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# A comparative anatomical study on two closely related *Astragalus* L. taxa (Fabaceae) from the central part of the Balkan Peninsula

*Original Article*

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*Abstract:*

The present study involves species *A. monspessulanus* L. and *A. spruneri* Boiss. section *Incani* DC. from the area of central Balkans. Due to high polymorphism, this section is taxonomically the most problematic group and delimitations of its species are not completely clear. The main aims of the study were to determine the micro-morphological and anatomical variability of populations of these species based on quantitative characters of leaflet and leaf petiole, and also to show the potential degree of differentiation within species. Additional goal was to determine anatomic parameters that may be used in taxonomy of studied species and their encompassing section. The results indicate high variability of analyzed characters and anatomical differentiation of populations of each species. Results of statistical analyses indicate that characters which refer to petiole anatomy have higher taxonomic value than characters which refer to leaflet anatomy. Anatomical features described in this paper mostly agree with characters found in previous studies of other *Astragalus* species, and they have potential taxonomic significance.

*Key words:*
*Astragalus monspessulanus*, *A. spruneri*, anatomical characters, taxonomy

*Apstract:*

**Uporedna anatomska istraživanja dva veoma srodna *Astragalus* L. taksona sa centralnog dela Balkanskog poluostrva**

U ovom radu su analizirane vrste *A. monspessulanus* L. i *A. spruneri* Boiss., sekcije *Incani* DC. sa područja centralnog Balkana. Sekcija *Incani* DC. predstavlja taksonomski složenu sekciju, sa velikim brojem endemičnih vrsta, u okviru koje, zbog preklapanja morfoloških karaktera i velike fenotipske plastičnosti, odnosi između vrsta još uvek nisu u potpunosti razjašnjeni. Glavni cilj rada bio je utvrđivanje mikro-morfološke i anatomske varijabilnosti populacija ovih vrsta na osnovu kvantitativnih karakteristika lamine i lisne drške, kao i prikaz potencijalnog stepena diferencijacije unutar vrsta. Cilj je bio i otkriti anatomske karaktere koji se mogu koristiti u taksonomiji proučavanih vrsta, kao i sekcije kojoj one pripadaju. Rezultati ukazuju na visoku varijabilnost analiziranih karaktera i anatomsku diferencijaciju populacija svake vrste. Rezultati statističkih analiza takođe pokazuju da karakteristike koji se odnose na anatomiju lisne drške imaju veću taksonomsku vrednost od karakteristika koji se odnose na anatomiju lamine. Anatomske karakteristike opisane u ovom radu uglavnom se slažu sa karakteristikama opisanim u prethodnim istraživanjima drugih vrsta roda *Astragalus* i imaju potencijalni taksonomski značaj.

*Ključne reči:*
*Astragalus monspessulanus*, *A. spruneri,* anatomska diferencijacija, taxonomy

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## Introduction

The genus *Astragalus* L. is one of the largest genera of vascular plants, with 2500-3000 species (Podlech, 1986; Maassoumi, 1998; Ranjbar & Karamian, 2002). This genus is widespread, mostly in the Northern Hemisphere and in South America, with the diversity centers in arid and semiarid mountainous areas. The place of the greatest diversity and evolutionary differentiation is in Southwestern Asia (Maassoumi & Ranjbar, 1998). The region of Southern Europe, including Balkan Peninsula, contains a particularly large number of *Astragalus* species (60). This is one of the taxonomically most interesting polymorphic genera of the Balkan flora. There are 17 species in

**Table 1.** Species analysed, with voucher numbers, and collection sites and dates

| Taxon | Collection site | Collection date | Voucher number |
|---|---|---|---|
| *Astragalus monspessulanus* | Serbia: village Petačinci | 11-May 2012 | HMN-7311 |
| *Astragalus monspessulanus* | Serbia: *G*orge of the river Jerma, village Vlasi | 11-May 2012 | HMN-7312 |
| *Astragalus monspessulanus* | Montenegro: Morača River Canyon, Bioče | 20-May 2012 | HMN-7306 |
| *Astragalus monspessulanus* | Bulgaria: Kyustendil, village Staro selo | 21-April 2012 | HMN-7314 |
| *Astragalus spruneri* | *Macedonia:* Matka Canyon, Monastery „Matka“ | 15-April 2012 | HMN-7313 |
| *Astragalus spruneri* | *Macedonia:* Mariovo, village Zović | 14-April 2012 | HMN-7309 |

flora of Serbia according to Diklić (1972). Additional floristic research also provided evidence of species *A. exscapus* L. (Bože, 1979), so with addition of the recently recorded species *Astragalus wilmottianus* the genus *Astragalus* is presently represented in Serbia with 19 species (Ranđelović et al., 2002). Many of which are narrow-range endemics.

In spite of numerous studies, genus *Astragalus* is still characterized by insufficiently resolved taxonomic issues. The goal of the previous studies was to evaluate interspecies relationships within the genus *Astragalus,* including both taxonomy and phylogeny and using morphological, phytogeographical, molecular systematics, palynological and karyological characters (Podlech, 1986; Sharawy et al., 2003; Osaloo, 2003; Taeb et al., 2007; Mourad & Sharawy, 2010; Meher et al., 2012; El-Sahhar et al., 2013; Amini et al., 2018). This genus shows high morphological variability and complexity. Certain characters, including characteristics of trichomes and leaves, were used in subgeneric classification of genus *Astragalus*, introducing subgenera (Podlech, 1982; Zarre, 2000; Zarre, 2003; Meher et al., 2012). There is high significance of the overall shape of hairs and detailed micromorphological studies on hairs. Zarre (2003) has shown that several characters of hairs may be used for phylogeny reconstruction in *Astragalus*. The anatomy of vegetative organs of *Astragalus* species has not been studied extensively. On the other hand, the anatomical parameters have been shown to greatly contribute to solving of significant taxonomic issues within various taxonomic groups (Zarre et al., 2010; Jušković et al., 2016, 2017; Raca et al., 2017; Stojanović et al., 2019).

