



Morphological and anatomical studies on mountain clover (*Trifolium montanum*) (Fabaceae) from Sharri Mountain, Kosovo

¹Kimete Lluga-Rizani, ²Dubravka Šoljan, ¹Kemajl Kurteshi, ¹Kasum Letaj

¹ Department of Biology, Faculty of Mathematical Natural Sciences, University of Prishtina, Kosovo; ² Department of Biology, Faculty of Mathematical Natural Sciences, University of Sarajevo. Corresponding author: K. Lluga-Rizani, kimete.lluga@uni-pr.edu

Abstract. The object of our research was *Trifolium montanum* (Linnaeus, 1758). The aim was to ascertain the level of morphological variability between different populations, and put more light on taxonomy of this species. The research was carried out on the vertical profile of Sharri Mountain, Kosovo. One part of the collected plant material was dried and placed in herbarium, whereas the other part was preserved in alcohol of 70% for preparation of microscope slides. A comparative-morphometric method was used to evaluate the morphological variability of individuals and populations. For examination of variability of stomatal apparatus, trichome length, vascular bundle diameter, palisade and sponge cell lengths, SWIFT M10L SERIES microscope with a measurement software, was used. 26 morphological traits have been investigated. From the results obtained during this research it can be concluded that there is significant difference between 10 locations from Sharri Mountain for most of micro and macro morphological traits. Also the results lead to the suggestion for the taxonomic revision of this taxon.

Key Words: morphological variability, *Trifolium montanum*, locations, taxonomic revision.

Abstrakt: Objekat našeg istraživanja bio je *Trifolium montanum* L. Cilj je bio utvrditi razinu morfoloških varijacija među 10 različitih populacija iz Šar Planine i staviti više svjetla na taksonomiju ove vrste. Istraživanja su obavljena na vertikalnom profilu Šar Planine, Kosovo. Jedan dio sakupljenog biljnog materijala se osušilo i pohranio se u herbarij, dok se drugi dio se konzervisao u alkoholu od 70% za pripremu mikroskopskih preparata. U procjeni morfološke varijabilnosti individua i populacija primijenjena je uporedno-morfometrijska metoda. Za sagledavanje varijabilnosti: stomatalnog aparata, dužine trihoma, dijametara kolateralnog snopića, dužine palisadnih i sponderasnih stanica upotrijebljen je SWIFT M10L SERIES mikroskop sa softverom za mjerenje. U tu svrhu istraženo je 26 morfoloških svojstava. Iz rezultata dobivenih u ovom istraživanju može se zaključiti da postoji značajna razlika između 10 lokacija iz Šar Planine za većinu mikro i makro morfoloških svojstava. Rezultati također dovode do prijedloga za taksonomsku reviziju ove vrtse.

Ključne riječi: morfološka varijabilnost, *Trifolium montanum*, lokacije, taksonomska revizija

Introduction. The mountain clover (*Trifolium montanum*) is a perennial, predominantly outcrossing and diploid species bearing one to four inflorescences, with up to 150 small zygomorphic yellowish-white flowers (Gillet & Taylor 2001), that are primarily visited by honeybees and occasionally by bumblebees (Pettersson & Sjodin 2000; Schleuning et al 2009).

T. montanum belongs to the Fabacea family, the third largest family of Angyospermae, after Asteraceae and Orchidaceae. This family knows a worldwide distribution. By whatever criteria are used to measure evolutionary success, the legume family (Leguminosae) is one of the most successful lineages of flowering plants, with 751 genera and 19500 species (Lewis 2005). It has a global distribution spanning in all major biomes and forming ecologically important constituents of temperate, Mediterranean, tropical, arid, seasonally dry, rain forest and savanna ecosystems (Schrire et al 2005). According to Diklić et al (1972), *Trifolium* L. genus has about 30 taxa (species and subspecies) in Kosovo.

The aim of this research was to investigate the morphological and anatomical traits of this species, determine the type, frequency and variability of the stomatal apparatus and the structure of the epidermis. Furthermore, it aimed at identifying the

forms and levels of individual, intrapopulation and interpopulation variability of *T. montanum*, depending on the ecological factors of the environment in which they grow and develop. The most variable and most consistent morphological traits can serve in more complete and critical taxonomic analyses and in the revision of this variable taxon. Since the plant material was collected not only in horizontal but also in vertical profile of the Sharri Mountain, the added value of this research consists also in the assessment of the impact of altitude elevation in the investigated parameters of this species. Elevation gradients are among the most powerful 'natural experiments' for testing ecological and evolutionary responses of biota to geophysical influence (Korner 2007).

Material and Method. The research plant material consisted of samples of *T. montanum* L. species. Thirty plants were collected in ten different topo-geographical-ecological localities, on vertical profile of Sharri Mountain (Figure 1). The populations were nominated by numbers (from 1 to 10), where 1 represents the population from the lowest altitude (891 m), while 10 represents the population from the highest altitude (1415 m).

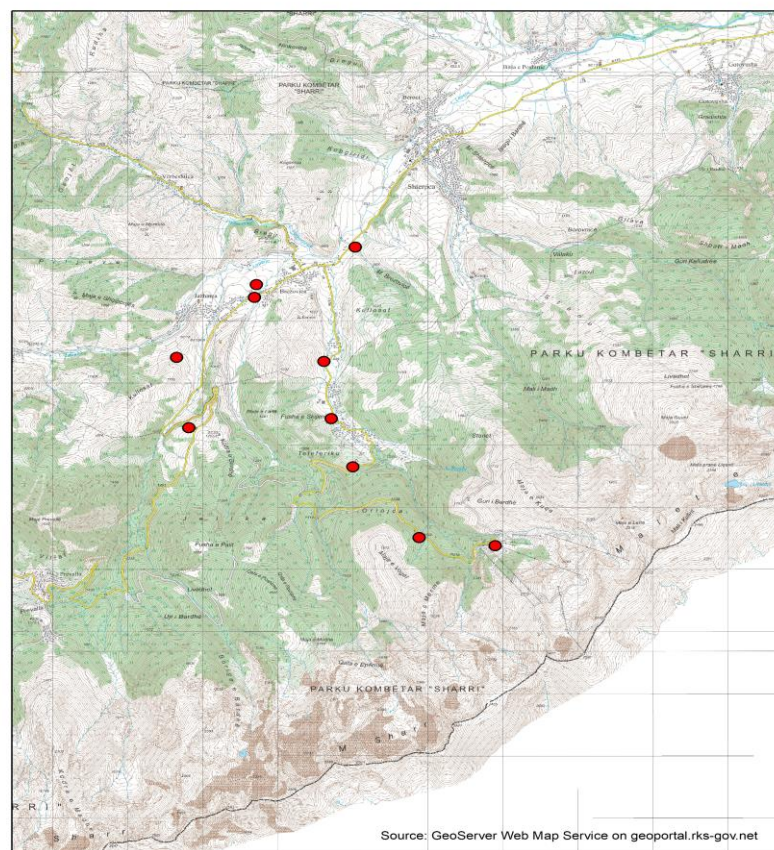


Figure 2. Topographic map of Sharri Mountain, with the *Trifolium montanum* collecting points (source: GeoServer Web Map Service).

