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

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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 *a priori* distinguished taxa. Altogether, 25 characters were measured or scored. Morphometric (canonical discriminant analysis) data separated five taxa, evaluated here at the species rank: *H. chrysostyloides, H. decipiens, H. nigrescens* (all from the Sudety Mountains), *H. jarzabczynum,* and *H. vapenicanum* (the Western Carpathians). A distinct local population from Mount Babia hora (the Western Carpathians) comprised a further possible taxon, given the preliminary name '*H. babiagorense*'. Genetic diversity was studied in 17 populations of H. chrysostyloides, H. decipiens, *H. jarzabczynum*, *H. nigrescens*, *H. vapenicanum* and '*H. babiagorense*' using five enzyme systems. All *a priori* recognized species were proved to be genetically homogeneous, each consisting of one unique multilocus allozyme genotype, except '*H. babiagorense*' which shared the same genotype with *H. jarzabczynum*. For the first time, a chromosome number is reported for *H. vapenicanum* ($2n = 3x = 27$) and previously published numbers were confirmed for *H. chrysostyloides* ($2n = 5x = 45$), *H. decipiens* ($2n = 4x = 36$), *H. jarzabczynum* ($2n = 4x = 36$), *H. koprovanum* ($2n = 4x = 36$), and *H. nigrescens* ($2n = 4x = 36$). All species have been shown to be endemic to either the Sudety Mountains or the Western Carpathians. Except for the species studied, two further ones (*H. apiculatum, H. nivimontis*) are recognized in the area, giving a total of seven species from the *Hieracium nigrescens* group in the area studied. The morphologically slightly different local population from Mount Babia hora/Babia Góra ('*H. babiagorense*') requires further study. Two new combinations are proposed: *Hieracium jarzabczynum* (Pawł. & Zahn) Mráz & Chrtek f. and *Hieracium vapenicanum* (Lengyel & Zahn) Chrtek f. & Mráz.

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

INTRODUCTION

The genus *Hieracium* L. in the narrow sense (*Hieracium* subgen. *Hieracium*; Sell, 1987) contains herbs distributed mainly in the temperate regions of the northern hemisphere (Zahn, 1921–23; Sell & West, 1976). It is well known as a genus with widespread

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agamospermy (seed apomixis), giving rise to a very large number of variants that have been described as species (narrow species concept) or subspecies (broad species concept). Agamospermy is closely associated with polyploidy; the great majority of taxa are either triploids ($2n = 27$) or tetraploids ($2n = 36$) (Schuhwerk, 1996). Sexuality is rare and is confined to a few diploid species (e.g. Merxmüller, 1975; Chrtek, Mráz & Sevéra, 2004).

Two kinds of species in the broad sense (= species groups) are traditionally distinguished: (1) basic species ('species principales', 'Hauptarten') with a unique set of morphological characters; (2) intermediate species ('species intermediae', 'Nebenarten', 'Zwischenarten') sharing morphologically intermediate position between two or more basic species (Nägeli & Peter, 1885; Zahn, 1921–23). They are supposed to be a result of past hybridization between two or more basic species.

The *Hieracium nigrescens* group (*H. nigrescens s.l.*), together with the *H. atratum* group, occupies an intermediate position between the *H. alpinum* and *H. murorum* groups (species in the broad sense). Worldwide Zahn (1921–23) recognized 154 subspecies within *H. nigrescens* (in the broad sense), primarily on the basis of differences in leaf shape and dentation, the indumentum of peduncles and phyllaries, the number of stem leaves and the style colour. Since then, many new taxa mostly at the species rank have been described from the British Isles (e.g. Pugsley, 1948; Sell, West & Tennant, 1995) and from north-west Russia (Üksip, 1960; Shlyakov, 1966, 1989). The total distribution area of the group includes Greenland, Iceland, the British Isles, and through the Scandinavian mountains extending to north-west Russia. In Central Europe the group has a discontinuous distribution at high-altitude locations: the Alps, the highest Sudety Mountains, the Carpathians, and an isolated population in the Harz Mountains (Germany) (cf. Hultén & Fries, 1986; map incomplete). Plants of this group are typically found in high mountain grasslands (usually open-canopy), rocky knolls and ledges in gullies and on cliffs.

There have been relatively few chromosome studies of the group. All previously examined plants were polyploid. Tetraploids seem to prevail, while triploids are less common; two pentaploid species are unique within the genus *Hieracium (s.s.)* (e.g. Chrtek, 1994; Stace *et al.*, 1995; Chrtek, 1996). Agamospermy has been reported for *H. decipientiforme* from the Ukrainian Carpathians (Chrtek, 1997) and is supposed to be a common mode of reproduction in the group. It is widely supposed that the group includes hybridogeneous taxa originated from past crosses between *H. alpinum* and *H. murorum* (or their hybrids). It is unknown whether the group is monophyletic or whether particular taxa of this group have originated repeatedly through polytopic hybridization events between different taxa of both putative parental groups.

Both the Sudety Mountains and the Western Carpathians belong to genetic and diversity centres of the *Hieracium nigrescens* group. In the Sudety Mountains, members of the group occur in the Krkonoše Mountains (the western Sudety), the Hrubý Jeseník Mountains and Mount Králický Sněžník (both the eastern Sudety). Results of long-term research (the first studies in the Krkonoše Mountains go back to second half of the 19th century) of *Hieracium* are summarized in Zahn's monographs (Zahn, 1921–23; Zahn, 1936–38). Zahn (1936–38) recognized five subpecies of *H. nigrescens* in the Krkonoše Mountains, and four in the eastern Sudety Mountains Later on, Zlatník (1938, 1939) recognized in the Krkonoše Mountains four more or less sympatrically occurring species (in the narrow sense) within the 'group IV'; delimitation of the group is nearly identical to that of the *Hieracium nigrescens* group. Diversity of high mountain *Hieracium* species in the eastern Sudety is lower; recent revision of the *H. nigrescens* group revealed two endemic taxa treated at the species level here (Chrtek, 1995). More recent taxonomic treatment of the *H. nigrescens* group in the Western Carpathians is still lacking. Zahn (1936–38) distinguished 11 subspecies within the highest Western Carpathian mountain ranges. Local diversity centres of *Hieracium* sect. *Alpina* are situated mostly in the Tatra Mountains. Considering the total distribution range, important studies (apart from Zahn's monographs) come from the British Isles (e.g. Pugsley, 1948; Sell *et al.*, 1995), Scandinavia (e.g. Elfstrand, 1893, 1894; Norrlin, 1912; Omang, 1928) and north-west Russia (e.g. Üksip, 1960; Shlyakov, 1966, 1989).

The *Hieracium nigrescens* group is considered here to consist of eight *a priori* recognized species in the Sudety Mountains and the Western Carpathians. They are as follows: *Hieracium apiculatum* Tausch, *H. decipiens* Tausch, *H. nigrescens* Tausch, *H. chrysostyloides* (Zahn) Chrtek f., *H. nivimontis* (Oborný & Zahn) Chrtek f. (all the Sudety Mountains), *H. jarzabczynum* (Pawł. & Zahn) Mráz & Chrtek f. (formerly placed by Zahn in *H. pietroszense* Degen & Zahn) and *H. vapenicanum* (Lengyel & Zahn) Chrtek f. & Mráz (both from the Western Carpathians). Morphologically distinct plants from Mount Babia hora/Babia Góra (the Oravské Beskydy Mountains, Western Carpathians) are preliminarily treated as a separate unit and named '*H. babiagorense*'. The *a priori* concept proposed here is based on the revision of herbarium specimens (including most of the original collections of previously described taxa) and our own field observations.

Two recognized species, namely *H. apiculatum* and *H. nivimontis* were excluded from our analyses. Both of them are very rare and any sampling might seriously influence their survival. *Hieracium koprovanum* (Rech. f. & Zahn) Mráz & Chrtek f., *ined*., previously treated by Zahn (1936–38) as a subspecies of *H. nigrescens*, is considered here to belong to either the *H. rohacsense* or *H. pietroszense* group (owing to stellate hairs on phyllaries). It was therefore excluded from our analyses, except for allozyme analysis (to show its relationship with the *H. nigrescens* group) and chromosome number counts (necessary for a proper interpretation of the allozyme banding patterns).

The aims of this paper are: (1) to examine the overall morphological variation of the *H. nigrescens* group in the study area, (2) to assess genetic variation and diversity using allozyme analysis, and (3) to determine chromosome numbers/ploidy levels, and mode of reproduction.