In the present paper, micro-morphological and anatomical variability of populations *A. monspessulanus* L. and *A. spruneri* Boiss. were analyzed according to quantitative characters of leaflet and leaf petiole. Intraspecies variability plays a key role in both long-term and short-term responses of species toward variations in environmental factors. Species *A. monspessulanus* and *A. spruneri* belongs to the section *Incani* DC. This is one of the most species-rich sections of *Astragalus,* with 140 species. Due to extensive overlaps in morphological characters and high phenotype plasticity, section *Incani* DC. represents a taxonomically interesting group. The selection of studied anatomical characters was based on previous research on *Astragalus* taxa by Haddad & Barnett, 1989; Zarre, 2003; Pirani et al., 2006. The analyzed characters were sampled in three populations each of *A. monspessulanus* and *A. spruneri* from the central part of the Balkan Peninsula. The goal was to determine anatomic characters that may be used in determining taxonomy of the studied species as well as the section to which they belong.

## Materials and Methods

The specimens of *A. monspessulanus* L. and *A. spruneri* Boiss. were collected during the flowering season, from six native populations from the Balkan peninsula. Voucher specimens were deposited in the Herbarium Moesiacum Niš, Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Serbia (HMN) (**Tab. 1**). The collected plant material was either placed in the herbarium or fixed in 50% ethanol. The anatomical analysis of leaflet and leaf petiole was performed on permanent and temporary slides prepared by the standard histological method for light microscopy (Ruzin, 1999). Manual

microtome (Gligorijević & Pejčinović, 1983) was utilized in order to make cross-sections. The cross sections of the petiole were taken in the middle, between the stem an lamina, following the observation by Howard (1979). 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 morphoanatomic measurements were performed on the microscope Leica DM 2500-Leica DFC490-Leica Qwin Standard (Leica Microsystem, Germany).

In the present study, statistical analyses were carried out for 31 quantitative characters related to the leaflet and leaves petiole anatomy. Statistical significance of differences between the species in regard to the analyzed characters was assessed by t-test for independent groups. The degree of variability and morpho-anatomical differentiation on level of population and species were established using principal component analysis (PCA), canonical discriminant analysis (CDA) and agglomerative hierarchical classification (Single Linkage method). These analyses were performed on the three data sets. One of them was included all characters analysed (leaflet and petiole anatomy), while the other two data sets included only the characters related to leaflet anatomy or petiole anatomy, respectively. All statistical analyses were done in statistical package STATISTICA 8.0 (StatSoft, 2007).

## Results and discussion

### *Leaflet anatomy of A. monspessulanus*

The leaflets from all populations in species *A. monspessulanus* have shown similar anatomical patterns. Internal leaflet tissues are organized in

