

Populations of *Knautia* in ecologically distinct refugia on the Hercynian massif belong to two endemic species

Chrastavce (*Knautia*) z hadcových a subalpínských hercynských refugií představují dva samostatné endemické druhy

Filip Kolář^{1,2,3}, Zdeněk Kaplan³, Jan Suda^{2,3} & Milan Štech⁴

¹National Centre for Biosystematics, Natural History Museum, University of Oslo, NO-0318 Oslo, Norway, e-mail: filip.kolar@gmail.com; ²Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 00 Prague, Czech Republic, e-mail: zdenek.kaplan@ibot.cas.cz; ³Institute of Botany, The Czech Academy of Sciences, Zámek 1, CZ-252 43 Průhonice, Czech Republic; ⁴Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-370 05 České Budějovice, Czech Republic, e-mail: stech@prf.jcu.cz

Kolář F., Kaplan Z., Suda J. & Štech M. (2015): Populations of *Knautia* in ecologically distinct refugia on the Hercynian massif belong to two endemic species. – Preslia 87: 363–386.

Comprehensive taxonomic studies in which a combination of molecular, cytogenetic, morphological and ecological approaches are used have resulted in remarkable discoveries even in well-known floras. In particular, recognition of new local endemics has important implications for conservation and management of plant diversity. Due to Quaternary climatic oscillations, the vascular flora of the Czech Republic only includes a few endemic taxa, usually microspecies with an apomictic mode of reproduction. Here we re-evaluate the taxonomy of *Knautia arvensis*, an intricate eco-geographically differentiated diploid-polyploid complex, and identify two new sexual species endemic to central Europe, which were previously included in the polymorphic *K. arvensis*. While *K. serpentinicola* Smejkal ex Kolář, Z. Kaplan, J. Suda et Štech is a diploid and tetraploid species restricted to four isolated serpentine areas in the Czech Republic and Germany, diploid *K. pseudolongifolia* (Szabó) Žmuda is known from a single subalpine site in the Krkonoše Mts. Our investigation of 38 populations of *K. arvensis* s. str. and the two newly recognized species sampled across eastern central Europe revealed a distinct yet incomplete (i.e. confounded by phenotypic plasticity) morphological differences between the three species. These results together with available data on cytological (distinct nuclear genome size), genetic (independent evolutionary histories) and ecological (distinct ecological preferences) variation support an independent taxonomic status for the newly described species. Our study highlights the importance of ecologically stable habitats where plant competition is not severe (Holocene refugia) for preserving unique plant diversity. In addition, it demonstrates the value of multi-disciplinary taxonomic research even in botanically well-known areas.

Key words: central Europe, endemic species, *Knautia*, multivariate morphometrics, polyploidy, postglacial relict, refugium, serpentine, speciation, taxonomy

Introduction

The rapid developments in molecular, cytogenetic and statistical tools have greatly influenced many research fields, including plant taxonomy and biosystematics. Detailed investigations have resulted in new discoveries and taxonomic re-assessments, even in well-researched floras such as that of the Czech Republic. The last decade has seen an increase in comprehensive studies of groups of plants with locally distributed species (putative endemics or subendemics). These have considerably changed our view of the

ecology (e.g. *Cerastium alsinifolium*: Vít et al. 2014) and distribution (e.g. *Sorbus bohemica*: Lepší et al. 2009; and *S. eximia*: Vít et al. 2012) of some Czech endemics, which resulted in a re-assessment of their taxonomy. While some central-European endemics have lost their species status after careful taxonomic revision (e.g. *Melampyrum bohemicum*: Štech 2006; *Sorbus querneae*: Lepší et al. 2013), new (sub)endemics are still being described, mainly in agamic complexes such as *Rubus* (Trávníček et al. 2005, Lepší & Lepší 2006, 2009, Trávníček & Žíla 2011), *Sorbus* (Lepší et al. 2008, 2009, 2013, Velebil 2012, Vít et al. 2012) and *Taraxacum* (Vašut & Trávníček 2004, Trávníček et al. 2008). In contrast, newly recognized sexual endemics are rare. Out of the 27 endemic taxa with a sexual mode of reproduction currently recognized in the Czech flora (Kaplan 2012), seven were described during the last five decades, namely *Campanula gelida* (Kovanda 1968), *Carex derelicta* (Holub 1960, 1965, Štěpánková 2008), *Dianthus moravicus* (Kovanda 1982), *Euphrasia corcontica* (Smejkal 1963, Dvořáková 1999a), *Minuartia corcontica* (Dvořáková 1999b), *M. smejkalii* (Dvořáková 1988) and *Scilla bifolia* subsp. *rara* (Trávníček et al. 2010). All putative Czech endemics were mainly delimited on the basis of their morphology, occasionally also karyological data, while information on their genetic variation and phylogenetic relationships inferred from molecular markers is largely missing, leaving ample room for taxonomic uncertainties and confusions (see Kaplan 2012). This is very different from the situation in zoology, where new taxa, including endemics in the Czech Republic and adjacent areas, are supported by evidence obtained from many different sources (e.g. Řezáč et al. 2008, Khatib et al. 2014).

In the present study, we investigate the variation of the polymorphic species *Knautia arvensis* (*Dipsacaceae*, or *Caprifoliaceae* – *Dipsacoideae*) in eastern central Europe as delimited in the Flora of the Czech Republic (Štěpánek 1997). We place special emphasis on the unique diversity preserved at ecologically distinct natural sites such as serpentine outcrops and subalpine glacial cirques that are known to harbour numerous Holocene plant relicts (e.g. Chytrý 2007, Kaplan 2012). *Knautia arvensis* belongs to the highly polymorphic, ploidy-variable and taxonomically challenging *Knautia* section *Trichera* (Schrad. ex Roem. et Schult.) DC., which occurs from western Asia to western Europe, with centres of diversity in the Balkans and Southern Alps (Ehrendorfer 1962, 1981, Rešetnik et al. 2014, Frajman et al. 2015). The evolutionary history of this section is complex, being shaped by several interacting processes. First, different species and/or cytotypes are often eco-geographically differentiated, with diploids usually growing in open less competitive habitats and polyploids at more competitive and/or man-disturbed sites (Ehrendorfer 1962, 1981; but widespread ruderal diploids are known from south-eastern Europe: Rešetnik et al. 2014). Island-like distribution of diploid populations may lead to considerable allopatric divergence potentially resulting in speciation. Divergent diploids may independently undergo genome duplication and the resulting polyploid derivatives frequently hybridize introgressively with other species of the same ploidy level, blurring species boundaries (Ehrendorfer 1962, Breton-Sintes 1974). Finally, great phenotypic plasticity in relation to ecological conditions makes morphology-based taxonomic conclusions uncertain. Interestingly, in contrast to frequent homoploid hybridization, strong reproductive barriers exist between diploid and tetraploid *Knautia* plants (Ehrendorfer 1962, Breton-Sintes 1974, 1975, Kolář et al. 2009, Hanzl et al. 2014).

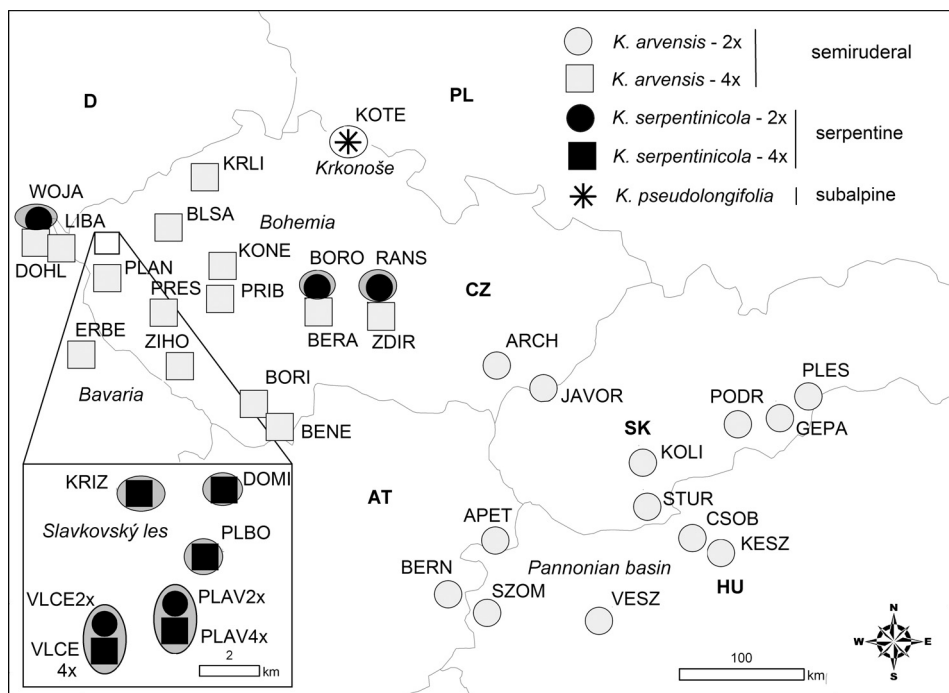


Fig. 1. – Geographic distribution, habitat preferences and ploidy level of the populations of *Knautia arvensis*, *K. serpentinicola* and *K. pseudolongifolia* analysed in this study. Circles – diploid populations, squares – tetraploid populations, white oval – subalpine grasslands, grey ovals – serpentine pine forests; the remaining populations occur in semiruderal grasslands (based on data of Kolář et al. 2009, 2012). The square inset displays the situation in the diploid-tetraploid serpentine area in the Slavkovský les Mts. See Table 1 for population codes.

Four subspecies of *Knautia arvensis* are traditionally recognized in the Czech Republic and adjacent areas, based on phenotypic, ecological, geographic and karyological differences (Štěpánek 1997, Kolář et al. 2012). Widespread diploids [$2n = 2x = 20$, subsp. *pannonica* (Heuff.) O. Schwarz] occur mainly in the southern part of central Europe, whereas widespread tetraploids ($2n = 4x = 40$, subsp. *arvensis*) occupy the northern half of the region (Štěpánek 1997, Fischer 2008, Király 2009). Both cytotypes are morphologically very similar and prefer semiruderal mesophilous grasslands (Štěpánek 1997). In addition, there are a few spatially isolated diploid populations in areas otherwise occupied by tetraploids (Fig. 1). These populations inhabit ‘Holocene refugia’, i.e. sites with a low level of competition where they most likely survived periods of forest expansion during the Holocene (Štěpánek 1989, Kaplan 1998, Kolář et al. 2012). First, a single morphologically distinct population from the subalpine carbonate outcrop in the Kotelní jámy glacial cirque in the Krkonoše Mts has long been recognized as subsp. *pseudolongifolia* (Szabó) O. Schwarz (Štěpánek 1989, Štěpánek & Procházka 1999, Krahulec 2006, Kaplan 2012). Second, three pure diploid populations and one diploid-tetraploid (meta)population occur in open pine forests growing on serpentine outcrops in Bohemia and northern Bavaria, and have been tentatively referred to as subsp. *serpentinicola* nom. inval. (Štěpánek 1997, Kaplan 1998, Danihelka et al. 2012; Fig. 1). Morphological

assessment of five characters of stem leaves and capitula indicate the distinctness of serpentine populations, however, limited sampling preclude robust taxonomic conclusions (Kaplan 1998). Importantly, the isolated diploid serpentine and subalpine populations form a distinct genetic lineage within all European *Knautia* diploids (i.e. the ‘Northern Arvensis Group’ in Rešetnik et al. 2014). The lineage itself shows a further slight genetic differentiation, most likely reflecting long-term isolation in distinct refugia (Kolář et al. 2012). Finally, serpentine tetraploids occurring in the Slavkovský les Mts (Fig. 1) originated *in situ* from their still present serpentine diploid counterparts and are only distantly related to surrounding non-serpentine tetraploids of *K. arvensis* subsp. *arvensis* (although both tetraploid cytotypes may hybridize; Kaplan 1998, Kolář et al. 2009, 2012).

