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COMPARATIVE ANALYSIS OF POPULATIONS OF THE BALKAN ENDEMIC SPECIES *DAPHNE MALYANA* BLEČIĆ (THYMELEACEAE)

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Abstract - *Daphne malyana* Blečić (Thymeleaceae) is an endemic species of the western part of the Balkan Peninsula, distributed in the mountains, canyons and gorges of N. Montenegro, E. Bosnia and W. Serbia. The comparative morphoanatomic investigations have included four distantly separated populations of the species *D. malyana*, i.e. two from Serbia, from the ravines of Sokoline and Vranjak on Mt. Tara, and two from Montenegro, in the canyons of the Tara and Piva rivers. Comparative morphoanatomical studies have shown the presence of general adaptive characteristics of a specific, conservative xeromorphic type, slightly differing in each population. Principal component analysis (PCA) and canonical discriminant analysis (CDA) of 20 morphoanatomical characteristics of the leaves and stems have shown a clear distinction between the populations from the river Piva canyon (Montenegro) and those from the Sokoline ravine (Serbia), on one side, and those of Vranjak gorge (Serbia) and of the river Tara canyon (Montenegro) on the other side. It may be assumed that the mild morphological variability of the isolated populations of the Balkan endemic species *D. malyana* in the canyons and gorges seem to have been affected by the microclimate conditions in their habitats.

Key words: *Daphne malyana*, Balkan Peninsula, endemic, anatomy, morphology

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INTRODUCTION

The genus *Daphne* belongs to the family Thymelaeaceae and includes approximately 70 species (Brickell and Matthew, 1978), which are mostly distributed in Europe and temperate and subtropical Asia, with several representatives in N. Africa. There are 17 recorded species in the flora of Europe (Webb and Ferguson, 1968). The Balkan endemorelict species *Daphne malyana* Blečić belongs to section *Daphnanthes* C.A. Mayer, subsection *Oleoides* (Keissler, 1898), together with *D. jasminea* from central and southern Greece (including *D. jasminea* subsp. *jarmilae* Halda from northern Libya) and the species *D. oleoides,* which is distributed in the mountains of S. Europe and SW Asia (Stevanović and Zlatković, 2006). Blečić (1953), and Stevanović and Mitrović (1996) have pointed out the greater morphoanatomical similarity between *D. malayna* and *D. jasminea* despite their having distant ranges, than between the species *D. malayna* and *D. oleoides*, which have overlapping ranges.

D. malyana was first recorded and described from the limestone rocks in the canyon of the Piva river in Montenegro (Blečić, 1953). Afterwards, it was recorded in several localities in eastern Bosnia and Herzegovina (Mts. Maglić and Zelengora, the canyon of the Sutjeska river), as well as in northern Montenegro (Mts. Durmitor, Sinjajevina and Lukavica) and in canyons of the Tara and Komarnica rivers (Lakušić and Pulević, 1980; Pulević, 2005). The presence of the species *D. malyana* has been recently recorded also in Serbia (Stevanović and Zlatković, 2006). The localities in Serbia are the northernmost while those in Bosnia and Herzegovina are the westernmost boundaries of the species range (Fig. 1).

