

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

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Communicating Editor: Chrissen Gemmill

**Abstract**—The present paper provides a large-scale taxonomic revision of *Picris hieracioides*, a highly polymorphic and taxonomically controversial species in Europe. Altogether, 104 populations were sampled and examined using multivariate morphometrics and genetic amplified fragment length polymorphism (AFLP) data. Two morphotypes are delimited, the ‘higher altitude’ and the ‘lower altitude’ morphotypes, which are congruent with the two main genetic groupings revealed by AFLP markers. It is concluded that two subspecies should be recognized within this species, *P. hieracioides* subsp. *hieracioides*, comprising annual to biennial plants occupying dry, sunny, often man-made habitats at low altitudes, and *P. hieracioides* subsp. *umbellata*, being mostly a short-lived perennial that grows in mesic, semi-natural or natural habitats at higher altitudes. This infraspecific treatment strongly contrasts with the traditional taxonomic concepts, which recognize up to 10 subspecies of *P. hieracioides* in Europe. An identification key and a nomenclatural account are presented, including the designation of lectotypes. *Picris hieracioides* harbors large genetic variation, and two lineages can be recognized within each subspecies, most likely reflecting their glacial survival and postglacial colonization routes. Long-distance dispersals, anthropogenic introductions and recent spread are suggested to have shaped their genetic structure as well. A Balkan endemic *P. hispiddissima*, although morphologically and ecologically well defined, appears to be genetically close to *P. hieracioides*.

**Keywords**—AFLPs, Asteraceae, infraspecific variation, multivariate morphometrics, taxonomy.

Polymorphic plant species with large intraspecific variation and complicated evolutionary histories are undoubtedly a challenge for taxonomic research. Because of the ambiguity of the variation patterns observed within such species (e.g. continual morphological variation, discrepancies between genetic and morphological data), sound taxonomic concepts are difficult to establish, and these taxa are traditionally surrounded by taxonomic controversies. In recent decades, however, significant advances have been made in species-level systematics, mainly due to the large-scale sampling of material and comparative approaches employing several methods and techniques simultaneously (e.g. morphometric, molecular, karyological, or genome size data). These data, along with the detailed knowledge on distribution patterns, ecological requirements and mating systems, facilitate the delimitation of closely related taxa and help to unravel complex patterns and species histories (e.g. Jørgensen et al. 2006; Pimentel and Sahuquillo 2008; Smith and Waterway 2008; Schönswetter et al. 2009; Bardy et al. 2010, 2011).

The genus *Picris* L. (Compositae, Lactuceae) comprises approximately 40–50 species distributed in Eurasia, Africa, Australia, and New Zealand, with putative evolutionary centers in the Mediterranean Basin and Asia Minor (Holzapfel and Lack 1993; Holzapfel 1994; Chaudhary 2000; Greuter 2006–2009). *Picris* includes several taxonomically critical groups of closely related species, as well as polymorphic species with complex intraspecific variation. *Picris hieracioides* L. is certainly one of the taxonomically most critical and highly polymorphic species of this genus in Europe. It is widespread across Europe, extending to Asia, and has been introduced to North America, southern Africa, and Australia (Lack 1974, 1979; Holzapfel 1994; McMullen 2002). Its exact distribution range and morphological variation in Asia, however, are still poorly known, as they seem to be complicated by the sympatric occurrence of two morphologically similar and often confused taxa, *P. japonica* Thunb. and *P. nuristanica* Bornm. (Lack 1974, 1979).

Extensive morphological variation (probably including large phenotypic plasticity), a broad distribution area and ecological amplitude (from Mediterranean xerothermous, lowland habitats to alpine mesic communities) of *P. hieracioides* have been the main factors hampering a sound intraspecific concept to date. Linnaeus (1753) originally described *Picris hieracioides*; since that time, a number of taxa, both at the species and intraspecific levels, have been described. Presently, the complex is usually treated as a single species, but several highly controversial intraspecific treatments can be found in European Floras and in checklists, with a total of ten subspecies recognized (see Table 1). The peduncle length, the size and color of the involucre and the involucreal indument are among the characters most commonly used to distinguish these subspecies. A critical evaluation of the species variation across a large geographic area and using advanced taxonomic tools, however, is still missing. In the recent morphometric investigation of populations from the western Carpathians and adjacent Pannonia, two morphological types were recognized and informally named as the ‘lower altitude’ (LA) and ‘higher altitude’ (HA) types (Slovák and Marhold 2007). The two morphotypes apparently differ in their ecological preferences and life forms: the former (LA) is usually annual to biennial, occupies dry, sunny, often man-made habitats in lowlands (or at low altitudes in mountains), whereas the latter (HA) is often a short-lived perennial, occurring predominantly in mesic, semi-natural or natural habitats (such as tall herb meadows) at higher altitudes (Slovák and Marhold 2007).

A recently undertaken karyological survey of numerous populations sampled across Europe showed that *P. hieracioides* is strictly diploid with  $2n = 10$  (Slovák et al. 2008). However, flow-cytometric screening of 54 populations revealed significant heterogeneity in the genome size. Approximately 1.37-fold variation in  $2C$  values was found in the material examined, suggesting also some geographic patterns. It was hypothesized that the high intraspecific variation in nuclear DNA amounts may reflect the taxonomic heterogeneity and

TABLE 1. Intraspecific taxonomic concepts of *Picris hieracioides* in recent European literature. References are accompanied by the area covered (in parentheses). \* only the European part of Russia is considered here. If specified in the particular reference, areas reported for the individual subspecies are also indicated: A – Alps, AP – Apennine Peninsula, BP – Balkan Peninsula, CE – Central Europe, EE – eastern Europe, IP – Iberian Peninsula, M – Mediterranean Basin, P – Pyrenees, SE – southern Europe, WA – the whole area of the species, WC – Western Carpathians, WE – western Europe, SEm – mountain regions of southern Europe.

Source	Intraspecific Taxa Recognized Within <i>Picris hieracioides</i>					
Sell 1975, 1976 (Europe)	subsp. <i>grandiflora</i> CE, BP, AP	subsp. <i>hieracioides</i> WA	subsp. <i>longifolia</i> IP	subsp. <i>spinulosa</i> CE, SE	subsp. <i>villarsii</i> CE, WE	
Hayek 1979 (Central Europe)	subsp. <i>auriculata</i> A, WE	subsp. <i>hieracioides</i> WA	subsp. <i>paleacea</i> A	subsp. <i>sonchoides</i> A	subsp. <i>spinulosa</i> M	
Pignatti 1982 (Italy)	subsp. <i>auriculata</i>	subsp. <i>crepoides</i>	subsp. <i>paleacea</i>	subsp. <i>spinulosa</i>		
Geltman 1989 (Russia)*	subsp. <i>hieracioides</i>					
Dostál and Červenka 1992 (former Czechoslovakia)	subsp. <i>grandiflora</i>	subsp. <i>crepoides</i> WC	subsp. <i>hieracioides</i> WA	subsp. <i>paleacea</i> WC	subsp. <i>spinulosa</i> M	
Bolós and Vigo 1995 (Catalonia)	subsp. <i>hieracioides</i> WA	subsp. <i>longifolia</i> IP	subsp. <i>riellii</i> P	subsp. <i>spinulosa</i> M		
Kerguélen 1999 (France)	subsp. <i>hieracioides</i>	subsp. <i>spinulosa</i>	subsp. <i>villarsii</i>			
Haeupler and Muer 2000 (Germany)	subsp. <i>auriculata</i>	subsp. <i>hieracioides</i>	subsp. <i>villarsii</i>			
Rothmaler 2002 (Germany)	subsp. <i>grandiflora</i> CE, EE, WE	subsp. <i>hieracioides</i> WA	subsp. <i>spinulosa</i> SE, M	subsp. <i>villarsii</i> CE, EE, WE		
Štěpánek 2004 (Czech Republic)	subsp. <i>hieracioides</i>					
Aeschmann et al. 2004 (Alps)	subsp. <i>grandiflora</i> SEm	subsp. <i>hieracioides</i> WA	subsp. <i>spinulosa</i> M	subsp. <i>villarsii</i> SEm		
Greuter 2006–2009 (Europe)	subsp. <i>grandiflora</i> AP, BP, CE, WE	subsp. <i>hieracioides</i> WA	subsp. <i>longifolia</i> IP	subsp. <i>riellii</i> IP+France	subsp. <i>spinulosa</i> CE, M, WE	subsp. <i>villarsii</i> AP, BP, CE, IP, WE

the complex evolutionary history of this species (Slovák et al. 2009a). The study also called for further investigations with a multi-method approach. Reproduction modes also play a significant role in shaping the intraspecific variation. The previously reported apomictic seed formation in *P. hieracioides* (Bergman 1935) was recently rejected, and it was shown that the species is strictly allogamous. *Picris japonica* and *P. nuristanica*, on the other hand, display a certain level of selfing (Slovák et al. 2008).

This study presents a large-scale taxonomic revision of *P. hieracioides* in Europe by employing genetic amplified fragment length polymorphism (AFLP) and morphometric data. The main objectives addressed here are as follows: (1) to examine the patterns of morphological variation in field-sampled populations; (2) to investigate whether morphological differences, potentially diagnostic for field-collected samples, retain stability after cultivation under similar environmental conditions; (3) to explore genetic variation patterns and relate them to the observed morphological groups; and (4) to suggest a taxonomic treatment of *P. hieracioides* reflecting these inferred patterns and relationships. With a restricted sampling of its close relatives, *P. japonica*, *P. nuristanica*, and *P. hispidissima* (Bartl.) W. D. J. Koch (a west Balkan endemic, recently proven to be ecologically and morphologically well-differentiated from *P. hieracioides*; Slovák et al. 2009b), we also aimed to describe the morphological and genetic delimitation of *P. hieracioides* from these related, and often confused, species. The combination of a multivariate morphometric approach with molecular fingerprinting (AFLPs) employed here has proven efficient in several other cases of complex intraspecific patterns, typical for recently diverged taxa (e.g. Lihová et al. 2003; Kučera et al. 2006; Mereda et al. 2008; Smith and Waterway 2008; Bardy et al. 2010).

## MATERIALS AND METHODS

**Plant Material**—Plant material of *Picris hieracioides* was collected in the field at 104 localities, covering a significant part of its distribution in Europe (Fig. 1A: Appendix 1, Supplementary Table 1). The populations spanned a large geographic area, from the Sierra Nevada Mts. in Spain to the southern Carpathians in Romania, and they comprised a wide altitudinal range, from lowland to alpine habitats. The sampling also included all currently recognized subspecies, and, whenever possible, samples were also collected from the respective type localities (Appendix 1, Supplementary Table 1). In addition, a few individuals of the closely related taxa, *P. japonica* (Japan, plants grown from seeds), *P. nuristanica* (Kirgizia, plants grown from seeds), and *P. hispidissima* (Croatia and Montenegro, plants sampled from two populations) were included in the genetic analyses. The first two taxa were also included in the morphological study. Nevertheless, due to few plants available of these relatives, the analyses of their variation and relationships to *P. hieracioides* should be regarded as illustrative only, and need to be taken with caution. A detailed morphological comparison of *P. hispidissima* with *P. hieracioides* has been performed and published elsewhere (Slovák et al. 2009b).

For morphometric analyses, 101 population samples of *P. hieracioides* were collected in the field (1,861 individuals in total) and conserved as herbarium specimens (hereafter referred to as field-collected plants) (Appendix 1, Supplementary Table 1, Fig. 1A). Before the specimens were dried, three outer involucral bracts, three inner involucral bracts, and three ligules taken from a single well-developed capitulum per plant were attached by adhesive translucent tape to paper to preserve their size and shape and to allow a detailed character scoring and measurements. Each population sample consisted typically of 15–20 plants; only on exception were fewer plants sampled.

To examine the extent of the phenotypic plasticity and to eliminate characters largely influenced by environmental conditions from further analyses, mature seeds of *P. hieracioides* were collected across a wide geographic area (32 populations, Appendix 1, Supplementary Table 1) and sown; the plants were cultivated under similar conditions (resembling lowland conditions) in a common garden experiment at the Institute of Botany, Slovak Academy of Sciences, Bratislava (48°10'15"N, 17°04'15"E; 180 m) for three years (2003–2006). Flowering individuals were harvested, preserved as herbarium specimens and used for

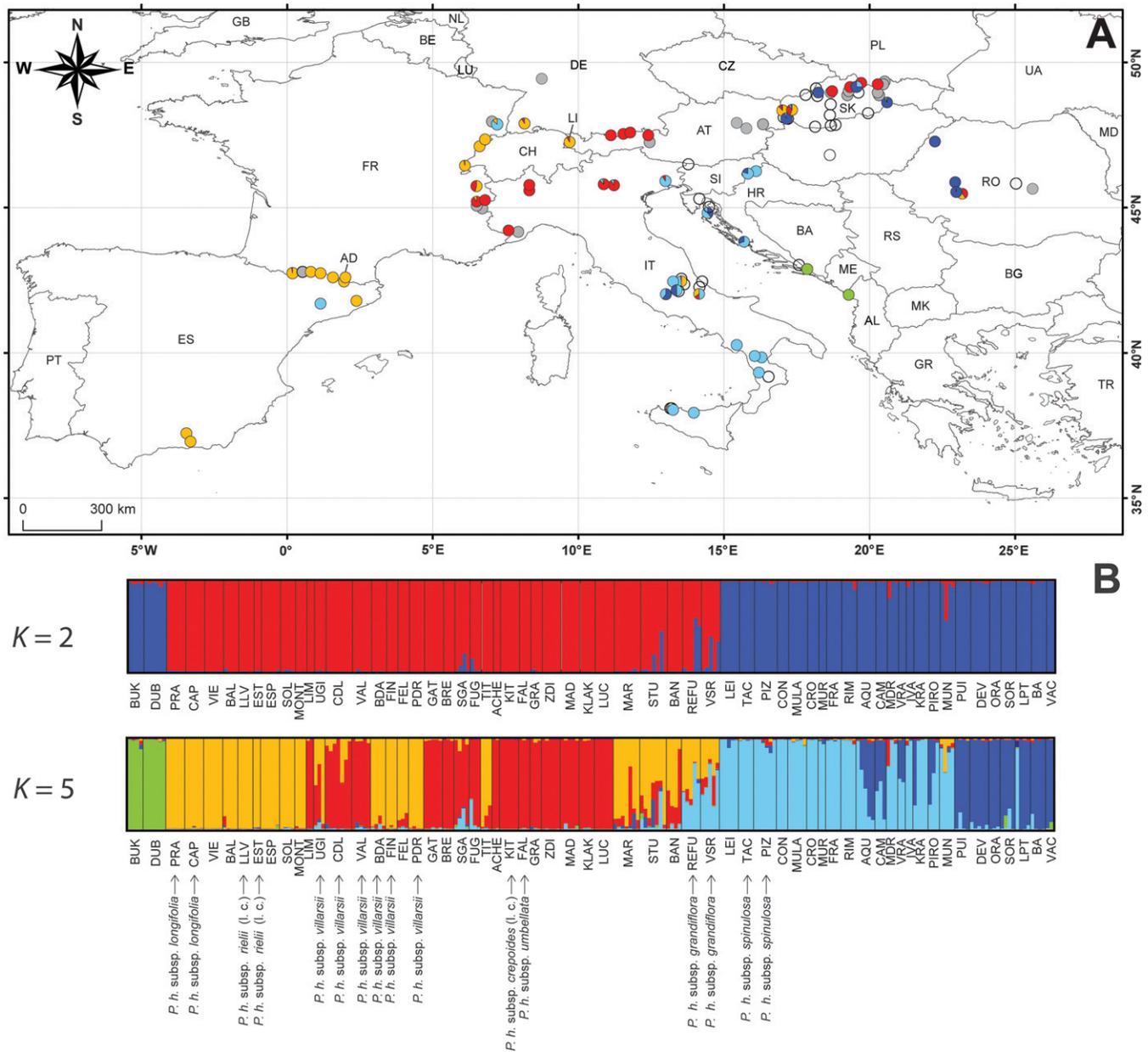


FIG. 1. Sample sites and genetic structure of *Picris hieracioides* and *P. hispidissima*. A. A map depicting sample sites of the analyzed populations. Dark/light blue circles correspond to *P. hieracioides* subsp. *hieracioides* (i.e. 'LA morphotype'), red/yellow circles to *P. hieracioides* subsp. *umbellata* ('HA morphotype'), and green circles to *P. hispidissima*, all included in the AFLP analyses. Empty circles indicate *P. hieracioides* subsp. *hieracioides*, gray circles are for *P. hieracioides* subsp. *umbellata* included in the morphometric analyses only. Two genetic lineages resolved within each subspecies are indicated by different colors (yellow – SW-HA lineage, red – NE-HA, light blue – SW-LA, dark blue – NE-LA). Genetic admixture observed in some populations is shown by dividing the circles into sectors of different colors, illustrating the proportion of genetically admixed individuals (according to the STRUCTURE results, part B). Country codes: AD – Andorra, AT – Austria, DE – Germany, ES – Spain, FR – France, HR – Croatia, HU – Hungary, IT – Italy, ME – Montenegro, RO – Romania, SI – Slovenia, SK – Slovakia. B. Genetic structure in *Picris hispidissima* and *P. hieracioides* based on AFLP markers as inferred with the Bayesian clustering using STRUCTURE at  $K = 2$  and  $K = 5$ . Replicate runs at  $K = 2$  gave stable results, whereas those at  $K = 5$  inferred two different clustering outputs and the more common is shown here. Each individual is represented by a vertical bar, colored proportionally according to the cluster assignment. The population localities follow Appendix 1 and Supplementary Table 1. Populations attributable to traditionally recognized subspecies (according to the type localities – l. c., or regions specified in the protologues) are also indicated.

morphometric analyses (306 individuals; hereafter referred to as cultivated plants) in the same manner as the field-collected ones.

