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Differentiation of the taxa of the genus *Taraxacum* sect. *Erythrosperma* on the basis of morphological characters on achenes and outer bracts

MATEJ DUDÁŠ [1], RÓBERT ŠUVADA [2], LENKA MÁRTONFIOVÁ [3] & PAVOL MÁRTONFI [1]

[1] Institute of Biology & Ecology, Faculty of Science, P. J. Šafárik University, Mánesova 23, SK-041 54, Košice, Slovakia; dudas.mato@gmail.com, pavol.martonfi@upjs.sk

[2] Administration of the Slovak Karst National Park, Hámosiho 188, SK-049 51, Brzotín, Slovakia; robert.suvada@sopsr.sk

[3] Botanical Garden of P.J. Šafárik University, Mánesova 23, SK-043 52 Košice, Slovakia; lenka.martonfiova@upjs.sk

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Abstract: The genus *Taraxacum* sect. *Erythrosperma* is represented by one diploid species and several triploid microspecies in the territory of Slovakia. In comparison with the sect. *Ruderalia* it is not widespread and its occurrence is limited to areas with xerophilous and thermophilous vegetation. Besides some morphological characters on leaves particular microspecies within the section *Erythrosperma* differ by position and size of outer bracts as well as size and colour of achenes. Morphometric analyses with employment of multivariate statistic methods in the sample of 745 achenes and 447 outer bracts showed no substantial differences in the studied characters between diploid *Taraxacum erythrospermum* and triploids (*T. cristatum, T. danubium, T. prunicolor*). In the case of analysis of sole triploid microspecies, these microspecies can be distinguished from each other on the basis of characters on achenes and outer bracts quite well. These characters, when combined with other morphological characters, can play an important role in determination of particular taxa.

Keywords: *Taraxacum* sect. *Erythrosperma*, morphology, outer bracts, achene, taxonomy.

Introduction

The genus *Taraxacum* F. H. Wigg. comprises large number of species which is related to its taxonomic complexity. The genus consists of 46 – 55 sections and new sections are still being described (KIRSCHNER & ŠTĚPÁNEK 1997, 2004, KIRSCHNER et al. 2002). It comprises up to 2500 microspecies (UHLEMANN 2007). The first taxonomic study of the section *Erythrosperma* (H. Lindb.) Dahlst from Slovakia was carried by KLÁŠTERSKÝ (1937), who described a new taxon *Taraxacum slovacum* Klášt. from the village of Zádiel in Slovak Karst. Large study of the section *Erythrosperma* from the Pannonian region was published by RICHARDS (1970). It includes descriptions of new microspecies from the territory of Slovakia: *Taraxacum danubium* A. J. Richards and *Taraxacum punctatum* A. J. Richards. DOLL (1973) in his revision of European section *Erythrosperma* mentioned 28 species in the territory of former Czechoslovakia, however, most of them were not correctly determined (VAŠUT 2003). At the present, 22 microspecies of the section *Erythrosperma* were found in Moravia (and new species *T. maricum, T. cristatum, T. prunicolor* and *T. princeps* were described) (VAŠUT 2003), approximately 10 in Poland (MARCINIUK et al. 2009) and at least 9 microspecies in Slovakia (ŠUVADA 2010).

Taraxacum sect. *Erythrosperma* (H. Lindb.) Dahlst. is taxonomically well distinguished section. The present concept of the section is based on the existence of one diploid, sexually reproducing species *Taraxacum erythrospermum* Andrz. ex Besser and approximately 150 polyploid microspecies (VAŠUT 2003). Diploid plants (2n = 16) reproduce sexually, while the microspecies are of higher ploidy level and reproduce by apomixis. The most widespread polyploid level is triploid (2n = 24), less frequent is tetraploid (2n = 32) (DEN NIJS 1997, DE KOVEL 2001).

The microspecies of the section have large variation of the shape of leaves and leaf lobes, as well as of the position and shape of outer bracts, the size of cornicula and the width of a hyaline margin. Differences between individual microspecies of the section are found also in the shape and colour of achenes, and the length of a cone (ŠUVADA 2010).

In the section *Erythrosperma* outer bracts are adpressed to capitulum, erect, spreading or recurved, in comparison with inner bracts they are shorter, ovate to elongate. Hyaline margin is usually present, 0,1 – 0,3 mm wide, but sometimes difficult to observe. The apex of outer bracts can be markedly corniculate, or only with small cornicula, or with a small tubercle. Achenes are small, 3 – 4 mm long with a 0,6 – 1,1 mm long cylindrical cone. Colour is red, reddish-brown, or rarely brown. Rarely some diploid plants can produce grey achenes (ŠUVADA 2010).

The plants produce pollen grains of approximately the same size (diploids), or markedly different sizes (diploids and triploids), or they do not produce any pollen grains at all (triploids) (MÁRTONFIOVÁ et al. 2010).

This study tries to discover morphological characters on the achenes and outer bracts which would discriminate not only between particular ploidy levels, but among individual microspecies as well. By means of morphometric analyses we tried to find out, if there are some differences between diploid and triploid

plants in the measured quantitative and qualitative characters on the achenes and outer bracts. By further analyses of these characters we tried to found out, if there are some differences between individual triploid microspecies too.