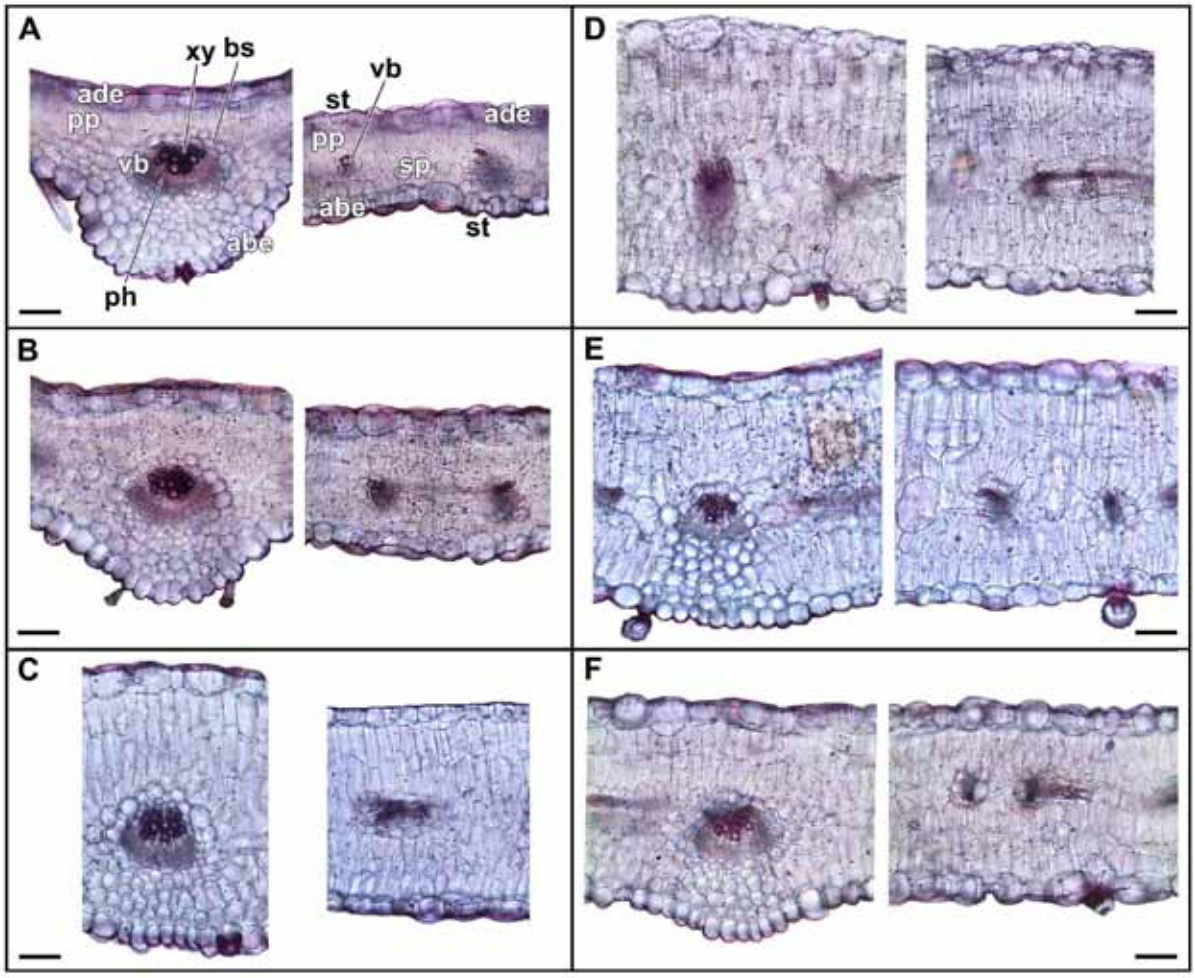


**Fig. 1.** Cross sections of the leaflet midrib (left) and lamina (right) in investigated *Astragalus* species. **A** – *A. monspessulanus* (Vlasi), **B** – *A. monspessulanus* (Petačinci), **C** – *A. monspessulanus* (Bioče), **D** – *A. spruneri* (Monastery „Matka"), **E** – *A. spruneri* (Staro selo), **F** – *A. spruneri* (Zović). Scale bars = 50 μm. Abbreviations: **ade** – adaxial epidermis, **abe** – abaxial epidermis, **ph** – phloem, **pp** – palisade parenchyma, **sp** – spongy parenchyma, **st** – stomata, **vb** – vascular bundle, **vs** – vascular sheath, **xy** – xylem.

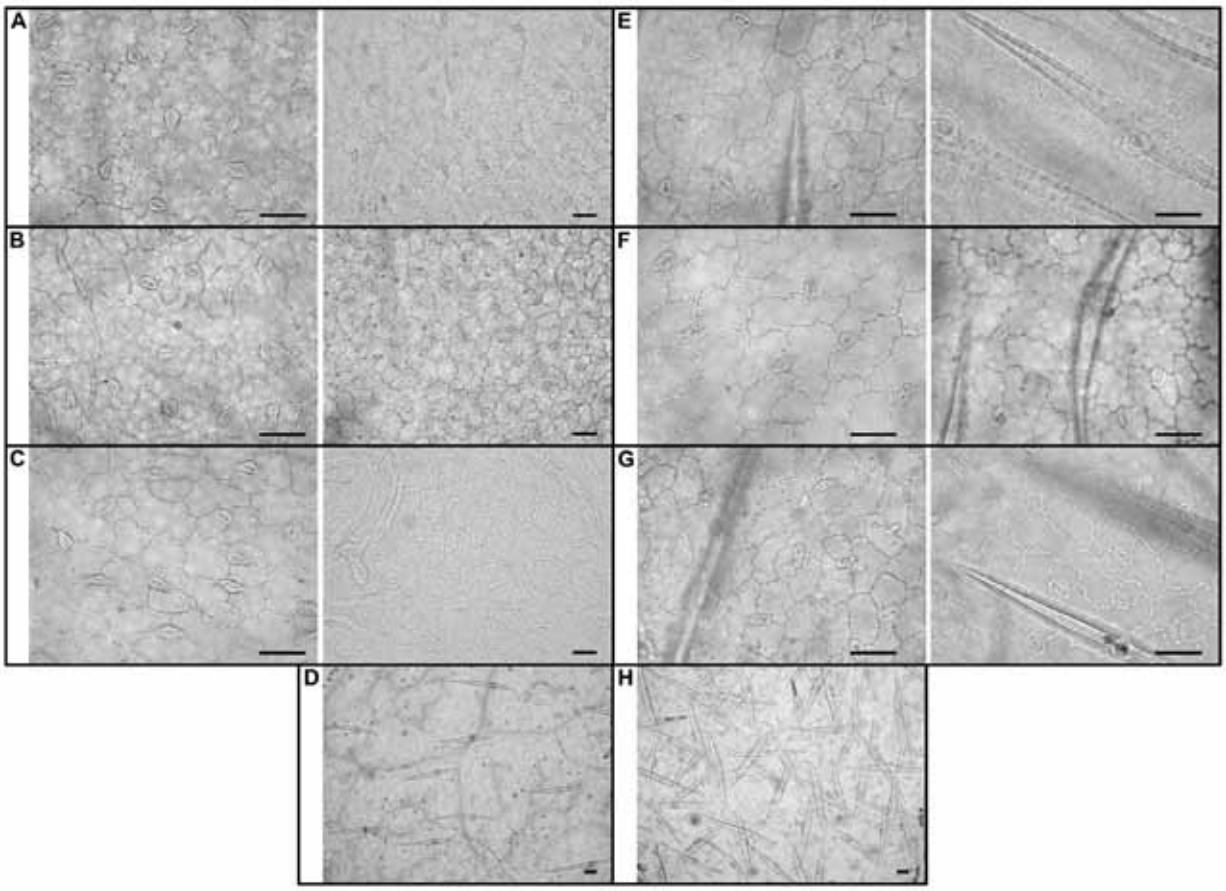


**Fig. 2.** Comparative overview of adaxial (left) and abaxial (right) epidermis (A-C; E-G) and trichomes on abaxial epidermis (D, H) in investigated *Astragalus* species. **A, D** – *A. monspessulanus* (Vlasi), **B** – *A. monspessulanus* (Petačinci), **C** – *A. monspessulanus* Bioče, **E, H** - *A. spruneri* (Monastery „Matka"), **F** - *A. spruneri* (Staro selo), **G** – *A. spruneri* (Zović). Scale bars = 50 μm.

an isolateral structure (**Fig. 1**). The leaflets are densely hairy, with bifurcate hairs with two equal or unequal arms, usually opposed in the same plane, tip pointed; arms with verrucose surface are observable particularly on the major vein surface (**Fig. 2**). The presence of bifurcate hairs is a common character in the *Astragalus*. Number of hairs at the abaxial side of the leaflet is 0-12 and number on the adaxial side is 0-11.

