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Evolutionary mechanisms and relationships among taxa of genus *Pilosella*

Evoluční mechanismy a vztahy mezi taxony
rodu *Pilosella*

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Content

Papers included in the thesis

Hereby I declare that I made this thesis independently, using the mentioned references. I have not submitted or presented any part of this thesis for any other degree or diploma.

Tomáš Urfus (in Prague 8. 8. 2011)

Author contribution statement

I declare that I made substantial part of the thesis papers (manuscripts). Contribution to each paper is specified below:

1. Tomáš Urfus, František Krahulec and Anna Krahulcová - Hybridization within *Pilosella* population: a morphometric analysis: collection of all morphometric data, their analysis, ca 50% of paper text and corrections.

2. Patrik Mráz, Barbora Šingliarová, Tomáš Urfus and František Krahulec - Cytogeography of *Pilosella officinarum* (Compositae): Altitudinal and Longitudinal Differences in Ploidy Level Distribution in the Czech Republic and Slovakia and General Pattern in Europe: majority of data originating from Czech Republic (field work, flow cytometry - 65%), distribution maps and methodical chapter production, a part of discussion and entire text corrections.

3. Tomáš Urfus and František Krahulec - Morphological variation of *Pilosella officinarum*: whole dataset (fieldwork, cytometry, morphometrics, multivariate analyses, reproductive modes analyses) and majority of text.

4. Marek Slovák, Petr Vít, Tomáš Urfus and Jan Suda - Complex pattern of genome size variation in a polymorphic member of the Asteraceae and **5. Marek Slovák, Tomáš Urfus, Petr Vít and Karol Marhold - The Balkan endemic *Picris hispidissima* (Compositae): morphology, nuclear DNA content and relationship to the polymorphic *P. hieracioides***: substantial contribution to experiments planning, flow cytometric part, methodical part writing, methodical optimalizations and text writing.

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Summary

Several case studies at different levels (population, regional, comparative etc.) were carried out to evaluate the importance of different sources of variation and to follow microevolutionary traits in *Pilosella* (Asteraceae). The gradient of different spatial levels and comparative studies of closely related taxa group (characterised by partly different type of ongoing microevolution - genus *Picris*) revealed some common processes while at the same time highlighted the uniqueness of genus *Pilosella*. Microevolutionary potential was studied at the population level using morphometric approach (hybridization tendencies of highly complex hybrid swarm in Prague - Praha Vysočany). Cytogeography of *Pilosella officinarum* was then investigated at the regional scale (Central Europe). Tetraploid cytotype was detected almost exclusively in Bohemia region and further to the west whereas pentaploid and hexaploid cytotypes prevailed in Moravia and Slovakia and further to south-east. Cytogeography was followed by morphometric evaluation of three most abundant ploidy levels (4x, 5x and 6x) of *P. officinarum* combined with the analyses of reproduction mode. Results confirmed the possibility to distinguish the particular cytotypes (intermediate pentaploids partly overlapping with the tetra- and hexaploids). Different morphological traits of sexual vs. apomictic hexaploids were uncovered. Finally the microevolutionary processes of *Pilosella* were compared to those observed in *Picris hieracioides* group. The existence of two distinct morphological groups of *P. hieracioides* was confirmed by differences in their absolute genome size. Genome size and morphology of Balkan endemic *Picris hispidissima* were found to be significantly different compared to the closely related taxon to *P. hieracioides.* The comparative case studies of *Picris* revealed highly distinct microevolutionary pattern compared to genus *Pilosella.*

General introduction

The tribe Cichorieae Lam. & DC. (syn. Lactuceae Cass.) represents one the most studied groups of Asteraceae family especially thanks to several genera such as *Crepis* L., *Hieracium* L., *Pilosella* Hill. or *Taraxacum* Wigg. and economically important genera as *Lactuca* L. Cichoriaea is well morphologically and anatomically defined (occurrence of milky latex and homogamous capitula with 5-dentate, lingulate flowers have been constantly respected characters since the time of its description; Tournefort 1964). Taxonomic situation inside of the tribe is more complicated, i.e. that the majority of above mentioned genera are generally well known because of polyploidy, hybridization and apomixis frequently resulting in highly intricated taxonomic classifications. The aim of a taxonomic treatment is to mirror real situation in nature usually expressed by numerous "microspecies" representing particular apomictic lineages[1]. Nevertheless, system of these complex taxonomic treatments can hardly be natural as hybridization (even an ancient one) results in reticulate evolution.

Hieracium **s.l.**

Formal genus *Hieracium* sensu lato has recently been more frequently treated as two distinct genera (*Hieracium* and *Pilosella*; Sell & West 1975, Bräutigam & Greuter 2007), which is why genus ranks *Hieracium* and *Pilosella* are used separately in further text. Genera *Hieracium* and *Pilosella* comprise perennial herbs with 1 to numerous stems (foliaged or leafless) terminated by 1 to numerous capitula. Leaves are shaped from entire to deeply dentate or lobed. Involucres are formed by several irregularly imbricate rows of linear to lanceolate bracts. Individual ligules are usually yellow (sometimes with a red stripe on the outer face or rarely orange, reddish, green or white). Achenes are covered in 10 to 13 ribs of narrowly obconical shape (never beaked) carrying 1 or 2-rowed pappus (e.g. Zahn 1921, 1930, Sell & West 1976, Bräutigam & Schuhwerk 2002, Chrtek 2002, 2004).

Pilosella

Genus *Pilosella* differs from *Hieracium* in several important characters. Cypselas length is up to 2.5 mm (genus *Hieracium* 2.5 - 5mm) and they are covered in ca 10 denticular ribs. Pappus hair forms mainly 1 row (in case of *Hieracium* 2 rows). Apart from the mentioned characters genus *Pilosella* may be discriminated by several morphological tendencies such as presence of stolons or red veins on the abaxial ligule surface. Genus *Pilosella* significantly differs also at the level of other biosystematical characters. Monoploid

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[1] The highest number of taxa was described in *Hieracium* s.l. compared to any other genus in the European flora (Sell & West 1976).

genome size (Cx-value) is approximately twice lower in *Pilosella* than in *Hieracium* (Bräutigam & Bräutigam 1999, Vladimirov & Greilhuber 2003, Chrtek et al. 2009). Also apomixis type is essentially different (*Pilosella* - apospory vs. *Hieracium* - diplospory; Rosenberg 1906, 1907, Pogan & Wcisło 1989, 1995). Molecular markers (nuclear - ITS and chloroplast haplotypes; Fehrer et al. 2007a) brought better understanding of the whole group. Molecular study based on the analyses of the internal transcribed spacer of the nuclear ribosomal DNA (nrDNA ITS) and *trn*T-*trn*L intergenic spacer and *mat*K gene of the chloroplast DNA (Fehrer et al. 2007) had great impact for the understanding of the whole group. All markers confirmed the monophyly of the clade composed of *Hieracium* s.l. *Andryala* L. and *Hispidella* Lam. However incongruence among the results based on chloroplast and nuclear data was observed in respect of the evolutionary history of *Pilosella.* Whereas the nuclear data (ITS) mainly confirm the hitherto taxonomical concepts with *Pilosella*/*Hispidella* being a sister group to *Hieracium* s.str./C*hionoracium* and *Andryala* clades, *Pilosella* was split into two major lineages according to the cpDNA. Moreover neither of them was composed of *Pilosella* accessions solely. In the first one *Andryala* clustered together with part of the *Pilosella* species, the other clade comprised *Hispidella* in addition. These data suggest ancient hybridization events among these genera. However, higher number of representative individuals should be added to potential consequent analyses as recent biosystematical studies at region scale clearly confirm highly different microevolutionary patterns (hybridization direction, ploidy levels, breeding modes etc.) even from very close mountain ranges (Krahulec et al., unpublished).

Generally accepted taxonomic concept of the genus *Pilosella* is structured into group of basic (Hauptarten) and intermediate species (Nebenarten or later called Zwischenarten; Nägeli & Peter 1885). Basic species comprise taxa characterized by unique morphological traits, whereas intermediate ones combine basic species features indicating their hybridogenous nature. The concept is supported by numerous hybridization experiments of Nägeli and Peter (1885) and its robustness has been corroborated by many studies (i. e. Suda et al. 2007, Fehrer et al. 2007a etc.). The basic vs. intermediate species distinction also corresponds well with the patterns of ploidy level and breeding mode differentiation (basic species being frequently sexual diploids vs. predominantly polyploid apomictic intermediates; Krahulcová et al. 2000). However, problems of particular hybridogenous species age cannot be sufficiently determined either by molecular markers or by paleobotanical research (most of Asteraceae taxa do not differ in pollen morphology, phytolites or any other known paleobotanical marker; e.g. Beug 2004, Petr & Urfus unpub.). Nevertheless, the majority of hybridogenous intermediate species do evolve so they are already distinguishable (e.g. by progeny structure) from recent hybrids (e.g. Krahulec et al. 2004, 2008). Number of basic species varies depending on the species rank concept between 20 - 30 (i.e. 20 with numerous taxa treated at subspecific level; Bräutigam & Greuter 2007). On the contrary, number of described intermediate species reaches place value of hundreds to thousands. Detailed taxonomic concept probably reflects the real situation more naturally (especially in case of application of biological species concept; e.g. Juxip 2002, Tyler 2005).

On the other hand, morphological delimitation of the microspecies is often doubtful. Vast number of previously described intermediate taxa represents apomictic lineages artificially separated from partly sexually and apomicticaly breeding populations which hybridize occasionally. Thus a high number of taxa described for example a hundred years ago does not even exist (they vanished via recurrent hybridization events; e.g. Krahulec et al. 2004, 2008[2]). The situation, obviously irresolvable by traditional taxonomic approaches, has therefore resulted in resignation on detailed taxonomic concept [i.e. Bräutigam and Greuter (2007) mention 122 intermediate species].

Most of the *Pilosella* species distribution areas does not spread far over the European geographic borders (Bräutigam 1992, Fehrer et al. 2007b), but in many cases their eastern distribution borders are simply unknown. Several species distribution areas reach South Siberia (i. e. *Pilosella echioides* (Lumn.) F. W. Schultz & Sch. Bip., *P. onegensis* Norrl.; Schischin 2002) or middle Asian mountains (*Pilosella aurantiaca* (L.) F. W. Schultz & Sch. Bip.; Krahulec unpubl.). The majority of genus *Pilosella* species are mainly stress tolerant plants. Basic species tend to occur mostly in primary mainly forest-free habitats (rocky outcrops, steppes, sandy habitats, various transition mires margins etc.), whereas intermediate species mainly occur in secondary habitats (e.g. Zahn 1921, 1930, Sell & West 1976, Bräutigam & Schuhwerk 2002, Chrtek 2002, 2004).

The vast number of described taxa, various taxonomic concepts and frequent resignation on detailed plant determination evidently imply question of the variation sources. There are several main sources of enormous variation of the *Pilosella* species: hybridization accompained by polyploidisation (detected cytotypes: 2x, 3x, 4x, 5x, 6x, 7x, 8x; x= 9), variation in reproductive mode (apomixis combined with sexual reproductive mode often within the same capitulum) as well as vegetative reproduction via stolons (Gadella 1987, 1991, Krahulcová et al. 2000). These sources are accompanied by production of fertile pollen (even in case of apomicts and completely sterile plants such as F1 triploids) and occurrence of haploid parthenogenesis or autogamy via mentor effect (Krahulcová et al. 1999, Krahulcová et al. 2000, Krahulcová & Krahulec 2000). Moreover, all the above mentioned phenomena are usually combined in an intricate way. Apomictic lineages are facultative thus they subsequently hybridize with other cytotypes or taxa resulting in enormous number of different morphological lineages and cytotypes (Krahulcová & Krahulec 2000). In addition, several other important variation sources have not yet been sufficiently studied and, for example, the frequency of unreduced gametes formation has not yet been assessed. Nothing is known about the importance of further genom duplication of polyhaploids frequently resulting in highly distinct lineages (Krahulec et al. 2011).

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[2] Detailed study of *Pilosella* species in Krkonoše mountains illustrates intricacy of microspecies concept (e.g. number of described taxa is some cases higher than number of clones; Krahulec et al. 2004).

Polyploidy

Polyploidy represents one of the fundamental evolutionary phenomena frequently causing single step speciation, so called saltation (Grant 1981). Polyploidisation is supposed to be more frequent in plants than in animals (Mable 2004). Essential role of polyploidisation may be illustrated by the fact that ancient polyploidisation was confirmed for all plants (except for the *Amborella*; Soltis et al. 2009). Although polyploidy was for the first time detected at the beginning of the twentieth century (e.g. Strasburger 1910), it has been extensively studied only during last several decades (in connection with developing flow - cytometry and molecular methods).

Depending on polyploid genome composition two polyploidy types are distinguished: autopolyploidy and allopolyploidy. Autopolyploidy is a result of a single genome duplication or of unreduced gamete participation (including polyploidisation within individuals of one population of a particular species; Ramsey & Schemske 1998). Surprisingly, autopolyploids seem to be not so common and not many examples have been properly proven (for details see Soltis et al. 2007). Fusion of two more differentiated genomes, which is typically a product of interspecific hybridization results in allopolyploidy. Besides the simple genome duplication polyploidisation via unreduced gametes is considereded to be the most common mechanism. Higher production of unreduced gametes was especially detected in case of hybrids, thus polyploidy is evidently closely connected with hybridization (Ramsey & Schemske 1998). Polyploidisation via unreduced gametes raises either by combination of two unreduced gametes or by participation of one unreduced and reduced gamete followed by complicated passing of triploid block (e.g. Ramsey & Schemske 1998, Burton & Husband 2000, Bretagnolle 2001, Husband 2004, Köhler et al. 2010). Process of polyploidisation is apparently limited either by simple selection against higher polyploids or by more complex mechanisms (i.e. polyhaploidisation - high polyploids are generating haploid progeny, *Pilosella*; Krahulec et al. 2011). Polyploidy is obviously accompanied by numerous selection predispositions. Many restrictions or potential advantages are induced by bigger cell size (proportions of whole organism are bigger) followed by lower growing speed (due to more complex mitosis; Leitch & Bennett 2007). Polyploid species also tend to form specific geographical patterns. Obvious geographical pattern is represented in deglaciated holoarctic regions where polyploids markedly prevail (Stebbins 1957, also in case of *Pilosella*, Krahulcová et al. 2004, 2008).

Genus *Pilosella* is deeply influenced by polyploidisation. Broad spectrum of ploidy levels was recorded within the species (2x, 3x, 4x ,5x, 6x, 7x, 8x - basic chromosome number x=9; Schuhwerk 1996, Schuhwerk & Lippert 1997, 1998, Krahulcová & Krahulec 1999, Krahulcová et al. 2000, Rotreklová et al. 2002, 2005 etc.). During experimental hybridization even higher ploidy levels were detected (up to 12x; Skalińska 1976). Almost half of *Pilosella* taxa are supposed to consist of more ploidy levels (even within one population; Schuhwerk 1996, Schuhwerk & Lippert 1997, 1998, Krahulcová & Krahulec 1999, Rotreklová et al. 2002, 2005 etc.). Diploid level is characteristic mostly for basic species (even though e.g. basic species *P. aurantiaca* does not have any diploid cytotype, only dihaploid plants were recorded as a result of parthenogenesis during experimental crossing; Skalińska 1971). Polyploidisation in the genus is probably induced via unreduced gametes, which fuse with usually reduced gametes (rarely unreduced gametes) resulting in addition hybrids. According to results of experimental crossing addition hybrids represent the first step for subsequent polyploidisation (Skalińska 1973, 1976, Gadella 1988, Krahulcová & Krahulec 2000, Krahulcová et al. 2004, 2011). The importance of addition hybrids as a source of variation and as a basic step for generation higher polyploids is probably still not valued enough (i.e. *P. rubra* in Krkonoše mountains was proved to be an addition hybrid between *P. aurantiaca* - 2n and *P. officinarum* Vaill. - n; Krahulcová et al. 2004, Suda et al. 2007). Moreover, species of genus *Pilosella* differ in absolute DNA content at the monoploid level and intermediate contents of intermediate species are confirming the hybridogenous character (Suda et al. 2007).

Problems of aneuploidy (abnormal number of chromosomes) are obviously connected with plolyploidisation (and hybridization). In case of genus *Pilosella* aneuploidy was recorded several times: *P. aurantiaca* (2n=30; Pashuk 1987), 5 individuals of *P. piloselliflora* (Nägeli & Peter) Soják (2n=48; Krahulcová & Krahulec 1999, Krahulcová et al. 2000), or in hybrid swarm between *P. officinarum* and *P. piloselloides* subsp. *bauhini* (Schult.) S. Bräut. & Greuter (Krahulcová et al. 2009) etc. Even in cases of experimental crossings aneuploidy is quite a rare phenomenon known either as a triploid progeny of tertraploid and diploid crosssing (*P. officinarum, P. aurantiaca*; Skalińska 1971, Gadella 1991) or relatively frequently as a result of pentaploid (pollen donor) and diploid or tetraploid crossing (Krahulcová & Krahulec 2000).

Reproduction modes

Apomixis (= apomixis s.str., i.e. agamospermy - asexual reproduction via seeds) is considered to be closely correlated with polyploidy (only diploid exception seems to be *Boechera* A. Löve & D. Löve, Brassicaceae; Kantama et al. 2007) and hybridization. Apomixis clearly prevails in certain families (Poaceae, Rosaceae and Asteraceae; Bicknell & Koltunow 1993, Koltunow et al. 2000, Richards 2003). At the taxonomic level apomixis is the reason for discussions regarding species definition (high number of apomictic microspecies as a more natural solution, but many botanists could hardly use it; Dickinson 1998, Hörandl 1998, Kirschner 1998, Stace 1998). Apomixis has been reported in 126 angiosperm genera (divided into ten-thousands of described taxa; Carman 1997). The mechanism of apomixis is extensively studied due to potential economic utilization. If apomixis could be induced in agricultural crop-plants to fix characters of the most productive F1 hybrids (mostly heterozygous), the increase of the yields would start a true agricultural revolution (Koltunow 1993, Ramulu et al. 1999, Koltunow et al. 2000). Nevertheless, control mechanisms of apomixis are probably more complex because despite being studied for many years by several teams, the apomixis control has not yet been figured out. The apomixis mechanism involves three elementary steps: embryo sac formation without meiotic reduction, spontaneous (fertilisation independent) development of embryo and endosperm formation (Koltunow 1993). Apomixis occurs in many specific types but principally is divided according to embryo sac origin. Diplosporic type is less derived from sexual process because the megaspore mother cell still gives rise to the embryo sac. On the other hand, in aposporous type the embryo sac is formed out of somatic cell of nucleus (e.g. Richards 1997, Koltunow et al. 2000). Although the embryo development is autonomous the pollination is frequently needed to form endosperm via fertilizing the central cell by a male gamete. Such apomixis type is called pseudogamy (Asker & Jerling 1992, Koltunow 1993, Richards 1997). Apomixis represents absolutely prevailing mode of breeding (obligatory apomixis) in some cases, but more frequently apomixis is combined with sexual reproduction (facultative apomixis). Even in case of obligatory apomicts residual sexuality still might be expected and several studies already proved incomplete genetic uniformity of apomictic populations (Bayer 1989, Noyes & Soltis 1996, Hörandl et al. 2001, Kashin et al. 2005). Therefore apomictic taxa comprise either lineages consisting of several up to many genotypes or uniclonal cases (Richards 2003, Krahulec et al. 2004 etc.). Majority of agamic complexes tend to occur in similar geographic pattern - geographical parthenogenesis. Geographical parthenogenesis is a pattern comprising sexual lower (usual diploid) ploidy levels occurring in the centre of distribution area and higher apomictic cytotypes several times exceeding the distribution of their sexual relatives. Geographical parthenogenesis mirrors obvious advantage of clonal species during colonising new areas (e.g. deglaciated; Hörandl 2006, Hörandl et al. 2006).

Apomixis in genus *Pilosella* is, as mentioned above, of aposporous type (i.e. type *Antenaria* L.; Rosenberg 1907, Pogan & Wcisło 1995). Essential role of genus *Pilosella* in apomixis research is underlined by the first detection of aposporous type directly in the genus (Pogan & Wcisło 1989). Unreduced embryo sacs are frequently mixed with the standard reduced ones resulting in mixed progeny (facultative apomixis, e.g. Krahulcová et al. 2000, 2004, 2011, Krahulec et al. 2004). Both types of embryo sacs are competing until one of them prevails (but only little is so far known about factors influencing one of the embryo sacs final dominance).

The essential source of genus enormous variation is not apomixis itself, but especially the combination of various reproductive modes (frequently within single capitulum; Krahulcová et al. 2000, 2004). Breeding modes are partly fixed to particular ploidy levels. The higher ploidy level is, the higher is the probability of apomixis (diploids are sexual). Odd ploidy levels are usually characterized by apomictic breeding (or sterile; Gadella 1987, 1991, Krahulec & Krahulcová 1999, Krahulcová et al. 2000, Krahulcová et al. 2001, Rotreklová et al. 2002, 2005). Nevertheless, many exceptions occur (e.g. rare sexual triploids, apomictic tetraploids and sexual pentaploids or large groups of sexual hexaploids; Pogan & Wcisło

1995, Rotreklová et al. 2002, Peckert 2005, Peckert & Chrtek 2006, etc.). But at least the diploid cytotypes were proved to be sexual (*P. lactucella* - Skalińska 1967, Gadella 1984, Krahulcová & Krahulec 1999; *P. peleteriana* (Mérat) F. W. Schultz & Sch. Bip. and *P. hoppeana* (Schult.) F. W. Schultz & Sch. Bip. - Gadella 1984, *P. officinarum* - Gadella 1984; *P. onegensis* - Skalińska 1967, Skalińska & Kubień 1972, Krahulcová & Krahulec 1999; *P. echioides* - Kašin & Černiškova 1997, Peckert 2001, Rotreklová et al. 2002). Despite that, the apomixis was confirmed in case of dihaploid individuals (Bicknell 1997, Bicknell et al. 2000, Krahulec et al. 2011).

Sexual forms were proved to be allogamous (Gadella 1988, 1991, Krahulcová & Krahulec 1999). However, self-compatibility is still possible via stimulation of stigma by foreign pollen - mentor effect (Krahulcová et al. 1999, subsequently confirmed in case of *Hieracium* s.str.; Mráz 2003). Such process is probably common in the field, as more than one species usually grow together; thus self-pollination probably occurs quite frequently.

Indispensable source of genus *Pilosella* variation is clonal growth (Krahulcová et al. 2000) via stolons (above or below ground). Due to clonal growth a particular genotype is able to spread at local scale even in case of otherwise sterile plants. Long-time persistence takes the sterile genotype back to microevolutionary process because even sterile plants of genus *Pilosella* species were proved to produce viable pollen. The ability to produce viable pollen (of probably all cytotypes) is in contrast to *Hieracium* s.str. because some of its cytotypes produce aborted pollen grains (Gadella 1987, Krahulcová & Krahulec 2000).

Hybridization

Last, but not least, an essential source of genus *Pilosella* variation is hybridization (Krahulcová et al. 2000). Hybridization is supposed to be the fundamental process of whole genus speciation (Skalińska 1967, Turesson 1972, Krahulcová et al. 2000). For a long time, hybridization was not considered to be an important and frequent phenomenon which could markedly influence evolution. The process was regarded as an evolutionary error (Arnold 1997), yet clearly hybridization is very common and its evolutionary impact is obviously substantial. Its frequency at the level of individuals is usually very low (often less than 0.1% individuals per generation) but at the level of species the impact increases because single individual can provide sufficient interspecific gene flow (e.g. Rieseberg 1997, Seehausen 2004, Mallet 2007). Abundance of hybridization may be illustrated with an example of British flora, in which hybridization was detected in 25% of species (Mallet 2005). Real hybridization rate can hardly be assessed because many hybrids are for instance indistinguishable in their morphology (only modern biosystematic methods are helpful; Mallet 2008). During hybridization already stabilised genomes are combined and restructured. Thus a fitness of hybrid may be either lower or higher than that of the parental taxa (Arnold & Hodges 1995). Hybrids are often less fertile (frequently sterile due to incompatibility at chromosomal level) than parental individuals and that is why they were considered to have very low evolutionary impact (Grant 1981).

In some cases, hybrids back cross with the parental species (introgressive hybridization or introgression). Gene flow influences the parental species differently, frequently in positive way due to the increase of their adaptability (Arnold 1997) although it can also diminish the genome of one of the parental species. Thus introgression endanger many species and even lead to their extinction (especially if they occur in small area; Wolf et al. 2000, Seehausen 2004). Introgression is frequently abundant in zones of sympatry and it sometimes interconnects the parental taxa by phenotypic continuum (clinal variation; Zimmerman 1964).

Hybridization is very common in genus *Pilosella* and the whole group represents a typical example of hybrid speciation in many respects. Solitary hybrids, or at least signs of introgression occur in majority of populations and even large hybrid swarms are quite frequent, because the most sympatric taxa are able to hybridize (almost no hybridization barriers are established; Gadella 1987). Introgression is obviously a widespread phenomenon in the genus *Pilosella* species and even triple hybrids production happens (Gadella 1988, 1992). Hybridization also knocks down barriers between different ploidy levels or breeding modes. Hybridization between different ploidy level species was reported many times (e.g. Skalińska 1967, Gadella 1987). Even apomictic individuals frequently participate in crossing either as pollen donors (e.g. Skalińska 1976, Gadella 1982, 1987) or surprisingly often as mother plants (naturally apomicts are facultative in case of genus *Pilosella*; e.g. Skalińska, Krahulec et al. 2004, 2008).

Current high rate of genus *Pilosella* hybridization is substantially increased due to human activities. Artificially preserved forest-free areas (anthropic steppe) markedly contribute to genus *Pilosella* species spreading. Broad-spectrum of variously disturbed secondary habitats frequently combines species whose niches would not otherwise connect, resulting in an antropohybridization (Wójcicki 1991). Montane meadows are a classic example of long time established anthropic habitat type, which is regularly disturbed by cutting for hay and many less competitive species occur there. Species from alpine forest-free habitats and lower altitudes grow there together, forming hybrid swarms and resulting in many well established hybridogenous taxa (Krahulcová & Krahulec 1999, Krahulec et al. 2004).

Pilosella officinarum **and** ***P. piloselloides*** **subsp.** ***bauhini*** **under investigation**

Pilosella officinarum is probably the most abundant species of the whole genus *Pilosella.* Together with several mostly diploid species it forms a section *Piloselina*, which is distinctly defined by one headed (single capitulum) stem and dense cover of stellate hairs at abaxial side of leaf (e.g. Zahn 1923, Sell & West 1976). *Pilosella officinarum* is the only abundant polyploid of the section (except for a rare tetraploid *P. saussuroides*); it is an extremely variable species. The complexity of its variation may illustrate 624 subspecies mentioned or directly described by the monograph of genus *Hieracium* s.l. K. H. Zahn (Zahn 1923).

Sources of *P. officinarum* variation are identical to the ones in the whole genus (polyploidy, different reproductive modes, hybridization and clonal growth; Krahulcová et al. 2000). According to high number of ploidy levels (2x, 4x, 5x, 6x, 7x), various combinations of reproductive modes to different cytotypes and extensive tendency to hybridize (the most frequent participant of all hybridization events and also most frequent parental species of hybridogenous species; e.g. Zahn 1923) *Pilosella officinarum* represents a model species crucial for understanding the problems of the entire genus *Pilosella* and even for possible reconstructing the ancient history of many other agamic complexes, where apomixis absolutely prevails (such as *Alchemilla*). Different ploidy levels relate to particular reproductive modes. Tetraploids tend to be mostly sexual (only rarely a partly apomictic exceptions were detected too; e.g. Gadella 1984). Pentaploids, on the contrary, tend to be apomictic (e.g. Gadella 1984), but partly sexual individuals have been already proven (Turesson & Turesson 1960, Tureson 1972, Pogan & Wcisło 1995) and even fully sexual ones (Krahulcová et al. 2000, Rotreklová et al. 2002). Hexaploids form both apomictic and sexual lineages. Sexual plants were documented from mountainous habitats (especially the Alps; Gadella 1984) and recently also from Central European regions from river canyons (Urfus 2006). Rare heptaploid individuals are either apomictic or sterile (Gadella 1984, 1991). *Pilosella officinarum* diploid cytotype (described by Delcourt 1972 and Gadella 1984) requires perhaps further studies as its status seems to be obscure.

Pilosella piloselloides subsp. *bauhini* (further in text *P. *bauhini*) does not belong to such markedly morphologically distinct group compared to *P. officinarum* - section *Praealtina* (e.g. related *P. piloselloides* subsp. *piloselloides* differs only in the absence of stolons; e.g. Gottschlich 1987). As well as *P. officinarum, P. *bauhini* consists of many intraspecific taxa (e.g. Zahn 1923, 1930). *Pilosella *bauhini* is also not as diversified in ploidy levels (four were detected - 4x, 5x, 6x and 7x; Rotreklová 2004, Krahulcová et al. 2009). Cytotypes of *P. *bauhini* are negatively distributed in Central Europe compared to *P. officinarum* (Roteklová 2004). Tetraploid cytotype breeds mainly sexually, but some apomictic tetraploid populations were also documented (Rotreklová 2004, Krahulcová et al. 2009). Only apomictic pentaploids and hexaploids were detected. *Pilosella *bauhini* also frequently participates in hybridization. The species forms huge hybrid swarms especially with *P. officinarum* [resulting in broad spectrum of morphological lineages more or less close to *P. brachiata* (DC.) F. W. Schultz & Sch. Bip. and *P. leptophyton* (Nägeli & Peter) S. Bräut. & Greuter - intermediate species between both parental species; e.g. Krahulcová et al. 2009].

To understand the problematics of genus *Pilosella*, a comparison with a related group characterised by evolution at the diploid level might by helpful. *Picris hieracioides* L. and very close *P. hispidissima* (Bartl.) Koch represent species group of closely related and almost exclusively diploid taxa (Sell 1975, 1976, Bolòs & Vigo 1990, Slovák et al. 2007). According to recent phylogenetic studies (Klilian et al. 2009) *Pilosella* and *Picris* rank among genera forming sister clades (clade 4 and 5). *Picris hieracioides* group may be characterised by high morphological variation despite being strictly diploid and allogamous sexual (Sell 1976). High number of intraspecific taxa confirms the real variation (currently recognised at the base of peduncle length, involucrum proportions, colour and hair characteristics - very similar set of characters is also crucial in entire genus *Hieracium* and *Pilosella* determination; Sell 1975, 1976). Different and incompatible taxonomic concepts of *Picris hieracioides* were established (analogous to different taxonomic treatments in genus *Hieracium* and *Pilosella*; Sell 1975, 1976, Bolòs & Vigo 1990, Haeupler & Muer 2007). Generally *Picris hieracioides* (according to study in the Western Carpathians; Slovák & Marhold 2007) occurs either in mountaineous primary habitats ("higher altitude type") or in widespread secondary habitats ("lower altitude type").

Aims of the thesis

I. The detection of processes within hybrid swarm by morphometric comparisson of maternal and filial generation

II. Ploidy levels distribution pattern on different scales

III. Morphometric evaluation of cytotypes defined by ploidy level and reproductive mode

IV. Comparison of different variation patterns in related taxa

Hybridisation within the *Pilosella* population: a morphometric analysis

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Abstract Hybridisation processes that occur within the population of *Pilosella piloselloides* subsp. *bauhini* and *P. officinarum* were detected by means of a morphometric analysis. We analysed a set of maternal plants that occurred in the field and set of their progeny that originated in the field. The analysis showed common hybridisation of both parents as well as of their hybrids (*P. brachiata* and *P. leptophyton*). All of the plants with different reproductive modes (sexual, facultative apomictic and variable) participated in these hybridisations.

Introduction

One of the key features of the genus *Pilosella* (*Hieracium* subgen. *Pilosella*) is common hybridisation, which is mentioned in many books and papers (Zahn 1921-1923, 1922-1930, Sell & West 1975, 1976, Krahulcová et al. 2000, Tyler 2001, Fehrer et al. 2007). The studies of *Pilosella* led to the descriptions of basic and hybridogenous species; these are formed not only between basic species but also between individual hybridogenous species. Therefore, new types are formed by both one-step and multi-step hybridisation processes. This finding results in a rather complicated system within particular taxa (e.g., Nägeli & Peter 1885, Schuhwerk & Fischer 2003, and a contrasting approach in Tyler 2001). The studies of *Pilosella* hybrids were performed at the beginning of modern genetics (Mendel 1869) and at the beginning of the discovery of apomixis (Ostenfeld & Raunkier 1903, Ostenfeld 1906). Surprisingly, there are very few studies in the field regarding topics such as the population level, analyses of hybridisation and its related processes including introgression or progeny analyses (Krahulcová et al. 2009, 2011). Evidently, the possibility of the detection of hybrids and/or their description led to a uniform scientific approach. In geographically limited areas, the use of molecular markers, such as chloroplast haplotypes, revealed which species were the mother plants. Surprisingly, apomictic plants were found to be the mother plants of about half of the hybrids and hybridogenous species (Krahulec et al. 2004, 2008). In a detailed study of two model populations, Krahulcová et al. (2009) showed that apomictic maternal plants produced more variation with respect to the ploidy level than sexual maternal plants. However, we did not consider different morphological types of hybrids. In the present paper, we studied one of these populations to demonstrate the directions of hybridisation by examining the morphological characters, which are used for the description and the determination of particular taxa. These processes are related to the reproductive mode of maternal plants that produce seed progeny in the field.

The studied population

The studied population consisted of two basic species, *Pilosella officinarum* and *P. piloselloides* subsp. *bauhini* (subsequently referred to as *P.*bauhini*). These species have contrasting morphology; *P. officinarum* has one large capitulum and a dense cover of stellate hairs underleaf, while *Pilosella *bauhini* is a tall plant and has many small capitula in its cymose inflorescence, but its leaves do not contain any stellate hairs. These characters allow for the easy identification of their hybrids. Both species hybridise freely and often. Based on the morphology, they form two types of hybrids. *P. brachiata* (*P. *bauhini* ≤ *P. officinarum*) is characterised by its range of scattered to abundant stellate hairs underleaf, its relatively few capitula and its deeply branched short stem (e.g., Chrtek 2004). It corresponds to the primary n+n hybrids or to different products of back crosses with *P. officinarum*. *Pilosella leptophyton* (*P. *bauhini* > *P. officinarum*) has more abundant but smaller capitula in the cymose inflorescence and scattered stellate hairs underleaf.

At the studied locality (Krahulcová et al. 2009), *P. officinarum* was mostly tetraploid and sexual, although several plants were found to be pentaploid and sexual. *Pilosella *bauhini* was determined to be tetra-, penta-, hexa- and heptaploid at this locality, and plants of all ploidy levels were apomictic. The ploidy and reproductive mode of the hybrids were complex, with many aneuploids and combinations that displayed different reproductive modes; this finding was especially apparent for *P. brachiata*. In addition to sexual and apomictic reproduction, we distinguished a variable mode, which combines both modes. The apomictic plants usually retain a level of residual sexuality of up to 10%. The specimens with a variable mode, which were usually the 2n + n hybrids that resulted from an apomictic and a sexual parent, retain up to 80% of their sexuality. Therefore, such hybrids produce more variation when compared to their apomictic parent because they form a low proportion of progeny that are identical to the mother plant, and they often form a high proportion of polyhaploid and/or hybrid progeny.

Material and methods

We used herbarium specimens (deposited in the Herbarium PRA), which originated from the hybrid population in Prague (Praha-Vysočany; Krahulcová et al. 2009), both as mature plants that were established at the locality and for their seed progeny. These progeny were cultivated from seeds, and their morphological characters were evaluated together with their maternal parent. The population was formed by *Pilosella officinarum*, *P. bauhini* and various morphotypes of their hybrids (*P. brachiata* = *P. bauhini* < *P. officinarum, P. leptophyton* = *P. bauhini* > *P. officinarum*). The following two sets of plants, which corresponded to the material that was sampled and processed in the previous study (Krahulcová et al. 2009, locality 1 in Fig. 1), were used in this analysis:

(i) Maternal data set. Seventy-nine plants that were collected in the field were chosen for subsequent cultivation in the experimental garden of the Institute of Botany, ASCR Průhonice. The chromosomes of all 79 plants were counted. The DNA ploidy levels of the progeny were determined using flow cytometry, and the chromosomes of the progeny class representatives were counted to confirm the accuracy of the flow cytometry. The reproductive mode (parthenogenetic versus sexual) of all plants was confirmed using a routine emasculation test (Gadella 1987, Krahulcová and Krahulec 1999). Consequently, the karyological analysis of the progeny (the detection of ploidy and the chromosome number variation) distinguished between a variable and an apomictic reproductive mode within those plants that produced some parthenogenetic seeds in the emasculation test.

(ii) Filial data set. Seeds from the maternal plants, which were collected in the field or during a period of 10 days after transplantation (which definitely originated in the field) were sown, and the seedlings were cultivated to the rosette stage.

> The ploidy (or chromosome number) was determined to distinguish between an apomictic and a variable reproductive mode of the corresponding maternal plants. In total, 223 plants were cultivated to maturity and collected for the herbarium. These plants were characterised by the taxonomic identity of their maternal plant, its reproductive mode and its chromosome number.

In total, 21 morphological characters (quantitative or binary) of 302 herbarium specimens were measured or scored (see Table 1). The characters that were measured or scored included traits widely used for the species group determination. The characters of the vegetative portions of the plants comprised the leaf length (LL), the leaf width (LW), the length from maximum leaf width to the tip (WT), the shape of leaf tip (acuteness, TA), the stem height (up to the highest capitulum, SH or to the first branch of the inflorescence, SB), and the length of the peduncle of the terminal capitulum (PH). The characters of the generative organs were the involucral bract length (BL) and width (BW), which were determined by the average of 5 measurements; the terminal capitulum diameter (CD); and the rate of dark trichome density (TD). Several characters dealt with either the hair density of the leaves, which were the presence of single hairs covering the adaxial side (SL) and stellate hairs on the adaxial side (SA); the hair density on the stem, which included simple hairs (SS), stellate (STS) and glandulate (ES); or the hair on the involucrum, which was single (SI), stellate (STI) or glandulate (EI). The occurrence of stolons (SO) and flagellae (FO) were also determined.

The basic statistical parameters, such as the mean value, standard deviation, and the 1, 5, 95 and 99 percentiles, were computed for each of the characters. The correlative relationship among the characters was investigated using Pearson's correlation and the non-parametric Spearman's rank coefficients to detect a high correlation (>0.95) to avoid the distortion of the multivariate analysis.

The principal component analysis (PCA, Krzanowski 1990), which is based on a correlation matrix, was applied to reduce the multidimensional nature of the character space. The variation pattern was then expressed by the first three components. To compute the distance matrix, the Euclidean distance was applied.

Consequently, discriminant analyses (canonical discriminant analyses (CDA) and classificatory discriminant analyses; Klecka 1980) were performed. We used the taxonomic identity and the reproductive mode of the maternal plants as a discrimination character. The discriminant function that was computed from the maternal individuals' dataset was applied to the progeny dataset to determine the identity of the progeny. Finally, the non-parametric k-nearest neighbour classificatory discriminant analysis was used to determine the percentage of progeny individuals from particular taxonomic groups.

The multivariate analyses of the morphometric data set were performed with the SAS 9.1.3 (SAS Institute 2000) and PAST software (Hammer et al. 2001).

Results

PCA: The analysis of the maternal data set was rather clear (Fig. 1): the first axis was identical to the gradient between *P. *bauhinii* (and its morphologically closer hybrid *P. leptophyton*) on one side and *P. officinarum* on the other side. The most common hybrid, which was *P. brachiata*, occupied most of space and overlapped on both sides with the parental taxa. No overlap between the parental taxa occurred in the plane of the first two axes. The same analysis that was performed with the progeny of maternal specimens (Fig. 2) showed some differences. The progeny of both of the maternal species in the reciprocal hybridisations, *P. *bauhini* and *P. officinarum*, overlapped to a greater degree with the progeny of *P. brachiata*. This finding indicates the presence of two processes, which could occur simultaneously. At least a portion of the progeny of the maternal species was of a hybrid origin, and *Pilosella brachiata* was pollinated by both parental species, i.e., a portion of its progeny are backcrosses. It seems that *P. brachiata*, which displays a sexual and variable system, produced hybrids that were closer to *P. officinarum*, but the plants that displayed apomictic reproduction produced plants that were closer to *P. bauhini*. This result is even more significant when the progeny of *P. brachiata* were treated separately with respect to the reproductive mode of the mother plant (Fig. 3). The primary gradient was correlated with the sexual and apomictic parents, while the progeny of the specimens with a variable reproductive system were scattered between the two, and they showed a greater overlap with the progeny of the sexual maternal plants.

Discriminant analysis. A discriminant analysis (Fig. 4) of the maternal data set revealed the clear separation of particular taxa. The first canonical axis separated both of the parental species (*P. *bauhini* and *P. officinarum*), and the second axis separated both of the species of hybrid origin (*P. leptophyton* and *P. brachiata*) in addition to *P. officinarum* and *P. brachiata*. These results are not surprising because these characters were chosen based on their ability to differentiate between these species. The filial data set that was based on the discriminant function of the maternal specimens (Fig. 5) showed a complete overlap between the progeny of *P. *bauhini* and *P. leptophyton* and the penetration of the progeny of *P. brachiata* to the progeny of *P. officinarum* and also partially to *P. *bauhini*.

The percentage of the determination of the progeny of individual maternal plants by the discriminant function that was based on the parents revealed the processes within the population (Table 2). *Pilosella officinarum* produced a high percentage of *officinarum* progeny, and it was followed by *brachiata*, which corresponds to n+n hybrids. Surprisingly, some progeny were of the *leptophyton* type. The apomictic *P. *bauhini* primarily produced *bauhini*, but it also produced both types of the hybrids. Most of the *P. brachiata* progeny were classified as *P. officinarum*; this result suggests that common backcrosses and introgression occurred with this parent. Some progeny were also of the *P. leptophyton* or *P. *bauhini* type, which also suggests that backcrosses with the second parent occurred. Most of the *P. leptophyton* progeny were determined to be *P. *bauhini*.

The filial generation displayed different proportions with respect to its origin. The numbers (not percentages) are evident in the columns of the Table 2.

The most diverse reproductive mode of the maternal generation was that of *P. brachiata,* which displayed all three types, the sexual, apomictic, and variable modes. The progeny of the mother plants with respect to the reproductive mode differed (Table 3). Surprisingly, a greater proportion of the progeny that were classified as identical to the mother plant was found from the plants with variable a reproduction mode than from those of the apomictic plants. The plants with a variable mode produced more progeny that were classified as *P. officinarum* than the apomictic plants; the latter produced a high proportion that was classified as *P. *bauhini*.

Evaluation of characters: Table 4 presents the data regarding the correlations of the characters with a particular axis in the PCA and a canonical axis in the CDA. The characters that significantly correlated with the first axis of the PCA were leaf length (positively correlated with *P. *bauhinia*) and the density of stellate trichomes underleaf (a character of *P. officinarum*). The second axis correlated with the leaf width and the number of simple trichomes on the involucrum. The best combination of character that could be used to discriminate the parental species and the hybrids within the maternal data set were again leaf length, the length of the leaf from the widest point to the tip, the stem height and the height to the first branch (all of the characters positively correlated with *P. *bauhini*). The characters that positively correlated with *P. officinarum* were the length of terminal capitulum peduncle (in *P. officinarum* it was identical with the stem height), the length and width of the involucral bracts and the diameter of the terminal capitulum. The density of stellate hairs underleaf and the shape of leaf tip were correlated with the second canonical axis, and these characters can, in fact, discriminate between both of the hybrid types.

Discussion

The PCA of the maternal data set revealed that there is a morphology gradient; both of the parental species are on the ends, and both of the hybrid taxa are located between them. The only overlaps that were present were small. The PCA of the filial data set revealed the same gradient, but there were greater overlaps between the progeny of the individual maternal specimens. This finding is understandable because of the formation of new hybrids by the parental species and due to the backcrosses and the segregation of hybrids.

A more detailed discriminant analysis was used to determine which plants from the maternal set form the discriminant function and the best combination of characters to distinguish between them. Four individual groups were well defined even in the plane of the first and second axis; this result is in agreement with the data from the hybrids between the two species. When we used the discriminant function that was obtained from the analysis of the maternal set for the filial data set, the same phenomenon that was observed in the PCA was clearly visible. This result showed that there was greater overlap between the groups. The percentage of misidentifications was high and it suggests that there were hybridisations and/or segregation within the progeny of the hybrids.

The progeny of the sexual *P. officinarum* was of primarily of the *officinarum* type; although both types of hybrids occurred, the *brachiata* type, which corresponds to n+n hybrids, prevailed. This result suggests that about one-third of the progeny were derived from the hybridisation with *P. *bauhini* or one of the interspecific hybrids.

The *P. brachiata* progeny were diverse. Surprisingly, the greatest portion of these progeny was determined to be *P. officinarum*. This result suggests the common presence of backcrosses and introgression to *P. officinarum*. This hybridisation may be a source of the pentaploid sexual type of *P. officinarum*, which was observed at this locality (Krahulcová et al. 2000, 2009). A small proportion of the progeny was also determined to be *P. *bauhini,* which also suggests the possibility of introgression with *P. *bauhini,* but it occurred at a ten-fold lower frequency. It seems that the rare hybrids that were produced by the plants with an apomictic reproduction mode were more similar to *P.* *bauhini (Figs. 2 and 3, Table 3). However, the progeny of the plants with a sexual reproduction mode were more similar to *P. officinarum*. With respect to the space occupied along the first PCA axis (Fig. 2), the progeny of the plants with an apomictic reproduction mode were more variable than the progeny of the sexual plants; this result was also found in our previous study with respect to ploidy levels. However, we are aware that the number of the progeny of the apomictic plants was rather low due to their low residual sexuality.

The composition of the progeny of the second hybrid, *P. leptophyton*, also suggests introgression with a closely related parental species, *P. *bauhini,* but the low number of analysed plants weakens this conclusion.

The progeny of the apomictic maternal *P. *bauhini* showed a relatively high proportion of hybrids, at more than 20%.

All of the results show that interspecific hybridisation is very common and involves both the parental taxa and their hybrids.

The only morphotype that displayed all three types of reproductive modes, i.e., sexual, apomictic, and variable, was *P. brachiata*. The explanation of the progeny composition of individual plants with their various reproductive modes is not easy; evidently, it is necessary to analyse a greater number of mother plants to suggest a definite conclusion. The frequency of the hybrids and the high variation in their chromosome numbers, including aneuploids (Krahulcová et al. 2009), showed that at least at studied locality, the hybridisation and subsequent introgression to the sexual parent are common. The data presented in this paper showed that hybridisation is present in almost all directions despite the fact that one parental species (*P. *bauhini*) and a portion of the hybrids have apomictic reproduction modes. The high proportion of both hybrids and aneuploids was not detected only in seeds, but it was also found directly in the locality. This result confirms that the rates of both hybridisation and establishment are rather high. This finding is in agreement with our field observations, where the open soil surface consisting of fine gravel and sand allowed the common growth of seedlings.

Several of the characters that were important for differentiating the species, and especially the size parameters, are usually not used in keys; however, in this specific case, they were efficient. Characters as the shape of the leaf tip and the position where the leaf is widest are easily observable and could be used directly in the field.

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Tab. 1. List of morphological characters used.

Continuous quantitative characters	
leaf length	LL
leaf width	LW
distance between the widest point of leaf to the tip	WT
rate of leaf tip acuteness (1-sharp, 2-obtuse, 3-rounded)	TA
density rate of stellate trichomes at adaxial side of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs)	SA
density rate of single trichomes at upper surface of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs)	SL
stem height (up to terminal capitulum)	SH
stem height (up to the first branching)	SB
lenght of terminal capitulum peduncle	PH
density rate of single trichomes at stem (1-glabrate, 2-single trichomes, 3-dense hairs)	SS
density rate of stellate trichomes at stem (1-glabrate, 2-single trichomes, 3-dense hairs)	STS
density rate of eglandulate trichomes at stem (1-glabrate, 2-single trichomes, 3-dense hairs)	ES
average of 5 involucral bracts lenght	BL
average of 5 involucral bracts width	BW
terminal capitulum diameter	CD
rate of dark trichomes density at involucrum (1-bright, 2-dark bases, 3-dark trichomes)	TD
density rate of simple trichomes at involucrum	SI
density rate of stellate trichomes at involucrum	STI
density rate of eglandulate trichomes at involucrum	EI
binary characters	
stolon occurrence	SO
flagella occurrence	FO

Tab. 2. Number of filial generation specimens with respect to determination of mother plants.

Progeny of	Determined as				
	P. officinarum	_P. brachiata_	_P. leptophyton_	_P. *bauhini_	sum
P. officinarum	45 (62,5%)	22 (30.6%)	5 (6.9%)	0	72
P. brachiata	42 (48,3%)	36 (41.4%)	2 (2.3%)	7 (8%)	87
P. leptophyton	0	1 (14.3)	0	6 (85.7%)	7
P. *bauhini	0	2 (3.5)	10 (17.5%)	45 (78.9%)	57

Tab. 3. Composition of filial generation with respect to reproductive mode of mother plants.

Mother plant	Progeny determined as				Sum
	P. officinarum	_P. brachiata_	_P. leptophyton_	_P. *bauhini_	
P. officinarum					
- sexual	44 (62%)	22 (31%)	5 (7%)	0	71
- apomictic	1 (100%)	0	0	0	1
P. brachiata					
- sexual	33 (57.9)	22 (38.6%)	1 (1.75%)	1 (1.75%)	57
- apomictic	2 (14.3%)	5 (35.7%)	1 (7.1%)	6 (42.9%)	14
- variable	7(43.75%)	9 (56.25%)		0	16
P. leptophyton					
- apomictic	0	1 (14.3%)	0	6 (85.7)	7
P. *bauhini					
- apomictic	0	2 (3.5%)	10 (17.5%)	45 (79%)	57
					223

Tab. 4. Correlations of particular axis in PCA and CDA, both maternal data set. Significant values (p) are given in bold.

	PCA			CDA – maternal data set	
Character	Prin 1	Prin 2	Prin 3	Can 1	Can 2
LL	-0,293	0,274	0,235	**-0,730**	0,112
LW	-0,097	**0,485**	**0,344**	-0,102	0,170
WT	-0,270	0,255	0,298	**-0,568**	0,041
TA	0,235	0,063	0,047	0,368	**0,547**
SA	**0,315**	-0,095	0,103	0,783	0,116
SL	0,165	0,135	-0,146	0,357	0,230
SH	-0,322	0,154	0,096	**-0,827**	0,004
SB	**-0,304**	-0,044	0,117	**-0,774**	-0,009
PH	0,233	0,171	0,280	**0,592**	0,294
SS	-0,002	0,223	**-0,417**	-0,077	-0,103
STS	0,186	-0,083	0,076	0,391	0,355
ES	0,170	0,126	0,007	0,479	0,242
BL	0,293	0,233	0,198	**0,698**	0,309
BW	0,271	0,229	0,006	**0,711**	0,171
CD	0,271	0,235	0,119	**0,714**	0,399
TD	-0,035	0,037	-0,055	-0,255	-0,084
SI	0,006	**0,363**	**-0,447**	0,105	-0,251
STI	0,237	-0,052	0,187	0,374	**0,561**
EI	0,123	-0,205	0,272	0,209	-0,237
SO	0,172	0,212	-0,169	0,351	0,065
FO	-0,049	0,286	-0,171	-0,078	-0,130

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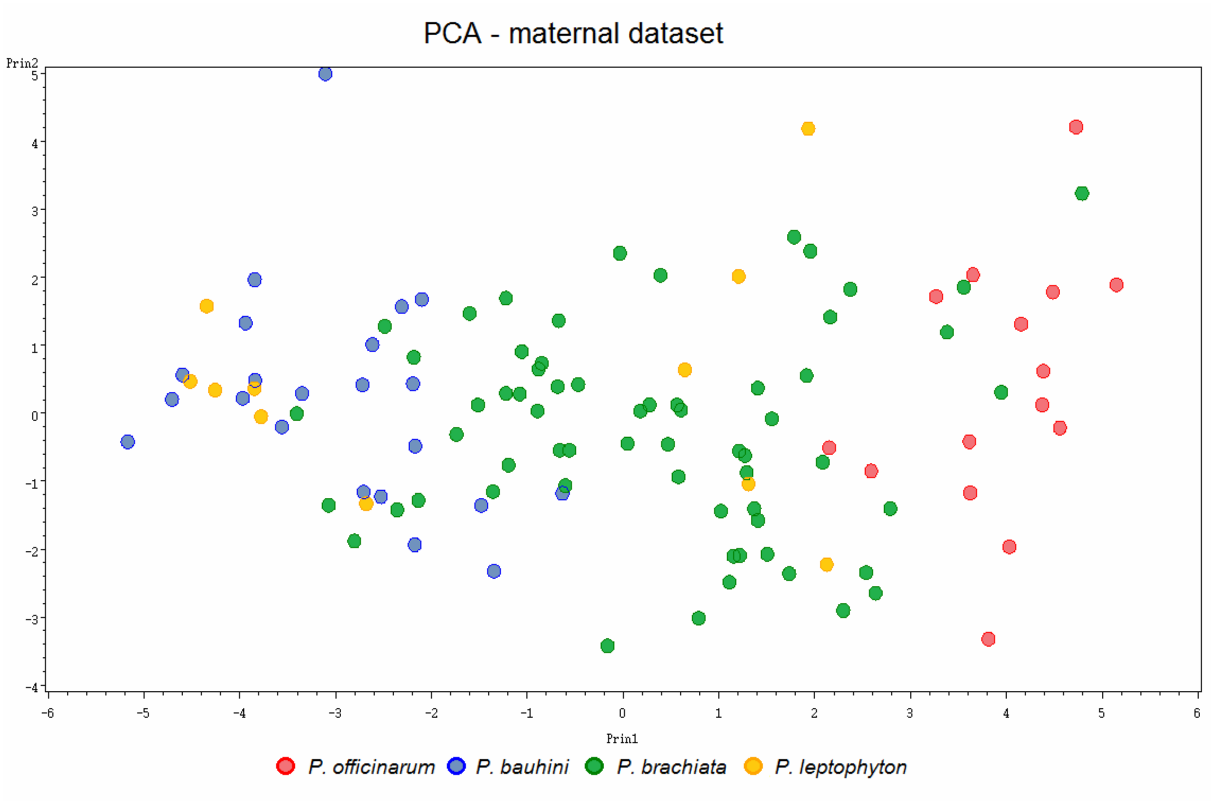

Fig. 1 Principal component analysis (PCA) ordination diagram (maternal dataset of 114 *Pilosella officinarum, P. bauhinii, P. brachiata* and *P. leptophyton* individuals based on 21 characters) illustrating the partial separation of particular taxa (the first component axis expresses 33.5% of the total variation, and the second expresses 12.3% of the total variation)

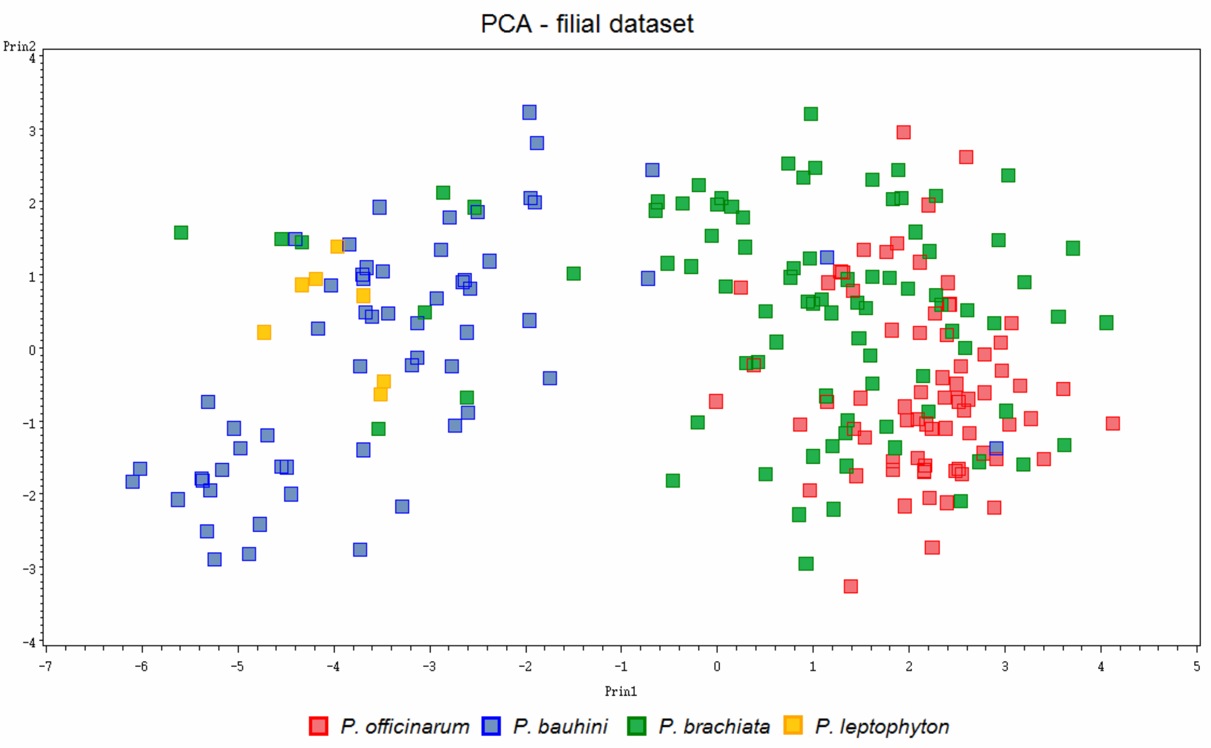

Fig. 2 Principal component analysis (PCA) ordination diagram (filial dataset of 223 progeny plants of *Pilosella officinarum, P. bauhinii, P. brachiata* and *P. leptophyton* based on 21 characters) illustrating only the partial separation of individuals and progeny of particular taxa (the first component axis expresses 36.4% of the total variation, and the second expresses 10% of the total variation)

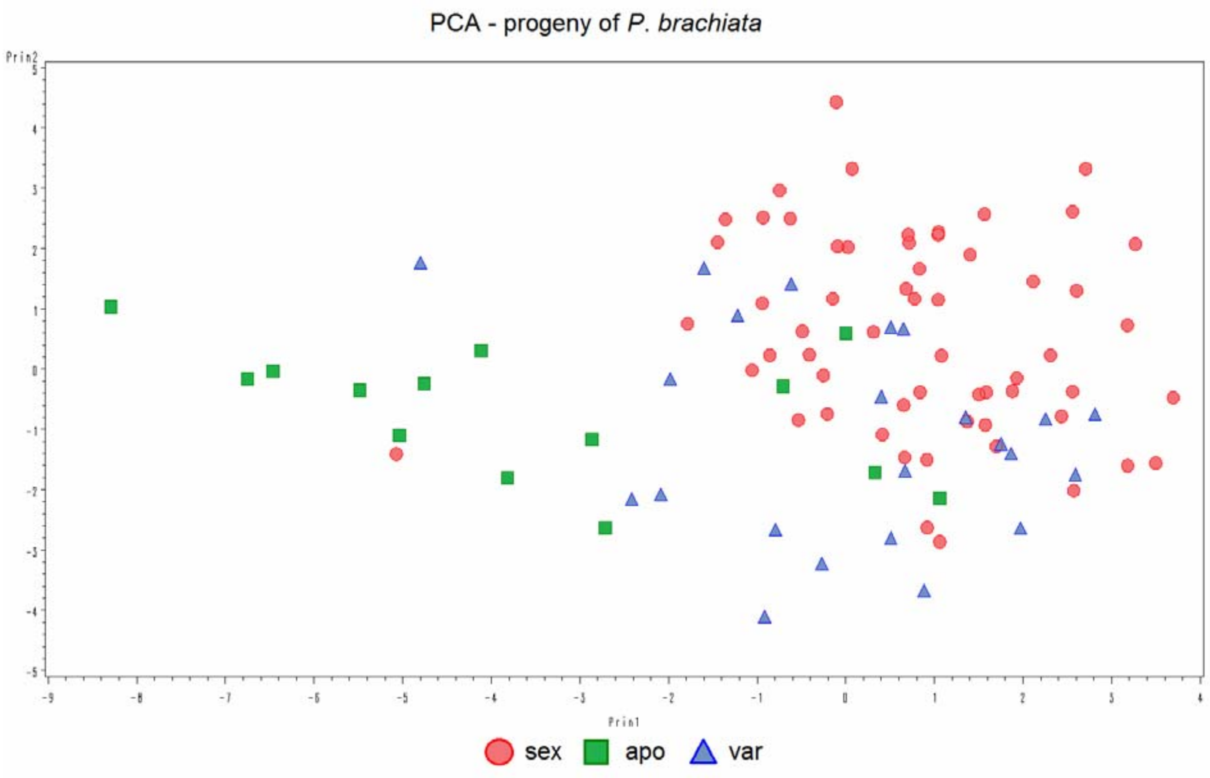

Fig. 3 Principal component analysis (PCA) ordination diagram (the progeny of the *P. brachiata* dataset contained 104 individuals and was based on 21 characters) illustrating the partial separation of particular taxa (the first component axis expresses 27.1% of the total variation, and the second expresses 14.6% of the total variation)

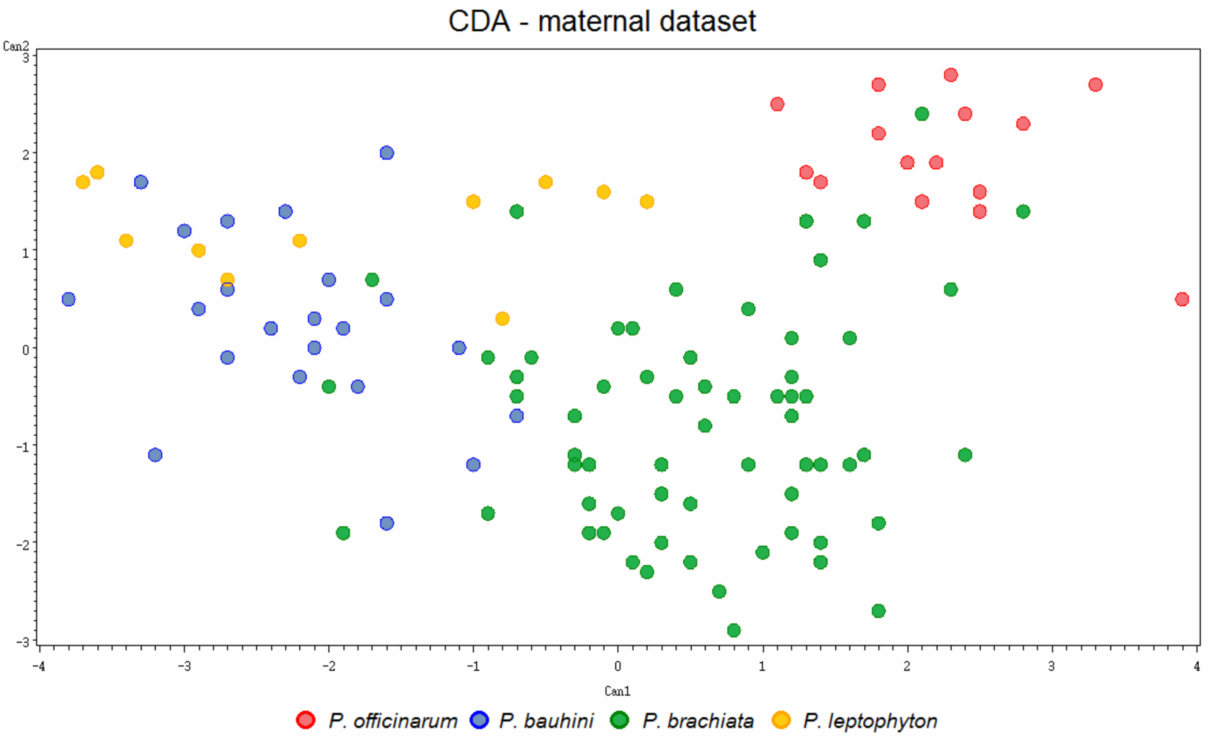

Fig. 4 Canonical discriminant analysis (CDA) diagram (a maternal dataset of 114 *Pilosella officinarum, P. bauhinii, P. brachiata* and *P. leptophyton* individuals based on 21 characters; the first canonical axis expresses 62.3% of the total variation, and the second expresses 30.7% of the total variation)

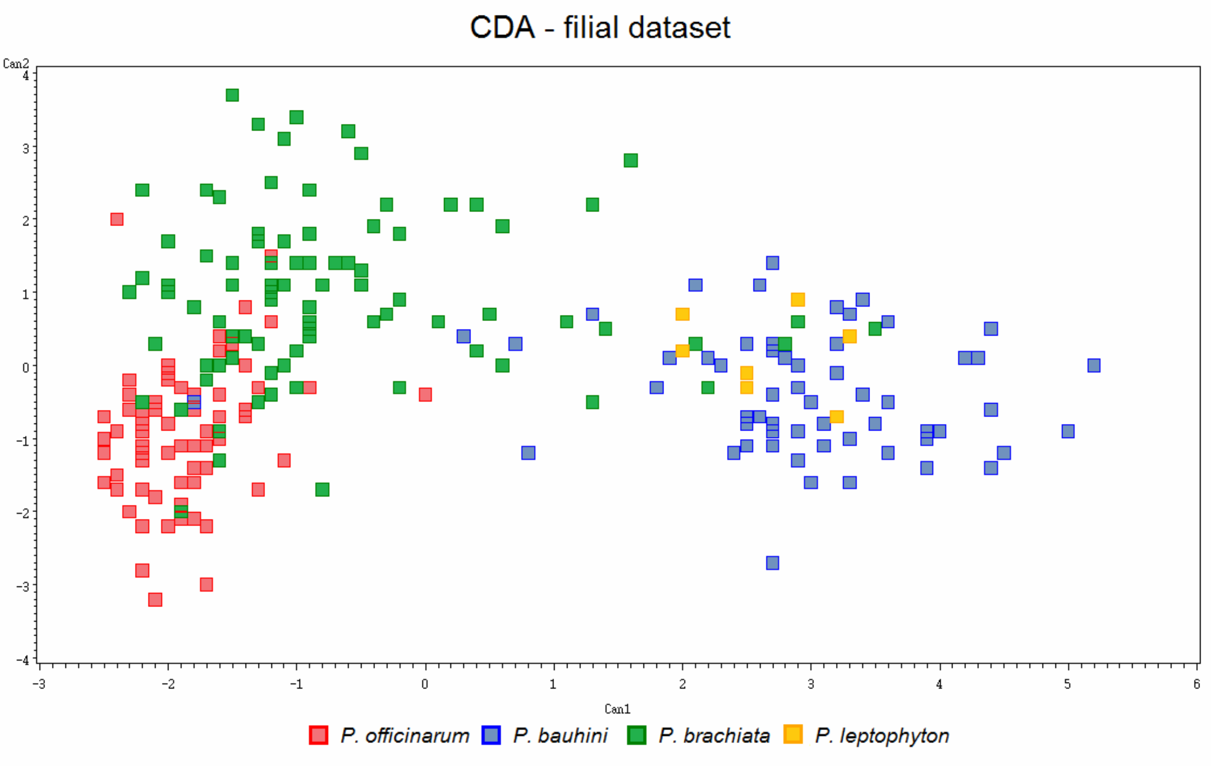

Fig. 5 Canonical discriminant analysis (CDA) diagram (the filial dataset of 114 individuals contained the progeny of *Pilosella officinarum, P. bauhinii, P. brachiata* and *P. leptophyton* and was based on 21 characters; the first canonical axis expresses 80.7% of the total variation, and the second expresses 14.8% of the total variation)

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Cytogeography of *Pilosella officinarum* (Compositae): Altitudinal and Longitudinal Differences in Ploidy Level Distribution in the Czech Republic and Slovakia and the General Pattern in Europe

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- *Background and Aims Pilosella officinarum* (syn. *Hieracium pilosella*) is a highly structured species with respect to the ploidy level, with obvious cytogeographic trends. Previous non-collated data indicated a possible differentiation in the frequency of particular ploidy levels in the Czech Republic and Slovakia. Therefore, detailed sampling and ploidy level analyses were assessed to reveal a boundary of common occurrence of tetraploids on one hand and higher ploids on the other. For a better understanding of cytogeographic differentiation of *P. officinarum* in central Europe, a search was made for a general cytogeographic pattern in Europe based on published data.
- *Methods* DNA-ploidy level and/or chromosome number were identified for 1059 plants using flow cytometry and/or chromosome counting on root meristem preparations. Samples were collected from 336 localities in the Czech Republic, Slovakia and north-eastern Hungary. In addition, ploidy levels were determined for plants from 18 localities in Bulgaria, Georgia, Ireland, Italy, Romania and Ukraine.
- *Key Results* Four ploidy levels were found in the studied area with a contrasting pattern of distribution. The most widespread cytotype in the western part of the Czech Republic is tetraploid (4*x*) reproducing sexually, while the apomictic pentaploids and mostly apomictic hexaploids (5*x* and 6*x*, respectively) clearly prevail in Slovakia and the eastern part of the Czech Republic. The boundary between common occurrence of tetraploids and higher ploids is very obvious and represents the geomorphologic boundary between the Bohemian Massif and the Western Carpathians with the adjacent part of Pannonia. Mixed populations consisting of two different ploidy levels were recorded in nearly 11% of localities. A statistically significant difference in a vertical distribution of penta- and hexaploids was observed in the Western Carpathians and the adjacent Pannonian Plain. Hexaploid populations tend to occur at lower elevations (usually below 500 m), while the pentaploid level is more or less evenly distributed up to 1000 m a.s.l. For the first time the heptaploid level (7*x*) was found on one site in Slovakia. In Europe, the sexual tetraploid level has clearly a sub-Atlantic character of distribution. The plants of higher ploidy level (penta- and hexa-) with mostly apomictic reproduction prevail in the northern part of Scandinavia and the British Isles, the Alps and the Western Carpathians with the adjacent part of Pannonia. A detailed overview of published data shows that extremely rare records on existence of diploid populations in the south-west Alps are with high probability erroneous and most probably refer to the closely related diploid species *P. peleteriana*.
- *Conclusions* The recent distribution of *P. officinarum* in Europe is complex and probably reflects the climatic changes during the Pleistocene and consequent postglacial migrations. Probably both penta- and hexaploids arose independently in central Europe (Alps and Carpathian Mountains) and in northern Europe (Scandinavia, Great Britain, Ireland), where the apomictic plants colonized deglaciated areas. We suggest that *P. officinarum* is in fact an amphidiploid species with a basic tetraploid level, which probably originated from hybridizations of diploid taxa from the section *Pilosellina*.

