The agamic complex of *Pilosella* (*Asteraceae*) in Bulgaria and SW Romania: variation in ploidy levels and breeding systems. Part 2.

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Abstract: Chromosome numbers and breeding systems are given for a set of *Pilosella* species occurring in Bulgaria and SW Romania (Banat). All diploids and tetraploid accessions of *P. bauhini* and *P. cymosa* subsp. *sabina* were found sexual, and tetraploid *P. pavichii* both sexual and apomictic. One hexaploid accession of *P. bauhini* was found sexual, but semisterile. Other polyploids were apomictic. Ploidy levels are published for the first time for the following taxa: *P.* ×*bodewigiana* (3*x*), *P.* ×*georgieffiana* (5*x*, 6*x*), *P.* ×*byzantina* (2*x*), *P.* ×*pintodasilvae* (4*x*), *P.* ×*pavichiodes* (5*x*), and for an undescribed hybrid *P. bauhini* × *P. onegensis* (2*x*, 6*x*). *Pilosella* ×*byzantina* and *P.* ×*pavichiodes* are given for the first time for Bulgaria, and *P.* ×*pintodasilvae* is reported for the first time from the Balkan Peninsula.

Key words: breeding systems, Bulgarian flora, chromosome numbers, *Hieracium* subg. *Pilosella*, Romanian flora

Introduction

Pilosella Vaill. (syn. *Hieracium* subgen. *Pilosella*) is a huge agamic complex with a typical co-occurrence of three factors causing its taxonomic complexity: hybridisation, polyploidy and facultative apomixis (Fehrer & al. 2007). Investigations into variation in the breeding systems, hybridisation and ploidy level have proved the agamic complex of *Pilosella* a suitable model for studies at population level (Fehrer & al. 2007). This approach allows better understanding of the microevolutionary processes that generate variation in the field. Studies in *Pilosella,* carried out at local population level, have started in Germany (Bräutigam & Bräutigam 1996) and continued up to the present mostly in Central Europe (Krahulec & al. 2004, 2008; Fehrer & al. 2005; Krahulcová & al. 2009a, b, 2012, 2014). The first study focused on the populations in Southeast Europe (involving altogether 21 populations of *Pilosella* in Bulgaria and Southwest Romania) confirmed an expected higher proportion of sexual diploid species as compared with Central Europe. This study also presented four apomictic polyploid species and revealed rather frequent apomictic polyploid hybrids of putatively recent origin (Krahulcová & al. 2009b). The present work continues the investigations into co-occurring taxa in Southeast Europe, involving 58 localities in Bulgaria and Southwest Romania. The results obtained from individual populations represent the breeding systems and ploidy levels of co-occuring taxa/cytotypes and their hybrids, allowing a more general comparison of the observed patterns in Central and Southeast Europe.

Material and methods

Taxonomic concept follows the classification of taxa proposed by Bräutigam & Greuter (2007–2009), except for *P.* ×*georgieffiana*, which we consider an interspecific hybrid. The putative hybrid origin of plants is indicated by the symbol '×' in the respective hybrid formula; in some cases the signs <, >, >>, << are used to indicate the level of morphological similarity of the hybrid plant to any of the presumable parental species. In our opinion, almost all types of hybrid origin sampled in the field and presented in this paper are recent hybrids (see also Krahulcová & al. 2009b). Voucher specimens of all plants studied at Průhonice by A. Krahulcová and F. Krahulec were deposited in the Herbarium of the Institute of Botany, Průhonice (PRA); voucher specimens of plants studied by V. Vladimirov were deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia (SOM).

During this continuing study of *Pilosella* populations in Bulgaria and Romania, we treated the whole population as one entity, following the proved approach that has been used for the first set of plants (Krahulcová & al. 2009b). The sampled populations occupied areas variable in size. Most populations covered a relatively small area, but some of them were scattered nearby each other (Fig. 1). Such neighbouring small populations were sampled and labelled separately. The geographic coordinates of localities (Appendix 1) imply the distance among individual populations.

The plants coming from Bulgaria were collected either by F. Krahulec, V. Vladimirov and A. Krahulcová during common field trips in 2009 and 2010, or by F. Krahulec, V. Vladimirov and V. Mahelka during a field

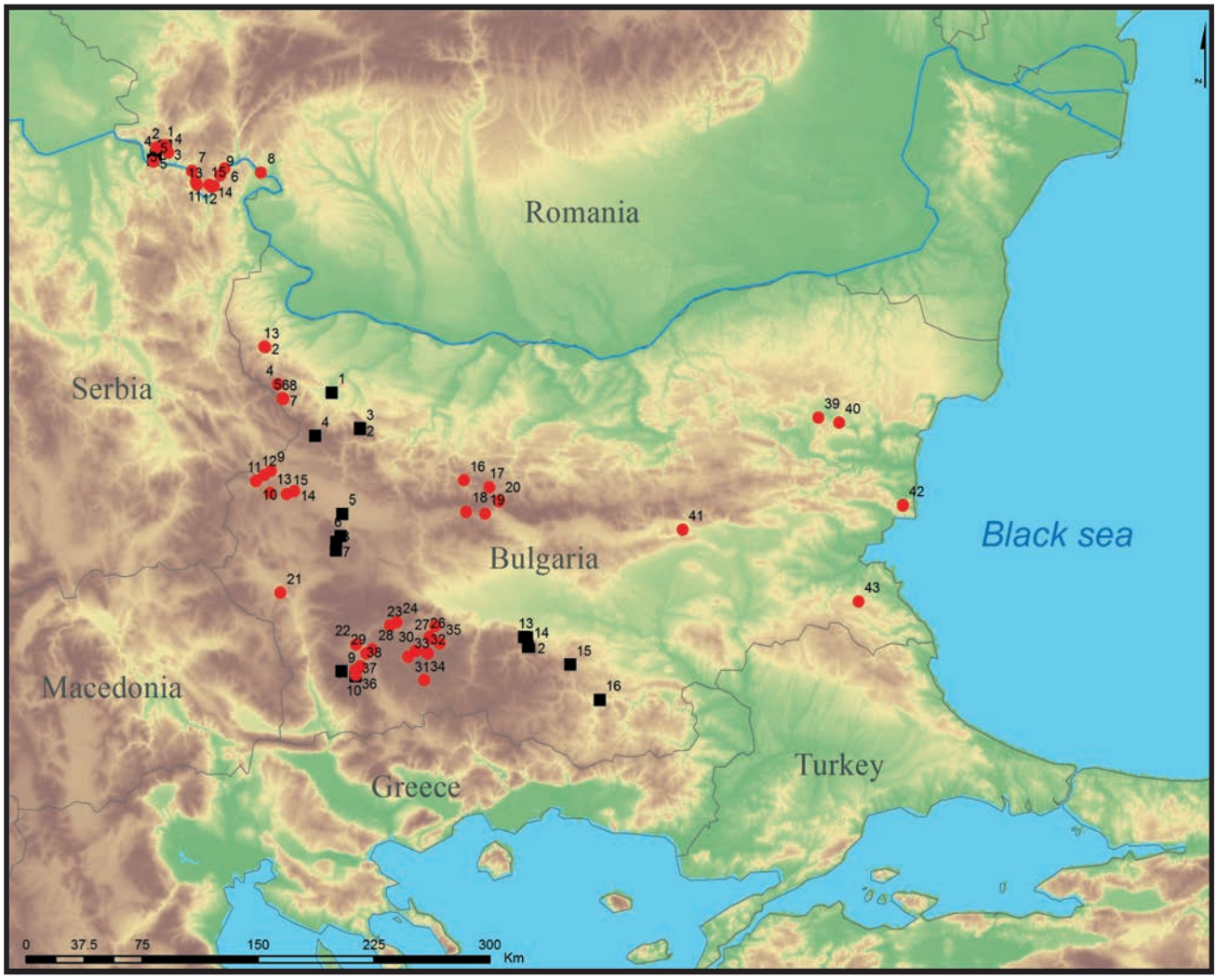

Fig. 1. Map of localities of the studied *Pilosella* species (circles indicate localities mentioned in the present article; squares indicate localities from Krahulcová & al. 2009b).

trip in 2008. The plants from Romanian Banat were collected in 2009 by F. Krahulec, H. Skálová, V. Mahelka, and J. Hadinec, and in 2014 by A. Krahulcová, Z. Skála, H. Skálová, and F. Krahulec. The localities of Bulgarian (**Bu**) and Romanian (**Ro**) populations are described in Appendix 1: they are numbered consecutively, according to their location within each country, from north to south and from west to east (Fig. 1).

To determine the ploidy level/chromosome number and the reproductive system in all morphologically distinguishable plants sampled in the field, we used the same methods as we have used for the first set of plants coming from Bulgaria and Romania (Krahulcová & al. 2009b). Furthermore, the reproductive system in this second set of plants was determined using two methods. We have combined the method used before, namely comparison of the seed-set in at least three emasculated *versus* three open-pollinated capitula (Gadella 1987; Krahulcová & al. 2009a), with the method of Flow Cytometric Seed Screen (FCSS, Matzk & al. 2000). Using the latter method, we analysed 20 achenes sampled from open-pollinated capitula. Thus, we screened the sexually/apomictically derived embryos according to endosperm/embryo ploidy ratio (for the method modified for *Pilosella*, see, for example, Krahulcová & al. 2011).

Results and discussion

An annotated list of species and hybrids is provided below. The symbols for locality and floristic region of the Bulgarian plants are supplemented for each cytotype. Full description of the localities is given in Appendix 1. The studied specimens are numbered by AK & FK and kept in PRA or by VV and kept in SOM.

Basic species

Pilosella bauhini (Schult.) Arv.-Touv. subsp. ***bauhini***

2*n* = 4*x*, sexual

Ro 5: 1 plant 2180.

2*n* = 5*x*, apomictic

Bu 14: 1 plant 1800.

2*n* = 6*x*, sexual

Bu 27: 1 semisterile plant 1674.

2*n* = 6*x*, apomictic

Bu 3: 1 plant 1872; **Bu 9**: 1 plant 1788; **Bu 13**: 1 plant 1775; **Bu 15**: 1 plant 1769; **Bu 17**: 1 plant 1836A; **Bu 19**: 3 plants 1469, 1818, 1826; **Bu 25**: 2 plants 1676A, 1676B; **Bu 30**: 1 plant 1677; **Bu 32**: 1 plant 1685; **Bu 38**: 2 plants 1718, 1719; **Bu 41**: 2 plants 1461, 1463; **Bu 43**: 1 plant 1455; **Ro 1**: 1 plant 2179; **Ro 9**: 1 plant 2177.

Pilosella bauhini subsp. ***magyarica*** (Peter) S. Bräut.

2*n* = 5*x*, apomictic

Bu 6: 1 plant 1842 with chromosome number determined as 2*n* = 45 (AK); **Bu 17**: 1 plant 1837; **Bu 40**: 2 plants 1442, VV4611.

