Acta Bot. Croat. 75 (1), 67–73, 2016
DOI: 10.1515/botcro-2016-0002

CODEN: ABCRA 25
ISSN 0365-0588
eISSN 1847-8476

Anatomical characteristics of two *Ornithogalum* L. (Hyacinthaceae) taxa from Serbia and Hungary and their taxonomic implication

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Abstract – Anatomical characters of two morphologically similar *Ornithogalum* taxa, *O. umbellatum* and *O. divergens*, were investigated. An analysis of leaf and scapus cross-sections was performed on plants from ten populations from Serbia and Hungary, using light microscopy. The aim of this research was to give data about the qualitative and quantitative anatomical characteristics of these taxa, in order to evaluate their taxonomic significance and single out distinctive anatomical features, as well as to contribute to the knowledge of the genus *Ornithogalum* in the studied region. On the basis of the variability of anatomical characters, similar populations formed two clusters, joining the plants previously determined as *O. divergens* and *O. umbellatum.* The two taxa significantly differed for most of the quantitative leaf and scapus characters. Since only quantitative differences were recorded in this research, anatomical characters could not be solely used to separate these two taxa. However, the results of anatomical investigations are consistent with the results of previous morphological and genetic analyses; therefore anatomical parameters could be useful as additional taxonomic characters.

Key words: anatomy, leaf, *Ornithogalum*, scapus

Introduction

Genus *Ornithogalum* L. is one of the most abundant in species and taxonomically the most interesting in the family Hyacinthaceae Batsch ex Borckhausen 1797 (Order Asparagales Bromhead, subfamily Ornithogaloideae) (Manning et al. 2009, Martínez-Azorín et al. 2011). The definition of taxa within this group has troubled taxonomists for a long time (Martínez-Azorín et al. 2011); an accurate number of belonging species is still a controversial issue. *Ornithogalum* is native in Europe, Asia (as far as Afghanistan in the East) and Africa, where it is widely distributed (Martínez-Azorín et al. 2010). The Mediterranean and South Africa are considered to be the centers of distribution (Zahariadi 1980, Cullen 1984).

Natural habitats of these bulbous monocots are very heterogeneous; they are in bloom in spring and summer and the flowers are entomophilous. These plants are important hosts for insects, especially for some groups of hoverflies, whose adults feed on pollen and nectar and larvae develop in the bulbs of these geophytes (Vujić et al. 2012). Some species of the genus are of importance in the flower trade, being grown as cut flower or potted plant crops, while some are poisonous to livestock and considered weeds (Obermeyer 1978, Littlejohn and Blomerus 1997).

Eleven subgenera and 34 species of the genus *Ornithogalum* have been described in the Flora Europaea (Zahariadi 1980) and *Ornithogalum umbellatum* L. 1753 and *Ornithogalum divergens* Boreau 1887 are given as separate species. Thirteen *Ornithogalum* species were recorded in the Flora of Serbia (Diklić 1975), whilst eight were recorded in Hungarian flora (Soó 1973). *O. divergens* was described as a subspecies of *O. umbellatum* in both of them.

Ornithogalum umbellatum is a type species of the genus *Ornithogalum*. It is widespread in Europe, North Africa and Southwestern Asia and was introduced in North America (EuroPlusMed 2006, eFloras 2008). The species rank of this taxon is not questionable; however, the intraspecific systematics are often interpreted differently. The taxonomic position of *Ornithogalum divergens* has had lots of different interpretations (Martínez-Azorín et al. 2009); it was considered a hexaploid form of the polyploid complex of *O. umbellatum* (Moret et al. 1991, Moret 1992), a subspecies, a variety and it has also been widely misidentified as

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O. umbellatum. Nevertheless, Martínez-Azorín et al. (2009, 2010) presented its nomenclature and taxonomy as well as its ecology and distribution and described this species as clearly different from *O. umbellatum* s. s., primarily for its ploidy level, morphology of bulbils and inflorescence structure.

The need for redefinition of the infrageneric taxa of the genus *Ornithogalum* was suggested by Garbari et al. (2003, 2008) in Italy. Herrmann (2002) deals with *Ornithogalum umbellatum* s. l. in Central Europe. A biometric study of *O. umbellatum* s. l. was done by Moret et al. (1991) in France and Moret (1992) investigated ploidy levels using numerical taxonomy within this genus, concluding that species from *O. umbellatum* s. l. from France exhibit considerable variability. Systematic studies had been made previously on this complex by Van Raamsdönk (1986) who singled out *O. umbellatum* as the most widespread species among European representatives of the genus and a variable wide-ranging taxon with populations showing different morphological character combinations. The nomenclature and taxonomy of *O. umbellatum* s. l. continue to be investigated in recent years in Spain (Martínez-Azorín et al. 2009), and taxonomic revision of subgenus *Ornithogalum* was also done for the Spanish species including *Ornithogalum divergens* (Martínez-Azorín et al. 2010).

Many authors consider the genus *Ornithogalum* inconvenient from the standpoint of systematics stating that its morphology is insufficiently connected with variations in karyotype (Martínez-Azorín et al. 2010). Since this additionally complicates taxonomic delimitations, anatomical data could make a particularly useful contribution to the solution of taxonomical problems (Meriç et al. 2011).

Studies regarding anatomical characteristics referring to these taxa have not been numerous. A comprehensive view on leaf anatomy in relation to the systematics of the whole Hyacinthaceae family was given by Lynch et al. (2006). Morpho-anatomical variability of the leaves among different taxa of the genus *Ornithogalum* was also investigated by Peruzzi et al. (2007). Differences in leaf anatomy between *Ornithogalum nutans* L. and *Ornithogalum narbonense* L. from Bulgaria were presented by Popova and Anastasov (1997). Morphological and anatomical analyses were conducted for leaf and scapus of *O. nutans* and *Ornithogalum boucheanum* (Kunth) Aschers from Turkey (Meriç et al. 2011). Comparative anatomical studies of leaf and scapus features of twelve *Ornithogalum* species, belonging to the subgenera *Ornithogalum* and *Beryllis*, were recently done in Central Anatolia (Öztürk et al. 2014). Types of crystals are especially interesting from a taxonomic point of view and Tilton and Lersten (1981) described idioblasts with calcium oxalate crystals as a type of specialized cells in the gynoecium of *Ornithogalum caudatum* Aiton.

