

# **Od počiatkov uplatňovania moderných metód taxonómie a systematiky na Botanickom ústave SAV ku komplexným mikro-evolučným štúdiám**

**Karol Marhold**



# Začiatky štúdií na základe populačných vzoriek šesťdesiate roky minulého storočia – Mária Jasičová



Botanický ústav SAV, oddelenie systematiky rastlín,  
Bratislava, Dúbravská 26

Mária Jasičová

*Anthyllis vulneraria* L. s. l. na Slovensku

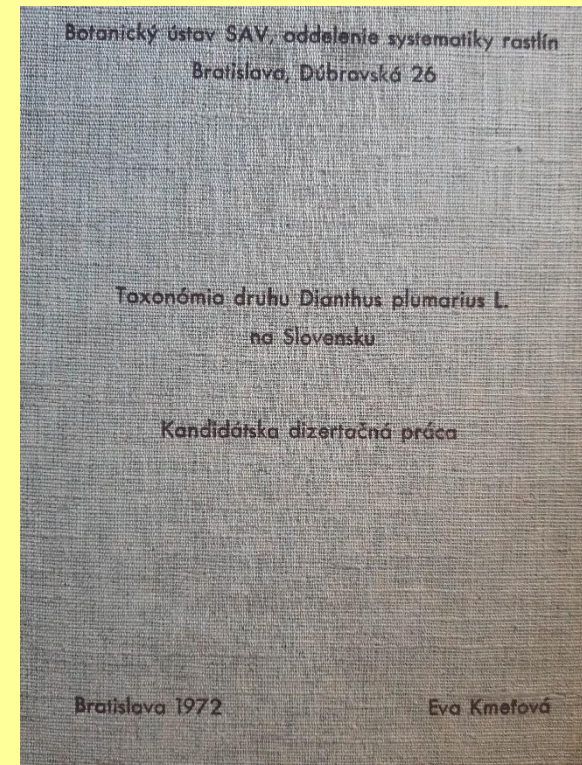
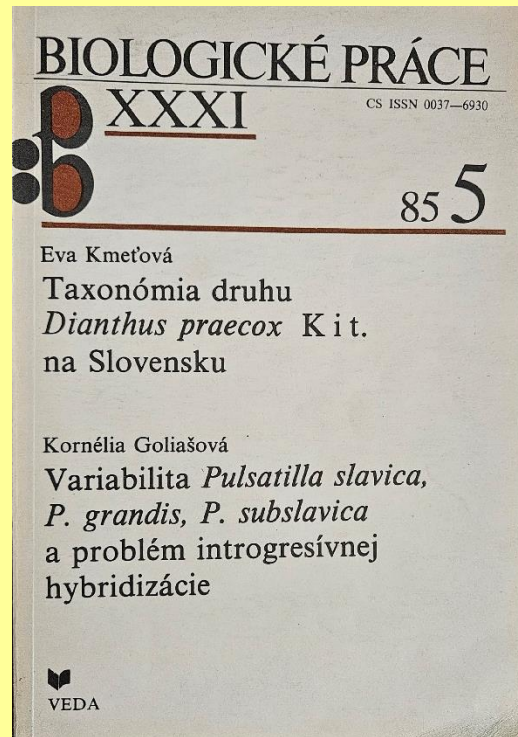
Kandidátska dizertačná práca

Bratislava, 1969





**Nástup dôkladnejších štúdií, analýzy peľových zŕn, mikromorfologických znakov, počtov chromozómov, dôraz na kvalitné spracovanie nomenklatúry a synonymiky taxónov – Eva Kmet'ová**



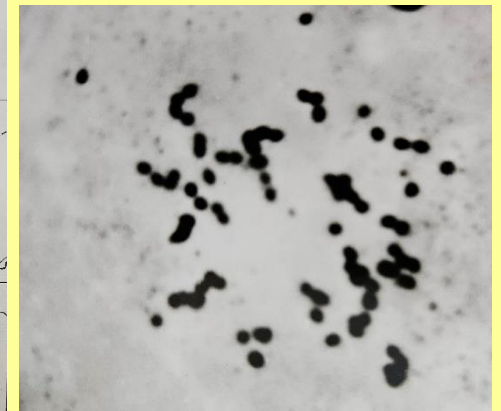
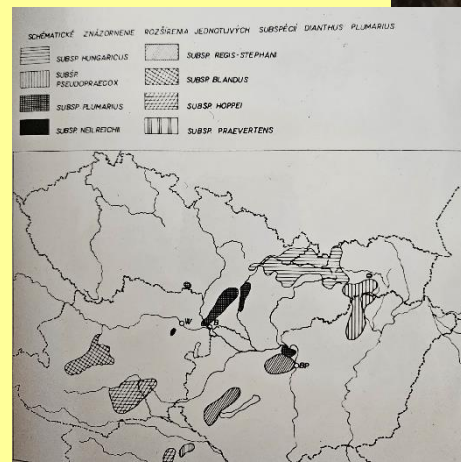
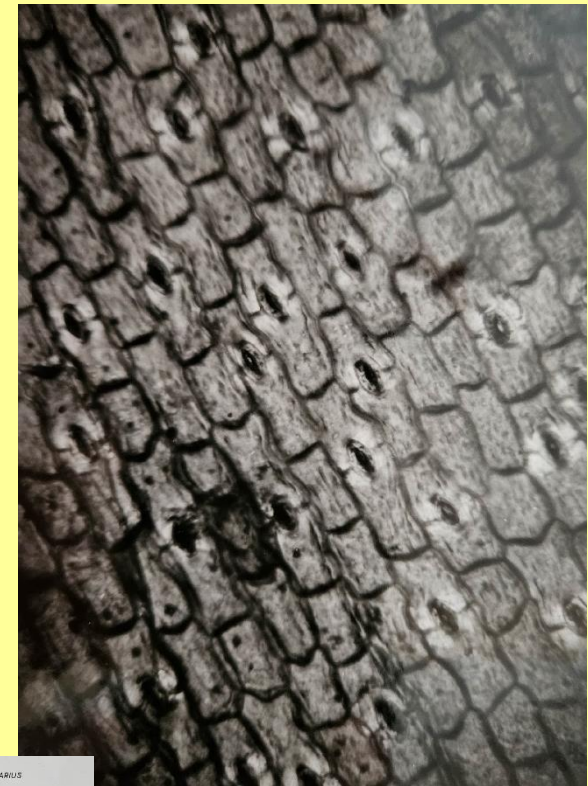
# Nástup dôkladnejších štúdií ... – Eva Kmet'ová

Tabuľka priemerných hodnôt habituálnych znakov.

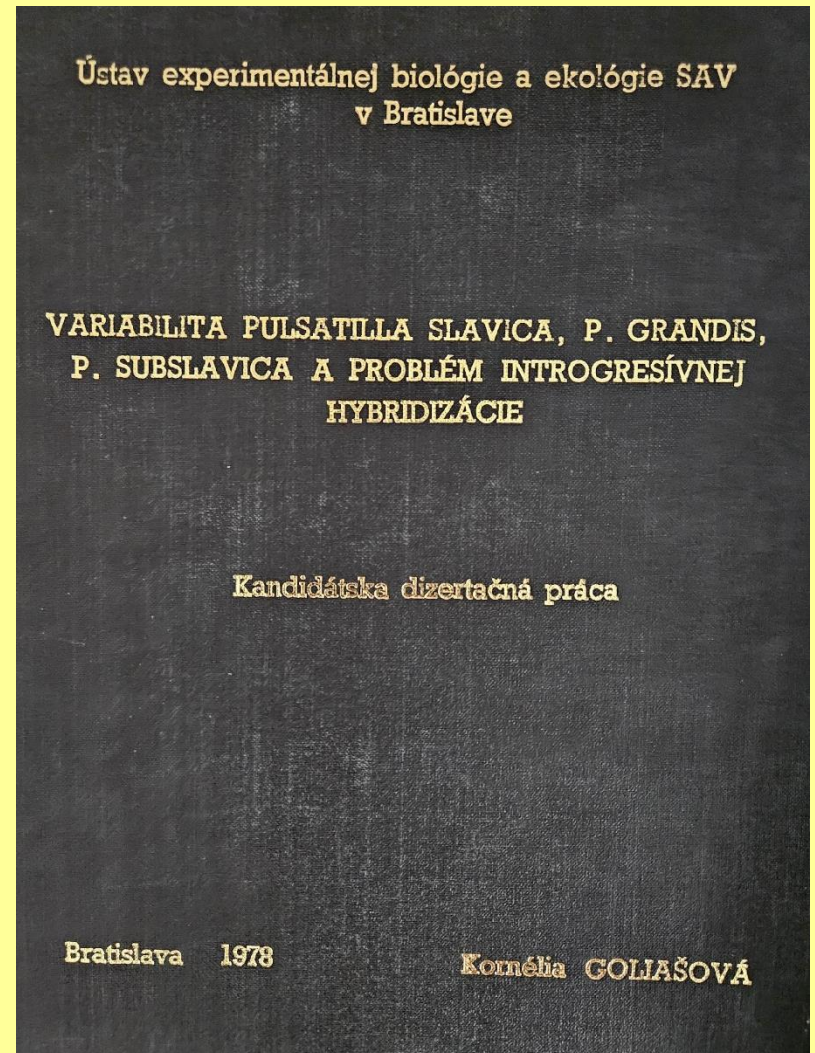
číslo štúdie	lokalita	taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
			šírka stonky	podiel šírky stonky	číslo rastlín	veľkosť listu	postavenie listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu	šírka listu
3	Zatĺska dolná	<i>Dianthus plumarius</i> ssp. <i>pseudopraecox</i>	15,8	4-5 / 3-6/	S=16 Z=14	S=12 e=18	20-30°	1/3-18 1/2-12	1:12 2:14 3:6 4:12	3,35	0,10	1/3-30	4,34	0,26	4:17 2:2 2/2-11	PE=19 Z=11	0,30	0,57	2,33	0,33	V=30	PE=18 Z=12	1,04	2,64	0,37	S=30
3	Slička plošina sály nad Bratislavou	..	15,2	3-5 / 2-3/	S=30	S=10 e=20	15-30°	1/3-13 1/2-17	1:12 2:12 3:5	3,31	0,17	1/3-30	3,80	0,23	4:22 4/2-1 3/2-6	PE=26	0,36	0,55	2,37	0,44	V=30	PE=25 Z=1	1,07	2,55	0,40	S=30
5	Devínsky kameň vrch	<i>Dianthus plumarius</i> ssp. <i>plumarius</i>	17,2	5 / 4-6/	S=19 Z=11	S=21 e=9	5-35°	1/3-27 1/2-3	1:12 2:7 3:2 4:12	3,19	0,19	1/3-24 1/2-6	4,06	0,28	2/2-12 4:25 4/2-3	F=2 Z=16 PE=14	0,42	0,73	2,18	0,43	V=20 K=6	PE=18 Z=13	0,87	1,79	0,26	S=30
10	Travná kopce - vŕšky	<i>Dianthus plumarius</i> ssp. <i>plumarius</i> < ssp. <i>hungaricus</i>	26,7	4-5 / 3-6/	S=28 Z=2	S=10 e=20	15-45°	1/3-18 1/2-13	1:12 2:12 3:6	2,60	0,14	1/3-30	3,54	0,24	4:15 2:10 2/2-3	PE=30	0,35	0,51	2,30	0,40	V=28 K=22	PE=28 Z=1	0,91	2,57	0,26	S=20 S=10
11	Travná kopce - líšce	<i>Dianthus plumarius</i> ssp. <i>plumarius</i> > ssp. <i>hungaricus</i>	12,3	4-5 / 3-5/	S=28 Z=2	S=25 e=5	5-35°	1/3-28 1/2-2	1:12 2:6 3:6	2,72	0,18	1/3-26 1/2-4	3,02	0,21	2/2-1 4:3	F=3 Z=16 PE=11	0,38	0,63	2,08	0,43	V=22 K=6	PE=23 Z=7	0,93	2,13	0,25	S=30
13	Sčítava skaly	<i>Dianthus plumarius</i> ssp. <i>hungaricus</i> > ssp. <i>plumarius</i>	15,7	4-5 / 3-5/	S=16 Z=14	S=4 e=20	5-45°	1/3-12 1/2-10	1:20 2:6 3:6	2,23	0,17	1/3-30	3,08	0,20	4:25 4/2-12 2:1	PE=24 Z=3 F=3	0,36	0,57	2,06	0,41	V=30	PE=23 Z=4 F=3	0,90	2,29	0,25	S=27 S=3
21	Prosecká dolina	<i>Dianthus plumarius</i> ssp. <i>hungaricus</i>	15,1	4 / 3-5/	S=7 Z=23	S=16 e=14	20-30°	1/3-21 1/2-9	1:22 2:6 3:6	2,47	0,15	1/3-30	3,06	0,21	4:17 2:12 2/2-1	PE=25 Z=1	0,37	0,48	2,15	0,37	V=30	PE=29 Z=1	0,92	2,18	0,38	S=30
	Paršovská kopce	<i>Dianthus plumarius</i> ssp. <i>plumarius</i> var. <i>palatinensis</i>	12,0	3 / 2-4/	S=30 Z=30	S=30 e=30	15-45°	1/3-17 1/2-13	1:12 2:12 3:6	2,19	0,15	1/3-30	3,26	0,23	4:10 2:19 2/2-3	PE=23 Z=6 F=1	0,42	0,62	2,12	0,39	V=24 K=6	PE=25 Z=5 Z=1	1,31	2,41	0,31	S=21 S=5
	Skaly pri Bôdliagu	<i>Dianthus plumarius</i> ssp. <i>palatinensis</i>	15,0	4-5 / 3-6/	S=30 Z=2	S=2 e=28	5-45°	1/3-19 1/2-1	1:12 2:12	3,17	0,20	1/3-30	3,65	0,25	6:1 5/2-6 2/2-3	Z=1 PE=9	0,44	0,60	2,18	0,46	V=24 K=6	PE=24 Z=1 PE=27	1,10	2,53	0,35	S=3 S=25
6	Čance	<i>Dianthus serotinus</i>	25,8	4-10 / 2-4/	S=28 Z=2	S=10 e=30	15-45°	1/3-26 1/2-4	1:12 2:12	2,04	0,17	1/3-21 1/2-9	1,75	0,17	4:28 2:24	PE=6 Z=24	0,36	0,43	2,11	0,40	V=13 K=17	PE=1 Z=29	1,04	2,44	0,32	S=30