For AFLPs, we sampled 234 individuals of *P. hieracioides* originating from 61 populations (two to seven individuals per population; Appendix 1, Supplementary Table 1), which were chosen to be a representative sample of the observed morphological variation, ecological types, genome size variants (reported by Slovák et al. 2009a) and distribution of the species in Europe.

Voucher specimens of all field-collected and cultivated plants are deposited in the herbarium SAV (Holmgren et al. 1990).

**Morphometric Analyses**—The morphological characters included characters traditionally used for the delimitation of infraspecific taxa in *P. hieracioides* in the literature, mainly those reported by Holzapfel (1994), and characters that were inferred as useful during the field sampling. Altogether, 25 characters (17 quantitative, two semi-quantitative, and six binary) were measured or scored on each herbarium specimen (Table 2). Selected morphological characters are illustrated in Fig. 2. The ligules and involucre bracts attached to the paper were scanned using a Microtek ScanMaker 9800XL scanner, and the characters were measured with CARNOY 2.0 (Schols et al. 2002).

TABLE 2. List of morphological characters used in morphometric analyses. <sup>1</sup>Mean values of three measurements of ligules or involucre bracts on one randomly chosen capitulum are given; <sup>2</sup>counted from three randomly chosen capitula; <sup>a</sup> brown-black, red-black or black hairs; <sup>b</sup> white or pale colored hairs; <sup>c</sup> green-brown, light brown, bi- or multi-colored hairs. If DHP and DHI were recorded as 1 (i.e. absence of hairs), the characters DH, PH, IH recording the hair color were given the value of 0.

**NBr** – Number of stem branches, **LBr** – Length of the longest stem branch (cm), **NL** – Number of stem leaves, **LP** – Length of the longest peduncle (cm), **NBP** – Maximum number of bracts per peduncle, **DHP** – Distribution of hairs on peduncle (1 – no hairs, 2 – sparse hairs, 3 – dense hairs) (see Fig. 2D), **DHI** – Distribution of hairs on involucre (1 – no hairs, 2 – sparse hairs, 3 – dense hairs) (see Fig. 2E), **DH** – Dark<sup>a</sup> hairs on the involucre and peduncle (0 – absent, 1 – present), **PH** – Pale<sup>b</sup> hairs on the involucre and peduncle (0 – absent, 1 – present), **IH** – Color-intermediate<sup>c</sup> hairs on the involucre and peduncle (0 – absent, 1 – present), **DC** – Distribution of the capitula on stem branches (0 – along the whole stem branch, 1 – only in upper 1/2–1/3 of stem branch) (see Fig. 2A), **NBr** – Maximum number of the capitula per stem branch, **NCP** – Maximum number of the capitula per peduncle, **NoB** – Average number of the outer involucre bracts<sup>2</sup>, **NiB** – Average number of inner involucre bracts<sup>2</sup>, **LoB** – Length of the outer involucre bracts<sup>1</sup> (mm), **WoB** – Width of the outer involucre bracts<sup>1</sup> (mm), **LiB** – Length of the inner involucre bracts<sup>1</sup> (mm), **WiB** – Width of the inner involucre bracts<sup>1</sup> (mm), **LL** – Length of the ligules<sup>1</sup> (mm), **LCT** – Length of the corolla tubes<sup>1</sup> (mm), **WL** – Width of the ligules<sup>1</sup> (mm), **LTL** – Length of the longest teeth on the ligule<sup>1</sup> (mm), **RSL** – Red longitudinal strip on the outer ligules (0 – absent, 1 – present) (see Fig. 2B), **BML** – Brown marks on the upper part of ligules (0 – absent, 1 – present) (see Fig. 2C).

Pearson (parametric) and Spearman (non-parametric) correlation coefficients were first computed to reveal pairs of highly correlated characters (Legendre and Legendre 1998) that may distort the computations, especially the discriminant analyses. Principal component analysis (PCA; Sneath and Sokal 1973; Krzanowski 1990) and canonical discriminant analysis (CDA; Klecka 1980; Krzanowski 1990) were performed, the former both with individual plants and with populations (represented by mean values of each character) as objects. These ordination techniques are efficient tools that summarize large amounts of information in only a few dimensions and, in contrast to cluster analyses, do not impose a hierarchical structure or artificial groupings when the variation is largely continuous. Whereas PCA extracts most of the overall variation among the individuals and displays it in a two- or three-dimensional space, CDA differentially weights all characters to achieve the best separation between predefined groups. Discriminant analyses generally require multivariate normal distribution of the characters, but have been shown to be considerably robust against deviations in this respect (Thorpe 1976; Klecka 1980).

Several data matrices were assembled, and analyses were performed as listed below. All morphometric data matrices used in this study are available from the first author upon request.

- (1) Principal component analysis 1, based on population means (101 populations) of all field-collected plants and the correlation matrix of all 25 characters, was performed. The aim was to obtain the first insights into the overall variation patterns in the material sampled, irrespective of the risk of the inclusion of characters that may have been influenced by environmental heterogeneity of their natural habitats.
- (2) Principal component analysis 2, based on all cultivated plants (306 individuals) from the common garden experiment and the correlation matrix of all 25 characters, was performed. The analysis displayed an overall variation pattern among the plants cultivated under similar ecological conditions, where the potential plasticity of characters was eliminated. Differentiation between the two groupings suggested by PCA 2 (attributable to the 'LA' and 'HA morphotype,' respectively; see Results) was tested by CDA 1, which was based on the same data matrix.
- (3) To compare the phenotypes of the field-collected and cultivated plants and to identify characters most influenced by environmental variables (that should not be taken into taxonomic consideration, or at least should be interpreted carefully), CDA and box-plots (displaying standard statistical parameters, such as the median, the 10th, 25th,

75th, and 90th percentiles and the extreme values) were computed. The two groupings revealed in steps 1 and 2 ('HA morphotype' and 'LA morphotype') were analyzed separately. Whereas in CDA we included only populations that were available both as field-collected and cultivated material, for the descriptive statistics and box-plots, we analyzed all of the available material (Appendix 1, Supplementary Table 1). Thus, CDA 2 was based on individuals of the 'HA morphotype,' with two groups predefined, HA field-collected plants (396 individuals) and HA cultivated plants (178 individuals), and all 25 characters. Canonical discriminant analysis 3 was based on individuals of the 'LA morphotype,' with two groups predefined, LA field-collected plants (208 individuals) and LA cultivated plants (85 individuals), respectively. This analysis was based on 24 characters; the binary character DH (Table 2) was invariable in this dataset and therefore excluded from CDA 3. As a result, we identified three characters that contributed most to the separation between the field-collected and cultivated plants (i.e. largely influenced by environment; see Results).

- (4) Field-collected plants were again subjected to PCA and CDA but with the exclusion of characters identified as environmentally plastic, i.e. PCA 3 was based on population means (101 populations) of all field-collected plants and the correlation matrix of 22 characters (excl. LBr, WiB, DH, Table 2). Canonical discriminant analysis 4 was based on individual plants (1,861 individuals from 101 populations), the same set of 22 characters, and two groups predefined (the two morphotypes suggested by PCA 3).
- (5) To get deeper insights into the variation patterns and potential differentiation within the main morphological types found ('HA' and 'LA morphotypes'), two additional PCA were performed on field-collected plants, separately for each morphotype: PCA 4 was based on the population means (53 populations) of the 'HA morphotype,' while PCA 5 was based on the population means (48 populations) of the 'LA morphotype,' both based on the correlation matrix of 22 characters.
- (6) Finally, CDA 5 was performed to explore the morphological differentiation between *P. hieracioides* from Europe (306 cultivated plants) and its close relatives: *P. nuristanica* (eight cultivated plants) and *P. japonica* (14 cultivated plants). The analysis included only quantitative characters (i.e. 17 characters); binary characters were excluded (and analyzed separately by means of the character-state-frequency) due to their invariance in at least one group. Box-plot graphs depicting variations in selected characters were also drawn. All analyses were performed using the SAS 8.2 package (SAS Institute 2000).

**AFLP Fingerprinting**—Genomic DNA was extracted from silica gel-dried leaves using the DNeasy plant mini kit (Qiagen, Hilden, Germany). AFLP analyses followed the general procedure described by Vos et al. (1995) and the protocol provided by Applied Biosystems (Applied Biosystems 2005) with some modifications, as described below. Genomic DNA was double-digested using *EcoRI* and *MseI* enzymes at 37°C for 3 hrs. The reaction mix of 10 µL volume contained 5 U *EcoRI* (Fermentas, St. Leon-Rot, Germany), 2 U *MseI* (New England BioLabs, Ipswich, Massachusetts), 2 µL 10 × Tango buffer (Fermentas) and 5 µL of the DNA extract (500–1,000 ng). After the digestion, a ligation mix was added in a volume of 5 µL per sample, and the reactions were incubated for an additional 12 hrs at 16°C. The ligation mix contained 1 U T4 DNA ligase (Fermentas), 1.5 µL T4 DNA ligase buffer (including ATP) and 1 µL of each adaptor pair (Applied Biosystems, Foster City, California). Ligated DNA fragments were diluted 1:10 with TE (10 mM Tris, 0.1 mM EDTA) buffer. Preselective amplifications were performed in 10 µL reaction volumes containing 1 µL 10 × PCR Buffer II (Applied Biosystems), 0.6 µL MgCl<sub>2</sub> (25 mM), 0.2 µL dNTPs (10 mM each), 0.5 µL of each preselective primer (Applied Biosystems), 0.04 µL AmpliTaq DNA polymerase (5 U/µL, Applied Biosystems) and 2 µL of diluted restriction-ligation product. The PCR cycle profile was 72°C for 2 min, 30 cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 2 min, which were followed by a final extension at 72°C for 10 min and cooling to 4°C (Mastercycler ep Gradient S, Eppendorf). Products of the preselective amplification were diluted approximately 1:15 with TE buffer.

We searched for optimal selective primer pairs that resulted in clear and well-scorable AFLP profiles of an appropriate level of polymorphisms. In total, 27 primer pairs were screened using eight individuals of *P. hieracioides*, originating from four different geographic regions. Four pairs of selective primers that repeatedly produced the most reliable profiles were chosen: *EcoRI*-ATC-(6-FAM)/*MseI*-CAG, *EcoRI*-ACG-(VIC)/*MseI*-CTC, *EcoRI*-ACC-(NED)/*MseI*-CAT and *EcoRI*-AGG-(PET)/*MseI*-CTC. The *EcoRI* primers were fluorescently labeled at the 5' end. The

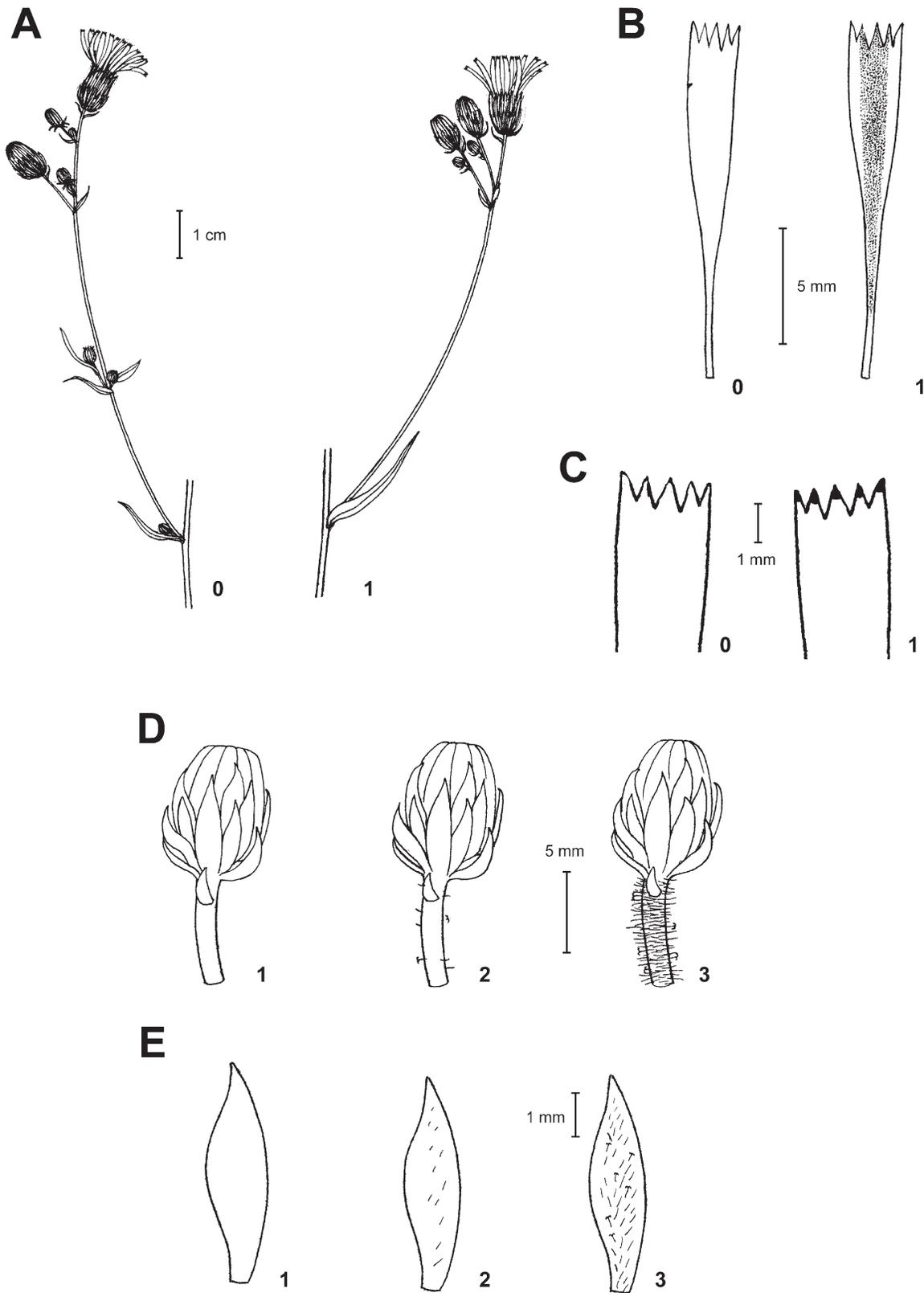


FIG. 2. Selected morphological characters used in multivariate morphometric analyses of *Picris hieracioides*. A. Distribution of the capitula on stem branches – DC (0 – along the entire stem branch, 1 – only in upper 1/2–1/3 of stem branch). B. Red longitudinal strip on the outer ligules – RSL (0 – absent, 1 – present). C. Brown marks on the upper part of ligules – BML (0 – absent, 1 – present). D. Distribution of hairs on peduncle – DHP (1 – no hairs, 2 – sparse hairs, 3 – dense hairs). E. Distribution of hairs on involucre – DHI (1 – no hairs, 2 – sparse hairs, 3 – dense hairs).

reaction mix of selective amplifications (10  $\mu$ L reaction volume) contained 1  $\mu$ L 10  $\times$  PCR Gold buffer (Applied Biosystems), 1  $\mu$ L MgCl<sub>2</sub> (25 mM), 0.2  $\mu$ L dNTPs (10 mM each), 0.5  $\mu$ L of each primer (*EcoRI* at 1  $\mu$ M, *MseI* at 5  $\mu$ M), 0.08  $\mu$ L AmpliTaq Gold DNA polymerase (5 U/ $\mu$ L, Applied Biosystems), and 2  $\mu$ L of diluted preselective PCR product. The PCR cycle profile was 95°C for 10 min, 13 cycles of 94°C for 30 sec, 65–55.9°C (each cycle decreasing by 0.7°C) for 1 min, 72°C for 1 min, 23 cycles of 94°C for 30 sec, 56°C for 1 min, 72°C for 1 min, followed by 72°C for 10 min and cooling to 4°C (Mastercycler ep Gradient S, Eppendorf). The products obtained with primers labeled with four different fluorescent dyes were pooled and analyzed using an ABI 3100 Avant DNA sequencer (BITCET Consortium, Department of Molecular Biology, Comenius University, Bratislava). Size calibration was achieved using the internal size standard, GeneScan –500 LIZ© (Applied Biosystems).