One part of the research material collected from nature was stored and dried in paper, whereas the other part was preserved in alcohol 70%, for later preparation of microscope slides for anatomical investigations. Material was collected during the flowering and fruiting phenophases. A manual microtome was used to prepare leaf cross sections for further investigation. Epidermis was prepared with the manicure method for stomata observation. The microscopic slides were observed and parameters were measured with a SWIFT M10L SERIES digital Microscope.

To investigate the impact of habitat variability in populations, the following parameters were determined: total plant height, total leaf length, length and width of the first and middle leaflet, length of the leaf petiole, corolla length, total calyx length, length

of the calyx tube, number of flowers in inflorescence, length of the short and long calyx teeth, fruit length, fruit width and weight, number of stomata per mm² on the upper and lower leaf epidermis, length and width of stomata on the upper and lower leaf epidermis, the length of trichomes on the lower leaf epidermis, diameter of the midrib vascular bundle, the length of palisade and spongy tissue layers.

The values obtained during measurements were analyzed using statistical methodologies. The evaluation and descriptive interpretation of the above-mentioned morphological parameters, the degree of correlation, respectively connection between certain features and ecological parameters were performed with the Sigma software.

Results and Discussion. The species was found in meadows with relatively high humidity, places surrounded by trees of *Cornus mass* and *Corylus avellana*, but sometimes they have been found to inhabit dry places, alongside the road and hilly meadows, protected under the shadow of other bushy plants, in order to cope better with the influence of high radiation and low humidity. The first visible response of certain species to environmental conditions of different habitats is the change of the plant height. The results in Table 1 show that mean values between populations vary from 37.4 to 62.1 cm.

Table 1

Trifolium montanum - plant height (cm)

Locations	1	2	3	4	5	6	7	8	9	10
AV	37.8	37.7	37.4	47	52.5	50	48.51	62.1	52.3	45.2
SD	6.41	7.44	6.25	3.81	5.73	6.08	1.9	9.88	8.20	9.48
Max	47.6	50.0	52.6	53.2	62.5	59.0	67.0	81.0	65.3	64.0
Min	30.5	27.4	31.0	41.0	44.2	38.5	32.0	50.0	40.0	34.0

Note: AV - mean; SD - standard deviation; Max - maximum value; Min - minimum value.

The leaf morphology and anatomy. The leaf of *T. montanum* is a compound leaf with three leaflets. The investigations covered the total leaf length, leaf petiole length, width and length of the first and the middle leaflet and trichomes length (Table 2).

The mean values of the leaf length, based on the results obtained from the measurements range from 3 to 4.38 cm. The leaf petiole presents very low variability to different environmental conditions of various habitats. The mean values of the leaf petiole range from 1.09 to 2.33 cm. The mean values of the first leaflet width vary between 0.87 and 1.08 cm, whereas the mean values of the length of the first leaflet vary between 2.92 and 4.16 cm. The investigation results of the first and middle leaflet show that middle leaflet in all investigated populations is longer and wider than first leaflet. The mean values of the middle leaflet length vary between 3.09 and 4.60 cm, whereas the mean values of the width range from 0.92 to 1.14 cm. The leaf epidermis of *T. montanum* is covered by trichomes, on both sides. Trichomes are single-celled outgrowths, thin and long with no living content (Figure 2).

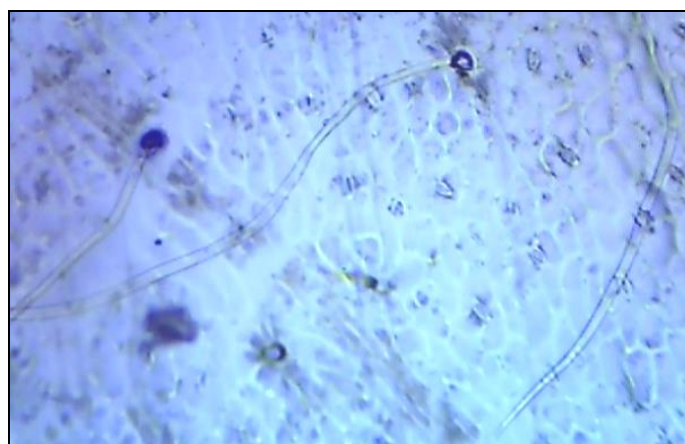


Figure 2. *Trifolium montanum* trichomes.

