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Morphological variability of leaf and shoot traits of four barberry taxa (*Berberis* L.) from the Balkan Peninsula and Sicily

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ABSTRACT:
Leaf and shoot characteristics of the following four European barberry taxa from the Balkan Peninsula and Sicily were investigated in the present study: *Berberis croatica*, *B. vulgaris*, *B. aetnensis* and *B. cretica*. Analyses were based on 10 populations of *B. croatica,* five of *B. vulgaris* and two populations of both *B. aetnensis* and *B. cretica*. Populations were randomly selected within the natural distribution area of these species. Eight leaf traits, three shoot traits and the blade length/width ratio were analysed. Multivariate analysis (principal component analysis, canonical discriminant analysis and cluster analysis) distinguished *B. cretica* and *B. aetnensi*s populations and, to a lesser extent, the populations of *B. croatica* and *B. vulgaris.* ANOVA showed that the analysed populations of both *B. aetnensis* and *B. cretica* were homogeneous within the species. All populations of both *B. croatica* and *B. vulgaris* showed different degrees of intraspecies variability. Lack of complete separation, the observed grouping of populations and high intraspecies variability in *B. vulgaris* and *B. croatica* may reflect the fact that the sampled *B vulgaris* and *B.croatica* populations were located at environmentally variable sites (unlike *B. aetnensis* and *B. cretica*), resulting in high phenotypic plasticity in those populations. Even though the observed patterns of morphological variation support the idea of four barberry taxa on the Balkan Peninsula and in Sicily, because of suspected adaptive phenotypic plasticity of the analysed *Berberis* taxa, the true taxonomic status of these taxa needs to be additionally confirmed by molecular methods.

Keywords:
Berberidaceae, *Berberis aetnensis*, *Berberis cretica*, *Berberis croatica*, *Berberis vulgaris*, morphology, morphometry, multivariate analysis, taxonomy

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INTRODUCTION

The genus *Berberis* L. includes about 500 species native to Asia, North Africa, America and Europe (Ahrendt 1961). Taxonomically, *Berberis* is considered to be a very complex genus and has even been called a "taxonomic black hole" (Landrum 1999), with variable characters in its species (Sodagar *et al.* 2012). Many authors used morphological traits to examine inter- and intraspecies varibility of *Berberis*, mostly on species growing in South America (Bottini *et al.* 1998; Landrum 1999; Arena *et al.* 2011; Radice & Arena 2015; Giordani *et al.* 2017), but also in Europe and elsewhere (Rivas-Martínez *et al.* 1985; Karlović *et al.* 2009; Jannatizadeh & Khadivi-Khub 2016). As a result of the large size of the genus and its ubiquitous distribution, the number of barberry species in the world and in Europe remains controversial. In 1931, Dermen cited *Berberis vulgaris* L. as the only European species, while Rikli (1946) mentioned four subspecies in the European Mediterranean area: *B. vulgaris* subsp. *hispanica*, *B. vulgaris* subsp. *aetnensis*, *B. vulgaris* subsp. *cretica* and *B. vulgaris* subsp. *crataegina*. Later Webb (1964) recognised four European barberry species: *B. vulgaris* L., *B. aetnensis* C. Praesl, *B. hispanica* Boiss. & Reut. and *B. cretica* L. Around the same time, some authors suggested that *Berberis croatica* Horvat, was a separate species occurring in Croatia (Kušan 1969), Bosnia and Herzegovina (Šilić 1996), the Republic of North Macedonia (Trinajstić 1973) and Montenegro (Grlić 1979). Other authors, in contrast, recognised *B. croatica* as a subspecies of *B. vulgaris* (Anić 1946) or *B. aetnensis* (Anić 1946; Forenbacher 1990). More recently, Akeroyd & Webb (1993) included only *B. vulgaris* and *B. cretica* as European barberry species, while considering *B. aetnensis* and *B. hispanica* as subspecies of *B. vulgaris*. *Berberis croatica*, on the other hand, was not even listed as a European barberry species by Akeroyd & Webb (1993), while a database like IPNI (2020) does not list it and the Euro+Med (2006) database lists it as a synonym for *B. vulgaris*.

In this way, the number and classification of *Berberis* taxa in Europe remains controversial due to their morphological diversity and extensive distribution. *Berberis vulgaris* seems to be the only European barberry species whose taxonomic status is not in doubt. To clarify uncertainties in the classification of other barberry species, the present study analysed leaf and shoot morphological traits of the barberry taxa from the Balkan Peninsula and Sicily, i.e., *B. croatica*, *B. vulgaris*, *B. aetnensis* and *B. cretica*.