**AFLP Data Analyses**—Raw AFLP data were analyzed using GENESCAN 3.7 software (Applied Biosystems), subsequently imported into GENOGRAPHER 1.6.0 (available at <http://hordeum.msu.montana.edu/genographer/>) and scored. Only well-scorable and unambiguous markers in the size range 50–500 bp were recorded and coded as present (1) or absent (0). To estimate the reproducibility of the AFLP data, 22 individuals (9% of the final dataset, originating from different populations and representing all taxa and morphological types analyzed) were re-extracted and used as replicated samples, which were then analyzed and scored independently (Bonin et al. 2004). The complete AFLP data matrix is available from the first author upon request.

The overall genetic structure and relationships among the studied taxa and populations were explored by principal coordinate analysis (PCoA; Krzanowski 1990) and cluster analyses based on a neighbor-joining algorithm (NJ; Saitou and Nei 1987) or by using a network-generating NeighborNet method (Huson and Bryant 2006), both including and excluding the two Asian taxa (*P. japonica* and *P. nuristanica*). Principal coordinate analysis, using Jaccard's coefficient for calculating pairwise genetic similarities, was performed in FAMD 1.108 beta (Schlüter and Harris 2006). The NJ tree based on Nei and Li's (1979) genetic distances was constructed using PAUP\* version 4.0b10 (Swofford 2001). The support of each node in the tree was assessed by bootstrap analyses (Felsenstein 1985) with 5,000 replicates. The NeighborNet diagram was produced in the program SPLITSTREE 4 (Huson and Bryant 2006), using Nei and Li's (1979) genetic distance matrix calculated in PAUP\* version 4.0b10 (Swofford 2001). Furthermore, a Bayesian clustering method implemented in STRUCTURE 2.2 (Falush et al. 2007) was employed for the data matrix including only *P. hieracioides* and *P. hispidissima* to obtain additional insights into the genetic patterns at the individual, population and regional levels. In STRUCTURE computations, based on a Markov chain Monte Carlo (MCMC) algorithm, we used a recessive allele

model, assuming admixture and independence of allele frequencies among populations. Individuals were assigned into  $K$  clusters; the  $K$ -value, which is a user-defined number of clusters, was set from 1–10. For each  $K$ , 10 replicate runs were performed to test the stability of the resulting clustering patterns. We used runs of 10<sup>6</sup> MCMC iterations after a burn-in period of 10<sup>5</sup> iterations. The analysis was carried out at the Bioportal of the University of Oslo (<http://www.bioportal.uio.no/>). The following statistics were computed to identify the optimal number of clusters ( $K$ ) that best reflect the genetic structure present in the dataset: 1) similarity coefficients between the replicate runs, 2) mean  $L(K)$  – the means of the estimated log posterior probability of the data over the replicate runs for each  $K$  value, and 3)  $\Delta K$  – a quantity based on the second order rate of change with respect to  $K$  of the likelihood function (see Evanno et al. 2005 for more details). All these statistics parameters were calculated in the R-script Structure-sum-2009 (part of AFLPdat; Ehrich 2006). Graphical output was generated using CLUMPP ver. 1.1.1 (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004) software.

Analyses of molecular variance (AMOVA), based on Euclidean pairwise distances, were computed to reveal partitioning of total genetic variance within and among populations, as well as among population groupings (ARLEQUIN 3.11; Excoffier et al. 2005). The significance of differentiation was tested using permutation tests (10,000 permutations).

For each population or population grouping that resulted from the clustering analyses, we estimated the genetic diversity by calculating the percentage of polymorphic markers ( $P\%$ ) and the average proportion of pairwise differences between individuals (Nei's gene diversity,  $D_{Nei}$ ) using the R-script AFLPdat (Ehrich 2006). The genetic divergence was assessed by calculating the number of rare markers (those present at a frequency < 10%), private markers (those confined to a certain population or a population grouping and not necessarily present in all of its individuals), private fixed markers (those confined to a certain population or a population grouping and present in all its individuals) (calculated in FAMD; Schlüter and Harris 2006) and by the frequency-down-weighted marker values (DW; Schönswetter and Tribsch 2005), as implemented in AFLPdat (as DW1 in Ehrich 2006).

## RESULTS

**Morphometric Analyses**—The correlation coefficients did not exceed 0.75 for any character pair; thus, all measured characters were retained in further analyses.

- (1) Principal component analysis 1, based on 101 field-collected populations of *P. hieracioides* and all 25 examined characters,

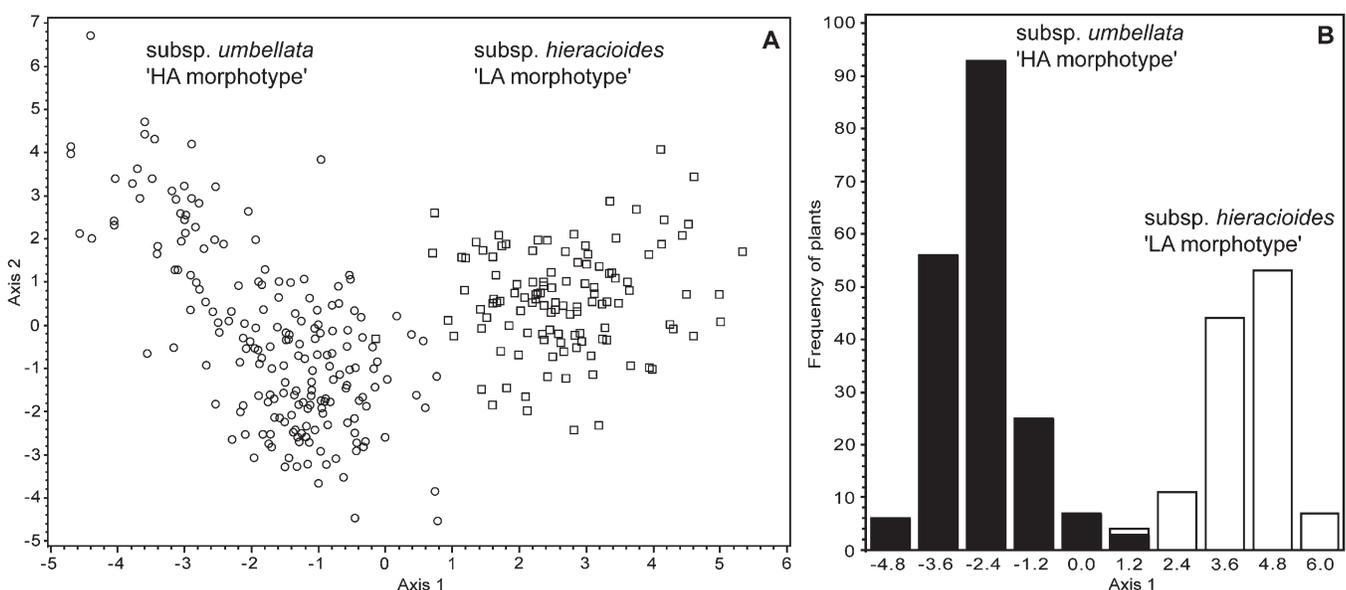


FIG. 3. Morphometric analyses of cultivated plants of *Picris hieracioides* (306 plants), based on 25 morphological characters. A. Principal component analysis (PCA 2): squares – *P. hieracioides* subsp. *hieracioides* ('LA morphotype'), circles – *P. hieracioides* subsp. *umbellata* ('HA morphotype'). The two ordination axes explained 23.50% and 13.72% of the total variation. B. Canonical discriminant analysis (CDA 1) with two pre-defined groups: white – *P. hieracioides* subsp. *hieracioides* ('LA morphotype'), black – *P. hieracioides* subsp. *umbellata* ('HA morphotype').

including those that are potentially environmentally influenced, resulted in two distinct groupings, separated along the first axis (extracting 20.34% of the total variation; figure not shown). One of the groupings included populations growing typically in dry, sunny, and man-made habitats at lower altitudes and comprising mainly annual to biennial plants (further referred to as the 'lower altitude morphotype' (LA) following Slovák and Marhold (2007)), while the other was formed by populations found in mesic, semi-natural to natural habitats at higher altitudes and being mostly short-lived (further referred to as the 'higher altitude morphotype' (HA)).

- (2) Both PCA 2 and CDA 1, based on the set of 306 cultivated plants (obtained from seeds collected in 32 natural populations) from the common garden experiment and all 25 characters, showed two groupings formed along the first axis (Fig. 3), which corresponded to the same two morphotypes as found in PCA 1. Three binary characters (the presence of pale and color-intermediate hairs on the involucre and peduncle (PH, IH) and the distribution of capitula on stem branches (DC)) and four quantitative characters (the number of stem branches (NBr), number of stem leaves (NL), length of ligules (LL), and number of outer involucre bracts (NoB)) showed the highest correlations with the discriminating axes of both PCA and CDA, thus contributing most to the morphotype distinction. In addition, the presence of a red longitudinal strip on the outer ligules (RSL) was suggested by CDA 1 as another discriminating character (Table 3). Populations of the 'HA morphotype' apparently have fewer stem branches, stem leaves, outer involucre bracts, but longer ligules when compared with the 'LA morphotype' (Supplementary Table 2). The 'HA morphotype' is also characterized by capitula that are typically distributed

only in the upper 1/2–1/3 of stem branches (vs. being distributed along the whole branch in the 'LA morphotype'), the presence of mainly color-intermediate hairs on the involucre and peduncle (vs. mainly pale hairs in the 'LA morphotype'), and a scarce occurrence of red strips on the outer involucre bracts (vs. frequent one in the 'LA morphotype') (Supplementary Table 3).

- (3) Canonical discriminant analysis 2, performed on the individuals of the 'HA morphotype' and the sets of cultivated and field-collected plants as two predefined groups, was focused on the identification of characters that are largely influenced by environmental variables, and thus should not be considered taxonomically relevant. The analysis identified two characters that most strongly contributed to the shift between the cultivated and field-collected plants: the width of inner involucre bracts (WiB) and the presence of dark hairs on the involucre and peduncle (DH) (Supplementary Fig. S1A). In CDA 3, which was performed on the individuals of the 'LA morphotype' (Supplementary Fig. S1B), two characters contributing to the shift between the cultivated and field-collected individuals were revealed: the width of the inner involucre bracts (WiB), and the length of the longest stem branch (LBr). Thus, three characters were identified as environmentally plastic: WiB, DH, and LBr. Indeed, whereas dark hairs were observed in as many as 43% of the field-collected plants of the 'HA morphotype,' such hairs were found only in 12% of the cultivated plants of this morphotype (Supplementary Table 3). Exploratory data analyses of the characters WiB and LBr (Supplementary Table 2; Supplementary Fig. S2) also confirmed the results of CDA; substantial shifts in the values observed for the cultivated and field-collected plants were apparent.

TABLE 3. The numerical output of morphometric analyses: PCA 2, PCA 3, PCA 4 – eigenvectors expressing correlations of the examined characters with the principal components (axis 1, axis 2); CDA 1, CDA 4, CDA 5 – total canonical structure expressing correlations of the morphological characters with the canonical axes (axis 1, axis 2). For details on the different PCA and CDA, see Materials and Methods. For character explanation, see Table 2. The higher values of eigenvectors and total canonical structure, referred to in the text, are in bold.

Char.	PCA 2 Fig. 3A		PCA 3 Fig. 4A		PCA 4 Fig. 5		CDA 1 Fig. 3B	CDA 4 Fig. 4B		CDA 5 Fig. 6	
	axis 1	axis 2	axis 1	axis 2	axis 1	axis 2	axis 1	axis 1	axis 1	axis 2	
NBr	<b>0.295</b>	0.069	<b>-0.285</b>	0.161	0.209	0.316	<b>0.580</b>	<b>-0.563</b>	0.606	-0.258	
LBr	0.125	0.138	-	-	-	-	0.220	0.026	0.265	0.037	
NL	<b>0.325</b>	0.005	<b>-0.281</b>	0.070	-0.144	0.212	<b>0.673</b>	<b>-0.608</b>	0.670	-0.432	
LP	-0.066	0.090	<b>0.270</b>	0.003	0.109	0.285	-0.152	<b>0.506</b>	-0.110	0.259	
NBP	-0.148	0.070	0.244	-0.064	-0.144	0.245	-0.355	0.453	-0.341	0.278	
DHP	0.057	0.320	-0.136	0.414	0.417	-0.080	0.280	-0.229	-	-	
DHI	0.150	0.263	0.010	0.331	0.342	-0.106	0.436	-0.029	-	-	
DH	-0.112	0.063	-	-	-	-	-0.228	0.571	-	-	
PH	<b>0.358</b>	0.129	<b>-0.329</b>	0.130	0.272	-0.020	<b>0.923</b>	<b>-0.795</b>	-	-	
IH	<b>-0.271</b>	-0.079	0.207	-0.016	0.239	-0.044	<b>-0.658</b>	0.343	-	-	
DC	<b>-0.324</b>	-0.117	<b>0.335</b>	0.034	-0.049	0.101	<b>-0.842</b>	<b>0.857</b>	-	-	
NCBr	0.141	0.127	0.064	0.060	0.095	0.385	0.229	0.072	0.221	-0.176	
NCP	0.167	0.126	-0.086	-0.016	-0.042	0.427	0.343	-0.160	0.363	-0.135	
NoB	<b>0.258</b>	0.096	<b>-0.269</b>	0.080	0.029	0.280	<b>0.661</b>	<b>-0.620</b>	<b>0.788</b>	0.353	
NiB	0.019	0.115	-0.110	0.175	0.215	0.210	0.032	-0.106	0.019	-0.070	
LoB	-0.101	0.346	0.147	0.429	0.181	0.272	-0.020	0.191	0.033	0.219	
WoB	0.061	0.318	0.019	0.339	0.030	0.261	0.348	-0.079	0.420	0.100	
LiB	-0.273	0.295	<b>0.296</b>	0.161	0.062	0.157	-0.472	<b>0.612</b>	-0.537	-0.024	
WiB	-0.139	0.278	-	-	-	-	-0.166	0.223	-0.153	0.152	
LL	<b>-0.303</b>	0.215	0.246	0.104	-0.049	0.178	<b>-0.559</b>	0.484	-0.542	0.414	
LCT	-0.033	0.308	0.153	0.011	-0.199	0.151	0.067	0.234	0.022	-0.211	
WL	-0.131	0.305	0.170	0.318	0.364	-0.001	-0.124	0.243	-0.021	0.439	
LTL	-0.215	0.172	0.213	0.245	0.144	0.065	-0.349	0.338	-0.297	0.418	
RSL	0.204	0.206	-0.233	0.285	0.324	-0.043	<b>0.594</b>	-0.457	-	-	
BML	-0.007	0.077	0.090	0.214	0.289	-0.054	-0.021	0.183	-	-	



