

Rare hybrid swarm of *Pilosella polymastix* × *P. officinarum*: cytotype structure and modes of reproduction

Vzácný hybridní roj *Pilosella polymastix* × *P. officinarum* – skladba cytotypů a způsoby reprodukce

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Dedicated to Siegfried Bräutigam on the occasion of his 70th birthday

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We studied a small, spatially limited population of *Pilosella* plants, rich in morphological types, in the southwestern part of central Bohemia, Czech Republic. The following tetraploid parental *Pilosella* species putatively gave rise to the hybrid swarm analysed: sexual *P. officinarum* and apomictic *P. polymastix* (*P. bauhini*–*P. caespitosa*). In addition, the swarm consisted of (i) a stabilized tetraploid apomictic *P. melinomas* (*P. officinarum* × *P. polymastix*) represented by two isozyme phenotypes (one dominating), and (ii) tetraploid and sexual hybrids between *P. officinarum* × *P. polymastix*, with 16 isozyme phenotypes in the 18 plants analysed. We also found pentaploid *P. bauhini* (three plants comprising one isozyme phenotype), one hexaploid plant corresponding to *P. melinomas* (putative $2n + n$ hybrid) and one pentaploid plant (probably a hybrid between hexaploid and unknown tetraploid). The single triploid plant detected in the hybrid swarm is probably of polyhaploid origin. Both *P. polymastix* and *P. melinomas* are rare hybrid species, which because they had not been recorded for many years were considered as probably extinct in the Czech Republic.

Key words: chromosome number, hybrid swarm composition, *Pilosella*, ploidy level, rare species, reproductive modes

Introduction

The genus *Pilosella* (*Hieracium* subgen. *Pilosella*) is one of the most taxonomically complicated in Europe. This complexity is caused by a combination of various breeding systems accompanied by a high incidence of hybridization and polyploidy (Krahulcová et al. 2000, Fehrer et al. 2007). During last few years we have developed a novel approach to studying populations consisting of sexual and apomictic species by analysing the ploidy levels and breeding systems of plants growing in the field, including also those of their progeny (Krahulcová et al. 2009, 2014). Some of the field studies were supplemented by experimental hybridizations. The main results of our previous studies were that facultative apomicts are involved in hybridization not only as pollen donors, but also as mother plants and serve as drivers of polyploidization, because they produce a significant percentage of unreduced female gametes. Hybrid populations consist of recent hybrids that originated from $n + n$ gametes, of the less common primary $2n + n$ hybrids (mostly an unreduced female gamete comes from a facultatively apomictic parent) and rare plants, which seem to be of polyhaploid origin from $2n + n$ hybrids (the progeny nomenclature according to Harlan & de Wet 1975).

So far we have only studied hybridization between a facultatively apomictic and a sexual parent (the latter represented by tetraploid *P. officinarum*) in the following parental combinations: *Pilosella rubra* and *P. officinarum* (Krahulcová et al. 2004); *P. aurantiaca* and *P. officinarum* (Krahulec et al. 2008, Krahulcová et al. 2012); *P. bauhini* and *P. officinarum* (Krahulcová et al. 2009, 2014, Urfus et al. 2014); *P. floribunda* and *P. officinarum* (Krahulec et al. 2004).

In 2010, Rudolf Hlaváček found a population of unknown *Pilosella* plants near the train station of Milín in the southwestern part of central Bohemia. This population was small, spatially limited and rich in morphological types. We determined the plants as a hybrid swarm formed by *P. polymastix* and *P. officinarum*. Hybrids included a type close to *P. polymastix*, i.e. *P. melinomelas* and many morphological types connecting this hybrid with *P. officinarum*. Both *P. polymastix* and *P. melinomelas* are extremely rare in the Czech Republic: they have not been recorded for a century (Chrtek 2004) and are categorized as A2 (missing taxa, probably disappeared) in the last version of the Red List (Grulich 2012). These types are also rare in other countries in central Europe (Bräutigam 2011). Because of the rarity of the taxa, the spatial limitation of their populations and, also, because the hybrid swarm provided a chance to study a different system from those previously studied (Krahulcová et al. 2014), we decided to study this population in detail.

The aim of the present paper was to (i) document the occurrence of rare types of *Pilosella* together with their morphological characters (they were considered as very rare, and there are no illustrations of them in, e.g., our Flora, cf. Chrtek 2004); and (ii) test whether conclusions drawn from the composition of populations of facultative apomict and sexual species are valid also for the parental combination not yet analysed.