Key words: Amphidiploidy, apomixis, Asteraceae, flow cytometry, geographical parthenogenesis, *Hieracium*, postglacial migration, polyploidy.

INTRODUCTION

The genus *Pilosella* Hill., often treated as a subgenus of *Hieracium* L. [syn. *Hieracium* subgen. *Pilosella* (Hill) Gray], is one of the taxonomically most intricate vascular plant groups of the temperate flora. The reticulate pattern of morphological variation reflected in several thousands of taxa described from the species level to the form (Zahn, 1921–1923) complicates taxonomic treatement. Widespread polyploidy, various modes of reproduction (sexuality, obligate and facultative apomixis of aposporous type, haploid parthenogenesis, vegetative propagation), inter- and intraspecific hybridization within the same and across different ploidy levels are the most important processes involved in microevolution of the genus (Krahulcová *et al.*, 2000). The ploidy level occurring in natural populations varies from diploid ($2n = 2x = 18$) to octoploid ($2n = 8x = 72$). The most frequent cytotypes are tetraploids, pentaploids and hexaploids. Diploids are less frequent, and triploids, heptaploids, octoploids and aneuploids are rather rare (Schuhwerk and Lippert, 1997;

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Krahulcová *et al.*, 2000). Higher ploidy levels up to dodecaploid ($2n = 12x = 108$) were found in plants obtained by experimental hybridization (Skalińska, 1976). In approximately half of the taxa of the genus *Pilosella* analysed more than one ploidy level was found, even in plants growing together in one locality (Schuhwerk, 1996 and references therein).

Sell and West (1976) recognize 63 'numbered' species (including nothospecies) of *Pilosella* (treated as subgenus of *Hieracium*) in Europe. Six species having only one capitulum per stem (with the exception of hybrids) are members of the section *Pilosellina* Zahn. All but one are diploids occurring mainly in western, southern and central parts of Europe: *P. argyrocoma* (Fries) (southern and central Spain), *P. castellana* (Boiss. & Reuter) F. W. Schultz & Sch. Bip. (Spain and northern Portugal), *P. hoppeana* (Schult.) F. W. Schultz & Sch. Bip. (central and eastern Alps), *P. pseudopilosella* (Ten.) F. W. Schultz & Sch. Bip. (southern Europe, from Portugal and Spain to Bulgaria, Turkey and Romania), *P. peleteriana* (Mérat) F. W. Schultz & Sch. Bip. (northern and western Europe and the western part of central Europe). In addition, some authors distinguish a lowland form of *P. hoppeana* as a distinct diploid taxon – *P. macrantha* (Ten.) F. W. Schultz & Sch. Bip. (central and southern Europe) (e.g. Holub, 1986; Chrtek, 1998, 2002; Gottschlich, 1998; Schuhwerk and Fischer, 2003). The only almost exclusively polyploid species of section *Pilosellina* is *Pilosella officinarum* F. W. Schultz & Sch. Bip. (syn. *Hieracium pilosella* L.). It is distributed much wider than the diploid taxa mentioned above (cf. Hultén and Fries, 1986; Bräutigam, 1992). It extends from the British Isles across the whole of Europe, except the Arctic parts, to western Asia and north-western Siberia. Moreover, it has been introduced into New Zealand, North America and Patagonia, and has become an invasive and troublesome weed (Hultén and Fries, 1986; Chapman *et al.*, 2000; Cárdenas Vergara, 2005; Wilson *et al.*, 2006). *Pillosella officinarum* usually grows on dry, permeable and nutrient-poor soils from sea level to the sub-Alpine belt. The obligate heliofilous species occurs in tussock grassland communities usually with regular disturbance (grazing, mowing). Due to its low competitive ability, it tends to establish itself on open, sparsely vegetated, sites (roadside dykes, eroded slopes, landslides, etc.). Morphologically, *P. officinarum* may be distinguished from other representatives of *Pilosellina* section by long and slender stolons bearing small distant leaves decreasing in size towards the apex, involucral bracts (0·5–)1–2 mm wide, covered by stellate, glandular and eglandular trichomes (Sell and West, 1976). Despite its more or less easy identification in the field, a great phenotypic plasticity has been recorded within the species (Turesson and Turesson, 1960; Gadella, 1987, 1991). A high level of morphological variation is reflected in numerous infraspecific taxa described from the entire distribution range (Nägeli and Peter, 1885; Zahn, 1921–1923). The correlation between some phenotypic characters on one hand (e.g. rosette size, the number and length of stolons) and ploidy level on the other was revealed by Gadella (1991). In total, five cytotypes (2*x*, 4*x*, 5*x*, 6*x* and 7*x*) have been recorded in natural populations of *P. officinarum* (cf. Table 1). The mode of seed reproduction of each particular cytotype depends on the ploidy level. Diploids are sexual. Tetraploid plants reproduce sexually, but several apomictic populations deviate from this general rule (Gadella, 1984, 1987). Pentaploid *P. officinarum* is almost exclusively

TABLE 1. *Ploidy levels found in* Pilosella officinarum *in Europe according to the literature and present records*

Country	Ploidy level	References
Armenia	4*x*	Nazarova and Ghukasyan, 2004
Austria	4*x*, 5*x*, 6*x*	Turesson and Turesson, 1960; Gadella, 1972, 1984; Schuhwerk and Lippert, 1997
Belarus	4*x*, 5*x*	Dmitrieva, 1987; Parfenov and Dmitrieva, 1988
Belgium	4*x*	Gadella, 1972, 1984
Bulgaria	4*x*, 5*x*, 6*x*	Mráz *et al.*, this study
Croatia	6*x*	Gadella, 1984
Czech Republic	4*x*, 5*x*, 6*x*, 7*x*	Měsíček and Jarolímová, 1992; Krahulcová and Krahulec, 1999; Krahulcová *et al.*, 2001; Rotreklová *et al.*, 2002, 2005; Košt'álová, 2004; Mráz *et al.*, this study
Denmark	4*x*	Turesson and Turesson, 1960; Gadella, 1972, 1984
Finland	4*x*, 5*x*	Turesson and Turesson, 1960; Jalas and Pellinen, 1985
France	2*x*, 4*x*, 5*x*, 6*x*	Delcourt, 1972; Auquier and Renard, 1979; Natarajan, 1981, 1988; Gadella, 1972, 1984
Georgia	4*x*, 5*x*	Mráz *et al.*, this study
Germany	4*x*	Turesson and Turesson, 1960; Gadella, 1972; Bräutigam and Bräutigam, 1996; Schuhwerk and Lippert, 1997, 2002; Albers and Pröbsting, 1998; Rotreklová *et al.*, 2005
Hungary	5*x*, 6*x*	Mráz *et al.*, this study
Ireland	4*x*, 5*x*, 6*x*	Gadella, 1972, 1984; Finch, 2005; Watson, 2005; Mráz *et al.*, this study
Italy	2*x*, 4*x*, 5*x*, 6*x*	Gadella, 1972, 1984; Mráz *et al.*, this study
Luxembourg	4*x*	Gadella, 1972, 1984
Macedonia	6*x*	Gadella, 1972
Netherlands	4*x*, 5*x*, 7*x*	Gadella and Kliphuis, 1963; Gadella, 1972, 1984
Norway	4*x*, 5*x*	Gadella, 1972
Poland	4*x*, 5*x*, 6*x*	Skalińska, 1967; Skalińska *et al.*, 1971; Gadella, 1972; Pogan *et al.*, 1987; Pogan and Wcisło, 1989; Rotreklová *et al.*, 2005
Portugal	4*x*	Fernades and Queirós, 1971; Gadella, 1972
Romania	5*x*, 6*x*	Gadella, 1972; Mráz *et al.*, this study
Russia	6*x*	Lavrenko and Sereditov, 1991
Slovakia	4*x*, 5*x*, 6*x*, 7*x*	Májovský *et al.*, 1970; Uhríková and Feráková, 1977; Mičieta, 1982; Murín, 1986; Píšt'anský and Mičieta, 2000; Rotreklová *et al.*, 2002, 2005; Mráz *et al.*, this study
Spain	4*x*	Gadella, 1984
Sweden	4*x*, 5*x*, 6*x*, 7*x*	Turesson and Turesson, 1960; Turesson, 1972; Lövkvist and Hultgård, 1999
Switzerland	4*x*, 5*x*, 6*x*	Gadella, 1972, 1984
United Kingdom	4*x*, 5*x*, 6*x*	Turesson and Turesson, 1960; Gadella, 1972, 1984; Morton, 1974; Moore, 1982; Edmonds *et al.*, 2005; Finch, 2005; Grime *et al.*, 2005; Watson, 2005
Ukraine	4*x*, 6*x*	Pashuk, 1987; Mráz *et al.*, this study

apomictic, although a rare sexual seed production was also reported (Turesson and Turesson, 1960; Turesson, 1972; Gadella, 1984). Facultative apomixis in pentaploids was later confirmed embryologically by Pogan and Wcisło (1995). Recently, two accessions of fully sexual pentaploids have been found in the Czech Republic (Krahulcová *et al.*, 2000; Rotreklová *et al.*, 2002). Hexaploids are either sexual or apomictic, while very rare heptaploids are either apomictic or sterile (Gadella, 1984, 1991). Vegetative reproduction by means of over-ground stolons is common for all cytotypes and, together with apomixis, it might contribute to the uniclonal structure of populations.

Diploid plants of *P. officinarum* are rare and their distribution is considered to be of a relict character (e.g. Gadella, 1984). They were reported from the Valley of Aosta (Italy) (Gadella, 1972) and south-eastern France (Delcourt, 1972), respectively (but see the Discussion below). In most of Europe, the tetraploid and pentaploid populations of *P. officinarum* are by far the most common cytotypes (Gadella, 1984). Tetraploids are widespread in the lowlands of west and central Europe (e.g. Turesson and Turesson, 1960; Gadella, 1972, 1984; Pogan and Wcisło, 1989; Schuhwerk and Lippert, 1997, 2002; Krahulcová and Krahulec, 1999), while the pentaploids occur chiefly in regions that were covered by the Pleistocene glaciation – Scandinavia, the British Isles (Turesson and Turesson, 1960; Gadella, 1972, 1984, 1987; Finch, 2005; Watson, 2005). Several hexaploid populations of *P. officinarum* were found mainly in the Alps, Scandinavia, Balkan Peninsula (e.g. Turesson and Turesson, 1960, Gadella, 1972, 1984, 1991; Lavrenko and Sereditov, 1991; Schuhwerk and Lippert, 1997) and the Western Carpathians (see below). The rare occurrence of heptaploids was reported from only three localities in Sweden (Turesson and Turesson, 1960), one site in the Netherlands (Gadella, 1984) and one population in the Czech Republic (Košťálová, 2004).

Four ploidy levels (tetra-, penta-, hexa- and heptaploid) have been recorded in the Czech Republic and Slovakia (Májovský *et al.*, 1970; Uhríková and Feráková, 1977; Mičieta, 1982; Murín, 1986; Měsíček and Jarolímová, 1992; Krahulcová and Krahulec, 1999; Píšťanský and Mičieta, 2000; Krahulcová *et al.*, 2001; Rotreklová *et al.*, 2002, 2005; Košťálová, 2004). Recently, Píšťanský and Mičieta (2000) recorded tetraploids in approx. 30 localities mainly in southern and western Slovakia, while other authors reported pentaploid and hexaploid plants mostly from eastern, northern and central Slovakia. Most of the chromosome counts coming from the Czech Republic that had been published indicated that the plants analysed were tetraploids.

Almost all published data on ploidy level of *P. officinarum* are based on classical chromosome counting. This precise method is, however, considerably time-consuming. Since routine introduction of the flow cytometry in plant science in the nineties of the last century (Doležal, 1991), this approach has rapidly became popular for estimating DNA-ploidy level (Doležel, 1991). This is mainly due to the very easy sample preparation and the possibility of screening large numbers of individuals in a very short time. Here, the search which was carried out for a boundary between the area of distribution of the tetraploid cytotype and the range of penta- and hexaploids of *P. officinarum* in the territory of Slovakia and the Czech Republic, using mostly a flow cytometric approach, is reported. Moreover, an attempt was made to find out if there was a correlation between the distribution of particular ploidy levels on one hand and the altitude on the other. To understand better the cytogeographic differentiation of *P. officinarum* in central Europe, a search, based on published data, was made for a general cytogeographic pattern in Europe.

The area studied

Research has been carried out in the area of the Czech and Slovak Republics with an adjacent part of north-eastern Hungary. The area studied belongs to two different biogeographic regions, the mountain range of the Western Carpathians and the Bohemian Massif. The border between both regions is situated in the eastern part of the Czech Republic, lying north-north-east to south-south west. These two regions differ in a variety of environmental and historical parameters. In this respect, differences in the cytotype distribution cannot be explained in any easy way. On the other hand, this area covering their border can show that the pattern in cytotype distribution can be very contrasting even across a very narrow zone.

The Bohemian Massif has an old Paleogenic relief, younger areas being only canyons, those areas with Tertiary volcanism in the northern part of Bohemia, and glacial cirques in the Sudetes and the Šumava Mountains. The highest point is Mt Sněžka (1602 m), the lowest is the valley of the River Elbe on the German border at 115 m. Mostly acid Varisian parts were later covered with Permian-Carboniferous or Mesozoic sediments. Base-rich bedrocks are concentrated at lower altitudes. Vegetation cover has a coarser grain (homogeneous on a larger scale) in comparison with the Carpathian Mountains.

The Western Carpathians, including the Intra-Carpathian (Pannonian) Basin, represent the north-west part of the Carpathian arc extending from north-east Austria and south-east Czech Republic to north-east Slovakia and south-east Poland. The relief is young, of Tertiary age, similar to the Alps. The highest point is Gerlach Peak (2655 m). The bedrock is more complicated, mostly of Mesozoic and Tertiary ages. Calcium-rich substrates occur from lowland to the high mountains; e.g. in central Slovakia almost consistant limestone substrates can be found from the xerothermic Slovak Karst to the highest altitudes of the Belaer Tatra with altitudes above 2000 m. Some areas are very continental, with climatic conditions which do not allow the growth of *Fagus sylvatica* as in the area between the High and Low Tatra Mountains. On the other hand, some not distant areas are more oceanic, as in north-west Slovakia. For all these reasons, the vegetation cover is fine-grained (homogeneous in small areas but, on a larger scale, heterogeneous). Large regions with homogeneous vegetation are rare.

The area of the Czech Republic has a rather uniform climate; the warmest month is July and it is also the month of highest rainfall. This contrasts with Slovakia, where the same condition applies only at higher altitudes. At lower altitudes, the warmest month is also July, but the highest rainfall is distributed from May to September, depending on the exact geographic position (Vesecký, 1961). In this way, the same area is rather oceanic in May–June and more continental in September and vice versa. Slovakia (the Carpathian Mountains with the Pannonian Plain) is therefore fine-grained and more diverse with respect to relief, bedrock and climate.

MATERIALS AND METHODS

Material collection

Plants of *Pilosella officinarum* F. W. Schultz & Sch. Bip. (syn. *Hieracium pilosella* L.) for the present study were collected in 2003–2006 in their natural habitats throughout Slovakia and the Czech Republic, to a lesser extent also in the north-eastern part of Hungary to cover all geographic regions. They were cultivated in pots in the Botanical Garden of P. J. Šafárik University, Košice and in the experimental field of the Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice. For the complete list of localities see Supplementary Information 1 (available online). Besides the plants from the region above mentioned, some plants from a further 18 localities from different parts of Europe have also been analysed (Supplementary Information 4, available online).

As a rule, three or five plants from each population were sampled, three from the pure populations, five in the case of co-occurrence of other potentially hybridizing species of the genus *Pilosella*. Efforts were made to avoid collecting samples originating from one clone. If it was apparent that plants at the collecting site did originate from one plant, they belonged to one clone (usually plants growing very close together in a very small area of several square cm), only one individual plant *per* locality (population) was dug up. In some cases however, several cultivated plants died before analysis. For both these reasons, some populations are represented by only one plant. To determine the proportion of mixed cytotypes in populations, only those populations with two and more plants analysed were involved. Despite the fact that the ploidy level of only one plant had been estimated by us, some localities (marked in Supplementary Information 1, available online) can be considered as collecting sites with two or more analysed plants because the chromosome number of other plant/plants from the same locality was published earlier (see Supplementary Information 2, available online). Therefore, in addition to the data collected for this research, a few previous accounts from the literature (Rotreklová *et al.*, 2005) were used to search for some localities consisting of two different ploidy levels. These plants were not included in the total number of plants analysed in this present study. The voucher specimens have been deposited recently in the herbarium of Patrik Mráz, at the Institute of Biology and Ecology, P. J. Šafárik University, Košice and in the herbarium of the Institute of Botany, Průhonice (PRA).

Chromosome counts

The chromosome counts are based on the somatic mitosis in the root-tip cuttings of pot-cultivated plants. The material was pre-treated at room temperature with a 0·5% solution of colchicine for 1·5–3 h and then fixed in a cold mixture of ethanol and acetic acid (3 : 1) for at least 1 h. The fixed material was stored in 70 % ethanol at 4 °C until processed. The root tips were macerated in 1 N HCl at 60 °C for 7–10 min. The squash and smear method with cellophane replacing the glass covers (Murín, 1960) and with Giemsa solution in a phosphate buffer was used. Selected permanent slides are deposited at the Institute of Biology and Ecology, P. J. Šafárik University in Košice.

Estimation of ploidy level

Flow cytometry was used to detect the DNA-ploidy level (Suda *et al.*, 2006) for most of the plants. An analysis of relative DNA content was performed with a PA II ploidy analyser (Partec GmbH, Münster, Germany) equipped with an HBO-100 mercury arc lamp in the Flow Cytometry Laboratory, Institute of Botany, Academy of Sciences, Průhonice, Czech Republic and FACSCalibur instrument (Becton Dickinson, USA) equipped with an argon-ion laser excitation at 488 nm in the Flow Cytometry Laboratory, Institute of Biology and Ecology, P. J. Šafárik University, Košice. Sample preparations were carried out in a two-step procedure (Otto, 1990; Doležel and Göhde, 1995). Approximately 1 cm^2 of leaf tissues from both the sample and the reference internal standard were ground together for about 30 s in a Petri dish containing 1 ml of ice-cold Otto I buffer (4·2 g citric acid monohydrate + 1 mL 0·5 % Tween 20 adjusted to 200 mL and filtered through a 42-μm filter). Filtration through a 42-μm nylon mesh was followed by centrifugation at 150 *g* for 5 min. The supernatant was removed and 100 μL of fresh Otto I buffer was added. The nuclei in the pellet were resuspended and stored for 30 min at room temperature for incubation. For DNA staining 1 mL of Otto II buffer (0·4 M disodium hydrogenphosphate dodekahydrate) including 50 μL of propidium iodide, 50 μL ribonuclease, 2 μL mercaptoethanol (FACSCalibur, Becton Dickinson) or DAPI (4′,6-diamidino-2-phenylindole) at a concentration of 4 μg ml^{-1} (PA II flow cytometer, Partec GmbH) was used. The clones of previously cytologically studied diploid ($2n = 2x = 18$) plants of *Pilosella lactucella* (Wallr.) P. D. Sell & C. West (Rotreklová *et al.*, 2002, 2005) were used as an internal reference standard for the relative DNA content measurements. Moreover, one tetraploid and several pentaploid and hexaploid plants of *P. officinarum* with known chromosome numbers were used in separate and mixed flow cytometry analysis to determine the exact position of peaks of known polyploids in relation to the diploid standard peak (Fig. 1). Histograms were accumulated at a flow rate of about 20–50 particles per second for a total count of 3000–5000 nuclei. The resulting values were expressed as a peak ratio, which is a ratio of the mean position of the G_0/G_1 peak in the DNA histogram of the tested plant

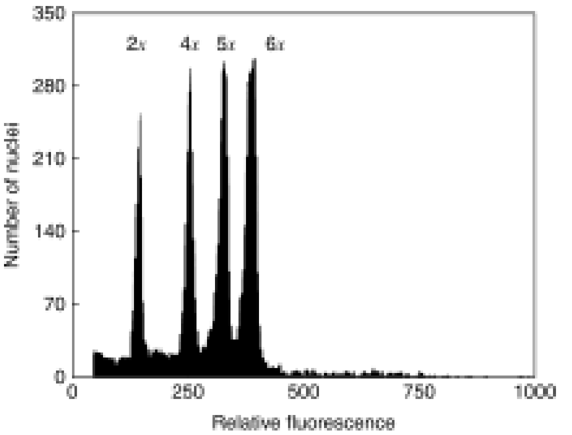

FIG. 1. Histogram of relative DNA content of DAPI-stained nuclei from a diploid plant of *Pilosella lactucella* (2*x*) used as a reference plant, with tetraploid (4*x*), pentaploid (5*x*) and hexaploid (6*x*) plants of *Pilosella officinarum.*

to the mean position of the G_0/G_1 peak in the histogram of the reference plant.

Maps

The distribution maps of cytotypes/ploidy levels in the Czech Republic and Slovakia are based on the co-ordinates determined by a GPS receiver, or found *ex post facto* from the tourist maps at a scale of 1 : 50 000 (usually old literature data). For most references from Europe for which the appropriate geographical co-ordinates were not given in original sources, the geographical position of collecting sites was estimated using *Microsoft Encarta World Atlas* (1998 Edition) and GeoNet Name Server (http://gnswww.nga.mil/geonames/GNS/index.jsp; accessed in December 2005). However, for approx. 10 % of references, estimation of co-ordinates failed (marked by an asterisk in Supplementary Information 3, available online) usually due to the absence of the name of the nearest village/town, or the existence of two or more villages/towns with the same name. Most of the chromosome numbers of the plants from the British Isles were obtained from the online version of Cytological database of the Botanical Society of the British Isles (accessed in February 2005). Distributional maps were prepared using distribution mapping software DMAP (Morton, 2004).

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's pairwise comparison (using Minitab for Windows Release 11) were applied to determine the significance ($P < 0{\cdot}05$) of the difference in the altitudinal distribution between pentaploid, hexaploid and mixed populations in the West Carpathians and adjacent Pannonia (Slovakia and the eastern part of the Czech Republic).

RESULTS

Ploidy level distribution in the Czech Republic and Slovakia

The DNA-ploidy levels and/or chromosome numbers were detected for 1059 plants sampled at 336 localities throughout the Czech Republic and Slovakia. Some plants were sampled also in north-eastern Hungary, along the Slovak–Hungarian state border. In total, 1055 plants were analysed by flow cytometric analysis. For eight plants the ploidy level was found using two approaches – by classical counting and by flow cytometry – while another four plants were counted only (cf. Supplementary Information 1, available online).

Altogether, four ploidy levels, tetra-, penta-, hexa- and heptaploid, were revealed in the area on which the study focused. The tetraploid level (4*x*; altogether 426 plants which represent 40·2 % of all plants analysed) was found to be the most common, followed by pentaploid (5*x*; 389 plants, 36·7 %) and hexaploid (6*x*; 241 plants, 22·8 %). Three heptaploid plants (7*x*) were discovered in a mixed population with one pentaploid plant at only one site in western Slovakia (Fig. 2). The record of heptaploid ploidy level is the first for *P. officinarum* in the territory of the Western Carpathians. The effort made to determine the chromosome number of heptaploid plants was not successful (the plants died), thus the new ploidy level should be considered merely as a DNA-ploidy level, i.e. not based on an exact chromosome count. Estimations of ploidy levels given for the plants from the Hungarian part of the Western Carpathians are the first records of ploidy level for *P. officinarum* for this area. In 32 localities out of 302 (10·6 %), from which at least two plants were analysed, mixed populations consisting of two different ploidies were found.

The distribution of ploidy levels in the Czech Republic is not proportional to that in Slovakia. While tetraploids are the most widespread in the Czech Republic, specifically in its western part, penta- and hexaploids predominated in Slovakia and in the eastern part of the Czech Republic (Fig. 2). The boundary between a common occurrence of the tetraploid cytotype and higher ploids is very conspicuous and corresponds well with the natural geological and geomorphological boundary between the Bohemian Massif and the Western Carpathians with the adjacent Pannonian Plain (Král, 1999). If the proportion of the particular ploidy level for each geographic region is taken into account separately, i.e. the Bohemian Massif on one hand and the Western Carpathians with Pannonia on the other, then the differences are very striking (Fig. 3).

Apart from latitudinal differentiation in ploidy level distribution in the Czech Republic and Slovakia, a statistically significant difference was also found between the proportion of pentaploids and hexaploids across the altitudes in the territory of the Western Carpathians and adjacent Pannonia (Slovakia, north-east Hungary and the eastern part of the Czech Republic) (Table 2). Generally, pure hexaploid populations tend to occur at lower elevations (usually below 500 m), while the pentaploids are very common above 500 m a.s.l. Mixed populations consisting

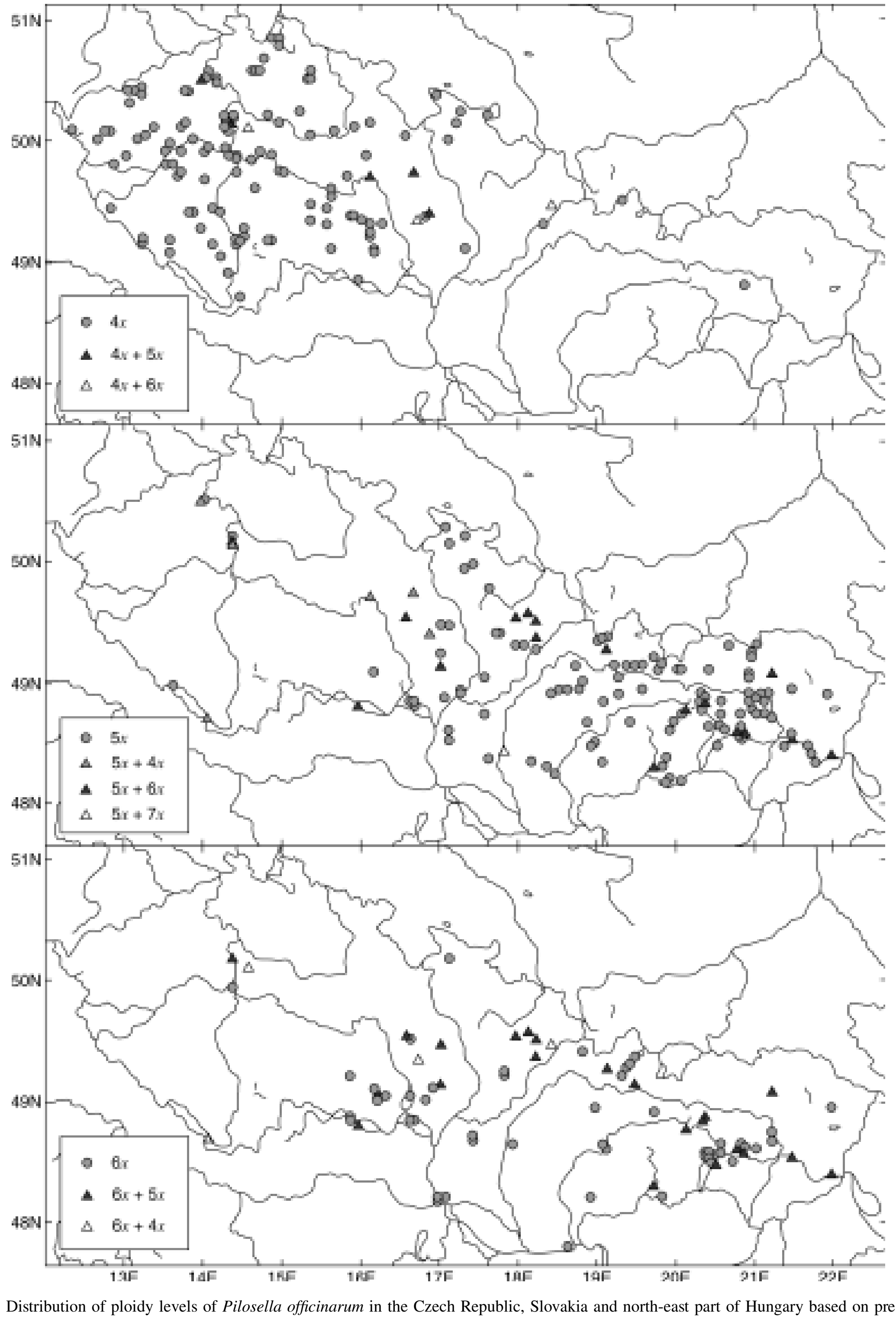

FIG. 2. Distribution of ploidy levels of *Pilosella officinarum* in the Czech Republic, Slovakia and north-east part of Hungary based on present data.

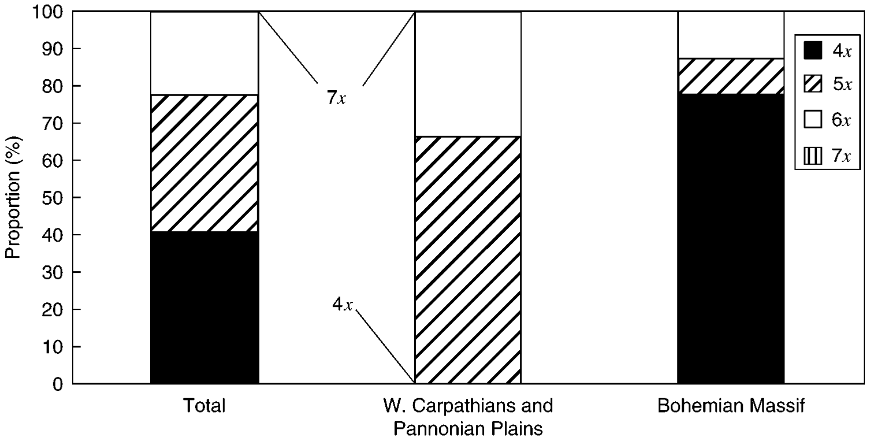

FIG. 3. Proportions of the ploidy level of *Pilosella officinarum* in the Czech Republic, north-east Hungary and Slovakia based on present data, expressed as a portion of plants of a particular ploidy level compared with the total plants analysed. The proportions over the whole area studied are given (total), together with the proportions found in two different geomorphological regions – the Bohemian Massif and the Western Carpathians with the adjacent part of Pannonia.

of two different ploidy levels were found relatively evenly along the altitudinal gradient up to 1000 m (Fig. 4).

General pattern of ploidy level distribution in Europe

Pilosella officinarum is the European taxon most examined by karyology. Chromosome numbers were counted in plants originating from 655 localities, excluding present data, across the whole continent (refer to Supplementary Information 3, available online, and Table 1). It was possible to localize geographically nearly 600 sites (Fig. 5). The most common cytotype, tetraploid (without present data), was reported in 284 localities (43 %), followed by pentaploid found at 257 collecting sites (40 %). The hexaploid ploidy level is obviously rarer, i.e. detected in 74 cases (11 %). Mixed populations consisting of two or more different cytotypes were found on 40 sites (6 %). However, in most publications the number of plants analysed per population was not given and therefore the proportion of mixed populations would be probably higher if only populations with at least two or more analysed plants per locality were taken in consideration. Tetraploids are distributed mostly in western Europe and the western part of central Europe, being the only cytotype detected in Denmark and Germany. It prevails considerably in the Netherlands (82 %), Poland (71 %) and France (65 %). The pentaploids have two main centres of distribution: at high latitudes in northern Europe (Sweden, 70 %; the British Isles, 64 %) and in major orophytic systems in Europe – the Alps (30 %) and the Carpathian Mountains with the adjacent part of Pannonia (present data for the Western Carpathians indicate 66 % of pentaploids). The predominant ploidy level in the Alps is hexaploid (59 %, in Switzerland even 84 %). The records on diploids and heptaploids are extremely scarce. The former

TABLE 2. *Means and standard deviations of altitudes of pure pentaploid (5x), pure hexaploid (6x) and mixed populations (5x + 6x) of* Pilosella officinarum *in the Western Carpathians and adjacent part of Pannonia*

Ploidy level	*N*	*X* ± s.d.
5*x*	86	546 ± 292^{a}
6*x*	40	370 ± 189^{b}
5*x* + 6*x*	21	513 ± 253^{ab}

Only populations with two or more analysed plants were included. The tetraploids and heptaploids were, according to their comparatively low abundance, excluded from this analysis. Altitudinal ranges and means are given in metres above sea level. *N*, Total number of populations; *X*, mean; s.d., standard deviation. Means in columns sharing the same superscript letters are not significantly different (Tukey's pairwaise comparisions, $P = 0{\cdot}003$, $F = 6{\cdot}19$).

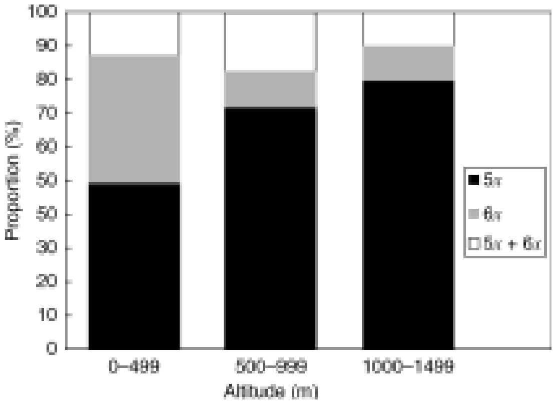

FIG. 4. Proportions of pure pentaploid (5*x*), pure hexaploid (6*x*) and mixed populations (5*x* + 6*x*) of *Pilosella officinarum* found at different altitudinal ranges in the Western Carpathians and the adjacent part of Pannonia, expressed as a number of populations of the particular ploidy level compared with the total number of populations analysed in the particular altitudinal range.

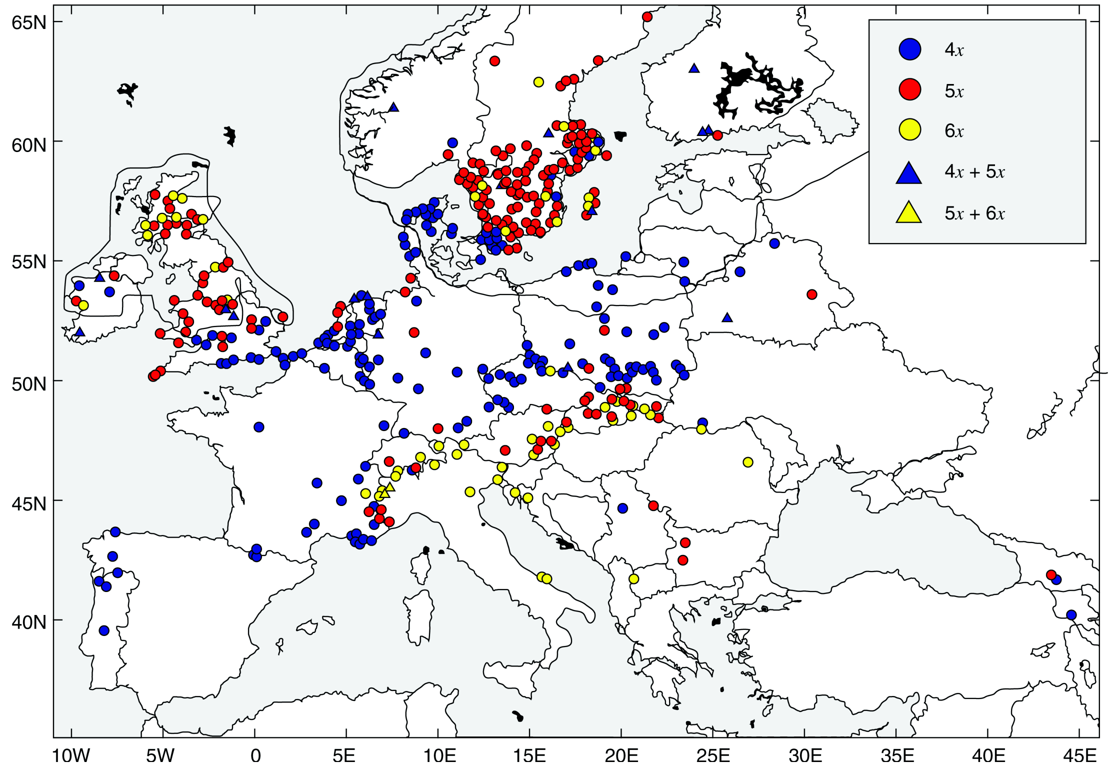

FIG. 5. Cytotype distribution of *Pilosella officinarum* in Europe (based on data from Supplementary Information 2–4, available online; present data from the Czech Republic, Slovakia and Hungary are not included). The rare heptaploid level and rare mixed cytotype combinations are not given in the map. The bold black line indicates the boundary of Last Glacial Maximum in northern Europe according to Adams (1997).

were found only in the south-west Alps (but see Discussion), the latter mostly in northern and central Europe (Sweden, Netherlands and the Czech Republic; heptaploids are not included in Fig. 5). The new data from 18 European localities confirm this general pattern: Bulgaria 4x, 5x and 6x; Georgia 4x and 5x; Ireland 5x and 6x; Italy 6x; Romania 5x, 6x and mixed 5x + 6x; Ukraine 6x (cf. Table 1 and Supplementary Information 4, available online).

DISCUSSION

Ploidy level distribution in the Czech Republic and Slovakia

According to the results of the present research, the tetraploids strongly prevail in the western part of the Czech Republic, while the pentaploids and hexaploids represent two main cytotypes in Slovakia and the eastern part of the Czech Republic. This corresponds well with scattered data published in previous studies, with the exception of the tetraploids. Our results contradict the data published by Píšt'anský and Mičieta (2000), who reported a significant predominance of tetraploid populations in the Western Carpathians, but only a rare occurrence of penta- and hexaploid plants (cf. Supplementary Information 2, available online). As preliminary results did not confirm any common occurrence of tetraploids in Slovakia (Šingliarová and Mráz, 2004), in 2004 five localities were visited from which the tetraploids were published by Píšt'anský and Mičieta (2000). However, no tetraploid was detected in any of them. Based on these observations, all data of Píšt'anský and Mičieta (2000) were considered as dubious.

The present study revealed a new heptaploid ploidy level in *P. officinarum* in the territory of the Western Carpathians. So far, the heptaploids had been detected only in three localities in Sweden (Turesson and Turesson, 1960), in one site in the Netherlands (Gadella, 1984) and one plant in a population of hexaploid plants near Prague in the Czech Republic (Košt'álová, 2004). In the Western Carpathians locality, from four analysed plants three were heptaploid and only one pentaploid. Although pentaploids reproduce the most often via aposporic apomixis, there are some data on facultative apomixis (Turesson and Turesson, 1960; Turesson, 1972) or even full sexuality (Krahulcová *et al.*, 2000; Rotreklová *et al.*, 2002). Moreover, apomictic pentaploids usually produce 2x to 3x pollen grains (Gadella, 1987; Krahulcová and Krahulec, 2000). A possible explanation of an increased ploidy level may be the fusion of reduced and unreduced gametes, as was suggested in the case of a large Dutch heptaploid population situated between two localities – the first occupied by tetra- and the second by pentaploid plants (Gadella, 1988).

A sympatry of two ploidy levels within one population was confirmed in nearly 11 % of populations. The presence of cytotype mixtures is pronounced especially in the Western Carpathians (16·4 %), whereas in the Bohemian Massif a co-occurrence of different ploidy levels is rarer (5·9 %) and confined only to the warmest regions in relict river valleys or in the zone adjacent to the Western Carpathians and the Pannonian Plain. Higher numbers of mixed populations in the Western Carpathians might be explained by a high presence of two different ploidy levels (5*x* and 6*x*) in this territory. However, this is not the case for the Bohemian Massif which has only one completely dominating tetraploid cytotype. Whether the presence of mixted cytotypes is mainly due to the more or less stochastic co-existence of different clones with different ploidy levels or to the local formation from one dominant ploidy level is yet unknown. Undoubtedly, the production of fully or partially reduced or unreduced gametes and gene flow between plants in the locality may contribute to the presence of a cytotype mixture in populations (cf. Krahulcová *et al.*, 2000). Mixed cytotype populations were found previously also in other parts of Europe in 37 localities (cf. Supplementary Information 3, available online), as well as in the Western Carpathians (Skalińska, 1967).

Surprisingly, the boundary between a common occurrence of tetraploids and higher ploids of *P. officinaum* is rather sharp and corresponds well to the natural boundary of two geomorphological units: the Western Carpathians with the adjacent Pannonian Plain and the Bohemian Massif. From the cytological point of view, a similar boundary between two cytotypes of *P. bauhini* was recorded by Rotreklová (2004), albeit, with the reverse pattern in comparison with *P. officinarum*. The tetraploids of *P. bauhini* are more frequent in Slovakia and Hungary and rare in the Czech Republic, Poland or Germany. On the other hand, pentaploid populations prevail in the Czech Republic and Germany. The border between Hercynian (including the Bohemian Massif) and Carpathian regions seems to be an important biogeographic boundary in central Europe as is also seen in the distribution patterns of many plant species. There is a whole set of species reaching this border from the east, from the Carpathian Mountains, e.g. *Cardamine glandulosa, Dianthus latifolius, Euphorbia serrulata, Galium rivale, Luzula luzulina, Salvia glutinosa etc.* (Hendrych, 1987). On the contrary, some species have migrated eastwards but did not enter, or only rarely, the Carpathian Mountains, like *Campanula rotundifolia s.str.* (Kovanda, 1977) and *Cirsium heterophyllum*. Such strong cytological and chorological differentiation between neighbouring geomorphological regions is difficult to explain. Differences in floristic composition suggest that historical processes, such as migration and expansion from different refugial areas using various migration corridors, might have played an important role (see also below). Moreover, the mountainous areas of the Bohemian Massif and the Western Carpathians are separated from each other by the north-west part of the Pannonian Plain (the Intra-Carpathian Basin) and so-called rather narrow Moravia gate, connecting the Pannonian lowland with lowlands of Silesia, Poland. This natural geographic and climatic barrier might have contributed to the different floristic and cytological patterns of these regions.

A significant difference in the proportion of penta- and hexaploid populations across altitude was found within the Western Carpathians and adjacent Pannonia. While pentaploids are more or less evenly present up to 1000 m, the hexaploids usually grow in regions with a warmer climate, usually below 500 m a.s.l. However, there are several regional deviations. Prevailing populations of the pentaploid level are present in the Zemplínske vrchy Mountains (south-east Slovakia) belonging to the warmest region of the Western Carpathians situated in the neighbouring zone with the Pannonian Plain. On the other hand, several hexaploid populations were recorded at a high elevation with a cold and humid climate in the Oravská Magura Mountains. In the Bohemian Massif, rare penta- and hexaploids or mixed populations are confined mostly to the warm, low-elevated, regions, such as river valleys, or to the adjacent zone with the Pannonian Plain (Fig. 2). One hexaploid population was found on the top of the Hrubý Jeseník Mountains (eastern part of the Sudetes range, Czech Republic). It seems that there are at least two hexaploid types in the area studied, differing in distribution and breeding systems (T. Urfus, unpubl. res.). The first one is confined mostly to thermophilous vegetation in the Carpathian Mountains and has an apomictic breeding system; it is probably related to apomictic hexaploids occurring throughout the Carpathian Mountains to the Balkan. The second hexaploid type is confined to relict river valleys in the Bohemian Massif and is sexual; this type is probably related to sexual hexaploids of the Alps (Gadella, 1984).

Amphidiploid origin of Pilosella officinarum*?*

Diploids of *P. officinarum* that are considered to have a relict distribution were found by Delcourt (1972) and by Gadella (1972) only in the south-western Alps. However, 16 records of data from the French Alps published by the former author were doubted later due to mis-identification as a closely related but different diploid species from the section *Pilosellina – P. peleteriana* (Gadella, 1984). Nevertheless, two diploid plants of *P. officinarum* counted by Gadella might belong to this species, as it is obvious from the photograph of these plants (Gadella, 1972: 362). These diploids originating from a very widely defined locality ‘the valley of Aosta’ (north-west Italy) have long stolons with decreasing leaf size towards the stolon apex. On the other hand, these plants could also represent the hybrids between true *P. officinarum* and some diploid taxon from *P.* section *Pilosellina*. These questionable data on existence of diploids of *P. officinarum* may suggest that a well-established diploid cytotype within *P. officinarum* does not really exist in nature. Another fact supports this hypothesis: most of the diploid species of *Pilosella* that had been counted up to the present, including the closely related taxa from the section *Pilosellina*, have been found usually in several if not many localities and occupied much wider ranges (e.g. Zahn, 1921–1923; Bräutigam, 1992; Schuhwerk, 1996). Moreover, polyploidy in diploid taxa of section *Pilosellina* is either unknown or very scattered records of polyploids might be regarded as mis-identifications with *P. officinarum* or interspecific hybrids. The almost exclusive presence of polyploid populations with the tetraploid ones being the commonest leads to the hypothesis that *P. officinarum* is likely to be an amphidiploid species originated from one or more crosses between diploid members of section *Pilosellina*. Both place and time of this hybridization are difficult to estimate. Analysis of ITS sequences showed low differentiation between diploid taxa (Fehrer *et al.*, 2007*a*), which suggests a relatively low age of the particular members of this group. With respect to chloroplast haplotypes, diploid members of the section *Pilosellina* share both main types. *Pilosella hoppeana*, *P. macrantha* and *P. peleteriana* share the haplotype typical of steppe and mountain species such as *P. onegensis*, *P. alpicola*, *P. glacialis, P. echioides*, etc., i.e. those species, which occurred together during the Glacial Period in steppic and tundra-like habitats in the area of central Europe. The other diploids confined to southern Europe (*P. castellana*, *P. argyrocoma* and *P. pseudopilosella*) share the haplotype with *P. lactucella*, *P. vahlii* and *P. breviscapa* (Fehrer *et al.*, 2007*a*). Central European populations of *P. officinarum* exhibit the same haplotype as *P. hoppeana* and *P. macrantha* (Krahulec *et al.*, 2004, Fehrer *et al.*, 2007*a, b*). The close relationship of diploid *P. peleteriana* and *P. officinarum* has been proven by an allozyme pattern observed in the plants originating from Scandinavia (Tyler, 2005). The possible polyphyletic and polytopic origin of polyploid populations of *P. officinarum* is supported by its enormous morphological variation. Zahn, a monographer of the genus, distinguished about 600 subspecies (Zahn, 1921–1923, 1922–1930). Morphologically, the tetraploid plants of *P. officinarum* found recently in Bulgaria resemble hybrids between hexaploid *P. officinarum* and diploid *P. macrantha*. To understand the origin of *P. officinarum* it is necessary to know more about the detailed distribution of haplotypes in the whole distribution area of *P. officinarum* and its diploid relatives. Recently Trewick *et al.* (2004) included several plants from their natural European range of distribution in their study on the origin of the introduction of *P. officinarum* into New Zealand. They found a mixed distribution of two common chloroplast haplotypes with no clear geographic pattern. However, three rare haplotypes were distributed mainly in the Alps, Sudeten Mountains, the Carpathian Mountains and Finland.

General pattern of ploidy level distribution in Europe and its relationship with polyploidy and apomixis

Gadella (1984, 1987, 1991) studied the distribution of particular cytotypes of *P. officinarum* in Europe and tried to explain its pattern. The revision presented in this paper (Fig. 5) showed that this pattern is more complex and fine grained (as was shown in the area of the Slovak and Czech Republics) than that suggested by Gadella. Despite the fact that *P. officinarum* is the most karyologically studied vascular plant species, it is realized that the published data cover only some parts of its natural distribution range sufficiently (western, central and northern Europe). Large areas in southern, south-eastern and eastern Europe have scarce or almost no data.

In total, four different cytotypes of *P. officinarum* were found in Europe (see Table 1 and Fig. 5). The records on diploids are highly questionable (see above). The most common ploidy levels are 4*x*, 5*x* and 6*x*. The range of sexual tetraploid cytotypes clearly separates the higher ploidy levels (5*x* and 6*x*) into two groups occurring in geographically different regions – into northern Europe and the mountains of central and south-east Europe (the Alps and the Carpathian Mountains, mountains in Bulgaria). Such a pattern of ploidy level distribution suggests an independent origin of penta- and hexaploids. Concerning the results from the Czech Republic and Slovakia, it seems that they match the general pattern in central Europe. While the prevailing tetraploid cytotype in the Bohemian Massif shows linkage to the tetraploid populations in the western part of central Europe, penta- and hexaploid populations in the Western Carpathians and the Pannonian Plain are likely to be related to the high ploids found in the Alps and in the Balkans (cf. Fig. 5).

It was hypothesized that the prevailing occurrence of high ploids (5*x* and 6*x*) correlates with either high latitudes or high altitudes and that their common distribution in northern Europe and in the Alps may be the result of the last Pleistocene glaciation (Gadella, 1984, 1987, 1991). The detailed map given in Fig. 5 shows that the tetraploid level is confined to western Europe and the western part of central Europe and that it has a sub-Atlantic distribution character. Northwards, sexual tetraploids are rare or completely missing and they are replaced by apomictically reproducing penta- and hexaploids (cf. Turesson and Turesson, 1960). The boundary of tetraploids and high ploids matches well with the border of the ice-sheet during the Last Glacial Maximum (cf. Adams, 1997) in the British Isles and it is very close to this geographic position in Scandinavia. Interestingly, the same pattern of cytotype distribution was found in *Parnassia palustris* L. (Parnassiaceae), where the boundary between diploids and tetraploids more or less correlates with the limit of Last Glacial Maximum (Gornall and Wentworth, 1993; Borgen and Hultgård, 2003). It therefore seems that the relationship between the presence of high ploid apomictic plants (5*x* and 6*x*) in northern Europe and glaciations might have a real basis and suggests evolutionary advantages of polyploidy associated with apomixis in the colonization of deglaciated areas in Scandinavia (cf. Asker and Jerling 1992). Merxmüller (1975) pointed out that diploid, sexually reproducing taxa of the closely related genera *Hieracium* and *Pilosella* are mostly confined to the southern latitudes, while there was a tendency for polyploids, mostly apomictic species, to prevail in northerly situated regions. Such geographically limited parthenogenesis is known also in other sexual–apomictic genera and was summarized by Bierzychudek (1985) (for thorough recent revision on complex causality of geographical parthenogenesis, see Hörandl, 2006). The diploid members of section *Pilosellina* have a more restrained range of distribution in comparison with polyploid *P. officinarum* and are confined mostly to southern and central Europe (see Introduction). This recalls the situation of several other groups of polyploid vascular plants associated with apomixis, e.g. *Antennaria* L. (Bayer and Stebbins 1987), *Ranunculus auricomus* group (Hörandl, 2006) and *Taraxacum* (den Nijs *et al.* 1990), where polyploid apomicts tend to have larger ranges than sexuals. In the present case, the colonizing success of *P. officinarum* might be attributed to the combinations of different factors, such as its probable allopolyploid origin (see above), increased heterozygosity and the existence of a high number of genetically different clones, the presence of an apomictic mode of reproduction in high ploids (5*x* and 6*x*) with the occurrence of residual sexuality, vegetative reproduction via above-ground stolons, the possibility of long-distance dispersal via achenes with a pappus, and the opportunity of recurrent formation of novel genotypes via hybridization. It is possible that all these factors have played an important role in shaping the present cytogeographic patterns of *P. officinarum*.

SUPPLEMENTARY INFORMATION

Supplementary information is available online at www.aob.oxfordjournals.org and contains the lists of *Pilosella officinarum* localities accompanied by geographical co-ordinates, ploidy levels and/or chromosome numbers (and references) for data (1) presented in this study for the areas of the Czech Republic, Slovakia and Hungary; (2) for previously reported data from the Czech Republic and Slovakia; (3) for previously published data from the rest of European area; and (4) for new data from Europe outside of Slovakia, Czech Republic and Hungary.

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SUPPLEMENTARY INFORMATION 1

List of the localities of *Pilosella officinarum* investigated by flow cytometric analyses or karyologically in the Czech Republic, Slovakia and Hungary. Number/abbreviation under which the plant was cultivated or collected is given in the first column. Abbreviations used: Sk = Slovakia, Hu = Hungary, Cz = Czech Republic, TU = Tomáš Urfus, BŠ = Barbora Šingliarová, PM = Patrik Mráz, VM = Viera Mrázová, DB = Drahoš Blanár, KC = Kateřina Čiháková, MD = Michal Ducháček, PL = ploidy level detected by flow cytometry. Chromosome numbers of the plants marked by '!' have been previously published in Rotreklová *et al.* (2005). All Slovak and Hungarian localities belong to the Western Carpathians and/or Panonnian plain. Czech localities are indicated by B.M.= Bohemian Massif or W.C. = Western Carpathians.