MATERIAL AND METHODS

PLANTS

Plants for morphological studies

Altogether, 12 population samples, of 15 plants each, were collected during 1995–2002. The populations were selected according to previous studies in order to: (1) include all *a priori* recognized taxa in the area, except for those very rare and extremely endangered (*H. apiculatum, H. nivimontis*), i.e. *H. chrysostyloides, H. decipiens, H. jarzabczynum, H. nigrescens, H. vapenicanum* and '*H. babiagorense*'; and (2) to cover the geographical range of the recognized taxa. A list of the population samples is given in Table 1. The number of populations collected per species ranged from one to three, and reflects the geographical distribution and population abundance. In most cases, only the aboveground part of the plant was carefully removed to allow the plant to survive at the locality. All morphological characters were measured (scored) on herbarium specimens. Voucher specimens are deposited in PRA (plants collected by JC) and in private herbarium of P. Mráz (plants collected by PM).

Plants for allozyme analysis, and karyological and breeding system studies

Living plants from the same localities as above (ten plants from each, except of *H. koprovanum*; see Table 1) were collected, transferred to the experimental garden of the Institute of Botany, Průhonice, and cultivated in pots under field conditions. Only leaves were collected in the field for the endangered species *H. chrysostyloides* and *H. nigrescens*. In contrast to the morphometric studies, *H. koprovanum* was also included in the allozyme and karyological (chromosome counting) analysis.

PLOIDY LEVEL AND NUMBER OF CHROMOSOMES

Either the chromosome number or the ploidy level was estimated for all the cultivated plants used for the allozyme and breeding system studies (five plants per population, a total of 65 plants). Chromosome counts were made for at least two plants per population. Root tip cuttings of mature plants were used. The material was pretreated with a saturated solution of p-dichlorobenzene, fixed in a mixture of ethanol and acetic acid (3:1) and stored in 70% ethanol. The squash method with staining by lacto-propionic orceine followed Dyer (1963). Determination of ploidy level by flow cytometry followed Krahulcová, Papoušková & Krahulec (2004).

BREEDING SYSTEM

The mode of reproduction was tested in ten cultivated plants per species; if more than one population per species was collected, the plants studied originated proportionally from all such populations. The top portions of unopened flower buds containing anthers and stigma were sliced off with a razor. Heads were than bagged to prevent loss of achenes. Fully developed achenes were considered to be the result of agamospermous mode of reproduction. Differences in the percentage of fully developed (viable) achenes were assessed by one way ANOVA.

MORPHOLOGICAL ANALYSIS

Altogether 25 quantitative characters (Table 2) were measured or scored on herbarium material from our own collections. Two datasets were used in the analyses: (1) a complete dataset that included all plants and all variables (matrix A), and (2) a reduced dataset that included plants attributed *a priori* to *H. chrysostyloides*, *H. decipiens* and *H. vapenicanum* and all variables (matrix B). Data analysis was performed in the following steps:

1. The normality of the distribution of all characters was tested using the Shapiro-Wilks test. Pearson and nonparametric Spearman correlation coefficients were calculated on the matrix of the whole material and on the individual matrices of taxa distinguished *a priori*.
2. Two canonical discriminant analyses (CaDA) were performed based on individual plants and populations as groups. The first one was based on the full dataset (matrix A) and the second one on the reduced dataset (matrix B).

Table 1. List of samples of the *Hieracium nigrescens* group studied. M, number of plants for morphometric analysis; A, number of plants for allozyme analysis, CR, number of plants for chromosome counting (C, counted by J Chrtek; M, counted by P Mráz, counts were originally published in Mráz, 2001); PL, determination of ploidy level by flow cytometry (by A. Krahulcová)

		No of individuals		
Population code	Origin and sampling data	M	A	CR/PL
H. jarzabczynum				
1/1, Jarz 1	Sk: Západné Tatry Mts., Zuberec: Roháčske plesá mountain lakes, 10.5 km south-east of village,1620 m a.s.l., 49°12′30″N, 19°44′08″E, 22.vii.1997 & 7.vii.2000, *J. Chrtek & M. Odvodyová.*	15	10	5/5 (C)
1/2, Jarz 2	Sk: Západné Tatry Mts., Zuberec: Mt. Lúčna, south-west slopes, 11.5 km east-south-east of village, 1400–1620 m a.s.l., 49°13′46″N, 19°45′40″E, 23.vii.1997 & 8.vii.2000, *J. Chrtek.*	15	10	4/6 (C)
1/3, Jarz 3	Sk: Západné Tatry Mts., Pribylina: Mt. Hrubý vrch, western slopes, 10.5 km north of village, 1820 m a.s.l., 49°11′54″N, 19°47′20″E, 25.vii.1998 & 27.vii.2001, *J. Chrtek & D. Vaňková.*	15	10	5/5 (C)
'H. babiagorense'				
2/1, Babiag 1	Sk: Oravské Beskydy Mts., Oravská Polhora: Mt. Babia hora, eastern slopes, 8 km north-west of village,1700 m a.s.l., 49°34′15″N, 19°31′40″E, 23.vii.1998, *J. Chrtek.*	15	10	3/7 (C)
H. decipiens				
3/1, Dec 1	Cz: Krkonoše Mts., Pec pod Sněžkou: Mt. Sněžka, western slopes, 5 km north of village, 1390 m a.s.l., 50°44′15″N, 15°43′45″E, 24.vi.2000, *J. Chrtek.*	15	10	8/2 (C)
3/2, Dec 2	Cz: Krkonoše Mts., Špindlerův Mlýn: Mt. Kotel, 5.5 km west-north-west of village, 1420 m a.s.l., 50°45′05″N, 15°31′45″E, 15.vii.2001, *J. Chrtek & M. Odvodyová.*	15	10	5/5 (C)
3/3, Dec 3	Cz: Krkonoše Mts., Špindlerův Mlýn: Mt. Vysoké kolo, south-east slopes, 6 km south-west of village, 1460 m a.s.l., 50°46′32″N, 15°33′20″E, 4.viii.2000, *J. Chrtek.*	15	10	5/5 (C)
H. chrysostyloides				
4/1, Chrys 1	Cz: Hrubý Jeseník Mts., Karlova Studánka: Petrovy kameny rocks, 5.5 km west of village, 1430 m a.s.l., 50°03′57″N, 17°14′40″E, 4.viii.2000, *J. Chrtek & D. Vaňková.*	15	10*	4/0 (C)
H. vapenicanum				
5/1, Vapen 1	Sk: Nízke Tatry Mts., Hel'pa: Mt. Vel'ká Vápenica, 5.5 km north-east of village, 1690 m a.s.l., 48°54′30″N, 19°59′00″E, 17.vii.2001, *J. Chrtek.*	15	10	6/4 (C)
5/2, Vapen 2	Sk: Západné Tatry Mts., Roháče, Zuberec: mountain ridge between Mt. Osobitá and Mt. Lúčna, 8.5–11 km east-south-east of the village, 1500–1550 m a.s.l., 49°15′12″N, 19°43′50″E–49°14′20″N, 19°45′30″E, 15.vii.1995, 8.vii.2000 & 28.vii.2001, *J. Chrtek.*	15	10	6/4 (C)
5/3, Vapen 3	Sk: Západné Tatry Mts., Roháče, Zuberec: Zábrat' saddle, 10.5 km east-south-east of village, 1650 m a.s.l., 49°13′15″N, 19°44′58″E, 7.vii.2000 & 27.vii.2001, *J. Chrtek.*	15	10	5/5 (C)
H. nigrescens				
6/1, Nigres 1	Cz: Krkonoše Mts., Pec pod Sněžkou: Mt. Sněžka, W slopes, 5 km north of village, 1390 m a.s.l., 50°44′15″N, 15°43′45″E, 17.vii.1997 & 24 June 2000, *J. Chrtek.*	15	10*	5/0 (C)
H. koprovanum				
7/1, Koprov 1	Sk: Západné Tatry Mts., Podbanské: Zadná Tichá dolina valley, 9 km north east of village, 1550 m a.s.l., 49°12′30″N, 19°59′10″E, 24.viii.1997, *P. Mráz (cult. nos. 491, 493, 494, 496).*	–	4	–

Table 1. *Continued*

Population code	Origin and sampling data	No of individuals M	A	CR/PL
7/2, Koprov 2	Sk: Vysoké Tatry Mts., Starý Smokovec: Vel'ká Studená dolina valley, 4.5 km north-north-west of village, 1550 m a.s.l., 49°10′32″N, 20°11′50″E, 13.viii.1996, *P. Mráz (cult. nos. 211, 213, 225).*	–	10 + 3	4/6 (C) 2/0 (M)
7/3, Koprov 3	Sk: Vysoké Tatry Mts., Štrbské Pleso: Važecká dolina valley (Zahandel), 1 km south-east of Mt. Kriváň, 5.5 km north-west of village, 1720 m a.s.l., 49°08′43″N, 20°00′29″E, 16.vii.1998, *P. Mráz & V. Jurkovičová (cult. nos. 517, 519).*	–	2	1/0 (M)
7/4, Koprov 4	Po: Tatry Wschodnie Mts., Łysa Polana: south-east slopes of Mt. Swistowa Czuba, 0.3 km south-east of Mt. Swistowa Czuba, 7.8 km south-west of village, 1840 m a.s.l., 49°12′50″N, 20°03′44″E, 23.vii.1997, *P. Mráz (cult. nos. 462, 464).*	–	2	2/0 (M)
7/5, Koprov 5	Sk: Nízke Tatry Mts., Mýto pod Ďumbierom: Demänovské sedlo saddle, 10 km north of village, 1750 m a.s.l., 48°56′25″N, 19°37′21″E, 15.vii.1996, *P Mráz (cult. no. 13).*	–	1	2/0 (M)

Cz, Czech Republic; Po, Poland; Sk, Slovakia. *see Material and methods.