Table 2

Trifolium montanum leaf morphological traits

Morphological trait		Locations									
		1	2	3	4	5	6	7	8	9	10
The total leaf length (cm)	AV	3.21	3.005	3.065	3.77	3.5	3.47	3.37	4.38	3.30	3.64
	SD	0.78	0.53	0.48	0.39	0.66	0.47	0.37	0.89	0.19	0.99
	Max	4.35	4.15	3.8	4.35	4.7	4.3	4.3	5.95	4.40	5.85
	Min	1.9	2.3	2.3	3.15	2.15	2.8	2.8	3.30	1.85	2.55
The length of leaf petiole (cm)	AV	1.36	1.8	2.33	1.6	1.67	1.34	1.18	1.86	2.08	1.09
	SD	1.33	1.20	1.41	1.0	1.10	0.90	0.81	0.62	1.35	1.31
	Max	4.6	4.6	4.8	3.0	2.8	3.0	6.5	5.0	4.2	3.8
	Min	1.0	0.8	0.4	1.5	0.6	0.2	0.5	0.5	0.2	0.5
The length of medium leaflet (cm)	AV	3.35	3.09	3.1	3.84	3.66	3.59	3.56	4.6	3.5	3.61
	SD	0.92	0.57	0.56	0.40	0.72	0.53	0.33	0.89	1.01	0.79
	Max	4.6	4.3	3.9	4.5	5.0	4.6	4.3	6.2	4.6	4.7
	Min	1.8	2.5	2.2	3.3	2.2	2.8	3.1	3.6	2.0	2.6
Maximum width of medium leaflet (cm)	AV	1.12	0.98	1.0	1.11	0.92	0.96	1.05	1.14	1.11	1.03
	SD	0.18	0.18	0.23	0.12	0.17	0.11	0.16	0.17	0.23	0.30
	Max	1.3	1.2	1.2	1.3	1.2	1.2	1.4	1.5	1.5	1.5
	Min	0.8	0.7	0.5	0.9	0.6	0.8	0.9	0.9	0.8	0.7
The length of the first leaflet (cm)	AV	3.07	2.92	3.03	3.7	3.34	3.35	3.19	4.16	3.11	3.68
	SD	0.63	0.50	0.41	0.44	0.61	0.43	0.45	0.91	0.84	1.32
	Max	4.1	4.0	3.7	4.3	4.4	4.0	4.0	5.7	4.3	7.0
	Min	2.0	2.2	2.4	3.0	2.1	2.8	2.4	3.0	1.7	2.5
Maximum width of the first leaflet (cm)	AV	0.92	0.87	1.01	1.08	0.88	0.9	1.0	1.04	1.02	0.91
	SD	0.13	0.17	0.16	0.15	0.12	0.14	0.21	0.17	0.29	0.23
	Max	1.1	1.2	1.2	1.3	1.0	1.1	1.4	1.4	1.5	1.2
	Min	0.7	0.6	0.60	0.8	0.6	0.7	0.7	0.8	0.6	0.6
The length of trichoma on lower epidermis (μm)	AV	667	732	810	787	794	811	825	833	785	910
	SD	118.7	95.93	375.6	175.7	209.2	200.3	279.2	160.3	248.6	207.4
	Max	843	859	1747	1146	1318	1303	1344	1089	1410	1406
	Min	463	520	559	467	510	593	501	595	450	690

Note: AV - mean; SD - standard deviation; Max - maximum value; Min - minimum value.

The length of the trichomes from the lower leaf epidermis was measured. Mean values of this parameter vary between 667 and 910 μm (Table 2). The lowest value was noted in population 1 from the lowest altitude, whereas the highest mean value was found to be in population 10 from the highest altitude.

The leaf of *T. montanum* is dorsoventral, both sides being covered by a single cell protective layer, the epidermis. The cells of the upper leaf epidermis are mostly hexagonal and smaller than those of the lower leaf epidermis (Figure 3A). The lower epidermis cells are also hexagonal, but larger and elongated (Figure 3B). Leaves of *T. montanum* are amphistomatic (Figure 3).

The investigation of stomata number, width and length on the upper and lower leaf epidermis was conducted (Table 3). It was found that the number of stomata in the upper leaf epidermis is regularly higher than the number of stomata on the lower leaf epidermis. Also, the length and width of the stomata in the upper leaf epidermis is regularly smaller than the length and width of stomata in the lower leaf epidermis. The number of stomata on the upper epidermis varies between 114 and 156 per mm^2 . On the lower epidermis the investigation results indicate that the number of stomata is lower and varies between 39 and 52 per mm^2 . The mean values of stomata length on the upper leaf epidermis vary between 15.42 to 17.74 μm , whereas the stomata width ranges from 9.88 to 11.87 μm .

Table 3

Trifolium montanum stomata

Morphological trait		Locations									
		1	2	3	4	5	6	7	8	9	10
The length of stomata on upper epidermis (μm)	AV	15.42	16.72	15.69	17.74	16.34	16.03	16.81	16.71	15.47	15.7
	SD	0.86	1.42	1.56	1.55	1.68	1.60	1.31	0.83	0.65	1.43
	Max	16.9	18.5	18.0	20.2	19.2	18.7	18.0	17.9	16.5	17.4
	Min	13.9	14.3	3.4	14.7	13.7	13.8	13.6	15.5	14.2	13.1
The length of stomata on the lower epidermis (μm)	AV	24.28	25.69	24.6	24.9	23.7	23.8	25.0	24.4	23.2	23.6
	SD	1.53	1.60	1.88	1.61	1.87	2.90	2.16	1.39	2.32	1.83
	Max	26.9	28.3	27.4	27.1	26.5	27.8	28.9	26.7	27.6	26.8
	Min	22.0	23.5	21.9	22.6	20.9	17.16	22.7	22.1	19.9	22.0
The maximum stomata width on upper epidermis (μm)	AV	10.75	11.87	9.88	11.7	11.26	11.1	11.3	11.52	10.45	10.72
	SD	0.34	2.72	0.94	0.96	1.20	0.90	0.80	0.69	0.77	1.18
	Max	11.1	19.4	11.4	13.0	12.5	12.1	12.1	12.3	11.5	13.0
	Min	10.0	9.7	8.4	10.0	9.7	9.7	9.5	10.6	9.3	8.7
The maximum width of stomata on the lower epidermis (μm)	AV	15.95	16.52	16.07	16.57	16.02	15.27	15.99	15.56	15.63	15.47
	SD	1.19	0.74	0.69	1.14	0.99	1.64	0.88	0.35	1.51	1.21
	Max	17.2	17.5	17.6	18.1	17.9	16.7	17.2	16.1	18.6	18.0
	Min	13.9	15.1	15.1	14.7	4.5	10.9	14.4	15.0	13.8	14.0
The number of stomata per mm^2 on upper epidermis	AV	140	146	132	125	139	156	127	114	149	141
	SD	19.93	16.24	14.68	10.54	24.20	22.72	14.29	11.17	23.24	22.37
	Max	172	173	154	143	170	191	150	134	184	163
	Min	112	126	111	111	93	129	105	99	110	95
The number of stomata per mm^2 on lower epidermis	AV	46	43	43	41	46	49	42	39	49	52
	SD	8.73	10.86	8.48	7.60	12.44	9.08	9.20	8.19	10.49	9.66
	Max	65	61	61	53	67	67	59	58	59	63
	Min	35	30	31	30	27	39	33	29	26	31

Note: AV - mean; SD - standard deviation; Max - maximum value; Min - minimum value.

Stomata of the lower leaf epidermis were regularly longer and wider compared with stomata of the upper leaf epidermis. The mean values of stomata length range from 23.21 to 25.69 μm , whereas the mean values of stomata width vary between 15.27 and 16.57 μm . Therefore, there is a negative correlation between the number of stomata and length/width of stomata, because in the lower epidermis, the number of stomata is lower but the length and width of stomata present higher values.

