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TAXONOMIC IMPLICATIONS OF MULTIVARIATE MORPHOMETRICS IN SELECTED TAXA OF THE GENUS *ASTRAGALUS* SECT. *DISSITIFLORI* (FABACEAE)

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Abstract: The taxonomy of *Astragalus* sect. *Dissitiflori* from Europe is shaped by a controversial nomenclatural history and considerable morphological variation that requires numerical methods to facilitate species delimitation. Besides molecular methods, multivariate analysis of morphological traits has long been recognised to offer important and often complementary evidence in taxonomic research. We applied Multiple Discriminant Analysis based on 14 field-measured morphological characters to test the phenetic distinctiveness of *A. pseudoglaucus*, *A. tarchankuticus*, *A. vesicarius* s.l., *A. peterfii* and *A. pallescens*. In addition, we attempted to find the most discriminative morphological traits that separate the taxa investigated. Distinctness of *A. tarchankuticus* from *A. pseudoglaucus* and *A. vesicarius* is clear, whereas the latter two taxa are fairly close to each other. *A. peterfii* and *A. pallescens* separated from the other three species, but were found to be close morphologically. Five morphological characters proved to be useful traits to distinguish the species surveyed. Our findings contribute to a refined taxonomy of *Astragalus* and may serve as a starting point in a molecular systematic study of sect. *Dissitiflori*.

Keywords: *Astragalus peterfii*, *A. tarchankuticus*, distinctive characters, MDA, morphometrics.

Introduction

With an estimated total of 2500 species, *Astragalus* L. is the most species-rich genus among angiosperms [11]. The species are classified into more than 240 sections worldwide [2, 16]. Sect. *Dissitiflori*, which comprises nearly 160 species [18], is one of the largest sections of the genus. In Europe, it is represented by approximately 50 species, whereas the Flora of Romania lists six species and two subspecies [6]. Relationships (either phylogenetic or morphological) are scarcely understood within the European taxa of sect. *Dissitiflori*, and the taxonomy of the section has been blurred by the controversial nomenclatural history of several of the taxa. One of the taxonomically problematic species groups is the *A. vesicarius* L. aggregate, which, according to its most recent circumscription in Europe (but disregarding the former Soviet Union), includes three subspecies: *A. vesicarius* subsp. *vesicarius* (incl. *A. albidus* Waldst. & Kit.), *A. vesicarius* subsp. *pastellianus* (Pollini) Arcang. and *A. vesicarius* subsp. *carniolicus* (A. Kern.) Chater [17].

A. vesicarius subsp. *vesicarius* has the widest but more dispersed distribution, extending from the Iberian Peninsula to Ukraine, whereas subsp. *pastellianus* is endemic to north-eastern Italy, and subsp. *carniolicus* confined to north-eastern Italy, Croatia and Slovenia. Two other taxa, *A. pseudoglaucus* Klokov and *A. tarchankitucus* Boriss., were repeatedly affiliated with (or

even subsumed within) the *A. vesicarius* group. Another species, *A. glaucus* M.-Bieb., has been reported from the coastal region of Romania and Bulgaria [8, 13], but its presence in Romania has been questioned recently [6]. Our field surveys from 2009–2011 suggest the morphological similarity of the dispersed populations traditionally identified as *A. glaucus* to the population of the type locality of *A. pseudoglaucus* near Odessa [9], supporting evidence that *A. glaucus* is indeed absent from the Romanian flora. *A. tarchankitucus*, another coastal species, grows exclusively in the Crimea, on the Tarkhankut Peninsula, from where it was originally described [4]. In *Flora Europaea*, *A. pseudoglaucus* and *A. tarchankitucus* were treated as "perhaps to be included" under *A. vesicarius* subsp. *pastellianus* [5]. Later, *A. pseudoglaucus* was placed in the synonymy of *A. vesicarius*, whereas *A. tarchankuticus* was treated as a synonym of *A. albicaulis* DC. [18]. More recently, the new combination *A. vesicarius* subsp. *pseudoglaucus* (Klokov) Ciocârlan has been introduced, thus corroborating previous views on this taxon based on morphology [7].

