Biosystematic study of the *Cyanus triumfetti* group in Central Europe

Biosystematická štúdia skupiny *Cyanus triumfetti* v strednej Európe

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Multivariate morphometrics and an assessment of genetic diversity obtained using amplified fragment length polymorphism (AFLP) were used to determine the variability of the polymorphic group *Cyanus triumfetti* in Central Europe. The ploidy level of the populations studied was also determined; all individuals from the *C. triumfetti* group were diploid (2n ~ 2x ~ 22) and all those of the related *C. montanus* group were tetraploid (2n ~ 4x ~ 44). A multivariate morphometric study of 71 populations revealed that three species from the *C. triumfetti* group occur in Central Europe, namely ‘*Cyanus axillaris*’, *C. strictus* and *C. dominii*. Three subspecies are recognized within the latter species, namely *C. dominii* subsp. *dominii*, *C. dominii* subsp. *slovenicus* and *C. dominii* subsp. *sokolensis*. Morphological characters of leaves are the best features for delimiting these taxa; a shift in characters caused by cultivation did not affect the value of key characters and differences among the taxa remained. AFLP analysis of 38 populations from the *C. triumfetti* group and two from the *C. montanus* group revealed a contrasting pattern of genetic variation that was related to the geographic distribution of the populations rather than the morphological variation in the *C. triumfetti* group. The AFLP data revealed the following three genetically differentiated and allopatric groups: (i) *C. triumfetti* s.s. and *C. montanus* from the Western Alps, (ii) ‘*C. axillaris*’ from Austria and the Czech Republic (except the Carpathians) and (iii) ‘*C. axillaris*’, *C. strictus* and *C. dominii* from the Western Carpathians and Pannonia. The striking genetic gap between the Austrian-Czech and the W Carpathian-Pannonia groups and the high genetic diversity and weak genetic differentiation within the latter group are discussed in the light of potential glacial refuges, postglacial migration routes and/or the probability of hybridization events occurring during the evolutionary history of this group. An identification key for the taxa of the *C. triumfetti* group in Central Europe is presented.

Keywords: *Asteraceae*, *Cyanus* sect. *Perennes*, flow cytometry, genetic variation, morphology, Pannonia, taxonomy, Western Carpathians

Introduction

The *Cyanus triumfetti* group is one of the many species-rich and taxonomically complicated aggregates of perennial knapweeds in the subtribe *Centaureinae* (Cass.) Dumort. There are two taxonomic opinions about the classification of *Cyanus* taxa: first, they should be included in *Centaurea* s.s. (e.g. Susanna & Garcia-Jacas 2007) or secondly, placed in a separate genus, *Cyanus* Mill. (e.g. Greuter 2006–2009). The genus *Cyanus* comprises 25 (Hellwig 2004) to 50 (Dostál 1969) species classified into two sections: *Cyanus* sect. *Cyanus* and *C.* sect. *Perennes* (Boiss.) (valid combination at the sectional level in the genus *Cyanus* is not available), differing in pollen type, basic chromosome number and life form. *Cyanus* sect. *Cyanus* consists of annual species with pollen type ‘*Cyanus*’ and basic chromosome numbers of x = 8, 9 or 12. *Cyanus* sect. *Perennes* consists of perennial species with pollen type '*Montana*' and basic chromosome number of x = 10 or 11. The molecular, karyological and morphological evidence indicate that *C.* sect. *Perennes* is monophyletic and originated from annual species of *C.* sect. *Cyanus* (Garcia-Jacas et al. 2001, Hellwig 2004, Löser et al. 2009).

Within *C.* sect. *Perennes*, the three following unranked groups of morphologically similar taxa are proposed in the taxonomic literature: the *C. triumfetti* group, the *C. montanus* group and the *C. napulifer* group (cf. Dostál 1931b, 1976b, Stefanov & Georgiev 1931, Borhidi 1957, Bancheva & Raimondo 2003, Greuter 2006–2009). However, these three groups have not yet been delimited using comparative biosystematic methods and several species of uncertain taxonomic position remain in this section.

The *C. triumfetti* group was first defined by Borhidi (1957), who included six species in this group, which in terms of the nomenclature used in the present paper correspond to: *C. triumfetti* (All.) Dostál ex Á. Löve et D. Löve, *C. graminifolius* (Lam.) Olšavská, *C. baldaccii* (Bald.) Holub, *C. pindicola* (Griseb.) Soják, *C. achtarovii* (Urum.) Holub and *C. pinnatifidus* (Schur) Holub. An additional 10 infraspecific taxa, which were included in the *C. triumfetti* group by Borhidi (1957), have been recently recognized as separate species and in terms of the nomenclature used in the present paper are listed as follows: *C. adscendens* (Bartl.) Soják; *C. albofimbriatus* (Stef. et T. Georgiev) Greuter; *C. angelescui* (Grinţ.) Holub; *C. axillaris* auct.; *C. bourgaei* (Boiss.) Wagenitz et Greuter; *C. dominii* (Dostál) Holub; *C. fuscomarginatus* (K. Koch) Greuter; *C. lingulatus* (Lag.) Holub; *C. matthiolifolius* (Boiss.) Wagenitz et Greuter; and *C. strictus* (Waldst. et Kit.) Soják (Czerepanov 1995, Greuter 2006–2009, this paper). The taxa of the *C. triumfetti* group differ from the remaining perennial species of *C.* sect. *Perennes* as they lack thickened roots, in which they differ from the *C. napulifer* group, and have pale fimbriae, which are longer than the width of the margin of the appendages of involucral bracts, in which they differ from the *C. montanus* group (cf. Dostál 1976b, Bancheva & Raimondo 2003, Štěpánek 2004). Taxa of the *C. triumfetti* group occur from Morocco across Europe (except the northern parts) to Iran (Greuter 2006–2009). Several taxa are widespread while others are narrow endemics restricted to certain geographical areas. The ecological differentiation among the taxa is substantial and they grow on sunny steppes or in rocky habitats, ranging from lowland to the subalpine belt.

The taxonomic treatment of the *C. triumfetti* group in floras and identification keys is often inconsistent and the delimitation of the taxa within the group is difficult because of their weak morphological differentiation. Moreover, intermediate morphotypes among some taxa and considerable intra-population variability are also reported (Millionová 2000, Olšavská et al. 2009). Such a pattern of morphological variation could be the result of a short time of speciation and/or weak reproductive isolation within the *C. triumfetti* group. Similar taxonomic complexity due to extreme morphological variation and a high degree of phenotypic plasticity also exists in other polymorphic groups in the Compositae, e.g. *Senecio nemorensis* agg. (Hodálová 1999), *Doronicum* L. (Álvarez Fernández & Nieto Feliner 2001), *Centaurea phrygia* agg. (Koutecký 2007), *Picris hieracioides* L. (Slovák & Marhold 2007) and *Centaurea stoebe* L. (Španiel et al. 2008).

Diploids and tetraploids with two basic chromosome numbers (x = 10, x = 11) are reported for taxa of the *C. triumfetti* group (Dostál 1976b, Goldblatt & Johnson 1979–2010, Olšavská & Perný 2009). Ongoing karyological analyses of European populations (Olšavská et al. 2009, K. Olšavská, unpublished) and the majority of previous studies on chromosome numbers (ca 75 reports; Goldblatt & Johnson 1979–2010, Millionová 2000, Bancheva & Greilhuber 2006, Marhold et al. 2007) report diploids with a basic chromosome number of x = 11 (2n = 2x = 22) for the *C. triumfetti* group. In addition to diploid chromosome numbers, there are four tetraploid counts with x = 11 (2n = 4x = 44) for the *C. triumfetti* group from southern Europe (Guinochet 1957, Lovrić 1982, Sharkova 1996). One of these tetraploid counts is for *Cyanus graminifolius* (Guinochet 1957, as for *Centaurea triumfetti* var. *seusana* Gugler). This count was not confirmed by the current analyses of plants from the same locality and the chromosome number corrected (x = 10; 2n = 4x = 40) indicates that *C. graminifolius* occupies a separate position within the *C. triumfetti* group (Olšavská & Perný 2009). Diploid counts with both the basic chromosome numbers x = 10 and x = 11 (2n = 2x = 20, 2n = 2x = 22) are reported for *C. lingulatus* and *C. pindicola*, Mediterranean taxa from the *C. triumfetti* group (Morales 1974, Strid & Franzen 1981, Montserrat Martí 1987, Baltisberger 1991). However, further studies on chromosome numbers, with special emphasis on determining the basic chromosome numbers, are needed to bring new insights to the relationships among perennial taxa of the genus *Cyanus*.

Four taxa from the *C. triumfetti* group, treated by various authors either as species or subspecies or varieties, are reported from Central Europe; they are listed as follows: *C. axillaris* auct., *C. dominii*, *C. strictus* and *C. triumfetti* s.s. (Dostál 1989, Marhold & Hindák 1998, Štěpánek 2004, Olšavská et al. 2009). However, a recent multivariate morphometric analysis revealed that *C. triumfetti* s.s. does not occur in this area (Olšavská et al. 2009).

Cyanus axillaris auct. is the most widespread taxon of the *C. triumfetti* group occurring almost throughout the whole distribution range of the group (except for the southernmost parts of Europe and Western Asia; Hellwig 2004, Greuter 2006–2009). *Cyanus axillaris* auct. is characterized by decurrent leaves, which are broadly lanceolate on the lower part of stem and non-silver fimbriae on the appendages of the involucral bracts. Because the nomenclature of this taxon is unresolved (see Nomenclature notes) it is hereinafter referred to as '*C. axillaris*'.

Cyanus strictus is morphologically similar to '*C. axillaris*' and it is difficult to distinguish between these two species (Štepánek 2004). *Cyanus strictus* is characterized by long erect stems branching mainly in the upper part and narrowly lanceolate leaves with a narrow and long decurrent part, and silver fimbriae that are twice as long as the width of the appendage's margin (Waldstein & Kitaibel 1805, Dostál 1976b). This species was described from an area on the border between Slovakia and Hungary (Zempléni-hegység Mts; Waldstein & Kitaibel 1805) and is reported from other countries in Central, eastern and southern Europe (Czech Republic, Croatia, Montenegro, Romania, Bulgaria and Ukraine; Horváth et al. 1995, Marhold & Hindák 1998, Mosyakin & Fedoronchuk 1999, Nikolič 2000, Oprea 2005, Greuter 2006–2009). Outside Central Europe, the two taxa are considered to be closely related to *Cyanus strictus*: taxon from western Ukraine originally described as *Centaurea ternopoliensis* Dobrocz, and *Cyanus angulescui* from south-eastern Romania and Moldavia (Dobrochayeva 1949, Dostál 1976b, Mosyakin & Fedoronchuk 1999, Ciocârlan 2000). However, their taxonomic relationships need to be studied in more detail.

Cyanus dominii was described by Dostál (1931a) as *Centaurea triumfetti* subsp. *dominii* Dostál from the Western Carpathians. This species can be clearly distinguished from '*C. axillaris*' and *C. strictus* by its non-decurrent or very short-decurrent stem leaves that are all approximately the same size (Dostál 1976b). There are different opinions on the distribution of this species depending on its treatment in current Floras and other works. *Cyanus dominii* is either considered to be endemic to the Western Carpathians (Dostál 1989, Marhold and Hindák 1998, Kliment 1999) or to have a wider distribution, including Bulgaria and Ukraine (Dostál 1976b, Andreev et al. 1992, Mosyakin & Fedoronchuk 1999, Greuter 2006–2009).

Five varieties are described within *Centaurea triumfetti* subsp. *dominii* by Dostál (1931a, b). However, these taxa are not mentioned in his later publications (Dostál 1950, 1976b, 1989) and only a few authors recognized them (e.g. Borhidi 1957). These five varieties are described as follows: (i) The nominal variety '*Centaurea triumfetti* subsp. *dominii* var. *eu-dominii*' described from Branisko Mts (Slovakia) is characterized by small cylindrical involucre and narrowly lanceolate or linear rigid leaves with a revolute margin and a glabrous upper surface and white-tomentose lower surface. (ii) *Centaurea triumfetti* subsp. *dominii* var. *slovenica* Dostál described from the Western Carpathians has oblong or lanceolate leaves that are tomentose both above and beneath. (iii) *Centaurea triumfetti* subsp. *dominii* var. *densifolius* Dostál was described from Slovenský kras Karst and is reported also from the Západné Tatry Mts in Slovakia. This variety is characterized by tomentose linear-lanceolate or linear leaves, a short pedunculus and a tiny capitulum. Populations from the Západné Tatry Mts were formerly described as *Centaurea axillaris* f. *sokolensis* Pawłowski (Pawłowski 1930). (iv) *Centaurea triumfetti* subsp. *dominii* var. *romanica* Dostál was described from the Domogled Mts in Romania (Dostál 1931b). Typical characters of this taxon are oblong to ovate, subglabrous leaves with serrate margins and stem foliose up to the capitulum. This variety is no longer reported from Romania (Oprea 2005). (v) *Centaurea triumfetti* subsp. *dominii* var. *perfoliosa* Dostál was described from the Čornohora Mts in Ukraine and is very similar to *Centaurea triumfetti* subsp. *domini* var. *romanica,* but differs in having tomentose leaves according to Dostál's description (Dostál 1931a).

Our previous analysis revealed complex morphological variation in the *Cyanus triumfetti* group in the Western Carpathians and a need for further taxonomic studies was emphasized (Olšavská et al. 2009). The aim of this study is to determine the morphological variation and relationships of Central European populations of the *C. triumfetti* group using morphometric analyses based on field-collected plants and plants grown in an experimental garden. The ploidy level of all populations studied was determined using flow cytometry because taxonomic conclusions based on multivariate morphometrics are often supported by ploidy level analysis (e.g. Suda & Lysák 2001, Marhold et al. 2005, Ekrt & Štech 2008). Morphometric and karyological analyses were supplemented by a pilot study of the genetic variability using amplified fragment length polymorphism (AFLP) markers. AFLP is a powerful method for assessing inter- and intra-specific genetic variation, especially in species groups that are closely related and/or have diverged recently (Bussell et al. 2005, Meudt & Clarke 2007). An approach combining AFLP and morphometric data has been successfully applied to a range of polymorphic plant groups, e.g. *Cardamine* L. (Lihová et al. 2004, Perný et al. 2004), *Festuca* ser. *Psammophilae* Pawlus (Šmarda et al. 2007), *Viola suavis* M. Bieb. (Mereďa et al. 2008), *Papaver alpinum* L. s.l. (Schönswetter et al. 2009) and *Prunus* L. sect. *Prunus* (Depypere et al. 2009). In order to confirm that *C. triumfetti* s.s. does not occur in the Western Carpathians and adjacent parts of Pannonia, which is indicated by a previous morphometric study (Olšavská et al. 2009), samples of this species from the Western Alps were included in the AFLP analyses.

The present study is a part of a broader investigation that aims to fill the gap in our knowledge about the taxonomy of the perennial taxa of the genus *Cyanus*. There are only a few biosystematic studies on the morphological and karyological diversity of *Cyanus* taxa (Millionová 2000, Bancheva and Raimondo 2003, Bancheva & Greilhuber 2006, Olšavská et al. 2009, Olšavská & Perný 2009). In addition, in the two preliminary studies of *Cyanus,* cpDNA and nrDNA sequences or isozymes were used (Löser et al. 2009, Bancheva et al. 2009; respectively) and some molecular phylogenetic studies on the subtribe *Centaureinae* using ITS and cpDNA were based on results for only one or two individuals of *Cyanus* sect. *Perennes* (Sussanna et al. 1995, Garcia-Jacas et al. 2001, 2006).

The specific questions addressed in this study are: (i) Which of the taxa from the *C. triumfetti* group occur in Central Europe? (ii) Which of the characters used in morphometric analyses can be used to separate the revealed taxa and which are not strongly affected by environmental conditions? (iii) Is the AFLP fingerprinting pattern related to the morphological variation of populations from the *C. triumfetti* group? (iv) Do molecular analyses confirm the results of previous morphological analyses (Olšavská et al. 2009), which indicate that *C. triumfetti* s.s. does not occur in Central Europe?

Material and methods

Plant material

Between 2005 and 2009, five to 20 plants were collected from 71 populations of the *C. triumfetti* group in Central Europe. In addition, samples from three populations of *C. triumfetti* s.s. and two populations of *C. montanus* from the Western Alps were collected for AFLP analyses (Table 1, Fig. 1). The number of plants collected per locality depended on their abundance. When plants grew in clusters (i.e. each cluster corresponds to a single genet), only one stem from a cluster was collected. Roots/rhizomes from 5–10 flowering plants (the same plants used for morphometric analyses of field-collected population samples) per population were transferred from the field and cultivated under similar environmental conditions in an experimental garden at Višňové (49°09'40"N, 18°47'13"E, 470 m a.s.l., Žilina district, northwestern Slovakia). Herbarium specimens from plants cultivated in an experimental garden were used to examine the genetically determined variation in morphological traits. Fresh leaf material from cultivated plants was used for flow cytometric analyses and leaves from selected cultivated plants were dried and preserved in silica gel for AFLP analyses. Consequently, all analyses were conducted on the same individuals. The aim of the AFLP analyses was to elucidate genetic variation over a wide distribution range of all taxa rather than to assess intrapopulation variation of a few populations. Therefore, one or two individuals from selected populations of the *C. triumfetti* group were chosen so as to cover the whole study area. Selected samples of *C. montanus* were used as the outgroup in the AFLP analyses because it is closely related to the *C. triumfetti* group.