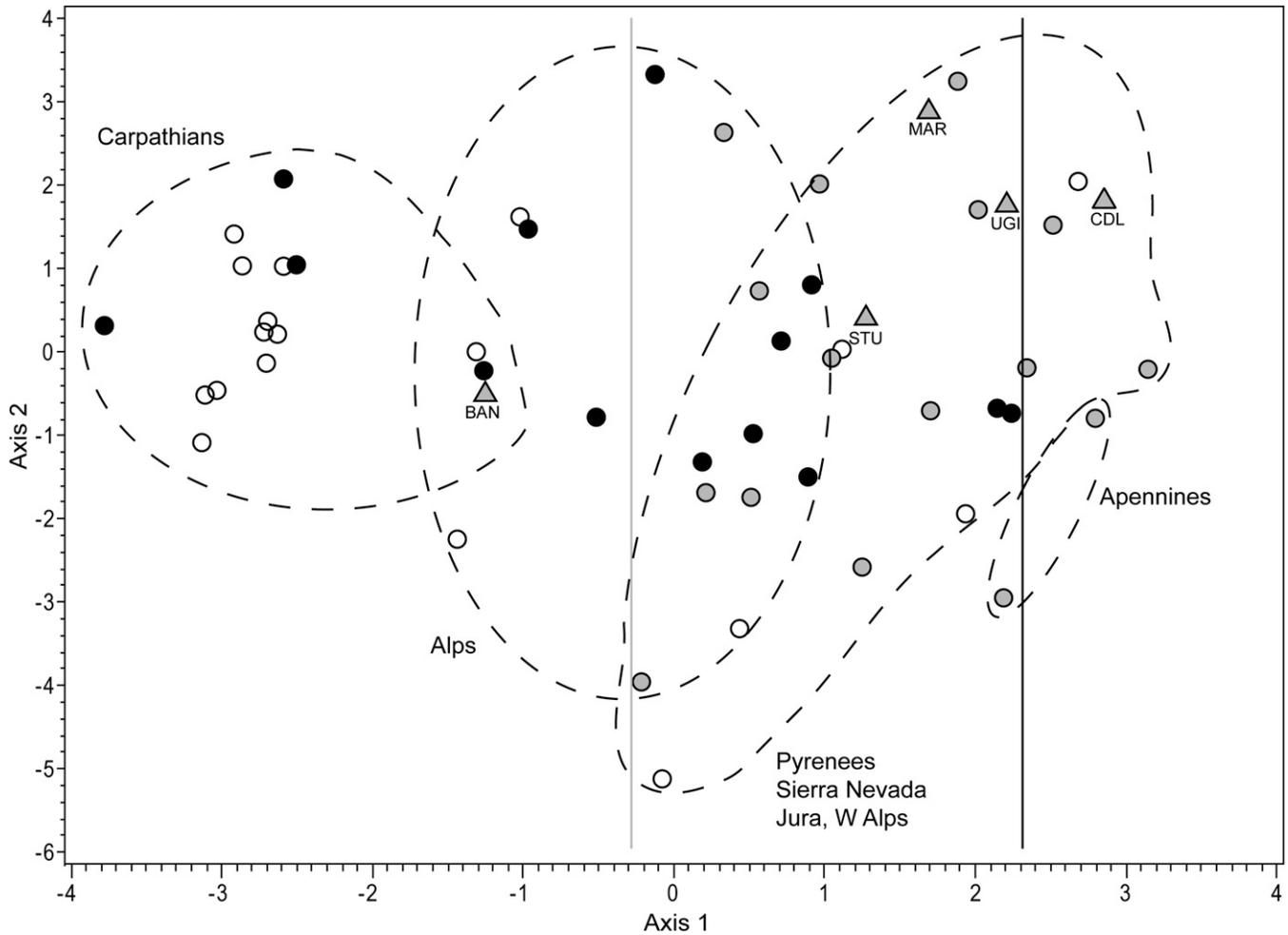


FIG. 5. Principal component analysis (PCA 4) of *Picris hieracioides* subsp. *umbellata* ('HA morphotype') based on 53 field-collected populations samples and 22 morphological characters. The two genetic lineages are indicated; full black circles – NE-HA, full gray circles – SW-HA; two vertical lines show the zone of overlap. Empty symbols are populations not included in the AFLP analyses. Populations exhibiting some genetic admixture (gray triangles) between the NE-HA and SW-HA lineages are labeled by their codes. Geographic regions are also indicated. The two ordination axes explained 18.25% and 14.51% of the total variation.

in positions clearly separated from *P. hispidissima* and *P. hieracioides* (figure not shown). Further analyses presented below were performed on the dataset with the exclusion of the Asian taxa, to resolve the genetic structure of *P. hieracioides* and *P. hispidissima* in more detail.

In the STRUCTURE analyses, the mean  $L(K)$  increased with the increasing number of clusters,  $K$ . The highest  $\Delta K$  was achieved at  $K = 2$ , but a small increase in the plot of  $\Delta K$  vs.  $K$  was visible also at  $K = 5$ . Replicate runs yielded stable clustering results with a coefficient of similarity of 1.0 only for  $K = 2$ , although similarity among the runs was also reasonably high for  $K = 5$  (0.67). Thus, the STRUCTURE analyses indicated that the recognition of two ( $K = 2$ ) and five ( $K = 5$ ) clusters best reflects the genetic structure present in the data. At  $K = 2$ , one cluster included the populations of the 'HA morphotype,' and the other was composed of the populations of the 'LA morphotype' together with *P. hispidissima* (Fig. 1B). A few populations included individuals with uncertain assignments, indicating genetic admixture (pop. STU, Malé Karpaty, SW Slovakia; REFU, VSR, both Abruzzo, central Italy; and MUN, Alsace, NE France).

At  $K = 5$ , two clustering outcomes were obtained (in two vs. eight runs, respectively); the more common and more

likely clustering is interpreted here. It illustrates the distinction of *P. hispidissima* and two genetic lineages within both the 'HA' and 'LA morphotypes' of *P. hieracioides* indicating certain geographic structure. The five clusters were resolved as follows (Fig. 1): 1) *P. hispidissima*; 2) populations of the 'HA morphotype' mainly from the southwestern (SW) range (denoted as SW-HA), including the Sierra Nevada, Pyrenees, Jura, and SW Alps; 3) populations of the 'HA morphotype' mainly from the northeastern (NE) range (NE-HA), comprising the Carpathians and Alps; 4) populations of the 'LA morphotype' from the SW range (SW-LA), Spain, France, Italy, and Croatia; and 5) populations of the 'LA morphotype' from the NE range (NE-LA), Slovakia and Romania. Certain populations, however, harbored genetically intermediate individuals, indicating admixture between these lineages. Admixture between the two 'HA' lineages was observed in two western (W) Alpine populations (CDL, UGI), and also in the Carpathians (pop. MAR and BAN). Admixture between the two 'LA' lineages was evident in some central Italian (AQU, CAM) and Croatian (VRA, KRA, PIRO) populations and also in one individual in the population LPT from Slovakia. Three populations from northern Italy and one from NE France showed a low, but still detectable, level of admixture between

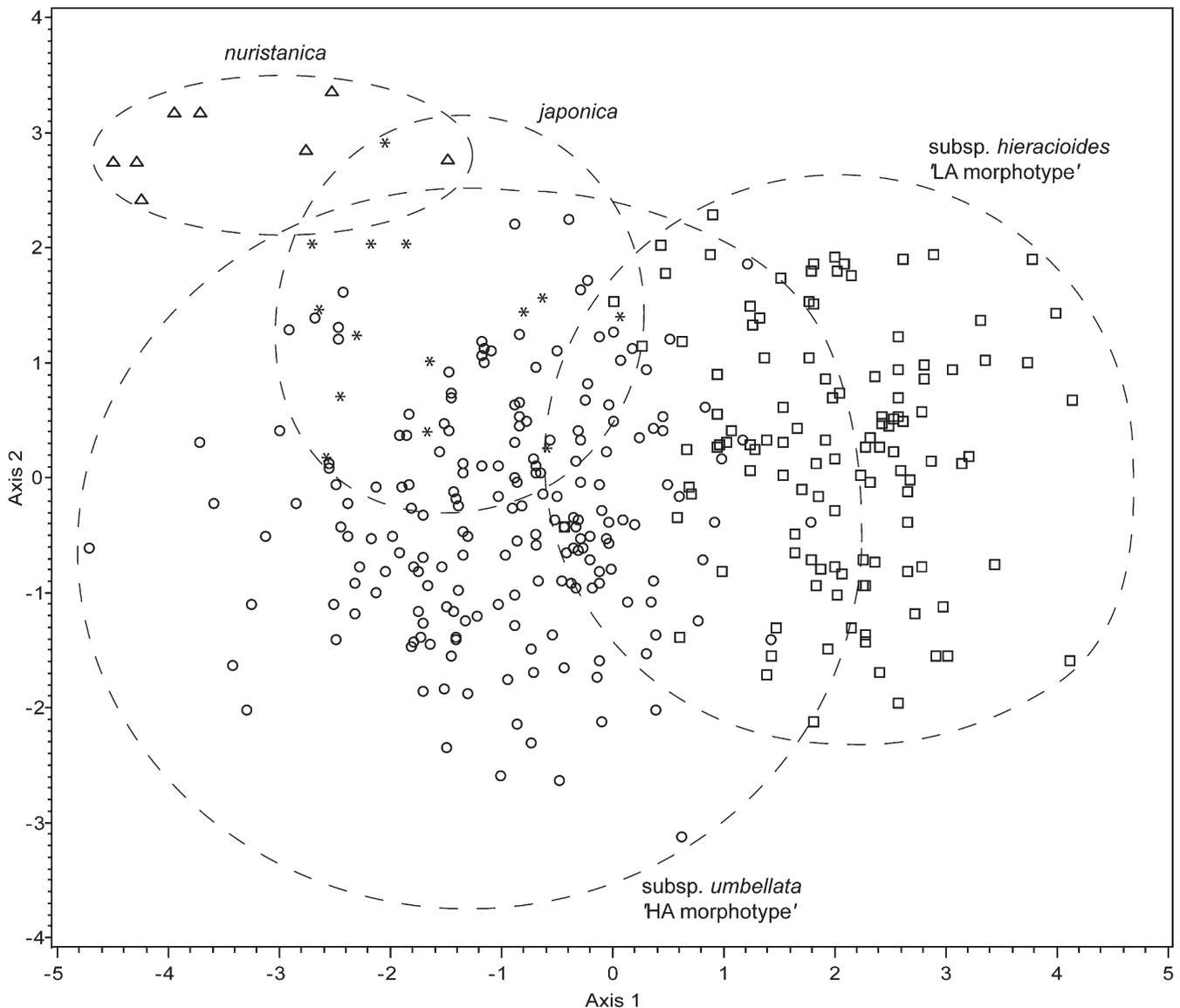


FIG. 6. Canonical discriminant analysis (CDA 5) based on cultivated plants of *Picris hieracioides* and its close relatives, with 17 quantitative characters used and four groups pre-defined: squares – *P. hieracioides* subsp. *hieracioides* ('LA morphotype';  $n = 116$ ), circles – *P. hieracioides* subsp. *umbellata* ('HA morphotype';  $n = 190$ ), asterisks – *P. japonica* ( $n = 14$ ), triangles – *P. nuristanica* ( $n = 8$ ).

the NE-HA and NE-LA lineages (populations SGA, FUG), between the SW-HA and SW-LA lineages (pop. MUN), and also between the SW-LA and NE-HA lineages (pop. MDR). In addition, two populations from Abruzzo, central Italy (REFU and VSR) were resolved as completely intermediate between the SW-HA and SW-LA lineages. Interestingly, the population STU (SW Slovakia) exhibited a contribution from all four genetic lineages of *P. hieracioides* (see Fig. 1).

The NeighborNet diagram (Fig. 8) supported the Bayesian clustering well, also with regards to the genetically admixed populations. The three clusters corresponding to the *P. hispidissima*, 'HA' and 'LA' populations of *P. hieracioides* can be clearly delimited. The cluster of *P. hispidissima* was located in an intermediate position, between the 'LA' and 'HA' populations of *P. hieracioides*. The 'HA' populations formed several clusters: one large cluster comprised populations of the NE-HA lineage, while the populations of SW-HA were found in several smaller clusters. The two

remarkably intermediate populations (VSR, REFU) clustered together in a position that was shifted towards the cluster of the 'LA' populations. Individuals from the peculiar STU population were found scattered across different 'HA' clusters. The 'LA' populations were divided into three clusters; one corresponded to the NE-LA lineage, and the other two comprised populations of the SW-LA lineage. Genetic admixture in the MDR and MUN populations, as detected in the STRUCTURE analyses, was apparent also here; these individuals appeared to be somewhat shifted towards the 'HA' clusters.

In line with the results given above, PCoA revealed three distinct groups, matching *P. hispidissima* and the two morphotypes of *P. hieracioides*. Populations revealed as genetically admixed (FUG, MDR, MUN, REFU, SGA, STU, and VSR as well as some individuals from the populations STU, MUN, and FUG) were placed just in the center of the graph, between the ordination space occupied by the 'LA' and 'HA' populations (figure not shown). Separate PCoA

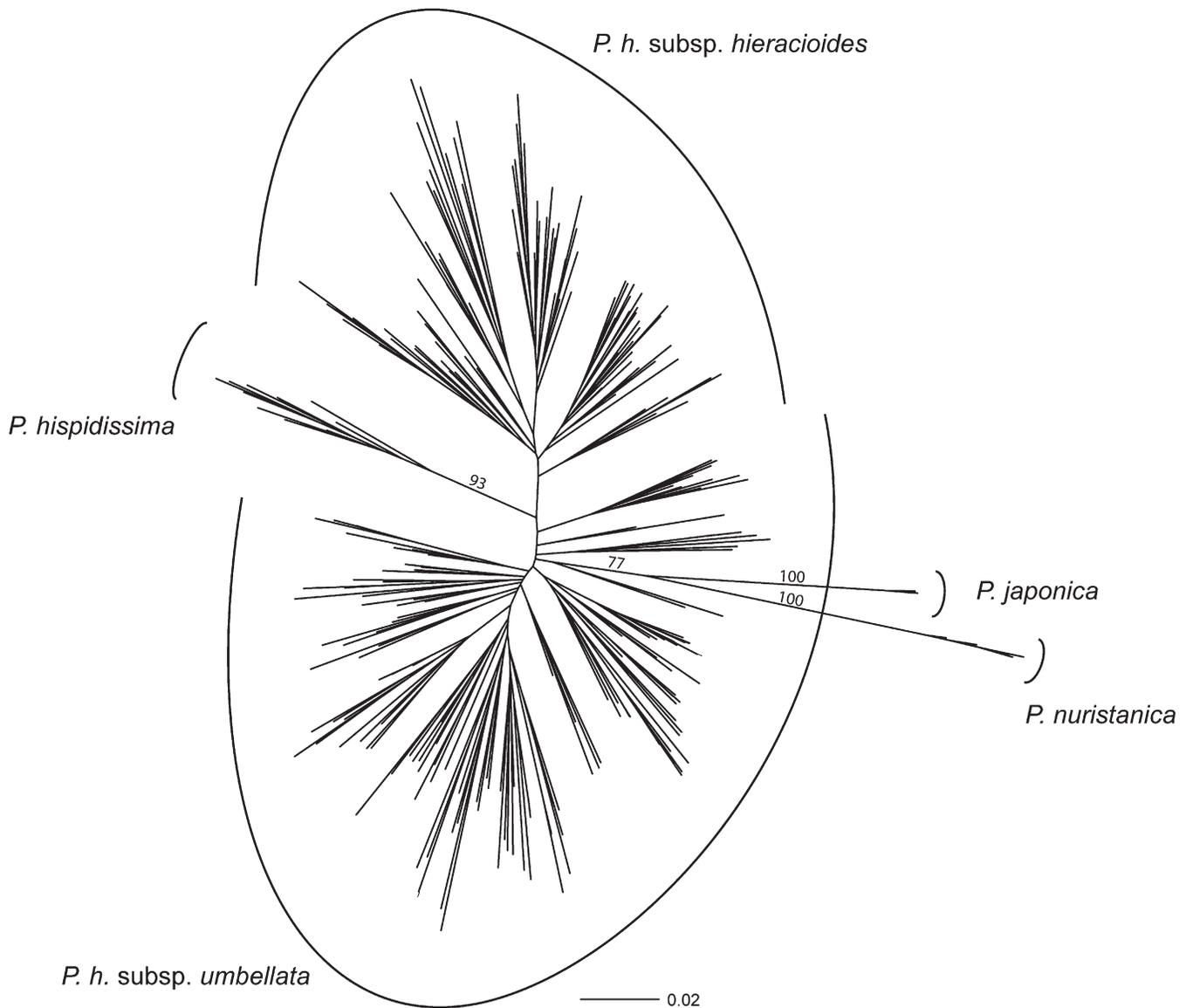


FIG. 7. Unrooted neighbor-joining tree based on the AFLP data of *Picris hieracioides*, *P. hispidissima*, *P. nuristanica* and *P. japonica*. Numbers along branches indicate bootstrap support greater than 50% (but not shown for terminal nodes within populations). Two subspecies, delimited as a result of this study, *P. hieracioides* subsp. *hieracioides* ('LA morphotype') and subsp. *umbellata* ('HA morphotype') are also indicated. Tree branches are proportional to Nei and Li's (1979) genetic distances (see scale bar).

analyses including only the 'HA' or 'LA' populations showed further geographic structuring, as outlined above, with considerable overlap involving the populations revealed as genetically admixed (figure not shown).