Number	PL	2*n*	Locality	Latitude, longitude
886		36	Sk, Volovské vrchy Mts, village of Prakovce, *loco dicto* Hutno, rocks, 400 m a.s.l., PM, 19 November 2000	N 48°48′48.24′′, E 20°53′53.16′′
871	4*x*	36	Sk, Javorníky Mts, Mt Malý Javorník, 1018 m a.s.l., PM & VM, 14 October 2000	N 49°18′24′′, E 18°18′15′′
923	4*x*		Sk, Oravské Beskydy Mts, Mt Pilsko, near the path from the village of Sihelné, 1250 m a.s.l., PM, August 2000	N 49°31′34′′, E 19°20′31′′
1463, 1464, 1465	5*x*		Sk, Juhoslovenská kotlina basin, village of Ožďany, slope above the road in S part of the village, 272 m a.s.l., PM & BŠ, 1 June 2004	N 48°22′33.4′′, E 19°53′56.8′′
1467, 1468, 1469, 1470, 1471	5*x*		Sk, Juhoslovenská kotlina basin, village of Šávoľ, pasture, 251 m a.s.l., PM & BŠ, 1 June 2004	N 48°18′06′′, E 19°49′30′′
1473, 1474, 1475,1476	5*x*		Sk, Cerová vrchovina Mts, village of Šurice, pasture ca 300 m SE of Soví hrad castle, 171 m a.s.l., PM & BŠ, 1 June 2004	N 48°13′31.5′′, E 19°55′00′′
1477	5*x*		Hu, Cserhát Mts, Somosko castle, S slope, 405 m a.s.l., PM & BŠ, 1 June 2004	N 48°10′19.7′′, E 19°51′28.5′′‘
957	5*x*		Sk, Slovenský kras Mts, village of Silica, ca 4.5 km NE of Mt Delené (500.7), 480 m a.s.l., PM, 13 June 2001	N 48°34′47′′, E 20°31′02′′
1323, 1324, 1325	5*x*		Sk, Slovenský kras Mts, Plešivecká planina plateau, Mt Veľký vrch, 721 m a.s.l., coll. PM, 5 June 2003	N 48°38′39′′, E 20°26′09′′
1328, 1329,1330	5*x*		Sk, Slovenský kras Mts, Plešivecká planina plateau, the margin of the road between Mt Veľký vrch and Mt Gerlašská skala, 748 m a.s.l., PM, 5 June 2003	N 48°38′58.5′′, E 20°25′54.1′′
1333, 1334, 1335	5*x*		Sk, Slovenský kras Mts, Zádielska planina plateau, 1 km SE of the elevation point 780.8, 700 m a.s.l., PM, 12 June 2003	N 48°38′38′′, E 20°49′46′′
1726, 1727	5*x*		Sk, Slovenský kras Mts, village of Lipovník, Horný vrch plateau, *loco dicto* Soroška, in the saddle, 530 m a.s.l., PM & VM, 28 August 2004	N 48°37′03′′, E 20°37′57′′
ZP2	5*x*		Sk, Slovenský kras Mts, village of Zádiel, Zádielská planina plateau, 597 m a.s.l., PM, 29 October 2004	N 48°37′58′′, E 20°50′05′′
947	5*x*		Sk, Slovenský kras Mts, village of Krásnohorské Podhradie, lower part of the castle hill "Krásna Hôrka", W slope, 380 m a.s.l., PM, 29 May 2001	N 48°39′40′′, E 20°35′51′′

1191, 1192	5*x*		Sk, Slovenský kras Mts, village of Krásnohorské Podhradie, W slopes of the castle hill “Krásna Hôrka”, 423 m a.s.l., PM, 29 May 2002	N 48°39′26′′, E 20°35′55.5′′
1520, 1521	5*x*		Sk, Borská nížina lowland, village of Studienka, fallow, 190 m a.s.l., BŠ, 3 June 2004	N 48°32′10.6′′, E 17°08′03.4′′
1530	5*x*		Sk, Borská nížina lowland, village of Studienka, ca 3 km SW of the village, margin of the road, 188 m a.s.l., BŠ, 5 June 2004	N 48°30′10′′, E 17°07′01′′
1596, 1597, 1598	5*x*		Sk, Borská nížina lowland, village of Šaštín-Stráže, *loco dicto* Gazárka, blowing sands, 191 m a.s.l., BŠ, 8 June 2004	N 48°37′51.4′′, E 17°08′20′′
1571, 1572, 1574, 1575	5*x*		Sk, Podunajská pahorkatina upland, village of Mlyňany, mowned meadow near the cemetery, 139 m a.s.l., BŠ, 7 June 2004	N 48°18′49′′, E 18°21′49′′
1582,	5*x*	45	Sk, Podunajská pahorkatina upland, village of Mochovce, slope near the road to power-station, 137 m a.s.l., BŠ, 7 June 2004	N 48°15′09′′, E 18°27′19′′
1581, 1583, 1584, 1585	5*x*			
1611, 1612, 1613	5*x*		Sk, Podunajská pahorkatina upland, city of Trnava, the garden of the family house, 171 m a.s.l., BŠ, 9 June 2004	N 48°22′11′′, E 17°36′31′′
906	5*x*		Sk, Východoslovenská nížina lowland, village of Veľký Kamenec, Tarbucka Hill, 268 m a.s.l., PM, 16 April 2004	N 48°21′32′′, E 21°47′31′′
910,	5*x*		Sk, Zemplínske vrchy Mts, village of Veľká Bara, former vineyards, ca 100 m N of Mt Piliš (277.6), 240 m a.s.l., PM & VM, 15 April 2001	N 48°25′47.07′′, E 21°42′28.30′′
909!		45		
914!	5*x*	45	Sk, Zemplínske vrchy Mts, village of Veľká Tŕňa, 1.5 km NNW of village, pasture, 180 m a.s.l., PM & VM, 14 April 2001	N 48°28′35.04′′, E 21°40′24.53′′
1541, 1542, 1543	5*x*		Sk, Myjavská pahorkatina upland, town of Myjava, the margin of the road, 338 m a.s.l., BŠ, 5 June 2004	N 48°45′11′′, E 17°35′18′′
730	5*x*		Sk, Malé Karpaty Mts, city of Bratislava, the areal of the Institute of Botany SAS, Dúbravská cesta No. 14, 200 m a.s.l., PM, 28 April 2000	N 48°10′21′′, E 20°03′21′′
1567, 1568, 1569, 1570	5*x*		Sk, Podunajská pahorkatina upland, village of Žírany, slope, 250 m a.s.l., BŠ, 7 June 2004	N 48°20′32.8′′, E 18°10′19′′
CAV1, CAV2, CAV3	5*x*		Sk, Strážovské vrchy Mts, village of Čavoj, ca 5 km W of the village, 511 m a.s.l., BŠ, 11 September 2004	N 48°54′00′′, E 18°26′57′′
CIC1, CIC2, CIC3, CIC4	5*x*		Sk, Strážovské vrchy Mts, village of Čičmany, rocks in the W part of the village, 655 m a.s.l., BŠ, 12 September 2004	N 48°57′03′′, E 18°30′28′′
889		45	Sk, Kremnické vrchy Mts, village of Lúčky, meadow near the road at the end of the village towards to the town of Kremnica, 580 m a.s.l., PM & VM, 2 January 2001	N 48°41′48.24′′, E 18°53′53.16′′
POL1, POL2, POL3	5*x*		Sk, Poľana Mts, village of Kyslinky, meadow, *loco dicto* Majerová, 700 m a.s.l., BŠ, 17 July 2003	N 48°40′11′′, E 19°25′48′′
CS1, CS2, CS3	5*x*		Sk, Štiavnické vrchy Mts, town of Banská Štiavnica, *loco dicto* Červená studňa, crossroad, the	N 48°28′38′′, E 18°54′25′′

			margins of the path, 791 m a.s.l., BŠ, 10 July 2004	
HN1, HN2, HN3	5*x*		Sk, Štiavnické vrchy Mts, village of Banská Belá, slope at the shore of the Halčianska nádrž reservoir, 472 m a.s.l., BŠ, 11 July 2004	N 48°28′01′′, E 18°56′58′′
KOZ1, KOZ2	5*x*		Sk, Štiavnické vrchy Mts, village of Kozelník, mouth of the Radošovo brook, 390 m a.s.l., BŠ, 11 July 2004	N 48°30′40′′, E 18°58′59′′
1554, 1555, 1556, 1557	5*x*		Sk, Krupinská planina plateau, town of Krupina, clearing, 248 m a.s.l., BŠ, 6 June 2004	N 48°21′10′′, E 19°04′26′′
944	5*x*		Sk, Volovské vrchy Mts, village of Krásnohorské Podhradie, dry meadow under Sitárka Hill, 420 m a.s.l, PM, 29 May 2001	N 48°39′39′′, E 20°35′14′′
878	5*x*		Sk, Volovské vrchy Mts, village of Smolnícka Huta, ca 0.7 km N of Mt Lastovičí vrch, forest road, 975 m a.s.l., PM, 14 October 2000	N 48°44′45.31′′, E 20°48′42.53′′
951, 952	5*x*		Sk, Volovské vrchy Mts, Jahodná, end of the ski-lift, 720 m a.s.l., PM & VM, 9 June 2001	N 48°45′41′′, E 21°07′22′′
JAH	5*x*		Sk, Volovské vrchy Mts, Jahodná, ski-lift, 720 m a.s.l., PM, 11 August 2004	N 48°45′41′′, E 21°06′55′′
954	5*x*		Sk, Volovské vrchy Mts, village of Zlatá Idka, the margins of the forest, 700 m a.s.l., PM & VM, 9 June 2001	N 48°45′01′′, E 21°00′51′′
958,	5*x*		Sk, Volovské vrchy Mts, village of Čučma, ca 200 m SW of the top of Mt Skalisko, 1250 m a.s.l., PM & VM, 12 June 2001	N 48°44′38.4′′, E 20°34′35.36′′
959!		45		
1431,	5*x*		Sk, Volovské vrchy Mts, town of Gelnica, part Turzov, 455 m a.s.l., PM, 24 August 2003	N 48°51′32.5′′, E 20°54′32.5′′
1432	5*x*	45		
1433, 1434,	5*x*		Sk, Revúcka vrchovina Mts, village of Brdárka, pasture NE of the village, 500 m a.s.l., PM, 26 August 2003	N 48°46′11.9′′, E 20°20′13.3′′
1435, 1436	5*x*	45		
1438, 1439	5*x*		Sk, Volovské vrchy Mts, village of Hnilčík, part Roztoky, *loco dicto* Poľana, 735 m a.s.l., PM, 24 August 2003	N 48°51′32.4′′, E 20°33′42′′
1441, 1442	5*x*		Sk, Volovské vrchy Mts, village of Kojšov, Mt Kojšovská hoľa, the margin of the road near the Erika chalet, 1174 m a.s.l., PM, 19 September 2003	N 48°46′52.3′′, E 20°58′46.8′′
1458, 1459, 1460, 1462	5*x*		Sk, Revúcka vrchovina Mts village of Španie Pole, pasture, 353 m a.s.l., PM & BŠ, 1 June 2004	N 48°43′43.3′′, E 21°13′56.7′′
MARG1, MARG2, MARG3, MARG4	5*x*		Sk, Volovské vrchy Mts, village of Margecany, *loco dicto* Margecianska transgresia, 330 m a.s.l., PM & BŠ, 6 October 2004	N 48°53′12.7′′, E 21°00′21.5′′
1624, 1625, 1626	5*x*		Sk, Muránska planina plateau, village of Muráň, *loco dicto* Biele Vody, pasture, 475 m a.s.l., DB, 11 June 2004	N 48°44′46′′, E 20°04′58′′
1628, 1629, 1630, 1631	5*x*		Sk, Muránska planina plateau, town of Tisovec, *loco dicto* Podhrad, pasture, 475 m a.s.l., DB, 9 June 2004	N 48°40′23′′, E 19°57′59′′
1636, 1637, 1638, 1639, 1640	5*x*		Sk, Muránska planina plateau, town of Tisovec, *loco dicto* Podhrad, pasture, 470 m a.s.l., DB, 9 June 2004	N 48°37′33′′, E 19°54′58′′

1643, 1644	5*x*		Sk, Slovenský raj Mts, Veľký Sokol valley, 685 m a.s.l., BŠ, 18 June 2004	N 48°55´49.1´´, E 20°20´08.6´´
1645, 1649, 1652	5*x*		Sk, Slovenský raj Mts, another locality in Veľký Sokol valley, 685 m a.s.l., BŠ, 18 June 2004	N 48°55´26.5´´, E 20°20´30.9´´
1653	5*x*		Sk, Slovenský raj Mts, village of Dedinky, the shore of the Palcmanská Maša dam, 814 m a.s.l., BŠ, 19 June 2004	N 48°52´22.6´´, E 20°21´39.8´´
1670, 1672, 1674	5*x*		Sk, Slovenský raj Mts, village of Stratená, Stratená valley, *loco dicto* “Pod Hanisej”, pasture, 900 m a.s.l., BŠ, 19 June 2004	N 48°51´24´´, E 20°18´42´´
928		45	Sk, Čierna hora Mts, city of Košice, Podhradová, grass-plot near road to the village of Kavečany, 236 m a.s.l., PM, 20 May 2001	N 48°44´44´.02´´, E 21°14´43.01´´
1443, 1444, 1445, 1446	5*x*		Sk, Čierna hora Mts, the margin of the road to Mt Sivec, 400 m a.s.l., BŠ, 18 May 2004	N 48°51´54´´, E 21°06´07´´
LAC1, LAC2, LAC4	5*x*		Sk, Branisko Mts, village of Lačnov, 625 m a.s.l., PM & BŠ, 6 October 2004	N 49°04´04.3´´, E 20°55´47´´
KLUK1, KLUK2, KLUK3, KLUK4	5*x*		Sk, Šarišská vrchovina Mts, village of Kluknava, 428 m a.s.l., PM & BŠ, 6 October 2004	N 48°55´26.6´´, E 20°55´58.08´´
LIP1, LIP2, LIP3	5*x*		Sk, Branisko Mts, village of Lipovce, 587 m a.s.l., PM & BŠ, 6 October 2004	N 49°03´17´´, E 20°56´40´´
VIT2, VIT3, VIT4	5*x*		Sk, Šarišská vrchovina Mts, village of Víťaz, 455 m a.s.l., PM & BŠ, 6 October 2004	N 48°54´48.1´´, E 21°05´07.4´´
1721, 1722, 1723, 1724	5*x*		Sk, Slanské vrchy Mts, vilage of Kalša, E shore of the Izra lake, 437 m a.s.l., PM, 21 October 2004	N 48°34´13´´, E 21°29´42´´
ZB1, ZB2, ZB3	5*x*		Sk, Slanské vrchy Mts, village of Zlatá Baňa, Pusté pole saddle, 650 m a.s.l., PM & BŠ, 6 October 2004	N 48°57´15´´, E 21°27´12´´,
926!	5*x*	45	Sk, Vihorlat Mts, village of Jasenov, ca 0.5 km NE of the village, pasture, 150 m a.s.l., PM, 13 May 2001	N 48°54´14.71´´, E 21°54´36.57´´
F1, F2, F3, F4	5*x*		Sk, Malá Fatra Mts, village of Fačkov, Fačkovské sedlo saddle, the margin of the path, 802 m a.s.l., BŠ, 3 August 2003	N 48°57´48´´, E 18°36´48´´
KPZ1, KPZ2, KPZ3, KPZ4	5*x*		Sk, Malá Fatra Mts, village of Kláštor pod Znievom, slope in the spruce forest, 570 m a.s.l., BŠ, 11 September 2004	N 48°57´54´´, E 18°47´41´´
RT1, RT2, RT3	5*x*		Sk, Malá Fatra Mts, village of Rajecké Teplice, part “Poluvsie”, margin of the road to the city of Žilina, 450 m a.s.l, BŠ, 12 September 2004	N 49°57´49´´, E 18°42´55´´
VAL1, VAL2, VAL3	5*x*		Sk, Malá Fatra Mts, village of Valča, Valčianska dolina valley, dry slope under the pine forest, 510 m a.s.l., BŠ, 11 September 2004	N 49°01´25´´, E 18°48´33´´
1370, 1371, 1372, 1373	5*x*		Sk, Veľká Fatra Mts, town of Ružomberok, *loco dicto* Baničné, margin of the road, 495 m a.s.l., BŠ, 9 July 2003	N 49°03´46´´, E 19°17´33´´
1374, 1375, 1376	5*x*		Sk, Veľká Fatra Mts, town of Ružomberok, *loco dicto* Baničné, rocks, 500 m a.s.l., BŠ, 9 July 2003	N 49°03´45´´, E 19°16´35´´
TUR1, TUR2,	5*x*		Sk, Veľká Fatra Mts, village of Turecká, meadow, ski-lift, 805 m a.s.l., BŠ, 11 July 2004	N 48°51´30´´, E 19°05´42´´

TUR3, TUR4				
1391, 1392, 1393	5*x*		Sk, Veľká Fatra Mts, village of Žaškov, near the forest road ca 3 km SE of the village, 595 m a.s.l., BŠ, 27 June 2003	N 49°09´56´´, E 19°12´00´´
1381, 1382, 1383	5*x*		Sk, Chočské vrchy Mts, village of Valaská Dubová, Mt Veľký Choč, *loco dicto* Stredná poľana, the meadow in the spruce forest, 1260 m a.s.l., BŠ, 29 June 2003	N 49°09´04´´, E 19°19´43´´
LUC1, LUC2, LUC3	5*x*		Sk, Chočské vrchy Mts, village of Lúčky, slope near the road to the village of Malatiná, 620 m a.s.l., BŠ, 13 July 2004	N 49°08´19´´, E 19°23´22´´
JAS1, JAS2	5*x*		Sk, Nízke Tatry Mts, Demänovská dolina valley, tourist centre Jasná, margin of the path, 1320 m a.s.l., BŠ, 14 July 2004	N 48°57´52´´, E 19°35´17´´
KOR1, KOR2, KOR3, KOR4	5*x*		Sk, Nízke Tatry Mts, village of Korytnica, meadow in the Malá Krivá valley, 800 m a.s.l., BŠ, 11 July 2004	N 48°54´07´´, E 19°16´30´´
972		45	Sk, Západné Tatry Mts, Račkova dolina valley, crossroad of the road and brook, 1143 m a.s., VM, 18 June 2001	N 49°09´36.15´´, E 19°48´29.30´´
976	5*x*		Sk, Západné Tatry Mts, village of Pribylina, pasture near the river of Belá, Kokavský most bridge, 800 m a.s.l., VM, 20 June 2001	N 49°06´21´´, E 20°25´04´´
979 978!	5*x*	 45	Sk, Západné Tatry Mts, Račkova dolina valley, meadow near the chalet of the Agriculture University of Nitra, 940 m a.s.l., VM, 18 June 2001	N 49°07´57.04´´, E 19°46´59.55´´
1385, 1386, 1387	5*x*		Sk, Západné Tatry Mts, village of Zuberec, Roháčska dolina valley, the margins of the road to the Ťatliakova chata chalet, 1250 m a.s.l., BŠ, 6 July 2003	N 49°12´36´´, E 19°44´47´´
1388, 1389, 1390	5*x*		Sk, Západné Tatry Mts, village of Zuberec, Roháčska dolina valley, the margin of the road ca 20 m from the parking place, 1200 m a.s.l., BŠ, 6 July 2003	N 49°13´28´´, E 19°43´47´´
838!	5*x*	45	Sk, Vysoké Tatry Mts, village of Tatranská Štrba, ca 1.5 km NW of Štrbské pleso tarn, 1400 m a.s.l., PM & VM, 7 July 2000	N 49°07´47.95´´, E 20°02´26.70´´
974	5*x*		Sk, Vysoké Tatry Mts, Štrbské pleso tarn, near the hotel Patria, 1355 m a.s.l., VM, 19 June 2001	N 49°07´22.37´´, E 20°04´09.35´´
LA1, LA2	5*x*		Sk, Liptovská kotlina basin, village of Liptovská Anna, forest road above the village, 690 m a.s.l., BŠ, 13 July 2004	N 49°09´22´´, E 19°28´01´´,
MH1, MH2, MH3	5*x*		Sk, Liptovská kotlina basin, village of Liptovské Matiašovce, *loco dicto* Matiašovské háje, the margin of the spruce forest, 600 m a.s.l., BŠ, 14 July 2004	N 49°08´36´´, E 19°33´51´´
869!	5*x*	45	Cz, Javorníky Mts, ca 0.5 km of the Portáš chalet, 900 m a.s.l., M & VM, 14 October 2000	N 49°17´47.29´´, E 18°14´40.22´´
BES1, BES2, BES3, BES4	5*x*		Sk, Oravské Beskydy Mts, village of Oravská Lesná, Beskyd saddle, margin of the lane, 900 m. A.s.l., BŠ, 4 July 2004	N 49°23´19´´, E 19°07´27´´
NB1, NB2, NB3, NB5	5*x*		Sk, Oravské Beskydy Mts, village of Nová Bystrica, ca 0.5 km of the dam, 526 m a.s.l., BŠ, 3 July 2004	N 49°20´39´´, E 19°02´31´´
VYCH1, VYCH2,	5*x*		Sk, Oravské Beskydy Mts, village of Vychylovka, lane near the bus-stop, 615 m a.s.l., BŠ, 3	N 49°22´23´´, E 19°04´06´´

VYCH3			July 2004	
LEN1, LEN2	5*x*		Sk, Čergov Mts, village of Lenartov, mowned meadow near the bus-stop, 477 m a.s.l.,BŠ, 10 June 2004	N 49°18´38´´, E 21°00´55´´
STL1, STL2, STL3	5*x*		Sk, Spišská Magura Mts, town of Stará Ľubovňa, castle hill, meadow between pines, 710 m a.s.l., BŠ, 11 June 2004	N 49°18´48´´, E 20°41´43´´
LUB1, LUB2, LUB4	5*x*		Sk, Šarišská vrchovina Mts, village of Ľubovec, 332 m a.s.l., PM & BŠ, 6 October 2004	N 48°54´53´´, E 21°09´20.2´´
SAK1, SAK2, SAK3	5*x*		Sk, Šarišská vrchovina Mts, village of Sedlice, between the villages of Klenov and Sedlice, 477 m a.s.l., PM & BŠ, 6 October 2004	N 48°54´48.1´´, E 21°05´07.4´´
CMM1, CMM2, CMM3	5*x*		Sk, Čergov Mts, village of Lenartov, Mt Malý Minčol, meadow in the spruce forest, 1054 m a.s.l., BŠ, 10 June 2004	N 49°15´39´´, E 20°58´37´´,
CVM1, CVM2	5*x*		Sk, Čergov Mts, village of Kyjov, Mt Veľký Minčol, the margin of the tourist path, 1150 m a.s.l., BŠ, 11 June 2004	N 49°13´55´´, E 20°59´31´´
1478, 1480	6*x*		Sk, Cerová vrchovina Mts, village of Radzovce, xerothermic slope with pines, 191 m a.s.l., PM & BŠ, 1 June 2004	N 48°13´07.3´´, E 19°50´11.2´´
932, 933	6*x* 6*x*	54	Sk, Slovenský kras Mts, Plešivecká planina plateau, ca 1 km of the town of Plešivec, a margin of the road, 240 m a.s.l., PM, 30 May 2001	N 48°33´23.82´´, E 20°24´37.04´´
935, 934!	6*x*	54	Sk, Slovenský kras Mts, Plešivecká planina plateau, on the forest route ca 0.5 km NE from the Zbojnícka priepasť chasm, 545 m a.s.l., PM, 30 May 2001	N 48°34´22.37´´, E 20°25´09,35´´
965, 966	6*x* 6*x*	54	Sk, Slovenský kras Mts, village of Silická Brezová, 0.2 km NE of Mt Delené (500,7), 490 m a.s.l., PM, 13 June 2001	N 48°32´24.20´´, E 20°29´39.20´´
1201, 1200!	6*x*	54	Sk, Slovenský kras Mts, village of Kečovo, Silická planina plateau, xerothermic slopes on the N margin of the village, 350 m a.s.l., PM, 30 May 2002	N 48°29´45´´, E 20°29´20´´
1513,1514, 1515	6*x*		Sk, Slovenský kras Mts, village of Pašková, Koniarska planina plateau, 518 m a.s.l., PM, 8 June 2004	N 48°34´06´´, E 20°22´52´´
1516, 1517, 1518, 1519	6*x*		Sk, Slovenský kras Mts, town of Plešivec, the margin of the path to the Koniarska planina plateau, 350 m a.s.l., PM, 8 June 2004	N 48°32´56´´, E 20°23´30´´
ZP1	6*x*		Sk, Slovenský kras Mts, village of Zádiel, Zádielská planina plateau, 617 m a.s.l., PM, 29 October 2004	N 48°38´07´´, E 20°49´50´´
ZP3	6*x*		Sk, Slovenský kras Mts, village of Zádiel, Zádielská planina plateau, 591 m a.s.l., PM, 29 October 2004	N 48°37´34´´, E 20°50´05´´
ZP4	6*x*		Sk, Slovenský kras Mts,village of Zádiel, Zádielská planina plateau, 613 m a.s.l., PM, 29 October 2004	N 48°37´16´´, E 20°50´18´´
ZP5	6*x*		Sk, Slovenský kras Mts, village of Zádiel, Zádielská planina plateau, 573 m a.s.l., PM, 29 October 2004	N 48°37´07´´, E 20°50´41´´

1309, 1310, 1311	6*x*		Sk, Slovenský kras Mts, village of Krásnohorské Podhradie, castle hill Krásna Hôrka, W slope, 423 m a.s.l., PM, 14 May 2003	N 48°34′35′′, E 20°35′45′′
1314,1316	6*x*		Sk, Rožnavská kotlina basin, W part of the village of Krásnohorské Podhradie, slope near the road, 393 m a.s.l., PM, 16 May 2003	N 48°39′04.5′′, E 20°34′49.6′′
1315	6*x*	54		
BOD1, BOD3	6*x*		Hu, Aggteleki Karszt Mts, village of Bodvaszilas, S of the village, 157 m a.s.l., BŠ, 19 October 2004	N 48°31′43.5′′, E 20°43′48′′
1586, 1587, 1588,1590	6*x*		Sk, Chvojnícka pahorkatina upland, town of Skalica, mowned meadow near the vineyards, 313 m a.s.l., BŠ, 8 June 2004	N 48°13′22′′, E 17°03′07′′
1522, 1523, 1524, 1525	6*x*		Sk, Malé Karpaty Mts, city of Bratislava, Mt Devínska Kobyla, meadow, 206 m a.s.l., BŠ, 5 June 2004	N 48°12′04′′, E 16°58′46′′
1526, 1527, 1528	6*x*		Sk, Malé Karpaty Mts, city of Bratislava, Mt Devínska Kobyla, sandy slope, 274 m a.s.l., BŠ, 5 June 2004	N 48°11′55.3′′, E 16°58′35.5′′
1544, 1545, 1546	6*x*		Sk, Podunajská pahorkatina upland, town of Štúrovo, ca 2 km W of the town, vineyards, 146 m a.s.l., BŠ, 6 June 2004	N 47°48′56′′, E 18°38′39′′
931	6*x*		Sk, Košická kotlina basin, village of Čečejovce, dry slope along the road, ca 1 km of the Moldavský most bridge, 210 m a.s.l., PM, 1 June 2001	N 48°36′20′′, E 21°00′43′′
1312, 1313	6*x*		Sk, Košická kotlina basin, village of Turňa nad Bodvou, 204 m a.s.l., PM, 16 May 2003	N 48°36′19′′, E 20°52′26.6′′
1500, 1501, 1502, 1503, 1504	6*x*		Sk, Košická kotlina basin, city of Košice, near the road between city of Košice and village of Pereš, 250 m a.s.l., PM, 9 June 2004	N 48°41′20′′, E 21°13′24′′
912	6*x*		Sk, Zemplínske vrchy Mts, village of Luhyňa, N slopes of Ondrejský kopec Hill, margin of oak forest, 160 m a.s.l., PM & VM, 14 April 2001	N 48°30′03.80′′, E 21°38′01.37′′
1599, 1601, 1602, 1603, 1604	6*x*		Sk, Myjavská pahorkatina upland, village of Podbranč, stone pit, 420 m a.s.l., BŠ, 8 June 2004	N 48°43′47′′, E 17°26′28′′
1532, 1533, 1534, 1535	6*x*		Sk, Malé Karpaty Mts, village of Prietrž, ca 1 km W of the village, slope near the road, 328 m a.s.l., BŠ, 5 June 2004	N 48°40′37′′, E 17°26′30′′
1537, 1538, 1539, 1540	6*x*		Sk, Malé Karpaty Mts, village of Krajné, dry slope in front of family house, 250 m a.s.l., BŠ, 5 June 2004	N 48°11′57.5′′, E 16°58′40′′
1591, 1592, 1593, 1594, 1595	6*x*		Sk, Malé Karpaty Mts, village of Záhorská Bystrica, margin of the path to the stone pit, 183 m a.s.l., BŠ, 8 June 2004	N 48°15′31.2′′, E 16°59′39′′
1605, 1606, 1608, 1609, 1610	6*x*		Sk, Považský Inovec Mts, village of Stará Lehota, xerothermic slope at the S margin of the village, 349 m a.s.l, BŠ, 8 June 2004	N 48°38′52′′, E 17°56′38′′
KOV1, KOV2	6*x*		Sk, Zvolenská kotlina basin, village of Kováčová, gravely slope near the road between the village of Kováčová and city of Zvolen, 300 m a.s.l., BŠ, 11 July 2004	N 48°36′41′′, E 19°07′23′′
S1, S2	6*x*		Sk, Kremnické vrchy Mts, village of Sielnica, margin of the road, ca 3 km NW of the village, 400 m a.s.l., BŠ, 12 September 2003	N 48°38′20′′, E 19°05′27′′

1558, 1559, 1560, 1561	6*x*		Sk, Krupinská planina plateau, village of Dvorníky, xerothermic meadow at the end of the village, 175 m a.s.l., BŠ, 6 June 2004	N 48°12´08´´, E 18°56´15´´
1317, 1318, 1319, 1320, 1321, 1322	6*x*		Sk, Volovské vrchy Mts, city of Košice, *loco dicto* Červený breh, near the ski-lift, 362 m a.s.l., BŠ, 21 May 2003	N 48°44´23.2´´, E 21°13´48.2´´
ZŠ 1	6*x*		Sk, Vihorlat Mts, village of Zálužice, the shore of the Zemplínska Šírava dam, 200 m a.s.l., BŠ, 18 August 2003	N 48°56´32´´, E 21°58´20´´
GD1, GD2, GD3	6*x*		Sk, Veľká Fatra Mts, village of Blatnica, Gaderská dolina valley, near the road, ca 1 km NE of the castle, 610 m a.s.l., BŠ, 20 September 2003	N 48°56´53´´, E 18°57´22´´
1087	6*x*		Sk, Nízke Tatry Mts, Čertovica saddle, ca 300 m W of the saddle, 1250 m a.s.l., PM & VM, 14 July 2001	N 48°54´24´´, E 19°44´07´´
1377, 1378, 1379, 1380	6*x*		Sk, Oravská vrchovina Mts, town of Dolný Kubín, dry slope near the road, N periphery of the town, 470 m a.s.l., BŠ, 9 July 2003	N 49°12´27´´, E 19°18´17´´
KNK1, KNK2, KNK3, KNK4	6*x*		Sk, Kysucká vrchovina Mts, village of Krásno nad Kysucou, mowned meadow under the bridge, 364 m a.s.l., BŠ, 3 July 2004	N 49°24´04´´, E 18°50´40´´
OMI1, OMI2, OMI3	6*x*		Sk, Oravská Magura Mts, village of Oravský Podzámok, margin of the forest road, ca 2 km N of the village, 540 m a.s.l., BŠ, 17 July 2003	N 49°16´41´´, E 19°21´03´´
OMII 1, OMII 2, OMII 3, OMII 4	6*x*		Sk, Oravská Magura Mts, village of Babín, ca 3 km E of the village, meadow in spruce forest, 910 m a.s.l., BŠ, 17 July 2003	N 49°19´32´´, E 19°25´05´´
OMIII 1, OMIII 2, OMIII 3	6*x*		Sk, Oravská Magura Mts village of Ťapešovo, Magurka transmitter, slope near the path, 1107 m a.s.l., coll. BŠ, 17 July 2003	N 49°22´05´´, E 19°29´13´´
1483 1481	5*x* 6*x*		Sk, Juhoslovenská kotlina basin, village of Fiľakovské Kľačany, sunny and dry slope near the road, 130 m a.s.l., PM & BŠ, 1 June 2004	N 48°18´34.1´´, E 19°42´45.7´´
1448, 1449 1451	5*x* 6*x*		Sk, Slovenský kras Mts, planina Horný vrch plateau, ca 1.5 km W of the elevation point 801.3, 780 m a.s.l., PM, 25 May 2004	N 48°37´16´´, E 20°45´03´´
TH2 TH1	5*x* 6*x*		Sk, Slovenský kras Mts, village of Turňa nad Bodvou, in the saddle W of the castle hill, 300 m a.s.l., PM, 1 October 2004	N 48°36´43´´, E 20°52´05´´
1580 1577, 1578, 1579	5*x* 7*x*		Sk, Podunajská pahorkatina upland, town of Hlohovec, Mt Soroš, meadow, 257 m a.s.l., BŠ, 7 June 2004	N 48°26´53.3´´, E 17°49´23.7´´
HOS1, HOS3 HOS2	5*x* 6*x*		Sk, Bodvianska pahorkatina upland, village of Hosťovce, 174 m a.s.l., PM & BŠ, 19 October 2004	N 48°35´06.7´´, E 20°51´08.2´´
904! 905	5*x* 6*x*	45	Sk, Východoslovenská nížina lowland, town of Kráľovský Chlmec, Veľký kopec Hill, 260 m a.s.l., PM & VM, 17 April 2001	N 48°25´01.43´´, E 21°57´44.68´´
1453,1454, 1455 1456	5*x* 6*x*		Sk, Volovské vrchy Mts, village of Rudná, elevation point 421, ca 1 km SE of the village, 421 m a.s.l., PM, 27 May 2004	N 48°29´35´´, E 20°30´01´´
1620, 1621, 1622	5*x*		Sk, Stolické vrchy Mts, village of Muránska Huta, slope near the church, 695 m a.s.l., DB, 11	N 48°46´38´´, E 20°06´31´´

1623	6*x*		June 2004	
1505, 1508, 1510	5*x*		Sk, Volovské vrchy Mts, town of Dobšiná, Mt Dobšinský kopec, the margin of the tourist path, 890 m a.s.l., PM, 9 June 2004	N 48°50´53´´, E 20°22´51´´
1506, 1509	6*x*			
1664	5*x*		Sk, Slovenský raj Mts, village of Stratená, the path to the pastures “pod Hanisej”, 895 m a.s.l., BŠ, 19 June 2004	N 48°51´24´´, E 20°18´42´´
1667	6*x*			
1687	5*x*		Sk, Slovenský raj Mts, village of Dedinky, planina Geravy plateau, towards to Mt Gačovská skala, 1000 m a.s.l., PM, 20 June 2004	N 48°52´35´´, E 20°23´08´´
1683, 1684, 1685	6*x*			
TER1, TER2	5*x*		Sk, Malá Fatra Mts, village of Zázrivá, “Rovná hora” saddle, pasture, 700 m a.s.l., BŠ, 12 September 2004	N 49°16´02´´, E 19°06´18´´
TER3	6*x*			
1076!		45	Sk, Chočské vrchy Mts, village of Prosiek, 0.5 km N of the village, pasture, 644m a.s.l., PM, 15 July 2001	N 49°09´29.9 ‘‘, E 19°29´54.4´´
1075	6*x*			
LS3	5*x*		Sk, Spišsko-Šarišské medzihorie Mts, village of Hubošovce, NW slope of the Lysá stráž Hill, 670 m a.s.l., PM & BŠ, 6 October 2004	N 49°05´26.6´´, E 21°13´17.7´´
LS1, LS2, LS4	6*x*			
HGA1, HGA3, HGA4	5*x*		Hu, Aggteleki Karszt Mts, village of Hidvégardó, 162 m a.s.l., PM & BŠ, 19 October 2004	N 48°33´56.3´´, E 20°50´45.3´´
HID1, HID2, HID3, HID4	5*x*		Hu, Aggteleki Karszt Mts, SE of the village of Hidvégardó, 173 m a.s.l., PM & BŠ, 19 October 2004	N 48°32´34.2´´, E 20°50´27.9´´
JOS3	5*x*		Hu, Aggteleki Karszt Mts, village of Josvafő, 300 m a.s.l., PM & BŠ, 19 October 2004	N 48°28´15´´, E 20°31´56´´
FUZ1, FUZ4	5*x*		Hu, Zemplényi hegység Mts, village of Füzér, meadow under the castle hill, 445 m a.s.l., PM & BŠ, 19 October 2004	N 48°32´40.6´´, E 21°27´44.2´´
FUZ2, FUZ3	6*x*			
TEL4	5*x*		Hu, Zemplényi hegység Mts, village of Telkibánya, the slope under the church, 292 m a.s.l., PM & BŠ, 19 October 2004	N 48°29´00.6´´, E 21°21´38.3´´
869!	5*x*	45	Cz, W.C., Javorníky Mts, ca 0.5 km of the Portáš chalet, 900 m a.s.l., M & VM, 14 October 2000	N 49°17'47.29", E 18°14'40.22"
TU1 (1,2,3)	4*x*		Cz, B.M., Český kras Mts, village of Lištice, diabase rocks 100 m of the village, near the road to the town of Beroun, 284 m a.s.l., TU, 15 May 2004	N 49°57'41.9", E 14°05'55.3"
TU2 (1,2)	4*x*		Cz, B.M., Cidlinská pánev basin, village of Činěves - the main road to Poděbrady, 500 m S of the side cut to the village of Činěves, 207 m a.s.l., TU, 18 May 2004	N 50°14'05.0", E 15°13'18.5"
TU3 (1,2,3,4)	4*x*		Cz, B.M., Pražská plošina plain, Motolská kalvárie calvary, 30 m S of the stone sideway cross, 297 m a.s.l., TU, 21 May 2004	N 50°04'58,8", E 14°19'39,2"
TU4 (1)	6*x*		Cz, B.M., Dolní Povltaví river basin, town of Roztoky- heather moor above the left bank of the Vltava river, between the towns of Roztoky and Levý Hradec, 223 m a.s.l., TU, 22 May 2004	N 50°10'13,7", E 14°22'49,0"
TU5 (1,2,3,4)			Cz, B.M., Moravian foothills of Vysočina Mts, *loco dicto* Pašerácká stezka (SW of the village	N 48°52'57,1", E 15°51'00,9"

	6*x*	of Čížov), 50 m below the road, 385 m a.s.l., KC, 29 May 2004	
TU6 (1,2,3)	4*x*	Cz, B.M., Moravian foothills of Vysočina Mts, 2 km SE of the village of Podmolí, yellow-marked hiking path, oak forest near the path, 408 m a.s.l., MD, 30 May 2004	N 48°50'21,4", E 15°57'10,8"
TU7 (1,2,3,4,5)	4*x*	Cz, B.M., Moravian foothills of Vysočina Mts, *loco dicto* Malšovické nivky, near the crossroad of the hiking paths, 387 m a.s.l., TU, 30 May 2004	N 48°50'35,0", E 15°58'28,8"
TU8 (1,2,3)	4*x*	Cz, B.M., Křivoklátsko region, Baba Hill, above the Berounka river, 373 m a.s.l., TU, 1.6.2004	N 50°01'31,2", E 13°52'45,7"
TU9 (1,2,3,4)	4*x*	Cz, B.M., Slavkovský les Mts., Tajga moor, NE of Lázně Kynžvart spa, 100 m N of the crossroad with Rota brook, 827 m a.s.l., TU, 2 June 2004	N 0°01'57,2", E 12°41'25,3"
TU10 (1,2,3,4)	4*x*	Cz, B.M., Slavkovský les Mts., *loco dicto* Dominova skalka, 3 km W of the village of Bečov nad teplou, 751 m a.s.l., TU, 2 June 2004	N 50°04'16,6", E 12°47'10,0"
TU11 (1,2,3,4)	4*x*	Cz, B.M., Slavkovský les Mts., *loco dicto* Bečovské louky, 2 km NE of the village of Bečov n.Teplou, 643 m a.s.l., 2 June 2004	N 50°05'10,7" E 12°49'22,0"
TU12 (1,2,3,4)	4*x*	Cz, B.M., Lounsko-labské středohoří Mts, Oblík Hill, 400 m SW below the top, meadow with Stipa zalesskii, 357 m a.s.l., TU, 3 June 2004	N 50°24'31,3" E 13°48'19,3"
TU13 (1,2,3)	4*x*	Cz, B.M., Ralsko-bezdězská tabule table, near the Břehyňský rybník lake, side cut to the village of Strážov from the road Doksy-Mimoň, the margin of the road, 281 m a.s.l., TU, 4 June 2004	N 50°35'10,7" E 14°43'41,9"
TU14 (1,2,3)	4*x*	Cz, B.M., Střední Polabí region, *loco dicto* Hrabanovská černava, sand dune, 187 m a.s.l., TU, 5 June 2004	N 50°13'07,8" E 14°49'51,5"
TU15 (2,3)	4*x*	Cz, B.M., Křivoklátsko region, near the Baba Hill, slope with the pines, ca 3 km of the road to the Baba Hill, 400 m a.s.l., TU, 1 June 2004	N 50°01'31,3" E 13°52'19,3"
TU16 (1) (2,3,4,5)	5*x* 4*x*	Cz, B.M., Lounsko-labské středohoří Mts, SW slope of the Mt Boreč, 50 m W of the path, 428 m a.s.l., TU, 11 June 2004	N 50°30'49,1" E 13°59'14,3"
TU17 (1,2,3,4)	4*x*	Cz, B.M., Lounsko-labské středohoří Mts, Holý vrch Hill near the Sutom - rock 50 m below the top, 447 m a.s.l., TU, 11 June 2004	N 50°30'10,1" E 13°58'41,2"
TU18 (1,2,3)	4*x*	Cz, B.M., Středočeská tabule table, Tuchoměřická hůrka Hill, near the village of Tuchoměřice, 292 m a.s.l., TU, 23 June 2004	N 50°07'45,9" E 14°16'36,6"
TU19 (1,2,3,4)	4*x*	Cz, B.M., Středočeská tabule table, village of Svrkyně, the rock above the football field, 309 m a.s.l., TU, 23 June 2004	N 50°10'20,5" E 14°18'02,8"
TU20 (1,2,3)	4*x*	Cz, B.M., Říčanská plošina plain, village of Pecerady, the crossroed of the blue-marked hiking path and the route in the village, near the lane, 302 m a.s.l., TU, 24 June 2004	N 49°50'07,8" E 14°37'05,3"
TU21 (1,2,3)	4*x*	Cz, B.M., Říčanská plošina plain, village of Sázava, the crossroad of the hiking paths, 340 m a.s.l., TU, 24 June 2004	N 49°52'16,3" E 14°53'57,0"
TU22 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, village of Horní Kněžeklady-Hněvkovice, the margin of the road, 294 m a.s.l., TU, 26 June 2004	N 49°11'40,4" E 14°27'59,9"
TU23 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, village of Hrušov, 484 m a.s.l., TU, 26 June 2004	N 49°12'33,1" E 14°31'50,3"

TU24 (1,2,3,4)	4*x*	Cz, B.M., Střední Povltaví river basin, village of Horní Kněžeklady, 492 m a.s.l., TU, 26 June 2004	N 49°11'37,7" E 14°28'50,8"
TU25 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, dam in the village of Hněvkovice, the meadow in front of the chalet, 387 m a.s.l., TU, 26 June 2004	N 49°11'56,5" E 14°26'32,3"
TU26 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, village of Jeznice, near the road, 374 m a.s.l., TU, 27 June 2004	N 49°09'13,6" E 14°26'29,8"
TU27 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, village of Litoradlice, near the former shooting-gallery, 474 m a.s.l., TU, 27 June 2004	N 49°10'38,9" E 14°25'11,4"
TU28 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, lake 1,5 km S of the village of Zvíkovského podhradí, margin of the road, 397 m a.s.l., TU, 27 June 2004	N 49°24'48,8" E 14°12'33,2"
TU29 (1,2,3)	4*x*	Cz, B.M., Podbrdsko region, gas station in the village of Řitka, 359 m a.s.l., TU, 27 June 2004	N 49°53'53,0" E 14°19'14,7"
TU30 (1,2,3,4,5)	4*x*	Cz, B.M., Český ráj Mts, village of Ploužnice, near the village of Lomnice nad Popelkou, near the road, 514 m a.s.l., TU, 1 July 2004	N 50°30'43,0" E 15°23'09,5"
TU31 (1,2,3)	4*x*	Cz, B.M., Český ráj Mts, village of Rváčov, near the village of Lomnica, Krčkův kostel church, 564 m a.s.l., TU, 1 July 2004	N 50°31'51,9" E 15°19'58,8"
TU32 (1,2,3)	4*x*	Cz, B.M., Jesenicko-rakovnická plošina plain, periphery of the town of Rakovník, direction to village of Lišany, near the sculpture of St. Adalbert, 369 m a.s.l., TU, 6 July 2004	N 50°06'36,8" E 13°44'02,1"
TU33 (1,2,3)	4*x*	Cz, B.M., Jesenicko-rakovnická plošina plain, between the Hlaváčov stone pit and *loco dicto* Na cikámce, 304 m a.s.l., TU, 6 July 2004	N 50°07'20,6" E 13°44'13,4"
TU34 (1,2,3)	4*x*	Cz, B.M., Jesenicko-rakovnická plošina plain, 100 m of the loco dicto Sládkov kříž, blue-marked hiking path, near the village of Stará Lužná , 394 m a.s.l., TU, 6 July 2004	N 50°09'09,0" E 13°46'58,8"
TU35 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, 200 m S of the village of Zborná (close to the town of Jihlava), margin of the road, 559 m a.s.l., TU, 7 July 2004	N 49°26'06,5" E 15°34'22,6"
TU36 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, village of Štoky, Smilov settlement, near the bus stop, 475 m a.s.l., TU, 8 July 2004	N 49°32'11,8" E 15°37'53,0"
TU37 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, lane near the main road between the villages of Šlapanov and Smilov, 474 m a.s.l., TU, 8 July 2004	N 49°32'11,1" E 15°38'29,3"
TU38 (1,2,3,4)	5*x*	Cz, B.M., Hrubý Jeseník Mts, village of Revíz, meadows S of the village, cairn of the rocks, 754 m a.s.l., TU, 11 July 2004	N 50°13'31,5" E 17°18'13,6"
TU39 (1,2,3,4)	5*x*	Cz, B.M., Hrubý Jeseník Mts, *loco dicto* Vřesová studánka, foundations of the church, 1286 m a.s.l., TU, 12 July 2004	N 50°08'44,9" E 17°08'04,9"
TU40 (1,2,3,4,5)	6*x*	Cz, B.M., Hrubý Jeseník Mts, tourist chalet Šerák, 1324 m a.s.l., TU, 12 July 2004	N 50°11'13,1" E 17°06'31,4"
TU41 (1,2,3)	4*x*	Cz, B.M., Hanušovicko-rychlebská vrchovina Mts, 1 km NE of the castle Rychleb, near the village of Uhelná , 442 m a.s.l., TU, 14 July 2004	N 50°22'00,9" E 16°59'30,3"
TU42 (1,2,3)	5*x*	Cz, B.M., Hanušovicko-rychlebská vrchovina Mts, former limestone pit above the village of	N 50°16'59,5" E 17°05'32,0"

		Vápenná, 448 m a.s.l., TU, 15 July 2004	
TU43 (1,2,3)	4*x*	Cz, B.M., Hrubý Jeseník Mts, village of Chebzí, meadows, 533 m a.s.l., TU, 16 July 2004	N 50°14'44,4" E 17°15'42,8"
TU44 (1,2,3)	4*x*	Cz, B.M., Horažďovická pahorkatina Mts, periphery of the town of Blatná, near the crossroad Praha-Písek, 452 m a.s.l., TU, 28 July2004	N 49°25'26,4" E 13°51'45,2"
TU45 (1,2,3,4)	4*x*	Cz, B.M., Horažďovická pahorkatina Mts, lane 4 km of the town of Blatná, green-marked hiking path, 495 m a.s.l., TU, 28 July 2004	N 49°24'56,2" E 13°50'07,5"
TU46 (1,2,3,4,5)	4*x*	Cz, B.M., Horažďovická pahorkatina Mts, Velký Pálenec lake, meadow over the field hydrologic station, 472 m a.s.l., TU, 28 July 2004	N 49°24'56,1" E 13°49'01,6"
TU47 (1,2,3)	4*x*	Cz, B.M., Horažďovická pahorkatina Mts, reserve so called Smyslovské louky meadows, near the Smyslov lake, 455 m a.s.l., TU, 28 July 2004	N 49°25'07,3" E 13°48'04,6"
TU48 (1,2,3)	4*x*	Cz, B.M., Podbrdsko region, periphery of the town of Příbram, road to towns of Milín and Písek, 491 m a.s.l., TU, 23 August 2004	N 49°40'25.0" E 14°00'19.1"
TU49 (1,2,3)	4*x*	Cz, B.M., Střední Povltaví river basin, 500 m W of the Varvažovský most bridge across the Skalica river, 303 m a.s.l., TU, 23 August 2004	N 49°26'20.1" E 14°06'53.2"
TU50 (1) (2,3)	5*x* 6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, village of Dukovany,Templštejn castle, W slope above the Jihlava river, 359 m a.s.l., TU, 6 September 2004	N 49°05'23,9" E 16°14'52,3"
TU51 (1,2,3,4,6) (5)	4*x* 5*x*	Cz, B.M., Žďárské vrchy Mts, *loco dicto* Pustá Rybná, 12 km of the town of Polička, meadow NW of the Damašek settlement, 635 m a.s.l., TU, 11 September 2004	N 49°42'49.3" E 16°07'24.4"
TU52 (1,2,3)	6*x*	Cz, W.C., Zlínské vrchy Mts, village of Zádveřice u Vizovic, in front of the evangelical church, 271 m a.s.l., TU, 21 September 2004	N 49°12'58,0" E 17°98'11,8"
TU53 (1,2) (3)	5*x* 5*x* 45	Cz, Střední pobečví basin, town of Vsetín, near the main road to Valašské Meziríčí, 353 m a.s.l., TU, 21 September 2004	N 49°19'55,1" E 17°59'41,1"
TU54 (1,2,3)	4*x*	Cz, B.M., Horní Poohří basin, Komorní hůrka Hill, bottom of the exploited pit, 481 m a.s.l., TU, 22 September 2004	N 50°05'58,9" E 12°20'23,3"
TU55 (1,2,3)	4*x*	Cz, B.M., Ralsko-bezdězská tabule plateau, town of Doksy, near the railway station, 275 m a.s.l., TU, 3 October 2004	N 50°34'25,2" E 14°38'54,5"
TU56 (1,2)	4*x*	Cz, B.M., Ralsko-bezdězská tabule plateau, pine forest below the Havířov vrch Hill, between the Máchove jezero lake and Břehyňský rybník fishpond, 292 m a.s.l., TU, 3 October 2004	N 50°35'11.1" E 14°40'29.7"
TU57 (1,2,3,4,5,6,7,8,9,12)	6*x*	Cz, B.M., Dolní Povltaví river basin, town of Roztoky, heather moor above the left bank of the Vltava river, between the town of Roztoky and Levý Hradec, 223 m a.s.l., TU, 9 October 2004 (vide TU 004)	N 50°10'13,7" E 14°22'49,0"
TU58 (1,2,3,4,6,7,8) (5)	4*x* 5*x*	Cz, B.M., Dolní Povltaví river basin, rocks between the village of Zámky and Drahaňské údolí valley, 229 m a.s.l., TU, 12 October 2004 (vide TU 016)	N 0°08'38,3" E 14°23'59,5"
TU59 (1,2,3,4)	4*x*	Cz, B.M., Lounsko-labské středohoří Mts, Boreč Hill, SW slope, 50 m W of the path, at the top of scree, 428 m a.s.l., TU, 17 October 2004	N 50°30'49,1" E 13°59'14,3"

TU60 (1,2,3,4)	4*x*		Cz, B.M., Dolní Povltaví river basin, *loco dicto* Větrušické skály, 500 m N of the village of Větrušice 255 m a.s.l., TU, 22. October 2004	N 50°11'53,3" E 14°22'31,9"
TU61 (1,2,3)	5*x*		Cz, B.M., Dolní Povltaví river basin, Větrušické skály, another locality, edge of oak forest and rocky heath, 246 m a.s.l., TU, 22 October 2004	N 50°12'02,0" E 14°22'15,2"
TU62 (1,2,3)	4*x*		Cz, B.M., Dolní Povltaví river basin, village of Otvovice, *loco dicto* Otvovická skála rock, top of the rock, 259 m a.s.l., TU, 23 October 2004	N 50°12'57,4" E 14°17'00,7"
TU63 (1,2,3)	4*x*		Cz, B.M., Střední Povltaví river basin, rocks between village Živohošť and Živohošťský most bridge, 200 m below the green-marked hiking path, 329 m a.s.l., TU, 30 October 2004	N 49°45'18,8" E 14°26'00,3"
TU64 (1,2,3)	4*x*		Cz, B.M., B.M., Terezínská kotlina fold, hill in the village of Oleško, near the tlen of Terezín, 151 m a.s.l., TU, 30 October 2004	N 50°31'00,0" E 14°09'00,0"
TU65 (1,2,3)	4*x*		Cz, B.M., Český kras Mts, *loco dicto* Krásná stráň hillside, E margin, the beginning of the river terrace, 327 m a.s.l., TU, 23 April 2005	N 49°56'34,4" E 14°16'41,5"
TU66 (1,2,3)	4*x*		Cz, B.M., Střední polabí river basin, village of Písty, near the sand dune, 274 m a.s.l., TU, 30.4.2005	N 50°09'44,7" E 14°59'41,7"
TU67 (1,2,3,4)	4*x*		Cz, B.M., Střední Povltaví river basin, village of Štěchovice, 218 m a.s.l., TU, 6 April 2005	N 49°51'16,5" E 14°24'38,1"
TU69 (1,2,3)	4*x*		Cz, B.M., Český kras Mts, Koukolova hora Hill, 200 m N of the stone pit, yellow-marked hiking path, 443 m a.s.l., TU, 7 May 2005	N 49°55'13,7" E 14°01'28,0"
TU70 (1,2,3,4,5)	6*x*		Cz, B.M., Moravian foothills of Vysočina Mts, village of Dukovany,Templštejn castle, W slope above the Jihlava river, 359 m a.s.l., TU, 12 May 2005 (vide TU 050)	N 49°05'23,9" E 16°14'52,3"
TU71 (1,2,3)	4*x*		Cz, B.M., Lounsko-labské středohoří Mts, N margin of the village of Dolné Zálezly, green-marked hiking path, in the chalet colony , 218 m a.s.l., TU, 16 May 2005	N 50°35'55,5" E 14°03'32,8"
TU72 (1,2,3,4)	4*x*		Cz, B.M., Lounsko-labské středohoří Mts, lower top of Lovoše Hill, 473 m a.s.l., TU, 17 May 2005	N 50°31'43,8" E 14°01'25,0"
TU73 (1)	5*x*		Cz, B.M., Lounsko-labské středohoří Mts, top of Lovoš Hill, the crossroad of blue- and green-marked hiking path, 543 m a.s.l., TU, 17 May 2005	N50°31'38,4" E014°01'08,2"
(2,3)	5*x*	45		
TU74 (1,2,3)	4*x*		Cz, B.M., Střední Povltaví, N margin of the tlen of Davle, right bank of the river, the rock cca 30 m above the Vltava River, 235 m a.s.l., TU, 21 May 2005	N49°53'53,7" E014°24'08,2"
TU75 (1,2,3)	6*x*		Cz, B.M., Střední Povltaví river basin, 500 m N of the village of Vranné, road near the Vltava River, 214 m a.s.l., TU, 21 May 2005	N49°56'40,0" E014°23'49,3"
TU76 (1,3,5)	5*x*	45	Cz, B.M., Šumava Mts, margin of the road in the village of Františkov, 929 m a.s.l., TU, 2 June 2005	N48°59'59,8" E013°37'41,6"
(2,4, 6)	5*x*			
TU77 (1,2,3,4,5)	6*x*		Cz, B.M., Moravian foothills of Vysočina Mts, village of Dukovany,Templštejn castle, W slope above the Jihlava river, 359 m a.s.l., TU, 5 June 2005	N 49°05'23,9" E 16°14'52,3"
TU78 (1,2,3)	4*x*		Cz, B.M., Českomoravská vrchovina Mts, E periphery of the vilage of Měřín, margin of the road near the cemetery, 509 m a.s.l., TU, 8 June 2005	N 49°23'09,2" E 15°53'14,8"

TU79 (1,2,3,4,5)	6*x*	Cz, B.M., Českomoravská vrchovina Mts, town of Třebíč, rock above the jewish ward, 414 m a.s.l., TU, 8 June 2005	N 49°13'04,6" E 15°52'48,6"
TU80 (1,2,3,4)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, rock near the road above Oslava River, between the villages of Naloučovice and Jedovský mlýn, 350 m a.s.l., TU, 8 June 2005	N 49°13'40,9" E 16°08'31,3"
TU81 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, margin of the road 1 km N of the village of Naloučany, 436 m a.s.l., TU, 8 June 2005	N 49°14'57,1" E 16°08'08,9"
TU82 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, periphery of the town of Velké Meziříčí, rock above the bus stop "Lihovar", 422 m a.s.l., TU, 8 June 2005	N 49°20'21,6" E 16°01'53,4"
TU83 (1,2,3,4)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, small chapel between the villages of Blízkov and Měřín, 501 m a.s.l., TU, 8 June2005	N 49°23'52,2" E 15°54'09,1"
TU84 (1,2,3)	4*x*	Cz, B.M., Moravian foothills of Vysočina Mts, blue-marked hiking path from the Mohelnská step heath to the Jihlava River bank, 200 m of the edge of the heath, 286 m a.s.l., TU, 11 June 2005	N 49°06'23,3" E 16°10'57,3"
TU85 (1,3)	4*x*	Cz, B.M., Moravian foothills of Vysočina Mts, 400 m N of the Mohelno dam, rock near the trail, 305 m a.s.l., TU, 11 June 2005	N 49°05'58,0" E 16°10'41,1"
TU86 (1,2,3,4,5)	6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, *loco dicto* Kozí stezky, rock above the Dyje River, 391 m a.s.l., TU, 12 June 2005	N 48°51'41,5" E 15°52'15,2"
TU87 (1,2,3,4) (5)	6*x* 5*x*	Cz, B.M., Znojemsko-brněnská pahorkatina Mts, Havraníky heather moor , 339 m a.s.l., TU, 12 June 2005	N 48°48'43,1" E 15°59'38,6"
TU88 (1,2,3,4)	6*x*	Cz, W.C., Mikulovská pahorkatina-Pálava Mts, blue-marked hiking path to the Děvín Hill, edge between oak forest and the heath, 371 m a.s.l., TU, 13 June 2005	N 48°51'52,2" E 16°39'07,9"
TU89 (1,2,3,4)	6*x*	Cz, W.C., Mikulovská pahorkatina-Pálava Mts, canyon „Soutěska“ near the Děvín Hill, 100 m below the country-sight, 429 m a.s.l., TU, 13 June 2005	N 48°51'51,9" E 16°38'36,8"
TU90 (1,2,3,4,5,6)	5*x*	Cz, W.C., Mikulovská pahorkatina-Pálava Mts, canyon near the Děvín, another locality, 200 m of the previous locality, 383 m a.s.l., TU, 13 June 2005	N 48°51'47,2" E 16°38'40,0"
TU91 (1,2,3)	5*x*	Cz, W.C., Jihomoravský úval ravine, reserve „Důbrava u Hodonína“, near the town of Hodonín, near the former main road to the Brno city, 235 m a.s.l., TU, 14 June 2005	N 48°53'05,9" E 17°05'13,2"
TU92 (1,2,3,4)	5*x*	Cz, W.C., Jihomoravský úval ravine, village of Bzenec, reserve „Váté písky“, sand dunes, pine forest near the railway station, 199 m a.s.l., TU, 14 June 2005	N 48°56'01,3" E 17°16'58,3"
TU93 (1,2,3,4)	5*x*	Cz, W.C., Jihomoravský úval ravine, village of Bzenec, another locality, 300 m of the previous locality, near the train line, 194 m a.s.l., TU, 15 June 2005	N 48°55'50,4" E 17°16'37,5"
TU95 (1,2,3)	4*x*	Cz, B.M., Hrubý Jeseník Mts, village of Bělá pod Pradědem, convalescent home, near the bridge across the brook, 643 m a.s.l., TU, 17 June 2005	N 50°08'10,1" E 17°12'29,3"
TU96 (1,2,3)	5*x*	Cz, B.M., Hrubý Jeseník Mts, village of Rejvíz, margin of the road, 775 m a.s.l., TU, 17 June 2005	N 50°13'45,9" E 17°18'07,8"

TU97 (1,2,3,4,5,6)	6*x*	Cz, B.M., Hrubý Jeseník Mts, tourist chalet Šerák, 1324 m a.s.l., TU, 24 June 2005 (videTU 40)	N 50°11'13,1" E 17°06'31,4"
TU98 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, 1 km W of the village of Strašice (direction: town of Rokycany), 496 m a.s.l., TU, 27 June 2005	N 49°44'06,3" E 13°44'27,0"
TU99 (1,2,3,4)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, near the road from the village of Mirošov to the town of Rokycany, 432 m a.s.l., TU, 27 June 2005	N 49°43'10,2" E 13°39'17,8"
TU100 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, near the road from the village of Březina to the village of Bezděkov, 481 m a.s.l., TU, 27 June 2005	N 49°49'04,2" E 13°36'09,2"
TU101 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, village of Kozojedy, 50 m of the church, 365 m a.s.l., TU, 27 June 2005	N 49°55'57,9" E 13°32'39,1"
TU102 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, village of Všehrdy, rocks below the village, 372 m a.s.l., TU, 27 June 2005	N 49°58'01,8" E 13°35'07,4"
TU103 (1,2,3)	4*x*	Cz, B.M., Křivoklátsko region, stone pit near the village Mléčice, direction: village of Zbiroh, 368 m a.s.l., TU, 27 June 2005	N 49°54'44,1" E 13°42'06,1"
TU104 (1,2,3)	4*x*	Cz, B.M., Hornosázavská pahorkatina Mts, periphery of the town of Havlíčkuv Brod, the bus stop near the main exit road to the town of Pardubice, 436 m a.s.l., TU, 29 June 2005	N 49°36'39,3" E 15°36'49,2"
TU105 (1,2,3,4)	4*x*	Cz, B.M., Hornosázavská pahorkatina Mts, 300 m E of the town of Ždírce nad Doubravou, near the road, TU, 29 June 2005	N 49°42'01,4" E 15°49'12,7"
TU106 (1,2,4) (3)	4*x* 5*x*	Cz, B.M., Českomoravské mezihoří upland, rocks between the village of Linhartice and the town of Moravská Třebová, 349 m a.s.l., TU, 29 June2005	N 49°45'39,5" E 16°40'37,4"
TU107 (1,2,3,4)	5*x*	Cz, W.C., Jesenické podhůří foothills, rocks 1 km E of the town of Budišov nad Budišovkou, near the road direction: Vítkov, 527 m a.s.l., TU, 29 June 2005	N 49°47'42,5" E 17°38'50,4"
TU108 (1,2,3) (4)	5*x* 6*x*	Cz, W.C., area called Moravská brána, village of Straník, above the chapel, 382 m a.s.l., TU, 29.6.2005	N 49°32'55,5" E 17°59'20,4"
TU109 (1,2,3)	5*x*	Cz, W.C., Haná region, town of Prostějov, lawn over the cinema Metro70, 221 m a.s.l., TU, 30.6.2005	N 49°28'24,7" E 17°06'36,7"
TU110 (2,4) (1,3)	5*x* 6*x*	Cz, W.C., Drahanská vrchovina Mts, village of Mostkovice, W periphery of the village, slope near road direction: Blansko, 269 m a.s.l., TU, 30 June 2005	N 49°28'21,6" E 17°02'03,6"
TU111 (1,2,3,4)	5*x*	Cz, W.C., Drahanská vrchovina Mts, village of Plumlov, rock between the fishpond and the football field, 280 m a.s.l., TU, 30 June 2005	N 49°28'05,1" E 17°00'22,9"
TU112 (1,3,4) (2)	4*x* 5*x*	Cz, B.M., Drahanská vrchovina Mts, village of Otiněves, rocks between fishpond and the garages, 560 m a.s.l., TU, 30 June 2005	N 49°25'02,0" E 16°51'58,8"
TU113 (1,2,3,4)	4*x*	Cz, B.M., Drahanská vrchovina Mts, village of Rozstání, rock in the centre of the village, 546 m a.s.l., TU, 30 June 2005	N 49°23'45,0" E 16°50'52,5"
TU114 (1, 3) (2,4,5)	4*x*	Cz, B.M., Moravský kras Mts, village of Lažánky, limestone rock above the village, 456 m	N 49°21'03,8" E 16°42'30,0"