This article provides a taxonomic evaluation of those central-European populations inhabiting natural habitats with a relict flora that were traditionally assigned to *Knautia arvensis*. We summarize the recently recorded karyological, genetic and ecological evidence and provide a detailed morphological assessment of the group studied. Our synthesis shows that the two lineages from isolated Holocene refugia are sufficiently distinct to merit the status of separate species. *Knautia serpentinicola* and *K. pseudolongifolia* are described at the end of the article, but their names are for the sake of clarity used hereafter.

Materials and methods

Field sampling

Plant material was collected from 2005 to 2008 in Austria, the Czech Republic, Germany, Hungary and Slovakia. Because our study focused on populations inhabiting natural habitats with a relict flora the serpentine ‘archipelago’ in the Slavkovský les Mts, in western Bohemia was sampled more intensely (Kolář et al. 2009, Hanzl et al. 2014). In total, we sampled 38 populations (Table 1), including (i) 13 populations of 2x *K. arvensis* subsp. *pannonica*, (ii) 14 populations of 4x *K. arvensis* subsp. *arvensis*, (iii) all known serpentine populations of *K. serpentinicola* (five diploid and five tetraploid, the metapopulation from the Slavkovský les Mts was further subdivided) and (iv) the single known subalpine population of *K. pseudolongifolia* in the Krkonoše Mts. Diploid and tetraploid subpopulations at the two mixed-ploidy serpentine sites (Table 1) were treated as separate entities due to strong inter-ploidy reproductive barriers (Breton-Sintes 1974, 1975, Kolář et al. 2009).

At each locality intact stems from 20 individuals on average (range 10–25; three and 35 individuals were collected in two exceptional cases, see Table 1) were sampled together with information on the habitat and geographic coordinates. Due to concerns about the need to conserve these species, we sampled only one fertile stem per individual, leaving basal leaf rosettes and underground parts. We aimed to cover the entire morphological variation present at each locality (except for plants lacking flowering stems or otherwise damaged and/or parasitized). Ploidy levels of all the individuals analysed were taken from Kolář et al. (2009). Genetic data (plastid and AFLP variation) were available for a subset of plants (Table 1; see Kolář et al. 2012). Vouchers are deposited in the herbarium of the Faculty of Science, University of South Bohemia, České Budějovice (CBFS). Isotypes of the newly described *K. serpentinicola* are also deposited in BRNM, BRNU, PR and PRC.

Table 1. – Details of 38 populations of the three *Knautia* species (*K. arvensis*, *K. pseudolongifolia* and *K. serpentinicola*, sorted according to ploidy and population code) subjected to multivariate morphometrics and the proportion of individuals correctly classified in the two modifications of the discriminant analysis. Code 1 – population code as in Kolář et al. (2012); Code 2 – population code as in Kolář et al. (2009), where locality details are provided. * Populations for which the classification success to a particular species is below 50%. Dataset 1 – all 16 characters were analysed in a subset of 675 individuals with divided leaves. Dataset 2 – nine characters were analysed for all 747 individuals (with both divided and undivided leaves). ** coordinates in Kolář et al. (2012) are inaccurate, the correct ones are provided in the description of *K. pseudolongifolia*.

Population code	Code 1	Code 2	Locality name, country	Habitat	Ploidy level	Number of individuals		Correct classification (%)	
						Dataset 1	Dataset 2	Dataset 1	Dataset 2
<i>K. arvensis</i>						501	537	81	75
APET	P12	2	AT – Apetlon	semiruderal	2x	19	20	47*	60
ARCH	P13	31	CZ – Archlebov	semiruderal	2x	20	20	95	100
BERN	P18	1	AT – Bernstein	semiruderal	2x	20	20	75	75
CSOB	P14	50	HU – Csobánka	semiruderal	2x	20	20	80	80
GEPA	–	60	SK – Gemerská Panica	semiruderal	2x	13	18	100	100
JAVOR	P15	19	CZ – Javorník	semiruderal	2x	20	20	100	90
KESZ	–	51	HU – Kesztole	semiruderal	2x	20	20	75	55
KOLI	–	56	SK – Kolíňany	semiruderal	2x	19	22	79	63
PLES	P10	61	SK – Plešivec	semiruderal	2x	10	20	90	90
PODR	P09	58	SK – Podrečany	semiruderal	2x	13	20	85	65
STUR	–	54	SK – Štúrovo	semiruderal	2x	20	20	80	90
SZOM	P17	49	HU – Szombathely	semiruderal	2x	19	19	95	94
VESZ	P16	48	HU – Veszprém	semiruderal	2x	20	20	100	90
BENE	P35	126	CZ – Benešov n. Černou	semiruderal	4x	20	20	70	80
BERA	P37	216	CZ – Bernartice	semiruderal	4x	19	20	74	50
BLSA	P32	225	CZ – Blšany	semiruderal	4x	19	20	84	70
BORI	P34	144	CZ – Křemže	border-serpentine	4x	18	20	44*	15*
DOHL	P27	242	D – Döhlau	semiruderal	4x	20	20	90	95
ERBE	–	240	D – Erbendorf	semiruderal	4x	20	20	80	70
KONE	P33	223	CZ – Koněprusy	semiruderal	4x	19	20	74	55
KRLI	–	229	CZ – Krásná Lípa	semiruderal	4x	20	20	75	70
LIBA	P28	224	CZ – Libá	semiruderal	4x	19	20	74	55
PLAN	P29	221	CZ – Planá	semiruderal	4x	20	20	90	80
PRES	P31	215	CZ – Přeštice	semiruderal	4x	20	20	80	75
PRIB	P30	217	CZ – Příbram	semiruderal	4x	20	20	85	80
ZDIR	P38	218	CZ – Ždírec	semiruderal	4x	14	18	79	94
			n. Doubravou						
ZIHO	–	181	CZ – Žihobce	semiruderal	4x	20	20	85	75
<i>K. serpentinicola</i>						174	185	87	81
BORO	P02	263	CZ – Borovsko	serpentine	2x	33	35	94	80
PLAV2x	P04	278	CZ – Planý vrch (2x)	serpentine	2x	7	10	100	90
RANS	P01	71	CZ – Staré Ransko	serpentine	2x	20	20	75	70
VLCE2x	P05	277	CZ – Vlček (2x)	serpentine	2x	2	3	100	66
WOJA	P03	279	D – Woja	serpentine	2x	19	19	89	100
DOMI	P24	261	CZ – Dominova skalka	serpentine	4x	20	20	80	80
KRIZ	P23	260	CZ – Křížky	serpentine	4x	20	20	70	60
PLAV	P20	278	CZ – Planý vrch (4x)	serpentine	4x	16	19	94	94
PLBO	P22	259	CZ – Pluhův bor	serpentine	4x	19	20	95	90
VLCE	P21	277	CZ – Vlček (4x)	serpentine	4x	18	19	89	73
<i>K. pseudolongifolia</i>						–	25	–	100
KOTE	P06	72**	CZ – Krkonoše	subalpine	2x	–	25	–	100
Total/average						675	747	82	77

Morphometric analyses

We assessed 16 morphological characters (12 primary and four ratios) of stems, stem leaves and the terminal inflorescence of air-dried herbarium vouchers (Table 2); ploidy level of all individuals investigated was inferred from fresh material prior to desiccation (data published in Kolář et al. 2009). Missing values of the width of the terminal head for three individuals were replaced by population means. Because seven of the 16 characters could only be evaluated on divided or lobate leaves, two datasets were generated and analysed separately. Entire or shallowly-lobed leaves were a feature of nearly all individuals at the subalpine site and also of a few plants in other populations. Dataset 1 included 675 plants with divided leaves and all 16 characters; Dataset 2 included all 747 plants but only nine characters. No pair of characters was highly correlated (Spearman's r always below 0.9). It should be noted that some characters previously used to identify *Knautia* species (e.g. characteristics of daughter rosettes and fruits) could not be statistically evaluated by us due to non-destructive sampling and a single visit to each locality (these characters are nonetheless addressed in the Discussion). Other characters were excluded because of their high environmentally determined plasticity (e.g. the length of involucral bracts, number and length of calyx bristles) as revealed by long-term experimental cultivation (Štěpánek 1979).

Delimitation of taxonomic groups for discriminant analyses was morphology-independent, based on patterns of ploidy and genetic variation (Kolář et al. 2009, 2012) and partly also on habitat-related distribution. Genetically highly divergent diploid populations with distinct monoploid genome size (cluster K1 of Kolář et al. 2012) formed one group (taxonomically corresponding to *K. arvensis* subsp. *pannonica*), while serpentine (*K. serpentinicola*) and subalpine (*K. pseudolongifolia*) diploids formed two additional taxonomic groups. At the tetraploid level, we distinguished two groups reflecting the serpentine/non-serpentine dichotomy (i.e. *K. serpentinicola* vs *K. arvensis* subsp. *arvensis*, respectively) and their independent evolutionary histories and partly also their genetic variation. The genetic structure at the tetraploid level was, however, less pronounced due to introgressive hybridization between different lineages (Kolář et al. 2012).

Data were analysed using a set of R-scripts morphotools 1.1 (Koutecký 2014) in R 3.1.1. Specifically, principal component analysis (PCA) was constructed to visualize main directions of variation of individuals and population means using the `prcomp` function in package `stats`. To test differences among the a-priori defined taxonomic groups we applied linear discriminant analysis using the `cca` function in `vegan` (Oksanen et al. 2013) and classificatory discriminant analysis using the `lda` function in `MASS` (Venables & Ripley 2002). We used four modifications of discriminant analyses: (i) a discrimination of three taxonomic groups regardless of ploidy (i.e. *K. serpentinicola*, *K. pseudolongifolia*, and *K. arvensis*) using only nine characters that were possible to score on plants with undivided leaves, (ii) a discrimination of two taxonomic groups regardless of ploidy using all characters (i.e. *K. serpentinicola* and *K. arvensis*), (iii) a discrimination of *K. serpentinicola* di- and tetraploids, and (iv) a discrimination of *K. arvensis* di- and tetraploids (using all 16 characters for both).