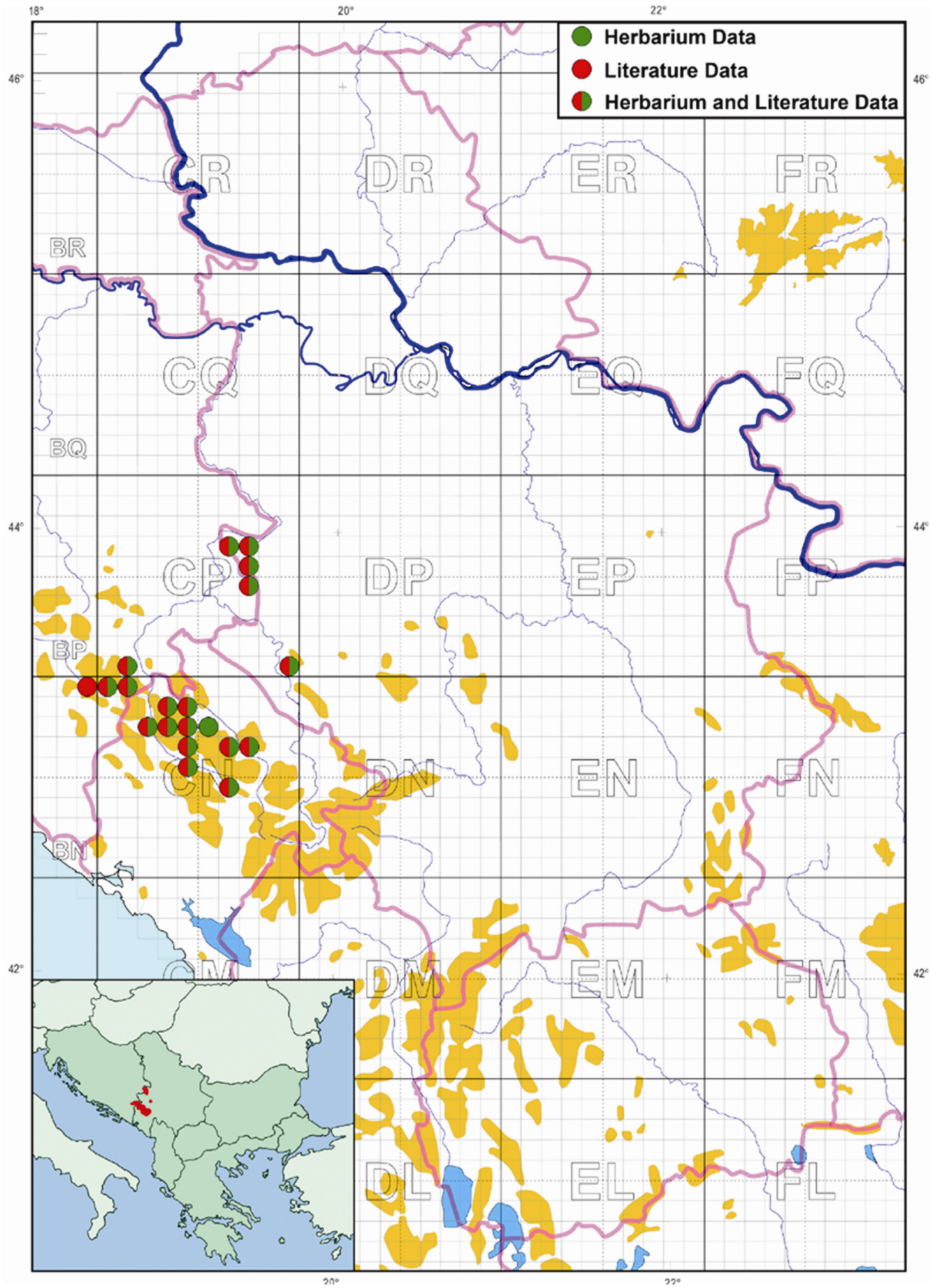

Fig. 1. Map of distribution of Daphne *malyana* Blečić.

Table 1. Ecological characteristics of the habitats of the analyzed populations.

	Mt. Tara, Sokoline (Serbia)	Mt. Tara, Vranjak (Serbia)	Canyon of the river Tara, (Montenegro)	Canyon of the river Piva, (Montenegro)
Substratum	Limestone	Limestone	Limestone	Limestone
Altitude	750-900 m	740-850 m	700-750m	550-700 m
Average annual temperature	7-8 °C	7-8 °C	8-9 °C	7-8 °C
Average January temperature	4-5 °C	1-2 °C	1-3 °C	-1-3 °C
Annual precipitation	900-1000 mm	700-900 mm	1500mm	1300-1500mm
References	http://www.hidmet.gov.rs/		http://www.meteo.co.me/	

D. malyana is an evergreen dwarf, densely branched shrub, 10-30 cm in height. It belongs to the life form of fruticose, caespitose chamaephytes. The plant grows in the rock crevices of inaccessible limestone cliffs as a typical chasmophyte. In the canyons it is mostly recorded on the northerly rocky slopes, while in the high-mountain regions, at the altitudes of 600-2000 m, it also occupies southerly sites.

The comparative morphoanatomical studies have dealt with two populations from W. Serbia, from two distant localities on Mt. Tara, and with two populations from Montenegro, from habitats in the canyons of the Tara and Piva rivers. All the studied populations of *D. malyana* grow under steady microclimatic canyon conditions on a thin soil of limestone substrate (Table 1). Low population density was a common characteristic of all these geographically isolated populations.

The aim of the present study was to determine whether, and to what extent, the isolation of *D. malyana* populations in the canyons, as refugial habitats, may have caused the morphoanatomical variability of this ancient endemic species.

MATERIALS AND METHODS

Plant material

The specimens of *D. malyana* were collected in May 2008, during the flowering season, in four different sites in W. Serbia and N. Montenegro. The collected plant material was either placed in the herbarium or fixed in 50% ethanol. Voucher specimens of the plants were deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology in Belgrade (BEOU).

Voucher specimens

1. Serbia: Mt. Tara, Sokoline, canyon of the river Rača, steep limestone slopes named Sokoline, 740-860 m a.s.l. (*Jušković, M., Milinković, N., 16383*, BEOU, 02-May-2008);
2. Serbia: Mt. Tara, Vranjak – Zaovine, N-facing limestone cliffs, 740-860 m a.s.l. (*Jušković, M., Stevanović, B., Stevanović, V., 16384*, BEOU, 29-May-2008);

3. Montenegro: canyon of Tara River, 732 m a.s.l. (*Jušković*, *M*., *Stevanović*, *B*., *Stevanović*, *V*., *16385*, BEOU, 30-May-2008);

4. Montenegro: canyon of Piva River, 650-850 m a.s.l. (*Jušković*, *M*., *Stevanović*, *B*., *Stevanović*, *V*., 16386, BEOU, 31-May-2008);

Morphoanatomical analysis

The anatomical analysis of leaves and stems was performed on permanent and temporary slides prepared by the standard histological method for light microscopy (Ruzin, 1999). The analysis was carried out on the mature leaves (taken under the inflorescence region) as well as on one-year-old stems. The leaves were fixed in Navashin's solution, dehydrated through a series of ethyl-alcohol and embedded in paraplast. Cross-sections of the leaves and stems, 7-10 µm thick, were cut using a Reichert sliding microtome, and double stained with safranin and hematoxylin.

Epidermal peels, for surface structure and stomata analyses, were prepared by using Jefferson's solution (10% nitric acid and 10% chrome-trioxide, 1:1), stained in safranin and alcian blue, and after the dehydration, mounted in Canada balsam.