2*n* = 6*x*, apomictic

Bu 4: 3 plants 1856, 1858B, 1860; **Bu 6**: 1 plant 1844; **Bu 9**: 1 plant 1789; **Bu 10**: 1 plant 1782; **Bu 12**: 2 plants 1786A, 1786B; **Bu 13**: 2 plants 1773, 1777; **Bu 14**: 1 plant 1796; **Bu 15**: 1 plant 1768; **Bu 16**: 1 plant 1838; **Bu 19**: 6 plants 1811, 1813, 1821A, 1821B, 1823, 1825; **Bu 20**: 2 plants 1831, 1833; **Bu 21**: 1 plant 1725; **Bu 24**: 1 plant 1699; **Bu 26**: 2 plants 1665, 1666; **Bu 36**: 1 plant 1708; **Bu 42**: 2 plants 1446, 1447; **Bu 43**: 3 plants 1450, 1453, 1457; **Ro 2**: 3 plants 1613A, 1613B, 1613C; **Ro 3**: 1 plant 1615; **Ro 6**: 2 plants 1619, 1620; **Ro 7**: 1 plant 1612; **Ro 8**: 1 plant 1621; **Ro 13**: 1 plant 2176.

Additionally, three hexaploid (2*n* = 6*x*) plants were sampled (**Bu 17**: 1836C, 1836D; **Bu 41**: 1462), in which the subspecies cannot be determined with certainty. These plants were classified as *P. bauhini* s.l.

So far, we have detected only pentaploid and hexaploid plants with apomictic reproduction mode within *P. bauhini*. Here we present a single hexaploid plant of *P. bauhini* subsp. *bauhini* that had formed in open-pollinated capitula only few viable seeds, exclusively of sexual origin (the reproduction mode was determined using the FCSS method). This is the first record of a sexual reproduction mode in *P. bauhini*, the ploidy level of which exceeds the tetraploid one. On the other hand, the presence of a tetraploid sexual plant in not surprising, because such types are known from Western Carpathians and Pannonia region, both from Slovakia and Hungary (Rotreklová 2005).

Pilosella cymosa subsp. ***sabina*** (Sebast.) H.P. Fuchs (Fig. 2)

2*n* = 4*x*, sexual

Bu 1: 1 plant 1862; **Bu 2**: 1 plant 1870 (perished); **Bu 5**: 1 plant 1852; **Bu 11**: 1 plant 1779 (perished); **Ro 12**: 1 plant 2168; **Ro 15**: 1 plant 1609.

Fig. 2. *Pilosella cymosa* subsp. *sabina*.

In the plant from locality close to our **Ro 12** (summit area of Mt Trescâvât) was recorded the tetraploid chromosome number (Mráz & Szeląg 2004, under the name of *P. cymosa*).

Pilosella hoppeana subsp. ***testimonialis*** (Peter) P.D. Sell & C. West (*P. hoppeana* subsp. *macrantha* auct. non Ten. 1830)

2*n* = 2*x*, sexual

Bu 1: 1 plant 1865; **Bu 4**: 1 plant 1857; **Bu 6**: 1 plant 1843; **Bu 7**: 1 plant 1849; **Bu 9**: 1 plant 1790 (perished); **Bu 13**: 1 plant 1776; **Bu 15**: 1 plant 1770; **Bu 18**: 1 plant 1806; **Bu 19**: 2 plants 1478, 1827; **Bu 20**: 3 plants 1829, 1830, 1834; **Bu 21**: 1 plant 1722; **Bu 24**: 1 plant 1687; **Bu 26**: 2 plants 1667, 1670; **Bu 33**: 1 plant 1680; **Bu 34**: 1 plant 1681; **Bu 43**: 1 plant 1452; **Ro 6**: 2 plants 1617, 1618; **Ro 13**: 1 plant 2175.

Pilosella officinarum Vaill.

2*n* = 5*x*, apomictic

Bu 6: 1 plant 1841; **Bu 7**: 1 plant 1846; **Bu 14**: 2 plants 1794, 1801; **Bu 20**: 1 plant 1835; **Bu 32**: 1 plant 1684 (a somatic mosaic with chromosome number determined by AK as 2*n* = 44/45), 1 aneuploid plant 1683B (hypopentaploid, with chromosome number determined by AK as 2*n* = 44), the reproductive system in these two perished plants remained undetermined; **Bu 33**: 1 plant 1679 with chromosome number determined as 2*n* = 45 (AK).

2*n* = 6*x*, apomictic

Bu 4: 1 plant 1859; **Bu 5**: 1 plant 1853; **Bu 19**: 1 plant 1470; **Bu 20**: 1 plant 1832; **Bu 24**: 2 plants 1686, 1694; **Bu 27**: 1 plant 1673; **Bu 28**: 1 plant 1705 with chromosome number determined as 2*n* =54 (AK); **Bu 31**: 1 plant 1682; **Bu 35**: 1 plant 1660; **Bu 39**: 1 plant 1440A with chromosome number determined as 2*n* = 54 (AK); **Bu 40**: 3 plants 1443A, 1443B, VV4620; **Bu 43**: 1 plant 1451; **Ro 10**: 1 plant 2178; **Ro 11**: 1 plant 2172.

The occurrence of aneuploid plants probably reflects the effect of residual sexuality within populations of the facultatively apomictic pentaploids.

Pilosella onegensis Norrl.

2*n* = 2*x*, sexual

Bu 5: 1 plant 1851; **Bu 8**: 1 plant 1845; **Bu 18**: 2 plants 1804, 1810; **Bu 19**: 6 plants 1464, 1472, 1812, 1816, 1820, VV4868; **Bu 35**: several specimens of seeds labelled 1659, seeds collected from plants of *P. onegensis* at this locality (the diploid ploidy level and sexual origin of embryos was determined using the FCSS method); **Ro 4**: 1 plant 1616C.

Pilosella pavichii (Heuff.) Arv.-Touv.

2*n* = 2*x*, sexual, with reduced seed fertility

Bu 4: 2 plants 1854, 1855A; **Bu 6**: 2 plants 1839, 1840; **Bu 22**: 1 plant 1703; **Bu 23**: 1 plant 1702; **Bu 29**: 1 plant 1704; **Bu 36**: 1 plant 1712; **Bu 37**: 1 plant 1716; **Bu 38**: 1 plant 1717; **Ro 11**: 1 plant 2174; **Ro 14**: 5 plants 1610A, 1610C, 1610E, 1611A, 1611B; **Ro 15**: 2 plants 1608B, 1608F.

2*n* = 4*x*, sexual

Bu 37: 1 plant 1714 with chromosome number determined as 2*n* = 36 (AK); **Bu 40**: 1 plant 1441 with reduced seed fertility and chromosome number determined as 2*n* = 36 (AK).

2*n* = 4*x*, apomictic

Bu 2: 2 plants 1867, 1871, both with chromosome number determined as 2*n* = 36 (AK), 1 plant 1868B; **Bu 24**: 1 plant 1693C with expressed residual sexuality; **Bu 26**: 1 plant 1668A.

2*n* = 5*x*, apomictic

Bu 24: 5 plants 1688, 1693A, 1693B, 1693D, 1697 with chromosome number determined as 2*n* = 45 (AK); **Bu 26**: 2 plants 1668B, 1669 with chromosome number determined as 2*n* = 45 (AK); **Bu 27**: 3 plants 1671 with chromosome number determined as 2*n* = 45 (AK), 1672A, 1672B with chromosome number determined as 2*n* = 45 (AK).

2*n* = 6*x*, apomictic

Bu 13: 1 plant 1774.

Additionally, a hexaploid (2*n* = 6*x*) plant was sampled (**Bu 1**: 1 plant 1864B), which perished before the reproductive system could be determined.

Mráz & Szeląg (2004) have recorded a diploid chromosome number in *P. pavichii* from a locality close to our Ro 11 and Ro 12 (summit area of Mt Trescâvât), whereas Yurukova-Grancharova & al. (2006) have reported diploid count from the same locality as our Bu 6.

Pilosella pavichii has a relatively small distribution area confined to the Balkan Peninsula, Romania and Turkey. We have found conspicuous variation with respect to reproduction modes and ploidy levels: diploids were found as sexual (confirming an earlier report by Yurukova-Grancharova & al. 2006), tetraploids as sexual and apomictic, pentaploids and hexaploids as apomictic. In this respect this is the most variable species of *Pilosella* found in Bulgaria. Identifying these types with described taxa seems impossible now.

Pilosella pseudopilosella (Ten.) Soják
2*n* = 2*x*, the reproductive system undetermined because of rare flowering

Bu 36: 1 plant 1706.

Plants of hybrid origin

P. bauhini* × *P. onegensis (Figs. 3, 4)
2*n* = 2*x*, sexual

Bu 19: 4 plants 1468A (semisterile plant), 1468B, VV4871, VV4872, and numerous diploid progeny cultivated from seeds sampled from diploid plants (1468A, 1468B) at the locality.

***2n* = 6*x*, apomictic**

Bu 19: 1 plant 1824.

Variation in the ploidy level of this hybrid seems mysterious, as co-existence of both diploid and hexaploid plants is difficult to explain. In spite of repeated sampling at this locality in 2008 and 2010 respectively, we did not find any diploid plant of *P. bauhini* s.l. All plants belonging to both subspecies (*P. b.* subsp. *bauhini*, subsp. *magyarica*) were hexaploid. Theoretically, the diploid hybrid plant sampled at the locality might have originated as a polyhaploid progeny of a primary tetraploid hybrid. However, the good growth and fertility of this diploid hybrid do not support such explanation.

The origin of the hexaploid hybrid is easier to explain: it might arise from combination of an unreduced gamete of pentaploid *P. bauhini* and a reduced gamete of diploid *P. onegensis*. Although, we did not record the pentaploid *P. bauhini* at this locality, we found it several times at other localities. Therefore, this pentaploid putative parent of the hexaploid hybrid could remain undetected within the huge amount of plants of *P. bauhini* present at the locality Bu 19, from where we have tested only nine plants belonging to both subspecies of *P. bauhini*. Our opinion is supported by the fact that the hexaploid hybrid plant is morphologically closer to *P. bauhini* than the diploid one. Both diploid and hexaploid hybrids combine characters of putative parents, but in a different way. Therefore, we suppose that the two cytotypes of this hybrid originate from two different hybridization events. Comparison of the morphological characters of these taxa is provided in Table 1.

This hybrid between *P. bauhini* and *P. onegensis* is parallel to the hybrid between *P. onegensis* and *P. praealta*, which has been described by Sennikov (2011) as *P.* ×*tambovica*. According to existing literature sources (e.g. Zahn 1922–1930, 1923; Bräutigam & Greuter 2007–2009), the hybrid found by us has no formal name. We cannot eliminate the possibility that it has already been described in the past, because the range of both putative parents has a large overlapping zone of their distribution in eastern Central Europe and the Balkan Peninsula. Because *P. onegensis* was considered as a subspecies of *P. caespitosa* (the most common

Table 1. Morphological comparison between *P. onegensis*, *P. bauhini* and their hybrids.

