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Morphometric studies of *Sideritis scardica* Grsb. and *S. syriaca* L. in their natural populations in Bulgaria

[Estudios morfométricos de *Sideritis scardica* Grsb. y *S. syriaca* L. en sus poblaciones naturales en Bulgaria]

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Abstract: The paper aimed at studying the morphometric variation in eight natural populations of two rare Sideritis species occurring in Bulgaria. Thirteen measured traits and four ratios were used to reveal the degree and distribution of variation. Most traits exhibited moderate to high variation and the most differentiating one was the length of the acumen. Cluster analysis and Principal component analysis revealed that the two taxa, *S. scardica* and *S. syriaca* are well distinguished but the population Chervenata stena, classified as *S. scardica* differed significantly from the remaining ones of the same species. Results of the morphometric study indicate the necessity of further studies for revealing the taxonomic relationships among the taxa.

Keywords: Medicinal plants; Phenotypic variation; Taxonomy; Section Empedoclia

Resumen: El manuscrito tenía como objetivo estudiar la variación morfométrica en ocho poblaciones naturales de dos especies raras de Sideritis que crecen en Bulgaria. Se utilizaron trece rasgos medidos y cuatro relaciones para revelar el grado y la distribución de la variación. La mayoría de los rasgos mostraron una variación de moderada a alta y la más diferenciadora fue la longitud del acumen. El análisis de conglomerados y el análisis de componentes principales revelaron que los dos taxas, *S. scardica* y *S. syriaca* están bien diferenciados, pero la población de Chervenata stena, clasificada como *S. scardica*, difería significativamente de las restantes de la misma especie. Los resultados del estudio morfométrico indican la necesidad de estudios adicionales para revelar las relaciones taxonómicas entre los taxones.

Palabras clave: Plantas medicinales; Variación fenotípica; Taxonomía; Sección Empedoclia

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INTRODUCTION

Genus *Sideritis* (Lamiaceae, Lamioideae) comprises more than 150 species distributed in the temperate and tropical area of the Northern hemisphere (Barber *et al.,* 2000; Barber *et al.,* 2002) and subdivided into two subgenera: *Sideritis* and *Marrubiastrum*. Southeastern Europe and Eastern Mediterranean with about 50 species represent a center of diversity with 45 species in Turkey (Huber-Morath, 1982), and about 10 species in Greece and in Balkans in general (Heywood, 1972; Koutsos & Chatzopoulou, 2009). Number of species depends on the taxonomic treatment and concepts, which are not straightforward, because of the high polymorphism, including ecotype diversity, and hybridization between species (Papanikolaou & Kokkini, 1982).

In Bulgaria *Sideritis* is represented by four species, two of them belonging to section *Hesiodia* Bentham (*S. montana* L. and *S. lanata* L.) and two – to section *Empedoclia* (Rafin.) Bentham (*S. scardica* Griseb. and *S. syriaca* L.) (Assenov, 1989). All but *S. montana* are rare species in Bulgaria and needs measures for conservation (Evstatieva, 2015a; Evstatieva, 2015b; Stoyanov, 2015). Moreover, *S. scardica* and *S. syriaca* are considered very important medicinal plants and are also subjected to cultivation (Aneva, 2016). While the two species of section *Hesiodia* are discrete and well distinguishable, there are still some taxonomic uncertainties with the species of section *Empedoclia* (Papanikolaou & Kokkini, 1982).

A typical characteristic of the *Sideritis* species of section *Empedoclia* is their high percentage of endemism (Huber-Morath, 1982; Aytaç & Aksoy, 2000). *S. scardica* is endemic for the Balkan Peninsula while *S. syriaca sensu lato* is believed to have wider distribution (Heywood, 1972). Both species occur naturally exclusively on limestone, although can be cultivated on a broader range of soil pH (Evstatieva, 2005).