3. principal component analysis (PCA; Sneath & Sokal, 1973; Krzanowski, 1990) based on a correlation matrix of characters and individual plants as OTUs (operational taxonomic units) was conducted (based on matrix A), to elucidate morphological homogeneity of the taxa recognized *a priori*.
4. Exploratory data analysis (EDA) was performed on the data matrices of the six recognized taxa. Within each group, the mean, standard deviation, and 5% and 95% percentiles were computed for each character.

The numerical analyses were computed using statistical packages SYN-TAX 2000 (Podani, 2001), SAS, v.8 (SAS Institute, 2000) and STATISTICA (StatSoft, 1998).

Allozyme analysis

Young leaves of cultivated plants were used. Plant material was ground in extraction buffer generally according to Kato (1987) with some modifications: 0.1 M Tris-HCl (pH 8.0), 70 mM mercaptoethanol, 26 mM sodium metabisulfite, 11 mM L-ascorbic acid, 4% (w/v) soluble PVP-40, pH-adjusted after the addition of the ascorbate. Crude homogenates were centrifuged for 10 min at 15 000 r.p.m. Clear supernatant was stored in a deep freeze at –75 °C. The PAGE was carried out using separating gel (8.16%) with the buffer 1.82 M Tris-HCl, pH 8.9; the stacking gel (4.0%) with the buffer (0.069 M Tris-HCl, pH 6.9); the electrode buffer was 0.02 M Tris, 0.24 M glycine, pH 8.3. The following enzymes were analysed: AAT (Aspartate aminotransferase, EC 2.6.1.1), LAP (Leucine aminopeptidase, EC 3.4.11.1), PGM (Phosphoglucomutase, EC 5.4.2.2), 6-PGDH (6-phosphogluconate dehydrogenase, EC 1.1.1.44), and SKD (Shikimate dehydrogenase, EC 1.1.1.25).

The staining procedures followed Vallejos (1983) to visualize 6-PGDH and SKD, and Wendel & Weeden (1989) for PGM and EST, with the following modifications: 6-PGDH (0.1 M tris-HCl, pH 8.4, 30 mg 6-phosphogluconic acid), SKD (0.1 M tris-HCl, pH 8.4), colorimetric EST (Na-phosphate buffer pH 6.45, 25 mg β-naphthyl phosphate, 50 mg Fast Blue BB), PGM (24 mg $MgCl_2$, 50 mg glucose-1-phosphate, 10 mg NADP). Visualization of LAP was performed using buffer 0.2 M tris-maleate pH 6. The gel was rinsed with the buffer and then incubated for 10 min in a solution of 30 mL of the buffer, 40 mg L-leucyl-β-naphthylamide, HCl (in 50% acetone) and 60 mg $MgCl_2$. Then 25 mg Fast Black K Salt in 30 mL of the buffer was added. The gel was incubated in the dark until bands appeared. Two staining solutions were prepared for AAT: A (20 mL 0.1 M tris-HCl, pH 8.4, 240 mg aspartic acid, 40 mg α-ketoglutaric acid) and B (20 mL 0.1 M tris-HCl, pH 8.4, 50 mg Fast Blue BB Salt, 50 mg Fast Violet B, 25 mg pyridoxal-5-phosphate). Solution A was prepared at least 15 min before application. The gel was rinsed in water and then in buffer tris-HCl pH 7. Solutions A and B were mixed and poured onto gel. The gel was incubated in the dark at 32 °C until bands appeared. It was then rinsed and fixed (1:1:3:5, glycerine, acetic acid, H_2O, methanol).

As a consequence of the complex 'nonsegregating' banding pattern in our agamospermous taxa, patterns were compared with those produced by closely related

Table 2. Variables used for morphometric analysis of *Hieracium nigrescens* group

Abbreviation	Description
PH	Plant height
NBL	Number of basal leaves
Longest basal leaf (L1), primordial leaves were excluded	
LL1	Length of L1
WL1	Width of L1
MWL1	Position of maximal width of L1 (distance between the widest part and the top)
TLL1	Length of longest tooth on L1
ATLL1	Mean length of three longest teeth on L1
MGL1	Total of teeth and mucronate glands on L1
HUL1	Number of simple eglandular hairs at 0.5 cm^2 of upper leaf surface (L1) (scored in the middle part of the leaf, central vein and margins are not included)
HLL1	Number of simple eglandular hairs at 0.5 cm^2 of lower leaf surface (L1) (scored in the middle part of the leaf, central vein and margins are not included)
Stem leaves	
NSL	Number of non bract-like stem leaves
BSL	Number of bract-like stem leaves
Lowest stem leaf (L2)	
LL2	Length of L2
WL2	Width of L2
TLL2	Length of longest tooth on L2
ATLL2	Mean length of three longest teeth on L2
MGL2	Total of teeth and mucronate glands on L2
Heads, akladium, peduncles	
NH	Number of heads
SEHP	Number of simple eglandular hairs (SEH) on 0.5 cm-long part of peduncle
GHP	Number of glandular hairs (GH) on 0.5 cm-long part of peduncle; microglands (gland usually shorter than 0.1 mm) were not scored
LSEH	Length of SEH on peduncle (mean of 10 consequently inserted SEH)
LGH	Length of GH on peduncle (mean of 10 consequently inserted GH)
LI	Length of involucrum
WB	Width of middle involucral bract (measured in the middle of their length, mean of 3 bracts)
LL	Length of outer ligules (mean of 3 ligules)

sexual *Hieracium alpinum* (Kirschner & Chrtek, unpubl. data). We used an approach indicating no increase in locus number for agamosperms; interpretation was accomplished by assigning bands within a prescribed 'zone' to a locus corresponding to 'zones' occupied by individual loci for sexual species. According to the migration distance, alleles at a particular locus were marked by lowercase letters (a,b…), corresponding to alleles identified in previous studies in *Hieracium* sect. *Alpina* (Mráz, Chrtek & Kirschner, 2001; Štorchová *et al*., 2002).

For each multilocus genotype (genotypes correspond to taxa delimited *a priori*) the average number of alleles per locus (A) and observed heterozygosity (H_O) were calculated. Nei's standard genetic distances between all pairs of multilocus genotypes were calculated using the program FREETREE (Pavlíček, Hrdá & Flégr, 1999). The distance matrix was subjected to principal coordinate analysis (PCoA), using the programs SYN-TAX 2000 (Podani, 2001) and STATISTICA (StatSoft, 1998). Furthermore, Euclidean distances between all pairs of genotypes were calculated. Based on this matrix, UPGMA clustering method was performed, using the STATISTICA (StatSoft, 1998) program.

RESULTS

Chromosomes and mode of reproduction

We report here for the first time the chromosome number for *H. vapenicanum* ($2n = 27$, 17 plants from three populations). In 13 other plants, the triploid ploidy level was determined by flow cytometry. Previously reported chromosome counts (Chrtek, 1994, 1996; Mráz, 2001) were confirmed for *H. decipiens* ($2n = 36$),

H. nigrescens ($2n = 36$), *H. chrysostyloides* ($2n = 45$), *H. koprovanum* ($2n = 36$) and *H. jarzabczynum* ($2n = 36$; the count for '*H. nigrescens s.l.*' in Mráz, 2001: 327 refers to this species as well). We have found no variation in chromosome number within a species.

The percentage of fully developed achenes ranges from 55.8 to 100 in *H. chrysostyloides*, 38.1–90.5 in *H. decipiens*, 66.3–97.1 in *H. jarzabczynum*, 62.5–90.5 in *H. nigrescens*, 39.1–93.8 in *H. vapenicanum* and 74.8–91.4 in '*H. babiagorense*' (Table 3). One-way ANOVA did not show statistically significant differences among species at the $P < 0.05$ level.