	6*x*	a.s.l., TU, 30 June 2005	
TU115 (1,2,3)	4*x*	Cz, B.M., Moravian foothills of Vysočina Mts, rock near the road between the villages of Pánov and Křoví, 487 m a.s.l., TU, 30 June 2005	N 49°18'48,3" E 16°16'33,5"
TU116 (1,2)	4*x*	Cz, B.M., Hornosázavská pahorkatina Mts, xeric balk near the lane 1 km W of the village of Černýš, 360 m a.s.l., TU, 30 June 2005	N 49°44'33,9" E 15°02'16,5"
TU117 (1,2,3)	4*x*	Cz, B.M., Hornosázavská pahorkatina Mts, E of the lumber-mill near the village of Psáře, in the bend of the road, 477 m a.s.l., TU, 30 June 2005	N 49°44'48,9" E 14°58'15,4"
TU118 (1,2,3)	4*x*	Cz, B.M., Třeboňská pánev basin, trail 20 m N of the road from the town of Kardašovy Řečice to the town of Jindřichuv Hradec, 491 m a.s.l., TU, 4 July 2005	N 49°10'28,7" E 14°53'32,4"
TU119 (1,2,3)	4*x*	Cz, B.M., Třeboňská pánev basin, rock in the field 100 m NE of the village of Nítovice, 482 m a.s.l., TU, 4 July 2005	N 49°10'35,3" E 14°49'32,1"
TU120 (1,2,3)	4*x*	Cz, B.M., Votická vrchovina Mts, margin of the road near the nature park between the villages of Nová ves and Sudoměřice u Bechyně, 334 m a.s.l., TU, 4 July 2005	N 49°16'18,0" E 14°32'23,8"
TU121 (1,2,3)	4*x*	Cz, B.M., Východní Polabí river basin, rock above the train line SEE of the village of Týnec nad Labem, 228 m a.s.l., TU, 6 July 2005	N 50°02'27,7" E 15°21'58,7"
TU122 (1,2,3)	4*x*	Cz, B.M., Východní Polabí river basin, hillside between the villages of Luže and Střemošice, W of the town of Chrudim, 451 m a.s.l., TU, 24 July 2005	N 49°53'28,7" E 16°04'30,3"
TU123 (1,2,3)	4*x*	Cz, B.M., Šumavsko-novohradaské podhůří foothills, 1km W of the village of Pražák, rock near the road opposite to fishpond, 396 m a.s.l., TU, 25 July 2005	N 49°08'29,7" E 14°06'55,7"
TU124 (1,2,3)	4*x*	Cz, B.M., Podkrkonoší Mts, xeric slope near the road on the E periphery of the village of Košťálov, 357 m a.s.l., TU, 4 August 2005	N 50°34'34,5" E 15°23'11,5"
TU125 (1,2,3)	4*x*	Cz, B.M., Lužická kotlina fold, ruin of the wind mill on the yellow-marked hiking path near the village of Machnín, NW of the town of Liberec, 345 m a.s.l., TU, 4 August 2005	N 50°47'14,3" E 14°58'30,9"
TU126 (1,2,3)	4*x*	Cz, B.M., Lužická kotlina fold, reconstructed castle called Wothanburg near the village of Horní Vítkov, 513 m a.s.l., TU, 4.8.2005	N 50°51'23,9" E 14°58'46,9"
TU127 (1,2,3)	4*x*	Cz, B.M., Lužická kotlina fold, settlement of Uhelná near the village of Václavice, 331 m a.s.l., TU, 4 August 2005	N 50°51'51,1" E 14°53'53,4"
TU128 (1,2,3)	4*x*	Cz, B.M., Ralsko-bezdězská tabule plateau, village of Noviny pod Ralskem, canyon of the Ploučnice River, 309 m a.s.l., TU, 4 August 2005	N 50°41'12,4" E 14°45'49,0"
TU129 (1,2,3,4)	4*x*	Cz, B.M., Lounsko-labské středohoří Mts, 300 m W below the top of the Mt Raná, 400 m a.s.l., TU, 9 August 2005	N 50°24'21,4" E 13°46'10,5"
TU130 (1,2,3)	4*x*	Cz, B.M., Krušné hory Mts, village of Výsluní between Vejprt and Chomutov, slope with heather near the road, 1 km W of the village, 775 m a.s.l., TU, 9 August 2005	N 50°27'41,8" E 13°13'30,8"
TU131 (1,2,3)	4*x*	Cz, B.M., Krušné hory Mts, village of Měděnec, W slope of the Mt Mědník, 896 m a.s.l., 9 August 2005	N 50°25'26,6" E 13°06'40,2"

TU132 (1,2,3)	4*x*	Cz, B.M., Krušné hory Mts, village of Klášterec nad Ohří, the village of Mýtinka, road below the village, 748 m a.s.l., TU, 9 August 2005	N 50°24'03,4" E 13°04'29,3"
TU133 (1,2,3)	4*x*	Cz, B.M., Krušnohorské podhůří Mts, chalet colony 2 km W of the town of Klášterec nad Ohří, rock near the Ohře River, 310 m a.s.l., TU, 9 August 2005	N 50°22'57,7" E 13°13'06,6"
TU134 (1,2,3,4)	4*x*	Cz, B.M., Šumavsko-novohradské podhůří Mts, 1.5 km E of the village of Křemže, 569 m a.s.l., TU, 13 August 2005	N 48°54'55,2" E 14°19'22,1"
TU135 (1,2,3)	4*x*	Cz, B.M., Šumavsko-novohradské podhůří Mts, village of Babice u Netolic, dike of the fishpond, NW of the village, 508 m a.s.l., TU, 13 August 2005	N 49°02'29,0" E 14°13'10,3"
TU136 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, village of Mysletín, N of the town of Humpolec, slope near the fishpond, 479 m a.s.l., TU, 17 August 2005	N 49°28'33,2" E 15°22'14,5"
TU137 (1,2,3)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, village of Řeženčice, rockery near the road, 634 m a.s.l., TU, 17 August 2005	N 49°21'50,2" E 15°21'16,8"
TU138 (1,2,3,4)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, heath-moor near the village of Suchá, 200 m of the village, yellow-marked hiking path, 569 m a.s.l., TU, 17 August 2005	N 49°18'03,6" E 15°34'54,0"
TU139 (1,2,3,4)	4*x*	Cz, B.M., Českomoravská vrchovina Mts, between the villages of Želetava and Krasonice, edge of the forest, lane joining to the main road, 549 m a.s.l., TU, 17 August 2005	N 49°07'13,4" E 15°37'56,8"
TU140 (1,2,3)	6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, the town of Moravský Krumlov, surrounding of stone pit near the village of Džbánice, 351 m a.s.l., TU, 17 August 2005	N 49°00'18,2" E 16°12'22,5"
TU141 (1,2)	6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, town of Moravský Krumlov, rock of the amphitheatre, below the chapel, 301 m a.s.l., TU, 17 August 2005	N 49°02'51,5" E 16°19'10,8"
TU142 (1,2,3)	6*x*	Cz, W.C., Jihomoravský úval ravine, village of Židlochovice, Výhon Hill, former orchard, 257 m a.s.l., TU, 17 August 2005	N 49°02'20,0" E 16°37'43,1"
TU143 (1,2,3,4)	6*x*	Cz, W.C., Jihomoravská pahorkatina Mts, the village of Velké Hostěrádky, near the crossroad of the roads Nr. 480 and 422, 256 m a.s.l., TU, 17 August 2005	N 49°00'54,9" E 16°49'55,5"
TU 144	6*x*	Cz, W.C., Středomoravské Karpaty Mts, Ždánický les, reserve "u Křížanovického potoka", slope between the villages of Heršpice and Rašovice, 240 m a.s.l., TU, 17 August 2005	N 49°07'19,3" E 16°56'10,4"
TU145 (1,2,3)	5*x*	Cz, W.C., Jihomoravská pahorkatina Mts, reserve near the village of Zouvalka, orchard, 319 m a.s.l., TU, 17 August 2005	N 49°14'55,7" E 17°01'10,3"
TU146 (1,2) (3)	5*x* 6*x*	Cz, W.C., Jihomoravská pahorkatina Mts, 500 m W of the village of Nesovice, xeric hillside near the stone pit ,236 m a.s.l., TU, 18 August 2005	N 49°08'16,4" E 17°01'51,3"
TU147 (1,2,3)	4*x*	Cz, W.C., Středomoravské Karpaty Mts, the Buchlov castle, edge of the forest, red-marked hiking path, 438 m a.s.l., TU, 18 August 2005	N 49°06'22,7" E 17°18'31,6"
TU148 (1,2,3)	5*x*	Cz, W.C., Bílé Karpaty Mts, village of Lhotka Hradčovice, orchard in the centre of the village, 217 m a.s.l., TU, 18 August 2005	N 49°02'57,9" E 17°35'17,2"
TU149 (1,2,3,4)	6*x*	Cz, W.C., Zlínské vrchy Mts, village of Slušovice, xeric slope near the road 0.5 km N of the	N 49°15'31,0" E 17°48'12,8"

		village, 309 m a.s.l., TU, 18 August 2005	
TU150 (1,2,3)	6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, village of Svitávka (near the town of Boskovice), slope on the E periphery of the village, 325 m a.s.l., TU, 18 August 2005	N 49°30'31,7" E 16°36'22,4"
TU151 (1,2) (3)	5*x* 6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, W periphery of the village of Letovice, pine forest and sandstone rocks, 361 m a.s.l., TU, 18 August 2005	N 49°32'25,4" E 16°33'47,3"
TU152 (1,2,3)	4*x*	Cz, B.M., Dolní Poorličí river basin, the village of Chvojenec, edge of the forest, 274 m a.s.l., TU, 18.8.2005	N 50°07'03,4" E 15°55'58,7"
TU153 (1,2,3,4)	4*x*	Cz, B.M., Říčanská plošina plain, the village of Mirošovice, N of the town of Benešov, highway D1, lawn, 381 m a.s.l., MD, 10 August 2005	N 49°54'49.00" E 14°42'38.00"
TU154 (1,2,3,4)	4*x*	Cz, B.M., Moravian foothills of Vysočina Mts, village of Lhotka, WNW of the village of Velká Bíteš, exit from the highway D1, meadow, 540 m a.s.l., MD, 11 August 2005	N 49°18'34.00" E 16°07'01.00"
TU155 (1,2,3,4)	5*x*	Cz, W.C., Střední Pobečví river basin, village of Losovy, N of the village of Husleneky, 478 m a.s.l., KC, 1 October 2005	N 49°19'02,0" E 18°05'48,0"
TU156 (1,2,3,4)	5*x*	Cz, Střední Pobečví river basin, village of Husleneky, settlement "u Sivků", below the cote with broadcaster, 684 m a.s.l., KC, 1 October 2005	N 49°19'29,0" E 18°04'43,0"
TU157 (1) (2,3)	6*x* 5*x*	Cz, W.C., Javorníky Mts, the village of Hustisko-Solanec, near the town of Rožnov pod Radhoštěm, 572 m a.s.l., KC, 1 October 2005	N 49°23'59,8" E 18°14'59,1"
TU158 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, village of Černošín u Stříbra, edge of the Černošínský bor forest, 543 m a.s.l., TU, 3 october 2005	N 49°48'41,5" E 12°52'29,5"
TU159 (1,2,3)	4*x*	Cz, B.M., Tepelské vrchy Mts, settlement Starý Mlýn near the village of Potín, near the town of Konstantinovy lázně , 496 m a.s.l., TU, 3 october 2005	N 49°53'25,2" E 13°01'09,8"
TU160 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, the town of Manětín, *loco dicto* Chlumská hora, 610 m a.s.l., TU, 3 October 2005	N 50°00'41,3" E 13°11'42,9"
TU161 (1,2,3)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, the town of Rabštejn nad Střelou, near the bridge across the Střela River, above the sculpture of St. Jan Nepomuk, 459 m a.s.l., TU, 3 October 2005	N 50°02'52,4" E 13°17'48,2"
TU162 (1,4,5) (2,3,6)	4*x* 6*x*	Cz, B.M., Pražská plošina plain, Prague periphery, town district Černý Most, E slope above the highway R11, 241 m a.s.l., TU, 15 October 2005	N 50°06'50,3" E 14°35'21,7"
TU163 (1,2,3)	4*x*	Cz, B.M., Votická vrchovina Mts, the village of Oldřichovec near the town of Votice, dike of the fishpond, 694 m a.s.l., TU, 1 November 2005	N 49°36'00,6" E 14°39'57,3"
TU164 (1,2,3)	4*x*	Cz, B.M., Doupovske vrchy Mts, 3 km E of the village of Jakubov, near the town of Ostrov nad Ohří, 636 m a.s.l., TU, 3 November 2005	N 50°18'14,0" E 13°04'30,1"
TU165 (1,2,3,4,5)	5*x*	Cz, B.M., Dolní Povltaví river basin, Větrušické skály, another locality, edge of oak forest and rocky heath, 246 m a.s.l., MD, 5 November 2005 (vide TU 61)	N 50°12'02,0" E 14°22'15,2"
TU166 (1,2,3)	5*x*	Cz, B.M., Dolní Povltaví river basin, 200 m N of the previous locality, 258 m a.s.l., MD, 5 November 2005	N 50°12'04,9" E 14°22'11,0"

TU167 (1,2,3,4,5,6)	4*x*	Cz, B.M., Dolní Povltaví river basin, rocky heath above the chalets near the Vltava River, 300 m N of the village of Zlončice, near the town of Kralupy nad Vltavou, 203 m a.s.l., TU, 5 November 2005	N 50°13'37,3" E 14°21'12,5"
TU168 (1,2,3)	4*x*	Cz, B.M. Ralsko-bezdězská table plateau, peak of Ralsko Mountain, 665 m a.s.l., TU, 14 April 2006	N 50°40'26,6" E 14°45'59,0"
TU169 (1,2,3)	4*x*	Cz, B.M. Jesenicko-rakovnická plošina plain, the village of Blatno near the town of Jesenice-dam of pond Blatno, 420 m a.s.l., TU, 15 April 2006	N 50°06'64,2" E 13°23'57,0"
TU170 (1,2,3)	6*x*	Cz, W.C., Mikulovská pahorkatina - Pálava Mts the heath-blue-marked hiking path, 700m N of the village of Klentnice, 393 m a.s.l., TU, 22 April 2006	N 48°51'29,5" E 16°38'16.5'
TU171 (1,2,3)	5*x*	Cz, W.C., Mikulovská pahorkatina - Pálava Mts limestone pit on the Mt Sv. Kopeček, 331 m a.s.l., TU, 22 April 2006	N 48°48'35,7" E 16°39'19,0"
TU172 (1,2)	4*x*	Cz, B.M., Plzeňská pahorkatina Mts, the village of Darová near the town Rokycany, 330 m a.s.l., TU, 4 May 2006	N 49°49'58,2" E 13°31'32,4"
TU173 (1,2)	4*x*	Cz, B.M., Hrubý Jeseník Mts, near the village of Sobotín, *loco dicto* Ztracenka, 750 m a.s.l., TU, 4 May 2006	N 50°00'25,8" E 17°08'05,5"
TU174 (1,2,3)	4*x*	Cz, B.M., foothills of Novohradské hory Mts, town Kaplice, pine wood near the petrol station, 562 m a.s.l., TU, 21 May 2006	N 48°43'47,2" E 14°28'57,0"
TU175 (1,2,3)	6*x*	Cz, B.M., Moravian foothills of Vysočina Mts, serpentine steppe near the town of Mohelno, 378 m a.s.l., TU, 26 May 2006	N 49°06'27,2" E 16°11'22,4"
TU176 (1,2,3)	4*x*	Cz, B.M., Východolabská tabule plateau, the village of Semtín, between towns Pardubice and Lázně Bohdaneč, 221 m a.s.l., TU, 3 June 2006	N 50°04'05,0" E 15°41'35,4"
TU177 (1,2,3)	4*x*	Cz, B.M., Orlická tabule table plateau, town of Týniště nad Orlicí, meadows SW of the village of Rašovice, 284 m a.s.l., TU, 15 June 2006	N 50°08'42,5" E 16°07'53,7"
TU178 (1,2,3)	4*x*	Cz, B.M., Orlická tabule table plateau, village of Nekoř - Bredůvka, xeric slope in the village, 253 m a.s.l., TU, 15 June 2006	N 50°02'40,4" E 16°33'55,6"
TU179 (1,2,3)	5*x*	Cz, B.M., Nízký Jeseník Mts, village of Malá Štáhle near the town of Rýmařov, next to column of the crucification in the centre of village, 606 m a.s.l., TU, 15 June 2006	N 49°57'06,5" E 17°20'28,6"
TU180 (1,2,3,4,5)	5*x*	Cz, B.M., Nízký Jeseník Mts, nearby town of Bruntál, puff-stone quarry in Uhlířský vrch, 640 m a.s.l., TU, 16 June 2006	N 49°58'19,8" E 17°26'28,6"
TU181 (1,2,3)	4*x*	Cz, B.M., Zlatohorská vrchovina Mts., village of Liptáň near the town of Třemešná, 345 m a.s.l., TU, 16 June 2006	N 50°13'51,2" E 17°37'01,4"
TU182 (1,2,4) (3)	5*x* 6*x*	Cz, W.C., Moravskoslezské Beskydy Mts, village of Trojanovice near the town of Frenštát pod Radhoštěm, xeric slope above road, 461 m a.s.l., 16 June 2006	N 49°30'58,7" E 18°12'32,5"
TU183 (1) (2,3,4)	4*x* 6*x*	Cz, W.C., Moravskoslezské Beskydy Mts, sinuous road next to dam Šance, between the village of Ostravice and Staré Hamry, stone pit, 503 m a.s.l., TU, 16 June 2006	N 49°29'00,1" E 18°25'20,6"

TU184 (1,2,3)	5*x*	Cz, W.C., Hostýnsko-vsetínská hornatina Mts., village of Podhradní Lhota near the town of Bystřice pod Hostýnem, xeric slope near the road, brook called Juhyně, 545 m a.s.l., TU, 16 June 2006	N 49°25'12,9" E 17°47'58,8"
TU185 (1,2,3)	5*x*	Cz, W.C., Hostýnsko-vsetínská hornatina Mts., village of Osíčko near the town of Bystřice pod Hostýnem, xeric slope in the centre of the village, 406 m a.s.l., TU, 16 June 2006	N 49°25'30,5" E 17°44'44,4"
TU186 (2) (1)	5*x* 6*x*	Cz, W.C., Podbeskydská pahorkatina Upland, saddle of Mt Kotouč near the town of Štramberk, 501 m a.s.l., TU, 18 June 2006	N 49°35'03,6" E 18°06'56.0"
TU187 (1,2,3)	4*x*	Cz, B.M., Podčeskoleská pahorkatina upland, village of Nový Pařezov near the town of Domažlice, stone pit next to road, 429 m a.s.l., TU, 15 July 2006	N 49°27'53,3" E 12°50'20,3"
TU188 (1,2)	4*x*	Cz, B.M., Šumava Mts.,Mt Špičák, ski slope, near the village of Železná Ruda, 868 m a.s.l., TU, 15 July 2006	N 49°09'54,1" E 13°13'12,7"
TU189 (1,2)	4*x*	Cz, B.M. , Šumava Mts., Mt Špičák, in the middle of ski slope, near the village of Železná Ruda, 938 m a.s.l., 15 July 2006	N 49°10'04,5" E 13°12'48,7"
TU190 (1,2,3)	4*x*	Cz, B.M., Šumava Mts., pasture by the village of Klepačka, near the town of Kašperské Hory, 777 m a.s.l., TU, 16 July 2006	N 49°10'20,0" E 13°35'10,0"
TU191 (1,2,3)	4*x*	Cz, B.M., Šumava Mts., village of Zhůří, near the town of Horská Kvilda, meadows near the cross-road, 1149 m a.s.l., TU, 16 July 2006	N 49°04'51,0" E 13°33'27,6"
TU192 (1,2,3)	4*x*	Cz, B.M., Šumava Mts., village of Kbelnice, xeric slope above road, 452 m a.s.l., TU, 16 June 2006	N 49°17'46,2" E 13°59'25,3"

SUPPLEMENTARY INFORMATION 2

Previously published data on chromosome numbers of *Pilosella officinarum* from the Western Carpathians, Pannonia Plain and the Czech Republic. Localities are given as in the original sources. Completed data based on our searching are given in square brackets. Localities marked by an asterisk were not included in the map of cytotype distribution (Fig. 5) for several reasons (see Material and Methods, Results and Discussion). Abbreviations used: Cz = Czech Republic, Pl = Poland, Sk = Slovakia.

2*n*	Locality	Latitude, longitude	Source
36	Pl, Beskid Wyspovy Mts, Rzeki, alt. c. 450 m, coll. A. Jankun	[N 49°35', E 20°13']	Pogan and Wcisło, 1989
36	Pl, Gorce Mts, slope of the Mt Mogielnica, coll. A. Joachimiak	[N 49°59', E 21°54']	
36	Sk, Slovenský raj, Gačovská skala, Geravy	[N 48°52'35", E 20°23'08"]	Murín, 1986
36	Sk, Ipeľsko-rimavská brázda, Antalova pustatina, 260 m n.m., 28,8,1998, leg. Píšťanský*		Píšťanský and Mičieta, 2000
36	Sk, Ipeľsko-rimavská brázda, Ratka, 200 m n.m., 27.8.1998, leg. Píšťanský*		
36	Sk, Ipeľsko-rimavská brázda, Petrovce, 240 m n.m., leg. Píšťanský*		
36	Sk, Ipeľsko-rimavská brázda, Hodejovec, 205 m n.m., 26.8.1998, leg. Píšťanský*		
36	Sk, Ipeľsko-rimavská brázda, Šiatorská Bukovinka, 240 m n.m., 26.8.1998, leg. Píšťanský *		
36	Sk, Záhorská nížina, Šaštín-Stráže, Gazárka, 190 m n.m., 4.4.1997, leg. Píšťanský*		
36	Sk, Záhorská nížina, Prietrž, 280 m n.m., 4.9.1997, leg. Píšťanský *		
36	Sk, Považský Inovec, Hlohovec, Soroš, 260 m n.m., 15.7.1997, leg. Píšťanský*		
36	Sk, Devínska Kobyla, vrch Devínska Kobyla, 300 m n.m., 29.5.1998, leg. Píšťanský*		
36	Sk, Malé Karpaty, Lančár, 260 m n.m., 9.8.1997, leg. Píšťanský *		
36	Sk, Malé Karpaty, Veľký Plešivec, 460 m n.m., 14.8.1997, leg. Píšťanský*		
36	Sk, Malé Karpaty, Záhorská Bystrica, 220 m n.m., 19.8.1997, leg. Píšťanský *		
36	Sk, Malé Karpaty, Dobrá Voda, 360 m n.m., leg. Píšťanský *		
36	Sk, Malé Karpaty, Brezová pod Bradlom, 320 m n.m., 4.9.1997, leg. Píšťanský*		
36	Sk, Malé Karpaty, Hradište pod Vrátnom, 300 m n.m., 3.9.1997, leg. Píšťanský*		
36	Sk, Považský Inovec, Lúka, Babia hora, 380 m n.m., 16.7.1997, leg. Píšťanský *		
36	Sk, Považský Inovec, Lúka, Pod Vinohrady, 320 m n.m., 16.7.1997, leg. Píšťanský*		
36	Sk, Považský Inovec, Havran, 400 m n.m., 12.8.1997, leg. Píšťanský*		
36	Sk, Považský Inovec, Pod Skalinami, 600 m n.m., 12.8.1997, leg. Píšťanský *		
36	Sk, Slovenské rudohorie, Španie pole, 370 m n.m., 24.8.1998, leg. Píšťanský *		
36	Sk, Javorníky, Čertov, 570 m n.m., 19.7.1998, leg. Píšťanský*		
36	Sk, Javorníky, Frňovské sedlo, 940 m n.m., 20.7.1998, leg. Píšťanský*		
36	Sk, Javorníky, Kohútka, 880 m n.m., 22.7.1998, leg. Píšťanský*		
36	Sk, Javorníky, Holýš, 440 m n.m., 18.7.1982, leg. Mičieta*		
36	Sk, Javorníky, Veľký vrch, pri obci Divinka, 360 m n.m., 15.6.1987, leg. Mičieta*		

36	Sk, Javorníky, Medzihony, pri obci Stupné, 400 m n.m., 12.8.1988, leg. Mičieta*		
36	Sk, Javorníky, Jakubovský vrch, 830 m.n.m., 16.8.1988, leg. Mičieta *		
36	Sk, Javorníky, Považský Chlmec, 370 m n.m., 370 m n.m., leg. Mičieta, (Mičieta, 1978) *		
45	Pl, Tatra Mts, Polana Biały potok, dry parts of the meadow, among low vegetation, alt. c. 940 m	[N 49°18', E 19°56']	Skalińska, 1967
45	Pl, Tatra Mts, sunny slope over the road from Przysłop Miętusi to Kobylarz, at the bottom of the young spruce wood, alt. c. 1170 m	[N 49°14', E 19°51']	
45	Pl, Tatra Mts, valley Jaworzynka, sunny slope over the road, near the entrace to the valley, alt. c. 1000 m	[N 49°34', E 20°00']	
45	Pl, Tatra Mts, Kasprowa Polana, over valley Jaworzynka, drier parts of a meadow, alt. c. 1100 m	[N 49°15', E 19°54']	
45	Pl, Tatra Mts, higher parts of valley Olczyska, near the road, alt. c. 1100 m	[N 49°19', E 20°00']	
45	Pl, Tatra Mts, Kopa Magury, among calcareous rocks in the top part, alt. c. 1700 m	[N 49°15', E 20°02']	
45	Pl, Tatra Mts, vicinity of Łysa Polana, in the meadow with low vegetation, alt. c. 960 m	[N 49°16', E 20°07']	
45	Pl, Tatra Mts, vicinity of Łysa Polana, a different part of the same meadow, alt. c. 960 m	[N 49°16', E 20°07']	
45	Pl, Pieniny Mts, Wąwóz Sobzański, in gorge, alt. c. 600 m*		Skalińska *et al.*, 1971
45	Pl, Gorce Mts, Lubomierz, alt. c. 500 m, coll. L. Przywara	[N 49°37', E 20°11']	Pogan and Wcisło, 1989
45	Pl, Gorce Mts, Polana Jaworzyna, alt. c. 1000 m, coll. L. Przywara *		
45	Pl, Gorce Mts, slope of the Mt. Jasień, alt. c. 600 m, coll. A. Joachimiak *		
45	Pl, Tatra Mts, Toporowa Cyrla, alt. c. 1100 m, coll. R. Izmailow, A. Jankun*		
45	Pl, Tatra Mts, Dolina Chochołowska, alt. c. 1100 m, coll. R. Izmailow	[N 49°14', E 19°49']	
45	Sk, Devínska Kobyla	[N 48°11', E 17°00']	Uhríková and Feráková, 1977
45	Sk, Južné Biele Karpaty, Myjava, Turá Lúka, 300 m n.m., 4.9.1997, leg. Píšťanský*		Píšťanský and Mičieta, 2000
45	Sk, Malé Karpaty, Bukovec, 400 m n.m., 4.9.1997, leg. Píšťanský *		
45	Sk, Považský Inovec, Hôrka nad Váhom, Medzihorie, 31. m n.m., 11.8.1997, leg. Píšťanský *		
45	Sk, Považský Inovec, Bezovec, 540 m n.m., 29.6.1998, leg. Píšťanský*		
45	Sk, Vtáčnik Mts, valley of Pokulský potok brook, in the grassy place1 km E of the village of Ostrý Grúň, 490 m a.s.l., coll. F. Krahulec, 18 September 1998	N 48°33'50", E 18°41'00"	Rotreklová *et al.,* 2002
45	Sk, Vtáčnik Mts, valley of Jastrebský potok brook, ca 2.1 km S of the church in the village of Prochoť, 500 m a.s.l., coll. F. Krahulec, 18 September 1998	N 48°35'30", E 18°42'40"	
45	Cz, distr. Zlín, meadow 1.2 km SE of the railway station in the village of Valašské Klobouky, 510 m a.s.l., coll. F. Krahulec, 17 August 1999	N 49°08'10", E 18°02'10"	
45	Sk, Volovské vrchy Mts, Cechy, on the medieval copper heaps ca 0.5 km N of the center of the town of Gelnica, 500 m a.s.l., coll. P. Mráz & M. Bačkor	N 48°51'28", E 20°55'24"	

45	Sk, Západné Tatry Mts, Pribylina village, Račkova dolina valley, meadow near the of Agriculture University of Nitra chalet, 940 m a.s.l., coll. V. Mrázová, 18 June 2001	N 49°07'57", E 19°46'59"	Rotreklová *et al.,* 2005
45	Sk, Zemplínske vrchy Mts, Kráľovský Chlmec village, Veľký kopec Hill (263.9), 260 m a.s.l., coll. P. Mráz & V. Mrázová, 17 April 2001	N 48°25'01", E 21°57'44.7"	
45	Sk, Zemplínske vrchy Mts, Streda nad Bodrogom village, ca. 1 km SE of the village centre, *loco dicto* Veterné piesky on the Tarbucka Hill massive, ca 140 m a.s.l., coll. P. Mráz & V. Mrázová, 16 April 2001	N 48°22'40", E 21°47'02.4"	
45	Sk, Západné Tatry Mts, Pribylina village, Račkova dolina valley, near the crossroad with Jamnická dolina valley, 960 m a.s.l., coll. V. Mrázová, 18 June 2001	N 49°07'57", E 19°46'59"	
45	Sk, Volovské vrchy Mts, Hnilčík village, on the phylite rocks near the crossroad to Hnilčík-Roztoky quarter, 640 m a.s.l., coll. P. Mráz & V. Mrázová, 3 July 2000	N 48°51'24.4", E 20°34'28.6"	
45	Sk, Zemplínske vrchy Mts, Veľká Bara village, former vineyards, ca 100 m N from Piliš Hill (277,6), 240 m a.s.l., coll. P. Mráz & V. Mrázová, 15 April 2001	N 48°25'47", E 21°42'28.3"	
45	Sk, Vysoké Tatry Mts, ca. 1.5 km NW of Štrbské pleso tarn, 1400 m a.s.l., coll. P. Mráz & V. Mrázová, 7 August 2000	N 49°07'48", E 20°02'27"	
45	Sk, Ostrôžky Mts, Polichno village, meadow on the top of the Mt Bralce, 817 m a.s.l., coll. P. Mráz & V. Mrázová, 17 April 2001	N 48°25'27", E 19°27'44"	
45	Sk, Chočské vrchy Mts, Prosiek village, 0.5 km N of the village, pasture, 644 m a.s.l., coll. P. Mráz, 15 July 2000	N 49°09'30", E 19°29'54"	
45	Sk, Vysoké Tatry Mts, on the tourist path between Štrbské pleso tarn and Jamské pleso tarn, 1450 m a.s.l., coll. P. Mráz & V. Mrázová, August 2001	N 49°07'56", E 20°02'28"	
45	Sk, Volovské vrchy Mts, Čučma village, ca 200 m SW of the top of Mt Skalisko, 1250 m a.s.l., coll. P. Mráz & V. Mrázová, 12 June 2001	N 48°44'38", E 20°34'35"	
45	Sk, Volovské vrchy Mts, Čučma village, S slopes of Mt Skalisko, *loco dicto* Doboška ca 1 km N from elevation marker 874.2, 890 m a.s.l., coll. P. Mráz & V. Mrázová, 12 June 2001	N 48°43'50", E 20°35'25"	
45	Sk, Volovské vrchy Mts, Úhorná village, on the forest route from the Panské sedlo saddle to Mt Biele skaly, NW of the village, 1100 m a.s.l., coll. P. Mráz, June 2002	N 48°43'19", E 20°39'01"	
45	Sk, Volovské vrchy Mts, Prakovce village, *loco dicto* Hutno on E margin of the village, near the forest road,400 m a.s.l., coll. P. Mráz, September 2000	N 48°49'08", E 20°55'25"	
45	Sk, Volovské vrchy Mts, Kojšov village, *loco dicto* Strieborná lúka ca 2.5 km NW of the village, 700 m a.s.l., coll. P. Mráz, 20 July 2000	N 48°50'25", E 20°59'20"	
45	Sk, Vihorlat Mts, Jasenov village, ca 0.5 km NE of the village, pasture, 150 m a.s.l., coll. P. Mráz & V. Mrázová, 13 May 2001	N 48°54'14,7", E 21°54'36"	
45	Sk, Zemplínske vrchy Mts, Veľká Tŕňa village, xerothermic margins of the road 1.5 km NNW of village, pasture, 180 m a.s.l., coll. Mráz & V. Mrázová, 14 April 2001	N 48°28'35", E 21°40'24"	

45	Cz, Javorníky Mts, on the main ridge near the Portáš chalet, 900 m a.s.l., coll. P. Mráz & V. Jurkovičová, 14 October 2000	N 49°17'47", E 18°14'40"	
45	Sk, Považský Inovec, Topoľčany, Podhradie	[N 48°34', E 18°10']	Májovský *et al.*, 1970
54	Sk, Považský Inovec, Nová Lehota – Dolina, 300 m n.m., 15.8.1998, leg. Píšťanský *		Píšťanský and Mičieta, 2000
54	Sk, Považský Inovec, Podhradie, 450 m n.m., 16.8.1998, leg. Píšťanský*		
54	Sk, Veľká Fatra Mts, Mt Kráľova studňa, meadow near the hiking path 150 m NW of the chalet, 7 km W of the village of Staré hory, 1270 m a.s.l., coll. F. Krahulec & A. Krahulcová, 24 July 1999	N 48°52'40", E 19°02'30"	Rotreklová *et al.,* 2002
54	Sk, Ostrôžky Mts, ca 0.7 km SW of village of Ľuboreč, along the road between the villages of Ľuboreč a Závada, 250 m a.s.l., coll. P. Mráz &V. Jurkovičová, 23 April 2000	N 48°18'08", E 19°30'35"	
54	Sk, Slovenský kras Mts, Zádiel village, Zádielska planina plateau, ca 0.5 km NW from elevation point 591.2	N 48°37'58", E 20°41'57"	Rotreklová *et al.,* 2005
54	Sk, Volovské vrchy Mts, Čučma village, S slopes of Mt Skalisko , 1220 m a.s.l., coll. coll. P. Mráz &V. Jurkovičová, 13 June 2000	N 48°44'47", E 20°34'33"	
54	Sk, Volovské vrchy Mts, Vyšný Klátov village, Mt Predná Holica, meadow bellow the Lajoška chalet, 900 m a.s.l., coll. P. Mráz, June 2002	N 48°45'47", E 21°04'38"	
54	Sk, Volovské vrchy Mts, Košice city, Botanical garden grounds, 236 m a.s.l., coll. P. Mráz, May 2000	N 48°44'03", E 21°14'15"	
54	Sk, Slovenský kras Mts, Plešivec town, Plešivecká planina plateau, on the forest route ca 0.5 km NE of Zbojnícka priepasť chasm, 545 m a.s.l., coll. P. Mráz, 30 May 2000	N 48°34'22", E 20°25'09"	
54	Sk, Slovenský kras Mts, Kečovo village, Silická planina plateau, xerothermic slopes on the N margin of the village, 350 m a.s.l., coll. P. Mráz, 30 May 2002	N 48°29'45", E 20°29'20"	
54	Sk, Zemplínske vrchy Mts, Luhyňa village, N slopes of Ondrejský kopec Hill, oak forest margin, 160 m a.s.l., coll. Mráz & V. Mrázová, 14 April 2001	N 48°30'03", E 21°38'01"	
54	Pl, Tatra Mts, vicinity of Łysa Polana, higher parts of the meadow, alt. c. 970 m	[N 49°16', E 20°07']	Skalińska, 1967
45, 54	Pl, Tatra Mts, along the road valley Kościeliska to valley Tomanowa, alt. c. 1100 m	[N 49°12', E 19°52']	
45, 54	Pl, Tatra Mts, vicinity of Łysa Polana, among rocks scattered in a meadow, alt. c. 960 m	[N 49°16', E 20°07']	
36	Cz, Jičín, town Hořice, meadow near the forest ca 0.5 km N of the town, 400 m a.s.l.	[N 50°23', E 15°38']	Měsíček and Jarolímová, 1992
36	Cz, Vítkovice, Horní Mísečky, along the road ca 0.5 km NNW of the settlement, 1070 m a.s.l., coll. F. Krahulec & A. Krahulcová, 15 July 1997	N 50°44'20", E 15°33'50"	Krahulcová and Krahulec, 1999
36	Cz, Pec pod Sněžkou, Velká Úpa, meadow near the settlement Janovy Boudy, 1.5 km E of the church,860 m a.s.l., coll. F. Krahulec, 20 June 1995	N 50°41'20", E 15°48'00"	
36	Cz, Pec pod Sněžkou, the grassland area Velká pláň in the N part of the villagein the northern part of the village, 830 m a.s.l., coll. F. Krahulec, 18 June 1999	N 50°41'50", E 15°43'50"	Krahulcová *et al.,* 2001

36	Cz, Horní Malá Úpa, Pomezní Boudy, the meadow beside the road opposite to bus stop, 160 m S of Padolská bouda chalet, 950 m a.s.l., coll. F. Krahulec & S. Bräutigam & J. Chrtek, 30 June 1999	N 50°44'10", E 15°48'50"	
36	Cz, Pec pod Sněžkou, Velká Úpa, the grassland area Přední Výsluní, close to woodland edge, ca. 1.3 km SEE of the chuurch, 890 m a.s.l., coll. F. Krahulec & S. Bräutigam & J. Chrtek, 1 July 1999	N 50°41'20", E 15°47'30"	
36	Cz, Špindlerův Mlýn, in the meadow beside the touring path, between Petrova bouda and Moravská bouda chalets, ca 100 m NE of Moravska bouda chalet, 1240 m a.s.l., coll. F. Krahulec & S. Bräutigam & J. Chrtek, 2 July 1999	N 50°46'10", E 15°36'30"	
36	Cz, Benecko, in the lawn in the central part of the village ca 0,4 km W of the parking place, 800 m a.s.l., coll. F. Krahulec & S. Bräutigam & J. Chrtek, 2 July 1999	N 50°40'00", E 15°33'00"	
36	Cz, Benecko, the upper part of the meadow bellow Jindrova skála hill, S slope, in the southwestern periphery of the village, 770 m a.s.l., coll. F. Krahulec & S. Bräutigam & J. Chrtek, 2 July 1999	N 50°39'50", E 15°32'40"	
36	Cz, distr. Louny, W edge of Vroutecký les wood near the village of Valov, 2.2 km S of the church in the town of Podborany, 350 m a.s.l., coll. F. Krahulec, 2. June 1998	N 50°12'50", E 13°25'00"	Rotreklová *et. al.,* 2002
36	Cz, distr. Cheb, nature reserve Pluhův bor, 1.7 km N of the church in the village of Mnichov, 700 m a.s.l., coll. O. Rotreklová & P. Šmarda, 13 July 2000	N 50°03'12", E 12°47'07"	
36	Cz, Bohemian Karst, distr. Beroun, grassland above the road N of the village of Srbsko, 1.3 km NE of the railwaz station, 310 m a.s.l., coll. F. Krahulec, 13 June 1998	N 49°56'40", E 14°08'50"	
36	Cz, Šumava, distr. Prachatice, slope above the railway in the village of Stožec,50 m E of the railwaz station, 780 m a.s.l., coll. F. Krahulec, 11 August 1999	N 48°51'30", E 13°49'20"	
36	Cz, distr. Náchod, grassland and the former sand pit in the N perifery of the village of Vršovka, 330 m a.s.l., coll. F. Krahulec, 21 May 1999	N 50°19'30", E 16°07'00"	
36	Cz, Krušné hory Mts, the former tin mine 3.5 km NNE of the village Přebuz, 890 m a.s.l., coll. F. Krahulec, 8 June 2000	N 50°24'00", E 12°30'00"	Rotreklová *et. al.,* 2005
36	Cz, Ještědský hřeben Mts, distr, Liberec, in the S periphery of the village of Krištofovo Údolí ca 5.5 km SSW of the town of Chrastava, 440 m a.s.l., coll. F. Krahulec & S. Bräutigam, 7 July 2001	N 50°46'04", E 14°55'57"	
36	Cz, Praha city, part Řeporyje	N 50º03.232´, E 14º18.619´	Košťálová, 2004
36	Cz, Praha city, part Hrdlořezy	N 50º05.747´, E 14º31.343 ´	
36	Cz, Praha city, part Kyje	N 50º05.769´, E 14º32.828´	
36	Cz, Praha city, part Braník	N 50º02.251´, E 14º27.004´	
36	Cz, Praha city, part Řevnice	N 49º55.204´, E 14º14.967´	
36	Cz, Praha city, part Šeberov	N 50º00.5´, E 14º30.55´	

54	Cz, distr. Rychnov nad Kněžnou, meadow at the edge of Halín wood, 0.5 km NW of the crossroads near the village of Běstviny, 310 m a.s.l., coll. F. Krahulec, 21 May 1999	N 50°19'30", E 16°08'40"	
36, 45	Cz, distr. Olomouc, grassland on the E slope of Malý Kosíř hill, 0,6 km SW of the church in the village of Slatinice na Hané, 270 m a.s.l., coll. F. Krahulec, 25 May 1998	N 50°33'30", E 17°05'40"	Rotreklová *et. al.,* 2002
36, 45	Cz, Praha, slope above the railway W of the railway station Praha-Vysočany, 210 m a.s.l., coll. F. Krahulec & J. Chrtek, 18 May 2000	N 50°06'45", E 14°29'50"	
54+63	Cz, Praha city, part Černý most*	N 50º06.874´, E 14º35.276´	Košťálová, 2004

SUPPLEMENTARY INFORMATION 3

Published chromosome numbers of *Pilosella officinarum* in Europe, with exception of the data given in Supplementary Information 2. Localities are given as in the original sources. Completed data based on our searching are given in square brackets. Localities marked by an asterisk were not included in the maps of cytotype distribution (Figure 5), for several reasons (see Material and Methods, Results and Discussion). CD-BSBI = Cytological database of the Botanical Society of the British Isles (accesed February 2005).

2*n*	Country	Locality	Latitude, longitude	Source
18	France	Htes-Alpes, Région de Guillestre, Lac du Séguret, pelouse á *Festuca spadicea*, alt. 1200 m*	[N 44°56', E 06°28']	Delcourt, 1972
18		Htes-Alpes, Région de Vars, au pied du Châteleret, alt. 2000 m*	[N 44°37', E 06°41']	
18		Htes-Alpes, Région de Ceillac, bords d´un torrent eu sous-bois de Mélézes, alt. 1900 m*	[N 44°40', E 06°46']	
18		B.-Alpes, Col de Larche, Réserves du Lausannier, bords d´un sentier, en sous-bois de Mélézes, alt. 1700-1800 m*	[N 44°27', E 06°51']	
18		Htes-Alpes, Val d'Escreins, sous-bois de *Pinus sylvestris*, alt. 1600 m*	[N 44°37', E 05°47']	
18		Htes-Alpes, Vars-les-Claux, derriére le village, tapis herbeux en sous-bois de Mélézes, alt. 1800 m*	[N 44°34', E 06°34']	
18		Htes-Alpes, Vars-les-Claux, en bordure d´une pelouse á Festuca spadicea, alt. 1800 m*	[N 44°34', E 06°34']	
18		Htes-Alpes, Col de Vars, pelouse á *Festuca spadicea*, alt. 2000*	[N 44°32', E 06°41']	
18		Htes-Alpes, Vars, St-Marcellin, au lieu dit Le Rocher du chateau, pelouses pâtureés, alt. 1800 m*		
18		Htes-Alpes, Région de Guillestre, lavandaie á *Lavandula vera*, sur cailloutis consolidés des alluvions de la Durance, alt. 1000-1100 m*	[N 44°39', E 06°37']	
18		Htes-Alpes, Plateau de Peyrol, Région de Vars, pelouses en pré-bois de Mélézes, alt. 2200 m*	[N 44°37', E 06°39']	
18		Htes-Alpes, Région de Vars. Au lieau dit Les Escondus, sur éboulis plus ou moins siliceux avec rares pâturages, alt. 2300 m*		
18		Htes-Alpes, Région de Vars, Les Couniets, pelouse á Fétuques, alt. 2000 m*		
18		Htes-Alpes, Région de Lautaret, pelouses á *Festuca spadicea*, alt. 2000 m*	[N 45°02', E 06°23']	
36	Armenia	Yerevan, Botanicheskij sad	[N 40°11', E 44°31']	Nazarova and Ghukasyan, 2004
36	Austria	Deutschlandsberg (Steiermark)	[N 46°49', E 15°13']	Gadella, 1972

36	Belarus	Grodnenskaija obl., Smorgonskij reg., okr. Rybaki	[N 54°32', E 26°24']	Dmitrieva, 1987
36		1 km JZ ot der. Domzheritsy*	[N 55°44', E 28°19']	Parfenov and Dmitrieva, 1988
36	Belgium	De Panne (W. Vlaanderen)	[N 51°06', E 02°35']	Gadella, 1972
36		Eupen (Liége)	[N 50°38', E 06°02']	
36		near Houffalize (prov. Luxembourg)	[N 50°08', E 05°47']	Gadella, 1984
36		near Vise, (prov. Liége)	[N 50°44', E 05°42']	
36	Denmark	Brömsholm, H. Nordenskiöld, 1951	[N 55°55', E 12°29']	Turesson and Turesson, 1960
36		Lögstör, H. Nordenskiöld, 1951	[N 56°58', E 09°15']	
36		Oserild, H. Nordenskiöld, 1951	[N 57°02', E 08°50']	
36		Ribe, H. Nordenskiöld, 1951	[N 55°19', E 08°50']	
36		Rödding, H. Nordenskiöld, 1951	[N 53°19', E 08°50']	
36		Skibsted, H. Nordenskiöld, 1951	[N 56°52', E 10°05']	
36		Viborg, H. Nordenskiöld, 1951	[N 56°27', E 09°22']	
36		Talne, S. O. Björkman, 1949*		
36		Millinge (Fyn)	[N 55°08', E 20°13']	Gadella, 1972
36		Agger (Jylland)	[N 56°47', E 08°15']	
36		Ebeltoft (Jylland)	[N 56°12', E 10°41']	
36		Gladved (Jylland)	[N 56°19', E 10°50']	
36		Helsingör (Sjaelland)	[N 56°02', E 12°37']	
36		Hennestrand (Jylland)	[N 55°44', E 08°10']	
36		Hvide Sande (Jylland)	[N 55°59', E 08°08']	
36		Klim (Jylland)	[N 57°06', E 09°10']	
36		Lakolk (Römö)	[N 55°08', E 08°30']	
36		Lögstör (Jylland)	[N 56°58', E 09°15']	
36		Lokken (Jylland)	[N 57°22', E 09°43']	
36		Rebild (Jylland)	[N 56°50', E 09°51']	
36		Slettestrand (Jylland)	[N 57°09', E 09°22']	
36		Stege (Mön)	[N 54°59', E 12°18']	
36		Stenbjerg (Jylland)	[N 56°55', E 08°22']	
36		Thorupstrand (Jylland)*	[N 57°08', E 09°07']	
36		Hjimmelberget near Silkeborg (Jylland)	[N 56°10', E 09°34']	Gadella, 1984
36	France	Bouches-du-Rhône, Région de Marseille, Vallée de l´Huveaune, Les Camoins, alt. 0-50 m	[N 43°18', E 05°30']	Delcourt, 1972

36		Bouches-du-Rhône, Vallée de la Durance, au niveau du Pont-de-Mirabeau, sur cailloutis consolidés par un petite pelouse xérophile, alt. 0-100 m	[N 44°55', E 04°44']	
36		Htes-Alpes, non loin de Guillestre, au Plan de Fazy, alt. 500-700 m	[N 44°40', E 06°39']	
36		Htes-Alpes, Région d'Embrun à Boseodon, sur pelouses séches de type mediterranéen	[N 44°34', E 06°29']	
36		Htes-Alpes, Vallée de la Durance, non loin de la Roche-de-Rame, alt. 800 m	[N 44°55', E 04°44']	
36		Bouches-du-Rhône, Bassin d'Aix-en-Provence, région des Milles, pelouses séches en bordure de route, alt. 50-100 m	[N 43°32', E 05°26']	
36		Var, Evenos, sur basalte, dans un pelouse avec Lavandula stoechas	[N 43°10', E 05°51']	
36		Var, entre Flassans et le Luc, bords de route sur pelouses á gramineés, terrains calcareo-marneux, alt. 100 m	[N 43°22', E 06°13']	
36		Ste-Victoire, Champ en friche non loin du barrage de Bimont	[N 50°33', E 01°54']	
36		Var, bassin de Bausset, sur marnes ligeriennes, alt. 100 m*	[N 43°12', E 05°47']	
36		Ste-Victoire – St. Marc, jaumegarde, aneiennes cultures abandonnées*	[N 43°33', E 05°32']	
36		Ste-Victoire, au lieu dit le "Domaine de l'Etang", pelouses patureés sous quelques chénes verts, terrains plus ou moins dolomitiques*	[N 43°31', E 05°36']	
36		Les Milles, , croisement de St.-Pons, dans un Deschampsietum mediae	[N 43°30', E 05°23']	
36		Echenerex (Ain)	[N 46°19', E 06°02']	Gadella, 1972
36		Virieu le Grand (Ain)	[N 45°51', E 05°39']	
36		Cap Blanc Nez (Artois)	[N 50°53', E 01°38']	
36		Nant (Aveyron)	[N 44°01', E 03°18']	
36		Plage de Loon (Flandres)	[N 51°00', E 02°14']	
36		Hohneck, Vosges, (Haut Rhin)	[N 48°02', E 07°01']	
36		Collobriéres, Massif de Maures (Var)	[N 43°16', E 06°23']	
36		Egliseneuve prés Billon (dep. du Puy-de-Dôme)	[N 45°43', E 03°24']	Aquier and Renard, 1979
36		France: garrique, environs de Montpellier	[N 43°37', E 02°50']	Natarajan 1981
36		St. Julien de Verdon (Ht. Prov.)	[N 43°55', E 06°31']	Gadella, 1984
36		Le Mans (Ht. Alpes)	[N 48°00', E 00°12']	
36		Col de Tourmalet, alt. 1700 m	[N 42°54', E 00°09']	
36		near Obéost (Pyren. Atlantiques)*		
36		Marlestein (Ht. Rhin)*		
36	Germany	Obermoss, Zugsp., H. Nordenskiöld, 1950	[N 49°40', E 08°55']	Turesson and Turesson, 1960

36		St. Blasien, Schwarzwald (Baden-Wűrttemberg)	[N 47°46', E 08°08']	Gadella, 1972
36		Borken (Nordrhein-Westfalen)	[N 51°03', E 09°16']	
36		Konzen (Nordrhein-Westfalen)	[N 50°35', E 06°15']	
36		Monschau (Nordrhein-Westfalen)	[N 50°34', E 06°15']	
36		Bluchertal, near Kaub*	[N 50°06', E 07°47']	Gadella, 1984
36		Regierungsbezirk Oberbayern, Kiesgrube rechts der Strasse von Kreuzstrasse nach Eching	[N 48°17', E 11°35']	Schuhwerk and Lippert, 1997
36		Regierungsbezirk Oberpfalz, landkreis Regensburg, Mittelberg nördlich Tegerheim	[N 50°20', E 11°02']	
36		Regierungsbezirk Oberbayern, Landkreis Traunstein, von der Thoraualm südöstlich des Hochfelln zur Nesselauer Schneid, Lippert, 19.6.1996	[N 48°01', E 11°05']	Schuhwerk and Lippert, 2002
36		Regierungsbezirk Niederbayern, Landkreis Straubing-bogen, Bayerischer Wald, Steinbruch im Südhang des Welchenberges bei Espern, 340 m, 27.5.1998, Schuhwerk	N 48°52'42", E 12°46'04"	
36		Saxonia, 12 km SSW of Görlitz	[N 51°03', E 14°54']	Bräutigam and Bräutigam, 1996
36		Sachsen, Niederspree village, on the margin of the forest route along the lake bank	N 51°24'10", E 14°52'60"	Rotreklová *et al.*, 2005
36	Ireland	Lanesborough (Ct. Roscommon)	[N 53°40', W 07°59']	Gadella, 1972
36		Longford (Ct. Roscommon)	[N 53°44', W 07°48']	
36		Furnace, Lough Feeagh (Ct. Mayo)	[N 53°57', W 09°35']	
36	Luxembourg	near Vianden	[N 49°56', E 06°12']	
36		Müllertal near Larochette	[N 49°46', E 06°13']	Gadella, 1984
36		Château de Beaufort near Beaufort	[N 49°50', E 06°17']	
36	The Netherlands	Utrecht, Maarn-Maarnsbergen	[N 52°04', E 05°23']	Gadella and Kliphuis, 1963
36		Friesland, Island of Ameland	[N 53°27', E 05°43']	Gadella, 1972
36		Friesland, Oosterwolde	[N 53°00', E 06°18']	
36		Drente, Diphoorn	[N 52°46', E 06°49']	
36		Drente, Emmen	[N 52°47', E 06°54']	
36		Drente, Noordsleen	[N 52°47', E 06°48']	
36		Drente, Oosterhesselen	[N 52°46', E 06°49']	
36		Drente, Schoonebeek	[N 52°40', E 06°43']	
36		Drente, Sleen	[N 52°46', E 06°48']	
36		Overijssel, Ommen	[N 52°31', E 06°25']	

36	Gelderland, Bennekom	[N 52°00', E 05°41']	
36	Gelderland, Culemborg	[N 51°57', E 05°14']	
36	Gelderland, Harderwijk	[N 52°21', E 05°38']	
36	Gelderland, Heelsum	[N 51°59', E 05°46']	
36	Gelderland, Speuld	[N 52°16', E 05°43']	
36	Utrecht, de Bilt	[N 52°07', E 05°11']	
36	Utrecht, Rhenen	[N 51°58', E 05°34']	
36	Noord Holland, Bergen	[N 52°40', E 04°42']	
36	Noord Holland, Hilversum	[N 52°14', E 05°11']	
36	Zuid Holland, Island of Goeree	[N 51°49', E 03°57']	
36	Noord Brabant, Cromvoirt	[N 51°39', E 05°18']	
36	Noord Brabant, Drunen	[N 51°41', E 05°10']	
36	Noord Brabant, Heesch	[N 51°45', E 05°32']	
36	Noord Brabant, Helvoirt	[N 51°39', E 05°17']	
36	Noord Brabant, Lage Mierde	[N 51°24', E 05°09']	
36	Noord Brabant, Ossendrecht	[N 51°24', E 04°20']	
36	Limburg, Bemelen	[N 50°51', E 06°46']	
36	Limburg, Brunssum	[N 50°57', E 05°57']	
36	Limburg, Epen	[N 50°47', E 05°55']	
36	Limburg, Eys	[N 50°55', E 05°46']	
36	Limburg, Gulpen	[N 50°54', E 05°38']	
36	Limburg, Heerlen	[N 50°55', E 06°02']	
36	Limburg, Kunrade	[N 50°52', E 05°56']	
36	Limburg, Maastricht	[N 50°51', E 05°41']	
36	Limburg, Slenaken	[N 50°46', E 05°51']	
36	Limburg, Waubach	[N 50°55', E 06°03']	
36	Limburg, Wittem	[N 50°50', E 05°59']	
36	Noord Holland, Laren	[N 52°15', E 05°14']	
36	Gelderland, Opheusden	[N 51°56', E 05°38']	
36	Zuid Holland, Voorne	[N 51°54', E 04°07']	
36	Zeeland, Oostkapelle	[N 51°34', E 03°33']	
36	Limburg, Tierziet*		
36	Loonse en Drunense Duinen (prov. Noord Holland)	[N 51°39', E 05°08']	Gadella, 1984

36		Noord Holland, Bulk near Hilversum	[N 52°14', E 05°11']	
36		Limburg, near Brunssum*	[N 50°57', E 05°58']	
36		Limburg, near Gulpen*	[N 50°49', E 05°54']	
36	Norway	near Oslo	[N 59°55', E 10°46']	Gadella, 1972
36	Poland	near Opole (Lublin)	[N 50°40', E 17°57']	
36		district Przemyśl, Huta Złomy, near Narol, coll. A. Jankun	[N 50°21', E 23°19']	Pogan and Wcisło, 1989
36		district Przemyśl, Wola Wielka, near Narol, coll. A. Jankun	[N 50°12', E 23°24']	
36		district Katowice, Płoki near Trzebinia, coll. R. Izmailow	[N 50°12', E 19°31']	
36		district Słupsk, in the vicinity of Reservation Ruchome Wydmy, coll. E. Kuta	[N 54°28', E 17°02']	
36		district Słupsk, Ulinia, near the Lake Sarbsko, coll. E. Kuta	[N 54°46', E 17°38']	
36		district Siedlce, Pecowiec near Siedlce, coll. E. Kuta	[N 52°10', E 22°17']	
36		dictrict Zamość, Roztocze, Zwierzyniec, coll. E. Kuta *	[N 50°37', E 22°57']	
36		district Gdańsk, Karwia, way to Czarny Młyn,	[N 54°50', E 18°13']	
36		district Gdańsk, Ostrowo	[N 54°50', E 18°15']	
36		district Gdańsk, Jastrzębia Gora	[N 54°50', E 18°22']	
36		district Gdańsk, Świecino near Wejherovo	[N 54°45', E 18°11']	
36		district Gdańsk, Rozewie	[N 54°50', E 18°20']	
36		district Toruń, Toruń	[N 53°01', E 18°37']	
36		district Suwałki, Wiersnie near Giby	[N 53°47', E 19°34']	
36		district Suwałki, way from Zelwy to the lake Pomorze in vicinity of Giby	[N 54°52', E 23°22']	
36		district Włocławek, Widoń near Włocławek	[N 52°34', E 19°04']	
36		district Częstochowa, Sułostowice	[N 50°49', E 19°07']	
36		district Częstochowa, Romanów near Częstochowa	[N 50°41', E 19°23']	
36		district Kielce, Chęciny	[N 51°54', E 21°47']	
36		district Kielce, Koniecmosty near Pińczów	[N 50°20', E 20°40']	
36		district Kielce, Kije near Pińczów	[N 50°37', E 20°36']	
36		district Tarnobrzeg, Staszów	[N 50°33', E 21°07']	
36		district Katowice, Chrząstowice near Wolbrom	[N 50°20', E 19°41']	
36		district Kraków, Kraków-Kostrze	[N 50°03', E 19°57']	
36		district Biełsko-Biała, Maków Podhalański; Kamienna Góra,	[N 44°38', E 20°03']	
36		district Tarnobrzeg, Świniary, coll. Z. Sawicka	[N 50°33', E 21°35']	
36		district Tarnobrzeg, Połaniec, coll. Z. Sawicka	[N 50°26', E 21°19']	
36		district Kraków, Ruszcza near Kraków	[N 50°05', E 20°09']	