Výslovnosť v tabuľke:  
 10 = 1/3 = list najširší v dolnej tretine /pri stonke/  
 23 = čísla v rázsochke určujú podkálné listové tvorenie ďalší pár šupín  
 14 = Z = vzostup; F = fialové; PE = fialovo-ružové  
 19 = Y = výškovitý; K = mierne kňožný; K = kňožný  
 20 = so štvrtou v číslach 14  
 24 = S = biela; Z = ružová

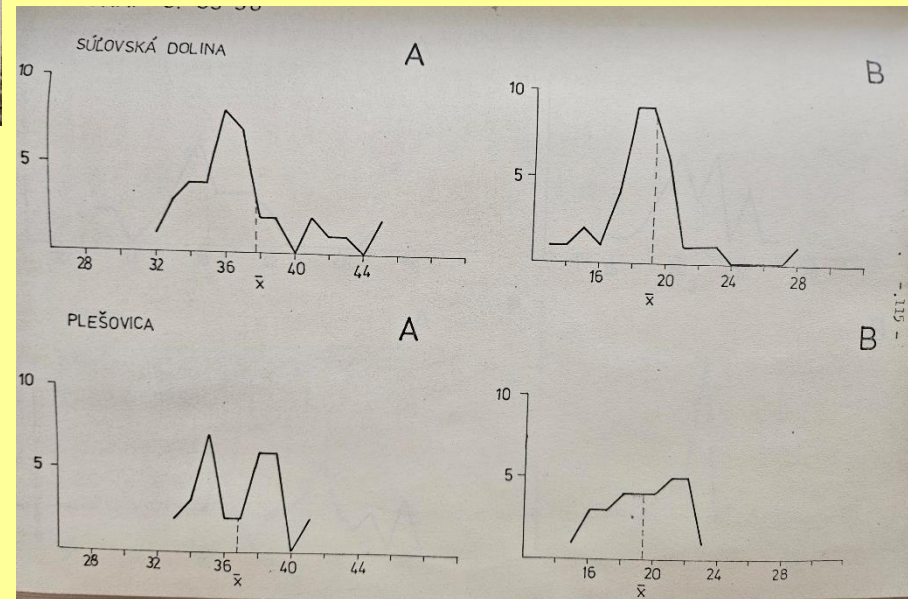
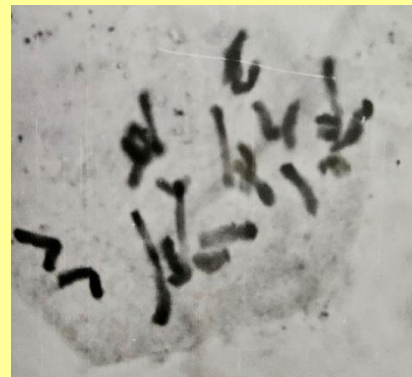
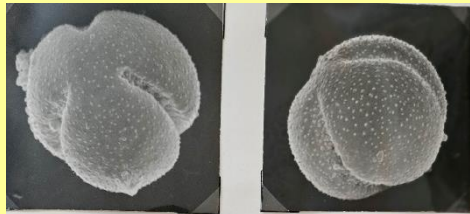
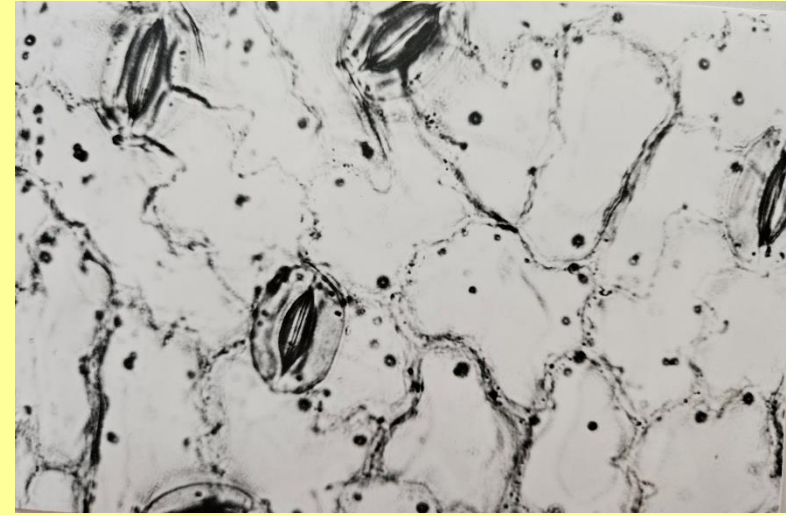
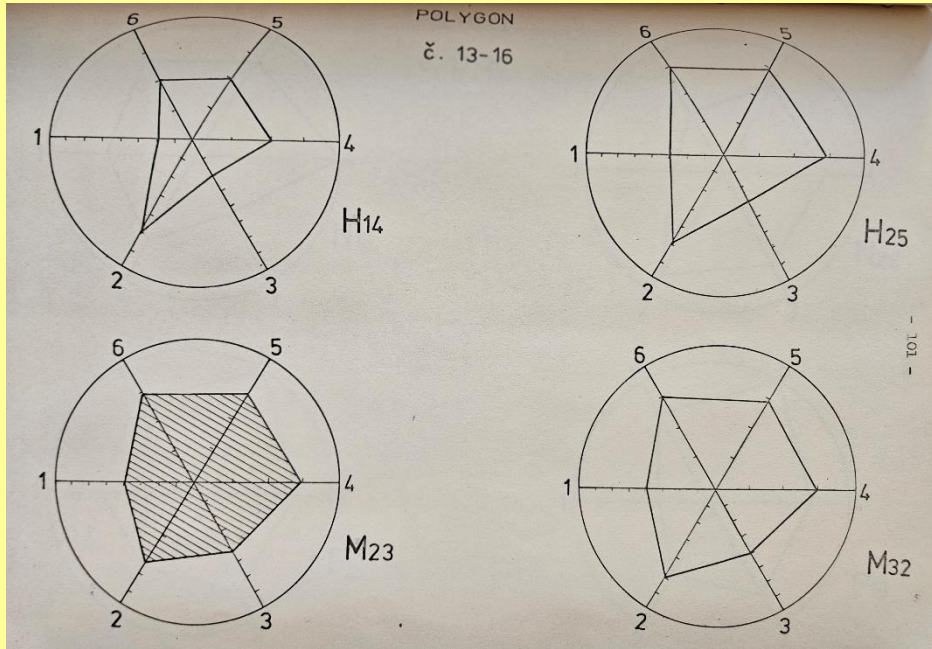
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# Detailné štúdium introgresívnej hybridizácie v rode *Pulsatilla* – Kornélia Goliašová

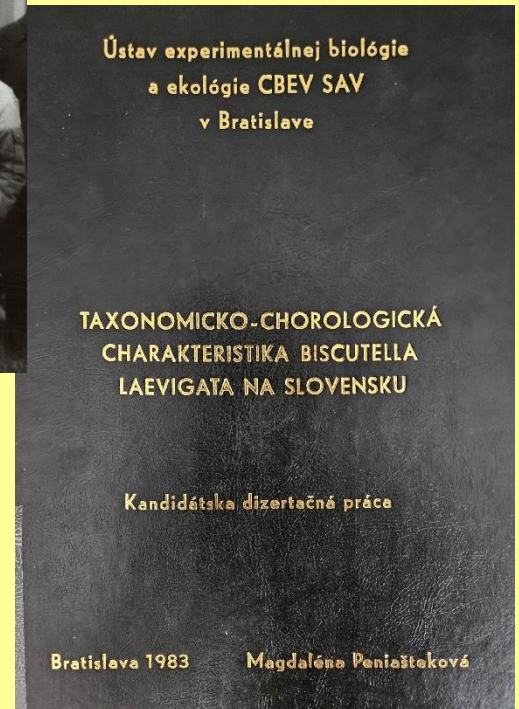
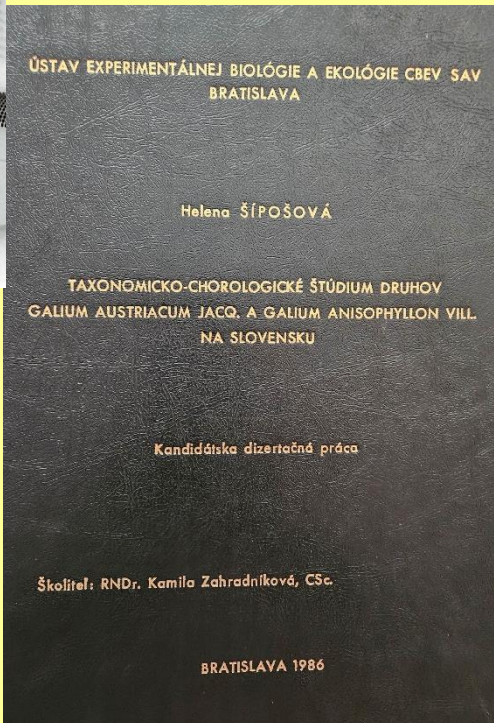


