mm	–
Length of carpels (**LC**)	4.12-6.93 mm	6-7 mm	–	6-7 mm	–

*expressed as a sum of the length of the lower part of the rosettes leaf (**LLR**) and length of the upper part of the rosettes leaf (**LUR**).

procedure that was conducted, morphological traits of the leaves pronounced high degree of variability, which indicates that even under the same condition leaves of populations of *S. ciliosum* subsp. *ciliosum* are very variable. Furthermore, in this study majority of analyzed traits of flowering region manifest low level of variability with exception of length of the main inflorescence (LI), width of the main inflorescence (WI) and number of flowers in the main inflorescence (NF) as well as the angle of branching of the main inflorescence (ABI) with higher pronounced degree of variability. Low extent of degree of morphological variability within individuals of the analyzed populations is characteristic for flower parts, such as width and length flower parts. It is interesting to emphasize that the width of the sepals (WS) is one of the least variable morphological trait, although very often sepals may exhibit a feature of succulence. One of the reasons for the explained difference in variability between morphological traits of vegetative and the flowering region is probably that morphology of vegetative parts is more ecologically determined, vegetative parts are more prone to store water, which, ultimately, affects on the variability of their morphology.

Values of the morphological traits of *S. ciliosum* s.s. represented in the literature (Hagemann, 1986; Parnell & Favarger, 1993; ‘t Hart, 2002; ‘t Hart et al., 2003) are compared with the corresponding morphological traits of S *ciliosum* subsp. *ciliosum* obtained in this study (**Tab. 5**). In comparison with data given in the literature, the obtained values of vegetative traits in this study are significantly higher, whit the exception of values diameter of the rosette (DR) which differs in narrower scale. The differences are most noticeable in the traits height of the plant (PH) and length of rosette leaves (expressed as a sum of the length of the lower part of the rosettes leaf (LLR) and length of the upper part of the rosettes leaf (LUR)). Those variations may be partially explained due to stable growth conditions in which analyzed individuals developed during the period of growing, i.e. amount of water that the plants received during this period were the same. But it is important to note that this is certainly not the only, and most important, cause of such differences. Regarding the morphological traits from the flowering region, more traits are available for comparison.

Generally, measured values of traits of flower parts are slightly higher, except for the length of carpels (LC) which proves to have lower values, than those presented in the literature (**Tab. 5**). Values of the diameter of the flower (DF), given in this study, do not deviate significantly, in a morphological sense, from those given in scientific sources. Measured values of length of petals (LP), as well as width of the petals (WP), are within the range, or could vary in narrower scale regarding the maximum value, of values given in scientific sources (Hagemann, 1986; Parnell & Favarger, 1993; 't Hart, 2002). Values of the length of filaments are difficult to compare to those stated in literature reports, because in this study variability of filaments was observed through two traits: length of shorter filaments (LSF) and length of longer filaments (LLF), while in literature this segregation is nonexistent i.e. all filaments were considered as equals. Regardless of this, maximal values of length of the shorter filaments (LSF) and length of the longer filaments (LLF) are slightly higher than those suggested by 't Hart (2002). It is interesting to note that, regarding morphological variability between analyzed populations, values of morphological traits from the flowering region are generally lower in individuals from Mt. Galičica while values of traits from the vegetative region are higher regarding values of traits of individuals from Mt. Rila and Mt. Orvilos. This morphological variation may be due to the fact that the population from Mt. Galičica belongs to *S. ciliosum* var. *galicicum*. As already explained Micevski (1998) considers previously mentioned taxon as *S. galicicum* but to confirm this statement more detailed studies are needed.

The number of morphological traits analyzed in this study is much greater than those presented in the referent scientific sources. As explained in the previous chapter, in the current literature, for the purposes of distinguishing *S. ciliosum* from related species, a greater number of characters of the flowering region are in use. Those traits are recognized as more stable i.e. traits in which morphological variability is less expressed, which was also confirmed by this study. Obtained differences in values of analyzed morphological traits in this study, from those mentioned in the available literature, may arise from the following reasons: the impact of growing conditions, especially on traits from vegetative region, enhanced phenotypic plasticity, insufficiency of more data of variability as well as that many of data present in literature are outdated and unresolved taxonomical position of taxa within *S. ciliosum* complex. Therefore, further research is necessary.