In a two-level AMOVA, 52.1% of the variation was within populations, and the rest (47.9%,  $F_{ST} = 0.48$ , d. f. = 60,  $p < 0.001$ ) of the variation occurred among them. In the three-level hierarchical AMOVAs we tested the degree of genetic differentiation among the groupings suggested by the above analyses (*P. hispidissima* vs. *P. hieracioides*; 'LA' vs. 'HA' populations of *P. hieracioides*), but excluding genetically strongly admixed populations (REFU, VSR, STU, FUG, SGA, MDR, and MUN) because of their unequivocal assignment to the predefined groups. These AMOVA computations supported the above clustering and ordination results. It revealed significant differentiation between *P. hispidissima*, the 'LA' and 'HA' populations of *P. hieracioides*, showing that 26.0% ( $F_{CT} = 0.26$ ,

d. f. = 2,  $p < 0.001$ ) of the total variance was explained by these three groups, whereas 29.6% ( $F_{SC} = 0.40$ , d. f. = 51,  $p < 0.001$ ) of the variation was among populations within groups and 44.4% ( $F_{ST} = 0.56$ , d. f. = 159,  $p < 0.001$ ) was within populations. When considering only *P. hieracioides* and the 'LA' and 'HA' populations as two groups, the level of differentiation was also highly significant; 24.8% ( $F_{CT} = 0.25$ , d. f. = 1,  $p < 0.001$ ) of total variance was explained by the two groups, whereas 30.4% ( $F_{SC} = 0.40$ , d. f. = 50,  $p < 0.001$ ) of the variation was found among populations within groups and 44.8% ( $F_{ST} = 0.55$ , d. f. = 151,  $p < 0.001$ ) within populations.

#### Comparison of the Morphological and Genetic Structure—

In the morphometric PCA 3 (Fig. 4A) of the field-collected population samples of *P. hieracioides*, we indicated their assignments to the two genetic clusters, as inferred by STRUCTURE analyses at  $K = 2$  (excluding *P. hispidissima*). It is evident that the two genetic clusters are congruent with the



the 'LA' and 'HA morphotypes,' they both yielded similar levels of genetic variation. The 'LA' populations were characterized by 37 private AFLP markers, whereas the 'HA' populations harbored 48 private ones (Supplementary Table 1).

#### DISCUSSION

##### *Patterns of Intraspecific Variation in Picris hieracioides*—

The analyses presented here demonstrated that the two morphotypes observed within *P. hieracioides* are congruent with the two main genetic groupings, as revealed by AFLP data. Thus, we conclude that two subspecies should be recognized within this species. Based on the study of the original material of several taxa described within *P. hieracioides* (see Taxonomic Treatment below), the 'HA' morphotype is attributed here to *P. hieracioides* subsp. *umbellata* and the 'LA' morphotype to *P. hieracioides* subsp. *hieracioides*.

The two morphologically well-differentiated entities were already suggested in a previous study based on populations from the W Carpathians and Pannonia and denoted in that study as the 'lower altitude (LA) morphotype' and the 'higher altitude (HA) morphotype' (Slovák and Marhold 2007). The large-scale sampling across Europe in the present study corroborates this main pattern. The two morphotypes largely overlap geographically, but they are characterized by different ecologies, life history traits (longevity) and, to a large extent, also by different genome sizes (Slovák and Marhold 2007; Slovák et al. 2009a; see also below). The genetic variation inferred from AFLP markers is congruent with the morphological differentiation, and it confirms the recognition of these two entities, most appropriately at the subspecies level, as *P. hieracioides* subsp. *hieracioides* and subsp. *umbellata*. Although the genetic patterns lack strong support (e.g. a lack of bootstrap support in the neighbor-joining tree, the presence of intermediate individuals), indicating some gene flow between the subspecies, all of the approaches employed (i.e. ordination analysis, Bayesian, neighbor-joining, and network generating clustering) reveal the same pattern of two genetic lineages. A few individuals showing uncertain assignments indicate the existence of genetically admixed genomes. These individuals were not randomly distributed, but were observed mainly in central and northern Italy and in the Western Carpathians (see Fig. 1A). The disturbance of mountain habitats through human activities (observed in some Italian localities) and the unintentional introduction by gardeners have most likely brought genetically differentiated individuals in close proximity and facilitated the gene flow among them.

The subdivision of *P. hieracioides* into two subspecies as presented here, strongly contrasts with the traditional taxonomic concepts that recognized numerous subspecies in Europe and were discordant (see Table 1). The assignments of the populations analyzed here to the previously described subspecies (based on their type localities and/or the areas of their description, see Figs. 1B and 4A) clearly illustrate this discrepancy. Several traditional subspecies were found to be morphologically heterogeneous, occupying a large phenetic space (e.g. *P. hieracioides* subsp. *villarsii*, subsp. *crepoides*, see Fig. 4A). At the same time, a compact morphological cluster on PCA 3 (Fig. 4A) comprised populations attributable to several traditional subspecies. The same picture can be seen in the results of genetic analyses (Fig. 1B). STRUCTURE analyses at  $K = 5$  showed that some genetic clusters (e.g. SW-HA and NE-HA lineages) comprised the populations of up to

three different traditional subspecies, while certain subspecies were dispersed between two different genetic clusters (e.g. *P. hieracioides* subsp. *villarsii*). For instance, the SW-HA lineage also included (besides two Iberian endemic subspecies *P. hieracioides* subsp. *longifolia* and subsp. *rieli*) some populations belonging to *P. hieracioides* subsp. *villarsii* that have been reported from nearly throughout Europe. Furthermore, *P. hieracioides* subsp. *grandiflora* was described from central Italy (Abruzzo) but also has been reported from the Alps and the W Carpathians. The populations sampled in Abruzzo, however, were revealed to be largely genetically admixed (see Fig. 1), which cannot be treated as the same subspecies together with the genetically different and homogenous populations from the Alps and central Europe.

A combination of several quantitative and binary characters differentiates the two subspecies of *P. hieracioides* defined here, and this finding applies not only to plants in their natural habitats, but also to those transferred to a garden and cultivated under the same environmental conditions. Important diagnostic characters are as follows: the color of hairs on the involucre and peduncle, the distribution of capitula on stem branches, the presence of red longitudinal strips on the outer ligules, the number of stem branches, the number of stem leaves, the length of ligules, the number of outer involucre bracts, and the length of inner involucre bracts (see also the key below).

Characters such as the length of the peduncles, color and length of the involucre bracts, color of hairs, and leaf shape have been reported as the most important characters for the delimitation of the subspecies within *P. hieracioides* (Sell 1975, 1976; Bolòs and Vigo 1995; Haeupler and Muer 2000). Results of the present study indicate that the length of the inner involucre bracts and the color of hairs contribute to the discrimination among the sampled populations, and, thus, can be taxonomically meaningful. Nevertheless, the color of hairs, although being a potential diagnostic trait, should be interpreted carefully: while the 'LA morphotype' (here defined as subsp. *hieracioides*) bears pale or at most (rarely) color-intermediate hairs, the whole color range can be found in the 'HA morphotype' (subsp. *umbellata*). In contrast, characters such as the number of stem leaves, stem branches, the length of ligules, panicle structure (distribution of capitula on branches), the number of the outer involucre bracts, all identified here as being diagnostic, have never been considered as important in identification keys. Apparently, previous taxonomic treatments and subspecies circumscriptions have relied on a few specimens only and have over-emphasized traits that are only part of the continuum of variation in *P. hieracioides*.

Based on AFLP markers, both Asian species, *P. japonica* and *P. nuristanica*, are shown to be well differentiated. The west Balkan endemic *P. hispidissima* seems to be a well-defined taxon as well (93% bootstrap support in the neighbor-joining tree, a separate genetic cluster at  $K = 5$  in STRUCTURE analyses), but it still appears very close to *P. hieracioides*. Whereas *P. hispidissima* is morphologically and ecologically well differentiated from *P. hieracioides* (see Slovák et al. 2009b), it was difficult to state clear morphological differences of the Asian taxa from *P. hieracioides*. Thus, before the final taxonomic conclusion is drawn, additional populations from all three taxa should be sampled to ascertain their genetic and morphological distinctness more precisely (Slovák et al. work in progress).

Australian representatives of the genus *Picris* (10 native species including one polymorphic species with three subspecies), which are morphologically and phylogenetically close to *P. hieracioides* (Lack 1979; Samuel et al. 2006), can be distinguished from each other by a comparable set of morphological characters as identified here (Holzapfel and Lack 1993; Holzapfel 1994). Conversely, morphometric studies on *Picris* species from the Arabian Peninsula and Socotra (Smalla 2000) have shown that the morphological variation in vegetative and floral characters is extremely complex and continuous, and only the characters on achenes contributed to the species separation. In a previous study on the Carpathian/Pannonian populations of *P. hieracioides* (Slovák and Marhold 2007), the achene width and length and the length of the pappus were also measured and analyzed, but their contribution to the morphotype separation was shown to be negligible.

Several other authors recently investigating polymorphic species by means of multivariate morphometrics have shown that, like our study, many taxa previously recognized at the specific or infraspecific levels in fact show a continuous or clinal variation and should be lumped into one or few taxonomic entities, such as in *Cineraria deltoidea* (Cron et al. 2007), *Hemizygia bracteosa* (Otieno et al. 2006), *Pedicularis bracteosa* (Robart 2005), or *Veronica chamaedrys* (Bardy et al. 2010). Combining morphometric data with highly variable DNA fingerprinting markers has also proven successful when revising taxonomic concepts and re-assessing species boundaries in taxonomically intricate plant groups, as demonstrated by the studies in *Veronica* (Martínez-Ortega et al. 2004; Bardy et al. 2010), *Carex roanensis* and allied species (Smith and Waterway 2008), *Cardamine amara* (Lihová et al. 2004), or the *Alyssum montanum* complex (Španiel et al. 2011).

**The Genetic Structure and Hypotheses on the Evolutionary History of *Picris hieracioides***—High overall genetic variation, with almost each individual representing a distinct multilocus phenotype, and considerable variation also within populations (the within-population variation component 52.1%, see AMOVA results) was observed in *P. hieracioides*. Three main factors related to the ecology and biology of this species apparently affect (increase) the variation and may have had strong impacts on the species' evolution and diversification: 1) self-incompatibility (Slovák et al. 2008) that promotes outcrossing and gene flow within and among populations; 2) the ability of long-distance dispersal through achenes bearing well-developed pappi (Sheldon and Burrows 1973; Andersen 1993; M. Slovák pers. obs.); and 3) high efficiency of spreading along 'anthropogenic corridors,' such as roads, highways, or abandoned fields (especially in *P. hieracioides* subsp. *hieracioides*), which allows the expansion and the rapid colonization of new sites, and, in addition, may bring differentiated populations into contact. Decreased genetic variation in some populations (e.g. those from the Sierra Nevada, a few from the Pyrenees, and from southern Italy or W Carpathians; see Appendix 1, Supplementary Table 1), however, can be explained by recent colonization via long-distance dispersal events or a recent, human-mediated spread. Indeed, almost all of these localities comprised a few individuals only and were strongly disturbed anthropogenically. On the other hand, high values of the divergence parameters (DW, rare fragments) in some of these genetically depauperate populations (LLV from the Pyrenees, LUC from the Western Carpathians, and MULA

from Calabria, southern Italy) are in contrast with the founder effect hypothesis and rather indicate a loss of genetic variation due to recent habitat disruption and a decrease in population size.

Conspicuous variation was recently observed in the genome size of *P. hieracioides* (Slovák et al. 2009a), which is extraordinarily high for an intraspecific range; it strongly indicated a taxonomic heterogeneity and/or complex evolutionary and colonization history of this species. Indeed, much of this genome size variation can be explained here by considering the two subspecies recognized and their internal genetic lineages. Whereas the populations of *P. hieracioides* subsp. *hieracioides* analyzed here exhibited 2C values in the range of 2.30–2.75 pg (2.30–2.46 pg in SW-LA lineage; (2.39 in LPT–) 2.74–2.75 pg in NE-LA), the 2C values of the populations of subsp. *umbellata* ranged from 2.73–3.11 pg (2.73–2.94 pg in SW-HA; 2.94–3.11 pg in NE-HA), thus, showing either small or no overlap between the subspecies and genetic lineages. The time and mode of the origin of the two subspecies of *P. hieracioides* cannot be ascertained with the present data, but we can speculate that these largely sympatric subspecies evolved through ecological adaptation to different habitats, which was accompanied by morphological differentiation, a shift in life-history traits (longevity) and the accumulation of genetic differentiation. The few intermediate individuals and populations found may document either secondary contact or initial and incomplete differentiation, but their concentration in certain, rather restricted areas geographically and the observation of disturbed habitats strongly favors the former scenario.

The two genetic lineages detected within *P. hieracioides* subsp. *umbellata* are largely allopatric. The SW-HA lineage occupies mainly high altitudes (552–2,370 m) in the Sierra Nevada, Montseny Mts., and Pyrenees, and extends to the Jura Mts. and the westernmost Alps, while the NE-HA lineage grows in the Carpathians and Alps (289–600 m). We assume that the (post)glacial history, past gene flow and colonization processes, have shaped this genetic structure. We did not observe distinct genetic lineages in each mountain range, as would be expected under a vicariance scenario of species survival in distinct and isolated refugia with restricted gene flow (e.g. Kropf et al. 2006; Mráz et al. 2007; Ronikier et al. 2008a). Conversely, the widespread gene pool we see both in the SW and NE ranges suggests that the populations may have survived the cold periods of the Pleistocene at lower altitudes across larger regions. Gene flow among the populations has probably not been severely restricted, and, thus, little internal structure has been maintained. A more recent spread and long-distance dispersal may also have contributed to the observed variation patterns. Genetic relatedness between the Iberian and western Alpine populations was revealed and discussed also for several other mountain species (e.g. Schönswetter et al. 2002; Ronikier et al. 2008b; Dixon et al. 2009; Lihová et al. 2009). Similarly, a close relationship and common evolutionary history of the Carpathian and eastern Alpine populations have also been documented (e.g. Kropf et al. 2003; Paun et al. 2008). The Alps have apparently been the major dispersal barrier for the two lineages of *P. hieracioides* subsp. *umbellata*, and genetic admixture, suggesting hybridization between them, has been observed there only in two populations from the westernmost Alps (CDL, UGI). Three other genetically admixed populations (STU, MAR, BAN) are found in the

Carpathians. These populations probably originated by long-distance events or, even more likely, by their unintentional introduction together with ornamentals (as all three populations grow in gardener's cottage communities) and subsequent hybridization. The morphological variation, however, is largely continuous within *P. hieracioides* subsp. *umbellata*, showing only slight morphological shifts in different mountain ranges.