MATERIALS AND METHODS

Plant material. Ten populations of *Berberis croatica* Horvat, five populations of *B. vulgaris* L., two populations of *B. aetnensis* C. Presl and two populations of *B. cretica* L. were randomly selected on the basis of published data (Kušan 1969; Phitos & Strid 2002; Karlović *et al.* 2009) within the area of natural distribution of these species (Fig. 1A). Species identification was based on descriptions and keys provided by Webb (1964), Kušan (1969), Trinajstić (1973), Pignatti (1982) and Akeroyd & Webb (1993). On the basis of morphological characters, two of the populations (one from Greece and one from Bosnia and Herzegovina) were assigned to different taxa after the identification procedure. The population from Greece that was reported as *B. vulgaris* in the literature was treated as *B. croatica* (Bc_Gr), and the population from Bosnia and Herzegovina that was reported to be *B. croatica* in the literature was treated as *B. vulgaris* (Bv_Os; Table 1).

Ten plants were sampled from each population, except for the small populations Bc_Cr and Bc_Sn, which included samples from seven and six plants, respectively. In total, 183 individuals were surveyed in the study. Voucher specimens of plant material were deposited in the "Fran Kušan" Herbarium, maintained by the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

The following eight leaf traits were measured on dry and pressed plant material: number of leaves on short shoots, blade length and width, number of teeth on the left and right sides of the blade, length of the longest teeth, the greatest distance between two teeth and petiole length. Blade length and width were used to calculate the blade length/width ratio. A higher ratio indicates a narrower blade, while a lower ratio indicates a rounder blade. In addition, three shoot traits were measured: number of spines on five consecutive nodes starting from the twig top, spine length and internode length.

Data analysis. Descriptive statistics of each morphological trait were calculated to obtain basic parameters of the studied populations/taxa. Morphological variation of the sampled taxa was evaluated using principal component analysis (PCA), cluster analysis (CA) and canonical discriminant analysis (CDA). Leaf and shoot traits were averaged for each individual to construct two data matrices: (1) the "population matrix" and (2) the "individual matrix". The "population matrix" was based on means of all morphological characters at the population level regardless of their taxonomic affiliation. The populations were used as units in PCA and CA in order to pre-specify their affinities for a taxonomic group. On the other hand, the "individual matrix" was based on individual means of morphological characters using individual plants as units in PCA and CDA. PCA performed on the "individual matrix" aimed at revealing the overall pattern of morphological variation and relationships among individuals originating from the specified groups. CDA based on the "individual matrix" with four groups was performed to determine morphological traits discriminating the studied taxa and to classify each individual into an *a priori* specified group (taxon). Prior to running analyses, all data were standardised due to different scales of character scoring (Quinn & Keough 2009). Spearman and Pearson correlation coefficients were calculated to find very highly correlated character pairs since they may distort the results of discriminant analysis (Legendre & Legendre 1998). Since the characters blade length and number of teeth on the left side were highly intercorrelated with blade width and number of teeth on the right side, respectively (r>0.8), the latter ones were excluded from CDA. The PCA of populations/individuals was computed on the correlation matrix of all scored traits, and the axes corresponding to principal components with Eigen values >1 were retained in analysis. Cluster analysis by the unweighted pair-group method with arithmetic means (UPGMA) was calculated using Euclidean distances (Sokal & Rohlf 2003). CDA computation was based on Mahalanobis distances of 10 variables. The relationships among individuals of the studied taxa were visualised on the bidimensional plot of discriminant function scores. The classification discriminant function was then derived and used to classify each individual into one of the *a priori* determined groups by the cross-validation procedure. Between-population variation was evaluated by analysis of variance (ANOVA) and Scheffe's post-hoc test, with $p<0.05$ defined as the threshold of significance.

Descriptive statistics, CDA and cluster analysis computations were performed in Statistica 7 (StatSoft Inc. 2004), while PCA was done in PAST, ver. 3.14 (Hammer 2016). The PCA and CDA graphs, phenogram and distribution map were edited for better performance in Adobe Illustrator CS6.

RESULTS AND DISCUSSION

From descriptive statistics of the analysed traits (Supplementary Material 1), it can be seen that *B. cretica* differed from the other taxa in having the smallest and narrowest leaves with almost no teeth. *Berberis aetnensis*, on the other hand, differed from the other taxa in having the longest spines. Populations of *B. croatica* were generally characterised by short spines; however, that was not a uniform trait since the Bosnian Kamešnica population (Bc_Ka) was an exception to the rule. Even though possession of the the largest leaves was a distinctive trait for some populations of *B. vulgaris*, that was not a uniform feature throughout *B. vulgaris* populations. Shoot traits were generally less variable than leaf traits. The most variable shoot trait was spine number, for which CV ranged from 25.2% in Bcre_O to 105.7% in Bv_Os. The least variable shoot trait was internode length, with CV ranging from 14.4% in Bae_MV to 37.0% in Bc_Bl. The most variable leaf trait was petiole length (ranging from CV = 38.8% in Bc_Cr to CV = 81.2% in Bv_RP), while the least variable leaf trait was the blade length/width ratio (ranging from CV = 15.3% in Bae_Li to CV = 28.2% in Bc_Ca).