Table 3. Percentage of fully developed (black) achenes after emasculation in *Hieracium chrysostyloides*, *H. decipiens*, *H. jarzabczynum*, *H. nigrescens*, *H. vapenicanum* and '*H. babiagorense*'

Taxon	Mean ± SD	Min. – max.
H. chrysostyloides	83.14 ± 15.86	55.8–100
H. decipiens	77.60 ± 15.48	33.1–93.5
H. jarzabczynum	84.94 ± 10.38	66.3–97.1
H. nigrescens	82.96 ± 11.55	62.5–90.5
H. vapenicanum	77.28 ± 16.88	39.1–93.8
'*H. babiagorense*'	81.86 ± 6.20	74.8–91.4

MORPHOLOGICAL ANALYSES

Normality of the distribution of morphological characters and character correlations

All but two (PH and LL1) variables showed certain deviations from the normal distribution in the Shapiro-Wilks test. A very high correlation (exceeding the value 0.95) was found between characters TLL1 and ATLL1 (Spearman $R = 0.98$, Pearson coefficient = 0.98) and TLL2 and ATLL2 (Spearman $R = 0.99$, Pearson coefficient = 0.98). Therefore, characters TLL1 and TLL2 were removed from further analyses.

Discriminant analysis

In CaDA, based on individual plants and populations as groups performed on matrix A (the complete dataset), rather distinct separation of populations attributed *a priori* to *Hieracium jarzabczynum, H. nigrescens* and '*H. babiagorense*' was achieved (Fig. 1). The first canonical axis represents 55% of the variation among the groups, with the variables strongly contributing to the separation of plants along this axis (in descending order): WB, WL1, LL, HUL1, LSEH, GHP, BSL and MGL1. The second canonical axis accounts for 16% of variation among the groups. The variables highly correlated with this axis are: SEHP, LGH, LI, GHP and Ll. Finally, the third axis

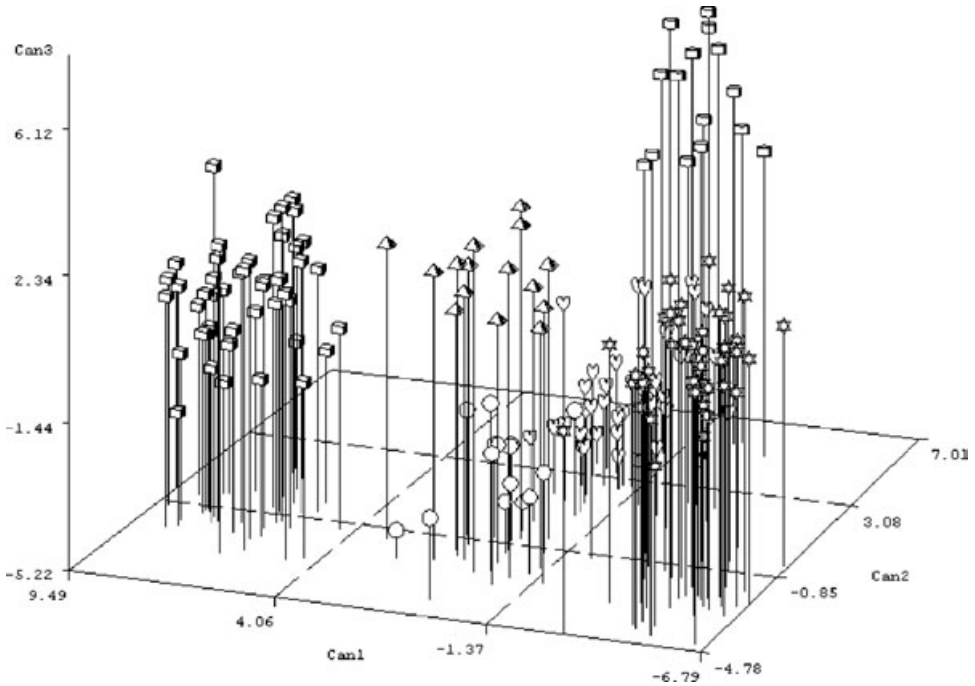

Figure 1. Canonical discriminant analysis based on 23 morphological characters of individuals of *Hiercium chrysostyloides* (○, $N = 15$), *H. decipiens* (▽, $N = 45$), *H. jarzabczynum* (□, $N = 30$), *H. nigrescens* (□, $N = 15$), *H. vapenicanum* (☆, $N = 45$), and '*H. babiagorense*' (△, $N = 15$).

represents 12% of variation, the highly correlated variables with this axis are LSEH and LI (Table 4).

CaDA performed on matrix B (all populations attributed *a priori* to *Hieracium chrysostyloides, H. decipiens* and *H. vapenicanum*) showed a good separation of populations attributed to the particular species (Fig. 2). *Hieracium vapenicanum* is well separated from *Hieracium chrysostyloides* and *H. decipiens* along the first canonical axis (most strongly correlated with LSEH, LGH, WB, LI, LL, WL1 and BSL), which accounts for 61% of variation among the groups. The population of *H. chrysostyloides* is separated along the second axis (most strongly correlated with SEHP, MWL1 and NBL, accounting for 18% of variation among the groups) (Table 5).

Principal component analysis

Two groups corresponding to the *a priori* recognized Carpathian species *H. jarzabczynum* and *H. vapenicanum* were revealed. However, the remaining samples did not show any clear structure, due to within-population variation (not shown).

Exploratory data analysis

The results of the evaluation of the variation of all characters in six taxa recognized on the basis of the previous analyses are given in Table 6. The taxa cannot be distinguished clearly by any single character: the ranges of characters overlap at least among some of the taxa studied. However, a combination of the characters does allow separation. *Hieracium vapenicanum* can be separated from the other taxa (except for a very small overlap with *H. decipiens*) by the width of the involucral bracts, *Hieracium nigrescens* by a very low number of simple eglandular leaves on the peduncles (except for a very small overlap with *H. decipiens*). *Hieracium nigrescens* and *H. jarzybczynum* have deeply dentate basal leaves (TLL1) in comparison with the shallowly dentate ones in *H. chrysostyloides, H. decipiens* and '*H. babiagorense*'.

Allozyme analysis

Five enzyme systems with five allelically interpretable loci (*Skd*, *Lap*-1, *Pgm*-1, *6Pgdh*-2 and *Aat*-2) were observed; allelic interpretation of the remaining loci

Table 4. Total canonical structure (correlation of the characters with canonical axes) obtained in the canonical discriminant analysis of plants *H. chrysostyloides, H. decipiens, H. jarzabczynum, H. nigrescens, H. vapenicanum* and '*H. babiagorense*'. Groups defined as populations

Character	Axis 1	Axis 2	Axis 3
PH	0.313	0.284	–0.094
NBL	–0.125	0.323	0.270
LL1	0.153	0.177	–0.163
WL1	0.766	0.207	0.098
MWL1	0.205	0.016	–0.108
ATLL1	0.439	0.241	0.455
MGL1	–0.504	0.359	0.431
HUL1	–0.652	0.201	–0.027
HLL1	–0.162	–0.088	0.191
NSL	–0.222	–0.046	–0.060
BSL	–0.526	–0.314	0.149
LL2	–0.128	0.020	–0.101
WL2	0.294	0.045	0.123
ATLL2	0.163	0.296	0.413
MGL2	–0.224	0.476	0.109
NH	0.229	0.039	0.074
SEHP	0.325	–0.701	–0.138
GHP	–0.556	0.522	0.079
LSEH	0.651	0.191	–0.607
LGH	0.261	0.664	–0.466
LI	0.232	0.609	–0.536
WB	0.952	0.102	0.003
LL	0.701	0.500	–0.049

Table 5. Total canonical structure (correlation of the characters with canonical axes) obtained in the canonical discriminant analysis of plants *H. chrysostyloides, H. decipiens,* and *H. vapenicanum*. Groups defined as populations

Character	Axis 1	Axis 2	Axis 3
PH	0.378	0.218	0.223
NBL	–0.157	0.626	–0.382
LL1	0.356	–0.305	0.189
WL1	0.701	0.171	0.157
MWL1	0.275	–0.532	0.101
ATLL1	0.061	0.091	0.198
MGL1	–0.324	0.241	0.317
HUL1	–0.218	–0.155	0.009
HLL1	–0.272	–0.071	–0.170
NSL	–0.053	–0.047	0.472
BSL	–0.688	0.236	–0.088
LL2	0.113	–0.255	0.344
WL2	0.203	–0.192	0.374
ATLL2	0.128	–0.219	0.369
MGL2	0.273	0.103	0.541
NH	0.154	–0.098	0.511
SEHP	–0.286	–0.688	0.053
GHP	–0.005	0.493	–0.164
LSEH	0.866	–0.172	–0.101
LGH	0.850	0.252	0.106
LI	0.816	0.156	–0.007
WB	0.843	–0.322	–0.218
LL	0.810	–0.075	0.308