Plant material and methods

Plant material

Plant material used in the analyses was collected from populations naturally occurring in the Zemplínske vrchy mountains, the Slovak Karst and the Východoslovenská nížina lowland. During the vegetative period in 2011 (April – May) totally 149 individuals belonging to the section *Erythrosperma* were collected. In the localities each species growing there was collected. From each taxon several plants were collected randomly. From each analysed plant pollen grains and 3 inner leaves from leaf rosette were taken. Herbarium specimens were prepared from the leaves and served for determination. Ploidy level was determined from fresh leaves by flow cytometry. Three outer bracts from a capitulum and the whole set of achenes was taken away from each plant. The outer bracts were taken during the full flowering.

For morphometrical analyses 5 achenes and 3 outer bracts from each individual were used. The achenes were chosen randomly. Totally 745 achenes and 447 outer bracts were evaluated. Since in the localities of *Taraxacum* sect. *Erythrosperma,* especially in the Zemplínske vrchy Mts. and the Východoslovenská nížina lowlands, particular apomictic microspecies occur quite rarely and with low frequency, the number of analysed plants of these microspecies is limited.

The herbarium specimens and herbarized achenes and outer bracts are deposited in KO.

Palynology

After collecting the plant from locality, mounts were prepared by rubbing the capitulum against clear adhesive tape, and the tape was stuck on a drawing paper. The mounts were observed under the microscope Meopta, magnification 10x and the presence of pollen and differences in the size of pollen grains were evaluated. The data were recorded as a qualitative character: 0 – pollen grains absent, 1 – pollen grains present, the same size, 2 – pollen grains present, different sizes.

Flow cytometry

The samples for flow cytometry analysis were prepared from young leaves of *Taraxacum* plants using a two-step procedure (OTTO 1990, DOLEŽEL et GÖHDE 1995), consisting of separate nuclear isolation and staining steps, using propidium iodide as DNA intercalator. Relative DNA contents were measured on CyFlow ML flow cytometer (Partec GmBH, Münster, Germany), in The Laboratory of Flow cytometry at the Institute of Biological and Ecological

Sciences of P.J. Šafárik University in Košice (Slovakia). Green laser (532 nm/150 mW) was used as exciting source.

Approximately 1 cm^2 of young leaf of sample and standard were chopped with a new razor blade in Petri dish about 30 sec in 1 ml of ice-cold Otto I buffer. This suspension was filtered through nylon mesh (42 µm) and centrifuged (5 min, 1250 rpm). Supernatant was removed and the pellet (about 100 µl) was resuspended in 100 µl of ice-cold Otto I buffer. After 30 min. of incubation at room temperature, fluorochrome solution was added. It consisted of 1 ml of Otto II buffer, 20 µg propidium iodide (PI), 20 µg RNase and 2 µl β-mercaptoethanol. After 10 min of incubation at 4°C the samples were measured in a flow cytometer. As a standard plant *Raphanus sativus* cv. Saxa was used.

Measurements of achenes and outer bracts

Five well-developed achenes from each individual were chosen randomly. Each of the achenes was placed under stereomicroscope OLYMPUS SZ61, 0.67x magnification and photographed by Artray Artcam 300 MI camera. Later each achene was measured by computer software QuickPHOTO CAMERA 2.1.

From each individual an arithmetic average was calculated for each measured character. Totally 149 sets of achenes were measured, i.e. 745 measurements were carried.

Outer bracts were measured in the same way. Three well-developed outer bracts from each individual were randomly chosen and these were photographed and measured. Arithmetic average was calculated for each individual. Totally 149 sets of outer bracts were measured, i.e. 447 measurements were carried.

On the achenes the following quantitative characters were evaluated: the length of body of achene (**Fig. 1a**), the length of cone (**Fig. 1b**), the length of rostrum (**Fig. 1c**) and the length of pappus (**Fig. 1d**). The colour of achenes was the only qualitative character. The definition of measured characters is given in **Tab. 1a**.

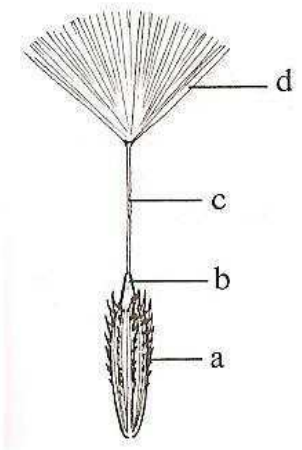

Fig. 1. Morphology of achene (KIRSCHNER et al. 2002):
a – body of achene;
b – cone;
c – rostrum;
d – pappus

On the outer bracts the following quantitative characters were evaluated: (**Fig. 2**): the length of outer bract (DZL), the width of outer bract in the widest place (SZL), the width of hyaline margin in the widest place (SBL) and the height of cornicula (VR). The position of outer bracts was the only qualitative character (**Fig. 3**). The definition of the measured characters is given in **Tab. 1b**.