Although the examined taxa have been research subjects in many fields worldwide, detailed investigations and publications on their anatomy in particular are very scarce, especially in this part of Europe. The main goal of this study is to contribute to the knowledge of the qualitative and quantitative anatomical characteristics of leaf and scapus of two *Ornithogalum* taxa, from several populations from Serbia and Hungary, with the aim of evaluating their taxonomic significance and singling out distinctive anatomical features potentially useful as diagnostic characters.

Materials and methods

Plant sampling was carried out during the flowering period, in April and May (2008–2013). Specimens for analysis were collected from ten different native populations in Serbia and Hungary (Tab. 1, Fig. 1). Plants were determined and voucher specimens deposited in the Herbarium BUNS (2-1632 – 2-1641) at the Department of Biology and Ecology, Faculty of Science, University of Novi Sad. Ten specimens from each population were fixed in 50% ethanol. Cross-sections of the middle parts of leaf and scapus, 60 μm thick on average, were made using a Leica CM 1850 cryostat at a temperature of – 20 °C. Anatomical characters were observed by light microscopy, using the image analyzing system Motic 2000. Ten cross-sections were examined for each population. In total, 31 quantitative characters were measured and compared, and relative proportions were calculated. The total cross-section area of leaf and scapus was measured and percentages of other parameters were determined considering total cross-section area as 100%. Palisade cell indices were calculated as the ratio of their height and width.

Data were statistically processed using STATISTICA for Windows version 12.0 (StatSoft, 2014). The significance of differences in the analysed parameters among all

Tab. 1. Analysed populations of *Ornithogalum umbellatum* and *O. divergens* in Serbia (SRB) and Hungary (HUN). Abb – abbreviation.

Taxon	Sampling locality	Abb	Coordinates	Sampling date	Voucher
O. umbellatum	Deliblatska Peščara, SRB	DE	N44°59’16” E20°56’42”	18.04.2008	2-1632
O. umbellatum	Vršačke Planine, SRB	VR	N45°07’22” E21°19’38”	26.04.2009	2-1633
O. umbellatum	Zlotska Klisura, SRB	ZL	N44°01’45” E21°57’28”	10.05.2009	2-1634
O. umbellatum	Valjevo, SRB	VA	N44°25’43” E19°54’35”	29.04.2012	2-1635
O. umbellatum	Mecsek, HUN	ME	N46°05’56” E18°13’07”	21.04.2013	2-1636
O. umbellatum	Szeged, HUN	SZ	N46°15’10” E19°38’36”	22.04.2013	2-1637
O. divergens	Titelski Breg, SRB	TI	N45°17’01” E20°14’29”	20.04.2012	2-1638
O. divergens	Susek, SRB	SU	N45°13’51” E19°28’38”	19.04.2012	2-1639
O. divergens	Novi Sad, SRB	NS	N45°14’51” E19°49’43”	26.04.2013	2-1640
O. divergens	Hódmezövásárhely, HUN	HO	N46°50’42” E20°31’47”	22.04.2013	2-1641

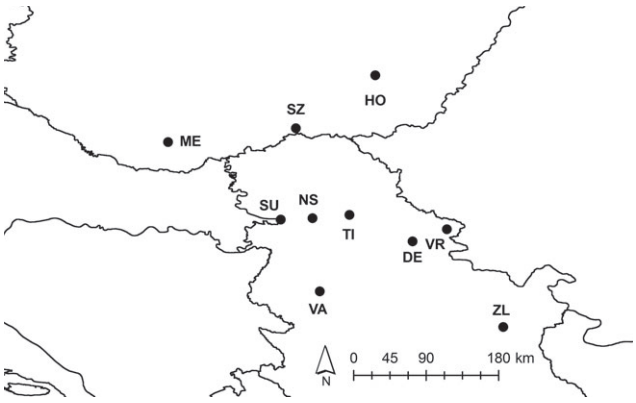

Fig. 1. Localities of the analysed populations in Serbia and Hungary. For abbreviations of sample localities see Tab. 1.

the populations was established using Duncan's test ($p \leq 0.05$), and between *Ornithogalum umbellatum* and *Ornithogalum divergens* applying t-test ($p \leq 0.05$). The general structure of sample variability was determined by principal component analysis (PCA), based on a correlation matrix. Multivariate discriminant function analysis (MDA) was done in order to test the hypothesis that the analysed sample was composed of groups which differed from each other according to anatomical features of leaf and scapus. Any characters that were not significantly different between the two taxa, according to the results of t-test, were not included in MDA.

Results

Leaf anatomy

Cross section of the leaf is U-shaped, slightly curved towards the dorsal side (Fig. 2). It is significantly smaller in *Ornithogalum umbellatum,* with an average total area of 1.2–2.2 mm^2, compared to 2.8–3.7 mm^2 in *Ornithogalum divergens*. Adaxial side is smooth while the abaxial has 6–8 or 8–10 more or less prominent ribs, in *O. umbellatum* and *O. divergens*, respectively. Epidermis is single layered, covered with a thin cuticle. Epidermal cells are round in shape, or slightly elongated, smaller and more elongated on the abaxial side. The leaves are amphistomatic, with fewer stomata on the adaxial side, positioned at the same level as epidermal cells. There are no trichoma on the epidermis. Mesophyll is composed of chlorenchyma differentiated into palisade and spongy tissue. Palisade tissue is single layered and present on both adaxial and abaxial sides. However, it is partly missing adaxially due to presence of large lacunae. Chlorenchyma cells are absent in the region of the main vein, and broad lacunae appear subepidermally, interspersed with irregularly shaped parenchyma cells.