36		district Kraków, Niepołomice, coll. L. Przywara	[N 50°02', E 20°16']	
36		district Suwałki, Sejny, coll. R. Czapik	[N 54°06', E 23°21']	
36		district Skierniewice, Budy Grabskie, Primeval forest-Puszcza Bolimowska, coll. R. Czapik	[N 52°01', E 20°17']	
36		district Włocław, Milicz, coll. J. Małecka	[N 51°32', E 17°16']	
36		district Gdańsk, near Jastrzębia Gora	[N 54°50', E 18°22']	
36		district Gdańsk, Losniewo*		
36		district Suwałki, Michnowce near Sejny, in the vicinity of Lake Płaskie	[N 53°47', E 19°34']	
36		district Tarnobrzeg, Ząrabki, coll. Z. Sawicka	[N 50°16', E 21°47']	
36		district Włocław, Ruda Malicka, coll. R. Izmailow*		Skalińska *et al.,* 1971
36		Stronie Śląskie, Sudetien Mts	[N 50°18', E 16°53']	
36		Wielogłowy near Pelplin	[N 53°55', E 18°42']	Pogan *et al.,* 1987
36		district Kielce, Pińczów	[N 50°32', E 20°32']	
36		Beskid Sląski Mts, grassland above chalet ca 3.8 km ESE of the of the station in the town of Ustroń	N 49°43'20", E 18°51'20"	Rotreklová *et al.*, 2002
36		Beskid Sląski Mts, grassland along the road 0.5 km SW of the chalet ca 3.25 km SE of the of the station in the town of Ustroń	N 49°43'00", E 18°50'40"	
36		Jizerské hory Mts, near the settlement of Orle 3.5 km W of the village of Jakuszyce	N 50°48'45", E 15°23'50"	Rotreklová *et al.*, 2005
36	Portugal	near Paradela (Tras os Montes)	[N 41°15', W 07°51']	Gadella, 1972
36		Serre do Gerês, Carris (no. 1385)	[N 39°30', W 08°06']	Fernades and Queirós, 1971
36		2km de Barracão vers Braga (no. 1386)	[N 41°33', W 08°26']	
36	Spain	Ordesa, alt. 1300 m	[N 42°38', E 00°02']	Gadella, 1984
36		Galicia, near Verin, alt. 650	[N 41°57', W 07°27']	
36		Galicia, Ria Euviande, N. of Chantada	[N 42°38', W 07°46']	
36		Galicia, Vieilro, alt. 850 m	[N 43°39', W 07°37']	
36		Ordesa Soaso, alt. 1750 m*	[N 42°40', W 00°04']	
36	Sweden	Scania, Ljungbyhed, G. Turesson, 1949	[N 56°04', E 13°13']	Turesson and Turesson, 1960
36		Scania, Dalby, G. Turesson, 1945	[N 59°32', E 17°21']	
36		Scania, Simrishamn, G. Turesson, 1945	[N 55°33', E 14°21']	
36		Scania, Gunnarp, G. Turesson, 1945	[N 57°19', E 12°39']	
36		Scania, Sätaröd, G. Turesson, 1945	[N 55°57', E 13°53']	
36		Scania, Ludvigsborg, B. Turesson, 1945	[N 55°54', E 13°36']	
36		Scania, S. Rörum, G. Turesson, 1945	[N 55°56', E 13°40']	

36		Scania, Stenestad, G. Turesson, 1945	[N 56°03', E 13°05']	
36		Scania, Kaffatorp, G. Turesson, 1945*		
36		Småland, Gränna, G. Turesson, 1945	[N 58°32', E 14°28']	
36		Småland, Forserum, G. Turesson, 1953	[N 57°43', E 14°19']	
36		Scania, Båstad, G. Turesson, 1953	[N 56°46', E 12°54']	
36		Scania, Landskrona, G. Turesson, 1949	[N 55°52', E 12°50']	
36		Scania, Axelvold, H. Nordenskiöld, 1951	[N 55°58', E 13°06']	
36		Scania, Barseback, G. Turesson, 1953	[N 55°46', E 12°54']	
36		Scania, Vankiva, G. Turesson, 1953	[N 56°11', E 13°44']	
36		Scania, Högestad, H. Nordenskiöld, 1951	[N 55°30', E 13°45']	
36		Scania, Falsterbo, G. Turesson, 1951	[N 57°39', E 16°22']	
36		Scania, Skanor, G. Turesson, 1951	[N 55°25', E 12°51']	
36		Scania, Tjornarp, G. Turesson, 1953	[N 56°00', E 13°38']	
36		Scania, Skillinge, G. Turesson, 1951	[N 55°28', E 14°17']	
36		Scania, Sjöbo, G. Turesson, 1951	[N 55°38', E 13°42']	
36		Scania, Hohög, G. Turesson, 1953	[N 55°26', E 13°05']	
36		Scania, Bjerred, G. Turesson, 1952	[N 55°42', E 13°02']	
36		Scania, Viktorshög, G. Turesson, 1952*		
36		Scania, Domsten, G. Turesson, 1952	[N 56°07', E 12°36']	
36		Scania, Forstavallen, G. Turesson, 1953*		
36		Scania, Klagshamn, G. Turesson, 1951	[N 55°32', E 12°55']	
36		Scania, Sanekulla, G. Turesson, 1951*		
36		Scania, Knutstorp, H. Nordenskiöld, 1951*		
36		Scania, Hovs Hallar, G. Turesson, 1954	[N 56°28', E12 42°']	
36		Scania, Malen, G. Turesson, 1949*		
36		Scania, Tunbyholm, G. Turesson, 1945	[N 55°36', E 14°07']	
36		Scania, Hylla, B. Turesson, 1945	[N 55°33', E 13°38']	
36		Scania, Östarp, G. Turesson, 1945	[N 55°36', E 13°33']	
36		Scania, Haväng, G. Turesson, 1956	[N 55°43', E 06°00']	
36		Halland, Högaskog, G. Turesson, 1949*		
36	Switzerland	Tessin: Val Verzasco near Sonogno, alt. 900 m	[N 46°15', E 08°35']	Gadella, 1984
36	Ukraine	Chornogora, v fitotsenozakh lednikovych kotlov Goverla-Breskul, Breskul-Pozhizhevskaja, Pozhizhevskaja-Dantser, Rebra-Gutin-	[N 48°10', E 24°24']	Pashuk, 1987

		Tomnatek, *Festucetum-rubrae,* 1430-1600 m		
36	United Kingdom	England, Sussex, H.G. Baker, 1950	[N 50°55', W 00°10']	Turesson and Turesson, 1960
36		England, Bristol	[N 51°28', W 02°36']	Finch, 2005 (CD-BSBI)
36		England, E. Sussex, Fairligh sand quarry	[N 50°54', E 00°13']	
36		England, E. Kent, Dungeness	[N 51°12', E 01°10']	
36		England, N. Wilts., Vale of Pewsey, Walker Hill	[N 51°21', W 01°47']	
36		England, W. Gloucs., Avon Gorge, N side, 0.5 mi below Clifton Bridge	[N 51°56', W 02°15']	
36		England, Cambs., Babraham Hall Estate	[N 52°08', E 00°10']	
36		England, S. Hants., Christchurch	[N 50°44', W 01°45]	
36		England, Glam., Caerphilly	[N 51°40', W 03°11']	
36		England, S. Devon, Berry Head	[N 50°'27, E 03°51']	
36		England, Berks., Wytham Wood, near Oxford	[N 51°45', W 01°14']	
36		England, Wight., Carisbrooke Castle	[N 50°'42, W 01°18']	
36		England, S. Hants., Portsmouth	[N 50°50', W 01°05']	
36		England, E. Gloucs., Birdlip	[N 51°55', W 02°07']	Watson, 2005 (CD-BSBI)
36		England, W. Suffolk, Lakenheath Warren	[N 52°25', E 00°31']	Edmond, *et al.*, 2005 (CD-BSBI)
45	Austria	Fischbach, Styria, B. Nordensiöld, 1950	[N 47°27', E 15°39']	Turesson and Turesson, 1960
45		Maria Trost, Graz, B. Nordensiöld, 1950	[N 47°05', E 15°26']	
45		Katschberg, B. Nordensiöld, 1953	[N 47°03', E 13°37']	
45		Bernstein (Burgenland)	[N 47°24', E 16°16']	Gadella, 1972
45		Oberwart (Burgenland)	[N 47°17', E 16°12']	
45		Niederösterreich, Retz, Windmühlenberg nördlich Retz	[N 48°45', E 15°57']	Schuhwerk and Lippert, 1997
45	Belarus	Mogilevskaja obl., Slavgorodskij, okr.Rabinovichi	[N 53°35', E 30°20']	Dmitrieva, 1987
45	France	Alpes-Maritimes, localités entre Berthemon et St-Martin V0subie, talus eu bordure de la route, alt. 1300 m	[N 44°04', E 07°15']	Delcourt, 1972
45		Alpes-Maritimes, entre St-Martin v0subie et Bor0on, talus en bordure de la route, pelouse pâtures avec Gentiana lutea, alt. 1500 m	[N 44°04', E 07°15']	
45		Alpes-Maritimes, St-Etienne-de-Tinée, sur cailloutis en bordure de la Tin0e, alt. 1170-1200 m	[N 44°15', E 06°55']	
45		B.-Alpes, Vallée de l'Ubaye, route de Maurin, alt. 1400 m	[N 44°33', E 06°53']	
45		B.-Alpes, Vallée de l'Ubaye, Tornoux, 1200 m	[N 44°28', E 06°18']	
45		Alpes-Maritimes, en descendant de la Madonede Fenestre, alt. 1800 m*	[N 44°07', E 07°21']	
45		Alpes-Maritimes, Thorene, alt. 1200 m*		

45	Finland	Nätö, Åland, E. Hesselman, 1948*		Turesson and Turesson, 1960
45		Ostersundom, B. Nordensiöld, 1947	[N 60°15', E 25°11']	
45		Vädö, B. Nordensiöld, 1947*		
45	Ireland	Knochnareah, Granche House (Co. Sligo)*	[N 54°16', E 08°28']	Gadella, 1984
45		Port Runny Bay, (Ct. Roscommon)*	[N 53°38', E 08°13']	Gadella, 1972
45		Ben Bulbin (Co. Sligo), H. McAllister 68/40		Watson (2005, CD BSBI)
45		Near Enniskillen (Co. Fermanagh)		Finch (2005, CD BSBI)
45	The Netherlands	Noord Holland, Camperduin	[N 52°44', E 04°34']	
45		Zuid Holland, Wassenaar	[N 52°09', E 04°24']	
45		Gelderland, near Winterswijk	[N 51°56', E 08°43']	
45		Noord Holland, Island Texel, de Muy	[N 53°05', E 04°50']	Gadella, 1984
45		Noord Holland, Island Texel, de Krim	[N 53°05', E 04°50']	
45	Norway	Oslofjord, Island of Boroya	[N 59°21', E 10°35']	Gadella, 1972
45	Poland	near Kuźnice	[N 52°02', E 19°04']	
45	Sweden	Gotland, Lojsta Hed, G. Turesson, 1957	[N 57°21', E 18°22']	Turesson and Turesson, 1960
45		Gotland, Vike, G. Turesson, 1957	[N 63°20', E 18°45']	
45		Gotland, Fårösund, O. Hedberg, 1949	[N 57°53', E 16°06']	
45		Scania, Kaffatorp, G. Turesson, 1951	[N 56°12', E 14°17']	
45		Scania, Skälderviken, G. Turesson, 1949	[N 56°25', E 12°42']	
45		Scania, V. Ströö, B. Lövkvist, 1949*		
45		Småland, Pataholm, B. Turesson, 1952	[N 56°55', E 16°25']	
45		Scania, Hallands Väderö, B. Lövkvist, 1949	[N 56°51', E 12°54']	
45		Scania, Bollerup, G. Turesson, 1951	[N 55°30', E 14°02']	
45		Scania, Sibbarp, G. Turesson, 1952	[N 57°21', E 12°42']	
45		Scania, Vittsjö, G. Turesson, 1953	[N 56°20', E 13°39']	
45		Scania, Osby, G. Turesson, 1953	[N 56°22', E 13°59']	
45		Blekinge, Bräkne-Hoby, G. Turesson, 1951	[N 56°13', E 15°07']	
45		Blekinge, Karlskrona, G. Turesson, 1956	[N 56°10', E 15°35']	
45		Halland, Tolarp, O. Gelin, 1952	[N 55°56', E 13°58']	
45		Halland, Skrea strand, G. Turesson, 1952	[N 56°53', E 12°30']	
45		Halland, Steninge, G. Turesson, 1952	[N 56°47', E 12°40']	
45		Halland, Åsa, G. Turesson, 1952	[N 57°21', E 12°08']	
45		Halland, Tönnersjö, G. Turesson, 1952	[N 56°38', E 13°04']	

45	Öland, Resmo, B. Turesson, 1952	[N 56°33', E 16°26']
45	Småland, Vassmolösa, B. Turesson, 1950	[N 56°35', E 16°10']
45	Småland, Jönköping, B. Nordensiöld, 1950	[N 57°47', E 14°10']
45	Småland, Tånnö, B. Nordensiöld, 1950	[N 57°07', E 14°00']
45	Småland, Almhult, G. Turesson, 1953	[N 57°14', E 14°17']
45	Småland, Markaryd, B. Nordensiöld, 1950	[N 56°27', E 13°36']
45	Småland, Bellö, G. Turesson, 1954	[N 57°35', E 15°20']
45	Småland, Svinshult, G. Turesson, 1954	[N 57°39', E 15°05']
45	Småland, Råshult, G. Turesson, 1953	[N 57°27', E 15°28']
45	Småland, Urshult, G. Turesson, 1953	[N 56°32', E 14°49']
45	Småland, Ravamåla, G. Turesson, 1953	[N 56°33', E 15°16']
45	Småland, Ryningsnäs, G. Turesson, 1953	[N 59°36', E 13°07']
45	Småland, Lenhovda, G. Turesson, 1953	[N 57°00', E 15°17']
45	Småland, Oskarshamn, G. Turesson, 1953	[N 57°16', E 16°28']
45	Småland, Vimmerby, G. Turesson, 1953	[N 57°40', E 15°51']
45	Småland, Eksjö, G. Turesson, 1953	[N 57°40', E 14°58']
45	Småland, Bankeryd, G. Turesson, 1953	[N 57°53', E 14°06']
45	Småland, Vrå, G. Turesson, 1954	[N 57°22', E 12°20']
45	Bohuslän, Munkedal, G. Turesson, 1948	[N 58°28', E 11°41']
45	Bohuslän, Stenungsund, G. Turesson, 1952	[N 58°04', E 11°49']
45	Bohuslän, Svanesund, G. Turesson, 1952	[N 58°08', E 11°50']
45	Bohuslän, Lysekil, G. Turesson, 1948	[N 58°16', E 11°27']
45	Őstergotland, Ringstad, G. Turesson, 1949	[N 59°36', E 13°07']
45	Őstergotland, Omberg, O. S. Björkman, 1949	[N 58°19', E 14°41']
45	Őstergotland, Grensholmen , A. Gustafsson, 1949	[N 58°31', E 15°48']
45	Őstergotland, Borghamn, A. Gustafsson, 1949	[N 58°23', E 14°41']
45	Västergotland, Läckö, O. S. Björkman, 1949	[N 58°41', E 13°13']
45	Őstergotland, Åtvidaberg, G. Turesson, 1954	[N 58°12', E 16°01']
45	Västergotland, Undenäs, G. Turesson, 1953	[N 58°39', E 14°25']
45	Västergotland, Hindås, B. Nordensiöld, 1950	[N 57°43', E 12°30']
45	Västergotland, Alingsås, B. Nordensiöld, 1950	[N 57°56', E 12°32']
45	Västergotland, Falköping, G. Turesson, 1951	[N 58°10', E 13°34']
45	Västergotland, Trollhättan, G. Turesson, 1952	[N 58°17', E 12°18']

45	Öland, Djupvik, Föra, N. Erlandsson, 1954	[N 57°18', E 18°09']
45	Bohuslän, Uddevalla, G. Turesson, 1952	[N 58°36', E 11°25']
45	Dalsland, Ed, Stora Le, S. Odén, 1951	[N 59°05', E 11°53']
45	Dalsland, Mellerud, G. Turesson, 1952	[N 58°42', E 12°27']
45	Dalsland, Bengtsfors, G. Turesson, 1952	[N 59°02', E 12°13']
45	Närke, Degerfors, S. Odén, 1951	[N 65°09', E 21°29']
45	Närke, Olshammar, G. Turesson, 1953	[N 58°45', E 14°48']
45	Närke, Askersund, G. Turesson, 1953	[N 58°53', E 14°55']
45	Södermanland, Kila, B. Nordensiöld, 1948	[N 57°53', E 15°58']
45	Södermanland, Tystberga, G. Turesson, 1949	[N 58°51', E 17°15']
45	Södermanland, Sjösa, B. Nordensiöld, 1949	[N 58°46', E 17°05']
45	Södermanland, Römora, Trosa, B. Nordensiöld, 1952	[N 58°54', E 17°34']
45	Södermanland, Sparreholm, B. Turesson, 1952	[N 59°04', E 16°48']
45	Södermanland, Simonstorp, G. Turesson, 1954	[N 58°47', E 16°10']
45	Västmanland, Harbo, B. Lövkvist, 1949	[N 60°07', E 17°13']
45	Södermanland, Klastorp, G. Turesson, 1954	[N 58°44', E 17°12']
45	Värmland, Valnäs, G. Turesson, 1952	[N 60°29', E 17°46']
45	Värmland, Karlstad, G. Turesson, 1954	[N 59°23', E 13°31']
45	Värmland, Brattfors, G. Turesson, 1954	[N 59°40', E 14°01']
45	Västmanland, Hjulsjö, G. Turesson, 1952	[N 59°47', E 14°46']
45	Västmanland, Arboga, G. Turesson, 1953	[N 59°24', E 15°21']
45	Uppland, Bäcklösa, G. Turesson, 1945	[N 60°22', E 17°23']
45	Uppland, Ärna, B. Nordensiöld, 1948*	
45	Uppland, Torslunda, G. Turesson, 1950	[N 59°32', E 17°56']
45	Uppland, Uppsala, B. Turesson, 1950	[N 59°51', E 17°38']
45	Uppland, Gårdskär, B. Lövkvist, 1950	[N 60°38', E 17°37']
45	Uppland, Biludden, B. Lövkvist, 1950*	
45	Uppland, Vittinge, G. Turesson, 1951	[N 59°54', E 17°03']
45	Uppland, Mångkarbo, G. Turesson, 1951	[N 60°14', E 17°28']
45	Uppland, Österbybruk, G. Turesson, 1951	[N 60°12', E 17°54']
45	Uppland, Älvkarleby, G. Turesson, 1951	[N 60°34', E 17°26']
45	Uppland, Norrtälje, G. Turesson, 1952	[N 59°46', E 18°42']
45	Uppland, Grisslehamn, B. Turesson, 1952	[N 60°06', E 18°48']

45	Uppland, Gottsunda, B. Turesson, 1952	[N 50°29', E 18°13']
45	Gästrikland, Storvik, G. Turesson, 1952	[N 60°35', E 16°31']
45	Gästrikland, Gävle, G. Turesson, 1952	[N 60°40', E 17°07']
45	Jämtland, Äre, G. Turesson, 1949	[N 63°24', E 13°06']
45	Dalarna, Säter, G. Turesson, 1948	[N 62°31', E 17°05']
45	Medelpad, Ange, A. Gustafsson, 1948	[N 62°31', E 15°37']
45	Medelpad, Norrhassel, A. Gustafsson, 1948	[N 62°16', E 16°43']
45	Småland, Ljungby, G. Turesson, 1954*	
45	Småland, Lindas, G. Turesson, 1952*	
45	Småland, Hornsborg, O. Gelin, 1952	[N 56°38', E 13°48']
45	Småland, Oskarshamn, Gränsö, G. Turesson, 1956	[N 57°16', E 16°27']
45	Småland, Oskarshamn, Gunarsö, G. Turesson, 1956	[N 57°16', E 16°27']
45	Småland, Oskarshamn, Rotvik, G. Turesson, 1956	[N 57°16', E 16°27']
45	Småland, Visingsö, G. Turesson, 1945	[N 58°09', E 14°20']
45	Öland, Vickleby, N. Erelandsson, 1949	[N 56°35', E 16°28']
45	Öland, Böda, B. Turesson, 1952	[N 60°15', E 18°23']
45	Scania, Nybroan, G. Turesson, 1949	[N 55°26', E 13°55']
45	Gotland, Lummelunda, B. Nordensiöld, 1950	[N 57°46', E 18°27']
45	Västergotland, Kleva, G. Turesson, 1951	[N 58°33', E 13°15']
45	Västergotland, Bjurum, G. Turesson, 1953	[N 57°14', E 12°15']
45	Västergotland, Mariestad, G. Turesson, 1953	[N 58°42', E 13°49']
45	Őstergotland, Näkna, G. Turesson, 1954	[N 58°43', E 16°09']
45	Småland, Väksjö, D. Bertman, 1948*	
45	Västergotland, Granvik, G. Turesson, 1953*	
45	Bohuslän, Svälte, G. Turesson, 1952	[N 58°25', E 11°17']
45	Scania, Gyllebo, G. Turesson, 1957	[N 55°35', E 14°13']
45	Gotland, Hoburg, G. Turesson, 1957	[N 56°58', E 18°08']
45	Bohuslän, Harleby, G. Turesson, 1952	[N 58°10', E 11°38']
45	Dalsland, Laxarby, S. Odén, 1951	[N 59°02', E 12°19']
45	Närke, Säbylund, B. Lövkvist, 1951	[N 59°10', E 15°10']
45	Södermanland, Vaxaker, B. Nordensiöld, 1949*	
45	Södermanland, Södertälje G. Turesson, 1951	[N 59°12', E 17°37']
45	Södermanland, Slemna, G. Turesson, 1954*	

45		Värmland, Blankheden, A. Gustafsson *		
45		Värmland, Noppen, A. Gustafsson	[N 47°57', E 10°00']	
45		Värmland, Bro, S. Odén, 1951*		
45		Värmland, Saxa, G. Turesson, 1954*		
45		Uppland, Lidingö, B. Nordensiöld, 1948	[N 59°22', E 19°11']	
45		Uppland, Udderböo, B. Lövkvist, 1949*		
45		Uppland, Hummeldal, G. Turesson, 1950	[N 60°15', E 18°26']	
45		Uppland, Hjällstaviken, B. Nordensiöld, 1950*		
45		Uppland, Funbo, G. Turesson, 1950	[N 59°51', E 17°49']	
45		Uppland, Hammarby G. Turesson, 1954*		
45		Uppland, Osby, B. Lövkvist, 1950*	[N 59°57', E 18°06']	
45		Uppland, Ultuna, G. Turesson, 1951	[N 59°49', E 17°40']	
45		Medelpad, Nordanede, A. Gustafsson, 1948	[N 62°33', E 17°21']	
45		Uppland, Frötuna, G. Turesson, 1952	[N 60°37', E 17°21']	
45		Uppland, Gottröra, G. Turesson, 1952	[N 59°44', E 18°09']	
45		Dalarna, Rämshytan, G. Turesson, 1952*		
45		Dalarna, Hösjo, G. Turesson, 1952*		
45		Brantevik	[N 55°31', E 14°21']	Lövkvist and Hutlgård, 1999
45	Switzerland	Val Verzasca, near Sonogno (Tessin)	[N 46°21', E 08°47']	Gadella, 1972
45		Breil (Graubünden)	[N 46°46', E 09°03']	
45		Jaunpass	[N 46°35', E 07°20']	
45	United Kingdom	England, W. Lancs., Bardsea Wood	[N 54°04', W 02°51']	Watson, 2005 (CD-BSBI)
45		England, Anglesey, Newborough Warren	[N 53°18', W 04°24']	
45		England, Angus, 1 mi NW of Forfar	[N 56°39', W 02°53']	
45		England, Angus, Auchmithie	[N 56°45', W 02°48']	
45		England, Carms., Llanovery, on A40	[N 51°59', W 03°47']	
45		England, Co. Durham, Quarrington	[N 54°43', W 01°48']	
45		England, Co. Durham, Sunderland	[N 54°53', E 01°26']	
45		England, Co. Durham, Teesdale, Slapstone Sike	[N 54°38', W 02°07']	
45		England, Derbys., Cressbrook	[N 53°16', E 01°43']	
45		England, Derbys., Derwent	[N 52°53', W 01°17']	
45		England, Derbys., Padley Woods	[N 53°12', W 01°12']	
45		England, E. Norfolk, Howeshill	[N 52°43', E 01°33']	

45	England, E.Gloucs., Opposite Crickley Hill	[N 51°51', W 01°39']	
45	England, Glam., Oxwich Burrows	[N 51°33', W 04°10']	
45	England, Glam., Whiteford Burrows*		
45	England, Cheshire, Northwich, Witton	[N 53°16', W 02°32']	
45	England, Merioneth, Dolgellau	[N 52°45', W 03°53']	
45	England, Monts., Llangurig	[N 52°24', W 03°36']	
45	England, N.W. Yorks, 1 mi SE of Middleton-in-Teesdale	[N 54°38', W 02°05']	
45	England, Pembs., near Trevine	[N 51°57', W 05°08']	
45	England, S. Lancs., Ainsdale	[N 53°36', W 03°02']	
45	England, S. Lancs., Formby	[N 53°33', W 03°04']	
45	England, Staffs., Cheddleton Hospital	[N 53°04', W 02°02']	
45	England, Staffs., Cheddleton, Canal Pool Bridge	[N 53°04', W 02°02']	
45	England, Staffs., Cheddleton, Pumphouse Field	[N 53°04', W 02°02']	
45	England, Staffs., Cheddleton, River Churnet	[N 52°56', W 01°52']	
45	England, Staffs., Leek, Leekbrook, Wallgrange railway embankment	[N 53°07', W 02°02']	
45	England, W. Cornwall, Newlyn East railway	[N 50°06', W 05°33']	
45	England, W. Cornwall, Perranporth	[N 50°21', W 05°09']	
45	England, W. Cornwall, Treskillard*		
45	England, W. Norfolk, Scolt Head	[N 52°33', W 00°11']	
45	Scotland, E. Perth, Cairnwell	[N 56°53', W 03°25']	
45	Scotland, E.Perth, Ben Vrackie	[N 56°27', W 03°39']	
45	Scotland, Mid Ebudes, Mull, near Dervaig	[N 56°23', W 06°00']	
45	Scotland, Mid Perth, Glen Lochay, near power station	[N 56°24', W 03°39']	
45	Scotland, Mid Perth, Loch Tummel	[N 56°43', W 04°02']	
45	Scotland, Mid Perth, River Coninish, near Tyndrum	[N 56°26', W 04°43']	
45	England, Westmorland, Scout Scar and Kendal	[N 54°20', W 02°46']	Finch, 2005 (CD-BSBI)
45	England, Fermanagh, near Enniskillen	[N 54°21', W 07°38']	
45	England, Cambs., Gamlingay, Hayley Wood	[N 52°10', W 00°11']	
45	England, Anglesey, Bywrdd Arthur	[N 53°18', W 04°21']	
45	England, Main Argyll, Oban	[N 56°'25, W 05°30']	
45	England, Main Argyll, Ledaig	[N 56°07', W 04°52']	
45	England, Burbage, Hathersage (Wiltshire)	[N 51°21', W 01°41']	Gadella, 1984
45	England, W. Cornwall, Gwinear road station	[N 50°11', W 05°22']	Gadella, 1972

45		Scotland, Glen Affric Valley (Inverness)	[N 57°29', W 04°42']	
45		Scotland, Loch Ness (Inverness)	[N 57°11', W 04°34']	
45		Scotland, St. Cyrus (Kincardines)	[N 56°04', W 03°43']	
45		Scotland, Ben Lawers Mt. (Perth)	[N 56°33', W 04°14']	
45		Scotland, Ben Eighe, Loch Maree (West Ross)	[N 57°42', W 05°28']	
54	Austria	Lanser Alm, Insbr., B. Nordensiöld, 1950	[N 47°17', E 16°25']	Turesson and Turesson, 1960
54		Glasshuten, Koralpe, B. Nordensiöld, 1950	[N 48°04', E 16°02']	
54		Stockacher, Insbr., B. Nordensiöld, 1949*	[N 47°16', E 11°24']	
54		Polster, Hochschwab., B. Nordensiöld, 1950	[N 47°32', E 15°03']	
54		Hebalm, NW from Deutschlandsberg (Burgenland)	[N 46°51', E 15°10']	Gadella, 1972
54		Ruster Hiigelland bei Oslip (Burgenland)	[N 47°50', E 16°37']	
54		Ötztal, Obergurgl, Tirol, alt. 1925 m	[N 46°52', E 11°01']	Gadella, 1984
54		Klein Walsertal, Vorarlberg, alt. 1350 m	[N 47°12', E 10°00']	
54		Ötztal, Untergurgl, Tirol, alt. 1800 m*	[N 46°53', E 11°02']	
54		between Zundorf and Nickelsdorf	[N 47°58', E 17°03']	Schuhwerk and Lippert, 1997
54	Croatia	between Senj and Kratnik, alt. 300 m	[N 45°00', E 14°55']	Gadella, 1984
54		Plensvika near Senj, alt. 220 m	[N 45°04', E 14°57']	
54		Vela Ucka, Istra, alt. 1000 m	[N 45°17', E 14°12']	
54	France	near Lac du Mont Cenis (Savoie), 1850 m	[N 45°15', E 06°55']	
54		near Bessans (Savoie), 1700 m	[N 45°19', E 07°00']	
54		Mont Cenis (Savoie), 2500 m	[N 45°15', E 05°64']	
54	Ireland	Strandhill near Sligo (Ct. Sligo)	[N 54°17', W 08°27']	
54	Italy	between S. Michele and Doberda (Venezia Giulia)*	[N 45°49', E 13°15']	
54	Macedonia	near Mavrovo	[N 41°39', E 20°43']	Gadella, 1972
54	Romania	near Bacau	[N 46°34', E 26°55']	Gadella, 1972
54	Russia	Komi Republic, Syktyvkar	[N 61°43', E 50°46']	Lavrenko and Sereditov, 1991
54	Sweden	Scania, Vankiva, G. Turesson, 1953	[N 56°11', E 13°43']	Turesson and Turesson, 1960
54		Småland, Vimmerby, G. Turesson, 1953	[N 57°40', E 15°52']	
54		Gotland, Vastergarn, G. Turesson, 1957	[N 57°27', E 18°09']	
54		Gotland, Eksta, G. Turesson, 1957	[N 57°17', E 18°12']	
54		Uppland, Bergshamra, G. Turesson, 1945	[N 59°38', E 18°38']	
54		Uppland, Hallstavik, G. Turesson, 1952	[N 60°03', E 18°35']	
54		G]strikland, Forsbacka, G. Turesson, 1952	[N 60°37', E 16°49']	

54		Medelpad, Ostavall, A. Gustafsson, 1947	[N 62°25', E 15°29']	
54		Öland, Karlevi, G. Turesson, 1952	[N 56°36', E 16°28']	
54		Gotland, Högklint, G. Turesson, 1957	[N 57°36', E 18°12']	
54	Switzerland	near Waltensburg	[N 46°47', E 09°07']	Gadella, 1972
54		Lej da Staz near St. Moritz, Graubünden, , alt. 1800 m	[N 46°30', E 09°50']	Gadella, 1984
54		Pontresina, Graubünden, alt. 1800 m	[N 46°29', E 09°52']	
54		Samedan, Graubünden, alt. 1725 m	[N 46°32', E 09°45']	
54		Bernina Lagalp, Graubünden, alt. 2000 m *		
54		Between Sils Maria and Crasta, Graubünden, alt. 1850 m	[N 46°27', E 09°45']	
54		Sils Maria, Graubünden, alt. 1800 m*		
54		Bernina Lagalp, Graubünden, alt. 2200 m	[N 46°23', E 09°54']	
54		Brigels, Graubünden, alt. 1250 m	[N 46°46', E 09°04']	
54		Morteratsch near Pontresina, Graubünden	[N 46°29', E 09°54']	
54		Alp Languard, Graubünden, alt. 2300 m*		
54		Val Roseg, Graubünden, alt. 2000 m*		
54		St. Moritz, Graubünden, alt. 1800 m	[N 46°30', E 09°50']	
54		Muottes Muragl, Graubünden, alt. 2450 m*		
54		Muottas da Pontraschigna, Graubünden, alt. 2200 m*		
54		Val Fex, Graubünden, alt. 1900 m*	[N 46°26', E 09°46']	
54		Crasta, Val Fex, Graubünden, alt. 1850 m	[N 46°25', E 09°46']	
54		Hohtschuggen near Grächen, Wallis, alt. 2000 m*		
54		Gäsenried near Grächen, Wallis, alt. 1800 m	[N 46°11', E 07°50']	
54		Niedergrachen, Wallis, alt. 1600 m*		
54		Flue, Wallis*	[N 46°01', E 07°46']	
54		Riedgleschel near Grächen, Wallis, alt. 1800 m *	[N 46°11', E 07°49']	
54	United Kingdom	Scotland, Loch Rescobie, (Perthshire)	[N 56°39', W 02°48']	
54		England, Mid Ebudes, Mull, Glengorm Castle	[N 56°26', W 05°58']	Watson, 2005 (CD-BSBI)
54		England, E. Ross, River Carron	[N 57°53', W 04°21']	
54		England, Co. Durham, Westgate-in-Weardale	[N 54°44', W 02°08']	Morton, 1974
54		Scotland, Westerness, Glengarry, between Loch Garry and Invergarry	[N 56°48', W 05°01']	Finch, 2005 (CD-BSBI)
54		Scotland, Main Argyll, Glencoe, W end	[N 56°02', W 05°52']	
54		Scotland, Westerness, Ben Nevis, Meall an Suidhe	[N 56°48', W 04°14']	
54		Scotland, Moray, between Nairn and Elgin, Findhorn	[N 57°35', W 03°53']	

63	The Netherlands	Friesland, Vuurboetsduin, Island of Vlieland	[N 53°16', E 04°58']	Gadella, 1984
63	Sweden	Scania, Stenshuvud, B. Turesson, 1956	[N 55°40', E 14°17']	Turesson, 1972
18,36	Italy	Valley of Aosta, near Ville sur Nus, alt. 1400 m	[N 45°45', E 07°20']	Gadella, 1972
36,45	Belarus	Brestskaija obl., der. Vygonoshchi	[N 52°39', E 25°43']	Dmitrieva, 1987
36,45	Finland	Vihti, Ojakkala-Otalampi, gravel pit	[N 60°25', E 24°20']	Jalas and Pellinen, 1985
36,45		Nurmijärvi, Kiljavannumi	[N 60°28', E 24°48']	
36,45		Porvoo, Linnanmäki	[N 63°00', E 23°55']	
36,45	Ireland	Killarney (Ct. Kerry)	[N 52°03', W 09°31']	Gadella, 1972
36,45		Elphin (Ct. Roscommon)	[N 53°03', W 05°01']	
36,45		Benbulbin (Ct. Roscommon)	[N 54°22', W 08°28']	
36,45	The Netherlands	Friesland, Island of Terschelling	[N 53°25', E 05°20']	
36,45		Friesland, Island of Schiermonnikoog	[N 53°30', E 06°12']	
36,45		Gelderland, Winterswijk	[N 51°58', E 06°43']	
36,45		Zuid Holland, Wassenaar	[N 52°09', E 04°23']	
36,45	Norway	near Skjolden	[N 61°30', E 07°36']	Gadella, 1972
36,45	Sweden	Őstergötland, Ǻby, G. Turesson, 1954	[N 58°40', E 16°11']	Turesson and Turesson, 1960
36,45		Broby, B. Lövkvist, 1949*		
36,45		Kungsmarken*		
36,45		Uppland, Mősseberg	[N 58°12', E 13°30']	
36,45	United Kingdom	England, Derbys., Dovedale	[N 52°55', W 01°30']	Watson, 2005 (CD-BSBI)
36,45		England, Staffs., Wetton Mill	[N 56°26', W 06°17']	Finch, 2005 (CD-BSBI)
36,45		England, Leics., Charnwood forest, Bradgate Park	[N 52°42', W 01°03']	Watson, 2005 (CD-BSBI)
45,54	France	Near Bonneval sur Arc (Savoie), 1850 m	[N 45°22', E 07°08']	Gadella, 1972
45,54		between Bonneval sur Arc and I'Ecot (Savoie), 1950 m	[N 45°22', E 07°08']	
45,54	Italy	National Park Gran Paradiso: near Rifugio Vittorio Sella	[N 45°30', E 07°19']	
45,54		between Valnontey and Rifugio Vittorio Sella, ca 2200 m	[N 45°30', E 07°19']	
45,54	Sweden	Västergötland, Göteborg, G. Turesson, 1948	[N 57°43', E 12°00']	Turesson et Turesson 1960
45,54		Uppland, Väddö Huvud, B. Turesson, 1952	[N 60°00', E 18°49']	
45,54		Dalarna, Hedemora, B. Turesson, 1949	[N 60°17', E 15°59']	
45,54		Uppland, Bogesund, G. Turesson, 1948	[N 59°24', E 18°17']	
45,54		Västergotland, Kleva, G. Turesson, 1951	[N 58°12', E 12°26']	
45,54		Ângermanland, Gansvik, G. Turesson, 1949	[N 57°08', E 18°25']	
45,54	United Kingdom	England, Main Argyll, Glencoe village	[N 56°02', W 05°52']	Finch, 2005 (CD-BSBI)

45,54		England, Sheffield area	[N 53°24', W 01°29']	Grime *et al.*, 2005 (CD-BSBI)
36,45,54		Island of Vlieland	[N 53°16', E 04°59']	Gadella, 1972
36,45,54		Island of Texel	[N 53°05', E 04°49']	
54,63	Sweden	Blekinge, Tjurkö, B. Turesson, 1956	[N 56°07', E 15°37']	Turesson and Turesson, 1960

SUPPLEMENTARY INFORMATION 4

New published data on DNA-ploidy level of *Pilosella officinarum* based on flow cytometric analysis from different countries of Europe. Abbreviations used: TU = Tomáš Urfus; AK, FK, VV = Anna Krahulcová, František Krahulec, Vladimir Vladimirov; ZS, HS = Zdeněk Skála, Hana Skálová; JK FR = Jan Kirschner, Frederick Rooks; JZ Jaroslav Zahradníček.

Number of plants	PL	Country	Locality	Latitude, longitude
1	6*x*	Bulgaria	Vrachanska Planina Mts., meadows along the road from Vratsa to Parshevitsa hut near the hotel “Kiparis”, ca. 6 km SW of the town of Vratsa, 1054 m a.s.l., 11 May2006, AK, FK, VV (No. 1093)	N 43°10'34", E 23°28'55"
2	5*x*	Bulgaria	Vratschanska Planina Mts., meadow above the road from Vratscha to Parshevitsa hut ca. 7.5 km SW of the town of Vratsa, 1059 m a.s.l., 11 May 2006, AK, FK, VV (Nos. 1086, 1091)	N 43°10'14" E 23°28'50"
4	4*x*	Bulgaria	wet meadow below the road in the saddle “Petrochan” (between Berkovska Planina Mts. and Koznica Mts.), ca. 12 km S of the town of Berkovitsa, 1394 m a.s.l., 12 May 2006, AK, FK, VV (Nos. 977, 978/2, 1102, 1104)	N 43°07'06" E 23°07'36"
2	5*x*	Bulgaria	in the valley between the Vitosha Mts. and the Verila Mts., old pasture above the road N of the village of Kovachevichi, 1074 m a.s.l., 30 June 2006, AK, FK, VV (Nos. 1142, 1143)	N 42°27'40" E 23°19'48"
3	4*x*	Georgia	Bakuriani, 1959 m a.s.l., 1 August 2005, JK, FR	N 47°56'11" E 24°19'31"
3	4*x*	Georgia	near Tabatskuri lake, 2014 m a.s.l., 3 August 2005, JK, FR	N 41°46'14" E 43°31’07"
3	5*x*	Georgia	Krestov pass, 2300 m a.s.l., 4 August 2005, JK, FR	N 41°39'15" E 43°41'44"
3	5*x*	Ireland	western Ireland, Galway, Greatman Bay, 12 m a.s.l., 23 May 2005, TU	N 53°14'40" W 9°37'24"
3	6*x*	Ireland	Burren, Carnsefin, western Ireland, 53 m a.s.l., 28 May 2005, TU	N 53°08'41" W 09°15'16"
5	6*x*	Italy	Colli Eugeniei-Monselice, Monte Gargano, 800 m a.s.l., 14 May 2006, TU	N 45°15'48" E 11°41'46"
8	6*x*	Italy	Monte St. Angelo, Monte Gargano, 722 m a.s.l., 16 May 2006, TU	N 41°42'32" E 15°56'48"
7	6*x*	Italy	San Marco in Lamis 2 km SZ, Monte Gargano, 523 m a.s.l., 18 May 2006, TU	N 41°43'60" E 15°36'04"
5	6*x*	Romania	Banat, village of Gârnic, valley on the NW part of the village, near “Filipova díra” cave, April 2005, ZS, HS (Nos. 1058, 1061, 1062, 1067, 1075)	N 44°45'16" E 21°46'48"
3 1	5*x* 6*x*	Romania	Banat, village of Gârnic, pastures on the karst plateau SW of the village, near the road to the village of Sfânta Helena, April 2005, ZS, HS (5x: 1066, 1071, 1072; 6x: 1076)	N 44°44'44" E 21°46'50"

1 4	*5x* *6x*	Romania	Banat, village of Gârnic, valley NE of the village, near “Petrův mlýn” mill, pastures along the brook, April 2005, ZS, HS (Nos. 5x: 1075; 6x: 1063, 1064, 1068, 1069)	N 44º45'35" E 21°47'56"
4	*5x*	Romania	Roumania, Banat, Sfânta Helena, valley S of the vilage to Dunarea in the vallye of the Ali Beg brook, April 2005, ZS, HS (Nos. 1043, 1045, 1046, 1050)	N 44º 40'23" E 21º42'12"
6	*6x*	Ukraine	Marmarosh Mts, 0,5 km S of the Mt. Pop Ivan, JZ	N 47°56'11" E 24°19'31"

Morphological variation of *Pilosella officinarum*

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Abstract

Pilosella officinarum is represented mainly by tetraploid (2n = 36), pentaploid (2n = 45) and hexaploid (2n = 54) cytotypes that are further combined by different breeding modes (4x - sexual, 5x - apomictic and 6x - sexual + apomictic). Its current intraspecific taxonomic treatment is based mainly on selected apomictic lineages, which is confusing and not generally applicable. Morphometrical analyses were performed to determine a set of characters that distinguishes particular lineages that are characterised by ploidy level and breeding mode. The results confirmed the morphological differences among the ploidy levels and the different breeding lineages of the hexaploid cytotype.

Introduction

The genus, *Pilosella* (sometimes considered as subgenus *Hieracium* subgen. *Pilosella*), is a member of the tribus Lactuceae of the Asteraceae family. Together with *Hieracium*, it belongs to the most complicated groups of European flora. In spite of its close relationship to *Hieracium*, the pattern of variation and the underlying mechanisms are different. These two groups differ in morphology, absolute genome size, apomixis (agamospermy) type, ITS sequences, and chloroplast markers (Bräutigam & Bräutigam 1999, Suda et al. 2007, Rosenberg 1907, Pogan & Wcisło 1995, Fehrer et al. 2007). It is mainly a common hybridisation and polyploidy that influences the complexity of *Pilosella* and differentiates it from *Hieracium*.

The *Pilosella* genus taxonomic concept is structured into groups of basic (Hauptarten) and intermediate species (Zwischenarten; Nägeli & Peter 1885). The basic species comprise taxa characterised by unique morphological traits, whereas the intermediate species combine basic species features, indicating their hybridogenous nature. The basic vs. intermediate species distinction also corresponds well to the patterns of ploidy level and breeding mode differentiation (basic species are frequently diploid sexual vs. the intermediate that are predominantly polyploid apomictic; Krahulcová et al. 2000).

The mechanisms causing the complexity of *Pilosella* were reviewed twice in recent years (Krahulcová et al. 2000, Fehrer et al. 2007) and include the following: hybridisation, facultative apomixis (apomixis combined with a sexual reproductive mode, often within the same capitulum), polyploidisation (cytotypes detected in the field: 2x, 3x, 4x, 5x, 6x, 7x, and 8x; x= 9) and vegetative reproduction via stolons (Gadella 1987, 1991, Krahulcová et al. 2000). These sources were found to be accompanied with the production of fertile pollen (even in cases of completely sterile plants, such as F1 triploids) and the occurrence of haploid parthenogenesis or autogamy via a mentor effect (Krahulcová et al. 1999, Krahulcová et al. 2000, Krahulcová & Krahulec 2000). Moreover, all of the abovementioned features are usually combined in an intricate way. Apomictic lineages are facultative; thus, they hybridise with other cytotypes or taxa, resulting in an enormous number of different morphological forms and cytotypes. For these reasons, the entire *Pilosella* genus represents an extremely reticulate group, and its species-level taxonomy cannot be treated as is usual for the other apomictic groups (i.e., each apomictic lineage is evaluated as a microspecies). Several substantially different taxonomic approaches (i.e., hundreds or thousands of described taxa; i.a., Zahn 1921, 1922, 1923, Sell & West 1976, Juxip 2002, Tyler 2001) mirror the real variation. The situation, which is clearly irresolvable by the traditional taxonomic approaches, has resulted in a current resignation on the detailed taxonomic concept (Bräutigam & Greuter 2007).

Pilosella officinarum F. W. Schultz et Sch. Bip. (*Hieracium pilosella* L., Asteraceae) may be considered as a typical species that represents the complexity of the entire genus. It belongs to section *Pilosellina*, which is characterised by a single capitulum per stem and dense stellate hairs covering the abaxial side of the leaf (Zahn 1923). This section is distinctly defined within the genus and comprises several species with rather limited variation, mostly diploids and tetraploid *P. saussuroides* and one extremely variable polyploidy, *P. officinarum*. *Pilosella officinarum* is the most frequently occurring species of the genus *Pilosella*; it is variable with respect to ploidy level (4x, 5x, 6x, and 7x; Gadella 1984, 1991) and reproductive mode (both apomictic and sexual) and is accompanied by a huge phenotypic plasticity. Tetraploid cytotypes occur in Western and Central Europe and the Iberian Peninsula, whereas the pentaploids and hexaploids prevail in deglaciated areas (including the Alps and the Carpathians) and in the Mediterranean (Gadella 1984, Mráz et al. 2008). The tetraploids are almost exclusively sexual in Europe, and apomictic tetraploids are known to have been introduced to New Zealand (Houliston & Chapman 2001). The pentaploids are mostly apomictic, with rare sexual individuals, whereas the hexaploid cytotype consists of sexual and apomictic lineages (Gadella 1984). The heptaploid cytotype is extremely rare, sterile or apomictic (only recorded a few times; Turesson & Turesson 1960, Gadella 1984, Mráz et al. 2008). Even higher ploidy levels, such as nonaploids and decaploids, were produced in experimental crosses (Gadella 1987); however, these have never been found in the field. The diploid cytotype from the Alps (the Aosta Valley) was described by Gadella (1972) and from Hautes-Alpes by Delcourt (1972); however, the identity of these plants has not been studied recently. It seems that, at least in Central Europe, *P. officinarum* has a crucial role in the evolution of the whole genus because it participates in many hybridisation events across *Pilosella* (Krahulec et al. 2004, Křišťálová et al. 2010).

Numerous morphological forms of *P. officinarum* were commonly classified at the subspecies level (i.e., the genus monographer, K. H. Zahn, mentioned 624 subspecies; Zahn 1923). Although such a vast number often precludes any intraspecific evaluation, the correlation of some phenotypic characters (e.g., rosette size and the number and length of stolons) and ploidy level has been indicated by Gadella (1991). Nevertheless, such indications were never subjected to any rigorous statistical evaluation. During a study on the cytotype distribution within the Czech Republic (see Mráz et al. 2008), we obtained rather representative material with determined ploidy levels. In the study presented here, we employed multivariate morphometrics to analyse whether the morphological variation correlated with any of the particular ploidy levels and reproductive mode within *Pilosella officinarum*.

We aimed to answer the following questions:

(1) Does the variation in the morphology of *P. officinarum* correlate with the ploidy level?

(2) Could the particular reproductive modes be used additively to discriminate the species further?

(3) Are there any group-specific morphological characters separating the studied cytotypes?

Material and methods

Material

We used a portion of the same plants that were used in a previous publication (see Mráz et al. 2008). The herbarium specimens (deposited in Herbarium PRA) had originated from 154 populations (384 individuals in total, mostly from Central European regions) were supplemented by plant material from several localities in Scandinavia, the Iberian Peninsula, the Alps, and the Carpathians. To exclude the influence of specific environmental conditions, all of the plants were cultivated under the conditions of an experimental garden of the Institute of Botany, Průhonice, Czech Republic (for one to three years).

The DNA ploidy level of all of the samples was determined using flow cytometry (Partec Ploidy Analyser PA-II and/or Partec CyFlow). *Glycine max* cv. Polanka (absolute genome size=2.50 pg; Doležel et al. 1992), tetraploid *P. officinarum* and diploid *P. lactucella* plants with verified chromosome counts were used as the internal controls. A standard two-step procedure (Suda et al. 2003) without centrifugation was used for the sample preparation. Fresh leaves of both the samples and the standards were chopped using a sharp razor blade in 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid and 0.5% Tween 20; Otto 1990), filtered through a 42-µm nylon mesh and incubated at room temperature for 20 min. An aliquot (1 ml) of staining solution (Otto II buffer [0.4 M Na2HPO4•12H2O], 50 µl/ml propidium iodide, 50 µl/ml RNase IIA, and 2 µl/ml 2-mercaptoethanol) was added (in the case of the PA-II instrument, the staining solution consisted of Otto II buffer, 2-mercaptoethanol (2 µl/ml) and DAPI [4 µg/ml]) and the mixture was shaken gently. The mixture was then analysed after 10 min. Symmetric peaks were analysed using Partec FlowMax Operating and Analysing Software (version 2.4d; Partec GmbH, Münster, Germany). For each measurement, the coefficient of variation (CV) for both an internal standard and the sample was calculated. To calibrate the flow cytometric analyses for each ploidy level, the chromosome numbers of 8 plants were counted.

The reproductive modes of 35 tetraploid plants, all of the pentaploid and the hexaploid plants were checked using a routine emasculation test, as described in Krahulcová & Krahulec 1999. The upper parts of the unopened capitula (containing the anthers and stigma) were sliced off with a razor, and the heads were bagged. Thus, fully developed achenes were considered to be the result of an apomictic mode of reproduction (Gadella 1987, Krahulcová & Krahulec 1999).

In total, 27 morphological characters of 384 herbarium specimens (from 154 populations) were measured or scored (see Table 1). The characters measured or scored included traits used for the subspecies determination. Several measured characters focused on the proportions and shape of the vegetative and generative parts as follows: the leaf length (LL) and width (LW); the distance between the widest point of the leaf to the tip (WT); the rate of leaf tip acuteness (TA); the average length (LS) and width (SW) of 3 stolon leaves; the average diameters of 3 stolons (SD); the rate of the stolon curve shape (SC); the average length (BL) and width (BW) of 5 involucral bracts; and the capitulum diameter (CD). Another group of characters measured or scored the characteristic hair density rate of the following: single trichomes on the upper surface of 5 leaves (in the centre [SL] and margins [SM]), stem (SS), involucrum (SI) and stolon (STS); glandular trichomes on the stem (ES), involucrum (EI) and stones (EST); stellate trichomes on the adaxial side of 5 leaves (SEL), stem (SES), involucrum (SEI) and stolons (SEST). We also measured the average lengths of 10 single trichomes at the centre of 5 leaf surfaces (SLC) and margins (SLM) and the lengths of 5 single trichomes on the stem (SLS). The rate of dark trichomes (all types) was scored in terms of the darkness character of the involucrum (ID).

Table 1 All of the measured characters. Except for the SEST two-state character, all of the characters are of the three-state continuous quantitative type.

LS	the longest leaf shape - (1-oblanceolate to 3-ovate)
LL	leaf length
LW	leaf width
WT	distance between the widest point of leaf to the tip
TA	rate of leaf tip acuteness (1-round, 2-obtuse, 3-acute)
SL	density rate of single trichomes in the centre of the upper surface of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs)
SLC	average length of 10 single trichomes at the centres of 5 leaf surfaces
SM	density rate of single trichomes at the margins of the upper surface of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs)
SLM	average length of 10 single trichomes at the margins of 5 leaf surfaces
SEL	density rate of stellate trichomes on the adaxial side of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs)
ES	density rate of glandular trichomes on the stem (1-glabrate, 2-single trichomes, 3-dense hairs)
SS	density rate of single trichomes on the stem (1-glabrate, 2-single trichomes, 3-dense hairs)
BL	average length of 5 involucral bracts
BW	average width of 5 involucral bracts
ID	rate of dark trichome density on the involucrum (1-bright, 2-dark trichomes)
EI	density rate of glandular trichomes on the involucrum (1-glabrate, 2-single trichomes, 3-dense hairs)
EC	glandular trichome colour - translucent to black
SI	density rate of single trichomes on the involucrum (1-glabrate, 2-single trichomes, 3-dense hairs)
SEI	density rate of stellate trichomes on the involucrum (1-glabrate, 2-single trichomes, 3-dense hairs)
CD	capitulum diameter
STS	density rate of single trichomes on the stolons (1-glabrate, 2-single trichomes, 3-dense hairs)
SEST	density rate of stellate trichomes on the stolons (1-glabrate, 2-single trichomes, 3-dense hairs)
SE	stolon leaves (1-diminishing, 2-same-sized, 3-enlarging)

SL	average length of 3 stolon leaves
SW	average width of 3 stolon leaves
SD	average diameter of 3 stolon
SC	rate of stolon curve shape (1-linear, 2-curved shape)

Statistical evaluation

The basic statistical parameters, such as the mean value, standard deviation, and the 1st, 5th, 95th and 99th percentiles, were computed for each of the characters. The correlation structure among the characters was evaluated using Pearson's correlation and non-parametric Spearman's rank coefficients to avoid very high correlations (> 0.95) and distortion of the multivariate analysis.

Principal component analysis (PCA; Krzanowski 1990) based on a correlation matrix was applied to reduce the multidimensional nature of the character space. The variation pattern was then expressed by the first three components.

Consequently, discriminant analyses (canonical discriminant analyses, CDA, and classificatory discriminant analyses; Klecka 1980) were performed. As a discrimination character, the DNA ploidy level and breeding mode (in the case of the hexaploids) was set. Finally, non-parametric k-nearest neighbours classificatory discriminant analyses were used to display the percentage of correctly assessed individuals to the predetermined groups.

The multivariate analyses of the morphometric data set were performed with SAS 9.1.3. (SAS Institute 2000) and PAST software (Hammer et al. 2001).

Results

Ploidy level analysis. The DNA ploidy level of 384 plants from 154 populations gave the following results: in the case of ploidy uniform populations, the tetraploid cytotype prevailed (79 populations), followed by the hexaploid (32) and pentaploid cytotype (26). In total, 159 tetraploid, 83 pentaploid and 142 hexaploid plants were analysed. Fifteen mixed ploidy populations were detected: 4x+5x – 3 populations, 4x + 6x - 3 pops and 5x + 6x – 10 pops. The histograms peaks coefficient of the variation (CV) ranged from 1.67% to 3.21% (2.52% on average, a standard deviation of 0.46).

All of the analysed tetraploid plants were sexual, whereas the pentaploids were apomictic, in accordance with the previous results from the studied areas. In contrast, the hexaploid individuals form apomictic (50 individuals) and sexual groups (92 individuals) of highly distinct geographical distribution patterns. The apomictic hexaploid plants occurred in many types of habitat in large areas (the West Carpathians), and the sexual hexaploid cytotype was strictly connected to the steep river canyons of the Czech Massif.

Morphometry. All of the included variables showed deviations from the normal distribution in the Shapiro-Wilks test (thus, non-parametrical methods were used). The correlation between the two characters exceeding a value 0.95 of the Spearman’s coefficient was not observed.

The principal component analysis indicated three partly overlapping groups of individuals. The tetraploid and hexaploid plants were separated along the first component axis (explaining 21.9% of the variation), whereas the second axis (explaining 8.23% of the variation) partly distinguished the pentaploid group (Fig. 1).

The canonical discriminant analysis further underlined that the tetraploid and hexaploid individuals were morphologically well separated, whereas despite being quite well defined, the pentaploids were still interconnected with two remaining groups (see Fig. 2). The first canonical axis separated the group of tetraploids and hexaploids, whereas the second canonical axis was responsible for the partial separation of the pentaploid individuals. Thus, the discriminant analysis confirmed the intermediary character of the pentaploid cytotype. The first canonical axis expressed 92.4% of the intergroup variation, and the second axis expressed 7.86% of the intergroup variation.

The stolon curve shape (SC), stellate hair density on the stem (SES), average of 3 stolon diameters (SD) and average lengths of 5 involucral bracts (BL) were the most strongly correlated with the first canonical axis, whereas the characters based on glandular trichomes (the glandular trichome density rate on the stem, ES, and the rate of the darkness of the involucrum, ID) were the best correlates of the second canonical axis (Table 2).

Table 2 Results of the morphometric analyses

PCA (principal component analysis) - eigenvectors expressing the value of the characters correlated with the principal component axes (Prin) based on the entire dataset **CDA** (canonical discriminant analysis) - total canonical structure expressing the correlation of particular characters with the total canonical structure axes based on the entire dataset with the DNA ploidy level as the discriminatory character (1. CDA) and the dataset of the hexaploid individuals with the breeding mode as the discriminatory character (2. CDA; values marked in bold are the most correlated with the particular axis).

	PCA			1. CDA		2. CDA
	Prin 1	Prin 2	Prin 3	Can 1	Can 2	Can 1
LS	-0,013	-0,016	0,175	-0,402	-0,035	0,081
LL	**0,308**	0,013	0,299	**0,561**	-0,104	0,038
LW	0,180	-0,009	**0,388**	0,115	0,052	0,270
WT	**0,316**	0,044	0,253	**0,587**	-0,061	0,113
TA	0,207	0,186	-0,025	**0,708**	0,122	-0,139
SL	0,003	0,275	-0,028	0,144	-0,242	0,332
SLC	0,137	**0,431**	0,213	0,398	-0,236	0,117
SM	0,033	0,256	-0,029	0,184	-0,228	0,373
SLM	0,169	**0,418**	0,140	**0,561**	-0,152	0,069
SEL	0,190	-0,023	-0,012	0,277	0,209	0,241
ES	0,162	-0,016	-0,010	0,242	**0,817**	0,351
SS	0,247	0,065	-0,024	**0,552**	-0,012	0,347
BL	**0,301**	-0,008	-0,007	**0,644**	0,446	0,325
BW	0,204	-0,015	-0,015	0,404	0,218	0,087
ID	0,107	-0,026	-0,022	0,174	0,433	**0,649**
EI	-0,004	-0,019	0,107	-0,189	0,227	0,435
EC	-0,002	-0,002	0,031	-0,046	0,061	-0,219
SI	0,258	0,071	-0,013	**0,591**	-0,031	**0,404**
SEI	0,011	0,211	0,105	0,060	-0,201	0,176
CD	0,145	-0,013	0,007	0,310	0,213	0,122
STS	0,087	-0,004	0,236	-0,023	-0,086	0,398
SEST	-0,007	0,123	0,245	-0,110	-0,248	-0,148
SE	-0,008	-0,004	0,242	-0,259	0,152	0,156
SL	**0,314**	-0,015	-0,002	**0,527**	0,044	0,175
SW	0,236	-0,022	0,074	0,225	0,037	0,315
SD	**0,314**	-0,004	0,037	**0,678**	0,110	0,055
SC	0,189	0,236	-0,004	**0,714**	-0,191	**-0,456**

Classification discriminant analysis resulted in the correct classification of 84% of the individuals, and a correct classification was very unequal for particular cytotypes. The tetraploids and hexaploids were, for the most part, correctly classified (91.9%, and 90.3% of correct classifications for the tetraploids and hexaploids, respectively), whereas the classification of the pentaploids failed in almost half of the cases (55.8% of the correct classifications).

The reproductive mode was included to distinguish those plants belonging to the sexual and apomictic hexaploid cytotype. The canonical discriminant analysis of all four of the groups indicated a slightly different pattern of sexual vs. apomictic hexaploids. Therefore, the separated CDA was performed with the dataset reduced to the hexaploids only. Both of the groups were notably separated, with minimum overlap. Characters ID, SEI and SC were most closely correlated with the first canonical axis. Subsequently, classificatory discriminant analysis indicated the correct classification of 80.7% of individuals.

Discussion

Our morphometric analysis showed a distinct morphological differentiation of *P. officinarum* cytotypes and reproductive mode types in Europe. The cytotype morphological pattern demonstrates a different approach for the investigation of *P. officinarum* intraspecific variation. Previously, the taxonomic concept for this species used combinations of characters (e.g., long hairs and stellate hairs) but did not take into account the reproductive mode and ploidy level. The results of the morphometric analyses, in fact, confirmed that these more natural units can be distinguished by their morphology. Thus, a more natural classification seems to be possible. However, the knowledge of the reproductive mode is necessary because the evaluation of variation should be different for sexual types than for apomictic types. Such analysis has not yet been performed; it is impossible to give these more natural units names, as hundreds of them were used in the past.

The multivariate statistic analyses indicated that a combination of characters might be used for the determination of *P. officinarum* plants: it is relatively feasible for the tetraploid and hexaploid plants but not for the pentaploids.

In particular, the morphology of the sexual types could be influenced by the common hybridisation with some other *Pilosella* species than what might be further followed by introgression. This has been shown, for example, in the hybrid swarm between *P. aurantiaca* and *P. officinarum* in Westphalia (Krahulcová et al. 2011). The movement of genetic material is in the direction towards sexual species because they hybridise more freely: althought in the abovementioned hybrid swarm, *P. officinarum* had obtained the plastids of *P. aurantiaca*. For this reason, it seems impractical to distinguish local sexual types, as they could be influenced by hybridisation, which could have occurred recently and/or in the past.

The fact that it was possible to distinguish between the sexual and apomictic hexaploid types shows they represent different entities. In addition, they have a different distribution (Mráz et al. 2008) and different habitats. The apomictic type is distributed in low altitudes in the Carpathians and in a broad spectrum of habitats in Pannonia. Conversely, the sexual hexaploid type is strictly confined to canyons of rivers in Southern Moravia and to canyons of the Vltava River in Central Bohemia. These canyons are typical relict habitats, which host many light-requiring species of different migration groups. Some of them are evidently relicts of a glacial time (e.g., *Allium strictum, Saxifraga paniculata, S. rosacea,* and *Biscutella laevigata*, i.a.: Reisch et al. 2003, Parisod & Besnard 2007), whereas many others came later, probably during the early periods of the Holocene. Therefore, it is difficult to speculate about the origin of these hexaploid types without a deeper knowledge of the *P. officinarum* populations in broader geographic areas.

Several morphological characters capable of discriminating between particular cytotypes were identified in the morphometric analysis. The tetraploid and hexaploid plants differed in the leaf shape (the leaves of hexaploids were more acute and longer in the distance between the point where is leaf the widest, and the leaf tip was longer). The involucral bract length was also markedly longer in case of the hexaploids. The most important characters were on the stolons (the hexaploid plant stolons were of a larger diameter, and the stolon shape tended to curve). However, the determination of the pentaploid cytotype seems to be more difficult, as they differed in hair characters (a higher density rate of glandular trichomes on the stem).