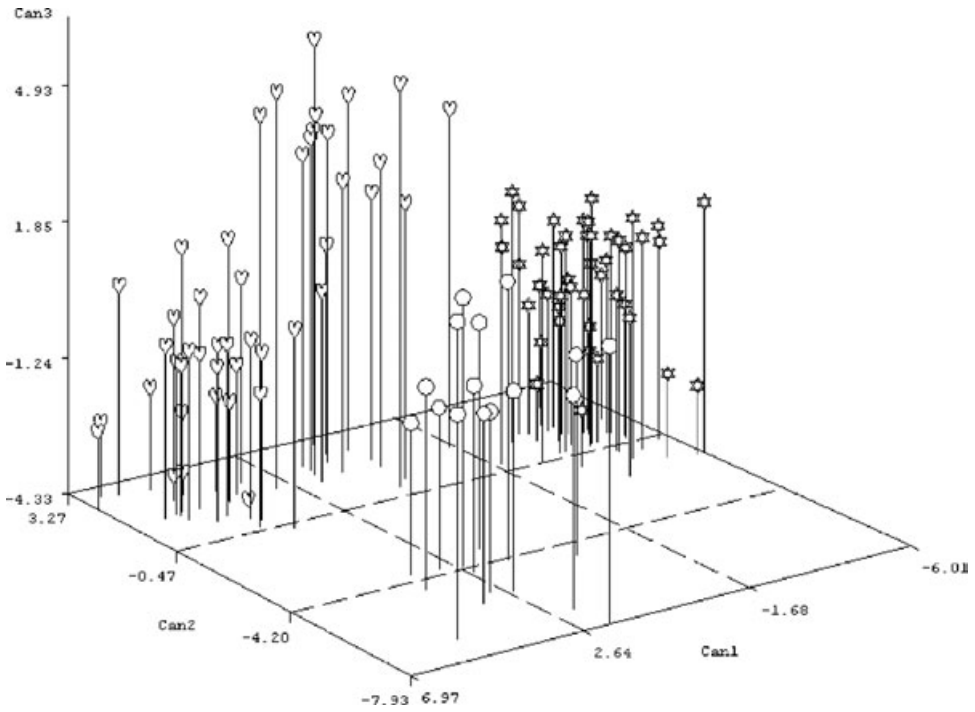

Figure 2. Canonical discriminant analysis based on 23 morphological characters of individuals of *Hieracium chrysostyloides* (♋, $N = 15$), *H. decipiens* (♈, $N = 45$), and *H. vapenicanum* (♎, $N = 45$).

was impossible due to either the complex banding pattern or insufficient staining. Among the interpreted loci, four were found to be polymorphic (*Skd,* *Lap*-1, *Pgm*-1, *6Pgdh*-2), while *Aat*-2 is monomorphic in all populations studied (Table 7). Six multilocus allozyme genotypes were detected corresponding to the taxa defined *a priori* (taxa of the *Hieracium nigrescens* group and *H. koprovanum*). Each taxon consists of a unique genotype, except '*H. babiagorense*' which shares the same genotype with *H. jarzabczynum*. Except for *H. decipiens*, each species has at least one unique allele: *H. nigrescens* (*Pgm*-1d), *H. chrysostyloides* (*Skd*-1d), *H. koprovanum* (*Lap*-1b, *Lap*-1d), *H. jarzabczynum* (*6Pgdh*-2b) and *H. vapenicanum* (*Lap*-1a). Assuming a geographical pattern of the variation, two alleles (*Skd*-1d, *Pgm*-1d) were only found in the Sudetic species, and four alleles (*Lap*-1a, *Lap*-1b, *Lap*-1d, *6Pgdh*-2b) in the Carpathian species. Measures of allelic variation (mean number of alleles per locus, and frequency of observed heterozygotes) are summarized in Table 8.

A dendrogram constructed from the corresponding Euclidean distances (Fig. 3) clearly separates *H. koprovanum*. In the remaining major cluster, two taxa from the Sudety Mountains (*H. decipiens* and *H. chrysostyloides*) are grouped together, and similarly also *H. vapenicanum* (the Carpathians) with *H. nigrescens* (the Sudety Mountains). The PCoA scatterplot based on Nei's standard distances (Fig. 4) shows a group formed by *H. nigrescens* and *H. vapenicanum*, separated along the first coordinate. *Hieracium jarzabczynum* is separated along the second coordinate; the remaining species are only loosely grouped.

DISCUSSION

CHROMOSOME NUMBERS, MODE OF REPRODUCTION

We confirmed the previously reported diversity of ploidy levels in the *H. nigrescens* group. A similar pattern of cytological diversity in the *H. nigrescens* group has been detected in the British Isles (Stace *et al*., 1995; further counts from the British Isles were published by Mills & Stace, 1974 and Morton, 1974). Both triploid and tetraploid counts were reported for *H. nigrescens* (agg.) from the North Ural (Komi Autonomous Republic, north-west Russia; Lavrenko, Serditov & Ulle, 1988; 1990) and from Iceland (Löve, 1970). Recently Schuhwerk & Lippert (1999) published $2n = 27$ for *H. nigrescens* ssp. *cochleare* (Huter) Zahn from the Bavarian Alps.

The percentage of fully developed achenes did not vary substantially among the species. Similar percentages have been reported by Štorchová *et al*. (2002) in several species of the *Hieracium fritzei* group. Empty

Table 6. Summary statistics for characters of '*Hieracium babiagorense*' (Babiag, *n* = 15), *H. chrysostyloides* (Chrys, *n* = 15), *H. decipiens* (Dec, *n* = 45), *H. jarzabczynum* (Jarz, *n* = 60), *H. nigrescens* (Nigres, *n* = 15), and *H. vapenicanum* (Vapen, *n* = 45). Upper line: mean ± SD, lower line 5% and 95% percentiles