The PCA performed on the "population matrix" showed separation of populations mostly corresponding to their taxonomic affiliation (Fig. 1 B). The first three components accounted for 82.24% of the total variance: 49.91% for PC1, 19.74% for PC2 and 13.29% for PC3 (Table 2). Several traits (none of them being highly correlated), viz., blade length, number of teeth on the right side and blade width, contributed the highest value for PC1. Spine length, number of leaves and distance between teeth contributed most to the second PC axis, while the maximum score for PC 3 was obtained for length of teeth and the blade length/width ratio (Table 2).

The PCA based on the "individual matrix" displayed a similar pattern as for the "population matrix" (Fig. 1D). In a biplot of PC1 and PC2, *B. aetnensis* and *B. cretica* showed a separation from *B. croatica* and *B. vulgaris* (Fig. 1B). Several individuals of *B. cretica* intermingled with individuals of *B. croatica* and vice-versa. Along the first axis, *B. croatica* differentiated from *B. vulgaris*, but these two groups were not clearly separated (Fig. 1D). Individuals mostly from the populations Bv_Pr, Bc_Me and Bv_CL were dispersed between the two main clusters.

Groupings of populations into taxa were also validated by cluster analysis based on Euclidean distances. Almost all populations were clearly defined as falling into the corresponding taxon. An exception was the Bv_Pr population (Fig. 1C), which was connected with *B. croatica* accessions. According to the conducted cluster analysis, the sampled populations could be divided into four main clusters. The first included *B. vulgaris* populations. The second cluster grouped populations of *B. cretica*, the third included populations of *B. aetnensis*. The fourth cluster was represented by *B. croatica* populations, including the Bv_Pr population. The constant intermixing (both in PCA and CA) of the Bv_Pr population with *B. croatica* accessions might be an indicator of spontaneous hybridisation and introgression in the past, which affected formation of the Prokike population. At the same time, the Prokike population grows in environmental conditions which are more similar to those of *B. croatica* (although at a low altitude of 593 m a.s.l., the population inhabits a slope on shallow and stony soil with southeastern exposition in drier conditions on an open, wind-exposed site with more sun available).

The CDA model and discrimination were significant (Wilks' $\lambda = 0.011$, df = 27, $p<0.001$). The first three canonical axes extracted 51.80, 28.70 and 19.50% of total variance among four groups (Table 3). The CDA based on nine morphological characters resulted in four groups separated along three canonical axes. The first two axes clearly separated *B. cretica* from the other species (Fig 1E). *Berberis croatica* and *B. vulgaris* represented two groups with some intermixing individuals, while *B. aetnensis* was weakly separated from *B. vulgar-*