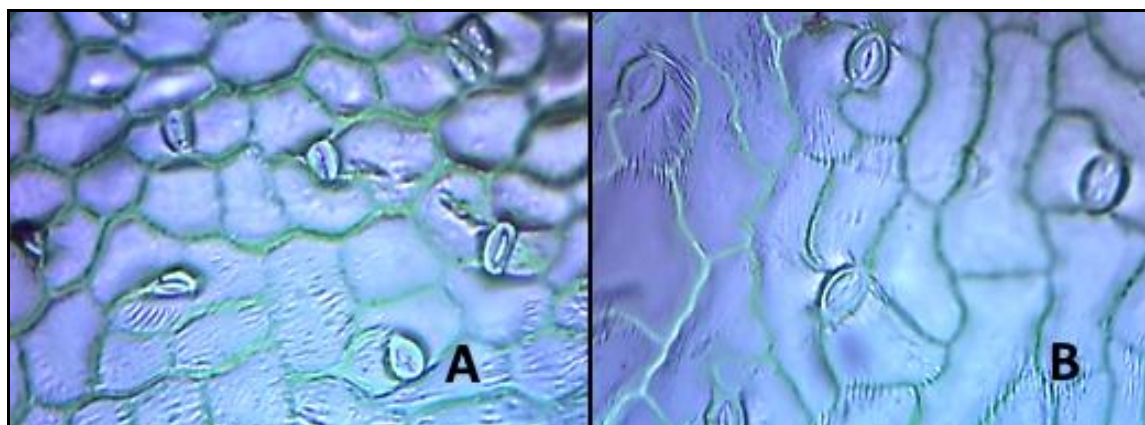


Figure 3. A - The upper leaf epidermis and stomata; B - the lower leaf epidermis.

The leaf mesophyll is differentiated in palisade and spongy tissue. The investigation of the length of the palisade and spongy layers was conducted (Table 4). The investigation of the midrib vascular diameter was also carried out (Table 4).

Table 4

Trifolium montanum leaf mesophyll

Parameters		Locations									
		1	2	3	4	5	6	7	8	9	10
The length of palisade tissue cells (μm)	AV	83.8	82.4	60.8	101.3	76.1	78.9	93.8	64.0	62.9	81.5
	SD	17.9	10.4	7.2	19.8	11.3	20.9	8.6	7.9	8.6	19.3
	Max	111.7	95.3	73.8	129.7	96.8	123.5	106	76.4	78.5	113
	Min	52.8	65.4	52.5	72.0	60.3	64.1	80	46.6	48.1	57
The length of spongy tissue cells (μm)	AV	59.6	50.0	44.6	60.0	44.0	55.0	56.4	46.8	47.3	55.9
	SD	8.1	5.0	6.1	8.28	5.9	4.0	6.77	4.53	4.39	10.3
	Max	71.5	59.4	51.1	75.4	57.9	61.6	65.1	54.8	52.7	70.6
	Min	48.0	41.2	34.8	46.5	37.7	49.0	43.4	41.2	39.8	42.3
The diameter of collateral vascular bundle (μm)	AV	227	221	224	206	265	251	262	290	219	239
	SD	53.6	40.9	46.5	59.5	38.4	42.2	66.8	64.2	58.5	39.3
	Max	297	281	296	319	305	320	373	418	380	284
	Min	152	145	145	153	194	183	131	201	143	167

Note: AV - mean; SD - standard deviation; Max - maximum value; Min - minimum value.

The investigation of the large number of microscope slides of the leaf cross section, from numerous population samples, indicates that palisade tissue is mostly constructed of two cell layers (Figure 4), but there are cases when there are three layers. Spongy tissue is constructed of four or five layers of cells. The palisade tissue cells are cylindrical and rich in chloroplasts. Spongy tissue cells are mostly round shaped or elliptical and sometimes cylindrical, elongated. The mean values of palisade layer length range from 60.8 to 101.3 μm . The spongy tissue layer length has mean values varying between 44.0 and 60.0 μm .

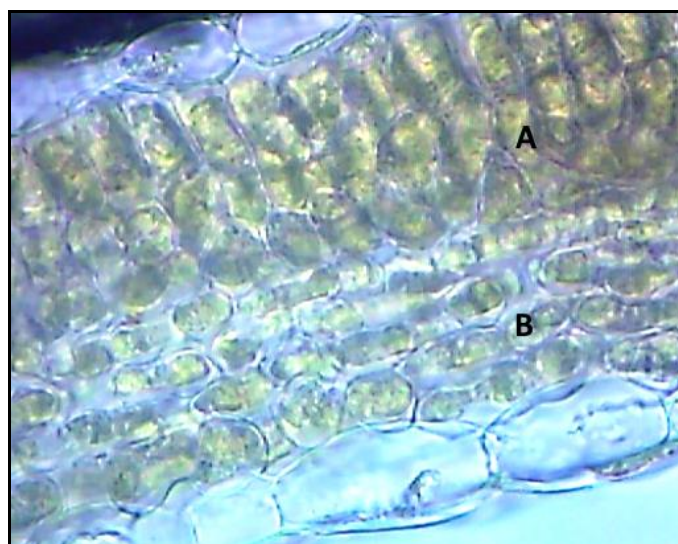


Figure 4. *Trifolium montanum* mesophyll; A - palisade tissue; B - spongy tissue.

Observations of the leaf cross section microscope slides of *T. montanum* showed that vascular bundles of the leaf midrib are very characteristic (Figure 5). In the majority of microscope slides was observed that the leaf midrib vascular bundle has experienced transformation in the way that it has two phloems and two xylems (Figure 5; Figure 6). It

seems that the creation of a secondary vascular bundle inside the primary vascular bundle has undergone through some phases. There was a phase that was observed in microscope slides, when at the beginning mechanical tissue covering phloem started to extend and penetrate inside, creating pressure on the xylem and pushing it towards the inner part, respectively toward the middle of the vascular bundle. Afterwards, the phloem and xylem had extend and created inside the primary vascular bundle the second phloem and xylem. Therefore, the collateral vascular bundle has two phloems and two xylems. In addition, the lateral collateral vascular bundles are well evolved. The mechanical tissue is very developed, constructed of round shaped cells with very thick walls (Figure 6). The mechanical tissue covers both poles of the vascular bundle. The part that covers the phloem is more developed than the one that covers the xylem, with the tendency to extend further and reach the other part of the mechanical tissue around the xylem. Mechanical tissue also exists on the poles of the secondary vascular bundle, being created inside the primary vascular bundle. The research results show that mean values of vascular bundle diameter vary between 206 and 290 μm .