The total of 20 quantitative characteristics subjected to statistical analysis was grouped into two categories: I - leaf anatomy and leaf shape characteristics (whole leaf and mesophyll thickness, thickness of palisade and spongy tissue, number of palisade layers, height and surface area of adaxial epidermal cells, height and surface area of abaxial epidermal cells, number of abaxial non-glandular hairs and stomata, surface area of abaxial stomata, leaf length, leaf surface area, length of leaf nervature, largest width of the leaf, distance between the largest leaf width and the leaf top); II - stem anatomy characteristics (stem diameter, stem periderm thickness, stem cortex thickness).

The morphoanatomic measurements were performed on the microscope Leica DM 2500-Leica DFC490-Leica Qwin Standard (Leica Microsystem, Germany).

Soil analysis

Soil samples were collected from 3 different sites of each of the four population localities. Each analyzed sample contained 300 g of dried, sieved soil. The pH value was determined in a soil suspension with potassium chloride and in one with water (10g : 25cm^3), using a potentiometer. The $CaCO_3$ content was determined by the volumetric method, using the Scheibler's calcimeter. The humus content was determined after Turin's method, while that of nitrogen was calculated on the basis of humus values. The available phosphorus was measured by the method of Allen (1940) using a spectrophotometer, whereas the available potassium was determined according to the method of Allen on a flame photometer.

SEM analysis

Leaf epidermis, trichomes and stomata were analyzed by a scanning electron microscope (SEM, JEOL 5300). The leaf samples were dried at the critical point of CO_2 and covered with gold in an ion evaporator.

Statistical analysis

The obtained results were processed using the statistical package SYSTAT 12 (Systat Software, Inc. 2007). Each of the studied characteristics was included in univariate statistical analysis that comprised the determination of the main statistic parameters: mean value and standard deviation for significance threshold $p \leq 0.05$. The general structure of sample variability was established by Principal Component Analysis (PCA), based on a correlation matrix. The canonical discriminant analysis (CDA) was performed to check the hypothesis that the analyzed sample groups differed from each other. Finally, the differences between the compared populations were confirmed through Mahalanobis distances.

Table 2. Analysis of variances on the level of individual morpho-anatomical characters.

	Mt. Tara, Sokoline (Serbia)	Mt. Tara, Vranjak (Serbia)	Canyon of the river Tara, (Montenegro)	Canyon of the river Piva, (Montenegro)	F	p
Leaf thickness (µm)	256.94±37.85	331.65±47.76	316.04±44.74	382.63±36.34	23.95	0.000
Thickness mesophyll (µm)	199.73±39.95	249.79±39.68	233.78±37.41	274.21±37.35	4.70	0.004
Height of adaxial epidermal cells (µm)	34.78±5.69	40.79±13.21	43.29±14.34	53.17±17.63	3.73	0.013
Thickness of palisade tissue (µm)	59.44±19.05	112.42±25.45	98.65±22.03	114.64±21.65	18.37	0.000
Thickness of spongy tissue (µm)	139.44±27.12	137.54±23.69	134.85±22.65	159.60±25.75	5.61	0.001
Height of abaxial epidermal cells (µm)	25.23±3.04	23.56±4.47	22.74±3.70	26.50±4.01	35.39	0.000
Number of abaxial stomata	106.47±40.49	102.82±11.68	95.15±11.76	164.17±34.48	101.69	0.000
Number of non-glandular abaxial hairs	19.27±5.75	3.60±3.29	9.87±3.81	4.83±1.20	38.48	0.000
Leaf length (mm)	17.93±2.16	20.98±2.28	19.84±2.20	13.90±3.25	34.25	0.000
Largest width of leaf (mm)	6.71±0.55	6.45±0.83	8.47±1.33	5.28±0.73	45.85	0.000
Leaf surface area(mm)	75.97±14.48	75.82±14.95	106.24±24.47	43.42±6.63	63.49	0.000
Stem diameter (µm)	1201.80±30.02	1220.43±254.54	1126.91±94.26	1223.06±95.58	2.22	0.089
Stem periderm thickness (µm)	39.24±1.48	26.17±4.40	58.75±8.64	31.36±4.65	92.74	0.000
Stem cortex thickness (µm)	288.13±7.10	253.89±40.84	277.34±43.02	271.51±37.81	4.06	0.009

RESULTS

Leaf morphology and anatomy

The leaves of *D. malyana* are simple, small, and elliptic to ovate, bluntly tipped, with a short stem or sessile. They are leathery, grey-green and dim, with a smooth margin that slightly curves toward the adaxial leaf side. Only the leaves of the population from the locality Vranjak (Mt. Tara, Serbia) were different in shape, being elongated and mildly pointed at the top.

The leaves of the analyzed plants from all localities were 10-24 mm long, 4-11 mm wide, with the surface area of between 30 and 165 mm^2. The greatest value of leaf surface area was recorded in the plants from the canyon of the Tara river (106.24±24.47 mm^2), while the smallest was in plants from the canyon of the Piva river (43.42±6.63 mm^2) (Table 2). On the surface of the leaves there are some mechanical, unicellular, short and straight hairs, more densely distributed only around the midrib. They are always on the abaxial leaf sides, except in plants from the Sokoline