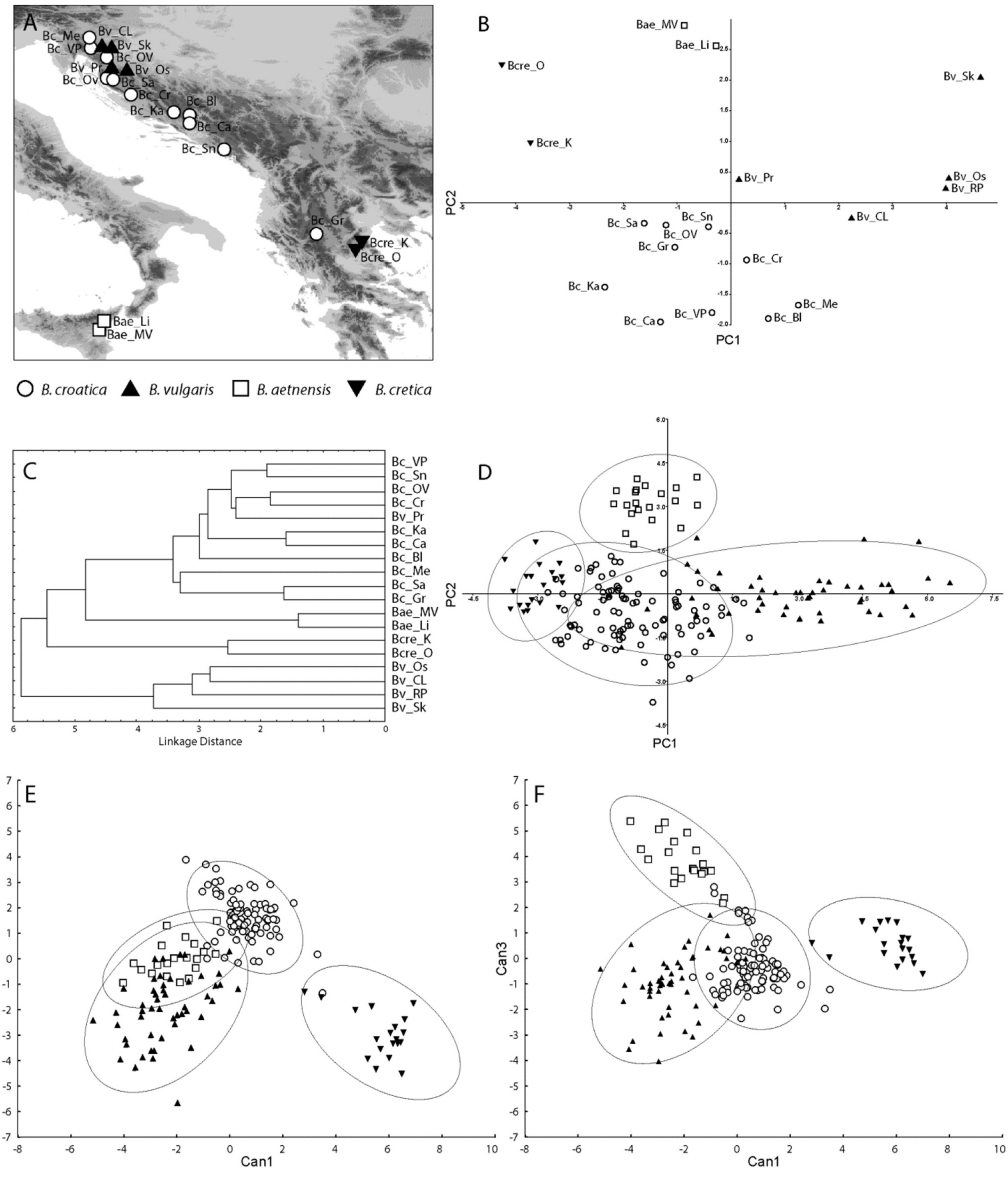

Fig. 1. Geographical distribution of the analysed *Berberis* populations (A); PCA ordination of the investigated *Berberis* populations (B); UPGMA dendrogram of the investigated populations of *Berberis* taxa (C); PCA ordination of the investigated *Berberis* spp. individuals (D); scatterplot of canonical scores on the first and second (E), and first and third (F) canonical axes for the investigated *Berberis* individuals.

Table 1. Origin and collection data of investigated *Berberis* L. spp. samples.

Population (Collectors´ names and sampling date)	Number of analysed plants	Voucher no.	Latitude; Longitude	Altitude m a.s.l.	Abbreviation
B. croatica – Vela Pliš, Croatia (Randić, Karlović, Kremer; July 2015)	10	HFK-HR-221–2015	N 45°23'; E 14°35'	1141	Bc_VP
B. croatica – Međuvrhi, Croatia (Randić, Karlović, Kremer; July 2015)	10	HFK-HR-222–2015	N 45°26'; E 14°34'	1396	Bc_Me
B. croatica – Obli Vrh, Croatia (Kremer, Kosalec; August 2015)	10	HFK-HR-223–2015	N 44°44'; E 15°01'	1515	Bc_Ov
B. croatica – Šatorina, Croatia (Kremer, Kosalec; August 2015)	10	HFK-HR-224–2015	N 44°38'; E 15°02'	1610	Bc_Sa
B. croatica – Crnopac, Croatia (Kremer, Kosalec; August 2015)	7	HFK-HR-225–2015	N 44°15'; E 15°50'	1350	Bc_Cr
B. croatica – Snježnica, Croatia (Karlović, Kremer; July 2015)	6	HFK-HR-226–2015	N 42°34'; E 18°21'	1125	Bc_Sn
B. croatica – Kamešnica, Bosnia and Herzegovina (Ballian, Bogunić; August 2016)	10	HFK-HR-142–2016	N 46°11'; E 15°54'	1421	Bc_Ka
B. croatica – Blidinje, Bosnia and Herzegovina (Ballian, Bogunić; August 2016)	10	HFK-HR-143–2016	N 45°54'; E 15°55'	1210	Bc_Bl
B. croatica – Čabulja, Bosnia and Herzegovina (Ballian, Bogunić; August 2016)	10	HFK-HR-144–2016	N 45°38'; E 15°56'	1626	Bc_Ca
B. croatica – Mt Grammos, Greece (Elefheriadou; July 2015)	10	HFK-HR-278–2015	N 40º18'; E 20°55'	1970	Bc_Gr
B. vulgaris – Rakov Potok, Croatia (Karlović, Kremer; July 2015)	10	HFK-HR-263–2015	N 45°44'; E 15°47'	130	Bv_RP
B. vulgaris – Skrad, Croatia (Karlović, Kremer; July 2015)	10	HFK-HR-264–2015	N 45°25'; E 14°54'	695	Bv_Sk
B. vulgaris – Crni Lug, Croatia (Randić, Karlović, Kremer; July 2015)	10	HFK-HR-265–2015	N 45°25'; E 14°42'	710	Bv_CL
B. vulgaris – Prokike, Croatia (Kremer; July 2015)	10	HFK-HR-266–2015	N 44°59'; E 15°04'	593	Bv_Pr
B. vulgaris – Ostrožac, Bosnia and Herzegovina (Ballian, Bogunić; August 2016)	10	HFK-HR-141–2016	N 45°40'; E 15°45'	370	Bv_Os
B. aetnensis – Mt. Vetore, Etna, Italy (Karlović; July 2015)	10	HFK-HR-75–2015	N 37°70'; E 14°98'	1700	Bae_MV
B. aetnensis – Linguaglossa, Etna, Italy (Karlović; July 2015)	10	HFK-HR-76–2015	N 37°83'; E 15°13'	1730	Bae_Li
B. cretica – Kanto Olympos, Kallipefki, Greece (Elefheriadou; July 2015)	10	HFK-HR-126–2015	N 39°58'; E 22°28'	1074	Bcre_K
B. cretica – Olympos, Kokkinopilos, Greece (Elefheriadou; July 2015)	10	HFK-HR-127–2015	N 40°05'; E 22°15'	1071	Bcre_O