Study site and population-forming taxa

The population studied occurs in an area just in the front of the railway station at Milín, district Příbram, central Bohemia (49°37'57.3"N, 14°01'22"E, altitude 548 m a.s.l.). The place was used for processing products and from which the surface layer of soil was removed. At present the area is overgrown with young plants of *Pinus sylvestris*, *Betula alba* and *Salix caprea*. The herbaceous plant layer consists mainly of various grasses, especially *Calamagrostis epigejos* and *Lupinus polyphyllus* (Fig. 1).

Based on morphological characters, two basic and three intermediate species were identified at this locality. The synonymy of these species is rather complicated and even different names for several of them are used in recent publications. The basic species were *Pilosella bauhini* (Schult.) Arv.-Touv. subsp. *bauhini* (syn. *P. piloselloides* subsp. *bauhini* (Schult.) S. Bräut. et Greuter, *Hieracium bauhini* Schult.) and *P. officinarum* Vaill. (syn.: *Hieracium pilosella* L.). The intermediate species were *P. polymastix* (Peter) Holub [*P. bauhini*–*P. caespitosa*] (syn. *Hieracium polymastix* Peter, *Hieracium obornyanum* Nägeli et Peter) and *P. melinomelas* (Peter) Holub [*P. officinarum*<*P. polymastix*] (syn.: *H. melinomelas* Peter, *P. acrothyrsa* (Nägeli et Peter) Soják, *H. acrothyrsus* Nägeli et Peter). In addition, there was also a hybrid swarm of plants, which were close to *P. officinarum* and corresponded to hybrid formula $P. officinarum \geq P. polymastix$.



Fig. 1. – Photograph of the locality studied taken in June 2010 and details of *Pilosella melinomeles*. Photo: R. Hlaváček.

Methods

Plant material

Living plants were collected and planted in the experimental garden of the Institute of Botany at Průhonice and later used for determining chromosome number/ploidy level and mode of reproduction. Voucher specimens of cultivated plants together with numerous specimens collected in the field are deposited in the herbarium PRA. Both the taxonomic concepts and nomenclature follow Bräutigam (2011).

Determination of chromosome number, DNA ploidy level and mode of reproduction

Chromosomes were counted in root-tip meristems of pot-grown plants following the method described in Krahulcová & Krahulec (1999). DNA ploidy level (Suda et al. 2006) was determined using flow cytometry, following the method of Krahulcová et al. (2004). As an internal standard karyologically examined plants cultivated in the experimental garden were used.

Mode of reproduction was determined by comparing the seed set of open pollinated and emasculated (cut) capitula (Gadella 1984, Krahulcová & Krahulec 1999). The plants, which did not produce any achenes when open pollinated, were considered to be sterile, those of which the open pollinated capitula produced achenes, but not the emasculated



Fig. 2. – *Pilosella polymastix* from the locality studied.

ones were considered to be sexual. The plants on which the emasculated capitula produced achenes were considered to be apomictic (agamospermous).

Isozyme analysis

Isozyme analysis was used to estimate the genotype (isozyme phenotype) structure of hybrid species. Methods used are described in detail by Krahulec et al. (2004); eight systems were used: 6-PGDH, ADH, DIA, IDH, LAP, ME, PGK and EST. Esterases are highly variable and are valuable for clone determination in the genus *Pilosella* (Krahulec et al. 2004).

Results

Taxonomic identity of plants comprising the hybrid swarm

The identity of two of the species found at this locality was evident: *P. bauhini* and *P. officinarum*. There was also a third type present, which in size and structure of its inflorescence resembled *P. caespitosa* (Dumort.) P. D. Sell et C. West, but it had slender and long stolons with small and rather narrow leaves. This plant was determined as *P. polymastix*, a hybrid between *P. bauhini* and *P. caespitosa* (*P. bauhini*–*P. caespitosa*). It is characterized by a combination of characters of both parental species: inflorescence is of the *P. caespitosa* type, stem is thick, with frequent long hairs. Stolons are more similar to those of *P. bauhini* (Table 1, Fig. 2).

Table 1. – Selected differential characters of *Pilosella caespitosa*, *P. bauhini* and *P. polymastix*.