Character	*P. onegensis*	hybrid 2*x*	hybrid 6*x*	*P. bauhini*
Inflorescence	dense	intermediate	dense	lax
Style	black	(blackish) brown	yellow	yellow
Involucre				
Involucral bracts (phyllaries)	broad, broad white margin	broad, broad white margin	broad, bright, with narrow white margin	broad, bright, with broad white margin
Long hairs	scattered, dark, only white ends	common, white, dark base	rare, white	very rare, white
Glandular hairs	many, dark	scattered, dark	scattered, dark base	scattered
Stellate hairs	scattered	scattered	scattered	very rare
Branches of inflorescence				
Long hairs	scattered, dark	scattered, white, dark base	rare, white, dark base	rare, white
Glandular hairs	dense, white, with dark base	dense, dark	rare, dark	very rare
Stellate hairs	many	many	dense, tomentose	dense, tomentose or very rare
Upper part of stem				
Long hairs	many, dark base	scattered, white, dark base	scattered, white	very rare
Glandular hairs	many	scattered	scattered	very rare
Stellate hairs	many	many	many	very rare
Stem base	many white hairs	many white hairs	rare stellate hairs, rare long hairs	glabrous
Stolons	short, with large broad leaves	bauhini like	bauhini like	long, with many small and narrow leaves

Fig. 3. *Pilosella bauhini* × *P. onegensis*, diploid.

Fig. 4. *Pilosella bauhini* × *P. onegensis*, hexaploid.

name was *Hieracium caespitosum* subsp. *silvicolum*), it is highly probable that such name may exist hidden in dozens of names published in that area. For this reason we cannot multiplicate the number of existing names, especially in the case of a hybrid known from a single locality.

P.* ×*bodewigiana (Zahn) Soják (Fig. 5)
[***P. cymosa*** subsp. ***sabina*** × ***P. pavichii***]
2*n* = 3*x*, the reproductive system undetermined due to fungal disease distorting the inflorescences
Ro 12: 1 plant 2169.

This rare hybrid has been described from the same part of Romania, where we have found it (Zahn 1922–30) and it has also been given exactly from the same locality that we have sampled it (Nyarády 1965). The hybrid has also been found in Bosnia and Hercegovina (Markgraf 1931; Beck 1983) and Bulgaria (Markgraf 1931; Stojanov & al. 1967), but it has not been mentioned in the *Conspectus of the Bulgarian Flora* by Assyov & Petrova (2012). Its triploid ploidy level corresponds to a primary hybrid between a diploid and a tetraploid parent.

Pilosella* ×*densiflora (Tausch) Soják
[***P. bauhini* × *P. cymosa***]
2*n* = 6*x*, apomictic, with considerably expressed residual sexuality and reduced seed fertility
Ro 3: 1 plant 1614.

The hexaploid ploidy level and apomictic reproduction combined with a high level of residual sexuality suggest that this hybrid has a recent origin. Variable reproductive mode is typical of such recent hybrids (see e.g., Krahulcová & al. 2014).

P.* ×*georgieffiana (Zahn) Soják (Figs. 6, 7a, b)
[***P. bauhini* × *P. pavichii***]
2*n* = 5*x*, apomictic
Bu 1: 1 plant 1866; **Bu 7**: 1 plant 1848; **Bu 18**: 1 plant 1807; **Bu 24**: 1 plant 1696.
2*n* = 6*x*, apomictic
Bu 1: 2 plants 1863, 1864A; **Bu 4**: 3 plants 1858A, 1858C, 1861; **Bu 19**: 1 plant 1467; **Bu 24**: 1 plant 1692.

Although both species occur often together, their hybrid has seldom been reported. Zahn (in Georgieff 1926; 1922–1930) described *P. georgieffiana* from Bulgaria, from the vicinity of Sofia, but it has been so far reported only once (Krahulcová & al. 2009b). Rarity of records of *P. georgieffiana* is probably caused by the morphological similarity of both putative parental species.

P. echioides* × *P. section ***Pilosellina*** (corresponding to *P. rothiana* (Wallr.) F.W. Schultz & Sch. Bip.)
2*n* = 4*x*, apomictic
Bu 21: 3 plants 1720, 1723, 1724, the last plant with chromosome number determined as 2*n* = 36 (AK);
Bu 41: 4 perished plants (reproductive system not determined) 1479B, 1479C, 1479D, 1479E.
2*n* = 5*x*
Bu 41: 1 perished plant 1479A (reproductive system not determined).

During our field studies in Bulgaria, we did not find any "pure" *P. echioides*. Concerning the other parent of the respective hybridogenous plants, it is impossible to say, which species of sect. *Pilosellina* participated in their origin.

P. hoppeana subsp. ***testimonialis* × *P. officinarum***
(corresponding to *P. hypeurya* (Peter) Soják)
2*n* = 5*x*, apomictic
Bu 10: 1 plant 1784; **Bu 13**: 1 plant 1778; **Bu 14**: 1 plant 1799; **Bu 18**: 1 plant 1805; **Bu 36**: 1 plant 1710 with chromosome number determined as 2*n* =45 (AK).
2*n* = 6*x*, apomictic
Bu 24: 1 plant 1700 (reproductive system not determined); **Bu 36**: 1 plant 1711.

Summarizing the results of our earlier (Krahulcová & al. 2009b) and present study, this hybrid between closely related parental taxa occurs in Bulgaria in three cytotypes, namely diploid, pentaploid and hexaploid.

P. hoppeana subsp. ***testimonialis/P. officinarum* × *P. bauhini*** (partly corresponding to *P. ruprechtii* (Boiss.) P. D. Sell & C. West, *P. acutifolia* (Vill.) Arv.-Touv. (= *P. brachiata* (DC.) F. W. Schultz & Sch. Bip.) and to *P. leptophyton* (Nägeli & Peter) S. Bräut. & Greuter)
2*n* = 5*x*, apomictic
Bu 13: 1 plant 1772 (*P. acutifolia*); **Bu 14**: 1 plant 1795 (*P. officinarum* > *P. acutifolia*), 1 plant 1803 (*P. ruprechtii*); **Bu 40**: 2 plants (*P. acutifolia*) 1445A, 1445B; **Bu 43**: 7 plants (*P. acutifolia*) 1448, 1449, 1454, 1456A, 1456B, 1458, VV4840;
2*n* = 6*x*, apomictic
Bu 10: 2 plants (*P. leptophyton*) 1781, 1783; **Bu 12**: 2 plants (*P. acutifolia*) 1785A, 1785B, 1 plant 1787 (*P. bauhini* >> *P. officinarum*); **Bu 14**: 2 plants (*P. acutifolia*) 1791, 1802; **Bu 15**: 2 plants (*P. acutifolia*) 1765, 1766, 1 plant 1767 (*P. bauhini* >> *P. hoppeana* subsp.

Fig. 5. *Pilosella ×bodewigiana*.

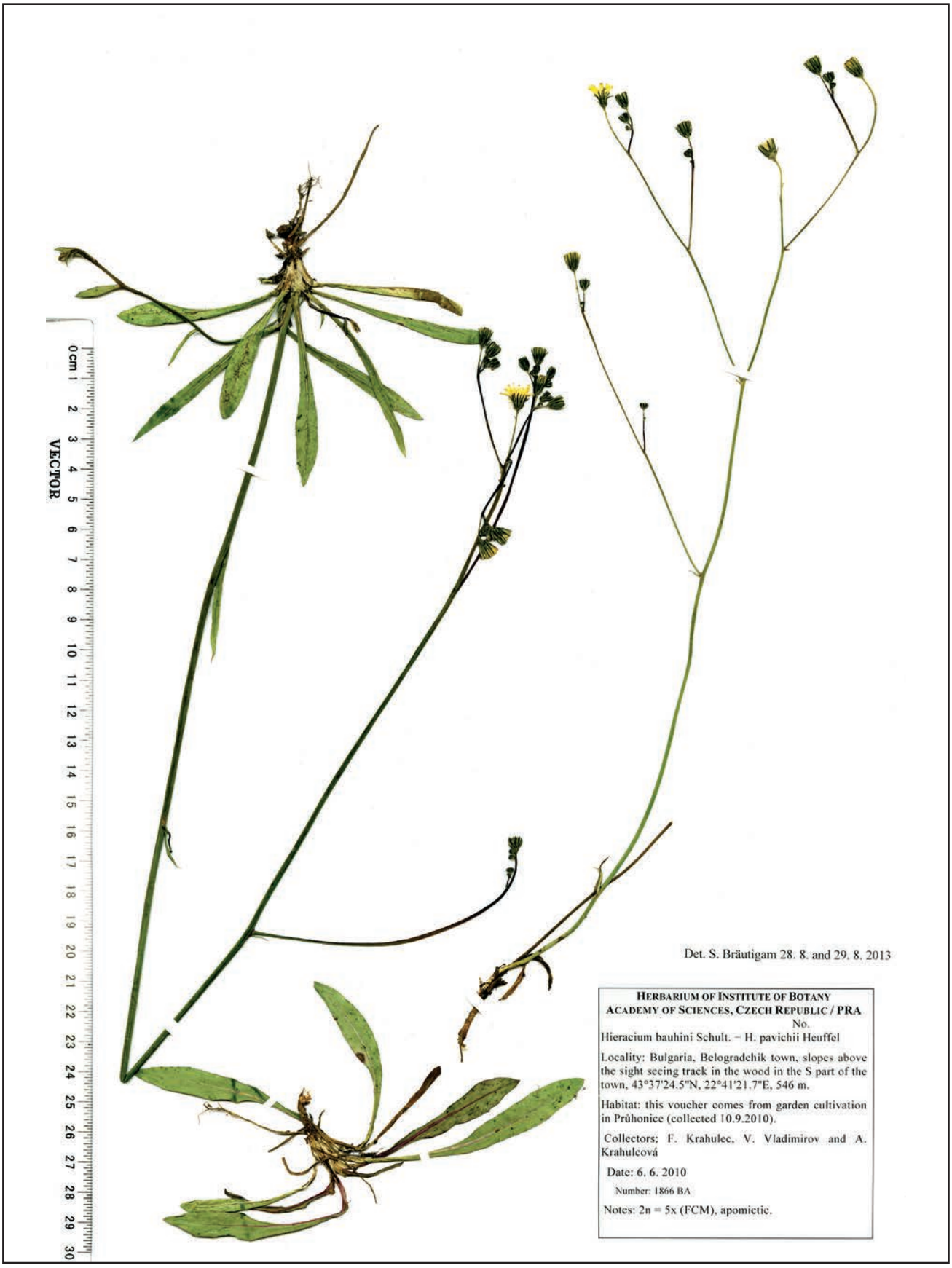

Fig. 6. *Pilosella ×georgieffiana*, pentaploid.