Table 2. – Morphological characters used in statistical analyses and their discrimination power in four separate linear discriminant analyses (LDA). * marginal effect, 0.003 < P < 0.05 (not passing Bonferroni correction), *** marginal effect, P < 0.003 (passing Bonferroni correction), +characters that were possible to score on plants with entire or shallowly lobed leaves. LDA 1 – discrimination of two taxonomic groups: *K. arvensis* and *K. serpentinicola* (Fig. 2B); LDA 2: discrimination of three taxonomic groups: *K. arvensis*, *K. serpentinicola* and *K. pseudolongifolia* (values for the first two canonical axes are shown; Fig. 3B); LDA 3: discrimination of two cytotype groups: 2x *K. arvensis* and 4x *K. arvensis*; LDA 4: discrimination of two cytotype groups: 2x *K. serpentinicola* and 4x *K. serpentinicola*.

Code	Description	LDA 1 axis 1	LDA 2 axis 1	LDA 2 axis 2	LDA 3 axis 1	LDA 4 axis 1
Height+	Plant height	0.69***	0.26***	0.82	−0.19*	0.03
BranchDg+	Degree of branching	0.35***	0.22***	0.42	−0.74***	−0.18
NLeaf+	Number of pairs of stem leaves	0.23***	0.14***	0.26	−0.15*	0.19
StemDiam+	Diameter of the stem 2 cm below the terminal head	0.39***	−0.62***	0.55	0.14*	0.52***
NGlands+	Number of glands on the stem 2–3 cm below the terminal head (semi quantitative)	0.18***	0.12***	0.21	−0.01	−0.10
HeadDiam+	Head diameter (distance between the tips of bracts in the terminal head)	0.06	−0.33***	0.13	0.24***	0.23*
LLength+	Length of the 2nd lowermost stem leaf	0.30***	−0.06***	0.39	−0.13*	−0.23*
LWidth+	Width of the 2nd lowermost stem leaf	0.28***	0.22***	0.29	0.01	−0.19
LatLength	Length of the longest lateral lobe of the 2nd stem leaf	0.31***	–	–	0.05	−0.02
LatWidth	Width of the longest lateral lobe of the 2nd stem leaf	0.45***	–	–	−0.16*	−0.25*
TerLength	Length of the terminal lobe of the 2nd stem leaf	0.15***	–	–	−0.08	−0.14
TerWidth	Width of the terminal lobe of the 2nd stem leaf	0.30***	–	–	−0.16*	−0.21
LL_LW+	Length / width of the 2nd stem leaf	−0.15***	−0.44***	−0.03	−0.12*	0.07
LatL_LatW	Length / width of the longest lateral lobe of the 2nd stem leaf	−0.20***	–	–	0.25***	0.18
TerL_TerW	Length / width of the terminal lobe of the 2nd stem leaf	−0.31***	–	–	0.07	0.11
LL_TerL	Total length / length of the terminal lobe of the 2nd stem leaf	0.11*	–	–	−0.07	−0.08

Results

PCA analysis of populations containing plants with divided leaves, i.e. *K. serpentinicola* and *K. arvensis*, revealed some morphological differentiation between these species (Fig. 2A). However, the phenotypic variation was rather continuous and *K. serpentinicola* largely overlapped *K. arvensis* in the PCA plot of individual plants (Electronic Appendix 1). Slender and less-branched individuals with shorter and narrower stem leaves in the latter species posed considerable problems (see inset in Fig. 2A). Discriminant analysis was highly significant (P = 0.001 with 1000 permutations; Fig. 2B) with 82% successfully classified individuals (Table 1). Plants of *K. arvensis* were slightly less well classified than those of *K. serpentinicola* (81% vs 87%). Most misclassified *K. arvensis* plants originated from populations inhabiting sites with distinct ecological conditions (e.g. pop. APET growing in subhalophilous steppe or pop. BORI at the margin of a serpentine pine forest, both with less than 50% of the individuals correctly classified; Table 1). The following characters contributed most to the discrimination of both taxonomic groups (*K. serpentinicola* plants always have lower character values): plant height, stem diameter, degree of branching, length and width of the second stem leaf, length and width of the longest lateral lobe and length/width ratio of the terminal lobe (Table 2).

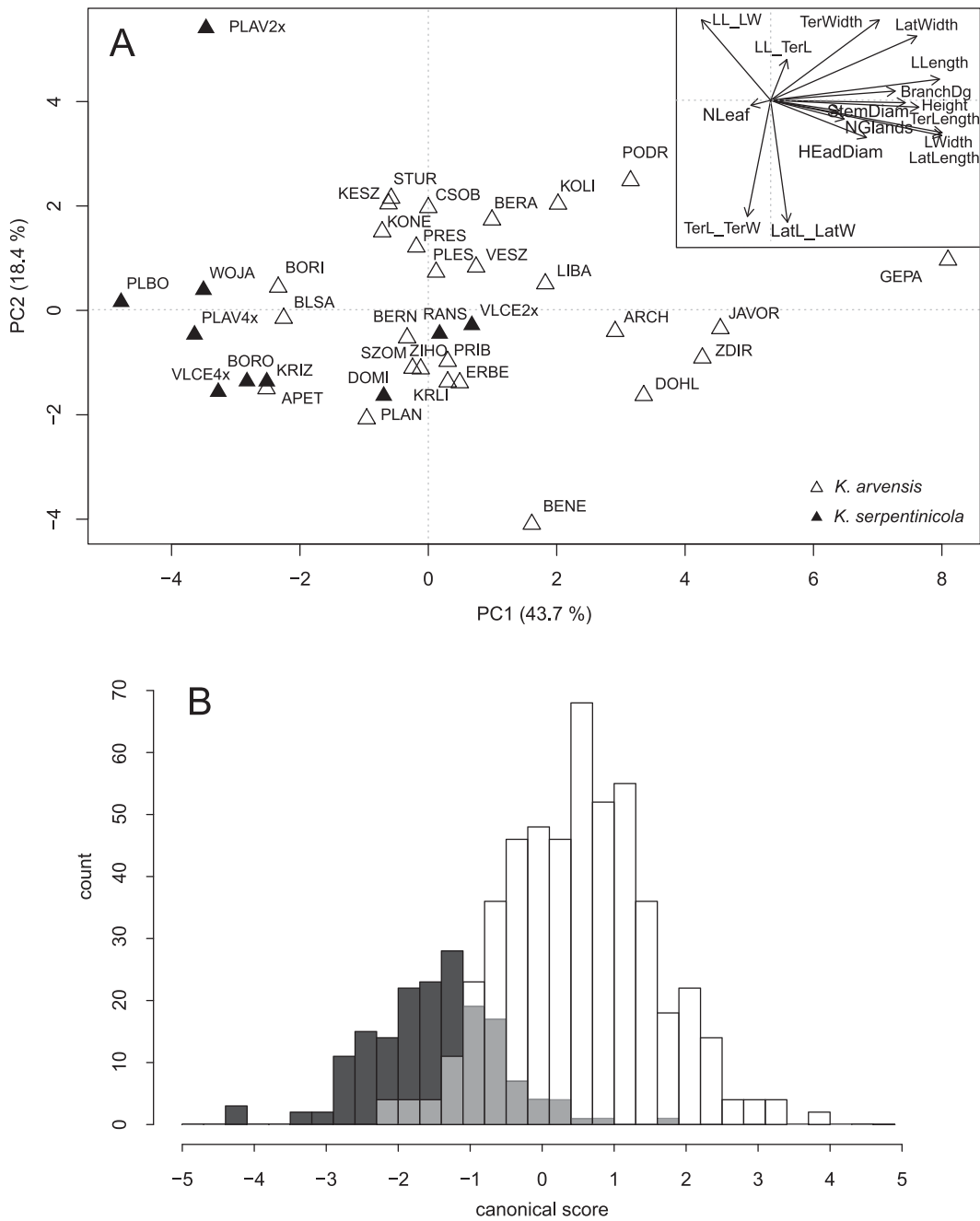


Fig. 2. – Morphological differentiation of *Knautia arvensis* (open triangles) and *K. serpenticicola* (solid triangles). (A) Principal component analysis of 37 populations based on mean values per population (only accessions with divided leaves were included). The inset displays ordination of 16 morphological characters used in the analysis (see Table 2 for code explanations). (B) Scores of individual plants of the two species on the first canonical axis of the linear discriminant analysis using the 16 characters (overlap of both species is indicated by grey colour).