Palisade tissue cells are elongated, narrower towards the the adaxial side, perpendicular to lamina surface (Fig. 3). Chloroplasts are more numerous closer to the cell walls that surround intercellulars. Spongy tissue cells are round or irregular in shape, with fewer chloroplasts than palisade cells. Intercellulars are larger in spongy tissue (Figs. 2, 3). The cells in the middle part of the mesophyll are larger, and do not contain chloroplasts. Some of the large mesophyll cells contain bundles of needle-shaped CaOx raphides (Figs. 3 A, C). Vascular bundles are arranged in two rows, the bigger ones centrally located, and the smaller ones towards abaxial side. *Ornithogalum umbellatum* has 7–10 vascular bundles of both types, whilst they are more numerous in *Ornithogalum divergens* (10–11 abaxial and 10–12 central vascular bundles). Vascular bundles are surrounded with one to two layers of parenchyma cells (Figs. 2, 3). Large lacunae are present in mesophyll, between the bundles (Figs. 3 A, B).

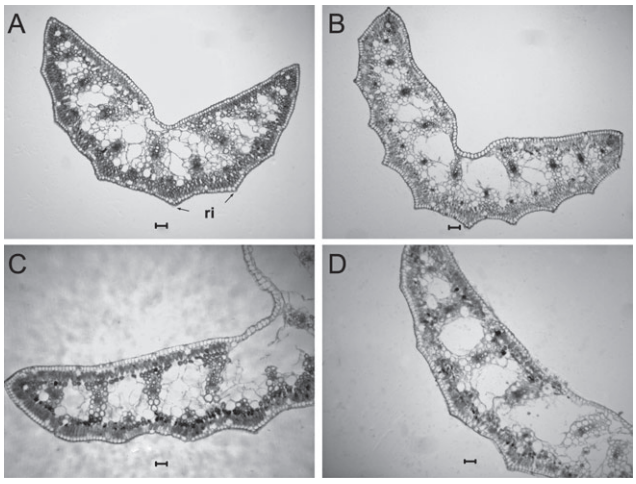

Fig. 2. Leaf cross section: A) *Ornithogalum umbellatum* (Vršačke Planine), B) *O. umbellatum* (Zlotska Klisura), C) *O. divergens* (Susek), D) *O. divergens* (Novi Sad). Abbreviations: ri – rib. Scale bars = 100 μm.

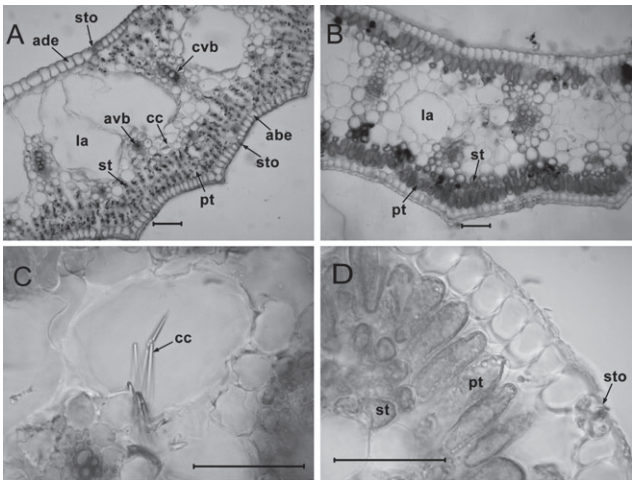

Fig. 3. Leaf cross section: A) *Ornithogalum umbellatum* (Deliblatska Peščara), B) *O. divergens* (Titelski Breg), C) *O. umbellatum* (Szeged), D) *O. divergens* (Novi Sad). Abbreviations: ade – adaxial epidermis, abe – abaxial epidermis, sto – stomata, pt – palisade tissue, st – spongy tissue, cvb – central vascular bundle, avb – abaxial vascular bundle, la – lacunae, cc – calcium oxalate crystals. Scale bars = 100 μm.

Scapus anatomy

Cross section of the scapus is round to oval in shape, with slightly wavy edges and larger total cross-section area in *Ornithogalum divergens* (8.7–10.4 mm^2) than in *Orni-*

thogalum umbellatum (3.0–7.7 mm^2) (Figs. 4 A, B). Epidermis is one-layered, with round shaped cells in cross section, glabrous, with thin cuticle and a few stomata (Figs. 4 C, D). Cortex consists of 4–5 layers of spherical parenchymatous cells, some of which contain chloroplasts. Idioblasts with CaOx raphide crystals are sometimes observed in cortex, beneath epidermis. Pith is predominantely composed of parenchymal tissue. In its peripheral part 1 to 3 layers of sclerenchyma occur. Small vascular bundles are located within sclerenchyma and just below it. Their number is very variable, (12–27 in *O. umbellatum* and 17–27 in *O. divergens*), depending on total cross-section area and the area of sclerenchyma, in sense that they are more numerous if these areas are larger. Larger, colateral vascular bundles, 10 to 20 of them in *O. umbellatum* and 17–24 in *O. divergens*, surrounded with parenchyma sheath, are randomly distributed in the pith. Their number is usually smaller than the number of small sclerenchyma bundles. The bundles become larger towards the central part of the scapus.