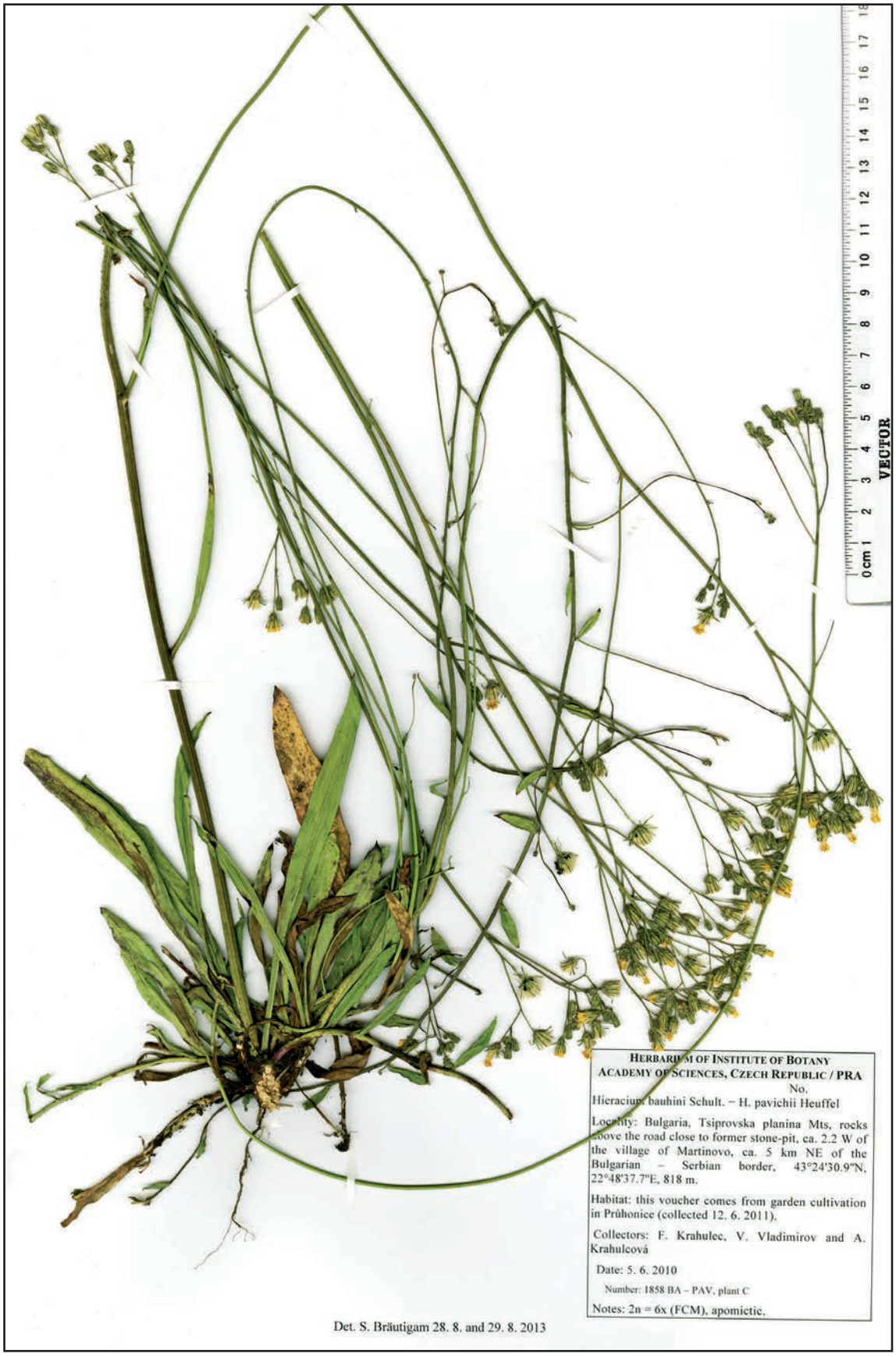

Fig. 7a. *Pilosella* ×*georgieffiana*, hexaploid no. 1858.

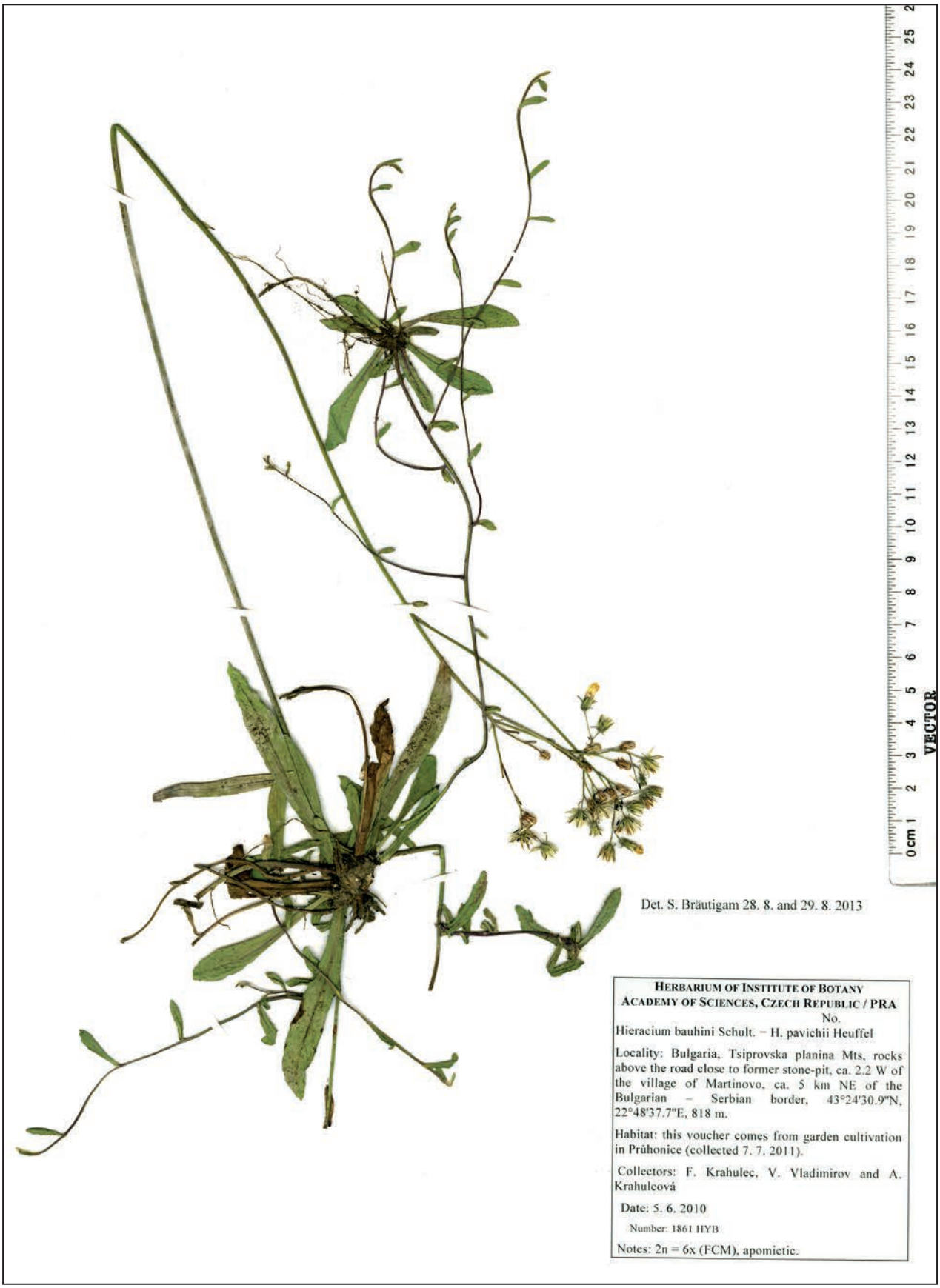

Fig. 7b. *Pilosella ×georgieffiana*, hexaploid no. 1861.

testimonialis / *P. officinarum*); **Bu 19**: 7 plants (*P. leptophyton*) 1465, 1466, 1473, 1475, 1814, 1817, 1819, 1 plant 1477 (*P. acutifolia*), 2 plants 1474, 1822 (*P. bauhini* >> *P. officinarum*), **Bu 24**: 3 plants 1689, 1690, 1691, 1 plant 1701 (*P. acutifolia*), 1 plant 1698 (*P. bauhini* > *P. officinarum*); **Bu 25**: 1 plant 1675B, 1 plant 1675A (*P. bauhini* >> *P. hoppeana* subsp. *testimonialis* / *P. officinarum*); **Bu 26**: 2 plants 1661, 1662, 1 plant 1663 (*P. bauhini* >> *P. officinarum*); **Bu 30**: 1 plant 1678 (reproductive system not determined); **Bu 32**: 2 plants (*P. bauhini* >> P. *hoppeana* subsp. *testimonialis* / *P. officinarum*) 1683A, 1683C; **Bu 37**: 2 plants 1713, 1715; **Bu 40**: 1 plant (*P. acutifolia*) 1444; **Bu 43**: 2 plants (*P. leptophyton*) 1459, 1460.

The most common hexaploid cytotype and less frequent pentaploid cytotype of this hybrid in Bulgaria correspond to findings presented in our earlier study (Krahulcová & al. 2009b). Furthermore, a rare tetraploid hybrid was found in a single locality (Krahulcová & al. 2009b).

P.* ×*byzantina (Boiss.) P.D. Sell & C. West (Fig. 8)
[*P. hoppeana* subsp. *testimonialis* × *P. pseudopilosella*]
2*n* = 2*x*, sexual
Bu 36: 1 plant 1709B.

Diploid ploidy level of the hybrid corresponds to both parental species. Apparently, we have found a primary hybrid, but not the hybridogenous species forming a more numerous population in the locality. *Pilosella* ×*byzantina* has been described from the European part of Turkey (Boissier 1875; Zahn 1922–1930; Sell & West 1975 gave list of specimens seen), and subsequently has been reported from Morocco (Jahandiez & Maire 1934) and Spain (Zahn, l.c., as *Hieracium pseudopilosella* subsp. *albarracinum*; Mateo Sanz 1988, 2005, 2006 as *P. macrantha*, but the name was missapplied). Undoubtedly, Mateo Sanz means *P.* ×*byzantina* and subsequently (Mateo Sanz 2007) he used this name.

This hybrid is characterized by presence of long hairs on the involucrum, but not as dense as in *P. pseudopilosella*; in comparison with the *P.* ×*pintodasilvae* (see the next hybridogenous species below) it has white margins on involucral bracts.

Pilosella ×*byzantina* has not been reported from Bulgaria yet (Stojanov & al. 1967; Assyov & Petrova 2012), in spite of the fact it has been described from a not very distant area.