Two epidermal layers develop on adaxial and abaxial surfaces of the leaflet. The cells of adaxial epidermis are visibly larger compared to the cells of abaxial epidermis. The outer walls are cutinized. Cuticle is thin on both sides of leaf: 1.3-5.6 μm (**Fig. 1**).

The epidermal cells of the leaflet are polygonal or irregular in shape. The outline of the anticlinal walls has been described as straight, sinuous or sinuate (**Fig. 2**). The stomata apparatus in *Astragalus* species is present at both surfaces of leaflet, so it is called an amphistomatic leaf. The stomata are tiny, at the same level or slightly above the level of the other epidermal cells. Stomata frequency varies, ranging from 57 to 181 per $mm^2$ at the abaxial side and 0 to 161 per $mm^2$ at the adaxial side. The greatest number of stomata (86-181 per $mm^2$ at the abaxial and 0-161 per $mm^2$ at the adaxial side) was recorded in plants from the village Vlasi, while the smallest (57-161 per $mm^2$ at the abaxial side and 60 – 128 per $mm^2$ at the adaxial side) was observed in plants from Bioče in Montenegro (**Tab. 2**). The dominant stomatal type in *Astragalus* is anisocytic (**Fig. 2**).

The average recorded thickness of leaflet ranged from 255 μm (population at Bioče and village Vlasi) to 276 μm (Petačinci population), with minimum recorded values of 178 μm and maximum recorded values of 329 μm (**Tab. 2**). Internal structure of leaflet does not differ significantly among the studied populations and species. The palisade parenchyma is 2-3 layered with cells arranged parallel to each other, where one layer is better developed. The spongy tissue is composed of 2-3 layers of chlorenchymatous cells with small intercellular spaces. There is a layer of atypical palisade tissue at the abaxial side in the

**Table 2**. Comparison of morphometric characteristics (mean ± SD and range) for *A. monsspesulanus* and *A. spruneri*

| Characteristics | Species | | t-test p value |
|---|---|---|---|
| | *A. monsspesulanus* mean±SD | *A. sprunerii* mean ±SD | |
| Leaf thickness (µm) | 249.026±33.263 | 264.208±38.386 | **0.005** |
| Palisade tissue thickness (µm) | 105.563±20.982 | 113.989±21.562 | **0.009** |
| Spongy tissue thickness (µm) | 72.518±12.567 | 84.512±17.181 | **0.000** |
| Largest thickness of the leaf blade (µm) | 328.854±31.797 | 325.899±41.043 | 0.590 |
| Height of adaxial epidermal cells (µm) | 38.647±7.729 | 35.984±5.643 | **0.009** |
| Height of abaxial epidermal cells (µm) | 35.894±6.347 | 34.354±4.939 | 0.071 |
| Cuticle thickness on the leaf adaxial epidermis (µm) | 2.649±0.703 | 2.489±0.306 | **0.049** |
| Cuticle thickness on the leaf abaxial epidermis (µm) | 2.475±0.7097 | 2.458±0.294 | 0.826 |
| Petiole surface area (µm²) | 829225.244±184868.229 | 756978.071±229110.570 | **0.021** |
| Petiole central cylinder surface area (µm²) | 484291.184±139401.760 | 404279.434±141626.392 | **0.000** |
| Petiole epidermis thickness (µm) | 39.304±4.727 | 35.997±3.944 | **0.000** |
| Petiole cortex thickness (µm) | 122.697±18.476 | 131.099±17.211 | **0.002** |
| Surface xylem area of the central vasculare bundle petiole (µm²) | 37483.420±9592.423 | 21245.137±8586.570 | **0.000** |
| Surface phloem area of the central vasculare bundle petiole (µm²) | 26875.076±7015.183 | 15728.974±6315.581 | **0.000** |
| Surface sclerenchym area of the central vasculare bundle of the petiole (µm²) | 33323.145±9757.989 | 15173.440±5963.925 | **0.000** |
| Surface xylem area of the left vasculare bundle petiole (µm²) | 9268.319±3833.309 | 6285.631±2333.143 | **0.000** |
| Surface phloem area of the left vasculare bundle petiole (µm²) | 10338.462±4893.622 | 5889.551±2500.453 | **0.000** |
| Surface sclerenchym area of the left vasculare bundle of the petiole (µm²) | 12358.751±4661.728 | 5589.495±2753.462 | **0.000** |
| Surface xylem area of the right vasculare bundle petiole (µm²) | 8630.900±3309.513 | 6230.853±2189.006 | **0.000** |
| Surface phloem area of the right vasculare bundle petiole (µm²) | 9294.905±2778.404 | 5772.199±2063.511 | **0.000** |
| Surface sclerenchym area of the right vasculare bundle of the petiole (µm²) | 11416.189±4396.266 | 5320.995±2563.347 | **0.000** |
| Number of adaxial stomata (/mm²) | 118.184±37.340 | 117.966±31.301 | 0.966 |
| Number of abaxial stomata (/mm²) | 105.274±27.850 | 106.452±21.090 | 0.749 |
| Number of adaxial hair (/mm²) | 3.354±2.619 | 5.110±2.585 | **0.000** |
| Number of abaxial hair (/mm²) | 3.302±2.447 | 5.257±2.640 | **0.000** |
| Length of adaxial stomata (µm) | 27.026±3.352 | 26.184±3.529 | 0.103 |
| Width of adaxial stomata (µm) | 25.161±3.088 | 24.589±2.391 | 0.166 |
| Surface area of adaxial stomata (µm²) | 538.831±122.565 | 510.127±113.029 | 0.104 |
| Length of abaxial stomata (µm) | 27.614±3.633 | 25.787±3.199 | **0.000** |
| Width of abaxial stomata (µm) | 25.418±3.265 | 24.250±2.293 | **0.006** |
| Surface area of abaxial stomata (µm²) | 557.974±134.615 | 494.227±100.144 | **0.000** |

mesophyll. Individual cells or a group of cells match palisade parenchyma in shape and arrangement. Intercellular spaces are present between cells in this part of mesophyll. The thickness of palisade tissue is 68-158 μm while thickness of spongy tissue is 48-118 μm. Differences related to dimensions of palisade and spongy tissues correspond to differences in the total thickness of leaflets. Mesophyll cells become rounded at median ribs (**Fig. 1**). Vascular bundles are abundant and surrounded by large cells of parenchyma. Mechanical tissue is poorly represented.