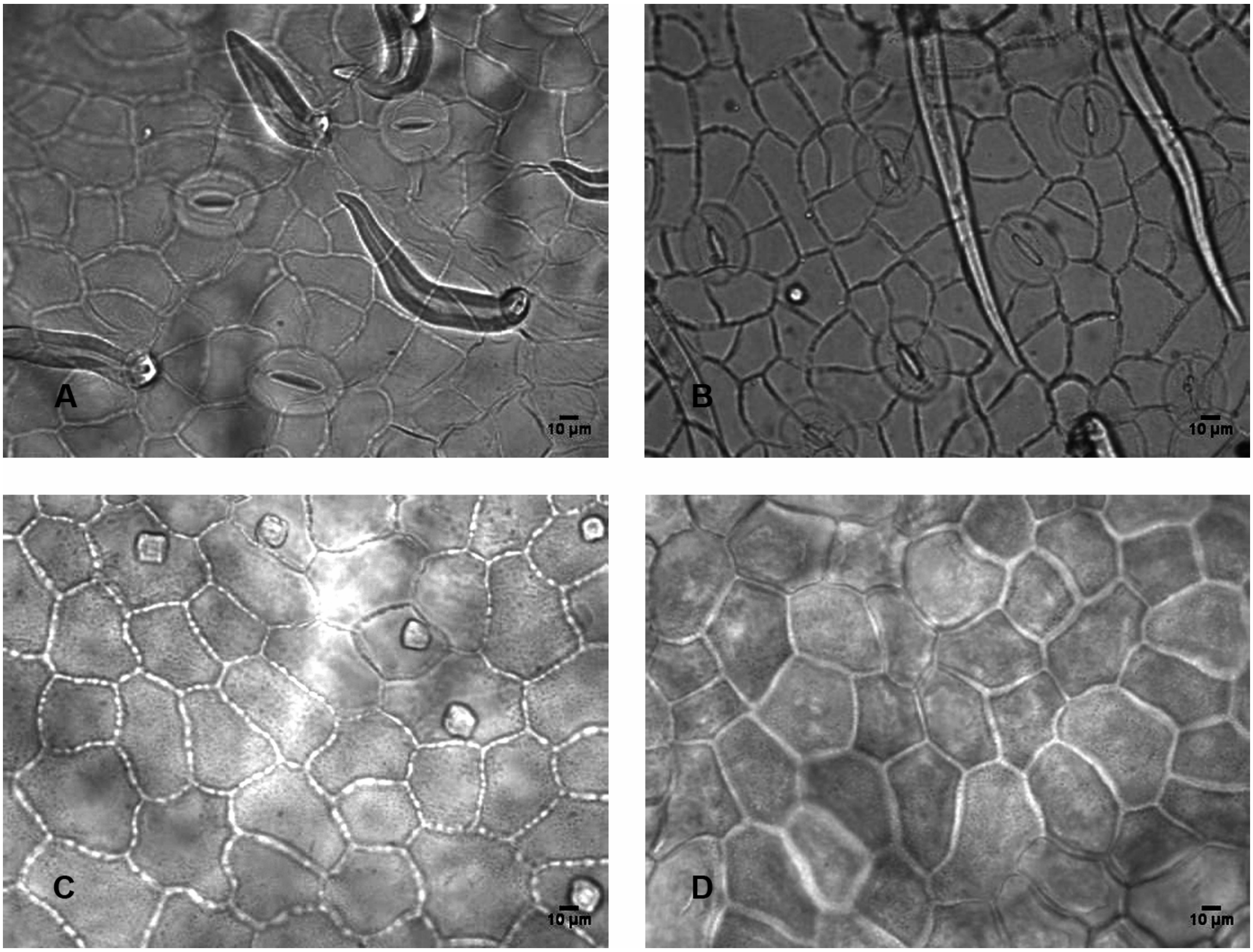

Fig. 2. Paradermal view of *Daphne malyana* leaves. Non-glandular hairs and anomocytic stomata on abaxial epidermis of the plants from: A - the Tara canyon and B - Sokoline. Straight anticlinal walls of adaxial epidermal cells of plants from: C - the Tara canyon and D – Vranjak.

locality (Mt. Tara) which have sparse hairs also on the leaf adaxial surface (Fig. 2A-B).

The leaves are bifacial or of dorsiventral symmetry. The mean thickness of the leaves was between 256.9 µm (Sokoline population) and 362.8 µm (the Piva canyon population) (Table 2). The leaves of the plants from all populations consist of one layer upper and lower epidermis, the mesophyll, differentiated into palisade and spongy parenchyma, and a number of vascular bundles. The epidermal cells are covered with well-developed, thick and strongly wrinkled cuticles both on the abaxial and adaxial sides of the leaf (Fig. 3A-B). The outer walls of the epidermal cells were also thickened. The anticlinal cell walls of both the upper and lower epidermis are mostly straight (Fig. 2C-D). The mean length (height) of the upper epidermal cells was between 53.2 µm (the Piva canyon population) and 34.8 µm (Sokoline population). On the other hand, the lower epidermal cells were smaller and more uniform in size (Table 2). In addition, the upper epidermal cells contained a greater amount of mucilaginous substances than the lower ones.