	Taxon					
Character	Babiag	Chrys	Dec	Jarz	Nigres	Vapen
PH (cm)	17.80 ± 3.63 12–24	20.03 ± 3.52 16–29	23.38 ± 4.43 17–30	23.91 ± 4.21 18.5–32.5	22.70 ± 3.13 17.5–27.5	18.93 ± 3.34 12.5–24
NBL	4.73 ± 1.28 2–6	2.73 ± 1.22 1–5	5.93 ± 1.64 3–8	5.51 ± 2.01 3–9	7.80 ± 2.31 4–12	6.11 ± 1.58 4–9
LL1 (cm)	7.65 ± 2.18 5.3–11	10.88 ± 2.30 7.5–15	9.22 ± 1.95 6.5–12.0	9.13 ± 2.03 6–12.5	9.80 ± 1.99 5.8–13	7.75 ± 2.14 3.5–10.8
WL1 (cm)	1.35 ± 0.29 1–2	1.27 ± 0.21 0.9–1.6	1.48 ± 0.24 1–1.8	2.18 ± 0.47 1.6–3	1.75 ± 0.38 1.3–2.6	1.01 ± 0.20 0.7–1.3
MWL1 (cm)	2.39 ± 0.58 1.4–3.5	3.43 ± 1.04 2–5	2.38 ± 0.55 1.4–3.3	2.62 ± 0.62 1.7–3.9	2.66 ± 0.79 1.5–4.5	2.16 ± 0.61 1.2–3.3
TLL1 (mm)	2.69 ± 1.27 1–5.5	1.47 ± 1.35 0–5	1.97 ± 1.43 0–5	4.68 ± 2.36 1.7–9	5.57 ± 2.93 1.8–12	1.55 ± 0.97 0–3
ATLL1 (mm)	2.09 ± 0.84 0.9–3.8	1.21 ± 1.18 0–4.4	1.50 ± 1.07 0–3.5	3.44 ± 1.81 0.9–6.3	4.35 ± 1.93 1.2–8.3	1.24 ± 0.78 0–2.3
MGL1	8.73 ± 2.12 5–12	6.33 ± 3.52 0–13	8.09 ± 2.54 4–12	5.31 ± 1.95 3–9	13.80 ± 2.08 10–18	9.16 ± 2.54 5–13
HUL1	4.93 ± 3.39 0–13	16.73 ± 4.99 11–28	13.42 ± 5.04 6–22	3.07 ± 4 0–10	20.07 ± 7.46 10–34	16.84 ± 7.65 8–35
HLL1	16.60 ± 6.37 7–28	15.47 ± 4.69 9–24	13.09 ± 5.49 6–24	13.93 ± 5.98 5–23	17.33 ± 7.67 7–37	17.91 ± 8.73 6–32
NSL	1.13 ± 0.35 1–2	1.73 ± 0.70 1–3	1.64 ± 0.77 1–3	1.27 ± 0.62 0–2	1.33 ± 0.49 1–2	1.51 ± 0.66 0–2
BSL	2.40 ± 0.63 1–3	2.00 ± 0.66 0–3	2.82 ± 0.91 2–4	2.47 ± 0.87 1–4	2.73 ± 0.70 2–4	4.49 ± 1.10 3–6
LL2 (cm)	4.47 ± 1.86 1.5–8.3	7.06 ± 3.36 2–13.5	5.28 ± 2.70 2–11	4.08 ± 2.51 0–8.3	4.99 ± 2.43 2–10	4.46 ± 2.17 0–7.2
WL2 (mm)	7.13 ± 3.18 3–15	8.20 ± 3.85 1.5–14	6.59 ± 3.54 2–12	9.05 ± 5.82 0–21	8.13 ± 5.60 2–16	4.90 ± 2.39 0–9
TLL2 (mm)	1.29 ± 1.25 0–3.5	1.09 ± 1.57 0–5.5	0.58 ± 0.97 0–2.5	1.71 ± 2.39 0–6.5	3.95 ± 3.48 0–11	0.29 ± 0.77 0–2
ATLL2 (mm)	1 ± 1.04 0–3.3	0.93 ± 1.32 0–4.4	0.46 ± 0.76 0–2	1.18 ± 1.75 0–5	3.14 ± 2.78 0–8.2	0.19 ± 0.50 0–1.3
MGL2	3.33 ± 3.09 0–10	3.87 ± 3.52 0–11	5.00 ± 3.13 0–10	1.67 ± 1.61 0–4	7.80 ± 4.40 0–14	2.22 ± 2.52 0–7
NH	1 ± 0 1–1	1.33 ± 0.49 1–2	1.22 ± 0.56 1–3	1.44 ± 0.87 1–3	1.20 ± 0.41 1–2	1 1
SEHP	42.07 ± 11.17 19–56	54.07 ± 13.10 31–72	21.29 ± 9.43 9–37	46.13 ± 13.87 24–66	4.47 ± 3.52 0–12	36.67 ± 16.08 15–61
GHP	138.40 ± 21.61 103–171	125.07 ± 22.58 93–166	191.80 ± 49.85 117–271	112.18 ± 24.83 79–156	247.47 ± 49.58 183–365	182.67 ± 33.10 133–231
LSEH (mm)	1.32 ± 0.18 1.05–1.67	2.41 ± 0.50 1.65–3.69	2.26 ± 0.44 1.41–2.73	2.49 ± 0.38 1.89–3.09	1.55 ± 0.18 1.28–1.86	1.34 ± 0.22 1.01–1.78
LGH (mm)	0.38 ± 0.05 0.33–0.50	0.44 ± 0.07 0.32–0.57	0.54 ± 0.07 0.42–0.65	0.44 ± 0.05 0.37–0.54	0.47 ± 0.05 0.36–0.56	0.34 ± 0.03 0.3–0.39
LI (mm)	13.27 ± 0.96 12–15	14.63 ± 1.01 13–16	15.98 ± 1.59 13–18	14.36 ± 1.03 13–16	14.80 ± 0.68 14–16	12.69 ± 1.13 11–15
WB (mm)	1.19 ± 0.16 1–1.5	1.23 ± 0.09 1.1–1.4	1.09 ± 0.17 0.8–1.3	1.65 ± 0.13 1.4–1.8	1.20 ± 0.12 1–1.5	0.82 ± 0.08 0.7–0.9
LL (mm)	15.97 ± 0.93 15–18	16.55 ± 1.01 14–18	16.69 ± 1.59 14.7–19.3	18.63 ± 1.91 16.3–22	18.73 ± 1.55 16–20.7	12.19 ± 1.77 9.7–15.7

Table 7. Allelic frequencies for all scored loci for *Hieracium chrysostyloides*, *H. decipiens*, *H. jarzabczynum*, *H. koprovanum*, *H. nigrescens* and *H. vapenicanum*. Allelic frequencies for *'H. babiagorense'* are the same as for *H. jarzabczynum*

	Skd-1				*Lap*-1				*Pgm*-1			*Aat*-2		6-*Pgdh*-2		
Species	a	b	c	d	a	b	c	d	b	c	d	a	c	a	b	c
H. chrysostyloides	1	1	0	1	0	0	1	0	1	1	0	1	1	1	0	1
H. decipiens	1	0	0	0	0	0	1	0	1	1	0	1	1	1	0	1
H. jarzabczynum	1	0	1	0	0	0	1	0	1	1	0	1	1	0	1	1
H. koprovanum	1	1	1	0	0	1	1	1	1	1	0	1	1	1	0	1
H. nigrescens	1	0	1	0	0	0	1	0	1	0	1	1	1	1	0	1
H. vapenicanum	1	0	1	0	1	0	1	0	1	0	0	1	1	1	0	1

Table 8. Measures of allelic variation of *Hieracium chrysostyloides*, *H. decipiens*, *H. jarzabczynum*, *H. koprovanum*, *H. nigrescens*, *H. vapenicanum* and *'H. babiagorense'* (each species consists of unique multilocus allozyme genotype, *'H. babiagorense'* shares the same one with *H. jarzabczynum*). *N*, number of plants, *A*, mean number of alleles per locus, H_o, observed frequency of heterozygotes

	N	*A*	H_o
H. chrysostyloides	10	2.0	0.8
H. decipiens	30	1.6	0.6
H. jarzabczynum + *'H. babiagorense'*	40	1.8	0.8
H. koprovanum	22	2.2	1.0
H. nigrescens	10	1.8	0.8
H. vapenicanum	30	1.8	0.8

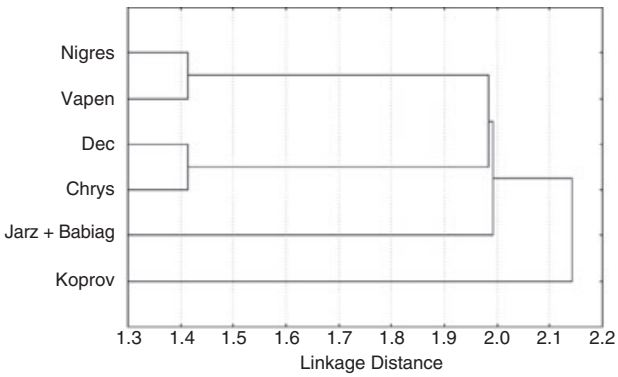

Figure 3. Cluster analysis, UPGMA method, of Euclidean distances between *Hieracium chrysostyloides* (Chrys), *H. decipiens* (Dec), *H. jarzabczynum* (Jarz), *H. koprovanum* (Koprov), *H. nigrescens* (Nigres), *H. vapenicanum* (Vapen) and *'H. babiagorense'* (Babiag) using four polymorphic allozyme loci (each species consists of one unique multilocus allozyme genotype).

achenes can be explained in several ways. Besides the possibility of meiotic development of the archesporial cell and a lack of fertilization, the empty achenes are much more likely explained by environmental conditions or the improper cutting of the upper part of the flowering buds.

GENETIC DIVERSITY

We have found no allozyme variation within species distinguished *a priori*: each consists of one unique allozyme genotype. '*Hieracium babiagorense*' shares the same allozyme genotype with *H. jarzabczynum*. However, we are aware that more sensitive methods/markers might reveal genetic differences. Thus, the morphologically distinguishable taxa (treated here at the species rank) represent single genotypes fixed through agamospermy, which became rather widespread or at least scattered in the Sudety Mountains and Western Carpathians. Except for *H. decipiens*, each species has at least one unique allele; two alleles were found to be unique to the Sudety Mountains and four to the Carpathians.

Genetic homogeneity seems to be a case of many *Hieracium* species (in the narow sense), mostly of those supposed to be of hybrid origin (Shi *et al*., 1996; Stace, Gornall & Shi, 1997; Gornall, 1999; Mráz *et al*., 2001; Štorchová *et al*., 2002). On the other hand, considerable genetic variation was found in nonhybrid 'basic' species *Hieracium alpinum* (triploid, agamospermous) in the Carpathians (Štorchová *et al*., 2002), the Krkonoše Mountains (the Sudety Mountains; Chrtek & Plačková, 2005), the Alps and the British Isles (Shi *et al*., 1996; Stace *et al*., 1997). Some level of genetic variation has also been found in several hybrid species of *Hieracium* sect. *Alpina* (Shi *et al*., 1996; Mráz *et al*., 2001; Štorchová *et al*., 2002) and in the progeny of three endemic *Hieracium* microspecies from Wales (Lledó & Rich, 2004).