Table 2. Component loadings for the first three principal components.

Variable	PC1	PC2	PC3
Number of leaves	0.089	0.477	0.148
Blade length	0.393	0.043	-0.138
Blade width	0.394	-0.047	-0.043
Blade length/width	-0.182	0.358	-0.450
Petiole length	0.357	0.037	-0.121
Number of teeth – left	0.387	0.128	0.072
Number of teeth – right	0.390	0.121	0.065
Length of teeth	0.129	0.053	0.725
Distance between teeth	0.070	-0.462	0.231
Number of spines	-0.271	0.281	0.173
Spine length	-0.067	0.536	0.255
Internode length	0.343	0.153	-0.232
Eigenvalue	5.90	2.36	1.59
% variance	49.21	19.74	13.29

Table 3. Standardised coefficients for canonical variables.

Variable	DF1	DF2	DF3
Internode length	-0.43	-0.63	-0.28
Spine length	-0.39	-0.38	0.79
Length of teeth	-0.24	0.35	0.26
Distance between teeth	-0.89	0.71	-0.12
Number of teeth – left	-1.28	0.47	0.19
Blade length	0.76	-0.64	0.13
Petiole length	0.23	-0.07	-0.46
Blade length/width	-0.12	-0.26	0.17
Number of spines	0.27	0.04	0.05
Eigenvalue	5.71	3.28	2.04
Cumulative proportion (%)	51.80	80.50	100

Table 4. Classification matrix of correctly classified individuals (N).

	B. croatica	*B. vulgaris*	*B. aetnensis*	*B. cretica*	% of correctly classified cases
B. croatica	90	0	2	1	96.7
B. vulgaris	4	45	1	0	90
B. aetnensis	1	0	19	0	95
B. cretica	0	0	0	19	100
Total	95	45	22	20	–

is (Fig. 1E). However, ordination of the first and third axes displayed clear separation of *B. aetnensis* and *B. cretica* from the two other species (Fig. 1F). Although *B. croatica* and *B. vulgaris* represented two groups morphologically, a portion of individuals from Bv_Pr intermixed within *B. croatica*. The first function was mostly determined by the following traits: number of teeth on the left side, distance between teeth and blade length. The second was mostly determined by internode length, blade length and distance between teeth, and the third by spine length (Table 3).

Classificatory discriminant analysis confirmed the CDA results and yielded a high rate of correct classification (≥90%). Only *B. cretica* resulted in a 100% correct classification, while 95% (N = 19) of *B. aetnensis* individuals were correctly determined (Table 4). A higher percentage of misclassified individuals was evident for *B. vulgaris* (10%, N = 5) and *B. croatica* (13%, N = 3). The greatest number of misclassified individuals originated from the Bv_Pr population.

It is evident that the Greek population of *B. croatica* (Bc_Gr), which according to literature sources belongs to *B. vulgaris*, noticeably clustered with *B. croatica* populations. This fact might be interpreted in two ways. Firstly, *B. croatica* has a larger natural distribution than is currently believed (Kušan 1969; Trinajstić 1973; Grlić 1979; Šilić

1996). Secondly, if we traditionally treat this population as *B. vulgaris*, then it inhabits contrasting environmental conditions, which presumably induced phenotypic traits of the plants. Such changes commonly occur in nature under different selection pressures (Gratani 2014). The Bc_Gr population featured small leaves with few leaves per shoot, fewer teeth on the blade edge and short internodes. These traits are typical of extreme harsh climates such as those in mountainous areas. Climate is considered to be a primary selective force inducing leaf morphology change (Wilf 1997). Plants growing in drier climates (such as alpine environments) tend to have smaller leaves (Bonan 2002) to reduce evaporative cooling, while larger leaves are common in more humid climates because the resulting water loss is less critical (Givnish 1984). Svriz *et al.* (2014) confirmed differential action of light on the growth of *B. darwinii* Hook. To be specific, the authors found that shoot internode length was smaller in plants growing in forest gaps than in those growing at the forest edge or under the tree canopy. Regarding the leaf margin, plants with toothed leaves have growth advantages, especially in non-optimal environments. Leaves with more teeth show more active photosynthesis; however, they are disadvantageous in xeric, water-stressed environments because of higher transpiration (Xu *et al.* 2008). The size and number of teeth correlate negatively with mean annual temperature (Royer *et al.* 2005, 2009; Royer & Wilf 2006).