# Detailné štúdium introgresívnej hybridizácie v rode *Pulsatilla* – Kornélia Goliašová



# Zavedenie metód multivariačnej morfometriky do analýz polyploidných komplexov – Magdaléna Peniašteková a Helena Šípošová v spolupráci s Ladislavom Mucinom

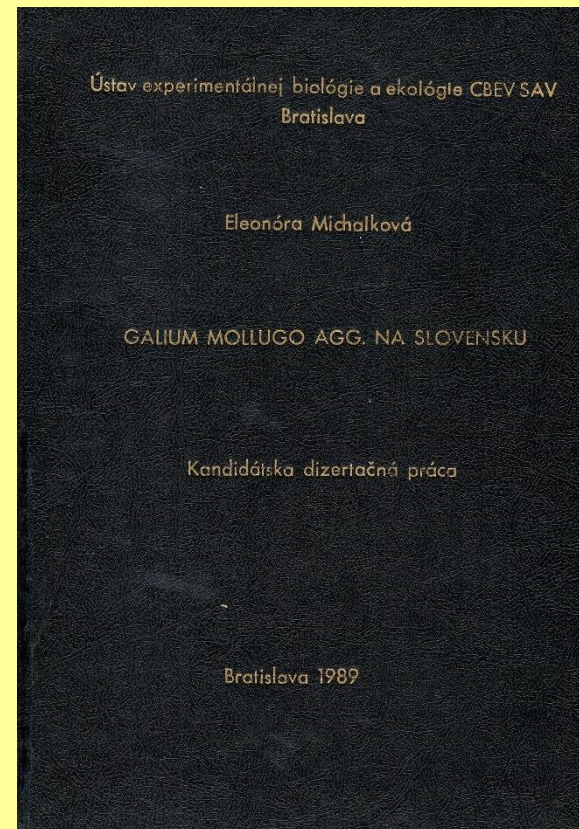
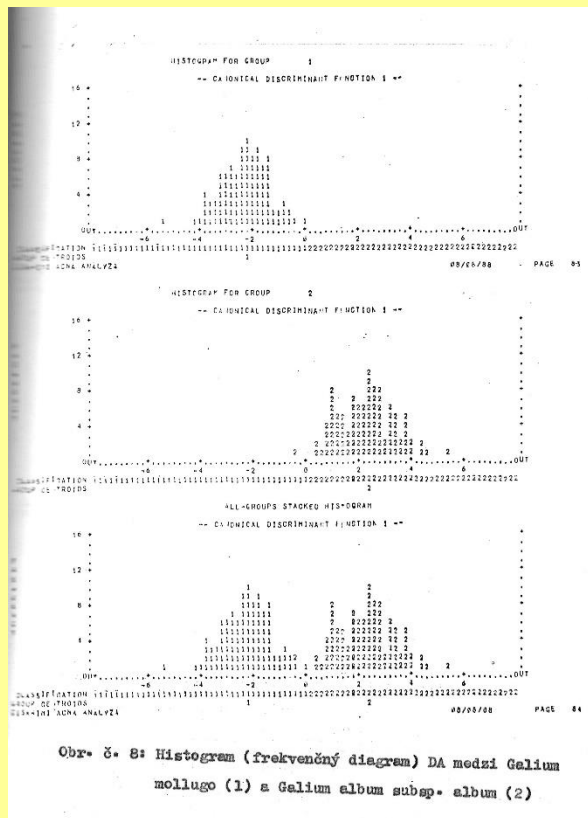
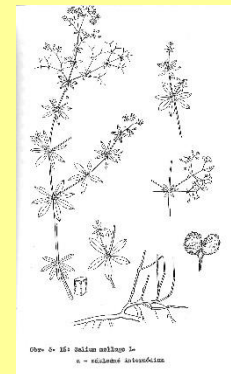
## *Biscutella laevigata, Galium anisophyllum*







Obdobie, keď dizertačné práce boli založené na kombinácii karyologických, morfometrických a chorologických metód sa završilo dizertačnými prácami Eleonóry Michalkovej (*Galium mollugo*), Karola Marholda (rod *Cardamine*), Ivy Hodálovej (*Senecio nemorensis*) a Romana Letza (*Sempervivum*)



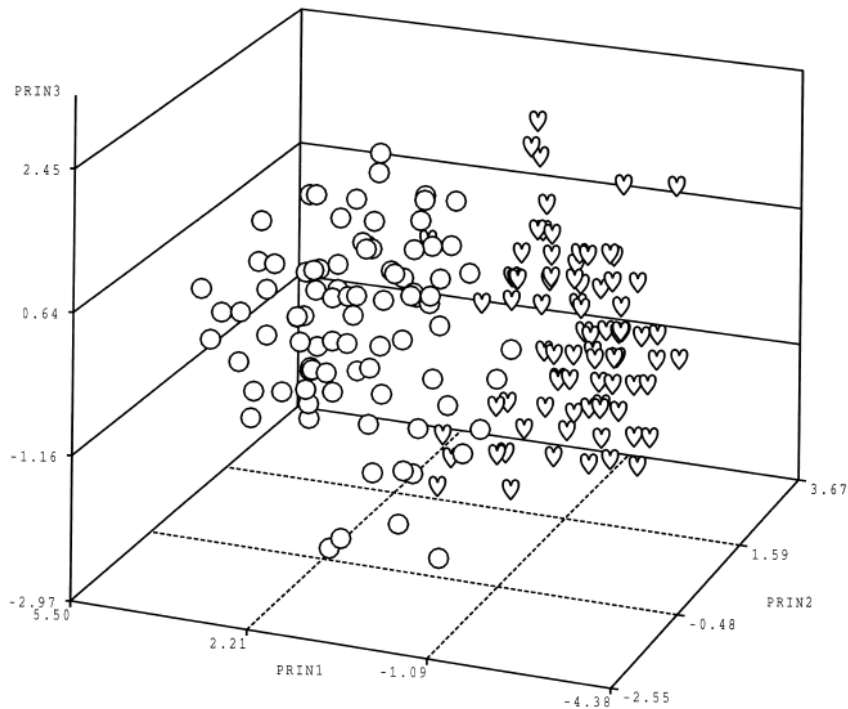


Fig. 6. Principal components analysis (PCA) of the individual plants of the *Senperivum montanum* group in the West Carpathians. Balloon – the “lowland” taxon, heart – the “upland” taxon.

## V morfometrike sme pokročili k náročnejším metódam

Results of the classificatory discriminant analysis of the two groups of populations of the *S. montanum* group in the West Carpathians.

Actual group		Predicted group membership (number of observations/percentage classified into groups)	
		“lowland” taxon	“upland” taxon
“lowland” taxon	Parametric method	85/94.44	5/5.56
	Nonparametric method	84/93.33	6/6.67
“upland” taxon	Parametric method	7/8.75	73/91.25
	Nonparametric method	2/2.50	78/97.50

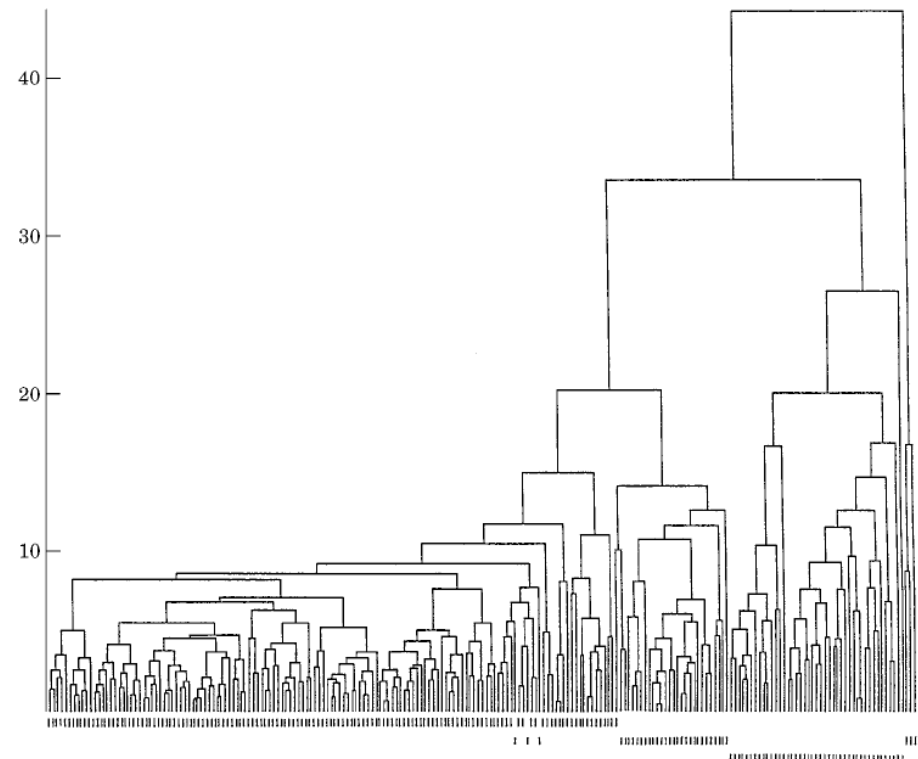
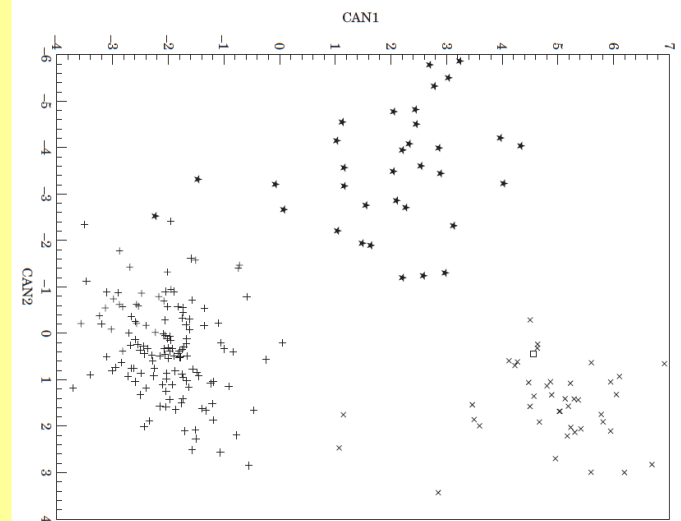


Figure 1. Cluster analysis (UPGMA) of *Senecio germanicus* (upper line), *S. dacicus* sp. nov. (bottom line) and *S. nemorensis* (middle line).



## A multivariate morphometric study of the *Cardamine amara* group (Cruciferae) in the Carpathian and Sudeten mountains

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Received December 1991, accepted for publication July 1992

MARHOLD, K., 1992. A multivariate morphometric study of the *Cardamine amara* group (Cruciferae) in the Carpathian and Sudeten mountains. A multivariate morphometric study of the *Cardamine amara* group based on 55 population samples, collected throughout the Carpathian and Sudeten mountains in Czechoslovakia, Poland, Ukraine and Romania, is presented. Among the multivariate methods, principal component analysis, cluster analysis, classificatory, canonical and discriminant analysis were used. The analysis of chromosome numbers from all studied populations showed wide variation. Together with the results of the present study, the best way of classifying this group into *C. amara* subsp. *amara* and *C. amara* subsp. *matthioli* is proposed. The Alps require further study.

ADDITIONAL KEY WORDS: Brassicaceae – Czechoslovakia

Introduction . . . . .  
Material and methods . . . . .  
Results . . . . .  
Discussion . . . . .  
Acknowledgements . . . . .  
References . . . . .  
Appendix . . . . .

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## Multivariate morphometric study of the *Cardamine pratensis* group (Cruciferae) in the Carpathian and Pannonian area

K. MARHOLD

Received November 12, 1992; in revised version November 3, 1993

**Key words:** Cruciferae, Brassicaceae, *Cardamine*. – Numerical taxonomy, principal component analysis, cluster analysis, discriminant analysis, chromosome numbers. – Carpathians, Pannonia.