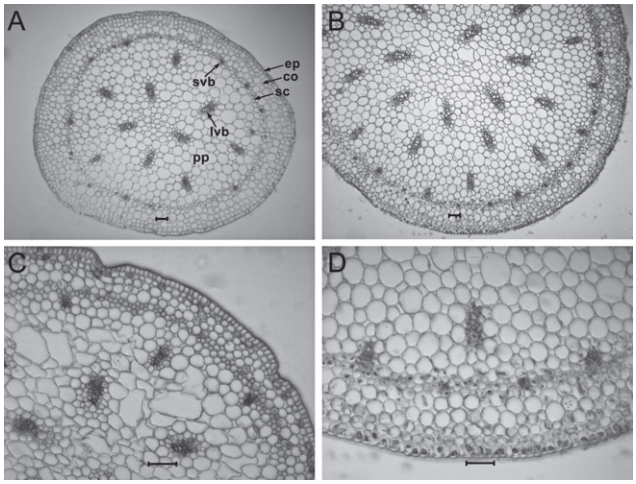

Fig. 4. Scapus cross section: A) *Ornithogalum umbellatum* (Valjevo), B) *O. divergens* (Titelski Breg), C) *O. umbellatum* (Zlotska Klisura), D) *O. divergens* (Titelski Breg). Abbreviations: ep – epidermis, co – cortex, sc – sclerenchyma, svb – small vascular bundle, lvb – large vascular bundle, pp – pith parenchyma. Scale bars: 100 μm.

Interpopulation variability of the anatomical characters

Duncan's test and t-test showed that the plants previously determined as *Ornithogalum divergens* (populations HO, NS, TI and SU) significantly differed from those determined as *Ornithogalum umbellatum* (DE, VR, ZL, VA, ME and SZ), for most of the quantitative characters (Tab. 2, Online Suppl. Tabs. 1, 2). Considering leaf characters, the two taxa had a similar number of stomata, percentage of vascular tissue and size of adaxial epidermal cells. *O. umbellatum* had s significantly smaller leaf cross-section area, with better developed palisade tissue, composed of significantly larger and more elongated cells, than *O. divergens.* Although *O. umbellatum* had a significantly lower number of vascular bundles, the total percentage of vascular tissue was not statistically different between the two taxa. Comparative analysis of scapus characters revealed that *O. umbellatum* had a significantly smaller scapus cross-section area, with better developed sclerenchyma and vascular tissue, than *O. divergens.* However, the percentage of pith parenchyma was lower in *O. umbellatum* and parenchyma tissue was composed of significantly smaller cells. Significant differences between the two taxa were not recorded in percentages of scapus cortex and pith, size of epidermal cells and the number of small vascular bundles.

The variation of the anatomical parameters was examined by PCA (On-line Suppl. Tab. 3). The first principal component accounted for 25.86% of total variation. Parameters that contributed the most to the total variability were percentages of palisade and spongy tissue, scapus cross-section area and percentage of scapus epidermis. The second component represented 18.91% of variation and was defined by the percentage of scapus cortex. The cumulative contribution percentage of the first three PCs was 53.74%. The projection of the cases of the first two components demonstrated that the investigated specimens could be separated into groups according to the variability of anatomical parameters (Fig. 5). Although the groups representing populations were heterogeneous, *Ornithogalum divergens* and *Ornithogalum umbellatum* populations clustered, but were not completely distinctive from each other.

Characters showing no significant differences between the two taxa were not included in MDA. The results of the MDA (On-line Suppl. Tab. 4) indicated similarities in anatomical characters between the populations from the same group. *Ornithogalum divergens* and *Ornithogalum umbellatum* populations were clearly separated along the first discriminant axis (Fig. 6). The parameter that contributed most to the discrimination of the populations, as well as of the two taxa, was leaf cross-section area, which was significantly higher in *O. divergens.* Percentages of palisade and spongy tissue and percentages of scapus epidermis and pith parenchyma singled out as characters that also had a share in separation of populations and taxa.

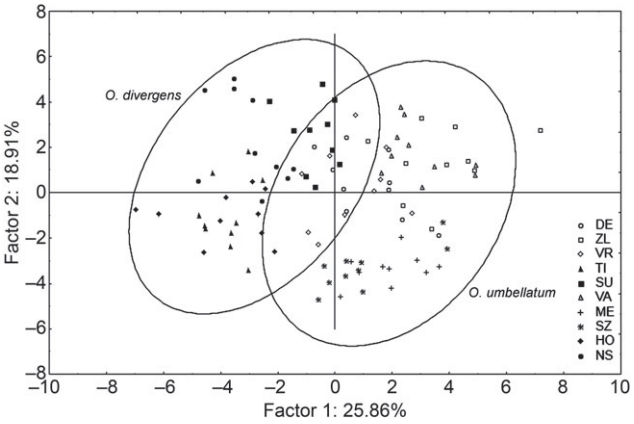

Fig. 5. The projection of the specimens of the first two components of the principal component analysis based on anatomical characteristics. Ellipses indicate populations of different taxa (right – *Ornithogalum umbellatum*, left – *O. divergens*) and represent 95% of each sample. For abbreviations of sample localities see Tab. 1.

Tab. 2. Anatomical characteristics of *Ornithogalum umbellatum* and *O. divergens*: mean value ± standard error and coefficient of variation % (in parenthesis). Asterisk (*) indicates significant differences between the two taxa and "ns" stands for "not significant", according to t-test ($p \leq 0.05$).