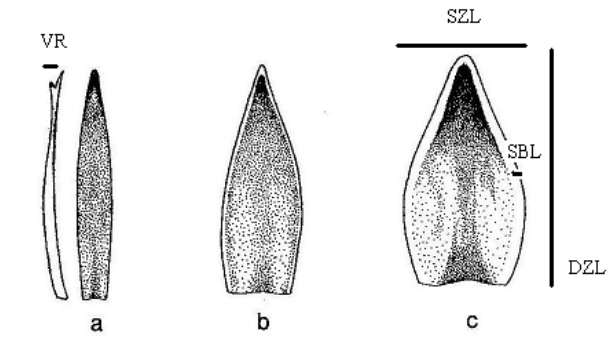

Fig. 2. Morphology of outer bract (TRÁVNÍČEK & VAŠUT 2011):
a – edge view with small cornicula (on the left) and front view without cornicula (on the right);
b – with narrow hyaline margin;
c – with wide hyaline margin with gradual cross-over to central grow thick part.
Abbreviation in Tab. 1b.

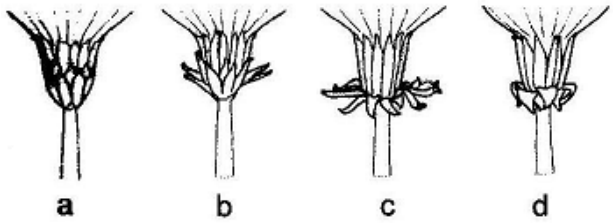

Fig. 3. Position of outer bracts (TRÁVNÍČEK & VAŠUT 2011):
a – adpressed;
b – erect;
c – spreading;
d – recurved.

Basic descriptive statistic

For basic descriptive statistic the following characteristics were chosen: minimum value, maximum value, standard deviation, arithmetic average and percentile (5 and 95). The Microsoft Office Excel 2007 was used for calculation. The descriptive values are calculated from the measured values for particular characters on achenes and outer bracts. The colour of achene was evaluated visually according to four colour scale defined prior to evaluation (**Tab. 1a**). The position of outer bracts (**Tab. 1b**) was determined in the course of fieldworks.

Multivariate statistic methods

For all morphometric analyses 8 quantitative and 2 qualitative characters were measured on the achenes and outer bracts. For the evaluation, the following multivariate methods were employed: principal coordinates analysis – PCoA (DAVIS 1986) using Gower´s similarity coefficient (GOWER 1971), linear discriminant analysis – LDA (FISHER 1936) and canonical discriminant analysis – CDA (KLECKA 1980). Analyses were performed using the PAST version 1.97 statistical package (HAMMER et al. 2001).

Tab. 1a. Definition of measured characters on the achenes of the studied microspecies of *Taraxacum* sect. *Erythrosperma*.

Achenes			
Characters –	**Abbreviation**	**Description of character**	**Scale**
quantitative			
Length of body of achene	DT	The average length of five bodies of achene	µm
Length of cone	DP	The average length of five cones	µm
Length of rostrum	DZ	The average length of rostrum of five achenes	µm
Length of pappus	DCH	The average length of pappus of five achenes	µm
qualitative			
Achene colour	FN	Evaluation of colour by visual assessment	1 – brown 2 – red 3 – murrey 4 – grey

Tab. 1b. Definition of measured characters on the outer bracts of studied microspecies of *Taraxacum* sect. *Erythrosperma*. (VZL – outer bract).

Outer bracts (VZL)			
Characters –	**Abbreviation**	**Description of character**	**Scale**
quantitative			
Length of VZL	DZL	The average length of three VZL	µm
Width of VZL	SZL	The average width of three VZL	µm
Width of hyaline margin	SBL	The average width of hyaline margin of three VZL in the most wide place of VZL	µm
Height of cornicula	VR	The average height of three cornicula	µm
qualitative			
Position		Evaluation of position of VZL to a capitulum	1 – adpressed 2 – erect 3 – spreading 4 – recurved

Results

Ploidy level and determination of the material

Flow cytometric analyses of all 149 plants included in the analyses detected 130 diploid individuals belonging to *Taraxacum erythrospermum* and 19 triploid individuals. One tetraploid plant, which was not included in this study, was detected, too. The triploids were classified with the following microspecies on the basis of palynological records and herbarized material study: 6 triploid plants belonged to *T. cristatum* Kirschner, Štěpánek & Vašut, 7 triploids to *T. danubium* Richards and 6 triploids to *T. prunicolor* Mart.Schmid, Vašut & Oosterv.

The evaluation of qualitative characters

The character colour of achene was evaluated in all of the studied diploid and triploid individuals. Diploids *Taraxacum erythrospermum* are showing wide variability, from the predominant red, via brown to grey colour of achene. On the contrary, triploid microspecies do not show large plasticity in this character and in the most cases the colour of achenes corresponds with the description of the species (**Tab. 5**).

However, the correlation between the position of outer bracts and ploidy level is very interesting. In diploid level the position of outer bracts is very variable, but in the most cases outer bracts are adpressed and spreading. Whereas in diploids recurved position of outer bracts is rare, in triploids it is very common and only occasionally other position of outer bracts was observed. In *Taraxacum prunicolor* outer bracts are typically recurved and violet colored.

The evaluation of quantitative characters

Diploid *Taraxacum erythrospermum* shows the largest variation in the measured values for particular characters on achenes and outer bracts. The variation of characters on the achenes and outer bracts in triploids is not so large (**Tab. 2**). *Taraxacum erythrospermum* shows the large variation also in the length and the width of outer bracts. In comparison with the group *Taraxacum scanicum* (*T. cristatum* and *T. prunicolor*), the individuals of *T. erythrospermum* and *T. danubium* produce quite high cornicula which differentiate them well (**Tab. 3**). The descriptive statistics of quantitative characters of particular species are presented in **Tab. 4**.