Character	<i>P. caespitosa</i>	<i>P. polymastix</i>	<i>P. bauhini</i> subsp. <i>bauhini</i>
Inflorescence	many medium sized capitula, cymose-corymbose, rather compact	many medium sized capitula, compact	many small capitula, lax, with longer branches
Hairs on the pedicels	simple hairs: common; dark glandular hairs: numerous; dark stellate hairs: numerous	simple hairs: common, white or grey, with dark basis; glandular hairs: common, dark; stellate hairs: dense (pedicels tomentose)	simple hairs: rare; glandular hairs: rare; stellate hairs: dense (pedicels tomentose)
Above ground stolons	thick, short	slender, long	slender, long
Stolon leaves	large, oblanceolate or broadly elliptical, acute	small, narrowly elliptical, acute	small, narrowly elliptical, acute
Leaf shape	oblanceolate or oblong-lanceolate, acute	narrowly elliptical acute	narrowly elliptical, acute
Leaf colour	green	green	glaucous
Stellate hairs on leaf surface	common on lower surface, rare on upper surface	rare on lower surface, common only on main nerve and leaf margin	absent
Hairs on the base of the stem	simple hairs: numerous; stellate hairs: rare	simple hairs: numerous; glandular hairs: absent; stellate hairs: very rare	simple hairs: few
Hairs on the upper part of the stem	simple hairs: common, black; glandular hairs: numerous; stellate hairs: sparse	simple hairs: common, black; glandular hairs: numerous; stellate hairs: rare	simple hairs: sparse, white, with black base; glandular hairs: rare; stellate hairs: rare

On the stems of the plants in this hybrid swarm there are frequent long simple hairs, of the same type as on the stems of *P. polymastix*. This fact and comparison with hybrids between *P. bauhini* and *P. officinarum* led us to the conclusion that the population originated from the hybridization of *P. officinarum* and *P. polymastix*. This hybrid swarm contained a lot of individuals with variable morphology; directly in the field two types of hybrids were evident. The first type was morphologically homogeneous, more similar to *P. polymastix* (Fig. 3). Its name is *P. melinomas* and corresponds with the hybrid formula $P. officinarum < P. polymastix$. The rest was a mixture of morphological types, some of them very close to *P. officinarum* (Figs 4 and 5). They have no binomial name, and we gave them the hybrid formula $P. officinarum \geq P. polymastix$. The characters distinguishing particular members of hybrid swarm are summarized in Table 2. *Pilosella caespitosa* was not found at this locality.

The structure of the hybrid swarm

The taxa, cytotypes and reproduction systems detected are summarized in Table 3. There are two basic species; *P. bauhini* is represented by one pentaploid and apomictic clone, which grew in one small polycormone on the edge of this locality. We did not detect any plants that indicate recent hybridization with *P. officinarum*. *Pilosella officinarum* is tetraploid and sexual. Numerous representatives of *P. officinarum* were found at this locality. Our previous studies provide sufficient knowledge of this species, which is regularly present in the hybrid swarms of this species previously studied (Krahulcová et al. 2009, 2012, 2014, Urfus et al. 2014); sexual tetraploids are regularly present in many genotypes. Therefore, only three plants of *P. officinarum* were collected and analysed for ploidy level and reproductive mode (Table 3). *Pilosella polymastix* is tetraploid ($2n = 36$ with a long hemizygous chromosome), apomictic and very rare; in fact, only one polycormone was found. It occurs here as a stabilized, hybrid species, because the parental species are either absent at this locality (*P. caespitosa*) or they occur there only occasionally (*P. bauhini*). The genetical variation in the hybrids between *P. polymastix* and *P. officinarum* is high and depends on the supposed proportions of the parental sexual (*P. officinarum*)/apomictic (*P. polymastix*) genomes. Whereas *P. melinomas* ($P. officinarum < P. polymastix$) is tetraploid, apomictic and is represented by two isozyme phenotypes (corresponding to two genotypes): one dominating and another one represented by a single plant. The rest of the hybrid swarm ($P. officinarum \geq P. polymastix$) is represented by mostly tetraploid sexual plants and many genotypes (16 isozyme phenotypes in the 18 tetraploid plants studied) – almost each plant with its own genotype. We found three plants with ploidy levels other than tetraploid: triploid (Fig. 6A, B), hexaploid (Fig. 6C) and pentaploid. The hexaploid plant, corresponding in morphology to *P. melinomas* is probably the product of hybridization between *P. polymastix* and *P. officinarum*, which involved one unreduced and one reduced gamete (most likely a $2n + n$ hybrid). In terms of the cytotype structure of the population, we suggest a polyhaploid origin of the triploid plant, namely from a hexaploid mother via haploid parthenogenesis. This plant had smaller capitula, but it grew well. However, the only hexaploid plant found at this locality could not be the maternal parent of this trihaploid plant, because the isozyme phenotype of the triploid plant has one allele that is absent in the hexaploid. The pentaploid plant is probably a result of hybridization between a hexaploid parent and any tetraploid member of the hybrid swarm.