Character / Taxon	*O. umbellatum*	*O. divergens*	t-test
total leaf cross-section area (mm^2)	1.7 ± 0.1 (34)	3.2 ± 0.1 (27)	*
% leaf epidermis	11.9 ± 0.3 (20)	8.7 ± 0.3 (19)	*
% palisade tissue	20.7 ± 0.5 (20)	13.0 ± 0.7 (36)	*
% spongy tissue	64.3 ± 0.8 (9)	75.8 ± 0.8 (7)	*
% vascular tissue	2.7 ± 0.2 (44)	2.5 ± 0.1 (18)	ns
cross-section area of adaxial epidermis cells (μm^2)	704 ± 34.5 (38)	784 ± 38.7 (31)	ns
cross-section area of abaxial epidermis cells (μm^2)	504 ± 21.4 (33)	600 ± 25.2 (27)	*
cross-section area of adaxial palisade tissue cells (μm^2)	1499 ± 49.2 (25)	1179 ± 95.4 (51)	*
cross-section area of abaxial palisade tissue cells (μm^2)	1578 ± 40.4 (20)	1243 ± 100 (51)	*
leaf thickness (mm)	0.6 ± 13.2 (16)	0.7 ± 16.6 (14)	*
adaxial palisade cells index	2.5 ± 0.1 (26)	1.5 ± 0.1 (40)	*
abaxial palisade cells index	3.1 ± 0.1 (25)	1.9 ± 0.1 (40)	*
number of ribs on abaxial side	7 ± 0.2 (18)	9 ± 0.3 (23)	*
number of abaxial vascular bundles	9 ± 0.2 (14)	10 ± 0.3 (18)	*
number of central vascular bundles	9 ± 0.2 (15)	11 ± 0.3 (18)	*
number of stomata on adaxial epidermis on cross-section	7 ± 0.4 (45)	7 ± 0.4 (38)	ns
number of stomata on abaxial epidermis on cross-section	12 ± 0.5 (34)	13 ± 0.7 (38)	ns
total scapus cross-section area (mm^2)	5.7 ± 0.3 (45)	9.6 ± 0.5 (32)	*
% scapus epidermis	4.8 ± 0.2 (31)	3.1 ± 0.2 (32)	*
% scapus cortex	20.3 ± 0.9 (33)	20.5 ± 0.6 (19)	ns
% scapus pith	74.8 ± 1.0 (10)	76.4 ± 0.7 (6)	ns
% scapus sclerenchyma	6.3 ± 0.2 (26)	4.4 ± 0.2 (22)	*
% scapus small vascular bundels	0.6 ± 0.03 (33)	0.3 ± 0.02 (29)	*
% scapus large vascular bundles	2.6 ± 0.1 (26)	2.2 ± 0.1 (24)	*
% scapus pith parenchyma	65.3 ± 1.1 (13)	69.5 ± 0.7 (7)	*
cross-section area of scapus epidermal cells (μm^2)	725 ± 26.7 (29)	738 ± 38.2 (33)	ns
cross-section area of scapus cortex parenchyma cells (μm^2)	1176 ± 39.2 (26)	1493 ± 55.1 (23)	*
cross-section area of pith parenchyma cells (μm^2)	5001 ± 196 (30)	6652 ± 269 (26)	*
number of scapus cortex cell layers	4 ± 0.1 (17)	5 ± 0.1 (12)	*
number of small vascular bundles (in sclerenchyma)	20 ± 1.0 (37)	22 ± 1.0 (26)	ns
number of large vascular bundles (in parenchyma)	16 ± 0.7 (35)	20 ± 0.9 (30)	*

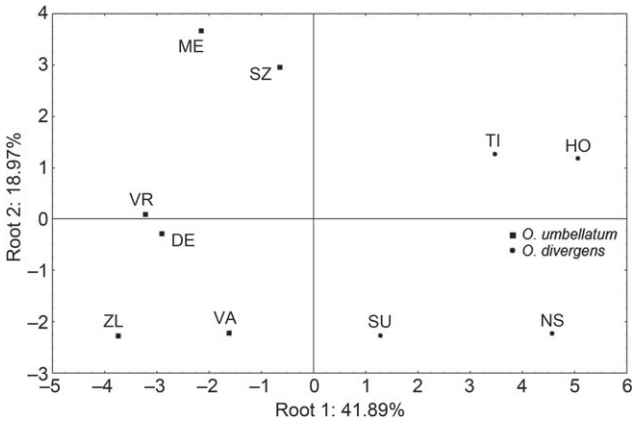

Fig. 6. The projection of the populations of the first two factors of the multivariate discriminant analysis based on anatomical characteristics. For abbreviations of sample localities see Tab. 1.

Discussion

In order to contribute to currently insufficient knowledge about the genus *Ornithogalum* in the studied region, leaf and scapus anatomical characteristics of *Ornithogalum umbellatum* and *Ornithogalum divergens* were analysed. This type of research represents an addition to previous and a foundation for future *Ornithogalum* studies, especially when it comes to problematic taxa such as these; however there are different opinions on the potential taxonomical importance of this approach. Peruzzi et al. (2007) concluded that leaf anatomical characteristics of *Ornithogalum* are useful for grouping similar species, while they are usually not sufficient for the characterization of every taxon individually. Thus they are the most valuable for general phylogenetic recapping and as a supplement to other types of analyses. Investigating leaf and scapus anatomy of species