Table 1. – Samples from the *Cyanus triumfetti* and *C. montanus* groups used for morphometric analyses (Mor F/G: number of field-collected/cultivated plants), karyological analyses (Kar 2x/2n: number of plants used for the measurement of the DNA ploidy level/for chromosome counting; * published in a previous study, Olšavská et al. 2009) and AFLP analyses (AFLP). Collection data are as follows: population code, country (CZ – Czech Republic, F – France, HU – Hungary, IT – Italy, SK – Slovakia); description of locality; geographic coordinates (WGS84); altitude; date of collection; name of collector (DD – D. Dítě, IH – I. Hodálová, PE – P. Eliáš Jr., KO – K. Olšavská, MO – M. Olšavský, MP – M. Perný, MV – M. Valachovič, RŠ – R. Šuvada, VK – V. Kolarčik).

Population code	Collection data	Mor F/G	Kar 2x/2n	AFLP
'*Cyanus axillaris*'				
TRI 1	SK; Devínska Kobyla Mts, Mt. Sandberg near Devínska Nová Ves; 48°11'55" N, 16°59'00" E; 340 m; 2 June 2005; KO	20/–	–/–	–
TRI 2	SK; Malé Karpaty Mts, S slope of Mt. Pohánska; 48°29'00" N, 17°15'55" E; 320 m; 3 June 2005; KO, IH & MP	20/–	–/–	–
TRI 4	SK; Považský Inovec Mts, S slope below Tematín Castle; 48°40'15" N, 17°57'45" E; 580 m; 17 June 2005; KO & MO	20/–	6*/1*	–
TRI 7	SK; Podunajská nížina Lowlands, SW slope of Mt. Zobor near Nitra; 48°20'49" N, 18°05'40" E; 362 m; 9 June 2006; KO & MP	20/4	3*/–	2
TRI 8	SK; Podunajská nížina Lowlands, Mt. Šipka near Plášťovce; 48°09'58" N, 18°59'47" E; 315 m; 10 June 2006; KO & MP	20/3	3*/–	1
TRI 16	SK; Považský Inovec Mts, Mt. Kamienka near Modrová; 48°38'37" N, 17°54'00" E; 245 m; 27 June 2006; MP	8/4	5*/4*	1
TRI 23	SK; Východoslovenská rovina Lowland, Mt. Veľký vrch near Brehov; 48°29'36" N, 21°48'35" E; 240 m; 5 July 2006; KO & MO	20/7	5*/–	–
TRI 34	SK; Belianske kopce Mts, Mt. Veľký vrch near Štúrovo; 47°49'45" N, 18°37'35” E; 240 m; 18 May 2007; KO & MO	15/3	5*/–	–
TRI 41	AU; Hainburger berge Mts, SW slope of Mt. Braunsberg; 48°09'15" N, 16°57'07” E; 291 m; 24 May 2007; KO & MO	17/6	6*/–	–
TRI 42	AU; Wienerwald Mts, Rauhenstein Castle near Baden; 48°00'46" N, 16°12'27” E; 324 m; 24 May 2007; KO & MO	14/6	8*/–	2
TRI 43	AU; Drosendorf Stadt, view point 1.5 km N of the town; 48°52'27" N, 15°37'52” E; 434 m; 25 May 2007; KO & MO	20/8	6*/–	2
TRI 45	CZ; Moravská vrchovina Mts, rocks NE of Moravský Krumlov; 49°03'39" N, 16°19'40” E; 283 m; 26 May 2007; KO & MO	18/3	5*/–	–
TRI 46	CZ; Moravská vrchovina Mts, rocks near Ivančice; 49°05'20" N, 16°20'25” E; 258 m; 26 May 2007; KO & MO	20/7	7*/–	–
TRI 47	CZ; Pavlovské vrchy Mts, Mt. Děvín near Horní Věstonice; 48°51'53" N, 16°38'37” E; 424 m; 26 May 2007; KO & MO	20/6	7*/–	2
TRI 48	CZ; Pavlovské vrchy Mts, Svatý kopeček hill near Mikulov; 48°48'24" N, 16°38'44” E; 356 m; 27 May 2007; KO & MO	16/5	6*/–	–
TRI 49	CZ; Biele Karpaty Mts, Žerotín hill near Radějov; 48°51'47" N, 17°19'38” E; 321 m; 28 May 2007; KO & MO	14/5	6*/–	1
TRI 56	SK; Volovské vrchy Mts, meadow near Trebejov; 48°50'06" N, 21°13'21" E, 253 m; 7 July 2006; KO & MO	15/–	4/–	1
TRI 70	SK; Záhorská nížina Lowlands, loc. Široká near Malacky, 48°24'44" N, 17°04'14" E; 193 m; 22 June 2007; MP & MV	9/4	4/–	1
TRI 75	HU; Gerecse Mts, meadows above Tatabánya; 47°34'51" N, 18°24 59" E; 290 m; 12 May 2008; KO, MP & IH	13/–	4/–	–
TRI 76	HU; Visegrádi-hegység Mts, Mt. Vaskapu near Esztergom; 47°47'10" N, 18°46'20" E; 292 m, 13 May 2008, KO, MP & IH	13/–	4/–	–
TRI 77	HU; Mecsek Mts, road to television tower (Misina) near Pécs, 46°05'59" N, 18°13 03" E; 522 m; 14 May 2008; KO & MP	13/–	4/–	–
TRI 78	HU; Mecsek Mts, viewpoint Dömörkapu near Pécs, 46°05'59" N, 18°14'01" E; 404 m; 14 May 2008; KO & MP	15/–	5/–	1
TRI 79	HU; Mecsek Mts, along the road to Tubes near Pécs, 46°06'38" N, 018°11'53" E; 407 m; 14 May 2008; KO & MP	17/–	5/–	–

Population code	Collection data	Mor F/G	Kar 2x/2n	AFLP
TRI 80	HU; Vértes Mts, meadow by the road to Köhányás; 47°26'33" N, 18°23'34" E; 15 May 2008, KO & MP	20/–	2/–	–
TRI 81	HU; Tési-fennsík Mts, Mt. Vár-Berek near Várpalota, 47°14'15" N, 18°06'04" E, 455 m; 15 May 2008; KO & MP	18/–	3/–	1
TRI 82	HU; Veszprémfajsz, Mt. Király-hegy, 47°02'02" N, 17°53'20" E; 370 m; 15 May 2008; KO & MP	12/–	5/–	–
TRI 83	HU; Budai-hegység Mts, Mt. Széchenyi-hegy near Budapest; 47°29'21" N, 18°58'52" E; 441 m; 16 May 2008; KO & MP	14/–	4/–	2
TRI 84	HU; Budai-hegység Mts, SW slope of Mt. Kis-hárs-hegy; 47°31'46" N, 18°57'55" E; 357 m; 16 May 2008; KO & MP	20/–	5/–	–
TRI 87	AU; Wiener Wald Mts, Mt. Leopoldsberg near Klosterneuburg; 48°16'41" N, 16°20'38" E; 397 m; 22 May 2008; KO & MO	20/–	2/–	–
TRI 88	AU; Wiener Wald Mts, slope above Bisamberg; 48°19'09" N, 16°21'39" E; 360 m; 22 May 2008; KO & MO	13/–	3/–	–
TRI 89	AU; Leiser Berge Mts, Dörfles, above ZOO garden; 48°32'40" N, 16°21'06" E; 371 m; 22 May 2008; KO & MO	20/–	4/–	1
TRI 90	AU; Wachau, meadow E of Dürnstein; 48°23'36" N, 15°31'55" E; 275 m; 22 May 2008; KO & MO	20/–	4/–	2
TRI 92	CZ; Český Kras Karst, Radotínske údolí valley near Radotín; 49°26'13" N, 14°19'47" E; 240m; 24 May 2008; KO & MO	20/–	4/–	1
TRI 93	CZ; České Středohoří Mts, E margin of Hlinná; 50°34'23" N, 14°06'44" E; 464 m; 24 May 2008; KO & MO	20/–	3/–	1
TRI 94	CZ; Moravský Kras Karst, Mt. Hády near Brno; 49°13'14" N, 16°40'29" E; 422 m; 25 May 2008; KO & MO	20/–	1/–	1
TRI 95	SK, Biele Karpaty Mts, Mt. Veterník near Skalica; 48°48'48" N, 17°43'58" E; 312 m; 25 May 2008; KO & MO	13/–	5/–	–
TRI 96	CZ; Český Kras Karst, above abandoned quarry in Velká Chuchle; 50°00'56" N, 14°22'24" E; 279m; 24 May 2008; KO & MO	5/–	2/–	–
Cyanus strictus				
TRI 22	HU; Zempléni-hegység Mts, hill with a cross above Tállya; 48°16'09" N, 21°11'35" E; 322 m; 5 July 2006; KO & MO	16/7	5*/–	1
TRI 35	HU; Bükk Mts, SE part of Mt. Bel-kö near Bélapátfalva; 48°02'30" N, 20°22'10" E; 630 m; 19 May 2007; KO & MO	14/4	5*/–	1
TRI 36	HU; Zempléni-hegység Mts, hill NW of Mád; 48°11'51" N, 21°16'00" E; 174 m; 20 May 2007; KO & MO (locality very close to the type locality of *Centaurea stricta* Waldst. et Kit)	20/3	4*/–	1
TRI 37	HU; Zempléni-hegység Mts, hill with a cross above Erdöbénye; 48°15'40" N, 21°21'40" E; 227 m; 20 May 2007; KO & MO	20/6	5*/–	2
TRI 38	HU; Zempléni-hegység Mts, in vineyards by Tolcsva; 48°16'13" N, 21°23'18" E; 159 m; 20 May 2007; KO & MO	20/7	6*/–	–
TRI 39	SK; Zemplínske vrchy Mts, E slope of Mt. Piliš near Veľká Bára; 48°25'52" N, 21°42'45" E; 224 m; 21 May 2007; KO & MO	15/6	6*/–	–
TRI 40	SK; Zemplínske vrchy Mts, Mt. Šimonov vrch near Malá Tŕňa; 48°26'28" N, 21°41'23" E; 173 m; 21 May 2007; KO & MO	15/1	5*/–	–
TRI 112	HU; Bükk Mts, rocks on the top of Három-kó hill; 48°03'34" N, 20°28'26" E; 905 m; 9 July 2008; KO, MO & MP	14/–	4/–	–
TRI 113	HU; Bükk Mts, meadow on the top of Tár-kó hill; 48°03'22" N, 20°27'42" E; 944 m; 9 July 2008; KO, MO & MP	16/–	5/–	2
Cyanus dominii subsp. *dominii*				
TRI 25	SK; Volovské vrchy Mts, Mt. Humenec near Veľká Lodina; 48°51'34" N, 21°09'33" E; 280 m; 6 July 2006; KO & MP	15/5	4*/2*	1
TRI 57	SK; Volovské vrchy Mts, rocks near Malá Lodina; 48°51'51" N, 21°05'54" E; 950 m, 5 July 2008, KO & MO	14/8	7/–	–

Population code	Collection data	Mor F/G	Kar 2x/2n	AFLP
TRI 110	SK; Branisko Mts, pine forest on the W slope of Mt. Rajtopíky; 49°00'00" N, 20°51'37" E; 950 m; 5 July 2008; KO & MO (locality cited in the protologue of ‘*Centaurea triumfetti* subsp. *dominii* var. *eu-dominii*’ Dostál)	15/–	5/–	2
TRI 111	SK; Volovské vrchy Mts, Mt. Folkmarské skaly near Veľký Folkmar; 48°49'37" N, 21°0'46" E; 836 m; 5 July 2008, KO & MO	20/–	7/–	2
Cyanus dominii subsp. *slovenicus*				
TRI 3	SK; Podtatranská kotlina Basin, Mt. Mních near Ružomberok; 49°05'15" N, 19°19'55" E; 500 m; 11 June 2005; KO & MO	20/6	6*/–	–
TRI 6	SK; Turčianska kotlina Basin, rocks by the road to Šútovo; 49°08'50" N, 19°05'30" E; 500 m; 28 May 2006; KO & MO	20/5	5*/–	–
TRI 13	SK; Veľká Fatra Mts, central part of Gaderská dolina valley; 48°56'40" N, 19°00'35" E ; 580 m; 17 June 2006; KO & MO	10/4	5*/–	–
TRI 14	SK; Horehronské podolie Basin, Mt. Horné lazy near Valaská; 48°49'40" N, 19°36'00" E; 620 m; 20 June 2006; KO & MO	20/2	4*/2*	1
TRI 18	SK; Spišsko-Gemerský kras Karst, ravine by Vernár; 48°56'00" N, 20°17'15" E; 760 m; 28 June 2006; KO, IH & MP	11/1	3*/1*	–
TRI 19	SK; Chočské vrchy Mts, Prosiecká dolina valley near Prosiek; 49°09'00" N, 19°29'55" E; 640 m; 1 July 2006; KO & MO	13/2	3*/–	–
TRI 27	SK; Nízke Tatry Mts, Mt. Vachtárová near Kráľová Lehota; 49°01'25" N, 19°48'05" E; 540 m; 14 July 2006; KO & MO	11/4	5*/1*	–
TRI 53	SK; Súľovské vrchy Mts, cottage Súľovčanka near Súľov; 49°10'05" N, 18°34'35” E; 410 m; 2 June 2007; KO & MO	17/2	5*/–	1
Cyanus dominii subsp. *sokolensis*				
TRI 5	SK; Slovenský kras Karst, hill above Turňa nad Bodvou; 48°36'43" N, 20°52'20" E; 349 m; 29 May 2007; KO, DD, RŠ & PE	14/5	5*/–	1
TRI 21	SK; Slovenský kras Karst, E slope above Slavec; 48°35'59" N, 20°27'59" E; 312 m; 22 June 2008, KO & MO	20/1	3/–	–
TRI 33	SK; Západné Tatry Mts, Mt. Mních near Bobrovec; 49°10'40" N, 19°38'45" E; 1380 m; 3 August 2006; KO & MO (locality cited in the protologue of *Centaurea axillaris* var. *sokolensis* Pawł.)	9/2	6*/–	–
TRI 44	SK; Západné Tatry Mts, Mt. Sokol near Bobrovec; 49°10'29" N, 19°38'17” E; 1325 m; 21 July 2007; KO & DD (locality cited in the protologue of *Centaurea axillaris* var. *sokolensis* Pawł.)	15/1	8*/–	2
TRI 50	SK; Slovenský kras Karst, Zádielská dolina valley near Zádiel; 48°37'37" N, 20°50'01” E; 567 m; 29 May 2007; KO, DD, RŠ & PE (locality cited in the protologue of *Centaurea triumfetti* subsp. *dominii* var. *densifolia* Dostál)	20/6	6*/–	2
TRI 51	SK; Slovenský kras Karst, Mt. Zajačia strana hill; 48°35'15" N, 20°43'40” E; 250 m; 30 May 2007; KO, DD, RŠ & PE	12/4	4*/–	–
TRI 109	SK; Slovenský kras Karst, Mt. Stráň near Jelšavská Teplica; 48°36'20" N, 20°16'36" E; 340 m, 22 June 2008, KO & MO	20/–	3/–	1
Intermediate morphotype between ‘*Cyanus axillaris*’ and *Cyanus dominii*				
TRI 9	SK; Slovenský kras Karst, limestone rocks above Domica Cave; 48°28'40" N, 18°28'15" E; 359 m; 12 June 2006; KO & MP	11/6	6*/–	–
TRI 10	SK; Slovenský kras Karst, SW slope below Krásna Hôrka Castle; 48°39'31" N, 20°35'55" E; 427 m; 12 June 2006; KO & MP	20/5	5*/–	–
TRI 20	SK; Slovenský kras Karst, meadow W of Gemerská Hôrka; 48°32'25" N, 20°21'01" E; 282 m; 22 June 2008, KO & MO	20/2	3/–	1
TRI 31	SK; Pieniny Mts, SW part of Mt. Holica near Lesnica; 49°24'35" N, 20°26'20" E; 540 m; 1 August 2006; KO & DD (locality cited in protologue of *Centaurea axillaris* var. *pieninica* Pawł.)	12/4	1*/1*	1