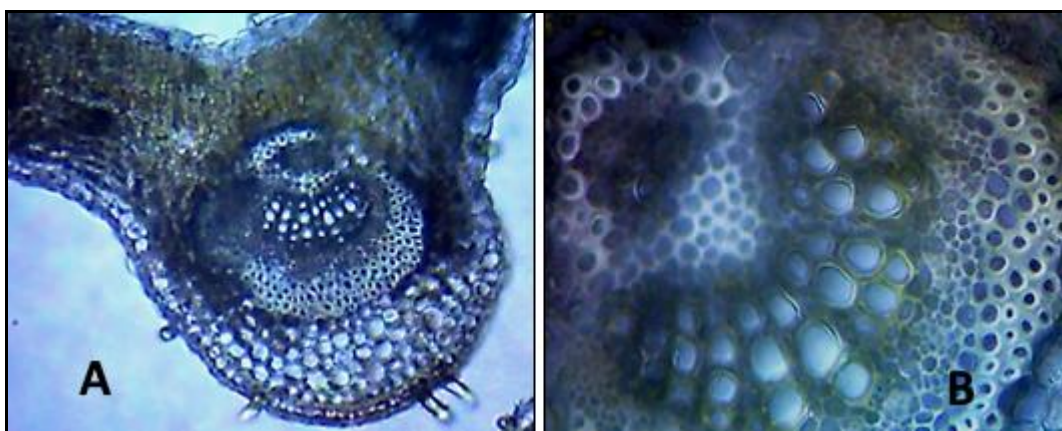


Figure 5. A - *Trifolium montanum* leaf midrib collateral vascular bundle; B - *T. montanum* secondary vascular bundle.

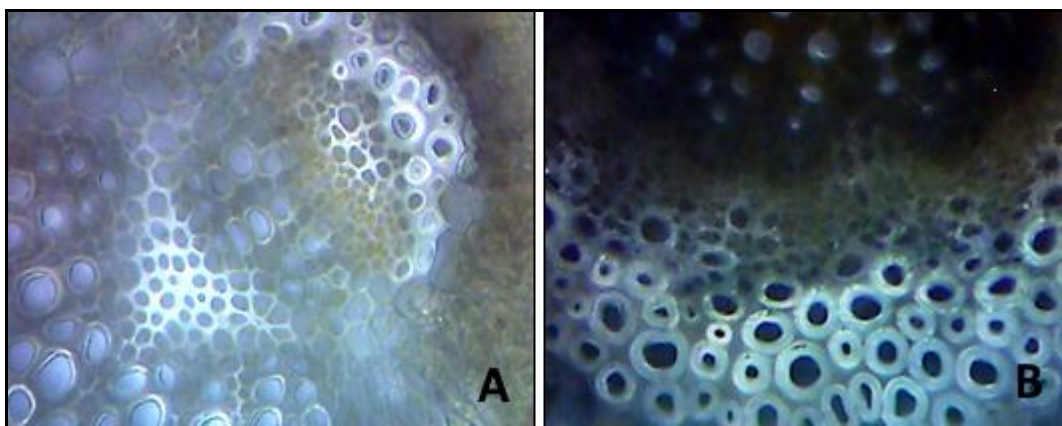


Figure 6. A - *Trifolium montanum* secondary vascular bundle; B - *T. montanum* mechanical tissue.

The flower morphology. The flowers of *T. montanum* are grouped in inflorescences called capitulum, or head, usually two per plant. The corolla has a white or yellowish color. The calyx is made of the calyx tube and five calyx teeth. The length of the teeth of the calyx is unequal; therefore, measurements for the long and short calyx teeth have been conducted separately. The investigation of the length of corolla, calyx tube, calyx teeth, total calyx length, peduncle length is presented in Table 5.

Table 5

Trifolium montanum flower morphological traits

Morphological trait		Locations									
		1	2	3	4	5	6	7	8	9	10
The peduncle length (cm)	AV	4.0	5.5	5.0	6.7	6.2	7.0	5.0	6.17	6.0	5.6
	SD	1.05	2.31	0.93	1.43	1.6	1.12	1.34	1.63	1.42	1.72
	Max	6.0	9.0	6.0	9.0	9.0	9.0	7.0	8.0	8.5	8.0
	Min	3.0	2.0	3.0	4.0	4.0	6.0	3.0	3.0	4.0	3.5
The total calyx length (mm)	AV	4.85	4.3	5.3	4.8	4.2	4.7	3.95	4.45	4.2	4.25
	SD	0.47	0.48	0.53	0.48	0.53	0.35	0.55	0.49	0.78	0.35
	Max	6.0	5.0	6.5	5.5	5.5	5.0	4.5	5.0	6.0	5.0
	Min	4.5	3.5	5.0	4.0	3.5	4.0	3.0	3.5	3.5	4.0
The length of calyx tube (mm)	AV	2.35	2.35	2.7	2.55	2.3	2.2	2.1	2.4	2.15	2.05
	SD	0.33	0.33	0.42	0.36	0.35	0.25	0.21	0.39	0.33	0.15
	Max	3.0	3.0	3.0	3.0	3.0	2.5	2.5	3.0	3.0	2.5
	Min	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
The length of long calyx teeth (mm)	AV	2.45	1.95	2.5	2.25	2.0	2.55	2.0	2.1	2.05	2.3
	SD	0.28	0.36	0.47	0.35	0.62	0.43	0.62	0.51	0.49	0.48
	Max	3.0	2.5	3.0	3.0	3.5	3.0	3.0	2.5	3.0	3.5
	Min	2.0	1.5	2.0	2.0	1.0	2.0	1.0	1.0	1.5	2.0
The length of short calyx teeth (mm)	AV	2.25	1.9	2.3	1.95	1.95	2.1	1.85	2.0	1.75	2.05
	SD	0.26	0.31	0.3	0.28	0.49	0.21	0.58	0.47	0.35	0.36
	Max	2.5	2.5	3.0	2.5	3.0	2.5	3.0	2.5	2.0	3.0
	Min	2.0	1.5	2.0	1.5	1.0	2.0	1.0	1.0	1.0	1.5
The total corolla length (mm)	AV	6.4	6.2	5.6	6.4	5.7	6.5	6.75	6.35	6.7	7.0
	SD	0.45	0.50	0.56	0.56	0.35	0.62	0.63	0.33	0.91	0.57
	Max	7.0	7.0	6.5	7.5	6.0	7.5	8.0	7.0	8.0	8.0
	Min	5.5	5.5	4.5	5.5	5.0	5.5	6.0	6.0	5.0	6.0
Number of flowers in inflorescence	AV	147	137	129	148	119	150	148	118	139	144
	SD	24.71	28.19	7.10	18.45	27.62	30.54	66.19	16.03	33.67	31.47
	Max	184	197	140	173	183	200	274	138	207	203
	Min	102	98	120	125	84	114	71	95	94	102

Note: AV - mean; SD - standard deviation; Max - maximum value; Min - minimum value.