Another seemingly well differentiated species, *A. peterfii* Jáv., endemic to Câmpia Transilvaniei (Transylvanian Lowland, Romania), has been similarly linked with *A. vesicarius* [5, 1] and even treated as a synonym of *A. vesicarius* subsp. *pastellianus* [12]. *A. peterfii* is of special importance, as this narrow endemic species, with only two known occurrences in Câmpia Transilvaniei, appears on many conservation lists, including Annex II of the EU Habitats Directive. This high conservation importance implies that any conservation action would require established taxonomic status. During our field trip in Ukraine in 2007, we recognised the apparent morphological similarity of *A. pallescens* Jáv. to *A. peterfii*, making it a new candidate as a close relative of this Transylvanian endemic.

The multivariate comparison of morphological traits has long been accepted as a powerful tool in taxonomic research [20]. This approach can visualise morphometric data for many variables simultaneously, and can separate morphological groups – if they exist – based on these variables. In this paper, we apply Multiple Discriminant Analysis (MDA) to test the morphological distinctiveness of *A. pseudoglaucus, A. tarchankuticus* and *A. vesicarius* s. l. Morphometric patterns alone could not be sufficient to make final taxonomic decisions, but they may represent additional evidence in a molecular phylogenetic study. Additionally, we analyse the difference between these *Astragalus* taxa, and try to find the most discriminative traits. Furthermore, we explicitly test a putative close morphological relationship between *A. peterfii* and *A. pallescens* M. Bieb.

Material and Methods

Six European taxa of *Astragalus* section *Dissitiflori* were included in our study. *Astragalus ucrainicus* Popov & Klokov, a species clearly diverged from our target species, was included as 'outgroup' to check morphological separation within the studied species complex compared to this evolutionary separate species.

As for the taxonomic position of taxa, we have maintained specific rank for both *A. pseudoglaucus* and *A. tarchankuticus* according to their original descriptions [4, 9]. In the case of *A. vesicarius* s. l., measurements were carried out on the population from the type locality of *A. albidus* which, according to the currently accepted taxonomic treatment [17], is synonymous with *A. vesicarius* subsp. *vesicarius*. Nonetheless, the eastern populations (i.e. those described as *A. albidus*) have whitish wings and keel, whereas plants from the western part of the range have purplish to violet petals [17]. This difference in petal colour may indicate separation of the eastern and western populations into two different taxa, and therefore we treat these populations as *A. vesicarius* s. l. throughout the paper. Nonetheless, this taxonomic question definitely requires further investigation.

Table 1: List of vegetative and floral morphological characters measured in the field

ShootHeight – height of one floral shoot from the ground to the apex of inflorescence (cm)
BasalLeafletWidth – width of the lowest leaflet of the leaf (mm)
BasalLeafletLength – length of the lowest leaflet of the leaf growing from the axil of the peduncle (mm)
CentralLeafletWidth – width of the middle leaflet of the leaf growing from the axil of the peduncle (mm)
CentralLeafletLength – length of the middle leaflet of the leaf growing from the axil of the peduncle (mm)
ApicalLeafletWidth – width of the highest leaflet of the leaf growing from the axil of the peduncle (mm)
ApicalLeafletLength – length of the highest leaflet of the leaf growing from the axil of the peduncle (mm)
LeafBladeLength – length of the leaf blade growing from the axil of the peduncle (cm)
LeafPetioleLength – length of the leaf petiole growing from the axil of the peduncle (cm)
LeafletNumber – number of leaflets of the leaf growing from the axil of the peduncle
CalyxWidth – width of the calyx of one fully anthesed flower (mm)
CalyxLength – length of the calyx of one fully anthesed flower (mm)
CalyxToothLength – length of the lower (odd) calyx-tooth of one fully anthesed flower (mm)
BractLength – length of the bract from under the calyx measured (mm)