belonging to two different subgenera (*Ornithogalum* and *Beryllis*), Öztürk et al. (2014) came to a similar conclusion. One of the latest anatomical researches of *Ornithogalum* was done by Meriç et al. (2011) on *Ornithogalum boucheanum* and *Ornithogalum nutans* in Turkey. It is difficult to distinguish these two species morphologically, thus anatomical characters could be particularly useful. They discovered no differences in scapus cross-sections, except in total sectional area and in the number of vascular bundles, features that were not considered of anatomical importance (Meriç et al. 2011). Similarly, Öztürk et al. (2014) found no significant variability on interspecies level, although they noted that plants belonging to subg. *Beryllis* had larger scapus diameter than members of subg. *Ornithogalum*, as well as more numerous vascular bundles. Our research has shown that *O. umbellatum* has a smaller scapus total cross-section area than *O. divergens*, with better developed sclerenchyma and vascular tissue, and a lower percentage of parenchyma tissue. However, no significant qualitative distinctions were found among *O. umbellatum* and *O. divergens* samples regarding scapus characteristics. On the other hand, it has been recorded that leaves differ in their anatomy in different species of this genus. Namely, leaves of *O. nutans* and *Ornithogalum narbonense* are presented as epistomatic (Popova and Anastasov 1997), while the *O. umbellatum* and *O. divergens* studied here have amphistomatic leaves, as was shown for all 12 species investigated by Öztürk et al. (2014), including *O. umbellatum*. Mesophyll of *O. narbonense* consists of two types of cells, which form palisade tissue and spongy parenchyma, while *O. nutans* mesophyll is composed of three cell types of palisade cells: elongated, rounded and irregularly shaped (Popova and Anastasov 1997). Meriç et al. (2011) have found mesophyll of *O. nutans* to be thinner, unifacial and to consist of monotypic chlorenchyma cells, while *O. boucheanum* has thicker equifacial mesophyll differentiated into palisade and spongy tissue, with large lacunae between vascular bundles and spongy tissue cells (Meriç et al. 2011). Leaves of the *O. umbellatum* and *O. divergens* studied here, like those of *O. narbonense* (Popova and Anastasov 1997), *O. boucheanum* (Meriç et al. 2011) and various members of *Ornithogalum* and *Beryllis* subgenera (Öztürk et al. 2014), have chlorenchyma differentiated into palisade and spongy tissue. Palisade tissue is one-layered and present on both adaxial and abaxial sides of the *O. umbellatum* and *O. divergens* leaves analysed in our study. Uniseriate upper and lower palisade parenchyma was also shown in some species belonging to subg. *Ornithogalum*, including *O. umbellatum*, while members of subg. *Beryllis* had only abaxial palisade tissue layer (Öztürk et al. 2014). Well defined, large lacunae appear in spongy tissue. Popova and Anastasov (1997) noted that the adaxial and abaxial epidermis become contiguous marginally at the edges of leaf blade in *O. narbonense* and considered this a typical feature of the species. *O. umbellatum* and *O. divergens* have cells of the upper and lower epidermis clearly separated by palisade tissue to the very edge, as shown in *O. nutans* (Popova and Anastasov 1997). In most of the representatives of the subfamily Ornithogaloideae vascular bundles in the leaf are distributed in two rows and bigger alternate with smaller (Lynch et al. 2006), which is also the case in our taxa.

There is a wide range of variations regarding shapes of crystals in the Hyacinthaceae family. Prychid and Rudall (1999) noted that different calcium oxalate crystal types, their presence or absence in monocotyledons, may represent useful taxonomic characters in certain groups, due to their specificity and constancy. They observed the presence of raphides in this family. Lynch et al. (2006) found solitary styloides in leaves, which are considered to be a diagnostic feature for certain families; they noted raphides and transitional forms between styloids and raphids, as well as crystal druses. In *Ornithogalum nutans* and *Ornithogalum boucheanum*, only raphides, immersed in the mucus of the parenchyma cells of scapus and leaves, were detected (Meriç et al. 2011). The presence of needle-like crystals in parenchyma cells of leaves and scapus, singly as well as in small groups of a few, was observed in *Ornithogalum umbellatum* and *Ornithogalum divergens* in our study. They are considered to be transitional forms between styloides and raphides, already mentioned in related taxa (Prychid and Rudall 1999, Lynch et al. 2006). It has been noted that raphides and styloids could be mutually exclusive, but if both types are present then intermediate forms occur, with two or three crystals per cell (Prychid and Rudall 1999). Chiappini (1962) recorded raphides in the parenchymatous tissues of the leaf and in several parts of the floral region of *O. caudatum*. Crystals, their presence in plants, their morphology and distribution, were noted to be very important features (Franceschi and Nakata 2005), thus constancy of the type and characteristics of crystals might be considered potential taxonomic characters. Variety of shapes of calcium oxalate crystals seems to be repeatable throughout the generations, illustrating consistency of genetic and physiological parameters controlling them (Prychid and Rudall 1999) and further studies of this aspect could be usefull for systematic analysis of this group.

Statistical analyses pointed out that most of the analysed anatomical characters have shown significant differences among observed populations. Observed differences were mostly quantitative, not qualitative. The most variable scapus characters among populations were total cross-section area and percentages of epidermis and cortex. Among the leaf characters, percentages of palisade and spongy tissue contributed most to the total interpopulational variability. The type of variability of these characters separated specimens that belong to two taxa, *Ornithogalum umbellatum* and *Ornithogalum divergens*, into different groups.

The size of leaf cross-section area proved to be the most important discriminative character between the two taxa. Moreover, the percentage of palisade tissue is significantly higher in *Ornithogalum umbellatum* and that of spongy tissue in *Ornithogalum divergens*; these are the leaf characters that contribute to discrimination of taxa with somewhat lower proportions.

According to the PCA and MDA results, the ten populations could be classified into two groups: one joining the plants previously determined as *Ornithogalum divergens* and the other one comprising *Ornithogalum umbellatum* populations. T – test showed significant differences between

the two taxa in most of the analysed characters. These results are consistent with the studies of morphological characteristics and ploidy levels (Garbari et al. 2003, 2008, Martínez-Azorín et al. 2009, 2010). However, anatomical characters alone could not be used to separate these morphologically similar taxa, due to the fact that only quantitative, not qualitative anatomical differences were recorded between them. These parameters could be a useful additional tool in resolving taxonomical problems, yet not for the characterization of a single species. They enabled a successful grouping of similar populations, but still remained insufficiently reliable for precise delimitation of *O. divergens* from *O. umbellatum*.

Acknowledgements

The authors would like to thank Tijana Nikolić, Sonja Mudri-Stojnić and Dušanka Krašić, colleagues who helped us with this work. The study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant No. 173002.