Population code	Collection data	Mor F/G	Kar 2x/2n	AFLP
TRI 52	SK; Slovenský kras Karst, Mt. Veľký Paklán; 48°35'18" N, 20°43'30" E; 253 m; 30 May 2007, KO, DD, RŠ & PE	20/5	2/–	1
TRI 74	SK; Pieniny Mts, Mt. Haligovské skaly near Paluby; 49°22'55" N, 20°27'35” E; 680 m; 20 July 2007; KO & DD	7/3	3*/–	1
Cyanus triumfetti s.s.				
TRI 60	IT; Valle del Chisone valley, Forte Fenestrellum Fort; 45°01'58" N, 07°03'35” E; 1303 m; 13 June 2007; KO, MP & DD	–/–	5*/–	1
TRI 63	IT; Valle del Chisone valley, meadow close to Sestriere; 45°05'15" N, 06°26'09” E; 1980 m; 14 June 2007; KO, MP & DD	–/–	10*/–	2
TRI 67	F; Alpes Maritimes Mts, road to Colle di Tende saddle; 44°06'03" N, 07°31'24” E; 2013 m; 17 June 2007; KO, MP & DD	–/–	6*/–	2
Cyanus montanus				
MON 3	F; Massif du Mţ. Cenis Mts, Lac Du Mont Cenis lake; 45°14"38' N, 6°56"49' E; 2108 m; 18 June 2007 ; KO, MP & DD	–/–	4/–	1
MON 6	F; Queyras Mts; meadow near Ceillac; 44°40"23' N, 6°46"10' E; 1706 m; 4 June 2008; KO, MO & VK	–/–	3/–	2

Plant material also included samples from populations at localities (or wider areas) from which the taxa studied were described. TRI 110 is referred to in the description of ‘*Centaurea triumfetti* subsp. *dominii* var. *eu-dominii*’ (Dostál, 1931a) as “Slovakia centralis, in rupibus trachyticis montis Bránisko, altitudine 800 m s. m., leg Jos. Dostál, 1928”; TRI 50 is referred to in the description of *Centaurea triumfetti* subsp. *dominii* var. *densifolius* (Dostál 1931a) as “Slovakia meridionalis, in rupibus calcareis vallis Zadielská rokle apud opp. Košice, leg Dostál, 1928”; TRI 31 and TRI 74 are referred to in the description of *Centaurea axillaris* var. *pieninica* Pawł. (Szafer et al. 1924) as “Pien., Tatry Bielskie”; and TRI 33 and TRI 44 are referred to in the description of *Centaurea axillaris* f. *sokolensis* Pawł. (Pawłowski 1931) as “Skały wapienne Sokol 1235–1320 m; Mnich 1462 m”. No typical population could be chosen for *Centaurea triumfetti* subsp. *dominii* var. *slovenica* because Dostál (1931a) did not mention any specific locality in the description of this variety (just “Slovakia, in Carpatis occidentalibus”) and this name was not typified. Material from the type locality of *C. strictus* [indicated as “auf Tockayer Berg war fast ausgeblüht auf Anfang Septembris”; lectotypified by Chrtek & Skočdopolová (1982)] is not included in this study for technical reasons. Nevertheless, population TRI 36 is located very close to the type locality of *C. strictus* and localities of populations TRI 36 and TRI 37 are mentioned on labels of the original herbarium material of *C. strictus* collected by Waldstein and Kitaibel and deposited in Herbarium Kitaibelianum in BP [indicated as “in collibus et montibus vitiferis, Hegyallya dictis, velut ad Máda, Erdöbénye”; “von Erdöbénye”; “Von Máda … Gebürg” (Jávorka 1926)]. Furthermore no original material of ‘*C. axillaris*’ was included in this study for two reasons. First, the illegitimate name coined by Willdenow (*Centaurea axillaris* Willd.) and all combinations based on this name are according to the Code of Botanical Nomenclature (McNeill et al. 2006) connected to the type of *C. graminifolius*. However, a recent karyological study revealed that *C. graminifolius* differs from Central European populations of the *C. triumfetti* group traditionally treated as ‘*C. axillaris*’ (Olšavská & Perný 2009, see the Introduction and

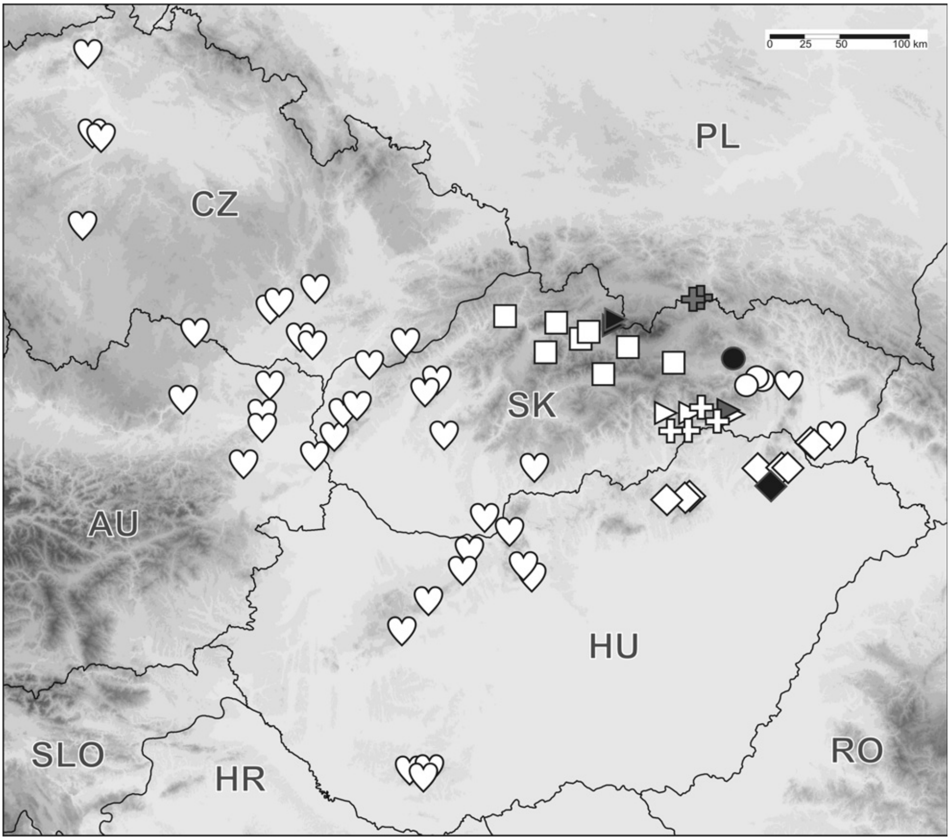


Fig. 1. – Map showing the distribution of the populations of the *Cyanus triumfetti* and *C. montanus* groups analysed in this study: ♡ '*C. axillaris*', ◇ *C. strictus* (◆ area close to the type locality of *Centaurea stricta*), ○ *Cyanus dominii* subsp. *dominii* (● area close to the locality cited in the protologue of '*Centaurea triumfetti* subsp. *dominii* var. *eu-dominii*'), □ *Cyanus dominii* subsp. *slovenicus*, ▷ *C. dominii* subsp. *sokolensis* (▶ the locality cited in the protologue of *Centaurea triumfetti* subsp. *dominii* var. *densifolia*; ▶ the localities cited in the protologue of *Centaurea axillaris* var. *sokolensis*) and ✙ intermediate morphotype between '*Cyanus axillaris*' and *C. dominii* (✚ localities close to the area cited in the protologue of *Centaurea axillaris* var. *pieninica*). For sample site details see Table 1.

Nomenclature notes). The second reason is that Willdenow's (1803) description of *Centaurea axillaris* lacks a specific geographical location ["Habitat in montibus Austriae, Hungariae, Helvetiae, et Galliae australis. (v.v.)" is in the protologue; "Habitat in collinis Austriae" is on the label of Willdenow's original herbarium material deposited in B]. Thus, it was not possible to identify the origin of plants that fit Willdenow's description of '*C. axillaris*'.

Plant material from the field and experimental garden was treated similarly for the morphometric analyses. The selected morphological characters of the terminal capitulum (length and width of involucre, number of interior and exterior florets) were measured or scored on fresh plants. Then herbarium specimens of the plants were prepared, with florets and involucral bracts attached to the paper by adhesive tape; the remaining morphological characters were measured or scored on these specimens (for more details see Olšavská et al. 2009). Voucher specimens are deposited in the Herbarium SAV.

Morphometric analyses

Altogether, 52 morphological characters were measured or scored on fresh or herbarium material from field-collected and cultivated plants for the multivariate morphometric study. The characters included one semiquantitative, four binary and 35 quantitative characters and another 12 computed ratios (Table 2). The semiquantitative character, colour of fimbriae of appendages of involucral bracts (AFCO) was initially coded as three binary characters (Table 2); then the first two binary characters (AFCO1, AFCO2) were included in the matrix. The characters include those used in a previous study (Olšavská et al. 2009) as well as additional characters obtained by a preliminary examination that were deemed useful for distinguishing the Central European taxa of the *C. triumfetti* group.

Two matrices were prepared for the morphometric analyses. The first was composed of a total of 1148 field-collected individuals from 71 populations. The second matrix consisted of plants cultivated in an experimental garden and comprised of a total of 195 individuals from 43 populations. Depending on the analyses listed below, the values of the characters of the individual plants or the average values of the characters for the populations were used as operational taxonomic units (OTUs). Shapiro-Wilk test (W; Shapiro & Wilk 1965) with associated probability (Prob < W), measures of skewness and kurtosis were computed to detect departures from normality of characters within the groups of individuals or groups of populations (groups were based on results of PCA and PCoA analyses). These tests showed that all variables more or less deviated from a normal distribution. Spearman correlation coefficients (Sneath & Sokal 1973, Krzanowski 1990) were computed for each matrix of field-collected and cultivated individuals to detect pairs of highly correlated characters and to exclude one character from a pair of highly correlated characters from further analyses.

Principal component analysis (PCA; Krzanowski 1990) of all field-collected populations (average values for populations were used as OTUs) was used to provide insights into the overall pattern of morphological variation and to create hypotheses about population groupings. Principal coordinate analysis (PCoA1; Krzanowski 1990) was computed on one of the groups of field-collected populations revealed by PCA. PCoA was used because the number of populations within the revealed group was lower than the number of characters. A separate PCoA of field-collected populations of '*C. axillaris*' (PCoA4) was computed to compare the grouping of populations resulting from the AFLP analyses (see below) according to morphological pattern and geographical origin of the populations. In both PCoA1 and PCoA2, the average values for the populations were used as OTUs. The characters in the above mentioned PCA and PCoA analyses were standardized to have zero mean and unit standard deviation and the Euclidean distance was used to compute the secondary distance matrix.

Three canonical discriminant analyses (CDA; Klecka 1980) using field-collected individuals as OTUs were computed to determine the characters that mostly contribute to the separation of the following groups (these characters were used for the identification key): CDA1 – *C. dominii* vs the group of '*C. axillaris*' and *C. strictus*; CDA2 – '*C. axillaris*' vs

Table 2. – List of characters scored or measured for morphometric analyses. Characters marked with an asterisk were measured three times from three different floral parts or involucre bracts of the same inflorescence.

Plant part	Code	Character	Character explanation/ measurement unit
Stem	SI	indument of stem	0 – hairy, 1 – glabrous
	BRN	number of branches	–
	LN	number of leaves	–
	SL	stem length	mm
	SBRL	stem length up to branching	mm
	PEL	pedunculus length	mm
	Ratios:	SBRL/SL, (SL–PEL)/LN	–
Stem leaves	LAI	indument on upper surface of leaves	0 – hairy, 1 – glabrous
	LBI	indument on lower surface of leaves	0 – hairy, 1 – glabrous
	LLN	maximum number of leaf lobes or indentations	–
	LLD	depth of maximum leaf lobe or indentation	mm
	LUL	length of the uppermost stem leaf	mm
	LUW	width of the uppermost stem leaf	mm
	LUD	distance of the widest part of the uppermost stem leaf from leaf base	mm
	LUBW	width of base of the uppermost stem leaf	mm
	LUDL	length of decurrent part of the uppermost stem leaf	mm
	LML	length of the middle stem leaf	mm
	LMW	width of the middle stem leaf	mm
	LMD	distance of the widest part of the middle stem leaf from leaf base	mm
	LMBW	width of base of the middle stem leaf	mm
	LMDL	length of decurrent part of the middle stem leaf	mm
	Ratios:	LUW/LUL, LUD/LUL, LMW/LML, LMD/LML, LUL/LML, PEL/LUL	–
Involucre	BN	number of involucral bracts	–
	AFN	number of fimbries or teeth on appendage of involucre bract	–
	AMCO	colour of margin of appendages	0 – black to dark brown, 1 – pale brown
	AFCO	colour of appendage fimbriae or teeth	1 – black to dark brown (AFCO 1), 2 – pale brown (AFCO 2), 3 – white
	IL	involucre length	mm
	IW	involucre width	mm
	ID	distance of the widest part of involucre from base	mm
	*BL	average lenght of involucral bract	mm
	*BW	average width of involucral bract	mm
	*BD	average distance of the widest part of involucral bract	mm
	*AMAW	average width of margin of appendage of involucral bract at the apex	mm
	*AMMW	average width of margin of appendage of involucral bract in the middle	mm
	*AFAL	average length of fimbria or teeth at apex of appendage of involucral bract	mm
	AFML	average length of fimbria or teeth in the middle of appendage of involucral bract	mm
	Ratios:	IW/IL, ID/IL, AFAL/AMAW, AFML/AMMW	
Floret	FEN	number of exterior florets	–
	FIN	number of interior florets	–
	*FEL	average length of exterior floret	mm
	*FIL	average length of interior floret	mm
	*PL	average length of petal	mm
	*PW	average width of petal	mm

C. strictus; and CDA3 – separation among three subspecies of *C. dominii*. To determine which characters were not strongly influenced by the environment and would therefore be useful for identification, CDA4 was computed using individuals as OTUs and two groups: field-collected plants vs cultivated plants. Finally, to determine differences in ecological plasticity among revealed taxa, four CDA analyses using individuals as OTUs were performed: CDA5 – field-collected vs cultivated plants of '*C. axillaris*'; CDA6 – field-collected vs cultivated plants of *C. strictus*; CDA7 – field-collected vs cultivated plants of *C. dominii* subsp. *slovenicus* and CDA8 – field collected vs cultivated plants of *C. dominii* subsp. *sokolensis*. Only the populations for which both field-collected and cultivated plants (Table 1) were available were used in the CDA4 to CDA8 analyses. Field-collected vs cultivated plants of *Cyanus dominii* subsp. *dominii* were not analysed by separate CDA because of insufficient number of plants. Classification discriminant analysis (DA; Klecka 1980), using field-collected individuals as OTUs, was performed to calculate the degree of separation; the five groups were designated according the results of PCA and PCoA. Given that the distribution of some characters within the groups was not normal, a non-parametric k-nearest-neighbour method (with k = 2) DA was used.

Univariate statistics (mean, 5 and 95 percentiles) of quantitative characters and frequencies of qualitative characters of field-collected plants were calculated for groups revealed by PCA, PCoA, CDA and DA. Box-and-whisker plots of the six most discriminant characters of field collected and cultivated plants were also plotted.

The SYNTAX package (Podani 2001) was used to calculate PCoA and the remaining morphological analyses were conducted using the SAS 8.2 statistical package (SAS Institute 2000).

Flow cytometry

The ploidy level was newly estimated for 123 individuals from 32 populations using the PI flow cytometry and Beckton Dickinson FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) or DAPI flow cytometry and Partec Cyflow ML instrument (Partec GmbH, Münster, Germany) equipment with an HBO-100 mercury arc lamp. When PI staining was used, samples were prepared using the method described by Olšavská et al. (2009). The following simplified two-step protocol (Doležel et al. 2007) was used for DAPI flow cytometry: young intact leaf tissue of the analyzed plant was chopped together with an internal standard in 1 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20), then the sample was filtered throught 42-μm nylon mesh and 1 ml of a solution containing Otto II buffer (0.4 M $Na_2HPO_4 \cdot 12H_2O$), 2-mercaptoethanol (2 μl/ml) and DAPI (4 μg/ml) was added to the flow-through fraction and stained for 1–2 min; flow cytometric histograms were evaluated using Partec FloMax software (v. 2.7d; Partec GmbH Münster, Germany). *Lycopersicum esculentum* Mill. 'Stupnické polní tyčkové rané' was used as an internal standard in all flow cytometric analyses. Relationship between chromosome number and DNA content was verified by using published chromosome counts (Table 1; Olšavská et al. 2009).