S. syriaca has caused many taxonomic controversies, which is illustrated by its rich synonymy (Heywood, 1972). The author treated the species in broader sense, including the following taxa within it: *S. cretica* Boiss. non L., *S. raeseri* Boiss. et Heldr., *S. sicula* Ucria, and *S. taurica* Stephan ex Willd. (Heywood, 1972). Most of these taxa were considered separate species by other taxonomists before (Hayek, 1931; Yuzepchuk, 1954) and after (Huber-Morath, 1982; Papanikolaou & Kokkini, 1982; Baden, 1991).

In Bulgaria, Stojanov *et al*. (1967) treated the populations in Strandzha Mountains as belonging to *S. taurica*, while Assenov (1989), accepted the treatment in Flora Europaea (Heywood, 1972) and assigned the same populations to *S. syriaca*.

Evidently, in spite of the thorough taxonomic revision of Papanikolaou and Kokkini (1982), there are still some uncertainties concerning the taxonomic scheme of the Balkan representatives of *Siderits* section *Empedoclia*. This outlines the necessity of further studies both on the phenotypic and genetic variation of the taxa. The markers applied include both secondary metabolites (e.g. diterpenoids and flavonoids), which are considered good chemotaxonomic markers (Bojović *et al*., 2011; Ibraliu *et al.,* 2015; Stanoeva *et al.,* 2015), and DNA-based genetic markers (Barber *et al.,* 2000; Barber *et al.,* 2002; Çinar *et al.,* 2009; Ince *et al.,* 2009). Recently, Kalivas *et al*. (2014) applied successfully DNA barcode ITS2 as a marker for identification of species occurring in Greece. Stanoeva *et al.* (2015) analyzed polyphenolic profiles of four taxa of section *Empedoclia* on Balkans and revealed that there was considerable similarity between *S. scardica*, *S. raeseri* and Bulgarian *S. syriaca*. They further found that Bulgarian *S. syriaca* is very similar to Turkish *S. taurica* and suggest further verification of its taxonomic status.

Traditional morphometric methods are still valuable and particularly in combination with other modern methods, including with application of molecular markers. The two groups of methods could well complement each other and could produce reliable results that could help the proper taxonomic decisions. Recently, an extensive study of 19 *Sideritis* species was performed by using 26 morphological characters (Dülgeroğlu, 2017). The study showed that section *Empedoclia* can be regarded as an ancestral group within the subgenus *Sideritis* and that morphological characters can still be useful in phylogenetic and taxonomic studies.

Therefore, the objective of the resent study was to perform a morphometric analysis of phenotypic traits in representative natural populations of *Sideritis scardica* and *S. syriaca* in Bulgaria. The questions addressed were: i) what is the level of variation within and among the populations, and ii) how the grouping of populations reflects their taxonomic position. We consider this morphometric survey as a first stage of a large-scale study on the taxonomy of Sideritis in Bulgaria and on the Balkans.

Table N° 1
Populations studied and sample size

Population (abbreviation)	Mountain ridge	Altitude	Geographic coordinates	Sample size
Sideritis scardica				
Sveshtnik (SVE)	Pirin	1930	41°31' N 23°37' E	8
Orelek (ORE)	Pirin	1950	41°34' N 23°37' E	20
Mugla (MUG)	Rhodopes	1650	41°38' N 24°30' E	10
Trigrad (TRI)	Rhodopes	1350	41°36' N 24°22' E	35
Chervenata stena (CHE)	Rhodopes	1300	41°53' N 24°51' E	32
Slavyanka (SLA)	Slavyanka	2000	41°23' N 23°36' E	14
Sideritis syriaca				
Golemiya Valog (GVL)	Strandzha	500	41°59' N 27°29' E	15
Dokuzak (DOK)	Strandzha	400	42° 0'19" N 27°31'39.78" E	12

Table N° 2
Traits measured

Trait	**Abbreviation**
Length of the uppermost leaf[a)]	L1
Width of the uppermost leaf [a)]	W1
Length of the second upper leaf [a)]	L2
Width of the second upper leaf [a)]	W2
Length of the third upper leaf [a)]	L3
Width of the third upper leaf [a)]	W3
Length of bracts in the middle part of the inflorescence[b)]	BH
Width of bracts in the middle part of the inflorescence [b)]	BW
Length of the acumen of the bracts in the middle part of the inflorescence [b)]	ACU
Number of leaf pairs	NL
Length of the inflorescence	LI
Length of the lowermost bract	L4
Width of the lowermost bract	W4
Ratio L1:W1	R1
Ratio L2:W2	R2
Ratio L3:W3	R3
Ratio BH:BW	R4

[a)] taken as average of the two opposite leaves; [b)] measured on one well-developed and visibly the largest bract.