The mean values of the peduncle length range from 4 to 7 cm. The mean values of the total calyx length vary between 3.9 and 5.3 mm. This morphological trait shows a slight negative correlation with increasing altitude, from location 1 (4.85 mm) to location 10 (4.25 mm). The investigation results regarding the calyx tube length vary between 2.05 and 2.7 mm. The mean values for the long calyx teeth length range from 1.95 to 2.55 mm, whereas the mean values of the short calyx teeth length range from 1.75 to 2.3 mm. The mean values of the corolla length range from 5.6 to 7 mm. The number of flowers in the inflorescence of *T. montanum* was also investigated (Table 5). The mean values vary between 118 and 150. The flower number slightly declines with increasing altitude.

The fruit morphology. The fruit of *T. montanum* is a legume with an ovoid shape and one seed (Figure 7). The seeds have a yellow-brownish color (Figure 7). The investigation of the fruit width, length and weight can be seen in Table 6.



Figure 7. A - the legume fruit of *Trifolium montanum*; B - the seed of *Trifolium montanum*.

Table 6

Trifolium montanum - fruit morphological traits

Morphological traits		Locations									
		1	2	3	4	5	6	7	8	9	10
The maximum fruit width/mm	AV	1.0	1.05	1.0	1.0	1.15	1.0	1.2	1.0	1.0	1.0
	SD	0.0	0.1	0.0	0.0	0.24	0.0	0.2	0.0	0.0	0.0
	Max	1.0	1.5	1.0	1.0	1.5	1.0	1.5	1.0	1.0	1.0
	Min	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
The maximum fruit length/mm	AV	2.1	2.5	3.4	2.0	2.5	2.1	2.3	2.4	2.0	2.3
	SD	0.21	0.52	1.07	0.0	0.52	0.31	0.42	0.51	0.0	0.67
	Max	2.5	3.0	5.0	2.0	3.0	3.0	3.0	3.0	2.0	3.0
	Min	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.0
The fruit weight (g)	AV	0.0054	0.0052	0.005	0.0013	0.0011	0.01	0.009	0.0092	0.014	0.013

Note: AV - mean; SD - standard deviation; Max - maximum value; Min - minimum value.

The research results regarding the maximum fruit width show that the mean values vary between 1.0 and 1.22 mm, whereas the maximum fruit length presents mean values from 2.0 to 3.4 mm. The mean values of the fruit weight range from 0.005 to 0.0134 g. It can be seen that there is a positive correlation between increase of altitude and fruit weight (location 1 – 0.0054 g; location 10 - 0.0134 g).

The correlation between the 26 investigated micro and macro morphological parameters was analyzed with the Spearman methodology. Between most of the parameters was established a strong positive or a negative correlation (Tables 7 to 11). High positive correlation was found between the: total plant height and total leaf length, middle and first leaflet length, peduncle length; total leaf length and middle and first leaflet length, peduncle length; petiole length and calyx tube length; middle leaflet length and first leaflet length; maximum width of the middle leaflet and maximum width of the first leaflet; first leaflet length and peduncle length; total calyx length and calyx tube length (Table 7). High negative correlation was found between the total plant height and total calyx length. Medium negative correlation was found between the petiole length and the first leaflet length. Low negative correlation was found between the total leaf length and total calyx length, peduncle length and total calyx length (Table 7).

Table 8 shows the following results: the high negative correlation between the total plant height and total calyx length; the medium negative correlation between the petiole length and the first leaflet length; the low negative correlation between the total leaf length and total calyx length, peduncle length and total calyx length. A high positive correlation was found between the total calyx length and long calyx teeth length, short calyx teeth length. Medium positive correlation was found between the stomata length on the upper leaf epidermis and total leaf length, middle leaflet length, first leaflet length, peduncle length; the calyx tube length and short calyx tube length, stomata length on the lower epidermis.

Table 7

The results of the correlation analysis for the investigated parameters of *Trifolium montanum* (r values)

Parameter	LL	PL	MLL	MLW	FLL	FLW	PEL	CAL	CTL
PH	0.661	0.0182	0.721	0.134	0.636	0.200	0.648	-0.5111	-0.267
LL		-0.297	0.988	0.267	0.988	0.406	0.661	-0.146	-0.0790
PL			-0.236	0.103	-0.333	0.309	-0.0788	0.256	0.632
MLL				0.219	0.964	0.382	0.697	-0.164	-0.0304
MLW					0.280	0.760	-0.243	0.180	0.189
FLL						0.418	0.685	-0.0912	-0.0912
FLW							0.0667	0.237	0.371
PEL								-0.140	-0.0486
CAL									0.762

Note: PH - total plant height; LL - total leaf length; PL - petiole length; MLL - middle leaflet length; MLW - maximum width of middle leaflet; FLL - first leaflet length; FLW - maximal width of the first leaflet; PEL - peduncle length; CAL - total calyx length; CTL - calyx tube length.

Table 8

The results of the correlation analysis for the investigated parameters of *Trifolium montanum* (continuation)

Parameter	LCTL	SHCTL	COL	FRW	FRL	FLN	SLUE	SLLE	SWLE
PH	-0.243	-0.395	0.164	-0.239	0.0969	-0.176	0.176	-0.430	0.176
LL	0.0790	-0.0304	0.261	-0.270	-0.201	-0.0303	0.382	-0.261	0.261
PL	-0.128	-0.0486	-0.693	0.319	-0.186	-0.697	-0.188	0.0545	-0.139
MLL	0.0243	-0.0608	0.164	-0.233	-0.127	-0.0788	0.406	-0.248	0.297
MLW	0.0732	-0.0457	0.259	-0.455	-0.441	-0.0243	-0.0912	0.0365	0.0365
FLL	0.170	0.0122	0.322	-0.331	-0.276	0.0667	0.370	-0.248	0.248
FLW	0.201	-0.0547	0.109	-0.417	-0.485	-0.0182	0.0909	0.0424	-0.0788
PEL	0.0365	-0.195	0.0669	-0.325	-0.127	0.103	0.394	-0.273	0.321
CAL	0.723	0.796	-0.451	0.0308	-0.658	0.0122	-0.261	0.213	-0.152
CTL	0.201	0.396	-0.802	0.277	-0.292	-0.389	0.122	0.474	0.271

Note: PH - total plant height; LL - total leaf length; PL - petiole length; MLL - middle leaflet length; MLW - maximum width of middle leaflet; FLL - first leaflet length; FLW - maximal width of the first leaflet; PEL - peduncle length; CAL - total calyx length; CTL - calyx tube length; LCTL - long calyx teeth length; SHCTL - short calyx teeth length; COL - total corolla length; FRW - maximum fruit width; FRL - maximum fruit length; FLN - number of flowers in inflorescence; SLUE - stomata length on the upper leaf epidermis; SLLE - stomata length on the lower leaf epidermis; SWLE - maximum width of stomata on the lower leaf epidermis.