ANOVA was carried out for leaf traits (Table 5) and shoot traits (Table 6) to gain insight into differences between populations for the investigated characters. In general, leaf traits differed among populations to a greater extent than shoot traits. The morphological variability noted in this research was also confirmed by Goodarzi *et al.* (2018), who documented high variations among the studied accessions of *B. vulgaris* var. *asperma* Willd. for most of the phenotypic, pomological and chemical traits analysed. The differences observed in the present analysis may be due to the small number of analysed *B. cretica* and *B. aetnensis* populations and their distribution at similar altitudes and under similar environmental conditions, in contrast to the larger number of sampled *B. croatica* and *B. vulgaris* populations, which are found over a wider range of altitudes and growing conditions. As Giordani *et al.* (2017) noted for *B. microphylla* G. Forst., short geographic distance and similar site characteristics can influence plant morphology. To support this thesis, there are reports of plants with characters intermediate between *B. aetnensis* and *B. vulgaris* found in the Alps and Southern France, while some results even distinguish the typical *B. aetnensis* found in Italy and Sicily from plants growing in Corsica and Sardinia (Webb 1964).

The number of teeth on the blade edge was the trait showing the greatest difference among populations, while tooth length showed the smallest difference. Most of the *B. croatica* and *B. vulgaris* populations showed significant differences in the majority of analysed shoot traits. The shoot trait showing the greatest difference among the investigated populations was spine length, while the number of spines showed the smallest difference.

Our results indicate that the population Bv_Os, first described as *B. croatica* by Kušan (1969), on the basis of leaf and shoot traits belongs to *B. vulgaris* and not *B. croatica*. Conversely, the population Bc_Gr (traditionally considered as *B. vulgaris*) on the basis of the analysed morphological traits belongs to *B. croatica* and not *B. vulgaris*. However, the finding that the population collected in Greece (Bc_Gr) grouped with the other *B. croatica* populations and not with *B. vulgaris* may also reflect the fact that Bc_Gr grows at 1970 m a.s.l. under environmental conditions that are more similar to those of *B. croatica* populations, which grow above 1000 m a.s.l., than to those of the other *B. vulgaris* populations in the present research, all of which grow at 130–710 m a.s.l. The Prokike population of *B. vulgaris* (Bv_Pr), which grows at a lower altitude (593 m a.s.l.) but in drier conditions on open wind-exposed ground with more available sunlight, differed from the other *B. vulgaris* populations and clustered with *B. croatica* populations. The Bv_Pr population, just like Bc_Gr, featured more compact leaves with fewer teeth and shorter internodes than the other *B. vulgaris* populations. The clustering of populations and species observed here probably reflects high phenotypic plasticity of the examined *Berberis* taxa. Phenotypic plasticity is the capability of a genotype to produce diverse phenotypic expression under different environmental conditions (Radice & Arena 2018), acting to increase the performance of plants under stress (Xu *et al.* 2008). *Berberis microphylla* grown under environmental conditions of higher temperatures and weaker sunlight showed modifications in leaf morphology and structure (Radice & Arena 2015). In the more recent research conducted by Radice *et al.* (2018), *B. microphylla* showed pronounced phenotypic plasticity in most of the variables studied. These findings were further reinforced by Radice & Arena (2018), who reported adaptive plasticity of *B. microphylla* in regard to the adjustment of its morphological but also physiological characteristics to the environmental conditions of growth. Giordani *et al.* (2017) reported significant correlation among some morphological characters of *Berberis microphylla* with environmental factors. For example, light intensity exerted significant influence on leaf roundness and compactness, altitude affected leaf elongation and wind influenced leaf compactness. Even inclination of a terrain influenced morphological traits: leaf elongation was higher in plants sited in an inclined area as opposed to flat areas. Following this line of thought, we feel that marked elongation of the spines of *B. aetnensis* could also be an expression of phenotypic plasticity. Spine length has often been linked with the protection of leaves. According to Kariyat *et al.* (2017), spines may play a significant role in defence

Table 5. Between-population variability for leaf traits: blade length (A), blade width (B), blade length/width ratio (C), number of teeth on right side (D) and length of teeth (E). The presence of a letter indicates significance at p≤0.05. For abbreviations, see Table 1.