Tab. 2. Comparison of measured values for particular characters on achenes with the values described in description (DT – length of body of achene, DP – length of cone).

	Description range of values (VAŠUT 2003, SCHMID et al. 2004, VAŠUT et al. 2005)	Measured range of values
T. erythrospermum		
DT+DP	2,5 – 3,5 mm	1,8 – 4,3 mm
DP	0,6 – 0,8 mm	0,2 – 1,5 mm
T. danubium		
DT+DP	3,2 – 3,5 mm	2,2 – 3,8 mm
DP	0,6 – 0,9 mm	0,4 – 1,0 mm
T. cristatum		
DT+DP	3,7 – 4,2 mm	2,3 – 4,1 mm
DP	0,9 – 1,1 mm	0,4 – 1,2 mm
T. prunicolor		
DT+DP	3,8 – 4,1 mm	3,4 – 3,9 mm
DP	0,7 – 1,0 mm	0,8 – 1,1 mm

Tab. 3. Morphological characteristics of outer bracts of studied microspecies of *Taraxacum* sect. *Erythrosperma*

	Length of outer bract	Width of outer bract	Width of hyaline margin (average)	Height of cornicula (average)
T. erythrospermum	3,6 – 8,2 mm	0,9 – 3,1 mm	0,12 mm	0,3 mm
T. cristatum	6,1 – 8,1 mm	1,9 – 2,5 mm	0,14 mm	0,15 mm
T. danubium	4,5 – 5,9 mm	1,8 – 2,4 mm	0,17 mm	0,4 mm
T. prunicolor	7,4 – 8,0 mm	2,2 – 2,6 mm	0,13 mm	0,14 mm

Tab. 4. Descriptive statistics of quantitative characters according to the particular species (DT – the length of body of achene, DP – length of cone, DZ – length of rostrum, DCH – length of pappus, DZL – length of outer bracts, SZL – width of outer bracts, SBL – width of hyaline margin, VR – height of cornicula).

	T. erythrospermum N = 130			*T. cristatum* N = 6			*T. danubium* N = 7			*T. prunicolor* N = 6		
	Min	**X**	**Max**	**Min**	**X**	**Max**	**Min**	**X**	**Max**	**Min**	**X**	**Max**
	5%	**±SD**	**95%**	**5%**	**±SD**	**95%**	**5%**	**±SD**	**95%**	**5%**	**±SD**	**95%**
DT	1600	2308,93	2848	1908	2562,90	2842	1817	2174,11	2785	2567	2676,47	2818
(µm)	1855,34	272,03	2693,70	2039,9	350,83	2832	1864,92	318,31	2656	2577,8	96,35	2802
DP	227	763,62	1533	409	756,20	1233	399	647,14	1068	836	963,22	1129
(µm)	411,55	209,79	1085,07	472,8	267,55	1117	427,90	214,66	962,14	845,35	107,25	1105
DZ	1948	6173,12	9988	3801	6895,93	8519	3337	5086,34	6474	6458	6837,80	7449
(µm)	4195,78	1283,96	8248,09	4241,15	1833,97	8410	3453,14	1272,05	6426,42	6485	359,77	7337
DCH	2938	4207,51	5765	4784	5493,93	6041	4146	4567,11	5258	3356	4716,88	5753
(µm)	3199,22	570,78	5071,53	4901,35	447,94	5970	4154	424	5150,38	3517	931,43	5683
DZL	3631	5093,53	8225	6183	7128,94	8103	4555	5388,57	5944	7474	7719,11	8032
(µm)	4030,62	830,19	6410,33	6245,33	708,02	7934	4738,87	453,23	5876,87	7510	196,45	7984
SZL	922	2085,41	3174	1955	2329,72	2571	1817	2163,48	2422	2216	2360,11	2632
(µm)	1474,65	416,90	2798,25	2021,17	223,93	2557	1859,87	240,41	2420,80	2218	188,59	2616
SBL	1	127,63	309	112	141,14	179	132	170,76	232	95	130,94	196
(µm)	78,28	43,99	198,47	113	29,67	178	139,23	30,68	214,47	96,58	36,74	182
VR	10	343,55	1428	152	245,28	298	149	400,71	572	10	143,50	469
(µm)	10	220	668,93	163,5	57,56	294	199,10	147,02	565,60	10	180,45	401

Tab. 5 Comparison of evaluated qualitative characters for particular microspecies with values described in description.

	Evaluated characters	Description range of values (VAŠUT 2003, SCHMID et al. 2004, VAŠUT et al. 2005)
T. erythrospermum		
Colour of achenes	brown, red, murrey, grey	red, rarely grey
Position of outer bracts	adpressed, erect, spreading, recurved	adpressed, erect, spreading, recurved
T. cristatum		
Colour of achenes	red, murrey	brown
Position of outer bracts	adpressed, erect, recurved	spreading, recurved
T. danubium		
Colour of achenes	red	dark brownish-red
Position of outer bracts	erect, recurved	spreading, recurved
T. prunicolor		
Colour of achenes	brown, murrey	brown
Position of outer bracts	recurved	recurved

Comparison of the variability of diploid and triploid individuals

In the two analyses PCoA1 and LDA all of 149 diploids and triploids were analyzed in order to verify if it is possible to assign the ploidy level to the plant unequivocally on the basis of characters on the achenes and outer bracts.