The discernment of the groups (i.e., tetraploid/pentaploid vs. hexaploid/pentaploid) may be effective in practice. Thus, for certainty, it would be better to determine a particular plant negatively (i.e., not hexaploid vs. not tetraploid), followed by a subsequent approximate pentaploid determination.

Remarkably, the apomictic vs. sexual groups of the hexaploid cytotype were distinguishable according to the rate of dark hairs on the involucrum (the apomictic plants tended to have a higher rate of dark trichomes on the involucrum) and the density of single trichomes (apomictics were more dense). The apomictic hexaploid individuals seemed to be less characterised by a curved stolon shape than the sexual ones.

Several circumstances did not allow us to solve the taxonomic consequences sufficiently according to the above-presented results. In particular, the enormously high number of described subspecies was problematic, and they should be typified and, subsequently, classified to newly distinguished entities. However, we did not evaluate all of them; this is a task for detailed research in the future. Our data are valid for a relatively small area within Central Europe.

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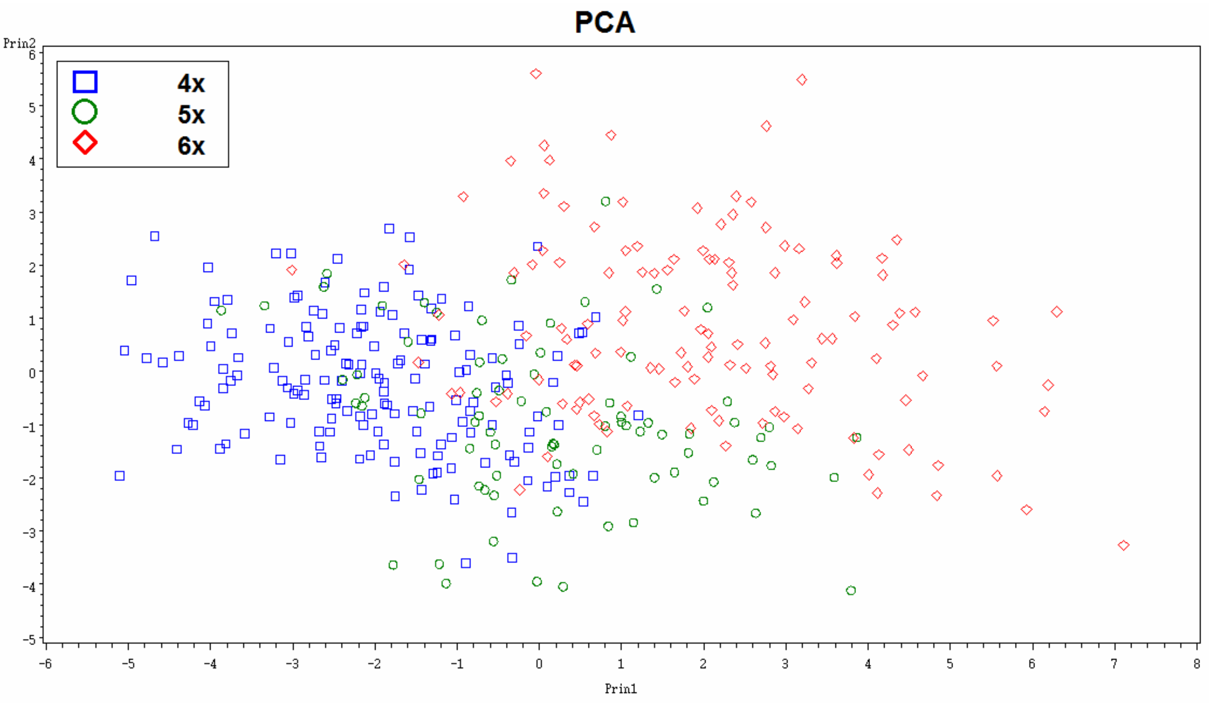

Fig. 1 Principal component analysis (PCA) ordination diagram (the entire dataset of 384 *Pilosella officinarum* individuals based on 31 characters) illustrating the partial separation of particular *P. officinarum* cytotypes (4x, 5x and 6x; the first component axis expresses 21,9%, and the second expresses 8,23%)

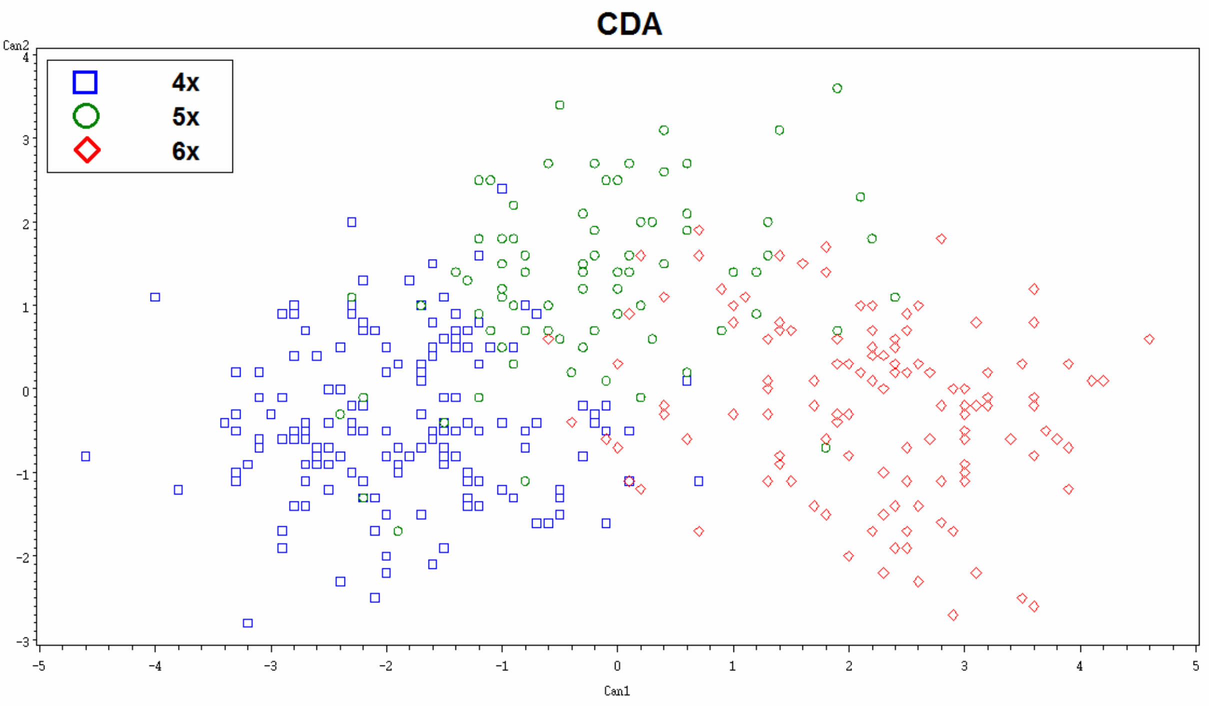

Fig. 2 Canonical discriminant analysis diagram (the entire dataset of 384 *Pilosella officinarum* individuals based on 31 characters; the first canonical axis expresses 92,4% of the intergroup variation, and the second axis expresses 7,86% of the intergroup variation)

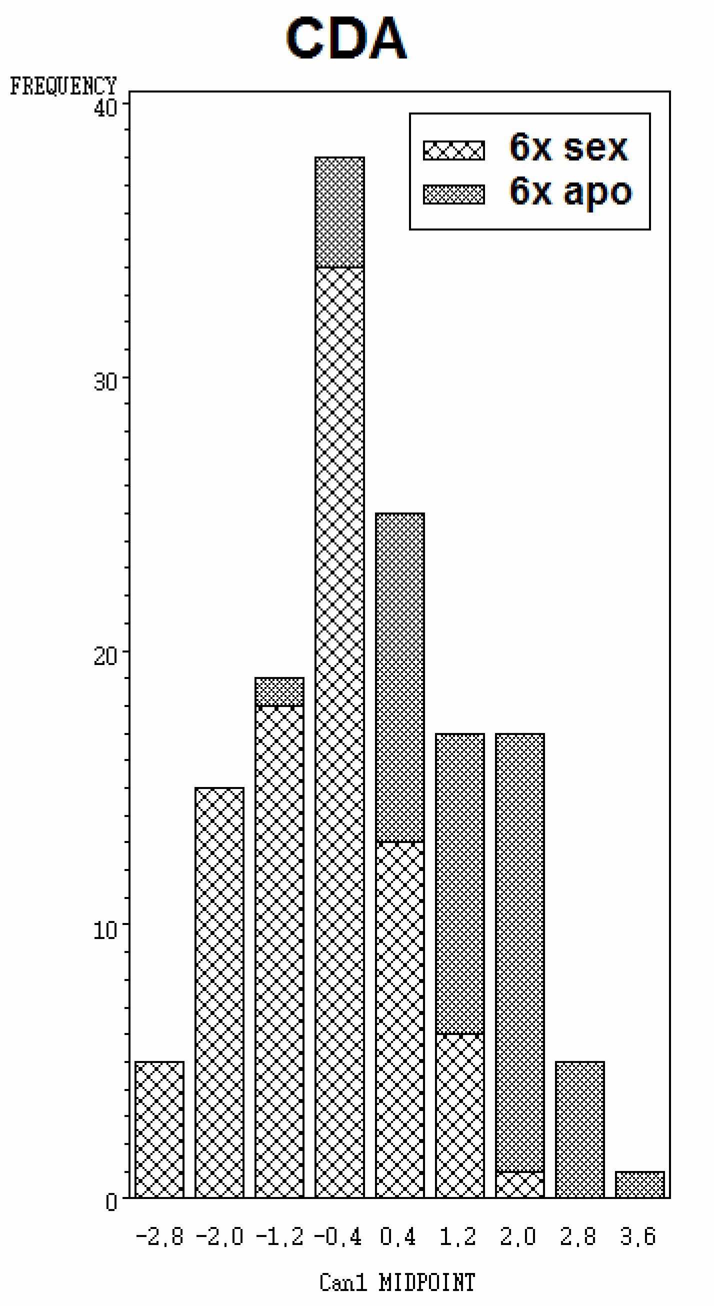

Fig. 3 Canonical discriminant analysis histogram (dataset of 92 sexual and 50 apomictic hexaploid *Pilosella officinarum* individuals based on 31 characters)

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ORIGINAL ARTICLE

Complex pattern of genome size variation in a polymorphic member of the Asteraceae

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ABSTRACT

Aim Although divergences in nuclear DNA content among different species within a genus are widely acknowledged, intraspecific variation is still a somewhat controversial issue. The aim of this study was to assess genome size variation in the polymorphic species *Picris hieracioides* L. (Asteraceae) and to search for potential interpretations of the size heterogeneity.

Location Europe.

Methods The genome sizes of 179 plants of *P. hieracioides* collected from 54 populations distributed across 10 European countries were determined by propidium iodide flow cytometry. Differences in nuclear DNA content were confirmed in simultaneous analyses.

Results 2C-values (population means) at the diploid level varied from 2.26 to 3.11 pg, spanning a 1.37-fold range. The variation persisted even after splitting the whole data set into two recently distinguished morphotypes (i.e. the 'Lower altitude' type and the 'Higher altitude' type) that possess significantly different nuclear DNA contents. Cluster analysis revealed the presence of three major groups according to genome size, which exhibited a particular geographical pattern. Generally, the genome size of both morphotypes increased significantly from south-west to north-east. A new cytotype, DNA triploid, was found for the first time.

Main conclusions High intraspecific variation in the amount of nuclear DNA in *P. hieracioides* correlates with the extensive morphological variation found within the taxon. Despite the complex pattern that was presented, genome size variants were non-randomly distributed and reflected palaeovegetation history. We suggest that the complex evolutionary history of *P. hieracioides* (e.g. the existence of several cryptic lineages with different levels of cross-interactions) is the most plausible explanation for the observed heterogeneity in genome size.

Keywords

Asteraceae, C-value, distribution, DNA ploidy, Europe, flow cytometry, genome size variation, *Picris hieracioides*, propidium iodide, triploid.

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INTRODUCTION

The last decade has seen significant advances in our understanding of the causes and consequences of variation in genome size in plants (e.g. Leitch & Bennett, 2007). Currently, genome sizes are known for about 5000 angiosperm species, with 1C-values ranging from 0.065 picograms (pg) of DNA in *Genlisea margaretae* Hutch. (Lentibulariaceae) to 127.4 pg in *Fritillaria assyriaca* Baker (Liliaceae). The interest in genome size data has been fuelled by the fact that nuclear DNA content can affect various characteristics at the cellular, tissue and organismal levels (i.e. the 'nucleotype theory'; Bennett, 1972). In addition, nuclear DNA content can have important ecological and evolutionary consequences. For example, it has been documented that the developmental lifestyle and life strategy (i.e. whether annual or perennial, herbaceous or

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woody), and the range of ecological environments that an organism can occupy may all be related to genome size (e.g. Knight *et al.*, 2005). Genome size has also attracted an ever-increasing amount of attention from plant taxonomists. In this field, nuclear DNA content may often facilitate the delimitation of taxa at various taxonomic ranks and may therefore influence taxonomic decisions (Kron *et al.*, 2007).

The existence and amount of variation in genome size below the species level has been debated for nearly two decades and is still somewhat controversial. The debate surrounding these topics was driven by numerous early reports of intraspecific variation that were dismissed by subsequent investigations using best-practice methodology (Greilhuber, 2005). For example, about 1.1- and 1.15-fold differences in genome size were reported in the legumes *Glycine max* Merr. (Rayburn *et al.*, 1997) and *Arachis hypogaea* L. (Singh *et al.*, 1996), respectively, but later attempts to confirm such results were largely unsuccessful (Obermayer & Greilhuber, 1999; Temsch & Greilhuber, 2000). The most striking (and very likely erroneous) case of intraspecific variation in DNA content concerns *Collinsia verna* Nutt. (Scrophulariaceae), which was reported to exhibit up to 288% divergence among individual accessions (Greenlee *et al.*, 1984). Over time, the concept of stable genome size within species gained increasing support. However, in recent years there has been an increase in the number of studies reporting genome size variation, using meticulous methodology (Schmuths *et al.*, 2004; Obermayer & Greilhuber, 2005; Leong-Škorničková *et al.*, 2007; Suda *et al.*, 2007a). Interestingly, intrapopulational variability in nuclear DNA content has also been documented (Šmarda & Bureš, 2006; Šmarda *et al.*, 2007). Species adapted to various climates, long-term-isolated populations, crops under long-lasting human selection, and allopolyploids with multiple origins are some of the candidate groups for which variation in genome size could potentially be detected. From a taxonomic standpoint, variation in nuclear DNA content may be used to indicate incipient speciation or taxonomic heterogeneity (Greilhuber & Speta, 1985).

One of the more taxonomically challenging plant groups of the European flora is hawkweed oxtongue (*Picris hieracioides* L., Asteraceae). This species grows in almost all of Europe, extending from the Iberian Peninsula, Italy and the Balkan Peninsula in the south to the Scandinavian countries in the north. The area of distribution continues to the Near East and Russia (Lack, 1974; Sell, 1975, 1976). The large morphological variation observed across this range has triggered a continuous dispute concerning the number of intraspecific taxa and their boundaries. Up to five distinct subspecies are currently recognized, based on peduncle length, size and colour of involucre, and characteristics of the involucral indumentum (e.g. Sell, 1975, 1976). However, the lack of any distinct phenotypic discontinuities (the size of the floral parts and the density of the indumentum, for example, vary tremendously) hinders unambiguous recognition of taxa. Several taxonomic concepts have therefore been adopted for recent treatments of the group (see Sell, 1975, 1976; Bolòs & Vigo, 1990; Haeupler & Muer, 2007). Targetted investigation of variation in *P. hieracioides* in the Western Carpathians and adjacent Pannonia revealed two morphological groups (the so-called 'Lower altitude' type and the 'Higher altitude' type), which were, at least partially, supported by ecological preferences and life-form (Slovák & Marhold, 2007). Whereas the former morphotype is usually annual to biennial and occupies dry, sunny, often man-made habitats in lowlands (or at lower altitudes in mountains), the latter is often a short-lived perennial, occurring predominantly in mesic, semi-natural or natural habitats (such as tall herb meadows) at higher altitudes. The most conspicuous, discriminating characteristics are the distribution of capitula along stem branches, the colour of the involucral indumentum, the length of ligules, the size of inner bracts and the presence/absence of red stripes on the outermost ligules (Slovák & Marhold, 2007).

Previous attempts to elucidate the taxonomy of *P. hieracioides* using conventional karyological counts were in vain, because all individuals examined exclusively contained a diploid number of somatic chromosomes ($2n = 2x = 10$; Slovák *et al.*, 2007). Another cytogenetic character, genome size, has not been widely studied; the Plant DNA C-values database (Bennett & Leitch, 2005) harbours the only estimate ($1C = 1.58$ pg), as determined by Feulgen microdensitometry. Because nuclear DNA content is often a taxonomically informative marker, and its value in resolving complex homoploid groups has been acknowledged repeatedly (e.g. Dimitrova *et al.*, 1999; Mishiba *et al.*, 2000; Mahelka *et al.*, 2005), we initiated a large-scale survey of genome size variation in a representative set of *P. hieracioides* plants. Five main questions were addressed, as follows. (1) What are the levels of intra- and inter-population variation in genome size? (2) Are there any historical or ecogeographical factors that may explain the divergence? (3) Does genome size correlate with recently distinguished morphotypes? (4) Can genome size be used as a supportive marker for taxonomic decision-making? (5) Is *P. hieracioides* uniform across its geographic range with respect to ploidy level?

MATERIALS AND METHODS

Plant material

Samples of *P. hieracioides* were collected in the field during 2004–07. The area sampled covered Andorra, Austria, Croatia, France, Germany, Italy, Romania, Slovakia, Spain and Ukraine, spanning the geographic range 36°58′–49°17′ N and 03°21′ W–27°14′ E, and the elevational range 5–2065 m a.s.l. (Fig. 1; Table 1). Altogether, 179 individuals from 54 populations were included in the study. Multiple accessions (i.e. two to six plants per locality) were available for 46 populations, and eight populations were represented by one accession only. Considering the results of previous morphometric studies (Slovák & Marhold, 2007; M. Slovák *et al.*, in preparation), samples were divided into two subsets, corresponding to 'Higher altitude' (H; 93 individuals from 27 populations;

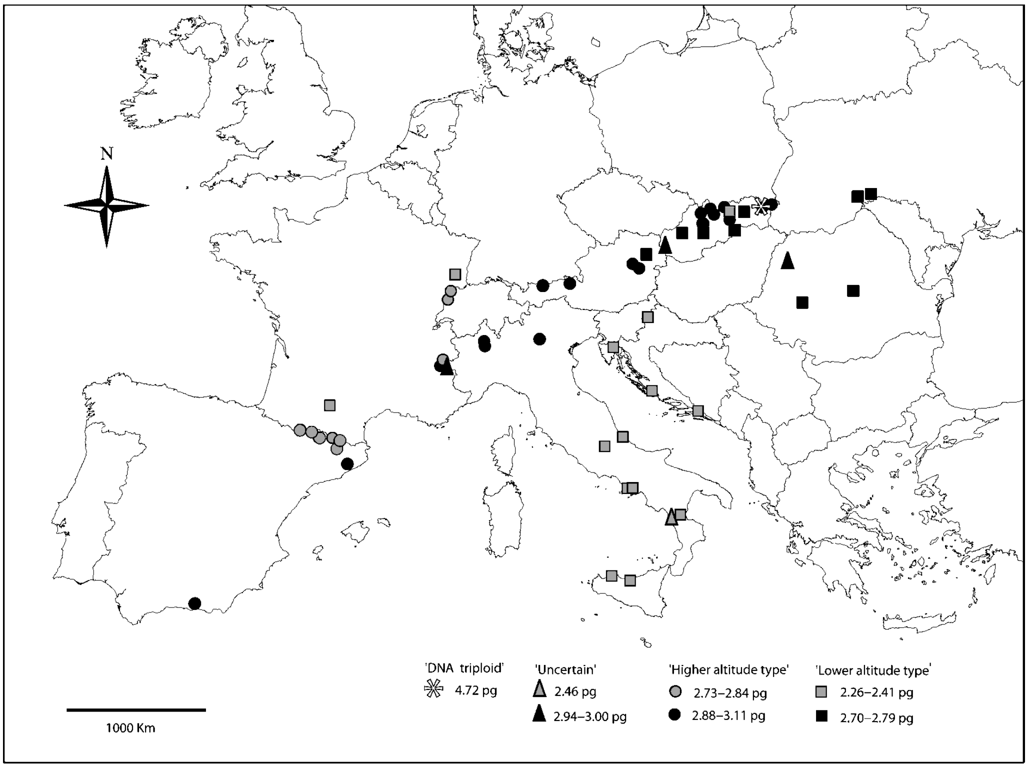

Figure 1 Geographical distribution of the populations of *Picris hieracioides* studied. The plants were separated into two morphotypes (the 'Lower altitude' type and the 'Higher altitude' type), in addition to a group of uncertain systematic position, following the results of our previous studies (Slovák & Marhold, 2007; M. Slovák *et al.*, in preparation). Genome size categories (2C-values) were determined on the basis of a cluster analysis (see Results).

altitude usually above 500 m a.s.l.) and 'Lower altitude' (L; 70 individuals from 23 populations; altitude usually below 500 m a.s.l.) types. Four populations harboured plants that could not be reliably determined, and were therefore regarded as of uncertain taxonomic status. Mature plants were transferred to the experimental field garden of the Institute of Botany of the Slovak Academy of Sciences in Bratislava, Slovakia (48°10′15″ N, 17°04′15″ E; 180 m a.s.l.) and grown under uniform conditions. In addition, achenes collected in the field were germinated on wet paper tissues in Petri dishes and grown in the same way as mature plants. Well-developed individuals were subjected to flow cytometric analyses in April–September 2007. Voucher specimens were deposited in the herbarium of the Slovak Academy of Sciences (SAV).

Genome size estimation

Genome sizes (C-values *sensu* Greilhuber *et al.*, 2005) and DNA ploidy levels (Suda *et al.*, 2006) of the analysed plants were estimated by flow cytometry (FCM). Measurements were taken on a CyFlow SL cytometer (Partec GmbH, Münster, Germany) equipped with a green solid-state laser (Cobolt Samba 532 nm, 100 mW; Cobolt, Stockholm, Sweden) as an excitation source. Fresh intact leaf tissues from the analysed sample and internal reference standard were chopped together (in a sandwich-like fashion) in 0.5 mL of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20; Otto, 1990). The crude suspension was filtered through a 42-μm nylon mesh and incubated at room temperature for 20 min. Nuclei were stained with 1 mL of Otto II buffer (0.4 M $Na_2HPO_4.12H_2O$), supplemented with propidium iodide (PI) + RNase IIA (both at final concentrations of 50 μg mL^{-1}) and 2-mercaptoethanol (2 μg mL^{-1}). After 10 min incubation at room temperature, samples were run on a flow cytometer and the fluorescence intensity of 5000 particles was recorded. Flow histograms were evaluated using the FloMax software (ver. 2.4d; Partec GmbH, Münster, Germany). At least three independent measurements on different days were performed for each sample. Only histograms with symmetrical peaks and with a coefficient of variation (CV) of the sample G_1 peak below 3% were considered. *Glycine max* Merr. 'Polanka' (2C = 2.50 pg; Doležel *et al.*, 1992) was selected as the appropriate primary

Table 1 Locality details, including geographic coordinates and altitude, morphotype, ploidy level/DNA ploidy level, genome size (mean 2C-value with standard deviation, intra-population range, and variation), range of coefficients of variation, number of plants investigated and internal reference standard for 54 populations of *Picris hieracioides*.

Population number	Morphotype*	Locality details†	Geographic coordinates‡	Altitude (m a.s.l.)‡	Ploidy level/DNA ploidy level§	Mean 2C-value ± SD (DNA pg)¶	2C-value range (min–max) (DNA pg)	Intra-populational variation (%)	CV range (%)	No. plants	Standard**
1	H	ES: Sierra Nevada Mts, village of Capileira	36°57.703′ N 03°21.457′ W	1605	2x	2.94 ± 0.03 EF	2.90–2.98	2.7	1.47–2.44	4	G
2	H	ES: Pyrenees Mts, town of Vielha	42°41.190′ N 00°47.241′ W	1230	2x!	2.84 ± 0.01 GHI	2.83–2.86	1.1	1.83–2.80	4	G
3	H	ES: Montseny Mts, between villages of Montseny and el Brull	41°46.702′ N 02°23.780′ E	740	2x!	2.90 ± 0.02 FG	2.87–2.93	1.9	1.66–2.69	4	G
4	H	ES: Pyrenees Mts, village of Baños di Panticosa	42°45.029′ N 00°14.557′ W	1575	2x	2.81 ± 0.02 HIJK	2.79–2.84	1.7	1.37–2.51	5	G
5	H	ES: Pyrenees Mts, village of Espot	42°33.547′ N 01°05.416′ E	1575	2x!	2.77 ± 0.01 IJKLM	2.75–2.78	1.3	1.59–2.64	5	G
6	H	AD: Pyrenees Mts, the town of Soldeu	42°34.170′ N 01°40.691′ E	1900	2x!	2.73 ± 0.02 LM	2.69–2.74	1.9	1.55–2.77	4	G
7	H	FR: Pyrenees Mts, town of Llívia	42°26.633′ N 01°56.531′ E	1170	2x	2.81 ± 0.01 HIJKL	2.79–2.83	1.5	1.73–2.86	4	G
8	H	FR: Pyrenees Mts, village of Estavar	42°29.915′ N 02°00.815′ E	1640	2x!	2.79 ± 0.02 IJKL	2.76–2.80	1.5	1.63–2.68	4	G
9	L	FR: Haute-Garonne, city of Toulouse, near the Canal du Midi	[43°35.000′ N 01°27.000′ E]	150	2x	2.41 ± 0.01 NO	2.40–2.43	1.2	1.65–2.71	4	S
10	H	FR: Savoie Alps Mts, between villages of Valloire and la Rivine	[45°10.000′ N 06°25.600′ E]	1555	2x!	2.81 HIJ	–	–	1.94–2.15	1	G
11	H	FR: Hautes-Alpes Mts, mountain pass Col du Lautaret	45°02.092′ N 06°24.239′ E	2065	2x!	2.88 FGH	–	–	1.79–1.99	1	G
12	U	FR: Hautes-Alpes Mts, town of Briançon	44°55.184′ N 06°37.261′ E	1310	2x!	2.95 ± 0.05 DEF	2.88–2.99	4.0	1.84–2.65	3	G
13	H	FR: Jura Mts, village of Les Fins	47°05.181′ N 06°38.356′ E	910	2x!	2.82 ± 0.01 GHIJ	2.81–2.84	0.8	1.50–2.81	5	G
14	H	FR: Jura Mts, village of Pont-de-Roide	47°22.612′ N 06°46.015′ E	385	2x	2.82 ± 0.01 HIJ	2.80–2.83	0.9	1.46–2.36	3	G
15	L	FR: Vosges Mts, town of Munster	47°54.537′ N 08°09.700′ E	855	2x	2.41 ± 0.02 NO	2.39–2.43	1.6	1.71–2.63	3	S

Table 1 Continued.

Population number	Morphotype*	Locality details†	Geographic coordinates‡	Altitude (m a.s.l.)‡	Ploidy level/DNA ploidy level§	Mean 2C-value ± SD (DNA pg)¶	2C-value range (min–max) (DNA pg)	Intra-populational variation (%)	CV range (%)	No. plants	Standard**
16	L	IT: Sicily, city of Palermo, Monte Cuccio Mt.	38°06.938′ N 13°14.543′ E	610	2x!	2.31 ± 0.01 PQR	2.31–2.32	0.7	1.58–2.50	3	G
17	L	IT: Sicily, national park Madoniae, Piano Zucchi	[37°54.000′ N 13°59.300′ E]	[1100]	2x!	2.29 QR	–	–	1.52–2.35	1	G
18	L	IT: Abruzzi, town of Pescara	42°27.489′ N 14°12.596′ E	5	2x	2.26 R	–	–	1.63–1.93	1	G
19	L	IT: Abruzzi, town of L′Aquila	42°12.103′ N 13°24.256′ E	420	2x	2.30 ± 0.01 QR	2.29–2.32	1.2	1.74–2.58	3	G
20	L	IT: Calabria, village of Frascineto	39°49.899′ N 16°15.305′ E	455	2x	2.31 ± 0.01 PQR	2.30–2.32	0.8	1.51–2.86	3	G
21	L	IT: Campania, city of Neapol, Campi Flegrei railway station	[40°49.173′ N 14°11.361′ E]	[20]	2x	2.35 ± 0.02 OPQ	2.33–2.37	1.5	1.99–2.87	2	S
22	L	IT: Campania, Vesuv Mt.	[40°49.287′ N 14°25.221′ E]	[1100]	2x	2.35 ± 0.01 OPQ	2.34–2.37	1.1	1.61–2.98	4	S
23	U	IT: Calabria, Mula Mt.	[39°45.000′ N 16°01.000′ E]	[1900]	2x	2.46 ± 0.02 N	2.44–2.48	1.8	1.55–2.79	4	S
24	H	IT: Piemonte, village of Breia	45°45.897′ N 08°18.300′ E	800	2x!	3.07 ± 0.02 AB	3.05–3.09	1.4	1.66–2.74	3	G
25	H	IT: Piemonte, village of Gattinara	45°36.520′ N 08°19.434′ E	290	2x	3.06 ± 0.01 AB	3.05–3.07	0.9	1.38–2.52	3	G
26	H	IT: Trento, village of San Giacomo – Brentonico	45°47.963′ N 10°54.831′ E	1160	2x	2.96 ± 0.02 CDEF	2.94–2.98	1.4	1.63–2.71	2	G
27	H	DE: Bavarian Alps Mts, town of Grassach	47°28.217′ N 11°07.114′ E	770	2x!	2.94 EF	–	–	1.64–2.57	1	G
28	H	AT: Tirolian Alps Mts, town of Kitsbühel	47°28.200′ N 12°23.807′ E	930	2x!	2.95 ± 0.01 EF	2.94–2.96	0.6	1.52–2.32	3	G
29	H	AT: Lower Austria, near the village of Annaberg	47°54.984′ N 15°26.399′ E	520	2x!	3.03 ± 0.03 ABCD	3.00–3.07	2.4	1.74–2.81	5	G
30	H	AT: Lower Austria, Schneeberg Mt.	47°44.225′ N 15°44.086′ E	545	2x	3.05 ± 0.01 AB	3.04–3.06	0.7	1.94–2.87	5	G
31	L	AT: Lower Austria, parking place Heiligen Kreuz	46°59.210′ N 16°15.390′ E	760	2x	2.74 ± 0.02 JKLM	2.72–2.76	1.7	1.48–2.83	5	S
32	L	HR: Zadar county, town of Pirovac	43°49.340′ N 15°40.140′ E	25	2x!	2.32 ± 0.01 PQR	2.30–2.33	1.2	1.71–2.30	3	G
33	L	HR: Dubrovacko-Neretvanska county, village of Ploce	43°00.937′ N 17°33.083′ E	15	2x	2.31 QR	–	–	1.81–2.37	1	G

Table 1 Continued.

Population number	Morphotype*	Locality details†	Geographic coordinates‡	Altitude (m a.s.l.)‡	Ploidy level/DNA ploidy level§	Mean 2C-value ± SD (DNA pg)¶	2C-value range (min–max) (DNA pg)	Intra-populational variation (%)	CV range (%)	No. plants	Standard**
34	L	HR: Istarska county, near the village of Lupoglav	45°19.305′ N 14°09.420′ E	385	2x	2.36 ± 0.01 OPQ	2.35–2.37	0.9	1.73–2.47	4	S
35	L	HR: Krapinsko-Zagorska county, village of Krapina	46°09.390′ N 15°52.440′ E	225	2x	2.33 ± 0.02 OPQR	2.30–2.35	2.1	1.66–2.84	4	S
36	H	SK: Malá Fatra Mts, Vrátna dolina valley	[49°12.563′ N 19°02.159′ E]	[700]	2x	3.04 ± 0.01 ABC	3.03–3.05	0.4	1.75–2.79	6	G
37	H	SK: Bukovské vrchy Mts, village of Nová Sedlica	49°03.010′ N 22°30.480′ E	415	2x	3.08 ± 0.01 AB	3.07–3.10	0.9	1.68–2.92	5	G
38	H	SK: Ondavská vrchovina hills, village of Adidovce	49°01.290′ N 22°03.100′ E	220	2x	3.11 ± 0.01 A	3.10–3.11	0.5	1.59–2.73	2	G
					3x	4.72 ± 0.02	4.70–4.74	0.9	1.30–2.34	2	
39	H	SK: Velká Fatra Mts, Krížna Mt.	48°52.350′ N 19°04.260′ E	1520	2x	2.94 ± 0.01 EF	2.93–2.96	0.8	1.62–2.81	3	G
40	H	SK: Západné Beskydy Mts, village of Podbiel	49°17.050′ N 19°32.230′ E	620	2x	3.06 ± 0.01 AB	3.04–3.08	1.0	1.69–2.79	3	G
41	H	SK: Belianske Tatry Mts, Ždiarska dolina valley	49°16.099′ N 20°14.991′ E	890	2x!	3.11 ± 0.00 A	3.10–3.11	0.3	1.78–2.82	3	G
42	H	SK: Nízke Tatry Mts, Jánska dolina valley	49°05.480′ N 19°40.505′ E	770	2x!	3.04 ABC	–	–	1.84–1.98	1	G
43	H	SK: Slovenský raj Mts, Dobšínsky kopec hill	48°50.920′ N 20°22.570′ E	870	2x	3.10 ± 0.02 A	3.08–3.12	1.5	1.66–2.34	2	G
44	U	SK: Malé Karpaty Mts, village of Záhorská Bystrica	48°14.086′ N 17°03.231′ E	220	2x	2.94 ± 0.04 EF	2.90–2.99	3.1	1.56–242	6	G
45	L	SK: Považský Inovec Mts, village of Modrová	48°39.460′ N 17°58.140′ E	525	2x	2.78 ± 0.01 IJKLM	2.77–2.80	1.0	1.42–2.64	3	G
46	L	SK: Štiavnické vrchy Mts, village of Kozelník	48°33.430′ N 19°00.300′ E	275	2x	2.78 ± 0.05 IJKLM	2.72–2.84	4.3	1.75–2.95	3	G
47	L	SK: Popradská kotlina basin, village of Liptovský Trnovec	49°06.926′ N 20°20.932′ E	590	2x!	2.39 ± 0.03 NOP	2.35–2.43	3.5	1.90–2.92	4	S
48	L	SK: Slovenský kras karst, near the Domica cave	48°28.650′ N 20°28.130′ E	345	2x	2.73 ± 0.01 LM	2.71–2.74	1.1	1.48–2.86	5	S
49	L	SK: Šarišská vrchovina Mts, village of Uzlovské Peklany	48°05.650′ N 20°59.180′ E	540	2x	2.70 ± 0.01 M	2.60–2.71	0.8	1.66–2.65	3	S
50	L	RO: Braşov province, village of Fagaraş	45°49.935′ N 25°01.951′ E	415	2x	2.79 IJKL	–	–	1.58–2.62	1	G
51	U	RO: Bihor province, between the town of Oradea and village of Chilaş	47°16.129′ N 22°13.658′ E	180	2x	3.00 ± 0.01 BCDE	2.99–3.01	0.7	2.03–2.72	3	G

Table 1 Continued.

Population number	Morphotype*	Locality details†	Geographic coordinates‡	Altitude (m a.s.l.)‡	Ploidy level/DNA ploidy level§	Mean 2C-value ± SD (DNA pg)¶	2C-value range (min–max) (DNA pg)	Intra-populational variation (%)	CV range (%)	No. plants	Standard**
52	L	RO: Hunedoara province, town of Deva	45°50.012′ N 22°56.341′ E	220	2x!	2.75 ± 0.01 JKLM	2.74–2.76	0.8	1.37–2.72	3	G
53	L	UA: Khmel'nyts'ka oblast', village of Smotrych	48°39.030′ N 26°35.050′ E	180	2x	2.73 ± 0.01 KLM	2.72–2.74	0.7	1.6–2.34	4	S
54	L	UA: Khmel'nyts'ka oblast', village of Chrebtyev	48°37.550′ N 27°14.420′ E	260	2x	2.79 ± 0.02 IJKL	2.77–2.81	1.5	1.89–2.50	3	G

*H, 'Higher altitude' morphotype; L, 'Lower altitude' morphotype; U, uncertain.

†Country codes: AD, Andorra; AT, Austria; DE, Germany; ES, Spain; FR, France; HR, Croatia; IT, Italy; RO, Romania; SK, Slovakia; UA, Ukraine.

‡Coordinates and altitudes taken from maps are given in brackets.

§Populations for which chromosome counts were available (see Slovák *et al.*, 2007) are marked with !

¶Letters indicate group of taxa that are not significantly different at α = 0.05.

**G, *Glycine max* 'Polanka' (2C = 2.5 pg, primary reference standard); S, *Solanum esculentum* 'Stupické polní rané' (2C = 2.05 pg, secondary reference standard).

reference standard. To avoid overlap between sample and standard peaks, a secondary reference standard (*Solanum esculentum* L. 'Stupické polní rané') was employed in some analyses (see Table 1). The genome size of the secondary standard (2C = 2.05 pg) was calibrated against the primary standard, based on 12 replicates performed on different days. Counted diploid plants of *P. hieracioides* (2n = 2x = 10; Slovák *et al.*, 2007) were used as reference material for DNA ploidy inference. To confirm the reliability of the estimated C-values, simultaneous analyses of *P. hieracioides* samples differing by more than 4.5% in genome size were performed. In addition, numerous accessions were subjected to FCM analyses using 4′,6-diamidino-2-phenylindole (DAPI), which binds selectively to adenine and thymine (AT) sites (see Suda *et al.*, 2007a; for protocol details). DAPI is less sensitive to the chromatin state than is PI and shows a high DNA selectivity and a higher increase in quantum efficiency after binding (Shapiro, 2003); it is therefore particularly suitable for the detection of small differences in the amount of nuclear DNA.

Statistical analyses

Genome size data were analysed with the SAS 8.1 statistical package (SAS Institute, 2000) using CORR, GLM and NPAR1WAY procedures. Differences among populations were tested by the general linear model, and Tukey's grouping was applied to compare mean values. The Spearman rank correlation coefficient was used to test whether mean genome sizes were related to the geographic location of populations. Differences in genome size between H and L morphotypes were analysed with the Kruskal–Wallis test. SYN-TAX 2000 module HIERCLUS (Podani, 2001) was used in cluster analyses [squared Euclidean distance being chosen as the dissimilarity coefficient and UPGMA (unweighted pair group method with arithmetic mean) being chosen as the clustering algorithm].

RESULTS

Flow cytometric analyses of genome size resulted in high-resolution histograms with mean CVs of G_1 peaks of *P. hieracioides* samples and internal reference standards of 2.13% (range 1.30–2.98) and 2.33% (range 1.37–3.20), respectively. Between-day variation caused by random instrument drift and/or non-identical sample preparation was very low, and the max./min. values of three repetitions of the same sample did not exceed a two-percent threshold. The data regarding the ploidy levels/DNA ploidy levels and 2C nuclear DNA contents (means and ranges) for 54 *P. hieracioides* populations are shown in Table 1. All but one population (number 38) were uniform in ploidy level and consisted of diploid plants only. A new cytotype (DNA triploid with 2C = 4.72 pg) was revealed in the mixed ploidy population from Slovakia, Ondavská vrchovina.

The mean 2C-values for diploid populations ranged from 2.26 pg (population number 18) to 3.11 pg (nos 38, 41),

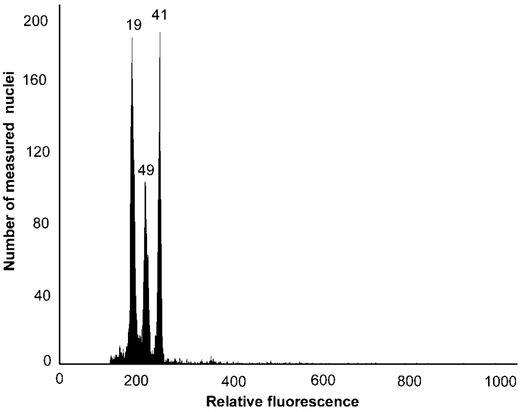

Figure 2 Differences in genome size among *Picris hieracioides* plants originating from three different populations. The nuclei from all individuals were isolated, stained with propidium iodide and analysed simultaneously. The peak ratios were 1 : 1.166 : 1.344. The mean 2C-values estimated for the populations were 2.30 pg (population number 19), 2.70 pg (number 49) and 3.11 pg (number 41). See Table 1 for population details.

giving an overall variation above 37%. This variation was genuine, as it was repeatedly confirmed in simultaneous analyses of two or three individuals with different fluorescence intensities (Fig. 2). In addition, distinct peaks were also always obtained for DAPI-stained samples (results not shown). Intrapopulation variation in genome size was usually low (< 3%), with exceptions being population numbers 12, 44, 46 and 47, in which a between-plant divergence of up to 4.3% was detected.

To obtain insights into the relationships among populations characterized by average genome sizes, a cluster analysis was performed. Populations clustered into two non-overlapping groups (differing by nearly 10% in genome size between their closest members), one group of which was further differentiated at a lower dissimilarity level (Fig. 3). With the exception of population number 23 (from the Mula, Italy), the small-genome group consisted solely of plants that matched well the ‘Lower altitude’ (L) morphotype. The large-genome group consisted only of the ‘Higher altitude’ (H) morphotype, along with three populations of uncertain taxonomic position (nos 12, 44, and 51). Both the L and the H morphotypes were present in the cluster that consisted of populations with an intermediate genome size. Nevertheless, their distribution was non-random, and L plants generally occurred in the lower half of the range. Overall, the genome size (population means) for L and H morphotypes ranged from 2.26 to 2.79 pg (1.23-fold variation), and from 2.73 to 3.11 pg (1.14-fold variation), respectively. The differences between the two groups were highly significant ($P < 0.001$), either at the level of individual plants ($n = 161$), or population means ($n = 50$).

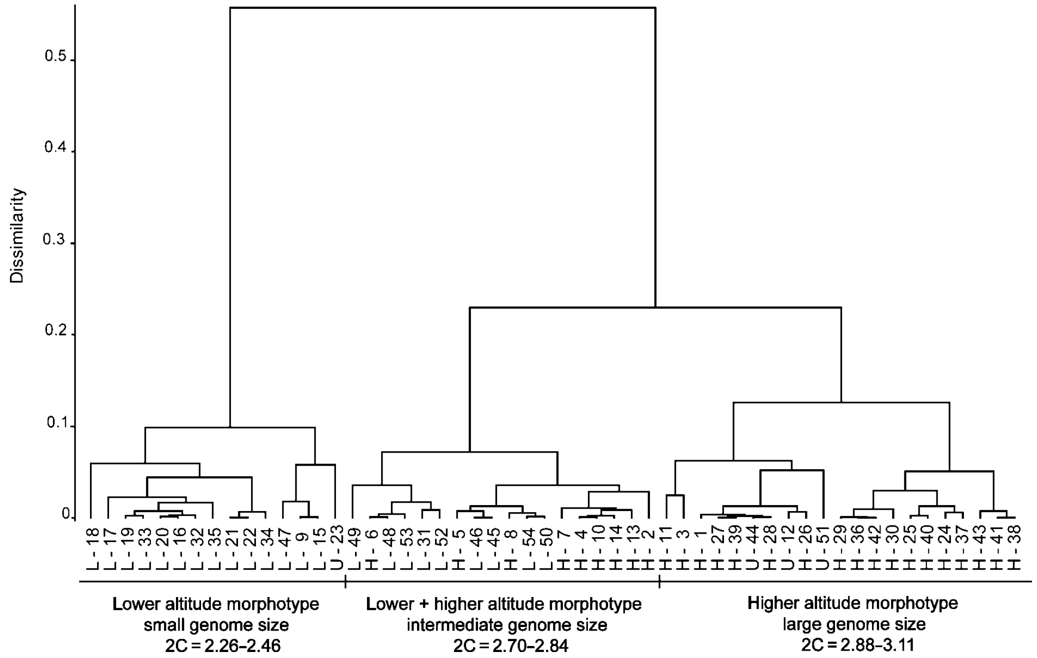

Figure 3 Cluster analysis (UPGMA: unweighted pair group method with arithmetic mean) of genome size data (population means) for 54 diploid populations of *Picris hieracioides*. Plant morphotypes (H, ‘Higher altitude’; L, ‘Lower altitude’; U, uncertain), together with population numbers (see Table 1) are given. Three major clusters are delimited. Triploid plants were omitted from the analysis.

The geographical distribution of plant morphotypes/genome size variants is shown in Fig. 1. Although the general pattern is quite complex, it is clear that, within each morphotype, plants with larger genomes predominate in the north-eastern part of the area studied. The genome size of the H plants increased significantly both from west to east ($r = 0.825$, $P < 0.0001$, $n = 27$) and from south to north ($r = 0.732$, $P < 0.0001$, $n = 27$). The same was true for L morphotypes (longitudinal change of genome size: $r = 0.655$, $P = 0.0007$, $n = 23$; latitudinal change: $r = 0.753$, $P < 0.0001$, $n = 23$).

DISCUSSION

Ploidy-level variation

Apparently, genome duplication has not played a major role in the evolutionary history of the genus *Picris*. The majority of species, including *P. hieracioides*, are known to have only diploid chromosome numbers, based on $x = 5$ (Slovák *et al.*, 2007). The exceptions are *P. hispanica* (Willd.) P. D. Sell, which has 2x, 4x and 6x cytotypes (Humphries *et al.*, 1978; Galland, 1988; Oberprieler & Vogt, 1993), and *P. aculeata* Vahl, which has 2x and 4x cytotypes (Brullo *et al.*, 1977; Guittonneau, 1978). In addition, one tetraploid count was reported for the closely related *P. japonica* Thunb. (Stepanov, 1994); however, this result may require confirmation, as other authors have observed only diploids (see Slovák *et al.*, 2007; for a review). Fluorescence intensities indicating a diploid level were also prevalent in our study (i.e. 177 out of 179 analysed plants). However, one mixed population (number 38) of the H morphotype was found in Slovakia and harboured two individuals differing by about 1.5-fold in genome size. Despite the fact that these FCM results are not accompanied by exact chromosome counts, we are convinced that the plants with distinct genome size (mean 2C-value = 4.72 pg) correspond to triploids. The most plausible mechanism for triploid genesis is a fusion of unreduced and reduced gametes. Our report contains the first evidence for ploidy differentiation in *P. hieracioides*, as well as the first incidence of triploidy in the whole genus. In general, this observation clearly indicates that large population samples are required for representative cytotype screening, and, in this respect, FCM has changed the perception of the magnitude of ploidy variation in wild species (Kron *et al.*, 2007; Suda *et al.*, 2007b).

Intraspecific genome size variation

Although divergences in genome size among different species within a genus are widely acknowledged (see the Plant DNA C-values database; Bennett & Leitch, 2005), our knowledge of variation in the frequency and range of DNA content below the species level is still fragmentary. Early reports of intraspecific variation in genome size in numerous gymnosperms and angiosperms (see Ohri, 1998; for a review) led to the theory of a 'plastic genome', which became popular around the 1990s (e.g. Cremonini *et al.*, 1994). The proponents assumed that certain fluid domains exist in the genome that may undergo rapid, programmed and reversible changes in copy number from one generation to the next, thus substantially altering nuclear DNA content (e.g. in the range of hundreds of mega-base pairs (Mbp) per haploid genome) (note that fluid domains should not be confused with transposable elements, whose role for genome inflation is being increasingly better understood; Bennetzen *et al.*, 2005). Greilhuber and his co-workers steadily criticized this concept, however, and, after a set of meticulous experiments, showed that methodological inaccuracies were largely responsible for the observed variation in genome size (Greilhuber, 1998, 2005). In addition to instrumental and methodological errors, several other sources of artefactual variation have been identified, such as the disturbing effects of secondary metabolites with potential seasonal fluctuations (e.g. Walker *et al.*, 2006), differences in measurements among different laboratories (Doležel *et al.*, 1998), and taxonomic heterogeneity of the material under investigation (Murray, 2005). As a result, genuine intraspecific variation in genome size is considered to be rather rare (but see Schmuths *et al.*, 2004; Obermayer & Greilhuber, 2005; Šmarda & Bureš, 2006; Leong-Škorničková *et al.*, 2007; Suda *et al.*, 2007a).

In our study, about 1.37-fold variation in 2C-values was found among 54 European populations of *P. hieracioides*. The variation in this polymorphic species persisted even after splitting the whole data set into two phenetic groups (Slovák & Marhold, 2007), namely the 'Lower altitude' type (divergence 23.3%) and the 'Higher altitude' type (divergence 13.8%). Although genome size variation in the latter group was more or less continuous, two very different genome types could be distinguished in the former (see Fig. 3). Considering the maximum genome size heterogeneity reported in other species [e.g. 7.4% in *Hieracium piloselliflorum* Nägeli & Peter, Asteraceae (Suda *et al.*, 2007a), *c.* 11% in *Dasypyrum villosum* (L.) P. Candargy, Poaceae (Obermayer & Greilhuber, 2005) and 16.6% in *Festuca pallens* Host, Poaceae (Šmarda & Bureš, 2006)], the variation revealed in *P. hieracioides* is rather large.

We are convinced that the observed genome size divergence in *P. hieracioides* was not caused by methodological shortcomings or differences in the number of somatic chromosomes. First, low CVs (2.13% on average), together with symmetrical G_1 peaks are not compatible with the presence of disturbing secondary metabolites (Loureiro *et al.*, 2006). In addition, repeated measurements of the same sample resulted in highly similar nuclear DNA content values. More importantly, simultaneous measurements of individuals with divergent genome sizes (> 4.5%) always gave histograms with two or more distinct peaks (see Fig. 2), which is regarded as robust proof of genuine genome size variation (Greilhuber *et al.*, 2007). Karyological heterogeneity should also be excluded as a potential source of variation, because chromosome counts (exclusively $2n = 10$) were available for 20 populations and covered virtually the whole range of fluorescence intensities (Table 1; Slovák *et al.*, 2007).

Perhaps the most precarious issue concerns the taxonomic identity of the plant material that was investigated in this study. Our intention was to obtain groups that were as homogenous as possible, based on the results of our previous study (Slovák & Marhold, 2007) and on further unpublished morphometric analyses (M. Slovák *et al.*, in preparation). However, it should be noted that, despite the fact that phenotypic discontinuities are still, for practical reasons, the most common markers used for taxonomic decision-making, they may not accurately reflect the evolutionary history of a particular taxon/lineage. This may also be true for *P. hieracioides*. We hypothesize that the complex evolutionary history of this polymorphic taxon may be responsible for the observed heterogeneity in genome size. Molecular data are required to elucidate exact evolutionary relationships; however, one scenario may involve the separate evolution of H morphotypes in major European mountain ranges, possibly including differentiation in genome size by means of processes such as the activity of retrotransposons. Vegetation changes triggered by Quaternary climate oscillations (Frenzel *et al.*, 1992; Lang, 1994) brought different H populations and/or H and L morphotypes into contact (the latter having evolved independently in lowlands). Subsequent hybridization among different lineages of this strictly allogamous species, coupled with further shifts in geographical distribution (e.g. range expansion after glaciation), gave rise to phenotypic complexities and blurred the pattern of genome size. Long-distance dispersal (Nathan *et al.*, 2002), both in the past and as a consequence of anthropogenic activities over the last few centuries, may have complicated the overall picture even more.

Geographic and life-history correlates of genome size

Despite presenting a complex geographic pattern, the distribution of particular genome size variants of *P. hieracioides* is far from random (Fig. 1). In general, within each morphotype, plants with lower DNA amounts are confined to the south-western part of the area studied, whereas their larger-genome counterparts predominate in the north-east. More precisely, all but one small-genome (2C = 2.26–2.41 pg) population of the L morphotype (i.e. the most distinct genome size variant) occur in Mediterranean countries, namely France, Italy and Croatia. The only exception applies to population number 47 from Slovakia, which probably represents a casual introduction from southern Europe. L morphotypes possessing larger genome sizes (2C = 2.70–2.79 pg; intermediate group in the cluster analysis) are from Austria, Slovakia, Ukraine and Romania. The same trend, although more shallow, is mirrored by the H morphotype. Although populations with a large nuclear DNA content (2C = 2.88–3.11 pg) are distributed throughout the area (i.e. from the Sierra Nevada in Spain, population number 1, to the Bukovské vrchy in Slovakia, number 37), those with a lower genome size (2C = 2.73–2.84 pg; intermediate group in the cluster analysis) were recorded only in south-west Europe (the Pyrenees, Jura and the Savoie Alps).

It would be tempting to attribute the observed variation to the different ecological conditions that predominate in particular areas (i.e. the south–north gradient and/or in oceanic vs. continental climate). Several studies have found associations between genome size and environmental parameters, including temperature and precipitation (summarized by Knight *et al.*, 2005). Although we do not reject the theory that changes in genome size in *P. hieracioides* may have been, at least partly, driven by the multifaceted effect of the environment, we are more inclined to the opinion that the taxon encompasses several independent lineages with unique evolutionary histories that evolved under different conditions. In fact, noteworthy relationships can be observed when the distribution of genome size variants is superimposed onto a map of palaeovegetation cover during the Last Glacial Maximum (about 20,000 years ago). Although the small-genome variants of the L morphotype occur in regions that were formerly covered with typical dry steppes, their counterparts with a larger genome size grow mainly in areas where tundra-steppes or transient communities were mapped in the Last Glacial Maximum (Frenzel *et al.*, 1992; Lang, 1994). An identical ecological pattern of genome size variants according to palaeovegetation types was found in *Festuca pallens* (Šmarda & Bureš, 2006). Despite a clear divergence in the amount of nuclear DNA between southern and Central European L morphotypes, the amount of phenotypic differentiation is negligible, if indeed any exists at all, between these morphotypes (M. Slovák *et al.*, in preparation). Whether these genome size variants should be formally recognized, and whether existing intraspecific names can be used for this purpose [e.g. subsp. *spinulosa* (Bertol.) Arcang. for southern populations], remains to be determined. The search for historical interpretations of genome size heterogeneity in H morphotypes is more challenging because there is more continuous variation in this taxon. Plants with smaller genomes appear to be confined to the Pyrenees and the western Alps, whereas those with slightly larger amounts of nuclear DNA are distributed throughout the area of investigation. This pattern may be influenced by the level of maintenance vs. disruption of gene exchange between different mountain regions during the Quaternary period (see Kropf *et al.*, 2006).

Variation in nuclear DNA content in *P. hieracioides* (in particular, the significant differences between the H and L morphotypes) provides us with the opportunity to search for relationships between genome size and life-history traits. Whereas L morphotypes are mostly annual or biennial, H plants have a longer life span, which may be best described as short-lived perennial. In addition, the former morphotype generally prefers various man-made habitats, and in some localities it shows a tendency towards invasive behaviour (M. Slovák, personal observations). All of these observations are in agreement with previous studies that documented a correlation between small genome size and a short developmental lifestyle (see Leitch & Bennett, 2007; for a review) and 'weediness' (Bennett *et al.*, 1998).

In summary, our targetted survey of genome size variation in the polymorphic species *P. hieracioides* revealed a complex pattern of variation, which, however, showed certain associations with phenotypic differentiation and geographic distribution. Genome size data provide some support for the two main morphotypes that were previously recognized (i.e. the 'Lower altitude' and the 'Higher altitude' types, which possess significantly different amounts of nuclear DNA). However, interpretation of the within-group variation is still a challenge (e.g. the two non-overlapping genome size variants in L vs. the rather continuous pattern of variation in H morphotypes). It is possible that several different evolutionary lineages may be hidden within each morphological group. Therefore, a multi-method approach, with an emphasis on molecular analyses, will be necessary in order to gain deeper insights into the evolutionary history of *P. hieracioides* and to elucidate its taxonomy. In addition, the causes and dynamics of genome size variation deserve further targetted study using a set of cytogenetic tools (e.g. analysis of heterochromatin blocks; Laurie & Bennett, 1985).

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ORIGINAL ARTICLE

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

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Abstract The only Balkan endemic of the genus *Picris*, *Picris hispidissima*, was studied in detail using morphological and karyological methods. The species was shown to be morphologically distinct from the closest taxon, *Picris hieracioides*, by the pectinate–ciliate indumentum of involucral bracts, dilatation of the peduncle, length of the outer and inner bracts, and indumentum colour. No morphological variation that would require taxonomic classification was found within this species. Despite the diploid chromosome number ($2n = 2x = 10$) being confirmed for *P. hispidissima*, variation of up to 9.5% in genome size was found. The likely explanation for this variation is hybridization and introgression with closely related *P. hieracioides*. The most convincing evidence for this hypothesis is the detection of plants with two clearly different DNA contents arising from a single capitulum found in the location where *P. hispidissima* and *P. hieracioides* co-occur.

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Introduction

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

Our study focuses on the western Balkan endemic species *Picris hispidissima* (Bartl.) W. D. J. Koch, which is among the less known species of the genus and is morphologically similar to the widespread and polymorphic species *Picris hieracioides* L. In fact, the entire Mediterranean represents one of the main centres of diversity of *Picris*, and is considered to be a plausible phylogenetic centre (Lack 1974, 1979; Holzapfel and Lack 1993). The genus comprises about 50 species (Chaudhary 2000), of which approximately 24 occur in the Mediterranean (Greuter 2005–2007). Four species, namely *P. hieracioides*, *P. rhagadioloides* (L.) Desf., *P. pauciflora* Willd. and *P. hispidissima* occur on the Balkan Peninsula [members of the closely related genus *Helminthoteca* Vaill. ex Juss. (Samuel et al. 2006) that were previously included in the genus *Picris* are not taken into account here], but only the last of these is endemic (Greuter 2005–2007).

Picris hispidissima inhabits xerothermous calcareous coastal areas along the seaside of Slovenia, Croatia, Bosnia and Herzegovina, and Montenegro; in the south it reaches Albania (Hayek 1931; Sell 1976; Nikolić 1997; Martinčič et al. 1999). Unlike *P. hieracioides*, which is annual to short-lived perennial, *P. hispidissima* is either biennial or monocarpic taxon (Lack 1974; M. Slovák, personal observation). Interestingly, contrary to most *Picris* species, which usually inhabit open grasslands, steppes and ruderal areas, *P. hispidissima* prefers rock crevices on calcareous cliffs and gorges.

In the 1930s, Degen (1938) studied intraspecific variation in *P. hispidissima* and recognized three intraspecific taxa at the level of variety: *P. hispidissima* var. *typica*, *P. hispidissima* var. *reichardioides*, and *P. hispidissima* var. *velebitica*. According to Degen, they differ in the density and quality of indument, the size of the floral parts and the size of the achenes. Lack (1974) in his monograph of the genus *Picris* in the eastern Mediterranean did not comment on Degen's taxonomic concept and regarded *P. hispidissima* as a taxon without any conspicuous intraspecific variation. No data on chromosome number, ploidy level or nuclear DNA content are currently available for *P. hispidissima*. The same holds true for other biological characteristics (mating systems, longevity, etc.).

Morphologically, *Picris hispidissima* is closely related to the widespread and highly variable *P. hieracioides*. Both species co-occur in the western part of the Balkan Peninsula. Conspicuous morphological variation in *P. hieracioides* throughout its range led to several contradicting concepts of its classification (Sell 1975, 1976; Pignatti 1982; Dostál and Červenka 1992; Bolòs and Vigo 1995; Haeupler and Muer 2000; Aeschimann et al. 2004; Rothmaler 2005; Greuter 2005–2007). Currently, two morphological types are recognized in the western Carpathians and adjacent Pannonia: the "lower altitude type" and "higher altitude type" (Slovák and Marhold 2007). They differ, at least partially, with respect to their ecological requirements and life forms: the "lower altitude type" is mostly annual to biennial, occupying dry, sunny, often man-made habitats at lower altitudes, whereas the "higher altitude type" is mostly short-lived perennial and occurs in mesic, natural or semi-natural habitats at higher altitudes (typically in tall herb meadows). This concept seems to be applicable to a much wider area (Slovák et al. in preparation). Strict self-incompatibility and a diploid ploidy level of $2n = 2x = 10$ were confirmed for *P. hieracioides* (Slovák et al. 2007) with the exceptional occurrence of triploid plants (Slovák et al. 2009). Nuclear DNA content varies considerably within European populations of *P. hieracioides* (Slovák et al. 2009): $2C$ values for individuals range 1.37-fold from 2.26 to 3.11 pg. This variation persists even after splitting the dataset into the "lower altitude" and "higher altitude" types that possess significantly different nuclear DNA contents. Generally, the genome sizes of both morphotypes increase significantly from southwest to northeast (Slovák et al. 2009).

As mentioned above, besides *P. hieracioides* and *P. hispidissima*, two other southern European *Picris* species, namely *P. pauciflora* and *P. rhagadioloides*, also occur in the Balkan Peninsula (Lack 1974; Sell 1976). Both are clearly distinguishable from *P. hieracioides* and *P. hispidissima* by their life forms, being strict annuals. Furthermore, *P. rhagadioloides* is the sole *Picris* species in the Balkans that possess 2-, 3-, and 4-furcate and anchor-shaped hairs. A lower number of conspicuously smaller capitula, thickened peduncles after anthesis and short-beaked curved achenes are the unique determining characteristics of *P. pauciflora* (Lack 1974; Sell 1976).

According to Lack (1974) and Sell (1976), *Picris hispidissima* differs morphologically from *P. hieracioides* by the presence of laciniate (pinnatisect) leaves, dilated and slightly thickened peduncles below capitulum, pectinate–ciliate margins on the outer involucral bracts and larger capitulum. However, preliminary investigation of herbarium material revealed that specimens of these taxa were sometimes misidentified, most probably due to the low reliability of some diagnostic characters. A mixed population of both species, together with morphologically intermediate individuals, was found in Croatia. However, the origin of atypical individuals is unclear and remains to be resolved. Despite the sympatric occurrence and presumed close relationship between both taxa, no hybrids have been reported in the literature until now (e.g., Hayek 1931).