Genetic variation in the lowland, highly ruderal subspecies *P. hieracioides* subsp. *hieracioides* also indicates the occurrence of SW and NE genetic lineages. However, their differentiation is much weaker. Populations from southern Italy (Calabria, Sicily) and NE Spain form a genetically homogeneous group, as do most Carpathian populations, but a large contact zone with populations exhibiting genetic admixture is present in central Italy and the western Balkans. Although the SW-NE pattern may indicate distinct colonization routes from different glacial refugia as well, these ruderal populations can spread along 'anthropogenic corridors' (e.g. roads, motorways, and abandoned fields) and expand rapidly to new areas. Therefore, considering their colonization ability and outcrossing rate, the large contact zone and high levels of genetic admixture has, in fact, been expected.

**The Comparison Between Field-Collected and Cultivated Specimens – Phenotypic Plasticity in *Picris hieracioides***—Phenotypic plasticity, the ability of a genotype to display different phenotypes in response to different environmental conditions, is a well-known phenomenon in plants. It has been viewed as a functional response that maximizes fitness in variable environments, and is thus of great interest to ecologists and evolutionary biologists (e.g. Coleman et al. 1994; Via et al. 1995). Certainly, phenotypic plasticity should be of major concern in infraspecific taxonomic studies, so as to recognize whether the variation observed (and taxonomically interpreted) deserves formal taxonomic recognition. Nevertheless, only a few taxonomic studies have addressed this issue in detail, even though it may be highly critical in some plant groups (e.g. Kaplan 2002). Different approaches to the assessment of plasticity can be seen in taxonomic studies, such as examining correlations between environmental factors and qualitative morphological characters (*Anthoxanthum amarum*; Pimentel and Sahuquillo 2007), transplantation and cultivation experiments with exposure to different values of environmental factors (*Potamogeton*; Kaplan 2002), or morphometric evaluation with two datasets, one comprising field-collected specimens, the other using specimens obtained by cultivation in a common garden experiment (*Cerastium*, Brysting and Elven 2000; *Cardamine acris*, Perný et al. 2004). This last approach was also employed here. Using canonical discriminant analyses and exploratory data analyses on two datasets (field-collected and cultivated plants) of *P. hieracioides*, we examined if characters that are potentially diagnostic for field-collected samples retain stability after cultivation (i.e. if they are reliable taxonomic markers). Two quantitative characters (LBr – the length of stem branches, and WiB – the width of the inner involucre bracts) and one binary character (DH – presence of dark hairs) exhibited the most pronounced shifts in their values when cultivated and field-collected plants were compared. Shifts were observed also in some other characters, mainly referring to plant and organ sizes, but in all cases, including the LBr and WiB characters, the relative differences between the two morphotypes were maintained in both datasets. As

expected, the ordination analyses, based either on cultivated or field-collected samples, resulted in similar patterns; they both delimited the same two morphotypes and identified the same set of diagnostic characters. Regarding the dark pigmentation (DH), dark hairs on the involucre and peduncles were found almost exclusively and quite commonly (in 43% of field-collected individuals, Supplementary Table 3) in *P. hieracioides* subsp. *umbellata* ('HA morphotype'). However, when the plants were cultivated in lowland conditions (our common garden experiment), this value dropped to only 12%. Still, the character can have a complementary diagnostic value when interpreted carefully and bearing in mind this variation. Plants of *P. hieracioides* subsp. *umbellata* ('HA morphotype') are exposed to higher UV radiation than those from the lowlands, and thus the production of flavonoids is likely stimulated in them. Flavonoid synthesis, however, is regulated by an enormously complex network of genes and regulatory factors, both external and internal, and its understanding requires further research (Koes et al. 2005).

The size and shape of stem and rosette leaves have not been included among the characters examined here. Although these features have previously been incorporated in identification keys (e.g. Padalíková 1972; Zángheri 1976), Lack, in his studies (Lack 1974; Holzapfel and Lack 1993), has already rejected their taxonomic value, noting that all characters related to leaves strongly vary, even within populations. Two populations from the Pyrenees (LLV, EST), traditionally assigned to the Iberian endemic *P. hieracioides* subsp. *riellii* (Bolòs and Vigo 1995), seem to be characterized by strongly undulate leaf margins and maintain this feature when grown from seeds and cultivated. Nevertheless, tendencies to undulate margins have been observed in other Pyrenean populations as well, and there seems to be a continuous transition from flat to strongly undulate margins. No other morphological distinction or genetic differentiation of these two Spanish populations from adjacent mountain populations were seen in this study, so we confirm that this feature has no taxonomic value.

Extremely shortened peduncles, responsible for the appearance of a dense and congested inflorescence, have been considered to be one of the diagnostic features of the south European *P. hieracioides* subsp. *spinulosa* (e.g. Sell 1976). Populations sampled at lower altitudes in the Mediterranean area indeed have generally shorter peduncles, but individuals with clearly prolonged peduncles were observed to be intermingled in several of these populations, and most importantly, this trait was not maintained in cultivation (see Supplementary Table 2).

We can conclude that phenotypic plasticity in *P. hieracioides* certainly contributes to the total morphological variation of this species, but it has only a small impact on the characters identified here as taxonomically diagnostic.

#### TAXONOMIC TREATMENT

**Identification Key to the Two Subspecies of *Picris hieracioides***—The descriptions given below and the value ranges refer to field-collected individuals. The ranges of quantitative characters correspond to the fifth and 95th percentiles, and the first and 99th percentiles are given in brackets.

1. Capitula distributed along the whole stem branches. Peduncles and outer involucre bracts with mostly pale, rarely brownish to grayish (but never black) 2-furcate and anchor-shaped hairs; inner involucre bracts (5–)6.1–10.9(–11.6) mm long, ligules (6.2–)6.9–12.4(–14.2) mm long; outermost ligules mostly with red longitudinal strips in their upper part . . . . . *Picris hieracioides* L. subsp. *hieracioides* ('lower altitude' morphotype)
1. Capitula only in the upper 1/2–1/3 of stem branches. Peduncles and outer involucre bracts with color-intermediate (brownish to grayish) or black (rarely pale) 2-furcate and anchor-shaped hairs; inner involucre bracts (9–)10–15(–16) mm long, ligules (6.9–)7.4–15.8(–19.9) mm long; red longitudinal strips in the upper part of outermost ligules typically absent, only rarely present . . . . . *Picris hieracioides* subsp. *umbellata* (Schrank) Ces. ('higher altitude' morphotype)

*PICRIS HIERACIOIDES* L., Sp. Pl. ed. 1, 2: 792. 1753; *Crepis hieracioides* (L.) Lam., Fl. Franç. 2: 111. 1778; *Hedypnois hieracioides* (L.) Huds., Fl. Angl. ed. 3: 342. 1798; *Picris undulata* Dulac, Fl. Hautes-Pyrénées: 497. 1867, nom. illeg. (Art. 52).—TYPE: designated by Lack (1975: 113): *Hortus siccus Cliffortianus* 387, no. 2 (lectotype: BM!), EPITYPE: here designated: Italy, Calabria, village of Frascineto, ruderal xerothermous outfield, 453 m a.s.l., 39°49.899' N, 16°15.305' E, 7 Jul 2005, *Slovák & Repa s. n.* (SAV!).

#### 1A *PICRIS HIERACIOIDES* L. subsp. *HIERACIOIDES*

*Picris ruderalis* F. W. Schmidt ex Willd., Sp. Pl. ed. 4, 3/3: 1558. 1803.—TYPE: [CZECH REPUBLIC] In rupibus Bohemiae circa Pragam, s. a., F. W. Schmidt s. n. (holotype: B-W 14634–010!).

*Picris spinulosa* Bertol. ex Guss., Fl. Sicul. Syn. 2/1: 400–401. 1843; *Picris hieracioides* subsp. *spinulosa* (Bertol. ex Guss.) Arcang., Comp. Fl. Ital.: 418. 1882.—TYPE: designated here [ITALY] Palermo, 1839, [*Gussone s. n.*] (lectotype: NAP-herb. Gussone!).

*Picris corymbosa* Gren. & Godr., Fl. France 2: 304. 1852.—TYPE: [FRANCE] Environs de Perpignan, 1845, *Bernard s. n.* (holotype: P).

*Picris setulosa* Guss. ex Ces., Pass. & Gibelli, Comp. Fl. Ital. 2/20: 469. 1878; *Picris hieracioides* subsp. *setulosa* (Guss. ex Ces., Pass. & Gibelli) Arcang., Comp. Fl. Ital.: 418. 1882.—TYPE: [ITALY] Palermo (original material unknown).

*Picris angustissima* Arv.-Touv., Bull. Herb. Boissier, Ser. 2, 5: 331. 1905.—TYPE: designated here, [SWITZERLAND] Terrains vagues à Locarno, 10 Sept 1904, *Chenevard s. n.* (lectotype: G 74198!).

*Picris aspera* Gilib., Fl. Lit. Inch. 1: 227. 1782, nom. inval. (Art. 32.9).

*Picris hispidissima* Lecoq & Lamotte ex Boiss. Fl. Orient. 3: 735. 1875, nom. inval., pro syn.

*Picris longifolia* Andrzej. ex Trautv., Trudy Imp. S.-Peterburgsk. Bot. Sada 8: 520. 1883, nom. inval., nom nud.

*Picris hieracioides* subsp. *euheracioides* Hayek in Hegi, Ill. Fl. Mitt.-Eur. 4/2: 1038. 1929, nom. inval. (Art. 24.3).

Annual to biennial, vigorous, 15–150(–200) cm high plants with a simple taproot, usually bearing few to numerous lateral roots. Stem slightly longitudinally grooved, green to reddish-brown, moderately to densely hairy, more or less equally hairy along the entire length. Stem moderately to densely branched; stem branches distributed usually along the entire stem, number of branches (1–)2–26(–39). Leaves usually densely hairy with 2-furcate and anchor-shaped, and simple rigid spinulose hairs. Basal leaves numerous, arranged in leaf rosettes, linear-lanceolate to widely lanceolate in shape, obtuse to acute at apex, petiolate, shallowly to deeply dentate at margin, or pinnatifid. Rosette leaves mostly

shriveled during the flowering and fruiting stage. Stem leaves (6–)7–38(–49), linear-lanceolate to obovate, petiolate, narrowed at base, upwards becoming smaller and sessile. Uppermost stem leaves lanceolate, reduced, bract-like. Peduncles (0.2–)0.3–5.2(–6.5) long, more or less tomentose, with 2-furcate and anchor-shaped hairs absent to numerous, mainly pale or brownish to grayish (never black). Peduncles with (0–)1–4(–6) bracts below capitulum, peduncle bracts of similar shape as outer involucre bracts. Capitula few to numerous, arranged in corymbose to racemose panicles, (1–)2–9(–15) per stem branch, (1–)1–4(–5) per peduncle, distributed mostly along the entire stem branches. Involucre bracts greenish to brown-black. Outer involucre bracts (11–)12–20(–22) per capitulum, (2.9–)3.1–6.1(–6.7) × (0.7–)0.7–1.6(–1.9) mm, lanceolate to obovate, obtuse to acute at apex, arranged in three irregular rows, squarrose, appressed to recurved, becoming more upright towards inner rows; usually more or less pubescent, with 2-furcate and anchor-shaped hairs absent to numerous, hairs mainly pale or brownish to grayish (never black)-colored, arranged in two or more lines. Inner involucre bracts (10–)11–15(–16) per capitulum, arranged in two rows, (5–)6.1–10.9(–11.6) × (0.7–)0.7–1.6(–1.8) mm, lanceolate to linear-lanceolate, acute at apex. Capitula with ca. 35–70 flowers, ligules yellow, red longitudinal strips in upper part of outermost ligules mostly present. Ligules (6.2–)6.9–12.4(–14.2) × (1.5–)1.6–3.1(–3.3) mm, corolla tube (2.8–)2.8–5.9(–6.7) mm long, apical teeth of ligules (0.3–)0.4–1.3(–1.7) mm long. Receptacle alveolate, without scales. Achenes brown-black to black, fusiform, slightly transversely muricate between ribs, ending abruptly into a minute beak, (2.2–)2.3–3.3(–3.6) × 0.6–1.02 mm. Pappus plumose with 2 rows of deciduous hairs, (3.3–)3.4–6.5(–6.9) mm long.

**Phenology**—Flowering from June to November.

**Habitat and Distribution**—The subspecies is found in dry xerothermous grasslands, disturbed synanthropic habitats, abandoned sites along roads, railways, lowland river terraces, orchards, vineyards, etc., generally at lower elevations (lowlands to the sub-montane belt, rarely introduced to higher altitudes) across Europe.

**Nomenclatural Notes**—(1) The specimen selected as the lectotype of the name *Picris hieracioides* L. is incomplete and cannot be critically identified for purposes of the precise application of the name on the subspecies level. Therefore, in the interest of the stability of the nomenclature, an epitype is designated here. (2) Regarding the type for *Picris corymbosa*, an herbarium specimen in P bears the label with the description in French, identical to the protologue and entitled "*Picris corymbosa* nob." The specimen was donated by Grenier to the Paris herbarium in 1875.

1B *PICRIS HIERACIOIDES* subsp. *UMBELLATA* (Schrank) Ces. in Cattaneo, Not. Nat. Civ. Lombardia 1: 303. 1844; *Picris umbellata* (Schrank) Nees ex Bluff & Fingherh., Comp.