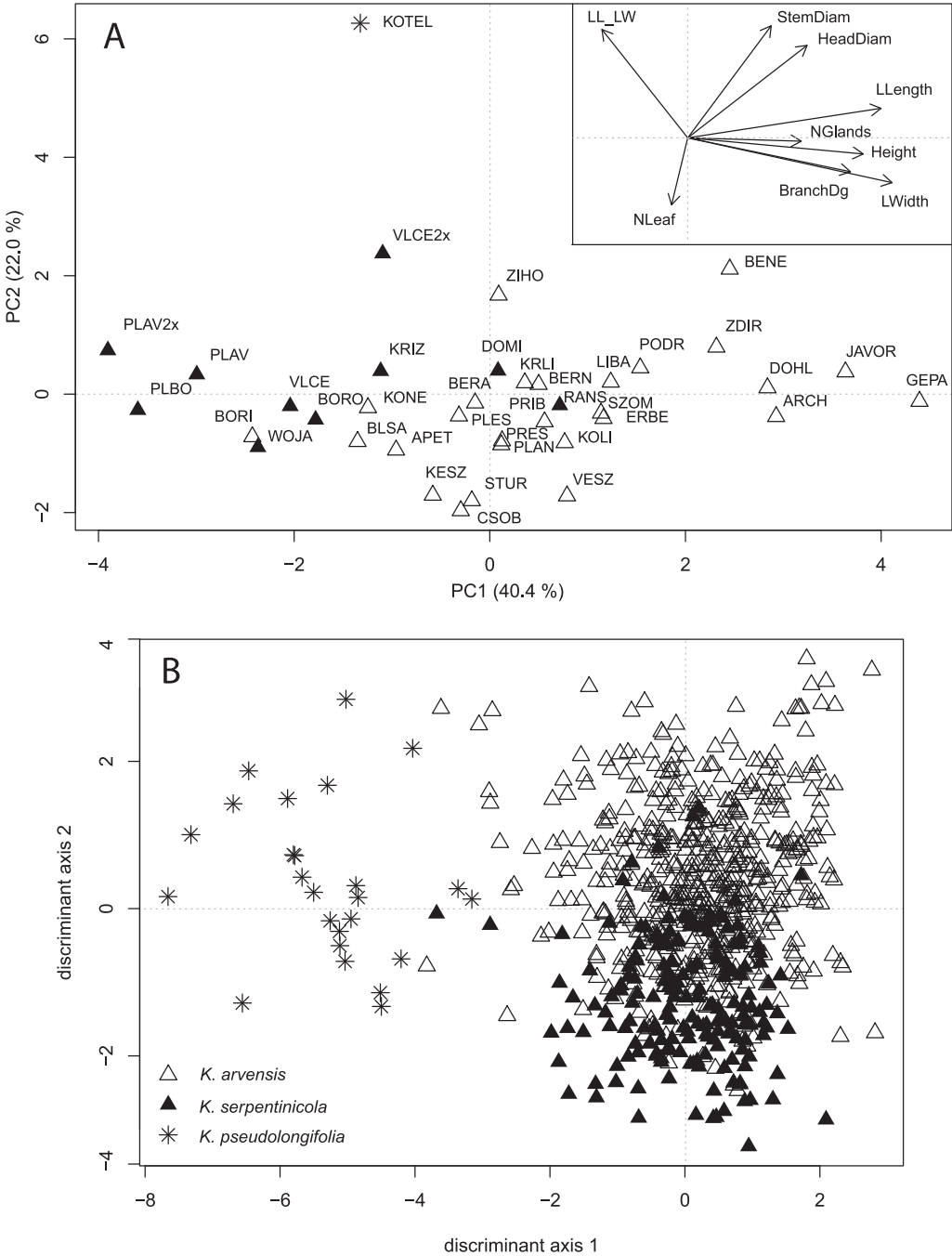


Fig. 3. – Morphological differentiation of *Knautia arvensis* (open triangles), *K. serpentinicola* (solid triangles) and *K. pseudolongifolia* (asterisks). (A) Principal component analysis of all 38 populations. The inset displays ordination of nine morphological characters used in the analysis (see Table 2 for code explanations). (B) Ordination of all 747 individuals along the two canonical axes of the linear discriminant analysis using the nine characters.

The subalpine diploid population of *K. pseudolongifolia* is morphologically distinct and well separated from the other two species (Fig. 3A). Discriminant analysis further confirmed considerable phenotypic differences ($P = 0.001$ with 1000 permutations; Fig. 3B) and resulted in 100% of the individuals being correctly classified (Table 1). In comparison with other *Knautia* species studied, *K. pseudolongifolia* has generally narrower stem leaves, higher leaf length/width ratio and stouter stems below the terminal inflorescence (Table 2).

Because the above analyses included both diploid and tetraploid individuals, we further investigated potential inter-ploidy differences in each of the ploidy-variable groups separately. Di- and tetraploids were phenotypically similar in both *K. serpentinicola* and *K. arvensis* (Electronic Appendix 2) although discriminant analysis revealed a significant effect of ploidy level ($P = 0.001$, 1000 permutations in both cases). Classification of individuals according to their ploidy level was largely unsuccessful for *K. serpentinicola* (59% success rate) but moderately successful for *K. arvensis* (72% success rate; see Table 3 for details).

Discussion

In this article we summarize many pieces of evidence that support the independent taxonomic status of two little known sexual lineages of *Knautia* endemic to the Hercynian massif, described here as *K. serpentinicola* and *K. pseudolongifolia*, which were assigned traditionally to polymorphic *K. arvensis*. We argue that the level of genetic, cytological, morphological and ecological differentiation of these central-European endemics is comparable to other currently recognized species in the genus *Knautia* (Ehrendorfer 1976, Rešetnik et al. 2014).

Genetic and cytological evidence

The separate evolutionary position of *K. serpentinicola* and *K. pseudolongifolia* (as against diploid *K. arvensis* subsp. *pannonica*) is indicated by both the nuclear genome size, which is significantly smaller in the two former species (Kolář et al. 2009) and by AFLP markers, which clearly distinguished the lineage containing *K. serpentinicola* and *K. pseudolongifolia* from that of *K. arvensis* subsp. *pannonica* (Kolář et al. 2012). Importantly, the group formed by *K. serpentinicola* and *K. pseudolongifolia* is genetically distinct in both ITS and AFLP analyses of all diploid members of the section *Trichera* (Rešetnik et al. 2014). *Knautia serpentinicola* and *K. pseudolongifolia* are closely related to *K. slovacica* Štěpánek, another local endemic of central Europe occupying somewhat similar habitats in open limestone pine forest (Štěpánek 1983); all three species form a separate lineage within the genus (Rešetnik et al. 2014). *Knautia slovacica*, however, differs from both the newly described species and *K. arvensis* in terms of their AFLP profiles (Kolář et al. 2012), dense and soft indumentum on both stem and leaves, relatively long terminal leaflets of stem leaves and pink to reddish-violet corollas (Štěpánek 1983, 1985). Diploids of *K. serpentinicola* and *K. pseudolongifolia* also differ genetically from the tetraploid *K. arvensis* subsp. *arvensis* (Kolář et al. 2012). In contrast, the 2x and 4x cytotypes of *K. serpentinicola* are genetically very similar, indicating a local autopolyploid origin of the tetraploids (Kolář et al. 2012).

Table 3. – Classification success of diploid and tetraploid individuals analysed separately for *Knautia arvensis* and *K. serpentinicola*.

	N	correct classification (%)
<i>Knautia arvensis</i>		
diploid	233	73
tetraploid	268	71
total	501	72
<i>Knautia serpentinicola</i>		
diploid	81	56
tetraploid	93	62
total	174	59

Reproductive evidence

Both endemic species seem to be well separated reproductively from each other and from other taxa. *Knautia serpentinicola* and *K. pseudolongifolia* are currently isolated by distinct eco-geographical barriers (although we are aware that this does not necessarily imply physiological reproductive barriers; Ehrendorfer 1962, Štěpánek 1979). *Knautia serpentinicola* occasionally meets tetraploid *K. arvensis*, which occurs at the borders of serpentine outcrops. Owing to strong inter-ploidy breeding barriers in the entire section *Trichera* (Ehrendorfer 1962, Breton-Sintes 1974, 1975), including the nearly total absence of triploids (i.e. mediators of possible unidirectional gene flow from tetraploids to diploids; Kolář et al. 2009, Hanzl et al. 2014), this spatial contact poses only a minimal threat to the genetic integrity of the serpentine diploids. In contrast, patterns of genetic and phenotypic variation indicate it is very likely that tetraploid cytotypes of *K. serpentinicola* and *K. arvensis* hybridize in the Slavkovský les Mts. However, the gene flow seems to be unidirectional from serpentine to non-serpentine populations (Kolář et al. 2012), ensuring the integrity of 4x *K. serpentinicola*. Low level of hybridization recorded in stands growing on serpentine may be, at least in part, associated with the reduced fitness of the non-serpentine *K. arvensis* under stressful serpentine conditions (Kolář et al. 2014).

Ecological and distributional evidence

The complex late Pleistocene/Holocene history of the populations investigated has resulted in a distinct spatio-ecological pattern in central Europe (Kaplan 1998, Kolář et al. 2012). While *K. arvensis* typically occupies various semiruderal grasslands and has a rather continuous distribution at low and middle altitudes, the two relict species presumably of late Pleistocene/early Holocene origin occur in spatially isolated, ecologically stable, low-competitive habitats, including open forests on serpentine soil (*K. serpentinicola*) and a carbonate outcrop in a subalpine glacial cirque (*K. pseudolongifolia*). Our cultivation experiments have shown that both cytotypes of *K. serpentinicola* are well adapted to growing in chemically stressful serpentine soils, whereas central-European populations of *K. arvensis* exhibit considerably reduced growth in serpentine conditions (Kolář et al. 2014).

Morphological evidence

Our morphometric analyses using fifteen vegetative and one generative character revealed clear trends in phenotypic variation that correspond with the genetic, cytological and ecological differentiation of the group studied. However, large phenotypic plasticity, particularly of *K. arvensis*, and probable gene flow at the tetraploid level account for the incomplete morphological segregation of the three species investigated.

Knautia pseudolongifolia is morphologically the most distinct taxon and is characterized by prolonged and usually undivided or only shallowly divided stem leaves that are glabrous or sparsely pubescent, large terminal heads born on stout stems and relatively large fruits (our data and that of Štěpánek 1982, 1989, 1997). Thus, these pronounced morphological and ecological differences justify the separation of *K. pseudolongifolia* from the genetically close *K. serpentinicola*.

Despite obvious trends in morphological data, *K. serpentinicola* is only slightly different from the widespread *K. arvensis* as there is considerable overlap in most of the characters examined. Inter-specific differences are usually quantitative and at least some characters seem to be dependent on soil conditions (see below). It should, however, be noted that our sampling intentionally covered the entire morphological variation present in each population and that extreme forms might have blurred boundaries between the species. In addition, we did not formally explore the value of potentially taxonomically informative characters, which are difficult to evaluate statistically or can hardly be assessed on herbarium vouchers, including the overall stature (more slender in *K. serpentinicola*; Štěpánek 1997, our field observations), flower colour (darker reddish-violet in *K. serpentinicola*; Štěpánek 1982, Kaplan 1998), shape of the lower corolla lobe (longer and narrower in *K. serpentinicola*; our field observations) and the frequent production of multiple lateral rosettes in *K. serpentinicola* (Štěpánek 1997). Importantly, several characters used to recognize *K. serpentinicola* (e.g. slender appearance, slender stem, dark flowers, smaller leaves) seem to be (epi)genetically fixed and independent of ecological conditions because they have remained stable in the same individual grown in garden soil over period of 17 years (Z. Kaplan, pers. observation; see Electronic Appendix 3). Thus, long-term common garden experiments combined with reciprocal transplants are needed to understand the interplay of ecological and genetic factors in shaping the phenotype of individual species of *Knautia*.

Knautia plants growing in atypical habitat for a given species (e.g. *K. arvensis* at the borders of serpentine areas and sub-halophilous sites, or *K. serpentinicola* at the margins of forest roads and grasslands on deforested serpentine outcrops) are particularly difficult to determine morphologically because they resemble the other taxon (see Figs. 2, 3). Such phenotypes are most likely manifestations of the great phenotypic plasticity of both species although genetic reasons (i.e. gene flow between serpentine and non-serpentine tetraploids; Kolář et al. 2012) cannot be excluded for certain populations in the Slavkovský les Mts.

Individuals of different ploidy levels within the same species only differ slightly morphologically. This is particularly true for *K. serpentinicola* (classification success below 60%), in which inter-ploidy morphological similarities are likely to reflect close genetic relationships between diploids and their local autotetraploid derivatives. As expected, classification success of different cytotypes of *K. arvensis* (2x subsp. *pannonica* and 4x

subsp. *arvensis*) was higher (~72%), which reflects the deep genetic split between both ploidy levels (Kolář et al. 2012) and their largely parapatric distribution in ecologically distinct regions on the Hercynian massif and in the Pannonian basin (Fig. 1).