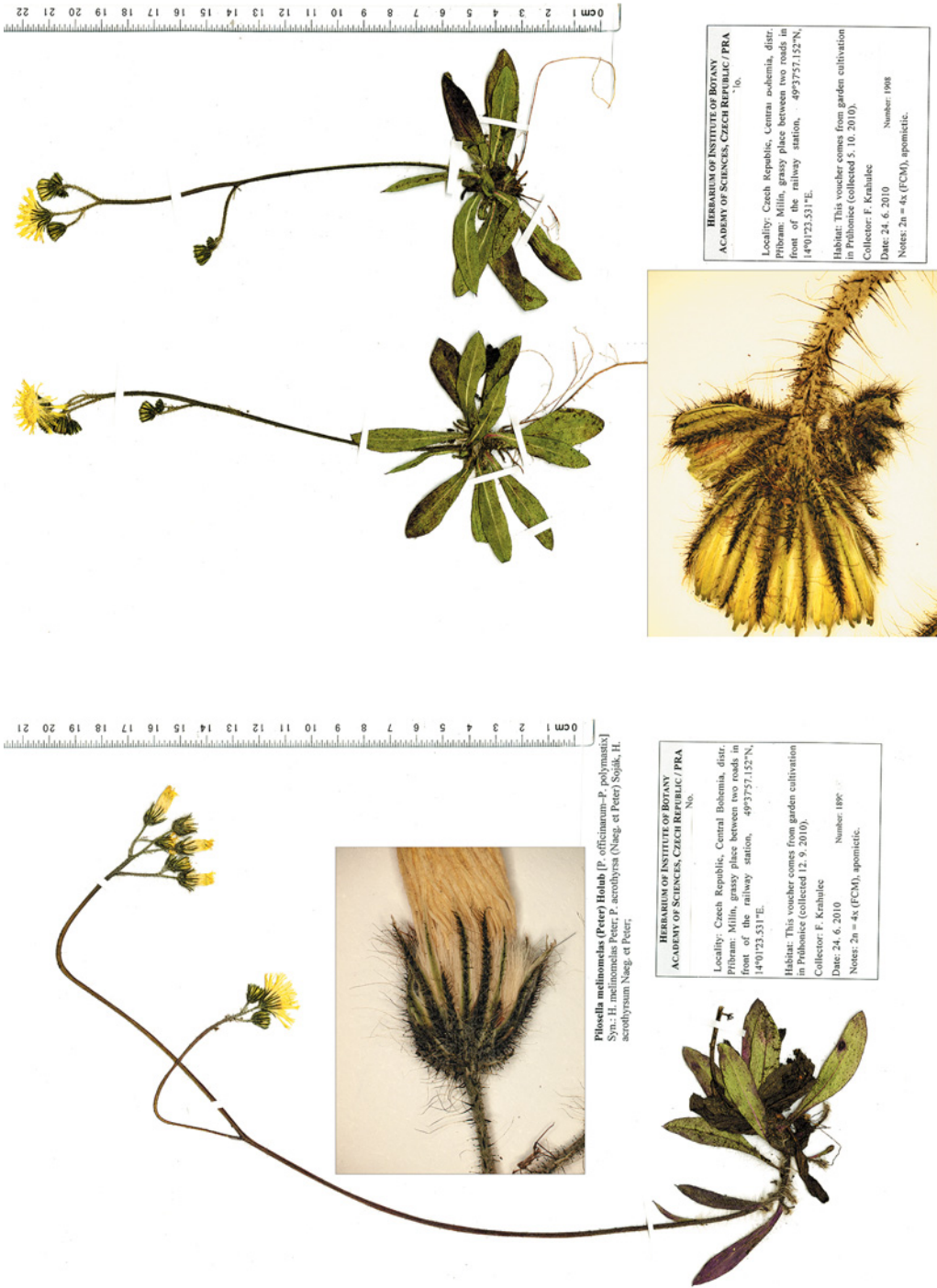
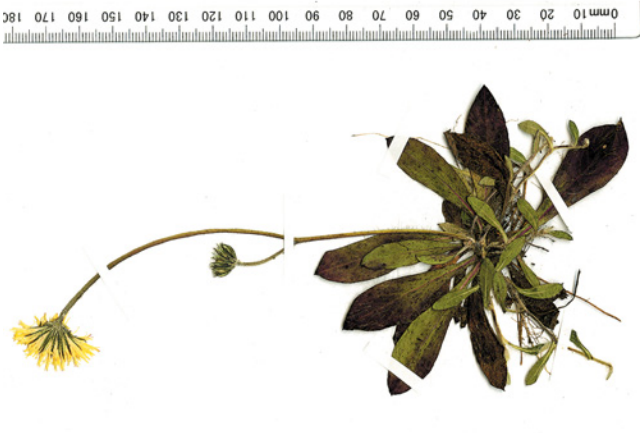