MATERIALS AND METHODS

Eight natural populations were included in the study, six of them belonging to *S. scardica* and two – to *S. syriaca*, according to Assenov (1989) (Table Nº 1). The populations selected cover the whole range of distribution of the two species in Bulgaria and are situated in four mountain ridges – Pirin, Slavyanka, Rhodopes (*S. scardica*) and Strandzha (*S. syriaca*). All populations grow on limestone, on generally shallow soils, but soil depth vary across the localities.

The sample size varied depending on the availability of individuals. There were some localities like Sveshtnik, where the species was represented by few individuals and sampling of many individuals could have affected negatively the population status. However, the attempt was to have at least 10

individuals per population. Representative voucher specimens were deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research (SOM, NoNo 172837, 172840-172846). Sampling was done after official permission by the National Service for Nature Conservation, affiliated with the Ministry of Environment and Waters of Bulgaria.

The traits measured and recorded are listed in Table Nº 2. Statistical processing of data included one-way ANOVA followed by post-hoc Tukey test for testing the significance of differences among means. Ratios were log-transformed before subjecting to ANOVA test because of violation of assumptions of the model. When commenting the results, non-transformed values were used.

Degree of variation was evaluated by the coefficient of variation (CV) following the approximate scale of Mamaev (1972). According to that scale the degree of variation is considered very low when CV is below 7%, low when CV is from 7 to 12%, moderate when CV is within the range 12-20% and high when CV is higher than 20%.

Inter-population differentiation was tested by revealing the grouping of populations using two approaches: Principal component analysis (PCA) and cluster analysis. PCA was done by using the software Clustvis (Metsalu and Vilo 2015). Cluster analysis was based on Euclidean distances between the population pairs. It was performed by UPGMA method, and dendrogram construction was done using the software DendroUPGMA (Garcia-Vallve *et al.*, 1999, genomes.urv.cat/UPGMA).

Table N° 3

Mean values of traits studied in the populations of *Sideritis scardica* and *Sideritis syriaca* (for abbreviations of populations and traits studied see tables 1 and 2)

Popu-lation	Traits studied								
	L1 [cm]	W1 [cm]	L2 [cm]	W2 [cm]	L3 [cm]	W3 [cm]	BH [cm]	BW [cm]	ACU [cm]
	Sideritis scardica								
SVE	2.79a[1)]	0.74bc	4.00ab	0.78b	4.10b	0.75b	1.88ab	0.84c	1.04a
ORE	2.71a	0.72c	3.55ab	0.68b	4.20b	0.72b	1.80ab	1.01bc	0.79ab
MUG	3.16a	0.81abc	4.03ab	0.73b	4.63ab	0.75b	2.09a	0.99bc	1.10a
TRI	3.13a	0.77abc	4.45a	0.77b	5.56a	0.86b	1.85ab	1.20a	0.66bc
CHE	2.17b	0.96a	2.93b	1.02a	4.34b	1.23a	1.59bc	1.10ab	0.49cd
SLA	3.27a	0.94ab	4.11a	0.80b	4.20b	0.79b	1.97a	1.12ab	0.85ab
CV[2)] range [%]	13-41	10-26	14-35	9-29	18-37	14-31	14-20	13-20	20-57
	Sideritis syriaca								
GVL	2.22b	0.75bc	2.81b	0.62b	3.67b	0.70b	1.33cd	1.05abc	0.28de
DOK	2.66ab	0.68c	3.49ab	0.70b	3.98b	0.74b	1.24d	1.09abc	0.15e
CV range [%]	14-25	16-18	10-20	16-17	4-23	18-20	12-14	11-14	57-68
Spec. diff.[2)]	0.040	0.011	0.009	<0.001	0.005	0.002	<0.001	0.501	<0.001