Principal coordinates analysis PCoA1

On the basis of the results depicted in ordination graph PCoA1 (**Fig. 4**) the studied objects could not be assigned to either diploid or triploid level with respect to quantitative and qualitative characters on the achenes and outer bracts. This method showed mutual overlap between particular ploidy levels. The characters the most correlated with the first axis were DZL (-0,73), DCH (-0,61) and DTN (-0,60). The characters the most correlated with the second axis were the position of outer bracts (-0,77), DTN (0,61) and DP (0,63). The characters the most correlated with the ploidy level were the position of outer bracts (0,52), DZL (0,49) and DCH (0,34).

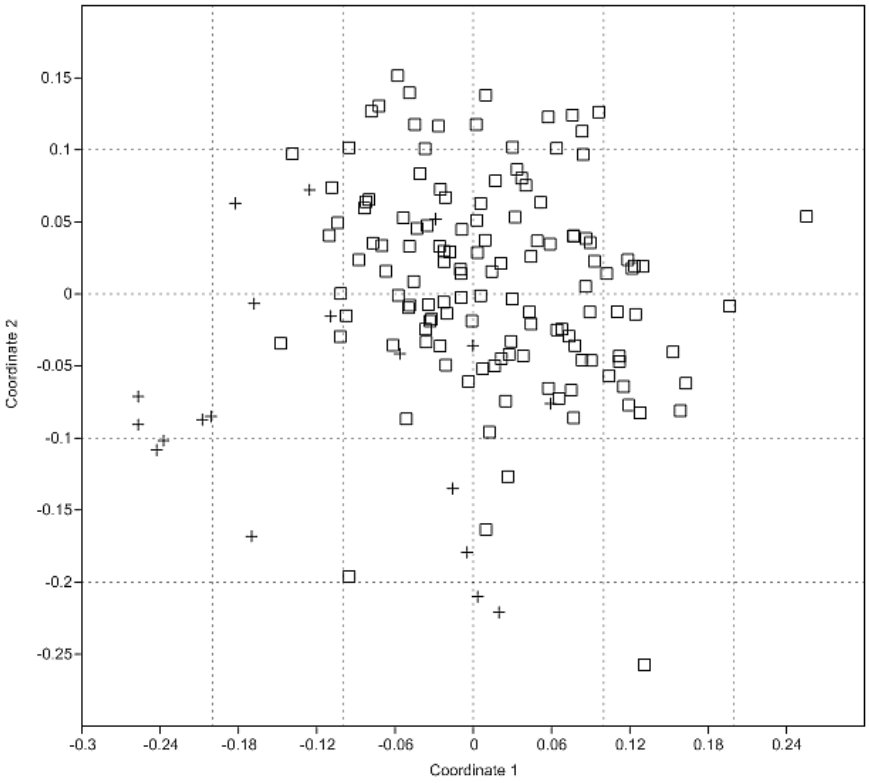

Fig. 4. Ordination graph of principal coordinates analysis (PCoA1), analysis of achenes and outer bracts based on quantitative and qualitative characters, diploids – square, triploids – cross mark

Linear discriminant analysis LDA

This method was used to prove the hypothesis stemming from the PCoA. The whole data set (149 individuals) was divided into the two groups according to the

ploidy level. The histogram LDA (**Fig. 5**) depicted clear overlap of the studied groups in characters on the achenes and outer bracts. Only quantitative characters on achenes and outer bracts were used. It was proved that in both groups the variation in achenes and outer bracts was so big that the individuals could not be assigned to one or the other group on the basis of these characters.

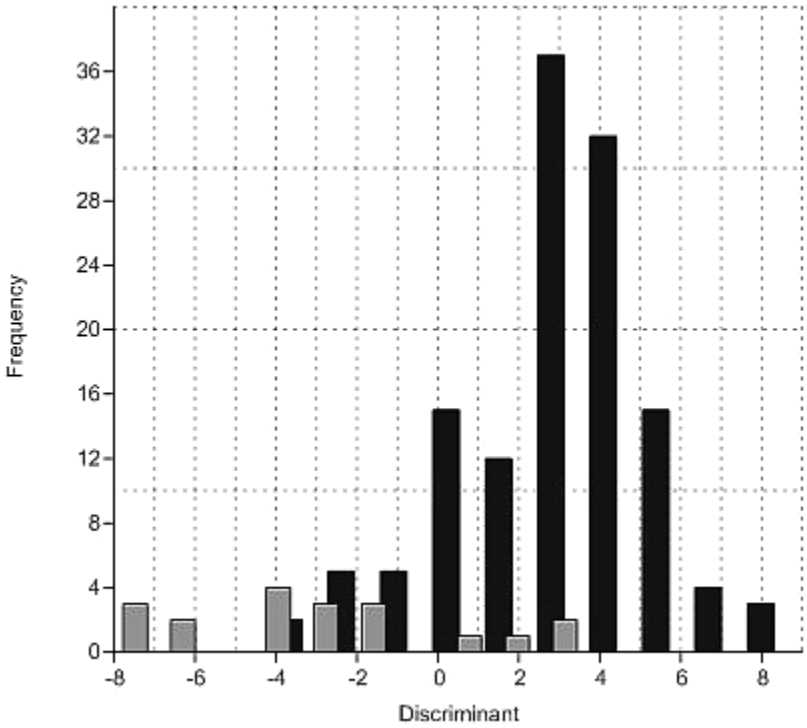

Fig. 5. Graph of linear discriminant analysis (LDA), analysis of achenes and outer bracts based on quantitative characters. Diploids – black colour, triploids – grey colour.