Fig. 3. – *Pilosella melinometas* from the locality studied.



Syn.: *H. melinomeles* Peter, *P. acrothyrsa* (Naeg. et Peter) Soják, *H. acrothyrsus* Naeg. et Peter; [*P. officinarum*-*P. polymastix*]

HERBARIUM OF INSTITUTE OF BOTANY
ACADEMY OF SCIENCES, CZECH REPUBLIC / PRA
No.

Locality: Czech Republic, Central Bohemia, distr. Příbram: Milín, grassy place between two roads in front of the railway station, 49°37'57.152"N, 14°0'123.531"E.

Habitat: This voucher comes from garden cultivation in Příbrnice (collected 10. 9. 2010).

Collector: F. Kráňálec
Date: 24. 6. 2010
Number: 1901
Notes: 2n = 4x (FCM).



***Pilosella melinomeles* (Peter) Holub** [*P. officinarum*-*P. polymastix*]
Syn.: *H. melinomeles* Peter, *P. acrothyrsa* (Naeg. et Peter) Soják, *H. acrothyrsus* Naeg. et Peter;

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Habitat: This voucher comes from garden cultivation in Příbrnice (collected 12. 6. 2011).

Collector: F. Kráňálec
Date: 24. 6. 2010
Number: 1889
Notes: 2n = 4x (FCM).



Fig. 4. – Two different morphological types of *Pilosella officinarum* ≧ *P. polymastix*.



HERBARIUM OF INSTITUTE OF BOTANY
ACADEMY OF SCIENCES, CZECH REPUBLIC / PRA
No.

Locality: Czech Republic, Central Bohemia, distr.
Příbram: Milín, grassy place between two roads in
front of the railway station, 49°37'57.152"N,
14°01'23.531"E.

Habitat: This voucher comes from garden cultivation
in Průhonice (collected 29. 5. 2011).

Collector: F. Krahulec

Date: 24. 6. 2010

Number: 1896

lant A

Notes: $2n = 4x$ (FCM), sexual.

Fig. 5. – Morphological type of *Pilosella officinarum* \geq *P. polymastix* closest to *P. officinarum*.