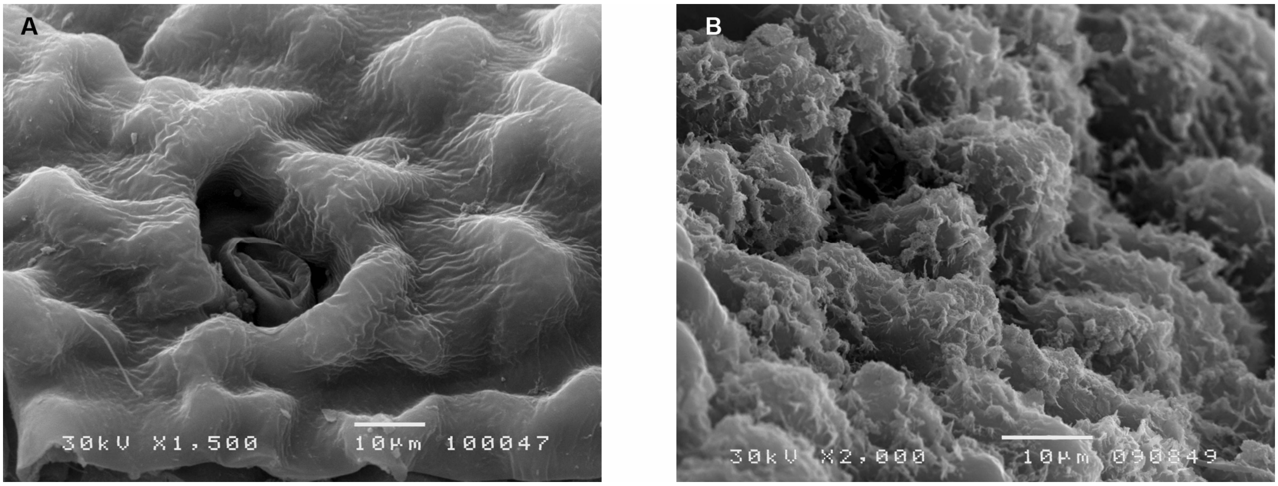

Fig. 3. Wrinkled cuticle and stomata on abaxial leaf surface of *Daphne malyana* from: A – Vranjak and B – the Tara canyon (SEM).

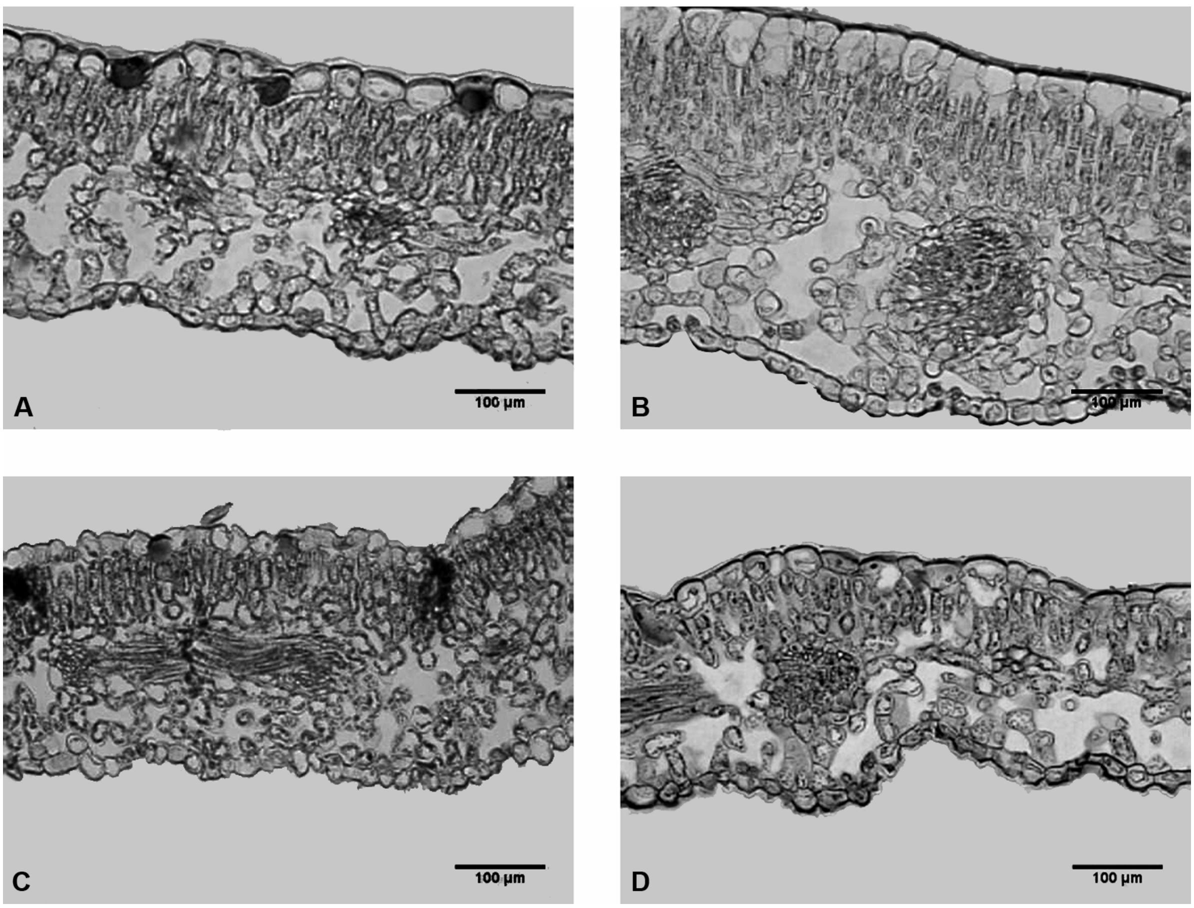

Fig. 4. Cross section of the leaves of *Daphne malyana* from: A - the Tara canyon, B – the Piva canyon, C - Sokoline, D - Vranjak.