Popula.	Bc_VP	Bc_Me	Bc_Ov	Bc_Sa	Bc_Cr	Bc_Sn	Bc_Ka	Bc_Bl	Bc_Ca	Bc_Gr	Bv_RP	Bv_Sk	Bv_CL	Bv_Pr	Bv_Os	Bae_MV	Bae_Li	Bcre_K
Bc_Me	ABCDE																	
Bc_Ov	BCDE	ABCDE																
Bc_Sa	ABD	ABDE	C															
Bc_Cr	ACD	ABCE	ABD	ABCD														
Bc_Sn	CE	ABCDE	AB	ABC	D													
Bc_Ka	ABCDE	ABCDE	CD	CD	ABD	ABD												
Bc_Bl	ABCDE	ACE	ABCE	ABCE	ABDE	ABDE	ABDE											
Bc_Ca	DE	ABCDE	BCD	D	ABCD	ACD	BC	ABCDE										
Bc_Gr	D	ABD	BCE	ABE	ACDE	CE	ABCDE	ABCE	DE									
Bv_RP	ABCDE	ABCDE	ABCDE	ABCDE	ABDE	ABDE	ABD	ABD	ABCDE	ABCDE								
Bv_Sk	ABD	ABCDE	ABCD	ABCD	ABD	ABD	ABD	ABDE	ABD	ABD								
Bv_CL	ABCD	BCDE	ABD	ABCD	AB	ABD	ABDE	ABDE	ABCDE	ABCD	ABDE	ABD						
Bv_Pr	ACDE	BCE	ABD	ABC	D	ACD	ABCD	ABCE	ABCD	ABCE	ABCDE	ABCD	BCD					
Bv_Os	ABD	ABCDE	ABCD	ABCD	ABCD	ABD	ABDE	ABDE	ABDE	ABD	DE	D	ABCD	ABCDE				
Bae_MV	CDE	ABC	DE	ACE	E	DE	ABDE	ABE	CDE	CE	ABDE	ABDE	ABDE	ABE	BCDE			
Bae_Li	CD	ABC	BDE	ACDE	E	DE	ABDE	ABDE	ACDE	CD	ABDE	ABDE	ABE	ADE	BDE	A		
Bcre_K	ABCD	ABCDE	ABCDE	BCDE	ABCDE	ABDCE	BCD	ABCD	BCDE	ABCDE	ABCD	ABCDE	ABCDE	ABCDE	BCDE	ABCDE	ABCDE	
Bcre_O	ABCD	ABCD	ABCD	BCD	ABCD	ABCD	BCD	ABCD	ABCD	ABCD	ABCD	ABCD	ABCD	ABCD	BCD	ABCD	ABCD	

Table 6. Between-population variability for shoot traits: number of spines (A), spine length (B) and internode length (C). The presence of a letter indicates significance at p≤0.05. For abbreviations, see Table 1.

Popula.	Bc_VP	Bc_Me	Bc_Ov	Bc_Sa	Bc_Cr	Bc_Sn	Bc_Ka	Bc_Bl	Bc_Ca	Bc_Gr	Bv_RP	Bv_Sk	Bv_CL	Bv_Pr	Bv_Os	Bae_MV	Bae_Li	Bcre_K
Bc_Me	AB																	
Bc_Ov	AB																	
Bc_Sa	A	A	A															
Bc_Cr		C		A														
Bc_Sn				A														
Bc_Ka	B	B	B	AB	B	B												
Bc_Bl		A	AB	A	C		B											
Bc_Ca		A	A	A			B											
Bc_Gr	AB	C			AB	A	A	ABC	A									
Bv_RP	C	AC	AC	AC	C	C	BC	C	C	ABC	C							
Bv_Sk	BC	ABC	ABC	ABC	BC	BC	C	BC	BC	AC	BC							
Bv_CL	BC	AC	AC	AC	AC	C	AC	BC	C	AC	C							
Bv_Pr	BC	BC	ABC	ABC	BC	BC	C	BC	BC	ABC	BC	C	ABC					
Bv_Os	BC	BC	BC	ABC	BC	BC	C	BC	BC	AC	B	C	C	C				
Bae_MV	AB	AB	AB	BC	ABC	AB	AB	AB	AB	BC	ABC	ABC	ABC	ABC	ABC			
Bae_Li	AB	B	B	AB	BC	B	B	AB	AB	BC	ABC	ABC	ABC	ABC	BC			
Bcre_K	AB	B	B	B	AB	AB	A	AB	AB		ABC	AC	AC	AC	AC	B	B	
Bcre_O	AB	B	B	BB	AB	AB	A	AB	AB	B	ABC	AC	ABC	AC	AC	B	B	

against insect herbivores by restricting herbivore movement and increasing the time taken to access feeding sites. Moreover, Gowda & Raffaele (2004) reported that spines are significantly longer in three *Berberis* species re-sprouting after fire compared to before fire. The combination of longer spines after fire with no elongation of leaves in burned plants results in a significantly higher portion of leaves protected by the spines. Because both investigated *B. aetnensis* populations are growing on Mt. Etna, in a region frequently crossed by lava flows, it is possible that one of the traits that distinguished *B. aetnensis* from the other taxa, i.e., long spines, is merely an expression of adaptive phenotypic plasticity. As reported by Sodagar (2012), spines and margins of leaves are very changeable in *Berberis*.