Molecular techniques

A total of 55 individuals from 40 populations were chosen for AFLP analyses. Genomic DNA was extracted from leaf fragments dried in silica gel using the NucleoSpin Plant II kit (Macherey-Nagel GmBH & Co KG, Düren, Germany). The quality of the extracted DNA was checked on 1% agarose gel and extracts were quantified with Spectrophotometer ND-1000 (NaNoDrop Technologies, Inc.). The extracts were diluted with UHP water (Rotisolv ® HPLC Gradient Grade; Roth, Karlsruhe, Germany) and the DNA concentrations of the aliquots were adjusted to 15 ng/µl. The AFLP procedure (Vos et al. 1995) followed the general protocol provided by Applied Biosystems (Applied Biosystems, 2005) with a few modifications. Restriction of the genomic DNA and ligation were performed separately. Restriction with *Tru*1I (equivalent with *MseI*) and *Eco*RI endonucleases was performed at 37 °C for 3 h and then at 65 °C for 10 min; the reaction mixture (total volume 15 µl) contained 2.5 U of *Eco*RI, 1.5 U of *Tru*1I, 2.5 µl of 10x Tango buffer (all reaction components were from Fermentas Inc., Ontario, Canada), 2.1 µl of UHP water (Roth) and 20 µl of DNA (ca 300 ng). Then, 5 µl of the ligation mixture containing 0.5 µl 1U T4 DNA ligase (Fermentas, Inc.), 0.5 µl of T4 DNA ligase buffer, 0.6 µl of ATP (10 mM), 2.4 µl of UHP water and 0.5 µl of each *Mse*I and *Eco*RI adaptors (both MWG Biotech) was added to each sample; the incubation continued overnight (for 16 h) at room temperature (20 °C) in a thermal cycler (Master® Gradient, Eppendorf, Hamburg, Germany). Both preselective and selective amplification followed the protocol of Vos et al. (1995). The amplifications were performed in two steps in a thermocycler (Master® Gradient, Eppendorf, Hamburg, Germany). For preselective amplification 1.5 µl of restriction-ligation product was used as the template; the pre-amplification mixture (total volume of 3 µl) further contained 0.7 µl of 10x PCR buffer (including 15 mM $MgCl_2$, Qiagen, Hilden, Germany), 0.14 µl of dNTPs (10 mM of each), 0.035 µl of AmpliTaq polymerase (5 U $µl^{-1}$; Qiagen), 4.225 µl of UHP water (Roth) and 0.2 µl of each primer [EcoRI-A, MseI-C (both 50 ng/µL); MWG Biotech, Ebersberg, Germany]. Preselective amplification was performed using the following cycle profile: initial hold at 94 °C for 2 min; 30 cycles at 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 2 min; the last step was performed at 64 °C for 30 min and then cooled to 4 °C. The products of preselective amplification were checked on 1% agarose gel and were quantified spectrophotometrically; the DNA concentrations of products were adjusted to 30 ng/µl prior to selective amplification by dilution with UHP water (Roth). Altogether, 12 primer combinations were tested on a small number of samples. The three combinations that gave the best results in terms of the clarity of the traces, the number of bands and the number of polymorphic bands were the following primer pairs: EcoRI-ACT/MseI-CAC, EcoRI-ACC/MseI-CAC and EcoRI-ACA/MseI-CTG (MWB Biotech); these were then used with all samples. Selective amplification was performed for each primer combination separately; the EcoRI primer was fluorescently labelled with WellRED D4-PA (Sigma-Aldrich Inc., Germany). The PCR reaction mixture (total volume of 4.21 µl) consisted of 3.14 µl of AFLP CoreMix (Applied Biosystems, Foster City, CA, USA), 0.65 µl of diluted preamplification product and 0.21 µl of each selective primer (*Eco*RI primers at 1 µM and *Mse*I primers at 5 µM). The PCR conditions for selective amplification were as follows: initial cycle at 94 °C for 10 min; 10 cycles at 94 °C for 20 s, 66–56 °C for 30 s (the temperature was reduced by 1 °C after each cycle) and 72 °C for 2 min; 25 cycles at 94 °C for 20 s, 56 °C for 30 s and 72 °C for 2 min; and the last step was performed at 60 °C for 30 min and then cooled to 4 °C. The PCR products were added by mixing 10 µl of a premix containing 1.4 µl of EDTA (100 mM), 1.4 µl of sodium acetate (3 M), 0.67 µl of Glycogen (20 mg/ml) and 6.7 of µl UHP water. The PCR products were purified by ethanol precipitation. The pellets was resuspended in 25 µl of

Sample Loading Solution (Beckman Coulter, Fullerton, CA, USA) followed by 0.15 μl of CEQ DNA Size Standard – 400 (PN 608098, Beckman Coulter). The samples were subjected to capillary electrophoresis on a CEQ^{TM} 8000 DNA Analysis System and raw AFLP data were collected and sized using the CEQ^{TM} 8000 Fragment Analysis System V.9 (both Beckman-Coulter). The AFLP profiles were scored using the GenoGrapher 1.6.0 (available at http://hordeum.msu.montana.edu/genographer). Amplified fragments between 50 and 500 base pairs (bp) were scored by visual inspection for the presence (1) or absence (0) of peaks in the output traces. Only distinct peaks were scored as present; the resulting binary matrices of AFLP bands were used to carry out genetic data analysis. Eight samples were analysed on two independent occasions to estimate the reproducibility of the AFLP data. The AFLP profiles of the replicates were scored and compared with each other to calculate the error rate (Bonin et al. 2004).

Genetic data analysis

Three methods were used on the entire dataset of 55 samples to obtain a general view of the variation in the AFLP pattern:

(i) Neighbour-joining tree analysis (NJ tree) was carried out using the software TREECON (version 1.3b; Van de Peer & De Wachter 1994) and Nei and Li's (1979) genetic distance. Three accessions of *C. montanus* were used to root the tree. Group support was assessed using the same software with repeated bootstrap analyses with 2000 replications.

(ii) The principal coordinate analysis using Jaccard's similarity coefficient was performed using SYN-TAX 2000 (Podani 2001). To achieve a better resolution two PCoA were performed; PCoA2 was based on the complete dataset and PCoA3 on one of the groups revealed by PCoA2.

(iii) In addition to phenetic clustering Bayesian clustering in the software BAPS 3.2 (Corander et al. 2006) was applied using the module clustering of individuals. For the complete dataset and the main groups revealed by PCoA, NJ and Bayesian cluster analyses the following parameters were calculated: the total number of fragments per taxon/group, the average, minimum and maximum number of fragments per individual and the number of polymorphic, private and private fixed fragments per taxon/group. The patterns in fragment sharing and the number of phenotypes were also computed.

Results

Morphometric analyses

One highly correlated pair of characters was found in both the matrix of the information on field-collected and cultivated plants, namely the number of leaf lobes or indentations and the depth of maximum leaf lobe or indentation (Spearman correlation coefficient = 0.965). Character depth of maximum leaf lobe or indentation was excluded from further multivariate analyses, which were thus based on 51 characters.

Principal component analysis of all the populations of the *C. triumfetti* group from Central Europe studied resulted in five main groups separated along the first and the second axis (Fig. 2, Table 3). (i) Populations corresponding to '*C. axillaris*' and *C. strictus*

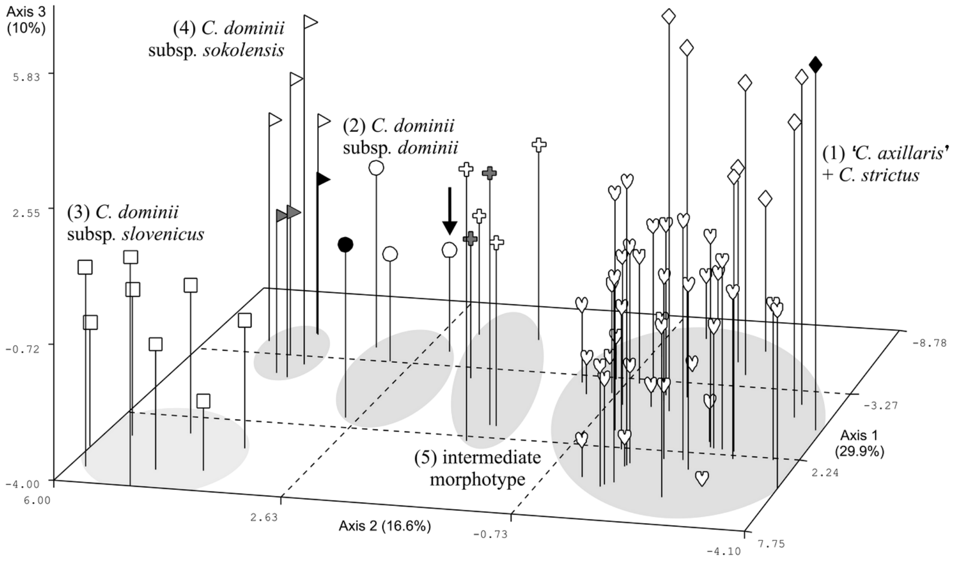


Fig. 2. – Principal component analysis based on 51 morphological characters of 71 field-collected populations from the *Cyanus triumfetti* group in Central Europe: ♡ '*C. axillaris*', ◇ *C. strictus* (◆ area close to the type locality of *Centaurea stricta*), ○ *Cyanus dominii* subsp. *dominii* (● area close to the locality cited in the protologue of '*Centaurea triumfetti* subsp. *dominii* var. *eu-dominii*'), □ *Cyanus dominii* subsp. *slovenicus*, ▷ *C. dominii* subsp. *sokolensis* (▶ the locality cited in the protologue of *Centaurea triumfetti* subsp. *dominii* var. *densifolia*; ▶ the localities cited in the protologue of *Centaurea axillaris* var. *sokolensis*) and ✙ intermediate morphotype between '*Cyanus axillaris*' and *C. dominii* (✚ localities close to the area cited in the protologue of *Centaurea axillaris* var. *pieninica*). The black arrow denotes population TRI 57, discussed in text.

formed a group on the right side of the PCA diagram. Because only a partial separation of '*C. axillaris*' and *C. strictus* was visible on the PCA diagram, this group was analysed separately using PCoA1. Three distinguishable groups on the left side of PCA diagram corresponded to three varieties described within *Cyanus dominii* by Dostál (1931a). These three groups were herein treated as (ii) *Cyanus dominii* subsp. *dominii*, (iii) *Cyanus dominii* subsp. *slovenicus* and (iv) *Cyanus dominii* subsp. *sokolensis* (= *Centaurea triumfetti* subsp. *dominii* var. *densifolia* Dostál, see also the Discussion). The inclusion of population TRI 57 (marked on the PCA diagram by an arrow) in the group of *Cyanus dominii* subsp. *dominii* was not clear from the PCA diagram, but we considered also geographic origin of this population and the fact that these plants correspond well to Dostál's description of *C. dominii* subsp. *dominii*. (v) On the PCA diagram, six populations remained in an intermediate position between the group of '*C. axillaris*' + *C. strictus* on the right and three groups of subspecies of *C. dominii* on the left; these populations, marked as "intermediate morphotype" in the analyses, were collected from two different areas. Four intermediate populations were collected in Slovenský kras Karst (southern Slovakia) where they occur in close vicinity to populations of *C. dominii* subsp. *sokolensis*. However, they differ in their ecological demands; the intermediate populations grow on sunny slopes in valleys, whereas *C. dominii* subsp. *sokolensis* grows on rocky

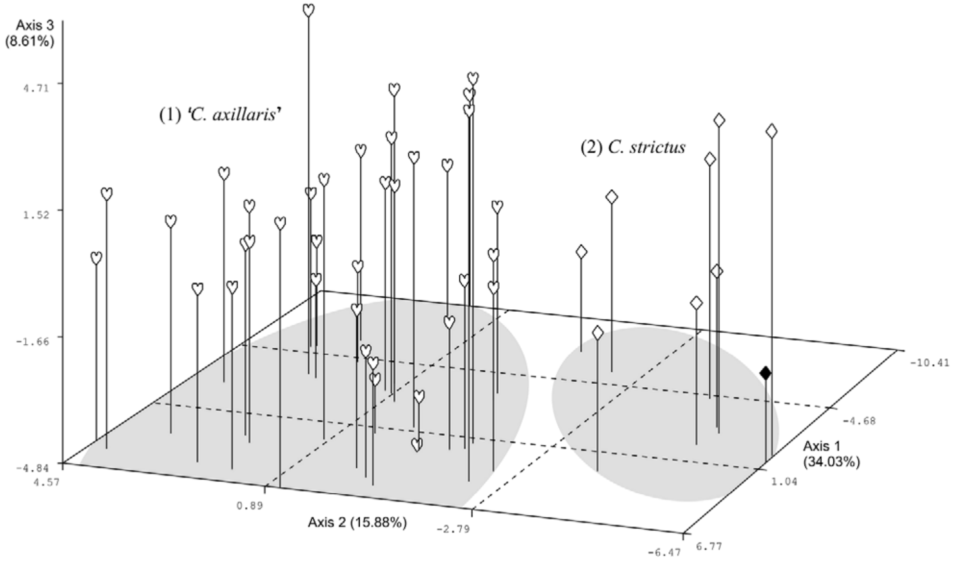


Fig. 3. – Principal coordinate analysis based on 51 morphological characters of 46 field-collected populations from the *Cyanus triumfetti* group in Central Europe: ♡ '*C. axillaris*', ◇ *C. strictus* (◆ area close to the type locality of *Centaurea stricta*).

margins of the karsts' plateaus. The two remaining intermediate populations were collected in the Pieniny Mts (northern Slovakia) from where *Centaurea axillaris* var. *pieninica* Pawł. was described (Szafer et al. 1924). It was not possible to resolve the taxonomic position of the populations from the Pieniny Mts because of the low number of plants included in the analyses as a consequence of their rarity.

The following characters were strongly correlated with the first ordination axis in PCA and thus had the highest influence on the differentiation within the group of '*C. axillaris*' and *C. strictus* and the delimitation of the three subspecies of *C. dominii*: pedunculus length, length of uppermost leaf on stem, involucre width, average width of margin of appendage of involucral bract at apex and in the middle. The separation of the groups along the second axis was influenced mainly by several leaf characters (distance of the widest part of uppermost and the middle leaves from their base, length of the decurrent part of the uppermost and the middle leaf on the stem, width of the base and length of the middle leaf on the stem) and the AFAL/AMAW and AFML/AMMW ratios. Samples of *C. strictus* were partially separated from '*C. axillaris*' also along the third axis with which the following characters were the most correlated: stem length, stem length up to branching and ratio LMW/LML (Table 3).

The results of PCoA1 presented in Fig. 3 confirmed that it is possible to distinguish between '*C. axillaris*' and *C. strictus* when the PCoA diagram reveals a clear separation of the populations into two groups. In the first group, the populations correspond to '*C. axillaris*', whereas in the second, the populations correspond to *C. strictus*.

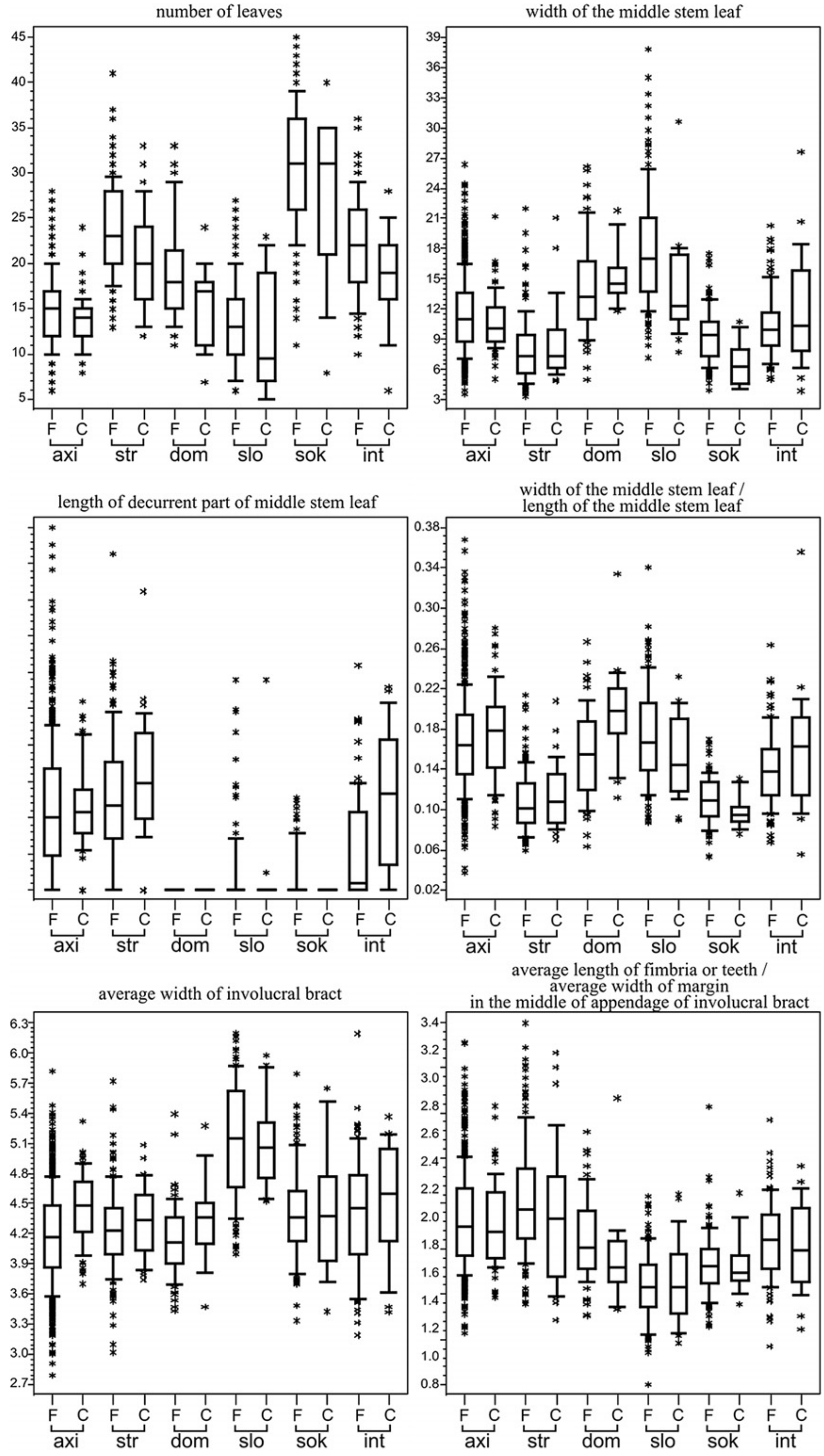
Table 3. – Eigenvectors showing the correlations of characters with the PCA axes (values that exceed the level of 0.2 are in bold type) and the total canonical structure expressing correlations of characters with canonical axes (CDA1 – CDA8; values that exceed the level of 0.4 are in bold type). The values were retrieved from PCA based on 51 morphological characters of 71 populations from the *Cyanus triumfetti* group (Fig. 2). CDA1 – CDA8 were based on 51 morphological characters of individuals expressed as OTUs. CDA1 with two groups pre-defined: *C. dominii* and '*C. axillaris*' + *C. strictus*; CDA2 with two groups pre-defined: '*C. axillaris*' and *C. strictus*; CDA3 with three groups pre-defined: *C. dominii* subsp. *dominii*, *C. dominii* subsp. *slovenicus* and *C. dominii* subsp. *sokolensis*; CDA4 with two groups pre-defined: field-collected and cultivated plants from the same populations; CDA5 with two groups pre-defined: field-collected and cultivated plants of '*C. axillaris*' from the same populations; CDA6 with two groups pre-defined: field-collected and cultivated plants *C. strictus* from the same populations; CDA7 with two groups predefined: field-collected and cultivated plants of *C. dominii* subsp. *slovenicus* from the same populations; CDA8 with two groups predefined: field-collected and cultivated plants of *C. dominii* subsp. *sokolensis* from the same populations. For character explanations see Table 1.