### *Leaflet anatomy of A. spruneri*

The internal tissue in leaflets of species *A. spruneri* is organized in the same way as in the leaflets of *A. monspessulanus* (**Fig. 1**). Densely packed T-shaped hairs with equal, tip-pointed arms are present at both adaxial and abaxial sides of the leaflet, and they are particularly densely distributed at the main vein (**Fig. 2**). Differences in indumentum in comparison to species *A. monspessulanus* include density and distribution of trichomes. Number of hairs varies in range of 1.7-12.4 at the abaxial side and 1.44-11.02 at the adaxial side.

Epidermis has single layers both on abaxial and adaxial sides, with thin cell walls and cuticle. Cuticle on both sides is less developed and thinner (2-3.2 μm) than in populations of species *A. monspessulanus.* The outer epidermal cells are larger than the inner ones (**Fig. 1**).

The epidermal cells of leaflets are polygonal or irregular in shape. The outline of the anticlinal walls was described as straight, sinuous or sinuate (**Fig. 2**). Stomata are present both on abaxial and adaxial side (amphistomatic leaves). Stomata frequency varies: 57-243 per $mm^2$ at the abaxial side and 60-161 per $mm^2$ at the adaxial side. The greatest number of stomata (86-181 per $mm^2$ at the abaxial side and 90-161 per $mm^2$ at the adaxial side) was recorded in plants from the Matka Canyon, while the lowest number, 57-161 per $mm^2$ at the abaxial side and 60 – 132 per $mm^2$ at the adaxial side) was observed in plants from Mariovo – Zović (**Tab. 2**). Stomata are small and similar in size on both sides of epidermis. They are of anisocytic type.

The mean thickness of the leaflets was 237 at Kyustendil - Old Village, 240 μm at Mariovo-Zović and 276 μm at Matka Canyon, with minimum at 187 μm and maximum at 336 μm (**Tab. 2**). Palisade tissue is usually composed of 1-2 layers of well-developed cells, and the spongy tissue has 2-3 cell layers. The thickness of the palisade tissue was 71-159 μm and thickness of spongy tissue was 46-110 μm. Lateral vascular bundles are present alongside the entire leaf. They are situated in the mesophyll, between spongy and palisade tissue (**Fig. 1**).

### *Comparation between A. monspessulanus and A. spruneri*

According to the results of t-test (**Tab. 2**), there is a significant statistical difference between *A. monspessulanus* and *A. spruneri* ($p<0.05$) in terms of the following leaf anatomy characters: leaf thickness, palisade tissue thickness, spongy tissue thickness, height of adaxial epidermal cells, thickness of cuticle at the adaxial side of the leaf, number of trichomes on abaxial and adaxial sides, length, width and surface area of stomata at the abaxial side of the leaf (**Tab. 2**). Although the results of t-test show statistically significant differences in 10 out of 18 characters related to leaf anatomy, results of PCA and CDA do not show clear separation of individuals of these two species on ordination diagrams (**Fig. 4**, **Fig. 5**). Separation of individuals along the first PCA ordination axis (surface area of stomata at the abaxial side of the leaf) and second PCA ordination axis (leaf thickness) is statistically significantly contributed to only by a single character (stated in brackets). Results of CDA and classification analysis (**Fig. 6**) indicate presence of clear differentiation in population of *A. monspessulanus* sampled in the canyon of Morača valley in comparison to all other studied populations. The greatest recorded degree of similarity in leaflet anatomy was present between populations of *A. spruneri* sampled in North Macedonia (Matka Canyon) and Bulgaria (Kyustendil) and between populations of species *A. monspessulanus* sampled in Serbia.

The anatomical results of this study are generally closely matching the referenced results for other species of genus *Astragalus* (Boughalleb et al., 2014). Leaflets of species *A. monspessulanus* and *A. spruneri* are characterized by certain xeromorphic characteristics – relatively tiny and soft leaves with hairs and tiny stomata present both on adaxial and abaxial sides of the leaf, well-developed palisade tissue both on adaxial and abaxial sides of the leaf, and a high number of vascular bundles. The results of statistical analyses are generally indicating low utilitarian value of characters related to leaf anatomy for taxonomy purposes. According to literature sources, micromorphology of hairs is an important taxonomic character for genus *Astragalus* and used in their systematics (Taeb et al., 2007; Zarre, 2000, 2003; Zarre & Podlech, 1996, 2001a,b,c; Pirani et al., 2006). Ghahremani-Nejad (2004) examined value of trichome characteristics for separation of bifurcating-hair genus *Astragalus* at the sectional level. The results point to a small range of hair character variation within the section *Incani* DC. According to that author, in order to illuminate