Comparison of morphological characters of particular triploid microspecies

Each of 19 triploids representing particular microspecies *Taraxacum cristatum, T. danubium* and *T. prunicolor* were evaluated in the following analyses.

Principal coordinates analysis PcoA2

In the ordination graph PCoA2 (**Fig. 6)**, with employment of both quantitative and qualitative characters, individuals of *Taraxacum danubium* were included in clearly separated cluster. A partial overlap was observed in individuals

determined as *Taraxacum cristatum* and *T. prunicolor*, since they are related microspecies from *Taraxacum scanicum* Dahlst. group. The characters the most correlated with the first axis were DTN (-0,87), DP (-0,82), DZ and DZL (both -0,81). The characters the most correlated with the second axis were the position of outer bracts (0,89), FN (-0,55) and VR (-0,54).

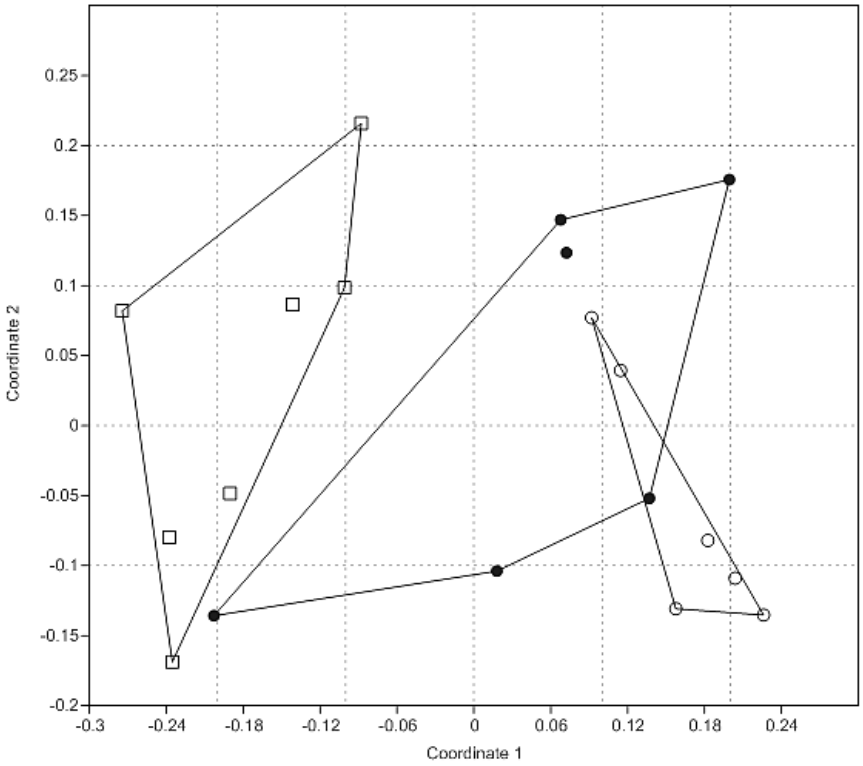

Fig. 6. Ordination graph of principal coordinates analysis PCoA2, analysis of achenes and outer bracts of sole triploids based on quantitative and qualitative characters. The first and second ordination axes are depicted. Full ring – *Taraxacum cristatum*, empty square – *T. danubium*, empty ring – *T. prunicolor*.

Canonical discriminant analysis CDA

An ordination graph of CDA analysis (**Fig. 7**) was employed to confirm the hypothesis on division of the studied triploid individuals into separate clusters on the basis of the morphological characters on achenes and outer bracts, and thus to verify the accuracy of taxonomic status of these microspecies. The characters the most correlated with the first axis were DZL (0,96), DTN (0,68) and VR (-0,64) whereas character the most correlated with the second axis was mainly DCH (0,70).