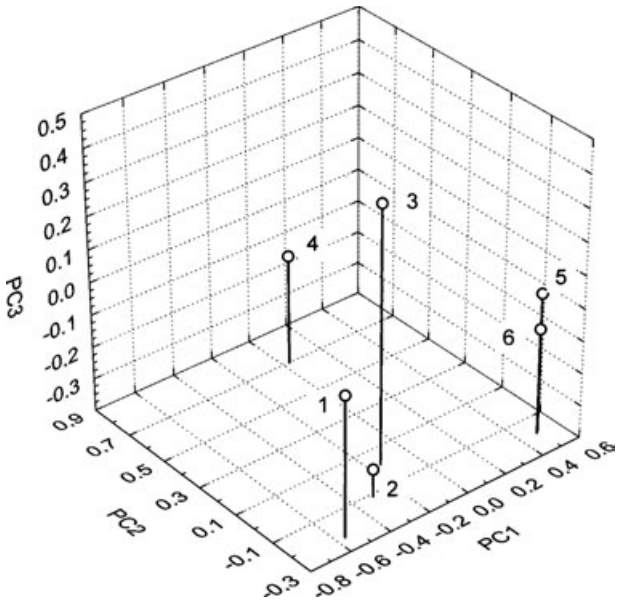

Figure 4. Principal coordinate analysis of allozyme data of *H. chrysostyloides* (3), *H. decipiens* (2), *H. jarzabczynum* and *'H. babiagorense'* (4, they share the same multilocus allozyme genotype), *H. koprovanum* (5), *H. nigrescens* (1) and *H. vapenicanum* (6). The first three coordinates explaining 43.42%, 29.65% and 12.50% of variation, respectively. Each species consists of one unique multilocus allozyme genotype, *'H. babiagorense'* shares the same one with *H. jarzabczynum*.

MORPHOLOGICAL ANALYSIS

Based on the results of CaDA computed on a complete dataset (matrix A), it is apparent that morphological differences between *Hieracium jarzabczynum, H. nigrescens* and '*H. babiagorense*' are sufficient to justify their separation into distinct taxa. *Hieracium vapenicanum* is also rather well separated along the first canonical axis. A second, more detailed, CaDA performed on a reduced dataset (matrix B, *H. chrysostyloides, H. decipiens, H. vapenicanum*) showed good separation of these taxa. Considering the geographical distribution of the target taxa, all taxa from the Western Carpathians (*H. jarzabczynum, H. vapenicanum* and '*H. babiagorense*') are well separated from each other. However, '*H. babiagorense*' fell rather close to a couple of Sudeten taxa, i.e. *H. chrysostyloides* and *H. decipiens*. *Hieracium nigrescens*, the third Sudeten taxon, seems to be well separated from both the Carpathian and remaining Sudetic taxa.

In the PCA of individual plants, the *a priori* recognized Carpathian taxa *H. jarzabczynum* and *H. vapenicanum* are rather separated from each other. The remaining samples belonging to *H. chrysostyloides, H. decipiens, H. nigrescens* and '*H. babiagorense*' did not show a clear structure. Partly separated along the second axis is *H. nigrescens*, which differs from *H. chrysostyloides, H. decipiens* and '*H. babiagorense*' first of all by the indumentum of the peduncles (only a few simple eglandular hairs in *H. nigrescens*, rather numerous in the remaining taxa; see Table 6), and the base of basal leaves (more or less truncate in *H. nigrescens*, cuneate in the remaining taxa). The lack of separation of some taxa in the PCA analysis reflects their overall similarity, while differences appear only in a few characters.

Apart from the characters used in the present morphometric studies, the colour of the styles and stigmas can be used for the reliable determination of some species. Yellow styles and stigmas were consistently observed in *H. chrysostyloides* and *H. vapenicanum*. In the rest of the species, brownish to dark styles and stigmas were observed (Chrtek, unpubl. data).

TAXONOMIC IMPLICATIONS

The combined morphological and allozyme study confirmed our preliminary concept of the six *a priori* recognized taxa treated here (except from '*H. babiagorense*', see below) at the species level. In the Sudety Mountains, the present study more or less confirmed the previously proposed taxonomic concepts (Zlatník, 1938; Zahn, 1936–38). In contrast, the taxonomic treatment of the group in the Western Carpathians deviates from previously proposed concepts (Zahn, 1936–38).

The Sudety Mountains. The three species treated in the present paper are recognized in this area, i.e. *Hieracium chrysostyloides, H. decipiens* and *H. nigrescens*. Two other species *H. apiculatum* and *H. nivimontis* have previously been recognized in this area (and are accepted by the present authors); thus, five species of the *Hieracium nigrescens* group are recognized in the Sudety Mountains.

The Western Carpathians. The two well-separated species, *Hieracium jarzabczynum* and *H. vapenicanum*, with rather overlapping geographical areas (the core of their distribution is in the Západné Tatry Mountains) can be recognized. A morphologically distinct population from Mount Babia hora/Babia Góra (named here '*H. babiagorense*') needs further study: we have refrained here from its formal taxonomic recognition.

All but one ('*H. babiagorense*') taxa clearly differ from each other in their multilocus allozyme genotypes (each consisting of one genotype/clone only). '*Hieracium babiagorense*' shares the same genotype with *H. jarzabczynum* and thus the nature of the morphological differences remains a puzzle that needs fur-

ther detailed examination. Based on the morphology, it is more or less grouped with the Sudeten taxa *Hieracium decipiens* and *H. chrysostyloides* (see above), which is also reflected in Zahn's monographs, where the plants from Mount Babia hora/Babia Góra refer to *H. nigrescens* ssp. *decipiens*. Geographically, Mount Babia hora/Babia Góra is situated between the highest parts of the Western Carpathians (where both *H. jarzabczynum* and *H. vapenicanum* occur) and the Sudety Mountains (with *H. decipiens, H. chrysostyloides* and *H. nigrescens*).

Differences in ploidy level support the separation of *H. chrysostyloides* ($2n = 45$) and *H. decipiens* ($2n = 36$). Generally, tetraploids are the most common ploidy level within the *Hieracium nigrescens* group. The chromosome number $2n = 3x = 27$ found in *H. vapenicanum* is rather rare in the group in Central Europe and contributes to the separation of this taxon.

Further differences were detected in pollen production and the size of pollen grains (J. Chrtek and P. Mráz, unpubl. data). The high production of pollen grains of a homogeneous size was found in *H. nigrescens* and *H. decipiens*, while rather small amounts of pollen of heterogeneous size were found in *H. chrysostyloides* and *H. jarzabczynum*; *H. vapenicanum* lacks any pollen.

Based on our results, two new combinations are proposed.

HIERACIUM JARZABCZYNUM (PAWŁ. & ZAHN) MRÁZ & CHRTEK F., **COMB. & STAT. NOV.**

≡*Hieracium pietroszense* ssp. *jarzabczynum* [*jarząbczynum*] Pawł. & Zahn in Zahn, Bul. Acad. Polon., Cl. Sci. Math. Nat., Sér. B, Sci. Nat. (Botanique) 1928: 210, 1929.

HIERACIUM VAPENICANUM (LENGYEL & ZAHN) CHRTEK F. & MRÁZ, **COMB. & STAT. NOV.**

≡*Hieracium nigrescens* ssp. *brachytrichellum* var. [γ] *vapenicanum* Lengyel & Zahn in Zahn, Magyar Bot. Lapok, 25 (1926): 369, 1927.

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REFERENCES

Chrtek J Jr, Mráz P, Severa M. 2004. Chromosome numbers in selected species of *Hieracium* s.str. (*Hieracium* subgen. *Hieracium*) in the Western Carpathians. *Preslia* **76:** 119–139.

Chrtek J Jr, Plačková I. 2005. Genetic variation in *Hieracium alpinum* (Asteraceae) in the Krkonoše Mts. (West Sudeten Mts., Czech Republic). *Biologia, Bratislava* **60:** 387–391.

Chrtek J Jr, 1994. Chromosome numbers in selected *Hieracium* species in the Krkonoše Mts. (the West Sudeten). *Folia Geobotanica & Phytotaxonomica* **29:** 91–100.

Chrtek J Jr, 1995. Notes on *Hieracium alpinum* and *Hieracium nigrescens* (section *Alpina* Fries) in the Eastern Sudeten (Mt. Králický Sněžník, the Hrubý Jeseník Mts.). *Preslia* **67:** 97–106.

Chrtek J Jr, 1996. Chromosome numbers in selected species of *Hieracium* (Compositae) in the Sudeten Mts. & the Western and Ukrainian Eastern Carpathians. *Fragmenta Floristica et Geobotanica* **41:** 783–790.