Table Nº 3 (continued)

Traits studied							
NL	LI [cm]	L4 [cm]	W4 [cm]	R1	R2	R3	R4
Sideritis scardica							
3.13c	2.91c	2.01abc	0.91c	3.76ab	4.87ab	5.17b	2.32a
3.55bc	5.08abc	2.03ab	1.14bc	3.82ab	5.25ab	5.78ab	1.81bc
3.71bc	2.97bc	2.51a	1.09bc	3.96ab	5.52ab	6.21ab	2.13ab
3.83bc	5.50ab	2.14a	1.39a	4.09a	5.76a	6.46a	1.56cde
5.13a	6.18a	1.75bcd	1.24ab	2.29c	2.90c	3.56c	1.45def
3.55bc	4.29abc	2.37a	1.37ab	3.48ab	5.14ab	5.32ab	1.76cd
19-26	24-54	12-28	8-29	13-40	10-26	12-23	11-26
Sideritis syriaca							
4.33ab	6.55a	1.39d	1.17abc	3.03bc	4.54b	5.27ab	1.28ef
4.89a	5.28abc	1.41cd	1.23abc	3.98ab	5.10ab	5.51ab	1.15f
17-21	27-48	4-15	18-24	29-44	14-24	15	12-15
0.021	0.065	<0.001	0.371	0.876	0.849	0.755	<0.001

[1] Population means within a column followed by the same letter are not significantly different at p≤0.05, as tested by one-way ANOVA and post-hoc Tukey test.

[2] CV – coefficient of variation

[3] Differences between the species: p-values, based on one-way ANOVA

RESULTS

Variation of traits studied

ANOVA test showed that the effect of population origin on the variation was highly significant for all traits (p<0.001; full results not shown).

The length of the uppermost leaf (L1) possesses highest the value in the population of Slavyanka – 3.27 cm, followed by Mugla and Trigrad - 3.16 and 3.13 cm, respectively (Table Nº 3). Chervenata stena has the lowest value of all populations – 2.17 cm. It is the only population differing significantly from the others within *S. scardica*. The populations of *S. syriaca* have on average shorter first leaf but the significance is weak (p=0.04). The variation of this trait is in most cases moderate to high, as indicated by the coefficient of variation (CV) (11 to 52%).

The width of the first leaf (W1) showed the highest mean value in Chervenata stena (0.96) and the lowest – in Dokuzak (*S. syriaca*). However, the differences are significant only among the highest and the lowest values. There is overlapping between the two taxa, but generally the difference is significant. The variation is lower - coefficient of variation (CV) is in most cases between 10 and 20%.

Length of the second leaf was highly variable – from 4.45 cm (Trigrad, S. scardica) to 2.81 (Golemiya Valog, *S. syriaca*). Even though there was overlapping of the range of this trait between the two taxa, generally the difference was significant (Table Nº 3). The width of the same leaf (W2) was the largest in Chervenata stena (1.02 cm), the other values being within the range 0.6-0.8. The trait had lowest values in *S. syriaca*. Coefficient of variation was lower than 20% indicating low to moderate degree of variation.

The third leaf (L3) had the highest mean value in the population of Trigrad (5.56 cm), and the lowest – 4.2 cm (Orelek and Slavyanka). *S. syriaca* had shorter third leaf (Table Nº 3). Degree of variation was moderate to high. The width of the same leaf had highest mean value in Chervenata stena (1.23 cm) while in the other populations (including *S. syriaca*) varied roughly between 0.7 and 0.8. Differences between means of the two taxa were significant in spite of overlapping the range of variation.

Mean values of the ratios length/width of the three leaf pairs (R1, R2 and R3) allowed two main inferences: first, that there are no significant

differences between the two taxa *S. scardica* and *S. syriaca*, and second, that the leaf shape of plants from the population Chervenata stena is significantly different from that of the remaining populations and requires further studies.