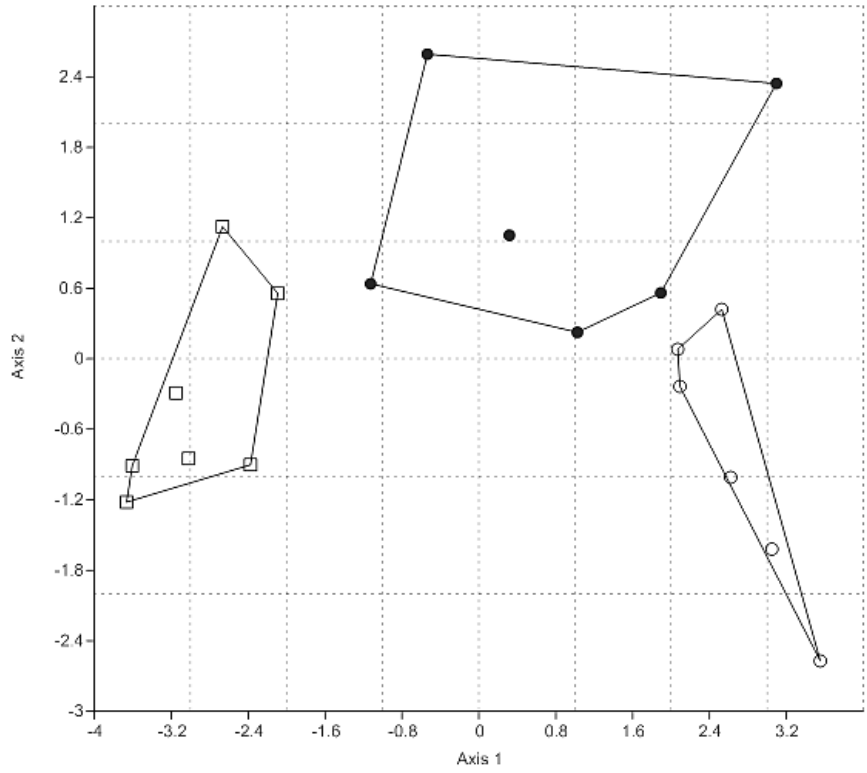

Fig. 7. Graph of canonical discriminant analysis CDA, analysis of achenes and outer bracts of sole triploids based on quantitative characters. The first and second axes are depicted. Full ring – *Taraxacum cristatum*, empty square – *T. danubium*, empty ring – *T. prunicolor*.

Discussion

Besides the 149 individuals included in the analyses (130 diploids and 19 triploid apomicts) a tetraploid plant was found on the hill near the village of Sirník (48°31'07" N, 21°47'25" E; 226 m, Slovakia) which is rather rare phenomenon in the sect. *Erythrosperma*. According to MÁRTONFIOVÁ et al. (2010) the tetraploids are the results of hybridization between diploids and triploids. This tetraploid represented the hybrid of *Taraxacum prunicolor* with characteristic terminal leaf lobe and recurved, violet coloured outer bracts.

Similarly to the studies of KIRSCHNER et al. (2002), VAŠUT (2003) and ŠUVADA (2010), our results proved that particular species in this section show different variation in the shape of leaves, position of outer bracts, height of cornicula, width of hyaline margin, shape and colour of achenes and length of cone. A comparison of our results with the studies of VAŠUT (2003), SCHMID et al. (2004)

and VAŠUT et al. (2005) shows that our data shift the limit of values for the length of body of achenes as well as the length of cone down and up (**Tab. 2**).

Diploid sexual species *Taraxacum erythrospermum* is the most common and the most widespread taxon in the sect. *Erythrosperma* in the studied area. It shows the biggest variability in the studied characteristics on achenes and outer bracts, the position of which may vary. Outer bracts are the most often adpressed to capitulum and erect, less often spreading and rarely recurved, on the contrary to triploids where the recurved position is the most common. This phenomenon was observed also for the position of outer bracts of diploids and triploids in the sect. *Ruderalia* (MIKOLÁŠ & MIHOKOVÁ, 1995). Frequently, *Taraxacum erythrospermum* f. *achyrocarpum* with grey achenes was present in the localities in Východoslovenská nížina lowland. VAŠUT (2003) points out possible confusion of *Taraxacum erythrospermum* with triploid microspecies, especially with *T. danubium* and *T. cristatum*, because *T. erythrospermum* shows a large spectrum of variation not only on achenes and outer bracts but also on leaves. This fact was confirmed also during our field sampling. The presence of pollen and the size of pollen grains was studied during the microspecies determination. Majority of diploid plants produced pollen grains of the same size. Two individuals from localities village of Ladmovce (48°24'53" N, 21°46'32" E, 149 m, Slovakia) and Streda nad Bodrogom (48°22'08" N, 21°47'09" E; 206 m, Slovakia) produced the pollen grains of different sizes, which corresponded with MÁRTONFIOVÁ et al. (2010), who pointed that *Taraxacum erythrospermum* can sporadically produce pollen grains of different sizes.

Triploid apomicts were rare and scattered in the Východoslovenská nížina lowland, however, in the Slovak Karst their occurrence was more abundant. *T. cristatum, T. danubium* and *T. prunicolor* were the most frequent.

Taraxacum danubium can form similar individuals to that of *Taraxacum erythrospermum*, which corresponds with the study of VAŠUT (2003). The individuals differ by position of outer bracts which are markedly recurved with narrow hyaline margin and small cornicula at the apex (ŠUVADA et al. 2012). Our measurements show that in comparison with other studied triploids, *T. danubium*, is characterized by shorter outer bracts. However, high values of width of a hyaline margin and high cornicula make this taxon well distinguishable.