Conservation value of the Hercynian Knautia endemics

A combination of intriguing evolutionary history, genetic and karyological distinctness and remarkable ecological and physiological adaptations make *K. serpentinicola* and *K. pseudolongifolia* valuable examples of likely Quaternary speciation and a high conservation priority. Both species are restricted to the Hercynian massif, representing distinct endemic elements in an otherwise endemic-poor Czech flora (Kaplan 2012). Their origin may serve as a textbook example of a complex evolutionary scenario that occurred during a succession of changes in the central-European vegetation during the Holocene, involving spatial isolation in low-competitive Holocene refugia, allopatric differentiation and local polyploidization (Štěpánek 1989, Kaplan 1998, Kolář et al. 2012). The ability of these endemic species to adapt to harsh environments such as subalpine habitats or serpentine outcrops (experimentally proven for *K. serpentinicola*; Kolář et al. 2014) indicates that this group is highly ecologically plastic and highlights the key role of ecological conditions in the origin of these species. Populations of *K. serpentinicola* in the Slavkovský les Mts deserve the particular attention of conservation authorities because they represent a rare case where an edaphically-specialized diploid co-occurs with its auto-tetraploid derivative. Such systems serve as unique ‘natural laboratories’ offering possibilities for further research on general mechanisms of polyploid origin and establishment (Hanzl et al. 2014). This fact together with indications that the tetraploid genotype can spread beyond the borders of its original refugium imply that serpentine relicts are not evolutionary dead-ends but still have the potential to shape the surrounding biota (Kolář et al. 2012).

All of the (meta)populations of *K. serpentinicola* known are sufficiently large (reaching several hundreds to thousands of individuals), occur in stable habitats and regularly set fertile seeds (although vegetative reproduction via lateral rosettes seems to be frequent; Hanzl et al. 2014). This species would qualify as endangered (EN following IUCN) and in category C2 (see Grulich 2012) in the Czech Republic and critically endangered (CR) in Bavaria, Germany because only the smallest population (Woja) occurs there. *Knautia serpentinicola* should be brought under legislative protection in both countries. The high inter-population genetic differentiation (Kolář et al. 2012) suggests that optimally populations in all the disjunct areas should be protected in order to preserve the major part of this species’ genetic diversity. Actually, this is already the case because significant parts of all *K. serpentinicola* populations lie within small-scale protected areas. It is desirable to consider the ecological requirements of this species in the management plans for these sites. Dramatic and large-scale changes in forest management (e.g. establishing dense spruce plantations or the destruction of patches of heliophilous vegetation) represent the greatest potential threat to *K. serpentinicola*.

In contrast, there is a maximum of only 100–200 plants of *K. pseudolongifolia* (authors’ pers. obs.), restricted to a small part of a single glacial cirque (Štěpánek 1989). Although there seems to be no immediate threat to this species, we consider the category C1 (critically endangered, CR following IUCN), suggested in previous red lists (Holub & Procházka 2000, Grulich 2012), appropriate given the rarity of *K. pseudolongifolia*.

Descriptions of the two newly recognized species

Values of quantitative characters in the following morphological description are expressed as (minimum–) 5 percentile–95 percentile (–maximum); sizes of the stomatal guard cells, pollen grains and fruits are those cited by Štěpánek (1997).

Knautia serpentinicola Smejkal ex Kolář, Z. Kaplan, J. Suda et Štech, **spec. nova**

T y p e: Czech Republic, Středočeský kraj, Bernartice: pine forest next to the highway bridge; alt. 400 m a.s.l.; lat: 49°41'17.1N", long 15°06'19.8"E; 26.6.2006; leg. F. Kolář & M. Štech (holotype: CBFS, No. 5310, Fig. 4; isotypes: BRNM, No. 414224; BRNU, No. 634135; PR, No. 843328; PRC, No. 455079).

Description: Perennial herbs with a sympodial rhizome, forming numerous basal leaf rosettes. Flowering stems erect, slender, unbranched or sparingly branched, (14–) 24–61 (–89) cm high, (0.6–) 0.8–1.2 (–1.4) mm in diameter below the terminal head, with (1–) 2–6 (–7) pairs of opposite leaves. Indumentum consisting of numerous eglandular hairs and soft bristles, occasionally with glandular hairs below the inflorescence. Middle stem leaves sessile, lanceolate to oval, (3.2–) 4.3–12.1 (–17.7) cm × (1.0–) 1.4–6.9 (–8.3) cm, (1.4–) 1.6–3.9 (–6.2) times longer than wide, usually pinnatifid to pinnatisect with 1–2 (–4) lateral lobes on each side, rarely undivided, bristly hairy. The largest lateral lobe (6–) 10–36 (–48) mm long and (1.2–) 1.7–5.3 (–9.3) mm wide; terminal lobe (12–) 20–64 (–83) mm long and (1.5–) 2.9–12.7 (–21.7) mm wide, accounting for 2/5–1/2 of total leaf length. Stomatal guard cells on the adaxial surface of stem leaves (25–) 28–37 (–43) μm long. Terminal head relatively small, (10–) 15–27 (–36) mm in diameter (measured as the distance between the tips of involucral bracts), with rounded, bristly hairy base. Outer involucral bracts narrowly lanceolate to obovate. Flowers hermaphrodite, bilaterally symmetric, tetramerous, scentless. Calyx synsepalous, with a cup-shaped tube and several terminal bristles, hairy, shed at fruiting. Corolla sympetalous, violet to dark reddish-violet, with a short tube and four unequal lobes, the lower lobe elongated. Stamens four, adnate to corolla tube, protruding out of the flowers; pollen grains (80–) 84–94 (–105) μm in diameter. Ovary inferior, bicarpellate, style protruding from the corolla tube, stigma bilobed. Achenes elliptic, weakly compressed laterally, (3.5–) 4.1–4.7 (–5.4) mm long, hairy, greenish to dark brown, with persistent white fleshy pedicel (elaiosome). Flowers July – September. 2n = 20 (all regions), 40 (the Slavkovský les Mts). Figs 4, 6A.

D i a g n o s i s: *Knautia serpentinicola* resembles slender forms of *K. arvensis*, however, it differs in having numerous lateral rosettes, unbranched or sparingly branched flowering stems, which are more slender below the inflorescences. In addition, *K. serpentinicola* has shorter and narrower middle stem leaves, with less developed lobes. The colour of petals is somewhat darker than in *K. arvensis*.

Distribution, population size and genetic structure

Knautia serpentinicola was first recognized by Miroslav Smejkal who tentatively (“in schedis”) identified morphologically distinct serpentine plants from the Ranský Babylon hill as *K. arvensis* subsp. *serpentinicola* in 1967 and 1972 (vouchers deposited in BRNU). The likely independent taxonomic status of serpentine populations was then further discussed in a karyological study of *Knautia* in the former Czechoslovakia (Štěpánek



HOLOTYPE of
Knautia serpentinicola Smejkal ex Kolář, Z. Kaplan, J. Suda et Štech

Herbarium of the Faculty of Science, University of South Bohemia (CBFS)	
No. 5310	
<i>Knautia arvensis</i> subsp. <i>serpentinicola</i> Smejkal ined.	
Czechia, Středočeský kraj	
Bernartice: pine forest next to the highway bridge	
Coord. (WGS84): lat.: +49.6881 , long.: +15.1055	
Altitude: 400 m a. s. l. Mapping grid cell: 6356b	
Phytogeogr. region: 41. Střední Povltaví	
Date: 26.6.2006	Legit: Kolář Filip & Štech Milan
Note: DNA-2x (FCM), plant 23	

Fig. 4. – Holotype of *Knautia serpentinicola* deposited in CBFS.

1982) and also reflected (although still within *K. arvensis*) in the Flora of the Czech Republic using a provisional name (Štěpánek 1997).

The species occurs in four spatially isolated serpentine areas on the Hercynian massif, three of which are located in the Czech Republic and one in northern Bavaria, Germany. The largest population of the diploid cytotype, consisting of several thousands of individuals, grows in the eastern part of central Bohemia, in the serpentine area of Dolnokralovické hadce north and north-west of Bernartice village in the valley of the Želivka river (locus classicus). This population occupies nearly the entire serpentine body (population borders are located approx. at 49°41'17"N, 15°05'54"E; 49°41'00"N, 15°06'49"E; 49°41'00"N, 15°08'02"E; and 49°41'24"N, 15°06'40"E; all GPS coordinates for the Czech Republic taken from www.mapy.cz). Most plants occur in pine forests on the flat central area but some occur on the rocky serpentine slopes, avoiding only the most exposed and driest sites. The second diploid population occupies serpentine outcrops on the Ranský Babylon hill, south of the town Ždírec nad Doubravou in the Vysočina region. Here, *K. serpentinicola* occurs in open grassy patches along a paved forest road (approx. between 49°40'06"N, 15°49'46"E and 49°39'05"N, 15°49'02"E) from where it extends into forests on serpentine bedrock. The population size is nearly 1,000 individuals, although we cannot exclude the possibility that additional patches not recorded by us exist at this site. The last purely diploid population inhabits a small serpentine outcrop Wojaleite east of Wurlitz, in the Hof region in northern Bavaria. Plants occur in pine forest both in the flatland above serpentine rocks in the northern part of this site (50°15'14"N, 11°58'28"E) and, more often, in the southern part of the serpentine area (50°15'07"N, 11°58'21"E; coordinates taken from maps.google.com). The total population size was estimated to be several hundreds of individuals. Finally, diploid and tetraploid cytotypes co-occur in a large serpentine area at Mnichovské hadce in the Slavkovský les Mts, in the Karlovarský region. Diploids are less abundant (a few thousand individuals) and confined to several patches on the southern slopes of the Vlčí hřbet massif, between Vlček and Planý vrch hills (approx. between 50°01'47"N, 12°44'08"E and 50°02'17"N, 12°46'01"E). Interestingly, diploid plants almost exclusively occur at permanently forested sites and avoid new forest plantations (Hanzl et al. 2014). In contrast, tetraploids are much more widespread and abundant there (many thousands of individuals); they occupy extensive forested areas on serpentine bedrock between Vlčí hřbet hill (50°01'41"N, 12°43'25"E) and Pluhův bor (50°03'33"N, 12°47'15"E) and also occur on isolated rocky outcrops at Dominova skalka (50°04'17"N, 12°47'09"E) and Křížky (50°03'58"N, 12°44'55"E). For details on the distribution of cytotypes in the Slavkovský les Mts see Hanzl et al. (2014). It is likely that serpentine tetraploids hybridize with tetraploid *K. arvensis* subsp. *arvensis*. The extent of hybridization is currently unknown but considered in a separate study (M. Čertner et al., unpubl.). The altitudinal range of this species is from 380 m a.s.l. (Dolnokralovické hadce) to 883 m a.s.l. (top of Vlčí hřbet hill in the Slavkovský les Mts).

This species seems to be endemic to the above-listed serpentine sites on the Hercynian massif. No other populations were found during a thorough search of other serpentine outcrops and ecologically similar relict non-serpentine sites (e.g. open pine and oak-pine forests in rocky river valleys) (Kaplan 1998, Kolář et al. 2009). Although all known populations of *K. serpentinicola* occur at serpentine sites, this species is not an obligate serpentinophyte and the plants also thrive when planted in non-serpentine garden soil (F. Kolář and Z. Kaplan, pers. observ.).