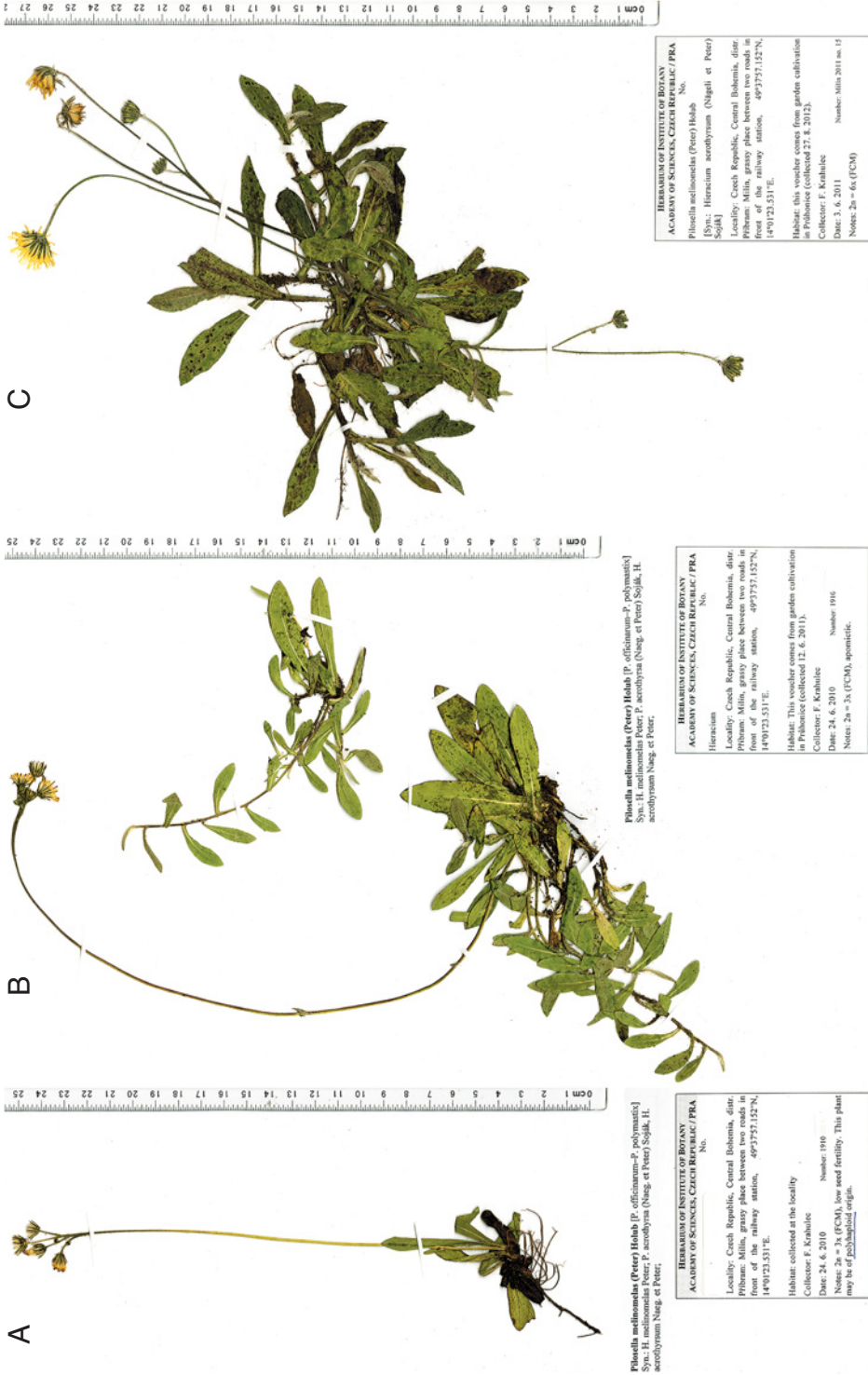


Fig. 6. – Triploid and hexaploid plants found in the *Pitose/ta* hybrid swarm. A – triploid (putative trihaploid) collected in the field; B – the same clone after one year of cultivation; C – hexaploid plant (cultivated in the garden).

Table 2. – Some differential characters of members of the *Pilosella officinarum*–*P. polymastix* hybrid swarm.