In the present study middle bract length (BH) varied from 2.09 cm (Mugla) to 1.59 cm (Chervenata stena) in *S. scardica* and in *S. syriaca* it was significantly shorter. The variation was low to moderate, as determined by CV values up to 20%. Middle bract mean width (BW) did not differ significantly between the two taxa and varied from 0.84 to 1.2 (Table Nº 3).

Another very important diagnostic trait is the acumen length. In *S. scardica* it varied from 1.1 (Mugla) to 0.49 cm (Chervenata stena), and in *S. syriaca* it was significantly shorter (average 0.22 cm). Mean number of leaf pairs varied broadly and was slightly higher in *S. syriaca* (mean 4.6, p=0.021), while in *S. scardica* it was within the range 3.13 (Sveshtnik) - 5.13 (Chervenata stena). The latter population differed significantly from the remaining ones.

Mean length of inflorescence was slightly higher in *S. syriaca*, but the differences were not statistically significant (Table Nº 3). In *S. scardica* this trait varied from 6.18 (Chervenata stena) to 2.91 cm (Sveshtnik). The degree of variation was very high for both taxa.

The last two directly measured traits, length (L4) and width (W4) of the lowermost bract did not show some particular trends of variation. L4 was significantly different in the two taxa (shorter in *S. syriaca*), while W4 was not.

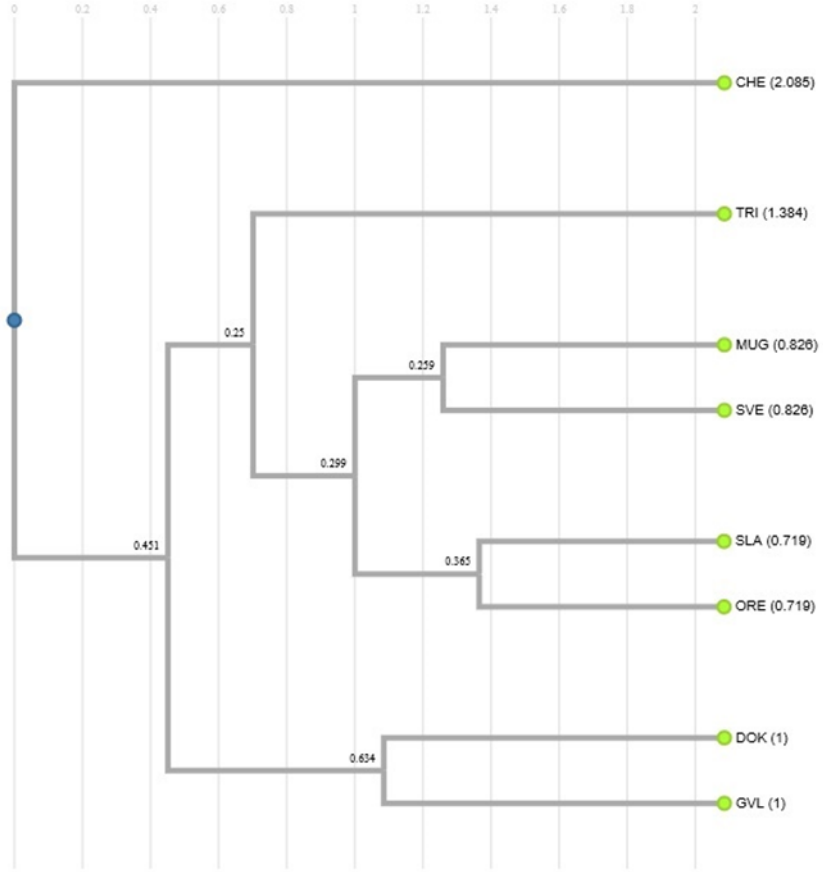

Figure N° 1
Cluster dendrogram expressing the grouping of populations based on the Euclidean distances.