Fourteen quantitative morphological characters expressed on the ratio scale (Table 1) were measured on living plants (one flowering shoot per individual) randomly selected from 1–2 populations of each taxon (Table 2). Spatially distinct (i.e. presumably separate genetic) individuals were chosen in order to minimise the inclusion of more than one ramet of the same genetic individual. In the case of *A. vesicarius* the small population size caused us to include a lower number of samples (10 shoots per population instead of 15).

The morphological characters measured were chosen as putatively variable among these taxa. The length of the inflorescence was not measured because it changes considerably over the flowering time on a given shoot [17], a variation that could have biased the analyses.

Multiple Discriminant Analysis (MDA) was used to analyse the difference between the *Astragalus* taxa surveyed and to find the most discriminative traits [15]. All variables were entered simultaneously. Cross-validated classification was used to determine the success of the discrimination. In cross validation, each case is classified by the functions derived from all cases other than that case. Classification has been achieved in two ways because of unequal sample size in two out of the six taxa (Table 2). One attempt was made by taking populations as units while a second attempt relied on groupings based on taxonomic affiliations. Wilks' lambda was used to evaluate the validity and significance of each discriminant function. Statistical analyses were carried out using SPSS v.16.0.

Results

The first five discriminant functions were highly significant ($p<0.001$). Character CalyxWidth has the largest absolute correlation with the first function that accounted for 64.2% of the variance (Wilks' lambda=0.001) between the groups. The second function accounted for 21.9% of the variance (Wilks' lambda=0.019); CentralLeafletLength, BasalLeafletLength, ApicalLeafletLength, ShootHeight, CentralLeafletWidth, BasalLeafletWidth, LeafBladeLength, ApicalLeafletWidth show the largest correlation with this function.

With the exception of *A. vesicarius*, population memberships have been retrieved with at least 69.7% overall accuracy (two-third of samples per population) during the cross validation procedure (Table 3). This might refer to slight differentiation of populations even between those belonging to the same taxonomic group.

Table 2: Details of the populations investigated regarding their location, date of measurements and number of individuals measured per population

Acronym	Locality and date of measurements	No. of individuals measured
A. vesicarius		
vesBud	Hungary, Budaörs, Odvas-hegy 47°28'6.12"N, 18°56'53.04" / 15.05.2011	10
A. pallescens		
palSof	Ukraine, Kherson Oblast, Sofiivka 46°35'51.09"N, 32°14'15.80"E / 10.05.2009	15
A. peterfii		
petSua	Romania, Suatu, Dealul Bánffy (*locus classicus*) 46°47'36.34"N, 23°57'41.12"E / 17.05.2009	15
petCai	Romania, Căianu Mic, Dealul Fogheu 46°46'24.38"N, 23°54'17.07"E / 17.05.2009	15
A. pseudoglaucus		
pseBab	Romania, Codru, Pădurea Babadag 44°49'3.41"N, 28°41'35.31"E / 16.05.2009	15
pseJur	Romania, Jurilovca, Capul Dolojman 44°45'34.46"N, 28°56'23.72"E / 16.05.2009	15
A. tarchankuticus		
tarArt	Ukraine, Crimea, Artemivka 45°28'53.55"N, 32°50'15.86"E / 13.05.2009	15
tarOle	Ukraine, Crimea, Olenivka 45°25'8.33"N, 32°29'54.01"E / 13.05.2009	15
A. ucrainicus		
ucrOle	Ukraine, Crimea, Olenivka 45°25'8.33"N, 32°29'54.01"E / 13.05.2009	15
A. ucrainicus		
ucrMyl	Ukraine, Kherson Oblast, Mylove 47° 2'24.54"N, 33°34'45.94"E / 11.05.2009	15

MDA classified correctly the *a priori* grouped specimens (according to taxonomic affinities) with 89% overall accuracy by means of the cross validation procedure (Table 4). All taxa can be considered as separate entities based on these morphological features, except *A. vesicarius*, for which only 50% of the specimens could be correctly classified. It is followed by *A. pallescens* with 86.6% success, though this value indicates high morphological distinctiveness. In other words, all taxa examined represent well-defined morphological groups, except for *A. vesicarius*, which greatly overlaps with *A. pseudoglaucus*.