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On-line Suppl. Tab. 1. Anatomical characteristics of the leaf presented as mean value, and coefficient of variation % (in brackets). Characters: 1 – total cross-section area (mm^2), 2 – % leaf epidermis, 3 – % palisade tissue, 4 – % spongy tissue, 5 – % vascular tissue, 6 – cross-section area of a daxial epidermis cells (µm^2), 7 – cross-section area of abaxial epidermis cells (µm^2), 8 – cross-section area of adaxial palisade tissue cells (µm^2), 9 – cross-section area of abaxial palisade tissue cells (µm^2), 10 – leaf thickness (mm), 11 – adaxial palisade cells index, 12 – abaxial palisade cells index, 13 – number of ribs on abaxial side, 14 – number of abaxial vascular bundles, 15 – number of central vascular bundles, 16 – number of stomata on adaxial epidermis on cross-section, 17 – number of stomata on abaxial epidermis on cross-section. Different superscripts indicate that differences between localities are significant according to Duncan's test ($p \leq 0.05$).

	O. umbellatum						*O. divergens*			
	Vršačke Planine	Zlotska Klisura	Deliblatska Peščara	Valjevo	Mecsek	Szeged	Susek	Novi Sad	Titelski Breg	Hódmezö vásárhely
1	2.2^{c} (29)	1.6^{cd} (29)	1.9^{c} (24)	1.9^{c} (33)	1.2^{d} (15)	1.3^{d} (15)	3.4^{ab} (19)	3.7^{a} (35)	3.0^{b} (16)	2.8^{b} (21)
2	12.1^{bc} (17)	15.0^{a} (11)	11.4^{bcd} (16)	12.5^{b} (17)	10.8^{cde} (10)	9.5^{efg} (16)	8.8^{fgh} (12)	8.2^{gh} (15)	10.3^{def} (20)	7.7^{h} (14)
3	17.3^{c} (17)	19.6^{bc} (20)	20.7^{b} (17)	20.7^{b} (14)	25.1^{a} (19)	20.7^{b} (13)	18.2^{bc} (18)	13.3^{d} (17)	12.3^{d} (33)	8.3^{e} (31)
4	66.6^{defg} (6)	59.9^{h} (18)	64.6^{efg} (6)	64.8^{fg} (6)	62.3^{gh} (9)	67.7^{def} (4)	70.7^{cd} (6)	76.4^{b} (3)	74.8^{bc} (5)	81.2^{a} (4)
5	4.0^{a} (34)	3.5^{ab} (31)	3.3^{bc} (23)	2.0^{f} (35)	1.8^{f} (25)	1.9^{f} (23)	2.3^{def} (15)	2.1^{ef} (18)	2.7^{cde} (15)	2.8^{bcd} (10)
6	814^{abc} (37)	884^{ab} (28)	824^{abc} (31)	746^{bc} (24)	496^{de} (23)	459^{e} (33)	1008^{a} (30)	668^{bcd} (21)	662^{cd} (26)	798^{abc} (21)
7	606^{ab} (22)	486^{bc} (24)	628^{ab} (30)	536^{ab} (26)	394^{c} (42)	378^{c} (19)	665^{a} (17)	658^{a} (26)	498^{bc} (20)	581^{ab} (33)
8	1883^{a} (18)	1496^{b} (24)	1715^{ab} (7)	1592^{ab} (16)	972^{c} (24)	1337^{b} (9)	1757^{ab} (27)	1495^{b} (32)	613^{d} (57)	852^{cd} (24)
9	1880^{a} (17)	1430^{b} (17)	1626^{ab} (18)	1716^{ab} (17)	1358^{bc} (17)	1457^{b} (13)	1856^{a} (16)	1641^{ab} (35)	658^{c} (51)	818^{c} (24)
10	0.7^{b} (18)	0.6^{c} (20)	0.7^{b} (14)	0.7^{b} (14)	0.6^{c} (8)	0.6^{c} (9)	0.8^{a} (9)	0.8^{a} (11)	0.6^{c} (10)	0.7^{b} (10)
11	2.2^{b} (21)	2.4^{ab} (32)	2.8^{a} (16)	2.6^{ab} (19)	2.2^{b} (25)	2.6^{ab} (35)	2.1^{b} (13)	1.7^{bc} (39)	1.2^{c} (30)	1.0^{c} (28)
12	2.5^{c} (15)	2.5^{c} (23)	3.0^{bc} (11)	3.8^{a} (16)	3.2^{b} (21)	3.6^{ab} (26)	2.8^{bc} (10)	2.1^{c} (31)	1.5^{d} (37)	1.3^{d} (46)
13	7^{b} (18)	8^{b} (19)	7^{b} (22)	8^{b} (9)	6^{b} (10)	6^{b} (13)	10^{a} (12)	10^{a} (28)	10^{a} (24)	8^{b} (17)
14	9^{bc} (10)	10^{b} (9)	10^{b} (9)	9^{bc} (12)	8^{c} (8)	7^{d} (15)	10^{b} (15)	11^{a} (15)	11^{a} (19)	10^{b} (16)
15	10^{bc} (10)	10^{bc} (10)	9^{c} (8)	8^{cd} (11)	8^{cd} (13)	7^{d} (13)	10^{bc} (17)	11^{b} (16)	12^{a} (14)	10^{bc} (20)
16	8^{ab} (60)	8^{a} (36)	8^{abc} (33)	8^{a} (31)	5^{c} (25)	5^{bc} (41)	7^{abc} (41)	8^{ab} (33)	6^{abc} (44)	6^{abc} (34)
17	13^{ab} (32)	12^{ab} (28)	13^{ab} (35)	15^{a} (26)	8^{c} (24)	10^{bc} (32)	14^{ab} (39)	14^{ab} (32)	12^{ab} (39)	10^{bc} (40)

On-line Suppl. Tab. 2. Anatomical characteristics of the scapus presented as mean value, and coefficient of variation % (in brackets). Characters: 1 – total cross-section area (mm^2), 2 – % scapus epidermis, 3 – % scapus cortex, 4 – % scapus pith, 5 – % scapus sclerenchyma, 6 – % scapus small vascular bundels, 7 – % scapus large vascular bundles, 8 – % scapus pith parenchyma, 9 – cross-section area of scapus epidermal cells (µm^2), 10 – cross-section area of scapus cortex parenchyma cells (µm^2), 11 – cross-section area of scapus pith parenchyma cells (µm^2), 12 – number of scapus cortex cell layers, 13 – number of small vascular bundles (in sclerenchyma), 14 – number of large vascular bundles (in parenchyma). Different superscripts indicate that differences between localities are significant according to Duncan's test ($p \leq 0.05$).