Grouping of populations

The cluster analysis (Figure Nº 1) revealed that the populations studied form three distinguishable groups - the first one contains all populations of *S. scardica* but Chervenata stena, which forms the second group and the third group is formed by the two populations of *S. syriaca*.

Principal component analysis confirmed the grouping achieved by cluster analysis. The projection on a coordinate system of the first two principal axes explained 72% of the total variation (Figure Nº 2). It can be seen that the population Chervenata stena is again rather distant both from *S. scardica* and *S. syriaca*.

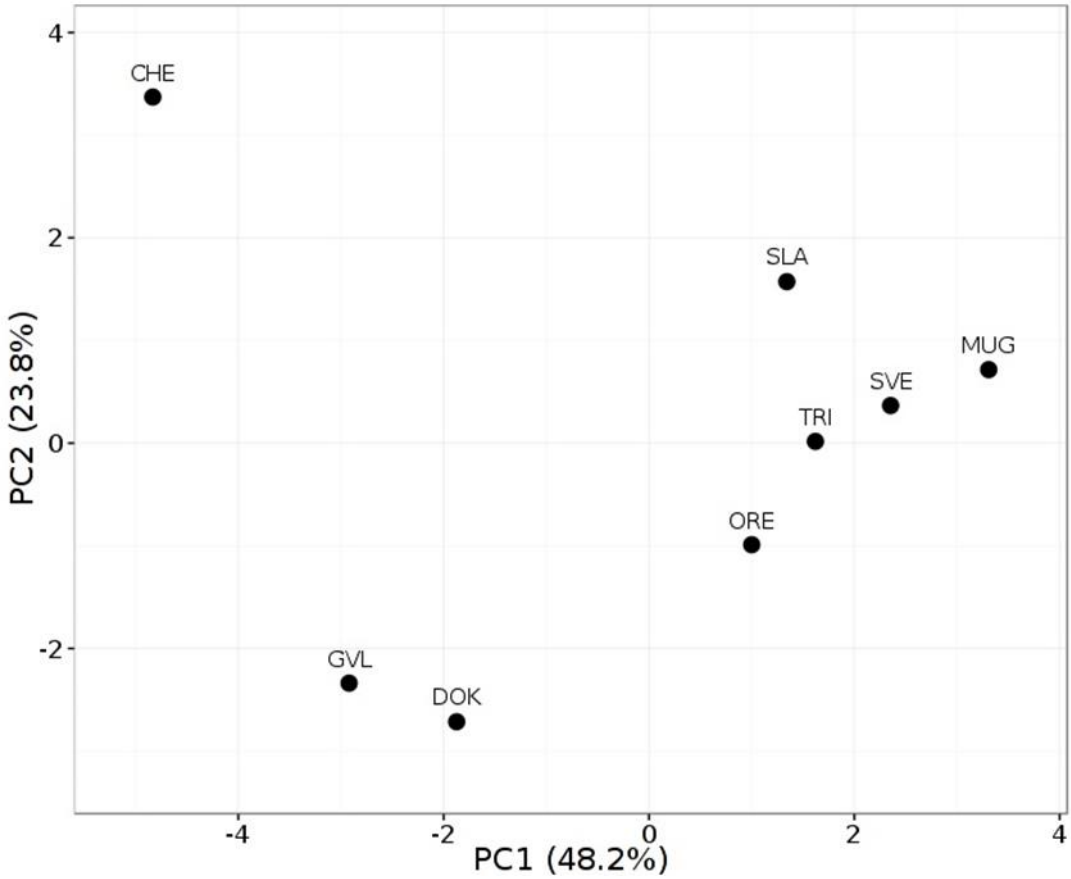

Figure N° 2
Principal Component Analysis (PCA) based on the morphometric traits.