Table 3: Classification matrix of the MDA cross-validation. Predicted population memberships are in columns and *a priori* classification is given in rows (for population abbreviations see Table 2)

	Group of specimens of the *Astragalus* populations examined										
	pseBab	pseJur	tarArt	tarOle	palSof	petCai	petSua	ucrMyl	ucrOle	albBud	total
pseBab	11	4	0	0	0	0	0	0	0	0	15
pseJur	4	9	0	1	0	0	0	0	0	1	15
tarArt	0	0	10	2	2	1	0	0	0	0	15
tarOle	0	0	4	10	1	0	0	0	0	0	15
palSof	0	0	0	0	13	1	1	0	0	0	15
petCai	0	0	1	0	0	9	5	0	0	0	15
petSua	0	0	1	0	1	3	10	0	0	0	15
ucrMyl	0	0	0	0	0	0	0	13	2	0	15
ucrOle	0	0	0	0	0	0	0	1	14	0	15
albBud	1	3	1	0	0	0	0	0	0	5	10

The above finding is mirrored on the scatterplot of the first two canonical variates (Fig. 1.). The outgroup taxon, *A. ucrainicus*, is well separated from the rest of the taxa. The remaining taxa form two non-overlapping data-clouds: one containing specimens of *A. peterfii* and *A. pallescens*, and a second that includes specimens of *A. vesicarius*, *A. pseudoglaucus*, and *A. tarchankuticus*. Within these groups, *A. pallescens* overlaps with *A. peterfii*, whereas *A. vesicarius* widely overlaps with *A. pseudoglaucus*, and *A. tarchankuticus* is connected to them by one outlier of both former taxa in the multivariate space (Fig. 1).

Table 4: Classification matrix of the MDA cross-validation. Predicted taxonomic memberships are in columns and *a priori* classification is given in rows

	Group of specimens of the *Astragalus* species examined					
	pseudoglaucus	*tarchankuticus*	*pallescens*	*peterfii*	*ucrainicus*	*vesicarius*
A. pseudoglaucus	93.3%	3.3%	0%	0%	0%	0%
A. tarchankuticus	0%	90%	10%	0%	0%	0%
A. pallescens	0%	0%	86.7%	13.3%	0%	0%
A. peterfii	0%	3.3%	6.7%	86.7%	0%	3.3%
A. ucrainicus	0%	0%	0%	0%	100%	0%
A. vesicarius	40%	10%	0%	0%	0%	50%

Discussion

A substantial morphological overlap between *A. pseudoglaucus* and *A. vesicarius* s. l. corroborates previous opinions [5, 7, 18] on the subspecific status of the former taxon within *A. vesicarius*. *A. albidus* was already considered to be the closest relative of *A. pseudoglaucus* in the original description of the latter [9]. As compared with *A. vesicarius*, *A. pseudoglaucus* has lower-growing stems, smaller and more elongated leaflets, narrower stipules, more compact inflorescences, shorter corollas, and narrower legumes [9]. The separation of *A. tarchankuticus* from both *A. pseudoglaucus* and *A. vesicarius* might be in agreement with its current taxonomic position [18], i.e. conspecificity with *A. albicaulis*. Further investigations, involving living *A. albicaulis* specimens, are needed to confirm the taxonomic position of these species. Unfortunately, we have not yet had access to material of *A. albicaulis* s. str.