**Abstract:** A multivariate morphometric study of the *Cardamine pratensis* group is presented, based on 84 population samples collected from the Carpathian and Pannonian area in the Czech Republic, Slovakia, Poland, Ukraine, Hungary, and Romania. Among the multivariate methods, principal component analysis, cluster analysis, and classificatory and canonical discriminant analysis were used. The analysis of chromosome numbers from all populations studied showed wide variation. The morphometric study showed that not all groups of populations characterised by their chromosome numbers and geographical criteria are morphologically, and thus taxonomically, distinguishable. Besides the morphologically well characterised species *Cardamine dentata* and *C. rivularis*, the following species were recognised in the area studied: *C. matthioli*, *C. majovskii* and *C. pratensis*. Within the last species, besides the typical populations, two diploid "types" are provisionally recognised: type "ucranica" and type "rivularis auct."

JONES (1964) recognised four species of the *Cardamine pratensis* group (Cruciferae) – *Cardamine pratensis* L., *C. matthioli* MORETTI, *C. dentata* SCHULT. [= *C. palustris* (WIMM. & GRAB.) PETERM.] and *C. rivularis* SCHUR – from the Carpathian and Pannonian area. In addition, *C. majovskii* MARHOLD & ZÁBORSKÝ was described from Eastern Slovakia (MARHOLD & ZÁBORSKÝ 1986). However, there are many unsolved taxonomic problems in this group which result in inconsistencies in local Floras. The *Cardamine pratensis* group has been studied in Europe by LÖVkvIST (1956), URBANSKA-WORYTKIEWICZ & LANDOLT (1974), and SPASSKAJA (1978, 1979), but Carpathian and Pannonian populations were considered only very briefly in these works.

Jones in *Flora Europaea* and Eastern Europe two Presl within the range of (1986), *Cardamine barb. barbareoides* (Halácsy) Str *amara* group. The author two entities as separate *Cardamine amara* L. subsp. Presl) Čelak. However, c

0024-4074/92/100121+15 \$08.00

## Sympatric populations of *Senecio ovatus* subsp. *ovatus*, *S. germanicus* subsp. *germanicus* (Compositae) and their hybrid in the Carpathians and the adjacent part of Pannonia

### I. Multivariate morphometric study

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Accepted: November 14, 1995

### Summary

A multivariate morphometric study of *Senecio germanicus* subsp. *germanicus*, *Senecio ovatus* subsp. *ovatus* and their hybrid, *Senecio xfutakii* based on 19 population samples, collected throughout the Carpathians in Slovakia, Ukraine and Romania, is presented. Multivariate methods used include principal components analysis, cluster analysis, classificatory and canonical discriminant analysis. As a complete classification of certain plants into "proven."

**Key words:** *Senecio ovatus* subsp. *o* introgressive hybridization, multivar

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0253-1453/02/020137-15  
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Botanica Helvetica

### Introduction

The *Senecio nemorensis* group in adjacent part of Pannonia is represented by: *Senecio hercynicus* HERB. *S. ovatus* (P. GÄRTNER, B. M. subsp. *ovatus*, *S. germanicus* WAL. *S. nemorensis* auct. non L.: HER 97, 1987, and by the populations to the first author as *S. ucranicus* I (Hodálová 1994). The first result and synecological study of this group indicated that many population hybrid swarms having its origin hybridization (KUCOWA 1976, H. During the present study we sympatric populations of *S. ova* *S. germanicus* subsp. *germanicus* hybrid remained unnoticed either by HERBERG (1987), who studied group in South Poland and in

## A multivariate morphometric study of *Senecio paludosus* L. (Asteraceae) in Central and Western Europe

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Manuscript accepted August 13, 2002



Hodálová I., Grulich V. and Marhold K. 2002. A multivariate morphometric study of *Senecio paludosus* L. (Asteraceae) in Central and Western Europe. Bot. Helv. 112/2:

A multivariate morphometric study of *Senecio paludosus* L. from Central and Western Europe, with additional material from other neighbouring areas is presented. The species, namely *S. paludosus* L. subsp. *paludosus*, *S. paludosus* subsp. *angustifolius* and *S. paludosus* subsp. *lanatus* Holub are recognised. Principal coordinate analysis confirmed good morphological separation of the above-mentioned taxa and qualitative characters with only a few intermediates. The recognition of *S. paludosus* subsp. *bohemicus* (Tausch) Čelak. as a separate subspecies was not confirmed.

**Key words:** *Senecio paludosus*, Asteraceae, Europe, taxonomy, distribution.

### Introduction

The distribution area of *Senecio paludosus* L. ranges from Western Europe to Western Asia. Some European Floras report this species as present also in North America (Nyárády 1964), but there are no concrete data for this area and neither Kartesz (1994) nor Kartesz and Meacham (1999) include this taxon in their works. It is a highly vari-

# A počty publikácií v medzinárodných časopisoch narastali ...

## Multivariate Morphometric Study of the *Sempervivum montanum* Group (*Crassulaceae*) in the West Carpathians.

By

Roman LETZ \*) and Karol MARHOLD \*\*)

With 8 Figures

Received April 16, 1998

**Key words:** *Crassulaceae*, *Sempervivum montanum*. – Cluster analysis, morphometrics, principal components analysis. –

### Summary

Multivariate morphometric study of the *Sempervivum* group in the West Carpathians. – *Phyton* (Horn, Austria) 38 (2): 323–336, 1998. German summary.

A multivariate morphometric study of the *Sempervivum montanum* group based on 12 morphological characters is presented. Methods used include principal component analysis and discriminant analysis. The study compares two taxa on the subspecific level in the area of the West Carpathians, both different from *S. montanum* L. s. str., and proposes to reject the name *S. carpathicum* WETST. The taxa are treated as subspecies at present under informal designations, as the proposed subspecies differ in respect of the size and shape of leaves, the colour of leaves. Both differ from *S. montanum* L. s. str. by sparse, short glandular hairs, and in the petals (Fig. 1).

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## Lectotypification of some names in *Jovibarba* and *Sempervivum* (*Crassulaceae*)

Roman Letz & Karol Marhold<sup>1</sup>

### Summary

Letz, R. & Marhold, K.: Lectotypification of some names in *Jovibarba* and *Sempervivum* (*Crassulaceae*). – *Taxon* 45: 111–116, 1996. – ISSN 0040-0262.

Lectotypes are designated for four Linnaean names for taxa belonging at present to the genera *Jovibarba* and *Sempervivum*: *S. globiferum* (= *J. globifera*), *S. hirtum* (= *J. globifera* subsp. *hirta*), *S. arachnoideum*, and *S. montanum*. In addition, epitypes are designated for *S. arachnoideum* and *S. montanum* as well as a lectotype for *S. sibiriferum* Sims, a synonym of *J. globifera*.

During work by the first author on the genera *Jovibarba* and *Sempervivum* in the Carpathians and E. Alps it was necessary to consider the typification of four Linnaean names for taxa belonging at present to these genera. The name *S. tectorum* L., providing the type of *Sempervivum*, had already been lectotypified (Jarvis & al., 1993: 87).

*Sempervivum globiferum* L., Sp. Pl.: 464, 1753 = *Jovibarba globifera* (L.) J. Parn. in Bot. J. Linn. Soc. 103: 219, 1990. – Lectotype (designated here): Herb. Linn. No. 632.1 (LINN).

= *Sempervivum sibiriferum* Sims in Bot. Mag.: ad t. 1457, 1812. – Lectotype (designated here): [icon in] Bot. Mag.: t. 1457, 1812.

The protologue (Linnaeus, 1753: 464) reads:

4. *Sempervivum (globiferum)* foliis ciliatis, propaginibus globosis.

*Sempervivum* foliis radicalibus in globum congestis ciliatis, propaginibus globosis. *Hort. cliff.* 180. *Roy. lugdb.* 457.

*Sedum majus vulgari simile, globulis decidentibus. Moris. hist.* 3. p. 472. s. 12. t. 7. f. 18.

*Sedum vulgari magno simile. Bauh. hist.* 3. p. 688.

*Habitat in Rutheno D. Gmelin.* 2]

The final line refers to a specimen Linnaeus received from J. G. Gmelin, collected in Ruthenia [southern European Russia]. There is a specimen in Linnaeus's own herbarium (No. 632.1, LINN; see Savage, 1945: 87) bearing the symbol for the western edge of Asia (cf. Savage, 1945: vii; Stearn, 1957: 106), the species number in *Species plantarum* ("4"), the specific epithet "*globuliferum*" at the bottom of the sheet, and an annotation "*Sempervivum hexagynum. Sempervivum 2dum Hort. Etiss.*" (cf. Savage, 1945: 87) on the verso of the sheet in Linnaeus's handwriting. There is a slight difference in the spelling of the epithet, but no species number in the epithet "*globuliferum*" appears to have been published by Linnaeus. The specimen seems to be original material, collected by Gmelin. As pointed out by Tjaden (1969: 168), it appears from Linnaeus's letter to Gmelin of 1744 that the latter had sent a specimen to Linnaeus as "*Sempervivum hexagynum*" which Linnaeus had identified

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**Koniec 90tych rokov a obdobie po roku 2000 znamenalo príchod mladej generácie ...  
A tiež nástup cytometrie a molekulárnych metód**



# Najprv sme experimentovali s chemotaxonomiou a s izozýmami (Patrik Mráz)



Pergamon Biochemical Systematics and Ecology 30 (2002) 1037–1049  
www.elsevier.com/locate/biochemsyssec

biochemical  
systematics  
and ecology

## Chemotaxonomic significance of flavonoids and phenolic acids in the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*; Lactuceae, Compositae)

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Received 4 July 2001; accepted 25 January 2002



For their phenolic compounds in the leaves: chlorophyll 4'-O-β-D-glucosyl only quantitative taxonomic marker. Based on these related and still undetermined species—*H. rateracense*—morphological and seasonal variation of the substances

phenolic acids; HPLC

Botanical Journal of the Linnean Society, 2007, 153, 287–300. With 4 figures

## Morphological and allozyme diversity in the *Hieracium nigrescens* group (Compositae) in the Sudety Moun and the Western Carpathians

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KAROL MARHOLD<sup>1b,c,d</sup>, IVANA PLÁČKOVÁ<sup>1</sup>, ANNA KRAHULCOVÁ<sup>1</sup>  
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The overall pattern of morphological variation and genetic diversity (allozyme analysis) was studied in the *Hieracium nigrescens* group (*H. nigrescens* s.l., *H. alpinum* > *H. murorum*) in the Sudety Mountains and the Western Carpathians. A morphological analysis was performed on 180 plants from 12 populations belonging to six distinguished taxa. Altogether, 25 characters were measured or scored. Morphometric (canonical discriminant) data separated five taxa, evaluated here at the species rank: *H. chrysostyloides*, *H. decipiens*, *H. nigrescens* (the Sudety Mountains), *H. jarzabeczynum*, and *H. vepenicicum* (the Western Carpathians). A distinct population from Mount Babia hora (the Western Carpathians) comprised a further possible taxon, given the name '*H. babiagorensis*'. Genetic diversity was studied in 17 populations of *H. chrysostyloides*, 1 *H. jarzabeczynum*, *H. nigrescens*, *H. vepenicicum* and *H. babiagorensis* using five enzyme systems. Recognized species were proved to be genetically homogeneous, each consisting of one unique multilocus genotype, except '*H. babiagorensis*' which shared the same genotype with *H. jarzabeczynum*. For the chromosome number is reported for *H. vepenicicum* ( $2n = 3x = 27$ ) and previously published but not confirmed for *H. chrysostyloides* ( $2n = 5x = 45$ ), *H. decipiens* ( $2n = 4x = 36$ ), *H. jarzabeczynum* ( $2n = 4x = 36$ ), and *H. nigrescens* ( $2n = 4x = 36$ ). All species have been shown to be endemites of the Sudety Mountains or the Western Carpathians. Except for the species studied, two further ones (*H. nitimontis*) are recognized in the area, giving a total of seven species from the *Hieracium nigrescens* area studied. The morphologically slightly different local population from Mount Babia hora ('*H. babiagorensis*') requires further study. Two new combinations are proposed: *Hieracium jarzabeczyni* (Zahn) Mráz & Chrtěk f. and *Hieracium vepenicicum* (Lengyel & Zahn) Chrtěk f. & Mráz. © 2007 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2007, 153, 287–300.