Character	<i>P. polymastix</i>	<i>P. melinomelas</i>	<i>P. officinarum</i> ≥ <i>P. polymastix</i>	<i>P. officinarum</i>
Height	tall	medium	variable	small
Inflorescence	compact, many medium sized capitula	furcate in upper half, lax, with about 4–12 medium sized capitula	very variable, deeply furcate, with 1–3 capitula	one big terminal capitulum
Hairs on the pedicels	simple hairs: numerous, white or grey, with dark bases; glandular hairs: numerous, dark; stellate hairs: dense (pedicels tomentose)	simple hairs: numerous white or grey, with dark bases; glandular hairs: numerous, dark; stellate hairs: numerous (pedicels tomentose)	simple hairs: white or grey with dark bases, numerous; glandular hairs: numerous; stellate hairs: numerous	simple hairs: absent; glandular hairs: numerous; stellate hairs: numerous
Above ground stolons	slender, long	slender, long	slender, long	slender, long
Stolon leaves	small, narrowly elliptical, acute	small, narrowly elliptical to oblanceolate	Small, spatulate to oblanceolate	small, oblanceolate to spatulate
Leaf shape	narrowly elliptical, acute	elliptical to oblanceolate, acute, outer obtuse	variable, oblanceolate to spatulate, acute to obtuse	oblanceolate or spatulate, obtuse
Stellate hairs on leaves	rare on lower surface, frequent only on the main nerve and leaf margin	scarce on lower surface of leaf	usually many on lower surface of leaf, sometimes numerous, leaf surface tomentose	numerous, dense (lower surface of leaf tomentose)
Hairs on base of stem	simple hairs: numerous; glandular hairs: absent; stellate hairs: very rare	simple hairs: common; glandular hairs: absent; stellate hairs: numerous	simple hairs: present, but variable in number; glandular hairs: scarce to numerous; stellate hairs: numerous	simple hairs: absent; glandular hairs: scarce; stellate hairs: numerous
Hairs on upper part of stem	simple hairs: common; glandular hairs: common; stellate hairs: numerous	simple hairs: common, black; glandular hairs: numerous; stellate hairs: numerous	simple hairs: present, but variable in number; glandular hairs: rare; stellate hairs: numerous	simple hairs: absent; glandular hairs: scarce; stellate hairs: numerous

Table 3. – Characteristics of members of the *Pilosella* hybrid swarm.

Taxon	DNA ploidy level	Reproductive system	Number of genotypes/ number of plants studied
<i>P. bauhini</i>	5x	apomictic	1/3
<i>P. polymastix</i>	4x, 2n = 36	apomictic	1/3
<i>P. melinomelas</i>	4x	apomictic	2/12
<i>P. officinarum</i> ≥ <i>P. polymastix</i>	4x	sexual	16/18
<i>P. officinarum</i>	4x	sexual	3/3
Putative trihaploid	3x		1/1
2n + n hybrid	6x		1/1
n + n hybrid	5x		1/1

Discussion

Finding of Pilosella polymastix and P. melinomeles

Both *P. polymastix* and *P. melinomeles* are recorded in the Czech Republic under the names *Hieracium obornyanum* or *H. polymastix* for the first species and *H. acrothyrsum* for the other. The data mentioned by Chrtek (2004) is that cited in older literature (e.g. Zahn 1922–1930). As there was no new data on both these species they were considered as probably extinct by Grulich (2012). Both of the hybrid species, *P. polymastix* and *P. melinomeles*, rediscovered by us at the locality in Milín, are facultative apomicts that produce seeds apomictically and at this locality also spread by means of stolons. Therefore, it is likely they will be found at other localities in the surrounding area. In Germany, *P. polymastix* is included in the Flora (Buttler & Hand 2008) and Bräutigam (2011); Gottschlich in Raabe et al. (2011) record it as extinct in Nordrhein-Westfalen. In Austria several localities of *P. polymastix* and *P. melinomeles* are recorded (Zahn 1922–1930) and they are cited as primary hybrids (Schuhwerk 2008). Just recently, both species were published as new plants for the Salzburg region (Brandstätter 2013). Both species are not included in the flora of Slovakia (Marhold & Hindák 1998) and in Poland, only *P. polymastix* (as *H. obornyanum*) is present (Mirek et al. 2002). Zając & Zając (2001: 288) mapped it in six quadrats in southern Poland, especially in the Carpathian region. In Romania it is recorded at several localities (as *H. obornyanum*) by Nyárády (1965). *P. polymastix* is recorded in the area of Kaliningrad in Russia and the Lviv region in the Ukraine and *P. melinomeles* in the Lviv region (Schlyakov, 1989). Both are also mentioned by Zahn (1922–1930) as occurring in these regions, which he refers to as Galicia and Lemberg. All these facts show that both species are generally rare in central Europe. This publication may lead to the discovery of new localities for these species.

The data on the occurrence of tetraploids in both *P. polymastix* and *P. melinomeles* and the corresponding chromosome number ($2n = 36$) recorded in *P. polymastix* are the first on this subject to be published. The long hemizygous chromosome in the tetraploid karyotype of *P. polymastix* is the same as that in both putative parental species, the tetraploid *P. caespitosa* (Krahulcová & Krahulec 1999) and pentaploid *P. bauhini* (Krahulcová et al. 2001, Rotreklová et al. 2002).