High positive correlation was found between the long calyx teeth length and short calyx teeth length; total corolla length and number of flower in inflorescence; maximum fruit length and stomata length on the upper leaf epidermis; maximal width of stomata on the upper leaf epidermis and stomata length on the upper and lower epidermis (Table 9). High negative correlation was found between the fruit length and long and short calyx teeth length; long calyx teeth length and maximal stomata width on the upper leaf epidermis; total corolla length and maximal fruit width; fruit width and flowers number (Table 9).

Table 10 presents the high positive correlation found between the trichomes length and total leaf length, middle and first leaflet length; calyx tube length and maximal stomata length on the lower epidermis; total plant height and the diameter of midrib vascular bundle. High negative correlation was found between the petiole length and the length of palisade and spongy layer; number of stomata on the upper leaf epidermis and maximal width of the first leaflet; calyx tube length and stomata number on the lower leaf epidermis.

Table 9

The results of the correlation analysis for the investigated parameters of *Trifolium montanum* (continuation)

<i>The parameter</i>	<i>SHCTL</i>	<i>COL</i>	<i>FRW</i>	<i>FRL</i>	<i>FLN</i>	<i>SLUE</i>	<i>SLE</i>	<i>SWUE</i>
LCTL	0.838	0.0732	-0.215	-0.763	0.328	-0.498	-0.286	-0.559
SHCTL		-0.296	0.252	-0.539	-0.0182	-0.456	-0.0851	-0.413
COL			-0.658	-0.0972	0.657	-0.0365	-0.328	-0.146
FRW				0.408	-0.650	0.0736	0.313	0.0307
FRL					-0.0373	0.500	0.395	0.440
FLN						0.115	0.0909	0.0182
SLUE							0.661	0.855
SLE								0.648

Note: LCTL - long calyx teeth length; SHCTL - short calyx teeth length; COL - total corolla length; FRW - maximum fruit width; FRL - maximum fruit length; FLN - number of flowers in inflorescence; SLUE - stomata length on the upper leaf epidermis; SLE - stomata length on the lower leaf epidermis; SWUE - maximum width of stomata on the upper leaf epidermis.

Table 10

The results of the correlation analysis for the investigated parameters of *Trifolium montanum* (continuation)

<i>Parameter</i>	<i>SWLE</i>	<i>TRL</i>	<i>PALL</i>	<i>SPOL</i>	<i>VBD</i>	<i>STNUE</i>	<i>STNLE</i>
PH	-0.430	0.321	-0.224	-0.297	0.552	-0.139	-0.0669
LL	-0.309	0.600	0.115	0.103	0.406	-0.467	-0.201
PL	0.358	-0.345	-0.685	-0.661	-0.261	-0.127	-0.249
MLL	-0.248	0.539	0.0909	0.0303	0.442	-0.491	-0.243
MLW	-0.103	-0.0486	0.152	0.395	-0.109	-0.462	-0.354
FLL	-0.382	0.624	0.127	0.164	0.382	-0.406	-0.182
FLW	0.0909	0.127	-0.0545	0.200	-0.236	-0.500	-0.426
PEL	-0.200	0.236	-0.0303	-0.0909	0.115	0.0788	0.000
CAL	0.170	-0.249	-0.0547	0.148	-0.316	-0.128	-0.174
CTL	0.578	-0.328	-0.134	-0.164	-0.249	-0.492	-0.619

Note: PH - total plant height; LL - total leaf length; PL - petiole length; MLL - middle leaflet length; MLW - maximum width of middle leaflet; FLL - first leaflet length; FLW - maximal width of the first leaflet; PEL - peduncle length; CAL - total calyx length; CTL - calyx tube length; SWLE - maximum width of stomata on the lower leaf epidermis; TRL - Trichomes length; PALL - The length of palisade layer; SPOL - The length of spongy layer; VBD - the diameter of midrib vascular bundle; STNUE - number of stomata on the upper leaf epidermis; STNLE - stomata number on the lower leaf epidermis.

Table 11 presents the high positive correlation was found between the length of palisade layer and number of flowers in inflorescence, stomata length on the upper leaf epidermis, stomata width on the upper leaf epidermis; stomata length on the lower leaf epidermis and maximal stomata width on the lower leaf epidermis; the length of the spongy layer and the length of palisade layer, total corolla length, number of flowers in inflorescence; trichomes length and the diameter of the midrib vascular bundle; stomata number on the upper leaf epidermis and stomata number on the lower leaf epidermis. High negative correlation was found between the total corolla length and maximal width of stomata on the lower epidermis; maximal fruit width and the length of spongy layer; stomata number on the lower leaf epidermis and the length and width of stomata on the upper and lower epidermis; trichomes length and stomata width on the lower leaf epidermis.

Plants responded to the changes of environmental condition with a high variability. This is confirmed for different plants by Mekonnen et al (2015), Grozeva et al (2016) and others. Significant morphological variability for the leaflet length is found by Poljak et al (2015). Variability of the leaf length is usually a response to different environmental conditions. Environmental heterogeneity is a major cause of variation in leaf morphology (Greeske et al 1994; Velasquez-Rosas et al 2002; Mcpherson et al 2004). The correlation analysis between the total leaf length and the first and middle leaflet length has shown significant positive correlation. Similar results are obtained during the investigation of *Quercus robur* by Batos et al (2017), when the length of lamina had a strong positive

correlation with the total length of lamina ($r=0.995$). On the other hand, the maximum width of the first and middle leaflets are more stable characters and do not respond to the change of environmental conditions; therefore, this two morphological traits can be used as a valuable trait for the taxonomic analysis of this taxon. The investigation of the leaf petiole length did not indicate a significant difference between populations and can serve as a valuable trait for the taxonomic analyses of this taxon. According to Kost et al (2003), some metric characters, such as the length of petiole, could be also used for identification purposes.