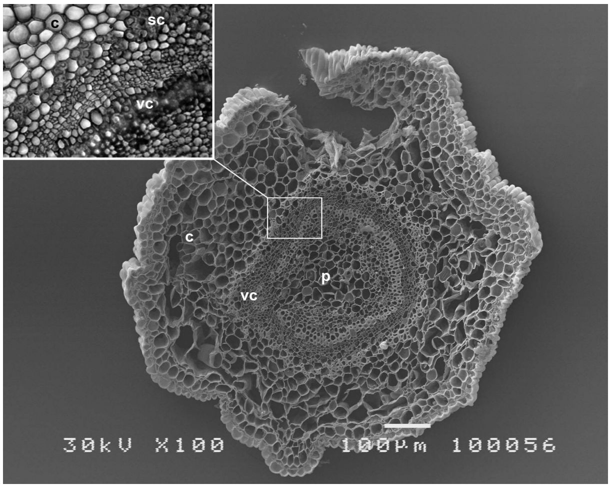

Fig. 5. Cross section of the one-year old stem of *Daphne malyana* from the Piva canyon: c – stem cortex, sc – sclerenchyma cells, vc – vascular cylinder, p – pith.

Table 3. pH values and and contents (in % or mg/g dw) of some elements in the soils from studied localities.

	Mt. Tara, Sokoline (Serbia)	Mt. Tara, Vranjak (Serbia)	Canyon of the river Tara, (Montenegro)	Canyon of the river Piva, (Montenegro)
pH	6.54	7.14	7.41	7.18
$CaCO_3$ (%)	15.17	42.73	79.47	73.06
Humus (%)	10.06	10.01	3.25	11.75
N (%)	0.5	0.5	0.16	0.56
P_2O_5 (mg/g)	2.8	16.4	5	5.4
K_2O (mg/g)	23.6	35	7.8	18

The plants from all the populations studied have hypostomatous leaves except those from Sokoline whose leaves are amphistomatous. The stomata are anomocytic, deeply sunken, thereby leveling the first layer of spongy parenchyma cells. Their frequency varies greatly, ranging between 57 and 218 per mm^2. The greatest number of stomata (106-218 per mm^2) was recorded in plants from the Piva canyon, while the smallest (81-115 per mm^2) was observed in plants from Tara canyon (Table 2).

In the differentiated mesophyll the differences between the palisade and the spongy parenchyma are pronounced (Fig. 4). The palisade tissue consists of two or three layers, seldom of four layers of short, densely packed cells. The spongy tissue is composed of irregular, more or less round-shaped cells, separated by large intercellular spaces. The ratio of palisade to spongy tissue varied from 1:1 (Vranjak) to 1:2 (Sokoline) (Fig. 4D and 4C). Sporadically several cells or groups of cells of spongy tissue resemble palisade cells in shape and pattern of arrangement. The mesophyll also includes rare sclerenchymatous idioblasts.

Stem anatomy

The general morphoanatomic characteristics of one-year-old stems and branches varied only insignificantly among the representatives of the four studied populations. The internodes of the stunted stems and branches are very short even when the shrub growing upwards reaches the height of over 30 cm. It should be noted that the plants from the populations of the Piva canyon are distinguished by dwarfishness. These short shrubs, 5-10 cm tall, are almost creeping, strongly attached to the rocky cliff. The stems bear knob-like leaf scars.

On the cross section, one-year-old stems are round in shape. The single layered epidermal cells have a thick cuticle and cutinized outer walls. At some parts of the stem the initiation of periderm formation was observed (Fig. 5). The wide cortex of the stem contains two or three layers of collenchyma and about a dozen layers of round to elliptical parenchyma cells.

The vascular tissue commonly forms a (more or less complete) cylinder between the stem cortex and the pith. The sclerenchyma cells at the outer side of phloem form caps or semi-rings. The pith parenchyma was compact, composed of parenchyma cells, with no cavity present. The dimensions of the stem in cross-section have similar values in all analyzed populations (Table 2). The correlation coefficient of the cortex and the whole stem diameter ranges is similar throughout this species, with values from 0.42 (Vranjak) to 0.50 (Tara canyon).

Soil analysis

The results of soil analysis of the four different sites inhabited by *D. malyana* are presented in Table 3. The pH of soil varied from neutral to slightly alkaline (6.5-7.4), and due to significant $CaCO_3$ content ranged mainly between 15% (Sokoline) and 79% (the canyon of the river Tara). The greatest values of organic matter were recorded in the rocky crevice soil in the Piva canyon (11.75%), while the smallest values were in the soil deriving from the Tara canyon (3.25%). The available nitrogen in the soils from the studied localities varied from rather low to sufficient (0.5-0.56%). The amount of phosphorus and potassium ranged widely, i.e. from low to adequate accessibility (2.8 to16.4 mg/g dw, and 7.8-35 mg/g dw, respectively).