P.* ×*pintodasilvae (de Retz) Mateo (Fig. 9)
[*P. officinarum* × *P. pseudopilosella*]
2*n* = 4*x*, apomictic
Bu 36: 1 plant 1709A.

The ploidy level of this hybrid corresponds to a primary hybrid between a hexaploid *P. officinarum* and diploid *P. pseudopilosella* that did not form a population in the respective locality. *Pilosella* ×*pintodasilvae* has been so far reported only from the Iberian Peninsula, namely Spain and Portugal (de Retz 1974, Mateo Sanz 2005, 2006).

P.* ×*pavichiodes S. Bräut. & Greuter (Fig. 10)
[*P. pavichii* × *P. officinarum*]
2*n* = 5*x*, apomictic
Bu 19: 1 plant 1476, 1 plant 1471 (*P. pavichii* > *P. officinarum*) with chromosome number determined as 2*n* = 45 (AK).

We have visited this locality twice (see discussion above related to the hybrid *P. bauhini* × *P. onegensis*), but we did not find there any plant belonging to the first putative parent *P. pavichii*.

This hybrid has been described and reported from Romania (Nyárádi 1965; Popescu & Sanda 1998), it has not been reported from Bulgaria yet (Stojanov & al. 1967: Assyov & Petrova 2012), in spite of common co-occurrence of both parental species.

Unclassified hybrid of ***P. officinarum***
2*n* = 6*x*, apomictic
Bu 24: 1 plant 1695; **Ro 11**: 2 plants 2171, 2173.

General discussion

Chromosome numbers of all basic species and of most hybrids presented here are in agreement with the earlier published data. Summarizing our earlier and present study, we have found the evident hybrids between hexaploid *P. officinarum* and diploid *P. hoppeana* subsp. *testimonialis*, which were diploid, pentaploid and hexaploid. This diversification of the hybrid cytotypes suggests their rather complicated origin. In the present study, we have found a similar puzzle in another hybrid between diploid *P. onegensis* and hexaploid *P. bauhini*: in the respective locality, the hybrids were diploid and hexaploid, respectively.

We detected also hexaploid ploidy level in *P. pavichii*. This species was not thoroughly studied. As has already been published by Mráz & Szeląg (2004), we

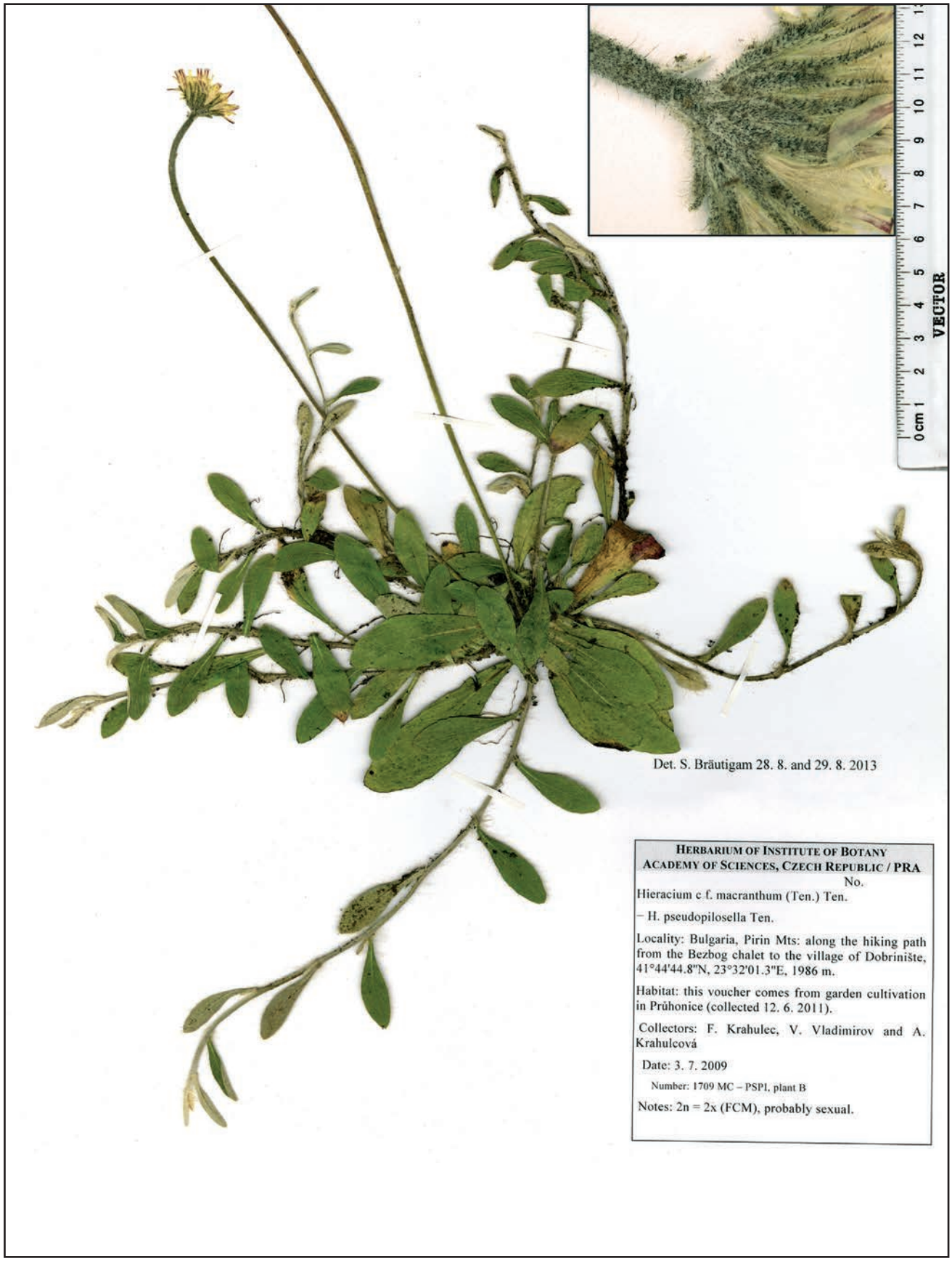

Fig. 8. *Pilosella ×byzantina*.