ADDITIONAL KEYWORDS: apomixis – Asteraceae – chromosome numbers – flow cytometry –

### INTRODUCTION

The genus *Hieracium* L. in the na (*Hieracium* subgen. *Hieracium*; Sell, 198 herbaria distributed mainly in the temperate northern hemisphere (Zahn, 1921–23; 1976). It is well known as a genus with

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DOI: 10.1007/s00606-002-0213-1

Plant Systematics  
and Evolution  
Printed in Austria

## Evolutionary history of the polyploid complex of *Cardamine amara* (Brassicaceae): isozyme evidence

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**Abstract.** In samples from 56 populations of *Cardamine amara* subsp. *pyrenaea* is monomorphic for a unique allele, and subsp. *balcanica* has a unique allele, too which, however, is not fixed in all populations of the taxon. Both taxa seem to be relic ones, although otherwise subsp. *balcanica* in respect of allelic spectrum much resembles subsp. *amara*. The other two diploid subspecies, subsp. *amara* and subsp. *opicii*, are not characterised by presence of unique alleles but differ in allele frequencies. The two tetraploid subspecies have different evolutionary histories. *C. amara* subsp. *austriaca* seems to be an autopolyploid derivative of subsp. *amara* which colonised open space offered by retreating glaciers in the Eastern Alps. *C. amara* subsp. *oleensis* from the Iberian Peninsula represents most probably a polyploid of preglacial time.

**Key words:** *Cardamine amara*, Large Bitter-cress, isozymes, polyploidy, glaciation, relic taxa.

### Introduction

*Cardamine* L. comprises several polyploid complexes in its European distribution area, e.g. the *C. pratensis* group, *C. amara* L. and the *C. raphanifolia* group. Until now detailed attention has been paid mainly to the *C. pratensis* complex, which consists of several diploid taxa and higher polyploids up to dodecaploid level, including diploids and aneuploids (e.g. Lövkvist 1956; Urbanska-Worytkiewicz and Landolt 1974; Marhold 1994a,b, 1996; Marhold and Ančev 1999; Franke and Hurka 2000).

Recent studies of populations of *C. amara* in various parts of Europe revealed an interesting pattern of karyological and morphological variation classified at the subspecific level (Lihová et al. 2000; Marhold 1992, 1999; Marhold et al. 1996). Four diploid subspecies are currently recognised within *C. amara* subsp. *amara*, widespread in most of Europe



**Pomoc s molekulárnymi metódami prišla z Viedne za veľa vd'áčime Todovi Stuessymu a Andimu Tribschovi**





# December 2005 – Prvé laboratórium molekulárnej systematiky – v budove na GÚ SAV na Valašskej ulici



# 2009 – Nové a väčšie laboratórium molekulárnej systematiky – v novej budove BÚ SAV





## Molecular study of the *Cardamine maritima* group (Brassicaceae) from the Balkan and Apennine Peninsulas based on amplified fragment length polymorphism

Jaromír Kučera · Karin Tremetsberger · Jaroslav Vojta · Karol Marhold

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**Abstract** The amphio-Adriatic region, and especially the Western Balkan Peninsula, belongs to the most biodiversity hotspots in the temperate region. New detailed phylogeographic and molecular systematic studies in the Western Balkans are rare due to sporadic surveys, where access has been, until recently, very rare. The *Cardamine maritima* group, which is the subject of this study, comprises not only the currently recognised species *C. maritima* and *C. montelucii*, but also other taxa which have been rendered to synonymy by macro- and micro-geographical florists. Molecular data and the amplified fragment length polymorphism showed a clear pattern within the group. Italian populations of *C. montelucii* are well separated from Balkan populations in a step forward from previous taxonomic

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# Kombinácia molekulárnych a morfológických metód sa stala štandardom (Jaromír Kučera) a pridalo sa aj meranie obsahu DNA (Marek Slovák)

Botanical Journal of the Linnean Society, 2006, 152, 169–195. With 7 figures

## Taxonomy and phylogeography of *Cardamine impatiens* and *C. pectinata* (Brassicaceae)

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Received November 2005; accepted for publication March 2006

The taxonomic position of *Cardamine pectinata*, a controversial taxon treated either as infraspecific to *C. impatiens* or as a separate species, was studied. Forty-nine populations were sampled and used in multivariate morphometric and molecular (amplified fragment length polymorphism) analyses. Our results showed that *C. impatiens* and *C. pectinata* represent two well-differentiated taxa in terms of both molecular and morphological data, and should be treated as two separate species. We present the taxonomic conspectus for both species, including the list of synonyms, information on type specimens, morphological descriptions, ecological characteristics, and distribution area. Type specimens for several names are designated here. The detailed geographical distribution of *C. pectinata* is presented based on the survey and revision of herbarium material. It covers the area from the Balkans through Turkey and the Caucasus to Iran. As an introduction to the phylogeography of both species, the distribution of genetic diversity within and between the studied populations and geographical regions was assessed. In *C. pectinata*, the highest genetic diversity was observed in northern Turkey, suggesting the location of glacial refugia along the Black Sea coast. Populations in Bulgaria were less diverse, and this area has most probably been colonized postglacially. *C. impatiens*, on the other hand, did not display clear phylogeographical structure, most probably as a result of the efficient spread and mixing between different colonization routes. © 2006 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2006, 152, 169–195.

**ADDITIONAL KEYWORDS:** amplified fragment length polymorphism – Balkan – Caucasus – chromosome – Cruciferae – genetic diversity – glacial refugia – multivariate morphometrics – nomenclature

### INTRODUCTION

*Cardamine* L. (Brassicaceae) comprises species occurring indigenously on all continents, including Antarctica (Al-Shehbaz, 1988), as well as polyploid taxa up to high ploidy levels (reviewed by Lihová & Marhold, 2006). Several detailed studies have been published on the taxonomic complexes of *Cardamine* in Europe (e.g. *C. maritima* group: Lovkvist, 1956; Marhold, 1994a; Marhold *et al.*, 1997; Franzke & Hurka, 2000; Lihová, 2002; Tribsech, 2003; Lihová, Tribsech & Stuessy, 2004; *C. maritima* group: Lovkvist, 1957; Marhold, 1992, 1994; Marhold & Neuffer, 2000; Lihová *et al.*, 2005; *C. hirsuta* group: Perný *et al.*, 2005a), less

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## The Morphological and Genetic Variation in the Polymorphic Species *Picris hieracioides* (Compositae, Lactuceae) in Europe Strongly Contrasts with Traditional Taxonomical Concepts

Marek Slovák,<sup>1,3</sup> Jaromír Kučera,<sup>1</sup> Karol Marhold,<sup>1,2</sup> and Judita Zozomová-Lihová<sup>1</sup>

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ORIGINAL ARTICLE

## The Balkan endemic *Picris hispida* (Compositae): morphology, nuclear DNA content and relationship to the polymorphic *P. hieracioides*

Marek Slovák · Tomáš Urfus · Petr Vít · Karol Marhold

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© Springer-Verlag 2009

**Abstract** The only Balkan endemic of the genus *Picris*, *P. hispida*, was studied in detail using morphological and karyological methods. The species was shown to be morphologically distinct from the closest taxon, *P. hieracioides*, by the pectinate-gilliate indumentum of involucral bracts, dilatation of the peduncle, length of the outer and inner bracts, and indumentum colour. Nonmorphological variation that would require taxonomic classification was found within this species. Despite the diploid chromosome number ( $2n = 2x = 10$ ) being confirmed for *P. hispida*, variation of up to 9.5% in genome size was found. The likely explanation for this variation is hybridization and introgression with closely related *P. hieracioides*. The most convincing evidence for this hypothesis is the detection of plants with two clearly different DNA contents arising from a single capitulum found in the location where *P. hispida* and *P. hieracioides* co-occur.

**Keywords** Balkan Peninsula · Genome size · Multivariate morphometrics · *Picris hispida* · *P. hieracioides* · Ploidy level

### Introduction

The Balkan Peninsula represents one of the most significant biodiversity hotspots in Europe (Turrill 1929; Griffiths *et al.* 2004), and is sometimes considered to be the most important hotspot (Kryštofek & Reed 2004). This is due to its long-term environmental stability, topographic and climatic diversity, and by the fact that it acted as a glacial refugium for at least ca. 1.8 million years in the Quaternary period (Tzedakis 2004). Despite this richness, knowledge of the flora of the Peninsula remains inadequate. In particular, there is a lack of studies that use cross-border sampling (challenging because of limited access in the past years due to armed conflict) and that apply modern karyological and/or molecular methods to elucidate the patterns and dynamics of plant biodiversity (however, see Mugnier & Siljak Yakovlev 1987; Bogunic *et al.* 2003; Siljak-Yakovlev *et al.* 2005; Park *et al.* 2006; Kryštofek *et al.* 2007; Frajman & Oxelman 2007).

Our study focuses on the western Balkan endemic species *Picris hispida* (Bartl.) W. D. J. Koch, which is among the less known species of the genus and is morphologically similar to the widespread and polymorphic species *Picris hieracioides* L. In fact, the entire Mediterranean represents one of the main centres of diversity of *Picris*, and is considered to be a plausible phylogenetic centre (Lack 1974, 1979; Holzapfel & Lack 1993). The genus comprises about 50 species (Chaudhary 2000), of which approximately 24 occur in the Mediterranean (Greuter 2005–2007). Four species, namely *P. hieracioides*,



## Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations

Stanislav Španiel · Karol Marhold ·  
Barbora Filová · Judita Zozomová-Lihová

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**Abstract** The genus *Alyssum* is least known genera of the Brassicaceae study focusses on the Central Europe. *A. montanum*, a species that is hijacked by its morphology, ploidy levels and taxonomic concepts. This variation is naturally recognised infraspecific taxa, and with regards to their value and amplified fragment length polymorphism as morphometric and flow-cytometry contrast here that the variation current taxonomic concepts held support the division of the analysis following groupings: (i) the ‘core’ of the Central European diploid (ii) the diploid ‘Swiss–SW German’ population from the type locality to the hexaploid *A. montanum* subspecies venian and Croatian endemic silesian origin; and (iv) the diploid ‘Serbian dunes of Deliblatska Peščara. A.

**Electronic supplementary material**  
article (doi:10.1007/s00606-011-0438-y) material, which is available to authorized users.