Some peculiar forms of *Knautia*, with a tetraploid number of chromosomes, were previously recorded from the serpentine area at Křemžské hadce in southern Bohemia (Kaplan 1998). However, following a detailed survey they turned out to be $4x$ *K. arvensis*, which occasionally spread out from surrounding non-serpentine habitats and a distinct hexaploid species *K. dipsacifolia* (Kolář et al. 2009, 2012).

The two diploid populations in central Bohemia (Dolnokralovické hadce and Ranský Babylon) are genetically distinct from their western-Bohemian and Bavarian counterparts. Intra-population genetic diversity is relatively high and very comparable to central-European populations of the widespread *K. arvensis*. Most of these diploid populations have a high proportion of rare genetic markers, most likely reflecting their long-term spatial isolation (Kolář et al. 2012).

Ecology

Knautia serpentinicola occurs exclusively on serpentine outcrops, mostly in open pine forests of the *Dicrano-Pinion sylvestris* alliance (assoc. *Asplenio cuneifolii-Pinetum sylvestris* and *Vaccinio myrtilli-Pinetum sylvestris*; the nomenclature follows Chytrý 2013) and in different types of secondary forests with dominant pine and spruce (however, it never grows in dense spruce or pine plantations). At Dolnokralovické hadce, *K. serpentinicola* occasionally occurs in relict pine forests with *Sesleria* of the *Erico carnaeae-Pinion* alliance (*Thlaspi montani-Pinetum sylvestris*). It prefers open and slightly moister patches in forests, forest clearings, old forest roads and/or grassy roadsides. Tetraploids have a broader ecological niche and also occur in secondary grasslands and heathlands on serpentine bedrock. *Knautia serpentinicola* often co-occurs with several other heliophilous species, which are probably also relicts from the last glaciation and/or early Holocene, including *Cerastium alsinifolium*, *Erica carnea*, *Polygala chamaebuxus* and *Thesium alpinum* in the Slavkovský les Mts, *Armeria vulgaris*, *Dianthus carthusianorum*, *Minuartia smejkalii*, *Potentilla crantzii*, *Sesleria caerulea* and *Thlaspi montanum* at Dolnokralovické hadce, and *Armeria vulgaris*, *Dianthus gratianopolitanus*, *Festuca pallens* and *Saxifraga rosacea* at Wojaleite.

Knautia pseudolongifolia (Szabó) Žmuda, Bull. Acad. Sci. Cracov., sci. natur., 1916: 171, 1917.

Syn.: *Knautia arvensis* var. *pseudolongifolia* Szabó, Math. Termesztud. Közlem. 31: 244, 1911; *K. arvensis* subsp. *pseudolongifolia* (Szabó) O. Schwarz, Mitt. Thüring. Bot. Ges. 1: 118, 1949; *Trichera arvensis* subsp. *pseudolongifolia* (Szabó) Holub, Preslia 51: 282, 1979.

Lectotype (designated here): [Czech Republic:] Flora des Riesengebirges, Kl. Kessel an der Kesselkoppe, d. 28. Juli 1889, E. Fiek (WRSL; Fig. 5).

Note on typification: Two collections (syntypes) used for the description of *K. arvensis* var. *pseudolongifolia* by Szabó (1911) are mentioned in the protologue: “Riesengebirge: in cacumine Kesselkoppe (Engler!, Fiek!)”. None of these syntypes were located in BP where the main herbarium of Szabó is kept. When working on his monograph of *Knautia*, Szabó also used specimens borrowed from museums in Berlin and Wrocław (see Szabó 1911: p. 7). Collections of H. G. A. Engler were preserved in B but were destroyed during World War II. Collections of E. Fiek are preserved in WRSL and one specimen (Fig. 5) that matches both the type citation and the description given in the protologue was found there. This is apparently the only extant specimen that meets the definition of the Code for original materials and is thus designated as the lectotype.

Description: Perennial herbaceous plants with sympodial rhizomes, forming several basal leaf rosettes. Flowering stem erect, relatively robust, usually unbranched (rarely sparingly branched), (18–) 28–58 (–64) cm high, 1.4–2.0 (–2.2) mm in diameter below the terminal head, with 3–6 pairs of stem leaves. Indumentum consisting of numerous eglandular hairs and soft bristles, occasionally with glandular hairs below the inflorescence. Middle stem leaves sessile, narrowly lanceolate to lanceolate, (7.1–) 7.5–13.1 (–14.8) cm × (1.3–) 1.4–3.9 (–5.4) cm, (2.4–) 2.8–7.3 (–7.5) times longer than wide, usually undivided, serrate, rarely pinnately-lobed to pinnately-parted with 2–6 usually unequal lobes on each side, scarcely bristly hairy to glabrous, relatively thick. Terminal lobe (if present) short, accounting for 1/3–2/5 of total leaf length. Stomatal guard cells on the adaxial surface of stem leaves (28–) 31–37 (–40) µm long. Terminal head relatively large, (22–) 23–33 (–35) mm in diameter (measured as the distance between the tips of involucre bracts), with rounded bristly hairy inflorescence base. Outer involucre bracts lanceolate to ovate. Flowers hermaphrodite, bilaterally symmetric, tetramerous, scentless. Calyx synsepalous, with a cup-shaped tube and several terminal bristles, hairy, shed at fruiting. Corolla sympetalous, pink to pinkish-violet, with a short tube and four unequal lobes. Stamens four, adnate to corolla tube, protruding out of the flowers; pollen grains (88–) 90–100 (–108) µm in diameter. Ovary inferior, bicarpellate, style protruding from the corolla tube, stigma bilobed. Achenes elliptic, weakly compressed laterally, (4.8–) 5.2–5.7 (–5.8) mm long, hairy, greenish to dark brown, with persistent white fleshy pedicel (elaiosome). Flowers August – September. $2n = 20$. Figs 5, 6C.

Diagnosis: *Knautia pseudolongifolia* differs from both *K. arvensis* and *K. serpentinicola* in having narrow and usually undivided or only shallowly-lobed stem leaves, which are scarcely pubescent to almost glabrous. In addition, flower heads of *K. pseudolongifolia* are larger, stem below the terminal inflorescence is stouter and ripe fruits are larger.

Distribution, population size and genetic structure

Knautia pseudolongifolia was described at the rank of a variety at the beginning of the 20th century (Szabó 1911) and recently usually recognized as a subspecies of *K. arvensis* (Štěpánek 1989, 1997). This species is known from a single population confined to the ridge between Malá and Velká Kotelní jáma glacial cirques in the Krkonoše Mts, ~1320–1390 m a.s.l. (50°45'06"N, 15°31'57"E). The population comprises 100–200 individuals at maximum, most of which occur in an area of approx. 30 × 30 m (Štěpánek 1989). Several plants were recently found by us (FK) ca 150 m away at 50°45'10", 15°31'57". Genetic diversity is relatively high despite the long-term isolation and small number of individuals (Kolář et al. 2012).

Ecology

This species grows in neutral soils on carbonate outcrops in subalpine grassland (Šourek 1969). *Knautia pseudolongifolia* usually occurs in relatively basiphilous and moist subalpine grassland communities of the *Agrostion alpinae* alliance (assoc. *Saxifrago oppositifoliae*-*Festucetum versicoloris*; the nomenclature follows Chytrý 2007; Fig. 6D), occasionally occurring in surrounding dwarf shrub vegetation with dominant *Calluna*

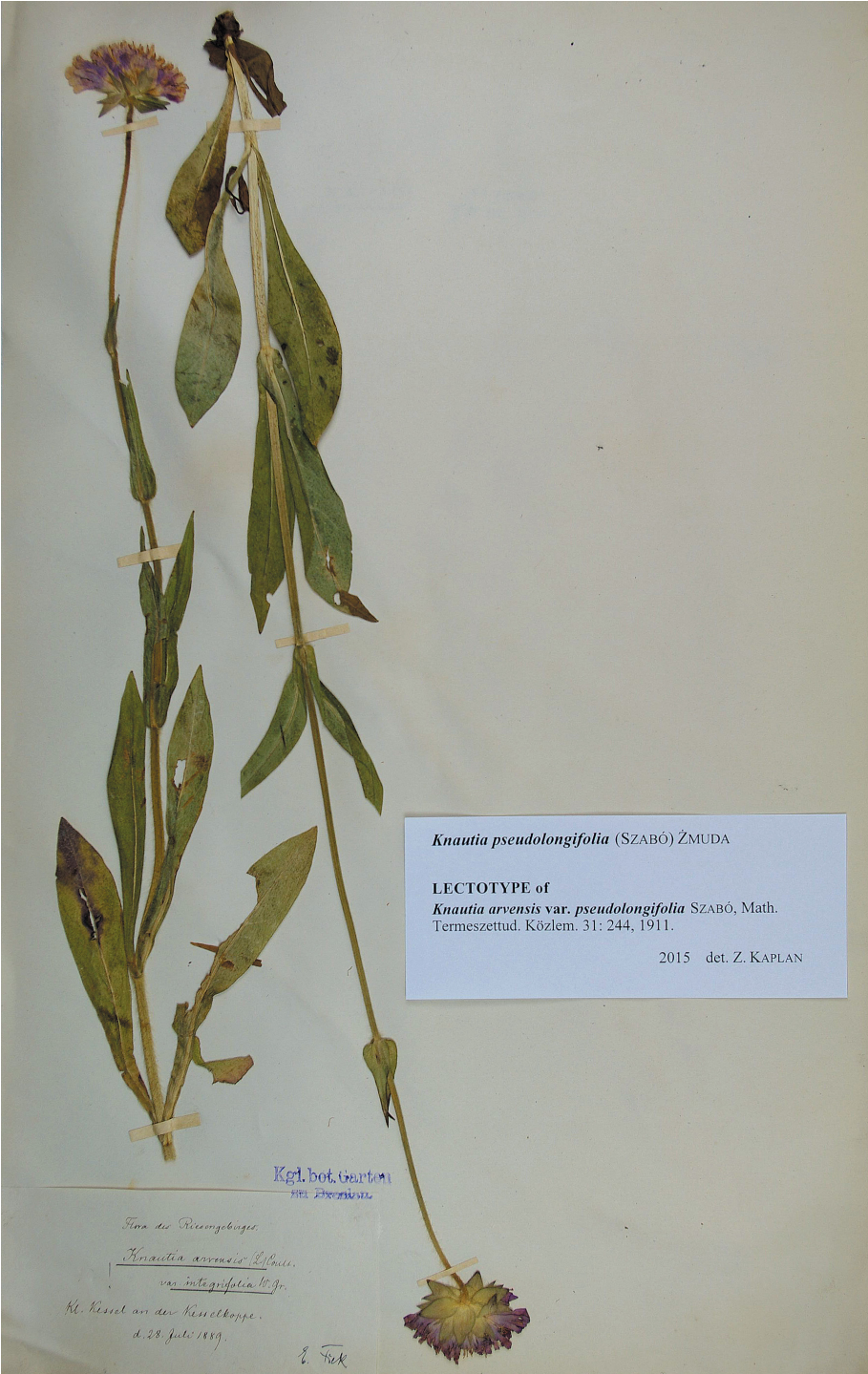


Fig. 5. – Lectotype of *Knautia pseudolongifolia* deposited in WRSL.