Genetic structure of the hybrid swarm and comparison with similar systems in Pilosella

Krahulcová et al. (2014) compare seven populations of hybrids between *P. officinarum* as one parent and *P. bauhini* or *P. aurantiaca* as the other parent. Their composition is similar in terms of chromosome numbers and breeding systems: The hybrid swarms consist of $n + n$ hybrids and the type closest to the apomictic parent is usually apomictic. There are also $2n + n$ hybrids, with the unreduced gamete coming from the apomictic maternal parent. Exactly the same structure was recorded in the population studied. Several tetraploid hybrids co-occurred in the hybrid swarm and the type closest to the apomictic parent (*P. melinomeles*) was apomictic and the numerous plants except one sampled at this locality all belonged to the same clone. The hybrids closest to *P. officinarum* were sexual and represented by many genotypes (isozyme phenotypes). One hexaploid plant evidently originated as a $2n + n$ hybrid from tetraploid parents, namely by the conjugation of one unreduced tetraploid gamete and one reduced diploid gamete. The pentaploid possibly originated by the hybrid-

ization of hexa- and tetraploid plants. In addition, we recorded a triploid plant, which is very probably a trihaploid that originated via haploid parthenogenesis ($n + 0$ progeny) from a hexaploid parent (see above). This is difficult to prove, because when the maternal parent is unknown there is no way of detecting the polyhaploid origin of putative polyhaploids in the field. However, there would appear to be no other way of accounting for the existence of a triploid plant within a generally tetraploid population as was recorded in this study, as no diploid plants were detected at this locality. Krahulec et al. (2008) found a similar putative trihaploid plant, which originated from hexaploid *P. rubra*.

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Souhrn

V roce 2010 nalezl Rudolf Hlaváček hybridní populaci chlupáčků (*Pilosella*) u železniční stanice Milín na Příbramsku. Ukázalo se, že jde o *P. polymastix* (*P. bauhini*–*P. caespitosa*) a produkty hybridizace tohoto hybridního druhu s *P. officinarum*: *Pilosella melinomeles* (*P. officinarum*<*P. polymastix*) a hybridy *P. officinarum*≥*P. polymastix*. *Pilosella polymastix* je typem stejně vysokým jako oba rodiče, řadou znaků je mezi nimi intermediární: typem květenství a častými dlouhými černými chlupy na stonku se podobá *P. caespitosa*, typem výběžků, které jsou tenké a s drobnými úzkými listy, se podobá *P. bauhini*. Hybridy s *P. officinarum* se od sebe liší množstvím hvězdotvých trichomů na rubu listů, počtem úborů a stupněm větvení: *Pilosella melinomeles* je poměrně vysoký a málo větvený typ s drobnějšími a početnějšími úbory. Téměř všechny studované rostliny byly tetraploidní: *P. polymastix* a *P. melinomeles* jsou fakultativní apomikty a na lokalitě se vyskytují v jednom, resp. dvou genotypech. Ostatní tetraploidní hybridy blízké sexuálnímu druhu *P. officinarum* byly sexuální a na lokalitě se vyskytovaly v mnoha genotypech. Tvořily tak souvislou řadu přechodů k *P. officinarum*. Velmi vzácné byly na lokalitě nalezeny ještě další tři cytotypy hybridního původu: hexaploidní rostlina pravděpodobně vznikla z neredukované gamety *P. polymastix* a redukované gamety *P. officinarum* (morfologicky patří k *P. melinomeles*). Druhý vzácný cytotyp byl triploid, který vznikl s největší pravděpodobností parthenogeneticky z redukované gamety hexaploida (trihaploid); zcela určitě to ale nebyla hexaploidní rostlina námi sebraná, což vyloučilo porovnání izozymových fenotypů. U pentaploidní rostliny je velmi pravděpodobný původ v křížení mezi hexaploidem a tetraploidem. *Pilosella polymastix* a ještě více *P. melinomeles* jsou v celé střední Evropě velmi vzácné, na území České republiky nebyly nalezeny po dlouhou dobu a byly pokládány za nezvěstné. Vzhledem k tomu, že jde o apomikty a lokalita se nachází v těsné blízkosti železnice, je velmi pravděpodobný jejich výskyt na dalších lokalitách, zejména ve středních či jižních Čechách.

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