S. Španiel (✉) · K. Marhold · B. Filová

Plant Systematics and Evolution (2017) 303:1443–1466  
https://doi.org/10.1007/s00606-017-1470-3

ORIGINAL ARTICLE

## The polyploid *Alyssum montanum*-*A. repens* complex in the Balkans: a hotspot of species and genetic diversity

Stanislav Španiel<sup>1,2</sup> · Karol Marhold<sup>1,2</sup> · Judita Zozomová-Lihová<sup>3</sup>

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### Abstract

The Balkan Peninsula is an important diversity and speciation centre for many species groups. The high genetic, karyological and morphological complexity of the *Alyssum montanum*-*A. repens* species group (Brassicaceae) in the Balkans makes it a challenging study subject for exploring different speciation mechanisms and their taxonomic consequences. In the present study, ploidy level and genetic (AFLPs and chloroplast DNA sequences) data were examined and confronted with recent taxonomic concepts. Remarkable genetic and morphological variation, which is often geographically structured, and high incidence of polyploids suggest a very complex evolutionary history in this area, involving allopatric differentiation and past hybridisation and polyploidisation events. A new taxonomic treatment, differing substantially from recent concepts, is suggested. Several previously recognised taxa, including many endemics, such as *A. austrodalmaticum*, *A. handelii*, *A. moellendorffianum*, *A. pirinicum* and *A. wierzbickii*, are confirmed as distinct. Several other taxa are suggested to be resurrected or elevated to the species level, namely, *A. bosniacum*, *A. montenegrinum*, *A. reiseri* and *A. vernale*. Phylogenetic relationships among populations from the central Balkans and Greece are still partly blurred apparently due to more extensive reticulations, and their taxonomic classifications remain provisional. They are treated here under the tentative name *A. spraneri*. The patterns of variation revealed in the present study highlight the importance of the Balkan Peninsula for the persistence and diversification of vascular plants.

...entiation · Balkan Peninsula · Carpathians · Disjunction · Polyploidy

and northern Europe and the centre of endemism (Tzedakis 2004; Stevanović et al. 2007). A large part of the Balkan Peninsula belongs to the Mediterranean, which harbours highly variable habitats with stable environmental conditions over longer periods of time (Hewitt 2011; Nieto Feliner 2014). The Balkan Peninsula is also characterised by geological heterogeneity and outstanding topographic diversity, as almost 70% of its relief is formed by mountains (Reed et al. 2004). Environmental barriers, such as vast mountain ranges or deep valleys, contribute to the geographic isolation of populations, enable the diversification and preservation of infraspecific lineages and act as a trigger for allopatric speciation (Thompson 2005; Nève and Verlaque 2010). This phenomenon is responsible for the generation of phylogeographic splits and the ‘refugia within refugia’ patterns recognised by Gómez and Lunt (2007) for the Iberian Peninsula, and later inferred for many taxa also in the Balkan Peninsula (e.g. Kučera et al. 2010; Surina et al. 2011, 2014; Kutnjak et al. 2014). The Balkan biota probably also benefits from its geographic position in the centre of the Mediterranean and

## Morphological Characters Useful for the Delimitation of Taxa Within *Viola* Subsect. *Viola* (Violaceae): A Morphometric Study from the West Carpathians

Iva Hodálová · Pavol Mereďa Jr. ·  
Pavol Mártonfi · Lenka Mártonfi  
Jiří Danihelka

© Institute of Botany, Academy of Sciences of

**Abstract** Forty-nine morphological characters (individuals) of *Viola* subsect. *Viola* from the Republic of Austria and Hungary. The presence of *V. ambigua*, *V. collina*, *V. hirta*, *V. odorata* is cytological and morphometric analyses. The aim is to identify the taxa and most common hybrids presented. Chromosome counting and flow cytometry populations studied. All individuals of *V. all* tetraploid, while those of *V. ambigua* and *V. su*

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Systematics and Biodiversity (2011), 9(3): 211–231

## Research Article

### Genetic and morphological variation in *Viola suavis* s.l. (Violaceae) in the western Balkan Peninsula: two endemic subspecies revealed

PAVOL MEREĎA JR., IVA HODÁLOVÁ, JAROMÍR KUČERA, JUDITA ZOZOMOVÁ-LIHOVÁ, DOMINIK R. LETZ & MAREK SLOVÁK

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(Received 23 March 2011; revised 10 June 2011; accepted 28 June 2011)

The Balkan Peninsula, with many endemic species, is known as one of the most important speciation and diversification centres in Europe. Here, we present a study of the western Balkan populations of the polymorphic European species, *Viola suavis* s.l., which have been reported under the name *V. adriatica*, but their taxonomic status and position within the genus have remained uncertain. *Viola suavis* s.l. and nine close relatives sampled across Europe were subjected to molecular (sequencing of nuclear ribosomal internal transcribed spacers and amplified fragment length polymorphism), karyological and morphometric analyses. Our results revealed the presence of four allopatric, genetically and morphologically differentiated lineages within *V. suavis* s.l. in Europe, which are suggested here to be recognized at the subspecific rank. Populations from the western Balkans were segregated into two distinct entities: (1) those from north-western Croatia correspond to the previously recognized taxon, *V. suavis* subsp. *adriatica* and (2) those from southern Dalmatia (southern Croatia, southern Bosnia and Herzegovina, and south-western Montenegro) are described here as *V. suavis* subsp. *austrodalmatica* subsp. nov. The other two lineages of *V. suavis* s.l., which both harbour blue- and white-flowered morphotypes, occur in central and eastern Europe (*V. suavis* subsp. *suavis*) and in north-eastern Spain (plants provisionally treated as *V. suavis* ‘Spain’). The AFLP and morphological data indicate gene flow between the nominate subspecies and *V. suavis* subsp. *adriatica* in a few localities. The distribution of the two western Balkan subspecies is discussed and an identification key to the *V. suavis* subspecies in Europe is presented.

**Key words:** AFLP, flow cytometry, ITS sequences, multivariate morphometrics, *Viola* subsect. *Viola*, western Balkans

... important centres of European and Mediterranean

... important centres of European and Mediterranean  
... is the Balkan Peninsula (Turrill 1929;  
... rýstufek & Reed 2004), which harbours  
... diversified fauna and flora (for exam-  
... et al. 2004). Approximately 6530 plant  
... species in the area, of which about one-  
... (Horvat et al. 1974; Polunin 1997).  
... diversity can be explained by several  
... of this area (Polunin 1997; Reed et al.,  
... et al. 2008): (1) a geographic position  
... of different floral and faunal provinces; (2)  
... and topographically very diverse terrain,  
... geological and edaphic complexity; and  
... high environmental stability throughout  
... during the Pleistocene glaciations,  
... asula served as one of the most impor-

... Pavol Mereďa Jr. E-mail: pavol.mereda@

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# Kombinácia molekulárnych a morfometrických prístupov s cytometriou sa osvedčila (Stanislav Španiel, Pavol Mereďa)

## Biosystematic study of the diploid–polyploid *Pilosella alpicola* group with variation in breeding system: Patterns and processes

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**Abstract** Members of the *Pilosella* group (Asteraceae) are common in mountainous regions and represent a complex taxonomic group. We studied the taxonomic concept of *P. alpicola* (Serbia) among 557 plants and found that it is a complex cytogenetic group. We recorded in *P. rhodopea* a clear morphological origin of all studied ecotypes from a hybrid or data strongly support sometimes treated as and ITS polymorphism evolutionary and taxonomic circumscription.

## Abstract Members of the *Pilosella* group (Asteraceae) are common in mountainous regions and represent a complex taxonomic group. We studied the taxonomic concept of *P. alpicola* (Serbia) among 557 plants and found that it is a complex cytogenetic group. We recorded in *P. rhodopea* a clear morphological origin of all studied ecotypes from a hybrid or data strongly support sometimes treated as and ITS polymorphism evolutionary and taxonomic circumscription.

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Populations of sympatric diploid and autopolyploid cytotypes provide a unique opportunity to study early stages of polyploid evolution. *Pilosella rhodopea* is a diploid–autopolyploid complex forming frequent mixed-ploidy populations, most probably representing the largest primary contact zone documented so far in angiosperms. Our aims were to elucidate: (1) the origin of autopolyploids (single vs. multiple); (2) cytotype distribution patterns at various spatial scales; and (3) potential ecological differentiation of the cytotypes by measuring several habitat variables along two elevational transects. In total, five cytotypes were found across the species range. Triploids were the most frequent (50%), followed by diploids (29%) and tetraploids (15%), whereas pentaploids and hexaploids were highly amplified fragment length polymorphism and cytotype distribution patterns and frequent intercytotype gene flow. Cytotype diversity and allele dependence. At a local scale (5 m × 5 m), cytotype co-occurrence (1 × 1 m) were cytotypically more homogeneous. We did not find any cytotypes. Our results show that *P. rhodopea* is a rare example of a and ongoing polyploid formations in primary contact zones. Recurrent intercytotype gene flow among spatially close cytotypes might explain, at least in part, the observed patterns.

Fragment length polymorphism – aneuploidy – autopolyploidy – cytotype diversity – primary contact zone – recurrent polyploidization

Polyploidization is still an ongoing process in many plant groups (neopolyploidy) (Soltis, 2005; Van de Peer et al., 2009). Within genera, different ploidy levels often represent separate taxonomic entities (species, e.g. Soltis et al., 2007; Catalán et al., 2012; Borges et al., 2012; Dančák et al., 2012); however, intraspecific ploidy variation also appears to be a rather common phenomenon (Rice et al., 2015).

Mixed-ploidy populations harbouring two or more cytotypes occur when polyploids emerge directly within a diploid or lower-level polyploid population (primary contact zone) or when previously parapatric distributions of different cytotypes overlap owing to a range expansion (secondary contact zone; Petit et al., 1999; Kolář et al., 2017). Coexistence of several cytotypes might be only a transitional stage,

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

Biosystematická štúdia skupiny *Cyanus triumfetti* v strednej Európe

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Oľšavská K., Perný M., Kučera J. & Hodálová I. *Cyanus triumfetti* group in Central Europe. – Preslia 83:

Multivariate morphometrics and an assessment of microsatellite length polymorphism (AFLP) were used to study the *Cyanus triumfetti* group in Central Europe. The ploidy level was determined for all individuals from the *C. triumfetti* group related to *C. montanus* group were tetraploid (2n = 4x) populations revealed that three species from the *C. Cyanus caillardi*, *C. strictus* and *C. domini*. The species, namely *C. domini* subsp. *domini*, *C. de skolosteni*. Morphological characters of leaves as well as characters caused by cultivation did not affect the taxa remained. AFLP analysis of 38 populations *C. montanus* group revealed a contrasting geographic distribution of the populations rather than the group. The AFLP data revealed the following three (i) *C. triumfetti* s.s. and *C. montanus* from the Western Carpathians and Pannonia, and (iii) Western Carpathians and Pannonia. The striking geographic distribution of the populations rather than the latter group are discussed in the light of contact and/or the probability of hybridization events in the group. An identification key for the taxa of the *C.*

**Keywords:** Asteraceae, *Cyanus* sect. *Perenne*, Pannonia, taxonomy, Western Carpathians

## Introduction

The *Cyanus triumfetti* group is one of the many species-rich aggregates of perennial knapweeds in the



Botanical Journal of the Linnean Society, 2013, 173, 230–257. With 5 figures

## Cytogeography of European perennial species of *Cyanus* (Asteraceae)

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To reveal the general cytogeographical pattern of *Cyanus* section *Protocyanus* in Europe, DNA ploidy and/or chromosome numbers were newly examined for 160 populations by flow cytometry (450 plants) and/or chromosome counting (30 plants). Furthermore, previously published karyological data were revised (236 records). Our analyses confirmed chromosome counts of 2n = 22 for all newly investigated samples of the *C. triumfetti* group (the records for *C. semidecurans* and *C. ternopoliensis* are new), *C. diopoliensis* and *C. ochotovi*; 2n = 44 for *C. montanus* and *C. mollis*; and 2n = 20 for *C. linguatus*, *C. napulifer*, *C. nissanus*, *C. orbiculus*, *C. thirkei*, *C. tuberosus* and *C. velenovskyi*. The chromosome count of 2n = 20 is the first report for *C. epirotus*. The cytotype 2n = 40 was newly recorded for the Crimean endemic *C. fuscomarginatus* and Calabrian and Greek populations of *C. graminifolius*. The cytotypes 2n = 20 and 2n = 40 were confirmed for *C. pindicola*. For the first time triploidy (2n–3x=30) was found in *C. nissanus*, *C. thirkei* and in a newly discovered hybrid, *C. epirotus* × *C. graminifolius*. Two contrasting cytogeographical patterns emerged: cytotypes derived from the base chromosome number x = 11 (2n = 22, 44) are widespread in northern latitudes and ecologically diverse, whereas cytotypes with x = 10 (2n = 20, 30, 40) are confined to mountains in southern Europe. In general, tetraploids have smaller ranges than diploids. The new combinations *Cyanus* section *Protocyanus* (Dobrocz), Oľšavská comb. nov. and *Cyanus ternopoliensis* (Dobrocz), Oľšavská comb. nov. are provided. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, 173, 230–257.