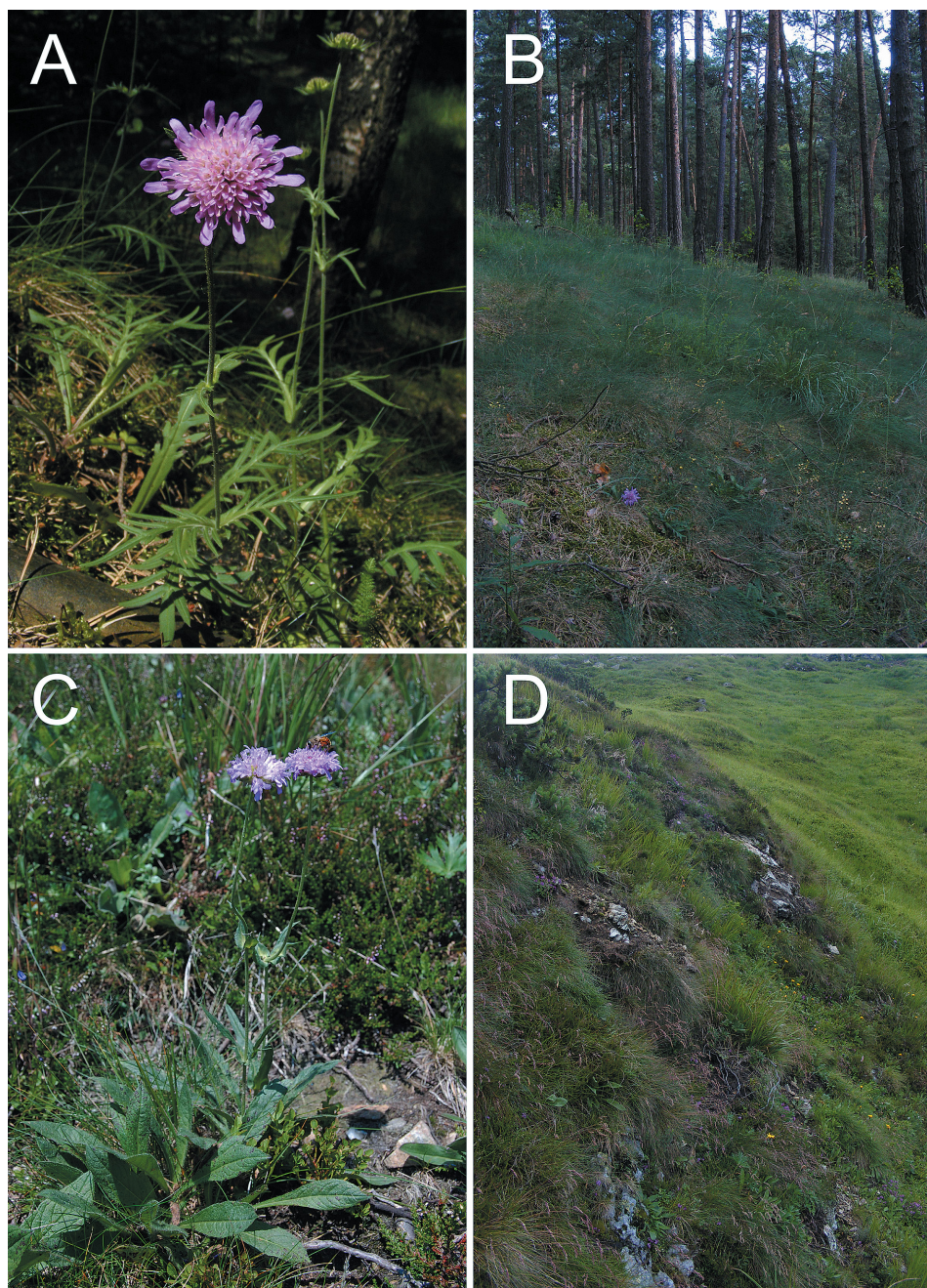


Fig. 6. – General appearance and typical habitat of *Knautia serpentinicola* (A and B) and *K. pseudolongifolia* (C and D) (photographs F. Kolář and J. Suda).

vulgaris (Štěpánek 1989). This species is allogamous, however, only 5–15 % of the plants flower each year, suggesting a significant role of vegetative reproduction (Štěpánek 1989).

See www.preslia.cz for Electronic Appendices 1–3

Acknowledgements

Kristýna Šemberová and Markéta Dortová kindly made the morphometric measurements. We are grateful to Friedrich Ehrendorfer and Božo Frajman for insightful comments on earlier versions of the manuscript, to Jan Štěpánek for his valuable comments on *Knautia* taxonomy and the curators of herbaria, particularly Zoltán Barina (BP), Krzysztof Świerkosz (WRSL) and Jiří Danihelka (BRNU) for searching for specific specimens. The present work was partly funded by Charles University in Prague (project GAUK 418411) and partly by the Ministry of Education, Youth and Sports of the Czech Republic (bilateral Czech Republic–Austria project 7AMB13AT015). Additional support was provided by The Czech Academy of Sciences (long-term research development project RVO 67985939 and project ROZE of the Strategy AV21), institutional resources of the Ministry of Education, Youth and Sports of the Czech Republic for the support of science and research and the Czech Science Foundation (project 14-36079G, Centre of Excellence PLADIAS).

Souhrn

Taxonomické studie založené na syntéze morfologických, cytogenetických, molekulárních a ekologických přístupů stále přinášejí nové poznatky i v relativně dobře prozkoumaných územích. Mezi nejvýznamnější patří objevy nových lokálních endemitů, které mají přímý praktický dopad pro ochranu biodiverzity. Flóra cévnatých rostlin v České republice je v důsledku čtvrtohorních klimatických změn velmi chudá na endemity. Většinu českých endemitů navíc představují málo morfologicky diferencované, apomikticky se rozmnožující druhy (mikrospécie). Náš příspěvek představuje v tomto ohledu výjimku a shrnuje informace o dvou nových sexuálních endemických druzích, dosud nesprávně zahrnovaných do ekogeograficky diferencovaného a ploidně variabilního druhu chřastavce rolního (*Knautia arvensis*). Morfometrická analýza 38 populací *K. arvensis* a obou nově vylišených druhů, pocházejících z východní části střední Evropy, odhalila zjevné, byť neúplné mezidruhové morfologické odlišnosti (rozdíly patrně stírá velká fenotypová plasticita). Tyto výsledky spolu s již publikovanými cytologickými (odlišná velikost jaderného genomu), genetickými (jiná evoluční historie) a ekologickými daty (výrazně rozdílné stanovištní nároky) podporují nezávislý druhový statut hadcových a vysokohorských populací Hercynského masivu. Chřastavec hadcový (*Knautia serpentinicola*) zahrnuje diploidní a tetraploidní populace vázané na čtyři hadcové oblasti, konkrétně Dolnokralovické hadce, okolí Starého Ranska a Slavkovský les v České republice a Wojaleite v severním Bavorsku. Od *K. arvensis* se uvedený druh liší především gracilnějším vzrůstem, celoroční přítomností postranních listových růžic, užšími lodyžními listy i jejich úkrojky a tmavší červenofialovou barvou květů. Vyskytuje se výhradně v otevřených borech na hadcových substrátech, na stanovištích s malou konkurencí ostatních rostlin. Chřastavec krkonošský (*K. pseudolongifolia*) naproti tomu osidluje subalpínské trávníky na jediné lokalitě v Kotelních jamách v Krkonoších. Od ostatních druhů agregátu se odlišuje nedělenými nebo jen nejvýše laločnatými a výrazně protaženými lodyžními listy, velkými terminálními strbouly a delšími plody. *Knautia serpentinicola* i *K. pseudolongifolia* představují vzácné doklady postglaciální evoluce květeny ve střední Evropě a ukazují na významnou úlohu holocenních ekologicky podmíněných refugií v uchování vzácné biodiverzity. Náznaky dalšího rozrůžňování v rámci téže ploidie v geograficky izolovaných oblastech (alopatrická diferenciace) i vznik nové tetraploidní linie chřastavce hadcového (která se zřejmě šíří i za hranice původního hadcového refugia prostřednictvím hybridizace) dokládají nečekaně velký evoluční potenciál těchto reliktních druhů. Evolučně-historický význam obou druhů spolu s jejich maloplošným výskytem ukazuje na nutnost cílené ochrany, především v případě dosud ochrannýsky přehlížené *K. serpentinicola*. V neposlední řadě naše studie ukazuje na významný přínos komplexních taxonomických studií pro poznání biodiverzity i v již zdánlivě dobře prozkoumaných oblastech.