Table 11

The results of the the correlation analysis for the investigated parameters of *Trifolium montanum* (continuation)

Parameter	SWLE	TRL	PALL	SPOL	VBD	STNUE	STNLE
LCTL	-0.413	0.182	-0.182	0.182	-0.0608	0.140	0.308
SHCTL	-0.219	0.146	-0.201	-0.0243	0.152	-0.0365	0.137
COL	-0.559	0.340	0.371	0.596	-0.0182	0.274	0.405
FRW	0.252	0.215	-0.393	-0.669	0.405	-0.215	-0.215
FRL	0.365	-0.0224	0.291	-0.142	0.336	-0.112	-0.239
FLN	-0.176	-0.0182	0.636	0.794	-0.236	0.297	0.195
SLUE	0.442	0.224	0.527	0.212	0.0909	-0.491	-0.705
SLE	0.648	-0.139	0.479	0.261	-0.127	-0.455	-0.796
SWUE	0.394	-0.0909	0.588	0.248	0.0909	-0.345	-0.699
SWLE		-0.515	0.297	0.0424	-0.491	-0.394	-0.529
TRL			-0.176	-0.139	0.612	-0.285	-0.0182
PALL				0.867	-0.176	-0.176	-0.286
SPOL					-0.358	-0.0424	-0.0790
VBD						-0.261	-0.116
STNUE							0.802

Note: LCTL - long calyx teeth length; SHCTL - short calyx teeth length; COL - total corolla length; FRW - maximum fruit width; FRL - maximum fruit length; FLN - number of flowers in inflorescence; SLUE - stomata length on the upper leaf epidermis; SLE - stomata length on the lower leaf epidermis; SWUE - maximum width of stomata on the upper leaf epidermis; SWLE - maximum width of stomata on the lower leaf epidermis; TRL - trichomes length; PALL - the length of palisade layer; SPOL - the length of spongy layer; VBD - the diameter of midrib vascular bundle; STNUE - number of stomata on the upper leaf epidermis; STNLE - stomata number on the lower leaf epidermis.

The leaf epidermis is single-layered in both sides. Single layered epidermis was also observed by Pereira et al (2009) in the leaves of other plant species. The protection ability of the leaf epidermis is enhanced with the presence of trichomes. According to the results of Lluga et al (2014), this morphological trait has also found to be consistent in *Trifolium pratense* (Linnaeus 1753). Therefore, it is recommended as a credible micro morphological trait for the taxonomic analysis of this taxon, as it is used like the leaf base shape in the taxonomy of *Alnus* sp. (Sabeti 1965).

The leaf of *T. montanum* is amphistomatous. According to Čortan et al (2017), the poplars are also known as amphistomatous species. It was found that the number of stomata in the upper leaf epidermis is regularly higher than the number of stomata on the lower leaf epidermis. Our results are consistent with results of Zoric et al (2008). On the lower epidermis, the investigation results indicate that the number of stomata ranges from 39 – 52 per mm². The stomata length and width are in negative correlation with stomatal density (number of stomata per mm²). Similar results are obtained by Wang et al (2014) during the investigation of other plants species. The stomata length on the upper leaf epidermis shows a slight increasing trend of mean values in relation to the increasing altitude. Our results are in concordance with results of Holland & Richardson (2009).

The flower number slightly declines with the increase of altitude. Similar results were obtained by Roblek et al (2008), where reproductive success was much lower in plants growing at high altitudes, because they develop fewer flowers. The fruit of *T. montanum* is a legume with an ovoid shape, with one seed. The seed has yellow brownish color. Our results are in accordance with Zoric et al (2010).

The research results clearly indicate in which population the maximum mean values. The micro morphological traits such as the number of stomata per mm² on the lower epidermis, length of trichomes on the lower epidermis, the length of palisade and spongy tissue layers present higher values in location 10. This location is a place rich in moisture, with shadow from *Betula pendula* at 1415 m. The length and width of stomata on the lower leaf epidermis and stomata width on the upper leaf epidermis are better developed and arrive at their maximum mean values in location 2. This location is characterized by floristically rich meadows with a high diversity of species, situated close to the river and with relatively high humidity, at 922 m altitude.

The macro morphological traits of the total plant height, the length and width of the middle and first leaflet, the maximum fruit length have maximum mean values in location 7. This location is dry, fully exposed to sun radiation, at 1070 m. From this results we can conclude that *T. montanum* has optimum ecological parameters in location 7, as *T. montanum* inhabits dry grassy places (Tutin 1968; Domac 1973; Forenbacher 1990). The total calyx length, the length of the calyx tube, the length of the long and short calyx teeth, the corolla length and the maximum fruit width have reached their maximum mean values in location 3 (962 m), in a relatively humid place surrounded by trees of *Corylus avellana*.

Phenotypic plasticity is highly important for plants since they cannot move and have to deal with changing ambient conditions. It further allows for faster adjustment to a changing environment (Sultan 2000; Hoffman & Sgro 2011). However, a certain number of morphological traits (length of the leaf petiole, maximum width of the first and middle leaflet, number of stomata per mm² on the lower leaf epidermis, maximum length and width of stomata on the lower leaf epidermis, trichomes length on the lower leaf epidermis, short calyx teeth length and number of flowers in inflorescence) in various populations and habitats remain very stable, and can serve as valuable traits in taxonomic studies of this taxon.

Conclusions. *Trifolium montanum* is a perennial plant that inhabits dry, grassy places, floristically rich meadows with relatively high humidity, areas alongside the road, pastures, and areas inside forests where the trees were cut. It was found that this species likes to live in most cases under the shadow of other bushes or trees, but it can also be found in open areas, fully exposed to the sun radiation. From the results obtained during this research it can be concluded that *T. montanum* from Sharri Mountain presents high plasticity for most of micro and macro morphological traits. The results also lead to the suggestion for the taxonomic revision of this taxon. The vertical distribution is very narrow in our study place, from 891 to 1415 m altitude. Therefore, morphological variability cannot be fully attributed to the altitude change, because it can also be the result of the influence of local climate and other environmental conditions.

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Authors:

Kimete Lluga-Rizani, University of Prishtina, Faculty of Mathematical Natural Sciences, Department of Biology, Mother Theresa Str., Prishtina 10000, Kosovo; e-mail: kimete.lluga@uni-pr.edu

Dubravka Šoljan, University of Sarajevo, Faculty of Mathematical Natural Sciences, Department of Biology, Zmaja od Bosne 33-35, Sarajevo 71000, Bosnia and Herzegovina, e-mail: dr.dsoljan@gmail.com

Kemajl Kurteshi, University of Prishtina, Faculty of Mathematical Natural Sciences, Department of Biology, Mother Theresa Str., Prishtina 10000, Kosovo, e-mail: kemajl.kurteshi@uni-pr.edu

Kasum Letaj, University of Prishtina, Faculty of Mathematical Natural Sciences, Department of Biology, Mother Theresa Str., Prishtina 10000, Kosovo, e-mail: kasum.letaj@uni-pr.edu

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