**ADDITIONAL KEYWORDS:** *Centaurea* – chromosome numbers – Compositae – cytotaxonomy – environmental conditions – flow cytometry – polyploidy.

## INTRODUCTION

*Cyanus* Mill. [sometimes treated as *Centaurea* L. section *Cyanus* (Mill.) DC.] is confined to the Old World and includes 25–50 species depending on taxonomic classification (Dostál, 1969; Hellwig, 2004). The number of species continues to increase as new species have recently been described (Bancheva & Solyanov, 2009; Kaya & Bancheva, 2009). The genus has been divided into *Cyanus* section *Cyanus* containing five annual species and *Cyanus* section *Protocyanus* (Dobrocz.) Oľšavská comb. nov. (see Discussion)

composed of 45 perennial species (Table 1). The sections are not only distinguished by pollen morphology (annuals have the ‘*Cyanus*’ pollen type, perennials have the ‘*Montana*’ pollen type), but also differ in important life-history traits (Wagenitz, 1955). In annuals the variability in base chromosome number (x = 8, 9, 11, 12; Wagenitz & Hellwig, 1996; Bancheva, 1998; Gornitzgen & Adigüzel, 2001; Romaschenko et al., 2004; Chaffari & Keilich, 2006) reflects their tendency towards increased selfing, rapid gain of reproductive isolation and fixation of karyotypic changes. Perennials have a presumably outcrossing breeding system (Gonnet, 1993; Oľšavská & Loser, 2013) and often reproduce vegetatively by stolons or

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# Rody *Pilosella* a *Cyanus* priniesli nové výzvy a potrebu odlišných prístupov (Barbora Šingliarová, Katarína Skokanová)

## Research Article

**Tracking the expanding distribution of *Solidago ×niederederi* (Asteraceae) in Europe and first records from three countries within the Carpathian region**

 Katarína Skokanová<sup>1</sup>, Barbora Šingliarová<sup>1\*</sup>, Stanislav Španiel<sup>1,2</sup>, Iva Hodálová<sup>1</sup> and Pavol Meroďa Jr.<sup>1</sup>
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**Abstract**

Besides the well-known negative effects of invasive plant species on autochthonous plant communities, the breakdown of genetic integrity of indigenous species via alien-to-native hybridisation represents an additional direct threat to native flora which should not be underestimated. Our aim was to survey the current distribution of *Solidago ×niederederi*, a hybrid that has originated through spontaneous hybridisation between the native European *S. virgaurea* and allochthonous (North American) *S. canadensis*. Although this hybrid was first recorded at the very end of the 19<sup>th</sup> century, most occurrences have been reported during the last decades. It is only known to grow in Europe and its current distribution is still not well explored. Based on field research in the Carpathians, we list five new localities of *S. ×niederederi*, which represent the first records of this hybrid in Slovakia, Hungary, and Romania. The present paper documents existing reports from 409 sites in 17 European countries. We provide a detailed list of all records from the literature, freely available databases and webpages as well as a summary map of *S. ×niederederi*'s known distribution. In addition, records are analysed regarding a time context and habitat preferences. Finally, we discuss a potential threat that the hybrid could pose to the genetic integrity of the native European populations of the *Solidago virgaurea* complex.

**Key words:** plant invasion, hybrid, *Solidago canadensis*, new chorological records, range expansion

**Introduction**

The worldwide distributed genus *Solidago* L. includes 120–133 species. North America, with the occurrence of roughly 150 *Solidago* species and infraspecific taxa, is considered to be the centre of genus diversity (Nesom 2000; Semple and Cook 2006; Semple 2020). The genus is divided into two sections, *S.* sect. *Solidago* and *S.* sect. *Parmicoidei* (House) Semple & Gandhi. The first section is taxonomically complex, rich in species and further subdivided into eleven subsections and one nothosubsection, while *S.* sect. *Parmicoidei* includes only 7 species and is not further subdivided (Semple and Cook 2006; Gudžinskas and Žalneravičius 2016; Semple 2020). In Europe, only *S.* sect. *Solidago* is represented in nature by the

**Relative DNA content differences reliably identify *Solidago ×niederederi*, a hybrid between native and invasive alien species**

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**Abstract:** Hybridization between native and alien congeners may pose a serious threat to biodiversity and negatively affect native flora. Here we study *Solidago ×niederederi*, which originated and became established in Europe as a result of a cross between the alien *S. canadensis* and native *S. virgaurea*. The recent increase in the number of records of *S. ×niederederi* in Europe has highlighted the need to monitor its occurrence, spread and behaviour. In the present study, we tested the effectiveness of flow cytometry for detecting hybrid plants of *S. ×niederederi*. Sequences of the ITS region of nrDNA and the *rpS15-ycf1* spacer of cpDNA were used to confirm the hybrid origin of analysed plants and to identify the maternal species. Our study included 60 single-species populations of *S. canadensis*, *S. gigantea* and *S. virgaurea*, and 16 mixed populations with the presence of hybrid *S. ×niederederi* sampled from six countries in central Europe and adjacent areas. All individuals of *S. canadensis*, *S. ×niederederi* and *S. virgaurea* investigated were diploid (2n=2x=18) but differed in their relative DNA content values. The DNA content of *S. ×niederederi* was intermediate between *S. canadensis* and *S. virgaurea* with no overlaps, with the differences between the species being statistically significant. Therefore, we conclude that flow cytometry is a reliable and efficient method for detailed screening for hybrids within mixed *Solidago* populations and for identifying non-flowering individuals. The method was applied on a large extent and was also inferred to other species. Hybridization has occurred in

**Keywords:** homoploid, hybridization, relative DNA content

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## Pervasive Introgression During Rapid Diversification of the European Mountain Genus *Soldanella* (L.) (Primulaceae)

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Marek Slovák and Andrea Melichárková contributed equally to the study.

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**Abstract.**—Hybridization is a key mechanism involved in lineage diversification and speciation, especially in ecosystems that experienced repeated environmental oscillations. Recently radiated plant groups, which have evolved in mountain ecosystems impacted by historical climate change provide an excellent model system for studying the impact of gene flow on speciation. We combined organellar (whole-plastome) and nuclear genomic data (RAD-seq) with a cytogenetic approach (rDNA FISH) to investigate the effects of hybridization and introgression on evolution and speciation in the genus *Soldanella* (snowbells, Primulaceae). Pervasive introgression has already occurred among ancestral lineages of snowbells and has persisted throughout the entire evolutionary history of the genus, regardless of the ecology, cytotype, or distribution range size of the affected species. The highest extent of introgression has been detected in the Carpathian species, which is also reflected in their extensive karyotype variation. Introgression occurred even between species with dysploid and euploid cytotypes, which were considered to be reproductively isolated. The magnitude of introgression detected in snowbells is unprecedented in other mountain genera of the European Alpine System investigated hitherto. Our study stresses the prominent evolutionary role of hybridization in facilitating speciation and diversification on the one hand, but also enriching previously isolated genetic pools. [chloroplast capture; diversification; dysploidy; European Alpine system; introgression; nuclear-cytoplasmic discordance; ribosomal DNA.]

Hybridization is a key evolutionary process shaping diversification and speciation processes (Soltis and Soltis 2009; Taylor and Larson 2019; Nieto Feliner et al. 2020). Recent methodological developments allowed uncovering numerous, often unexpected, cases of hybridization and introgression (Mallet 2007; Whitney et al. 2010; Abbott et al. 2013, 2016; Mallet et al. 2016; Taylor and Larson 2019). Identification of historical hybridization in various groups of organisms has opened avenues to investigate the long-term consequences of gene flow in evolution, including hybrid speciation (Greig et al. 2002; Harris and Nielsen 2016; vonHoldt et al. 2016; Taylor and Larson 2019), adaptive introgression (Choler et al. 2004; Suarez-Gonzalez et al. 2018), but also adaptive radiation (e.g., Meier et al. 2017). A direct consequence of speciation induced by gene exchange is genomic mosaicism (Jiggins et al. 2008). The substantially increased allelic diversity

of introgressed populations can facilitate their adaptation to novel and challenging environments. Hence, this mechanism can directly trigger the emergence of new species and fuel adaptive radiations (Pease et al. 2016; Meier et al. 2017). On the other hand, excessive introgression can reintegrate previously separated gene pools into a single biological entity, and thus hampering diversification (Bog et al. 2017; Kearns et al. 2018).

Key extrinsic triggers facilitating hybridization are dynamic changes in the environment, which enable previously allopatric populations to shift their ranges and come into secondary contact (Stebbins 1985). Mountain landscapes are highly diverse, forming steep altitudinal gradients featuring mosaics of niches with specific microclimates and bedrock. Such fine-scale, ecologically-heterogeneous systems promote secondary contacts of originally allopatric or parapatric species and hence provide ample opportunities for gene exchange (e.g.,



- |   |   |  |  |
|---|---|--|--|
| 1 <i>S. alpina</i> ssp. <i>alpina</i>       | 2 <i>S. alpina</i> ssp. <i>cantabrica</i> | 3 <i>S. minima</i> ssp. <i>minima</i>    | 4 <i>S. minima</i> ssp. <i>austriaca</i> |
| 5 <i>S. minima</i> ssp. <i>sarmatica</i>    | 6 <i>S. pusilla</i> ssp. <i>pusilla</i>   | 7 <i>S. pusilla</i> ssp. <i>alpicola</i> |  |
| 8 <i>S. angusta</i>                         | 9 <i>S. calabrella</i>                    | 10 <i>S. carpatica</i>                   | 11 <i>S. chrysostricta</i>               |
| 12 <i>S. hungarica</i>                      | 13 <i>S. major</i>                        | 14 <i>S. marmarossiensis</i>             | 15 <i>S. montana</i> ssp. <i>montana</i> |
| 16 <i>S. montana</i> ssp. <i>gubalowkae</i> | 17 <i>S. oreodoxa</i>                     | 18 <i>S. pindicola</i>                   | 19 <i>S. rhodopaea</i>                   |
| 20 <i>S. rugosa</i>                         | 21 <i>S. sacra</i>                        | 22 <i>S. tabricola</i>                   | 23 <i>S. villosa</i>                     |

FIGURE 1. Sampling map of *Soldanella* accessions used in the present study. Taxa specialized in alpine zone are represented by squares, while species that grow in forests or have a large ecological amplitude are represented by circles. The bold line on the symbol depicts species with a dysploid cytotype, whereas the dashed line denotes species revealed to be non-monophyletic through inferred tree analyses (Supplementary Figs. S2 and S3). Photos with their credits from left to right: *S. alpina* subsp. *alpina* (Jaromír Kučera—JK), *S. minima* subsp. *minima* (JK), *S. angusta* (Eliška Gbúrová Štubňová), *S. carpatica* (Marek Slovák), *S. pindicola* (JK) and *S. villosa* (JK).