Our results clearly indicate a close morphological relationship between *A. peterfii* and *A. pallescens*. Although morphometric similarities do not necessarily reflect phylogenetic relationships but simple morphological convergence, the present contribution suggests inclusion of *A. pallescens* in a molecular study aiming at the reconstruction of phylogenetic affinities of this enigmatic endemic species of the Transylvanian Lowland. In fact, a close relationship between the octoploid *A. peterfii* [10] and the tetraploid *A. pallescens* [14] extending to Moldavia [21] would be more plausible than between *A. peterfii* and the Italian endemic *A. vesicarius* subsp. *pastellinus*. The present morphometric comparison, however, lacks data from the latter species, under which *A. peterfii* has been placed in synonymy [12].

Among the morphological traits surveyed, the width of the calyx, the length of the leaf blade, the height of shoot and length and width of leaflets turned out to have the most discriminative power in separation of taxa. These traits should be given preference in future analyses of other groups in the genus and when new data are added to the present dataset.

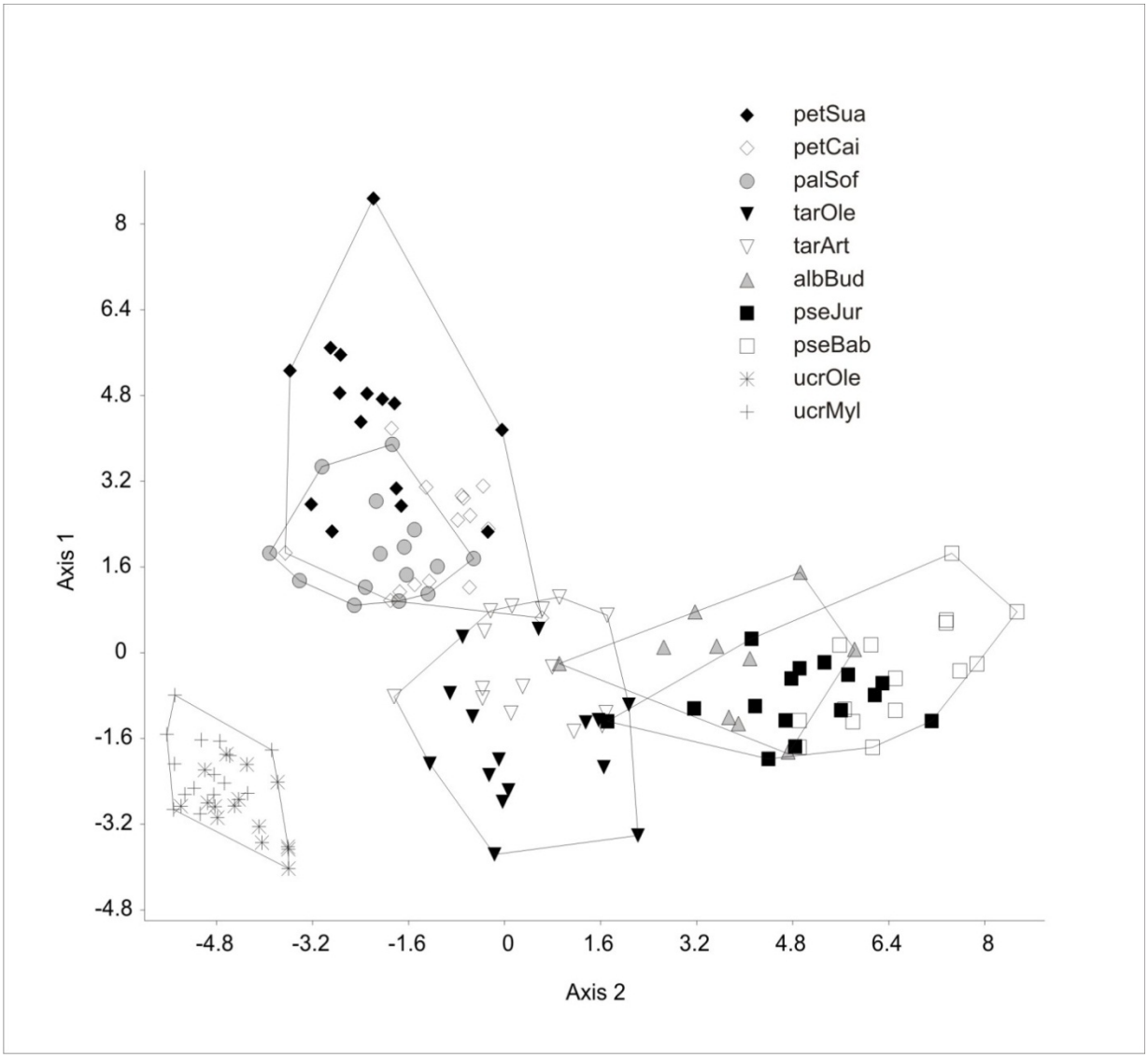

Fig. 1: Ordination of *Astragalus* samples on the first two discriminant functions that together account for 89.0% of the total variance between taxonomic groups. Convex hulls are superimposed to clarify separation of taxa (for population abbreviations see Table 2).

Multivariate morphometric analysis of quantitative traits can be a useful and promising source of information in addressing taxonomic classification within *Astragalus* sect. *Dissitiflori*. Taxa of this section frequently show substantial morphological variation [22] that can complicate species delimitation. Thus explicit methods are needed to answer the taxonomic questions. In spite of the usually higher resolution power of molecular methods compared with morphology, most authors of recent and comprehensive taxonomic studies [e.g. 3, 19] agree in drawing conclusions based on different lines of evidence, often morphology combined with molecular analyses. The results presented above, therefore, may corroborate other observations from karyology, genome size and molecular data.