Statistical analysis

The analysis of variance (ANOVA) has shown statistically significant differences in all characteristics except for the stem diameter of all the four investigated populations (Table 2). Principal component analysis (PCA) indicated that the pattern of variability of the analyzed populations was very complex, as judged by a number (20) of important components. However, out of these 20 the five main components account for 65.9 % of the total variability (Table 4). It should be pointed out that out of these 5 the first two components (29.1% and 13.8%, respectively) contributed most significantly to the structural differentiation of the leaves of the plants from the different populations. The morphoanatomical variability was primarily related to the following characteristics: leaf, mesophyll, palisade and spongy tissue thickness, abaxial epidermal cells height, number and surface of stomata at the abaxial leaf side, and the distance between the largest leaf width and the leaf top. The three other main components constitute only about 23% of the total variability, including both leaves and stems of the plants studied. Both principal component analysis (PCA) and canonical

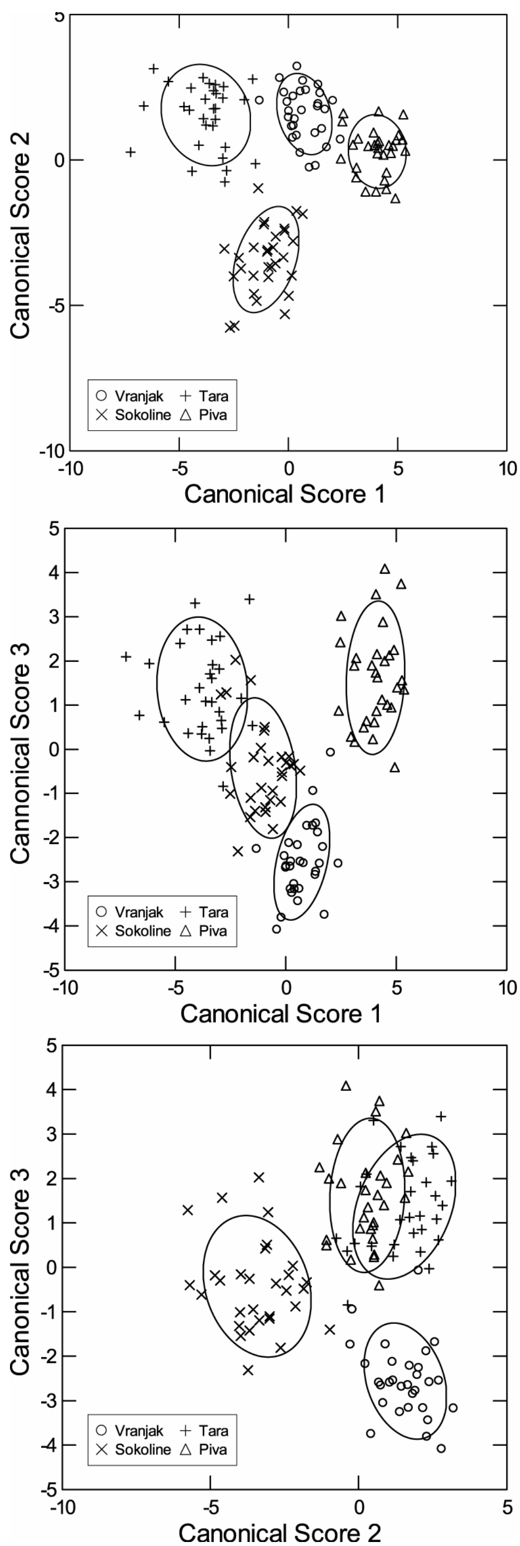

Fig. 6. Canonical discriminant analysis (CDA).

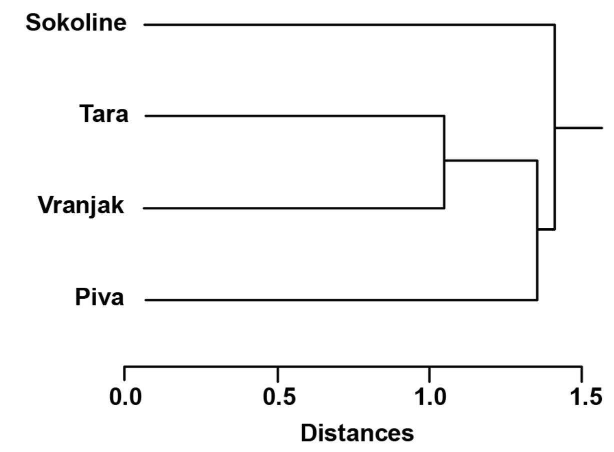

Fig. 7. Mahalnobius distances between four populations of *Daphne malyana.*

discriminant analysis (CDA) approved structural variability and differentiations among the plants from the four different localities: the population from Sokoline (Mt. Tara) and that of the Piva canyon are clearly distinguished from the populations of Vranjak (Mt. Tara) and the Tara canyon (Fig. 6). In addition, morphoanatomical differentiation among the four populations of *D. malyana* was clearly confirmed by the results of the cluster analysis performed on the basis of Mahalanobis distances (Fig. 7). The above-mentioned results point to the correlation between plant structural traits and the ecological, especially microclimatic, conditions of their habitats.

DISCUSSION

The endemo-relict species *D. malyana* is an obligate calciphile, growing on thin layers of organo-mineral soil on limestone rocky crevices. The populations of this chasmophyte are characteristically small and scarcely distributed in the shaded canyon cliffs of the central part of the Balkan Peninsula, at altitudes of 600 to 2000 m. The leaves of *D. malyana* are small, microphyllous when compared with those of other species of same genus from the Balkans (Blečić, 1972; Webb and Ferguson, 1968). Its evergreen leaves are distinguished by the combination of xeromorphic and mesomorphic traits. The reduced leaf size and prominent peripheral structures, comprising a thick wrinkled cuticle, mucilaginous epidermis, a thickened outer wall of the epidermal cells, straight anticlinal walls and deeply sunken stomata, are typical xeromorphic features that provide protection and prevent rapid water loss (Fahn and Cutler, 1992). All these plants, and particularly those from the Piva canyon population, have mucilaginous epidermal cells, which could be regarded as an adaptive xeromorphic trait, otherwise being one of the main characteristics of plants from Thymeleaceae family (Metcalfe and Chalk, 1950). However, the internal leaf anatomy presents a rather mesomorphic organization owing to the palisade and spongy tissue arrangement and their ratio that varied from 1:1 to 1:2. Namely, the palisade tissues consisted of two or three layers of palisade tissues, the cells of which are short and not so tightly packed, whereas among the round-shaped spongy cells were large intercellular spaces. It is worth mentioning that this type of leaf anatomical adaptation, combining xeromorphic and mesomorphic traits, is suitable to *D. malyana* growing on a thin soil layer in shady rock crevices where adequate humidity prevails. Similar morphoanatomical characteristics were observed in some other relict and/or endemic species, such as *Satureja horvatii*, some species of the genera Edraianthus or Teucrium from the Balkan Peninsula, especially from humid oro- and/or sub-Mediterranean environment (Stevanović and Stevanović 1984; Todorović and Stevanović, 1994; Lakušić et al., 2007, 2010).