The morphological analysis of leaves and shoots described here differentiated populations of *B. cretica* and *B. aetnensis*, but also *B. croatica* and *B. vulgaris* populations, even though to a lesser extent. The observed partial overlap of *B. croatica* and *B. vulgaris* populations and the grouping of Bv_Pr and Bc_Gr populations with *B. croatica* likely reflects environmental factors and site adaptation. There are two possible explanations for the case of the Bc_Gr population from Greece, which belongs to *B. vulgaris* according to published sources and to *B. croatica* according to morphological traits: either the distribution of *B. croatica* is broader than conventionally considered, or else it is possible that *B. vulgaris* shows pronounced adaptability to environmental conditions, as reflected in high phenotypic plasticity. Because of the suspected adaptive plasticity of the analysed *Berberis* taxa, morphological characters are probably not enough or could even be misleading for recognition of the given taxa without using appropriate molecular methods. In order to test the hypothesis presented here, further investigation should concentrate on cultivation of the analysed taxa under the same environmental conditions to assess how much of the observed variability is site-related and how much is species-specific.

CONCLUSIONS

Multivariate analyses of leaf and shoot traits successfully distinguished four investigated *Berberis* taxa from the Balkan Peninsula and Sicily. It clearly differentiated the *B. cretica* and *B. aetnensis* populations and, to a lesser degree, those of *B. croatica* and *B. vulgaris*. ANOVA revealed that two populations of *B. cretica* and two populations of *B. aetnensis* were similar within species limits, while all populations of both *B. croatica* and *B. vulgaris* varied to a greater or lesser extent. Similar site characteristics could be a factor influencing the observed uniformity of *B. aetnensis* and *B. cretica*. Equally, diverse phenotypic expression of the *B. vulgaris* and *B. croatica* populations under different environmental conditions could represent a manifestation of adaptive plasticity of these taxa in order to improve performance in a stressful environment. Definitive conclusions about taxonomic relationships between these taxa require further genetic analysis using appropriate molecular methods, but also cultivation of the researched taxa under the same environmental conditions.

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REZIME

Morfološka varijabilnost lista i izbojaka četiri taksona žutike (*Berberis* L.) sa Balkanskog poluostrva i Sicilije

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Istraživane su karakteristike lista i izdanaka četiri taksona roda žutika s područja Balkanskog poluostrva i Sicilije, *Berberis croatica*, *B. vulgaris*, *B. aetnensis* i *B. cretica*. Analiza je urađena na deset populacija *B. croatica*, pet populacija *B. vulgaris* te po dve populacije *B. aetnensis* i *B. cretica*. Populacije su nasumično odabrane unutar prirodnog područja rasprostranjenja istraživanih vrsta. Analizirano je osam karakteristika lista, tri svojstva izdanaka i odnos širine/dužine lista. Multivarijantna analiza (analiza glavnih komponenata, kanonička diskriminativna analiza i klaster analiza) ukazuje na razlike populacije vrsta *B. aetnensis* i *B. cretica* te, u manjoj meri, populacije vrsta *B. croatica* i *B. vulgaris.* ANOVA je pokazala unutarvrsnu homogenost populacija *B. aetnensis* i *B. cretica* dok su sve populacije *B. vulgaris* i *B. croatica* pokazale različite stepene unutarvrsne varijabilnosti. Nedostatak potpunog razdvajanja, obrazac grupisanja populacija i visoka unutarvrsna varijabilnost kod *B. vulgaris* i *B. croatica* mogli bi da odražavaju činjenicu da su uzorkovane populacije ovih dvaju taksona bile locirane na sredinski različitim mestima (za razliku od *B. aetnensis* i *B. cretica*) što je rezultovalo visokom fenotipskom plastičnošću populacija. Premda uočeni način morfološke varijacije podupire ideju o postojanju četiri taksona roda *Berberis* na području Balkanskog poluostrva i Sicilije, adaptivna fenotipska plastičnost je razlog zbog čega bi taksonomski status ovih entiteta trebalo dodatno potvrditi budućim molekularnim istraživanjima.

Ključne reči: Berberidacae, *Berberis aetnensis*, *Berberis cretica*, *Berberis croatica*, *Berberis vulgaris*, morfologija, morfometrija, multivarijantna analiza, taksonomija