Character	PCA1			CDA1	CDA2	CDA3		CDA4	CDA5	CDA6	CDA7	CDA8
	Axis1	Axis2	Axis3	Axis	Axis	Axis1	Axis2	Axis	Axis	Axis	Axis	Axis
SI	–0.105	0.100	0.135	0.314	–0.382	–0.274	0.231	–0.227	0.008	**–0.530**	0.082	0.000
BRN	–0.055	0.131	0.143	0.403	–0.323	**–0.567**	0.282	0.119	**0.419**	0.180	–0.158	0.024
LN	–0.138	0.102	**0.260**	0.344	**–0.794**	**–0.818**	–0.004	–0.217	**–0.461**	–0.216	0.113	0.123
SL	0.074	–0.068	**0.345**	–0.096	**–0.501**	–0.095	0.043	–0.161	–0.201	0.104	0.281	0.281
SBRL	–0.001	–0.111	**0.337**	–0.211	**–0.607**	–0.181	–0.228	–0.373	**–0.457**	–0.186	0.362	0.362
PEL	**0.212**	0.036	0.019	0.111	0.284	0.580	0.076	0.487	**0.485**	**0.727**	–0.173	–0.170
SBRL/SL	–0.163	–0.131	0.073	–0.308	–0.329	–0.240	**–0.525**	**–0.452**	**–0.729**	–0.138	0.351	**0.415**
(SL–PEL)/LN	0.173	–0.133	0.074	–0.309	0.078	0.621	0.069	–0.089	–0.086	0.214	0.178	0.282
LAI	0.112	0.015	–0.124	0.008	0.283	0.781	**–0.419**	0.230	0.245	**0.440**	0.001	0.000
LBI	–0.106	0.111	0.107	0.321	–0.346	–0.194	–0.016	–0.295	–0.071	**–0.710**	0.115	0.000
LLN	0.097	–0.104	–0.182	–0.256	0.362	0.190	0.107	0.220	0.250	0.233	0.038	–0.107
LUL	**0.202**	0.158	–0.001	0.382	0.393	0.641	0.366	0.167	0.230	**0.594**	0.259	–0.141
LUW	0.198	0.104	–0.136	0.233	**0.535**	0.716	0.314	0.096	0.220	**0.435**	0.342	–0.257
LUD	0.174	**0.203**	–0.032	0.449	0.431	0.495	0.401	0.146	0.192	**0.491**	0.087	–0.128
LUBW	0.147	–0.186	–0.011	–0.394	0.145	0.294	0.314	0.114	0.085	**0.423**	0.235	0.188
LUDL	0.141	**–0.221**	0.058	**–0.425**	0.042	0.262	0.283	0.255	0.253	**0.448**	0.151	0.179
LML	0.117	**0.211**	0.139	0.552	–0.120	0.483	0.097	–0.156	–0.102	0.198	0.356	**0.420**
LMW	0.138	0.141	–0.149	0.308	**0.440**	0.677	0.069	–0.075	–0.015	0.157	0.340	**0.410**
LMD	0.062	**0.282**	0.060	0.606	0.134	0.197	0.109	–0.209	–0.190	0.110	0.187	**0.447**
LMBW	0.088	**–0.231**	0.051	**–0.549**	–0.036	0.092	0.212	0.141	0.202	0.102	0.146	0.137
LMDL	0.100	**–0.256**	0.086	**–0.562**	–0.105	0.047	0.203	0.234	0.272	0.227	0.064	0.145
LUW/LUL	0.078	–0.051	**–0.269**	–0.104	**0.408**	0.556	0.100	0.017	0.063	0.001	0.255	–0.191
LUD/LUL	–0.016	0.177	–0.123	0.249	0.262	–0.218	0.173	0.023	0.009	0.068	–0.136	0.005
LMW/LML	0.061	–0.025	**–0.326**	–0.089	**0.574**	0.560	–0.005	0.053	0.075	0.076	0.188	0.274
LMD/LML	–0.094	0.194	–0.163	0.255	0.320	–0.396	0.064	–0.159	–0.159	–0.033	–0.098	0.212
LUL/LML	0.197	0.003	–0.149	–0.057	**0.577**	0.457	0.420	0.463	**0.412**	**0.606**	–0.085	**–0.533**
PEL/LUL	0.158	–0.067	0.036	–0.057	0.185	0.349	–0.284	0.540	**0.488**	**0.608**	–0.334	–0.124
BN	0.086	–0.065	0.097	–0.050	–0.194	0.218	0.051	0.117	0.216	–0.021	0.072	–0.149
AFN	0.176	0.009	–0.077	–0.003	0.242	0.262	0.211	0.309	**0.449**	**0.408**	–0.360	0.099
AMCO	0.173	–0.064	–0.043	–0.154	0.374	0.476	0.155	0.265	0.275	0.395	–0.006	0.101
AFCO 1	0.069	0.007	–0.102	–0.049	0.236	–0.061	0.062	0.170	0.387	0.000	0.022	0.116
AFCO 2	0.019	0.177	–0.156	0.330	0.350	0.220	–0.204	–0.054	–0.130	0.167	–0.010	0.226
IL	0.106	0.195	**0.234**	0.479	–0.207	0.251	0.394	0.088	0.127	**0.419**	0.281	–0.037
IW	**0.216**	0.005	0.049	0.039	0.057	0.587	0.490	0.181	**0.440**	–0.042	0.159	0.097
ID	0.126	0.083	0.190	0.209	–0.055	0.148	0.257	0.080	0.112	0.393	0.266	0.044
BL	0.156	0.125	0.123	0.337	0.105	0.405	0.109	0.433	**0.512**	**0.548**	–0.165	**–0.420**
BW	0.145	0.168	0.103	0.370	–0.076	0.507	0.505	0.100	0.286	0.072	0.024	0.013
BD	0.028	0.024	0.137	0.086	0.030	–0.018	–0.182	0.103	0.078	0.280	–0.018	–0.365
AMAW	**0.214**	–0.034	0.022	–0.104	0.150	0.630	0.416	0.192	0.344	0.057	–0.061	–0.074
AFAL	0.160	–0.193	0.060	**–0.444**	0.032	0.546	0.313	0.257	**0.414**	0.158	0.027	0.059

Character	PCA1			CDA1	CDA2	CDA3		CDA4	CDA5	CDA6	CDA7	CDA8
	Axis1	Axis2	Axis3	Axis	Axis	Axis1	Axis2	Axis	Axis	Axis	Axis	Axis
AMMAW	**0.211**	–0.002	0.057	0.024	0.048	0.632	0.452	0.332	**0.519**	0.267	–0.171	–0.037
AFML	0.153	–0.189	0.116	**–0.439**	–0.144	0.535	0.186	0.383	**0.577**	0.196	–0.221	–0.026
IW/IL	0.165	–0.127	–0.096	–0.277	0.189	0.519	0.347	0.167	0.396	–0.261	–0.034	0.127
ID/IL	0.049	–0.113	–0.016	–0.154	0.086	–0.020	–0.002	0.027	0.044	0.202	0.112	0.066
AFAL/AMAW	–0.052	**–0.227**	0.038	–0.359	–0.090	–0.202	–0.183	0.049	0.019	0.154	0.074	0.168
AFML/AMMW	–0.074	**–0.223**	0.059	**–0.472**	–0.211	–0.225	**–0.460**	–0.012	–0.046	–0.009	–0.065	0.014
FEN	0.162	–0.085	–0.038	–0.145	0.007	0.306	**0.434**	0.205	0.262	–0.152	–0.109	0.019
FIN	0.127	–0.058	–0.112	–0.102	0.135	0.582	0.400	0.135	**0.431**	–0.110	0.054	0.130
FEL	0.181	0.121	0.071	0.282	0.120	0.556	0.429	0.306	0.368	**0.448**	–0.158	–0.097
FIL	0.164	0.056	0.036	0.140	0.193	0.132	**0.453**	0.390	**0.520**	0.365	–0.140	–0.150
PL	0.173	0.154	0.136	0.390	–0.027	0.646	0.509	0.068	0.255	0.329	0.242	–0.047
PW	0.190	0.028	0.076	0.048	0.054	0.459	0.596	0.272	0.356	0.288	–0.059	–0.301

CDA1 based on field-collected plants was calculated to reveal characters differentiating the two groups: (i) *C. dominii* and (ii) '*C. axillaris*' + *C. strictus.* Several characters of the leaf on the middle of the stem, e.g. its length, distance of the widest part from leaf base, width of base and length of the decurrent part were the most important for separating taxa along the axis (diagram not shown; Table 3). When the two taxa '*C. axillaris*' and *C. strictus* were defined as groups, CDA2 based on individuals as OTUs revealed striking separation by the number of leaves, stem length up to branching and the ratios LMW/LML and LUL/LML (diagram not shown; Table 3). Finally, CDA3 of individuals of three subspecies of *C. dominii* confirmed their clear separation with minor overlap. The number of leaves, average distance between leaves, indumentum on upper surface of leaves, width of the middle leaf on the stem and length and width of the uppermost leaf on the stem were the most important characters for the separation of *C. dominii* subsp. *sokolensis* from the other two subspecies along the first axis. Involucre width, average length and width of petal in addition to the ratios SBRL/SL and AFML/AMMW contributed to the separation of *C. dominii* subsp. *dominii* along the second axis (diagram not show; Table 3).

CDA4 of field-collected plants and cultivated plants defined as two groups revealed only a slight shift between these groups. Cultivated plants differed from field-collected plants mainly in pedunculus length and average length of involucral bract, and the ratios SBRL/SL, LUL/LML and PEL/LUL (diagram not shown, Table 3). Of the most useful characters for taxa determination identified by analyses CDA1, CDA2 and CDA3 only the ratio LUL/LML was affected by cultivation (Fig. 4). CDA5, CDA6, CDA7 and CDA8 showed differences in ecological plasticity of studied taxa: *C strictus* and '*C. axillaris*' are more influenced by cultivation than *C. dominii* subsp. *sokolensis* and *C. dominii* subsp. *slovenicus* (diagrams not shown, Table 3). Cultivated plants differed from field-collected plants in various characters, depending on the particular taxon. In comparison to field-collected plants of '*C. axillaris*', cultivated plants of this taxon (CDA5) had shorter stems, fewer leaves, more branches, occurrence of branching in lower part of the stem, longer interior florets, wider margins (AFML) and longer fimbria or teeth (AMMW) on the appendage of the involucral bract in the middle (but the ratio of the last two characters, AFML/AMMW, did not change). Cultivated plants of *C. strictus* differ from field-collected plants (CDA6) in the type of indumentum on stem and leaves, and characters of the uppermost leaves (ratios that define the shape of uppermost leaves, LUW/LUL and



number of leaves

width of the middle stem leaf

length of decurrent part of middle stem leaf

width of the middle stem leaf / length of the middle stem leaf

average width of involucral bract

average length of fimbria or teeth / average width of margin in the middle of appendage of involucral bract

LUD/LUL, did not change). Cultivated plants of *C. strictus* are less glabrous and have larger uppermost leaves than field-collected plants. CDA7 of *C. dominii* subsp. *slovenicus* showed that no character is strongly affected by cultivation. Cultivated plants of *C. dominii* subsp. *sokolensis* had smaller leaves on the middle of the stem (LML, LMW, LMD) than field-collected plants (CDA8), but the shape of these leaves (ratios LMW/LML, LMD/LML) did not change. The difference between field-collected and cultivated plants may be partially caused by the low number of plants cultivated and the short period of time for which they were cultivated.

Non-parametric classificatory discriminant analysis of the five groups identified by the ordination analyses – (i) '*C. axillaris*', (ii) *C. strictus*, (iii) *C. dominii* subsp. *dominii*, (iv) *C. dominii* subsp. *slovenicus* and (v) *C. dominii* subsp. *sokolensis* – showed that more than 90% of the individuals were correctly classified. Some morphological overlap among the taxa was exhibited by 25 plants of '*C. axillaris*' (4.1%) that were misclassified as *C. strictus* and by nine of *C. dominii* subsp. *slovenicus* (7.4%) that were misclassified as *C. dominii* subsp. *dominii* (Table 4).

Table 4. – Results of non–parametric discriminant analysis of individuals from the *Cyanus triumfetti* group with the following five pre–defined groups: '*C. axillaris*' (axi). *C. strictus* (str). *C. dominii* subsp. *dominii* (dom). *C. dominii* subsp. *slovenicus* (slo) and *C. dominii* subsp. *sokolensis* (sok).

Actual group	Group membership predicted (number of observations and percentage classified into groups)				
	axi	str	dom	slo	sok
axi	579 (94.61%)	25 (4.08%)	3 (0.49%)	1 (0.16%)	4 (0.65%)
str	0 (0%)	149 (99.33%)	0 (0%)	0 (0%)	1 (0.67%)
dom	0 (0%)	0 (0%)	64 (100%)	0 (0%)	0 (0%)
slo	0 (0%)	0 (0%)	9 (7.38%)	111 (90.98%)	2 (1.64%)
sok	0 (0%)	0 (0%)	1 (0.91%)	0 (0%)	109 (99.09%)

Box-and-whisker plots of the six groups of field-collected and cultivated plants revealed by multivariate morphometric analyses showed only a slight overlap in the depicted discriminant characters (Fig. 4). Univariate statistics of all quantitative characters and frequencies of all qualitative characters also showed that the recognized taxa differed in several characters. The univariate analyses showed that *C. dominii* differ from '*C. axillaris*' and *C. strictus* in having very short decurrent leaves (there is an even smaller overlap in this character between cultivated plants of these taxa); '*C. axillaris*' has fewer stem leaves than *C. strictus* and also the width and the ratio expressing the shape of the middle leaf on the stem differ in these two species (LMW/LML; Electronic Appendix 1, Fig. 4). Examination of qualitative characters showed that most plants of *C. dominii* subsp. *sokolensis* have leaves with a tomentose upper surface (99.1%) while most plants of

Fig. 4. – Box-and-whisker plots displaying the variation in selected morphological characters among five taxa from the *Cyanus triumfetti* group in Central Europe and comparison of field-collected (F) and cultivated plants (C); '*C. axillaris*' [axi; n(F) = 612, n(C) = 71], *C. strictus* [str; n(F) = 150, n(C) = 34], *C. dominii* subsp. *dominii* [dom; n(F) = 64, n(C) = 13], *C. dominii* subsp. *slovenicus* [slo; n(F) = 122, n(C) = 26], *C. dominii* subsp. *sokolensis* [sok; n(F) = 110, n(C) = 19] and intermediate morphotype between '*C. axillaris*' and *C. dominii* [int; n(F) = 90, n(C) = 25]. Boxes define the 25th and 75th percentiles, horizontal lines show the median, whiskers are from the 10th to 90th percentiles and asterisks indicate outliers.