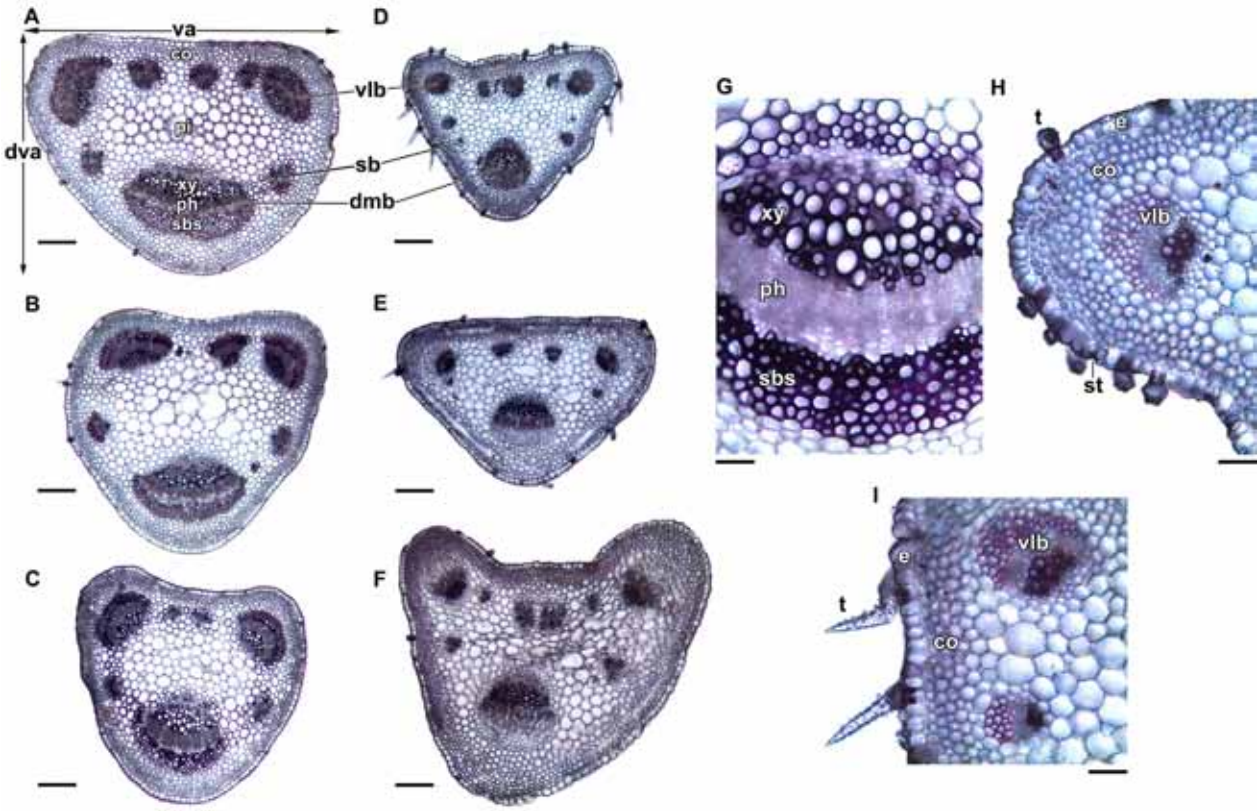


**Fig. 3.** Cross sections of the petiole in investigated *Astragalus* species. **A** – *A. monspessulanus* (Vlasi), **B** – *A. monspessulanus* (Petačinci), **C** – *A. monspessulanus* (Bioče), **D** – *A. spruneri* (Monastery „Matka"), **E** – *A. spruneri* (Staro selo), **F** – *A. spruneri* (Zović), **G** – dorsal median vascular bundle in *A. monspessulanus* (Bioče), **H**, **I** – details of the spine petiole anatomy in *A. spruneri* (Monastery „Matka"). Scale bars = 200 µm (A-F); 50 µm (G-I). Abbreviations: **co** – cortex, **dva** – dorsal ventral axis, **dmb** - dorsal median vascular bundle, **e** – epidermis, **ph** – phloem, **pi** – pith, **sb** – subsidiary vasculare bundle, **sbs** – sclerenchymatous bundle sheath, **st** – stomata, **t** – trichoma, **va** – ventral axis, **vlb** – ventral lateral vascular bundle, **xy** – xylem.

intersectional relationships, bifurcate hair characters should be studied more closely.

### *Petiole anatomy of A. monspessulanus*

In cross section, the outline of the petiole in species *A. monspessulanus* varies among populations from suborbicular to elliptic (**Fig. 3**). The epidermis of all populations consists of a single layer of subcircular to subrectangular cells. Cortex is situated below the epidermis, in form of a continuous ring composed of 3–5 layers of collenchymatous cells and parenchymatous cells. All populations have three prominent, primary vascular bundles, one dorsal median bundle and two ventral lateral bundles. The dorsal median bundle (DMB) is larger than the ventral lateral bundles. Two to six smaller, secondary bundles accompany the three primary bundles. The vascular bundles are collateral and arranged in a circle, separated from one another by parenchymatic tissue. Each vascular bundle is surrounded by a thick sclerenchymatous sheath composed of very thick-walled extraxylary fibers. The pith in all species is composed of large parenchyma cells (**Fig. 3**).

### *Petiole anatomy of A. spruneri*

The outline of cross section of the petiole in species *A. spruneri* has a semi-triangular outline in which their abaxial sides were convex and their adaxial sides either flat or only slightly convex (**Fig. 3**). Just as in species *A. monspessulanus,* 2/3 of the petiole surface is composed of the central cylinder and 1/3 of the primary cortex. Single–layered epidermis is covered with denser indumentum than the petiole epidermis of *A. monspessulanus* petiole. The cortex is composed of 2-3 layers of collenchyma and several layers of parenchyma cells. The central cylinder includes several vascular bundles, the largest main bundle in the center and two lateral, somewhat smaller vascular bundles. The number and size of vascular bundles varied between individuals of the same population as well as among populations: 5-11 bundles were recorded in different individuals. The vascular bundles are surrounded by well-developed sclerenchyma tissue. The central region is composed