This study aimed to gather data concerning karyological and phenotypic variation in *P. hispidissima* and to use these data for taxonomic classification. The key questions are as follows:

(1) What is the extent of variation in morphology, ploidy level, chromosome number and nuclear DNA content in *P. hispidissima* over the main part of its distribution area?
(2) What are the species-specific morphological characters separating *P. hispidissima* and *P. hieracioides*?
(3) What it the most plausible source of variation within the mixed population of *P. hispidissima* and *P. hieracioides*?

Materials and methods

Material

To evaluate morphological variation in *P. hispidissima*, 211 individuals (12 population samples; see Table 1; Fig. 1) from Croatia and Montenegro were collected. These included population samples from the localities (or wider areas) from where *P. hispidissima* and all three of Degen's varieties (Degen 1938; see "Introduction") were described. To identify species-specific morphological characters for *P. hispidissima* and *P. hieracioides*, representative population samples (22 population samples, 421 individuals in total) of *P. hieracioides* from central and southwestern Europe were included in the morphometric analyses (Table 1). In addition, an intermingled population of both species from the village of Zaton Doli in Croatia (ZAD, 29 individuals) was analysed.

Material was collected at localities from where *P. hispidissima* and one of the Degen's varieties were described [KRK—referred to in the description of *P. hispidissima* (Bartling and Wendland 1825) as "in insulae Vegliae (Krk) litore depresso arenoso prope oppidum Castel Muschio (Omišalj)"; PAK—referred to in the description of *P. hispidissima* var. *velebitica* (Degen 1938) as "in Felsspalten tiefer. Schluchten, 150–700 m, S. Velika Paklenica", for details of localities see Table 1]. No material corresponding to *P. hispidissima* was found at the localities of *P. hispidissima* var. *reichardioides* (Degen 1938) mentioned in the manuscript as "Zwischen Senj und Sveti Juraj, Vranjak, … im Liburnischen Karst zwischen Bunica, Sv. Ilja und Čupina". Nevertheless, our localities BAK and KAR are located close to the first two of these localities.

To detect ploidy level and chromosome number in *P. hispidissima*, 13 individuals from four populations (Table 2) were analysed. In addition, 28 individuals originating from nine populations (Table 2) were screened for DNA ploidy level (Suda et al. 2006) and absolute DNA content. Voucher specimens of all analysed individuals are deposited in the herbarium of the Institute of Botany, Slovak Academy of Sciences, Bratislava (SAV).

Morphometric analyses

Twenty-four morphological characters were measured or scored for morphological evaluation (see Table 3). These included (1) characters referred to in guides and identification keys as important for distinguishing *P. hispidissima* and *P. hieracioides*, (2) characters recognized by Degen (1938), and (3) those that appeared to be useful during the evaluation of the material during our study. All morphological characters were measured on dried specimens collected in field with the exception of floral characters (ligules and involucral bracts). In the field, these floral parts were attached by adhesive tape to a paper sheet, dried, scanned on a Microtek 9800XL scanner, and subsequently measured using the program Carnoy (Schols et al. 2002). *P. hispidissima* leaf shape (deeply toothed to laciniate) has in the past been treated as an important differential character for this species (Lack 1974; Sell 1976). However, on the base of our preliminary observations and analyses of herbarium specimens and collected plant material, leaf shape shows considerable variation in *P. hispidissima*, ranging from almost entire to deeply laciniate leaves (Fig. 2). In addition, there is a conspicuous overlap in leaf shape between *P. hispidissima* and *P. hieracioides*. Therefore, this character was omitted from the present study. Similarly, we did not study variation in achenes because our previous investigations showed no differences in this character between morphotypes of *P. hieracioides* (see Slovák and Marhold 2007 for values from *P. hieracioides*).

Several data matrices were used for morphometric analyses (some characters were excluded from certain matrices due to a lack of variation; for details see Table 3).

Matrix A comprised population samples tentatively identified as both *P. hispidissima* and *P. hieracioides* characterized by mean values of characters; the putatively mixed population, ZAD, was excluded (24 population samples × 21 characters). *Matrix B* included all individuals tentatively identified as *P. hispidissima* and *P. hieracioides*, including population ZAD (661 individuals × 21 characters). All individuals tentatively identified as *P. hispidissima* and *P. hieracioides* with the exception of population ZAD (632 individuals × 21 characters) comprised *Matrix C*. The last data set, *Matrix D*, was composed of individual plants from all populations of *P. hispidissima* (211 individuals × 19 characters). The composition of matrix D resulted from the previous steps of the analyses, where the differences between *P. hispidissima* and *P. hieracioides* were established.

The following analyses were performed to reveal patterns of morphological variation within *P. hispidissima* and differences between this taxon and *P. hieracioides*. First, Spearman correlation coefficients (Legendre and Legendre 1998) were computed from the matrix of all plants studied to eliminate pairs of highly correlated characters from further analyses. Spearman correlation coefficients were used because the distribution of characters mostly deviated from normality. As a second step, principal component analyses (PCA) based on the correlation matrices between morphological characters were used (Krzanowski 1990). This method enables the reduction of the original character space into three coordinates representing the major part of variation included in the original data set, which can be visually controlled. PCA was applied to matrix A and subsequently to matrix B.

Table 1 List of localities of *Picris hispidissima* and *P. hieracioides* analysed in this study

ABB	Co.	Locality	Latitude, longitude	Altitude (m a.s.l.)	N/M	N/GS
Picris hispidissima						
PLO	HR	Istarska county, village of Plomin	45°07.388′N 14°12.145′E	225	19	2
BAC	HR	Primorsko-Goranska county, village of Bakarac	45°16.828′N 14°34.341′E	12	15	–
BAS	HR	Island Krk, village of Baška near the Jurani Dvor settlement	45°01.682′N 14°40.448′E	280	15	5
KRK	HR	Island Krk, near the village of Omišalj	45°14.355′N 14°33.562′E	53	19	5
PAK	HR	Lika and Senj county, Velika Paklenica valley	[44°17.000′N 15°27.000′E]	[30]	21	3
KAR	HR	Lika and Senj county, village of Karlobag	44°31.722′N 15°7.529′E	695	21	2
GRA	HR	Karlovac county, village of Gračac	44°15.868′N 16°0.529′E	756	22	–
ZAD	HR	Dubrovacko-Neretvanska county, near the village of Zaton-Doli	42°48.989′N 17°6.683′E	124	29	4
DUB	HR	Dubrovacko-Neretvanska county, town of Dubrovnik	42°38.559′N 18°7.296′E	145	19	3
STS	ME	Budva county, village of Sveti Stefan	42°18.601′N 18°3.621′E	757	19	–
CET	ME	Cetinje county, village of Cetinje	42°22.961′N 18°6.870′E	641	4	–
KLO	ME	Podgorica county, village of Klopot	42°24.142′N 19°3.526′E	682	20	1
BUK	ME	Bar county, village of Bukovik	42°13.260′N 18°7.973′E	614	16	3
Picris hieracioides						
CAP	ES	Sierra Nevada Mts., village of Capileira	36°57.703′N 03°1.457′W	1,606	20	4
VIE	ES	Pyrenees Mts., town of Vielha	42°41.190′N 00°47.241′W	1,230	20	4
MON	ES	Montseny Mts., between villages of Montseny and el Brull	41°46.702′N 02°23.780′E	740	–	5
BAL	ES	Pyrenees Mts., village of Baños di Panticosa	42°45.029′N 00°14.557′W	1,576	–	5
ESP	ES	Pyrenees Mts., village of Espot	42°33.547′N 01°05.416′E	1,576	–	5
SOL	AN	Pyrenees Mts., the town of Soldeu	42°34.170′N 01°40.691′E	1,900	20	5
LLV	FR	Pyrenees Mts., village of Llívia	42°26.633′N 01°56.531′E	1,170	20	4
EST	FR	Pyrenees Mts., village of Estavar	42°29.915′N 02°00.815′E	1,640	–	4
TUL	FR	Haute-Garonne, city of Toulouse, near the Canal du Midi	[43°35.000′N 01°27.000′E]	150	–	4
VAL	FR	Savoie Alps Mts., between villages of Valloire and la Rivine	45°10.000′N 06°25.600′E	1,556	–	1
CDL	FR	Hautes-Alpes Mts., mountain pass Col du Lautaret	45°02.092′N 06°24.239′E	2,067	22	1
BRI	FR	Hautes-Alpes Mts., town of Briançon	44°55.184′N 06°37.261′E	1,308	–	3
FIN	FR	Doubs, Jura Mts., village of Les Fins	47°05.181′N 06°38.356′E	911	16	5
BDA	FR	Doubs, Jura Mts., village of Pont-de-Roide	47°22.612′N 06°46.015′E	384	–	3
MUN	FR	Alsace, Vosges Mts., town of Munster	47°54.537′N 08°09.700′E	853	–	3
SAMT	IT	Sicily, city of Palermo, Monte Cuccio	38°06.938′N 13°14.543′E	611	20	3
PIZ	IT	Sicily, national park Madoniae, Piano Zucchi	[37°54.000′N 13°59.300′E]	[1,100]	–	1
VSR	IT	Abruzzi, Majella Mt., Valle di Selva Romana	42°07.632′N 14°07.381′W	1,575	29	–
PES	IT	Abruzzi, town of Pescara	42°27.489′N 14°12.596′E	6	–	1
AQU	IT	Abruzzi, town of L'Aquila	42°12.103′N 13°24.256′E	421	20	3
FRA	IT	Calabria, village of Frascineto	39°49.899′N 16°5.305′E	453	15	3
NAP	IT	Campania, city of Neapol	[40°49.173′N 14°11.361′E]	[20]	–	2
VES	IT	Campania, Mt. Vesuv	[40°49.287′N 14°25.221′E]	[1,098]	–	4
MUL	IT	Calabria, Mt. Mula	[39°45.000′N 16°01.000′E]	[1,900]	–	4
BRE	IT	Piemonte, Verceli, village of Breia	45°45.897′N 08°18.300′E	799	–	3
GAT	IT	Piemonte, Verceli, village of Gatinara	45°36.520′N 08°19.434′E	289	–	3
SGD	IT	Trento, Veneto, village of San Giacomo - Brentonico	45°47.963′N 10°54.831′E	1,162	–	2
GRA	DE	Bavaria, Bavarian Alps Mts., town of Grassach	47°28.217′N 11°07.114′E	768	16	1
KIT	AT	Tirolia, Tirolian Alps Mts., town of Kitzbühel	47°28.200′N 12°23.807′E	930	20	3
ANN	AT	Lower Austria, village of Annaberg	47°54.984′N 15°26.399′E	519	–	5
SCH	AT	Lower Austria, Mt. Schneeberg	47°44.225′N 15°44.086′E	543	20	5
HAK	AT	Lower Austria, parking place Heiligen Kreuz	46°59.210′N 16°15.390′E	761	–	5

Table 1 continued

ABB	Co.	Locality	Latitude, longitude	Altitude (m a.s.l.)	N/M	N/GS
PIR	HR	Zadar county, town of Pirovac	43°49.340′N 15°40.140′E	24	20	3
PLM	HR	Dubrovacko-Neretvanska county, village of Ploce	43°00.937′N 17°33083′E	17	15	1
LEP	HR	Istarska county, near the village of Lupoglav	45°19.305′N 14°09.42′E	386	14	4
KRA	HR	Krapinsko-Zagorska county, village of Krapina	46°09.390′N 15°52.440′E	224	–	4
VRD	SK	Malá Fatra Mts., Vrátna dolina valley	[49°12.563′N 19°02.159′E]	[700]	–	6
NOS	SK	Bukovské vrchy Mts., village of Nová Sedlica	49°03.010′N 22°30.480′E	413	–	5
ADI	SK	Ondavská Vrchovina hills, village of Adidovce	49°01.290′N 22°03.100′E	219	–	2
KRI	SK	Veľká Fatra Mts., Mt. Krížna	48°52.350′N 19°04.260′E	1,522	–	3
POD	SK	Západné Beskydy Mts., village of Podbiel	49°17.050′N 19°32.230′E	621	–	2
ZDI	SK	Belianske Tatry Mts., Ždiarska dolina valley	49°16.099′N 20°14.991′E	890	–	3
JAN	SK	Nízke Tatry Mts., Jánska dolina valley	49°05.480′N 19°40.505′E	771	–	1
DOB	SK	Slovenský raj Mts., Dobšínsky kopec hill	48°50.920′N 20°22.570′E	870	–	2
CEK	SK	Pieniny Mts., village of Červený Kláštor	49°23.341′N 20°25.325′E	468	20	–
ZAH	SK	Malé Karpaty Mts., village of Záhorská Bystrica	48°14.086′N 17°03.231′E	220	–	6
MOD	SK	Považský Inovec Mts., village of Modrová	48°39.460′N 17°58.140′E	525	–	3
KOZ	SK	Štiavnické vrchy Mts., village of Kozelník	48°33.430′N 19°00.300′E	273	–	3
LPT	SK	Popradská kotlina basin, village of Liptovský Trnovec	49°06.926′N 20°20.932′E	588	–	4
DOM	SK	Slovenský kras karst, near the Domica cave	48°28.650′N 20°28.130′E	345	–	5
UZP	SK	Šarišská vrchovina Mts., village of Uzlovské Pekľany	48°05.650′N 20°59.180′E	538	–	4
SOR	SK	Slovenský kras karst, on the top of Soroška pass	48°37.053′N 20°37.805′E	544	20	
FAG	RO	Braşov province, village of Fagaraş	45°49.935′N 25°1.951′E	415	–	1
ORA	RO	Bihor province, between the town of Oradea and village of Chilaş	47°16.129′N 22°13.658′E	180	–	3
DEV	RO	Hunedoara province, town of Deva	45°50.012′N 22°56.341′E	220	–	3
TIM	RO	Braşov province, city of Braşov	45°39.516′N 25°34.636′E	600	19	–
SMO	UA	Khmel'nyts'ka oblast', village of Smotrych	48°39.030′N 26°5.05′E	180	–	4
CHR	UA	Khmel'nyts'ka oblast', village of Chrebtyev	48°37.550′N 27°4.420′E	260	–	3

Localities are arranged from west to east. GS values of *P. hieracioides* are taken from a previous study (Slovák et al. 2009). Coordinates and altitudes in square brackets were estimated from maps

ABB, Abbreviations of population samples under which samples are collected, cultivated and specimens stored; Co., country of an origin of population sample; AN, Andorra; AT, Austria; HR, Croatia; DE, Germany; FR, France; IT, Italy; ME, Montenegro; RO, Romania; SK, Slovakia; ES, Spain; UA, Ukraine; N/M, number of plants per population analysed by multivariate morphometrics; N/GS, number of plants (per population) in which genome size (GS) was estimated

The third step involved canonical discriminant analysis (CDA) and *k* nearest neighbour non-parametric classificatory discriminant analyses, performed using matrix C; the definition of the groups, namely *P. hispidissima* and *P. hieracioides*, was based on the results of the second step of this analysis. CDA based on individual plants was applied to find the most important characters for identification of these two taxa, allowing for the best classification of individuals into the predicted groups (Klecka 1980; Krzanowski 1990). Then, matrix D was subjected to PCA with the aim of determining whether there is any morphological variation within *P. hispidissima*, especially with respect to the varieties described by Degen, but also with respect to the potential geographical differentiation between populations from Croatia and Montenegro, which seemed to be slightly different in the field. As a last step, basic statistical parameters (mean values, standard deviations, medians, 5 and 95% percentiles) were calculated for the recognized taxonomic units. Calculations of multivariate analyses were run using SAS 9.1.3 software (SAS Institute 2007).

Karyological analyses

Chromosome number was determined from mitotic metaphases in root tips of germinated seeds obtained from natural populations (for details see Slovák et al. 2007). The permanent slides are deposited at the Institute of Botany of the Slovak Academy of Sciences in Bratislava. To assess genome size and DNA ploidy level of analysed taxa, flow cytometry was used. Absolute DNA content was estimated

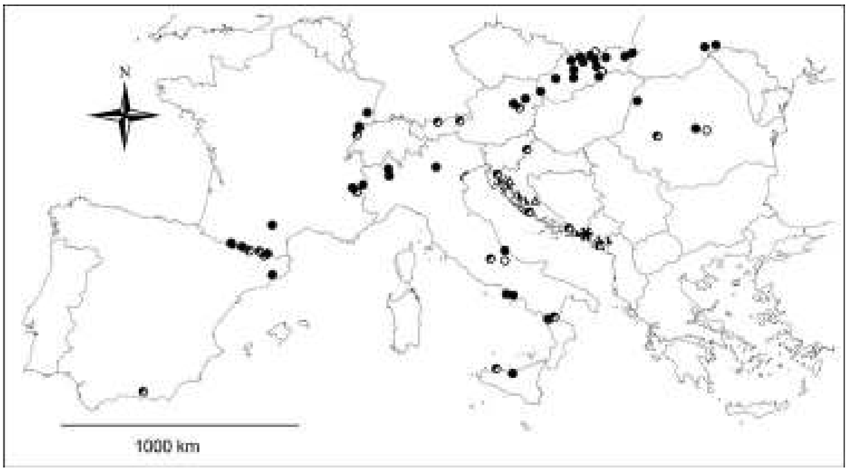


Fig. 1 Distribution map of populations of *P. hieracioides* and *P. hispidissima* analysed in this study. *Circles* populations of *P. hieracioides*, *full circles* populations used for genome size analyses, *open circles* populations used for morphometric analyses, *semi-full circles* populations used for both types of analyses. *Triangles* populations of *P. hispidissima*, *semi-full triangles* populations used for both types of analyses, *open triangles* populations used for morphometric analyses. *Asterisk* mixed population ZAD

Table 2 List of absolute DNA content (propidium iodide) analysed populations of *Picris hispidissima*

Locality code	PL/DPL*		Max. BPV (%)	Range GS (pg)	Max. IPV (%)	CvSa min./max.	CvSt min./max.
	($2n = 10$)	$2C$ (pg) ± SD					
PLO	3/2*	3.03 ± 0.023	2.4	3.03–3.04	0.3	1.74–2.43	1.04–2.78
BAS	0/5*	2.96 ± 0.068	–	2.90–3.06	5.5	2.19–2.98	2.39–2.93
KRK	0/5*	3.12 ± 0.060	5.4	3.03–3.20	5.6	1.48–2.56	1.71–2.90
PAK	2/3*	3.08 ± 0.023	4.1	3.07–3.10	0.1	1.53–2.48	1.08–2.79
KAR	3/2*	3.07 ± 0.017	3.7	3.05–3.08	0.1	1.79–2.80	1.79–2.74
ZAD	0/4*	3.19, 3.21, 2.31, 2.80**	8.1	2.30–3.22	***	1.87–2.80	2.05–2.93
DUB	2/3*	3.24 ± 0.020	9.5	3.22–3.25	0.9	1.88–2.47	1.96–2.79
KLO	0/1*	3.12	5.4	–	–	1.73–2.19	2.07–2.55
SVS	3/0*	–	–	–	–	–	–
BUK	0/3*	3.14 ± 0.026	6.1	3.12–3.17	1.6	1.69–2.45	1.74–2.92

First column: locality code; second column: number of analysed individuals; PL, ploidy level explored by chromosome counting; DPL*, ploidy level estimated by flow cytometry; third column: $2C$ ± SD (pg), $2C$ nuclear DNA content in picograms (mean value ± standard deviation; **, individual measurements are presented for the mixed population ZAD); fourth column: Max. BPV, maximum between population variability, all population means are compared to population with lowest GS, BAS fifth column: Range GS, range of minimum and maximum $2C$ nuclear DNA content within analysed populations; sixth column: Max. IPV, intrapopulation variability (*** not counted for mixed population ZAD); seventh column: CvSa min./max., minimum and maximum coefficient of variation of samples; eighth column: CvSt min./max., minimum and maximum coefficient of variation of internal standard (*Glycine max* cv. 'Polanka', $2C = 2.50$ pg; Doležel et al. 1992)

by a Partec CyFlow flow cytometer (Partec GmbH, Münster, Germany) with a green solid state laser (Cobolt Samba 532 nm, 100 mW, Cobolt, Stockholm, Sweden) as the excitation source. Symmetric peaks were analysed using Partec FlowMax Operating and Analysing Software (version 2.4d; Partec GmbH, Münster, Germany). *Glycine max* cv. 'Polanka' ($2C = 2.50$ pg; Doležel et al. 1992) was used as an internal standard due to its possessing a genome size close to that of analysed plants to minimize potential instrument non-linearity. Young fresh leaf tissue was extracted from the cultivated plants for sample preparation. A standard two-step procedure (Suda et al. 2003) without centrifugation was used for sample preparation. Fresh leaves of both samples and the standard were chopped using a sharp razor blade in 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20; Otto 1990), then filtered through a 42-μm nylon mesh and incubated at room temperature for 20 min. One ml of staining solution [Otto II buffer (0.4 M $Na_2HPO_4{\cdot}12H_2O$), 50 μl/ml propidium iodide, 50 μl/ml RNase IIA, and 2 μl/ml 2-mercaptoethanol] was added, the mixture was shaken gently, and after 10 min the mixture was analysed. For each population, one to five individuals were analysed. To confirm the precision of measured DNA content, three independent measurements on different days were performed for each individual. For each measurement, the coefficient of variation (CV) for both an internal standard and the sample was calculated. In addition, genome size data for *P. hieracioides*

Table 3 List of 24 morphological characters measured or scored in morphometric analyses for each herbarium specimen

Morphological character	1	2
1. NBr: number of stem branches	+	+
2. NL: number of stem leaves	+	+
3. LP: length of the longest peduncle (cm)	+	−
4. DP: presence or absence of dilatation of peduncles below capitulum (0, absent; 1, present)	+	+
5. NBP: maximum number of bracts per peduncle	+	+
6. DHP: distribution of hairs on peduncle (1, no hairs; 2, sparse hairs; 3, dense hairs)	−	+
7. DHI: distribution of hairs on involucre (1, no hairs; 2, sparse hairs; 3, dense hairs)	+	+
8. PCM: presence or absence of pectinate-ciliate margins of outer involucral bracts (0, absent; 1, present)	+	+
9. DH: dark^{c} hairs on involucre and peduncle (0, absent; 1, present)	−	+
10. PH: pale^{d} hairs on involucre and peduncle (0, absent; 1, present)	−	+
11. IH: colour-intermediate^{e} hairs on involucre and peduncle (0, absent; 1, present)	−	+
12. DC: distribution of capitula on stem branches (0, along the whole stem branch; 1, only in upper 1/2 to 1/3 of stem branch)	+	+
13. NoB: average number of outer involucral bracts^{b}	+	−
14. NiB: average number of inner involucral bracts^{b}	+	−
15. LoB: length of outer involucral bracts^{a} (mm)	+	+
16. WoB: width of outer involucral bracts^{a} (mm)	+	+
17. LiB: length of inner involucral bracts^{a} (mm)	+	+
18. WiB: width of inner involucral bracts^{a} (mm)	+	+
19. LL: length of ligules^{a} (mm)	+	+
20. LCT: length of corolla tubes^{1} (mm)	+	+
21. WL: width of ligules^{1} (mm)	+	+
22. LTL: length of the longest teeth on ligule^{1} (mm)	−	+
23. RSL: red longitudinal strip on outer ligules (0, absent; 1, present)	+	+
24. BML: brown marks on upper part of ligules (0, absent; 1, present)	+	+

1: Characters used in analysis of pure *P. hispidissima* populations; characters 6, 9, 10, 11, 22 were omitted due to lack of variation in analysed set (matrix D); 2: for comparative analyses of both *P. hispidissima* and *P. hieracioides* 21 characters were used; characters 3, 13, 14 were excluded due to low variation (matrices A, B, and C)

[a] Mean values of three measurements of ligules or involucral bracts on one randomly chosen capitulum are given

[b] Counted from three randomly chosen capitula

[c] Brown-black, red-black or black hairs

[d] White or pale coloured hairs

[e] Green-brown, light brown, bi- or multi-coloured hairs. If DHP and DHI were recorded as 1 (i.e. absence of hairs), the characters DH, PH, IH recording the hair colour were given the value of 0

recently reported by Slovák et al. (2009) from southwestern and central Europe were used for comparison.

Mean values and standard deviations of absolute DNA content were calculated for both individuals and populations. To show different patterns of genome size in *P. hieracioides* and *P. hispidissima*, box-plots and histogram were prepared. Box-plots show the median, interquartile ranges, and extreme data points (stars). Common descriptive statistics were calculated using Excel (Microsoft). To explore differences among populations, the Tukey–Kramer test was used (Tukey test for unequal sample sizes; Sokal and Rohlf 1998, calculated with SAS 9.1.3 software, SAS Institute 2007).

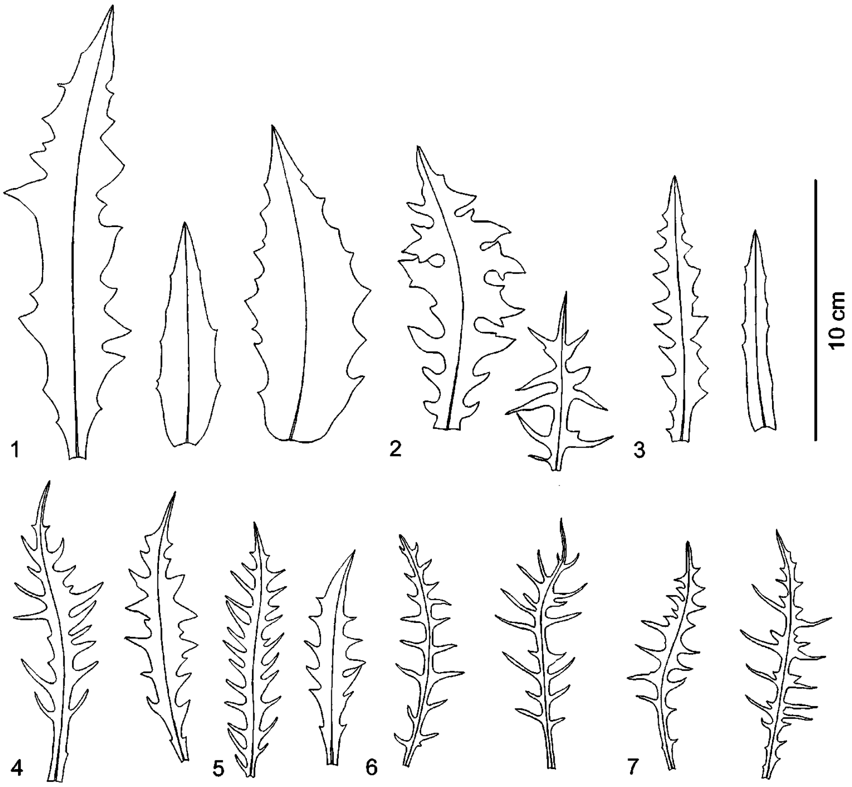
Results

Morphometric analyses

Morphological differentiation between P. hispidissima *and* P. hieracioides *s.l*

Principal component analysis based on 34 population samples of *P. hispidissima* (12 populations) and *P. hieracioides* (22 populations), characterized by mean values of 21 morphological characters (matrix A), resulted in two well-separated groups of populations visible on the two-dimensional ordination diagram. The groupings restricted

Fig. 2 Variation in leaf shape in *P. hispidissima* from Croatia. 1, PAK; 2, BAK; 3, DUB; 4, PLO; 5, KAR; 6, KRK; 7, GRA. For locality details, see Table 1



to the bottom right portion of the diagram (Fig. 3) correspond to populations traditionally classified as *P. hispidissima*. Populations of strongly polymorphic *P. hieracioides* appeared on the diagonal and were loosely divided into two sub-groupings more or less corresponding to the "lower altitude" morphological type (bottom left part of the diagram) and the "higher altitude" type (upper part of the diagram) recognized by Slovák and Marhold (2007) and Slovák et al. (in preparation). Therefore, populations of *P. hispidissima* were separated from *P. hieracioides* along both the first and second component axes. The following characters were tightly correlated with the first axis (mostly separating *P. hispidissima* and the "lower altitude morphotype" of *P. hieracioides*): length of inner and outer bracts, presence/absence of pale and colour-intermediate hairs on the involucrum, number of stem leaves, dilatation of peduncles below the capitulum, presence/absence of pectinate–ciliate margins on outer involucral bracts, distribution of capitula on stem branches, number of stem branches, and ligule length (Table 4). The second axis (mostly separating *P. hispidissima* and the "higher altitude" morphotype of *P. hieracioides*) correlated strongly with the presence/absence of dark hairs on the involucrum, maximum number of bracts on peduncle, presence/absence of brown marks on the upper part of ligules, dilatation of peduncles below the capitulum, presence/absence of pectinate–ciliate margins on outer involucral bracts, and distribution of hairs on the peduncle (Table 4).

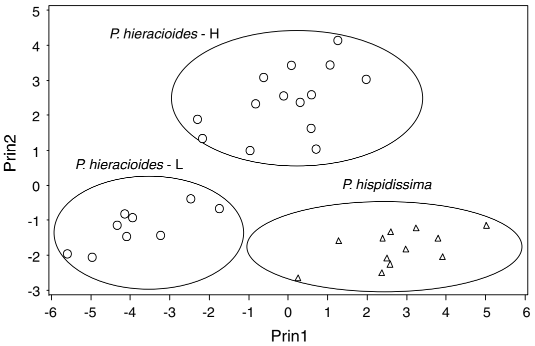


Fig. 3 Ordination diagram of principal component analyses based on 34 populations (OTUs). Twelve populations of *P. hispidissima* (*triangles*) and 22 populations of *P. hieracioides* (*circles*) were included in this analysis. Twenty-one morphological characters were measured and scored. The first two ordination axes expressed 38 and 19.3% of the overall variation

Table 4 Principal component analysis (PCA) based on 12 populations (OTU's) of *Picris hispidissima* and 22 populations (OTU's) of *P. hieracioides* (A), PCA based on 211 individuals of *P. hispidissima* as OTU's (B); Canonical discriminant analysis (CDA) of the individuals of *Picris hispidissima* and *P. hieracioides*

Morphological character	A			B	
	PCA		CDA	PCA	
	Axis 1	Axis 2	Axis 1	Axis 1	Axis 2
NBr	**0.596**	−0.267	0.283	0.342	−0.393
NL	**0.673**	−0.250	0.357	0.429	**−0.532**
LP	–	–	–	−0.158	0.013
DP	**−0.667**	**−0.580**	**−0.952**	0.100	−0.441
NBP	0.081	**0.625**	0.440	0.102	0.162
DHI	0.271	0.224	0.421	0.049	−0.037
DHP	−0.252	**−0.513**	−0.488	–	–
PCM	**−0.671**	**−0.586**	**−0.930**	0.238	−0.243
DH	0.062	**0.651**	0.332	–	–
PH	**0.741**	−0.233	0.455	–	–
IH	**−0.715**	−0.309	**−0.678**	–	–
DC	**−0.587**	0.406	−0.194	0.223	0.048
NoB	–	–	–	0.411	−0.461
NiB	–	–	–	0.301	−0.238
LoB	**−0.732**	−0.144	**−0.573**	**0.828**	−0.055
WoB	−0.399	0.063	−0.182	**0.798**	−0.064
LiB	**−0.742**	0.255	−0.319	**0.840**	0.116
WiB	−0.131	0.461	0.210	**0.637**	−0.108
LL	**−0.641**	0.320	−0.245	**0.741**	**0.504**
LCT	−0.486	0.077	−0.267	**0.718**	0.399
WL	**−0.560**	0.179	−0.296	0.329	**0.692**
LTL	−0.242	0.394	0.064	–	–
RSL	0.411	−0.248	0.162	−0.421	0.337
BML	0.323	**0.514**	**0.534**	−0.360	−0.072

For PCA, eigenvectors show correlation of the characters with the components (axis 1, axis 2). For CDA, correlations of characters with canonical axis are given. Values higher than 0.5 are set in *bold*

Principal component analyses based on individual plants (matrix B) showed a very similar pattern (Fig. 4). Once again, plants corresponding to *P. hispidissima* were located in the lower right part of the diagram, while plants corresponding to *P. hieracioides* appeared on the diagonal. Only a few plants were intermingled between the groups. The plants from the mixed population ZAD appeared in both the *P. hispidissima* and *P. hieracioides* groupings, as well as in between them. Characters with the highest correlation with the first two component axes are identical to those of the PCA analysis based on population means.

The results of CDA based on matrix C (all individual plants except those from population ZAD) and groupings corresponding to the results of PCA confirmed the clear differentiation of *P. hispidissima* and *P. hieracioides*. Only negligible overlap between the groups can be observed on the histogram (Fig. 5). Dilatation of peduncles below capitulum and presence/absence of pectinate–ciliate margins of outer involucral bracts showed the highest correlation with the canonical axis (Table 4). In non-parametric *k* nearest neighbours classificatory discriminant analyses (*k* = 6) more than 99% of individuals (100% for *P. hispidissima* and 99.52% for *P. hieracioides*) from both tested groups were classified correctly.

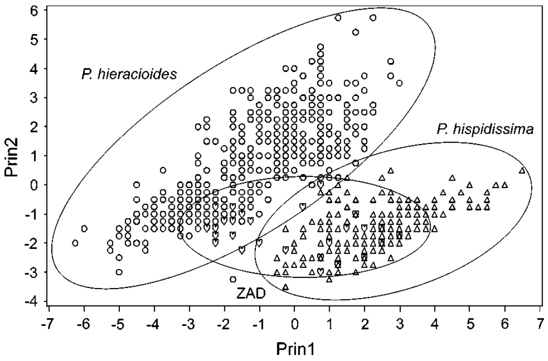


Fig. 4 Ordination diagram of principal component analyses (PCA) based on 661 individuals (OTUs), including 211 individuals of *P. hispidissima* (*triangles*), 421 individuals of *P. hieracioides* (*circles*) and 29 individuals from the mixed population ZAD (*hearts*). Twenty-one morphological characters were measured and scored

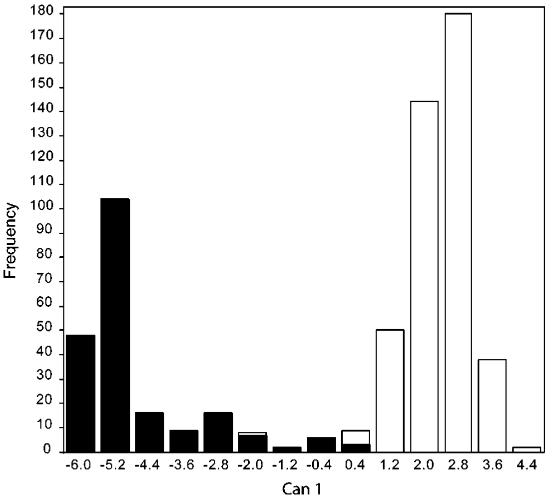


Fig. 5 Result of canonical discriminant analysis of 632 plants (OTUs), involving 211 individuals of *P. hispidissima* (*white*) from Croatia and Montenegro and 421 individuals of *P. hieracioides* (*black*) from central and southwestern Europe. Twenty-one morphological characters were measured and scored

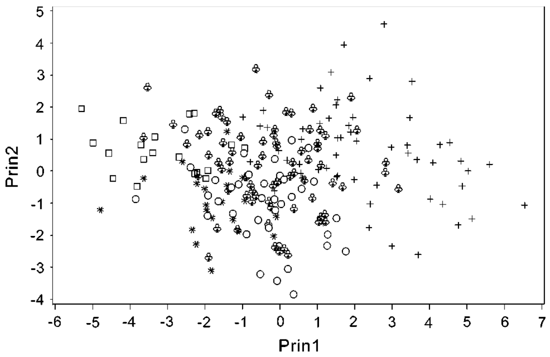


Fig. 6 Ordination diagram of principal component analyses (PCA) based on 211 individuals (OTUs) of *P. hispidissima*, using 19 morphological characters. The first two axes expressed 24.5 and 10.5% of the overall variation. The following groups are displayed and marked with different symbols: *squares* type locality of *Picris hispidissima* (KRK), *circles* area close to the type locality of *Picris hispidissima* var. *reicharioides* (BAK, KAR), *asterisks* type locality of *Picris hispidissima* var. *velebitica* (PAK), *clubs* another individuals from coastal areas of Croatia (BAS, DUB, GRA, PLO) and *crosses* plants from Montenegro (BUK, CET, KLO and SVS). For locality details, see Table 1. The mixed population (ZAD) was omitted from this analysis

Intraspecific phenotypic variation in P. hispidissima

Principal component analyses of *P. hispidissima* individuals (matrix D) did not reveal any clear differentiation among population samples (Fig. 6). Neither material from the localities of *P. hispidissima* var. *hispidissima*, nor from those of *P. hispidissima* var. *velebitica*, formed any distinct grouping. Similarly, plants from populations from *P. hispidissima* var. *reichardioides* localities collected in Croatian coast did not group together. The only slight shift in morphological characters observed was among the populations from Croatia and Montenegro. This is correlated with floral characters, particularly ligule length, perianth tubes, and outer and inner involucral bracts, as well as the width of outer and inner involucral bracts.

Karyological analyses

Chromosome numbers

A diploid chromosome number of $2n = 2x = 10$ was determined for all 13 individuals of *P. hispidissima* analysed (Table 2). These data represent the first published records of chromosome numbers for this species.

Flow cytometry

Flow cytometric analyses resulted in histograms with mean CV values (±SD) of 2.46 ± 0.30 (range 1.71–2.93) and 2.24 ± 0.32 (range 1.48–2.98) in the internal reference standard (*Glycine max* cv. 'Polanka') and *P. hispidissim*a, respectively, indicating high-quality measurements (Table 2). Between-day variation related to random instrument drift and/or non-identical preparation of samples was low, and max/min values of the three repetitions of the same sample did not vary more than 2%; in 97.5% of cases, they did not vary more than 1%.

Intraspecific genome size variation of P. hispidissima

Mean DNA content (2*C* values) in populations of *P. hispidissima* (except population ZAD) ranged from 2.96 to 3.24 pg, resulting in 9.5% variation (between populations BAS and DUB; Table 2; Figs. 7, 8). Intrapopulational variation did not exceed 5.6%, which, taking into account the above-mentioned CV values and the approximately 2% error in the equipment, falls within the measuring error. Results of Tukey–Kramer tests at the population level revealed significant differences only between populations with extreme 2*C* values, particularly populations BAS (mean 2*C* value = 2.96 pg) and DUB (mean 2*C* value = 3.24 pg). To confirm the differences, simultaneous analysis of the two samples with the highest and lowest DNA content was performed (Fig. 9), resulting in two clearly separated peaks. Other populations differed from 2 to 8% from the population with the smallest genome size (BAS), but these differences are not statistically significant and were not confirmed by simultaneous analyses. Nevertheless, they are slightly above the measuring error.

Genome size variation between P. hispidissima *and* P. hieracioides

Data for the genome size of *P. hispidissima* can be related to those for *P. hieracioides* recently acquired by Slovák et al. (2009). The range of intraspecific variation in individual plants of *P. hispidissima* (2*C* = 2.90–3.25 pg) was much lower than the corresponding values of *P. hieracioides* from central and southwestern Europe (2*C* = 2.26–3.11 pg, representing 1.37-fold variation; Slovák et al. 2009, Fig. 8). Nevertheless, *P. hieracioides* plants from the area of Croatia (where the distribution of both species overlaps) showed much more uniform genome sizes (2*C* = 2.30–2.37 pg). Thus, the genome size of *P. hieracioides* from Croatia is much lower and significantly different from that of *P. hispidissima*. Population ZAD, which comprises both *P. hispidissima* and *P. hieracioides*, was represented by four samples in the flow cytometric analyses (Table 2; Figs. 7, 8). The first two individuals fit within the upper limit of the genome size of *P. hispidissima*, having mean 2*C* values of 3.21 and 3.19 pg (ZAD3 and ZAD4). Two other individuals, the progeny of one plant (i.e., plants from seeds arising from

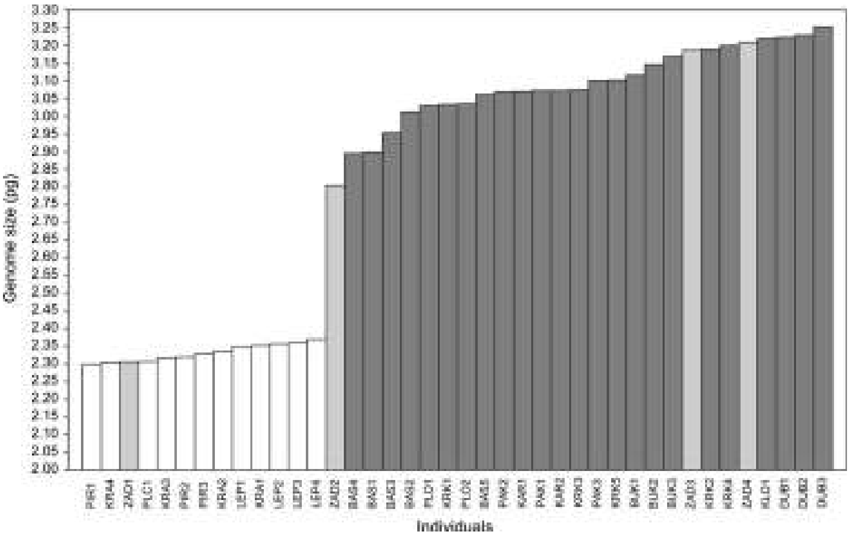


Fig. 7 Genome size variation (DNA pg) in individuals of the mixed population ZAD (*light grey columns*) and putative parental taxa. Only plant samples originating from Croatia were considered—*Picris hispidissima* (*dark grey columns*) and *P. hieracioides* (*white columns*). Genome size values of *P. hieracioides* are taken from our previous study (Slovák et al. 2009)

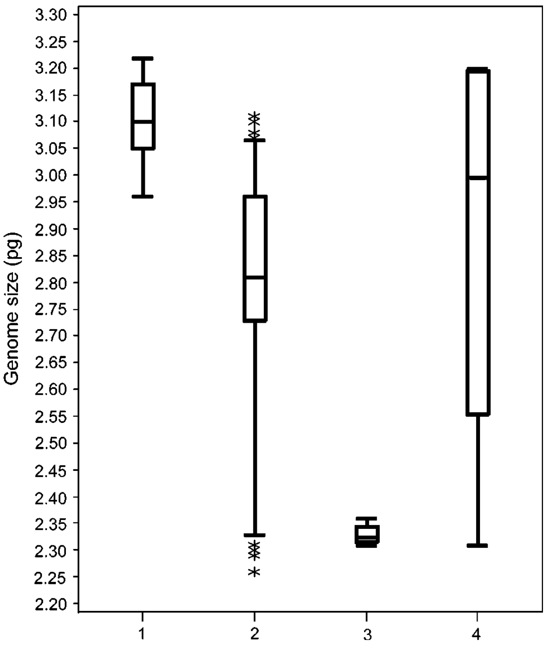


Fig. 8 Absolute DNA content variation of individuals of *Picris hispidissima* and *P. hieracioides*. Genome size values of *P. hieracioides* are from a previous study (Slovák et al. 2009). 1, *P. hispidissima*; 2, *P. hieracioides* (from central and southwestern Europe); 3, *P. hieracioides* (Croatia) and 4, individuals from the mixed population ZAD

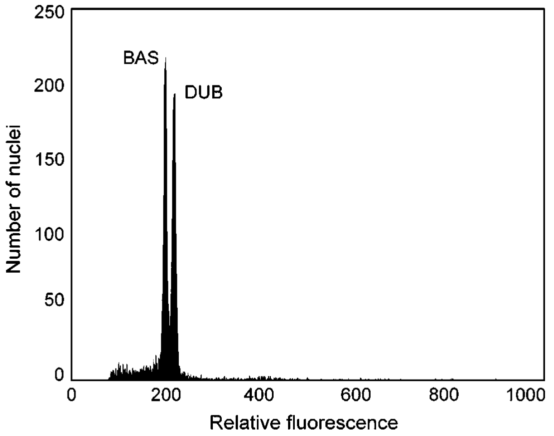


Fig. 9 Differences in genome size between the two most different *Picris hispidissima* samples from Croatia. Nuclei of all individuals were isolated, stained with propidium iodide and analysed simultaneously. Peak ratios are 1:1.114. Mean 2*C* values estimated for the populations were 2.90 (BAS) and 3.23 pg (DUB). See Table 1 for population details

the same capitulum), had mean 2*C* values of 2.31 (ZAD1) and 2.80 pg (ZAD2). Whereas the first value fits into the range of Croatian *P. hieracioides*, the genome size of 2.80 pg seems to be intermediate between *P. hieracioides* and *P. hispidissima*.

Discussion

Phenotypic relationships

Multivariate morphometric studies were successfully applied in numerous genera of the Compositae family with complicated patterns of morphological variation, based

Table 5 Summary of the main differences between *P. hispidissima* and *P. hieracioides*

	Taxon	
	P. hieracioides	*P. hispidissima*
Morphological characters[a]		
Dilatation of peduncles below the capitulum	Absent	Mostly present
Pectinate–ciliate margins on outer involucral bracts	Absent	Mostly present
Colour of hairs on involucrum and peduncle	Pale to dark colour	Colour-intermediate
Length of outer involucral bracts (mm)	3.1–7.9	3.5–11.5
Karyological characters		
Genome size (pg) (2*C* values, min–max values between individuals)[b]	2.26–3.11	2.90–3.25
Life form[c]	Annual to short lived perennial	Biennial to monocarpic
Ecology[c]	Open grasslands, steppes and xerothermous ruderal areas to mesophylous subalpine grasslands, usually not in coastal areas	Xerothermous rocky slopes, ruderal rocky biotopes, rock crevices on calcareous cliffs and gorges, mountain and coastal areas near Adriatic sea
Distribution	Europe, Asia	Western part of Balkan peninsula

[a] Only most important morphological characters separating *P. hispidissima* and *P. hieracioides* (both morphotypes included) are listed here. For character explanation see Table 3

[b] Genome size data for *P. hieracioides* follow Slovák et al. (2009)

[c] Life form and ecological data for *P. hieracioides* were partly taken from Slovák and Marhold (2007). Data on intermingled population ZAD are not included

especially on quantitative characters. Within the aggregate of *Senecio nemorensis* in Central Europe, morphological entities and their hybrids were successfully separated (Hodálová and Marhold 1996, 1998; Hodálová 1999). On the contrary, in the same genus, no clear morphological differentiation of tetraploid and octoploid individuals of *S. jacobaea* was detected (Hodálová et al. 2007). Morphology of the genus *Picris* was extensively studied by Holzapfel and Lack (1993) and Holzapfel (1994) in Australia, by Smalla (2000) in the Arabian Peninsula and Socotra, and by Slovák and Marhold (2007) in the West Carpathians, but only the latter two studies used multivariate approaches. Smalla (2000) concluded that *Picris* species occurring in the Near East can be reliably identified only in the fertile stages using achene morphology. Based on morphometric analyses, he also detected putative hybrids. Slovák and Marhold (2007) distinguished two morphotypes within the polymorphic *P. hieracioides* in the West Carpathians.

In the present study, multivariate morphometric analyses elucidated the degree of differentiation between the Balkan endemic *P. hispidissima* and the widespread *P. hieracioides* (see "Results", Tables 4, 5). The former species is clearly separated from all morphotypes of *P. hieracioides* by a number of characters (Table 4). The traditional ones involve the pectinate–ciliate indumentum of involucral bracts and the dilatation of the peduncle (Lack 1974; Sell 1976). In addition, several new characters with high discriminatory power were detected, including outer and inner bract length and indumentum colour. On the other hand, the shape of leaves of *P. hispidissima* considered to be laciniate (Lack 1974) as opposed to more or less toothed or entire in *P. hieracioides* was not particularly useful for the identification of these taxa. Leaf shape in *P. hispidissima* varies from pinnatisect to almost entire (Fig. 2) and overlaps considerably with that of *P. hieracioides*. Similarly, capitula size (expressed by ligule length, corolla tube length and by the length and width of inner and outer bracts) in *P. hispidissima*, considered by Lack (1974) to be the largest within the genus *Picris*, apparently overlaps with that of mountain populations of *P. hieracioides*. Surprisingly, individuals of *P. hispidissima* resemble the "higher altitude type" of *P. hieracioides* distributed in major European mountain ranges more than the "lower altitude type" that occurs in the studied area.

We found only one mixed population of *P. hispidissima* and *P. hieracioides* (locality ZAD). Plants matching the parental species were found together with morphologically intermediate individuals. We suppose that at least some of them are of hybrid origin. This hypothesis is partly supported by the discovery of achenes with two different genome sizes in one capitulum at the same locality. Nevertheless, further studies are needed to confirm hybridization between these two taxa and its extent.

There is no clear morphological diversification among populations of *P. hispidissima*, but slight morphological shifts between plants from Montenegro and Croatia were detected. We have not confirmed either the existence of the three different varieties recognized by Degen (1938) or other distinct morphological entities within this species. In populations from the Velebit Mountains (PAK, GRA), individuals without pectinate–ciliate indumenta of involucral bracts and/or dilatation of the peduncle were found, in addition to the typical plants. However, such plants were not separated from other individuals of *P. hispidissima* on the ordination diagrams. It should be emphasized that they merely represent individual variation within otherwise typical populations of *P. hispidissima*, and their hybridogenous origin is highly improbable.

Chromosome number

As expected, no variation in chromosome number ($2n = 2x = 10$) or ploidy level was found within *P. hispidissima* in the present study (Table 2). Similarly, the same chromosome number and ploidy level were detected in *P. hieracioides* in central and southwestern Europe (Slovák et al. 2007). These results are also congruent with other karyological studies of the genus *Picris*. Almost all representatives of the genus were found to be diploid (Lack 1974, 1979; Sell 1976; Holzapfel and Lack 1993; Holzapfel 1994; Smalla 2000). The only exceptions within the genus *Picris* are tetraploids and hexaploids recorded besides of diploid individuals in *P. hispanica* (Willd.) P. D. Sell (Humphries et al. 1978; Galland 1988; Oberprieler and Vogt 1993).

Absolute DNA content

Variation in absolute DNA content in progeny of *P. hispidissima* suggests possible diversification of genome size within this species. However, only two populations with extreme genome sizes (9.5% difference) were significantly different (Table 2). There are at least two plausible hypotheses to explain this divergence. First, intraspecific variation might have evolved under pressure from environmental conditions. Fragmentation of formerly continuous distribution areas might have led to microevolutionary events resulting in genome size variation among populations. However, narrow ecological amplitude, restricted distribution area, low intraspecific morphological variation, anemochory facilitating long distance dispersal and a tendency toward synanthropic behaviour do not support this hypothesis.

Another potential explanation for the genome size pattern found in *P. hispidissima* invokes interspecific hybridization with another species with a different genome size. The closely related *P. hieracioides* is perhaps the most plausible candidate. The ZAD locality harbouring mixed population of *P. hieracioides* and *P. hispidissima* supports hybridization hypothesis. The most convincing evidence is the detection of plants with two clearly different DNA contents arising from a single capitulum. Another population that might have undergone introgressive hybridization is BAS, which contained individuals with genome sizes on the lower edge of the *P. hispidissima* range. Nevertheless, plants of *P. hieracioides* were not found there. Similar hybridization (or introgression) scenarios could also plausibly explain other detected interpopulation genome size differences (up to 8%) within *P. hispidissima* (see "Results", Table 2). Identical ploidy levels, strict allogamy (Slovák et al. 2007, M. Slovák, unpublished data), sympatric occurrence, and the similar ecological niches of *P. hispidissima* and *P. hieracioides* also favour the hybridization hypothesis. Despite the above-mentioned pieces of evidence, hybridization requires further study using molecular markers (e.g., AFLP or chloroplast sequences).

Several studies have recently reported genuine intraspecific genome size variation. For example, variation up to 11% was reported in *Dasypyrum villosum* (Obermayer and Greilhuber 2005), 7.4% in *Hieracium piloselliflorum* (Suda et al. 2007), and 16.6% in *Festuca pallens* (Šmarda and Bureš 2006). In addition, hybridization events between closely related taxa were also successfully detected by flow cytometry [e.g., in Central European species of *Cirsium* (Bureš et al. 2004) and *Artemisia arborescens* complex (Garcia et al. 2006)]. The complex relationships of three *Helianthus* species and their natural and synthetic hybrids were at least partially resolved by flow cytometry analyses (Baack et al. 2005).

Genome size variation in *P. hispidissima* (whether or not it is caused by the hybridization) is lower than in *P. hieracioides* (Tables 2, 5). This fact agrees with the theory that genomes of plants inhabiting wide distribution areas and possessing larger ecological amplitude evolve and diversify more rapidly than those of species with limited distribution and ecological requirements (Vinogradov 2003). The larger genome of *P. hispidissima*, together with its restricted distribution and uniform, often relict, biotopes (e.g., the gorge of Velka Paklenica) also fit with the "large genome constraint hypothesis" (Knight et al. 2005). In other words, plants with larger genomes are less competitive in terms of ecology and evolve and diversify more slowly than those with smaller genome sizes. These trends are apparent within *P. hieracioides*, where the "higher altitude type" populations restricted to the higher mountain ranges have larger genomes than the "lower altitude type", which inhabits a wider range of biotopes and shows strong ruderal and synanthropic tendencies (Slovák et al., unpublished data).

Nomenclature

The first record possibly referring to *P. hispidissima* dates back to the beginning of the nineteenth century when Schkuhr (1803) described a new species, *P. laciniata*, on the basis of plants cultivated in his garden in Wittenberg. Later, in 1825, Bartling described *Crepis hispidissima* from Croatia (island Krk), a species later placed by Koch (1837) in the genus *Picris*. Finally, Visiani (1829) reported and described *P. laciniata* from Dalmatia, which he himself considered to be very similar or identical to the species described by Schkuhr (1803). Whereas, none of the specimens used by Schkuhr to describe *P. laciniata* are known to exist (Lack 1974), and there are some doubts as to the accuracy of the illustration, which is part of the protologue of this name (Schkuhr 1803, pp 14–15); the name *Picris hispidissima* (Bartl.) W. D. J. Koch has been generally accepted for this species (Lack 1974; Sell 1976). The name *P. hispidissima* was typified by Lack (1974, 1993) by specimen 1076 deposited in GOET and Visiani's *P. laciniata* was rendered as a synonym of this name. Nevertheless, the validly published name *P. laciniata* Schkuhr was never formally rejected. This nomenclatural problem should be dealt with in the future, most likely via the rejection of the name *P. laciniata*.

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Conclusions

To assess the importance of various sources of variation within genus *Pilosella* and to follow microevolutionary trends, several case studies at different levels were performed (population, regional, comparative etc.). The gradient of different spatial levels and comparative studies of closely related groups (characterised by partly different type of ongoing microevolution) clearly indicated common processes and at the same time underlined the uniqueness of genus *Pilosella*.

Microevolutionary potential was studied at the population level (hybridization tendencies of highly complex hybridization swarm in Prague - Praha Vysočany). Basic species *P. officinarum* and *P. piloselloides subsp. bauhini* (further in text *P. *bauhini*) hybridize there resulting in wide spectrum of various lineages (according to ploidy level, reproductive mode etc.). Aim of the above mentioned study was to focus on morphological manifestation of highly complex microevolutionary phenomena (especially hybridization accompanied by polyploidy, reproductive mode variation, parthenogenesis etc.).

The study was focused on two related datasets: maternal plants and their progeny. Both datasets were further structured to biosystematically characterised groups based on ploidy level and reproductive mode. Results of multivariate morphometric study confirmed morphological gradient in the hybrid swarm between *P. officinarum* and *P. *bauhini* which exists in maternal plants. Crossing resulted in the most frequent hybrid *P. brachiata* (and rare *P. leptophyton*). Analysis of filial plants showed shifts within the progeny: they indicate several microevolutionary traits such as formation of new hybrid combination, introgressive hybridization and hybrids segregation or most probably their combination. Hybridization was present in almost all directions despite the fact of apomictic reproductive mode presence (one of the parental species as well as part of hybrids) and *P. officinarum* was indicated to play dominant role in backcrossing (especially with *P. brachiata*), which may be considered to be the crucial result of the analyses.

Population level was followed by studies at regional scale (focused on Central Europe overlapping to whole distribution range). *Pilosella officinarum* was chosen as a model species. The taxon is characterised by enormous variation (in ploidy levels and reproductive modes) and at the same time it is the most frequent species of *Pilosella* in Europe. Further extrapolation of *P. officinarum* studies to the rest of genus *Pilosella* is adequate, because *P. officinarum* is the most frequent participant of hybridization events in the entire genus. *Pilosella officinarum* was sampled from the area of Central Europe (especially the Czech and Slovak Republic). DNA ploidy level analyses confirmed highly discrete pattern of cytotype (DNA ploidy level) group distribution including accurately determined cytotype distribution borders. Whole Europe cytotype distribution map was created out of published data and the Central European distribution maps were finally interpreted in whole European context.

Cytotype distribution pattern in Central Europe corresponds to geomorphologic/geologic regions. Tetraploid cytotype almost exclusively occurs in Bohemia and further to the West and North whereas pentaploid and hexaploid cytotypes prevail in Moravia and Slovakia and further to south-east. The distribution areas correspond to geomorphologic units of Czech Massif (≈Hercynicum) and Western Carpathians, probably reflecting different history (florogenesis), geological constitution and climatic gradient towards continental type. Tetraploid cytotype area has distribution centre in Central and Western Europe (incl. Baltic region) and Iberian Peninsula, whereas pentaploid and hexaploid cytotypes occur in surrounding areas (deglaciated regions of British Isles and Scandinavia, Central and Eastern Mediterranean and the Balkan Peninsula). Overall distribution pattern probably mirrors different processes in history, partially of postglacial recolonisation (geographic parthenogenesis).

When we included results of reproductive mode analysis the cytotypes were further divided. Tetraploid cytotype was confirmed to be sexual, whereas apomixis almost absolutely prevailed among pentaploids. Hexaploid plants formed two distinct groups in Central Europe. Apomictic hexaploids occur frequently in Moravia and Slovakia regions (Western Carpathians) and in Pannonia. On the other hand, sexual hexaploid cytotype is almost exclusively restricted to several steep slopes and rocky outcrops of river canyons in Southern Moravia and Central Bohemia (relic primary forest free habitats).

Morphological characters of most of the plants were then analysed using multivariate morphometrics. Results confirmed possibility to distinguish particular cytotypes (pentaploids partly overlapping with the tetra- and hexaploids). Different morphologic traits of sexual vs. apomictic hexaploids were indicated too. Nevertheless, the confirmed distinct groups could hardly be taxonomically treated for several reasons (over 600 already described subspecies, difficulties with their typifications, additional biosystematic data which would be needed for original types etc).

Comparative studies of related group were then performed. As microevolutionary comparable taxa *Picris hieracioides* group was chosen (less complex pattern, no apomixis, almost exclusively diploid). Variation is still evidently mirrored at the level of absolute genome size. Detailed absolute genome size study revealed two distinct groups with no overlaps. Each group occurs in different habitat according to altitude and rate of anthropic impact ("higher altitude type" - mountains, primary habitats vs. "lower altitude type" - lowland, secondary habitats) and therefore they are probably lineages with unique evolutionary history. Generally, the genome size of both morphotypes increased significantly from south-west to north-east. During genome size screening also a mixed population with two triploid plants was found (the first report of triploid incidence in the whole genus). *Picris hieracioides* was supposed to be exclusively diploid; thus triploid plants existence (probable via unreduced gamete participation) confirmed a theoretical polyploid potential even in this group.

Final comparative study was focused on *Picris hispidissima* - a Balkan taxon, which seemed to be already distinctly derived from the *P. hieracioides* group (probably due to isolation). Stability and discreetness of *P. hispidissima* as taxon was supported by much lower range of intraspecific genome size variation. Absolute genome size also separates *P. hispidissima* from *P. hieracioides* (significantly higher content of DNA). Multivariate morphometric studies finally confirmed markedly specific morphology compared to *P. hieracioides.*

The *Picris* comparative case studies revealed highly distinct microevolutionary pattern compared to genus *Pilosella.* Some crucial sources of genus *Pilosella* variation are evidently not presented here and therefore less reticulate and complex pattern is formed. Especially apomixis has not ever been recorded in *Picris.* Thus formation of discrete lineages (≈genotypes) characteristic of apomicts is not present. At the same time almost no polyploidy was recorded. Results also indicate relatively low rate of hybridization (especially compared to genus *Pilosella*). In addition, no clonal growth occurs and such rarity as long-time persistence of triploids is not possible. Microevolution in genus *Picris* seems to be rapidly ongoing but there still exist natural, partly discrete taxonomic units. On the contrary, during extremely rapid and reticulate microevolution within genus *Pilosella* natural taxonomic units merge together and the whole genus is evidently coming through deep changes.

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Current research

- flow cytometry
- biosystematics of polyploid and agamic groups (e.g. genus *Pilosella*, *Sorbus*)
- genome size microevolutionary aspects (e.g. genus *Picris*)
- hybridisation in natural environments (e.g. genus *Diphasiastrum*)

Grant projects

project leader of 2 grant project, member of research team in 4 current and 5 finished grant projects

2009-2011: Hybridization and microevolutionary relationships among Central European Diphasiastrum species (GAUK 90809/2009, project leader).
2008-2010: A molecular biosystematic study of the section Pilosellina and evolutionary aspects of its glacial origin (GAUK 98708/2008, project leader).