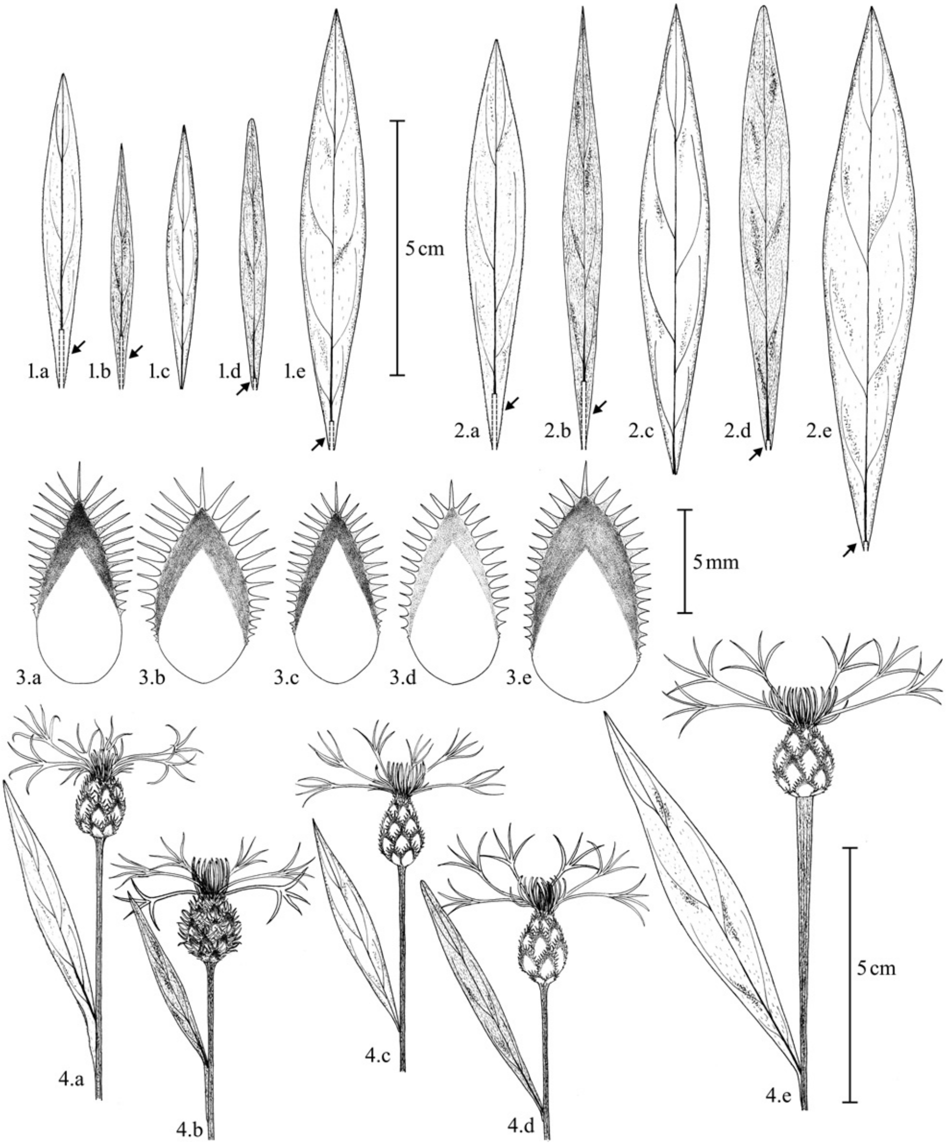


Fig. 5. – Shape of the (1) uppermost leaves on stem, (2) middle leaves on stem, (3) involucral bracts and (4) involucres of the *Cyanus triumfetti* group in Central Europe (a) '*C. axillaris*', (b) *C. strictus*, (c), *C. dominii* subsp. *dominii*, (d) *C. dominii* subsp. *sokolensis,* (e) *C. dominii* subsp. *slovenicus*. Drawings are based on the mean values of the measurements of the characters; the decurrent parts of the leaves are marked by arrows. Figures were drawn by Zlata Komárová and Katarína Olšavská.

C. dominii subsp. *dominii* and subsp. *slovenicus* have leaves with a glabrous upper surface (96.8% and 76.9% respectively). Most *C. strictus* plants have white fimbrie on the appendages of the involucral bracts (99.9%), whereas no more than 42% of the plants of other taxa have the same colour of appendages (Electronic Appendix 1). The differentiation among '*C. axillaris*', *C. strictus* and the three subspecies of *C. dominii* are also apparent when involucral bract, involucres, the uppermost and the middle leaf on the stem of the taxa are illustrated based on the mean values of the characters measured (Fig. 5).

Flow cytometry

The 123 individuals from 32 populations of '*C. axillaris*', *C. strictus* and *C. dominii* were diploid (2n ~ 2x ~ 22) (Table 1). Data for the populations of the *C. triumfetti* group reported here confirm and extend previous records (Olšavská et al. 2009). The seven plants from two populations of *C. montanus* were tetraploid (2n ~ 4x ~ 44) (Table 1), which accords with the chromosome number 2n = 44 already cited for this taxon in France (Guinochet 1957), Switzerland (Krahenbühl & Küpfer 1992) and Austria (Lipper & Heubl 1988).

AFLP analyses

Neighbour-joining tree (Fig. 6) of the *C. triumfetti* and *C. montanus* groups identified four main clusters. Three of them, (i) *C. montanus* from the Western Alps, (ii) *C. triumfetti* s.s. from Western Alps and (iii) cluster containing samples of '*C. axillaris*' from Austria and the Czech Republic (except the Carpathians; hereinafter referred to as Austria-Czech group) had high bootstrap support (100%, 100% and 92%, respectively). (iv) The fourth cluster containing the remaining samples of '*C. axillaris*', *C. strictus* and *C. dominii* from the Western Carpathians and Pannonia (hereinafter referred to as W Carpathians-Pannonia group) had only very low bootstrap support (< 40%). Within the latter cluster, individuals of *C. strictus* from the Zempléni-hegység Mts and the Bükk Mts formed two distinct clusters with intermediate bootstrap support (82% and 77%, respectively).

On the ordination diagram of the PCoA analysis of the entire dataset based on AFLP data (55 individuals; PCoA2; Fig. 7a), there are four groups identical to the clusters on the NJ tree: (i) *C. montanus* from the Western Alps, (ii) *C. triumfetti* s.s. from the Western Alps (iii) the Austria-Czech group of '*C. axillaris*' and (iv) the W Carpathians-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii*. Individuals of the *C. triumfetti* group were clearly separated along the first and second axis while those of the *C. montanus* group were divided from the remaining individuals mainly along the third axis. The extra PCoA based on 35 individuals from the W Carpathians-Pannonia group (PCoA3; Fig. 7b) showed only a tendency to form groupings of individuals corresponding to the groups identified by the morphometric analyses ('*C. axillaris*', *C. strictus*, *C. dominii* and intermediate morphotype). The samples of *C. dominii* together with those of the intermediate morphotype were situated mostly on the left side of PCoA3 diagram while most samples of '*C. axillaris*' and *C. strictus* were on the right side. The exception is one individual from population TRI 56 (marked on the diagram by an arrow), which, according to its morphology, belongs to '*C. axillaris*', but on the PCoA diagram of AFLP data appears on the left side in the *C. dominii* group. The samples of *C. strictus* formed two groups as on the NJ tree, reflecting their geographical origin (Zempléni-hegység Mts and Bükk Mts). Interestingly, three individuals of *C. strictus* from the Bükk Mts were clearly separated from all remaining samples of the W Carpathians-Pannonia group along the second axis.

The optimal partition with the highest log marginal likelihood (–4419.3013) produced by BAPS consisted of three clusters that corresponded to the following groups: (i)

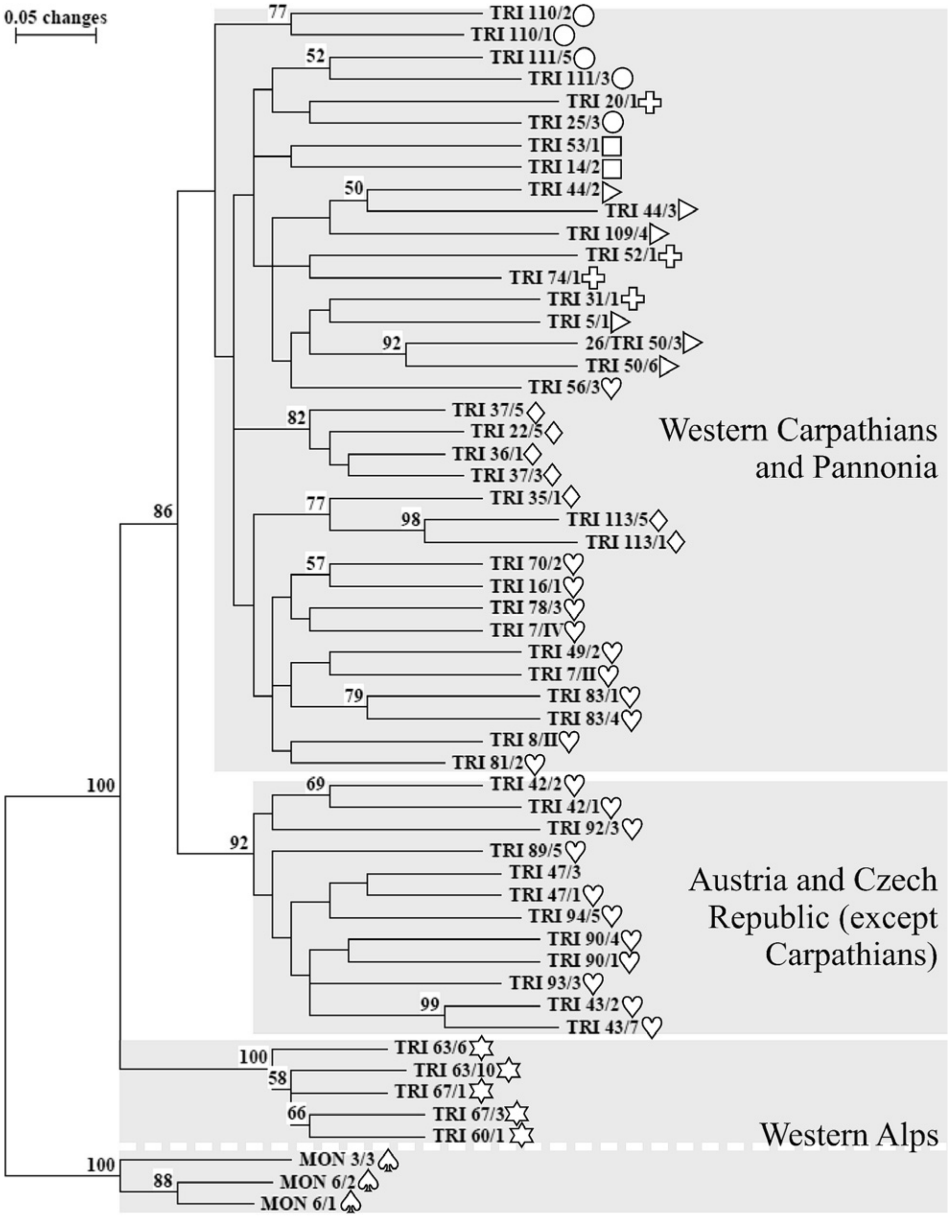


Fig. 6. – Neighbour-joining tree of AFLP data for 55 individuals from the *Cyanus triumfetti* and *C. montanus* groups: ♤ C. montanus, ✡ *C. triumfetti* s.s., ♡ '*C. axillaris*', ◇ *C. strictus*, ○ *C. dominii* subsp. *dominii*, □ *C. dominii* subsp. *slovenicus*, ▷ *C. dominii* subsp. *sokolensis* and ✙ intermediate morphotype between '*C. axillaris*' and *C. dominii* (cross). Bootstrap values above 40% based on 2000 replicates are shown.

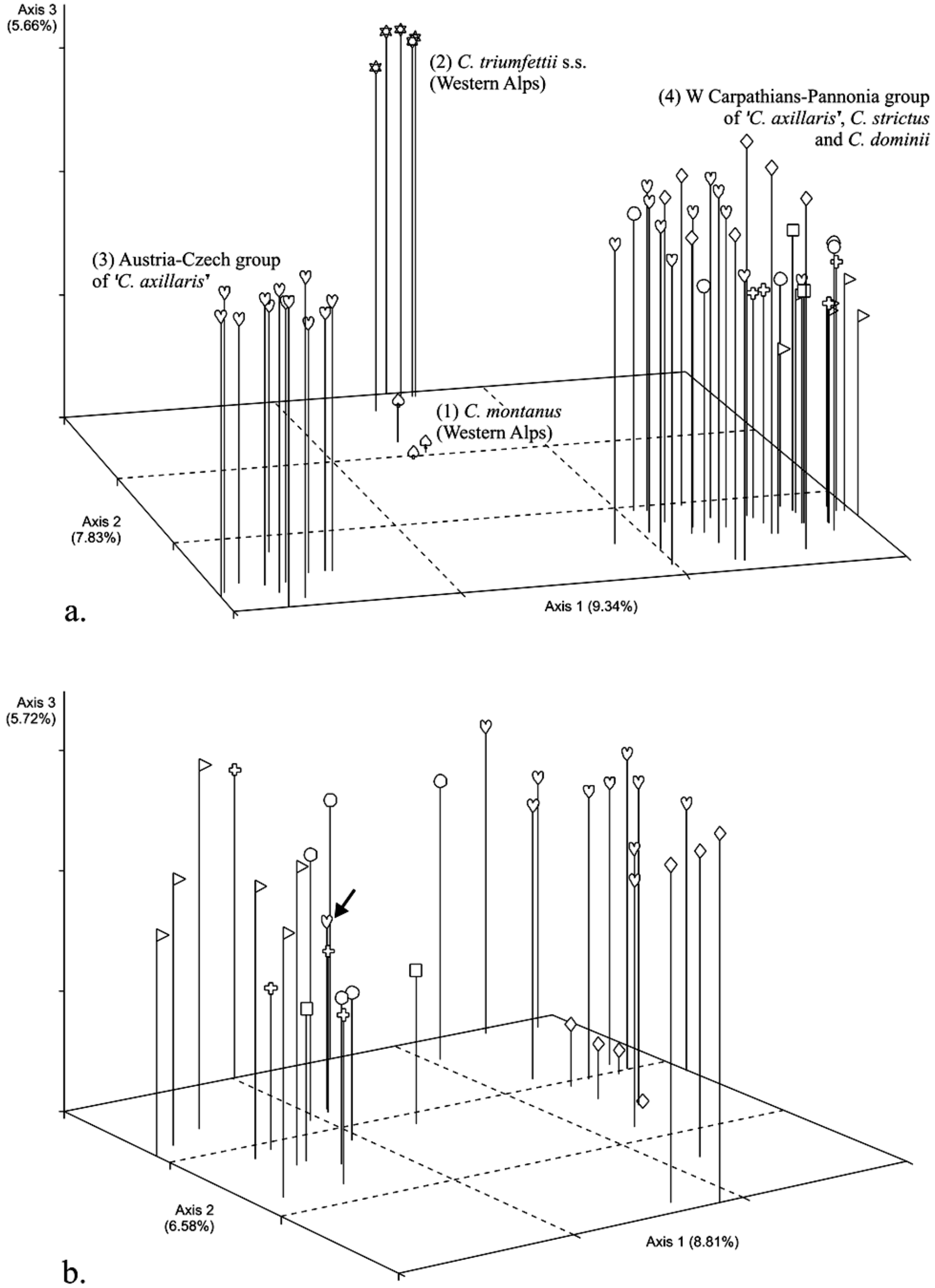


Fig. 7. – Ordination diagrams of principal coordinate analyses based on AFLP data; (a) PCoA2 calculated for 55 individuals from the *Cyanus triumfetti* and *C. montanus* groups; (b) PCoA3 calculated for 35 individuals from the *C. triumfetti* group from the Western Carpathians and Panonia: ♤ *C. montanus*, ✡ *C. triumfetti* s.s., ♡ '*C. axillaris*', ◇ *C. strictus*, ○ *C. dominii* subsp. *dominii*, □ *C. dominii* subsp. *slovenicus*, ▷ *C. dominii* subsp. *sokolensis* and ✣ intermediate morphotype between '*C. axillaris*' and *C. dominii* (cross). The black arrow denotes population TRI 56, discussed in text.

C. triumfetti s.s. + *C. montanus* from the Western Alps, (ii) '*C. axillaris*' from Austria and the Czech Republic (= Austria-Czech group of '*C. axillaris*') and (iii) '*C. axillaris*', *C. strictus* and *C. dominii* from the Western Carpathians and Pannonia (= W Carpathians-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii*). According to the results of Bayesian clustering, the species *C. triumfetti* and *C. montanus* from the Western Alps formed one cluster but the NJ tree and the PCoA analyses indicated two groups.

The AFLP analysis resulted in 264 scorable bands for 55 individuals with 24 (9%) monomorphic bands. Control replicates proved that the AFLP data were highly reliable (repeatability 99.79%). Across the whole dataset, 55 unique AFLP multilocus phenotypes were detected. No pair or group of individuals had an identical AFLP phenotype. The average total number of fragments per individual was highest for *C. montanus* (74) and lowest for *C. triumfetti* s.s. (67). Table 5 shows the statistics for the AFLP bands. The W Carpathians-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii* had the most polymorphic bands (186 out of 210; 89%) and *C. triumfetti* s.s. the lowest (65 out of 89; 73%). The number of private bands varied considerably from 74 in the W Carpathians-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii* to seven in *C. montanus*. No fragments were private fixed in either the Austria-Czech group of '*C. axillaris*' or W Carpathians-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii*, while three and nine were fixed in *C. triumfetti* s.s. and *C. montanus*, respectively. Presence of private fixed AFLP fragments in *C. triumfetti* s.s. and *C. montanus* may be a consequence of the low number of samples. Examination of AFLP bands that were shared in the whole AFLP data set revealed that there were no fragments that were shared between any taxon or group of taxa.