- Fl. German. 2: 273. 1825; *Leontodon umbellatus* Schrank, Baier. Fl. 2: 334. 1789.—TYPE: designated here, GERMANY, Kreis Miesbach, Tegernseer Berge, vom Wallberghaus über den Setzberg, 11 Jul 1981, *W. Lippert* 17 880 (neotype: M!).
- Picris sonchoides* Vest, Flora (Regensburg) 3: 6. 1820; *Picris hieracioides* subsp. *sonchoides* (Vest) Thell. in Schinz & Keller, Fl. Schweiz, ed. 3, 2: 357. 1914.—TYPE: designated here, [label 1] *Sonchus oleraceus asper* / [label 2] zu St. Veit / auch in Holland, s. a. [*Wulfen s. n.*] (lectotype: W!, plant on the right side of the sheet).
- Picris paleacea* Vest, Syll. Pl. Nov.: 78. 1824; *Picris hieracioides* subsp. *paleacea* (Vest) Domin & Podp., Klíč Květ. Rep. Českoslov.: 622. 1928.—TYPE: designated here, [AUSTRIA] ex Carinthia, s. a., *Vest s. n.* (neotype: TUB!).
- Picris virens* Desf., Tabl. École Bot. ed. 3 (Cat. Pl. Hort. Reg. Paris.): 399. 1829.—TYPE: designated here, [FRANCE] H. p. [Hortus Parisiensis], s. a., *ex herb. Desfontaines* (lectotype: FI-Herb. Webb 108388!).
- Picris crepoides* Saut., Flora (Regensburg) 13: 409. 1830; *Picris hieracioides* subsp. *crepoides* (Saut.) Nyman, Consp. Fl. Eur.: 467. 1879.—TYPE: designated here, [AUSTRIA] . . . infra Kitzbühl [?], s. a., *Sauter s. n.* (lectotype: W!).
- Picris grandiflora* Ten., Syll. Pl. Fl. Neapol.: 397, 1831; *Picris hieracioides* subsp. *grandiflora* (Ten.) Arcang., Comp. Fl. Ital.: 418. 1882.—TYPE: designated here, [ITALY] [label 1] Selve di Chiarino, e di Pietra Camelo / [label 2] illegible, s. a., [*Tenore s. n.*] (lectotype: NAP! largest fragment on the sheet), EPITYPE: designated here, ITALY, Abruzzo, national park Grand Sasso, loco dicto Prati di Tivo located above Pietra Camella, on the rocky slopes near the trail, 42°29'942" N, 13°33'825" E, 1,434 m, 9 Jul 2005 *Slovák and Repa s. n.* (SAV!).
- Picris villarsii* Jord., Cat. Graines Jard. Dijon: 29–30. 1848, nom. nov.; *Picris hieracioides* subsp. *villarsii* (Jord.) Nyman, Consp. Fl. Eur.: 467. 1879; *Picris pyrenaica* Vill., Hist. Pl. Dauphiné 3: 148. 1789, nom. illeg. (Art. 53), non L. 1753.—TYPE: designated here, *Picris pyrenaica* Gaert. Vill. 3 p. 148 h. n°1145 DC. Sup. n°2974a / *tuberosa* Laper / *hieracium pyrenaicum* y. Wild. / h. en Valgaudemard, s. a. [*Villars? s. n.*] (lectotype: GRM MHNGr.1837.27597!), EPITYPE: designated here, [FRANCE] Provence-Alpes-Côte d'Azur, mountain pass Col du Lautaret, grassland near the Alpine Botanical Garden, 45°02'092" N, 06°24'239" E, 2,067 m, 15 Aug 2004, *Slovák* (SAV!).
- Picris longifolia* Boiss. & Reut., Pugill. Pl. Afr. Bor. Hispan.: 69. 1852; *Picris hieracioides* subsp. *longifolia* (Boiss. & Reut.) P.D. Sell, Bot. J. Linn. Soc. 71: 248. 1975.—TYPE: designated here, [SPAIN] Sierra Nevada, région sous-alpine, au Cortijo de S. Geronimo, 4 Jul 1851, *Bourgeau Pl. d'Espagne*, no. 1281 (lectotype: G-Boiss!).
- Picris crinita* Reut., Compt.-Rend. Trav. Soc. Hallér.: 111. 1854–1856.—TYPE: designated here, [ITALY] La Grigna, in herbosis veg. media descenté sur Mondello, Aug 1854, *Reuter s. n.* (lectotype: G 74199!).
- Picris auriculata* Sch. Bip., Cichoriaceothesca Suppl., No. 124. 1863; *Picris hieracioides* subsp. *auriculata* (Sch. Bip.) Hayek in Hegi, Ill. Fl. Mitt.-Eur. 4/2: 1039. 1929.—TYPE: designated here, [FRANCE] Alsatia, in Vogesorum m. granitico Hoheneck, alt. 3,500', 10 Aug 1862 [flowering specimen], *Martin s. n.* (lectotype: P!).
- Picris oligocephala* Schur, Enum. Pl. Transsilv.: 361. 1866.—TYPE: designated here, [ROMANIA] In pascuis subalpinis Transsilv. . . . Piatra Mare, Kalk, Jul, s. a., *Schur s. n.* (lectotype: LW!).
- Picris monticola* Lamotte, Prodr. Fl. Plat. Centr. 2: 453. 1881.—TYPE: designated here, [FRANCE] Mont-Dore, vallé des bains, en allaut à . . . , 18 Jul 1849, [*Lamotte s. n.*] (lectotype: CLF!).
- Picris tatrae* Borb., Magy. Bot. Lapok 1: 318. 1902; *Picris hieracioides* subsp. *tatrae* (Borbás) Domin & Podp., Klíč Květ. Rep. Českoslov.: 622. 1928.—TYPE: designated here, [SLOVAKIA] in alpinis calcareis Tatrae ad Barlangliget [Tatranská Kotlina], 3,000'–4,000', m, 24 Jul 1892, *Borbás s. n.* (lectotype: BP 202560!).
- Picris senecioides* Beauverd, Bull. Soc. Bot. Genève Ser. 2, 18: 325. 1926.—TYPE: [FRANCE]. Garides de Lourdens, sur Montmélian, Bourges, 17 Sept 1926, *Beauverd s. n.* (holotype: G 74201!).
- Picris rielii* Sennen, Bol. Soc. Iber. Ci. Nat. 28 (11): 170. 1929; *Picris hieracioides* subsp. *rielii* (Sennen) Bolós & Vigo, Fontqueria 14: 9. 1987.—TYPE: designated here, [SPAIN] Cerdagne: Llivia et Estavar, marges, 1,220–1,320 m, 30 Aug, 2 Sept 1925, *Sennen, Plantes d'Espagne* 5353 (lectotype: MA 138382!, plant on the left side of the sheet).
- Picris tatrae* Borb., Természettud. Közl. 26: 498. 1894, nom. inval., nom. nud.
- Picris rielii* Sennen, Bull. Soc. Bot. France 73 (1926): 656. 1927, nom. inval., nom. nud.

Biennial to short-lived perennial, 15–100 cm high plants with a simple taproot, usually bearing few to numerous lateral roots. Stem slightly longitudinally grooved, green to reddish-brown, moderately to densely hairy, hair size and density decreasing upwards. Stem sparsely to moderately branched; stem branches distributed usually in upper half or third of the stem, number of branches (1)–2–12(–16). Leaves usually densely hairy with 2-furcate and anchor-shaped, and simple rigid hairs, rather soft. Basal leaves numerous, arranged in rosettes, linear-lanceolate, ovate to obovate in shape, obtuse to acute at apex, petiolate, entire to shallowly dentate, crenate, or rarely undulate at margin. Rosette leaves mostly persisting during the flowering and fruiting stage. Stem leaves (4)–5–20(–23), linear-lanceolate to obovate, petiolate, narrowed at base, upwards becoming smaller and sessile, semi-amplexicaul to amplexicaul, often auriculate. Uppermost stem leaves lanceolate, reduced, bract-like. Peduncles (0.2)–0.3–7.6(–10.1) long, more or less tomentose, with 2-furcate and anchor-shaped hairs absent to numerous, pale to black-colored. Peduncles with (1)–1–6(–9) bracts below capitulum, peduncle bracts of similar shape as outer involucre bracts. Capitula few to numerous, arranged in corymbose panicles, (1)–1–11(–15) per stem branch, (1)–1–4(–4) per peduncle, distributed mostly in upper half or third of stem branches. Involucre bracts greenish to brown-black. Outer involucre bracts (8)–8–18(–20) per capitulum, (2.7)–3.1–6.7(–7.3) × (0.6)–0.6–1.6(–2) mm, lanceolate to obovate, obtuse to acute at apex, arranged in three irregular rows, squarrose, appressed to recurved, becoming more upright towards inner rows; usually more or less pubescent, with 2-furcate and anchor-shaped

hairs absent to numerous, hairs pale, brownish, grayish to black-colored, arranged in two to more lines. Inner involucre bracts (9–)10–15(–16) per capitulum, arranged in two rows, (6.9–)7.4–12.7(–13.7) × (0.7–)0.7–1.8(–2) mm, lanceolate to linear-lanceolate, acute at apex. Capitula with ca 30–60 flowers, ligules yellow, red longitudinal strip in upper part of outermost ligules mostly missing, rarely present. Ligules (6.9–)7.4–15.8(–19.9) × (1.6–)1.8–3.5(–4.0) mm, corolla tube (2.5–)2.8–6.7(–9.7) mm long, apical teeth of ligules (0.3–)0.4–1.7(–2.2) mm long. Receptacle alveolate, without scales. Achenes red-brown to black-brown, fusiform, slightly transversely muricate between ribs, ending abruptly into a minute beak, (2.5–)2.5–3.7(–4.1) × 0.6–1.03 mm. Pappus plumose with 2 rows of deciduous hairs, (4.1–)4.2–7.7(–8.2) mm long.

**Phenology**—Flowering from May to October.

**Habitat and Distribution**—The subspecies is found in tall herb communities in the montane to alpine belt, tall grass species-rich communities on relatively dry and warm slopes of the supramontane and subalpine belt; subalpine communities of deciduous shrubs, penetrating also in non-forest synanthropic habitats in mountain valleys, along forest roads and in other secondary habitats in mountains. Generally it grows at higher altitudes (montane to alpine belt, rarely introduced to lower altitudes) across European mountain ranges.

**Nomenclatural Notes**—(1) No original material of the name *Leontodon umbellatus* Schrank was found in BR (Piet Stoffelen pers. comm.) nor M, where herbarium specimens by Schrank are known to be deposited. Therefore, a neotype is designated here for this name from the locality mentioned in the protologue. (2) There are three plant fragments on the sheet with the selected lectotype of the name *Picris sonchoides* Vest. Two of them belong to *P. hieracioides* subsp. *umbellata*, the third one is apparently a leaf of some *Sonchus* species. Although the specimen is undated, it belonged to Wulfen's herbarium and was apparently the one that Vest referred to in the protologue. (3) No dated original material or material collected by Vest from the locality mentioned in the protologue of *Picris paleacea* Vest is available. Therefore, a neotype was selected from the material collected by Vest in the region neighboring that mentioned in the protologue. (4) There are several badly damaged plant fragments on the herbarium sheet representing the original material of the name *Picris grandiflora* Ten. There are also three different localities on two labels on this sheet, one locality is illegible. Because of the extent of the damage, the specimen cannot be unequivocally identified for the purpose of the interpretation of the name. Therefore, apart from the lectotypification of the name by this specimen, an appropriate epitype is designated here. (5) Referring to the name *Picris pyrenaica* Vill., Lack (1975) wrote that its earlier homonym *P. pyrenaica* L. (1753) is related to a species of *Crepis*, but noted that it cannot be taken up there as the epithet was already occupied by *C. pyrenaica* (L.) Greuter, based on *Hieracium pyrenaicum* L. (Linnaeus 1753). There is another later homonym of *P. pyrenaica* Vill., namely *P. pyrenaica* (L.) Gaertn., based on *Hieracium pyrenaicum* L. (6) There are two specimens in Villars' herbarium that bear the name *P. pyrenaica*. The first of them, with a label in Villars' hand, was collected in 1811 in the Simplon Pass in Switzerland and does not belong to the original material. The second specimen bears the above mentioned label, which is, however, written by Artus de Miribel, a botanist who rearranged Villars' herbarium in

1827 (Vincent Poncet pers. comm.). In addition, this second specimen is badly damaged and is demonstrably ambiguous, as such, it cannot be identified for the precise application of the name. Therefore, as this specimen is designated here as a lectotype, an appropriate epitype is chosen. (7) There is no relevant specimen identified as *Picris longifolia* Boiss. & Reut. collected by Reuter (as referred to in the protologue) in Boissier's herbarium in G. Nevertheless, there are specimens collected by Bourgeau in 1851 at the type locality, which were distributed as exsiccate under the name "*Picris longifolia* Boiss. et Reut.!" This indicates that this collection belongs to the original material and the specimen from Boissier's herbarium is designated here as the lectotype.

**ACKNOWLEDGMENTS.** We would like to thank Fabio Conti (Barisciano), Giannantonio Domina (Palermo), Rolland Douzet (Grenoble), Iva Hodálová (Bratislava), Nicodemo Passalacqua (Cosenza), Marián Perný (Bratislava), Peter Repa (Bratislava), and Luis Villar (Jaca) for their assistance in the field and to the parents of M. S. for their general support. We also appreciate valuable comments of two anonymous reviewers. Special thanks go to Dušan Senko (Bratislava) for preparing the map of the sample sites and to Zlata Komárová for preparing the illustration of the morphological characters. This study was supported by the Research and Development Support Agency (project no. LPP-0239-09 to Karol Marhold).