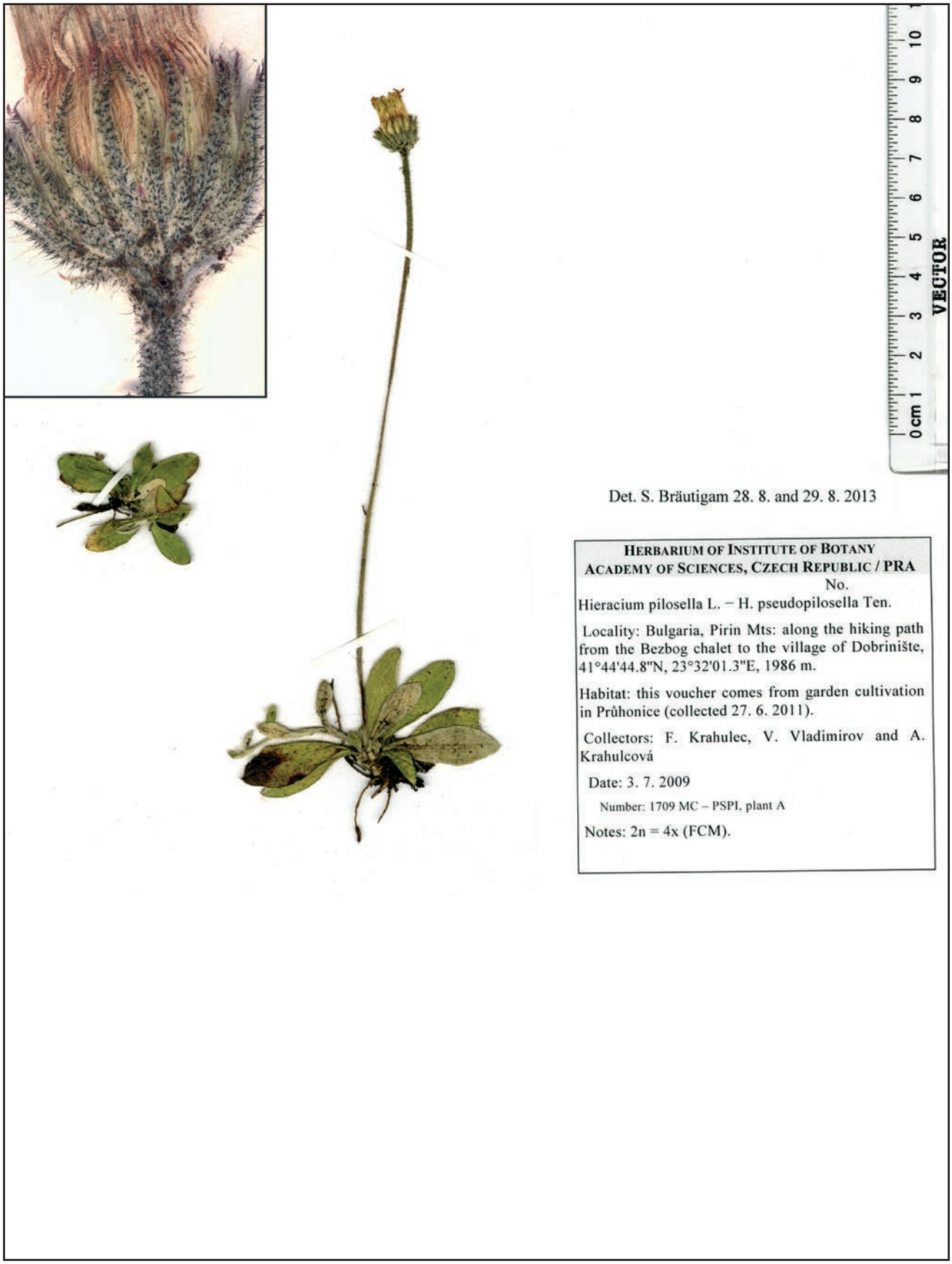

Fig. 9. *Pilosella ×pintodasilvae.*

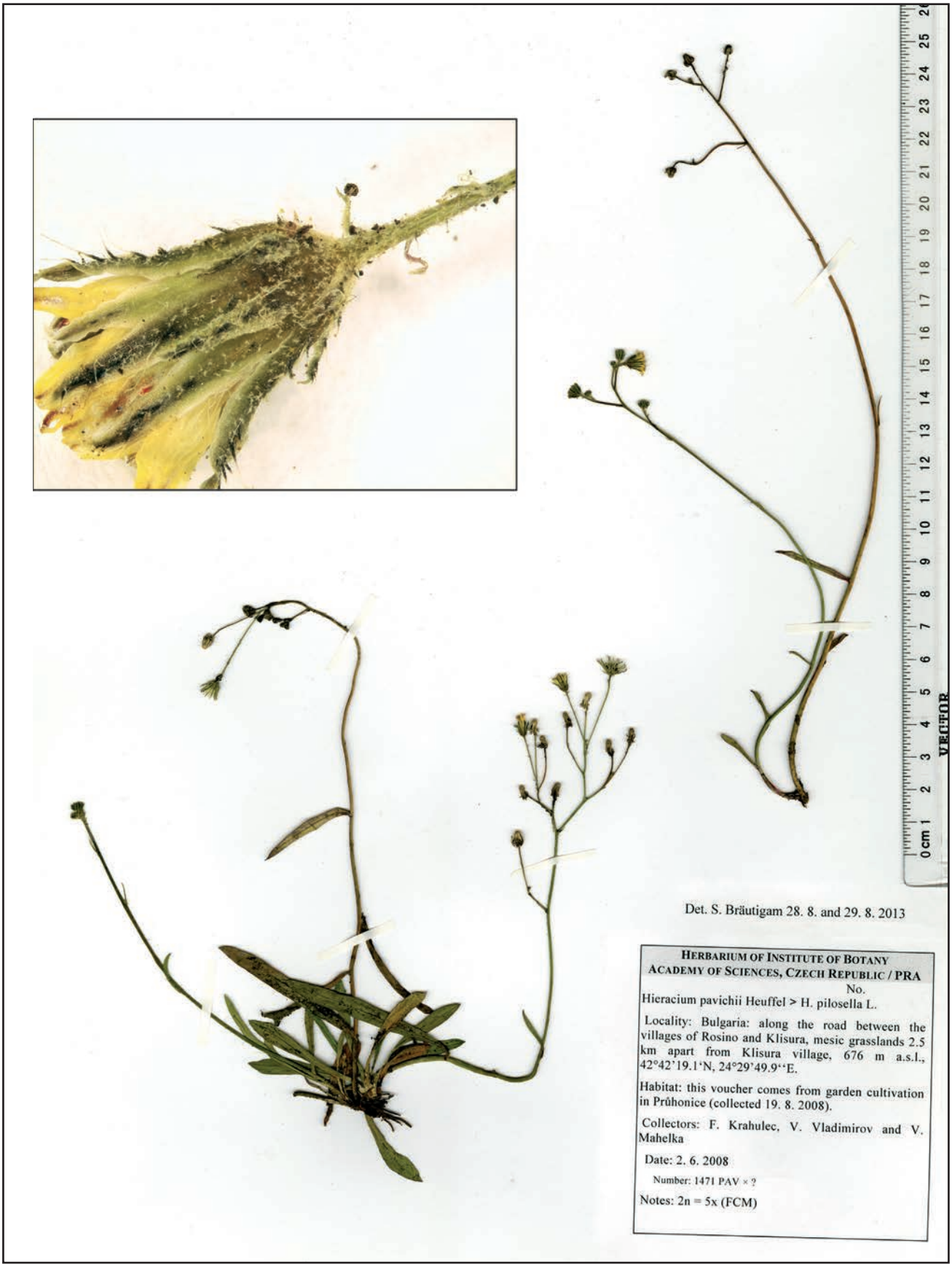

Fig. 10. *Pilosella ×pavichiodes.*

have found plants belonging to *P. pavichii* from a limited number of localities in Romania as exclusively diploid. On the contrary, in Bulgaria we have found diploids, tetraploids, pentaploids, and hexaploids. While the diploids were sexual, pentaploids and hexaploids were apomictic. The tetraploid plants comprised both sexual and apomictic biotypes. Thus, this species seems as most variable of all basic species studied in Bulgaria. In spite of the cytotype and reproductive diversity found in *P. pavichii*, the number of hybrids is not high: we detected the hybrids of *P. pavichii* with *P. bauhini*, *P. cymosa* subsp. *sabina*, and *P. officinarum*, all of them being rare. We think that rare hybridization of *P. pavichii* is caused by two reasons: *P. pavichii* has different ecological preferences than most other species: it occurs often in eroded and/or semishaded habitats. Seed fertility of the sexual diploid *P. pavichii* is commonly reduced, as compared with other sexual diploid species, for example, with *P. onegensis* or *P. hoppeana* subsp. *testimonialis* (Krahulcová & al. 2009b and this paper). Furthermore, the populations of *P. pavichii* have been studied by a limited number of specialists.

Most hybrids we have found were evidently of recent origin. Only one of the hybrids can be identified with hybridogenous species, namely with *P. rothiana*. Nevertheless, this respective hybrid was determined as *P. echioides* × unknown species of sect. *Pilosellina* (probably corresponding to *P. rothiana*), because the second parent remains uncertain. We have sampled this hybrid at two different localities, where we did not find the parental species *P. echioides*. A detailed study seems desirable, as this hybridogenous species comprised two ploidy levels at one of these localities.

This difference in the occurrence of stabilized hybridogenous species is at least partly connected with the presence of sexual and tetraploid *P. officinarum* in Central Europe. This cytotype of *P. officinarum* is involved in the origin of many stabilized apomictic hybridogenous species. Tetraploid *P. officinarum* still hybridizes easily with many other species. *Pilosella cymosa* subsp. *sabina* found in Bulgaria and South Romania also represents a tetraploid sexual species potentially able to hybridize, but it is not as common as *P. officinarum* in Central Europe. We have found a hybrid of *P. cymosa* subsp. *sabina* as a parent only once, namely the triploid *P.* ×*bodewigiana*. Another sexual tetraploid species, *P. pavichii*, was recorded only once in Bulgaria. Most apomictic species found in Bulgaria and Southwest Romania are pentaploid or hexaploid, while the sexual species are diploid. There are evidently some barriers preventing heteroploid hybridization of diploid and polyploid parents, as we have seldom found hybrids of such putative origin: certain hybrids were identified between *P. hoppeana* subsp. *testimonialis* and *P. bauhini* or *P. officinarum*, and between *P. pseudopilosella* and *P. officinarum*. Two of these three couples are closely related species of sect. *Pilosellina*; their hybridization is evidently easier than between other species.

Novelties in the distribution of hybrids

During our work, we have found several hybrids (or hybridogenous species) which have been seldom reported from the studied area, or not reported at all.

Pilosella ×*georgieffiana* (*P. bauhini – P. pavichii*) has been described and reported from Bulgaria by Zahn (in Georgieff 1926; 1922–1930), but it has never been reported again, except in our earlier paper (Krahulcová & al. 2009b). In the Euro-Med Database (Bräutigam & Greuter 2007–2009), it is not mentioned as a hybrid, but as a synonym of *P. bauhini*. Both parental species, namely *P. bauhini* and *P. pavichii*, occur together in various cytotypes. In our opinion, their hybrid is not rare, but not easily distinguishable because of the general similarity of both species. Nevertheless, the set of differentiating characters (presence/absence of stolons, shape of inflorescence, shape of stem leaves) is sufficient to distinguish the hybrid from both parental species.

Two other hybrids are those within the section *Pilosellina*. Although *P. pseudopilosella* is known from several countries on the Balkan Peninsula (Bulgaria, Greece, countries of former Yugoslavia, European Turkey) and Romania (see Bräutigam & Greuter 2007–2009), its hybrids were not studied except by Boissier, who described *P.* ×*byzantina* (*P. hoppeana – P. pseudopilosella*). Recent data on this hybrid are known from the Iberian Peninsula. Similarly, as in *P.* ×*georgieffiana* discussed above, the main reason of sparse data on *P.* ×*byzantina* is probably the general similarity of parents and also the fact that little attention has been paid to their hybridization. Another hybrid, *Pilosella* ×*pintodasilvae* (*P. officinarum – P. pseudopilosella*), is given for the first time from the Balkans; we have found both hybrids, namely *P.* ×*byzantina* and *P.* ×*pintodasilvae*, in the same place with co-occurrence of all putative parental species. Both hybrids can be

distinguished by presence of long hairs on the involucral bracts (Figs. 8 and 9).

The last case is a so far undescribed hybrid between *P. bauhini* and *P. onegensis*. It is an analogue of the recently described hybrid between *P. onegensis* and *P. praealta* (*P.* ×*tambovica*, Sennikov 2011). The putative origin of cytotypes found in this hybrid is discussed above.

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Appendix 1. List of localities and co-occurring species. Ploidy level detected in particular taxa and hybrids is given in parenthesis. Entries of recorded taxa without determination of ploidy level refer to those plants that were collected in the field for herbarium documentation, but were not cultivated in experimental garden for subsequent analyses. Collecting sites are ordered according to location from north to south and from west to east (Fig. 1).

Bulgaria (plants collected by *F. Krahulec*, *A. Krahulcová*, *V. Vladimirov* and *V. Mahelka*, determined by *S. Bräutigam*, *F. Krahulec* and *V. Vladimirov*):

Bu 1 Forebalkan (*Western*): Belogradchik town, on slopes above the sightseeing trail in a wood in the S periphery of the town, 43°37'24.5"N, 22°41'21.7"E, 546 m, 6.6. 2010.

Recorded taxa: *P. cymosa* subsp. *sabina* (4*x*), *P. piloselloides*, *P. pavichii* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrid: *P. bauhini* × *P. pavichii* (5*x*, 6*x*).

Bu 2 Forebalkan (*Western*): Belogradchik town, in grassy places and at the wood margin above the sightseeing trail near the Museum of Natural History in the S periphery of the town, 43°37'22.4"N, 22°41'40.9"E, 540 m, 6.6. 2010.

Recorded taxa: *P. cymosa* subsp. *sabina* (4*x*), *P. pavichii* (4*x*), *P. bauhini* subsp. *bauhini*.

Bu 3 Forebalkan (*Western*): Belogradchik town, at wood margin along the sightseeing trail near the Museum of Natural History in the S periphery of the town, 43°37'17.3"N, 22°41'38.6"E, 517 m, 6.6. 2010.

Recorded taxon: *P. bauhini* subsp. *bauhini* (6*x*).

Bu 4 Balkan Range (*Western*): Mt Chiprovska, on rocks above the road at the wood margin close to a former stone-pit, 2.2 km WNW of Martinovo village, *ca* 5 km NE of Bulgarian – Serbian border, 43°24'30.9"N, 22°48'37.7"E, 818 m, 5.6. 2010.

Recorded taxa: *P. pavichii* (2*x*), *P. bauhini* subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum* (6*x*);

hybrid: *P. bauhini* × *P. pavichii* (6*x*).

Bu 5 Balkan Range (*Western*): Mt Chiprovska, in a meadow near the chalets situated below the starting point of the hiking trail to a waterfall, *ca* 4.75 km W of Kopilovtsi village, 43°19'41.4"N, 22°51'16.1"E, 965 m, 5.6. 2010.

Recorded taxa: *P. onegensis* (2*x*), *P. cymosa* subsp. *sabina* (4*x*), *P. officinarum* (6*x*).