Table 5. – Distribution of AFLP fragments across the investigated *Cyanus* samples; N ind – number of individuals. N phen – number of AFLP multilocus phenotypes. N fragm – (min) average (max) number of fragments per individual. N pr – number of private fragments per taxa. N prf – number of private fixed fragments per taxa.

Taxon	N ind	N phen	(min) N fragm (max)	N pr	N prf
C. montanus	3	3	(71) 74 (77)	7	9
C. triumfetti s.s.	5	5	(65) 67 (71)	8	3
Austria-Czech group	12	12	(60) 70 (80)	20	0
W Carpathians-Panonian group	35	35	(63) 71 (77)	74	0

Morphometric and genetic variation of '*Cyanus axillaris*'

As the AFLP analyses revealed that populations of '*C. axillaris*' differentiate into two groups a detailed analysis of the variation in the infraspecific morphology of this species was undertaken. PCoA4 computed for 37 field-collected populations of '*C. axillaris*' revealed a certain amount of morphological differentiation within the species (Fig. 8). The populations were divided into two groups along the first axis. Within the group on the right side of the diagram another division of populations along the second axis was visible. However, this morphological variation of '*C. axillaris*' was incongruent with the geographic origin of the populations and AFLP data (Austria-Czech group of '*C. axillaris*' vs '*C. axillaris*' from the W Carpathians-Pannonia group; Fig. 8).

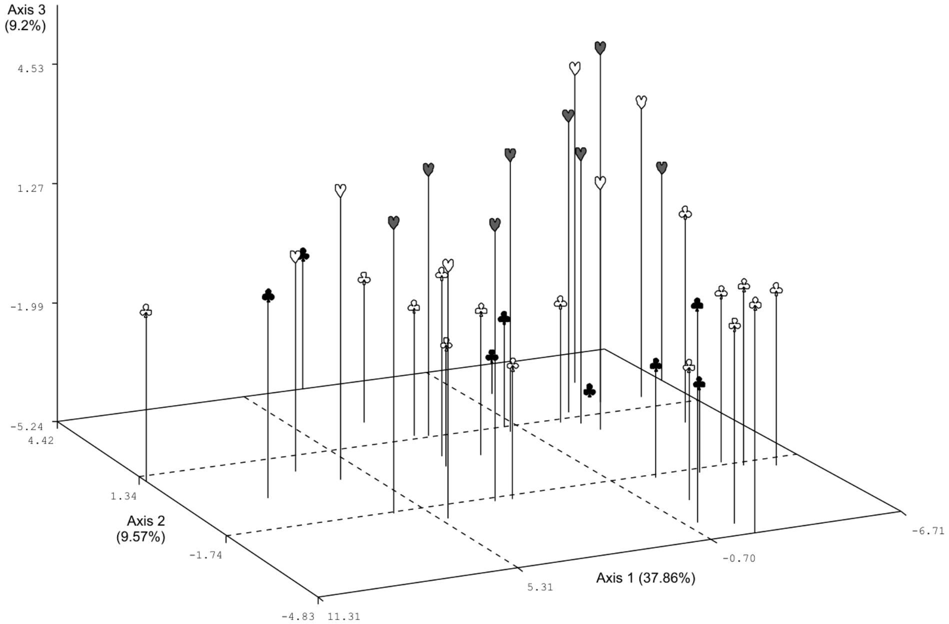


Fig. 8. – Ordination diagram of principal coordinate analysis based on 51 morphological characters calculated for 37 field-collected populations of ‘*Cyanus axillaris*’ in Austria and the Czech Republic except the Carpathians (♡, grey symbols indicate population assigned to the Austrian-Czech group by AFLP analyses) and from the Western Carpathians and Pannonia (♧, black symbols indicate population assigned to the W Carpathians-Pannonian group by AFLP analyses). The grey symbols indicate a population from which selected individuals were analysed by AFLP.

Discussion

Morphological differentiation of the Cyanus triumfetti group in Central Europe

The current morphological analyses suggest that the following three species of the *C. triumfetti* group in Central Europe can be distinguished: ‘*C. axillaris*’, *C. strictus* and *C. dominii.* These species can be recognized by a combination of morphological characters of which the leaf characters are the best for delimiting these species. *Cyanus dominii* can be clearly distinguished from both ‘*C. axillaris*’ and *C. strictus* as *C. dominii* has non-decurrent stem leaves, whereas ‘*C. axillaris*’ differs from *C. strictus* mainly in the number of leaves, stem length up to branching and in the ratio of width to length of the leaf on the middle of the stem (LMW/LML). Interestingly, the ratio of length of fimbria/width of margin of appendage of involucral bract that is considered as a crucial character in some identification keys to Central-European knapweeds (Dostál 1976b, 1989, Simon 1992) was not confirmed as suitable for classification of these species. The analyses indicate there is a large overlap in this character (measured in the current analyses as two ratios AFAL/AMAW, AFML/AMMW, see Table 2) and in other similar characters such as the length of the fimbria (AFAL, AFML) and width of the margin of the involucral bract (AMAW, AMMW); this result questioned the importance of these characters for delimitating these species (Fig. 8). However, the morphological analyses showed that differences among the taxa were mainly in the colour of the margin and the fimbriae of the appendages of the involucral bracts (see identification key below and Electronic Appendix 1).

As depicted by the multivariate morphometric analyses, infraspecific morphological variation within each of the three species was very conspicuous. This intraspecific variation has taxonomic implications for *C. dominii,* which includes three groups of populations recognized as subspecies based on their morphology and distribution: *C. dominii* subsp. *dominii*, *C. dominii* subsp. *sokolensis* and *C. dominii* subsp. *slovenicus* (see below).

Cultivation of plants of '*C. axillaris*', *C. strictus* and *C. dominii* showed that mainly leaf and stem characters were influenced by environmental conditions. However, the shift in characters caused by cultivation did not affect the determination value of key characters and differences among taxa remained. For example, the number of leaves was lower in cultivated plants of all taxa, but this character was still useful for identifying the taxa (Fig. 4).

Pattern of genetic variation

Principal coordinate analyses of the AFLP data revealed three genetically differentiated groups within the *C. triumfetti* aggregate corresponding to their geographic origin (Fig. 7a). The group of *C. triumfetti* s.s. from the Western Alps and the Austria-Czech group of '*C. axillaris*' also had high bootstrap support in the neighbour-joining tree, but there was no support for the internal node of the W Carpathians-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii* (Fig. 6). Thus, it was difficult to infer the genetic relationships of the latter group. In the case of *C. triumfetti* s.s., the genetic analyses confirmed the results of a previous study, which revealed the morphological separation of this Alpine taxon from Central European taxa (Olšavská et al. 2009). The pattern of AFLP variation of the *C. triumfetti* group in Central Europe is more remarkable considering the morphological differentiation of the taxa assessed in this study. Populations of the *C. triumfetti* group from Central Europe are divided into two genetically distant and allopatric groups, which do not correspond to the three species recognized by the morphometric analyses. All samples from the Austria-Czech genetic group should be treated as '*C. axillaris*' based on their morphology and those from the W Carpathian-Pannonia group are of the three morphologically well-differentiated species. This result was unexpected because the populations of three different species from the one area are genetically closer to each other than are populations of one species – '*C. axillaris*' – from two different areas. Such a pattern of genetic variation raises some evolutionary hypotheses. For example, the genetic variation recorded for '*C. axillaris*' might indicate that populations of this species from the Austria-Czech and W Carpathian-Pannonia groups may differ in their biogeographic history. The fact that '*C. axillaris*' is widely distributed throughout Europe could imply that this species survived periods of glaciation in several glacial refugia and/or it colonized Europe by more postglacial migration routes. A similar scenario of postglacial colonization of Europe by other groups from the family Compositae was revealed by phylogeographic analyses based on genetic markers (e.g. Chauvet et al. 2004, Mráz et al. 2007, Font et al. 2009). Based on the results of AFLP analyses, it is assumed that the '*C. axillaris*' populations from the Austria-Czech group are not connected by gene flow with populations of '*C. axillaris'* in the Western Carpathians and Pannonia. Such a genetic gap between the populations from different parts of Central Europe is recorded for other plant groups. For example, the *Dryas octopetala* L. populations in the Tatras are connected to northeastern Europe and Siberia, whereas the alpine populations are connected to northwestern Europe (Skrede et al. 2006). *Rosa pendulina* L. expanded into Central Europe from two refugia (roughly the Alps vs Southern Carpathians) and now its populations (circumscribed by their cpDNA haplotypes) are divided by a contact zone in the Danube valley (Fér et al. 2007). However, the results of AFLP analysis of the *C. triumfetti* group recorded in this study could not confirm or reject any phylogeographic scenario about '*C. axillaris*' in Central Europe due to the lack of material from other parts of its distribution, including potential refugia.

The low genetic differentiation of the W Carpathian-Pannonia group of '*C. axillaris*', *C. strictus* and *C. dominii* and high genetic variation within this group can be interpreted in two different ways. The first scenario suggests that *C. strictus* and *C. dominii* originated by the isolation of two populations of some widely distributed ancestor. A taxon that may be considered as the putative ancestor is '*C. axillaris*', which also currently occurs in this area. The distribution of these species suggests long-term isolation of the Western Carpathians populations that could be maintained over time by the specific substrate and vegetation requirements of this species (for a similar example, see Pečinka et al. 2006). *Cyanus dominii* and *C. strictus* grow exclusively on dolomites, limestones and tuffs, and they occur in relatively small spatial isolates of steppe and rocky-slope vegetation that are separated by large areas of continuous forest at least since the end of the last glaciation, i.e. approx. 10,000 years BP (Krippel 1986). The alternative scenario suggests occasional gene flow and/or hybridization events among previously diverged taxa occurring in this area. Our ongoing hybridization experiments show the possibility of crossing among '*C. axillaris*', *C. strictus* and *C. dominii*, as well-developed seeds were obtained in 48–83% of the hybrids (K. Olšavská et al., unpublished). These two scenarios (common ancestor and short time of speciation vs gene flow or hybridization) on their own or in combination could result in very low genetic differentiation of morphologically well-separated taxa.

Relative to the morphological delimitation of populations or taxa there are two patterns of genetic differentiation recognized in the studies on genetic differentiation at the species or below-species level published over the last decade. The first and more common pattern is the congruence between the genetic and morphological variation, resulting often in a definite taxonomic classification of the taxa (e.g. Lihová et al. 2004, Perný et al. 2004, Šmarda et al. 2007). The second and rarer conclusion is that the patterns of genetic and morphological variation are incongruent. This incongruence is attributed to various factors, such as the polygenic regulation of quantitative traits (Roldán-Ruiz et al. 2001, Archak et al. 2003), hybridization and introgression (Greimler & Jang 2007, Lihová et al. 2007), human selection and domestication (Depypere et al. 2009) or natural selection (Kamada et al. 2007, Pérez-Barrales et al. 2009). In the present study, the incongruence between the AFLPs and the morphology evident in the W Carpathians and Pannonia group and in '*C. axillaris*' may be attributed to past hybridization events and natural selection for some polygenic quantitative morphological characters in addition to biogeographic history as discussed above. These processes have yet to be identified.

Classification of the Cyanus triumfetti group

Analysing the morphological data and geographical distribution led to the conclusion that the rank of species is appropriate (according to the classical phenetic species concept;

Sneath & Sokal 1973, Stuessy 2009) for the following four groups of populations of the *C. triumfetti* group studied: *C. triumfetti* s.s., '*C. axillaris*', *C. strictus* and *C. dominii*. These four species are differentiated by several morphological characters and have non-overlapping distributions. The three subspecies within *C. dominii* provide an example of allopatric differentiation where the classification at the subspecies level is the most adequate (cf. Perný et al. 2004, Galbany-Casals et al. 2006, Conti 2007, Kropf 2008, Peruzzi & Passalacqua 2008). In the Western Carpathians, similar examples of infraspecific differentiation addressed by biosystematic studies include e. g. *Aconitum firmum* Rchb. (Mitka et al. 2007), *Cardamine amara* L. (Marhold et al. 2002) or *Scilla drunensis* (Speta) Speta (Kochjarová 2005). The three subspecies of *C. dominii* share a synapomorphic character – leaves are not decurrent or very shortly – that can be used to discriminate them from all other taxa of the *C. triumfetti* group.

***Cyanus triumfetti* s.s.** Results of AFLP analyses presented in this paper accord with previous multivariate morphometric analyses that excluded *C. triumfetti* s.s. from the Western Carpathians and adjacent parts of Pannonia (Olšavská et al. 2009). The PCoA2 diagram based on AFLP data (Fig. 7a) and results of Bayesian clustering indicate that *C. montanus* subsp. *montanus* may be genetically closer to *C. triumfetti* s.s. than to other taxa of the *C. triumfetti* group. However, the apparent clustering of *C. montanus* and *C. triumfetti* s.s. may be a consequence of uneven sampling of the main four groups (the largest portion of the variation is among the larger groups). In addition, *C. montanus* and *C. triumfetti* are distinctly separated by numerous private and private fixed fragments of both taxa and the absence of shared fragments.

'*Cyanus axillaris*'. The morphological analyses showed that '*C. axillaris*' is a taxonomically critical species with high intraspecific variation. The morphometric analyses that are presented indicate morphological differentiation within '*C. axillaris*', but this differentiation is incongruent with the AFLP data and geographical distribution. Therefore, no groups of populations were distinguished at any taxonomic level based on the results presented (Fig. 8). However, '*C. axillaris*' has a broad distribution, and therefore, additional studies of material from a broader range of areas are necessary to determine whether the morphological and genetic variation outlined for Central European populations may be applicable to neighbouring areas and if the variation of '*C. axillaris*' deserves some taxonomic delimitation. Until the problem of the intraspecific variation of this species is elucidated, nomenclatural questions can not be resolved (see Nomenclature notes).

Cyanus strictus. The NJ tree (Fig. 6) and PCoA3 (Fig. 7b) of the AFLP markers indicate that the samples of *C. strictus* form two separate groups that reflect their geographical origin (the Zempléni-hegység Mts and the Bükk Mts). This separation is also partially visible in the results of morphometric analyses (Fig. 2, 3) where three populations from the Bükk Mts appear closer to the populations of '*C. axillaris*' than the remaining populations of *C. strictus*. However, there are no clear groups in a separate morphometric analysis of individuals of *C. strictus* (results not shown); thus, the degree of infraspecific morphological differentiation was not sufficient to recognize any intraspecific taxa within this species.

Cyanus dominii. The considerable intraspecific variation of this species resulted in the description by Dostál (1931a) of five varieties in Europe. The morphometric analyses presented in the current paper confirmed the broad morphological variation of the Western Carpathians populations of *C. dominii* and support their classification into the three following morphologically distinct taxa: *C. dominii* subsp. *dominii*, *C. dominii* subsp. *slovenicus* and *C. dominii* subsp. *sokolensis*. These morphological analyses also indicate that the population from Slovenský kras Karst [where *Centaurea triumfetti* subsp. *dominii* var. *densifolius* was described (Dostál 1931a)] and from the Zapadné Tatry Mts [where *Centaurea axillaris* f. *sokolensis* Pawł. was described (Pawłowski 1931)] belong to the same subspecies *C. dominii* subsp. *sokolensis*. The three subspecies of *C. dominii* have distinct distributions restricted to certain mountains in Slovakia (Fig. 1). The results of AFLP analyses provide no genetic differentiation of these three subspecies, possibly because of the rather low number of samples. Overall, the results of this study may stimulate further research on populations from the *C. triumfetti* group in Bulgaria or Ukraine, where *C. dominii* is reported by several authors (Dostál 1976b, Andreev et al. 1992, Mosyakin & Fedoronchuk 1999, Greuter 2006–2009).