	O. umbellatum						*O. divergens*			
	Vršačke Planine	Zlotska Klisura	Deliblatska Peščara	Valjevo	Mecsek	Szeged	Susek	Novi Sad	Titelski Breg	Hódmezö vásárhely
1	7.2^{cde} (30)	3.0^{g} (48)	6.6^{de} (29)	4.1^{fg} (25)	5.7^{ef} (30)	7.7^{bcde} (42)	8.7^{abcd} (43)	10.1^{ab} (12)	10.4^{a} (22)	9.2^{abc} (48)
2	3.7^{cd} (20)	6.3^{a} (27)	4.4^{bc} (17)	6.3^{a} (14)	4.6^{b} (26)	3.7^{cd} (23)	4.4^{bc} (16)	3.0^{de} (12)	2.2^{e} (28)	2.8^{de} (22)
3	18.9^{bc} (14)	27.7^{a} (18)	19.9^{bc} (15)	27.4^{a} (16)	12.8^{e} (23)	15.4^{de} (31)	21.7^{b} (11)	25.0^{a} (11)	18.3^{cd} (13)	17.2^{cd} (15)
4	77.5^{b} (4)	66.0^{e} (9)	75.7^{bc} (4)	66.3^{e} (7)	82.6^{a} (4)	80.9^{ab} (7)	73.9^{cd} (3)	72.0^{d} (4)	79.5^{ab} (4)	80.0^{ab} (4)
5	6.2^{ab} (15)	6.9^{a} (23)	6.5^{ab} (19)	5.6^{b} (34)	7.3^{a} (24)	5.2^{bc} (27)	5.5^{b} (20)	3.8^{d} (16)	4.1^{cd} (14)	4.2^{cd} (18)
6	0.8^{a} (15)	0.6^{ab} (53)	0.6^{ab} (33)	0.5^{bc} (27)	0.7^{ab} (30)	0.6^{ab} (21)	0.4^{cd} (24)	0.4^{cd} (18)	0.4^{c} (28)	0.3^{d} (23)
7	3.4^{a} (18)	2.8^{b} (24)	2.8^{bc} (22)	2.1^{d} (19)	2.1^{d} (19)	2.5^{bcd} (13)	2.3^{cd} (36)	2.0^{d} (21)	2.3^{bcd} (19)	2.1^{d} (15)
8	67.1^{b} (4)	55.6^{c} (13)	65.8^{b} (5)	58.1^{c} (10)	72.5^{a} (6)	72.6^{a} (9)	65.9^{b} (5)	65.9^{b} (4)	72.7^{a} (5)	73.5^{a} (4)
9	570^{c} (22)	711^{bc} (29)	778^{b} (22)	964^{a} (25)	734^{bc} (13)	594^{c} (18)	974^{a} (32)	773^{b} (20)	613^{bc} (18)	593^{c} (19)
10	1222^{bc} (21)	1207^{bc} (29)	1405^{b} (24)	1349^{bc} (14)	948^{d} (20)	926^{d} (13)	1751^{a} (14)	1744^{a} (14)	1327^{bc} (18)	1150^{cd} (18)
11	4035^{ef} (21)	3609^{f} (26)	4788^{de} (20)	5638^{cd} (21)	5458^{cd} (23)	6481^{bc} (28)	5334^{cd} (18)	8386^{a} (20)	5489^{cd} (13)	7403^{ab} (12)
12	5^{a} (18)	4^{bcd} (14)	4^{abc} (19)	4^{cd} (0)	4^{d} (13)	4^{d} (17)	4^{abc} (12)	5^{a} (9)	4^{abc} (12)	5^{ab} (15)
13	27^{a} (32)	12^{e} (33)	25^{ab} (24)	15^{cde} (12)	19^{cd} (18)	24^{abc} (28)	17^{cde} (22)	21^{bc} (22)	27^{a} (12)	22^{bc} (26)
14	20^{b} (26)	10^{d} (26)	19^{bc} (27)	12^{d} (15)	14^{cd} (24)	18^{bc} (28)	17^{bc} (37)	18^{bc} (14)	24^{a} (10)	20^{b} (41)

On-line Suppl. Tab. 3. Principal component analysis: factor coordinates of the variables based on correlations. Only variables with factor values > 0.7, which significantly contribute to total variation, were presented.

Character	Factor 1	Factor 2	Factor 3
Percentage of palisade tissue	**0.724**	–0.213	0.363
Percentage of spongy tissue	**–0.779**	0.113	–0.082
Total scapus cross-section area	**–0.775**	–0.109	0.359
Percentage of scapus epidermis	**0.829**	0.260	–0.173
Percentage of scapus cortex	0.307	**0.718**	–0.365
% Total variance	25.86	18.91	8.97

On-line Suppl. Tab. 4. Multivariate discriminant analysis: standardized coefficients for canonical variables. Only variables with root values > 0.7, which significantly contribute to total variation, were presented.

Character	Root 1	Root 2	Root 3
Total leaf cross-section area	**1.346**	0.456	–0.416
Percentage of palisade tissue	0.071	0.132	**–1.336**
Percentage of spongy tissue	0.264	0.088	**–1.105**
Percentage of scapus epidermis	–0.400	–0.190	**–0.815**
Percentage of scapus pith parenchyma	–0.477	**0.999**	–0.058
Percentages of the vectors	41.89	18.97	16.48