Scientific societies membership

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Teaching experience:

since 2009: Methods in plant taxonomy I – Karyology, flow cytometry and palynology
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Publications (h-index = 5):

Hörandl E., Dobes Ch., Suda J, Vit P., **Urfus T.**, Temsch E., Cosendai A., Wagner J. & Ladinig U. (2011): Apomixis is not prevalent in subnival to nival plants of the European Alps. - Annals of Botany 108(2): 381-390

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Appendix 1

Primary morphometric data used in the paper no. 1 (Hybridization within *Pilosella* population: a morphometric analysis). Following chracters are included in the table below:

v1 - field number
v2 - internal number
v3 - plant group (filial: 1 *officinarum*, 2 *bauhini*, 3 *brachiata*, 4 *leptophyton*;
    paternal: 5 *officinarum*, 6 *bauhini*, 7 *brachiata*, 8 *leptophyton*; paternal)
v4 - leaf lenght - LL
v5 - leaf width - LW
v6 - distance between the widest point of leaf to the tip - WT
v7 - rate of leaf tip acuteness (1-sharp, 2-obtuse, 3-rounded) - TA
v8 - density rate of stellate trichomes at adaxial side of 5 leaves
    (1-glabrate, 2-single trichomes, 3-dense hairs) - SA
v9 - density rate of single trichomes at upper surface of 5 leaves
    (1-glabrate, 2-single trichomes, 3-dense hairs) - SL
v10 - stem height (up to terminal capitulum) - SH
v11 - stem height (up to the first branching) - SB
v12 - enght of terminal capitulum peduncle - PH
v13 - density rate of single trichomes at stem
    (1-glabrate, 2-single trichomes, 3-dense hairs) - SS
v14 - density rate of stellate trichomes at stem
    (1-glabrate, 2-single trichomes, 3-dense hairs) - STS
v15 - density rate of eglandulate trichomes at stem
    (1-glabrate, 2-single trichomes, 3-dense hairs) - ES
v16 - average of 5 involucral bracts lenght - BL
v17 - average of 5 involucral bracts width - BW
v18 - terminal capitulum diameter - CD
v19 - rate of dark trichomes density at involucrum
    (1-bright, 2-dark bases, 3-dark trichomes) - TD
v20 - density rate of simple trichomes at involucrum - SI
v21 - density rate of stellate trichomes at involucrum - STI
v22 - density rate of eglandulate trichomes at involucrum - EI
v23 - stolon occurrence - SO
v24 - flagella occurrence - FO

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
651BRAkastr/2	1	7	46	6	14	2	2	3	262	59	35	2	2	2	7,0	0,7	16	2	2	2	3	1	0
513BRAkastr/4	2	7	44	6	12	1	2	2	172	104	14	2	3	1	5,3	0,7	11	1	2	2	2	1	0
651BRAvsp/8	3	7	83	8	14	1	1	1	189	135	16	2	2	1	7,0	0,9	11	1	3	2	1	1	1
651BRAkastr/1	4	7	52	12	21	2	2	1	287	39	11	1	3	2	5,7	0,6	9	1	1	3	2	1	0
513BRAvsp/19	5	7	115	14	42	2	2	1	294	36	8	1	3	2	7,9	1,3	10	2	1	3	3	1	1
507BRAkastr/3	6	7	89	6	38	1	2	1	265	65	26	1	2	1	5,6	0,7	15	1	2	2	1	1	0
513BRAkastr/5	7	7	69	6	25	2	2	1	381	230	5	1	3	1	4,9	0,7	11	1	2	2	1	1	0
513BRAkastr/6	8	7	59	17	16	2	2	1	227	75	12	1	2	1	6,3	0,8	10	1	2	2	1	0	0
295Br1/6	9	7	41	5	11	1	3	1	114	34	34	1	3	1	8,8	1,0	20	1	2	2	1	1	1
295Br2/2	10	7	69	15	19	2	3	1	194	4	190	1	2	1	8,5	1,2	21	1	2	2	1	1	0
295Br1/9	11	7	99	12	22	2	2	2	447	105	185	1	3	1	9,2	1,1	15	1	3	2	1	1	1
295Br2/1	12	7	103	21	26	2	3	1	298	4	140	2	3	1	10,0	1,4	19	1	2	2	1	1	1
295Br1/7	13	7	50	10	12	2	2	2	190	0	190	2	2	2	7,3	0,9	12	1	2	2	1	1	1
295Br1/1	14	7	51	8	13	1	2	1	256	21	176	2	2	2	6,6	1,1	14	1	2	2	2	1	1
295Br2/5	15	7	74	13	21	2	2	2	283	44	238	2	3	2	9,3	1,0	16	1	3	2	1	1	1
295Br1/10	16	7	44	10	11	1	2	2	92	0	92	2	3	2	8,8	1,1	17	2	3	3	1	0	0
295Br2/3	17	7	61	9	10	1	3	3	183	0	183	2	3	1	10,5	1,0	24	1	2	3	1	1	1
295Br1/2	18	7	44	11	14	3	3	3	249	50	198	1	2	3	7,5	1,1	12	2	3	3	2	1	0
295Br1/5	19	7	33	5	14	2	2	2	100	0	100	2	3	2	9,9	1,1	12	1	3	3	1	1	1
295Br1/4	20	7	25	9	10	3	2	1	127	0	127	1	2	2	9,9	1,1	13	1	3	3	1	1	0
295Br2/6	21	7	100	11	24	2	2	2	235	0	235	2	2	1	7,8	1,3	14	1	2	3	1	1	0
295Br2/4	22	7	54	7	10	2	3	3	85	0	85	2	3	3	8,7	1,1	11	2	2	2	2	1	1
295Br2/2	23	7	48	8	15	1	3	1	186	0	186	2	3	2	8,3	1,0	10	2	2	3	1	1	1
295Br1/8	24	7	56	12	17	2	2	2	116	0	116	2	3	1	10,1	1,4	16	1	3	3	1	1	1
295Br1/3	25	7	43	10	13	1	2	2	71	36	35	2	3	3	9,5	1,2	16	2	3	3	2	1	1
295Br1/11	26	7	53	12	21	2	2	3	166	0	166	2	2	2	10,0	1,2	18	1	2	2	1	1	1
661-BRA/14	27	7	75	11	23	1	1	1	358	0	6	1	1	1	4,3	0,8	9	2	2	2	1	1	0
660BRA/16	28	7	38	7	11	1	3	2	162	0	162	1	2	2	6,5	0,7	11	1	1	3	2	1	0
660BRA/13	29	7	96	11	25	1	2	2	226	0	226	1	2	2	9,1	1,1	12	2	2	2	2	1	1
660BRA/9	30	7	87	16	25	1	2	1	241	54	187	1	2	2	9,9	1,1	12	1	3	2	1	1	1
660BRA/4	31	7	76	16	17	2	2	2	392	0	392	1	2	1	9,2	1,1	12	1	3	2	1	1	1
660BRA/11	32	7	89	20	29	2	2	2	260	0	260	2	3	1	9,8	1,1	13	1	3	3	1	1	0
660BRA/17	33	7	65	12	24	1	2	3	157	91	65	2	3	2	9,0	1,1	6	1	3	2	1	1	1
660BRA/21	34	7	71	14	22	2	3	2	107	67	40	1	3	2	8,8	1,0	13	1	2	2	2	1	1
660BRA/9	35	7	36	7	13	1	3	2	149	105	43	1	2	2	9,2	1,0	12	1	2	2	2	1	0
660BRA/18	36	7	68	11	13	2	3	3	216	0	216	2	3	1	10,1	0,9	16	2	3	2	1	1	1
660BRA/20	37	7	69	16	19	2	2	1	260	49	211	1	2	1	9,3	0,9	9	1	2	2	3	1	0
660BRA/23	38	7	65	14	19	1	2	2	214	71	143	1	3	1	8,3	0,9	10	1	2	3	3	1	1
660BRA/6	39	7	68	18	22	2	3	2	337	110	227	1	2	1	7,9	0,6	11	2	2	2	1	1	1
660BRA/3	40	7	85	23	28	2	3	1	253	56	170	2	3	1	8,3	1,0	14	1	3	2	1	1	0
648BRA/5	41	7	85	16	32	1	3	1	142	10	132	2	3	2	10,0	1,3	14	1	1	2	3	1	0
648BRA/9	42	7	40	8	19	2	3	2	115	0	115	1	3	2	8,9	1,0	15	2	1	3	3	1	0
648BRA/13	43	7	71	8	18	1	2	1	243	0	243	1	2	1	7,5	1,2	11	2	3	3	1	1	0
648BRA/10	44	7	27	8	12	1	2	2	56	0	56	1	3	1	7,3	1,1	9	2	1	3	2	0	0
648BRA/1	45	7	39	8	10	2	3	3	110	0	110	1	3	3	8,2	1,0	14	1	1	3	3	1	0
297Br/1-1,F2/3	46	7	53	14	21	1	1	3	244	92	10	1	3	2	5,2	0,6	6	2	1	2	1	1	1
297Br/1-1	47	7	58	8	20	1	2	3	228	98	6	2	3	1	5,5	0,9	8	2	2	2	1	1	1
297Br/1-2	48	7	67	4	15	1	2	2	351	53	9	1	2	1	6,7	0,7	8	1	2	2	1	1	1
297Br/1-4	49	7	114	19	36	1	1	2	473	409	20	1	3	1	8,4	1,0	9	2	2	2	2	1	1
515BRA/10	50	7	79	18	23	2	2	2	155	78	77	2	2	2	9,8	1,1	13	1	3	2	2	0	1
515BRA/1	51	7	78	10	16	2	3	3	162	40	42	1	2	3	7,5	1,1	11	1	2	2	2	1	1
515BRA/15	52	7	60	13	21	1	3	3	252	10	48	1	2	2	9,4	1,2	15	1	3	2	2	1	0
515BRA/13	53	7	48	11	16	2	3	3	123	0	123	1	3	3	8,5	1,0	16	2	1	3	3	1	0
515BRA/8	54	7	105	14	28	1	1	1	278	170	108	3	2	3	10,7	1,2	14	2	3	3	1	0	0
515BRA/4	55	7	36	8	16	1	2	3	148	49	52	1	2	1	7,3	0,8	9	1	1	2	2	0	1
515BRA/4	56	7	46	5	9	1	2	2	144	0	144	1	2	2	7,0	0,6	9	1	1	2	3	1	1
515BRA/10	57	7	36	5	12	2	3	3	216	0	216	1	2	3	8,3	1,0	11	1	1	3	3	1	0
515BRA/10	58	7	36	6	12	2	3	3	209	0	209	1	2	2	9,5	1,0	11	1	2	3	2	1	0

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
515BRA/6	59	7	88	14	29	1	1	1	315	27	9	1	3	3	8,1	1,2	14	2	2	3	2	1	1
513BRA/1	60	7	42	10	15	2	3	3	137	33	7	1	3	3	8,4	1,0	13	1	2	2	3	1	0
513BRA/3	61	7	37	5	9	2	1	1	138	74	5	3	2	3	4,7	0,8	7	1	3	2	3	1	0
513BRA/2	62	7	46	7	13	1	2	1	84	29	55	1	3	2	8,0	1,0	10	1	1	3	2	1	1
647LEP/8	63	8	130	14	22	2	1	2	594	239	11	1	1	1	5,1	0,6	6	1	2	1	1	1	1
647LEP/3	64	8	80	11	27	1	1	2	434	207	6	1	1	1	6,2	0,6	7	1	1	1	2	1	1
647LEP/4	65	8	109	15	24	1	1	1	508	74	15	1	1	2	6,2	0,6	7	1	2	1	2	1	1
647LEP/7	66	8	100	15	27	1	1	1	616	100	12	2	1	1	6,0	0,5	6	1	2	2	1	1	1
647LEP/5	67	8	93	13	34	1	1	2	553	488	23	1	1	2	5,4	0,5	6	1	2	1	2	1	1
647LEP/1	68	8	108	10	36	1	1	1	565	425	12	2	2	2	7,0	0,8	7	2	1	2	2	1	0
647LEP/2	69	8	118	19	31	2	1	1	601	35	9	1	2	2	6,0	0,6	6	1	2	1	1	1	1
643BRA/4	70	7	75	16	27	2	2	3	215	60	98	1	2	3	8,2	0,9	11	2	3	2	3	1	1
643BRA/5	71	7	67	13	31	2	3	3	277	65	149	1	2	1	9,3	0,9	14	2	1	3	2	1	1
643BRA/1	72	7	30	6	12	3	3	3	85	0	85	1	3	3	8,7	1,1	11	1	2	3	3	1	0
643BRA/2	73	7	53	6	13	2	2	3	301	84	217	1	2	2	9,0	0,8	13	1	1	2	2	1	0
651BRA/5	74	7	57	14	18	2	3	3	160	101	59	1	2	1	8,5	1,1	14	1	3	3	1	1	0
651BRA/1	75	7	64	15	20	2	1	3	323	83	102	3	2	2	7,6	1,0	11	2	2	1	1	1	0
651BRA/4	76	7	39	8	11	1	2	3	211	0	211	2	2	3	8,4	1,0	14	1	3	3	1	1	0
641BRA/7	77	7	68	17	22	2	3	2	189	19	170	1	2	2	9,8	0,9	13	1	1	3	3	1	1
641BRA/5	78	7	80	22	24	3	3	3	145	0	145	1	3	3	11,1	1,1	18	1	3	3	2	1	0
641BRA/3	79	7	90	22	22	2	3	3	193	46	147	1	3	2	9,8	1,2	16	1	1	3	3	1	1
653BRA/3	80	7	52	17	18	1	3	3	228	61	37	1	2	2	9,4	1,1	14	1	1	2	1	1	1
653BRA/12	81	7	70	14	21	1	1	2	280	223	11	3	2	2	7,4	0,9	8	2	3	2	2	1	0
653BRA/2	82	7	101	12	21	1	1	2	505	12	6	2	1	1	7,2	0,9	8	2	3	1	1	1	1
653BRA/6	83	7	105	14	26	2	1	2	555	60	11	2	1	2	6,1	0,9	9	1	2	2	1	1	1
653BRA/12	84	7	86	13	25	1	1	3	459	336	11	2	1	1	6,3	0,8	9	1	3	1	1	1	1
653BRA/4	85	7	84	11	20	1	1	3	605	203	8	1	1	1	6,3	0,8	10	2	1	1	1	1	1
653BRA/7	86	7	107	15	27	1	1	2	651	388	52	1	1	2	5,7	0,6	7	1	3	1	1	1	1
653BRA/10	87	7	122	17	21	1	1	1	687	252	8	1	1	2	6,5	0,7	8	2	2	1	1	1	1
653BRA/8	88	7	121	17	38	2	1	1	698	590	6	2	1	1	6,5	0,6	6	1	2	1	1	1	1
644BA/5	89	6	33	7	11	3	3	1	119	6	113	2	3	2	8,7	1,1	12	1	1	3	2	1	0
644BA/20	90	6	77	11	22	1	1	3	605	419	7	1	1	1	7,1	0,8	7	1	3	1	1	1	1
644BA/18	91	6	101	12	31	1	1	2	579	8	6	1	1	2	5,0	0,7	7	1	3	1	1	1	1
644BA/2	92	6	105	18	28	1	1	2	638	192	5	1	1	2	5,4	0,7	6	2	2	1	1	1	1
644BA/4	93	6	91	15	28	2	1	3	550	170	11	1	1	1	6,3	0,9	8	1	1	2	1	1	1
644BA/7	94	6	93	16	32	1	1	2	527	26	15	1	2	1	6,2	0,8	7	1	2	2	2	1	1
644BA/19	95	6	96	13	25	1	1	2	628	250	7	1	1	1	6,8	0,9	7	1	2	1	1	1	1
644BA/14	96	6	81	13	32	1	1	3	613	75	6	1	1	2	5,6	0,7	6	1	3	2	2	0	1
644BA/10	97	6	93	11	18	1	1	1	665	68	6	1	1	1	6,2	0,7	6	2	3	2	2	0	1
644BA/8	98	6	111	12	33	1	1	3	650	176	9	1	1	1	6,4	0,7	6	1	2	1	2	1	1
644BA/11	99	6	114	16	24	1	1	3	544	290	5	2	2	2	5,7	0,7	6	2	3	2	1	1	1
644BA/15	100	6	96	11	27	1	1	2	693	331	8	1	1	2	5,7	0,6	5	1	2	2	1	1	1
644BA/21	101	6	55	6	21	1	1	1	397	238	11	3	2	1	5,8	0,8	6	2	2	1	2	1	0
644BA/6	102	6	80	9	28	2	1	3	560	335	18	2	2	2	6,5	0,7	7	2	2	2	1	1	1
644BA/13	103	6	57	11	20	2	1	3	428	378	5	1	2	3	6,0	0,7	5	2	2	2	3	1	1
644BA/12	104	6	66	13	21	2	1	2	583	116	4	2	2	2	6,5	0,9	6	1	2	1	1	1	1
655BA/2	105	6	57	15	18	2	3	3	317	8	55	1	3	1	8,4	1,0	13	1	3	3	1	1	1
655BA/1	106	6	116	16	35	2	1	2	545	96	7	2	2	3	7,3	0,9	6	1	3	2	1	1	0
655BA/5	107	6	81	13	24	1	1	1	438	101	6	1	3	2	5,8	0,7	5	1	2	2	1	1	1
655BA/6	108	6	95	16	26	2	1	1	501	79	7	1	2	2	6,2	0,8	7	1	3	3	2	0	1
655BA/3	109	6	96	14	30	1	1	2	456	431	8	1	2	1	6,6	0,9	9	1	3	1	1	1	0
641BRA/14	110	7	74	12	24	1	1	1	473	405	13	1	2	1	7,2	0,9	6	2	3	1	2	0	0
641BRA/11	111	7	56	17	16	3	3	2	221	0	221	1	3	3	10,7	1,2	17	2	3	3	3	1	0
641BRA/12	112	7	57	15	15	3	3	3	164	0	164	1	3	3	11,2	1,0	14	2	2	3	3	1	0
641BRA/4	113	7	62	22	23	2	3	3	285	71	214	1	3	3	8,3	1,2	13	1	2	3	1	1	1
641BRA/6	114	7	67	15	19	2	3	3	209	0	209	1	1	3	9,8	0,9	11	2	1	3	3	1	1
641BRA/9	115	7	103	26	35	2	2	2	272	30	242	1	1	3	10,3	0,9	12	2	1	3	3	0	1

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
641BRA/8	116	7	67	17	18	2	3	3	125	15	110	2	3	3	9,2	1,1	12	1	1	3	3	1	0
641BRA/13	117	7	62	16	20	2	3	3	349	30	319	1	2	2	11,2	1,3	17	1	1	3	3	1	0
641BRA/11	118	7	64	12	19	2	3	3	411	0	411	1	3	3	10,8	1,0	11	1	1	3	3	1	0
641BRA/15	119	7	68	17	18	2	3	3	311	145	166	1	2	1	9,0	0,9	18	1	1	3	2	1	0
656BA/17	120	6	121	11	42	1	1	1	610	591	6	1	3	1	6,2	0,7	6	1	2	2	1	0	0
656BA/15	121	6	117	13	35	1	1	1	677	625	8	1	3	1	6,7	0,8	9	1	2	1	1	0	0
656BA/20	122	6	118	15	35	1	1	1	548	0	548	1	1	1	6,2	0,7	5	1	1	2	1	0	0
656BA/21	123	6	129	10	28	1	1	2	607	207	7	1	2	1	5,7	0,6	6	1	2	1	1	0	0
656BA/13	124	6	143	14	37	1	1	1	664	442	8	2	3	1	5,8	0,6	7	1	2	2	1	0	0
656BA/23	125	6	85	10	29	1	1	2	506	401	4	1	3	2	6,8	0,6	6	2	3	1	2	0	0
656BA/4	126	6	98	15	33	1	1	1	733	627	5	1	3	1	5,2	0,5	6	2	1	2	2	0	0
656BA/14	127	6	110	12	40	1	1	1	528	265	4	1	3	1	6,3	0,5	7	1	2	2	1	0	0
656BA/1	128	6	92	15	33	1	1	1	526	444	5	1	2	1	5,8	0,7	6	2	1	2	2	0	0
656BA/2	129	6	92	9	26	1	1	1	626	523	8	1	2	1	6,0	0,5	6	1	1	1	2	0	0
656BA/3	130	6	110	14	45	1	1	1	345	336	4	1	1	1	5,9	0,5	8	2	1	2	1	0	0
656BA/9	131	6	90	11	26	1	1	1	680	527	5	1	2	1	6,3	0,6	7	1	1	1	2	0	0
656BA/19	132	6	133	15	50	1	1	1	692	557	4	1	3	1	6,2	0,5	6	1	1	2	1	0	0
656BA/18	133	6	131	15	43	1	1	1	741	558	3	1	3	1	5,1	0,4	5	1	2	2	2	0	0
656BA/16	134	6	131	13	38	1	1	1	713	576	5	1	3	1	6,1	0,5	6	1	1	2	1	0	0
656BA/12	135	6	100	16	36	1	1	1	652	575	6	1	2	1	6,5	0,5	6	1	1	2	1	0	0
656BA/11	136	6	102	14	39	1	1	1	612	444	5	1	2	1	6,1	0,5	5	1	1	2	1	0	0
656BA/6	137	6	103	11	30	1	1	1	395	355	4	1	3	1	5,9	0,5	7	1	1	3	1	0	0
656BA/5	138	6	90	16	31	1	1	1	603	497	5	1	2	1	6,2	0,7	8	1	1	2	1	0	0
656BA/22	139	6	105	11	34	1	1	1	722	675	7	1	3	1	5,9	0,6	4	1	1	2	2	0	0
656BA/8	140	6	90	12	32	1	1	1	305	295	3	1	3	1	6,3	0,5	8	1	2	3	1	0	0
656BA/7	141	6	114	15	43	1	1	1	637	562	10	1	3	1	5,5	0,7	6	1	1	2	1	0	0
652BA/5	142	6	71	14	25	1	1	3	188	75	11	2	2	1	9,6	1,2	12	1	3	2	1	0	0
652BA/17	143	6	107	12	32	1	1	2	533	488	5	1	2	2	7,0	0,8	6	1	3	2	1	1	1
652BA/2	144	6	113	18	31	2	1	3	540	260	4	1	2	3	6,5	0,9	6	1	2	2	1	1	1
652BA/10	145	6	100	17	25	2	1	3	555	75	4	3	2	3	6,9	0,9	8	1	3	2	1	1	1
652BA/8	146	6	80	13	27	2	1	3	536	336	9	2	2	2	8,2	1,0	7	1	3	2	1	1	1
652BA/15	147	6	93	13	18	1	1	3	450	423	8	2	2	1	6,3	0,9	6	1	3	2	1	0	0
652BA/13	148	6	81	13	19	1	1	2	569	79	6	1	1	1	5,8	0,7	6	1	2	2	1	1	1
652BA/7	149	6	106	13	21	1	1	3	587	145	19	1	1	1	5,7	0,7	7	1	1	1	1	1	1
652BA/6	150	6	86	14	21	2	1	3	521	157	8	2	2	2	6,0	0,9	9	1	3	2	1	1	1
652BA/1	151	6	95	15	24	2	1	3	453	423	4	2	2	2	5,8	0,8	7	1	3	2	1	1	0
652BA/9	152	6	102	17	25	2	1	3	539	45	3	3	2	3	7,0	0,9	8	1	2	2	1	1	1
649BA/6	153	6	74	14	18	2	1	3	521	278	8	1	2	3	6,3	0,7	6	1	2	2	2	1	1
649BA/3	154	6	99	19	23	1	1	2	574	96	6	1	2	2	6,5	0,8	8	1	2	2	1	1	1
649BA/7	155	6	109	10	33	1	1	1	590	533	10	3	2	3	6,7	0,8	8	2	3	2	1	1	0
649BA/4	156	6	69	12	32	2	1	3	517	24	15	3	3	2	8,1	1,1	10	1	3	2	2	1	1
642BRA/3	157	7	57	18	22	2	3	3	194	25	169	1	3	1	9,4	1,1	15	1	3	3	1	1	1
661BRA/3	158	7	40	8	12	2	3	3	127	81	46	3	3	2	7,5	0,9	9	1	3	3	1	0	0
661BRA/2	159	7	68	11	24	1	1	2	235	21	10	2	3	2	8,0	1,0	12	1	3	3	3	1	1
292BA2a/3	160	6	84	7	28	1	1	2	519	172	7	1	2	1	6,3	0,8	6	1	2	2	1	1	0
643BRA/3	161	7	34	8	10	3	3	3	136	0	136	2	3	3	10,1	1,1	14	2	3	3	3	1	0
515BRA/12	162	7	52	8	15	2	3	2	184	11	77	2	3	3	8,7	1,1	9	2	1	3	3	1	1
653BRA/5	163	7	48	13	18	2	2	3	254	219	21	1	2	3	8,1	1,0	10	1	1	3	3	0	0
651BRA/2	164	7	41	7	12	2	2	3	143	12	57	1	2	3	6,3	0,7	10	2	2	3	3	0	0
515BRA/3	165	7	41	5	12	1	3	3	118	32	86	1	2	2	7,6	0,9	9	2	1	2	3	1	1
648BRA/7	166	7	44	11	17	2	3	1	216	16	200	1	2	3	8,1	0,9	11	1	1	3	3	1	0
646PI/11	167	5	55	8	22	2	3	3	142	0	142	1	3	3	8,8	1,0	12	2	2	3	3	1	0
654PI/5	168	5	20	7	7	3	3	3	91	0	91	1	3	1	8,4	0,9	8	1	3	3	1	1	0
654PI/13	169	5	30	6	10	3	3	2	114	0	114	3	3	2	9,4	1,2	9	1	3	3	1	1	0
660BRA/5	170	7	40	8	15	2	3	3	202	0	202	2	3	3	8,7	1,1	11	1	3	3	2	1	0
506PI/17	171	5	42	8	14	2	3	3	107	0	107	1	3	3	9,9	0,9	13	1	3	3	2	1	0
648BRA/17	172	7	49	7	17	1	2	1	156	21	135	1	3	3	8,0	1,1	12	2	1	2	3	1	0
648BRA/2	173	7	37	8	11	2	3	2	119	16	103	1	3	3	8,1	1,0	9	1	1	3	3	1	0
506PI/23	174	5	31	6	12	2	2	3	71	44	27	2	3	2	7,4	1,2	10	2	2	2	3	1	0

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
654PI/18	175	5	48	9	16	2	3	3	83	0	83	1	3	1	7,0	0,9	9	1	1	3	1	1	0
296PI/1	176	5	20	8	7	3	3	2	84	0	84	1	3	1	8,3	1,1	9	1	1	3	2	1	0
515BRA/14	177	7	26	7	11	2	3	3	70	0	70	3	3	2	7,3	0,9	9	1	3	3	2	0	0
505PI/24	178	5	32	7	12	1	3	2	129	0	129	1	2	1	8,9	1,2	9	1	1	3	1	1	0
505PI/18	179	5	42	9	12	2	3	2	99	0	99	1	3	2	8,6	1,0	13	1	3	3	1	1	0
505PI/19	180	5	19	6	5	1	3	1	122	0	122	1	2	1	6,9	0,8	8	1	1	3	3	1	0
505PI/2	181	5	28	7	8	2	3	2	110	0	110	1	3	2	9,4	1,3	13	1	1	3	2	1	0
505PI/7	182	5	27	8	9	2	3	3	87	0	87	1	3	3	8,3	1,0	10	1	3	3	3	1	0
505PI/6	183	5	36	7	11	2	3	3	173	0	173	1	3	3	9,4	0,9	11	1	3	2	3	1	0
505PI/23	184	5	26	9	11	2	3	3	81	0	81	1	3	1	8,1	0,8	8	1	3	3	3	1	0
505PI/9	185	5	40	8	12	2	3	3	61	0	61	1	3	1	6,5	1,0	10	1	1	3	2	1	0
505PI/10	186	5	45	12	15	2	3	3	281	0	281	1	2	1	10,0	1,0	11	1	1	3	3	1	0
505PI/10	187	5	37	12	16	2	3	2	135	107	28	1	3	2	7,3	0,8	10	1	1	3	3	1	0
505PI/18	188	5	62	10	15	2	3	2	175	0	175	1	3	3	7,9	1,1	13	1	2	3	3	1	0
505PI/20	189	5	49	9	19	2	3	2	221	0	221	1	3	2	9,8	0,9	12	1	3	3	3	1	0
505PI/1	190	5	59	10	14	2	3	3	129	0	129	1	2	3	10,0	0,9	13	1	1	2	3	1	0
505PI/8	191	5	53	9	14	2	3	2	166	0	166	1	2	1	10,1	0,9	14	1	1	3	2	1	0
505PI/22	192	5	29	8	10	2	3	3	82	0	82	1	3	3	10,0	1,2	13	1	1	2	2	1	0
505PI/21	193	5	32	10	9	2	2	2	144	0	144	1	3	3	9,7	1,1	14	2	1	3	2	1	1
505PI/11	194	5	74	12	23	1	3	3	246	66	8	1	3	2	7,0	1,0	8	1	2	2	2	1	0
506PI/18	195	5	43	10	17	1	3	2	166	0	166	1	3	1	9,4	1,2	13	1	1	3	1	1	0
506PI/25	196	5	65	11	20	1	3	3	151	61	90	1	3	1	9,4	1,0	12	2	3	3	1	1	1
506PI/12	197	5	52	10	17	2	3	2	206	0	206	1	2	1	8,4	1,2	11	1	3	3	1	1	0
506PI/21	198	5	79	15	22	2	3	3	250	0	250	1	2	2	9,7	1,1	13	1	2	2	1	1	0
506PI/6	199	5	30	9	11	2	3	3	70	0	70	1	3	2	8,6	1,1	12	1	1	3	2	1	0
506PI/7	200	5	85	7	13	1	3	2	179	0	179	1	2	3	10,2	1,2	10	1	3	3	2	1	0
506PI/28	201	5	84	15	26	2	2	2	374	0	374	2	2	2	10,7	1,4	15	1	3	3	1	1	0
506PI/5	202	5	68	11	20	2	3	2	123	0	123	1	2	1	7,8	1,1	6	1	1	3	1	1	0
506PI/20	203	5	39	7	13	1	3	2	140	0	140	1	3	3	9,0	1,1	14	1	3	3	1	1	0
506PI/24	204	5	32	9	10	3	3	2	77	0	77	1	3	1	8,0	1,1	10	1	1	3	1	1	0
511PI/7	205	5	29	7	7	3	3	2	141	0	141	1	3	3	9,1	1,3	11	1	1	3	2	1	1
511PI/12	206	5	19	4	6	3	3	3	88	0	88	1	2	1	8,3	0,9	9	1	3	3	2	1	0
511PI/12	207	5	40	8	13	2	3	3	232	0	232	1	2	2	9,9	1,1	12	1	1	2	2	1	0
511PI/1	208	5	48	13	17	3	3	3	160	0	160	1	2	3	8,3	1,1	13	1	2	3	3	1	0
511PI/4	209	5	58	10	18	2	3	2	208	0	208	1	2	3	10,0	1,0	11	1	3	2	2	1	1
511PI/7	210	5	27	5	8	2	3	3	162	0	162	1	3	3	9,0	1,1	13	1	1	3	2	1	1
511PI/18	211	5	46	7	10	2	3	3	192	0	192	2	2	2	9,5	1,1	13	1	3	2	1	1	0
511PI/19	212	5	47	8	10	2	2	2	241	0	241	1	2	3	10,0	1,1	14	1	1	2	3	1	1
511PI/11	213	5	46	15	11	3	2	1	98	0	98	1	2	1	9,5	1,1	14	1	1	3	2	1	0
511PI/14	214	5	34	6	11	2	3	3	167	0	167	1	3	3	10,4	1,2	16	2	1	3	3	1	0
511PI/5	215	5	39	7	11	2	2	3	117	0	117	1	2	3	9,1	1,1	14	2	1	2	3	1	0
511PI/8	216	5	55	11	17	1	3	3	157	0	157	1	2	2	10,3	1,2	16	1	3	2	1	1	0
511PI/1	217	5	57	10	16	2	2	3	201	0	201	2	2	3	9,3	1,3	15	1	3	2	1	1	0
511PI/20	218	5	65	8	15	2	2	2	203	0	203	1	2	3	8,2	1,1	16	1	3	1	1	1	0
511PI/13	219	5	61	10	18	1	3	3	139	0	139	1	2	3	10,2	1,3	13	1	2	2	2	1	0
646PI/2	220	5	18	6	6	2	3	3	109	0	109	1	3	1	8,6	0,9	12	1	1	3	1	0	0
646PI/13	221	5	20	7	6	3	3	1	116	0	116	1	3	1	8,2	0,9	12	1	1	2	1	1	0
646PI/12	222	5	33	6	8	3	3	3	146	0	146	1	3	3	9,2	1,1	11	1	2	2	1	1	0
646PI/22	223	5	27	8	7	2	3	3	108	0	108	1	2	1	8,8	1,1	11	1	1	2	3	1	0
646PI/15	224	5	47	11	15	3	3	2	263	0	263	1	3	2	9,6	1,0	15	1	1	2	1	1	1
646PI/13	225	5	38	9	10	2	3	2	126	0	126	1	3	1	8,0	0,9	13	1	1	2	2	1	0
646PI/3	226	5	45	10	12	2	3	2	161	69	92	1	3	1	8,4	1,2	10	1	1	2	1	1	0
646PI/7	227	5	31	5	6	2	3	2	104	0	104	1	3	3	8,4	0,9	10	1	1	2	2	1	0
646PI/20	228	5	21	5	5	2	3	2	100	0	100	1	3	2	9,4	0,9	10	1	1	2	3	1	1
646PI/1	229	5	69	14	21	2	2	1	375	68	307	1	1	3	8,7	0,8	12	2	1	2	3	1	1
646PI/5	230	5	73	13	17	2	0	0	242	0	242	1	2	3	9,8	0,9	12	2	1	2	3	1	0
646PI/18	231	5	27	4	5	2	3	2	225	40	185	1	1	1	7,1	0,7	10	2	1	2	1	1	0
646PI/10	232	5	31	6	8	2	3	2	160	0	160	1	1	1	9,3	0,9	11	1	1	3	2	1	0

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
646PI/9	233	5	45	8	11	2	3	3	149	0	149	1	2	1	9,3	1,0	13	1	1	2	2	1	0
646PI/14	234	5	40	7	10	2	3	1	141	0	141	1	2	1	9,3	1,0	13	1	1	3	1	1	0
646PI/19	235	5	49	11	11	2	3	3	136	0	136	1	2	3	8,1	0,8	10	1	1	2	1	1	0
646PI/2	236	5	25	6	11	2	3	1	140	0	140	1	3	3	8,6	1,1	9	1	1	2	1	1	0
654PI/7	237	5	30	7	10	2	3	3	62	0	62	1	3	2	8,0	0,9	10	1	3	2	1	1	0
654PI/12	238	5	25	6	6	2	3	3	105	0	105	1	2	2	7,5	0,7	8	1	2	3	1	1	0
654PI/19	239	5	48	15	18	3	3	2	228	0	228	1	1	1	8,4	1,1	12	1	2	2	1	1	0
654PI/8	240	5	50	15	16	2	3	3	262	0	262	1	2	1	7,9	1,1	10	2	3	2	1	1	0
654PI/3	241	5	54	21	16	3	3	2	189	0	189	1	2	2	10,1	1,2	15	2	3	3	2	1	1
654PI/17	242	5	52	9	16	2	3	3	221	45	176	1	2	1	7,9	0,8	9	1	1	3	2	1	1
654PI/14	243	5	57	20	20	3	3	3	332	33	276	1	2	1	9,5	1,0	13	2	2	3	1	1	0
654PI/29	244	5	37	9	12	2	3	3	137	0	137	1	1	3	8,9	1,2	12	1	3	2	2	1	0
654PI/26	245	5	21	6	6	3	3	2	77	0	77	1	2	2	7,4	1,1	11	1	2	2	1	1	0
654PI/27	246	5	33	8	9	2	2	1	91	0	91	1	2	2	8,7	1,0	10	2	2	2	2	1	0
656LE	247	4	124	7	36	1	1	1	466	433	20	1	1	1	5,9	1,0	6	2	1	2	1	0	0
649BA	248	2	67	9	17	1	1	1	489	319	9	1	1	2	6,1	0,9	6	2	2	2	1	1	0
492BA	249	2	153	35	39	1	1	2	695	420	15	1	2	1	6,9	0,9	9	1	2	2	1	1	0
662BA	250	2	112	10	24	1	1	1	485	396	6	1	1	1	5,3	0,8	8	1	2	1	1	1	0
652BA	251	2	60	7	19	1	1	2	239	0	5	1	3	1	5,6	0,8	6	1	3	2	1	1	0
659BA	252	2	62	7	13	2	1	1	456	302	7	1	3	1	6,6	0,7	6	2	3	1	2	1	0
491BA	253	2	106	6	25	1	1	1	438	0	5	1	2	1	5,3	0,8	9	2	1	1	1	0	0
494BA	254	2	72	10	23	1	2	1	454	351	4	1	2	1	5,8	0,6	7	1	1	3	2	0	0
494BA	255	2	94	15	26	1	2	2	788	662	6	1	1	1	6,4	0,5	6	1	1	1	1	0	1
492BA	256	2	142	9	26	1	1	1	761	651	8	1	1	1	6,2	0,5	7	1	1	1	2	0	0
VysočanyBA	257	2	92	12	27	2	1	1	485	395	5	1	2	1	6,2	0,7	10	1	3	2	1	0	0
292BA	258	2	140	14	41	1	1	1	497	272	5	1	2	1	7,1	0,7	6	2	2	1	1	0	0
BA	259	9	135	10	35	1	1	1	654	604	3	1	1	1	5,6	0,6	6	1	1	3	1	1	1
491BA	260	2	172	8	27	1	1	1	755	575	8	1	2	1	6,5	0,6	10	1	1	2	2	1	0
494BA	261	2	145	10	37	1	1	1	710	580	13	1	1	1	7,0	0,9	10	1	3	2	1	0	0
BA	262	9	128	9	26	1	1	1	567	523	6	1	1	1	6,3	0,8	6	1	1	2	2	1	0
659BA	263	2	117	8	38	1	1	2	404	351	11	3	2	1	6,2	0,7	9	2	1	2	2	1	0
656LE	264	4	129	12	41	1	1	1	504	473	11	2	1	1	6,5	0,7	6	1	1	3	2	0	0
1028BA	265	2	110	15	33	1	1	3	350	328	4	1	2	1	6,8	0,9	10	1	3	1	1	0	0
1017BA	267	2	77	8	27	1	1	1	424	389	7	1	2	1	6,7	1,0	6	2	1	2	2	0	0
VYS2005no.11 BA	268	9	122	12	29	1	1	2	543	329	8	1	1	1	7,1	0,9	11	3	1	2	2	1	0
VYS2005no.7 BA	269	9	68	8	19	1	2	1	355	18	3	1	3	1	6,2	0,6	6	1	1	2	1	0	0
VYS2005no.32 BA	270	9	108	15	35	2	1	1	313	188	8	1	1	1	6,4	0,7	8	1	3	1	1	1	0
1029LEP	271	4	55	6	15	2	2	1	172	5	21	1	3	1	7,4	0,8	11	2	3	3	1	1	0
VYS2005no.2	272	9	91	10	21	2	1	1	325	303	2	2	2	1	5,2	0,5	5	1	2	2	1	0	0
1015BRA-LEP	273	4	56	12	22	2	2	3	290	262	11	1	2	1	8,4	1,1	10	2	2	2	1	1	0
VYS05no.24 LEP	275	9	58	10	16	2	3	2	178	25	12	1	3	2	7,8	0,9	8	1	0	3	3	1	0
1022LEP	276	4	98	18	31	2	3	3	200	158	6	1	3	1	8,3	1,0	10	1	3	2	1	1	0
1022LEP	277	4	100	21	23	2	2	3	479	141	206	2	2	1	9,9	1,2	15	2	3	2	1	1	0
657LEP	278	4	156	12	30	2	1	1	568	260	13	1	1	1	5,7	0,7	5	1	1	1	1	0	1
647BA	279	2	121	11	26	1	1	1	572	302	5	1	1	1	6,3	0,8	6	2	2	1	1	0	0
657LE	280	4	142	13	35	1	1	1	644	574	4	1	2	1	6,1	0,6	6	1	1	2	1	0	0
657LE	281	4	140	15	49	1	1	1	586	555	8	1	2	1	6,6	0,7	7	1	1	2	1	0	0
302PI	282	1	46	13	19	2	3	3	108	0	108	1	3	3	10,0	1,2	18	1	3	3	1	1	0
303PI	283	1	61	13	20	2	3	3	103	0	103	1	3	2	10,2	1,1	17	1	3	3	1	1	0
1019PI-BRA	284	1	96	18	19	2	3	3	240	0	240	1	3	3	10,7	1,2	14	1	3	3	1	1	0
VYS2005no.1	285	9	45	7	15	2	2	1	191	0	191	1	3	1	7,8	0,9	8	1	1	2	2	0	0
1032PI	286	1	19	3	6	2	3	1	57	0	57	1	3	3	9,0	1,0	12	1	1	3	3	0	0
VYS2005no.27	287	9	43	6	13	2	3	2	44	0	44	1	3	2	7,1	1,1	9	1	1	3	2	0	0
500PI	288	1	57	12	15	2	3	1	185	0	185	1	2	1	7,9	0,8	11	1	1	3	1	1	0
301PI	289	1	49	10	15	2	3	3	182	0	182	1	3	2	9,4	1,2	13	1	2	3	1	1	0
503PI	290	1	53	10	15	1	3	1	131	0	131	1	2	3	10,2	1,1	14	1	1	3	3	1	0
646PI	291	1	23	5	7	2	3	3	88	0	88	1	3	1	8,9	1,1	13	1	1	3	2	1	0
497PI	292	1	36	7	11	2	3	3	90	0	90	1	3	1	8,3	1,1	13	1	1	3	1	1	0

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
305PI	293	1	47	9	15	2	3	3	107	0	107	1	3	2	9,3	1,3	15	1	1	3	2	1	0
300PI	294	1	54	14	19	2	2	1	173	0	173	1	2	2	8,8	1,2	15	1	3	3	1	1	0
654PI	295	1	41	10	9	3	3	3	71	0	71	1	3	1	8,5	1,3	14	2	3	3	1	1	0
516PI	296	1	98	16	25	2	3	2	128	0	128	1	3	3	9,0	1,2	13	1	2	3	1	1	0
509PI	297	1	65	13	21	2	3	2	162	0	162	1	3	1	9,8	1,4	16	1	1	3	1	1	0
496PI	298	1	90	21	37	2	3	3	210	0	210	1	3	3	12,1	1,4	15	1	2	2	2	1	0
497PI	299	1	56	12	17	2	3	3	101	86	15	1	3	1	7,5	1,0	13	1	1	3	2	1	0
1016BRA	300	3	81	10	29	1	2	2	201	42	159	1	2	1	7,1	1,0	10	1	1	1	2	1	0
1021BRA	301	3	80	18	32	1	1	1	236	54	17	1	2	1	9,0	1,1	11	1	3	2	1	1	0
1013BRA	302	3	64	12	20	1	1	1	246	132	7	1	1	1	7,5	1,2	7	2	1	1	3	1	0
1025BRA	303	3	78	12	30	1	2	1	262	119	8	1	2	1	6,6	0,8	10	2	2	2	1	1	0
1027BRA	304	3	57	11	23	1	2	2	98	28	47	1	3	1	8,0	1,0	11	1	1	2	2	1	0
295BRA	305	3	83	11	36	1	3	1	374	89	16	3	3	3	8,8	1,2	14	1	3	3	1	1	0
508BRA	306	3	90	11	28	1	2	1	361	111	15	1	1	2	7,1	0,8	9	1	2	2	2	1	1
493BRA	307	3	88	9	24	1	2	1	366	312	54	2	3	2	7,8	1,1	11	1	3	2	3	0	0
495BRA	308	3	134	11	35	1	2	2	314	259	55	1	3	2	7,3	0,9	9	1	1	2	3	1	0
498BRA	309	3	90	15	36	1	2	2	261	195	5	1	3	1	6,8	1,0	9	1	2	2	1	0	0
660BRA	310	3	64	12	22	2	2	2	250	129	120	1	3	1	9,0	1,1	13	1	3	2	1	1	0
501BRA	311	3	53	9	16	2	3	1	170	0	170	1	1	2	9,2	1,1	10	2	1	1	3	1	0
658BRA	312	3	123	7	18	1	1	1	617	457	9	1	1	1	6,8	0,9	8	2	1	1	1	1	0
517BRA	313	3	59	8	18	1	2	1	131	32	50	1	2	1	7,4	1,1	10	1	2	2	1	1	1
643BRA	314	3	79	9	24	1	3	1	149	7	47	1	3	1	9,2	0,9	11	1	3	2	1	1	0
650BRA	315	3	114	10	23	1	1	1	450	177	8	1	1	1	7,2	1,0	9	2	3	1	1	1	1
648BRA	316	3	72	10	22	1	2	1	273	0	273	1	2	1	8,7	1,2	9	1	2	3	2	1	0
514BRA	317	3	51	6	21	1	2	1	298	159	18	1	2	1	7,1	1,2	11	2	2	2	1	1	0
513BRA	318	3	72	11	21	1	2	1	225	171	20	1	2	1	7,6	0,9	8	2	2	2	2	1	0
508BRA	319	3	94	18	28	1	1	2	323	220	13	1	1	1	6,7	0,8	9	2	2	1	3	1	0
653BRA	320	3	44	5	16	1	1	1	403	365	5	1	1	1	6,0	0,8	7	1	2	1	1	1	0
515BRA	321	3	57	9	17	1	2	1	229	185	44	1	2	1	7,7	1,2	10	1	3	2	1	1	0
1024BRA	322	3	62	12	30	1	2	1	267	99	43	1	2	1	6,5	0,9	10	2	2	1	2	1	0
VYS2005no.4	323	9	41	9	16	2	3	1	98	0	98	1	2	1	6,8	0,9	10	1	2	1	1	0	0
VYS2005no.8	324	9	50	8	14	1	2	1	97	0	97	1	3	1	8,1	0,7	8	1	1	3	2	1	0
VYS2005no.10	325	9	42	7	11	2	2	1	102	96	6	1	2	1	7,9	0,9	9	1	3	3	2	1	0
VYS2005no.6	326	9	52	8	15	2	3	1	66	0	66	1	3	2	7,0	1,1	7	1	1	3	3	1	0
VYS2005no.20	327	9	45	8	14	1	3	3	46	32	14	2	3	1	7,8	1,0	8	1	1	3	2	0	0
VYS2005no.30	328	9	51	8	16	1	3	1	175	150	25	1	2	1	8,6	1,0	9	1	3	3	1	1	0
VYS2005no.5	329	9	58	9	23	1	1	1	310	293	17	1	2	1	8,3	0,9	10	1	3	2	1	1	0
VYS2005no.13	330	9	38	5	11	1	3	2	172	119	13	1	2	1	6,9	0,8	8	2	1	2	3	0	1
1023BRA	331	3	72	13	26	2	3	3	135	57	78	1	3	1	10,8	1,3	17	1	2	2	2	1	1
661/1BRA	332	3	60	9	15	2	1	2	442	398	9	1	1	1	6,4	0,8	8	1	1	1	1	0	0
651BRA	333	3	52	11	19	1	2	3	283	260	10	2	2	1	7,1	0,9	7	1	2	2	2	0	0
512BRA	334	3	46	8	15	1	3	3	131	120	11	1	2	1	6,8	0,9	10	1	3	3	1	1	0
295BRA	335	3	72	16	17	2	3	3	172	62	10	3	2	1	9,0	1,2	13	1	3	2	1	1	0
493BRA	336	3	91	17	29	1	2	3	256	50	64	1	2	2	8,4	1,0	10	2	3	1	2	1	0
641BRA	337	3	48	14	20	2	3	2	134	0	134	1	2	2	10,0	1,2	14	1	1	3	3	1	0
642BRA	338	3	100	11	33	2	2	3	199	0	199	1	1	1	9,4	1,1	12	1	3	2	1	1	0
514BRA	339	3	45	7	19	1	2	1	211	181	14	1	2	1	8,2	1,1	13	2	3	2	1	1	0
517BRA	340	3	59	10	18	2	2	2	137	67	36	1	2	2	7,0	1,1	11	1	2	2	1	1	0
504BRA	341	3	54	5	20	1	3	3	133	120	13	2	3	2	7,2	0,8	10	1	1	2	3	0	0
502BRA	342	3	20	6	14	2	3	1	107	41	66	1	2	2	8,6	0,9	10	1	3	3	2	1	0
512BRA	343	3	47	9	17	2	3	1	148	98	50	1	2	1	7,1	0,9	9	2	2	1	1	1	0
499BRA	344	3	66	12	21	2	3	1	165	40	125	1	3	2	8,5	1,1	15	1	3	3	1	1	0
495BRA	345	3	68	7	16	2	3	3	307	190	74	1	2	1	7,4	1,0	10	1	1	2	3	1	0
501BRA	347	3	39	8	11	2	3	2	89	39	50	1	3	3	7,1	0,9	8	1	1	2	3	0	0
645BRA	348	3	34	8	13	2	3	3	99	53	22	1	2	1	5,6	0,7	9	1	1	2	2	0	0
643BRA	349	3	32	5	7	1	3	2	115	85	30	1	3	1	7,5	0,9	10	1	3	1	1	0	0
508BRA	350	3	71	15	21	1	1	1	299	83	42	1	1	2	7,0	0,9	12	1	3	1	1	1	0
507BRA	351	3	77	12	29	1	2	1	224	145	32	1	1	1	6,8	1,0	9	1	3	1	1	1	0

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24
513BRA	352	3	71	8	18	1	2	1	275	234	10	2	1	1	7,8	1,1	9	1	3	2	2	0	0
513BRA	353	3	102	10	19	1	3	3	456	263	31	1	1	1	7,0	0,9	8	1	1	1	3	0	0
507BRA	354	3	81	16	22	1	2	3	378	45	29	1	1	1	6,3	0,6	8	1	2	1	2	0	0
BRA	355	9	109	16	32	2	2	1	484	185	7	2	1	2	6,6	0,9	8	2	3	1	3	1	0
BRA	356	9	89	12	31	1	3	1	350	244	16	1	1	2	7,3	0,9	10	1	2	1	2	1	0
515BRA	357	3	102	16	20	1	2	3	546	12	16	1	1	1	8,4	0,9	11	1	3	1	1	0	1
515BRA	358	3	71	10	20	1	2	2	431	258	34	1	1	2	7,6	0,9	7	2	3	1	1	0	0
658BRA	359	3	72	10	21	1	1	3	498	477	19	1	2	1	5,0	0,6	6	2	2	2	2	0	0
BRA	360	9	60	11	16	1	1	1	285	258	5	1	1	1	5,1	0,5	4	1	1	2	1	1	0

Appendix 2

Primary morphometric data used in the paper no. 3 (Morphological variation of *Pilosella officinarum*). Following chracters are included in the table below:
v1 - ploidy level (1-4x,2-5x,3-6x sex, 4-6x apo)
v2 - locality number
v3 - plant number
v4 - the longest leaf shape - (1-oblanceolate to 3-ovate) - LS
v5 - leaf length - LL
v6 - leaf width - LW
v7 - distance between the widest point of leaf to the tip - WT
v8 - rate of leaf tip acuteness (1-round, 2-obtuse, 3-acute) - TA
v9 - density rate of single trichomes in the centre of the upper surface of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs) - SL
v10 - average length of 10 single trichomes at the centres of 5 leaf surfaces - SLC
v11 - density rate of single trichomes at the margins of the upper surface of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs) - SM
v12 - average length of 10 single trichomes at the margins of 5 leaf surfaces - SLM
v13 - density rate of stellate trichomes on the adaxial side of 5 leaves (1-glabrate, 2-single trichomes, 3-dense hairs) - SEL
v14 - density rate of glandular trichomes on the stem (1-glabrate, 2-single trichomes, 3-dense hairs) - ES
v15 - density rate of single trichomes on the stem (1-glabrate, 2-single trichomes, 3-dense hairs) - SS
v16 - average length of 5 involucral bracts - BL
v17 - average width of 5 involucral bracts - BW
v18 - rate of dark trichome density on the involucrum (1-bright, 2-dark trichomes) - ID
v19 - density rate of glandular trichomes on the involucrum (1-glabrate, 2-single trichomes, 3-dense hairs) - EI
v20 - glandular trichome colour - translucent to black - EC
v21 - density rate of single trichomes on the involucrum (1-glabrate, 2-single trichomes, 3-dense hairs) - SI
v22 - density rate of stellate trichomes on the involucrum (1-glabrate, 2-single trichomes, 3-dense hairs) - SEI
v23 - capitulum diameter - CD
v24 - density rate of single trichomes on the stolons (1-glabrate, 2-single trichomes, 3-dense hairs) - STS
v25 - density rate of stellate trichomes on the stolons (1-glabrate, 2-single trichomes, 3-dense hairs) - SEST
v26 - stolon leaves (1-diminishing, 2-same-sized, 3-enlarging) - SE
v27 - average length of 3 stolon leaves - SL
v28 - average width of 3 stolon leaves - SW
v29 - average diameter of 3 stolon - SD
v30 - rate of stolon curve shape (1-linear, 2-curved shape) - SC

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31
1	1	2	1	1	67	13	17	2	1	4,6	1	3,4	1	3	2	9,2	1,1	2	2	3	3	1	14	2	2	3	19,0	5,7	1,0	1
1	1	3	1	1	75	16	20	2	2	5,9	2	4,2	1	3	2	8,1	1,1	2	2	3	3	0	16	3	2	3	21,3	6,3	1,4	1
1	2	1	1	1	35	8	12	3	2	4,3	2	3,9	1	2	2	8,5	1,2	1	2	3	2	1	22	3	2	3	12,7	3,3	1,1	1
1	3	1	1	1	33	7	10	2	2	3,8	2	2,2	1	2	1	7,1	1,0	3	3	1	3	1	21	2	2	3	7,7	2,0	0,6	1
1	7	2	1	1	42	13	8	1	1	4,2	1	2,8	1	2	1	8,2	1,1	2	2	3	3	1	25	2	2	2	22,0	5,0	0,7	1
1	8	1	1	1	51	14	11	2	2	4,3	2	3,4	2	2	1	7,8	0,9	1	2	3	1	0	16	3	2	2	28,3	7,3	1,1	1
1	8	2	1	2	67	17	19	1	2	3,3	2	3,0	1	2	1	8,5	1,3	2	2	3	1	0	27	3	2	3	26,0	8,3	0,9	1
1	9	2	1	1	55	12	18	3	2	4,1	2	3,5	1	3	2	7,4	1,1	3	2	1	3	0	16	3	2	2	24,3	5,7	1,5	1
1	11	1	1	1	57	14	16	2	3	5,2	3	4,3	1	2	1	7,9	1,0	2	1	3	1	1	21	3	3	3	22,3	5,0	1,3	1
1	11	2	1	1	41	9	12	2	1	4,2	2	3,2	1	2	1	7,6	1,1	2	2	3	1	1	22	3	2	3	16,7	3,7	0,6	1
1	11	3	1	1	40	7	10	3	2	3,3	2	2,3	1	3	1	7,7	1,1	2	1	3	1	0	26	2	2	3	18,0	3,7	0,6	1
1	11	4	1	2	51	12	17	2	2	5,2	2	4,9	1	2	2	7,1	1,1	1	1	1	3	1	13	2	2	3	17,3	3,3	0,9	1
1	12	2	1	3	41	15	15	2	3	4,9	3	4,2	1	2	1	6,3	1,2	2	1	3	2	1	18	2	2	2	13,7	2,3	0,3	1
1	13	1	1	2	48	12	12	2	1	4,2	1	3,6	1	2	2	7,5	1,1	2	1	3	2	1	23	2	2	2	16,0	2,3	0,5	1
1	14	1 I.	1	2	61	12	17	1	1	3,0	1	2,3	1	2	1	7,8	1,1	2	1	3	2	1	15	2	3	3	22,3	5,7	1,3	1
1	14	2	1	2	52	13	11	1	1	5,0	1	3,2	1	2	1	7,7	1,1	2	2	3	2	1	12	2	2	2	18,3	5,7	1,4	1
1	17	1 I.	1	1	92	9	13	2	1	2,8	2	2,0	2	3	1	7,7	0,9	2	2	3	1	0	23	2	2	2	22,3	5,0	1,1	2
1	21	1	1	1	25	6	7	3	2	3,7	2	2,5	1	2	1	7,3	1,1	2	2	1	1	1	11	2	2	2	16,7	6,0	0,3	1
1	22	1	1	1	31	7	12	3	2	2,9	2	2,6	1	2	1	6,1	1,0	1	1	3	2	1	18	2	2	2	14,3	4,3	0,4	1
1	23	1	1	3	40	12	14	1	1	3,4	2	2,5	1	2	1	7,2	1,2	2	2	3	1	1	16	3	2	2	22,7	7,0	1,0	1
1	23	3	1	1	28	7	7	2	3	3,6	3	3,4	1	2	1	6,8	1,1	2	2	3	1	1	20	2	3	2	16,0	6,0	0,8	1
1	24	1	1	1	46	14	15	2	1	2,5	1	2,2	2	3	1	8,9	1,2	3	2	1	1	0	25	3	2	2	24,0	6,0	1,2	1
1	24	3	1	1	44	10	13	2	2	4,6	2	3,9	1	3	1	7,6	1,1	3	2	1	1	0	15	2	3	2	16,0	4,7	1,1	1
1	26	2	1	1	30	11	10	3	1	3,2	1	2,7	2	2	2	7,5	1,2	2	1	1	2	0	26	2	2	2	12,0	3,3	0,7	1
1	27	2	1	1	41	11	13	3	3	4,3	3	3,8	1	2	2	7,3	1,1	2	2	3	2	1	15	3	2	2	21,7	6,0	0,8	1
1	29	3	1	4	58	17	18	1	1	4,6	1	3,8	2	2	2	8,6	1,2	2	2	3	3	1	27	3	2	2	37,0	11,0	1,3	1
1	31	2	1	2	62	12	13	1	2	5,9	2	3,9	1	2	2	8,0	1,0	1	1	3	2	1	24	3	3	2	43,0	13,7	1,3	1
1	31	3 II.	1	2	64	16	20	1	1	4,8	1	3,3	1	2	2	8,6	1,1	2	2	3	2	1	23	3	3	3	28,0	8,7	1,3	1
1	35	1	1	1	32	9	10	3	1	5,2	1	3,7	1	2	2	7,8	1,1	1	1	3	2	1	15	2	2	2	15,7	6,0	1,0	1
1	35	3	1	1	51	16	18	3	1	5,4	1	4,1	1	2	2	8,2	1,3	1	2	3	3	1	22	3	3	2	26,7	9,0	1,2	1
1	36	2	1	1	49	13	16	3	3	6,3	3	5,0	1	2	1	8,5	1,3	2	2	1	1	1	27	3	2	2	31,0	6,3	1,2	1
1	37	1	1	1	28	7	7	2	2	2,7	2	2,2	1	2	1	7,5	1,0	2	2	1	1	1	23	2	3	2	7,7	2,3	0,8	1
1	37	2	1	1	23	7	9	2	2	4,6	2	3,6	1	2	1	7,8	1,1	2	2	3	1	1	29	2	2	2	10,0	3,7	0,0	1
1	41	2	1	1	52	12	12	2	2	4,9	2	4,2	1	2	1	7,6	1,1	2	2	1	1	1	21	2	2	2	23,7	6,0	1,2	2
1	44	1	1	1	27	8	7	2	3	4,6	3	3,6	1	2	1	7,0	1,0	2	2	3	1	1	9	2	2	1	18,3	5,7	0,3	1
1	44	2	1	1	37	10	8	2	1	5,1	1	2,9	1	2	1	8,0	1,2	2	2	3	1	1	20	2	2	3	10,0	3,3	0,5	1
1	44	3 II.	1	3	38	11	12	2	1	3,7	1	3,1	1	3	1	7,1	1,3	2	2	1	1	1	19	3	2	2	17,7	5,3	1,1	1
1	45	2	1	4	24	8	8	3	3	3,7	3	2,8	1	2	2	8,8	1,1	2	2	1	2	1	18	2	2	2	13,3	4,0	0,6	1
1	46	1	1	4	21	7	7	1	3	3,9	3	2,3	1	2	1	7,4	1,0	2	1	1	2	0	13	2	3	2	13,7	5,3	0,4	1
1	46	2	1	2	52	14	14	2	2	5,0	2	4,1	1	3	1	8,7	1,1	2	1	3	1	1	21	3	2	3	15,7	5,0	1,1	1
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1	48	2 II.	1	1	56	10	15	2	1	3,3	1	2,5	1	2	1	7,8	1,1	1	1	3	1	1	23	3	2	3	28,0	6,7	0,7	1
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1	65	1	1	1	38	7	10	3	2	2,9	2	2,3	1	3	2	7,5	1,0	2	2	3	1	1	14	2	2	2	21,7	5,3	1,1	1

v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31
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1	66	2	1	1	39	11	13	2	1	4,9	2	2,8	1	2	2	8,9	1,0	2	1	3	2	1	17	2	3	2	13,7	3,3	1,2	1
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1	71	2 II.	1	2	83	18	15	2	1	5,1	2	3,5	1	3	2	8,9	1,0	2	2	1	3	1	20	3	2	3	39,7	11,3	1,4	1
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1	60	3	1	4	33	10	16	3	1	3,6	1	2,7	1	2	1	5,6	0,9	2	1	3	1	0	17	2	2	2	22,7	5,3	0,4	1
1	60	2 II.	1	1	59	13	23	3	2	4,8	2	3,4	1	2	1	7,7	1,0	1	1	3	1	0	16	3	2	3	22,7	6,3	1,9	1
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1	117	2	1	1	58	14	11	1	1	2,9	1	2,5	1	2	1	8,5	1,3	2	1	3	1	1	24	2	2	3	19,7	4,7	1,7	1
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1	158	3	1	2	85	22	18	2	1	3,5	2	2,7	1	2	1	8,3	1,3	1	2	3	1	1	15	3	3	2	18,3	7,7	1,6	1
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1	136	1	1	2	40	13	10	1	1	2,6	2	2,2	1	2	1	7,2	1,0	2	1	1	4	1	11	3	3	2	26,0	6,7	1,4	1
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v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31
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3	77	2 II.	3	1	49	12	12	3	1	6,4	2	5,1	1	3	2	10,4	1,1	2	2	3	3	1	31	3	3	2	26,3	6,0	1,6	2
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v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31
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v1	v2	v3	v4	v5	v6	v7	v8	v9	v10	v11	v12	v13	v14	v15	v16	v17	v18	v19	v20	v21	v22	v23	v24	v25	v26	v27	v28	v29	v30	v31
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3	196	6	3	1	98	19	24	3	1	6,9	2	5,4	1	3	1	8,9	1,3	2	2	3	2	1	20	3	3	3	22,7	4,3	1,6	2
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