Intermediate morphotypes. Based on the morphological analyses the intermediate plants from the Pieniny Mts (northern Slovakia) are similar in most characters to *C. dominii* but their leaves have a broad and long decurrent part that is typical of ‘*C. axillaris*’. Interestingly, Dostál (1931a) also mentions that plants from the Pieniny Mts are very similar to *C. triumfetti* subsp. *dominii* var. *slovenica* but differ in their distinctly decurrent leaves. Unfortunately, only a few plants were included in the current analyses due to their rare occurrence in this area. Hence, there is insufficient data to support or reject any hypotheses about their taxonomic position. Therefore, this study raises the question of whether populations from the Pieniny Mts are of hybrid origin or are part of the intraspecific variation of *C. dominii* or ‘*C. axillaris*’. Intermediate plants from Slovenský kras Karst may be more obviously attributed to a recent hybridization event. These intermediate plants have mixed morphological characters of both putative parental taxa. They have a high number of branches and stem leaves, which are typical of *C. dominii* subsp. *sokolensis*, but they have narrow lanceolate leaves and 60% of the plants with decurrent leaves on the middle of the stem similar to ‘*C. axillaris*’. These plants grow in Slovenský kras Karst in the vicinity of populations of *C. dominii* subsp. *sokolensis* (5–10 km), but they prefer different ecological conditions and grow on sunny slopes in valleys, which is more typical of ‘*C. axillaris*’ (whereas *C. dominii* subsp. *sokolensis* grows on rocky margins of karst plateaus). Hybrids between ‘*C. axillaris*’ and *C. dominii* are reported by Dostál (1950; described as *Centaurea triumfetti* subsp. hybr. *sillingeri* Dostál) from other parts of Slovakia, namely the Malé Karpaty Mts, Súľovské skaly Mts, Nízke Tatry Mts and Malá Fatra Mts. According to the results of the morphological analyses, the population TRI 2 from the Malé Karpaty Mts belongs to ‘*C. axillaris*’, whereas samples collected in the Súľovské skaly Mts (TRI 53) and Nízke Tatry Mts (TRI 27) belong to *C. dominii* subsp. *slovenica*.

Identification key of the taxa from the *Cyanus triumfetti* group in Central Europe

For confident identification, it is recommended that at least several plants from a locality should be identified using this key because of the high intrapopulation variation of the taxa. It is necessary to use a combination of characters (not just one) because of the morphological overlap between some taxa in some characters. The character values given in the key represent the 10–90 percentiles; the morphological characters of inflorescences and flowers were measured or scored for the terminal capitulum.

1a Middle stem leaves decurrent 2

1b Middle stem leaves not decurrent or rarely shortly decurrent (up to 7.5 mm) .... ***C. dominii*** (Dostál) Holub

  01a Stems with numerous branches (3–20); number of stem leaves 22–39; leaves tomentose on both surfaces; middle stem leaves linear (6.2–12.8 mm wide, ratio of width to length 0.08–0.14); endemic to Slovenský kras Karst and Západné Tatry Mts .... **_C. dominii_ subsp. _sokolensis_** (Pawł.) Olšavská

  01b Stems sparingly branched (0–4); number of stem leaves 7–29; leaves usually glabrous or tomentose above; middle stem leaves lanceolate to linear-lanceolate (8.9–25.8 mm wide, ratio of width to length 0.1–0.24) **02**

  02a Leaves usually glabrous above; middle stem leaves linear-lanceolate (8.9–21.3 mm wide); involucre cylindrical (6.9–10.5 × 12.3–14.9 mm); external floret 29.4–38.9 mm long with petals 8.3–10.9 mm long and 0.7–1.2 mm wide; endemic to Volovské vrch Mts and Branisko Mts .... **_C. dominii_ subsp. _dominii_**

  02b Leaves glabrous or tomentose above; middle stem leaves lanceolate (11.8–25.8 mm wide); involucre ovoid (11.8–25.8 × 13.5–16.4 mm); external floret 35.0–47.3 mm long with petals 11.1–16.8 mm long and 1.1–1.8 mm wide; endemic to mountains in Central Slovakia .... **_C. dominii_ subsp. _slovenicus_** (Dostál) Olšavská

2a Fimbriae white or silver; number of stem leaves 18–29; stems tomentose; stem leaves narrowly lanceolate; uppermost stem leaves 2.8–5.8 mm wide, middle stem leaves 4.6–11.6 mm wide; ratio of width to length of leaves 0.07–0.15; Zempléni-hegység Mts and Bükk Mts .... ***C. strictus*** (Waldst. et Kit.) Soják

2b Fimbriae pale to dark brown or white; number of stem leaves 10–20; stems tomentose to subglabrous; stem leaves lanceolate; uppermost stem leaves 4.1–11.2 mm wide, middle stem leaves 7.1–16.5 mm wide; ratio of width to length of leaves 0.1–0.22; Central Europe .... **‘*C. axillaris*’**

[Intermediate morphological types between ‘*C. axillaris*’ and *C. dominii* occur in Slovenský kras Karst and Pieniny Mts. Intermediate plants from the Pieniny Mts possess tomentose and lanceolate middle stem leaves (6.7–18.2 mm wide) and an ovoid involucre (8.6–10.4 × 12.8–15.1 mm) – both characters are similar to *C. dominii*; but their leaves have broad and long decurrent part – typical of ‘*C. axillaris*’. Plants from Slovenský kras Karst have many branches (2–11) and stem leaves (16–29) – both characters are typical of *C. dominii* subsp. *sokolensis*; but they have narrow lanceolate leaves and 60% of the plants have decurrent middle stem leaves – characters similar to those of ‘*C. axillaris*’.]

Nomenclature notes

Allioni (1774) named *Cyanus triumfetti* after Giovanni Battista Triumfetti (1658–1708), professor of botany and director of the Botanical Garden in Rome. In the original description Allioni used the epithet “*triumfetti*” as the appropriate Latin genitive to Latin form of the name Johannes Baptista Triumfettus. The other often used form “*triumfettii*” was formed by adding the appropriate genitive inflection (-i) to italian form of the name G. B. Triumfetti. According to the rules of the Code of Botanical Nomenclature (hereinafter the Code; Art. 60.; McNeill et al. 2006) both forms are correct, but the original spelling “*triumfetti*” has priority.

When Willdenow (1803) published the name *Centaurea axillaris* Willd., he cited in the synonymy two previously published names, *Centaurea variegata* Lam. and *Centaurea seusana* Chaix. According to the rules of the Code (Art. 52.1., 52.2.; McNeill et al. 2006), because Willdenow did not use the epithet of one of the cited names this name is illegitimate. Both names *Centaurea variegata* and *Centaurea seusana* belong to the taxon occurring in the south-eastern part of France; the correct name of this taxon in the genus *Cyanus* is *Cyanus graminifolius* (Lam.) Olšavská. The current karyological study indicates that *C. graminifolius* differs in its basic chromosome number and ploidy level from other taxa of the *C. triumfetti* group and is recognized as a separate taxon (Olšavská & Perný 2009). Thus, on the one hand, there are plants occurring in Central, eastern and southern Europe bearing the name *C. axillaris* according to numerous authors (e.g. Presl & Presl 1819, Čelakovský 1871, Velenovský 1891, Hayek 1901, Adamovič 1911, Jávorka 1924, Polívka et al. 1928, Hegi 1929, Dostál 1931a, 1931b, 1950, 1954, 1989, Hayek & Markgraf 1931, Stefanov & Georgiev 1931, Prodan & Nyárády 1964, Soó & Kárpáti 1968, Soó 1970, 1972, Marhold & Hindák 1998, Štěpánek 2002, 2004), whereas on the other hand, the name *C. axillaris* is typified by the type of *C. graminifolius* and belongs to the French plants according to the rules of the Code (Art. 7.5.; McNeill et al. 2006). Štěpánek (2004) tried to resolve this problem by introducing a new combination *Cyanus triumfetti* subsp. *axillaris* (J. Presl et C. Presl) Štěpánek. Štěpánek treated the name *Cyanus axillaris* J. Presl et C. Presl as the legitimate name of a new species because when Presl & Presl (1819) published this name, they did not refer to Willdenow or any other author. The most valid opinion in this case is that of Greuter which treats Presls' name as a combination based on an illegitimate name (Greuter 2006–2009) according to the rules of the Code (Art. 33.3.; McNeill et al. 2006). Article 33.3. of the Code should be applied here because Presls did not refer to the authors of the basionyms also for some other names [e.g. Presls did not refer to *Aira glauca* Schrad. (that they used in the genus *Koeleria* Pers.), to *Tussilago alba* L. (used in the genus *Petasites* Mill.), to *Centaurea nigrescens* Willd. (used in the genus *Cyanus*)]. Therefore, the name *Cyanus axillaris* J. Presl et C. Presl should be correctly cited as *Cyanus axillaris* (Willd.) J. Presl et C. Presl. As the basionymum for new combinations at the rank of subspecies, Greuter prefers to use the name *Centaurea montana* subsp. *axillaris* (Willd.) Čelak published by Čelakovský (1871). In some of the studies that concentrate on Central and eastern European flora, authors prefer to use the name *Centaurea triumfetti* subsp. *aligera* (Gugler) Dostál instead of *Cyanus axillari*s (Dostál 1976a, Soó 1980, Wagenitz 1987, Meusel & Jäger 1992, Simon 1992, Horváth et al. 1995, Ciocârlan 2000, Haupler & Muer 2000, Oprea 2005). This name was published by Gugler (1907) as *Centaurea variegata* var. *aligera* Gugler. While Gugler referred to Willdenow's name in the protologue and did not adopt the epithet "*axillaris*", the name *Centaurea variegata* var. *aligera* is also treated as nomen illegitimum (cf. Greuter 2006–2009) according to the rules of the Code (Art. 52.1., 52.2.; McNeill et al. 2006). As a result, all the available names, and any combinations based on them referred to Willdenow's name *Centaurea axillaris* Willd., have to be typified by the type of *Cyanus graminifolius*. Thus, the taxon that is now called *Cyanus axillaris* or *Centaurea triumfetti* subsp. *aligera* differs from the taxon to which those names were applied when following the rules of the Code. To resolve this problem, a new name needs to be chosen for European populations treated currently as *Cyanus axillaris*, auct.

Taxonomic treatment and typification of *Cyanus dominii*

Cyanus dominii (Dostál) Holub in Folia Geobot. Phytotax. 12: 308. 1977

Cyanus dominii (Dostál) Holub subsp. *dominii*

Basionym: *Centaurea triumfetti* subsp. *dominii* Dostál in Acta Bot. Bohem. 10: 71. 1931.
Ind. loc.: "Slovakia centralis, in rupibus trachyticis montis Bránisko, altitude 800 m s. m., leg. Jos. Dostál, 1928".
Neotype (designated here): "Slovakia, Volovské vrchy Mts, Mt. Humenec near Veľká Lodina village; 48°51'34" N, 21°09'33" E; 280 m; leg. & det. K. Olšavská, 26. 7. 2007" (SAV).

Synonymy:
≡ *Centaurea dominii* (Dostál) Dubovik in Bot. Zhurn. (Moscow & Leningrad)75: 1579. 1990.
≡ *Cyanus strictus* subsp. *dominii* (Dostál) Soják in Čas. Nár. Muz., Odd. Přír. 140: 131. 1972.
≡ *Cyanus triumfetti* subsp. *dominii* (Dostál) Dostál in Folia Mus. Rerum Nat. Bohemiae Occid., Bot. 21: 14. 1984.
– *Centaurea triumfetti* subsp. *dominii* var. *eu-Dominii* Dostál in Acta Bot. Bohem. 10: 71. 1931, nom. inval. (Art. 24.3.).

Note: Neotype of *Cyanus dominii* was designated here because the holotype cited in protologue of the name *Centaurea triumfetti* subsp. *dominii* and other original material belonging to this name is missing. The specimen collected at Veľká Lodina in the Volovské vrchy Mts fits the protologue as well as our results and will serve as the nomenclatural type of this taxon until Dostál's original material is rediscovered.

Cyanus dominii* subsp. *slovenicus (Dostál) Olšavská, **comb. nova**

Basionym: *Centaurea triumfetti* subsp. *dominii* var. *slovenica* Dostál in Acta Bot. Bohem. 10: 71. 1931.
Ind. loc.: "Slovakia, in Carpatis occidentalibus". **Lectotype (designated here):** "*Centaurea Triumfetti* All. ssp. *Dominii* Dostál var. *slovenica* Dostál, Lubochňa – před hotelem Bratislava, Det. Dr. Jos. Dostál, 27/VII. 1919" (PRC).

Note: The herbarium sheet from the surrounding of Lubochňa village in the north part of the Veľká Fatra Mts determined by Dostál in 1929 is selected here as a lectotype of *Cyanus dominii* subsp. *slovenicus*. In the protologue of *Centaurea triumfetti* subsp. *dominii* var. *slovenica* (Dostál 1931a) no specimens are cited, so the most representative specimen from the area mentioned in the protologue ("Slovakia, in Carpatis occidentalibus") was chosen.

Cyanus dominii* subsp. *sokolensis (Pawł.) Olšavská, **comb. nova**

Basionym: *Centaurea axillaris* var. *sokolensis* Pawł. in Kosmos (Lvov) 55: 70. 1930.
Ind. loc.: "Skały wapienne Sokol 1235–1320 m; Mnich 1462 m". **Lectotype (designated here):** "*Centaurea axillaris* W. var. *sokolensis* m. ad int., Tatry, pasmo Sivego Wierchu, Góra Sokol 1320 m, skały wap., B. Pawłowski., 27. 7. 1927" (KRAM 305241).
Synonymy:
≡ *Centaurea triumfetti* subsp. *sokolensis* (Pawł.) Dostál in Květena ČSR: 1686. 1950 (p. p.)
≡ *Centaurea triumfetti* subsp. *dominii* var. *densifolia* f. *sokolensis* (Pawł.) Dostál in Acta Bot. Bohem. 10: 72. 1931
= *Centaurea triumfetti* subsp. *dominii* var. *densifolia* Dostál in Acta Bot. Bohem. 10: 72. 1931
= *Centaurea triumfetti* subsp. *dominii* var. *densifolia* f. *densifolia* Dostál in Acta Bot. Bohem. 10: 72. 1931

Note: One of two syntypes cited in the protologue of the name *Centaurea axillaris* var. *sokolensis* Pawł. (Pawłowski 1931) is here selected as the lectotype of *Cyanus dominii* subsp. *sokolensis*. The specimen collected on Mt. Sokol is selected as lectotype because *Cyanus dominii* subsp. *sokolensis* was named after this mountain.

See http://www.preslia.cz for Electronic Appendix 1

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Súhrn

Morfometrická analýza 71 populácií (1148 jedincov) skupiny *Cyanus triumfetti* zo strednej Európy ukázala, že na tomto území možno rozlíšiť tri druhy: '*C. axillaris*', *C. strictus* a *C. dominii*. *Cyanus dominii* odlišujú od '*C. axillaris*' a *C. strictus* nezbiehavé alebo krátko-zbiehavé byľové listy; k tomuto druhu patria populácie zo stredného a východného Slovenska. *Cyanus strictus* a '*C. axillaris*' sa líšia farbou bŕv príveskov zákrovných listeňov, počtom a tvarom byľových listov. K druhu *C. strictus* boli priradené populácie zo severovýchodnej časti Maďarska a juhovýchodného Slovenska, k druhu '*C. axillaris*' patria všetky študované populácie z Rakúska a Českej republiky a niektoré populácie z Maďarska a Slovenska. Výsledky morfologických analýz potvrdili veľkú vnútrodruhovú variabilitu stredoeurópskych populácií *C. dominii* a možnosť ich klasifikácie do troch poddruhov: *C. dominii* subsp. *dominii* (Volovské vrchy, Branisko), *C. dominii* subsp. *slovenicus* (stredné Slovensko) a *C. dominii* subsp. *sokolensis* (Slovenský kras, Západné Tatry). Kultivačné experimenty ukázali, že '*C. axillaris*', *C. strictus* a *C. dominii* sa líšia v miere ekologickej plasticity, pričom najvýraznejšie ekologicky ovplyvnené boli byľové a listové znaky. Posun v analyzovaných znakoch neovplyvnil možnosť jednotlivé taxóny rozlíšiť a rozdiely medzi taxónmi zostali zachované aj pri pestovaní v rovnakých podmienkach. Analýza AFLP markerov 38 populácií zo skupiny *C. triumfetti* a dvoch populácií zo skupiny *C. montanus* (55 jedincov) odhalila tri geneticky odlišné a alopatrické skupiny: (1) *C. triumfetti* s.s. a *C. montanus* zo Západných Álp, (2) '*C. axillaris*' z Rakúska a Českej republiky (okrem Karpát), a (3) '*C. axillaris*', *C. strictus* a *C. dominii* zo Západných Karpát a Panónie. Genetická separácia vzoriek *C. triumfetti* s.s. potvrdila výsledky predchádzajúcich morfologických analýz, ktoré vylúčili výskyt tohto druhu na území Strednej Európy. Stredoeurópske vzorky skupiny *C. triumfetti* boli rozdelené do dvoch skupín, ktoré nekorelovali s taxónmi potvrdenými na základe morfologickej diferenciácie. Analýza AFLP markerov ukázala genetické odčlenenie vzoriek z Rakúska a Českej republiky patriacich k druhu '*C. axillaris*', ako aj veľkú genetická variabilitu a slabú genetickú diferenciáciu vzoriek zo Západných Karpát a Panónie patriacich druhom '*C. axillaris*', *C. strictus* a *C. dominii*. Získaný obraz genetickej variability je diskutovaný v súvislosti s potenciálnymi glaciálnymi refúgiami, cestami postglaciálnej migrácie a hybridizačnými udalosťami, ktoré sa mohli vyskytovať v evolučnej histórii skupiny. Ploidná úroveň študovaných populácií bola stanovená pomocou prietokovej cytometrie: diploidná ploidná úroveň (2n ~ 2x ~ 22) bola potvrdená pre jedincov zo skupiny *C. triumfetti* a tetraploidná (2n ~ 4x ~ 44) pre jedincov zo skupiny *C. montanus*.

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