According to VAŠUT et al. (2005), MARCINIUK et al. (2009) and ŠUVADA (2010) *Taraxacum cristatum* represents a microspecies which is well distinguishable on the basis of position of outer bracts and width of hyaline margin in combination with the shape of leaves. This is in accordance with our observations. It is well distinguishable from *T. prunicolor* by green, sometimes pinkish outer bracts, and red achenes. Ours measurements show that *T. cristatum* produces outer bracts longer than 6.1 mm and *T. danubium* outer bracts not longer than 5.9 mm. This makes the two taxa well distinguishable

Taraxacum prunicolor is clearly and well distinguishable triploid microspecies with characteristic shape of leaves and position of outer bracts. Outer bracts are always recurved with marked violet colour and small cornicula at the apex. This is in consent with studies of SCHMID et al. (2004) and ŠUVADA (2010). Our results

show that *T. prunicolor* produced the smallest cornicula in comparison with other studied apomicts. This makes the determination easier. Moreover the brown colour of achenes is in contrast with red achenes of *T. danubium*.

On the basis of the length of outer bracts *T. danubium* can be clearly distinguished from microspecies of the group *Taraxacum scanicum (T. cristatum* and *T. prunicolor)*. The group *Taraxacum scanicum* may differ also by the character "height of cornicula", because it produced cornicula markedly lower than *T. danubium* and *T. erythrospermum*. Within the frame of group *T. scanicum, T. prunicolor* and *T. cristatum* may be exactly distinguished on the basis of the colour of achenes and the colour of outer bracts and the shape of leaves.

The findings of this study are in accordance with previous knowledge on the complicated genus *Taraxacum*. In mixed diploid-polyploid populations, due to the presence of gene flow, triploids share gene pool with diploids (MÁRTONFIOVÁ 2006, ŠUVADA 2010). Sexually reproducing diploids show, owing to high genetic variability combined with phenotypic plasticity, wide range of values for the measured characters which overlap the values found in particular apomictic microspecies. Because of apomictic reproduction, triploid microspecies show very limited genetic variability and the differences between the individuals are due mostly to phenotypic plasticity. Reproductive isolation causing the barrier for gene flow in apomictic microspecies leads to characters allowing differentiation between selected microspecies. Triploid microspecies *T. cristatum, T. danubium* and *T. prunicolor* are well distinguishable on the basis of characters on achenes and outer bracts observed in this study in combination with characters on leaves.

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Appendix

The list of sampled localities. Locality number, country, locality description, geographic coordinates (Google Earth), altitude, date of collection, the occurring species, name of collector, name of determinator. Abbreviations of collectors and determinators: MD – M. Dudáš, RS – R. Šuvada.

1. Slovakia, village of Veľký Kamenec, surroundings of castle ruin in the village; 48°21'31" N, 21°48'28" E; 111 m; 23.4.2012; *T. erythrospermum, T. prunicolor;* col. MD; det. RS.
2. Slovakia, village of Malý Kamenec, a grassy vegetation on the top of the hill Tarbucka near the transmitter; 48°21'44" N, 21°47'20" E; 258m; 23.4.2012; *T. erythrospermum*; col. MD; det. RS.
3. Slovakia, village of Streda nad Bodrogom, north border of natural preserve Tarbucka, a grassy vegetation on sandy soil along field path; 48°22'08" N, 21°47'09" E; 206 m; 23.4.2012; *T. erythrospermum*; col. MD; det. RS.
4. Slovakia, village of Brehov, a grassy vegetation on the top of the hill Veľký vrch near the transmitter; 48°29'37" N, 21°48'45" E; 262 m; 22.4.2012; *T. erythrospermum, T. erythrospermum* f. *achyrocarpum*; col. MD; det. RS.
5. Slovakia, village of Sirník, a grassy vegetation on the top of the hill over the village; 48°31'07" N, 21°47'25" E; 226 m; 22.4.2012; *T. erythrospermum, T. prunicolor*; col. MD; det. RS.
6. Slovakia, village of Ladmovce, a termophilic grassy-herbaceous vegetation along field path in natural preserve Kašvár; 48°24'53" N, 21°46'32" E, 149 m; 23.4.2012; *T. erythrospermum, T. cristatum, T. danubium*; col. MD; det. RS.
7. Slovakia, village of Malá Bara, termophilic vegetation along field path on Hatfa, 900 m north-east from the village on the southern slope of the hill Brezina; 48°25'30" N, 21°44'31" E; 194 m; 20.4.2012; *T. erythrospermum*; col. MD; det. RS.
8. Slovakia, village of Veľká Bara, a grassy vegetation on the top of the hill Piliš near the transmitter; 48°25'42" N, 21°42'35" E; 267 m; 20.4.2012; *T. erythrospermum*; col. MD; det. RS.
9. Slovakia, village of Turňa nad Bodvou, a grassy vegetation in the natural preserve Turniansky hradný vrch, near the path to castle ruin; 48°36'35" N, 20°52'23" E; 300 m; 29.4.2012; *T. erythrospermum*; col. MD; det. RS.
10. Slovakia, village of Zádiel-Dvorníky, thermophilic grassy vegetation on northern slope of natural preserve Zemné hradisko; 48°36'32" N, 20°48'50" E; 260 m; 29.4.2012; *T. erythrospermum, T. cristatum*; col. MD; det. RS.
11. Slovakia, village of Krásnohorské Podhradie, Krásna Hôrka castle, a grassy hillside below the castle above the path leading to the castle; 48°39'29" N, 20°36'03" E; 477 m; 29.4.2012; *T. cristatum, T. danubium*; col. MD; det. RS.

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