DISCUSSION

Variation

The many studies on variation of different *Sideritis* species were focused in most cases to their taxonomic position (e.g., Dülgeroğlu, 2017; Erdem *et al.*, 2017). There is scarce information about the degree of variation, because most studies usually reported the mean values and range (min.-max.). Bilgin (2013) reported similar or higher degree of variation as determined by the coefficient of variation. In his study on *S. sipylea* Boiss. Coefficients of variation ranged from 25 to 86% for the different traits studied. However, even the information without proper statistical treatment could be useful for comparison with some taxa belonging to the same sections. Most relevant information is the one provided by Papanikolaou & Kokkini (1982), even though it concerns the species (in their treatment) as a whole. The authors reported values of upper and middle leaf size 30-60 x 6-8 mm, which concord with the results of the present study. The comparison only of mean vales is misleading because such a comparison masks

the variation found in the study. The variation in Bulgarian populations was very high, as illustrated by the range of the coefficients of variations (CVs) of different traits. In most cases the highest values of CVs were above 20%.

It is difficult to put the results of the present study into the context of other broader taxonomic analyses, because they concern different species and the characters used are not the same. However, there were successful applications of morphological and morphometric traits in solving or at least helping the solutions of taxonomic problems. Şahin *et al.* (2005) studied two species of section Empedoclia in Turkey - *S. bilgerana* P.H. Davis and *S. hispida* P.H.Davis. The same research group (Şahin et al. 2008) performed similar study *S. cilicica* Boiss. & Bal. and *S. niveotomentosa* Hub.-Mor. from Southern Anatolia. The important traits in distinguishing the species of section *Empedoclia* were usually the size of bracts and to a lesser extent - of leaves. Of course, direct comparison with the present study is not possible.

Papanikolaou & Kokkini (1982) reported leaf length of *S. syriaca* varying in wide range - 10-50 mm x 3-10 mm. Mean leaf sizes in Bulgarian populations fall within this range, but revealing relatively high variation. Inflorescences of Bulgarian populations of *S. syriaca* are somewhat shorter than these reported for Greek populations, while bracts are slightly wider (1-1.1 cm, Table 3).

Usually the shape and size of middle bracts are considered important diagnostic traits with taxonomic importance. Papanikolaou & Kokkini (1982) postulated that the middle bracts in *S. scardica* exceed the flowers, while in *S. syriaca* they are shorter or equal. They put the range of variation of middle bracts of *S. scardica* 16-23 x 14-19 mm. Acumen length is another important character used to distinguish the closely related species. Papanikolaou & Kokkini (1982) reported values 4-7 (10) mm for *S. scardica* and 2-4 mm for *S. syriaca*, which concords well with the results of the present study. The authors subdivided *S. scardica* into two subspecies: subsp. *scardica* and subsp. *longibracteata* and provided a diagnosis of the later subspecies, which is characterized, among others, with longer inflorescence.

The longest inflorescences in the set of populations classified as *S. scardica* was found in Chervenata stena (6.18 cm). However, it cannot be classified to subsp. *longibracteata*, because of the white lanate pubescent leaves and bracts, quite the opposite to subsp. *longibracteata*, as described in Papanikolaou & Kokkini (1982).

Taxonomic implications

Even though the molecular methods are becoming rapidly the most important part of taxonomic research, the variation of morphological characters is still very important, and in some particular cases, a decisive criterion. However, the two approach should complement each other and here we present only results based on morphometry.

The variation of the studied morphometric characters in Bulgarian populations of *S. scardica* and *S. syriaca* was generally consistent with the values reported for the two species in other studies. The most important conclusion was that one population of *S. scardica* - Chervenata stena - showed particular patterns of variation. This population differed from all other populations in several respects, which is expressed by its position in the cluster dendrogram and in PCA diagram (Figures 1 and 2). There were pronounced differences between this population and the remaining ones in many other characters.

One problem in the taxonomic revisions arises because of the fact that sometimes the specimens in herbarium collections do not cover the whole range of variation. Therefore, studying of individuals from natural populations could be more sound. Unfortunately, this is not always possible when making the taxonomic revisions and in most cases the researcher relies on incomplete set of herbarium specimens.

We believe the present study covers the substantial part of the morphological variation within the studied taxa. Still there are several problems to be solved, the most important being the taxonomic status and position of the plants from Chervenata stena.

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