Correlative analysis proved similarity in the variation in specified pairs of morphological traits. Among analyzed traits, all degrees, weak, moderate and strong, as well as forms, negative and positive, of correlation are expressed. A weak positive correlation is represented by the largest number of pairs, especially between pairs of traits from flowering region, followed by traits from vegetative part. This degree of linear correlation indicates that changes in morphology, i.e. increasing or decreasing, in a dimension of one morphological trait does not greatly affect the dimensions of correlated morphological trait, therefore those traits can be potentially used in examination purposes of morphological differentiation among analyzed populations. Strongest linear connection, according to results of this study is established in pairs of traits from the flowering region, such as numbers, lengths and widths of particular parts of a flower. This connection in morphological variation between traits of floral parts, may characterize them as ineligible for purposes of examining the extent of morphological variability between populations of *S. ciliosum*. But bearing in mind that the numbers of flower segments, sepals, petals, stamens, and carpels, usually changes simultaneously, values of those traits should not be considered as less important in determining degree of variability between analyzed populations, regardless to their high correlation. From vegetative region, high correlations are established between morphological traits of stem leaves, which, to some degree, can be expected. As the length of leaves increases, width and thickness of the leaves also increase, since leaves accumulate larger quantities of water.

To the best of our knowledge, Analysis of variance (ANOVA), same as Correlative analysis, was not conducted within researches of morphological variability of *S. ciliosum*. According to ANOVA results, the majority of morphological traits from the flowering region are highly statistically significant. The most important, for morphological differentiation, are traits: length of carpels (LC), length of petals (LP), number of carpels (NC), length of sepals (LS) and number of petals (NP). It is interesting to point out that Parnell & Favarger (1990) stated that within population of *S. ciliosum* s.s., great variation, especially in filaments size occurs. As demonstrated by this study as well, length of filaments, based on the differences in their length among individuals, is characterized as statistically not significant for delamination of analyzed populations. From a vegetative region, traits which highly reflect morphological differences among analyzed population are diameter of the stem (DS), length of the lower part of rosette leaf (LLR) and thickness of the leaves from the middle part of rosette (TLR). Previous claims again point to the significance of morphological traits from the flowering region, as more reliable to use in purposes of establishing the morphological differences between populations of *S. ciliosum* subsp. *ciliosum*.

Previously explained results of this study indicate the existence of difference in morphology within populations of typical subspecies of *S. ciliosum* from the Balkan Peninsula, which ultimately can be used to explain pronounced morphological variability of this and related species within *S. ciliosum* complex. In order to confirm this, the additional analysis (e.g. more detailed morphological, anatomical and other studies) should be conducted in the future.

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References

Berger, A. 1930: Crassulaceae. In: Engler, A., Prantl, K. (eds.), *Die Natürlichen Pflanzenfamilien*. 2nd edition, 18 A: 352–483. Leipzig.

Hagemann, I. 1986: *Sempervivum* L. In: Strid, A. (ed.), *Mountain Flora of Greece*, 1: 338. Cambridge University Press, Cambridge.

Karaer, F., Celep, F., Eggli, U. 2011: A taxonomic revision of the *Sempervivum davisii* complex (Crassulaceae). *Nordic Journal of Botany*, 29: 49–53.

Klein, J., Kadereit, J. 2015: Phylogeny, biogeography, and evolution of edaphic association in the european orophytes *Sempervivum* and *Jovibarba* (Crassulaceae). *International Journal of Plant Sciences*, 176 (1): 44–71.

Klein, J., Kadereit, J. 2016: Allopatric hybrids as evidence for past range dynamics in *Sempervivum* (Crassulaceae), a western Eurasian high mountain oreophyte. *Alpine Botany*. Swiss Botanical Society.

Micevski, K. 1998: *Flora na Republika Makedonija*, Tom I, sv. 4. Makedonska Akademija na Naukite i Umetnostite. Skopje. 1043 p.

Mort, M., O'Leary, T., Carrilo-Reyes, P., Nowell, T., Archibald, J., Randle, C. 2010: Phylogeny and evolution of Crassulaceae: past, present, and future. *Schumannia*, 6: 69–86.

Parnell, J., Favarger, C. 1990: Notes on *Sempervivum* L. and *Jovibarba* Opiz. In: Chater, A. O. (ed.), *Flora Europaea notulae systematicae, ser. 2, no. 3.* Botanical Journal of the Linnean Society, 103: 216–220.

Parnell, J., Favarger, C. 1993: *Sempervivum* L., *Jovibarba* Opiz. In: Tutin, T. G., Burges, N. A., Chater, A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S.M., Webb, D.A. (eds.), *Flora Europaea-Psilotaceae to Platanaceae*, 1: 426. Cambridge University Press, Cambridge.

't Hart, H. 2002: *Sempervivum* L. In: Strid, A., Tan, K. (eds.), *Flora Hellenica*, 2: 309. Ruggell (D): A.R.G. Gantner Verlag K.G.

't Hart, H., Bleij, B., Zonneveld, B. 2003: *Sempervivum.* In: Eggli, U. (ed.), *Illustrated handbook of succulent plants,* Vol 5: 338. Springer, Berlin.

Thiede, J., Eggli, U. 2007: Crassulaceae. In: Kubitzki, K. (ed.), *Families and Genera of Vascular Plants*. *Vol. 9. Flowering Plants Eudicots*, 83-118. Hamburg: Springer.