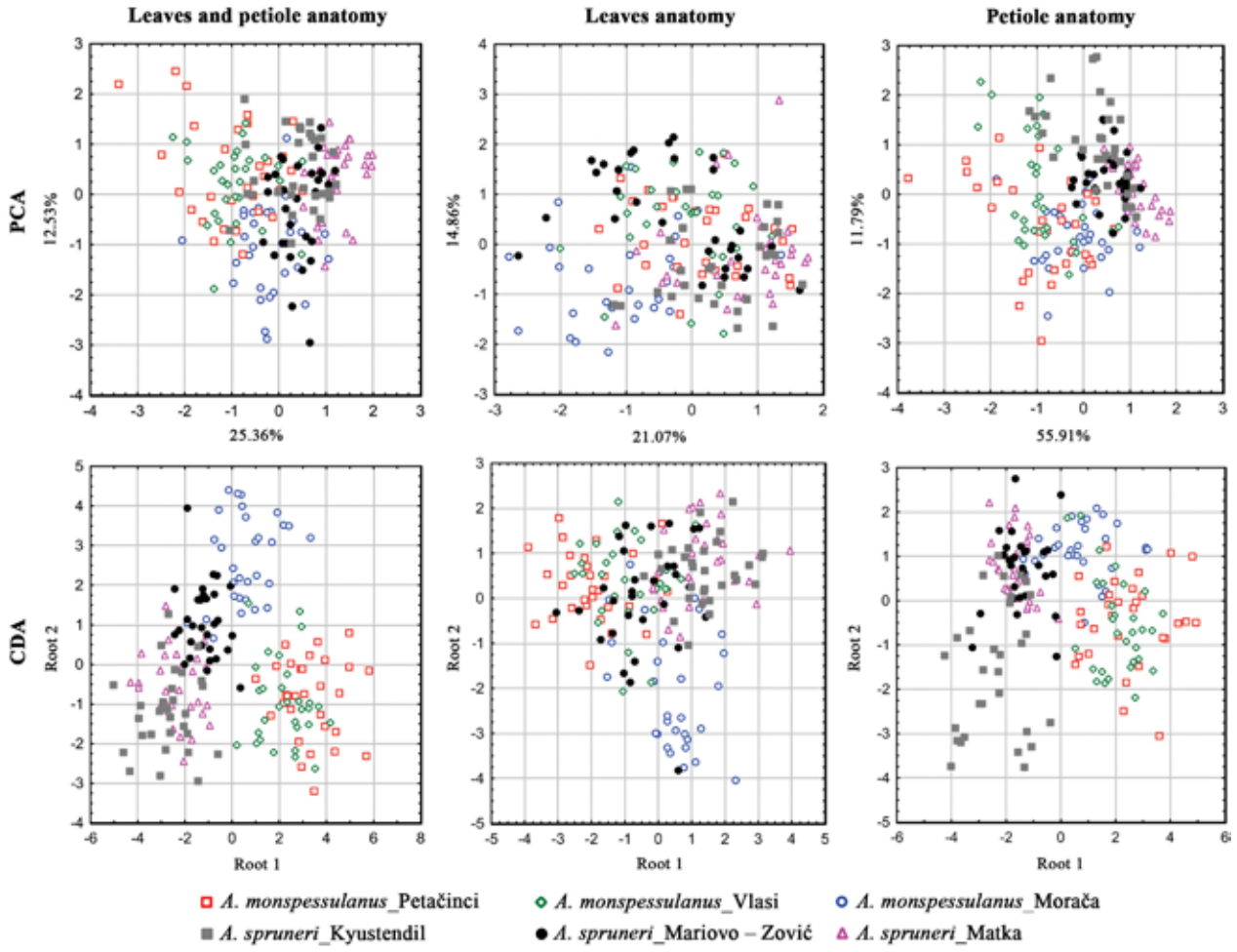


**Fig. 4.** Results of the principal component analysis (PCA) and canonical discriminant analysis (CDA) for populations of investigated *Astragalus* species based on: all characters of leaflet and petiole anatomy, characters of leaflet anatomy and characters of petiole anatomy.

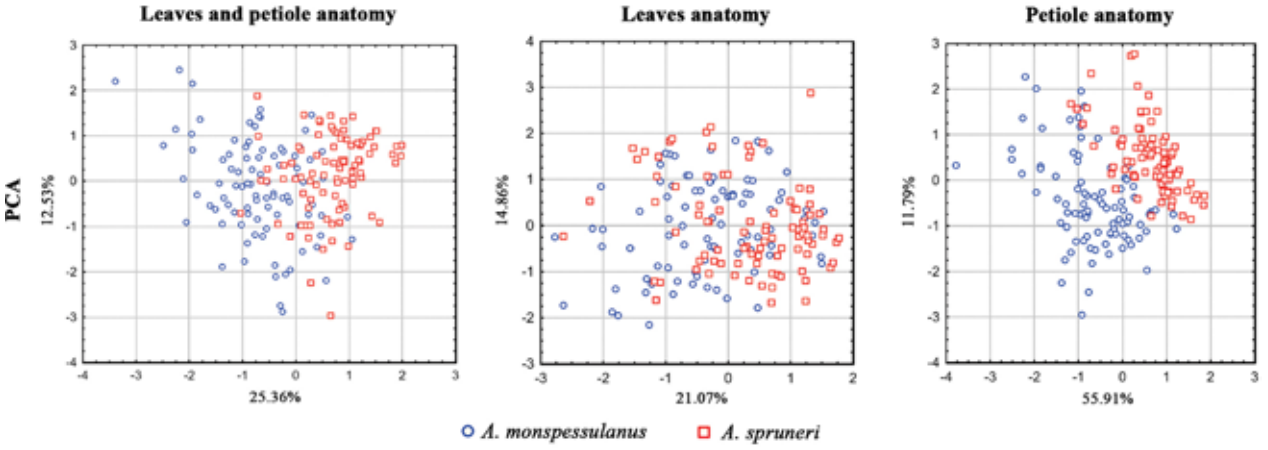


**Fig. 5.** Results of the principal component analysis (PCA) for populations of investigated *Astragalus* species based on: all characters of leaflet and petiole anatomy, characters of leaflet anatomy and characters of petiole anatomy.