Chrtek J Jr, 1997. *Hieracium decipientiforme* (the *H. nigrescens* group) – an interesting species of the Ukrainian Carpathians. *Preslia* **69:** 121–128.

Dyer AF. 1963. The use of lacto-propionic orcein in rapid squash methods for chromosome preparations. *Stain Technology* **38:** 85–90.

Elfstrand M. 1893. *Hieracia Alpina aus den Hochgebirgsgegenden des Mittleren Skandinaviens*. Uppsala: Almquist & Wiksells.

Elfstrand M. 1894. Archieracien aus Norwegisch-Finnmarken etc. *Kongliga Svenska Vetenskapsacademiens Handligar* **20**, **III** (1).

Gornall RJ. 1999. Population genetic structure in agamospermous plants. In: Hollingsworth PM, Bateman RM, Gornall RJ, eds. *Molecular systematics and plant evolution*. London: Taylor & Francis, 118–138.

Hultén E, Fries M. 1986. *Atlas of North European vascular plants II*. Königstein: Koeltz Scientific Books.

Kato T. 1987. Hybridization between *Dianthus superbus* var. *longicalycinus* and *D. shinanensis* evidenced by resolvable esterase isozymes from herbarium specimens. *Annals of the Tsukuba Botanical Garden* **6:** 9–18.

Krahulcová A, Papoušková S, Krahulec F. 2004. Reproduction mode in the allopolyploid facultatively apomictic hawkweed *Hieracium rubrum* (Asteraceae, *H.* subgen. *Pilosella*). *Hereditas* **141:** 19–30.

Krzanowski WJ. 1990. *Principles of multivariate analysis*. Oxford: Clarendon Press.

Lavrenko AN, Serditov NP, Ulle ZG. 1988. Chisla khromosom nekorykh vidov semeistv Asparagaceae, Asteraceae i Ranunculaceae s Severnogo Urala (Komi ASSSR) [Chromosome numbers in some species of Asparagaceae, Asteraceae and Ranunculaceae from the North Ural (Komi ASSR)]. *Botanicheskii Zhurnal* **73:** 605–607.

Lavrenko AN, Serditov NP, Ulle ZG. 1990. Chisla khromosom nekorykh vidov tsvetkovykh rastenii Urala (Komi ASSSR) [Chromosome numbers in some species of flowering plants of the Urals (Komi ASSR)]. *Botanicheskii Zhurnal* **75:** 1622–1624.

Lledó MD, Rich TCG. 2004. Genetic variation in progenies of three *Hieracium* microspecies endemic from Wales (United Kingdom). *Thaiszia* **14** (Suppl. 1)**:** 11–12.

Löve Á. 1970. *Íslenzk Ferðaflóra [Flora of Iceland]*. Reykjavík: Almenna Bókafélagid.

Merxmüller H. 1975. Diploide Hieracien. *Anales del Instituto Botaníco Cavanilles* **32:** 189–196.

Mills JN, Stace CA. 1974. Chromosome numbers of British plants, 2. *Watsonia* **10:** 167–168.

Morton JK. 1974. Chromosome numbers of British plants, 2. *Watsonia* **10:** 169.

Mráz P. 2001. Chromosome numbers in selected species of *Hieracium* sect. *Alpina* (Asteraceae) from Central and Eastern Europe. *Folia Geobotanica* **36:** 321–332.

Mráz P, Chrtek J Jr, Kirschner J. 2001. Genetic variation in the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*). *Phyton (Horn)* **41:** 269–276.

von Nägeli C, Peter A. 1885. *Die Hieracien Mittel-Europas. Monographische Bearbeitung der Piloselloiden mit besonderer Berücksichtigung der mitteleuropäischen Sippen.* München: R. Oldenbourg.

Norrlin JP. 1912. Nya nordiska Hieracia, II. [New Nordic Hieracia]. *Acta Societatia Pro Fauna et Flora Fennica* **36** (4)**:** 1–127.

Omang SOF. 1928. Hieracia alpina fra det nordlige Jemtland, samt fra Asele og Lycksele Lappmarker. *Arkiv for Botanik* **22A:** 1–31.

Pavlíček A, Hrdá S, Flégr J. 1999. Free-Tree freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia*. *Folia Biologica* **45:** 97–99.

Podani J. 2001. *SYN-TAX 2000. Computer programs for data analysis in ecology and systematics. User's manual*. Budapest: Scientia Publishing.

Pugsley HW. 1948. A prodromus of the British Hieracia. *Journal of the Linnean Society. Botany* **54**.

SAS Institute. 2000. *SAS OnlineDoc®*, v.8. *(available online)*. Cary, NC: SAS Institute.

Schuhwerk F. 1996. *Published chromosome counts in* Hieracium. *http://www.botanik.biologie.uni-muenchen.de/botsamml/projects/chrzlit.html.*

Schuhwerk F, Lippert W. 1999. Chromosomenzahlen von *Hieracium* (Compositae, Lactucaeae) Teil 3. *Sendtnera* **6:** 197–214.

Sell PD. 1987. An introduction to the study of the British Hieracia, 1. History and classification. *Watsonia* **16:** 365–371.

Sell PD, West C. 1976. *Hieracium* L. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europaea* **4:** 358–410. Cambridge, London: Cambridge University Press.

Sell PD, West C, Tennant D. 1995. Eleven new British species of *Hieracium* L. sect. *Alpina* (Fries) F. N. Williams. *Watsonia* **20:** 351–365.

Shi Y, Gornall RJ, Draper J, Stace CA. 1996. Intraspecific molecular variation in *Hieracium* sect. *Alpina* (Asteraceae), an apomictic group. *Folia Geobotanica & Phytotaxonomica* **31:** 305–313.

Shlyakov RN. 1966. Rod 355. Yastrebinka – *Hieracium* L. In: Klokov MV, Kuzeneva OI, Linchevskyi IA, Orlova NI, Poyarkova AI, Semenova-Tyan-Shanskaya NZ, Chernov EG, Shlyakov RN, eds. *Flora Murmanskoi oblasti [Flora of the Murmansk area] 5*. Moskva: Nauka, 268–460.

Shlyakov RN. 1989. Rod 32. Yastrebinka – *Hieracium* L. In: Tsvelev NN, ed. *Flora Evropeiskoi chasti SSSR [Flora of the European part of the U.S.S.R.] 8*. Leningrad: Nauka, 140–300.

Sneath PHA, Sokal RR. 1973. *Numerical taxonomy. principles and practice of numerical classification*. San Francisco: W.H. Freeman.

Stace CA, Gornall RJ, Shi Y. 1997. Cytological and molecular variation in apomictic *Hieracium* sect. *Alpina*. *Opera Botanica* **132:** 39–51.

Stace CA, Gornall RJ, Squirrell J, Shi Y. 1995. Chromosome numbers in *Hieracium* L. section *Alpina* (Fries) F. N. Williams. *Watsonia* **20:** 367–377.

StatSoft Inc. 1998. *STATISTICA for Windows*. Tulsa, OK: StatSoft, Inc.

Štorchová H, Chrtek J Jr, Bartish IV, Tetera M, Kirschner J, Štěpánek J. 2002. Genetic variation in agamospermous taxa of *Hieracium* sect. *Alpina* (Compositae) in the Tatry Mts. (Slovakia). *Plant Systematics and Evolution* **235:** 1–17.

Üksip AJ. 1960. *Hieracium* L. In: Shishkin BK, Bobrov EG, eds. *Flora SSSR [Flora of U.S.S.R.] 30.* Moskva: Izdatel'stvo Akademii Nauk SSSR.

Vallejos CE. 1983. Enzyme activity staining. In: Tanksley SD, Orton TJ, eds. *Isozymes in plant genetics and breeding, part A.* Amsterdam: Elsevier, 469–516.

Wendel JF, Weeden NF. 1989. Genetics of plant isozymes. In: Soltis DE, Soltis PS, eds. *Isozymes in plant biology*. Portland, OR: Dioscorides Press, 5–45.

Zahn KH. 1921–23. Compositae – *Hieracium*. In: Engler A, ed. *Das Pflanzenreich. IV/280.* Leipzig: Wilhelm Engelmann.

Zahn KH. 1936–38. *Hieracium* 3. In: Graebner P fil., eds. *Synopsis der mitteleuropäischen Flora 12.* Berlin: Gebrüder Borntraeger, 1–708.

Zlatník A. 1938. Hieracia Alpina Sudetorum Occidentalium. *Studia Botanica Čechoslovaca* **1:** 37–51, 105–242.

Zlatník A. 1939. Additamentum ad 'Hieracia Alpina Sudetorum Occidentalium'. *Studia Botanica Čechica* **2:** 64–96.