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IMPLICAȚII TAXONOMICE ALE MORFOMETRIEI MULTIVARIATE ÎN CADRUL UNUI GRUP DE SPECII DE *ASTRAGALUS* APARȚINÂND SECȚIEI *DISSITIFLORI* (FABACEAE)

(Rezumat)

Taxonomia secţiei *Dissitiflori* a genului *Astragalus* din Europa prezintă încă numeroase neclarităţi generate de nomenclatura controversată şi de variabilitatea morfologică ridicată a speciilor. Pentru a se facilita delimitarea acestor taxoni se impune utilizarea în mai mare măsură a unor metode numerice adecvate. Pe lângă metodele moleculare, analiza multivariată a caracterelor morfologice este larg acceptată ca furnizând informaţii complementare în studiile taxonomice. Pentru a testa diferenţierea fenetică dintre *A. pseudoglaucus, A. tarchankuticus, A. vesicarius* s.l., *A. peterfii* şi *A. pallescens* a fost aplicată analiza multiplă discriminantă, pe baza a 14 caractere morfologice măsurate pe teren. De asemenea, s-a încercat identificarea acelor caractere care separă cel mai bine taxonii studiaţi. Diferenţierea taxonului *A. tarchankuticus* de *A. pseudoglaucus* şi *A. vesicarius* este

evidentă, ultimele două specii prezentând afinităţi morfologice considerabile. *A. peterfii* şi *A. pallescens* sunt asemănătoare din punct de vedere morfologic, separându-se clar în analizele noastre de celelalte trei specii. În diferenţierea speciilor studiate, s-au dovedit a fi utile cinci caractere morfologice (lățimea caliciului, lungimea laminei, înălțimea tulpinii și lungimea, respectiv lățimea foliolelor). Rezultatele studiului nostru au contribuit la o cunoaştere mai detaliată a taxonomiei genului *Astragalus* şi pot reprezenta un ghid util pentru abordarea sistematicii moleculare a secţiei *Dissitiflori*.

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