In addition, in all the four populations even one-year stunted stems were remarkably tough. The well-developed cortex and subepidermal collenchyma, groups of sclerenchymatous elements in the pericycle and the mainly completely closed ring of vascular tissues (even more remarkable in older stems) indicated the obvious stem xeromorphic features. Moreover, the ratio coefficient of the cortex-to-stem diameter of 0.416-0.492, even in a one-year-old stem, fitted well with Fahn's data for xeromorphic stems ranging from 0.271-0.833 (Fahn and Cutler, 1992).

Table 4. Principal component analysis (PCA) of measured parameters.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Leaf thickness	0.714	0.450	0.139	0.107	-0.062
Thickness mesophyll	0.640	0.415	0.606	0.000	-0.102
Thickness of palisade tissue	0.535	0.520	0.353	-0.103	0.145
Thickness of spongy tissue	0.560	0.184	0.616	0.101	-0.318
Height of adaxial epidermal cells	0.331	0.362	-0.280	0.066	-0.359
Height of abaxial epidermal cells	0.546	-0.114	0.163	-0.018	0.138
Surface area of adaxial epidermal cells	0.201	0.311	-0.370	0.471	-0.053
Surface area of abaxial epidermal cells	-0.328	0.273	-0.183	-0.366	-0.230
Surface area of abaxial stomata	0.161	0.728	-0.258	-0.095	-0.017
Number of palisade layers	0.491	0.329	-0.439	0.305	0.271
Number of abaxial non-glandular hairs/mm^2	-0.420	-0.535	0.323	0.196	-0.363
Number of abaxial stomata/mm^2	0.650	-0.156	-0.203	0.152	0.045
Length of leaf nervature	-0.611	0.231	0.153	-0.274	-0.043
Leaf area	-0.821	0.354	0.167	0.207	0.096
Length of leaf	-0.658	0.373	0.251	-0.229	0.246
Width of leaf	-0.767	0.284	0.055	0.313	0.054
Distance between point of largest leaf width and leaf top	-0.738	0.547	0.054	0.055	0.150
Stem diameter	0.162	-0.171	0.275	0.215	0.672
Stem periderm thickness	-0.470	0.212	0.045	0.627	-0.308
Stem cortex thickness	-0.112	-0.223	0.214	0.571	0.085
Percent of Total Variance	29.079	13.820	9.069	7.962	5.992

The anatomical and micromorphological characteristics of the leaves and stems were very similar in all the populations studied, revealing stable conservative xeromorphic features, otherwise noticed in a number of relict and/or endemic plants from the Balkan Peninsula (Stevanović and Mitrović, 1996; Lakušić et al. 2006).

Based on PCA and CDA analyses of the morphoanatomic characteristics of leaves and stems, a certain distinction among the four populations has been established. Plants from the Piva canyon were characterized by pronounced xeromorphic features with the smallest and thickest leaves and the smallest surface area, four layers of palisade tissue and the greatest number of stomata per mm^2, as well as stunted stems with extremely short internodes. In contrast, the plants from Sokoline population had the thinnest, mostly amphistomatic leaves, and a ratio of palisade to spongy tissue of 1:2. A similar structure was observed in the plants from the Vranjak population, with elongated leaves, pointed at the top, and the ratio of palisade to spongy tissues 1:1. Finally, the leaves of plants from the Tara canyon had the largest surface area and a slightly smaller number of stomata per mm^2.

Over time and despite the isolation in the canyons, the populations of D. *malayana* were most similar in respect to morphoanatomical traits. However, the slight differences observed among them might have resulted from the response of the plants to the varying microclimatic conditions, though barely discernible, prevailing in each of the localities studied. Namely, the populations in the habitats from W. Serbia (the Mt. Tara ravines) grow under a transitional variant of temperate-continental to mountain climate, while the populations from the canyons of the rivers Piva and Tara develop under a transitional variant of sub-Mediterranean-Adriatic to temperate-continental humid climate (Stevanović and Stevanović, 1995). Therefore, it could be supposed that the observed morphoanatomical variations, however small, were triggered by micro-environmental conditions. In the warmer, less humid canyons of the Piva and Tara rivers in Montenegro the xeromorphic traits were more pronounced, particularly in individuals from the Piva canyon, whereas the plants from the cooler, humid microclimatic conditions of the Mt. Tara canyons in W. Serbia exhibited pronounced mesomorphic features, particularly evident in the ratio of palisade to spongy tissue, and in the loose arrangement of spongy cells with great intercellular spaces.

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