Bu 6 Balkan Range (*Western*): Mt Chiprovska, in the wood along the hiking trail near a parking place, *ca* 3 km SW of the western periphery of Kopilovtsi village, in the valley east of Mt Kopren, *ca* 2.5 km E of the Bulgarian – Serbian border, 43°19'30.2"N, 22°51'30.9"E, 953 m, 5.6. 2010.

Recorded taxa: *P. pavichii* (2*x*), *P. bauhini* subsp. *magyarica* (5*x*, 6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum* (5*x*).

Bu 7 Balkan Range (*Western*): Mt Chiprovska, in grasslands along the trail, *ca* 0.1 km E of a parking place, 4.25 km W of Kopilovtsi village (end of the local road from the village), 43°19'37.5"N, 22°51'41.4"E, 936 m, 5.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica*, *P. officinarum* (5*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrids: *P. bauhini* × *P. pavichii* (5*x*).

Bu 8 Balkan Range (*Western*): Mt Chiprovska, in mountain grasslands above the hiking trail 4.4 km WSW of Kopilovtsi village, in the valley east of Mt Kopren, *ca* 2.5 km E of the Bulgarian – Serbian border, 43°19'25.9"N, 22°51'35.4"E, 937 m, 5.6. 2010.

Recorded taxon: *P. onegensis* (2*x*).

Bu 9 Znepole Region: on rocks along the road leading from Tran to Dragoman towns, in the NE part of Krusha village, 6 km W of the Gaber town, *ca* 2 km SE of the Bulgarian – Serbian border, 42°54'12.0"N, 22°47'26.9"E, 589 m, 2.6. 2010.

Appendix 1. Continuation.

Recorded taxa: *P. bauhini* subsp. *bauhini* (6*x*), *P. bauhini* subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrid: *P. bauhini* × *P. cymosa* corresponding to *P. densiflora*.

Bu 10 Znepole Region: in a meadow on the southern foothill below the road between Filipovtsi village and Erma river gorge, close to a crossroad (branching to Bankya village), *ca* 2.3 km E of the Tran town, 42°50'24.9"N, 22°40'37.9"E, 810 m, 2.6. 2010.

Recorded taxon: *P. bauhini* subsp. *magyarica* (6*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (5*x*) corresponding to *P. hypeurya*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. leptophyton*.

Bu 11 Znepole Region: on limestone rocks on the northern slope above the road between Filipovtsi village and Erma river gorge, close to a crossroad (branching to Bankya village), *ca* 2.3 km E of the town of Tran, 42°50'23.7"N, 22°40'44.6"E, 810 m, 2.6. 2010.

Recorded taxon: *P. cymosa* subsp. *sabina* (4*x*).

Bu 12 Znepole Region: on a slope above the road leading from Tran to Dragoman towns, in the NE periphery of Vrabcha village, *ca* 0.9 km S of the Bulgarian – Serbian border, 42°52'37.5"N, 22°44'19.3"E, 950 m, 2.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini*;

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* corresponding to *P. leptophyton*, *P. bauhini* >> *P. officinarum* (6*x*).

Bu 13 Znepole Region: in a pasture above the road leading from Breznik to Tran towns, before Nedelkovo settlement, *ca* 10 km WNW of Breznik town, 42°46'26.3"N, 22°47'34.7"E, 836 m, 2.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini* (6*x*), *P. pavichii* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (5*x*) corresponding to *P. acutifolia*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* corresponding to *P. leptophyton*, *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (5*x*) corresponding to *P. hypeurya*.

Bu 14 Znepole Region: Mt Viskyar, in old pasture close to the southern wood margin, *ca* 0.2 km N of the SE periphery of Gurgulyat village, *ca* 8 km SW of Slivnitsa town, 42°47'28.6"N, 22°59'02.6"E, 847 m, 3.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini* (5*x*), *P. officinarum* (5*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* × *P. bauhini* (5*x*) corresponding to *P. ruprechtii*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. officinarum* > *P. acutifolia* (5*x*), *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (5*x*) corresponding to *P. hypeurya*.

Bu 15 Znepole Region: Mt Viskyar, in a former pasture below the highest point of the road passing through the saddle of the main ridge, leading from Rakita to Arzan villages, *ca* 4 km NNE of Breznik town, 42°46'23.5"N, 22°55'33.2"E, 904 m, 2.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini* (6*x*), *P. hoppeana* subsp. *testimonialis*;

hybrids: *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. bauhini* >> *P. hoppeana* subsp. *testimonialis* / *P. officinarum* (6*x*).

Bu 16 Balkan Range (*Central*): Zlatitsa-Teteven Divide, on rocks above the road between Ribaritsa village and Teteven town, *ca* 5 km NNW of Ribaritsa village, 42°53'40.6"N, 24°19'16.6"E, 492 m, 4.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini*.

Bu 17 Balkan Range (*Central*): Troyan Divide, on slope above the road between Shipkovo and Ribaritsa villages, *ca* 3.4 km SW of Shipkovo village, 42°51'26.9"N, 24°31'26.7"E, 1095 m, 4.6. 2010.

Recorded taxa: *P. bauhini* subsp. *magyarica* (5*x*), *P. bauhini* subsp. *bauhini* (6*x*), *P. bauhini* s.l. (6*x*).

Bu 18 Mt Sredna Gora (*Western*): in meadows along the road leading from Koprivshtitsa town to Sofia – Burgas motorway, *ca* 8.5 km N of Koprivshtitsa town, 42°42'47.8"N, 24°20'51.0"E, 873 m, 3.6. 2010.

Recorded taxa: *P. onegensis* (2*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum*.

Appendix 1. Continuation.

hybrids: *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (5*x*) corresponding to *P. hypeurya*, *P. bauhini* × *P. pavichii* (5*x*).

Bu 19 Balkan Range (*Central*): along Sofia – Burgas motorway, between Rozino and Klisura villages, in mesic grasslands 2.4 km E of Klisura village, 42°42'19.1"N, 24°29'49.9"E, *ca* 676 m, 2.6. 2008, 3.6.2010.

Recorded taxa: *P. onegensis* (2*x*), *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum* (6*x*);

hybrids: *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. leptophyton*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. bauhini* >> *P. officinarum* (6*x*), *P. bauhini* × *P. pavichii* (6x), *P. bauhini* × *P. onegensis* (2*x*, 6*x*), *P. pavichii* > *P. officinarum* (5*x*), *P. pavichii* × *P. officinarum* (5*x*).

Bu 20 Balkan Range (*Central*): Troyan Divide, the Troyan saddle (Troyan Pass) in the Beklemeto locality, in mountain grasslands along the road between Karnare village and Troyan town, *ca* 15 km SSW of Troyan town, 42°46'47.4"N, 24°36'22.7"E, 1528 m, 4.6. 2010.

Recorded taxa: *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum* (5*x*, 6*x*), *P. bauhini* subsp. *magyarica* (6*x*).

Bu 21 Valley of River Struma (*Northern*): at the road margin and slopes above the road in the valley of River Struma situated 1 km E the Pastuh village, *ca* 8.5 km NW of Boboshevo town, 42°11'56.0"N, 22°54'49.8"E, 450 m, 4.7. 2009.

Recorded taxa: *P. hoppeana* subsp. *testimonialis* (2*x*), *P. bauhini* subsp. *magyarica* (6*x*);

hybrid: *P. echioides* × *P.* section *Pilosellina* (4*x*) corresponding to *P. rothiana*.

Bu 22 Rila Mts: at the southern foothills, in a pasture above the road connecting the villages Banya and Dobarsko, S of Dolno Draglishte village, *ca* 5.5 km NE of Razlog town, 41°55'01.2"N, 23°31'16.8"E, 819 m, 2.7. 2009.

Recorded taxon: *P. pavichii* (2*x*).

Bu 23 Rhodopi Mts (*Western*): along the road between Yundola and Cherna Mesta villages (*ca* 6.5 km SW of Yundola village), *ca* 1.25 km before the road branch leading to Smolevo village, 42°02'05.9"N, 23°46'49.1"E, 1183 m, 2.7. 2009.

Recorded taxon: *P. pavichii* (2*x*).

Bu 24 Rhodopi Mts (*Western*): in a slightly disturbed habitat at the wood margin above the road, *ca* 2 km WSW of Yundola village, 42°03'25.4"N, 23°49'53.4"E, 1425 m, 2.7. 2009.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini*, *P. pavichii* (4*x*, 5*x*), *P. hoppeana* subsp. *testimonialis* (2*x*), *P. officinarum* (6*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*), *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. acutifolia*, *P. bauhini* > *P. officinarum* (6*x*), *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (6*x*) corresponding to *P. hypeurya*, *P. officinarum* × ? (6*x*), *P. bauhini* × *P. pavichii* (5*x*, 6*x*).

Bu 25 Rhodopi Mts (*Western*): in a pasture on the SE periphery of Dorkovo village, *ca* 11 km E of the Velingrad town, 42°02'26.3"N, 24°07'59.5"E, 852 m, 30.6. 2009.

Recorded taxon: *P. bauhini* subsp. *bauhini* (6*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*), *P. bauhini* >> *P. hoppeana* subsp. *testimonialis* / *P. officinarum* (6*x*).

Bu 26 Rhodopi Mts (*Western*): on the slope above the road (at the wood margin, former pasture) *ca* 1.25 km S of Rakitovo town, 10 km SE of Velingrad town, 41°58'43.5"N, 24°05'30.2"E, 870 m, 30.6. 2009.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. pavichii* (4*x*, 5*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*), *P. bauhini* >> *P. officinarum* (6*x*),.

Bu 27 Rhodopi Mts (*Western*): in a grassy place at the wood margin along the road, *ca* 2.5 km SE of Rakitovo town, 11 km SE of Velingrad town, 41°58'04.3"N, 24°06'12.5"E, 948 m, 30.6. 2009.

Recorded taxa: *P. bauhini* subsp. *bauhini* (6*x*), *P. pavichii* (5*x*), *P. officinarum* (6*x*).

Bu 28 Rhodopi Mts (*Western*): along the road, *ca* 4 km NE of Eleshnitsa village, *ca* 14 km E of Razlog town, 41°53'35.0"N, 23°38'53.0"E, 910 m, 2.7. 2009.

Recorded taxon: *P. officinarum* (6*x*).

Bu 29 Rhodopi Mts (*Western*): at southwestern foothills, 1 km ESE of Eleshnitsa village, *ca* 11 km E of the Razlog town, 41°52'03.8"N, 23°36'12.0"E, 763 m, 2.7. 2009.

Recorded taxon: *P. pavichii* (2*x*).

Appendix 1. Continuation.

Bu 30 Rhodopi Mts (*Western*): at the wood margin in the valley along the road between Velingrad and Sarnitsa towns, *ca* 15 km S of Velingrad town, 41°53'35.0"N, 23°59'11.5"E, 1620 m, 1.7. 2009.
Recorded taxon: *P. bauhini* subsp. *bauhini* (6*x*);
hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*).