References

- Breton-Sintes S. (1974): Etude biosystématique du genre *Knautia* (*Dipsacaceae*) dans le Massif Central français. II. Analyse morphologique et cytogénétique d'hybrides expérimentaux. – Ann. Sci. Nat. Bot., Sér. 12, 15: 277–319.
- Breton-Sintes S. (1975): Contribution cytogénétique à l'étude des *Knautia* (*Dipsacaceae*) du Plateau Central français. – Année Biol. 14: 45–68.
- Chytrý M. (ed.) (2007): Vegetace České republiky. 1. Travinná a keříčková vegetace [Vegetation of the Czech Republic. 1. Grassland and heathland vegetation]. – Academia, Praha.
- Chytrý M. (ed.) (2013): Vegetace České republiky. 4. Lesní a křovinná vegetace [Vegetation of the Czech Republic. 4. Forest and shrub vegetation]. – Academia, Praha.
- Danihelka J., Chrtek J. & Kaplan Z. (2012): Checklist of vascular plants of the Czech Republic. – Preslia 84: 647–811.
- Dvořáková M. (1988): *Minuartia smejkalii*, eine neue Art aus der *Minuartia gerardii*-Gruppe (*Caryophyllaceae*). – Preslia 60: 1–9.
- Dvořáková M. (1999a): *Euphrasia corcontica*, eine endemische Art aus dem Gebirge Krkonoše (Riesengebirge, Westsudeteten). – Preslia 71: 33–35.
- Dvořáková M. (1999b): Zwei neue zur Sektion *Polymechana* gehörende *Minuartia*-Arten (*Caryophyllaceae*). – Preslia 70 (1998): 335–338.
- Ehrendorfer F. (1962): Beiträge zur Phylogenie der Gattung *Knautia* (*Dipsacaceae*), I. Cytologische Grundlagen und allgemeine Hinweise. – Österr. Bot. Z. 109: 276–343.
- Ehrendorfer F. (1976): *Knautia* L. – In: Tutin T. G., Heywood V. H., Burges N. A., Valentine D. H., Walters S. M. & Webb D. A. (eds), Flora Europaea 4: 60–67, Cambridge University Press, Cambridge.
- Ehrendorfer F. (1981): Neue Beiträge zur Karyosystematik und Evolution der Gattung *Knautia* (*Dipsacaceae*) in den Balkanländern. – Bot. Jahrb. 102: 225–238.
- Fischer M. A. (2008): Exkursionsflora für Österreich, Liechtenstein und Südtirol. Ed. 3. – OÖ. Landesmuseen, Linz.
- Frajman B., Rešetnik I., Weiss-Schneeweiss H., Ehrendorfer F. & Schönschetter P. (2015): Cytotype diversity and genome size variation in *Knautia* (*Caprifoliaceae*, *Dipsacoideae*). – BMC Evol. Biol. 15: 140.
- Grulich V. (2012): Red List of vascular plants of the Czech Republic. – Preslia 84: 631–645.
- Hanzl M., Kolář F., Nováková D. & Suda J. (2014): Nonadaptive processes governing early stages of polyploid evolution: Insights from a primary contact zone of relict serpentine *Knautia arvensis* (*Caprifoliaceae*). – Am. J. Bot. 101: 935–945.
- Holub J. (1960): Nové nebo méně známé rostliny květeny ČSSR [New and poorly known elements of the flora of Czechoslovakia]. – Preslia 32: 423–426.
- Holub J. (1965): Určovací klíč k určování československých druhů okruhu *Carex flava* L. [Key to the species of the *Carex flava* group of Czechoslovakia]. – Východočes. Bot. Zprav. 3: 3–5.
- Holub J. & Procházka F. (2000): Red List of vascular plants of the Czech Republic. – Preslia 72: 187–230.
- Kaplan Z. (1998): Relict serpentine populations of *Knautia arvensis* s.l. (*Dipsacaceae*) in the Czech Republic and an adjacent area of Germany. – Preslia 70: 21–31.
- Kaplan Z. (2012): Flora and phytogeography of the Czech Republic. – Preslia 84: 505–573.
- Khatib F. A., Fusu L., Cruaud A., Gibson G., Borowiec N., Rasplus J.-Y., Ris N. & Delvare G. (2014): An integrative approach to species discrimination in the *Eupelmus urozonus* complex (*Hymenoptera*, *Eupelmidae*), with the description of 11 new species from the Western Palearctic. – Syst. Entomol. 39: 806–862.
- Király G. (2009): Új magyar fűvészkönyv. Magyarország hajtásos növényei. Határozókulcsok [New Hungarian herbal: the vascular plants of Hungary. Identification keys]. – Aggteleki Nemzeti Park Igazgatóság, Jászvafő.
- Kolář F., Dortová M., Lepš J., Pouzar M., Krejčová A. & Štech M. (2014): Serpentine ecotypic differentiation in a polyploid plant complex: shared tolerance to Mg and Ni stress among di- and tetraploid serpentine populations of *Knautia arvensis* (*Dipsacaceae*). – Plant Soil 374: 435–447.
- Kolář F., Fér T., Štech M., Trávníček P., Dušková E., Schönschetter P. & Suda J. (2012): Bringing together evolution on serpentine and polyploidy: spatiotemporal history of the diploid-tetraploid complex of *Knautia arvensis* (*Dipsacaceae*). – PLoS ONE 7: e39988.
- Kolář F., Štech M., Trávníček P., Rauchová J., Urfus T., Vít P., Kubešová M. & Suda J. (2009): Towards resolving the *Knautia arvensis* agg. (*Dipsacaceae*) puzzle: primary and secondary contact zones and ploidy segregation at landscape and microgeographic scales. – Ann. Bot. 103: 963–974.

- Koutecký P. (2014): MorphoTools: a set of R functions for morphometric analysis. – *Plant Syst. Evol.* 301: 1115–1121.
- Kovanda M. (1968): New taxa and combinations in the subsection *Heterophylla* (Witas.) Fed. of the genus *Campanula* L. – *Folia Geobot. Phytotax.* 3: 407–411.
- Kovanda M. (1982): *Dianthus gratianopolitanus*: variability, differentiation and relationships. – *Preslia* 54: 223–242.
- Krahulec F. (2006): Species of vascular plants endemic to the Krkonoše Mts (Western Sudetes). – *Preslia* 78: 503–516.
- Lepší M. & Lepší P. (2006): *Rubus kletensis*, a new species from South Bohemia and Upper Austria. – *Preslia* 78: 103–114.
- Lepší M. & Lepší P. (2009): *Rubus silvae-norticae*, a new species from Bohemia, Austria and Bavaria and the significance of brambles for regional migrations. – *Preslia* 81: 43–62.
- Lepší M., Lepší P. & Vít P. (2013): *Sorbus querneae*: taxonomic confusion caused by the naturalization of an alien species, *Sorbus mougeotii*. – *Preslia* 85: 159–178.
- Lepší M., Vít P., Lepší P., Boublík K. & Kolář F. (2009): *Sorbus portae-bohemicae* and *Sorbus albensis*, two new endemic apomictic species recognized based on a revision of *Sorbus bohemica*. – *Preslia* 81: 63–89.
- Lepší M., Vít P., Lepší P., Boublík K. & Suda J. (2008): *Sorbus milensis*, a new hybridogenous species from northwestern Bohemia. – *Preslia* 80: 229–244.
- Oksanen J., Blanchet F. G., Kindt R., Legendre P., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Stevens M. H. H. & Wagner H. (2013): vegan: community ecology package. – URL: <http://cran.r-project.org/web/packages/vegan>.
- Rešetník I., Frajman B., Bogdanović S., Ehrendorfer F. & Schönswetter P. (2014): Disentangling relationships among the diploid members of the intricate genus *Knautia* (*Caprifoliaceae*, *Dipsacoidae*). – *Mol. Phylogenet. Evol.* 74: 97–110.
- Řezáč M., Pekár S. & Johannesen J. (2008): Taxonomic review and phylogenetic analysis of central European *Eresus* species (*Araneae*: *Eresidae*). – *Zool. Scr.* 37: 263–287.
- Smejkal M. (1963): Taxonomické studie československých druhů rodu *Euphrasia* L. [Taxonomic study of Czechoslovak species of *Euphrasia*]. – *Biol. Práce Slov. Akad. Vied* 9/9: 1–83.
- Šourek J. (1969): Květena Krkonoš [Flora of the Krkonoše Mts]. – Academia, Praha.
- Štech M. (2006): Was sind *Melampyrum subalpinum*, *M. angustissimum* und *M. bohemicum*? – *Neilreichia* 4: 221–234.
- Štěpánek J. (1979): Příspěvek k řešení taxonomicko-chorologické problematiky komplexu *Knautia arvensis* v ČSSR [A contribution to taxonomic-chorological aspects of *Knautia arvensis* complex in Czechoslovakia]. – Ms. Thesis; depon. in: Knih. Kat. Bot. Přír. Fak. Univ. Karlovy Praha.
- Štěpánek J. (1982): Die Chromosomenzahlen von tschechoslowakischen Arten der Gattung *Knautia* L. (*Dipsacaceae*). – *Folia Geobot. Phytotaxon.* 17: 359–386.
- Štěpánek J. (1983): Eine neue Art der Gattung *Knautia* (*Dipsacaceae*) aus Westkarpaten. – *Preslia* 55: 1–8.
- Štěpánek J. (1985): *Knautia* L. – In: Bertová L. (ed.), Flóra Slovenska [Flora of Slovakia] 4/2: 154–177, Veda, Bratislava.
- Štěpánek J. (1989): Chrástavec rolní krkonošský – *Knautia arvensis* (L.) Coulter subsp. *pseudolongifolia* (Szabó) O. Schwarz. – In: Slavík B. (ed.), Vybrané ohrožené druhy flóry ČR [Selected endangered species of the flora of the Czech Socialist Republic], Studie ČSAV 10: 25–36, Academia, Praha.
- Štěpánek J. (1997): *Knautia* L. – chrastavec. – In: Slavík B., Chrtěk J. jun. & Tomšovic P. (eds), Květena České republiky [Flora of the Czech Republic] 5: 543–554, Academia, Praha.
- Štěpánek J. & Procházka F. (1999): *Knautia arvensis* (L.) Coulter subsp. *pseudolongifolia* (Szabó) O. Schwarz. – In: Čerňavský J., Feráková V., Holub J., Maglocký Š. & Procházka F. (eds), Červená kniha ohrožených a vzácných druhů rostlin a živočichů ČR a SR. Vol. 5. Vyšší rostliny [Red data book of endangered plant and animal species of the Czech Republic and Slovak Republic. Vol. 5. Higher plants], p. 205, Příroda, Bratislava.
- Štěpánková J. (2008): *Carex derelicta*, a new species from the Krkonoše Mountains (Czech Republic). – *Preslia* 80: 389–397.
- Szabó Z. (1911): A *Knautia* génusz monographiája [Monograph of the genus *Knautia*]. – *Math. Termeszettud. Közlem.* 31: 1–436, Tab. 1–54, 4 Maps.
- Trávníček B., Duchoslav M., Šarhanová P. & Šafářová L. (2010): Squills (*Scilla* s. lat., *Hyacinthaceae*) in the flora of the Czech Republic, with taxonomical notes on Central-European squill populations. – *Acta Mus. Morav., sci. biol.*, 94 (2009): 157–205.

- Trávníček B., Kirschner J. & Štěpánek J. (2008): Five new species of *Taraxacum* sect. *Ruderalia* from Central Europe and Denmark. – Preslia 80: 27–59.
- Trávníček B., Oklejewicz K. & Zieliński J. (2005): *Rubus ambrosius* (*Rubus* subsect. *Rubus*, *Rosaceae*), a new bramble species from the eastern part of Central Europe. – Folia Geobot. 40: 421–434.
- Trávníček B. & Žíla V. (2011): *Rubus silvae-bohemicae*: a new species of bramble from Bohemia and Bavaria. – Preslia 83: 99–110.
- Vašut R. J. & Trávníček B. (2004): *Taraxacum princeps* sp. nova, a new species of section *Erythrosperma* from Central Europe. – Thaiszia – J. Bot. 14: 37–46.
- Velebil J. (2012): *Sorbus omissa*, a new endemic hybridogenous species from the lower Vltava river valley. – Preslia 84: 375–390.
- Venables W. N. & Ripley B. D. (2002): Modern applied statistics with S. – Springer.
- Vít P., Lepší M. & Lepší P. (2012): There is no diploid apomict among Czech *Sorbus* species: a biosystematic revision of *S. eximia* and discovery of *S. barrandienica*. – Preslia 84: 71–96.
- Vít P., Wolfová K., Urfus T., Tájek P. & Suda J. (2014): Interspecific hybridization between rare and common plant congeners inferred from genome size data: assessing the threat to the Czech serpentine endemic *Cerastium alsinifolium*. – Preslia 86: 95–117.

Received 5 April 2015

Revision received 16 August 2015

Accepted 24 August 2015