Preparation of single-digest RAD libraries followed a protocol adapted from Brandrud et al. (2017). The starting amount of genomic DNA was 120 ng per individual. Considering genome size (1C = 1.5–2.0 pg, Štubňová et al. 2017), 48 barcoded samples were pooled together in one library. Final libraries were sequenced on an Illumina HiSeq 2500 at the sequencing facility of the Vienna BioCenter Core Facilities (VBCF; [https://](https://www.vbccf.org/)

[www.viennabiocenter.org/](https://www.viennabiocenter.org/)) as 100 bp single-end reads. Individuals with less than 1.5 million high-quality reads were re-sequenced.

### Filtering SNPs from RAD-seq Data

Raw Illumina reads were demultiplexed in two steps, first using BamIndexDecoder v.1.03 of the Picard Illumina2bam package (<http://ggq1.github.io/>

Zaviedli sme metódu RAD-Seq (Marek Slovák, Andrea Melichárková, Adam Kantor a ďalší)

## Evolution of hydrophytic plant species in the Anatolia–Caucasus region: insights from phylogenomic analyses of *Cardamine* perennials

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### Phylogenetic challenges in a recently diversified and polyploid-rich *Alyssum* (Brassicaceae) lineage: low divergence, reticulation, and parallel polyploid speciation

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S.Š. and M.Š. contributed equally to this work.

#### Abstract

Elucidating the evolution of recently diverged and polyploid-rich plant lineages may be challenging even with high-throughput sequencing, both for biological reasons and bioinformatic difficulties. Here, we apply target enrichment with genome skimming (Hyb-Seq) to unravel the evolutionary history of the *Alyssum montanum*-*A. repens* species complex. Reconstruction of phylogenetic relationships in diploids supported recent and rapid diversification accompanied by reticulation events. Of the 4 main clades identified among the diploids, 3 clades included species from the Alps, Apennine, and Italian peninsulas, indicating close biogeographic links between these areas. We further focused on the clade distributed from the Western Alps to the Iberian Peninsula, which comprises numerous polyploids as opposed to a few diploids. Using a recently developed PhyloSD (phylogenomic subgenome detector) pipeline, we successfully tracked the ancestry of all polyploids. We inferred multiple polyploidization events that involved 2 closely related diploid progenitors, resulting into several sibling polyploids. 2 autopolyploids and 6 allopolyploids. The skewed proportions of major homeo- and the occurrence of some minor homeo- and allopolyploids, both exhibiting geographic patterns, suggest introgression with the progenitors and other related diploids. Our study highlights a unique case of parallel polyploid speciation that was enhanced by ecological and geographic separation and provides an excellent resource for future studies of polyploid evolution.

**Keywords:** *Alyssum*, Brassicaceae, phylogenomics, polyploidy, reticulation, target enrichment

#### Introduction

Recently and rapidly diversified plant lineages present challenges for both taxonomic and evolutionary studies. They often form species complexes with problematic species delimitation and difficulties in resolving their relationships and evolutionary history. Reasons include low levels of genetic and morphological differentiation among taxa, incomplete lineage sorting (ILS), and permeable reproductive barriers with persistent interspecific gene flow, all of which can blur species boundaries, cause gene tree discordance, and complicate phylogenetic reconstructions even at the diploid level (Fernández-Mazuecos et al., 2018; Frajman et al., 2019; Larridon et al., 2020; Ott et al., 2022; Thomas et al., 2021). Moreover, polyploids frequently arise in such species complexes, further increasing their evolutionary and taxonomic complexity (Brandrud et al., 2020; Frajman et al., 2016; Kameva et al., 2017; López-González et al., 2021; Tomasello & Oberprieler, 2022). These can be autopolyploids formed through genome doubling from a single progenitor species, or allopolyploids, which combine different genomes via hybridization (Solís et al., 2003). Recurrent polyploid formation, gene flow between related, independently arisen (sibling)

polyploids, and genome evolution after polyploidization are commonly observed in polyploid lineages and contribute to their overall diversity and evolutionary complexity (Nieto Felner et al., 2020; Solís et al., 2009, 2014). Genome-scale data obtained by high-throughput DNA sequencing have had a profound impact on phylogenetic inferences. They provide an order of magnitude more data than in the past, but this also introduces more heterogeneity and higher levels of gene tree discordance (Bravo et al., 2019). Multispecies coalescent approaches that explicitly account for genealogical discordance resulting from ILS while incorporating hybridization and/or polyploidy have recently emerged and appear promising for addressing such evolutionarily complex lineages (Hilary & Ané, 2020; Solís-Lemus & Ané, 2016; Thomas et al., 2017; Wen et al., 2018; Yan et al., 2022).

Although hybridization and polyploidy are pervasive evolutionary processes that drive speciation (Rieseberg & Willis, 2007), there has been much debate about the frequency, speciation rate, and evolutionary success of polyploids from a long-term perspective (Arrigo & Barker, 2012; Radcliff et al., 2018; Bowers & Paterson, 2021; Landis et al., 2018; Mayrose et al., 2011). Immediate reproductive isolation from

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**Background and Aims** Southwestern Asia is a significant area for many plant groups, especially terrophytic elements. In clarification of its hydrophytic flora. To fill this gap, we focus on plants from a wide altitudinal range. We aimed to elucidate if gene flow between species, and draw inferences about intra- and inter-specific gene flow.  
**Methods** We applied the phylogenomic Hyb-Seq approach to a total of 85 *Cardamine* populations from the four to six species, and supplemented them with close relatives.  
**Key Results** Five diploids are recognized in the focus Black and/or Caspian Sea (*C. parvifolia*, *C. tenera*, *C. lazica* Lebanon and Iran (*C. alysinoides*), and one western Anatolian data suggest recent speciation during the Pleistocene, like and ecological divergence. With the exception of a single *wiedemanniana*, an endemic of southern Turkey, no signal were observed. Genetic variation within the studied species due to geographic and ecological barriers, but also glacial cycles.  
**Conclusions** This study highlights the importance of it both harbouring and generating hydrophytic species diversity of evolutionary links between Anatolia and the Balkans a minor evolutionary role here in contrast to the European region.

**Keywords:** Allopolyploidy, Anatolia, Caucasus, *Cardamine* flora, phylogenomics.

#### INTRODUCTION

Southwestern (SW) Asia is a significant centre of biodiversity in the Northern Hemisphere, located at the junction of three biogeographic regions, the Mediterranean, Euro-Siberian and Irano-Turanian (Mittermeier et al., 2011; Noroozi, 2020). Thanks to its complex topography and geology, diverse climate and a strategic crossroad position, it has acted as both a cradle of diversification and a migration corridor (Manafzadeh et al., 2014, 2017; Noroozi et al., 2018, 2019; Noroozi, 2020). Many vascular plant genera and even families (including Brassicaceae; Franzke et al., 2011) are thought to have originated and diversified in this area, as also supported by recent phylogenetic studies (e.g. Koch et al., 2017; Ortadoğru and Mummenhoff,

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### Allele Sorting as a Novel Approach to Resolving the Origin of Allotetraploids Using Hyb-Seq Data: A Case Study of the Balkan Mountain Endemic *Cardamine barbaraoides*

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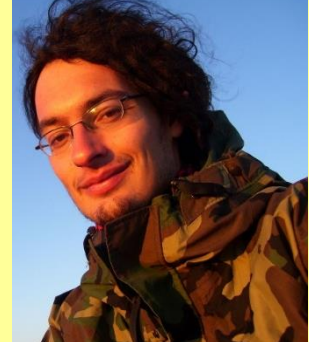
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Mountains of the Balkan Peninsula are significant biodiversity hotspots with great species richness and a large proportion of narrow endemics. Processes that have driven the evolution of the rich Balkan mountain flora, however, are still insufficiently explored and understood. Here we focus on a group of *Cardamine* (Brassicaceae) perennials growing in wet, mainly mountainous habitats. It comprises several Mediterranean endemics, including those restricted to the Balkan Peninsula. We used target enrichment with genome skimming (Hyb-Seq) to infer their phylogenetic relationships, and, along with genomic *in situ* hybridization (GIS-H), to resolve the origin of tetraploid *Cardamine barbaraoides* endemic to the Southern Pindos Mts. (Greece). We also explored the challenges of phylogenomic analyses of polyploid species and developed a new approach of allele sorting into homeologs that allows identifying subgenomes inherited from different progenitors. We obtained a robust phylogenetic reconstruction for diploids based on 1,168 low-copy nuclear genes, which suggested both allopatric and ecological speciation events. In addition, cases of plastid–nuclear discordance, in agreement with divergent nuclear ribosomal DNA (nrDNA) copy variants in some species, indicated traces of interspecific gene flow. Our results also support biogeographic links between the Balkan and Anatolian–Caucasus regions and illustrate the contribution of the latter region to high Balkan biodiversity. An allotetraploid origin was inferred for *C. barbaraoides*, which highlights the role of mountains in the Balkan Peninsula both as refugia and melting pots favoring species contacts and polyploid evolution in response to Pleistocene climate-induced range dynamics. Overall, our study demonstrates the importance of a thorough phylogenomic approach when studying

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# A už bežne využívame d'alšiu z metód celogenómového sekvenovania Hyb-Seq (Judita Zozomová, Marek Šlenker, Adam Kantor, Andrea Melichárková, Veronika Cetlová, Stanislav Španiel)

## Data and text mining MorphoTools2: an R package for multivariate morphometric analysis

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### Abstract

**Summary:** The package MorphoTools2 is intended for multivariate analyses of morphological data. Commonly used tools are missing or scattered across several R packages. The new package, in order to make the workflow convenient and fast, wraps available statistical and graphical tools and provides a comprehensive framework for checking and manipulating input data, core statistical analyses and a wide palette of functions designed to visualize results.

**Availability and implementation:** Stable version is available from CRAN: <https://cran.r-project.org/package=MorphoTools2>. The development version is available from the following GitHub repository: <https://github.com/MarekŠlenker/MorphoTools2>. The software is distributed under the GNU General Public Licence (v.3).

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**Supplementary information:** [Supplementary data](#) are available at *Bioinformatics* online.

### 1 Introduction

Systematics aims to understand the evolutionary history and reflect it in biological classification. Recent progress in molecular and genomic methods has enabled us to study deeper and finer phylogenetic relationships, but regardless of the methods used, classification based on morphology is crucial for many applications because it gives us the ability to identify biological entities.

Multivariate morphometrics represents a wide spectrum of methodological approaches aimed at evaluating patterns of morphological variation on the basis of simultaneous analysis of multiple variables (Marhold, 2011 and references therein). Statistical functions used for analyzing multivariate morphological data are missing or scattered in multiple packages, but a single package connecting all needed analyses, and providing tools for data import, export, manipulation and visualization is missing. As far as we know, the only attempt to connect all necessary functionalities using R was published as a set of functions named MorphoTools (Kouřtecký, 2015). The MorphoTools2 R package is neither an extension nor a mere version update. Although the naming of many features was inspired by the earlier MorphoTools functions, the MorphoTools2 package is a stand-alone implementation providing a new architecture, more features and a simpler workflow.

### 2 Description

#### 2.1 Data import, checking and manipulation

Data can be imported from text files using the `read.morphodata` function of a predefined format. The first three columns define the hierarchical structure of the data, namely levels of individuals, populations and taxa, enabling the further grouping of individuals into taxonomic units.

Descriptive statistics and boxplots are often used to gain a first insight into morphological differences between taxa. The normality of the distribution of data can be assessed numerically or graphically, and characters that deviate most from a normal distribution can be transformed to improve their distribution. Datasets commonly contain missing values, but a full matrix is needed by most of the functions. Therefore, functions for summarizing the distribution of missing data, removing samples or morphological characters and replacing missing values by the population averages of the respective characters are provided in order to make the dataset ready for downstream analyses.

#### 2.2 Statistical analyses

Integrated statistical methods can be divided into those used for generating hypotheses and those used for their testing. Hierarchical

Ale prispievame aj k ďalšiemu  
rozvoju morfometrických  
metód ... (Marek Šlenker)