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- 1,510 m, 42° 39.766'N, 00° 06.509'W, 19, -, -, -; **BAL**, ES, Aragón, Bañario de la Panticosa, 1,576 m, 42° 45.029'N, 00° 14.557'W, 20/6, 4/4, 0.0654, 0/17; **CAN**, ES, Aragón, Candanchú, 1,555 m, 42° 47.401'N, 00° 31.532'W, 20/2, -, -, -; **VIE**, ES, Catalonia, Vielha, 1,230 m, 42° 41.190'N, 00° 47.241'W, 20/8, 5/5, 0.0365, 0/11; **ESP**, ES, Catalonia, Espot, 1,576 m, 42° 33.547'N, 01° 05.416'E, 20/4, 5/5, 0.0451, 2/13; **MONT**, ES, Catalonia, between Montseny and el Brull, 740 m, 41° 46.702'N, 02° 23.780'E, 0/3, 3/3, 0.0277, 0/6; **LLV**<sup>6</sup>, ES, Catalonia, Llívia, 1,170 m, 42° 26.633'N, 01° 56.531'E, 20/5, 4/4, 0.0285, 0/11; **SOL**, AD, Andorra, Soldeu, 1,900 m, 42° 34.170'N, 01° 40.691'E, 20/4, 4/4, 0.0259, 0/6; **EST**<sup>6</sup>, FR, Languedoc-Roussillon, Estavar, 1,640 m, 42° 29.915'N, 02° 00.815'E, 20/8, 2/2, -, 1/13; **BDA**<sup>8</sup>, FR, Franche-Comté, Bois-d'Amont, 1,102 m, 46° 28.738'N, 06° 05.181'E, 20, 4/4, 0.0467, 1/8; **CDL**<sup>8</sup>, FR, Provence-Alpes-Côte d'Azur, Col du Lautaret, 2,067 m, 45° 02.092'N, 06° 24.239'E, 22/8, 7/7, 0.0579, 0/13; **VAL2**<sup>8</sup>, FR, Rhône-Alpes, la Rivine, 1,556 m, 45° 04.138'N, 06° 25.178'E, 20/4, 5/5, 0.0604, 1/17; **VAL1**<sup>8</sup>, FR, Rhône-Alpes, Valloire, 1,204 m, 45° 10.000'N, 06° 25.600'E, 15, -, -, -; **LAU**<sup>8</sup>, FR, Provence-Alpes-Côte d'Azur, Le Monétier-les-Bains, 1,700 m, 45° 00.573'N, 06° 28.179'E, 20, -, -, -; **UGI**<sup>8</sup>, FR, Rhône-Alpes, Ugine, 552 m, 45° 45.503'N, 06° 28.304'E, 15, 3/3, 0.0572, 1/9; **BR1**<sup>8</sup>, FR, Provence-Alpes-Côte d'Azur, Briançon, 1,308 m, 44° 55.184'N, 06° 37.261'E, 40/12, -, -, -; **FIN**<sup>8</sup>, FR, Franche-Comté, Les Fins, 911 m, 47° 05.181'N, 06° 38.356'E, 16, 3/3, 0.0387, 0/8; **PDR**<sup>8</sup>, FR, Franche-Comté, Pont-de-Roide, 384 m, 47° 22.612'N, 06° 46.015'E, 15, 4/4, 0.0353, 0/4; **ORM**, IT, Piemonte, Ormea, 750 m, 44° 09.296'N, 07° 55.431'E, 20, -, -, -; **LIM**, IT, Piemonte, Limonetto, 1,600 m, ca 44° 12'N, 07° 34'E, 20/25, 2/2, -, 0/12; **BRE**, IT, Piemonte, Breia, 799 m, 45° 45.897'N, 08° 18.300'E, 17, 3/3, 0.0516, 1/13; **GAT**, IT, Piemonte, Gatina, 289 m, 45° 36.520'N, 08° 19.434'E, 20, 5/5, 0.0631, 2/20; **SGA**, IT, Trentino-Alto Adige, San Giacomo – Brentonico, 1,162 m, 45° 47.963'N, 10° 54.831'E, 15, 4/4, 0.0747, 1/19; **FUG**, IT, Trentino-Alto Adige, Passo Piano delle Fugazze, 1,156 m, 45° 45.638'N, 11° 11.145'E, 15, 3/3, 0.0479, 0/13; **REFU**<sup>3</sup>, IT, Abruzzo, Prati di Tivo, 1,434 m, 42° 29.942'N, 13° 33.825'E, 25, 5/5, 0.0412, 1/12; **VSR**<sup>3</sup>, IT, Abruzzo, Valle di Selva Romana, 1,575 m, 42° 07.632'N, 14° 07.381'E, 29, 5/5, 0.0418, 2/11; **TIT**, DE, Baden-Württemberg, Titisee, 853 m, 47° 54.537'N, 08° 09.700'E, 19, 3/3, 0.0461, 0/7; **HEI**, DE, Baden-Württemberg, Heidelberg-Schlierbach, 100 m, 49° 24.470'N, 08° 44.554'E, 17, -, -, -; **GRA**, DE, Bayern, Graseck, 768 m, 47° 28.217'N, 11° 07.114'E, 16, 3/3, 0.0572, 0/10; **FAL**<sup>3</sup>, DE, Bayern, Fall, 780 m, 47° 34.000'N, 11° 31.400'E, 15, 3/3, 0.0498, 0/9; **ACHE**, AT, Tirol, Achenkirchen, 940 m, 47° 32.187'N, 11° 42.499'E, 9, 2/2, -, 0/3; **FEL**, AT, Vorarlberg, Feldkirch, 550 m, 47° 53.600'N, 11° 50.600'E, 17, 3/3, 0.0221, 0/7; **KIT**<sup>2</sup>, AT, Tirol, Kitzbühel, 930 m, 47° 28.200'N, 12° 23.807'E, 20, 5/4, 0.0405, 0/13; **HOL**<sup>2</sup>, AT, Salzburg, Hollersbach im Pinzgau, 898 m, 47° 16.48'N, 12° 25.081'E, 13, -, -, -; **ANN**<sup>5</sup>, AT, Niederösterreich, Annaberg, 519 m, 47° 54.984'N, 15° 26.399'E, 20/13, -, -, -; **SCHN**<sup>5</sup>, AT, Niederösterreich, Mt. Schneeberg, 543 m, 47° 44.225'N, 15° 44.086'E, 20/12, -, -, -; **OTS**, AT, Niederösterreich, Mt. Ötscher, ca 1,000 m, ca 47° 52'N, 15° 80'E, 0/9, -, -, -; **MAR**<sup>\*</sup>, SK, Malé Karpaty, Záhorská Bystrica, 220 m, 48° 14.086'N, 17° 03.231'E, 13, 7/7, 0.0613, 1/18; **STU**<sup>\*</sup>, SK, Malé Karpaty, Stupava, 224 m, 48° 16.894'N, 17° 03.594'E, 18, 7/7, 0.0769, 0/17; **FAC**<sup>\*</sup>, SK, Velká Fatra, Fačkovské sedlo saddleback, 1,220 m, 48° 58.660'N, 18° 37.063'E, 20/15, -, -, -; **KLAK**, SK, Velká Fatra, Mt. Klak, 1,290 m, 48° 58.466'N, 18° 38.319'E, -, 4/4, 0.0109, 0/3; **DON**<sup>\*</sup>, SK, Nízke Tatry, Donovaly, 867 m, 48° 52.624'N, 19° 12.156'E, 20, -, -, -; **VLK**<sup>\*</sup>, SK, Velká Fatra, Vlkolínec, 586 m, 49° 01.921'N, 19° 16.501'E, 20, -, -, -; **LUC**<sup>\*</sup>, SK, Chočské vrchy, Lúčky, 643 m, 49° 8.514'N, 19° 23.055'N, 20, 5/1, 0, 0/7; **JAN**<sup>\*</sup>, SK, Nízke Tatry, Jánska dolina valley, 771 m, 49° 05.480'N, 19° 40.505'E, 20, -, -, -; **ZDI**<sup>\*</sup>, SK, Belianske Tatry, Ždiarska dolina valley, 890 m, 49° 16.099'N, 20° 14.991'E, 20, 5/4, 0.0412, 1/11; **MAD**<sup>\*</sup>, SK, Západné Tatry, Mačie Diery, 900 m, 49° 15.525'N, 19° 40.234'E, 20/13, 5/5, 0.0491, 1/15; **SCHB**<sup>\*</sup>, SK, Slovenský Raj, Suchá Belá gorge, 942 m, 48° 56.081'N, 20° 22.853'E, 20, -, -, -; **GER**<sup>\*</sup>, SK, Slovenský Raj, Geravy, 860 m, 48° 52.194'N, 20° 23.476'E, 18, -, -, -; **CEK**<sup>\*</sup>, SK, Pieniny, Červený Kláštor, 468 m, 49° 23.341'N, 20° 25.325'E, 20/17, -, -, -; **SPH**<sup>\*</sup>, SK, Spišské vrchy, Spišské Hanušovce, 616 m, 49° 19.524'N, 20° 20.937'E, 20/11, -, -, -; **BAN**, RO, Hunedoara, Bănița, 540 m, 45° 28.387'N, 23° 11.951'E, 20, 4/4, 0.0545, 0/9; **TIM**, RO, Brașov, Brașov, 600 m, 45° 39.516'N, 25° 34.636'E, 19, -, -, -.

APPENDIX 1. Plant material analyzed in the present study. Localities are arranged geographically from the west to the east. Data are presented in the order of populational code (in bold), locality, altitude, latitude, longitude, the number of the field-collected/cultivated plants used for the morphometric analyses, the number of the plants used for AFLP analyses/the number of AFLP genotypes, Nei's gene diversity ( $D_{Nei}$ ), the number of private/rare AFLP markers per population. Country abbreviations: AD – Andorra, AT – Austria, DE – Germany, ES – Spain, FR – France, HR – Croatia, IT – Italy, JP – Japan, KG – Kyrgyz Republic, ME – Montenegro, RO – Romania, SI – Slovenia, SK – Slovakia. Super-scripts in populational codes indicate that the locality sampled corresponds to the type locality or a broader area specified in the protologue of the following names: <sup>1</sup> – *Picris auriculata* Sch. Bip., <sup>2</sup> – *P. crepidoides* Saut., <sup>3</sup> – *P. grandiflora* Ten., <sup>4</sup> – *P. longifolia* Boiss. & Reut., <sup>5</sup> – *P. paleacene* Vest., <sup>6</sup> – *P. rielii* Sennen, <sup>7</sup> – *P. spinulosa* Bertol., <sup>8</sup> – *P. villarsii* Jord., <sup>9</sup> – *P. umbellata* (Schrank) Ces. Asterisks in the codes indicate the population samples that were included in Slovák and Marhold (2007).

*Picris hieracioides* subsp. *umbellata* ('higher altitude' morphotype)—**PRA**<sup>4</sup>, ES, Andalusia, Pradolano, 2,370 m, 37° 05.686'N, 03° 23.516'W, 20, 5/5, 0.0232, 0/8; **CAP**<sup>4</sup>, ES, Andalusia, Capileira, 1,606 m, 36° 57.703'N, 03° 21.457'W, 20/11, 5/5, 0.0219, 0/6; **TOR**, ES, Aragón, Torla, Bujaruelo,

*Picris hieracioides* subsp. *hieracioides* ('lower altitude' morphotype)—**LEI**, ES, Catalonia, Tàrraga, ca 460 m, ca 41° 39'N, 01° 12'E, 19/5, 5/5, 0.0412, 1/14; **MUN**, FR, Alsace, Munster, 853 m, 47° 54.537'N, 07° 09.700'E, 18, 4/4, 0.0477, 0/9; **VOG**<sup>1</sup>, FR, Alsace, Linthal, 944 m, 47° 56.378'N, 07° 03.789'E, 15, -, -, -; **SAG**<sup>7</sup>, IT, Sicily, Sagana, 637 m, 38° 04.756'N, 13° 12.620'E, 20, -, -, -; **MTC**<sup>7</sup>, IT, Sicily, Palermo, Mt. Monte Cuccio, 611 m, 38° 06.938'N, 13° 14.543'E, 20, -, -, -; **TAC**<sup>7</sup>, IT, Sicily, Termini-Caccamo, 612 m, 38° 57.112'N, 13° 43.170'E, 20, 4/4, 0.0327, 0/7; **PIZ**<sup>7</sup>, IT, Sicily, Piano

Zucchi, ca 1,100 m, ca 37° 54'N, 13° 59.3'E, 0/21, 6/6, 0.0479, 0/10; **MDR**, IT, Friuli-Venezia Giulia, Madrisio, 10 m, 45° 51.694'N, 12° 59.000'E, 17, 3/3, 0.0424, 0/5; **RIM**, IT, Abruzzo, Fornace, 620 m, 42° 03.944'N, 13° 02.172'E, 14, 4/4, 0.0384, 0/10; **CAM**, IT, Abruzzo, Assergi Campo, 1,236 m, 42° 28.759'N, 13° 21.718'E, 11, 3/3, 0.0738, 0/18; **AQU**, IT, Abruzzo, L'Aquila, 421 m, 42° 12.103'N, 13° 24.256'E, 20/11, 5/5, 0.0551, 1/16; **CRO**, IT, Abruzzo, Campo Felice, 1,570 m, 42° 13.928'N, 13° 24.646'E, 20, 3/3, 0.0424, 0/11; **PIC**, IT, Abruzzo, Pietracamela, 778 m, 42° 32.323'N, 13° 32.877'E, 14, -, -, -; **STC**, IT, Abruzzo, San Colombo, 1,100 m, 42° 20.341'N, 13° 36.341'E, 20, -, -, -; **BLH**, IT, Abruzzo, Majella, Block House, 1,566 m, 42° 10.752'N, 14° 06.740'E, 20, -, -, -; **PES**, IT, Abruzzo, Pescara, 6 m, 42° 27.489'N, 14° 12.596'E, 20/7, -, -, -; **MUR**, IT, Basilicata, Muro Lucano, 750 m, 40° 15.573'N, 15° 27.420'E, 13, 2/2, -, 0/6; **POL**, IT, Calabria, Mt. Pollino, 1,450 m, 39° 53.605'N, 16° 04.732'E, 20, -, -, -; **SPI**, IT, Calabria, Spineto Manco, 1,292 m, 39° 21.404'N, 16° 13.718'E, 15, -, -, -; **MULA**, IT, Calabria, Mt. Mula, ca 1,900 m, 39° 45.000'N, 16° 01.000'E, -, 5/5, 0.0305, 2/8; **FRA**, IT, Calabria, Frascineto, 453 m, 39° 49.899'N, 16° 15.305'E, 15, 4/4, 0.0363, 0/4; **CON**, IT, Calabria, Cosenza, 225 m, 39° 10.340'N, 16° 32.706'E, 20, 3/3, 0.0332, 0/8; **SOL**, AT, Niederösterreich, Sollenau, 346 m, 48° 09.206'N, 16° 57.471'E, 20, -, -, -; **KRA**, SI, Gorenjska županija, Kranjska Gora, 864 m, 46° 28.324'N, 13° 47.105'E, 19, -, -, -; **LUP**, HR, Istarska županija, near Lupoglav, 386 m, 45° 19.305'N, 14° 09.420'E, 14, -, -, -; **VRA**, HR, Primorsko-goranska županija, Cres, Vrana, 158 m, 44° 48.329'N, 14° 25.265'E, 15, 2/2, -, 1/9; **VAB**, HR, Primorsko-goranska županija, Krk, Valbiska, 4 m, 45° 01.673'N, 14° 29.877'E, 5, -, -, -; **PIRO**, HR, Zadarska županija, Pirovac, 24 m, 43° 49.340'N, 15° 40.140'E, 20/1, 3/3, 0.0479, 0/11; **KRA**, HR, Krapinsko-zagorska županija, Krapina, 224 m, 46° 09.390'N, 15° 52.440'E, 20, 4/4, 0.0705, 0/19; **IVA**, HR, Varaždinska županija, Ivanec, 232 m, 46° 13.230'N, 16° 07.120'E, 6, 2/2, -, 0/6; **PLM**, HR, Dubrovačko-neretvanska županija, Ploče, 17 m, 43° 00.937'N, 17° 33 083'E, 15, -, -, -; **RET**, HU, Fejér, Rétság, 106 m, 46° 48.734'N, 18° 38.423'E, 20, -, -, -; **LAM**\*, SK, Podunajská nížina lowlands, Bratislava-Lamač, 202 m, 48° 11.951'N, 17° 02.672'E, 15, -, -, -; **CUN**\*, SK, Podunajská

nížina lowlands, Čunovo, 119 m, 48° 02.388'N, 17° 10.692'E, 19, -, -, -; **BA**, SK, Podunajská nížina lowlands, Bratislava-Petržalka, 132 m, 48° 05.578'N, 17° 05.556'E, -, 4/4, 0.0628, 0/18; **LOP**\*, SK, Biele Karpaty, Nová Bošáca-Lopeniček, 337 m, 48° 52.938'N, 17° 47.592'E, 20, -, -, -; **KOM**\*, SK, Podunajská nížina lowlands, Komárno, 275 m, 47° 46.652'N, 18° 08.735'E, 20, -, -, -; **VRS**\*, SK, Biele Karpaty, Mt. Vršatec, 730 m, 49° 04.172'N, 18° 09.089'E, 20, -, -, -; **BANB**\*, SK, Podunajská nížina lowlands, Bánovce nad Bebravou, 162 m, 48° 58.660'N, 18° 15.348'E, 19, -, -, -; **VAC**\*, SK, Strážovské vrchy, Mt. Vápeč, 543 m, 48° 56.475'N, 18° 18.916'E, 19, 2/2, -, 1/13; **VAP**\*, SK, Podunajská nížina lowlands, Vápnik, 260 m, 48° 11.022'N, 18° 38.639'E, 20/18, -, -, -; **VRT**\*, SK, Kováčovské kopce, Vršok, 221 m, 47° 49.351'N, 18° 39.169'E, 20/12, -, -, -; **PIL**\*, SK, Pohronský Inovec, Píla, 470 m, 48° 32.175'N, 18° 39.246'E, 0/10, -, -, -; **CHLA**\*, SK, Kováčovské kopce, Chlába, 142 m, 47° 50.071'N, 18° 49.053'E, 20, -, -, -; **DEM**\*, SK, Nízke Tatry, Demänovská dolina valley, 677 m, 49° 02.192'N, 19° 34.611'E, 29/8, -, -, -; **HAI**\*, SK, Cerová vrchovina, Hájnačka, 212 m, 48° 14.956'N, 19° 57.939'E, 20/10, -, -, -; **LPT**\*, SK, Popradská kotlina basin, Liptovský Tmovec, 588 m, 49° 06.926'N, 20° 20.932'E, 20/8, 4/4, 0.0887, 1/19; **SOR**\*, SK, Slovenský Kras, Soroška pass, 544 m, 48° 37.053'N, 20° 37.805'E, 20, 4/4, 0.0622, 2/17; **ORA**, RO, Bihor, Oradea and Chişlaz, 180 m, 47° 16.129'N, 22° 13.658'E, 19, 3/3, 0.0258, 0/8; **DEV**, RO, Hunedoara, Deva, 220 m, 45° 50.012'N, 22° 56.341'E, 20/5, 5/5, 0.0465, 0/9; **PUI**, RO, Hunedoara, Bara, 260 m, 45° 31.935'N, 23° 01.951'E, 19, 4/4, 0.0446, 0/12; **FAG**, RO, Braşov, Făgăraş, 415 m, 45° 49.935'N, 25° 01.951'E, 20, -, -, -

**Picris hispidissima**—**BUK**, ME, Bar, Bukovnik, 614 m, 42° 13.260'N, 18° 57.973'E, -, 4/4, 0.0420, 0/22; **DUB**, HR, Dubrovačko-neretvanska županija, Dubrovnik, 145 m, 42° 38.559'N, 18° 07.296'E, -, 6/6, 0.0503, 3/23.

**Picris japonica**—**JP106**, JP, Akita pref., Kitaakita-gun, Tashiro-cho, Hirataki, 339 m, 40° 22.387'N, 140° 26.331'E, -, 2/1, -, 0/13; **JP126**, JP, Hokkaido, Atsuta-gun, Atsuta-mura, Morai, 66 m, 43° 21.006'N, 141° 29.422'E, 0/14, 3/1, 0.0018, 0/13.

**Picris nuristanica**—**NUR**, KG, Tian-Schan, Fergana (Fergana Kyrka Toosu), 2,800 m, 41° 15.450'N, 73° 37.280'E, 0/8, 7/5, 0.0146, 5/22.