Bu 31 Rhodopi Mts (*Western*): in grassy places along the road near Beglika settlement, *ca* 12 km S of Rakitovo town, 41°53'03.3"N, 24°04'23.1"E, 1616 m, 1.7. 2009.
Recorded taxon: *P. officinarum* (6*x*).

Bu 32 Rhodopi Mts (*Western*): in grassy places along the road at the south of the locality Bu 31 near Beglika settlement, *ca* 12 km S of Rakitovo town, 41°52'48.8"N, 24°05'05.9"E, 1587 m, 1.7. 2009.
Recorded taxa: *P. bauhini* subsp. *bauhini* (6*x*), *P. officinarum* (5*x* and one hypopentaploid plant);
hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* << *P. bauhini* (6*x*).

Bu 33 Rhodopi Mts (*Western*): in a meadow along the road in the saddle between Velingrad and Sarnitsa towns, *ca* 15 km NNW of Sarnitsa town, 41°51'21.0"N, 23°55'49.7"E, 1503 m, 1.7. 2009.
Recorded taxa: *P. officinarum* (5*x*), *P. hoppeana* subsp. *testimonialis* (2*x*).

Bu 34 Rhodopi Mts (*Western*): in a pasture along the road between Sarnitsa and Dospat towns, 3.5 km ESE of Sarnitsa town, 41°43'25.7"N, 24°03'39.5"E, 1200 m, 1.7. 2009.
Recorded taxon: *P. hoppeana* subsp. *testimonialis* (2*x*).

Bu 35 Rhodopi Mts (*Western*): in a meadow on S shore of lake Batak, *ca* 3 km W of Batak town, 41°56'25.8"N, 24°10'29.0"E, 1125 m, 30.6. 2009.
Recorded taxa: *P. onegensis* (2*x*), *P. officinarum* (6*x*).

Bu 36 Pirin Mts (*Northern*): along the hiking trail from the uppermost lift station at Bezbog chalet to the lowermost lift station, 41°44'23.9" – 41°45'21.4"N, 23°31'42.9" – 23°31'49.0"E , 2124 – 1789 m, 3.7. 2009. This locality is identical with locality labeled Bu 11 in Krahulcová et al. (2009, p. 379).
Recorded taxa: *P. pseudopilosella* (2*x*), *P. bauhini* subsp. *magyarica* (6*x*), *P. pavichii* (2*x*), *P. hoppeana* subsp. *testimonialis*;
hybrids: *P. hoppeana* subsp. *testimonialis* × *P. pseudopilosella* (2*x*), *P. officinarum* × *P. pseudopilosella* (4*x*), *P. hoppeana* subsp. *testimonialis* × *P. officinarum* (5*x*, 6*x*) corresponding to *P. hypeurya*.

Bu 37 Pirin Mts (*Northern*): in grassy places in the wood along the hiking trail from Bezbog chalet to Dobrinishte village, near the lowermost lift station, 41°45'53.5" – 41°45'40.8"N, 23°31'28.7" – 23°32'25.3"E, 1635 – 1516 m, 3.7. 2009.
Recorded taxon: *P. pavichii* (2*x*, 4*x*);
hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*).

Bu 38 Pirin Mts (*Northern*): along the road leading from the lowermost lift station to Dobrinishte village, 3.2 km S of Dobrinishte village, 41°47'27.5"N, 23°33'45.7"E, 1080 m, 3.7. 2009.
Recorded taxa: *P. bauhini* subsp. *bauhini* (6*x*), *P. pavichii* (2*x*).

Bu 39 Northeast Bulgaria: on Madara Plateau, in grasslands between Madara village and Kaspichan town, 0.6 km SE of Kalugeritsa village, 43°17'28.4"N, 27°08'00.2" E, 284 m, 30.5. 2008.
Recorded taxon: *P. officinarum* (6*x*).

Bu 40 Northeast Bulgaria: in the southern periphery of Nevsha village, *ca* 13 km SE of Kaspichan town, 43°15'42.2"N, 27°17'58"E, 84 – 110 m, 30.5.2008.
Recorded taxa: *P. bauhini* subsp. *magyarica* (5*x*), *P. pavichii* (4*x*), *P. officinarum* (6*x*);
hybrid: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (5*x*, 6*x*) corresponding to *P. acutifolia*.
hybrid: *P. piloselloides* × *P. cymosa* corresponding to *P. ziziana*.

Bu 41 Tundzha Hilly Country: Sliven district, on S oriented dry slopes of the hill close to the road Burgas – Sofia, 1 km NE of Bliznets village, 42°38'12.8"N, 26°03'40.4"E, *ca* 259 m, 2.6.2008.
Recorded taxon: *P. bauhini* subsp. *bauhini* (6*x*), *P. bauhini* s.l. (6*x*).
hybrid: *P. echioides* × *P.* section *Pilosellina* (4*x*, 5*x*) corresponding to *P. rothiana*.

Bu 42 Balkan Range (*Eastern*): along the road 1.25 km W of Banya village, *ca* 15 km NNE of Nesebar town, 42°46'35.3"N, 27° 48'06.0"E, *ca* 238 m, 1.6.2008.
Recorded taxon: *P. bauhini* subsp. *magyarica* (6*x*).

Bu 43 Mt Strandzha, along the local road 2.4 km E of Indzhe Vojvoda settlement, 6.5 km SW of Krushevets village, *ca* 32 km S of Burgas town, 42°13'13.2"N, 27°26'53.7"E, *ca* 301 m, 1.6.2008.
Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. bauhini* subsp. *bauhini* (6*x*), *P. officinarum* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*);

Appendix 1. Continuation.

hybrids: *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (5*x*) corresponding to *P. acutifolia*, *P. hoppeana* subsp. *testimonialis* / *P. officinarum* × *P. bauhini* (6*x*) corresponding to *P. leptophyton*.

Romania (plants collected by *F. Krahulec* and *J. Hadinec* in 2009, by *F. Krahulec*, *A. Krahulcová*, *Z. Skála* and *H. Skálová* in 2014, determined by *S. Bräutigam* and *F. Krahulec*):

Ro 1 Banat, Gârnic, along the track in the northern periphery of the village, 44°45'45"N, 21°47'22"E, 560 m, 25.4.2014.

Recorded taxon: *P. bauhini* subsp. *bauhini* (6*x*).

Ro 2 Banat, on limestone rock along the road between Moldova and Gârnic villages, *ca* 2.8 km WSW of Padina Matei settlement, 44°44'44.1"N, 21°43'06.8"E, 300 m, 4.6.2009.

Recorded taxon: *P. bauhini* subsp. *magyarica* (6*x*).

Ro 3 Banat, along the road in the valley, 3.6 km SE of Gârnic village, 44°43'23.6"N, 21°48'50.1"E, 244 m, 4.6.2009.

Recorded taxon: *P. bauhini* subsp. *magyarica* (6*x*);

hybrid: *P. bauhini* × *P. cymosa* corresponding to *P. densiflora* (6*x*).

Ro 4 Banat, in a wet meadow with *Orchis coriophora* and *O. laxiflora* in the valley, 4.3 km SE of Gârnic village, 44°43'11.9"N, 21°49'17.0"E, 160 m, 4.6.2009.

Recorded taxon: *P. onegensis* (2*x*).

Ro 5 Banat, Sfânta Elena, along the path leading from the village to river Danube, above the bankside road, *ca* 1.8 km S of the church, 44°39'41"N, 21°42'26"E, 90 m, 26.4.2014.

Recorded taxon: *P. bauhini* subsp. *bauhini* (4*x*).

Ro 6 Banat, along the road 3.8 km NE of Dubova village, *ca* 12 km SW of Orsova town, 44°38'52.0"N, 22°17'16.4"E, 95 m, 5.6.2009.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*).

Ro 7 Banat, on a grassy slope above the local road, 2 km ESE of Cozla settlement, *ca* 6.5 km ESE of Berzasca village, 44°37'18.7"N, 22°01'29.4"E, 160 m, 3.6.2009.

Recorded taxon: *P. bauhini* subsp. *magyarica* (6*x*).

Ro 8 Banat, in a pine grove (on conglomerate base) along the main road close to river Danube, between Gura Vaii village and Drobeta-Turnu Severin town, *ca* 5 km W of Drobeta-Turnu Severin town, 44°38'09.6"N, 22°35'14.7"E, 62 m, 6.6. 2009.

Recorded taxon: *P. bauhini* subsp. *magyarica* (6*x*).

Ro 9 Banat, in the wood along the nature trail from Ponicova settlement to Ciucari Mare rocky plateau, *ca* 1.4 km S of Dubova village, 44°36'34"N, 22°15'18"E, 250 m, 22.4.2014.

Recorded taxon: *P. bauhini* subsp. *bauhini* (6*x*).

Ro 10 Banat, in a meadow in the western periphery of Ciucari Mare rocky plateau, *ca* 1.5 km S of Dubova village, 44°36'31"N, 22°15'28"E, 300 m, 22.4.2014.

Recorded taxon: *P.* cf *officinarum* (6*x*).

Ro 11 Banat, on shrubby and rocky slope along the hiking trail to Mt Trescâvât, *ca* 7.3 km NNW of Svinita village, 44°33'37.3"N, 22°03'40.5"E, 484 m, 21.4.2014.

Recorded taxa: *P. officinarum* (6*x*), *P. pavichii* (2*x*);

hybrid: *P. officinarum* × ? (6*x*).

Ro 12 Banat, in the wood along the hiking trail to Mt Trescâvât, *ca* 5.5 km NW of Svinita village, 44°32'39"N, 22°04'18"E, 115 m, 21.4.2014.

Recorded taxon: *P. cymosa* subsp. *sabina* (4*x*);

hybrid: *P. cymosa* subsp. *sabina* × *P. pavichii* (3*x*).

Ro 13 Banat, Eibenthal, in fallow land above the new road leading from the village to river Danube, 0.9 km ENE of the church, 44°32'48"N, 22°10'17"E, 440 m, 20.4.2014.

Recorded taxa: *P. bauhini* subsp. *magyarica* (6*x*), *P. hoppeana* subsp. *testimonialis* (2*x*).

Ro 14 Banat, on small rocks (exposed to southeast) in the wood on the west side of the valley, along the road 2.8 km ESE of Eibental village, *ca* 26 km SW of Orsova town, 44°32'13.4"N, 22°11'28.1"E, 235 m, 2.6.2009.

Recorded taxon: *P. pavichii* (2*x*).

Ro 15 Banat, on small rocks (exposed to southeast) in the wood on the east side of the valley, along the road 4.2 km E of Eibental village, *ca* 25 km SW of Orsova town, 44°32'32.6"N, 22°12'39.5"E, 103 m, 2.6.2009.

Recorded taxa: *P. pavichii* (2*x*), *P. cymosa* subsp. *sabina* (4*x*).

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