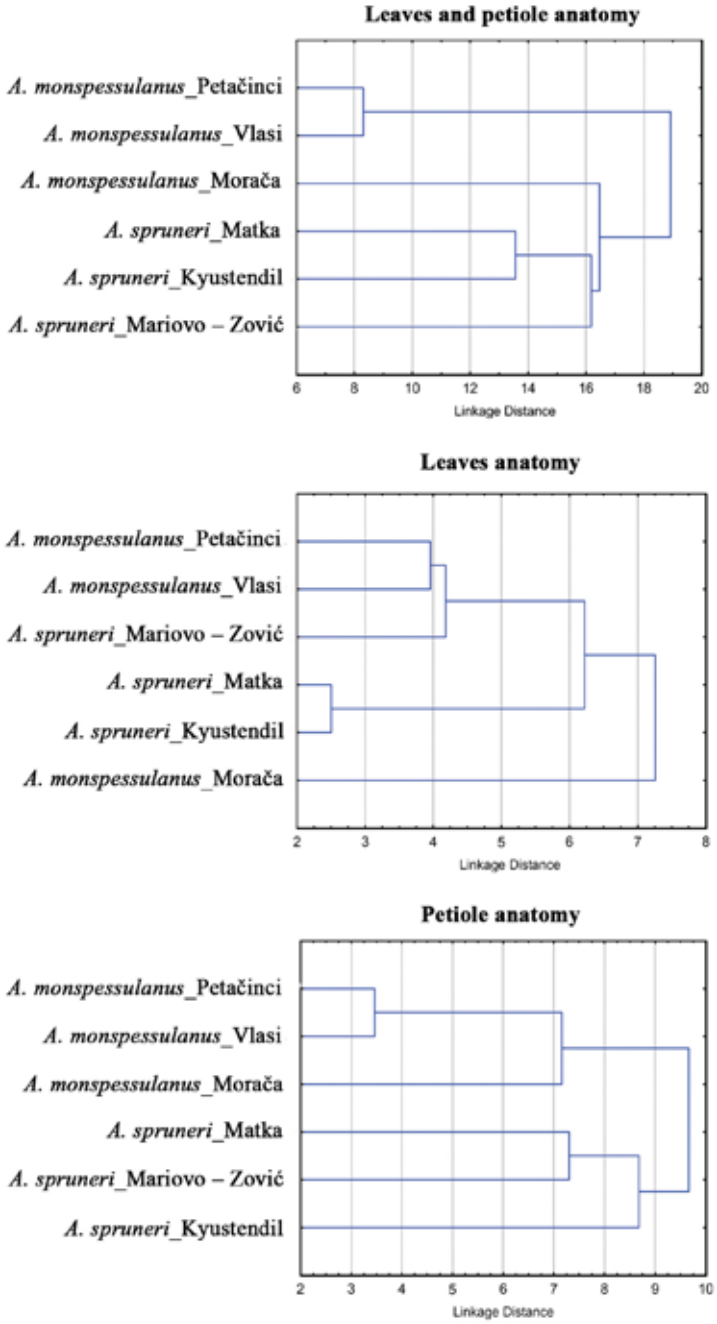


**Fig. 6.** Cluster analysis (Single Linkage method) for populations of investigated *Astragalus* species based on: all characters of leaflet and petiole anatomy, characters of leaflet anatomy and characters of petiole anatomy.

of large, thin-walled parenchyma cells, just as in species *A. monspessulanus* (**Fig. 3**).

### *Comparation between A. monspessulanus and A. spruneri*

According to the results of t-test, the studied species show statistically significant differences regarding the petiole anatomy (**Tab. 2**). According to PCA results, individuals of *A. monspessulanus* are almost completely differentiated from individuals of species *A. spruneri* along the first ordination axis (**Fig. 4**, **Fig. 5**). Only very few individuals of *A. monspessulanus* sampled in the canyon of river Morača show a more significant degree of similarity with individuals of species *A. spruneri*. On the other hand, a few individuals of *A. spruneri* sampled in Bulgaria (Kyustendil) show a significant degree of similarity with individuals of species *A. monspessulanus* (Serbia: village Vlasi). The list of characters making the highest contribution to differentiation along the first ordination axis of PCA diagram includes all the analyzed characters except for thickness of petiole epidermis and thickness of primary cortex of petiole. CDA diagram shows almost complete differentiation of species (**Fig. 5)**. Individuals of species *A. monspessulanus* are concentrated in the positive part of CDA diagram, while individuals of species *A. spruneri* are positioned in the negative part of the CDA diagram. The results of agglomerative hierarchical classification support the results of other statistical analyses, i.e. existence of clear interspecies differentiation regarding the leaf stalk anatomy (**Fig. 6**). Although the statistical analysis of the dataset including all studied characters of leaflets and petioles has also supported interspecies differentiation, analysis of dataset including only the characters of petioles has provided more illustrative results.

According to Metcalfe & Chalk (1950), the petiole has considerable taxonomic importance, as it is not heavily influenced by environmental changes. The general description of petiole cross-section is in accordance with previous data for other *Astragalus* species (Howard, 1979; Haddad & Barnett, 1989; Pirani et al. 2006). Research on European species of *Astragalus* has shown that anatomic characteristics of the petiole have a limited taxonomic value, while characters of type of tissue in parenchyma of central region and amount of collenchyma tissue around the vascular bundles have high taxonomic importance for recognizing the two main species groups (Haddad & Barnett, 1989). Species *A. monspessulanus* and *A. spruneri* belong to a group of taxa characterized by a relatively small number of thick-walled parenchyma cells in the central part of the petiole and a relatively high

amount of collenchyma tissue around the vascular bundles.

## Conclusions

Our results show which anatomical and micromorphological characteristics can be used for taxonomic differentiation of species from the *Astragalus* sect. *Incani*. Anatomical features described here largely agree with previous characters found in other *Astragalus* species. Anatomical and micromorphological characters described here have potential taxonomic and significance. Results of statistical analyses indicate that characters which refer to petiole anatomy have higher taxonomic value than characters which refer to leaflet anatomy.

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